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https://orcid.org/0000-0002-3020-8430

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Paulius Tušas

Analysis of Physicochemical and Biological Characteristics of Flowable Hydraulic Tricalcium Silicate-Based Root Canal Filling Materials

DOCTORAL DISSERTATION

Medicine and Health Sciences,
Odontology (M 002)

VILNIUS 2024

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Academic Supervisor – Prof. Dr. Vytautė Pečiulienė (Vilnius University, Lithuania, Medicine and Health Sciences, Odontology, M 002).

Academic Consultant – Prof. Dr. Josette Camilleri, B.Ch.D., M.Phil., PhD., FICD, FADM., FIMMM, FHEA (University of Birmingham, United Kingdom, Medicine and Health Sciences, Odontology, M 002).

This doctoral dissertation will be defended in a public meeting of the Dissertation Defence Panel:

Chairman – **Prof. Dr. Vygandas Rutkūnas** (Vilnius University, Lithuania, Medicine and Health Science, Odontology, M 002).

Members:
Prof. Dr. Jolanta Aleksejūnienė (University of British Columbia, Canada, Medicine and Health Sciences, Odontology, M 002),
Prof. Dr. Daiva Baltriukienė (Vilnius University, Lithuania, Natural Sciences, Biochemistry, N 004),
Prof. Dr. Vilma Brukienė (Vilnius University, Lithuania, Medicine and Health Sciences, Odontology, M 002),
Prof. Dr. Vita Mačiulskienė-Visockienė (Lithuanian University of Health Sciences, Lithuania, Medicine and Health Sciences, Odontology, M 002).

The dissertation shall be defended at a public meeting of the Dissertation Defence Panel at 14:00 on the 20th of June 2024 in the Main meeting hall of the Vilnius University Hospital Žalgiris Clinic.
Address: Žalgirio str. 117, LT-08215 Vilnius, Lithuania.
Tel. +370 5 2727589; email: mf@mf.vu.lt

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VILNIAUS UNIVERSITETAS

Paulius Tušas

Takių hidraulinių kalcio silikatinių dantų šaknų kanalų užpildų fiziko-cheminių ir biologinių savybių analizė

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Mokslinė vadovė – **Prof. dr. Vytautė Pečiulienė** (Vilniaus universitetas, Lietuva, medicinos ir sveikatos mokslai, odontologija, M 002).

Mokslinė konsultantė – **Prof. dr. Josette Camilleri**, B.Ch.D., M.Phil., Ph.D., FICD, FADM., FIMMM, FHEA (Birmingamo universitetas, Jungtinė Karalystė, medicinos ir sveikatos mokslai, odontologija, M 002).

Gynimo taryba:
Pirmininkas – **Prof. dr. Vygandas Rutkūnas** (Vilniaus universitetas, Lietuva, medicinos ir sveikatos mokslai, odontologija, M 002).

Nariai:
Prof. dr. Jolanta Aleksejūnienė (Britų Kolumbijos universitetas, Kanada, medicinos ir sveikatos mokslai, odontologija, M 002),
Prof. dr. Daiva Baltriukienė (Vilniaus universitetas, Lietuva, gamtos mokslai, biochemija, N 004),
Prof. dr. Vilma Brukienė (Vilniaus universitetas, Lietuva, medicinos ir sveikatos mokslai, odontologija, M 002),
Prof. dr. Vita Mačiulskienė-Visockienė (Lietuvos sveikatos mokslų universitetas, Lietuva, medicinos ir sveikatos mokslai, odontologija, M 002).

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Adresas: Žalgirio g. 117, 08215 Vilnius, Lietuva,
tel. +370 5 2727589; el. paštas mf@mf.vu.lt

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THE LIST OF ABBREVIATIONS

2D	– Two Dimensional
3D	– Three Dimensional
$\%OP_{VOL}$	– The Percentage Volume of Open Pores
$\%CP_{VOL}$	– The Percentage Volume of Closed Pores
µCT	– Micro-computed Tomography
ANOVA	– Analysis of Variance
CP_{VOL}	– Volume of Closed Pores
C_{VOL}	– Volume of Root Canal
DMSO	– Dimethyl Sulfoxide
ED	– Endodontology Specialist
EDTA	– Ethylenediaminetetraacetic Acid
fHCSC	– flowable Hydraulic Calcium Silicate-based Cement
F_{VOL}	– Volume of Filling Material
GDP	– General Dental Practitioner
GM	– Growth Medium
HCSC	– Hydraulic Calcium Silicate-based Cement
hDPSCs	– Human Dental Pulp Stem Cells
MF	– *MTA Flow*^{TM}
MFWhite	– *MTA Flow*^{TM} *White*
MTA	– Mineral Trioxide Aggregate
MTT	– 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
NegativeCG	– Negative Control Group
SC	– Single Cone
OP_{VOL}	– Volume of Open Pores
PI	– Propidium Iodide
PositiveCG	– Positive Control Group
ProRootCG	– Control Hydraulic Calcium Silicate-based Cement *ProRoot*® *MTA* group
PS	– Postgraduate Student
RPM	– Revolutions per Minute
UA	– Ultrasonic Agitation
US	– Undergraduate Student
V_{OI}	– Volume of Interest
V_{VOL}	– Volume of Voids/Pores
WL	– Working Length

CONTENTS

INTRODUCTION

Relevance of the study

Apical periodontitis is an inflammatory response related to pathogens and their toxins occupying the root canal system. [1] As apical periodontitis is usually asymptomatic, its diagnosis is mainly based on radiographic examination. [2] The worldwide prevalence of at least one tooth affected by apical periodontitis has been reported to range from 16% [3] to 86% [4,5]. A recent systematic review and meta-analysis revealed a high global prevalence of apical periodontitis: 52% of adults worldwide have at least one tooth affected by apical periodontitis. [6] Apical periodontitis was associated with several factors, such as country of residence, systemic diseases, radiographic examination for diagnostic purposes, and root canal filling. [6–8]

After detecting apical periodontitis, despite its primarily asymptomatic state, the goal is to perform a root canal treatment or retreatment, if necessary with surgical endodontic approaches, to promote the healing of periapical bone. [9] The complete resolution of apical periodontitis after initial endodontic treatment ranges from 85 to 97% [10,11] or retreatment from 74 to 82% [12–15], while the functionality of the treated teeth reaches 91-97%. [16,17] As root canal filling was shown to be a significant factor in the prevalence of apical periodontitis, the root canal treatment procedure is performed every day worldwide to retain natural teeth. A recent meta-analysis revealed the global prevalence of adult people with at least one root canal-filled tooth is 55.7%. [18]

Endodontic treatment aims to clean, disinfect, shape and seal the root canal system in three dimensions to eliminate or prevent (re)infection. [19] The quality of root canal obturation plays an essential role in ensuring the successful long-term outcome of endodontic treatment. [20] Studies show that approximately 60% of endodontic treatment failures can be associated with poor root canal obturation. [21] Root canals are considered adequately obturated if the filling material is uniformly homogeneous over the entire root canal working length and has an appropriate conical taper resembling the root canal's internal shape after preparation. [9] High-quality hermetical sealing of the root canal system should prevent the obturated root canals from recontamination, isolate remnant microorganisms, and prevent microorganisms and their metabolites from infecting periapical tissues by promoting periapical healing. [22] It has been shown that the homogeneity of the filling is directly related to the voids and pores inside the material. [23] Therefore, the materials' porosity can be used as one of the objective criteria for assessing the quality of the root canal obturation.

Various materials and filling techniques were suggested for root canal obturation to achieve hermetic, voids-free fillings and prevent microleakage. [24] However, it has been demonstrated that there is no technique for root canal obturation which can ensure the impeccable three-dimensional sealability of the root canal system. [25,26] It has been shown that the highest percentages of voids and poorer adaptation of the material to the root canal walls and irregularities are detected in the fillings when the cold lateral compaction technique was used for obturation. [25,27] Some studies demonstrated no advantages of the thermoplastic gutta-percha obturation techniques over cold lateral compaction in terms of the porosity of the fillings. In contrast, other studies claim that thermoplastic techniques ensure less porous fillings. [28,29] However, one of the main reasons that can lead to the formation of pores and voids in the softened gutta-percha mass is the materials' shrinking when they cool down. [30] Besides, it should be highlighted that the lateral compaction technique is complicated and time-consuming for the operator. In contrast, thermoplastic obturation techniques require expensive armamentariums and devices for obturation. [21,24]

Nowadays, the most widely suggested root canal filling, perforation closure and vital pulp therapy materials are hydraulic calcium silicate-based cement (HCSC) or sealers/fillers. [31,32] Primary HCSC materials have significant drawbacks, such as the final setting time taking more than an hour, which is too long clinically to wait, requiring the application of a protective provisional restoration that is afterwards replaced by a definitive restoration following visit [33,34], poor wash out resistance [35], primary dimensional stability [36], tooth colour changes [37] and problematic usage. [38] Nevertheless, these materials are highly biocompatible and bioactive and possess antibacterial activity, low solubility, shrinkage and long-term dimensional stability, enabling a higher volume of the sealer in conjunction with tapered gutta-percha points. [39–41] As a result, new generation Type IV and Type V formulations of HCSC were developed with improved characteristics: shorter setting time, premixed and improved consistency to be more conveniently used clinically, retaining comparable biological and bioactive properties. Therefore, newly introduced flowable hydraulic calcium silicate-based cement/fillers, such as *MTA Flow*^{TM} and *MTA Flow*^{TM} *White* (Ultradent Products Inc., South Jordan, UT, USA), must be tested *in-vitro* and *in-vivo* to recommend the usage of the new generation materials or even their usage over the widely tested older generations HCSC materials.

The simplified single cone (SC) root canal obturation technique, when a single gutta-percha point in conjunction with hydraulic calcium silicate-based sealer is used to fill the root canals, is rapidly gaining popularity among

clinicians. [42] In contrast to the previously discussed obturation methods, the SC obturation technique is simple, does not require a long learning curve, is clinically appealing, and requires no additional armamentarium or devices. During the obturation, no lateral or vertical compaction is used, minimising the risk of creating dentinal defects or cracks. [43] Numerous studies have demonstrated that the overall porosity of the SC fillings is comparable to or even less than that of other techniques. [26,27,44] Moreover, the hydraulic calcium silicate-based sealers used with a SC technique have enhanced adhesion to the root canal dentin compared to different sealers and ensure a dimensionally stable, tight, hermetic seal. [45,46] Additionally, due to the superior flowability, hydrophilicity and small particle size of the material, the excellent penetrability of the sealers can be achieved even without condensation pressure. [43] Recent clinical studies demonstrated the high clinical success rates when the SC technique was used for root canal obturation in cases of primary endodontic treatment or endodontic retreatment. [47,48]

It has been concluded that the success rates of endodontic treatment highly depend on the quality of root canal shaping, cleaning and eradication of the microorganisms. [20,21,39] Moreover, the impact of the root canal obturation quality and the operators' clinical experience has also been demonstrated. [24,48,49] The clinical success rates when a specialist endodontist performs treatment procedures can reach more than 90% or 95%. In contrast, in cases where general practitioners perform root canal treatment, the probability of clinical success can decrease even to 40–65%. [50–53] Moreover, an even lower success rate is observed when undergraduate students complete endodontic treatment. [54] Eskandarloo et al. (2017) demonstrated that dental students' quality of root canal obturation, detected on the periapical radiographs, is commonly poor and unacceptable. [55] Other investigations revealed that only 30.1–47% of the root canals obturated by undergraduate dental students are under acceptable quality standards. [51,56] Therefore, students in a postgraduate endodontic program achieved better obturation quality than undergraduates. [51] However, it has been claimed that the recently introduced simplified single cone root canal obturation technique potentially can be less related to the clinical experience of the operator. [26,57] Despite the simplicity and clinical appeal of the SC obturation technique, there is no information on how the clinical experience of the operator can determine the root canal obturation quality and porosity of these fillings.

Moreover, ultrasonic devices have been successfully used in endodontics for various clinical procedures, including root canal obturation. [58,59] It has been reported that ultrasonication of the sealers during the root canal filling procedure may increase their penetrability into the dentinal tubules and

improve the interfacial adaptation between the filling material and the root canal wall. [60,61] Additionally, ultrasonic energy can rearrange the material particles and remove the entrapped air, thus reducing the porosity. [62,63] Therefore, ultrasonic agitation has been recommended to improve the quality and homogeneity of root canal fillings. [63,64] However, most of the previous research has investigated the effect of ultrasonic agitation, applied to the sealers indirectly, and there are still no data available on the porosity distribution within the HCSC materials used in conjunction with gutta-percha point as SC technique and HCSC materials root canal fillings after the use of direct ultrasonication.

Finally, biological characteristics are one of the main advantages of HCSC materials. When in contact with tissues, these materials are set in a vet environment, forming hydroxyapatite and releasing calcium hydroxide as a by-product. [65–69] HCSC materials release ions long after setting and are the only materials ensuring continuous release in contact with moist dentine and bone. [70] The biological nature of HCSC promotes the proliferation and differentiation of cells. It demonstrates the formation of mineralised tissues during the development of the dentinal bridge in direct pulp capping [69,71] or hard-tissue barrier formation in the case of apical plug placement. [72,73] Most HCSC can be highly biocompatible, promoting pulp healing at minimal pulp inflammation or periapical bone structure healing. While the variation in methodology limits comparisons across studies, nearly all HCSCs seem to have favourable effects on hDPSCs. Studies have found comparable, but not superior, biological properties of some of the new generation – Type IV and Type V materials compared to the „gold standard“ *ProRoot® MTA White*. [67,74] A systematic review in 2018 concluded that all commercially available HCSCs are biocompatible, exhibit comparable and favourable effects on odontogenic differentiation of dental pulp cells *in-vitro* and can efficiently enhance dentin bridge formation with minimal inflammation. [74] However, another systematic review and meta-analysis in 2020 revealed that hDPSC viability when affecting the HCSC and bioactivity of HCSC differ depending on the tested material *in-vitro.* [75] The latter review suggested that original commercial resin-free hydraulic calcium-silicate cement, such as *ProRoot® MTA*, is the best option to provide a complete reparative bridge upon vital pulp therapy due to the vast *in-vitro* and long-term clinical studies. [75–77] On the other hand, other reviews could not recommend one specific material over the others. [74,78–80] Therefore, care should be taken when new materials are introduced into the market and are clinically applied in patients, as small changes in the materials' composition might significantly impact their clinical efficacy. As a first step, biological characteristics, such as cytotoxicity and

created cell proliferation analysis of current new flowable hydraulic calcium silicate-based materials is essential to use these new materials in clinical practice, for a rationale to use one material over the other and, as a result, to produce a reliable scientific background for future *in vivo* studies.

Aim of the study

Aim:

To evaluate physicochemical and biological properties of flowable hydraulic tricalcium silicate-based root canal filling materials and quality of root canal filling *in vitro*.

Objectives of the research

Objectives:

1. To evaluate and compare the quality of root canal obturation with flowable hydraulic calcium silicate-based material (BioRoot® RCS, Septodont, Saint-Maur-des-Fosses, France) used in single cone technique between undergraduate and postgraduate dentistry students, general practitioners and endodontology specialists.
2. To evaluate the quality and influence of activation methods/root canal morphology on root canal obturation with flowable calcium silicate-based materials used in single cone technique (BioRoot® RCS, Septodont, Saint-Maur-des-Fosses, France) or as apical plugs (MTA Flow™, Ultradent Products Inc., South Jordan, UT, USA) for the apical perforation repair in curved canals of extracted mandibular molars.
3. To evaluate the biological properties of flowable hydraulic calcium silicate-based cement (MTA Flow™ and MTA Flow™ White (Ultradent Products Inc., South Jordan, UT, USA) thick consistency) compared to the gold standard Portland cement-based HCSC (ProRoot® MTA White, Dentsply, Tulsa, OK, USA) on human dental pulp stem cells (hDPSCs) *in vitro*.

Novelty and significance of the study

This *in-vitro* study investigates the synergy of newly introduced root canal filling/dental pulp treatment methodologies and new generation Type IV dental HCSC materials in endodontology. The objectives of the study combine three main subjects which hold significant relevance in root canal filling/dental pulp treatment: the biocompatibility of new-generation HCSC dental materials, the impact of ultrasonic activation on HCSC and the resulting

quality of root canal fillings within clinicians with various clinical experience. These areas are relevant not only at the initial stages, as the root canal filling procedure, to produce hermetic, homogenous, and biocompatible root canal filling but also in ensuring the stability of the obturation and, as a result, reaching for the best possible long-term clinical outcome. The study highlights the rationale of analysing freshly mixed HCSC, their biological features as well, and the timesaving and ease of use of HCSC materials in root canal filling while at the same time endeavouring for advanced root canal filling methodologies which enable healing of periapical/dental pulp tissues.

In the current research articles, the evaluation and comparison of root canal obturation methodologies strongly depend on the research methodology and root canal filling material used, as one root canal filling technique may be superior and vice-versa. Nonetheless, an improved clinical outcome of the root canal (re)treatment, the versatility of the materials to be used in root canal filling and vital pulp treatment and the timesaving repeatability of the procedure in daily practice for all dental practitioners are what the new HCSC materials are aiming at. Due to the lack of comparison between differently clinically experienced operators, this study significantly complements the standardised evidence in the 3D μCT analysis of the root canal fillings depending on the operators' clinical experience. Most importantly, this study assesses various root canal filling quality factors, such as the total porosity of the filling material, dividing into open and closed pores distribution. Moreover, the currently trending single-cone root canal obturation technique with HCSC sealers/fillers created root canal fillings are compared to only HCSC fillings in 3D μCT analysis. Furthermore, the additional clinical step in root canal obturation protocol, such as ultrasonic activation on HCSC materials, is evaluated, enhancing the rationale for including or eliminating specific clinical steps and creating an evidence-based clinical protocol for dental clinicians.

New dental HCSC materials coming into the market urge the need for independent and thorough analysis as even minor changes in the materials' composition can significantly affect not only physicochemical and biological properties but ultimately lead to the different clinical behaviour of the HCSC and as a result, possibly implementing the clinical outcome. Therefore, this study also evaluates the biological properties of the new generation flowable HCSC in terms of human dental pulp stem cells. One of the key features contributing to the novelty of this study is the inclusion of freshly mixed HCSC into laboratory biological analysis and long-term proliferation analysis of hDPSCs. The primary cytotoxicity of the freshly mixed HCSC on hDPSCs reflects cell response to still hardening obturation materials, as this concept is

clinically relevant instead of analysing already set and less biointeractive HCSC materials. In addition to the previous publications, the new generation freshly mixed flowable Type IV HCSC materials are compared to the gold standard, widely analysed Type I HCSC material in terms of primary cytotoxicity to hDPSCs, flow cytometry and cell morphology analysis. This comprehensive approach to the biological properties of flowable HCSC allows for a more thorough understanding of the influence of the materials' composition compared to other HCSC. At the same time, the completed analysis provides for the decision of whether the new generation flowable HCSC materials can be recommended for use in daily clinical practice.

The study focuses on new-generation flowable HCSC materials in relation to the improved root canal filling quality between dental practitioners with different clinical experiences when analysed in accurate 3D μCT analysis while maintaining similar biological properties compared to primary HCSC. This research provides significant and repeatable results on HCSC materials while establishing their usage in providing evidence-based clinical protocols for dental practitioners.

Statements to defend

1. No operator or filling method can ensure void-free root canal filling when using the SC obturation technique in conjunction with hydraulic calcium silicate-based cement.
2. The quality and homogeneity of SC root canal fillings are similar between dentistry students and dentists with different clinical experiences.
3. Flowable HCSC *MTA Flow*^{TM} thin mixture is characterised by high porosity in all specimens compared to the SC and BioRoot® RCS HCSC technique.
4. Direct ultrasonic activation significantly increases porosity when filling root canals with *MTA Flow*^{TM} HCSC materials.
5. The flowable hydraulic calcium silicate-based materials *MTA Flow*^{TM} and *MTA Flow*^{TM} *White* significantly influence hDPSC viability, proliferation, and morphology.
6. *The biocompatibility of new-generation flowable HCSC materials MTA FlowTM and MTA FlowTM White is comparable to gold-standard HCSC ProRoot® MTA White.*

Original publications (Clarivate Analytics Web of Science):

1. Drukteinis S, Bilvinaitė G, Tušas P, Shemesh H, Pečiulienė V. Porosity Distribution in Single Cone Root Canal Fillings Performed by Operators with Different Clinical Experience: A microCT Assessment. J Clin Med. 2021 Jun 10;10(12):2569. doi: 10.3390/jcm10122569. PMID: 34200692; PMCID: PMC8230067.
2. Drukteinis S, Bilvinaitė G, Tušas P, Shemesh H, Peciuliene V. Microcomputed Tomographic Assessment of the Single Cone Root Canal Fillings Performed by Undergraduate Student, Postgraduate Student and Specialist Endodontist. J Clin Med. 2021 Mar 5;10(5):1080. doi: 10.3390/jcm10051080. PMID: 33807655; PMCID: PMC7961753.
3. Drukteinis S, Bilvinaitė G, Shemesh H, Tušas P, Pečiulienė V. The Effect of Ultrasonic Agitation on the Porosity Distribution in Apically Perforated Root Canals Filled with Different Bioceramic Materials and Techniques: A μCT Assessment. J Clin Med. 2021 Oct 27;10(21):4977. doi: 10.3390/jcm10214977. PMID: 34768498; PMCID: PMC8584978.

Conference abstracts published:

1. P. Tušas, J. Camilleri, S. Drukteinis, V. Pečiulienė. Characterisation of MTA Flow. Volume 52, Issue S1. Special Issue: European Society of Endodontology: Abstracts from the Biennial Congress 2019, 11-14 September 2019, Austria Center Vienna, Austria. https://doi.org/10.1111/iej.13172 (R075)
2. P. Tušas, M. Alksnė, J. Camilleri, S. Drukteinis, E. M. Jonaitytė, V. Bukelskienė, V. Rutkūnas, V. Pečiulienė. Cytotoxicity of Flowable Hydraulic Calcium Silicate-based Cement Leachates. The International Association for Dental Research (IADR)/CED Oral Health Research Congress. Brussels, Belgium. September 16-18, 2021. (0093). https://ced-iadr2021.com/abstract-book/

Oral presentations:

1. P. Tušas. Usage of hydraulic calcium silicate-based cement in clinical practice. Conference ‘Odontologijos Aktualijos‘. Stomatologija BD&MJ. 2022, Vilnius, Lithuania.
2. P. Tušas. Hydraulic calcium silicate-based cement: what can we do differently than before? Conference ‘EndoBlatic 2022‘ Lithuanian Society of Endodontology, 2022, Vilnius, Lithuania.

3. P. Tušas. Application of flowable hydraulic calcium silicate-based cement for root canal obturation. Conference ‘Praktinė Odontologija‘, LR Odontologų Rūmai. 2023, Kaunas, Lithuania.
4. P. Tušas. Can flowable hydraulic calcium silicate-based cement be applied in different clinical scenarios? Conference ‘Genialumas Paprastume’, Gydytojų Odontologų Draugija, 2023, Druskininkai, Lithuania.
5. P. Tušas. Usage of Flowable Hydraulic Calcium Silicate-Based Cements in Root Canal Filling Procedures. Conference ‚Klasikinės ir moderrnios odontologijos naujovės‘, VšĮ Odontologijos Studija, 2024, Vilnius, Lithuania.

1. LITERATURE REVIEW

1.1. History and terminology of hydraulic silicate cement materials

The first reported use of hydraulic cement root canal filling material dates back to 1878 when Dr. Witte in Germany used Portland cement to fill root canals [68]. Over a century later, in 1993, Dr. Mahmoud Torabinejad and Dean White at Loma Linda University obtained two patents for a Portland cement-based endodontic material, which became later known as mineral trioxide aggregate (MTA). [81,82]

MTA has rapidly gained acceptance in dentistry since its introduction in 1993. It was given approval by the US Federal Drug Administration for endodontic applications on 1997 February 10th and classified as 'root canal filling resin material' to 'be applied to a tooth to protect the pulp' and became commercially available as ProRoot MTA (Dentsply, Tulsa, OK, USA). [83] In 1998, the material's indications were broadened by adding the approval to use it as an apical plug during apexification, repair of root perforations, treatment of internal resorption and root-end filling material. [84,85] Also, the latter approvals stated that 'biocompatibility studies with the formulation are not necessary' since the material formula is identical to previously patented and approved MTA. [84,85]

MTA immediately gained high expectations with promising results for sealing root canal perforation defects with an excellent long-term prognosis. This material was even called a new material for the new millennium. [86] From 1993 to 1999, the first laboratory studies started on MTA analysis. For example, the sealing ability of MTA for the repair of lateral root perforations was compared to previously used amalgam and IRM fillings for perforations closure, where the MTA showed significantly less leakage. [87] A new root-end filling material's physical, chemical, and biological properties were analysed. [88–90] Finally, it discusses the clinical applications of MTA material by reviewing it as a biocompatible and bioactive material, preventing microleakage. [91] It was shown that the material promotes the regeneration of the tissues when placed in contact with the dental pulp or periradicular tissues. [92] The use of the material was also analysed in clinical procedures for application in pulp capping, apexification, non-surgical and surgical repair of root perforations and as root-end filling material. [92,93]

Until 2005, ProRoot MTA (Tulsa Dental Products, Tulsa, OK, USA) used two commercial forms in grey or white (tooth-coloured). [94] Since the approved patent restrictions expiry in 2013, many new materials with similar chemical properties have been introduced. Based on the results of already available

ProRoot MTA, the Brazilian company Angelus (Odonto-Logika, Ind. Prod. Odont. Ltda, Londrina, Parana, Brazil) chose a Portland cement with minimal arsenic content, bismuth oxide as a radio pacifier and this material became commercially available as MTA-Angelus (Angelus Solucoes Odontologicas, Londrina, Brazil). [95] It was one of the first materials to become commercially available after the original ProRoot MTA.

With more MTA-like materials coming into the market, no generic term for this class of materials appeared to be used. The original trade name 'Mineral trioxide aggregate' (MTA) appeared in scientific articles, books and other resources. The term 'MTA' was applied for a dental material containing Portland cement blended with a radiopaque additive. The similarity of MTA to ordinary Portland cement is not accidental; it is both the inspiration and preliminary trial material. However, Portland cement cannot be used in dental clinical practice due to concerns about its heavy metal constituents, inadequate radio-opacity, comparatively significant setting expansion, broad particle size distribution and relatively high solubility. Also, it does not have governmental approval for clinical purposes. [81] Since Portland cement is composed of three oxides: calcium, silica and alumina (CaO, SiO_2 and Al_2O_3), aggregation phases of the material emerge from minerals as the sources for the oxides, and the addition of the radiopaque powder makes the material as aggregate. Therefore, the primary term 'Mineral trioxide aggregate' seemed eligible. [68] However, due to the coherence of the term to its constitution, the term 'MTA' applied only to Portland-based dental cement.

Portland cement has been suspected to contain undesirable contaminating substances, one of which was thought to be arsenic, and thus is undesirable for use in humans. The first studies analysing the composition of Portland cement and MTA-like materials did not mention the presence of an arsenic component. [96,97] In contrast, later studies revealed that Portland cement and MTA showed evidence of heavy metal inclusion in the acid-soluble form and released in water and a physiological solution. [98–100] MTA contained levels of arsenic higher than the safe limit specified by international standards. [98,99] However, it is not known if the arsenic released in solution is detrimental to the health of the host because the ISO standards only specify the limits for the total arsenic content and not for the released species [98], thus demonstrating no contraindication for the use of these materials in clinical practice in terms of the presence of this chemical element. [101] However, new materials were coming into the market to overcome the drawbacks of the material, and with new materials, novel terms were presented. One of which was the introduction of the term 'bioceramic' into endodontic cement terminology. The first paper to mention bioceramics in endodontics relates to the laboratory-synthesized

water-based cement material called ‘BioAggregate’ (BioCeramix Inc, Vancouver, Canada), analysing its effects on cell viability. [102] The patent also refers to this invention as a bioceramic. [103] Since then, there has been some confusion with the material's nomenclature as bioceramics. In general, the term ‘bioceramics’ indicates engineered materials that are inorganic and nonmetallic and find their applications in medicine. [104]

In dentistry, the term bioceramics can be interpreted as a broader definition of all MTA-like cement, and it is related to a new constitution of the material and lack of aluminium in its composition. [105] The first articles present the clinical applications of the same materials. [106,107] Soon enough, the term ‘bioceramic’ became very popular among dental root canal filling materials manufacturers and dental clinicians. Also, bioceramics are sometimes misused to refer to all MTA-like cement, but his term is generally vague. It does not describe the chemistry and clinical behaviour of the current materials. [108]

The need for a general term for this group of materials standardisation purposes is crucial. Therefore, MTA-like cements were suggested to be classified as ‘Hydraulic Silicate Cement‘ (HSC) or ‘Hydraulic silicate’. [65] The essential features of HSC are based on their specific chemistry and constitution [109], the hydration setting reaction in wet environments [110] and hydraulic properties. [69] Therefore, the term ‘Hydraulic silicate cement’ is appropriate to adapt since it enhances the main aspects of the material.

Hydraulic cement is a group of materials that hydrate in contact with water and interact with environmental fluids, such as blood, tissue fluid, dentine, bone, irrigating solutions, and other restorative materials. [111] The term hydraulic derives from the Greek ‘hydra’, meaning water. [108] Most hydraulic cement used in endodontic procedures is based on tricalcium silicate. [112] Although hydraulic cement is assumed to be all calcium silicates, other types of hydraulic cement are currently in use. [108] For example, Calcium aluminate is a hydraulic cement. However, the material hydration process differs from the hydraulic calcium silicate cement. [113] Therefore, it has been suggested that MTA and MTA-like cement should be classified as ‘Hydraulic Calcium Silicate Cement’ (HCSC). [65] Recently, a classification of hydraulic cement used in dentistry was published, categorising them based on their chemistry and clinical use. [111]

1.2. Hydraulic calcium silicate-based root canal filling materials classification

Hydration, behaviour, and biological and mechanical properties of the hydraulic calcium silicate cement rely on its constitution and chemistry. [108,112]

Also, the material presentation depends on whether the material is supplied as a powder to be mixed with water or suspended in a non-aqueous vehicle. This property is related to the water resource necessary for hydration. Since significant hydraulic silicate cement modifications were developed during the past decades and many materials were introduced into the market, the systemic and precise distribution of hydraulic silicate cements was mandatory. Therefore, Dr. Josette Camilleri introduced a new classification system according to their clinical context and constitution in 2020. [111] Hydraulic calcium silicate cements were classified into five types based on their chemistry: cement base, vehicle, and modifiers. Classification of dental hydraulic calcium silicate-based materials are shown in Figure 1. Examples of commercially available HCSC endodontic materials are shown in Table 1, Table 2, and Table 3, while temporary HCSC-based endodontic materials are presented in Table 4.

- Type I – materials based on Portland cement, with a radiopacifier and no additives included. The first MTA material was available as a fine powder to be mixed with sterile or distilled water for a water-to-powder mass ratio of 0.35mL/g for ProRoot MTA (Dentsply, Tulsa, OK, USA) to be mixed on a sterilised glass slab for 30–60 s to obtain a "sandy" consistency. The only material is the original ProRoot MTA, one of the most laboratory and clinically-tested dental hydraulic cement materials. The material has been patented as composed of ASTM (American Standards for Testing Materials) Type I Portland cement (PC) with a 4:1 addition of bismuth oxide for radio opacity. [81] Both products, MTA and Portland cement, are similar. However, they are not equal in composition and exhibit noteworthy differences. Portland cement has been reported to contain more heavy metals, such as manganese and strontium, than MTA, which may induce rejection, inflammation, or allergic reactions. (54) Therefore, the well-considered structure and composition differences made MTA-like cements suitable for clinical dentistry.

- Type II – materials based on Portland cement, with a radiopacifier. Specific additives are included, which can aim at setting time reduction, like 2% calcium chloride [38], which is a hydration reaction accelerator, like in MM-MTA (Coltene µMega, Besancon, France). Methylcellulose is used as an anti-washout ingredient [38] , while calcium carbonate alters mechanical properties [114]. Calcium oxide in MTA Angelus (Angelus, Londrina, Brazil) increases the early release of calcium hydroxide. [115] Since the introduction of MTA Angelus in 2002, the primary setting time of the material was changed from 2.5 hours to 15 minutes by reducing calcium sulphate, which increases the setting time and by including

modifiers to achieve the shorter primary hardening of the material. Hydroxyapatite can enhance bioactivity [116], as in Bio MTA+ (Cerkamed, Stalowa Wola, Poland).

- Type III - materials based on Portland cement and water replacement by modified vehicle, with or without a radiopacifier. In 2017, Angelus company presented MTA Repair HP (Angelus, Londrina, Brazil). The material was changed from MTA Angelus by removing Bismuth oxide as a radiopacifier. Water was replaced with a specific liquid and organic plasticiser to improve material manipulation characteristics, such as increasing plasticity. One of the materials is EndoSeal MTA (Gangwon-do, South Korea), a pozzolan-containing calcium silicate-based material. Portland pozzolana cement is a variation of ordinary Portland cement, with fly ash and volcanic ash added as modifiers. In other materials, the vehicle can also be replaced by resin, such as salicylate resin. One of which is MTA-Fillapex. The material is presented as an auto-mix two paste, with a 13% calcium silicate charge in the product. One paste consists mainly of the base resin, silicone oxide and titanium dioxide, while the other includes salicylate resin, bismuth oxide as a radiopacifier and silicon oxide. [117,118] MTA Fillapex was among the first root canal sealers based on MTA. Several publications report the physical [119–121], chemical, and biological [122–124] properties of MTA Fillapex, which are close to MTA. However, the reported biocompatibility is lower than pure hydraulic calcium silicate sealers [125,126], and the cytotoxicity of the MTA Fillapex is usually comparable to the epoxy-based sealer with moderate inflammation [125], namely AH Plus (Dentsply, Konstanz, Germany), which is considered as the gold standard in endodontic sealers. [127] Some authors report higher cytotoxicity of the MTA Fillapex than AH Plus. [119] Although MTA Fillapex has Portland cement included in the material, as shown by the scanning electron microscopic/energy-dispersive spectroscopic analysis [117], a lack of the calcium hydroxide peak on X-ray diffraction analysis was evident. [117,128] TheraCal LC is a hybrid material comprising 45% Portland Type III cement and 43% resin. [129] Therefore, due to the low ratio of calcium silicates in the materials, their resinous base and lack of calcium hydroxide formation, it is doubtful whether these materials should be classified as hydraulic calcium silicate cement.

- Type IV and Type V materials are tricalcium silicate-based cements. The main goal of changing the composition to tricalcium silicate was eliminating the Portland cement. In MTA-like dental materials, an alternative base for hydraulic silicate cement was needed after concerns about aluminium and trace elements, such as chromium, arsenic, and

lead. [105] Including heavy metals in MTA-based materials is of concern because they come into direct contact with hard and soft tissues. [130] MTA-like materials released more arsenic than the amount specified in ISO 9917-1 (2007). [98] Dental materials based on tricalcium silicate cement, MTA Angelus and Ortho MTA release minimal quantities of trace elements, such as arsenic, chromium or lead, when in contact with simulated body fluids. [99,131] Therefore, the compositional changes of MTA-like materials were directed to more purified and safer use of hydraulic silicate cement. In 2006, using tricalcium silicate with the aluminium-free formulation of the hydraulic cement, it was patented as an alternative to Portland cement by BioCeramix Inc (Vancouver, Canada), patent application 7553362. [103]

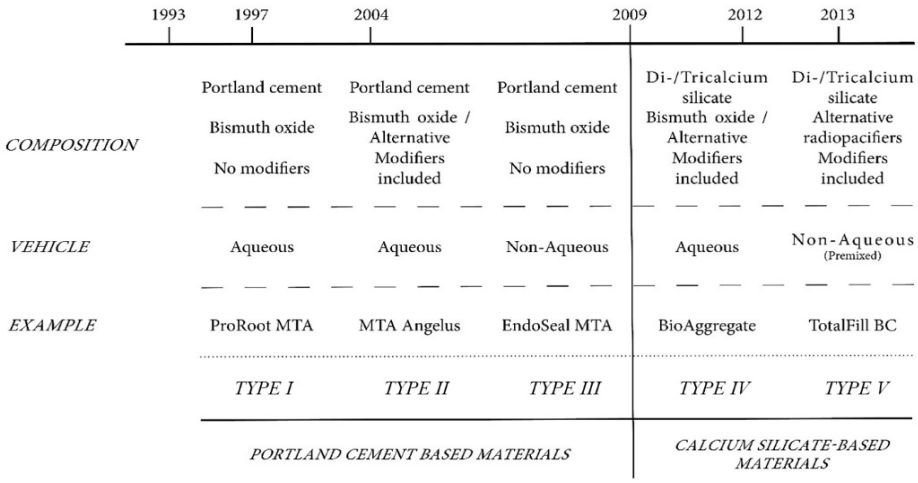
- Type IV hydraulic tricalcium silicate-based materials are presented as a powder and mixing liquid based on water. The original formulation of Type IV cement is BioAggregate (BioCeramix Inc., Vancouver, Canada), which was presented as a powder-to-liquid formulation. Better biocompatibility than MTA-like materials is also addressed in the patent due to an absence of aluminium and magnesium in the composition. [132,133] Septodont (Septodont, Saint-Maur-des-Fosses, France) also patented 'Preparation for producing a material used to restore a mineralised substance, particularly in the dental field' in 2003 [134], which material was improved and patented as 'Wear resistant dental composition' in 2012. [135] In 2009, Septodont presented materials known as Biodentine, a hydraulic calcium silicate-based cement, and BioRoot RCS, a hydraulic calcium silicate-based root canal sealer (Septodont, Saint-Maur-des-Fosses, France). Both materials are presented in two containers: powder intended to be mixed with an aqueous phase liquid, resulting in a dental cement. Also, these Type IV material patents of BioCeramix Inc. and Septodont include not only the calcium silicate-based solid/aqueous composition and preparation of the material but also refer to the use of specific additives to enhance the material properties. In BioCeramix Inc. inventions, calcium phosphate monobasic is added to strengthen the materials' mechanical properties. [132,133] In contrast, the properties of the Septodont cement are modified by adding calcium carbonate, a water-soluble polymer, and calcium chloride. [134,135] The radiopacifier is an alternative to bismuth oxide, such as zirconium oxide, for both material types.

  Recently, more Type IV materials came into the market. In 2017, Ultradent (Ultradent Products Inc., South Jordan, UT, USA) presented MTA Flow. The powder is based on di- and tricalcium

silicate and liquid composed of a water-soluble silicone-based gel. This material was developed to manipulate the gel/powder ratio, thus acquiring the desired consistency for the current clinical scenario. In 2022, Septodont released a new version of Biodentine, called Biodentine XP, available in two different cartridge sizes: 200 and 500. The powder and liquid are presented in one cartridge. However, they are in two different cartridge containers. Therefore, the clinician does not need to add a specific number of drops to the powder before mixing. XP 200 is designed for pulp capping, small restorations and endodontic indications, while XP 500 is suitable for deep caries, bulk filling and quadrant restorations (Septodont, Saint-Maur-des-Fosses, France). The system also comes with a specifically designed applicator gun for direct material placement and a mixer with a high rotation level (6200 rpm), which, according to the manufacturer, will reduce preparation time and facilitate material placement into the operative field.

- Type V hydraulic tricalcium silicate-based dental materials are described as premixed materials. However, the term premixed is considered a misnomer since the essential ingredient needed for hydration is missing. [105] To be premixed, the materials must have all the vital components. For example, the setting is prohibited by specific hydration blockers, which is not the case with the Type V materials. The materials are presented as one component with mixed powder and liquid phase, but no hydration reaction initiator is present. Therefore, the water must be absorbed from the tooth structure and body tissue fluids for the hydration reaction. Later, the same companies presented premixed versions of their Type IV cements. BioCeramix Inc. premixed materials were patented after Type IV materials. [136] These materials are now marketed as EndoSequence BC (Brasseler, Savannah AU, USA), TotalFill (FKG, La ChauxdeFonds, Switzerland) and iRoot (BioCeramix Inc., Vancouver, Canada). Although these materials have different labelling, they are chemically the same hydraulic calcium silicate-based root canal filling materials. Recently, other brands presented premixed Type V materials into the market, such as AH Plus Bioceramic by Dentsply (Dentsply Sirona, Charlotte, NC) and VDW.1Seal Bioceramic sealer, which are the same materials based on zirconium dioxide and tricalcium silicate. In 2019, company Angelus (Angelus, Londrina, Brazil) presented ready-to-use BioC Sealer Ion+ sealer and BioC Repair hydraulic calcium silicate-based cement. These materials are based on di- and tricalcium silicate and

zirconium oxide as a radiopacifier and were shown to have acceptable physicochemical properties: short setting time, alkalinisation ability, and adequate flow and radiopacity along with low volumetric change and cytocompatibility. [137,138] Lately, in 2022, Septodont patented more advanced hydraulic calcium silicate-based root canal filling materials 'Dental hydraulic cement comprising ultrafine calcium silicate particles having fast hardening and suitable mechanical properties' by modifying the dental hydraulic cement comprising of ultrafine calcium silicate in the presence of a limited amount of water, such that the hydraulic cement hardens quickly while providing a material with suitable mechanical properties for dental restoration, and in particular high compressive strength. [139] The material was recently presented as BioRoot® Flow (Septodont, Saint-Maur-des-Fosses, France).



1993 - Dr. Mahmoud Torabinejad & Dean White at Loma Linda University obtained two patents for a Portland cement-based endodontic material, which became later known as mineral trioxide aggregate (MTA); **1997** - Approval by the US Federal Drug Administration for endodontic applications in 1997 February 10th and classified as 'root canal filling resin material' with an intention to 'be applied to a tooth to protect the pulp' and became commercially available as ProRoot MTA (Tulsa Dental Products, Tulsa, OK, USA); **2004** – White version of Portland based mineral trioxide aggregate was presented; **2009** – The first formulation of di-/tricalcium silicate-based cement was presented as BioAggregate (BioCeramix Inc., Vancouver, Canada); **2009-2012** – Improved formulations of Type IV cement was patented, later became known as Biodentine and BioRoot (Septodont, Saint-Maur-des-Fosses, France); **2013** – Expiration of the first MTA patent in 1993; *Sealing ability of a mineral trioxide aggregate when used as a root end filling material. M. Torabinejad, T.F. Watson, T.R. Pitt Ford. Journal of Endodontics, December 1993, Volume 19, Issue 12, Pages 591–595.

Figure 1. Classification of commercially available radiopacified hydraulic calcium silicate-based dental cement.

Table 1. Examples of commercially available Type I, Type II and Type III MTA and MTA-like endodontic materials.

Material trade name	*Type of the cement**	*Manufacturer*	*Composition*
Grey ProRoot MTA	I	Dentsply Tulsa Dental Specialities, Johnson City, TN, USA	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulphate dihydrate (gypsum), calcium aluminoferrite and bismuth oxide Liquid: distilled water
White ProRoot MTA			Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulphate dihydrate (gypsum) and bismuth oxide Liquid: distilled water
MTA Angelus (Grey and White)	II	Angelus Dental Solutions, Londrina, Brazil	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, aluminium oxide, silicon dioxide and bismuth oxide Liquid: distilled water
MicroMega MTA	II	Coltene µMega, Besancon, France	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulphate dehydrate, magnesium oxide and bismuth oxide Liquid: distilled water
PD MTA White	II	Produits Dentaires Sa, Vevey, Switzerland	Powder: SiO_2, K_2O, Al_2O_3, Na_2O, Fe_2O_3, SO_3, CaO, MgO and bismuth oxide. Insoluble residues of CaO, KSO_4, $NaSO_4$ and crystalline silica. Liquid: distilled water
MTA Bio	II	Angelus Dental Solutions, Londrina, Brazil	Powder: Portland cement and bismuth oxide Liquid: distilled water
OrthoMTA	II	BioMTA, Seoul, Korea	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetra calcium aluminoferrite, free calcium oxide and bismuth oxide Liquid: distilled water
RetroMTA	II	BioMTA, Seoul, Korea	Powder: Calcium carbonate, silicon oxide, aluminium oxide and hydraulic calcium zirconia complex Liquid: distilled water
Bio MTA+	III	Cerkamed, Stalowa Wola, Poland	Powder: CaO, Hydroxyapatite, Oxides of Silicon, Iron, Aluminium, Sodium, Potassium, Bismuth, Magnesium, Zirconium, Calcium Phosphate. Liquid: Purified distilled water, calcium catalyst.
Endocem MTA	III	Maruchi, Wonju, Korea	Powder: Calcium oxide, Silicon dioxide, Aluminium oxide, other metallic oxides and bismuth oxide. Liquid: distilled water
Endocem MTA Zr	III	Maruchi, Wonju, Korea	Powder: Calcium oxide, Silicon dioxide, Aluminium oxide, other metallic oxides and zirconium oxide. Liquid: distilled water
MTA Repair HP	III	Angelus Dental Solutions, Londrina, Brazil	Powder: Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate, Calcium oxide and Calcium tungstate. Liquid: distilled water with plasticiser agent.

Table 2. Examples of commercially available Type IV and Type V Hydraulic calcium silicate-based root repair materials.

Material trade name	*Type of the cement**	*Manufacturer*	*Composition*
*Biodentine *Available in three forms: Original packaging, XP200 and XP500.*	IV	Septodont, Saint-MaurdesFosses Cedex, France	Powder: Tricalcium silicate, calcium carbonate, calcium oxide, iron oxide and zirconium oxide Liquid: aqueous solution of calcium chloride and a hydrosoluble polymer polycarboxylate.
MTA Flow™	IV	Ultradent Products Inc., South Jordan, UT, USA	Powder: Dicalcium silicate, tricalcium silicate, calcium sulphate, silica, bismuth oxide Liquid: Water-soluble silicone-based gel
MTA Flow™ White	IV	Ultradent Products Inc., South Jordan, UT, USA	Powder: Dicalcium silicate, tricalcium silicate, calcium sulphate, silica, tantalum oxide Liquid: Water-soluble silicone-based gel
BioAggregate	IV	Innovative BioCeramix, Vancouver, BC, Canada	Powder: Tricalcium silicate, Dicalcium silicate, Calcium phosphate monobasic, Amorphous silicon oxide and Tantalum pentoxides. Liquid: deionised water
*EndoSequence; iRoot / TotalFill BC RRM. *Available in three forms: Paste, Putty and Fast Set Putty.*	V	Brasseler, Savannah AU, USA; BioCeramix Inc., Vancouver, Canada (North America) / FKG, La ChauxdeFonds, Switzerland (Outside North America)	Premixed syringe: Tricalcium silicate, Calcium phosphate monobasic, Calcium hydroxide, Zirconium oxide, Tantalum oxide, Filler and thickening agents.
Bio-C Repair	V	Angelus dental solutions, Londrina, Brazil	Premixed syringe: Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate, Calcium oxide, Zirconium oxide, Silicon oxide, Polyethylene Glycol, Iron oxide.
EndoCem Regular	V	Maruchi, Wonju, Korea	Premixed syringe: Tricalcium silicate, Dodecacalcium heptaaluminate, Dimethyl sulfoxide and Zirconium oxide.

*According to the newly introduced classification system based on HCSC clinical context and constitution by Dr. Josette Camilleri in 2020. [111]

Table 3. Examples of commercially available Type IV and Type V Hydraulic calcium silicate-based sealers for root canal obturation.

Material trade name	*Type of the cement**	*Manufacturer*	*Composition*
BioRoot RCS (Root Canal Sealer)	IV	Septodont, Saint-MaurdesFosses Cedex, France	Powder: Tricalcium silicate, zirconium oxide and povidone. Liquid: aqueous solution of calcium chloride and polycarboxylate.
*EndoSequence; iRoot / TotalFill BC Sealer. *Available in two forms: Regular and HiFlow.*	V	Brasseler, Savannah AU, USA; BioCeramix Inc., Vancouver, Canada (North America) / FKG, La ChauxdeFonds, Switzerland (Outside North America)	Premixed syringe: Tricalcium silicate, Calcium phosphate monobasic, Calcium hydroxide, Zirconium oxide, Filler and thickening agents.
Bio-C Sealer ION+	V	Angelus Dental Solutions, Londrina, Brazil	Premixed syringe: Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate, Calcium oxide, Zirconium oxide, Silicon oxide, Polyethylene Glycol, Iron oxide.
EndoSeal MTA	V	Maruchi, Wonju, Korea	Premixed syringe: Tricalcium silicate, Calcium aluminate, Calcium aluminoferrite, calcium sulphates, bismuth oxide, zirconium oxide and a thickening agent.
BioRoot Flow	V	Septodont, Saint-MaurdesFosses Cedex, France	Premixed syringe: tricalcium silicate, propylene glycol, povidone, calcium carbonate, Aerosil (silica), zirconium oxide, acrylamide/sodium acryloyldimethyltaurate copolymer, isohexadecane and polysorbate.

Table 4. Examples of commercially available Hydraulic calcium silicate-based temporary root canal filling materials.

Material trade name	*Manufacturer*	*Composition*
EndoSequence® BC Temp™	Brasseler, Savannah AU, USA	Premixed syringe: Calcium Silicates and Calcium Oxide combine with the water naturally present in dentin to produce Calcium Hydroxide, which dissociates into Ca^{2+} and OH^-. The hydroxyl ions (OH^-) released significantly increase the pH of the surrounding tissues, making the environment unsuitable for bacterial growth.
Bio-C Temp	Angelus dental solutions, Londrina, Brazil	Premixed syringe: Calcium Silicates which, after being hydrated, produce Calcium Hydroxide that dissociates into Ca^{2+} and OH^-. Released Hydroxyl ions (OH^-) are responsible for a significant increase in the pH of the surrounding tissues, making the environment unsuitable for bacterial growth.

Also, hydraulic calcium silicate-based materials can be classified according to their clinical context: the specific indications and environment in which they are used. [111] This classification is based on the possible variation of material changes depending on the clinical environment in which the materials are placed. Materials are classified into three clinical indications (Figure 2):

- Intra-coronal. This category includes vital pulp therapy, which aims to maintain tooth vitality and create an environment for pulp to heal by performing indirect or direct pulp capping. [140] The goal of performing regenerative endodontic procedures is to regenerate tissues similar to a dental pulp by placing a coronal bioactive and biocompatible hydraulic calcium silicate-based barrier on a formed blood clot. [80,141]
- Intra-radicular. The materials are used for root canal filling, [142] internal root resorption treatment and closure of perforations, [143] and apical plugs, such as apexification. [144]
- Extra-radicular. It is indicated for root-end filling in the apical surgery [145] and perforation closure. [143]

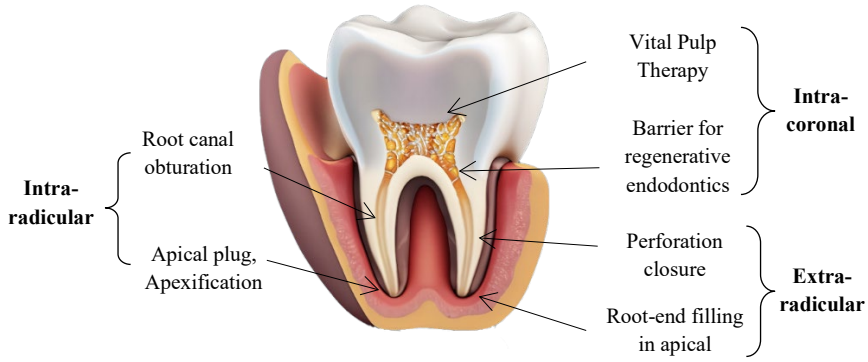


Figure 2. The illustrative drawing of a tooth and surrounding alveolar bone cross-sectional view with clinical classification indications to use hydraulic calcium silicate-based dental materials.

1.3. Hydraulic silicate cement materials: composition, manufacturing & setting reactions

1.3.1. Portland Cement-based dental materials composition

Portland cement-based Mineral Trioxide Aggregate (MTA) is a hydraulic cement (sets and is stable in wet conditions), which consists of a powder

composed of fine hydrophilic particles that rely on hydration reaction for setting when mixed with water. [146,147] According to the Original MTA US patent by Torabinejad et al. [81,82] and White MTA US patent [148], the principal component is Portland Cement, where Calcium oxide (CaO) is the main component with an average of about 65 weight per cent. However, the range can vary from 50 to 75% by weight. The second essential component is silicon dioxide (SiO_2), an average of 21 weight per cent, ranging from 15 to 25%. These materials constitute around 86% by weight, with a dedicated acceptable range from 70 to 95% of the cement. They produce tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetra calcium aluminoferrite when blended. The cement hydration setting reaction takes place by adding water, forming silicate hydrate gel. [81]

The chemical similarity between MTA and Portland cement has been demonstrated since the year 2000 in several comparative studies. [96,97,149] The similarity is not a coincidence since Portland cement was used as an inspiration and preliminary trial material. [65,95,150–152] Also, the original patent states that the principal component is Portland cement. [81] However, ordinary Portland Cement cannot be used in dental practice due to concerns about its heavy metal constituents [100,101], lack of radio-opacity [153,154], significant setting expansion [150], broad distribution of particle sizes [130] and relatively high solubility in some forms. [65,150] Also, this material has not been approved for clinical use. Therefore, the design of MTA has considered properties that need to be improved, such as particle size, setting rate, solubility, and reduction of heavy metal contents, being the material produced not in a kiln but in the laboratory. Also, adding Bismuth oxide or alternative radio pacifiers with or without calcium sulphate provides adequate radioopacity for MTA. [153,154]

In the construction industry, the influence of cement fineness is significantly investigated on the early-age properties of cement-based materials. [155] Blaine's fineness number determines the fineness of the material. This surface area-based parameter is quantified using the air permeability method and is essential to assess the quality of the cement. [156,157] During the analysis, the time taken for a fixed quantity of air to flow through a compacted cement bed of specified dimensions, called Pallets, and porosity are measured. The qualities considered during the measurements are cement particle size distribution, which determines the size and number of individual pores and, as a result, impacts the time for the specified air to flow. [155] The average Blaine fineness of modern cement ranges from 3000 to 5000 cm^2/g. [157] Mineral Trioxide Aggregate is also Portland Type I Cement, with a Blaine number fineness ranging from 4500 to 4600 cm^2/g. [146]

Magnesium oxide (MgO) in Portland cement was shown to have delayed hydration even in the cement phase, long after the cement hardened, accompanied by a volume expansion of up to 118%. [158] Therefore, the restriction on the maximum level of MgO in Portland Cement was introduced to less than 5%. [159] Thus, the MgO level was lowered in MTA production to less than 2%. In Portland Cement, approximately 75-90% of metals, like strontium (Sr), barium (Ba), manganese (Mn), chromium (Cr), nickel (Ni), vanadium (V), copper (Cu) and cobalt (Co) are added with raw material feed. The metals from the highest input rate are Sr > Ba >> Mn > Cr > Ni >V > Cu. [160] Only traces of heavy metals are found in the original MTA Portland-based cement. A detailed comparison of the composition of Ordinary Portland Cement and the Grey and White versions of MTA can be seen in Table 5. Even though some researchers reported the weight percentages of oxides, such as silicon oxide, calcium oxide and others from Energy Dispersive Analysis with X-ray, which give information on the elements present within the material [161,162], the apportionment of the oxides into the crystalline phases, such as tricalcium and dicalcium silicate, tricalcium aluminate or calcium carbonate are not taken into account. [68] Therefore, the XRD analysis of the cement can identify compound composition and diffraction patterns of cementitious materials and can provide phase, chemical, and crystal structure information data needed to understand cement performance. As verified by Rietveld's analysis, the calcium silicate hydrate phase is the main product of the hydration reaction. Also, Calcium hydroxide levels increased significantly after the hydration process. The detailed Portland Cement and MTA un-hydrated and hydrated phases detection comparison published by Dr. Camilleri in 2008 can be found in Table 6. [163]

Table 5. Composition comparison of Ordinary Portland Cement and Grey and White MTA.

Component	*Portland Cement*	*MTA*	
		Grey MTA	*White MTA*
CaO, mass%	50 - 60	50 - 75	50 - 75
SiO_2, mass%	29 - 25	15 - 25	15 - 29
Al_2O_3, mass%	2 – 10	<4	<2
Fe_2O_3, mass%	1 – 5	<5	0 – 0.5
MgO, mass%	<5	<2	<0.6
Bismuth Oxide, mass%	-	15 - 25	-
Fe, Mn chromophores	+	+	Negligible
Toxic heavy metals (Cu, Mn, Sr)	Appreciable, traces found	Low	Low
Calcium sulphate (weight % of the cement)	1 – 2 As anhydrite ($CaSO_4$), hemihydrate ($CaSO_4 \cdot ½H_20$), dihydrate	<1.5*	<2.2*
Particle size, distribution	Wide size range	Uniform, larger, ranging from < 50 µm to <3 µm	Uniform, smaller, ranging from < 25 µm to <3 µm

*Sulphate (half of Portland Cement) is reported as gypsum by some authors [130,164] but as anhydrite by others [165,166].

Table 6. Cement phases reported by Dr. Camilleri performed Rietveld X-ray diffraction analysis [163]

Phases	*Un-hydrated cement*		*Hydrated cement*	
	Portland Cement	*MTA*	*Portland cement*	*MTA*
Tri-calcium silicate	74.7	53.1	8.2	10.6
Di-calcium silicate	7.4	22.5	0	14.9
Tetra-calcium alumino ferrite	0	0	0	0
Tri-calcium alumino ferrite	3.6	0	0	0
Gypsum	1.1	0	0	0
Hemi-hydrate	1.1	0.7	0	0
Anhydrite	2.7	1.5	0	0
Calcium hydroxide	2.1	1.0	15.7	14.4
Calcium carbonate	5.0	1.4	3.2	0
Bismuth oxide	0	21.6	0	8.4
Ettringite	0	0	7.5	2.1
Calcium silicate hydrate	0	0	62.2	49.5

1.3.2. Portland Cement-based dental materials manufacturing process

The manufacturing of MTA is similar to that of ordinary Portland cement. [130,167–170] However, the phase proportions and the firing and grinding methods vary depending on the raw materials used and differ in every factory worldwide. [68] Generally, cement is a mixture of compounds created by burning raw materials (calcium carbonate blended with silica- and alumina-containing minerals) at a very high temperature, ranging from 1400 to 1600°C. [171,172] The fundamental requirements for the production of Portland cement are: (1) a source of lime for calcium oxide (CaO), such as limestone, shells or chalk; (2) sources of silica- and aluminium-containing materials for silicon oxide (SiO_2) and aluminium oxide (Al_2O_3); (3) source for iron ore. [170,173] Generally, the lime comes primarily from limestone, which is collected and crushed into small pieces. Rarely limestone is pure calcium carbonate; deposits can incorporate zinc, lead, fluoride, and chloride compounds. Therefore, the purification process is necessary to ensure the best quality of

the Portland-based cement. The crushed limestone is mixed with clay and ground to form a homogenous powder. However, microscopically, this powder remains heterogenous. Therefore, the ground mixture is heated in kilns that are long cylinders in shape, rotating on an incline. At the beginning of the kiln at the high end, the raw materials enter and slowly move along the length of the kiln. The fuel is injected and burned through the low end of the kiln, providing the necessary heat for the materials to react. While the mixture is moving down the cylinder, four transformation stages take place: (1) the first one is the dehydration stage, where any free water in the powder is evaporated; (2) calcination takes place, where decomposition because of the loss of bound water and carbon dioxide occurs; (3) clinkering takes over, where calcium silicates are formed; (4) the cooling stage finishes the production of clinker. Rapid clinker cooling is necessary to prevent powder decomposition and ensure that only dicalcium silicate's beta phase (β-C2S) is formed. [68] The beta phase is more hydraulic; therefore, the crystalline form of the dicalcium silicate is more ready to hydrate and forms hydrated calcium silicate, creating higher strength of the Portland Cement. Clinker can be described as marble-sized pieces produced by the kiln, with a smaller particle size than 0.5 cm in diameter. Therefore, after firing, the clinker is cooled, grounded to very fine particles, smaller than 80 microns [68], and mixed with desirable additives, such as calcium sulphate – gypsum ($CaSO_4 \cdot 2H_2O$), usually by grinding the materials together. Produced calcium silicate and calcium sulphate homogenous powder is called 'Ordinary Portland Cement' (OPC). The degree of grinding is related to the types of cement, with Type I, Type II and Type III being the most common. Type III cement has the finest powder. [68,167,170–173] A schematic drawing of the clinker production in a kiln can be found in Figure 3.

Iron ore forms a dark-coloured phase, creating the grey colour of Portland Cement. [68] Manganese, titanium copper, vanadium, or chromium can also colour the Portland Cement in grey. Raw materials used to produce white Portland Cement contain less than 0.5% of the iron content. However, this process requires higher temperatures or the addition of additives to replace iron oxide, such as sodium, alumina, or potassium oxides.

In dentistry, the manufacturing process is similar to that of ordinary Portland cement. However, the material is produced in a laboratory rather than a kiln. Also, Dr. Camilleri et al. in 2005 [109] demonstrated that the original MTA contains the same constituent elements verified by Energy Dispersive Analysis with X-ray (EDAX) under the Scanning Electron Microscope (SEM). Also, MTA has 80% of the same di- and tricalcium major phases as in Portland Cement, verified by X-ray diffraction analysis with the addition of

about 20% Bismuth oxide as a radiopacifier in MTA. [109,164] The clinker is ground to very fine and uniform particles, with a size of less than 50 μm [174], while in Portland cement, the particles vary in size. [130,175] The size of the particles included in the MTA cement influences the material's properties. Dental cements with finer particles are more likely to penetrate dentinal tubules due to the higher surface, a shorter setting time and, as a result, an earlier release of calcium hydroxide. [176] The patent of a white MTA describes the Portland Cement components in MTA as 90% of the particles finer than 25 μm, 50% of the particles finer than 9 μm, and 10% of the particles finer than 3 μm. [148] Studies in 2016 of the particle size of the original ProRoot MTA revealed a distribution of particles 10% sized below 1.13 μm, 50% of particles below 1.99 μm and 90% of particles below 4.3 μm. However, alternative Portland-based dental cement MTA Angelus showed distribution with much larger overall particle sizes, where 10% were <4.15μm, 50% <12.72μm and 90% <42.66μm. [177] Further research confirmed that ProRoot MTA has a wide range of particle sizes, from 0.42 to 41.43 μm. [178,179] Also, MTA Angelus particles have relatively low circularity and broad size distribution and are less homogeneous than ProRoot MTA. [179] Since Bismuth oxide powder particle size distribution is shown to vary up to 50μm [178], the original Grey MTA patent does not describe the resultant particle size distribution in the grey versions of MTA with bismuth oxide. However, SEM analysis of MTA indicated that particle size ranges from <1 μm to as large as 50 μm. [164,174] Bismuth oxide powder is shown to be insoluble in water. It is believed to be chemically inert. [180] At the same time, it is essential to identify the material radiographically. The inclusion of Bismuth oxide extends the setting time [181–185] and has an influence on the physical properties of the material, such as compressive strength [185–187], porosity [186], microhardness [181], and biocompatibility [188] and reduces reaction product formation timing, such as calcium silicate. [189] It was shown that Bismuth Oxide particles bigger than 10μm can have a negative impact on the performance of the MTA. [190] The overall size distribution of crystals observed in Grey MTA is significantly more extensive (range from 5 to 50 μm) than that observed in White MTA (from 5 to 25 μm). [174] Therefore, the strictly purified composition of the clinker phase and the addition of modifiers to improve the achieved Portland cement-based MTA dental cement characteristics are crucial. [81,191] It can be concluded that MTA is similar in nature to ordinary Portland Cement but is produced explicitly in the laboratory for dental use. [109,150,162,192]

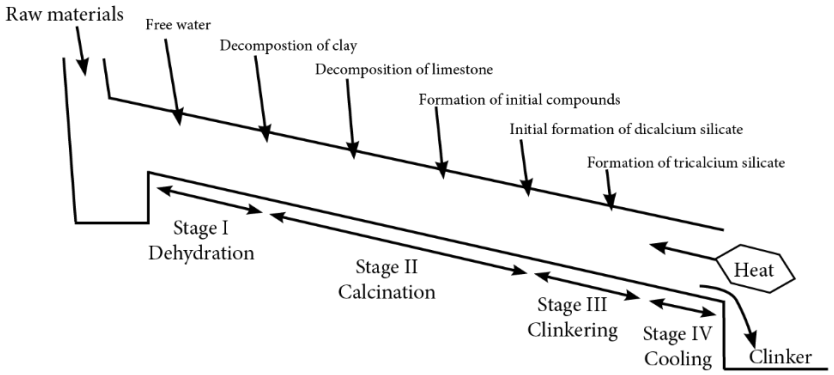


Figure 3. Schematic drawing of the clinker production in a kiln when manufacturing Portland-based cement.

1.3.3.Portland Cement Phases and Setting Reactions

After manufacturing the Portland Cement, the powder contains several phases: (1) alite – tricalcium silicate (C_3S); (2) belite – dicalcium silicate (C_2S); and (3) less in the amount of tricalcium aluminate (C_3A) and calcium aluminoferrite (C_4AF). Free CaO (lime) can be found, but it is reduced to the minimum amount since the hydraulic reactivity of lime is lower.

The alite crystals are the most reactive of the components with water and create around 45 – 70% of the Portland Cement, and microscopically its structure is elongated and hexagonal. The belite crystals are less reactive and usually present around 5 – 30% of Portland Cement powder, their structure being more rounded or equiaxed. Tricalcium aluminate and ferrite phases constitute less than 10% of Portland cement; although the hydration reaction is more exothermic than elite or belite, the shape of these phases is smaller and usually attached to alite or belite crystals.

Dr. Camilleri et al. [109] published the first hydration reaction of hydraulic calcium silicate cement in 2005. The analysis of the hydration mechanism was crucial to understanding the setting mechanism of MTA and the production of calcium hydroxide as a by-product of the hydration reaction since it forms in both Portland cement and MTA.

Calcium Silicate Portland Cement hydration reaction begins when the powder is mixed with water or water-containing liquids, usually by 0.3 – 0.7 weight water to cement ratio. Increasing the water-to-cement ratio generally increases porosity and permeability while also decreasing the compressive strength of the cement. The hydration reaction forms various hydration products, namely different phases of calcium silicate hydrate - a solid mass of

hydrated gel. The products include porous colloidal calcium silicate hydrate gel and radial acicular calcium silicate hydrate crystals, rhombohedral crystals of portlandite (calcium hydroxide), needle-like crystals of ettringite and calcium monosulphoaluminate or calcium monocarboaluminate. [69] The hydration process's reaction products are referred to as 'gels' but are currently described as 'amorphous reaction products'. These phases are impacted by the composition and impurities of the Portland cement phases, as well as the fineness of the powder and additives included in the Portland cement powder or water. [193] There are four stages described in setting and hydration of Portland Cement:

1. Preinduction;
2. Induction or dormant period;
3. Acceleration;
4. Post-acceleration.

The calcium sulphate, calcium aluminate, and calcium alumino-ferrite phases rapidly dissolve, and hydration occurs for the superficial alite phase particles. The calcium sulphate and calcium aluminate form an ettringite (a hexa-calcium aluminate tri-sulphate hydrate) – $(CaO)_6(Al_2O_3)(SO_3)_3{\cdot}32H_2O$. These crystals create a needle-like shape and grow into the liquid between the particles. As the cement transfers to the acceleration stage, the alite and belite react, forming ettringite. During stage II, the cement primary setting occurs - a gradual transition of the cement from a liquid to a rigid state. In stage III, alite crystal hydration proceeds by reducing free water, creating calcium hydroxide precipitates. In this phase, the calcium silicate hydrate has a layered structure. The layers grow radially from the calcium silicate particles, resulting in a fibrous needle-like complex structure alongside cuboidal calcium hydroxide crystals. [69,194,195] The hydration reaction of the tricalcium silicate phase resumes (Eq. 1). As the amount of non-hydrated material is reduced, the hydration process is slowed down and is controlled by the diffusion process of the alite and belite particles. The ettringite crystals can dissolve to release tricalcium aluminate and calcium mono-sulphate. In stage IV the belite phase continues hydration releasing less portlandite but forming the same reaction product (Eq. 2). Slower hydration reaction continues at a decreasing rate, when after about the four weeks terminal amount of hydration is reached. However, in some cases, the unreacted cement particles may remain in the solidified mass, surrounded by a layer of hydrated reaction products.

$$2Ca_3SiO_5 + 7H_20 \rightarrow 3CaO \cdot 2SiO_2 \cdot 4H_2O + 3Ca(OH)_2 \qquad (Eq.1)$$

$$2Ca_2SiO_4 + 5H_20 \rightarrow 3CaO \cdot 2SiO_2 \cdot 4H_2O + Ca(OH)_2 \qquad (Eq.2)$$

About one-third of the volume of the produced end products is $Ca(OH)_2$, enclosed in the form of complex gels or crystalline substances. The remaining two-thirds of the end products are calcium silicate hydrate and sulphate hydrate phases. In the produced gel, the colloidal particles are bound together by hydrogen bonds, Vander Waals forces, ionic attractions and covalent bonds, such as Si-O-Si bonds. [69,196] Most of the water is consumed during the hydration reaction. However, some are trapped in the pores due to non-reactional components, such as bismuth oxide. [186] Evaporation of the entrapped water may occur during or after the setting of the material. [196]

1.4. Characteristics of Hydraulic calcium silicate-based cement dental materials

1.4.1. Porosity

The porosity of the material occurs due to formed cavities - pores in the non-hydrated material (internal porosity / Cul-de-sac-type voids / blind pores) or at the contact of the material and surrounding tissue, like root canal wall dentine (external porosity / through-and-through voids / continuous pores).[197,198] This feature is essential because the porosity may affect the material's stability, integrity, and durability.[199] Unfilled zones in the filled root are of great concern, as such spaces may lead to the regrowth of microorganisms or allow their ingression by microleakage[200], which may be related to the clinical outcomes.[201]

The porosity of the tested HCSC can be analysed visually via a digital inverted phase microscope or SEM by observing the size and distribution of pores on the prepared and polished surface of the HCSC. However, this method has significant drawbacks due to the specimen preparation, polishing and qualitative evaluation.[197] Cutting the specimen for surface analysis may change the porosities, significantly influencing the number and size of pores.[202] Furthermore, only external or internal pores visible on the cutting edge of the material can be evaluated. Other methods include using Archimedes' principle to calculate the porosity. However, the methodology requires specific sample preparation and cannot reveal pore diameter, location, or type.[203] Therefore, there is a need to objectively and reproducibly evaluate the sealing ability of the root canal filling.[204] Micro-CT proved to be a powerful nondestructive 3D analysis tool for visualising the porous internal microstructure of dental/endodontic materials at the interface with dentine[198] and has been used in scientific research for porosity evaluation of different materials.[198,205,206] This testing modality is advantageous, as it provides data

on open and closed porosity and gives precise location and measurements of the pores without harming or changing the material.[207–209] However, the 3D analysis still lacks a clearly defined and comprehensive protocol.[210] For this purpose, a study by Kaan Orhan et al. was performed to determine the cutoff voxel size value for assessing root canal filling voids in micro-CT images and compare them to those obtained with nano-CT scans.[211] The results revealed that nano-CT was not superior to the micro-CT evaluation. A voxel size of 11.2 μm was suggested as a reasonable cutoff value in both imaging methods for assessing root canal filling voids.[211]

Analysing root canals filled with HCSC alone, J. Vergaças et al. found that different brands of putty consistency materials created similar percentual volumes of external voids with only ProRoot MTA White, resulting in fewer internal voids than pre-mixed tested materials.[212] J. Jung et al. compared retrograde root canal filling of two different consistencies of HCSC materials: the results revealed similar internal (1.2-1.3%) and external (0.13-0.19%) porosity when retrograde filling was performed using putty HCSC or putty and sealer HCSC.[213] Other studies found no differences between MTA Angelus, Biodentine and Neo MTA Plus root-end fillings.[214] In the JHO Wonkyung et al. study, ProRoot MTA White revealed significantly higher porosity in complex anatomy mesial canals of human mandibular molars than the continuous wave vertical compaction technique with AH Plus sealer.[215] However, the same study revealed no differences between the groups in mandibular molars' simpler-type distal root canals.

Some researchers have shown that the SC root canal filling technique creates equivalent and acceptable root canal filling in comparison to thermoplasticized filling techniques.[216,217] Furthermore, Daniele Angerame et al. concluded that the SC technique led to greater sealer thickness in partially oval canals. In contrast, the continuous wave condensation technique produced a thin sealer layer independent of the canal shape.[217] The root canal filling occupied 87 to 98.43% of the shaped root canal volume in SC and thermoplasticized filling techniques.[216–218] Some researchers have divided the unfilled areas of the root canal into 0.33% internal voids, 0.72% external voids and 0.52% combined voids.[216,217] However, these studies analysed the whole root canal filling as a unity without dividing the root canal filling into thirds. J. Pinto et al. analysed the root canal fillings in thirds with the highest porosity (7.7 to 9.3%), resulting in the middle root third; however, the filling was performed using a thermoplasticized continuous wave condensation technique.[219]

The first study to reveal systematic differences between the distribution of voids in roots treated with SC and conventional lateral condensation root

canal treatment techniques with micro-CT was by A.T. Monzadeh et al.[210] Betul Aycan Alim et al. have included four different root canal filling methodologies in their micro-CT analysis study: lateral compaction, SC, continuous wave and gutta-core.[220] However, the authors only included two-dimensional analysis at 2, 5 and 8 mm from the apex, analysing only the area in the section. The study has found less porosity at 2 and 5 mm in lateral condensation and gutta-core techniques groups. Regarding the coronal root canal third (8 mm point), all techniques are effective as long as the condensation is achieved.[220] Further studies have revealed that the quality of the fillings produced by the SC technique was similar to the cold lateral compaction using EndoSequence BC HCSC sealer in oval premolar root canals, with 13% of voids in the apical portion of the canal in the research by Poliana J. Penha da Silva et al.[214] and only 0.02% voids in the research conducted by B. Celikten et al.[27] Although both of the studies were conducted in premolars with oval canals and used the same evaluation method (micro-CT), differences in tooth selection and paring, apical preparation, the protocol of voids analysis, time for the sealer to set, scanning voxel size may be related to the differences in void percentage found.

A recent systematic review by S. Bhandi et al. analysed cold lateral condensation and thermoplastic techniques for the root canal filling quality, mainly concluding operators' skills as one of the major factors in research bias creation.[221] Therefore, the need to establish operators' skills in performing root canal filling and compare porosity distribution in root canal fillings performed by operators with different clinical experiences is crucial.

1.4.2. Alkalinity

MTA-like materials are known for an alkaline pH resulting from the setting reaction, where in the presence of water, calcium silicates undergo a hydration reaction, producing calcium hydroxide and calcium silicate hydrate.[115,222] Afterwards, in contact with physiological fluids, the HCSC reaction mainly results in hydroxyapatite formation at the surface of the setting material.[223] Therefore, the hydrated cement contributes to the presence of calcium hydroxide and an alkaline pH with an increase of up to 12-13.[189,223,224]

Multiple scientific articles evaluated the pH values of HCSC materials. pH is measured depending on the research methodology, at the baseline and during the specified period after the initiation of the hydration reaction by using specific pH meters or pH measuring tapes.[189] Depending on the material, most of the HCSC dental materials' pH ranges can be summarised as values between 9.65 and 13.6.[189,223] For example, Biodentine® pH ranges from 9.65

to 12.19, while MTA Flow was found to have a pH of 10.5 to 12.22. [223] Interestingly, the analysis by K. Kot et al. revealed a gradual increase in Biodentine and MTA Flow pH values over one year, with minor reductions in pH increase at one and three weeks. [223] A slightly higher pH increase over six hours was found in C. Chen et al. analysis, which showed that the pH of HCSC increased from 10.4 to 13.6. [189] BioRoot RCS was found to have a pH of 11 to 12 for the first 14 days[225], while pH decreased to 10.3 after six months.[226] Indeed, HCSC materials are known to have an initial pH of around 10.2 at the time of mixing. At the same time, it may increase during setting due to the calcium hydroxide that is additionally released. [88,227] However, the peak pH value was reported to be reached in 5 - 48 hours, depending on the material setting time. Nevertheless, for most HCSC materials, after 28 days, the pH tends to decrease due to the finalised setting and maturation of the material. [224]

During the formation of the calcium hydroxide and its dissociation, a continuous release of calcium and hydroxyl ions occurs[228], resulting in an alkaline environment conducive to mineralised tissue formation. [229,230] Furthermore, it has been shown that increased pH in the medium provides unfavourable conditions for bacterial growth, resulting in antimicrobial properties of the HCSC materials.[192] Additionally, the alkaline environment promotes moderate tissue damage by denaturation of the proteins, activating the alkaline phosphatase, an enzyme necessary for the inorganic phosphate release stimulation.[69] Alkaline phosphate enables the separation of the phosphoric esters and the release of phosphate ions; thus, calcium ions can react with free phosphate ions, forming calcium phosphate[231], the main component of hydroxyapatite. These formed calcium phosphate crystal structures are the initial matrix for further mineralisation.[232–234] At the same time, calcium ions react with the carbon dioxide in the surrounding tissues, forming a calcium carbonate precipitate.[235,236] Finally, calcium ions also participate in cell signalling for cell proliferation and the production of proteins that participate in the mineralisation process.[237] As a result, due to alkaline properties, HCSC has shown bioactive properties both *in vitro* and *in vivo*.[236,238,239]

1.4.3. Biocompatibility of HCSC materials on human dental pulp stem cells

Several research studies have analysed HCSC cytotoxicity on hDPSCs using cell viability assays. Colourimetric assay, which is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by

metabolically active cells (Figure 4), is used to measure cellular metabolic activity and as an indicator of cell viability, proliferation, and cytotoxicity.[240–242] In viable cells, NAD(P)H-dependent oxidoreductase enzymes reduce MTT to formazan.[243] Afterwards, the formed insoluble formazan crystals are dissolved using a solubilisation solution, like DMSO, and the resulting coloured solution (Figure 5) is measured in a multi-well spectrophotometer using absorbance at 500-600 nanometers. The darker the solution is, the greater the number of viable and metabolically active cells in the well. Despite the wide usage of this method, it still has the disadvantage of underestimating cell damage and detecting cell death only in the late stages when cell metabolism has significantly reduced.[244]

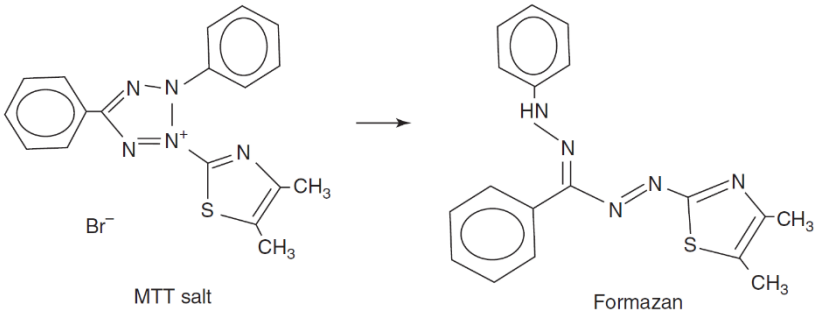


Figure 4. Schematic dissolution of MTT to formazan crystals.[245]

Figure 5. The colour gradient seen in the wells of a well plate after viable cells metabolise MTT to formazan crystals, as shown in the chemical reaction of Figure 4.

To analyze the hDPSCs in more detail after affecting with HCSC eluates, Flow cytometry analysis with Annexin V staining can be performed. Annexin V is a phospholipid-binding protein that binds to phosphatidylserine when it is translocated to the outer layer of the cellular membrane during apoptosis. A viability exclusion dye (like propidium iodide) is used when staining with Annexin V to confirm the binding is happening on the outer surface of the cellular membrane.[246] The Annexin V-FITC Apoptosis Detection Kit (for example, Invitrogen™) can be used to read the effects of material extracts on the hDPSCs and differentiate between early apoptotic cells (annexin V

positive, PI negative), necrotic cells (annexin V positive, PI positive), and viable cells (annexin V negative, PI negative). For example, as in S. Birant et al. research, flow cytometry analysis of hDPSCs after incubation with ProRoot MTA for different time intervals was performed (Figure 6).[247] After the Annexin V-FITC analysis, the cells are differentiated into early apoptotic (lower right quadrant), late apoptotic (upper right quadrant), necrotic (upper left quadrant) and live cells (lower left quadrant).

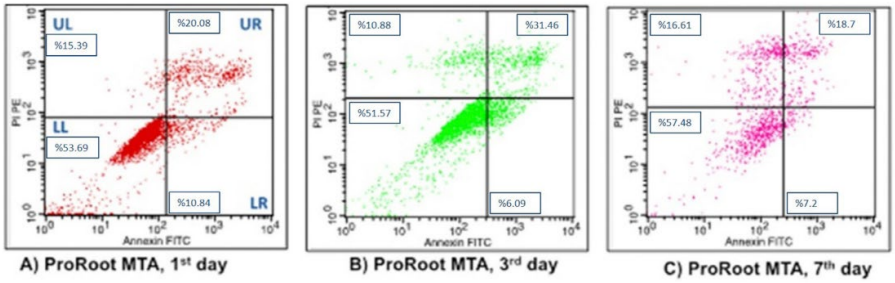


Figure 6. An example of flow cytometry analysis of hDPSCs after incubation with ProRoot MTA at different time intervals by S. Birant et al.[247]

Research studies have shown that HCSC materials possess biocompatible properties in various analyses and systematic reviews.[248–250] According to the systematic review by A. Yousefi-Koma et al., the most widely studied HCSC on hDPSCs currently are the golden standard ProRoot MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) and Biodentine™ (Septodont, Saint-Maur-des-Fosses, France). At the same time, the most used testing method is an indirect analysis of HCSC leachates by extracting supernatant from cement and creating a diluted medium.[249] Interestingly, viability and proliferation analysis performed by indirect methods were significantly more advanced and resulted in a substantially higher rate of higher results than the negative control group. Furthermore, the most widely used methodology included setting the HCSC material for 24 hours before placing and extracting leachate medium, resulting in significantly higher hDPSC viability and proliferation results than the negative control group. Extracting eluates from freshly mixed HCSC yielded significantly lower results than the negative control group.[249] In direct contact with the fully set cement, the medium remains in the incubator for a considerable amount of time to ensure all biocompatibility and regeneration-inducing molecules are released into the medium to make HCSC-enriched leachate.

Calcium hydroxide deposition after HCSC hydration is pivotal in initiating the consequential biological reactions of HCSC in contact with

hDPSCs.[164] In addition, other studies have found that the main ingredient of HCSC materials, tricalcium silicate, induces the proliferation of hDPSCs.[251] ProRoot MTA has shown its capacity to enhance osteogenic and dentinogenic markers and promote angiogenesis in hDPSCs.[252] Other studies have shown that calcium and silicon ions leached from HCSC, namely Biodentine, can potentially stimulate proliferation and odontoblastic-related gene expression in hDPSCs.[253] Also, the same study has found similar biocompatibility of Biodentine and Ortho-MTA, while cell viability was significantly higher than in the positive control (IRM) group. [253] A recent study by Y. Ha et al. analyzed hardened HCSC materials and found that ProRoot MTA and Biodentine created similar hDPSCs viability after 2, 4 and 6 days.[254] Analysing flow cytometry analysis of hDPSCs affected with hardened ProRoot MTA, S. Birant et al. study found the highest cell viability on the 7th day (57.5%), the most early apoptotic cells were detected on the 1st day (10.8%), late apoptotic on the 3rd day (31.5%) and necrotic cells on the 7th day (16.6%).[247] The same study found comparable highest hDPSCs ratios after affecting with hardened Biodentine with no statistically significant differences: viable – 75.2% (7th day), early apoptotic – 9.4% (1st day), late apoptotic – 23.8% (3rd day) and necrotic cells – 15.5% (7th day). In summary, Biodentine and ProRoot MTA were reported to have similar cytocompatibility.[255,256]

A few research studies have analysed BioRoot™ RCS (Septodont, Saint-Maur-des-Fosses, France) cytotoxicity on hDPSCs. L. Loison-Robert et al. have found that BioRoot RCS has caused a reduction in hDPSCs growth rate with minimal cytotoxic effect comparable to Biodentine[238] and in D. Seo et al. research BioRoot RCS resulted in significantly lower cytotoxicity on hDPSCs than resin-based sealer AH Plus.[257]

MTA Flow™ (Ultradent Products Inc., South Jordan, UT, USA) has been evaluated in several research articles on biocompatibility. One study evaluated MTA Flow on human gingival fibroblasts, revealing comparable biocompatibility to ProRoot MTA; however, the consistency of the tested MTA Flow still needs to be reported.[258] Another study by C. Bueno et al. revealed biocompatible properties of MTA Flow in the subcutaneous rat tissues and the ability to form biomineralised tissue, as conventional ProRoot MTA.[230] Nevertheless, the consistency tested of MTA Flow was also not reported. On the contrary, J. Mondelli et al. analysed thick and thin consistencies of MTA Flow, concluding that even though both tested MTA Flow consistencies and Biodentine were biocompatible, thick MTA Flow consistency resulted in a reduced inflammatory reaction and higher fibroblasts number around the material.[259] Also, a comparison between the materials showed a more intense inflammation induced by Biodentine than both MTA

Flow consistencies. Finally, L. Pelepenko et al. have analysed MTA Flow White thin consistency biocompatibility on human fibroblasts, revealing comparable MTA Flow White and ProRoot MTA cytotoxicity (80-100% cell viability in comparison to the control group).[260] In summary, MTA Flow and MTA Flow White all consistencies biocompatibility analysis is scarce and further analysis with different consistencies, cell lines and analysis methodologies are needed.

1.5. Grey and White versions of Hydraulic silicate cement dental materials and their relation to tooth discolouration

Since the introduction of MTA into the dental field, the first one to be presented was the Grey version of MTA [81], which powder contains iron oxide and manganese, and around 20% of the weight of bismuth oxide is added as a radiopacifier. It was shown that the grey version of MTA was associated with noticeable staining of dental structures in around 60% of cases when used for pulpotomy [261,262], thus indicating to avoid in the aesthetic region of the teeth. [263–265] Therefore, a new formulation of White MTA, also known as 'tooth coloured MTA', was introduced in 2002 as White ProRoot MTA (Dentsply, Tulsa, OK, USA) and in 2001 MTA-Angelus (Angelus, Londrina, Brazil / Clinician's Choice, New Milford, CT) was launched in Brazil, which received FDA approval in 2011, making it available in the US. [149,266] The new formulation of MTA was considered aesthetically acceptable in the front teeth region, even though a slight discolouration was still possible with White MTA, which can be managed by veneer if necessary. [267,268] Therefore, first case reports described coronal teeth discolouration even when using White MTA [269–271], and also, *in vitro* and *ex vivo* studies reported associated discolouration, where the colour changes were observed in-depth, on the surface and internally of the material, although minor when compared to Grey MTA. [272,273]

Types I and II, both Grey and White MTA, are based on Portland cement and manufactured from similar raw materials. According to the patent, the main components of the grey-coloured MTA are tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite and calcium sulphate. Both materials are composed of 50% to 75% CaO and 15% to 25% by weight of SiO_2; furthermore, 20% Bismuth oxide is added by weight. [82,148] Only in the White MTA production a fluxing agent is used to remove the ferrite phase during the clinkering process. [166] Therefore, most importantly, the tooth-coloured MTA contains less iron oxide and as a result, the tetracalcium aluminoferrite phase is reduced to a minimum. [94,148,166,274]

Analyses of the elements in MTA Grey and White cement, such as MgO, Al_2O_3, SiO_2, K_2O, CaO and TiO_2, tend to be colourless, white or pale-yellow, while Bi_2O_3 can cause discolouration due to its yellow colour oxide. Furthermore, oxides of iron are dark, particularly FeO is black. [174,275] Also, aluminium (Al_2O_3) and magnesium oxides (MgO) were reported to be significantly lower in the White version of MTA. These oxides have also been shown to be associated with the discolouration of the teeth. [109,174,276]

Furthermore, the White MTA cement was improved by increasing the amount of dicalcium and tricalcium silicate phases. [148] This should improve the material setting reaction and enable more early calcium hydroxide release. In both materials, White and Grey MTA, calcium and silicon were reported to be the predominant elements, followed by bismuth and oxygen. Only Grey MTA additionally revealed the presence of aluminium and iron. Predominant phases of White MTA were found to be tricalcium silicate and bismuth oxide, while Grey MTA was primarily composed of tricalcium silicate, dicalcium silicate and bismuth oxide. [146] A detailed comparison of the phases in ProRoot MTA Grey and White is presented in Table 7. [148] The results of the *Asgar S. et al.* study revealed that the crystal size distribution of Grey MTA is significantly larger, approximately eight times than that observed in White MTA. Also, a narrower range of particle size distribution was observed in White MTA. [174] Therefore, the White MTA provides a finer texture of the cement structure, which improves the material physical properties: smaller particle size increases the overall surface area of the cement and enables it to achieve quicker and more thorough hydration reaction of the material, therefore causing greater early strength. White MTA revealed better-handling characteristics, significantly higher solubility than Grey MTA [88] and lower solubility than Portland Cement. [150] Hydroxyapatite precipitation was shown to be related to the improved bone/cementum regeneration [277] and bioactivity of hydraulic calcium silicate-based cement results in stimulated hydroxyapatite formation at the surface of hydraulic calcium silicate-based cement and its surrounding tissue as shown by in vitro and vivo studies. [70,278–280] Subsequently, higher hydroxyapatite formation may result in improved clinical outcomes. Grey MTA was shown to produce more hydroxyapatite formation by 43% than White MTA; however, the Calcium released was similar. [281] This ability was theoretically attributed to the greater alkalinity of the Grey MTA. [88] Further studies revealed higher [281] or similar White MTA leakage when used as apical barrier or furcation perforation closure than Grey MTA. [274,282]. No teeth root canal hydraulic calcium silicate-based filling technique and materials are void-free.[221,283] During the setting reaction of MTA, the calcium ions are released into the pores and voids of MTA [180,284],

helping initiate hydroxyapatite crystal formation and potentially filling some cement voids. [281] Therefore, differences in leakage may be related to a higher amount of hydroxyapatite formed on Grey MTA.

The biocompatibility of White and Grey MTA was reported to be similar. [285] In primary molars, Grey MTA was reported to induce dentine bridge formation more efficiently after pulpotomy than White MTA, although both Grey and White MTA resulted in excellent clinical success. [286] Therefore, the Grey and White MTA were considered reasonable to use, only determining the material selection by aesthetic circumstances. [282]

Table 7. Composition of ProRoot MTA and ProRoot MTA White by presence of phases. [148]

Component phase	Grey MTA	White MTA
Tricalcium silicate ($3Ca{\cdot}SiO_2$)	62	68
Dicalcium silicate ($2Ca{\cdot}SiO_2$)	11	20
Tricalcium aluminate ($3CaO{\cdot}Al_2O_3$)	3	5
Tetracalcium aluminoferrite ($4CaO{\cdot}Al_2O_3$)	13	1
Total Crystalline Phases Calculated from Composition	89	94

The reduction of the mentioned components in MTA resulted in a white composition. However, the White MTA also caused tooth colour changes. Therefore, studies were conducted to detect components involved in this process and bismuth oxide was associated with tooth discolouration. [263] One of the requirements of the dental material is to be radiopaque, not less than 3 mm Al, to enable peri-/postoperative radiographic evaluation of the material placement in the operative field of the tooth. Adding 20% by weight of bismuth oxide creates the radiopacity of the material equal to 3 mm of aluminium at a 1 mm thickness of the cement as recommended by American National Standards Institute/American Dental Association no. 57/2012. The maximum amount of bismuth oxide in the material is up to 40%. However, Coomaraswamy et al. revealed a strong linear correlation between the addition of higher concentrations of bismuth oxide to Portland Cement as a radiopacifier and cement relative porosity, dry and strut densities. [186] Portland cement's mechanical stability is reduced by introducing flaws and increasing porosity, leaving more unreacted water and pores within Portland Cement due to the higher bismuth oxide concentration, which does not participate in the setting reaction, and relatively lowered Portland Cement active ingredients, such as di-/tricalcium silicate, which are responsible for the hydration reaction to occur. [186] Also, the yellow colour of bismuth oxide imparts a pale-yellow

colour to the cement mixture. [287] Reduction of bismuth oxide in bismuth and contact with the structure of the tooth results in the colour change of the material and, consequently, in the colour of the adjacent tooth structure. [112,287,288] There are two options to prevent colour changes due to bismuth oxide. The first method proposed replacing the bismuth oxide with calcium tungstate or 30% zirconium oxide as a radiopacifier. [287,289,290] These components were shown not to cause any tooth colour changes. [289,291,292] Therefore, the newer, Type IV and Type V hydraulic calcium silicate dental materials, such as Biodentine (Septodont, Saint-Maur-des-Fossés, France) or TotalFill BC Putty and BC Sealer (FKG Dentaire, La-Chaux-de-Fronds, Switzerland), include calcium tungstate or zirconium oxide as a radiopacifying agent. Marciano M. et al. proposed the second method: to include 5% of zinc oxide by weight into the composition of MTA. [291] Zinc oxide is included in the composition of other dental materials, like intermediate restorative materials or root canal sealers, and no reports of dental discolouration were noted. [293,294] Zinc oxide molecules react with bismuth oxide, stabilising it from phase changes and inhibiting its conversion to bismite, which is responsible for discolouration of the tooth. [112,287,295] It was shown that 5% of zinc oxide is preferable to prevent tooth colour changes and has no impact on radiopacity, setting time, volume change, pH and biocompatibility of the MTA. [291]

Further analysis revealed that bismuth oxide in contact with sodium hypochlorite exhibits colour changes from light yellow to dark brown. [296,297] In contrast, sodium hypochlorite does not influence zirconium oxide and calcium tungstate colour stability. [297] The discolouration has been attributed to the destabilisation of bismuth oxide when in contact with a strong oxidising agent with the formation of bismuth carbonate by reaction of the bismuth oxide with atmospheric carbon dioxide. Therefore, the hydraulic calcium silicate cement with bismuth oxide was reported not to be used after irrigation with sodium hypochlorite due to the colour alteration at the cement/dentine interface. [297]

In the presence of light, the bismuth carbonate forms a black precipitate. [288,298] After irradiation of a Bismuth oxide with a curing light wavelength of 300 to 500 nm or a fluorescent lamp in an oxygen-free environment resulted in dark discolouration of White MTA. The lights' intensity and wavelength were shown to influence the White MTA darkening speed. [298] Also, alterations in the MTA Angelus material microstructure and a depletion in calcium content were noted. Some authors reported dark marginal discolouration at the MTA–dentin interface, which spread into the dentine. [37,273,299] During the MTA setting reaction, hydration by-products are released, which penetrate dentinal tubules and create tag-like structures. [300,301] Han and Okiji [302]

suggested that during this biomineralisation process, when calcium ions are released from the setting MTA, they react with phosphate ions from surrounding tissue fluids, resulting in the precipitation of carbonated apatite. Some research speculated that some MTA components may bound to the phosphate ions or plasma proteins in the dentinal fluid. After a chemical reaction, the by-products might oxidise, resulting in a pigmented final by-product formation, accumulating in the dentinal mineral infiltration zone. [299] Also, it was reported that Bismuth Oxide undergoes thermal dissociation at high temperatures, forming metallic bismuth and oxygen. [303] The presence of these dark crystals was confirmed with x-ray diffraction analysis [304] and is thought to be responsible for the darkening of the tooth tissues. [295] Further analysis revealed that increasing the oxygen partial pressure at high temperatures avoids the formation of metallic bismuth, leaving the tooth structure without any colour changes. [295] Marciano et al. revealed the interaction between bismuth oxide and organic dentin matrix collagen, resulting in a black precipitate. [287] A similar conversion of bismuth oxide to a black precipitate was mentioned when reacted with sodium hypochlorite. When reacted with collagen, the amino acids originating from dentin collagen destabilise the bismuth oxide molecules, leading to the interaction and eventual black colour change. [287]

Contamination of MTA with blood can also significantly influence the materials' colour. [305] It has been reported that due to the MTA interaction with blood after usage for vital pulp therapy, revascularisation, or perforation repair had resulted in significant tooth discolouration. [306] The main mechanism associated with blood was the interaction between erythrocytes and still setting White MTA. [306] A similar mechanism takes place in discolouration of traumatised teeth, where sequential and/or concurrent haemolysis and catabolism of erythrocytes, haemoglobin, and hematin molecules occurs with subsequent release of iron from the haemoglobin superstructure with accumulation within dentinal tubules. [307,308] The slow hydration process of White MTA permits the absorption and haemolysis of erythrocytes from the surrounding dental pulp tissue, which finally results in the discolouration of the MTA material and tooth crown. [306] The main mechanisms noted in the literature on White MTA-associated tooth crown discolouration are presented in Table 8. Type IV and Type V hydraulic calcium silicate cement materials were considered safe alternatives to MTA in aesthetically compromising areas regarding colour stability. [297]

Table 8. The main mechanisms of White MTA-related teeth crown discolouration, reported in the literature.

Mechanism of action	Reactional component	References
Reaction between Bismuth Oxide and sodium hypochlorite, resulting in the formation of bismuth carbonate as a black precipitate.	*Bismuth Oxide*	288,292,297,298
Oxidation of Bismuth Oxide and incorporation of remaining iron molecules within MTA, resulting in calcium aluminoferrite phase when MTA is set.		306
Bismuth Oxide reduction to bismith when exposed to irradiation with curing lights and/or anaerobic conditions forming reduced dark crystals of metallic bismuth.		112,287,288,292,295,298,306,309
Oxidation of Bismuth oxide, when oxygen becomes unstable followed by reaction of the bismuth oxide with atmospheric carbon dioxide.		288,292
Organic dentin matrix collagen reacts with bismuth oxide, resulting in a greyish discoloration.		287
Released Calcium ions from MTA react with phosphate ions in the dentinal fluid, followed by precipitation of carbonated apatite in the presence of supplementary MTA by-products oxidation.	*MTA*	299
Interaction of Setting MTA and erythrocytes		306–308

1.6. Portland Cement-based MTA materials drawbacks in comparison to new generation hydraulic calcium silicate-based endodontic materials

Original Portland Cement-based MTA materials are classic hydraulic silicate cement with the addition of some heavy metals from Portland Cement. It is one of the most extensively researched and clinically used materials in the dental field. [88,97,110,150] Even though the original MTA has biocompatible [310–312] and bioactive properties [300,313,314], sets in a moist environment [111] and is

versatile as a dental material [76,88], it still has some drawbacks and space for improvement. The main disadvantages of Portland Cement-based MTA and MTA-like materials are discolouration potential, presence of toxic material traces, complex handling characteristics, high material cost, long setting time, and absence of a known MTA solvent, resulting in difficulty of the material removal after setting reaction is finished. [261,262,315–318]

Clinically, both Portland cement-based Grey and White MTA can cause tooth discolouration, as discussed previously. [271,298,319] New HCSC Type IV and Type V materials, such as Biodentine, OrthoMTA or EndoSequence/TotalFill Root Repair Material, manufacturers claim that due to purified composition, the shortcoming of the MTA-induced coronal tooth discolouration is overcome. Biodentine is based on tricalcium silicate and includes zirconium oxide as a radiopacifier. Therefore, the absence of bismuth oxide and traces of heavy metals in the material composition resulted in no coronal tooth discolouration compared to MTA. [320,321] Hence, a lack of bismuth oxide results in the lowered discolouration potential of the HCSC. [322] Further studies analysing Biodentine have shown lower discolouration potential than MTA or TotalFill BC Putty, even in the presence of blood after six months of evaluation. [147,323,324] However, some *in-vitro* studies authors revealed noticeable tooth colour changes after using Biodentine in the contamination with blood in one week to six months. [323–325] Further research resulted in even higher Biodentine discolouration in the presence of blood than premixed HCSCs, such as TotalFill BC Putty. [326] The authors suggested that discolouration is related to the preparation of the materials. Biodentine must be mixed in an amalgamator before usage, while TotalFill BC Putty material is premixed and ready to use, which may affect the materials' homogeneity. Due to higher Biodentine porosity after mixing, the cement may entrap more blood molecules, which can penetrate within the set material particles, resulting in increased discolouration potential in the presence of blood.

On the other hand, the Biodentine colour is like the natural tooth dentin colour, thus preventing the possible discolouration attributed to the translucency of the material through the tooth's hard tissues. In general, due to low discolouration potential and applicable properties, Biodentine was considered advisable to use as a dentine replacement material under light-cured composite restorations in anterior and posterior teeth. [327,328] Furthermore, all new generation Type IV and Type V materials are allowed and indicated to be used in the aesthetic region due to low discolouration potential.

Setting time was and still is one of the main issues related to the hydraulic calcium silicate placement. [33] Since ProRoot MTA has been available in the

dental market for more than two decades and has already been studied extensively in laboratory and *in vivo* studies, it was known that the original setting time of 228 – 261 minutes had to be improved. [329,330] The extended setting time of MTA is why the material cannot be applied in one visit. [331] Therefore, newer materials, such as MTA Angelus with Bismuth Oxide, reduced setting time to an average of 165 minutes. [181] White MTA Angelus without Bismuth Oxide reduced, even more, the setting time to an initial of 24 – 34 minutes and a final of 42 to 83 minutes [332,333], with an average initial setting time taking place around 31±4 minutes and final setting time of 67±1 minutes after mixing the cement. [310] Still, the final setting time taking more than an hour is clinically too long to wait, requiring the application of a protective provisional restoration that is afterwards replaced by a definitive restoration on the next visit. [33,34] Therefore, new generation Type IV and Type V formulations of HCSC were developed with even shorter setting times to be more conveniently used clinically. In 2016, Ultradent introduced the Grey version of MTA Flow (Ultradent Products Inc., South Jordan, UT, USA). According to the manufacturer, the initial setting time is 5 minutes, and the final setting is after 1 hour, while *Pelepenko et al.* [334] showed setting times of 8 minutes and 49 minutes, respectively. Recently, a White version of MTA Flow by Ultradent became available. The same authors analysed setting time, which resulted in a longer initial time of 16 minutes and a final 47 minutes than the Grey version. However, a complete HCSC cure and strengthening can occur in 4 weeks. [260] According to the manufacturer [335] and an *in-vitro* study [336], premixed TotalFill® BC RRM™ Fast Set Putty initial setting time was improved to an average of 20 minutes, while Biodentine was noted to have an initial average setting time of 9-13 minutes and final setting time of 29-45 minutes of Biodentine [35,260,333]. As it is known, a delayed root canal-treated tooth restoration is associated with a lower long-term clinical success rate [337] and a good quality coronal restoration, regardless of its type, is considered an integral part of root canal treatment to prevent postoperative infection. [338] Therefore, the shorter setting time of the material is more acceptable clinically, allowing for a one-visit final restoration if needed.

When first introduced, clinicians had difficulty handling HCSC endodontic materials due to their wet sand-like consistency, unlike most conventional dental materials. Material preparation is a concern attributed to the standardised and convenient mixing of the HCSC. [339] Since the introduction of MTA endodontic material, manufacturers and researchers have improved the presentation of the materials to dental clinicians. The Type I to Type IV materials bottles/containers with water or liquid-gel for mixing with HCSC powder were improved to give a precise and repeatable drop size

and volume to match the water-to-powder ratio, according to the manufacturer, as accurately as possible. Furthermore, Type V HCSC materials, like FKG TotalFill [340] or Bio-C [341], are already premixed in ready-to-use syringes in the form of syringeable paste as a root canal sealer or condensable putty with no required preparation of the material before use, easier handling and application when compared to original MTA.

Mooney GV and North S [318] observed messy MTA manipulation due to excessive moisture in the operative field, resulting in a soupy material consistency and, hence, difficulty to use. Proper delivery and condensation of the HCSC plug to the placement site are necessary to create a tight seal and ensure the best possible success of the treatment. [342] Placement of the original 'sand consistency' HCSC into the operative field is exceptionally demanding. It requires the operators' skill and direct visualisation of the operative field, and as a result, it requires costly and various endodontic equipment. [147,318,343] Therefore, for general dentists without a microscope or other magnifying equipment and limited skills, placement of the original 'sand consistency' MTA is usually hard to achieve. Moreover, direct visualisation of the perforations in the apical root thirds or open apices is generally impossible due to anatomical curvatures. [344] Therefore, even for skilled endodontists with the appropriate equipment, applying the MTA in narrow root canals is challenging, significantly beyond the apical curvatures. [267] Following the introduction of several customised application devices, like Micro Apical Placing - 'MAP system' from Swiss endodontic company PD or 'MTA Applicator' by Angelus Dental, Londrina, Brazil. With the invention of the equipment to use MTA, the handling and application of this material have become more predictable. [266] A recent study revealed that MAP System usage was superior to manual condensation of HCSC with prefabricated pluggers in terms of the sealing ability of apical plugs and the time required to form them. The study results revealed differences in the microleakage of apical plugs when developed with different techniques.

Using hand pluggers to condense HCSC in the apical third may deform the material mass and create voids and gaps. [342] However, the same authors discussed that these instruments may not be available in the dental clinic due to their higher price or difficulty obtaining them. Other research revealed that in immature roots with either parallel or convergent walls, MTA can be placed in an 'ideal' position significantly more often than in a divergent root canal. [73] The limited flowability and slow hydration bound of the HCSC to the surface of the dentin may be related to the problematic adaptation in the root canal system. [121,345] Also, irregular walls of the open apex or perforation dentin and divergent apices make it more challenging to adapt the HCSC. Therefore,

in some cases, the endodontic access cavity may be widened more to enhance the direct visualisation of the operative field and provide straight access for the placement of the HCSC. [346] In 2008 performed research, 86% of the interviewed paediatric dentists responded in favour of MTA usage in case of trauma for immature permanent teeth over traditional calcium hydroxide apexification. [318] However, almost half of the respondents in the same study were concerned about the difficulty of learning a new technique using MTA. On the other hand, some authors believe that MTA is an easy material to use. [347]

Several other concerns regarding HCSs were discussed in the literature. Firstly, the high cost of HCSC endodontic materials was found to be a concern among dentists. [318,331,346,348,349] 63% of the Mooney et al. study sample were concerned about the materials' price and the equipment required to use MTA. [318] Usage of MTA among postgraduate students in restorative dentistry was analysed in 2011, reporting MTA usage in permanent dentition by 100% of students, while only 32% of trainees in primary dentition. Limitations for using MTA were identified as material cost and lack of evidence in the primary dentition. [350] Secondly, complex retrieval of HCSC after setting from the root canal system was reported. [273] There is no known solvent for removing MTA. BioPure MTAD has been reported to have a dissolving effect on White MTA when remaining in contact with the material for 5 minutes. [351] Further research confirmed EDTA and BioPure MTADs' negative impact on the physical properties of MTA. However, no recommendations were noted for using these materials as a solvent for MTA. [352] However, it has been found that carbonation of the MTA reduces the tensile strength and resiliency of Portland Cement. [353] Therefore, carbonation occurs after affecting Portland cement-based HCSC materials with a carbonic acid, whose pH is around 5.5, converting portlandite to calcite. Furthermore, the long-term effect of carbonic acid on MTA results in the decomposition of the calcium silicate hydrate gel into calcium carbonate, acid-insoluble silica gel and water. Finally, complete carbonation finishes by decalcification of the calcium silicate hydrate gel, which reduces the strength of the HCSC. Calcium-depleting organic acids with a pH of 2, such as 10% citric and 20% tartaric acids, may potentially decrease the resilience of MTA by disrupting the crystallisation of calcium silicate hydrate gel. Calcium-depleting agents were found to hamper the formation of calcium silicate hydrate gel, reducing the strength of the MTA. [351] Further research revealed 2% carbonic acid's maximum efficacy in reducing the surface microhardness of partial and completely set MTA, followed by 10% citric acid and 20% tartaric acid. [354] Tartaric acid is a strong retarder for the hydration of Portland Cement. It affects cement mineral

phases, forming calcium tartrate hydrate and reducing tricalcium silicate, dicalcium silicate and tricalcium aluminate phases. Moreover, 5.25% NaOCl and 2% Chlorhexidine solutions were effective on partial set MTA 1 day after placement and had a negligible effect on completely set MTA 21 days after placement. However, caution should be exercised while using the mentioned carbonic acids; it should not exceed 10 minutes to prevent significant alterations in the mechanical properties of the tooth dentin during MTA retrieval. [354,355]

In the past decade, many shortcomings of HCSC have been improved, such as shortened setting time, handling properties, and material fineness. Furthermore, premixed endodontic materials with improved and more adaptable handling characteristics were introduced while adding or removing various additives to the material's composition, which can influence the HCSC's original physicochemical and biological properties. Therefore, comprehensive new materials formulation analysis *in vitro* and *in vivo* is crucial before usage in clinical practice.

1.7. Flowable Hydraulic Calcium Silicate-based Endodontic Materials

1.7.1. HCSC material interaction with the clinical environment

HCSC materials interact with the surrounding clinical environment. Therefore, the final root canal irrigation technique, solution and interaction with dentine and surrounding tissues are important. HCSC materials, when set, release calcium hydroxide as a by-product of the hydration reaction. Calcium hydroxide is antibacterial due to its high pH [356] and affects the dental pulp and dentine. [357,358] It was shown that the freshly mixed and placed into the root canal sealers become involved in a local elution process because of the direct contact with extracellular fluids. [359] Created alkaline pH neutralises acidic environment and promotes hard tissue formation by activating alkaline phosphatase. [360] Reparative dentine formation is a relatively complex event that requires progenitor cell recruitment within the pulp tissue, followed by the signalling of odontoblast-like cell differentiation. [361] High pH and the metallic ions released activate cellular events for dentine repair and regeneration. [362] On the other hand, HCSC materials were reported to affect the dentine collagen matrix, where some authors stated that prolonged contact with mineralised dentine may have an adverse effect on the dentine collagen matrix contact surface integrity due to extracted collagen. [363]

Type IV materials are mixed with a water-based vehicle, which induces a setting hydration reaction immediately after mixing. At the same time, Type

V flowable HCSC requires moisture from the dentinal tubules to trigger the setting reaction by initiating a cement/sealer hydration. Type V sealers were shown to require the smear layer removal due to the necessity of the fluid from the dentine for the hydration reaction to occur, while Type IV HCSC sealer was shown to interact with the dentine and provide antimicrobial activity independent of the presence of the smear layer. [364] The sealers absorb the residual root canal moisture to form hydroxyapatite, release calcium hydroxide as a by-product and ensure chemical bonding between the paste/cement and the dentin via the mineral infiltration zone. Interaction of the HCSC materials with dentine was shown to be mineral exchange at the interface of the cement and dentine. [365,366] Bioactive HCSC cement/sealers release by-products and components, such as calcium hydroxide and calcium ions, into the surrounding tissues, which triggers mineral deposition and leads to the formation of an interfacial layer with tag-like structures in the intratubular dentin called 'mineral infiltration zone'. [367–369] This zone is formed by the caustic effect of alkaline hydration products' on the calcium silicate cement by degrading the collagenous component of dentine in the interfacial zone. This degradation process of the collagen surface leads to the formation of a porous structure that facilitates the pervasion of calcium and carbonate ions, resulting in mineralisation of the HCSC-dentine interface. [369,370] Along the material-dentine interface, a tag-like structure was composed of crystalline deposits rich in calcium and phosphorus or the HCSC material itself. The silicon-rich layer increases over time at the contact zone. [302] During the setting stages of the material, selective diffusion of silicon, calcium and phosphorous across the cement/dentin interface. [371] However, other authors disputed this as there was no exchange of calcium and phosphorus [370], mostly silicon being diffused and the calcium phosphate deposited in the interfacial zone. [372,373] Several authors have reported the silicon migration from HCSC to the dentine interfacial mineral zone [365,366]; however, silicon's influence on the dentine and the pulpal responses remains unknown. [370] Reyes-Carmona et al. concluded that the constant formation of the precipitate at the HCSC–dentine surface contributes not only to the formation of the interfacial layer but also to the promotion of an intratubular mineralisation process in the dentine. [367]

The single-cone root canal obturation method is mainly based on the sealer. Therefore, the sealer setting, mechanical and biological properties are important. Due to the HCSC sealers' interaction with the surrounding tissues (substrate) after insertion into the root canal, the material was shown to be susceptible to environmental changes. [374] As a result, the final irrigation solution is essential for the further setting reaction and final properties of the flowable HCSC. [375,376] 17% ethylenediaminetetraacetic acid (EDTA) solution

is used during the root canal treatment to remove the inorganic compound–smear layer. [377] As the EDTA is a calcium chelator, it was shown to disrupt the hydration process of HCSC material, resulting in chemical alterations [378,379] and adversely affecting the micro-hardness, push-out bond strength [380], and reducing compressive strength and cell adhesion. [381] Furthermore, when EDTA was used as a final irrigant, it was shown to reduce the dislocation resistance of the HCSC materials. [382] Therefore, 17% EDTA solution should not be used as a final irrigation solution before root canal obturation with HCSC sealers. Chlorhexidine was speculated to improve the antimicrobial effect of the HCSC material. However, it may also cause alterations in the physical properties of the Type V HCSC materials [383,384] and induce discolouration. [385,386] Further investigation is required with Type IV materials, as the presence of Chlorhexidine had no influence on the antimicrobial properties of BioRoot RCS, and the sealer solubility was reduced. [387] Sodium hypochlorite (NaOCl) from 2% to 5.25% is used during the root canal chemo-mechanical preparation as a main irrigation solution due to the ability to solve organic and necrotic tissues, such as pulp, bacterial and biofilm. Even though some authors revealed an increase in the push-out bond strengths of some Type V HCSC materials [388,389], the same study revealed a reduction in push-out bond strength for Type IV HCSC. [388] Furthermore, NaOCl applied on the HCSC while setting may disrupt the Portlandite phase setting, resulting in reduced compressive strength. [288,375] Materials, including bismuth oxide in contact with NaOCl, are prone to colour changes. [37,297] Some authors recommend allowing HCSC materials to set for one week prior to exposure to irrigation solutions, which may improve microhardness. [390] Some authors recommend conditioning the dentine before filling the root canal with HCSC materials due to enhanced bonding of the material to the dentine [391], and it is even recommended by the European Society of Endodontology guidelines. [392] In contrast, other research did not find a significant influence of NaOCl on HCSC materials' compressive strength. [375] As it is impossible to draw a specific irrigation protocol when using HCSC sealers due to the lack of standardised research [393], removing traces of irrigation solutions from the root canal before using HCSC materials was recommended. [375] Some authors found that phosphate-buffered saline may alter the antimicrobial effect of these materials due to the interaction of the phosphate with released calcium hydroxide. [394] However, phosphate-buffered saline or distilled water has been suggested as a final irrigation solution to reduce the impact of root canal irrigation solutions on the HCSC materials and provide a reliable hydration setting reaction environment, especially for Type V materials. [376,395] Also, the HCSC in the presence of phosphate-buffered saline solution would result in

biomineralisation. [395] Furthermore, the highest bonding to the dentine was shown after inserting HCSC sealers into slightly moist root canals. [396] Therefore, it is crucial to not over-dry the root canal before filling with HCSC sealers.

1.7.2. Current Root Canal Filling Methods with Flowable Hydraulic Calcium Silicate-based Endodontic Cements

The final biological aim of root canal treatment is preventing or curing pulpal and periapical diseases. A systematic review has proven that the odds of healing apical periodontitis are increased when adequate root canal and restorative treatments are provided. [397] The obturation during the root canal treatment is carried out to provide a hermetic seal in the root canal from the root canal orifice to the apical foramen. [398] As long as none of the current root canal fillings can create a perfect void-free filling [399], the effort is targeted at reducing the porosity, increasing the stability of the root canal filling and evaluating the healing capacity of the apical periodontitis. Therefore, multiple root canal filling methodologies were adapted to fill root canals using flowable HCSC materials. However, the latest systematic review did not reveal any superiority of one filling method over the other regarding primary root canal treatment. [400]

1.7.3.Flowable HCSC materials delivery methods to the root canal system

After proper chemo-mechanical root canal preparation, several methods can be applied to deliver the root canal sealer to the root canal system. These include an injectable method, usage of special rotary endodontic instruments and transfer of the sealer with a hand file or gutta-percha cone. [401]

The injectable method is commonly used with premixed Type V flowable HCSC, presented in a syringe. After removing a protective cap from a syringe, a disposable delivery tip or cannula is placed on the syringe. Usually, cannulas are plastic to achieve the flexibility to reach deeper until the middle or apical third into the root canal. The sealer should be injected around 1-2 mm from the working length. According to the volume of the root canal system, the flowable HCSC is smoothly injected into the root canal. At the same time, the cannula is gently and evenly withdrawn until the sealer is visible at the canal orifice. The cannula or the tip is removed from the syringe, and a protective cap is placed back on the syringe to protect the material from the humidity. The storage should be in a foil pouch in a dry place at the appropriate temperature. The cannula is disposed of after each usage. Type IV materials,

such as BioRoot RCS, can also be used with a syringe delivery method. After preparing the material according to the manufacturer's instructions, a small disposable plastic syringe is filled with the mixed Type IV sealer, the cannula is placed, and the sealer is injected into the root canal system. Alternatively, the flowable Type IV and Type V HCSC sealers can be introduced into the root canal by coating the small hand file (size #15 or #20), special rotary endodontic instrument like a Lentulo spiral or pre-fitted gutta-percha cone with the sealer and transferring the flowable material on the walls of the root canal. [402] It was shown that using small syringes or hand files to deliver freshly mixed HCSC sealer can be clinically favourable to ensure better sealer distribution within the root canal. [403]

The sealer placement technique significantly affects the sealer's penetration into root canal isthmuses, irregularities and lateral canals, and sealing ability. [404,405] This aspect is essential when filling root canals with the single-cone method due to the higher volume of the sealer and reduced volume of the gutta-percha, which is necessary only to create the hydraulic pressure on the sealer. [406] The quality of the obturation is not affected so drastically when cold lateral compaction or warm obturation techniques are used. [406–408]

1.7.4. Root canal filling techniques with HCSC sealers

The cold lateral compaction technique is the standard root canal filling method. [24] It is probably the most commonly taught and practised root canal filling technique worldwide in the past two decades. [409–411] The method requires master cone adaptation and choice of sealer, spreader, and accessory gutta-percha cones. Even though the HCSC sealers were invented for cold root canal obturation techniques, the cold lateral compaction does not fit the idea when filling root canals using HCSC sealers. By actively compacting accessory gutta-percha in the root canal after inserting the master gutta-percha cone and packing it with a spreader, the total volume of the gutta-percha in the root canal is increased. Historically, the thinner layer of the sealer was preferred to decrease the sealer shrinkage during setting and dissolution over time, reducing the possibility of leakage. [412]

Thermoplasticized (warm) root canal filling techniques were adopted to shape the gutta-percha into the form as close as possible to the root canal dentin. The effectiveness of the thermoplasticized gutta-percha has been shown when used with epoxy resin-based sealers to maximise the volume and adaptation of the gutta-percha both *in-vitro* [117,216,217] and clinically. [413] However, when HCSC sealers were initially presented on the market, only cold obturation techniques were implemented to be used with these sealers

due to the possible physical alterations in the materials: reduction of the setting time and flowability. [414] Physicochemical changes were reported as significant, especially when used with Type IV HCSC sealers, such as BioRoot RCS, where heat resulted in reduced flowability, increased viscosity and weight loss of the material. [415] Therefore, with HCSC materials improving, only Type V HCSC sealers were recommended to be used with warm compaction root canal obturation techniques.

In contrast, HCSC sealers enable to increase the sealer's volume in the root canal and reduce the compacted gutta-percha volume. Therefore, passive lateral condensation or single-cone obturation/cold hydraulic/sealer-based root canal filling techniques were suggested for use with HCSC sealers. [402] After inserting the sealer into the root canal, a matched taper pre-fitted gutta-percha cone is slowly and gently inserted into the root canal to the working length to create hydraulic pressure, reduce the porosity of the sealer and create better adaptation between the root canal filling and the root dentin walls. HCSC sealers allow a shift in the way the root canals are filled. The studies demonstrated that using a matched gutta-percha cone to the diameter and taper of the chemo-mechanically prepared root canal results in similar root canal filling quality when compared to multi-step thermoplasticized gutta-percha techniques. [416,417] Gap volume and sealer penetration were found to be similar even in complex anatomy cases when filling C-shaped root canals with cold hydraulic technique compared to warm vertical compaction or cold lateral condensation. [418] The apical 2-3 mm root canal, despite the root canal filling method, is always filled with a pre-fitted gutta-percha cone and sealer. Therefore, the single-cone method is always filling the apical root third. Some studies report that when different root canal filling methodologies were used, the single-cone root canal filling with HCSC sealer resulted in the lowest apical leakage. [419] However, a recent review & meta-analysis did not find any significant differences between epoxy resin-based and HCSC sealers despite the filling methodology used. [420] Therefore, the single-cone root canal filling method demands a greater amount of sealer, leading to essential flowability and specific physicochemical properties of the material. [421]

1.7.5. Flowable Hydraulic Calcium Silicate-based Endodontic Materials and Their Features

HCSC materials can be divided into flowable and hard (putty) consistency endodontic materials. Flowable HCSC can be described as the materials that are delivered into the endodontic operative field via a syringe in the form of a paste or thin/thick consistency cement. Flowable HCSC materials are

available in premixed pastes (Type V) or powder/liquid formulations (Type IV). Examples and indications of currently available flowable HCSC in the market are presented in Table 2 The pre-mixed formulations are not water-based like powder/liquid sealers. Due to their different properties, these materials are intended to be used in various clinical applications, such as root canal fillers/sealers, along with gutta-percha points in several obturation methods. Therefore, the clinical application methods should be carefully selected according to the manufacturer's recommendations and the background of scientific evidence on the material. [402] The major advantages of these flowable HCSCs are high pH during setting [422], antimicrobial activity [423,424], biocompatibility [36,425], bioactivity [314,360], prolonged dimensional stability after setting [36,426], easy manipulation and application [427], the chemical bond between cement and dentin [367–369], inhibition of microorganism growth [424,428] and allowing conservative canal preparation for obturation. [429]

Most of the HCSC sealers currently in the market are presented in a pre-mixed single syringe, which can be classified as Type V HCSC materials. Only a few HCSC flowable HCSC Type IV materials are available, which are mixed in a dedicated powder-to-liquid ratio. A few examples of Type IV materials are BioRoot RCS (Septodont, Saint-Maur-des-Fosses, France), MTApex (Ultradent Products Inc., South Jordan, UT, USA) and MTA Flow™ and MTA Flow™ White (Ultradent Products Inc., South Jordan, UT, USA). The difference between the single syringe and powder/liquid presented materials is the active component source, which is necessary for the hydration setting reaction to initiate. Although Type V HCSC materials are termed ‘pre-mixed’ by the manufacturers, this is inaccurate as they require moisture from the environment for the setting reaction to start. [430] In contrast, Type IV materials start setting immediately after mixing two components. Furthermore, single-syringe HCSC sealers include water-soluble polymer vehicles that enable these materials to be used as sealers and interact with the fluids for the hydration reaction to occur. [431]

The vehicle of the flowable HCSC material is described as a medium in which the cement powder components are dispersed and additionally defines the reaction type of the material. [430] Manufacturers do not always state the specific vehicle which is used in the material, nor do they usually declare the complete composition of the HCSC sealer. Different vehicles have a significant influence on the physical material characteristics. [108]For example, BioRoot RCS and the new formulation BioRoot Flow by Septodont are identical materials in composition. However, the presentation of the materials is entirely different: one is powder/liquid, while the other is presented in the single syringe system. Therefore, the vehicles used in the BioRoot RCS and

BioRoot Flow HCSC sealers differ, where Type IV material uses water-based liquid, while Type V material includes non-aqueous water-soluble vehicles. (Septodont, Saint-Maur-des-Fosses, France) Also, the differences can be seen not only between different types of materials but also between the same type of materials. One of which is TotalFill and TotalFill HiFlow sealers by FKG. These materials are identical in composition. However, the modified vehicle in TotalFill HiFlow enables it to withstand higher temperatures. Both materials were proven to be safe to use with warm root canal obturation techniques. [415]

Radiopacity is an essential feature of the clinically used flowable HCSC. The radiopacifiers are included to enhance the radiopacity and be able to evaluate the root canal filling after the procedure. At first, HCSC materials contained bismuth oxide as a radiopacifier; however, this radiopaque additive was changed due to changes in tooth crown colour. [319,432] New generation flowable HCSC materials include zirconium oxide, tantalum oxide, niobium oxide, ytterbium trifluoride bismuth oxide or calcium tungstate as a radiopacifier. The amount of the radiopacifier may even exceed 50% of the material. [430] Such a high amount of radiopacifier affects the rest of the composition and characteristics of the HCSC material. For example, adding the niobium oxide decreases the setting time of the HCSC material. [433,434] However, the inclusion of calcium tungstate increases the setting time. [184,434] Therefore, the addition of both radiopacifiers affecting the hydration reaction is sometimes used to keep flowable HCSC characteristics unchanged.

All the flowable HCSC materials currently in the market are based on different compositions. [111] The main mentioned component is tricalcium silicate; dicalcium silicate is also included in some materials. These components are responsible for the formation of calcium silicate hydrate and calcium hydroxide as a by-product, resulting in the bioactive properties of the flowable HCSC. [164] However, according to the materials Safety Data Sheet (SDS) presented by some manufacturers, the amounts of tricalcium silicate do not exceed 35%; in some materials, it is as low as 5%. If dicalcium silicate is included, the total amount of calcium silicates may be up to 50%, as the amount of dicalcium silicate usually is 5 to 15%. While tricalcium silicate is responsible for the material's initial hydration setting reaction, dicalcium silicate reaction is slower, resulting in later hydration and delayed material strength. [430] As the quantity of the calcium silicate is important, a classification of HCSC materials according to the sum of the highest percentages of tricalcium silicate and dicalcium silicate stated by the manufacturer [430]:

- Low charged - <20%

- Medium charged - >20% but <40%
- High charged - >40%

Some root canal sealers may include calcium phosphate or tricalcium aluminate cementitious phases. [164] Tricalcium silicate hydration reaction was shown to be affected by the calcium phosphate, resulting in the reduction of calcium ion release, which may affect the biological characteristics of the HCSC cement. [435] Calcium aluminate may be found in small amounts (around 5%) in Portland cement-based HCSC materials. [164] When up to 10% by weight of tricalcium aluminate is added to the tricalcium silicate composition, it was found to enhance the short-term and long-term mechanical properties of the HCSC cement. [436] The hydration reaction of tricalcium aluminate has been reported to be instant, producing an amorphous phase which transforms further into tricalcium aluminate hexahydrate crystals, which may strengthen the HCSC material. The strength increase in the short-term is due to the tricalcium aluminate fast hydration reaction, which initially accelerates the hydration process and may strengthen the development of HCSC cement.[437] Despite the claimed improved resistance to the acids and reduced solubility of HCSC by the inclusion of calcium aluminate [374], there are doubts about these results in the biological environment and reduction of calcium ion release in relation to the modified biological properties of HCSC material. [430,438]

1.7.5.1. BioRoot™ RCS

Septodont (Saint-Maur-des-Fosses, France) was one of the first to introduce the Type IV hydraulic calcium silicate-based root canal sealer or biological filler in 2015, currently known in the market as BioRoot™ RCS (Root Canal Sealer), which is based on the Active Biosilicate Technology™. The technology facilitates the conversion of raw primary powder materials to pure tricalcium silicate hydrated cement without the presence of any additives, such as calcium sulphate and aluminate, in the final hardened cement. The Type IV material is available as a powder containing tricalcium silicate, povidone and zirconium oxide and an aqueous liquid containing calcium chloride and a water-soluble polymer polycarboxylate. [225]

BioRoot™ RCS is prepared by manually mixing the powder (1 spoon) and the liquid (5 drops) on a sterile glass plate with a spatula. The working time of the material is around 15 minutes, and the setting time is less than 4 hours after insertion into the root canal. The material was found to have an acceptable seal with the gutta-percha and the dentin. [439] However, micro-CT assessment revealed a significant difference in void percentage with more voids present in BioRoot RCS fillings with lateral gutta-percha compaction

technique compared to AH Plus. [440] However, no root canal fillings can show the absence of voids. [441] BioRoot RCS has proper radiopacity (more than 7mm of aluminium) with a time-dependent tendency to increase the radiopacity, reaching up to 8mm of Al, possibly due to favourable environmental conditions, particle rearrangement and deposition of hydration reaction products. [426,442] A root canal sealer with an acceptably high flow rate may fill root canal irregularities, accessory canals and isthmuses. [428] However, the more flowable the sealer is, the greater the risk of sealer extrusion to the periapical tissues. [422] The mixed BioRoot RCS has a smooth consistency with acceptable flowability: a film thickness of around 52 µm, a flow rate of 16 mm and a pH of 12 in 1 and 28 days. [443] The flowability may increase following placement into the root canal due to the body temperature. [402] The flowability of BioRoot RCS was found to be lower than that of TotalFill BC Sealer. [427] BioRoot™ RCS is a Type IV water-based sealer, which is not suitable to be used with warm gutta-percha obturation techniques. After heat application up to 200 °C at a rate of 20 °C/min, the lowest flow with the highest viscosity and greatest weight loss were observed in BioRoot RCS. [415] Heat treatment of BioRoot RCS above 77 °C leads to an immediate setting of the material. [415] Therefore, it is recommended to use this material only with cold lateral compaction or single-cone obturation techniques due to the heat production during warm vertical compaction can lead to the desiccation of the material, adversely affecting its film thickness and flowability. [426] Currently, the single-cone technique is highly recommended while using HCSC sealers/fillers [444], resulting in a similar percentage of voids in the root canal filling compared to other obturation techniques [28] and a clinical success rate of around 91% after one year. [47] Furthermore, BioRoot RCS was found to be applicable even as a pulp-capping material when a more fluid cement than hard, putty consistency, such as Biodentine, is required. [445]

The BioRoot RCS is biocompatible [439], has lower cytotoxicity than other conventional root canal sealers, such as Kerr's Pulp Canal Sealer (Kerr, Italy) or MTA-Fillapex (Angelus, Londrina, Brazil) and preserves the osteo-odontogenic intrinsic properties of pulp-derived stem cells, therefore, providing a more suitable environment to induce stem cells for hard tissue deposition. [445–447] Also, the material was shown to excel in antimicrobial activity and does not allow any biofilm accumulation. [423] Therefore, its antimicrobial properties can reduce clinical failures due to potential bacterial growth. BioRoot™ RCS is a hydraulic calcium silicate-based sealer, consequently, it is free of monomers. [402] As a result, the risk of any adverse tissue reactions is reduced. Additionally, the crystallisation of BioRoot™ RCS results in a hermetic seal for improved resistance to µleakage. The results of

the BioRoot RCS with the root canal dentin wall revealed excellent sealing ability in all root thirds through SEM analysis. [439] The material is bioactive and induces bone physiological processes and dentinal hard tissue mineralisation, which creates a suitable environment for periapical tissue healing. Its bioactive properties include biocompatibility, alkaline pH, and hydroxyapatite formation while setting and surrounding tissue mineralisation. [142,402]

1.7.5.2. Endo-Eze™ MTA Flow™ and MTA Flow™ White

Recently, Type IV flowable HCSC cement was introduced into the market by Ultradent as Endo-Eze™ MTA Flow™ and MTA Flow™ White (Ultradent Products Inc., South Jordan, UT, USA). Difficult handling characteristics, the necessity of proper delivery and condensation of HCSC to the placement site to ensure a hermetic and tight seal creating the best possible success of the treatment [318,342] led to the development of flowable materials in order to be able to manipulate in gel/powder ratio, thus acquiring the flowable consistency for the usage of the material as a root and pulp treatment material. In total, MTA Flow can be mixed into three consistencies: Thin, Thick, and Putty, two of which are flowable: Thick and Thin. Thin consistency includes the lowest amount of powder and the highest volume of liquid. Due to the highest flowability, it is intended to be delivered via a syringe with a NaviTip™ Tip (29ga). In contrast, Thick consistency includes more powder (by 0.7 grams more) than Thin consistency and should be delivered via a syringe with a Micro Tip (20ga). The material-allowed variation in the powder-to-gel ratio is innovative compared to the HCSC available in the market, which strictly states to maintain suggested manufacturers' recommended mixing ratio. Due to the variable mixing consistencies, MTA Flow is indicated to be used in various endodontic procedures: root-end filling, direct pulp capping or as a dressing over pulpotomies, perforation repair, apexification, and root resorption. Suggested approximated proportions of powder and gel to be used with different achieved consistencies of the MTA Flow White and MTA Flow by the manufacturer, together with dedicated indications, are presented in Table 9.

Table 9. Suggested approximated proportions of MTA Flow™ and MTA Flow™ White powder-to-gel ratio to achieve desired consistency by the manufacturer.

Indications	Pulp Capping, Pulp Chamber Perforation, Primary Dentition Vital Pulpotomy	Resorption, Apexification, Apical Plug	Root End Filling
Powder (measuring spoon)	2 big ends (0.26g)	1 big end + 1 small end (0.19g)	1 big end + 1 small end (0.19g)
Gel drops	3 drops	3 drops	1 drop**
Consistency	Thick	Thin	Putty
Delivery tip	Micro Tip (20ga)	NaviTip™ Tip (29ga)	Non-syringe delivery (non-flowable)

According to the manufacturer, MTA Flow consists of an extremely fine, radiopaque and inorganic powder of tricalcium and dicalcium silicate. After mixing, both MTA Flow repair cement should have a smooth consistency due to the ultrafine-grained powder and proprietary gel medium. The formulation was orientated to increase washout resistance [448], which is necessary to ensure the mixture remains in the placed operative field. MTA Flow was shown to exhibit hydroxyl ion release, resulting in an alkalising capability [223], low solubility, adequate radiopacity, biocompatibility and induced mineralisation when compared to other HCSC materials. [230,449]

1.7.5.3. TotalFill® BC Sealer™, EndoSequence® BC Sealer™ and iRoot®SP

Innovative BioCeramix Inc., Vancouver, a Canadian company, in 2008 introduced the first premixed and ready-to-use Type V HCSC material, iRoot SP injectable root canal sealer (iRoot®SP). The material has been available as EndoSequence® BC Sealer™ in North America from Brasseler, USA, since 2008 and as TotalFill® BC Sealer™ in Europe from FKG Dentaire, Switzerland. The sealers are presented as pre-mixed syringes with removable disposable tips. All three named products are chemically identical and are composed of tricalcium silicates, calcium phosphate monobasic, zirconium oxide and fillers while maintaining comparable biological and physicochemical characteristics and handling properties, such as flow, pH, shrinkage, setting time and radiopacity. [36] They were also found to be clinically effective. [47] TotalFill® BC Sealer™, EndoSequence® BC Sealer™ and iRoot®SP are easy-to-use, premixed and ready-to-use injectable white-

coloured HCSC pastes that are indicated to be used for permanent root canal filling applications in both pulpitis and periodontitis treatment cases.

These HCSC Type V sealers were found to have excellent biocompatibility even at high concentrations compared to epoxy resin-based sealers, such as AH Plus (Dentsply DeTrey Gmbh, Konstanz, Germany). [450] BC Sealers demonstrated better cytocompatibility regarding cell viability, migration, cell morphology, cell attachment and mineralisation capacity than AH Plus. [451] These sealers exhibit antimicrobial activity, particularly are associated with a significantly greater reduction of E. faecalis and eliminated C. albicans. [424] Although all root canal fillings contain voids to various degrees, with open porosity being a predominant type, the sealers tend to expand slightly during setting, thus reducing the porosity. [452] However, other authors revealed increased total porosity values after six months of incubation, especially in the apical third. [453] Nevertheless, reduction in porosity is noteworthy in order to maintain a tight seal between the sealer paste, core filling material, such as gutta-percha and the root canal dentin. Furthermore, these materials are highly flowable compared to Type IV BioRoot RCS [427], therefore ensuring superior penetrability into root canal irregularities, isthmuses and dentinal tubules when used with a single-cone gutta-percha matched point. [43]

The premixed and ready-to-use syringes of these HCSC sealers make the use of these materials clinically appealing, simple and fast. Direct delivery and immediate application of the sealers from the syringe into the root canal are possible with disposable tips or via traditional placement techniques, like transferring the sealer into the root canal with a gutta-percha cone.

Endosequence/TotalFill BC Sealers' working time is more than 4 hours at room temperature, and the setting time after insertion into the root canal is around 4 hours. The setting time depends on the moisture in the root canal dentine and can extend up to 10 hours in over-dried root canals. [426] An increase in body temperature may decrease the setting time and increase the flowability of the material. However, some authors reported higher temperatures up to 100°C did not significantly influence the setting time of these materials, even though flowability was found to decrease at 100°C. [454] However, other authors report increased setting time almost twice, from 24 to 48 hours, when subjected to 200°C heat up to 30 seconds. [415] Nevertheless, these sealers were designed to be used with cold and warm root canal obturation techniques. Endodontic retreatment was proven to be not more complicated or even less difficult, requiring less time and leaving less remaining sealer after root canal filling removal when these sealers are used along with gutta-percha points compared to epoxy resin-based sealers. The fillings can be removed using traditional root canal filling techniques. [455–457]

1.7.5.4. TotalFill® BC Sealer™ HiFlow and EndoSequence® BC Sealer™ HiFlow

HCSC materials setting reaction is based on water, which is necessary for the hydration reaction. Also, the HCSC sealers/cements were invented to be used with cold root canal obturation techniques due to the possibility of increasing the volume of the sealer and reducing the volume of the gutta-percha [458], as opposed to the root canal filling when epoxy resin-based sealers are used due to their shrinkage. [459] Scientific reports have proved that no root canal filling methods/materials can fill root canals completely. Both warm vertical compaction and single-cone technique provide comparable leakage and void presence in the fillings. [399,460] Other authors report warm vertical compaction as a superior root canal filling technique. [408,461] Nevertheless, after scientific reports, manufacturers have proposed that warm root canal obturation techniques created by heat application can significantly alter material properties. Therefore, warm obturation methods cannot be used with conventional HCSC sealers. As a result, the new formulations of Type V premixed HCSC TotalFill® BC Sealer and EndoSequence® BC Sealer™ were developed to be used with warm root canal obturation methods, as these materials can withstand heat up to 220° C. [402] Also, HiFlow versions of HCSC sealers have lower viscosity even after heat application [415] and are more radiopaque. However, after 28 days, it was shown not to differ significantly from the original EndoSequence® BC Sealer™. [442] According to the manufacturer, these mentioned properties optimise the usage of HiFlow sealer for warm vertical compaction techniques. However, the necessity of the novel HiFlow HCSC sealers is still questionable [402,462] since real-time intracanal temperature measurement techniques revealed a markedly lower temperature increase during the warm vertical compaction than stated by previous studies. The maximum temperature increase of 19.2°C was shown. [463] Nevertheless, even assuming temperatures reach higher temperatures, clinically relevant temperature levels and heating times do not result in relevant physical or chemical Type V HCSC sealers, such as TotalFill® BC or TotalFill® BC HiFlow. [462,464,465] Therefore, some authors suggest regular TotalFill® BC Sealer over HiFlow since the latter is cheaper and more effective. [462] However, when heat is applied to Type IV materials, such as BioRoot RCS, these materials' physical properties are significantly affected by drastically reducing setting time and flowability. [464] Therefore, while Type IV materials are intended to be used with cold root canal obturation techniques, Type V HCSC sealers, due to different characteristics, can be applied with both cold and warm root canal obturation methods regarding regular or HiFlow versions of these materials. Presentation, composition, indications and properties of

some commercially available Type IV and Type V flowable HCSC materials are shown in Table 10, Table 11 and Table 12.

Table 10. Indications and properties of some commercially available Type IV Flowable Hydraulic calcium silicate-based sealers and cement, according to the manufacturers.

Material trade name	Group	Indications according to the manufacturer	Properties and rationale to use, according to the manufacturer
BioRoot RCS™ (Root Canal Sealer) - Septodont	Sealer	- For root canal permanent filling using cold lateral compaction or single-cone obturation methods	- Based on the Active Biosilicate Technology™ that enables the hydration of raw powder material to pure tricalcium silicate hardened cement. - It is the water-based HCSC sealer, which is sensitive to heat and desiccation, therefore should not be used with the warm obturation techniques.
MTA Flow™ - Ultradent	Flowable cement (variable consistency)	Dental procedures contacting vital pulp tissues: - Primary dentition vital pulpotomy - Pulp capping Dental procedures possibly contacting the periradicular tissues: - Root-end filling - Apexification - Perforation repair - Root resorption *Grey version of MTA Flow should not be used in the aesthetic region (front teeth) due to the Bismuth oxide, which can cause tooth discolouration	- Based on di- and tri-calcium silicates, powder and liquid/gel system consisting of extremely fine, radiopaque, inorganic powder, which sets with a water-based gel for improved placement. - Flowable Thin and Thick consistencies are available in order to ease the delivery of the HCSC to the operative site (for example, placement beyond root curvatures to the apical root third or direct placement of the flowable HCSC onto the dental pulp in the molar teeth region) - MTA Flow HCSC is intended to be used without additional filling materials (like gutta-percha, etc.)
MTA Flow™ White - Ultradent	Flowable cement (variable consistency)		
MTApex™ - Ultradent	Sealer	- For root canal permanent filling using cold lateral compaction or single-cone obturation methods	- The proprietary gel and tricalcium/dicalcium silicate powder mixture releases calcium ions. - Deliverable via a NaviTip™ 29 ga single sideport tip or gutta percha.

Table 11. Indications and properties of some commercially available Type V Flowable Hydraulic calcium silicate-based sealers and cement, according to the manufacturers.

Material trade name	Group	Indications according to the manufacturer	Properties and rationale to use, according to the manufacturer
EndoSequence® BC Sealer™ - Brasseler; iRoot®SP - Innovaзve Bioceramix; TotalFill® BC Sealer™ – FKG.	Sealer	- For root canal permanent filling using cold lateral and warm compaction techniques or single-cone obturation methods	- Proved properties of bioactivity, biocompatibility and antibacterial activity - While setting, sealers tent to expand slightly, leading to a tighter seal between the root canal filling and root canal dentine
EndoSequence® BC Sealer™ HiFlow – Brasseler and *TotalFill® BC Sealer™ HiFlow - FKG*	Sealer	- For root canal permanent filling using warm compaction obturation techniques	- These sealers possess higher viscosity and have enhanced heat resistance for use with warm obturation techniques
Bio-C Sealer ION+ - Angelus	Sealer	- For root canal permanent filling using cold lateral and warm compaction techniques or single-cone obturation methods	- Sealers possess all main biological and physicochemical properties of the flowable HCSC materials - Bio-C Sealer ION + is more viscous and more suitable with warm obturation techniques
AH Plus Bioceramic - DentsplySirona	Sealer	- For root canal permanent filling using cold lateral and warm compaction techniques or single-cone obturation methods	- Low solubility and low film thickness sealer stably seals the root canal - High washout resistance - More radiopaque compared to current market leaders
Komet Bioseal - Komet	Sealer	- For root canal permanent filling using cold lateral and warm compaction techniques or single-cone obturation methods	- Excellent antibacterial effect to its initially high pH value during the curing phase - The most efficient but also the most economical bioceramic sealer
CeraSeal - MetaBiomed	Sealer	- For root canal permanent filling using cold lateral and warm compaction techniques or single-cone obturation methods	- It has a shorter setting time and is highly resistant to washout - Biological, physicochemical properties and clinical applicability are comparable to other HCSB sealers
BioRoot™ Flow - Septodont	Sealer	- For root canal permanent filling using cold lateral and warm compaction techniques or single-cone obturation methods	- The formulation from original BioRoot RCS was improved by changing vehicle to copolymer, therefore, the material is pre-mixed and can be used with warm obturation techniques - Maintaining biological and bioactive properties of BioRoot RCS

Table 12. Presentation and composition of some commercially available Type IV and Type V flowable Hydraulic Calcium silicate-based sealers/fillers and cement.

Material trade name	**Single syringe**	**Composition**						
		Cement			**Radiopacifier**	**Additves**	**Vehicle**	**Other**
		Tricalcium silicate	***Dicalcium silicate***	***Other***				
BioRoot™ RCS (Root Canal Sealer)	No, powder/ liquid	+		Povidone	Zirconium Oxide 25 – 50%	Calcium Carbonate 25 – 50%	Water based gel with Calcium Chloride dihydrate and polycarboxylate	
MTA Flow™	No, powder/ liquid	+	+		Bismuth Oxide			
MTA Flow™ White	No, powder/ liquid	+	+		Tantalum Oxide	Calcium Sulphate, Silica	Water, water soluble silicone-based gel	
MTApex™	No, powder/ liquid	+	+	Tricalcium aluminate	Tantalum Oxide		Water based Gel	
EndoSequence® BC Sealer™; iRoot®SP; TotalFill® BC Sealer™	Yes	20 – 35%	7 – 15%		Zirconium Oxide 35 – 45%			1 – 4% Calcium Hydroxide
EndoSequence® BC Sealer™ HiFlow and *TotalFill® BC Sealer™ HiFlow*	Yes	20 – 35%	7 – 15%		Zirconium Oxide 35 – 45%			1 – 4% Calcium Hydroxide

Continuation of Table 12. Presentation and composition of some commercially available Type IV and Type V flowable Hydraulic Calcium silicate-based sealers/fillers and cement.

Material trade name	**Single syringe**	**Composition**						
		Cement			**Radiopacifier**	**Additves**	**Vehicle**	**Other**
		Tricalcium silicate	***Dicalcium silicate***	***Other***				
AH Plus Bioceramic	Yes	5 – 15%			Zirconium Oxide 50 – 70%		Dimethysulphoxide 10 – 30%	Lithium Carbonate <0.5%, Thickening Agents <6%
BioRoot™ Flow	Yes	+			Zirconium Oxide 25 – 50%	Calcium Carbonate <5%	Propylene Glycol 25 – 50%	
Bio-C Sealer ION+	Yes	+	+	Tricalcium Aluminate		Silicon Oxide, Calcium Oxide	Propylene Glycol	Iron Oxide
CeraSeal	Yes	20 – 30%	1 – 10%	Tricalcium Aluminate 1- 10%	Zirconium Oxide 45 – 50%			
Komet Bioseal	Yes	20 – 30%	1 – 10%	Tricalcium aluminate 1- 10%	Zirconium Oxide 45 – 50%			

Information is based on manufacturers' provided Safety Data Sheets (SDS) and cited scientific articles [430]

1.8. Bioceramic-coated (BC) gutta-percha points for root canal obturation

Root canal obturation aims to create a perfect void-free filling, preventing infection from spreading in the root canal system. [466] It is known that none of the fillings provide gap-free and leakage-free filling, [210,467] and it is essential to prevent bacterial leakage between the root canal system and periapical tissues, as it may be associated with a root canal treatment failure. [201,468–470] As long as the root canal filling aims to reduce the porosity as much as possible and achieve a solid pore-free root canal filling. Therefore, using techniques to reduce the prevalence of porosity may improve the root canal treatment outcome.

As gutta-percha does not adhere well to the root canal sealers, in the 2000s, there was a similar idea from manufacturers to enrol adhesive mechanisms to create bonded interfaces from the sealer to the core obturation material, which is gutta-percha. This theoretical concept aimed at creating a monoblock in the root canal space, therefore eliminating interfacial gaps, producing ideal coronal and apical sealing and, most importantly, preventing post-treatment reinfection of the root canal system. [471,472] Examples of this concept include resin-coated gutta-percha cones with functional methacrylate groups for use with a hydrophilic self-priming methacrylate resin-based sealer system and bioactive glass powder-impregnated gutta-percha cones to be used with a glass ionomer-based sealers. [473,474] Theoretically, this concept of materials bonding should produce a tertiary monoblock with three adhesive interfaces: dentine and sealer, sealer and gutta-percha coating, and gutta-percha coating and gutta-percha. [471] However, there are manufacturing failures in creating a uniform gutta-percha coating over the entire surface. Frequently, the coating is damaged, or a non-uniform gutta-percha coating is present, revealing the gutta-percha cone itself. [204] Therefore, the results were doubtful due to the questionable quality of the gutta-percha coating and adhesive mechanisms.

After the introduction of HCSC sealers, manufacturers introduced „Bioceramic-coated“ gutta-percha to improve sealing ability by chemical bonding HCSC sealer to the bioceramic nanoparticles coated gutta-percha point; for example, EndoSequence® BC Points™. (Brasseler, Savannah AU, USA) The goal is again to achieve a monoblock root canal filling when using a single cone root canal filling technique with HCSC sealers. At first, some authors found that using bioceramic-coated gutta-percha points improved endodontically treated teeth fracture resistance to a level comparable to intact teeth. [475] However, further studies revealed conventional gutta-percha cones to be superior when compared to the bioceramic-coated gutta-percha cones

when used with HCSC sealer single-cone filling technique in terms of the interfacial gaps between the gutta-percha and the sealer. [476] The authors discussed possible reasons for the results, which could be attributed to the non-uniform coating of the gutta-percha, and the magnitude of stress created within the root canal during force application may increase with the number of adhesive interfaces present in the tertiary monoblock. [204,476,477] Despite the controversial scientific results, whether bioceramic-coated gutta-percha points, when used with HCSC sealers, provide better root canal sealing quality than conventional gutta-percha points, some manufacturers still recommend using coated gutta-percha points along with HCSC sealers for a tight and hermetic seal.

When warm root canal obturation techniques are used, extreme heat can reduce the hydration of the HCSC sealers and alter the material properties, affecting the hydration reaction, especially the Type IV sealers. [117] Therefore, when a clinician prefers to use a warm obturation technique with HCSC sealer, the use of 150 series bioceramic-coated gutta-percha points (Brasseler, Savannah AU, USA) and pellets could be beneficial. The 150 series gutta-percha contains bioceramic nanoparticles and melts at 150°C. Therefore, a lower melting temperature reduces the heat. However, these precautions should be applied only for the Type IV HCSC sealers, such as BioRoot™ RCS, as the Type V pre-mixed materials are heat and desiccation-resistant. [402]

2. MATERIALS AND METHODS

2.1. Microcomputed Tomographic Assessment of Flowable Hydraulic Calcium Silicate-based Cements

2.1.1.Porosity Distribution in Single-Cone Root Canal Fillings Performed by Operators with Different Clinical Experience

2.1.1.1. Specimen preparation

The sample size of experimental groups was calculated using G*Power 3.1 software (Heinrich Heine, Iniversität Düsseldorf, Düsseldorf, Germany) following a t-test family and the difference of two independent means with an alpha error probability of 0.05, and a power (1-beta error probability) of 0.95. Therefore, a total of 12 root canals was indicated as the minimum required sample size. One calibrated operator performed root canal chemomechanical preparation. In total, 28 standardised 3D plastic models of upper premolars (DRSK, Hassleholm, Sweden) with pre-opened endodontic accesses were used for the analysis. According to Weine's classification, the plastic teeth had two separate roots and a Type I canal configuration. The working length (WL) was determined by inserting a size 10 K-file (Dentsply Maillefer, Ballaiques, Switzerland) into the root canal until the instrument's tip was visible at the apical foramen. The WL was established 1 mm short of the apex. Root canals were subsequently shaped with HyFlex EDM (Coltene, Langenau, Germany) rotary NiTi endodontic files at the rotation speed of 400 rpm and the torque of 2.5 Ncm, powered by X-Smart (Dentsply Sirona, Ballaiques, Switzerland) endodontic motor. Instruments were used to the full working length in the following sequence: Glide-path file (size 10/0.05 taper), Preparation file (size 20/0.05 taper), OneFile (size 25/~taper) and Finishing file (size 40/0.04 taper).

After using each instrument, the root canals were repeatedly irrigated with 5 mL 3% sodium hypochlorite (Ultradent Products Inc., South Jordan, UT, USA). At the end of preparation, 5 mL of 17% ethylenediaminetetraacetic acid (Ultradent Products Inc., South Jordan, UT, USA) was used for two minutes. 5 mL of sterile distilled water was used as a final flush. Irrigation was performed using disposable syringes and 29-G NaviTip needles (Ultradent Products Inc., South Jordan, UT, USA). After irrigation, root canals were dried with size 40/0.04 taper paper points (Coltene, Langenau, Germany).

2.1.1.2. Root canal obturation

After preparation, plastic models were fixed into prefabricated A-silicone (3M Express, 3M ESPE, Seefeld, Germany) blocks up to the cementoenamel junction to ensure the blindness of the root canal filling procedure. In total, four groups were described in the study. Therefore, specimens were randomly divided into four experimental groups (7 teeth per group), according to the operator performing root canal obturation: undergraduate student (US), endodontology postgraduate student (PS), general dental practitioner (GDP) and endodontology specialist (ED). The single-cone (SC) obturation technique was theoretically introduced to all operators by an experienced academician and specialist endodontist before root canal obturation by giving a 1-hour presentation, following the 2 hours of hands-on practical training. The postgraduate student in the endodontology program had been working in daily clinical practice for two years, while the endodontist and general dental practitioner, both clinicians, had been working for over fifteen years in dental clinical practice.

A total of 14 canals (7 teeth) in each group were filled with BioRoot RCS (Septodont, Saint-Maurdes-Fosses, France) and one HyFlex EDM size 40.04 gutta-percha point (Coltene, Langenau, Germany), resulting in total to 56 filled root canals. The pre-fitted gutta-percha point was inserted into the root canal with a tug-back effect at the WL. The cone was coated with a small amount of the sealer mixed according to the manufacturer's instructions and slowly inserted into the root canal to coat the walls. The procedure was repeated twice to deliver the required amount of the sealer into the root canal. Finally, the last third time, the gutta-percha cone was recoated with the sealer and gently inserted to the full working length. The gutta-percha cone was subsequently cut with a heat carrier at the level of the orifice. The endodontic accesses were filled with temporary filling material (Cavit™-W; 3M ESPE, Seefeld, Germany) and submerged into the thermal bath (Thermo Scientific™Precision™; Fisher Scientific; Vantaa, Finland) containing 37° C water for one week to allow the filling material set completely before further analysis.

2.1.1.3. µCT Scanning and Analysis

All specimens were scanned with a high-resolution micro-CT scanner SkyScan 1272 (Bruker, Kontich, Belgium). All scans were performed using a 90 kV source voltage, 111µA beam current, 10 µm isotropic resolution, 0.2° rotation step and 1350 ms exposure time. A 0.5 mm aluminium and 0.038 mm copper filter were used for artefact reduction. Images obtained from the scan

were reconstructed using NRecon v.1.7.1.0 software (Bruker, Kontich, Belgium) with a ring artefact reduction factor of 3 and beam hardening correction of 20%.

The reconstructed images were analysed using CTAn v.1.14.4.1 software (Bruker, Kontich, Belgium). Selecting root canal contours, the grey scale range required to recognise the filling materials and voids was determined in a density histogram using a global threshold method. Comparisons between the original and segmented scans were performed to ensure segmentation accuracy. The root canal volume was selected as the volume of interest (V_{OI}). Subsequently, the voids (V_{Vol}), filling material (F_{Vol}), open pores (OP_{Vol}) and closed pores (CP_{Vol}) were determined by processing the segmented images with a custom-processing tool. The percentage volume of open pores ($\%OP_{Vol}$) and closed pores ($\%CP_{Vol}$) was calculated using the following formulas:

$$\% \, OP_{Vol} = OP_{Vol}/(V_{Vol} + F_{Vol}) \times 100$$
$$\%CP_{Vol} = CP_{Vol}/(V_{Vol} + F_{Vol}) \times 100$$

All images were examined by a single evaluator blinded to data regarding experimental groups and their specimens. The percentage volume of open and closed pores was calculated separately for the apical, middle and coronal thirds at intervals of 3 mm of each third, and a total V_{OI} of 9 mm was selected for assessment. The analysis did not include the last apical 1mm of the root. The CTVol v.2.2.3.0 software (Bruker, Kontich, Belgium) was used for 3D volumetric visualisation and qualitative evaluation of the fillings.

2.1.1.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, version 29 (IBM Corp., Armonk, N.Y., USA). The Shapiro–Wilk test revealed a non-normal distribution of the data. Therefore, the differences among experimental groups were compared using a non-parametric Kruskal–Wallis test. When statistically significant p-values were found, the Mann–Whitney test was applied for pairwise comparisons comparing the differences in the first part between US, PS, GDP and ED groups. The differences between the root canal thirds in the same group were determined using a non-parametric Friedman test followed by the Wilcoxon test for pairwise comparisons. The significance level was set at $p < 0.05$.

2.1.2. The Effect of Ultrasonic Agitation on the Porosity Distribution in Apically Perforated Root Canals Filled with Different HCSC Materials and Techniques

2.1.2.1. Specimen Selection and Preparation

A total of 40 human mandibular first molars were selected for this study, under the approval of the VšĮ Vilnius University Hospital Žalgiris Clinic local ethics committee, protocol EK-2. One calibrated operator performed all the procedures. The minimum sample size was calculated using G*Power 3.1.9.7 software (Heinrich Heine, Iniversität Düsseldorf, Düsseldorf, Germany) followed by t-test family, α error probability of 0.05 and 1-β error probability of 0.95. Therefore, the requirement of 16 root canals per group was determined. Teeth were extracted for medical reasons unrelated to the present study and were stored in a saline solution at room temperature until use. Only molars with two separate mesial root canals, fully developed root apices and moderately curved roots (10°–20°) were selected. The root curvature was determined on preoperative radiographs using Schneider's method. [478]

The orifices of root canals were accessed conventionally by preparing endodontic cavities with high-speed Endo Access burs (Dentsply Sirona, Ballaigues, Switzerland) under copious water-cooling. The presence of two separate mesial root canals was confirmed radiographically using the size 10 K-file (Dentsply Sirona, Ballaigues, Switzerland) inserted to the full working length (WL). The WL of both mesial canals was determined by inserting a size 10 K-file into the root canal until the tip approached the apical foramen and was visible under 10x magnification (OPMI Pico, Carl Zeiss, Oberkochen, Germany). Afterwards, the WL was increased by 2 mm to over-instrument the root canal and simulate apical perforation. All mesial canals were enlarged beyond the apical foramen. The glide path was created using size 15 and 20 K-Flexofiles (Dentsply Sirona, Ballaigues, Switzerland). The root canal shaping was performed with ProTaper NEXT (Dentsply Sirona, Ballaigues, Switzerland) nickel-titanium rotary instruments at the established WL in the following sequence: X1 (17/0.04), X2 (25/0.06), X3 (30/0.07), X4 (40/0.06) and X5 (50/0.06). Instruments were driven using an X-Smart (Dentsply Sirona, Ballaigues, Switzerland) endodontic motor at the rotation speed of 300 rpm and a torque of 1 Ncm.

After the use of each instrument, root canals were repeatedly rinsed with 5 mL of 3% sodium hypochlorite (Ultradent Products Inc., South Jordan, UT, USA), while 5 mL of 18% ethylenediaminetetraacetic acid (Ultradent Products Inc., South Jordan, UT, USA) followed by 5 mL of distilled water

was used for the final flush at the end of instrumentation. The irrigants were delivered using 29-G NaviTip needles (Ultradent Products Inc., South Jordan, UT, USA) attached to disposable syringes. Afterwards, the root canals were dried with paper points.

Imitating surrounding periodontal tissues and the alveolar bone was achieved using prefabricated A-silicone (3M ESPE, Seefeld, Germany) blocks. Specimens were fixed in these blocks up to the cement-enamel junction after the coverage of apices with a polytetrafluoroethylene tape (Tesa SE, Norderstedt, Germany).

2.1.2.2. Root Canal Obturation

A true randomness generator (www.random.org, accessed on 25 October 2021) was used for the random allocation of the samples into four equal experimental groups (10 teeth/20 canals per group) according to the material and technique selected and used for root canal obturation:

- BR/SC group—the root canals were filled with BioRoot RCS sealer and single Pro-Taper NEXT size X5 gutta-percha point (Dentsply Sirona, Ballaigues, Switzerland). The apical 4 mm of the gutta-percha point was cut with a sterile scalpel to fit the gutta-percha with a tug-back effect 2 mm shorter than the perforated apical foramen. The sealer was mixed according to the manufacturer's instructions, inserted into the Skini syringe (Ultradent Products Inc., South Jordan, UT, USA) and subsequently delivered into the root canal via attached plastic Capillary Tip cannula (Ultradent Products Inc., South Jordan, UT, USA). The tip was inserted approximately 2 mm shorter than the perforation site, and the syringe plunger was gently pressed while withdrawing the plastic cannula until it reached the orifice level. After the injection of BioRoot RCS, the pre-fitted gutta-percha point was coated with a thin amount of the sealer and gently inserted into the root canal 2 mm short of the perforated apex.

- BR/SC-UA group—the root canals were filled with BioRoot RCS sealer and single ProTaper NEXT size X5 gutta-percha point using ultrasonic agitation. The selection and adaptation of the gutta-percha point and the injection of the sealer were accomplished identically to the BR/SC group. After delivering the sealer into the root canal, an Ultrawave ET25 ultrasonic tip (Ultradent Products Inc., South Jordan, UT, USA) attached to an Ultrawave XS ultrasonic device (Ultradent Products Inc., South Jordan, UT, USA) was directly inserted into the root canal and BioRoot RCS sealer 2 mm short of the WL. The ultrasonic tip was activated for 10 seconds at medium power using

Reflex technology (Ultradent Products Inc.), capable of automatic real-time frequency adjustment of 28–36 kHz. The pre-fitted gutta-percha point was subsequently coated with a small amount of the sealer and slowly inserted into the root canal 2 mm shorter than the apical foramen.

- MF group—the root canals were filled with MTA Flow™ cement. A total of 0.19 g of powder and 3 drops of liquid were mixed according to the manufacturer's recommendations to get a thin consistency of the cement. The mixed material was inserted into the clear Skini syringe, and the flowability of the material was checked by extruding the small amount of the cement through the attached 29-G NaviTip needle. The filling material was delivered into the root canal by slowly pressing the syringe plunger and withdrawing the tip, which was inserted 2 mm short of the perforated apex.
- MF-UA group—the root canals were filled with MTA Flow™ cement using ultrasonic agitation. The filling material was prepared and injected into the root canal, like in the MF group. Afterwards, the Ultrawave ET25 ultrasonic tip was directly inserted into the root canal and MTA Flow cement, 2 mm short of the perforation site and activated for 10 seconds at the 28–36 kHz frequency and the power described previously.

Postoperative radiographs were made immediately after the obturation of the root canals to evaluate the filling quality. The obturation procedure was repeated when a lack of homogeneity or inadequate filling length was observed. New radiographs were taken to confirm the quality of the root canal fillings afterwards. The heat carrier was used to cut the gutta-percha point at the orifice level in the BR/SC and BR/SC-UA groups. The endodontic access cavities of all specimens were sealed with temporary filling material Cavit™-W (3M ESPE, Seefeld, Germany), and the teeth were stored at 37°C and 100% humidity for seven days to allow the filling materials to set completely.

2.1.2.3. µCT Analysis

Teeth were scanned before and after root canal obturation with a high-resolution micro-CT scanner SkyScan 1272 (Bruker, Kontich, Belgium). The scanning parameters were set at 100 kV source voltage, 100 µA beam current, 9.9 µm isotropic resolution, 0.11 mm copper filter, 1073 ms exposure time, 0.4° rotation step and 360° rotation angle. The obtained images were reconstructed using NRecon v.1.6.9.18 software (Bruker, Kontich, Belgium) under a beam hardening correction of 20% and a ring artefact reduction factor of 6.

The CTAn v.1.14.4.1 software (Bruker, Kontich, Belgium) was used to analyse the quality of root canal fillings in the apical 5 mm. All grayscale images from the selected region of interest were converted to binary images using a global threshold method in a density histogram. The original and segmented scans were thoroughly compared to confirm the segmentation accuracy before further analysis with a custom-processing tool. Images obtained from pre-obturation scans were used to quantify the root canal volume (C_{Vol}). In contrast, post-obturation images were used to determine volumes of filling material (F_{Vol}) and closed pores (CP_{Vol}). The total volume of pores (V_{Vol}) and volume of open pores (OP_{Vol}) were calculated using the following formulas, respectively:

$$V_{Vol} = C_{Vol} - F_{Vol},$$
$$OP_{Vol} = V_{Vol} - CP_{Vol}.$$

Afterwards, the percentage volume of open ($\%OP_{Vol}$) and closed ($\%CP_{Vol}$) pores was determined as follows:

$$\%OP_{Vol} = OP_{Vol}/C_{Vol} \times 100,$$
$$\%CP_{Vol} = CP_{Vol}/C_{Vol} \times 100$$

µCT images were evaluated by a single person blinded to data regarding the root canal filling material and technique.

2.1.2.4. Statistical Analysis

The porosity distribution between experimental groups was compared using a non-parametric Kruskal-Wallis test followed by the Mann-Whitney test due to a non-normal distribution of the data and validated with the Shapiro-Wilk test. All comparisons were performed using IBM SPSS Statistics, version 29 (IBM Corp., Armonk, N.Y., USA), with the significance level set at $p < 0.05$.

2.2. Biocompatibility Analysis of Flowable Hydraulic Calcium Silicate-based Cements

One calibrated operator performed the study in the Vilnius University Life Sciences Centre laboratory. The Lithuanian Bioethics Committee registered and approved this study, approval no. 158200-16-860-369, 2019 revision. The research was supported by a Research Project grant (Vilnius University, Lithuania), grant no. MSF-JM-4/2020 and MSF-JM-14/2021.

2.2.1. Groups and tested materials in the study

Groups in the analysis were divided as follows:

- Negative control group (NegativeCG) – hDPSCs cell culture, grown in a growth medium, used as a reference control group.
- Positive control group (PositiveCG) – hDPSCs cell culture grown in leachates extracted from intermediate restorative material (IRM, Dentsply, Tulsa, OK, USA). This group was used as a positive control group, where the desired effect – cell necrosis/apoptosis was achieved.
- Control hydraulic calcium silicate cement cell culture group (ProRootCG) – hDPSCs cell culture grown in leachates extracted from ProRoot MTA (Dentsply, Tulsa, OK, USA).
- Tested flowable hydraulic calcium silicate cement cell culture groups:
  - MTA Flow™ cell culture group (MF) – hDPSCs cell cultures grown in leachates extracted from MTA Flow™ 'Thick' consistency (Ultradent, USA).
  - MTA Flow™ White cell culture group (MFWhite) - hDPSCs cell cultures grown in leachates extracted from MTA Flow™ White 'Thick' consistency (Ultradent, USA).
- The PositiveCG, ProRootCG, and MF, MFWhite groups were divided into 100%, 50%, 25%, and 12.5% dilutions according to the leachate concentration. Table 13 shows the features of the hydraulic calcium silicate cement materials used in the study.
-

Table 13. Materials and their composition used in the biocompatibility analysis of flowable hydraulic calcium silicate-based cement.

Material Type	***Material trade name***	***Powder composition***	***Liquid***	***Manufacturer***
Portland cement	ProRoot MTA®	Portland cement, calcium sulfate dihydrate, tetracalcium aluminoferrite, gypsum, calcium oxide, bismuth oxide (radiopacifier)	Distilled water	Dentsply Tulsa Dental, USA
Flowable hydraulic calcium silicate cement	MTA Flow®	di- and tricalcium silicate, calcium sulphate, silica, bismuth trioxide (radiopacifier)	Water, Water soluble silicone-based gel	Ultradent, USA
	MTA Flow® White	di- and tricalcium silicate, calcium sulphate, silica, tantalum oxide (radiopacifier)		

2.2.2. Cell culture

Poietics™ Human Dental Pulp Stem Cells (hDPSC) (cat. no.: PT-5025, Lonza) were used for this study. The cells were cultured according to the manufacturer's protocol. Briefly, hDPSCs were maintained in growth medium (GM): alpha-medium essential (αMEM, Gibco, catalogue no. 12561056) supplemented with 10% fetal bovine serum (FBS; Gibco, catalogue no. A3160802) and 1% streptomycin/penicillin (Gibco, catalogue no. 10378016), cultured in a humified incubator at 37 °C with 5% CO_2. hDPSC monolayer was detached by incubating cells for 3 min at 37°C with EDTA-trypsin (0.25%) solution (Gibco, catalogue no. 25200056). Only passage number 3-5 cells were used in the experiments.

2.2.3. Material (eluate/leachate) preparation

Flowable hydraulic calcium silicate cements MTA Flow™ and MTA Flow™ White (Ultradent Products Inc., South Jordan, UT, USA) were prepared inside a laminar flow hood with sterile instruments following the respective manufacturer's recommendations to the „Thick" consistency: ratio of 2 big ends of powder (0.26g) and 3 drops of liquid. Briefly, 2.12 g in weight / 1.3 cm^3 in volume of the materials were prepared with a sterile 5 cm^3 syringe and applied to the bottom of sterile 50 mm diameter glass plates by delivery tip (Micro Tip 20 Ga). The cement was compacted using a sterile cotton swab until homogenously distributed on the whole plate surface. Immediately after preparing the cement, 13 ml GM was added. The ratio between the surface of the cement exposed and the amount of liquid added was 1638 mm^2 to 13 ml or 126 mm^2/ml, as stated in the ISO 10993-12:2021 standard. The medium was left in contact with the disks for 48 h at 37 ◦C and 95% humidity in CO_2 free atmosphere with soda lime before being collected. The leachate was collected, centrifuged at 15000 RPM (CL10 centrifuge Thermo Scientific) and filtered through the sterile filter with 0.22 µm size pores and the 100, 50, 25 and 12.5% dilutions were made with GM. Eluates were immediately used for further experiments.

2.2.4. pH measurement

pH measurements of all 100% leachates were made in triplicates before centrifugation and filtering through the sterile filter with test papers (*Johnson Test Papers Ltd, Tividale, UK*). pH was measured of the leachates collected in growth medium (GM), which consists of alpha-medium essential

supplemented with 10% fetal bovine serum and 1% streptomycin/penicillin (Gibco).

2.2.5. Cell cytotoxicity and proliferation

hDPSC (5000 cells/cm^2) of 3rd-5th passages were seeded in 96-well-plate and incubated for 24h. Afterwards, leachates were transferred onto hDPSC cultures and incubated for 0, 2, 24, 48, 72, 96 and 120 hours. At each endpoint, 100 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich Co.) solution (0.5 mg/mL in GM) was added to each well, and the specimens were incubated for 3 h at 37 °C with 5% CO_2. MTT solution was discarded, and 100 µL DMSO (≥99,5 %, *BioScience Grade*) was added to each well. The resulting suspension was then measured spectrophotometrically at 570 nm by a microplate reader (*Varioskan® Flash, Thermo Scientific Waltham, Massachusetts, MA, USA*). A standard cell viability curve was used, and the results were expressed as a percentage/ratio concerning the untreated cells. During the experiment, the leachates were renewed after three days by removing 50 µL of GM or leachate and adding 100 µL of new GM/leachate.

2.2.6. Annexin V FITC flow cytometry assay

To detect apoptotic hDPSCs after 24 hours of incubation with 50% fHCSC leachates, annexin V and propidium iodide (PI) double staining was performed using the Annexin V-FITC Apoptosis Detection Kit, according to the manufacturer instructions (Cat. No. 88-8005-72; eBioscience™). The cells stained with annexin V-FITC were analysed with a flow cytometer (*BD Bioscience FacsCanto II Flow Cytometer, Franklin Lakes, New Jersey, U.S.*) using FCS Express (De Novo Software, Los Angeles, CA) to read and analyse the effects of material extracts on the viable, necrotic, early and late apoptotic cell ratios. The three cell viability experiments were repeated independently, and the averages of the obtained values were evaluated.

2.2.7. Cell morphology assessment

hDPSC (10 000 cells/cm^2) of 3rd passage were seeded in 96-well plates and incubated for 24 h. After 24 hours, leachates were transferred onto hDPSC and incubated for 0, 2, 24, 48, 72, 96 and 120 hours. At each endpoint, the effects of leachates from flowable hydraulic calcium silicate cements on cell morphology changes were observed under an inverted phase contrast microscope (Olympus IX51). Differences in cells' morphology and shape

were evaluated by measuring cell width, length and the ratio between width and length using the image processing program ImageJ (ImageJ 1.8.0_172), as described previously by Alksnė et al. [479]

2.2.8. Statistical analysis

Data normality was evaluated using the Shapiro-Wilk test (normality assumption was met). The homogeneity of variances was assessed using Levene's test. The outliers were identified by plotting boxplot graphs. Three-way and Two-way ANOVA were performed to determine the interaction between hydraulic calcium silicate cement type, concentration and/or time. All simple pairwise comparisons were run with Tukey HSD for all HCSC concentrations and all time points with Bonferroni adjustments applied. In graphs, the bar heights represent means, with error bars representing the standard deviations (SD). Statistically significant differences were marked as following between the groups: * – $p<0.05$, ** - $p<0.01$ and *** - $p<0.001$. Each cement leachate was analysed in triplicates per test. IBM SPSS Statistics, version 29 (IBM Corp., Armonk, N.Y., USA) and GraphPad Prism, version 9 (GraphPad Software, San Diego, California, USA) were used for statistical analysis.

3. RESULTS

3.1. µCT Analysis of Porosity Distribution in Single Cone Root Canal Fillings Performed by Operators with Different Clinical Experience

The µCT evaluation revealed pores of various diameters and shapes inside the mass of the hydraulic calcium silicate-based sealer as well as in the interface of the sealer and root canal walls. In contrast, the open pores were the predominant type of porosity in all groups and thirds evaluated (Figure 7). No pores inside the gutta-percha points were detected, as might have been expected.

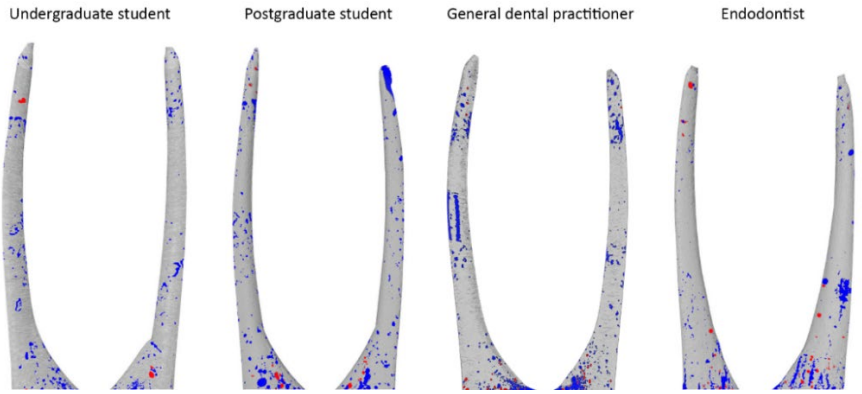


Figure 7. Three-dimensional reconstructions of the single cone root canal fillings in different experimental groups, demonstrating the distribution of open (blue) and closed (red) pores.

The results of the volumetric analysis of the porosity of root canal fillings are detailed in Table 14. The distribution of open and closed pores demonstrated statistically significant differences among all groups in the coronal third ($p=0.017$ and $p=0.02$, respectively). Regarding the results of pairwise comparisons, the percentage of open pores did not differ significantly only between the PS and ED groups and closed pores – between US and PS groups ($p > 0.05$). In the middle third, all groups exhibited a similar number of open pores ($p=0.06$). However, the distribution of closed pores remained significantly different ($p=0.014$). Even though the difference was not statistically significant, the GDP group had a higher mean value of open pores in the middle third, as was the distribution of the mean value in the group. The pairwise comparison revealed that the percentage of closed pores did not differ significantly only between US and PS groups ($p>0.05$). The apical third of root canal fillings had no statistically significant differences when comparing the amount of both open ($p=0.56$) and closed ($p=0.12$) pores.

Table 14. Mean values (%) and standard deviations (SD) of open and closed pores in the coronal, middle and apical thirds.

Group	*N*	*Coronal Third*		*Middle Third*		*Apical Third*	
		Open Pores	*Closed Pores*	*Open Pores*	*Closed Pores*	*Open Pores*	*Closed Pores*
US	14	2.415± 3.071^{A}	0.032± 0.029^{A}	2.970± 3.361^{A}	0.001± 0.003^{A}	8.140± 6.602^{A}	0.208± 0.191^{A}
PS	14	3.567± 2.181^{B}	0.026± 0.030^{A}	5.389± 3.158^{A}	0.003± 0.008^{A}	9.778± 6.324^{A}	0.261± 0.509^{A}
GDP	14	8.792± 7.973^{C}	0.369± 0.170^{B}	7.672± 7.051^{A}	0.053± 0.061^{B}	15.940± 10.001^{A}	0.169± 0.260^{A}
ED	14	3.535± 3.088^{B}	0.090± 0.094^{C}	2.592± 1.755^{A}	0.019± 0.029^{C}	10.861± 7.716^{A}	0.344± 0.378^{A}

Groups marked with the same superscript letter in the same column do not differ significantly (pairwise Mann-Whitney test; $p > 0.05$).

The cross-sectional 2D images of the four experimental groups at the different root canal thirds are shown in Figure 8. The μCT analysis revealed that all groups exhibited the highest percentage of open and closed pores in the apical third of root canal fillings. Furthermore, statistically significant differences were observed in the distribution of open and closed pores within the same group when comparing all root canal thirds ($p < 0.05$).

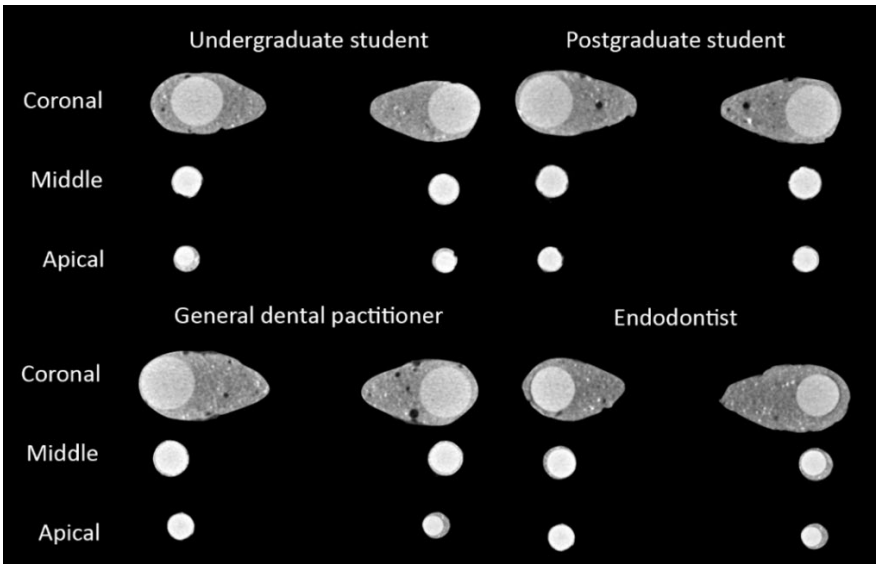


Figure 8. Two-dimensional cross-sectional images at the different thirds of the obturated root canals of random samples of US, PS, GDP, and ED groups demonstrate open and closed porosity inside the fillings.

Regarding the pairwise comparison results, summarised in Table 15, no statistically significant differences were detected only between the coronal and middle thirds of the open pores distribution, where the percentage of open pores remained similar in all experimental groups ($p > 0.05$).

Table 15. p-values from pairwise comparisons of the root canal thirds in the respective group.

Group	***Thirds***	***Open Pores***	***Closed Pores***
US	Coronal – Middle	0.363	0.001*
	Coronal – Apical	0.001*	0.004*
	Middle – Apical	0.001*	0.001*
PS	Coronal – Middle	0.056	0.007*
	Coronal – Apical	0.001*	0.024*
	Middle - Apical	0.006*	0.002*
GDP	Coronal – Middle	0.390	0.002*
	Coronal – Apical	0.034*	0.041*
	Middle - Apical	0.010*	0.208*
ED	Coronal – Middle	0.197	0.005*
	Coronal - Apical	0.002*	0.001*
	Middle - Apical	0.001*	0.001*

*Indicates a statistically significant difference (pairwise Wilcoxon test; $p < 0.05$).

Figure 9 represents the rendered three-dimensional reconstructions of the obturated root canals, demonstrating that open pores were the dominant porosity type in all groups.

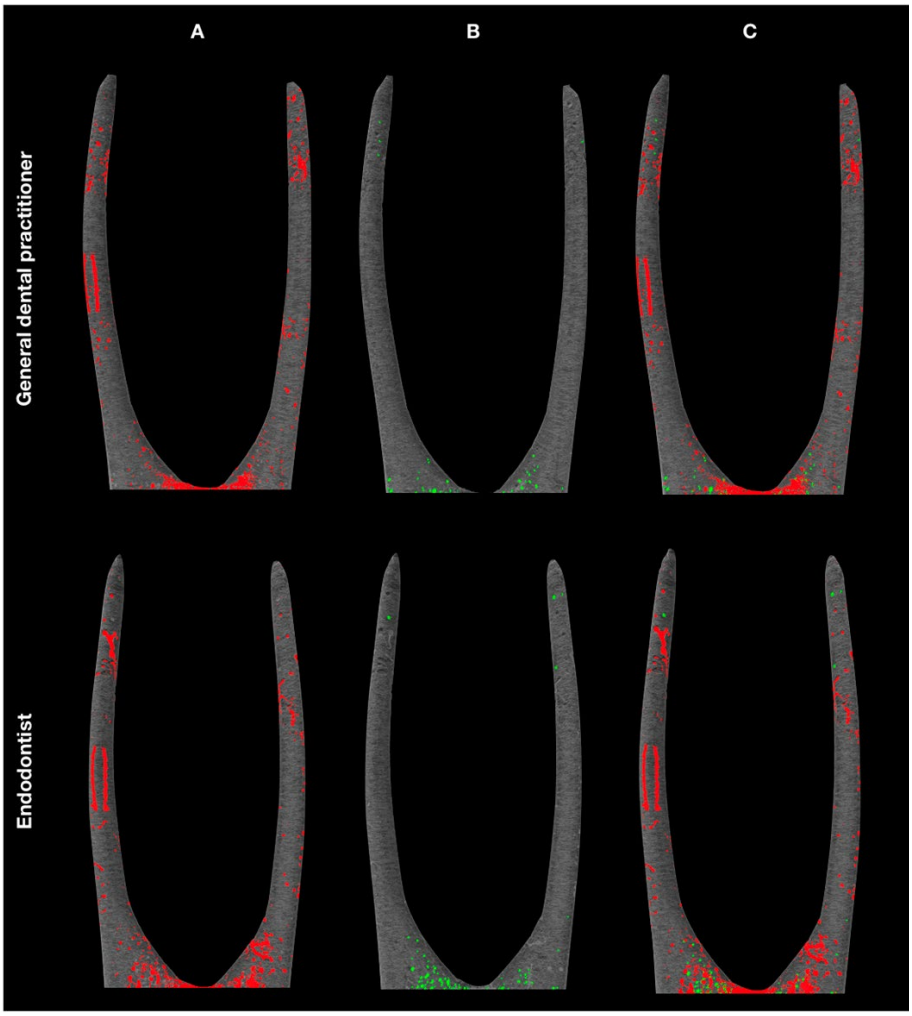


Figure 9. Three-dimensional reconstructions demonstrating the distribution of open (A, red) and closed (B, green) pores and overall porosity (C, superimposed picture) of root canal fillings in GDP and ED groups.

3.2. The Effect of Ultrasonic Agitation on the Porosity Distribution in Apically Perforated Root Canals Filled with Different HCSC Materials and Techniques

None of the techniques used was able to provide a pore-free root canal filling in the apical 5 mm pores; size and shape diversity were observed in all apical plugs, with open pores being the dominant type of porosity. The results of quantitative volumetric analysis of open and closed pores are summarised in Table 16. The μCT assessment revealed that volumes of prepared root canals had no considerable volumetric variances before the root obturation procedure ($p = 0.34$), indicating the initial equality among all experimental groups. However, the porosity distribution in root canal fillings significantly differed between all experimental groups evaluated ($p < 0.05$).

Table 16. Mean values (%) and standard deviations (SD) of open and closed pores in the respective groups.

Group	*N*	Open Pores	Closed Pores
BR/SC	20	3.374 ± 2.751^{A}	0.061 ± 0.080^{A}
BR/SC-UA	20	3.390 ± 3.428^{A}	0.066 ± 0.070^{A}
MF	20	18.832 ± 3.334^{B}	0.292 ± 0.226^{B}
MF-UA	20	29.075 ± 9.440^{C}	0.923 ± 0.684^{C}

Different superscript letters in the same column indicate significant differences between groups (pairwise Mann- Whitney test; $p < 0.05$).

A considerably higher quantity of open and closed pores was observed in both MF groups (with/without ultrasonic agitation) when compared to the fillings of BR/SC and BR/SC-UA ($p < 0.05$). The interaction between the MF and MF-UA groups was detected to be statistically significant ($p < 0.05$), with the highest porosity being in the MF-UA group (Figure 10).

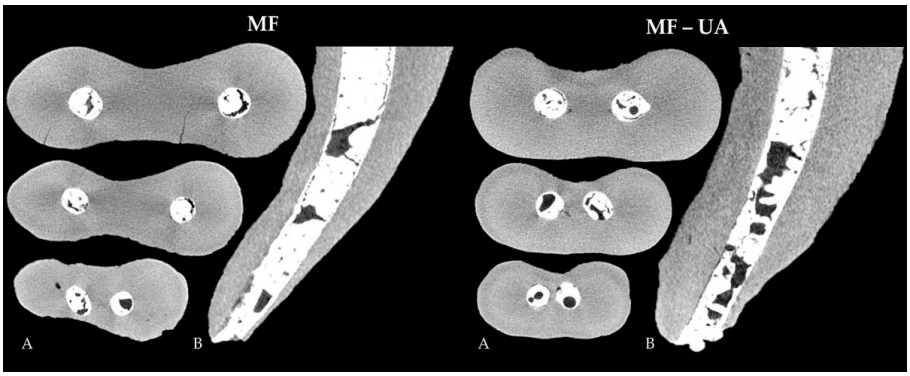


Figure 10. Representative cross-sections of random samples at the levels of 5 mm, 3 mm and 1 mm from the apex (A) and longitudinal sections (B) demonstrate the porosity distribution within the fillings of the MF (MTA Flow) and MF-UA (MTA Flow with ultrasonic agitation) groups.

However, no significant differences were observed between the specimens of the BR/SC and BR/SC-UA groups, where the quantity of open and closed pores within the fillings remained similar ($p = 0.82$ and $p = 0.57$, respectively) regardless of a lower mean porosity determined in the BR/SC group (Figure 11).

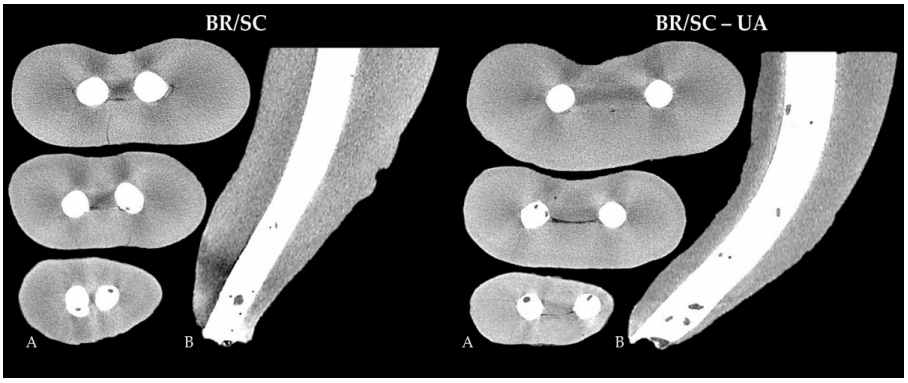
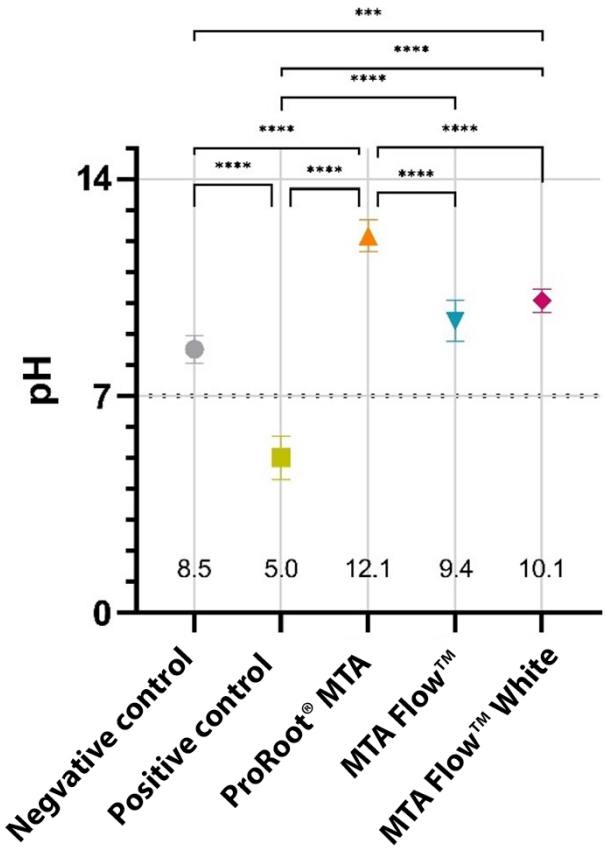


Figure 11. Cross-sectional images at the levels of 5 mm, 3 mm and 1 mm from the apex (**A**) and longitudinal images (**B**) representing the quality and homogeneity of BR/SC (BioRoot RCS/single cone) and BR/SC-UA (BioRoot RCS/single cone with ultrasonic agitation) apical plugs.

3.3. Biocompatibility Analysis of Flowable Hydraulic Calcium Silicate-based Cements

3.3.1. Characteristics of the HCSC leachates

Freshly extracted HCSC 100% leachates pH values of MFWhite and ProRootCG were statistically significantly different from NegativeCG ($p<0.05$). However, MF resulted in slightly lower pH alteration, which was not statistically significantly different from NegativeCG ($p>0.05$). Detailed pH values of freshly extracted HCSC 100% leachates and their comparison can be seen in Figure 12.



** - marks statistically significant differences between the groups (* – p<0.05, ** - p<0.01 and *** - p<0.001), N=3.*

Figure 12. Freshly extracted HCSC, 100% leachates, mean pH values.

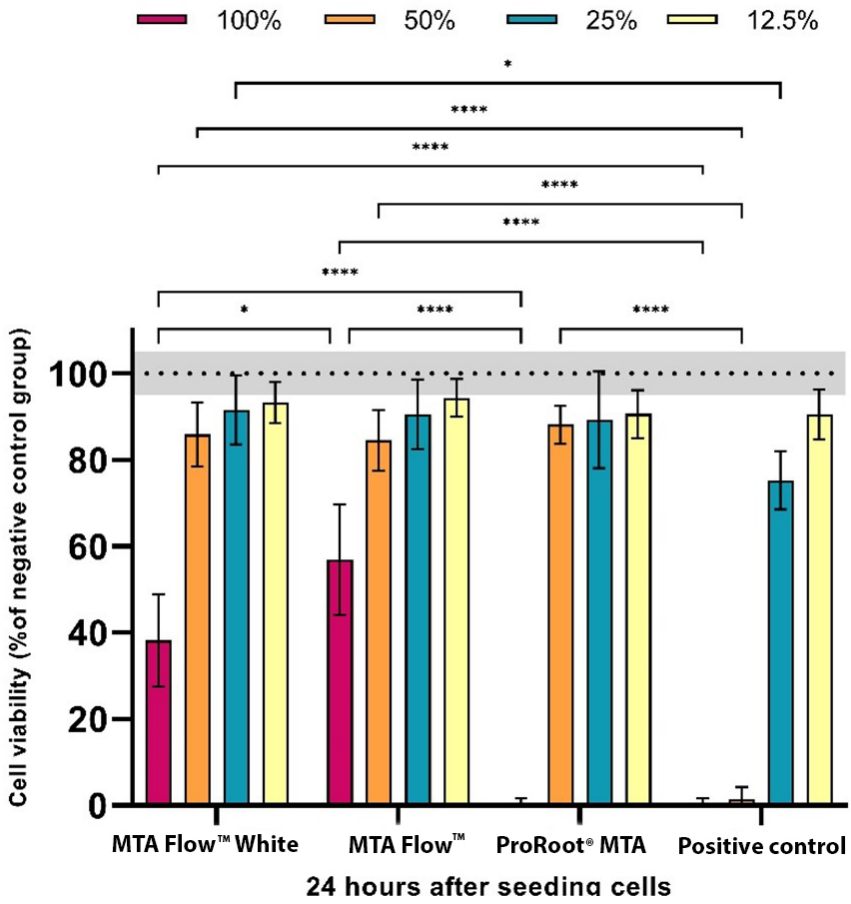
3.3.2. *MTA Flow*^{TM} and *MTA Flow*^{TM} *White* cytotoxicity (MTT) assay

The cell viability was significantly affected by the presence of the freshly mixed cement and to different degrees depending on the cement-leachate solution. Hardened flowable HCSC leachates at all concentrations did not influence cell viability ($p>0.05$). The cell-viability data of hDPCs exposed to freshly-mixed and hardened HCSC are summarised in Figures 13 and 14, accordingly.

At 12.5%, 25% and 50% concentration, all groups exhibited cell viability above 80%. At 100%, both fHCSC groups, *MF and MFWhite,* revealed lower cell viability ($p<0.05$), comparable to that of the NegativeCG. On the other hand, no viable cells were observed in a PositiveCG already at 50% dilution and statistically significantly reduced cell viability at 25% dilution compared to NegativeCG ($p<0.05$).

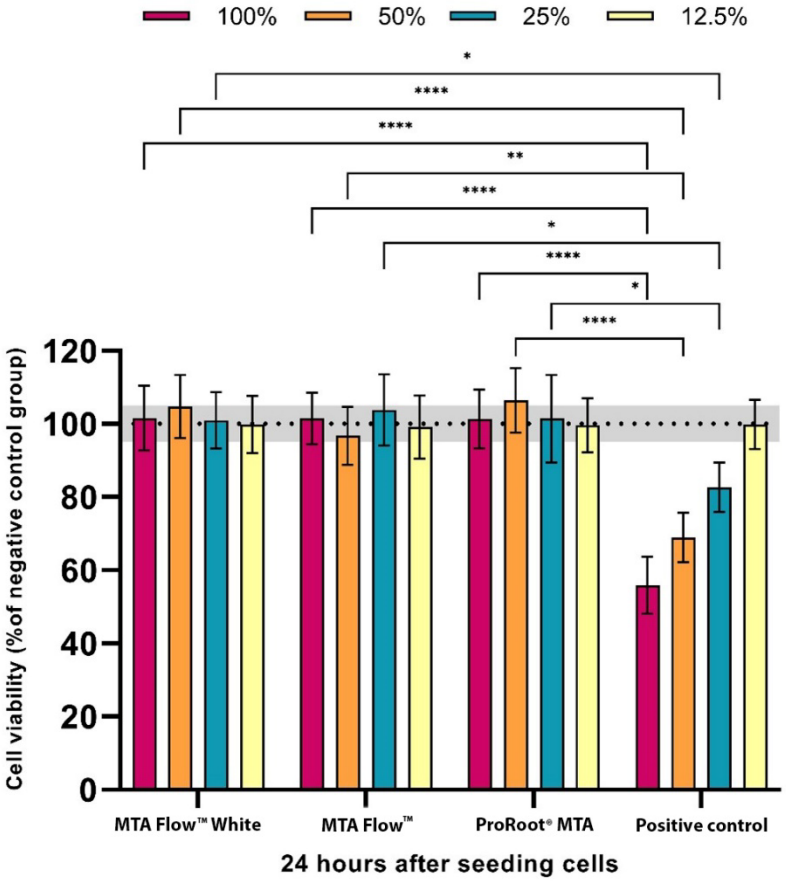
Moreover, no statistically significant differences were found between MF and MFWhite leachates at all concentrations after 24 hours. Both groups were moderately toxic to hDPSCs ($p<0.05$). However, both MF and MFWhite 100% leachate groups demonstrated statistically significantly lower cytotoxicity effects on hDPSCs than ProRootCG 100% leachate ($p<0.05$): hDPSC cell viability after the treatment with MF and MFWhite were 38-57%, while ProRootCG treated cell viability was 0.4-1%. No statistically significant differences were found between HCSC in 50, 25 and 12.5% concentration leachate groups. All groups and their comparison can be found in Figure 13.

When testing leachates collected from hardened HCSC, no differences were found between the tested cement groups and compared to NegativeCG ($p>0.05$). Hardened HCSC leachates cytotoxicity can be found in Figure 14.



The results are presented as a percentage of the negative control group (100% reference line with ±SD grey box). * - marks statistically significant differences between the groups (* – $p<0.05$, ** - $p<0.01$ and *** - $p<0.001$).

Figure 13. MTT cell-cytotoxicity assay: effect of the leachates derived from the freshly mixed fHCSC MTA Flow™ and MTA Flow™ White tested with different dilutions at 24h on the mitochondrial enzymatic activity of hDPCs.



The results are presented as a percentage of the negative control group (100% reference line with ±SD grey box). * - marks statistically significant differences between the groups (* – $p<0.05$, ** - $p<0.01$ and *** - $p<0.001$).

Figure 14. MTT cell-cytotoxicity assay: effect of the leachates derived from the hardened MTA Flow™ and MTA Flow™ White tested with different dilutions at 24h on the mitochondrial enzymatic activity of hDPCs.

3.3.3.Human dental pulp stem cells proliferation assay

A statistically significant three-way interaction between hydraulic calcium silicate cement type, concentration and time was found, $F(60, 360) =188.934$, $p<0.001$. Statistical significance was accepted at the $p<0.017$ level for simple two-way interactions and simple simple main effects. There was a statistically significant simple two-way interaction between hydraulic calcium silicate cement type and concentration for all time points: 2 hours – $F(12,360)=8.239$, $p<0.001$, 24 hours – $F(12,360)=47.357$, $p<0.001$, 48 hours – $F(12,360)=88.918$, $p<0.001$, 72 hours – $F(12, 360)=253.215$, $p<0.001$, 96

hours – F(12, 360)=713.184, p<0.001 and 120 hours – F(12, 360)=1341.966, p<0.001. There was a statistically significant simple simple main effect of hydraulic calcium silicate cement type for HDPSCs proliferation at all concentrations and time points. All simple pairwise comparisons were run for all HCSC concentrations and all time points with a Bonferroni adjustment applied. The proliferation rates of hDPCs mean, [CI] and pairwise comparisons according to the group, concentration and time exposed to HCSC leachates for 2, 24, 48, 72, 96 and 120 hours are summarised in Figure 15 (A-D), compared to the last day NegativeCG (100% reference point), and pairwise comparison is presented in Table 17 (A-D).

The proliferation of hDPSCs was significantly affected by the presence of the freshly mixed cement leachate. Overall, tested fHCSC leachates reduced cell proliferation at all times when exposed to 100 and 50% concentrations compared to NegativeCG (p<0.017). However, the strength of the inhibitory effect of the leachates depended on their concentration. PositiveCG at all time points showed statistically significantly more significant hDPSCs growth inhibition and/or cell death than NegativeCG (p<0.017).

At 100% leachate concentration, the proliferation pattern of the positive control group and *ProRoot MTA* was similar at all times. However, all groups at 100% concentration significantly affected cell viability, compared to NegativeCG (p<0.017). Although both of the tested MF and MFWhite leachates induced hDPSCs death and/or inhibited cell proliferation, the cell proliferation remained higher – from 2.3% up to 9.4%, compared to the PositiveCG and ProRootCG, which were less than 1% viable cells at 100% concentrations after 2 hours time point (p<0.017).

At 2 hours, MF's 100% leachate effect on hDPSCs proliferation was significantly lower by half than MFWhite (p<0.017). However, from the 24-hour time point, MF group cell proliferation was higher than MFWhite by around 3% (p<0.017) or remained similar. The same pattern resulted in 50, 25 and 12.5% MF groups at 48 hours, where lower cell proliferation excelled in the MFWhite group by 4.4%, 3.5% and 3.1%, accordingly (p<0.017).

At 50%, 25% and 12.5%, tested HCSC leachates showed similar hDPSC proliferation at all time points. However, at 48 hours, 50% and 12.5% MFWhite groups had 2.6 and 2.5% lower cell viability than MF (p<0.017). The same attribute was seen in 25% concentration. However, it was not statistically significant (p>0.017). At 120 hours, 25% of MF and ProRootCG groups revealed similar cell viability, around 70%. Meanwhile, 25% MFWhite group cell proliferation was higher, 74.8% (p<0.017). Nevertheless, cell proliferation remained statistically significantly higher from 24-hour time points in 50 and 25% tested fHCSC groups than in PositiveCG (p<0.017).

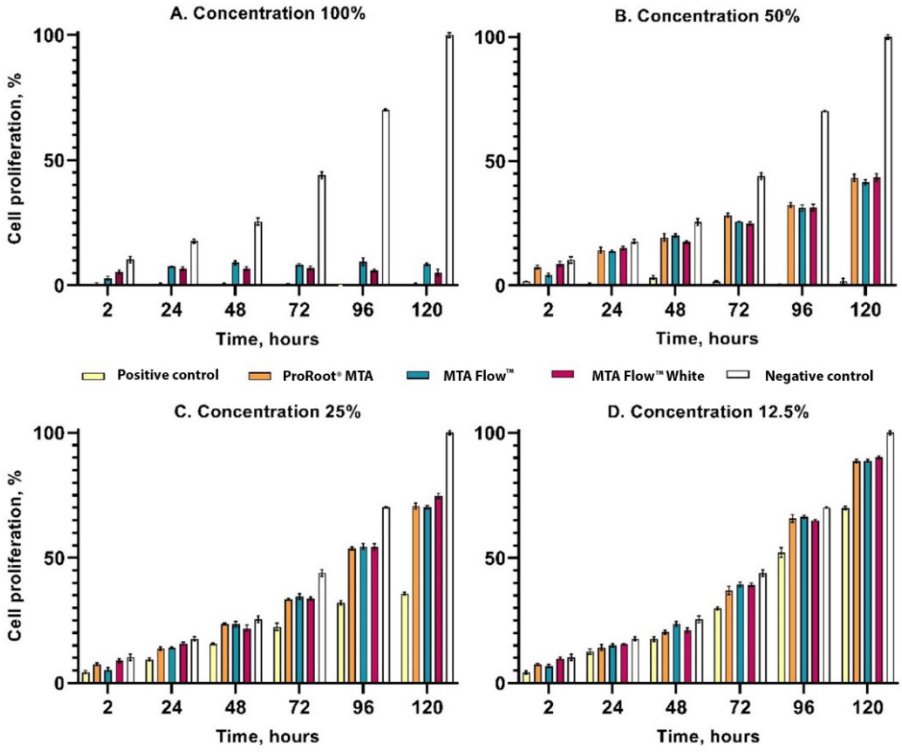


Figure 15. hDPSC proliferation assay using an MTT test at different time points (2, 24, 48, 72, 96, and 120h): The effect of the leachates derived from the freshly mixed HCSC tested at different time points on the mitochondrial enzymatic activity of hDPCs with different dilution factors: A—100%, B—50%, C—25%, and D—12.5% leachates. A pairwise comparison analysis of each group can be found in Table 17, A - D.

Table 17. A. Pairwise comparison analysis of hDPSCs proliferation simple simple main effect of hydraulic calcium silicate cement type for HDPSCs proliferation at 100% concentration and all time points.

Concentration 100%					
2h / 24h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		10.264* [8.36; 12.17)	9.670* [7.77; 11.57]	4.816* [2.91; 6.72]	7.367* [5.46; 9.27]
Positive control	17.641* [15.74; 19.54]		-0.595 [-2.5; 1.31]	-5.448* [-7.35; -3.55]	-2.897* [-4.8; -0.99]
ProRoot MTA	17.119* [15.22; 19.02]	-0.522 [-2.43; 1.38]		-4.854* [-6.76; -2.95]	-2.302* [-4.21; -0.4]
MTA Flow™ White	10.907* [9.00; 12.81]	-6.734* [-8.64; -4.83]	-6.212* [-8.11; -4.31]		2.551* [0.65; 4.45]
MTA Flow™	10.139* [8.24; 12.04]	-7.502* [-9.40; -5.60]	-6.980* [-8.88; -5.08]	-0.768 [-2.67; 1.14]	
48h / 72h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		25.368* [23.47; 27.27]	24.920* [23.02; 26.82]	18.720* [16.82; 20.62]	16.478* [14.58; 18.38]
Positive control	43.979* [42.08; 45.88]		-0.449 [-2.35; 1.45]	-6.648* [-8.55; -4.75]	-8.890* [-10.79; -6.99]
ProRoot MTA	43.420* [41.52; 45.32]	-0.559 [-2.46; 1.34]		-6.200* [-8.10; -4.30]	-8.441* [-10.34; -6.54]
MTA Flow™ White	37.003* [35.10; 38.91]	-6.976* [-8.88; -5.07]	-6.418* [-8.32; -4.51]		-2.242* [-4.14; -0.34]
MTA Flow™	35.766* [33.86; 37.67]	-8.213* [-10.12; -6.31]	-7.655* [-9.56; -5.75]	-1.237 [-3.14; 0.67]	
96h / 120h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		70.061* [68.16; 71.96]	69.986* [68.08; 71.89]	64.200* [62.30; 66.10]	60.696* [58.79; 62.60]
Positive control	99.945* [98.04; 101.85]		-0.076 [-1.98; 1.83]	-5.862* [-7.76; -3.96]	-9.366* [-11.27; -7.46]
ProRoot MTA	99.477* [97.57; 101.38]	-0.468 [-2.37; 1.44]		-5.786* [-7.69; -3.88]	-9.290* [-11.19; -7.39]
MTA Flow™ White	94.925* [93.02; 96.83]	-5.020* [-6.92; -3.12]	-4.552* [-6.46; -2.65]		-3.504* [-5.41; -1.60]
MTA Flow™	91.613* [89.71; 93.52]	-8.332* [-10.24; -6.43]	-7.864* [-9.77; -5.96]	-3.312* [-5.21; -1.41]	

Data are presented as mean and [CI]. Note: Bonferroni adjustments were made to the level at which statistical significance was declared by dividing the current level of statistical significance at ($p<0.05$) by the number of simple simple main effects analysed (3). Therefore, a simple simple main effect was declared statistically significant when $p<0.0.017$ ($p<0.05/3$).

Table 17. B. Pairwise comparison analysis of hDPSCs proliferation simple simple main effect of hydraulic calcium silicate cement type for HDPSCs proliferation at 50% concentration and all time points.

Concentration 50%					
2h / 24h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		8.599* [6.7; 10.5]	2.881* [0.98; 4.78]	1.651 [-0.25; 3.55]	6.038* [4.13; 7.94]
Positive control	17.024* [15.12; 18.93]		-5.717* [-7.62; -3.81]	-6.948* [-8.85; -5.04]	-2.561* [-4.46; -0.66]
ProRoot MTA	3.464* [1.56; 5.37]	-13.560* [-15.46; -11.66]		-1.230 [-3.13; 0.67]	3.156* [1.25; 5.06]
MTA Flow™ White	2.597* [0.69; 4.50]	-14.427* [-16.33; -12.52]	-0.867 [-2.77; 1.04]		4.387* [2.48; 6.29]
MTA Flow™	3.856* [1.95; 5.76]	-13.168* [-15.07; -11.27]	0.392 [-1.51; 2.29]	1.259 [-0.64; 3.16]	
48h / 72h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		22.312* [20.41; 24.22]	6.143* [4.24; 8.05]	8.057* [6.15; 9.96]	5.383* [3.48; 7.29]
Positive control	42.426* [40.52; 44.33]		-16.169* [-18.07; -14.27]	-14.255* [-16.16; -12.35]	-16.929* [-18.83; -15.03]
ProRoot MTA	15.740* [13.84; 17.64]	-26.686* [-28.59; -24.78]		1.913 [-0.10; 3.82]	-0.760 [-2.66; 1.14]
MTA Flow™ White	19.135* [17.23; 21.04]	-23.291* [-25.19; -21.39]	3.396* [1.49; 5.30]		-2.673* [-4.58; -0.77]
MTA Flow™	18.307* [16.40; 20.21]	-24.119* [-26.02; -22.22]	2.567* [0.66; 4.47]	-0.828 [-2.73; 1.07]	
96h / 120h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		69.845* [67.94; 71.75]	37.747* [35.84; 39.65]	38.779* [36.88; 40.68]	38.957* [37.05; 40.86]
Positive control	98.246* [96.34; 100.15]		-32.098* [-34.00; -30.20]	-31.066* [-32.97; -29.164]	-30.888* [-32.79; -28.99]
ProRoot MTA	56.736* [54.83; 58.64]	-41.510* [-43.41; -39.61]		1.033 [-0.87; 2.94]	1.210 [-0.69; 3.11]
MTA Flow™ White	56.642* [54.74; 58.55]	-41.604* [-43.51; -39.70]	-0.094 [-2.00; 1.81]		0.177 [-1.73; 2.08]
MTA Flow™	58.567* [56.6; 60.47]	-39.679* [-41.58; -37.78]	1.831 [-0.07; 3.73]	1.925 [-0.02; 3.83]	

Data are presented as mean and [CI]. Note: Bonferroni adjustments were made to the level at which statistical significance was declared by dividing the current level of statistical significance at ($p<0.05$) by the number of simple simple main effects analysed (3). Therefore, a simple simple main effect was declared statistically significant when $p<0.0.017$ ($p<0.05/3$).

Table 17. C. Pairwise comparison analysis of hDPSCs proliferation simple simple main effect of hydraulic calcium silicate cement type for HDPSCs proliferation at 25% concentration and all time points.

Concentration 25%					
2h / 24h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		5.908* [4.01; 7.81]	2.782* [0.88; 4.69]	1.393 [-0.51; 3.3]	4.890* [2.99; 6.79]
Positive control	8.237* [6.33; 10.14]		-3.126* [-5.03; -1.22]	-4.515* [-6.42; -2.61]	-1.018 [-2.92; 0.88]
ProRoot MTA	3.835* [1.93; 5.74]	-4.401* [-6.30; -2.50]		-1.389 [-3.29; 0.51]	2.108* [0.2; 4.01]
MTA Flow™ White	1.875 [-0.03; 3.78]	-6.362* [-8.26; -4.46]	-1.760 [-3.74; 0.06]		3.496* [1.59; 5.40]
MTA Flow™	3.592* [1.69; 5.50]	-4.644* [-6.55; -2.74]	-0.243 [-2.15; 1.66]	1.717 [-0.19; 3.62]	
48h / 72h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		9.913* [8.01; 11.82]	1.876 [-0.03; 3.78]	3.633* [1.73; 5.54]	1.985 [-0.08; 3.89]
Positive control	21.513* [19.61; 23.42]		-8.037* [-9.94; -6.13]	-6.280* [-8.18; -4.38]	-7.927* [-9.83; -6.02]
ProRoot MTA	10.590* [8.69; 12.49]	-10.922* [-12.83; -9.02]		1.757 [-0.15; 3.66]	0.110 [-1.79; 2.01]
MTA Flow™ White	10.263* [8.36; 12.17]	-11.250* [-13.15; -9.35]	-0.327 [-2.23; 1.58]		-1.647 [-3.55; 0.26]
MTA Flow™	9.396* [7.49; 11.30]	-12.117* [-14.02; -10.21]	-1.195 [-3.10; 0.71]	-0.867 [-2.77; 1.04]	
96h / 120h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		38.119* [36.22; 40.02]	16.447* [14.54; 18.35]	15.795* [13.89; 17.70]	15.614* [13.71; 17.52]
Positive control	64.308* [62.40; 66.21]		-21.672* [-23.57; -19.77]	-22.325* [-24.23; -20.42]	-22.506* [-24.41; -20.60]
ProRoot MTA	29.310* [27.41; 31.21]	-34.998* [-36.90; -33.09]		-0.653 [-2.74; 1.07]	-0.834 [-2.60; 0.93]
MTA Flow™ White	25.188* [23.29; 27.09]	-39.120* [-41.02; -37.22]	-4.122* [-6.03; -2.22]		-0.181 [-2.08; 1.72]
MTA Flow™	29.787* [27.88; 31.69]	-34.521* [-36.42; -32.62]	0.477 [-1.43; 2.38]	4.599* [2.70; 6.50]	

Data are presented as mean and [CI]. Note: Bonferroni adjustments were made to the level at which statistical significance was declared by dividing the current level of statistical significance at ($p<0.05$) by the number of simple simple main effects analysed (3). Therefore, a simple simple main effect was declared statistically significant when $p<0.0.017$ ($p<0.05/3$).

Table 17. D. Pairwise comparison analysis of hDPSCs proliferation simple simple main effect of hydraulic calcium silicate cement type for HDPSCs proliferation at 12.5% concentration and all time points.

Concentration 12.5%					
2h / 24h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		6.037* [4.13; 7.94]	2.893* [0.99; 4.80]	0.441 [-1.46; 2.34]	3.560* [1.66; 5.46]
Positive control	5.090* [3.19; 6.99]		-3.143* [-5.05; -1.24]	-5.596* [-7.50; -3.69]	-2.476* [-4.38; -0.57]
ProRoot MTA	3.417* [1.51; 5.32]	-1.672 [-3.58; 0.23]		-2.452* [-4.36; -0.55]	0.667 [-1.24; 2.57]
MTA Flow™ White	1.801 [-0.20; 3.70]	-2.988* [-4.89; -1.09]	-1.316 [-3.22; 0.59]		3.119* [1.22; 5.02]
MTA Flow™	2.566* [0.66; 4.47]	-2.523* [-4.43; -0.62]	-0.851 [-2.75; 1.05]	0.465 [-1.44; 2.37]	
48h / 72h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		7.890* [5.99; 9.79]	5.142* [3.24; 7.04]	4.348* [2.45; 6.25]	1.832 [-0.07; 3.74]
Positive control	14.107* [12.20; 16.01]		-2.749* [-4.65; -0.85]	-3.542* [-5.45; -1.64]	-6.058* [-7.96; -4.16]
ProRoot MTA	6.972* [5.07; 8.88]	-7.135* [-9.04; -5.23]		-0.793 [-2.70; 1.11]	-3.309* [-5.21; -1.41]
MTA Flow™ White	4.782* [2.88; 6.68]	-9.325* [-11.23; -7.42]	-2.190* [-4.09; -0.29]		-2.516* [-4.42; -0.61]
MTA Flow™	4.607* [2.70; 6.51]	-9.500* [-11.40; -7.60]	-2.365* [-4.27; -0.46]	-0.174 [-2.08; 1.73]	
96h / 120h	Negative control	Positive control	ProRoot MTA	MTA Flow™ White	MTA Flow™
Negative control		17.982* [16.08; 19.89]	4.325* [2.42; 6.23]	5.081* [3.18; 6.98]	3.680* [1.78; 5.58]
Positive control	30.086* [28.18; 31.99]		-13.657* [-15.56; -11.75]	-12.901* [-14.80; -11.00]	-14.302* [-16.21; -12.40]
ProRoot MTA	11.211* [9.31; 13.11]	-18.875* [-20.78; -16.97]		0.756 [-1.15; 2.66]	-0.645 [-2.55; 1.26]
MTA Flow™ White	9.761* [7.86; 11.66]	-20.325* [-22.23; -18.42]	-1.450 [-3.35; 0.45]		-1.401 [-3.30; 0.50]
MTA Flow™	11.174* [9.27; 13.08]	-18.912* [-20.82; -17.01]	-0.037 [-1.94; 1.87]	1.413 [-0.49; 3.32]	

Data are presented as mean and [CI]. Note: Bonferroni adjustments were made to the level at which statistical significance was declared by dividing the current level of statistical significance at ($p<0.05$) by the number of simple simple main effects analysed (3). Therefore, a simple simple main effect was declared statistically significant when $p<0.0.017$ ($p<0.05/3$).

3.3.4.Human dental pulp stem cells morphology assessment

The effects of leachates from freshly mixed flowable hydraulic calcium silicate cement on hDPSCs morphology changes observed under an inverted phase contrast microscope are shown in Figure 16. In the NegativeCG, hDPSCs were spindle-shaped and spread over the entire plate well surface area. They also contained pale, round or oval central nuclei with multiple nucleoli, indicating active DNA transcription and RNA synthesis. Multiple cytoplasmic vacuoles represented cellular secretory vesicles. In contrast, the 100% tested HCSC groups at all monitored time points determined more round cell morphology and decreased cell number. This hDPSCs shape change, compared to NegativeCG, can be associated with the ongoing cell death process after treatment with 100% leachates. Moreover, after treatment with 50% HCSC leachates, the cell length and width were higher than in NegativeCG. However, after incubation of hDPSCs with 25% HCSC leachates, cell morphology remained almost the same as in NegativeCG. However, cells were less frequently spread on the surface.

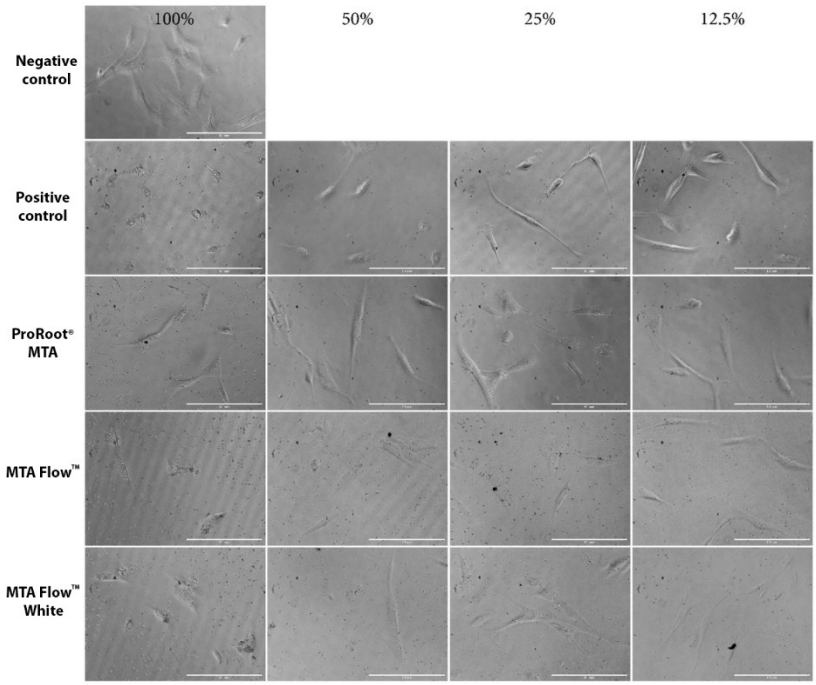
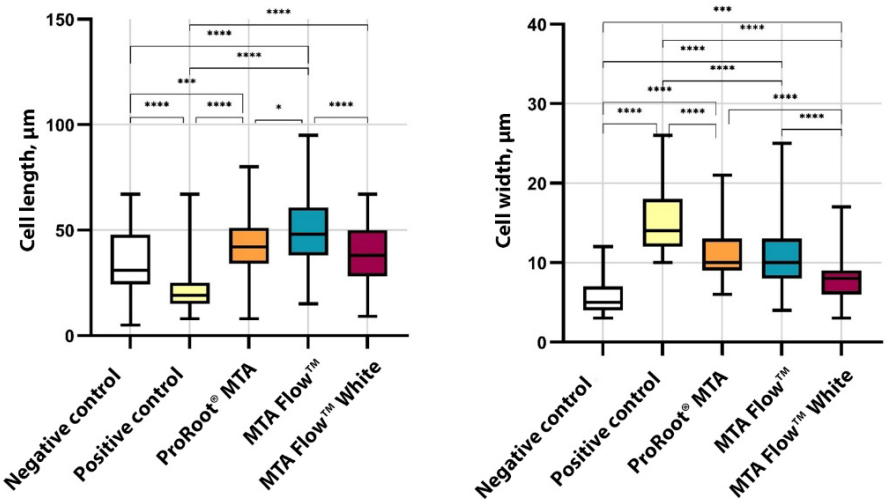


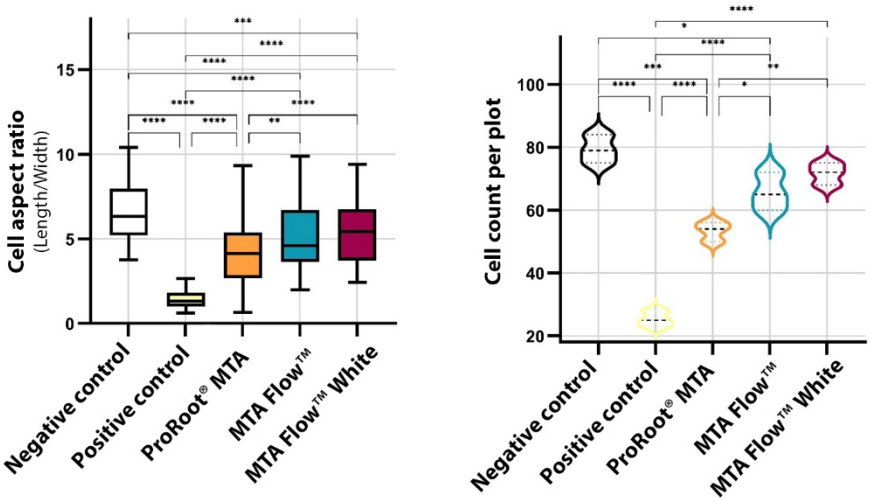
Figure 16. Representative hDPSC morphology assessment images after 24 hours of incubation with freshly mixed analysed fHCSC leachates. Also, hDPSCs morphology after incubation with freshly mixed control HCSC (ProRoot MTA), positive and negative control groups were observed.

The lowest cell length and width were observed in PositiveCG (Figure 17). In this group, the cells were the most rounded (aspect ratio) and the least in number (Figure 18). Nevertheless, hDPSCs affected by the tested MF 50% leachate were longer than those tested 50% MFWhite and ProRootCG. However, no significant differences in length were found between ProRootCG and tested MFWhite. The width of the cells from tested HCSC groups was the lowest in the MFWhite group, while the cell widths of MF and ProRootCG were not different. Overall, the hDPSCs remained longer and wider (lower in aspect ratio) in all HCSC groups than in NegativeCG. However, the mean number of cells was lower in all tested groups than in NegativeCG. The cell aspect ratio was not different between tested fHCSC MF and MFWhite groups' aspect ratio. A detailed analysis of hDPSCs' morphology after incubation with 50% leachates for 24 hours is presented in Figure 17 and Figure 18.



* - marks statistically significant differences between the groups (* – $p<0.05$, ** - $p<0.01$ and *** - $p<0.001$).

Figure 17. Assessment of hDPSCs morphology: length and width of hDPSCs after incubation for 24 hours with freshly mixed fHCSC 50% leachates and control groups.



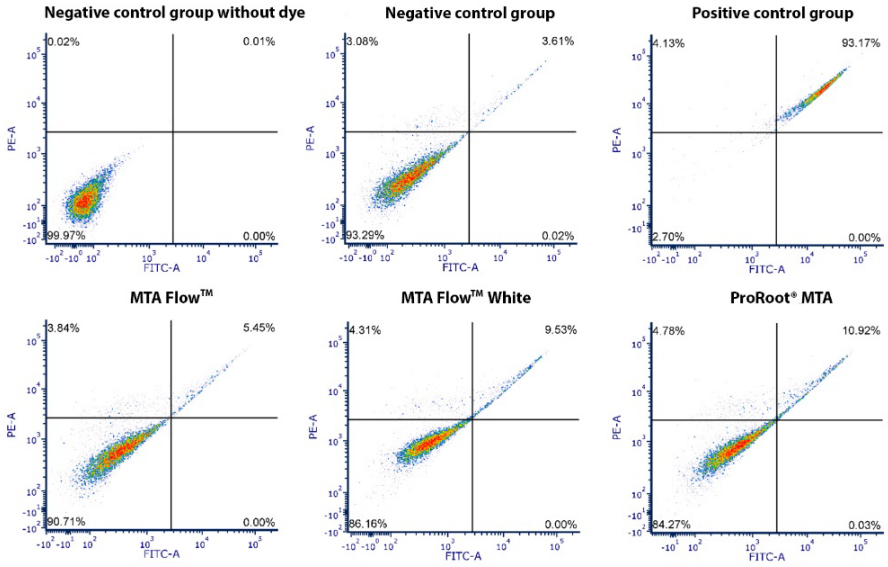
* - marks statistically significant differences between the groups (* – p<0.05, ** - p<0.01 and *** - p<0.001).

Figure 18. Assessment of hDPSCs aspect ratio (length/width) and cell mean count per standardised plot after incubation for 24 hours with freshly mixed fHCSC 50% leachates and control groups.

3.3.5.Flow cytometry Annexin V – FITC analysis of hDPSCs

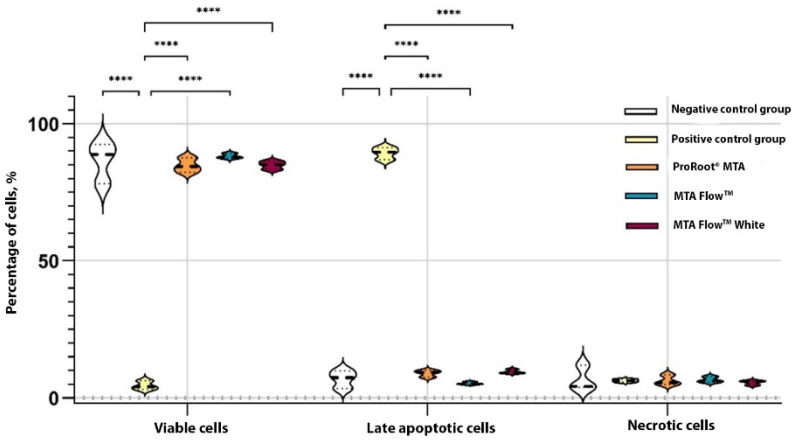
After hDPSCs treatment with 50% fHCSC MF and MFWhite, control HCSC 50% ProRootCG, 50% PositiveCG, and NegativeCG, the flow cytometry analysis was obtained. The data is presented as representative dot plot images in Figure 19. The lower left part of the flow cytometry graphs shows the live cell ratios, the lower right part shows the early apoptotic cell ratios, the upper right part shows the late apoptotic cell ratios, and the upper left part shows the necrotic cell ratios. The averages of the results were indicated in the relevant areas on the graphs as percentages.

All tested HCSC, including MF and MFWhite, ProRooCG, 50% leachates were associated with more than 84% viable cells after 24h. By contrast, PositiveCG decreased cell viability to only 4.5%, while NegativeCG viable cells remained around 87%. There were no significant differences between NegativeCG and all tested HCSC 50% leachates viable and late apoptotic cell ratios. In addition, no significant differences were found between necrotic cell ratios. A detailed analysis of Annexin V-FITC obtained results on apoptosis/necrosis flow cytometry can be found in Table 18, Table 19 and Figure 20.



The dot plots in each image represent the distribution of viable (lower left), early apoptotic (lower right), necrotic (upper left) and late apoptotic cells (upper right).

Figure 19. Representative dot plots images of flow cytometry Annexin-V FITC analysis and results of hDPSCs treated with MTA Flow™, MTA Flow™ White and ProRoot MTA 50% leachates for 24 hours and control groups.



Data are presented as median, first and third quartiles, minimum and maximum, and density plots. * - marks statistically significant differences between the groups (* – $p<0.05$, ** - $p<0.01$ and *** - $p<0.001$). No differences were found in the necrotic cell pairwise comparison.

Figure 20. Violin box plots of viable, late apoptotic, and necrotic hDPSCs treated with analysed cement 50% leachates for 24 hours.

Table 18. Evaluation of viable, late apoptotic, and necrotic hDPSCs apoptosis/necrosis analysis results among different groups after 24 hours of incubation with freshly mixed cement 50% leachates.

Group	*Viable cells*	*Late apoptotic cells*	*Necrotic cells*
Negative control no dye	99.97 ± 0.03 (99.88)	0.01 (0.01)	0.1 ± 0.04 (0.11)
Negative control	86.46 ± 6.07 (88.75)	6.87 ± 2.69 (7.37)	6.68 ± 3.75 (4.17)
Positive control	4.47 ± 1.32 (4.12)	89.24 ± 1.8 (89.59)	6.29 ± 0.49 (6.29)
ProRoot MTA	84.77 ± 2.15 (84.36)	8.93 ± 1.03 (9.24)	6.30 ± 1.51 (5.64)
MTA Flow™	88.15 ± 0.79 (87.88)	5.29 ± 0.41 (5.10)	6.56 ± 0.87 (6.26)
MTA Flow™ White	84.90 ± 1.21 (85.07)	9.55 ± 0.63 (9.11)	5.56 ± 0.69 (5.83)

Table 19. Pairwise comparison analysis of viable hDPSCs among different groups after 24 hours of incubation with freshly mixed cement leachates.

	Positive control	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Negative control	81.99* [72.70; 91.27]	1.69 [-7.60; 10.99]	1.56 [-7.72; 10.85]	-1.70 [-10.99; 7.59]
Positive control		-80.30* [-89.58; -71.01]	-80.42* [-89.71; 71.14]	-83.68* [-92.97; -74.39]
ProRoot MTA			-0.129 [-9.42; 9.16]	-3.385 [-12.67; 5.90]
MTA Flow™ White				-3.256 [-12.54; 6.03]

Data are presented as mean difference and [CI].

4. DISCUSSION

4.1. Porosity Distribution in Single-Cone Root Canal Fillings Performed by Operators with Different Clinical Experience

Conically shaped and homogeneous root filling can ensure the best long-term success of endodontic treatment if the filling material seals the entire root canal up to the apical constriction. [9,480,481] Also, the root canal fillings and the coronal seal prevent recontamination of the root canal system by the microorganisms and their metabolites from the oral cavity. [22] It creates unfavourable conditions for the micro-organisms remaining after chemo-mechanical root canal preparation and disrupts their penetration into periapical tissues by entombing them in the root canal system. [481,482] Apical extension of the filling in full WL and the homogeneity of the filling without gaps/pores were shown to be directly related to the success rate of root canal treatment. [483]

Various materials and obturation techniques were proposed and are nowadays implemented in clinical practice to avoid or minimise the formation of the pores inside the root canal fillings. [24] The most popular solid root canal-filling material is gutta-percha, which has good biocompatibility [484], plasticity and radiopacity. It is also dimensionally stable and easily inserted or removed from the root canal. [293,485,486] However, this material does not adhere to the root canal wall dentin. Therefore, a sealer is required to ensure a hermetical seal of the root canals. [22]

The lateral and vertical gutta-percha compaction techniques are the most widely investigated *in vitro* and *in vivo* studies. [22,24] Nevertheless, the impact of the root canal filling technique on the porosity distribution is highly controversial. [26,452] According to some authors, the porosity of the laterally condensed gutta-percha/sealer-created fillings is relatively high compared to the other obturation techniques, especially in curved root canals. [487] Similar results have been shown by Gupta et al., demonstrating worse homogeneity and higher porosity of the laterally condensed gutta-percha fillings than thermoplastic obturation methods. [25] The comparable findings were published in a recent systematic review, revealing significantly less porosity of the vertically compacted thermoplastic gutta-percha than cold lateral compaction. [221] However, the results of other studies did not demonstrate significant differences in the porosity distribution among these obturation techniques. [486,488] Moreover, endodontic treatment's clinical success and outcomes remained comparable when these techniques were used. [21,488]

Due to the physicochemical properties of the flowable hydraulic calcium silicate-based cement/sealers, they can be used as fillers instead of sealers, allowing the clinician to increase the material's volume inside the root canal. [118] The tapered gutta-percha points increase the hydraulic pressure inside the root canal, improve the sealer's distribution and make the single-cone filling removable if the retreatment is indicated. [110,489] Previous investigations have shown that the porosity distribution in single-cone fillings is comparable to that in other obturation techniques or even lower. [26,39,44,210,452] Even though some authors suggest that the single-cone technique creates a higher percentage volume of voids and gaps than the warm vertical compaction technique, the data concerning the porosity distribution in single-cone fillings is still controversial. [490] Previous investigations have found lower porosity of the single-cone fillings in the coronal and middle thirds of the root canal compared to laterally compacted gutta-percha or hybrid thermoplastic gutta-percha condensation. [26,210] However, other studies claimed that the single-cone fillings were more porous than hybrid thermoplastic gutta-percha condensation. [491] Nevertheless, many studies demonstrate comparable and similar porosity of single-cone fillings compared to other obturation techniques. [492,493] The controversy of the results may be related to root canal morphology, chemo-mechanical preparation, or physical properties of the filling materials, as well as the lack of standardisation of *in vitro* test models, data processing, and interpretation protocols. However, according to recent research data, there is no doubt that no obturation technique can ensure complete and void-free sealing of the root canal system. [218,494] On the other hand, the single-cone technique is effortless to use for clinicians [105] and, at the same time, demonstrates a high clinical success rate in both primary endodontic treatment and endodontic retreatment. [47,48,483] However, the data about single-cone fillings using modern type-four or type-five flowable hydraulic calcium silicate-based cement/sealers are still limited. [105]

Most studies reporting the success rates of endodontic procedures are based on treatments performed by highly trained and experienced professionals—endodontists or postgraduate students. [21,47] Even though it has been demonstrated previously that the clinical experience of the operator has a substantial impact on the quality of the endodontic procedures and clinical outcomes. [21,495] The same applies to experimental studies, as highly experienced operators usually perform laboratory work. [25–27] Whenever differently skilled clinicians perform endodontic treatment procedures, the quality of the endodontic treatment differs significantly. [496,497] Some authors have demonstrated that the quality of root canal obturation using conventional obturation techniques is related to the operators' experience. Bajawi et al. have

found that the quality of root canal obturation performed by endodontists was substantially better than that of general dental practitioners. [50] Indeed, the quality of root canal obturation performed by undergraduate students using lateral compaction of the gutta-percha varies from 46.6 % to 58.8 %. [495,498] Other studies reported similar or even worse results, indicating that only 25.2–66 % of the fillings were homogeneous and with acceptable quality. [499–501] However, the results gradually improved after graduation and the acquisition of clinical experience. [495,498] Therefore, the best results and the highest success rates were achieved when an endodontology specialist performed endodontic treatment, where an acceptable homogeneity of root canal fillings was reached from 86.1 % to 88.6 % of cases using lateral compaction technique. [502–505] However, it should be highlighted that these studies usually involved cases in which root canals were obturated using cold lateral or warm vertical compaction techniques and evaluated using two-dimensional radiographs. Nevertheless, these findings demonstrate the operators' experiences' direct impact on the root canals' obturation quality when lateral or vertical compaction of the gutta-percha is used. Finally, there is little information on how the clinical experience can impact the quality of the root canal obturation using the single-cone technique in conjunction with flowable hydraulic calcium silicate cement/sealer.

The quality of root canal obturation using a single-cone technique with hydraulic calcium silicate-based sealer and porosity distribution in these fillings performed by operators with different clinical experiences has not been previously evaluated. Therefore, our results cannot be directly compared to the findings of the previous investigations. Kharouf et al. demonstrated that the porosity distribution in root canal fillings performed by undergraduate students significantly dropped when the single-cone technique was used instead of lateral compaction of gutta-percha and acceptable root canal filling homogeneity was reached from 84.1 % to 90.9 % cases. [495] Few published articles evaluate the porosity in total single-cone root canal fillings. [26,57,210,490] However, there is no data about porosity distribution in the single-cone fillings performed by operators with different competencies and clinical experience. Therefore, due to the limited available data on the quality of single-cone fillings performed by undergraduate students, general dental practitioners, and endodontists, this study aimed to determine and analyse the porosity type and distribution in single-cone root canal fillings performed by the operators with a different clinical experience using μCT imaging. The null hypothesis tested was that the clinical experience of the operator has no significant impact on the quality of the root canal obturation.

It has been demonstrated that the fillings' homogeneity, tightness, and sealability highly depend on the pores inside the root canal filling material. [23] The two types of pores can be detected: open (external) and closed (internal). [488] The internal pores form inside the filling material and do not have any substantial adverse clinical impact. Also, the overall porosity is not a critical indicator. [488] Only the number of open pores plays a crucial role, as they are located between the filling material and the root canal walls and, therefore, create a through-and-through "network" of the pores, providing excellent conditions for the microleakage and negatively affecting the outcome of endodontic treatment. [210,452,481,483,506] Furthermore, the pores inside the mass of the materials can adversely affect their physical properties. [506] For these reasons, porosity is considered one of the main criteria for assessing the quality of root canal fillings. [25,507] B. Celikten et al. study revealed particularly low porosity of SC root canal filling: up to 1.58 %, including open and closed porosity. [508] Slightly higher porosity was found in M. Radwanski et al. study, where BioRoot® RCS and SC technique was used: porosity reached up to 4 %[453], while in E. Pedulla et al. study overall porosity was around 6.7 %.[494] According to the research, general porosity of the SC fillings with HCSC sealers was found to be 13±8 % of the whole root canal volume. [218]

When analysing porosity, particular focus should be paid to the apical third of the root canal, as the quality of its obturation may become a crucial factor in the outcome of endodontic treatment. [21,452] Therefore, this study assessed the porosity distribution in single-cone fillings in all three root canal thirds separately. The investigation has shown that the highest porosity was detected in the apical third of the root canal fillings in all experimental groups, ranging from 8.1 % to 15.9 % of open and from 0.17 % to 0.34 % of closed pores. The detected differences in the amount of porosity did not differ significantly ($p > 0.05$), indicating the same quality of obturation among US, PS, GDP and ED operators in the apical third. These findings are in concordance with A. Moizadeh et al. results, where the highest porosity volume was found in the apical thirds of the SC root canal fillings. The authors also evaluated overall root canal porosity reaching up to 5 %, including open and closed pores. [509] Depending on root canal thirds, the lowest porosity by M. Radwanski et al. study was found to be in the coronal root third (around 0.7 %), while the highest – in apical root third (around 2.24 %). [453] In this study, root canal filling porosity was found to be from 2.6 % (ES group middle third open and closed pores) to 16.1 % (GDP group, apical third open and closed pores). Results of the current study are in concord with previous reports, demonstrating the highest volume of the pores in the apical third of single-cone fillings, compared to middle and coronal thirds. [210] Even though,

the porosity was found higher in this study compared to A. Moizadeh et al. or M. Radwanski et al. studies. [509,453] It has been shown that the higher porosity in the apical third can be related to numerous factors, such as the irregular cross-sectional shape of the root canal, anatomical features, and the flowability of the sealer, which can be affected by inaccurate powder/liquid ratio, etc. [27,510] Meanwhile, in the middle third, a statistically significant difference was found only between the number of closed pores ($p < 0.05$). On the other hand, in the study by R. Castagnola et al. the SC technique was characterized by a statistically significantly lower open porosity in the apical third of the root than in the coronal or middle thirds, although the quantity of closed and mixed pores between root thirds did not differ significantly. [511] B. Celikten et al. identified a similar distribution of closed pores - up to 0.39%, however the distribution of open pores was significantly higher - up to 1.47% when using the SC filling technique. [508] Yan Huan et al. calculated open and closed pores throughout the root canal volume and found that closed pores constituted approximately 0.62 %, while open and mixed pores constituted about 1.56 % of the root canal volume. [512] Interestingly, separate analysis of root canal thirds individually conducted by B. Celikten et al. and Yan Huan et al. revealed that the lowest porosity was in the apical thirds, reaching approximately 0.21 % and 0.3 %, respectively. [508,512] Porosity of the filling significantly increased in the coronal third compared to the apical third, reaching 0.82 % and 0.93 %. M. Radwanski et al. analysis of the total porosity of root canals using BioRoot RCS and SC technique, and their distribution in root canal thirds using μCT analysis, showed that using BioRoot RCS resulted in significantly higher porosity in the apical third, reaching approximately 2.24 % of the total canal volume. [453] Assessing the overall porosity of root canals, open pores accounted for 1.13 % and closed pores for 2.86 %. Overall, the porosity of apical thirds of roots remains significant in terms of deficiencies in root canal fillings, indicating the need for techniques and/or materials to improve the homogeneity of fillings of the root canal. [509]

When evaluating open and closed pores separately, a statistically significant difference was found only in the quantity of closed pores in the middle third of the root. Interestingly, the significantly lowest quantity of closed pores was found in the US and PS groups, where root canals were filled by less clinically experienced dental students and endodontology resident doctors. According to the data from A. Moinzadeh et al., the porosity of middle thirds, although not statistically significant, was noticeably lower than that of coronal or apical thirds, reaching up to 1.5 %. [509] In the study by E. Pedulla et al., the porosity of BioRoot RCS in the middle third (0.78 %) was similar, although slightly higher than in the coronal third (0.6 %). [494]

Compared to the data from other authors, this study yielded higher average porosity values ranging from 2.6 % to 7.7 %.

Previous reports have shown that only through-and-through voids (continuous or open pores) have clinical significance, as they create the network of the pores and are related to an increased possibility of microleakage and worse clinical outcomes of endodontic treatment. [198,452] It has been demonstrated that regardless of the sealer type, interconnected or open voids can create an entire helicoidal or spiral-shaped space along with the fillings and act as a pathway for the diffusion of fluids and microorganisms. [198] Meanwhile, the *cul-de-sac*-type voids (closed or blind pores) are entrapped inside the material and can only affect the mechanical properties of the filler with no biologically substantial impact. [26,28,198,452] Therefore, from the clinical point of view, our findings demonstrate that clinically significant porosity distribution in apical and middle thirds of single-cone fillings was comparable between US, PS, GDP and ED groups. However, in this study, a significant difference in the amount of open and closed pores was observed only in the coronal third and GDP group, which had higher closed porosity than other groups ($p < 0.05$). Surprisingly, the porosity of the SC fillings in the coronal root canal third was higher in the ED and PS groups than in the US group fillings. Furthermore, the GDP group revealed more than twice as high open pores distribution as the ED and PS groups ($p<0.05$). It is difficult to explain this "phenomenon"; however, hypothetically, it could be related to the more accurate, gentle, and slower insertion of the gutta-percha point performed by the less experienced operator, such as the US. It has been demonstrated that the slow insertion of the tapered gutta-percha cone ensures better sealer distribution and possibly less material porosity. [27,371,452] The porosity of the filler in the coronal third of the canal could be related to the higher volume of the root canal and the subsequent increase in the volume of flowable HCSC sealer, in this case, BioRoot® RCS paste. Previous investigations revealed that the porosity of the fillings could be related to the sealers' type and their physical properties. [452,493,506] It has been demonstrated that flowable hydraulic calcium silicate-based sealers possess higher porosity immediately after obturation, with a substantial reduction in the pore volume within long-term observations compared to resin-based sealers. [452] To reduce the porosity of the single-cone fillings in the bigger and broader root canals, additional auxiliary gutta-percha points passively inserted along the master gutta-percha point were recommended. [513] At this moment, the higher hydraulic pressure created in the root canal improves the distribution of the sealer in the root canal and reduces the amount of paste and pores. The results of the study conducted by A. Moinzadeh et al. showed that

the total root canal porosity in the coronal third reached up to 2 %. [509] A slightly lower total porosity in the coronal third, up to 1 %, was found in the study by E. Bianco et al.. [441] The highest porosity of the coronal root canal third was identified by E. Pedulla et al.: approximately 5.3 % of the total root canal volume. [494] The data from these authors partially align with the results of the conducted study, as the identified porosity of coronal root thirds ranged from 2.4 % to 8.8 %, depending on the individual filling the root canal.

It should be highlighted that in many of the studies, the quality of root canal fillings was assessed based on dental X-rays, which cannot provide accurate information on the 3D homogeneity of the fillings. Indeed, the quality of root canal filling is usually assessed using periapical radiographs after root canal treatment to evaluate the length and homogeneity of the filling. However, the two-dimensional (2D) radiographic image does not always provide sufficient information about the fillings' real homogeneity, tightness, and sealability. [514] The visible pores in the fillings on the radiographs represent just 2D reality and do not provide 3D information. Therefore, the accuracy of the evaluation of the porosity of the fillings in clinical practice using radiographs is relatively limited. Still, at the same time, it is the only method available for clinicians. Previous investigations demonstrate that the percentage of pores in root canal fillings detectable by volumetric 3D μCT analysis is much higher than the 2D findings. [21,137,452,496] However, there is no clear evidence to determine which level of porosity is critical and can negatively impact the outcome of endodontic treatment. [21,210,506] μCT imaging is considered the technique of choice to assess the quality of root canal cleaning, shaping, and obturation *in vitro*. [26,210,452] The technique ensures the possibility of characterising filling materials and quantifying and qualifying the pores, voids, and gaps inside the material or at the material/root-canal wall interface. [452,488] The main advantage of the μCT method is its non-destructiveness, repeatability, and high accuracy. [26,49,515] As opposed to other methods, such as bacterial, glucose, radioactive isotopes, dye penetration tests, or SEM investigations, which have been used to evaluate the porosity and microleakage of the fillings with their significant limitations. [515] Therefore, the porosity of the single-cone fillings was assessed using μCT imaging. However, it should be mentioned that some drawbacks of the technique should be considered. Despite the high accuracy of μCT, tiny pores may still be undetectable due to the radiopacity of the materials and possible limitations of the thresholding procedure. [26,28] Moreover, Gandolfi et al. have demonstrated that the pixel size of the scanning can impact the accuracy of the results, affecting the possible exclusion of the smallest pores if lower resolutions are selected. [198] However, it should be mentioned that there is no

single protocol for optimal scanning resolution. There is no reliable scientific background if the pores of the size of 4 μm would have a different impact on the microleakage and outcome of endodontic treatment compared to the pores with a diameter of 10 μm. Therefore, the less time-consuming but still accurate and high resolution of the 9.99 μm was used in this study, as suggested by previously published reports. [26,28,40,452]

The standardised plastic 3D models were used in this study to optimise the homogeneity of the sample and ensure identical, uniform internal anatomy of the root canals and volumetric parameters in all groups. However, dentinal tubules, intratubular moisture, irregular shape, and diameter/volume of teeth root canals are the factors that could affect the hydration and behaviour of the flowable hydraulic calcium silicate-based sealers and their physical properties, including porosity. [26,371] As a result, plastic models cannot create a clinically identical environment and provide sufficient moisture needed for hydration and setting of the flowable hydraulic calcium silicate-based sealer. Therefore, the lack of hydration products, which can fill the gaps between non-hydrated cement particles and reduce filler porosity, may adversely affect the overall porosity. [371] On the other hand, the advantages of using the standardised models have already been discussed previously. [26] Therefore, if the same models are used under the same conditions, the assessment results are still comparable among the experimental groups. Furthermore, it should be highlighted that the primary aim of this investigation was to assess the 3D quality of the single-cone fillings performed by dentistry students, residents, general dental practitioners, and endodontists, using the clinical experience factor as the determinant for the comparison.

The data on the clinical outcomes of using a single-cone technique with a hydraulic calcium silicate-based sealer still needs to be improved. Previous clinical studies revealed high success rates of endodontic treatment when root canals were obturated by specialists endodontists using a single-cone technique with a hydraulic calcium silicate-based sealer: Chybowski et al. reported a success rate of up to 90.9 % after an average follow-up of 30.1 months [47], while the study conducted by Zavattini et al. demonstrated success rates varying from 84 % to 90 %. [48] Slightly lower success rates were revealed in a clinical study by Bardini and co-authors in which all treatment procedures, including root canal obturation using the single-cone technique, were performed by postgraduate students in an endodontology program: the complete healing rates over 12 months reached 76.92 %. [498] The results of a recent systematic review and meta-analysis showed that the absence of gaps in the filling reduced the chance of unhealed periapical lesions by 2.39 times when analysed in CBCT images after one year. [483] Nevertheless, the operators'

clinical experience must be considered. There are no observations and clinical outcome results at the level of general dental practitioners.

Based on the results of this study, it can be hypothesised that if undergraduate students, postgraduate endodontology students, general dental practitioners and endodontists were able to achieve comparable results, there is a potential possibility that the quality of the root canal obturation using the single-cone technique at the level of general dental clinical practice and even undergraduate students can create a comparable SC root canal filling to that of the endodontist. However, more clinical studies are needed to confirm the impact of the operators' experience on the long-term clinical results when the single-cone root canal obturation technique is used.

4.2. The Effect of Ultrasonic Agitation on the Porosity Distribution in Apically Perforated Root Canals Filled with Different HCSC Materials and Techniques

Root perforations are one of the most common complications observed in modern endodontology. [516] Regardless of recent advances in the field of endodontic instruments and devices, the mechanical preparation of curved root canals remains a significant challenge, even for experienced clinicians. [517] It has been reported that the risk of root perforation occurrence strongly correlates with the degree of root canal curvature, and the prevalence of apical root perforations is significantly higher in molars than in other teeth. [518,519] Therefore, mandibular first molars with a moderate curvature of mesial roots were selected in the present study to maximise their clinical relevance.

The management of root perforation is a time-dependent procedure, where a hermetic physical seal is crucial to improve the prognosis and survival of the affected tooth. [520] It has been reported that up to 52–79 % of the root canal may remain unprepared, regardless of the instruments or instrumentation technique used [521], and no currently available irrigation protocol can thoroughly disinfect the entire root canal system. [522] Therefore, the obturation phase of the endodontic treatment has undeniable importance in creating an unfavourable environment for the microorganisms left inside the root canal system after the preparation and preventing their penetration into periapical tissues. [22,516]

The homogeneity of root canal obturation highly depends on the porosity of the fillings [63], as open pores communicating with dentinal walls may create an excellent pathway for microleakage and eventually decrease the success rate and outcome of endodontic treatment. [40,452] Closed pores are considered less clinically relevant, representing empty spaces surrounded by filling material. [523] Nevertheless, it has been shown that this type of porosity may negatively affect

the material's physical properties, such as hardness and strength. [40,524] Therefore, quantifying both open and closed pores is necessary to properly evaluate the quality of root canal fillings. Previously, various porosity and leakage measuring approaches, such as dye staining, glucose or radioactive isotope penetration, protein loss, scanning electron microscopy, mercury and capillary flow porosimetry, were applied to assess the sealing feature of the material used. [525] However, the significant limitations of these methods, e.g., the need to section the samples and hence the creation of artefacts, led to µCT being the technique of choice for accurate 3D evaluation of root canal fillings. [58] Therefore, µCT analysis was used in the present study to quantify and qualify the pores within the apical plugs. The isotropic resolution was set at 9.9 µm, as it has been shown that a voxel size of 11.2 µm or less is a reliable cut-off value to assess the filling porosity [40,211], even though there is always a risk of tiny pores left undetected due to a high radiopacity of the material used.

Techniques and materials applied for root perforation repair have not been standardised. However, MTA is generally assumed to be a benchmark for sealing various types of root perforations. [520] MTA Flow™ is one of the newest MTA-based repair materials, surpassing traditional MTA in terms of clinical applicability due to its superior handling and delivery characteristics, faster setting time and, thus, increased washout resistance. [526] Moreover, according to this study, MTA Flow™ retains all desirable biological properties of the original MTA, such as biocompatibility, a crucial requirement for perforation repair material exposed to periodontal tissues. [230] The biocompatibility and bioactivity are attributed mainly to the continuous calcium ion release and the formation of calcium phosphate apatite crystals, which induce the regeneration and remineralization of adjacent hard tissues while reducing the filling material porosity. [230,452] Nevertheless, a previous study has shown that MTA Flow™ results in highly porous apical plugs despite all the improvements and advantageous characteristics. These results are in accordance with the present study, in which both MTA Flow™ groups (with/without ultrasonic agitation) exhibited a high porosity. The incidence of pores within MTA Flow™ fillings can be attributed to the increased water-to-cement ratio used during the mixing procedure to achieve a highly flowable consistency of the cement. It has been reported that excess water in the mixture eventually dries off and leaves pores that are not filled by hydration products. [527] Additionally, bismuth oxide added to the MTA Flow™ composition as a radiopacifier can negatively affect the sealing features by interfering with the hydration reaction and leaving more unreacted water within the filling. [186] Instead of bismuth oxide, some HCSC formulations, e.g., BioRoot® RCS,

contain zirconium oxide, which appears to have no impact on the material porosity. [528] These findings may correlate with the results of the present study, where significantly more homogeneous apical plugs were observed in both BR/SC groups than in the MTA Flow™ groups.

Sealing apical root perforations with BioRoot® RCS and a modified single-cone obturation technique was proposed mainly due to the simplicity and effectiveness previously reported in *in vitro* and *in vivo* studies. [48,498] The single-cone obturation technique refers to the desirable physicochemical properties of BioRoot® RCS [225,529], designed as a biological filler [530], and to the tapered gutta-percha cone, acting as a piston on the flowable sealer. [531] As reported previously, inserting the tapered gutta-percha cone creates hydraulic pressure, which improves the material distribution throughout the root canal. [532] Therefore, the gutta-percha cone may be the main factor in significant differences between the BR/SC and MTA Flow™ groups. No porosity associated with gutta-percha cones was observed in the present study through μCT analysis. Therefore, the superior overall homogeneity of BR/SC apical plugs can be attributed to solid gutta-percha cones.

Attempts to minimize the occurrence of pores within BR/SC and MTA Flow™ fillings by using ultrasonic agitation were made in this study, which demonstrated that neither of these techniques could produce pore-free apical plugs. The effect of ultrasonic application mainly refers to the acoustic energy transmission and the formation of cavitation bubbles, which eventually implode, increasing the temperature and the pressure inside the root canal. [59] According to previous investigations, which have reported significantly better results in terms of porosity after the use of indirect ultrasonication, the increased pressure may remove the entrapped air, disperse agglomerated particles, reduce their surface friction and provide more efficient incorporation of filler particles into the organic matrix, with no changes in particle size or material composition. [61,63,533] Additionally, the pressure generated during ultrasonic agitation may lead to superior interfacial adaptation between the filling material and the root canal wall, with better tubular penetration. [59,60] However, these advantageous effects of ultrasonic application did not provide more homogeneous apical plugs in the present study; the increased percentages of open and closed pores were observed in both BR/SC-UA and MF-UA groups. Therefore, the null hypothesis was rejected.

The lower overall homogeneity of ultrasonicated apical plugs could be attributed to the direct ultrasonic agitation, resulting in excessive vibratory forces. It has been reported that excessive ultrasonic energy can potentially lead to air incorporation into the filling material and thus contribute to higher porosity. [64,534] However, the use of direct ultrasonic agitation should not be

directly associated with less homogenous root canal fillings since it has been reported that indirect ultrasonication may also increase porosity. [58] Instead of the ultrasonication type, more attention should be paid to the agitation time, which is potentially directly related to both the rearrangement of cement particles and the heat generation. [58,535] The ultrasonic agitation of 10 seconds was selected in the present study in accordance with Sisli et al. [536], who agitated 5 mm apical plugs for 10 seconds and afterwards reported a lower incidence of pores. It has been reported that a short agitation time may create a shock-like effect, and a duration of 5 to 10 seconds is necessary to rearrange the cement particles and decrease the porosity. [58] On the other hand, the prolonged agitation time may be responsible for the increase in temperature, ultimately leading to water loss from flowable HCSC. [60,534] Even though the number of published studies evaluating the temperature changes in filling materials is still limited, there are few reports in the literature indicating that ultrasonic agitation is capable of raising the temperature inside the root canal by 2 ˚C [537], which can be sufficient to increase the water desorption occurring at temperatures as low as 20 ˚C. [538] The water loss may alter the rheological properties of the material and increase the porosity [537,538], which is considered the result of spaces between unhydrated cement particles. [527] Nevertheless, it can be speculated that indirect ultrasonic application is not prone to these adverse effects of temperature changes, as ultrasonic energy is transmitted to the material through the gutta-percha cone, plugger or another instrument. This would explain the contradictory findings regarding porosity obtained between the present study and previous investigations [62,536], which also performed ultrasonic agitation for 10 s. However, it is difficult to directly compare the results of the present study with the available literature, as they differ in too many aspects, including the type and properties of filling material, the application technique, ultrasonication type and duration, assessment method, etc.

The present study suggests that all apical plugs, regardless of the obturation technique used, may potentially lead to microleakage, as none of the fillings was pore-free, and the percentages of open pores surpassed the closed porosity in all experimental groups. Nevertheless, MTA Flow apical plugs (with/without ultrasonic agitation) demonstrated significantly higher percentages of open and closed pores than the BR/SC obturation technique. Therefore, reinforcing the findings of Benavides-García et al. [539], it can be concluded that MTA Flow prepared in a thin consistency should not be the material of choice for apical root perforation repair. Even though there is still no clear evidence of what porosity level is critical, the significantly higher

amount of pores observed in both MTA Flow™ groups may theoretically contribute to a worse outcome of endodontic treatment. [452,540] On the other hand, it has been shown that HCSC reduces their porosity with time in the presence of tissue fluids. [527] Therefore, the results of the present study should be evaluated with caution, as it is impossible to fully reproduce the clinical conditions using *in vitro* models. Further studies are needed to determine the clinical efficacy of BR/SC and MTA Flow™ obturation techniques in apically perforated and moderately curved roots and to confirm the adverse impact of direct ultrasonic agitation on the quality and homogeneity of root canal fillings.

4.3. Biocompatibility of Flowable Hydraulic Calcium Silicate-based Cements

Biocompatibility i*n vitro* studies with cell cultures is one of the first steps when analysing HCSC materials' biological features. These laboratory tests can be performed under controlled conditions, providing significant scientific knowledge on possible cytotoxicity effects of the material on tested cell culture. The biocompatibility of dental materials is essential for avoiding considerable inflammatory reactions and allowing repair. [541] Therefore, this study aimed to analyse the cytotoxicity of eluates extracted from freshly mixed flowable HCSC materials MTA Flow™ and MTA Flow™ White thick consistency on hDPSCs proliferation and morphology of the tested cell culture.

The majority of *in vitro* studies evaluate the hardened HCSC materials' eluates influence on cell cultures. [77,125,542,543] Extraction of eluates from HCSC after setting is one of the classic methods to evaluate biological properties of HCSC. This hardened HCSC eluates' analysis is important to analyse long-term HCSC influence on cells and/or periapical tissues. However, only freshly mixed and still setting cement/sealers are introduced into the teeth operative site. [544] Freshly mixed HCSC materials react with the environmental fluids and surrounding tissues, such as dentine, which in general are also called 'substrate' [32,391], resulting in the initial release of calcium-ions as well as the presence of leachable and toxic components from freshly mixed HCSC, increase of pH and, as a result, a more severe influence on surrounding cells. [32,75,314,425] After the setting reaction is complete and interaction with surrounding tissues is lower, the material's whole structure becomes more stable, resulting in lower cytotoxicity. [545] These thoughts were confirmed after a pilot study comparison of tested freshly mixed and hardened HCSC materials leachates, where hardened cement leachates had no significant influence on hDPSCs culture after 24 hours of incubation. At the same time, freshly mixed cement eluates, even

25% dilutions, resulted in significantly lower cell viability after 24 hours. Our findings are in agreement with other studies, which found lower or no cytotoxicity of hardened HCSC materials leachates. [545] As a result, a few recent studies changed or modified the methodology and also started analysing biocompatibility of freshly mixed HCSC leachates. [260,544] Therefore, to compare freshly mixed and hardened HCSC eluates effect on hDPSCs, both methodologies were used to analyse cytotoxicity after 24 hours of incubation. After detecting significant influence of freshly mixed HCSC eluates on hDPSCs, the latter were used for further analysis.

As the material is placed into the dental operative site, calcium hydroxide leaches into the surrounding tissues on hydration, clinically leading to alkalinization of the environment with the associated implications of interaction with the clinical environment and forming surrounding leachate. [32] The direct contact evaluation with the HCSC materials testing methods requires material sterilization, which can affect the material properties. [545] M. Pedano et al. discussed possible contamination of the specimens during the preparation of the leachates even though no contamination was observed. [544] Also, leachates (eluates) extracted from the HCSC materials were prepared inside a laminar flow cabinet with sterile instruments. Also, eluates enable easy sterilization by filtration through sterile filters. Furthermore, eluates enable the evaluation of the effect of the material in contact with and distant from the cell culture by simulating clinically periapically/in the dental pulp formed leachates. Finally, different leachate concentrations create a possibility to analyse a possible dose-related relationship and determine the ideal concentration for the sensitivity of the hDPSCs cells tested. [545,546] Indeed, only 100% leachates of tested HCSC resulted in clinically significantly lower cell viability after 24 hours and later time points than in the control group. While 50%, 25% and 12.5% concentration eluates maintained hDPSCs viability higher than 70%. As a result, 50% concentration leachates were selected for morphology and flow cytometry analysis of hDPSCs.

The results of the MTT cytotoxicity assay revealed biocompatible properties of tested Type IV flowable HCSC materials MTA Flow™ and MTA Flow™ White compared to the most widely analysed Type I HCSC ProRoot MTA. After 24 hours of incubation of hDPSCs with 100% HCSC leachates, the cell viability was around 40% in MFWhite and around 50% in MF groups, compared to the negative control group. The higher initial hDPSC proliferation inhibition of HCSC has already been reported in both *in vitro* [544,547] and *in vivo* studies. [541,548] Despite the primary lower cell viability than the control group cultured in the growth medium, the usage of HCSC cement/sealers *in vivo* showed similar or even better primary root canal

treatment clinical success, reaching around 89 % after 36 months. [549,550] Furthermore, similar and higher cell viability, maintaining above 80 % compared to the control group, was observed after 24 hours of incubation with 50 %, 25 % and 12.5 % concentrations of MTA Flow™ and MTA Flow™ White eluates. On the contrary, statistically significant differences were found between the MTA Flow™ leachates affected cell groups and the ProRoot MTA leachate cell group, where the cell viability was around 0 %, revealing severe cytotoxicity on hDPSCs and did not differ significantly from the positive control group. These results are comparable to the previous study by Chawan Maspon et al., who revealed similar 100% ProRoot MTA leachate cytotoxicity after 24 hours. [77] Nevertheless, these higher primary cytotoxicity results can be evaluated as biocompatible since ProRoot MTA was reported in many studies as biocompatible on hDPSCs. [77,551–553]

Primary higher ProRoot MTA cytotoxicity to the hDPSCs can be related to the higher primary wash-out of the setting material [554] and the resulting higher pH of the eluate. [555] Our pH analysis also showed a higher mean pH of the ProRoot MTA 100% leachate after 24 hours, reaching 12. Meanwhile, MTA Flow™ and MTA Flow™ White eluates pH were at around 10, which is more similar to the negative control group, which was 8.5. The higher pH results of Type I ProRoot MTA were also shown by another study where the pH of affected distilled water with ProRoot MTA was around 11.9. [556] Therefore, ProRoot MTA specimens' pH increases more quickly and drastically and reduces over time. [557] The MTA Flow™ pH analysis results are similar to those of the study by B. Guimares et al., who found MTA Flow™ pH after 24 hours to be around 10. It seems that the lower washout of the tested HCSC is related to the more steadily rising and lowering pH over time. [526] As a result, when the materials were set, cell viability after 24 hours of incubation with 100% leachates revealed no cytotoxicity of all three tested HCSC materials. Lower ProRoot MTA leachate concentrations resulted in mild cytotoxicity to the hDPSCs, maintaining cell viability above 80 %. According to the ISO 10993-5:2009 (revised in 2022) standard, the threshold determining whether a material is cytotoxic or not is when cell viability is lower than 70 %. [558] Performed flow cytometry Annexin V-FITC analysis of hDPSCs revealed higher than 84 % cell viability with no statistically significant differences between the groups after incubation with both tested 50% freshly mixed flowable HCSC leachates for 24 hours. According to the ISO 10993-5:2009 classification, the 50 % leachates created no cytotoxicity. Assuming the analysed proliferation analysis, 100% leachates resulted in 40% MFWhite and 50% MF hDPSCs cell proliferation compared to the negative control group. Indirectly comparing the hDPSCs proliferation results to the

viable, apoptotic, and necrotic cell analysis, the cytotoxicity of 100% leachates could be evaluated as mild and moderate, respectively.

The proliferation of hDPSCs was significantly affected by the freshly mixed HCSC leachates in all groups. However, the strength of the inhibitory effect of the leachates depended on their concentration. A few previous studies have analysed the biocompatibility of MF and MFWhite. [230,259,260,334] All the mentioned studies on MF used different methodologies to analyse biocompatibility. Bueno et al. [230] and Mondeli et al. [259] analysed HCSC in the subcutaneous tissue of rats at 7, 15, 30 and 60 days; both studies found moderate inflammatory reaction of MF 'Thick' consistency at 7 days with the reduction of inflammatory infiltrate at later time points. In the first study of MF analysis, L. Pelepenko et al. analysed MF 'thin' consistency cytotoxicity on periodontal ligament fibroblasts after 24 hours. [334] Even though this study used a more clinically relevant 3D cell culture associated with an in situ root-end filling experimental model by the previously mentioned methodology [559], the HCSC in the experiment was used after setting. Therefore, the cytotoxicity analysis of the MF leachate in the periapical model with periodontal ligament fibroblasts resulted in no cytotoxicity to the tested cell culture compared to the negative control group. The second study by L. Pelepenko et al. used freshly mixed MFWhite 'thin' consistency. [260] Consequently, the results shifted to reveal significantly different results from more biocompatible Biodentine in the mentioned study. ProRoot MTA and MFWhite resulted in lower cell viability, around 90 to 100 %, according to the negative control group, but did not differ significantly between the groups. The results of the study by L. Pelepenko et al. with MFWhite differ from our study results due to the different methodologies and cell cultures used. We found more severe and rapid cytotoxicity of MFWhite 100 % leachate on the hDPSCs, where the reduction of cell viability to 40 % after 24 hours was seen. These results could be attributed to the pre-preparation of 100 % leachates and the complete change of the growth medium to 100% leachate after 24 hours, while L. Pelepenko et al.'s study used a 3D model, where cells were emerged into the growth medium and, only afterwards was the specimen with freshly mixed HCSC introduced into the medium, resulting in a leachate formation and gradual medium pH changes. Furthermore, in the literature, hDPSCs are characterized as relatively slow-proliferating cells that quickly cease to grow in the culture. Meanwhile, periodontal ligament stem cells proliferate more rapidly and for more passages. [560] Therefore, the different cell cultures and methodology for analysing the cytotoxicity of freshly mixed flowable MFWhite could influence the results.

Analysing hDPSC proliferation over five days, the time-dependent phenomenon was seen: the increase in cell proliferation was observed in all tested HCSC materials 50 %, 25 % and 12.5 % concentration leachate groups at later time points. The proliferation of tested MF and MFWhite affected cell groups was comparable to the ProRoot MTA cell group in 50 %, 25 % and 12.5 % concentrations at all time points. However, no cell proliferation increase was seen in 100 % leachate groups during the whole observation period. Only MF and MFWhite maintained cell viability around 5 to 10 % compared to the negative control group. Our findings are comparable to those of previous studies, where freshly mixed 100 % leachate created the lowest cell viability, so reducing the eluate concentration and prolonged exposure in lower or equal to 50 % concentration leachates resulted in higher cell viability. [77]

The biological morphology reaction of hDPSCs in contact with these materials eluates was evaluated using an inverted phase contrast microscope. The cell phenotype in negative control and tested groups affected by lower leachate concentration were similar to the previously described mesenchymal stem cells with characteristic elongated fibroblastic morphology. [561,562] Compared to the negative control group, 100 % leachate-affected hDPSCs revealed more round cell morphology and decreased cell number. Moreover, a leachate concentration-dependent phenomenon was observed. Reduction in the eluate concentration created the morphology more similar to the negative control group, with the interesting exception at 50 % concentration, where morphology in the tested MF and MFWhite affected hDPSCs groups cells were longer and wider than in the negative control group. In the literature, the morphology of the hDPSCs, when cultured on the surface of MTA, was shown to be less fibrous and more differentiated than the negative control group. [563] This could be related to the previously mentioned bioactive potential of HCSC on hDPSCs. [564,565] Interestingly, the width and length of the hDPSCs affected by 50 % MF leachate were significantly higher than in the 50 % MFWhite group, while the aspect ratio was similar. Moreover, cells affected by the 50 % MFWhite leachate were more similarly shaped compared to the negative control group than the 50 % MF hDPSCs group. In contrast, other studies found different degrees of hDPSC shrinkage after affecting with different HCSC leachates [562] and superior spreading of the cells. [257] However, no direct comparison can be undertaken since no previous studies evaluated hDPSCs morphology after affecting with MF or MFWhite leachates. The results of this study suggest that hDPSC induction with 50 % MF leachate results in higher cell width and length; however, no statistically significant cell number increase was found between the tested flowable HCSC groups.

On the other hand, the results of the current study should be evaluated critically, as *in vitro* results cannot be directly transferred into *in vivo* situations. Isolated cell cultures do not represent the *in vivo* dental pulp tissues. These 2D cell culture models are simplistic, as the *in vivo* environment comprises a mixture of cell types and a blood flow that may affect the local response to the material. [566] Various buffering systems, immune system and other factors may influence hDPSC viability, proliferation and morphology. [567,568] Also, inflammation in the dental pulp may change the clinical scenario by gradually moving in an apical direction. [567] Nevertheless, exposing cell cultures to HCSC leachates provides valuable data on how the material may affect local tissues *in vivo.* Characterizing subsequent cellular responses can demonstrate the biocompatibility, cytotoxicity, proliferative and differentiation responses. [569] Therefore, this study revealed comparable biocompatibility of the new generation flowable HCSC materials MTA Flow™ and MTA Flow™ White Thick consistencies with hDPSC cultures to the original ProRoot MTA White. Further analysis is needed to recommend one tested flowable HCSC material over the other. However, easier usage, lower washout, quicker setting, and, as a result, more steady pH changes could suggest the use of new-generation MF materials. Also, considering the similar biocompatibility properties of the MTA Flow™ and MTA Flow™ White and the safety of not having tooth crown discolouration due to the elimination of bismuth oxide, the rationale would be to use the MTA Flow™ White. Nonetheless, combining the choice of flowable HCSC and its adaptability in clinical protocol may lead to a higher clinical success rate, which should be evaluated by future *in vivo* studies.

CONCLUSIONS

Considering the current *in-vitro* study limitations, the following conclusions can be stated:

1. Operators' skills could not ensure void-free root canal filling. Open pores being the predominant type in all root thirds of single cone root canal fillings, with exclusively higher porosity in the apical root third.
2. The quality and homogeneity of root canal fillings in the middle and apical root thirds showed comparable porosity between the operators with different clinical experiences. At the same time, significant differences were found in the coronal third among the groups.
3. Significantly higher porosity was observed in the flowable HCSC MTA Flow™ group than in the BioRoot RCS/single cone technique group.
4. Direct ultrasonic agitation did not considerably impact the porosity distribution in HCSC/single cone fillings. In contrast, flowable HCSC MTA Flow™ apical plugs/fillings demonstrated significantly higher overall porosity after direct ultrasonic agitation.
5. Freshly mixed new-generation flowable HCSC, MTA Flow™, and MTA Flow™ White leachates significantly influence hDPSCs' viability, proliferation, and morphology. The effect on hDPSCs is leachate concentration-dependent, resulting in the hDPSCs' viability below 70% (moderate reactivity/toxicity) after being affected with 100% concentration leachates.
6. The new generation flowable HCSC MTA Flow™ and MTA Flow™ White have a comparable cytotoxic effect on hDPSCs to that of the original ProRoot MTA.

PRACTICAL RECOMMENDATIONS AND FUTURE PERSPECTIVES

1. The single-cone root canal obturation technique with HCSC sealers/fillers is a repeatable and easily manageable filling methodology that results in comparable or even higher root canal filling quality and homogeneity despite the endodontic treatment skills of the operator. Therefore, SC technique could be recommend to use clinically.
2. Undergraduate dentistry students programme should include root canal obturation using single-cone technique with HCSC.
3. Single-cone obturation technique with HCSC, such as BioRoot® RCS, should be used in apically curved and perforated mandibular molar root canals to create a homogenous apical filling.

4. Direct ultrasonic agitation should not be used with MTA Flow™ HCSC material for root canal obturation.
5. HCSC biocompatibility studies should analyse freshly-mixed and hardened HCSC or their freshly-extracted leachates, and compare the results.
6. The new generation flowable HCSC materials MTA Flow™ and MTA Flow™ White are rational to use in clinical practice due to their ease of use, versatility and biocompatibility. These materials should be recommended for routine usage in case of indicated procedures.

CURRICULUM VITAE

NAME	Paulius
SURNAME	Tušas
DATE OF BIRTH	1994-08-15
PLACE OF BIRTH	Panevėžys, Lithuania
E-MAIL	paulius.tusas@gmail.com
RESEARCH ACTIVITIES WEBSITE	https://www.researchgate.net/profile/Paulius-Tusas

EDUCATION

2009 – 2013	Panevėžio Juozo Balčikonio gymnasium, Panevėžys, Lithuania *Certificate of Basic Education*
2013 – 2018	Lithuanian University of Health Sciences Faculty of Odontology, Kaunas, Lithuania *Master's Diploma in Dentistry*
2018 – 2021	Vilnius University, Faculty of Medicine, Institute of Odontology, Vilnius, Lithuania *Certificate of Specialist in Endodontology*

WORK EXPERIENCE

2018 – 2021	Dentist General Practitioner, Public practice
2020 – present	Junior assistant, Vilnius University, Institute of Odontology, Vilnius, Lithuania
2021 – present	Endodontology Specialist, Private practice

LICENCES

2018 – present	Dentist General Practitioner, OPL – 05355
2021 – present	Endodontology Specialist, OPL – 05876

SUPPLEMENTARY MATERIAL

Approvals of the Ethics Committee

VILNIAUS UNIVERSITETO MEDICINOS FAKULTETAS
Viešoji įstaiga, Universiteto g. 3, LT- 01513 Vilnius, tel. (8 5) 268 7001, faks. (8 5) 272 8646, el. p. infor@cr.vu.lt.
Duomenys kaupiami ir saugomi Juridinių asmenų registre, kodas 211950810.
Fakulteto duomenys: M.K. Čiurlionio g. 21/27, LT-03101 Vilnius, tel. (8 5) 239 8701, (8 5) 239 7800, faks. (8 5) 239 8705, el. p. mf@mf.vu.lt
VILNIAUS REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS
M.K. Čiurlionio g. 21/27, LT-03101 Vilnius, tel. (8 5) 268 6998, el. p. rbtek@mf.vu.lt

LEIDIMAS
ATLIKTI BIOMEDICININĮ TYRIMĄ

2016-07-12 Nr.158200-16-860-369

Tyrimo pavadinimas:
Protezinių medžiagų biosuderinamumo bei 3D spausdinimo technologijų audinių regeneracijai in vitro ir in vivo vertinimas

Protokolo Nr.:	1
Versija:	002
Data:	2016-06-27
Asmens informavimo ir informuoto asmens sutikimo forma:	In vitro
Versija:	2a
Data:	2016-06-28
Asmens informavimo ir informuoto asmens sutikimo forma:	In vivo
Versija:	2b
Data:	2016-06-28
Pagrindinis tyrėjas:	**Vygandas Rutkūnas**
Įstaigos pavadinimas:	VšĮ VUL Žalgirio klinika
Adresas:	Žalgirio g. 117, Vilnius
Įstaigos pavadinimas:	UAB „Prodentum“
Adresas:	Kalvarijų g. 128A, Vilnius
Leidimas galioja iki:	**2019-09-01**

Leidimas išduotas Vilniaus regioninio biomedicininių tyrimų etikos komiteto posėdžio (protokolas Nr. 158200-2016/07), vykusio 2016 m. liepos 12 d. sprendimu.

Vilniaus regioninio biomedicininių tyrimų etikos komiteto ekspertų grupės nariai			
Nr.	Vardas, pavardė	veiklos sritis	dalyvavo posėdyje
1	doc. dr. Laimutė Jakavonytė	filosofija	taip
2	prof.dr. Jolanta Dadonienė	epidemiologija, medicina	taip
3	doc.dr. Jaunius Gumbis	teisė	ne
4	Genovaitė Bulzgytė	slauga	taip
5	prof.dr. Augustina Jankauskienė	medicina	ne
6	dr. Laura Malinauskienė	medicina	taip
7	Eglė Zubienė	psichologija	taip
8	prof. Saulius Vosylius	medicina	taip
9	Ugnė Šakūnienė	pacientų teisės	ne

Pirmininkė Laura Malinauskienė

VILNIAUS UNIVERSITETAS Vilniaus regioninis biomedicininių tyrimų etikos komitetas MEDICINOS FAKULTETAS

Biomedicininio tyrimo „Protezinių medžiagų biosuderinamumo bei 3D spausdinimo technologijų audinių regeneracijai in vitro ir in vivo vertinimas"
pagrindiniam tyrėjui Vygandui Rutkūnui

2019-09-19 Nr. 2019-LP-37

Dėl leidimo Nr. 158200-16-860-369 papildymo Nr. 1

PRITARIMAS

Vilniaus regioninis biomediciniių tyrimų etikos komitetas išnagrinėjo Jūsų prašymą keisti/papildyti biomediciniio tyrimo *„Protezinių medžiagų biosuderinamumo bei 3D spausdinimo technologijų audinių regeneracijai in vitro ir in vivo vertinimas*", leidimą Nr. 158200-16-860-369, išduotą 2016-07-12 d. Ekspertai pritaria:

- Tyrimo pratęsimui iki 2025-09-01 d.;
- Atnaujintam protokolui (versijos Nr. 003, data 2019-06-27 d.);
- Atnaujintų informuoto asmens sutikimo formų teikimui (donorų grupė – versijos Nr. 4, data 2019-08-16 d. ir regeneracijos klinikinio tyrimo grupė - versijos Nr. 4, data 2019-08-16 d.).

Pirmininkas prof. dr. (HP) Saulius Vosylius

Viešoji įstaiga
Universiteto g. 3
01513 Vilnius

Duomenys kaupiami ir saugomi
Juridinių asmenų registre
Kodas 211950810

Komiteto duomenys:
M. K. Čiurlionio g. 21, LT- 03101 Vilnius
Tel. (8 5) 268 6998, el. p. rbtek@mf.vu.lt

VIEŠOJI ĮSTAIGA VILNIAUS UNIVERSITETO LIGONINĖS ŽALGIRIO KLINIKA

Committee of Ethics University Hospital Zalgirio clinics
Zalgirio 117, LT- 08217 Vilnius, tel/fax +37052727589,
email:info@zalgirioklinika.lt

Permission for biomedical study *ex vivo*
2014-06-03 No. EK-2

Biomedical study: Potential to induce dentinal cracks during retreatment procedures of teeth treated with "Russian red": an ex vivo study
Protocol No.: Date: 2014 05 01
Form of informed consent: Version: 1 Date: 2014 05 01
Main researcher: Egle Nedzinskiene
Place of biomedical study
University Hospital Zalgirio clinics

Permission was signed by committee members, 2014 – 06 - 01

No	Name, Surname	Participation during meeting
1	Laura Linkevičienė	yes
2	Kristina Landzbergienė	yes
3	Rūta Žaliūnienė	yes
4	Virginija Gailienė	yes

Chief of Committee of Ethics
University Hospital Zalgirio clinics

dr. phD Laura Linkevičienė

BIBLIOGRAPHY

1. Kakehashi, S., Stanley, H. R. & Fitzgerald, R. J. The Effects Of Surgical Exposures Of Dental Pulps In Germ-Free And Conventional Laboratory Rats. *Oral Surg. Oral Med. Oral Pathol.* **20**, 340–349 (1965).
2. Abbott, P. V. Classification, diagnosis and clinical manifestations of apical periodontitis. *Endod. Top.* **8**, 36–54 (2004).
3. Skudutyte-Rysstad, R. & Eriksen, H. M. Endodontic status amongst 35-year-old Oslo citizens and changes over a 30-year period. *Int. Endod. J.* **39**, 637–642 (2006).
4. Georgopoulou, M. K., Spanaki-Voreadi, A. P., Pantazis, N. & Kontakiotis, E. G. Frequency and distribution of root filled teeth and apical periodontitis in a Greek population. *Int. Endod. J.* **38**, 105–111 (2005).
5. Al-Zahrani, M. S., Abozor, B. M. & Zawawi, K. H. The relationship between periapical lesions and the serum levels of glycosylated hemoglobin and C-reactive protein in type 2 diabetic patients. *Saudi Med. J.* **38**, 36–40 (2017).
6. Tibúrcio-Machado, C. S. *et al.* The global prevalence of apical periodontitis: a systematic review and meta-analysis. *Int. Endod. J.* **54**, 712–735 (2021).
7. Kirkevang, L.-L., Vaeth, M., Hörsted-Bindslev, P., Bahrami, G. & Wenzel, A. Risk factors for developing apical periodontitis in a general population. *Int. Endod. J.* **40**, 290–299 (2007).
8. Kirkevang, L.-L., Ørstavik, D., Bahrami, G., Wenzel, A. & Vaeth, M. Prediction of periapical status and tooth extraction. *Int. Endod. J.* **50**, 5–14 (2017).
9. Löst, C. Quality guidelines for endodontic treatment: Consensus report of the European Society of Endodontology. *Int. Endod. J.* **39**, 921–930 (2006).
10. Sjögren, U., Figdor, D., Persson, S. & Sundqvist, G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int. Endod. J.* **30**, 297–306 (1997).
11. Paredes-Vieyra, J. & Enriquez, F. J. J. Success rate of single- versus two-visit root canal treatment of teeth with apical periodontitis: a randomized controlled trial. *J. Endod.* **38**, 1164–1169 (2012).
12. Torabinejad, M., Corr, R. & Handysides, R. Outcomes of Nonsurgical Retreatment and Endodontic Surgery : A Systematic Review. *J.*

Endod. **35**, 930–937 (2009).
13. Karaoğlan, F., Miçooğulları Kurt, S. & Çalışkan, M. K. Outcome of single- versus two-visit root canal retreatment in teeth with periapical lesions: A randomized clinical trial. *Int. Endod. J.* **55**, 833–843 (2022).
14. Sundqvist, G., Figdor, D., Persson, S. & Sjögren, U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **85**, 86–93 (1998).
15. de Chevigny, C. *et al.* Treatment Outcome in Endodontics: The Toronto Study-Phases 3 and 4: Orthograde Retreatment. *J. Endod.* **34**, 131–137 (2008).
16. Aquilino, S. A. & Caplan, D. J. Relationship between crown placement and the survival of endodontically treated teeth. *J. Prosthet. Dent.* **87**, 256–263 (2002).
17. Tsintsadze, N., Margvelashvili-Malament, M., Natto, Z. S. & Ferrari, M. Comparing survival rates of endodontically treated teeth restored either with glass-fiber-reinforced or metal posts: A systematic review and meta-analyses. *J. Prosthet. Dent.* (2022). doi:10.1016/j.prosdent.2022.01.003
18. León-López, M. *et al.* Prevalence of root canal treatment worldwide: A systematic review and meta-analysis. *Int. Endod. J.* **55**, 1105–1127 (2022).
19. Karamifar, K., Tondari, A. & Saghiri, M. A. Endodontic Periapical Lesion: An Overview on the Etiology, Diagnosis and Current Treatment Modalities. *Eur. Endod. J.* **5**, 54–67 (2020).
20. Goldberg, F., Cantarini, C., Alfie, D., Macchi, R. L. & Arias, A. Relationship between unintentional canal overfilling and the long-term outcome of primary root canal treatments and nonsurgical retreatments: a retrospective radiographic assessment. *Int. Endod. J.* **53**, 19–26 (2020).
21. Demirci, K. & C, M. K. A Prospective Randomized Comparative Study of Cold Lateral Condensation Versus Core / Gutta-percha in Teeth with Periapical Lesions. 1–5 (2016). doi:10.1016/j.joen.2015.10.022
22. Selem, L. C. *et al.* Quality of obturation achieved by a non-gutta-percha-based root filling system in single-rooted canals. *J. Endod.* **40**, 2003–2008 (2014).
23. Sijeria, P., Bhartia, R., Swamy Kv, N., Kulkarni, S. & Singla, S. Evaluation of Root Canal Filling in Primary Teeth by Volumetric Analysis: In Vitro Study. *Int. J. Clin. Pediatr. Dent.* **11**, 386–392

(2018).
24. Whitworth, J. Methods of filling root canals: principles and practices. *Endod. Top.* **12**, 2–24 (2005).
25. Gupta, R., Dhingra, A. & Panwar, N. R. Comparative Evaluation of Three Different Obturating Techniques Lateral Compaction, Thermafil and Calamus for Filling Area and Voids Using Cone Beam Computed Tomography: An Invitro study. *J. Clin. Diagn. Res.* **9**, ZC15-7 (2015).
26. Kim, S., Kim, S., Park, J.-W., Jung, I.-Y. & Shin, S.-J. Comparison of the Percentage of Voids in the Canal Filling of a Calcium Silicate-Based Sealer and Gutta Percha Cones Using Two Obturation Techniques. *Mater. (Basel, Switzerland)* **10**, (2017).
27. Celikten, B. *et al.* Evaluation of root canal sealer filling quality using a single-cone technique in oval shaped canals: An In vitro Micro-CT study. *Scanning* **38**, 133–140 (2016).
28. Iglecias, E. F. *et al.* Presence of Voids after Continuous Wave of Condensation and Single-cone Obturation in Mandibular Molars: A Micro-computed Tomography Analysis. *J. Endod.* **43**, 638–642 (2017).
29. Gok, T., Capar, I. D., Akcay, I. & Keles, A. Evaluation of Different Techniques for Filling Simulated C-shaped Canals of 3-dimensional Printed Resin Teeth. *J. Endod.* **43**, 1559–1564 (2017).
30. Küçükkaya Eren, S., Askerbeyli Örs, S. & Yılmaz, Z. Effect of Post Space Preparation on Apical Obturation Quality of Teeth Obturated with Different Techniques: A Micro-computed Tomographic Study. *J. Endod.* **43**, 1152–1156 (2017).
31. Duncan, H. F. Present status and future directions—Vital pulp treatment and pulp preservation strategies. *Int. Endod. J.* **55**, 497–511 (2022).
32. Camilleri, J. Present status and future directions : Hydraulic materials for endodontic use. **55**, 710–777 (2022).
33. Bolhari, B. *et al.* Calcium silicate cement interface with restorative materials through layering after different time intervals. *Odontology* **109**, 210–221 (2021).
34. Taha, N. A. & Khazali, M. A. Partial Pulpotomy in Mature Permanent Teeth with Clinical Signs Indicative of Irreversible Pulpitis: A Randomized Clinical Trial. *J. Endod.* **43**, 1417–1421 (2017).
35. Grech, L., Mallia, B. & Camilleri, J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. *Dent. Mater.* **29**, e20–e28 (2013).

36. Kwak, S. W. *et al.* Physicochemical Properties and Biocompatibility of Various Bioceramic Root Canal Sealers: In Vitro Study. *J. Endod.* **49**, 871–879 (2023).
37. Krastl, G. *et al.* Tooth discoloration induced by endodontic materials: A literature review. *Dent. Traumatol.* **29**, 2–7 (2013).
38. Ber, B. S., Hatton, J. F. & Stewart, G. P. Chemical Modification of ProRoot MTA to Improve Handling Characteristics and Decrease Setting Time. *J. Endod.* **33**, 1231–1234 (2007).
39. Krug, R., Krastl, G. & Jahreis, M. Technical quality of a matching-taper single-cone filling technique following rotary instrumentation compared with lateral compaction after manual preparation: a retrospective study. *Clin. Oral Investig.* **21**, 643–652 (2017).
40. Torres, F. F. E. *et al.* Solubility, Porosity, Dimensional and Volumetric Change of Endodontic Sealers. *Braz. Dent. J.* **30**, 368–373 (2019).
41. Milanovic, I. *et al.* Immediate and Long-Term Porosity of Calcium Silicate-Based Sealers. *J. Endod.* **46**, 515–523 (2020).
42. Guivarc'h, M. *et al.* An international survey on the use of calcium silicate-based sealers in non-surgical endodontic treatment. *Clin. Oral Investig.* **24**, 417–424 (2020).
43. Eymirli, A. *et al.* Dentinal Tubule Penetration and Retreatability of a Calcium Silicate–based Sealer Tested in Bulk or with Different Main Core Material. *J. Endod.* **45**, 1036–1040 (2019).
44. Celikten, B. *et al.* Micro-CT assessment of the sealing ability of three root canal filling techniques. **57**, 361–366 (2015).
45. Al-Hiyasat, A. S. & Alfirjani, S. A. The effect of obturation techniques on the push-out bond strength of a premixed bioceramic root canal sealer. *J. Dent.* **89**, 103169 (2019).
46. Kim, H., Kim, E., Lee, S.-J. & Shin, S.-J. Comparisons of the Retreatment Efficacy of Calcium Silicate and Epoxy Resin-based Sealers and Residual Sealer in Dentinal Tubules. *J. Endod.* **41**, 2025–2030 (2015).
47. Chybowski, E. A. *et al.* Clinical Outcome of Non-Surgical Root Canal Treatment Using a Single-cone Technique with Endosequence Bioceramic Sealer: A Retrospective Analysis. *J. Endod.* **44**, 941–945 (2018).
48. Zavattini, A., Knight, A., Foschi, F. & Mannocci, F. Outcome of Root Canal Treatments Using a New Calcium Silicate Root Canal Sealer: A Non-Randomized Clinical Trial. *J. Clin. Med.* **9**, 782 (2020).
49. Bouillaguet, S., Shaw, L., Barthelemy, J., Krejci, I. & Wataha, J. C. Long-term sealing ability of Pulp Canal Sealer, AH-Plus, GuttaFlow

and Epiphany. *Int. Endod. J.* **41**, 219–226 (2008).
50. Bajawi, A. M. *et al.* Radiographic Assessment of the Quality of Root Canal Treatments Performed by Practitioners with Different Levels of Experience. **11**, 1609–1616 (2018).
51. Santos, S. M. C. *et al.* Radiographic quality of root canal fillings performed in a postgraduate program in endodontics. *Braz. Dent. J.* **21**, 315–321 (2010).
52. Molander, A., Caplan, D., Bergenholtz, G. & Reit, C. Improved quality of root fillings provided by general dental practitioners educated in nickel-titanium rotary instrumentation. *Int. Endod. J.* **40**, 254–260 (2007).
53. Tavares, P. B. L., Bonte, E., Boukpessi, T., Siqueira, J. F. J. & Lasfargues, J.-J. Prevalence of apical periodontitis in root canal-treated teeth from an urban French population: influence of the quality of root canal fillings and coronal restorations. *J. Endod.* **35**, 810–813 (2009).
54. Awooda, E. M., Siddig, R. I., Alturki, R. S. & Sanhouri, N. M. Radiographic technical quality of root canal treatment performed by undergraduate dental students at the Academy Dental Teaching Hospital, UMST, Sudan. *J. Int. Soc. Prev. Community Dent.* **6**, 554–558 (2016).
55. Eskandarloo, A., Karkehabadi, H., Hoseini Hashemi, S. Z., Ahmadi, M. & Hendi, S. S. Radiographic Quality of Root Canal Obturation Performed By Fifth Year Students of Hamadan Dental School. *Iran. Endod. J.* **12**, 236–241 (2017).
56. Er, O., Sagsen, B., Maden, M., Cinar, S. & Kahraman, Y. Radiographic technical quality of root fillings performed by dental students in Turkey. *Int. Endod. J.* **39**, 867–872 (2006).
57. Angerame, D., De Biasi, M., Pecci, R. & Bedini, R. Filling ability of three variants of the single-cone technique with bioceramic sealer: a micro-computed tomography study. *J. Mater. Sci. Mater. Med.* **31**, 91 (2020).
58. El-Ma'aita, A. M., Qualtrough, A. J. E. & Watts, D. C. A micro-computed tomography evaluation of mineral trioxide aggregate root canal fillings. *J. Endod.* **38**, 670–672 (2012).
59. da Silva Machado, A. P. *et al.* Does the ultrasonic activation of sealer hinder the root canal retreatment? *Clin. Oral Investig.* **25**, 4401–4406 (2021).
60. Aguiar, B. A. *et al.* Influence of ultrasonic agitation on bond strength, marginal adaptation, and tooth discoloration provided by three coronary barrier endodontic materials. *Clin. Oral Investig.* **23**, 4113–

4122 (2019).
61. Wiesse, P. E. B. *et al.* Effect of ultrasonic and sonic activation of root canal sealers on the push-out bond strength and interfacial adaptation to root canal dentine. *Int. Endod. J.* **51**, 102–111 (2018).
62. Dinçer, A. N., Güneşer, M. B. & Sisli, S. N. Micro-CT analysis of the marginal adaptation and porosity associated with ultrasonic activation of coronally placed tricalcium silicate-based cements. *Aust. Endod. J. J. Aust. Soc. Endodontology Inc* **46**, 323–329 (2020).
63. Kim, J. A. *et al.* Root Canal Filling Quality of a Premixed Calcium Silicate Endodontic Sealer Applied Using Gutta-percha Cone-mediated Ultrasonic Activation. *J. Endod.* **44**, 133–138 (2018).
64. Kim, S. *et al.* Effects of Ultrasonic Activation on Root Canal Filling Quality of Single-Cone Obturation with Calcium Silicate-Based Sealer. 1–9 (2021).
65. Darvell, B. W. & Wu, R. C. T. MTA - An Hydraulic Silicate Cement: Review update and setting reaction. *Dent. Mater.* **27**, 407–422 (2011).
66. Rajasekharan, S., Martens, L. C., Cauwels, R. G. E. C. & Verbeeck, R. M. H. Biodentine™ material characteristics and clinical applications: a review of the literature. *Eur. Arch. Paediatr. Dent. Off. J. Eur. Acad. Paediatr. Dent.* **15**, 147–158 (2014).
67. Paula, A. *et al.* Biodentine™ boosts, WhiteProRoot®MTA increases and Life® suppresses odontoblast activity. *Materials (Basel).* **12**, (2019).
68. Camilleri, J. *Mineral trioxide aggregate in dentistry: From preparation to application*. *Mineral Trioxide Aggregate in Dentistry: From Preparation to Application* 9783642551, (Springer Berlin, Heidelberg, 2014).
69. Prati, C. & Gandolfi, M. G. Calcium silicate bioactive cements: Biological perspectives and clinical applications. *Dent. Mater.* **31**, 351–370 (2015).
70. Gandolfi, M. G. *et al.* Calcium silicate and calcium hydroxide materials for pulp capping: Biointeractivity, porosity, solubility and bioactivity of current formulations. *J. Appl. Biomater. Funct. Mater.* **13**, 1–18 (2015).
71. Yaemkleebbua, K., Osathanon, T., Nowwarote, N., Limjeerajarus, C. N. & Sukarawan, W. Analysis of hard tissue regeneration and Wnt signalling in dental pulp tissues after direct pulp capping with different materials. *Int. Endod. J.* **52**, 1605–1616 (2019).
72. Torabinejad, M., Parirokh, M. & Dummer, P. M. H. Mineral trioxide aggregate and other bioactive endodontic cements: an updated

overview - part II: other clinical applications and complications. *Int. Endod. J.* **51**, 284–317 (2018).
73. Moore, A., Howley, M. F. & O'Connell, A. C. Treatment of open apex teeth using two types of white mineral trioxide aggregate after initial dressing with calcium hydroxide in children. *Dent. Traumatol.* **27**, 166–173 (2011).
74. Emara, R., Elhennawy, K. & Schwendicke, F. Effects of calcium silicate cements on dental pulp cells: A systematic review. *J. Dent.* **77**, 18–36 (2018).
75. Pedano, M. S., Li, X., Yoshihara, K., Van Landuyt, K. & Van Meerbeek, B. Cytotoxicity and bioactivity of dental pulp-capping agents towards human tooth-pulp cells: A systematic review of in-vitro studies and meta-analysis of randomized and controlled clinical trials. *Materials (Basel).* **13**, 1–42 (2020).
76. Kunert, M. & Lukomska-Szymanska, M. Bio-Inductive Materials in Direct and Indirect Pulp Capping - A Review Article. *Materials (Basel).* **13**, (2020).
77. Manaspon, C. *et al.* Human dental pulp stem cell responses to different dental pulp capping materials. *BMC Oral Health* **21**, 1–13 (2021).
78. Paula, A. B. *et al.* Direct Pulp Capping: What is the Most Effective Therapy?-Systematic Review and Meta-Analysis. *J. Evid. Based. Dent. Pract.* **18**, 298–314 (2018).
79. Tran, X. V. *et al.* Reparative Mineralized Tissue Characterization after Direct Pulp Capping with Calcium-Silicate-Based Cements. *Mater. (Basel, Switzerland)* **12**, (2019).
80. Nie, E. *et al.* Effectiveness of direct pulp capping bioactive materials in dentin regeneration: A review. *Materials (Basel).* **14**, (2021).
81. Torabinejad M & White DJ. Tooth filling material and method of use. *US Pat. 5415547* (1995).
82. Al., T. et. United States patent 5415547. *United States Patent* (1995). doi:10.1016/0375-6505(85)90011-2
83. U.S. Food and Drug Administration. K964174.pdf. (1997).
84. U.S. Food and Drug Administration. K980332.pdf. (1998).
85. U.S. Food and Drug Administration. K981620.pdf. (1998).
86. Germain, L. P. Mineral trioxide aggregate: a new material for the new millennium. *Dent. Today* **18**, 66-67,70-71 (1999).
87. Lee, S., Monsef, M. & Torabinejad, M. Sealing Ability of a Mineral Trioxide Aggregate for Repair of Lateral Root Perforations. **19**, 541–544 (1993).
88. Torabinejad, M., Hong, C. U., McDonald, F. & Pitt Ford, T. R.

Physical and chemical properties of a new root-end filling material. *J. Endod.* (1995). doi:10.1016/S0099-2399(06)80967-2
89. Koh, E. T., Mcdonald, F., Ford, T. R. P. & Torabinejad, M. Cellular Response to Mineral Trioxide Aggregate. **24**, 0–4 (1998).
90. Mitchell, P. J. C., Ford, T. R. P., Torabinejad, M. & Mcdonald, F. Osteoblast biocompatibility of mineral trioxide aggregate. **20**, (1999).
91. Wu, M., Kontakiotis, E. G. & Wesselink, P. R. Long-Term Seal Provided by Some Root-End Filling Materials. **24**, 1–4 (1998).
92. Torabinejad, M. & Chivian, N. Clinical Applications of Mineral Trioxide Aggregate. **25**, (1999).
93. S, T. R. P. F. B. D. *et al.* Using Mineral Trioxide Aggregate as a pulp-capping material. *J. Am. Dent. Assoc.* **127**, 1491–1494 (1996).
94. Camilleri, J. & Pitt Ford, T. R. Mineral trioxide aggregate: A review of the constituents and biological properties of the material. *Int. Endod. J.* **39**, 747–754 (2006).
95. Antonio, M. *et al.* pH and calcium ion release of 2 root-end filling materials. 2002–2004 (2003). doi:10.1067/moe.2003.12
96. Estrela, C., Silva, R. S. & Pécora, J. D. Antimicrobial and Chemical Study of MTA , Portland Cement , Calcium Hydroxide Paste , Sealapex and Dycal. **11**, 8–12 (2018).
97. Di, S. & Wallace, J. A. A Comparative Analysis Of Mineral Trioxide Aggregate And Portland Cement. 43–44 (2003).
98. Schembri, M., Peplow, G. & Camilleri, J. Analyses of heavy metals in mineral trioxide aggregate and Portland cement. *J. Endod.* **36**, 1210–1215 (2010).
99. Camilleri, J., Kralj, P., Veber, M. & Sinagra, E. Characterization and analyses of acid-extractable and leached trace elements in dental cements. 737–743 (2012). doi:10.1111/j.1365-2591.2012.02027.x
100. Bramante, C. M., Cardoso, C., Demarchi, O., Antonio, M. & Duarte, H. Presence of arsenic in different types of MTA and white and gray Portland cement. *YMOE* **106**, 909–913 (1824).
101. Antonio, M. *et al.* Arsenic release provided by MTA and Portland cement. 648–650 (2005). doi:10.1016/j.tripleo.2004.09.015
102. De-deus, G. *et al.* Optimal Cytocompatibility of a Bioceramic Nanoparticulate Cement in Primary Human Mesenchymal Cells. *J. Endod.* **35**, 1387–1390 (2009).
103. Lu D, Zhou S. High strength biological cement composition and using the same. Patent number: 7553362; 2006. **2**, (2009).
104. Shackelford, J. F. *Bioceramics*. (Taylor & Francis, 1999).
105. Drukteinis, S. & Camilleri, J. *Bioceramic materials in clinical*

endodontics. *Bioceramic Materials in Clinical Endodontics* (2020). doi:10.1007/978-3-030-58170-1

106. Koch, K. A., Brave, D. G. & Nasseh, A. A. Bioceramic technology: closing the endo-restorative circle, Part I. *Dent. Today* **29**, 100–105 (2010).
107. Koch, K. A., Brave, G. D. & Nasseh, A. A. Bioceramic technology : closing the endo-restorative circle, part 2. *Dent. Today* **29**, 98,100,102-105 (2010).
108. Camilleri, J. Present status and future directions : Hydraulic materials for endodontic use. **55**, 710–777 (2022).
109. Camilleri, J. *et al.* The constitution of mineral trioxide aggregate. *Dent. Mater.* **21**, 297–303 (2005).
110. Trope, M., Bunes, A. & Debelian, G. Root filling materials and techniques: bioceramics a new hope? *Endod. Top.* **32**, 86–96 (2015).
111. Camilleri, J. Classification of Hydraulic Cements Used in Dentistry. *Front. Dent. Med.* **1**, 1–6 (2020).
112. Duarte, M. A. H. *et al.* Tricalcium silicate-based cements: Properties and modifications. *Braz. Oral Res.* **32**, 111–118 (2018).
113. Jefferies, S. R. Bioactive and Biomimetic Restorative Materials : A Comprehensive Review . Part I. **26**, 14–26 (2014).
114. Bernardi, A. *et al.* Effects of the addition of nanoparticulate calcium carbonate on setting time , dimensional change , compressive strength , solubility and pH of MTA. 1–9 (2015). doi:10.1111/iej.12594
115. Camilleri, J., Sorrentino, F. & Damidot, D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. *Dent. Mater.* **29**, 580–593 (2013).
116. Guerreiro-tanomaru, J. M. *et al.* Effect of addition of nano-hydroxyapatite on calcium silicate cements. **24**, 204–210 (2016).
117. Camilleri, J. Sealers and warm gutta-percha obturation techniques. *J. Endod.* **41**, 72–78 (2015).
118. Jafari, F. & Jafari, S. Composition and physicochemical properties of calcium silicate based sealers : A review article. **9**, 1249–1255 (2017).
119. Silva, E. J. N. L. *et al.* Evaluation of Cytotoxicity and Physicochemical Properties of Calcium Silicate-based Endodontic Sealer MTA Fillapex. **39**, 274–277 (2013).
120. Viapiana, R., Flumignan, D. L., Guerreiro-Tanomaru, J. M., Camilleri, J. & Tanomaru-Filho, M. Physicochemical and mechanical properties of zirconium oxide and niobium oxide modified Portland cement-based experimental endodontic sealers. *Int. Endod. J.* **47**, 437–448 (2014).

121. Zhou, H. *et al.* Physical properties of 5 root canal sealers. *J. Endod.* **39**, 1281–1286 (2013).
122. Marques, N. C. T. *et al.* Rat Subcutaneous Tissue Response to MTA Fillapex ® and Portland Cement. **24**, 10–14 (2013).
123. Yoshino, P., Nishiyama, C. K., Cristina, K., Santos, C. F. & Sipert, C. R. In Vitro Cytotoxicity of White MTA , MTA Fillapex ® and Portland Cement on Human Periodontal Ligament Fibroblasts. **24**, 111–116 (2013).
124. Güven, E. P., Yalvaç, M. E., Baybora, M. & Dnnõ, K. Human tooth germ stem cell response to calcium- silicate based endodontic cements. **21**, 351–357 (2013).
125. Benetti, F. Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. 1–9 (2019).
126. Zhou, H. M. *et al.* In Vitro Cytotoxicity of Calcium Silicate-containing Endodontic Sealers. *J. Endod.* **41**, 56–61 (2015).
127. Silva, E. J., Perez, R., Valentim, R. M., Belladonna, F. G. & Lima, I. C. Dissolution , dislocation and dimensional changes of endodontic sealers after a solubility challenge : a micro-CT approach. 1–8 (2016). doi:10.1111/iej.12636
128. Demirci, G. K. *et al.* Energy-Dispersive X-Ray Spectrometry Analysis and Radiopacity of Five Different Root Canal Sealers. *Braz. Dent. J.* **32**, 1–11 (2021).
129. Suh, B., Brook, O., Yin, R. & Grove, B. (12) Patent Application Publication (10) Pub. No.: US 2008/0318190 A1. **1**, (2008).
130. Scha, E., Dammaschke, T., Gerth, H. U. V & Zu, H. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. 731–738 (2005). doi:10.1016/j.dental.2005.01.019
131. Chang, S. *et al.* Heavy Metal Analysis of Ortho MTA and ProRoot MTA. *J. Endod.* **37**, 1673–1676 (2011).
132. Lu D, Zhou S. Hydraulic cement compositions and methods of making and using the same. Patent number: 7575628; 2006.
133. Lu D, Zhou S. Hydraulic cement compositions and methods of making and using the same. Patent number: 8343271; 2009.
134. Bergaya B, Bottero JY, Bottero MJ, Franquin JC, LeBlanc D, Marie O, Nonat A, Sauvaget C. Preparation for producing a material used to restore a mineralised substance, particularly in the dental field. Patent number: 7819663; 2003.
135. Richard G, Marie O, Bafounguissa L. Wear resistant dental composition. Patent number: 9427380; 2012.

136. Yang Q, Lu D. Premixed biological hydraulic cement paste composition and using the same. Patent number: 8475811; 2008. **1**, (2008).
137. Zordan-Bronzel, C. L. *et al.* Evaluation of Physicochemical Properties of a New Calcium Silicate–based Sealer, Bio-C Sealer. *J. Endod.* **45**, 1248–1252 (2019).
138. Mta, P. *et al.* Comparative Surface Morphology, Chemical Composition, and Cytocompatibility of Bio-C Repair, Biodentine, and ProRoot MTA on hDPCs. 1–13
139. Gilles RichardOlivier MarieClemence Co. Dental hydraulic cement comprising ultrafine calcium silicate particles having fast hardening and suitable mechanical properties. Patent number: US20220265518A1. 2022.
140. Islam, R. *et al.* Direct pulp capping procedures – Evidence and practice. *Jpn. Dent. Sci. Rev.* **59**, 48–61 (2023).
141. Fagogeni, I. *et al.* Materials used in regenerative endodontic procedures and their impact on tooth discoloration. **61**, 379–385 (2019).
142. Parirokh, M., Torabinejad, M. & Dummer, P. M. H. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview – part I: vital pulp therapy. *Int. Endod. J.* **51**, 177–205 (2018).
143. Clauder, T. Present status and future directions – - Managing perforations. **55**, 872–891 (2022).
144. Simon, S., Rilliard, F., Berdal, A. & Machtou, P. The use of mineral trioxide aggregate in one-visit apexification treatment : a prospective study. 186–197 (2007). doi:10.1111/j.1365-2591.2007.01214.x
145. Baek, S., Plenk, H. & Kim, S. Periapical Tissue Responses and Cementum Regeneration with Amalgam , SuperEBA , and MTA as Root-End Filling Materials. **31**, (2005).
146. Camilleri, J. The chemical composition of mineral trioxide aggregate. *J. Conserv. Dent.* **11**, 141–143 (2008).
147. Dawood, A. E., Parashos, P., Wong, R. H. K., Reynolds, E. C. & Manton, D. J. Calcium silicate-based cements: composition, properties, and clinical applications. *Journal of investigative and clinical dentistry* **8**, 1–15 (2017).
148. Primus, C. M. United States Patent Patent No .: US 7892342 B2. (2011).
149. Asgary, S., Parirokh, M., Eghbal, M. J. & Brink, F. A Comparative study of white mineral trioxide aggregate and white portland cements

using X-ray microanalysis. *Aust. Endod. J.* **30**, 89–92 (2004).
150. Islam, I., Kheng Chng, H. & Jin Yap, A. U. Comparison of the physical and mechanical properties of MTA and portland cement. *J. Endod.* **32**, 193–197 (2006).
151. Hwang, Y. C. *et al.* Chemical composition, radiopacity, and biocompatibility of Portland cement with bismuth oxide. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **107**, e96–e102 (2009).
152. Santos, A. D., Moraes, J. C. S., Araújo, E. B., Yukimitu, K. & Valério Filho, W. V. Physico-chemical properties of MTA and a novel experimental cement. *Int. Endod. J.* **38**, 443–447 (2005).
153. Bortoluzzi, E. A., Guerreiro-Tanomaru, J. M., Tanomaru-Filho, M. & Duarte, M. A. H. Radiographic effect of different radiopacifiers on a potential retrograde filling material. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **108**, 628–632 (2009).
154. Vivan, R. R. *et al.* Evaluation of the radiopacity of some commercial and experimental root-end filling materials. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **108**, e35–e38 (2009).
155. Program, N. C. H. R. Measuring Cement Particle Size And Surface Area by Laser Diffraction. *E. T. Harrigan* 1–10 (2013).
156. Gorai, D. K., Saida, S., Mehta, K. D. & Singh, B. K. Effect of Blaine Number on the Physical and Mechanical Properties of Iron Ore Pellets. *J. Inst. Eng. Ser. D* **104**, 131–141 (2023).
157. SriDevi, S.; Sakthivel, P. Data modelling. *Free Libr.* (2015). doi: https://www.thefreelibrary.com/Data%20modelling%20&%20analysis%20in%20real-time%20process%20control-a0420324592
158. L.S. Brown, M. A. S. *Rock Products*. (1938).
159. Rehsi, S. S. *Magnesium Oxide in Portland Cement*. *Advances in Cement Technology: Critical Reviews and Case Studies on Manufacturing, Quality Control, Optimization and Use* (Pergamon Press Ltd.). doi:10.1016/B978-0-08-028670-9.50019-3
160. Cipurkovic, A., Trumic, I., Hodžic, Z., Selimbašic, V. & Djozic, A. Distribution of heavy metals in Portland cement production process. *Adv. Appl. Sci. Res.* **5**, 252–259 (2014).
161. Lendini, M. *et al.* Caratterizzazione in microscopia SEM-EDS del cemento endodontico MTA: un confronto tra diversi prodotti. *G. Ital. Endod.* **25**, 33–40 (2011).
162. Asgary, S. *et al.* Comparison of Mineral Trioxide Aggregate's Composition with Portland Cements and a New Endodontic Cement. *J. Endod.* **35**, 243–250 (2009).

163. Camilleri, J. Characterization of hydration products of mineral trioxide aggregate. *Int. Endod. J.* **41**, 408–417 (2008).
164. Camilleri, J. Hydration mechanisms of mineral trioxide aggregate. *Int. Endod. J.* **40**, 462–470 (2007).
165. Belío-Reyes, I. A., Bucio, L. & Cruz-Chavez, E. Phase Composition of ProRoot Mineral Trioxide Aggregate by X-Ray Powder Diffraction. *J. Endod.* **35**, 875–878 (2009).
166. Song, J. S., Mante, F. K., Romanow, W. J. & Kim, S. Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and gray MTA-Angelus. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **102**, 809–815 (2006).
167. Scientific principles of Portland cement: from web site of the University of Illinois. Available at http://matse1.mse.uiuc.edu/concrete/prin.html.
168. Understanding cement web site. Available at http://www.understanding-cement.com/about.html.
169. Cement manufacturing: from web site of the Cement Association of Canada at https://cement.ca/the-cement-and-concrete-industry/how-cement-and-concrete-are-made/.
170. Hewlett, P., & Liska, M. *Lea ' s Chemistry of Cement and Concrete Edited by*. (2019).
171. Chattopadhyay, B. *Genetically-enriched microbe-facilitated self-healing nano-concrete*. *Smart Nanoconcretes and Cement-Based Materials: Properties, Modelling and Applications* (Elsevier Inc., 2019). doi:10.1016/B978-0-12-817854-6.00020-9
172. Singh, N. B. *Properties of cement and concrete in presence of nanomaterials*. *Smart Nanoconcretes and Cement-Based Materials: Properties, Modelling and Applications* (Elsevier Inc., 2019). doi:10.1016/B978-0-12-817854-6.00002-7
173. Portland Cements. *Constr. Mater.* 1–6 (2001). doi:10.4324/9780203478981.ch13
174. Asgary, S., Parirokh, M., Eghbal, M. J., Stowe, S. & Brink, F. A qualitative X-ray analysis of white and grey mineral trioxide aggregate using compositional imaging. *J. Mater. Sci. Mater. Med.* **17**, 187–191 (2006).
175. Joseph, R. Mineral Trioxide Aggregate : A review of the literature. *Researchgate.Net* **2**, 219–224 (2011).
176. Bentz, D. P., Garboczi, E. J., Haecker, C. J. & Jensen, O. M. Effects of cement particle size distribution on performance properties of Portland cement-based materials. *Cem. Concr. Res.* **29**, 1663–1671 (1999).

177. Ha, W. N., Kahler, B. & Walsh, L. J. Particle size changes in unsealed mineral trioxide aggregate powder. *J. Endod.* **40**, 423–426 (2014).
178. Ha, W. N., Shakibaie, F., Kahler, B. & Walsh, L. J. Deconvolution of the particle size distribution of ProRoot MTA and MTA Angelus. *Acta Biomater. Odontol. Scand.* **2**, 7–11 (2016).
179. Komabayashi, T. & Spångberg, L. S. W. Comparative Analysis of the Particle Size and Shape of Commercially Available Mineral Trioxide Aggregates and Portland Cement: A Study with a Flow Particle Image Analyzer. *J. Endod.* **34**, 94–98 (2008).
180. Fridland, M. & Rosado, R. Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. *J. Endod.* **29**, 814–817 (2003).
181. Grazziotin-Soares, R. *et al.* Effect of bismuth oxide on white mineral trioxide aggregate: Chemical characterization and physical properties. *Int. Endod. J.* (2014). doi:10.1111/iej.12181
182. Chiang, T. Y. & Ding, S. J. Comparative physicochemical and biocompatible properties of radiopaque dicalcium silicate cement and mineral trioxide aggregate. *J. Endod.* **36**, 1683–1687 (2010).
183. Camilleri, J. Evaluation of the physical properties of an endodontic Portland cement incorporating alternative radiopacifiers used as root-end filling material. *Int. Endod. J.* **43**, 231–240 (2010).
184. Hungaro Duarte, M. A. *et al.* Effect of different radiopacifying agents on the physicochemical properties of white portland cement and white mineral trioxide aggregate. *J. Endod.* **38**, 394–397 (2012).
185. Formosa, L. M., Mallia, B. & Camilleri, J. The effect of curing conditions on the physical properties of tricalcium silicate cement for use as a dental biomaterial. *Int. Endod. J.* **45**, 326–336 (2012).
186. Coomaraswamy, K. S., Lumley, P. J. & Hofmann, M. P. Effect of Bismuth Oxide Radioopacifier Content on the Material Properties of an Endodontic Portland Cement-based (MTA-like) System. *J. Endod.* **33**, 295–298 (2007).
187. Camilleri, J. The physical properties of accelerated Portland cement for endodontic use. *Int. Endod. J.* **41**, 151–157 (2008).
188. Min, K. S. *et al.* The Induction of Heme Oxygenase-1 Modulates Bismuth Oxide-induced Cytotoxicity in Human Dental Pulp Cells. *J. Endod.* **33**, 1342–1346 (2007).
189. Chen, C. C., Ho, C. C., David Chen, C. H. & Ding, S. J. Physicochemical Properties of Calcium Silicate Cements for Endodontic Treatment. *J. Endod.* **35**, 1288–1291 (2009).
190. Saghiri, M. A., Gutmann, J. L., Orangi, J., Asatourian, A. & Sheibani,

N. Radiopacifier particle size impacts the physical properties of tricalcium silicate-based cements. *J. Endod.* **41**, 225–230 (2015).

191. Torabinejad, M., Watson, T. F. & Pitt Ford, T. R. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J. Endod.* **19**, 591–595 (1993).
192. Estrela, C., Bammann, L. L., Estrela, C. R., Silva, R. S. & Pécora, J. D. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. *Braz. Dent. J.* **11**, 3–9 (2000).
193. Taylor, H. F. W. Cement chemistry. 2nd ed. *Acad. Press* **20**, 335 (1997).
194. Richardson, I. G. Tobermorite/jennite- and tobermorite/calcium hydroxide-based models for the structure of C-S-H: Applicability to hardened pastes of tricalcium silicate, β-dicalcium silicate, Portland cement, and blends of Portland cement with blast-furnace slag, metakaol. *Cem. Concr. Res.* **34**, 1733–1777 (2004).
195. Gandolfi, M. G. *et al.* Environmental Scanning Electron Microscopy Connected with Energy Dispersive X-ray Analysis and Raman Techniques to Study ProRoot Mineral Trioxide Aggregate and Calcium Silicate Cements in Wet Conditions and in Real Time. *J. Endod.* **36**, 851–857 (2010).
196. Steffen, R. & van Waes, H. Understanding mineral trioxide aggregate/Portland-cement: a review of literature and background factors. *Eur. Arch. Paediatr. Dent.* **10**, 93–97 (2009).
197. Khalil, I., Naaman, A. & Camilleri, J. Investigation of a novel mechanically mixed mineral trioxide aggregate (MM-MTA(^{TM})). *Int. Endod. J.* **48**, 757–767 (2015).
198. Gandolfi, M. G., Parrilli, A. P., Fini, M., Prati, C. & Dummer, P. M. H. 3D micro-CT analysis of the interface voids associated with Thermafil root fillings used with AH Plus or a flowable MTA sealer. *Int. Endod. J.* **46**, 253–263 (2013).
199. Mutal, L. & Gani, O. Presence of pores and vacuoles in set endodontic sealers. *Int. Endod. J.* **38**, 690–696 (2005).
200. Waltimo, T., Trope, M., Haapasalo, M. & Ørstavik, D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. *J. Endod.* **31**, 863–866 (2005).
201. Ng, Y. L., Mann, V., Rahbaran, S., Lewsey, J. & Gulabivala, K. Outcome of primary root canal treatment: Systematic review of the literature - Part 2. Influence of clinical factors. *Int. Endod. J.* **41**, 6–31

(2008).
202. Uyanik, M. O., Nagas, E., Cubukcu, H. E., Dagli, F. & Cehreli, Z. C. Surface porosity of hand-mixed, syringe-mixed and encapsulated set endodontic sealers. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **109**, e117-22 (2010).
203. Chang, S. W. Chemical Composition and Porosity Characteristics of Various Calcium Silicate-Based Endodontic Cements. *Bioinorg. Chem. Appl.* **2018**, 2784632 (2018).
204. Li, G.-H. *et al.* Ability of new obturation materials to improve the seal of the root canal system: a review. *Acta Biomater.* **10**, 1050–1063 (2014).
205. De Souza, E. T. G. *et al.* Tridimensional quantitative porosity characterization of three set calcium silicate-based repair cements for endodontic use. *Microsc. Res. Tech.* **76**, 1093–1098 (2013).
206. Torres, F. F. E., Guerreiro-Tanomaru, J. M., Bosso-Martelo, R., Chavez-Andrade, G. M. & Tanomaru Filho, M. Solubility, porosity and fluid uptake of calcium silicate-based cements. *J. Appl. Oral Sci.* **26**, e20170465 (2018).
207. Peters, O. A., Laib, A., Rüegsegger, P. & Barbakow, F. Three-dimensional analysis of root canal geometry by high-resolution computed tomography. *J. Dent. Res.* **79**, 1405–1409 (2000).
208. Ajina, M. A., Shah, P. K. & Chong, B. S. Critical analysis of research methods and experimental models to study removal of root filling materials. *Int. Endod. J.* **55 Suppl 1**, 119–152 (2022).
209. Aksoy, U., Küçük, M., Versiani, M. A. & Orhan, K. Publication trends in micro-CT endodontic research: a bibliometric analysis over a 25-year period. *Int. Endod. J.* **54**, 343–353 (2021).
210. Moinzadeh, A. T., Zerbst, W., Boutsioukis, C., Shemesh, H. & Zaslansky, P. Porosity distribution in root canals filled with gutta percha and calcium silicate cement. *Dent. Mater.* **31**, 1100–1108 (2015).
211. Orhan, K. *et al.* Evaluation of Threshold Values for Root Canal Filling Voids in Micro-CT and Nano-CT Images. *Scanning* **2018**, 9437569 (2018).
212. Vergaças, J. H. N. *et al.* Marginal gaps and voids of three root-end filling materials: A microcomputed tomographic study. *Microsc. Res. Tech.* **85**, 617–622 (2022).
213. Jung, J., Kim, S., Kim, E. & Shin, S.-J. Volume of Voids in Retrograde Filling: Comparison between Calcium Silicate Cement Alone and Combined with a Calcium Silicate-based Sealer. *J. Endod.* **46**, 97–102

(2020).
214. Jardine, A. P. *et al.* Marginal gaps and internal voids after root-end filling using three calcium silicate-based materials: A Micro-CT analysis. *Braz. Dent. J.* **32**, 1–7 (2021).
215. Jho, W. *et al.* Comparison of root canal filling quality by mineral trioxide aggregate and gutta percha cones/AH plus sealer. *Dent. Mater. J.* **35**, 644–650 (2016).
216. Somma, F. *et al.* Quality of thermoplasticized and single point root fillings assessed by micro-computed tomography. *Int. Endod. J.* **44**, 362–369 (2011).
217. Angerame, D. *et al.* Analysis of single point and continuous wave of condensation root filling techniques by micro-computed tomography. *Ann. Ist. Super. Sanita* **48**, 35–41 (2012).
218. Roizenblit, R. N., Soares, F. O., Lopes, R. T., Dos Santos, B. C. & Gusman, H. Root canal filling quality of mandibular molars with EndoSequence BC and AH Plus sealers: A micro-CT study. *Aust. Endod. J.* **46**, 82–87 (2020).
219. Pinto, J. C. *et al.* Filling Ability and Flow of Root Canal Sealers: A Micro-Computed Tomographic Study. *Braz. Dent. J.* **31**, 499–504 (2020).
220. Alim, B. A. & Garip Berker, Y. Evaluation of different root canal filling techniques in severely curved canals by micro-computed tomography. *Saudi Dent. J.* **32**, 200–205 (2020).
221. Bhandi, S. *et al.* Complete obturation—cold lateral condensation vs. Thermoplastic techniques: A systematic review of micro-CT studies. *Materials (Basel).* **14**, 1–15 (2021).
222. Camilleri, J. Characterization and hydration kinetics of tricalcium silicate cement for use as a dental biomaterial. *Dent. Mater.* **27**, 836–844 (2011).
223. Kot, K., Kucharski, Ł., Marek, E., Safranow, K. & Lipski, M. Alkalizing Properties of Six Calcium-Silicate Endodontic Biomaterials. *Materials (Basel).* **15**, 1–11 (2022).
224. Talabani, R. M., Garib, B. T. & Masaeli, R. Bioactivity and Physicochemical Properties of Three Calcium Silicate-Based Cements: An In Vitro Study. *Biomed Res. Int.* **2020**, 9576930 (2020).
225. Siboni, F., Taddei, P., Zamparini, F., Prati, C. & Gandolfi, M. G. Properties of bioroot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. *Int. Endod. J.* **50**, e120–e136 (2017).
226. Urban, K., Neuhaus, J., Donnermeyer, D., Schäfer, E. & Dammaschke,

T. Solubility and pH Value of 3 Different Root Canal Sealers: A Long-term Investigation. *J. Endod.* **44**, 1736–1740 (2018).
227. Rao, A., Rao, A. & Shenoy, R. Mineral trioxide aggregate--a review. *J. Clin. Pediatr. Dent.* **34**, 1–7 (2009).
228. Saghiri, M. A. *et al.* Calcium silicate-based cements and functional impacts of various constituents. *Dent. Mater. J.* **36**, 8–18 (2017).
229. Camilleri, J. Modification of mineral trioxide aggregate. Physical and mechanical properties. *Int. Endod. J.* **41**, 843–849 (2008).
230. Bueno, C. R. E. *et al.* Biocompatibility and biomineralization assessment of mineral trioxide aggregate flow. *Clin. Oral Investig.* **23**, 169–177 (2019).
231. Han, L. & Okiji, T. Bioactivity evaluation of three calcium silicate-based endodontic materials. *Int. Endod. J.* **46**, 808–814 (2013).
232. Benetti, F. *et al.* Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. *Braz. Oral Res.* **33**, e042 (2019).
233. Benetti, F. *et al.* In vivo biocompatibility and biomineralization of calcium silicate cements. *Eur. J. Oral Sci.* **126**, 326–333 (2018).
234. Benetti, F. *et al.* Cytotoxicity, Biocompatibility and Biomineralization of a New Ready-for-Use Bioceramic Repair Material. *Braz. Dent. J.* **30**, 325–332 (2019).
235. Holland, R. *et al.* Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. *Braz. Dent. J.* **12**, 3–8 (2001).
236. Estrela, C. *et al.* Mechanism of action of Bioactive Endodontic Materials. *Braz. Dent. J.* **34**, 1–11 (2023).
237. Cosme-Silva, L. *et al.* Biocompatibility and immunohistochemical evaluation of a new calcium silicate-based cement, Bio-C Pulpo. *Int. Endod. J.* **52**, 689–700 (2019).
238. Loison-Robert, L. S. *et al.* In vitro effects of two silicate-based materials, Biodentine and BioRoot RCS, on dental pulp stem cells in models of reactionary and reparative dentinogenesis. *PLoS One* **13**, e0190014 (2018).
239. Aminoshariae, A., Primus, C. & Kulild, J. C. Tricalcium silicate cement sealers: Do the potential benefits of bioactivity justify the drawbacks? *J. Am. Dent. Assoc.* **153**, 750–760 (2022).
240. Vistica, D. T. *et al.* Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. *Cancer Res.* **51**, 2515–2520 (1991).
241. Slater, T. F., Sawyer, B. & Straeuli, U. Studies On Succinate-

Tetrazolium Reductase Systems. Iii. Points Of Coupling Of Four Different Tetrazolium Salts. *Biochim. Biophys. Acta* **77**, 383–393 (1963).

242. Gorduysus, M. *et al.* Cytotoxic effects of four different endodontic materials in human periodontal ligament fibroblasts. *J. Endod.* **33**, 1450–1454 (2007).
243. Berridge, M. V & Tan, A. S. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. *Arch. Biochem. Biophys.* **303**, 474–482 (1993).
244. Wei, W. *et al.* Effects of an experimental calcium aluminosilicate cement on the viability of murine odontoblast-like cells. *J. Endod.* **38**, 936–942 (2012).
245. Inayat-Hussain, S., Rajab, N. F. & Siew, E. L. In vitro testing of biomaterials toxicity and biocompatibility. *Cell. Response to Biomater.* 508–537 (2009). doi:10.1533/9781845695477.3.508
246. McKinnon, K. M. Flow Cytometry: An Overview. *Curr. Protoc. Immunol.* **120**, 5.1.1-5.1.11 (2018).
247. Birant, S. *et al.* Cytotoxicity of NeoMTA Plus, ProRoot MTA and Biodentine on human dental pulp stem cells. *J. Dent. Sci.* **16**, 971–979 (2021).
248. Solanki, N. P., Venkappa, K. K. & Shah, N. C. Biocompatibility and sealing ability of mineral trioxide aggregate and biodentine as root-end filling material: A systematic review. *J. Conserv. Dent.* **21**, 10–15 (2018).
249. Yousefi-Koma, A.-A., Assadian, H., Mohaghegh, S. & Nokhbatolfoghahaei, H. Comparative Biocompatibility and Odonto-/Osteogenesis Effects of Hydraulic Calcium Silicate-Based Cements in Simulated Direct and Indirect Approaches for Regenerative Endodontic Treatments: A Systematic Review. *J. Funct. Biomater.* **14**, (2023).
250. de Oliveira, N. G., de Souza Araújo, P. R., da Silveira, M. T., Sobral, A. P. V. & Carvalho, M. de V. Comparison of the biocompatibility of calcium silicate-based materials to mineral trioxide aggregate: Systematic review. *Eur. J. Dent.* **12**, 317–326 (2018).
251. Du, R. *et al.* Role of the extracellular signal-regulated kinase 1/2 pathway in driving tricalcium silicate-induced proliferation and biomineralization of human dental pulp cells in vitro. *J. Endod.* **39**, 1023–1029 (2013).

252. Youssef, A.-R. *et al.* Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. *BMC Oral Health* **19**, 133 (2019).
253. Chang, S.-W., Lee, S.-Y., Ann, H.-J., Kum, K.-Y. & Kim, E.-C. Effects of calcium silicate endodontic cements on biocompatibility and mineralization-inducing potentials in human dental pulp cells. *J. Endod.* **40**, 1194–1200 (2014).
254. Ha, Y.-J., Lee, D. & Kim, S.-Y. The Combined Effects on Human Dental Pulp Stem Cells of Fast-Set or Premixed Hydraulic Calcium Silicate Cements and Secretome Regarding Biocompatibility and Osteogenic Differentiation. *Mater. (Basel, Switzerland)* **17**, (2024).
255. Attik, G. N. *et al.* In vitro biocompatibility of a dentine substitute cement on human MG63 osteoblasts cells: Biodentine™ versus MTA(®). *Int. Endod. J.* **47**, 1133–1141 (2014).
256. Tomás-Catalá, C. J. *et al.* Biocompatibility of New Pulp-capping Materials NeoMTA Plus, MTA Repair HP, and Biodentine on Human Dental Pulp Stem Cells. *J. Endod.* **44**, 126–132 (2018).
257. Seo, D.-G., Lee, D., Kim, Y.-M., Song, D. & Kim, S.-Y. Biocompatibility and Mineralization Activity of Three Calcium Silicate-Based Root Canal Sealers Compared to Conventional Resin-Based Sealer in Human Dental Pulp Stem Cells. *Mater. (Basel, Switzerland)* **12**, (2019).
258. Youssef, A.-R. & Elsherief, S. Evaluation of the cytotoxic effects of a new Harvard MTA compared to MTA Flow and ProRoot MTA on human gingival fibroblasts. *Saudi Dent. J.* **33**, 679–686 (2021).
259. Mondelli, J. A. S. *et al.* Biocompatibility of mineral trioxide aggregate flow and biodentine. *Int. Endod. J.* **52**, 193–200 (2019).
260. Pelepenko, L. E. *et al.* Physicochemical , antimicrobial , and biological properties of White-MTAFlow. (2020).
261. Maroto, M., Barbería, E., Planells, P. & García Godoy, F. Dentin bridge formation after mineral trioxide aggregate (MTA) pulpotomies in primary teeth. *Am. J. Dent.* **18**, 151–154 (2005).
262. Naik, S. & Hegde, A. H. Mineral trioxide aggregate as a pulpotomy agent in primary molars: an in vivo study. *J. Indian Soc. Pedod. Prev. Dent.* **23**, 13–16 (2005).
263. Antunes Bortoluzzi, E., Sivieri Araújo, G., Maria Guerreiro Tanomaru, J. & Tanomaru-Filho, M. Marginal Gingiva Discoloration by Gray MTA: A Case Report. *J. Endod.* **33**, 325–327 (2007).
264. Karabucak, B., Li, D., Lim, J. & Iqbal, M. Vital pulp therapy with

mineral trioxide aggregate. *Dent. Traumatol.* **21**, 240–243 (2005).
265. Bogen, G., Kim, J. S. & Bakland, L. K. Direct pulp capping with mineral trioxide aggregate: An observational study. *J. Am. Dent. Assoc.* **139**, 305–315 (2008).
266. Gelaye, B., Rondon, M., Araya, P. R. & A, P. M. MTA: A clinical review. **3**, 973–982 (2016).
267. Bogen, G. & Kuttler, S. Mineral Trioxide Aggregate Obturation: A Review and Case Series. *J. Endod.* **35**, 777–790 (2009).
268. Bakland, L. K. Revisiting Traumatic Pulpal Exposure: Materials, Management Principles, and Techniques. *Dent. Clin. North Am.* **53**, 661–673 (2009).
269. Jacobovitz, M. & De Lima, R. K. P. Treatment of inflammatory internal root resorption with mineral trioxide aggregate: A case report. *Int. Endod. J.* **41**, 905–912 (2008).
270. Belobrov, I. & Parashos, P. Treatment of tooth discoloration after the use of white mineral trioxide aggregate. *J. Endod.* **37**, 1017–1020 (2011).
271. Adel, M. *et al.* Comparison of Coronal Discoloration Induced by White MTA and CEM Cement. *J. Compos. Sci.* **6**, 1–7 (2022).
272. Watts, J. D., Holt, D. M., Beeson, T. J., Kirkpatrick, T. C. & Rutledge, R. E. Effects of pH and Mixing Agents on the Temporal Setting of Tooth-colored and Gray Mineral Trioxide Aggregate. *J. Endod.* **33**, 970–973 (2007).
273. Boutsioukis, C., Noula, G. & Lambrianidis, T. Ex Vivo Study of the Efficiency of Two Techniques for the Removal of Mineral Trioxide Aggregate Used as a Root Canal Filling Material. *J. Endod.* **34**, 1239–1242 (2008).
274. Ferris, D. M. & Baumgartner, J. C. Perforation repair comparing two types of mineral trioxide aggregate. *J. Endod.* **30**, 422–424 (2004).
275. Kaur, M., Singh, H., Dhillon, J. S., Batra, M. & Saini, M. MTA versus biodentine: Review of literature with a comparative analysis. *J. Clin. Diagnostic Res.* **11**, ZG01–ZG05 (2017).
276. Asgary, S., Parirokh, M., Eghbal, M. J. & Brink, F. Chemical differences between white and gray mineral trioxide aggregate. *J. Endod.* **31**, 101–103 (2005).
277. Shaikh, M. S., Zafar, M. S., Alnazzawi, A. & Javed, F. Nanocrystalline hydroxyapatite in regeneration of periodontal intrabony defects: A systematic review and meta-analysis. *Ann. Anat. = Anat. Anzeiger Off. organ Anat. Gesellschaft* **240**, 151877 (2022).
278. Pushpa, S. *et al.* Effect of pH on solubility of white Mineral Trioxide

Aggregate and Biodentine: An in vitro study. *J. Dent. Res. Dent. Clin. Dent. Prospects* **12**, 201–207 (2018).
279. Singh, S., Podar, R., Dadu, S., Kulkarni, G. & Purba, R. Solubility of a new calcium silicate-based root-end filling material. *J. Conserv. Dent.* **18**, 149–153 (2015).
280. Alazrag, M. A., Abu-Seida, A. M., El-Batouty, K. M. & El Ashry, S. H. Marginal adaptation, solubility and biocompatibility of TheraCal LC compared with MTA-angelus and biodentine as a furcation perforation repair material. *BMC Oral Health* **20**, 1–12 (2020).
281. Bozeman, T. B., Lemon, R. R. & Eleazer, P. D. Elemental Analysis of Crystal Precipitate from Gray and White MTA. *J. Endod.* **32**, 425–428 (2006).
282. Al-Hezaimi, K., Naghshbandi, J., Oglesby, S., Simon, J. H. S. & Rotstein, I. Human saliva penetration of root canals obturated with two types of mineral trioxide aggregate cements. *J. Endod.* **31**, 453–456 (2005).
283. Keleş, A. *et al.* Micro-CT evaluation of voids using two root filling techniques in the placement of MTA in mesial root canals of Vertucci type II configuration. *Clin. Oral Investig.* **22**, 1907–1913 (2018).
284. Sarkar, N. K., Caicedo, R., Ritwik, P., Moiseyeva, R. & Kawashima, I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. *J. Endod.* **31**, 97–100 (2005).
285. Al-Haj Ali, S. N., Al-Jundi, S. H. & Ditto, D. J. In vitro toxicity of grey MTA in comparison to white MTA on human periodontal ligament fibroblasts. *Eur. Arch. Paediatr. Dent.* **15**, 429–433 (2014).
286. Cardoso-Silva, C., Barbería, E., Maroto, M. & García-Godoy, F. Clinical study of Mineral Trioxide Aggregate in primary molars. Comparison between Grey and White MTA - A long term follow-up (84 months). *J. Dent.* **39**, 187–193 (2011).
287. Marciano, M. A. *et al.* Assessment of color stability of white mineral trioxide aggregate angelus and bismuth oxide in contact with tooth structure. *J. Endod.* **40**, 1235–1240 (2014).
288. Camilleri, J. Color stability of white mineral trioxide aggregate in contact with hypochlorite solution. *J. Endod.* **40**, 436–440 (2014).
289. Camilleri, J., Cutajar, A. & Mallia, B. Hydration characteristics of zirconium oxide replaced Portland cement for use as a root-end filling material. *Dent. Mater.* **27**, 845–854 (2011).
290. Tanomaru-Filho, M. *et al.* Compressive Strength and Setting Time of MTA and Portland Cement Associated with Different Radiopacifying Agents. *ISRN Dent.* **2012**, 1–4 (2012).

291. Marciano, M. A. *et al.* Zinc Oxide Inhibits Dental Discoloration Caused by White Mineral Trioxide Aggregate Angelus. *J. Endod.* **43**, 1001–1007 (2017).
292. Marconyak, L. J. *et al.* A comparison of coronal tooth discoloration elicited by various endodontic reparative materials. *J. Endod.* **42**, 470–473 (2016).
293. Vishwanath, V. & Rao, H. M. Gutta-percha in endodontics - A comprehensive review of material science. *J. Conserv. Dent.* **22**, 216–222 (2019).
294. Huang, F. M., Tai, K. W., Chou, M. Y. & Chang, Y. C. Cytotoxicity of resin-, zinc oxide-eugenol-, and calcium hydroxide-based root canal sealers on human periodontal ligament cells and permanent V79 cells. *Int. Endod. J.* **35**, 153–158 (2002).
295. Vallés, M., Mercadé, M., Duran-Sindreu, F., Bourdelande, J. L. & Roig, M. Influence of light and oxygen on the color stability of five calcium silicate-based materials. *J. Endod.* **39**, 525–528 (2013).
296. Camilleri, J. Tricalcium silicate cements with resins and alternative radiopacifiers. *J. Endod.* **40**, 2030–2035 (2014).
297. Marciano, M. A., Duarte, M. A. H. & Camilleri, J. Dental discoloration caused by bismuth oxide in MTA in the presence of sodium hypochlorite. *Clin. Oral Investig.* **19**, 2201–2209 (2015).
298. Vallés, M., Mercadé, M., Duran-Sindreu, F., Bourdelande, J. L. & Roig, M. Color stability of white mineral trioxide aggregate. *Clin. Oral Investig.* **17**, 1155–1159 (2013).
299. Jang, J. H. *et al.* Tooth discoloration after the use of new pozzolan cement (Endocem) and mineral trioxide aggregate and the effects of internal bleaching. *J. Endod.* **39**, 1598–1602 (2013).
300. Dreger, L. A. S. *et al.* Mineral trioxide aggregate and portland cement promote biomineralization in vivo. *J. Endod.* **38**, 324–329 (2012).
301. Chang, S.-W. Chemical characteristics of mineral trioxide aggregate and its hydration reaction. *Restor. Dent. Endod.* **37**, 188 (2012).
302. Han, L. & Okiji, T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine. *Int. Endod. J.* **44**, 1081–1087 (2011).
303. Sanz, O., Haro-Poniatowski, E., Gonzalo, J. & Fernández Navarro, J. M. Influence of the melting conditions of heavy metal oxide glasses containing bismuth oxide on their optical absorption. *J. Non. Cryst. Solids* **352**, 761–768 (2006).
304. Zhang, Y., Yang, Y., Zheng, J., Hua, W. & Chen, G. Effects of oxidizing additives on optical properties of Bi 2O3-B2O3-SiO2

glasses. *J. Am. Ceram. Soc.* **91**, 3410–3412 (2008).
305. Guimarães, B. M. *et al.* Color stability, radiopacity, and chemical characteristics of white mineral trioxide aggregate associated with 2 different vehicles in contact with blood. *J. Endod.* **41**, 947–952 (2015).
306. Felman, D. & Parashos, P. Coronal Tooth Discoloration and White Mineral Trioxide Aggregate. *J. Endod.* **39**, 484–487 (2013).
307. Marin, P. D., Bartold, P. M. & Heithersay, G. S. Tooth discoloration by blood: An in vitro histochemical study. *Endod. Dent. Traumatol.* **13**, 132–138 (1997).
308. Wang, S. *et al.* A colourimetric evaluation of the effect of bacterial contamination on teeth stained with blood in vitro: Evaluation of the efficacy of two different bleaching regimes. *Aust. Dent. J.* **63**, 253–260 (2018).
309. Ioannidis, K., Mistakidis, I., Beltes, P. & Karagiannis, V. Spectrophotometric analysis of crown discoloration induced by MTA- and ZnOE-based sealers. *J. Appl. Oral Sci.* **21**, 138–144 (2013).
310. Marciano, M. A. *et al.* Physical, chemical, and biological properties of white MTA with additions of AlF3. *Clin. Oral Investig.* 1–9 (2018). doi:10.1007/s00784-018-2383-4
311. Andrade, A. S. *et al.* Tissue Response and Immunoexpression of Interleukin 6 Promoted by Tricalcium Silicate–based Repair Materials after Subcutaneous Implantation in Rats. *J. Endod.* **44**, 458–463 (2018).
312. Damas, B. A., Wheater, M. A., Bringas, J. S. & Hoen, M. M. Cytotoxicity comparison of mineral trioxide aggregates and endosequence bioceramic root repair materials. *J. Endod.* **37**, 372–375 (2011).
313. Meschi, N. *et al.* Bioactivity potential of Portland cement in regenerative endodontic procedures : From clinic to lab. *Dent. Mater.* **35**, 1342–1350 (2019).
314. Niu, L. N. *et al.* A review of the bioactivity of hydraulic calcium silicate cements. *J. Dent.* **42**, 517–533 (2014).
315. Percinoto, C., de Castro, A. M. & Pinto, L. M. C. P. Clinical and radiographic evaluation of pulpotomies employing calcium hydroxide and trioxide mineral aggregate. *Gen. Dent.* **54**, 258–261 (2006).
316. Ni Chaollai, A., Monteiro, J. & Duggal, M. S. The teaching of management of the pulp in primary molars in Europe: a preliminary investigation in Ireland and the UK. *Eur. Arch. Paediatr. Dent. Off. J. Eur. Acad. Paediatr. Dent.* **10**, 98–103 (2009).
317. Ling, J., Xu, Q. & Wei, X. Microscopic management of teeth with open

apices using mineral trioxide aggregate. *Pract. Proced. Aesthet. Dent.* **20**, 49–51 (2008).
318. Mooney, G. C. & North, S. The current opinions and use of MTA for apical barrier formation of non-vital immature permanent incisors by consultants in paediatric dentistry in the UK. *Dent. Traumatol.* **24**, 65–9 (2008).
319. Eren, S. K., Örs, S. A., Aksel, H., Canay, Ş. & Karasan, D. Effect of irrigants on the color stability, solubility, and surface characteristics of calcium-silicate based cements. *Restor. Dent. Endod.* **47**, 1–11 (2022).
320. Shokouhinejad, N., Nekoofar, M. H., Pirmoazen, S., Shamshiri, A. R. & Dummer, P. M. H. Evaluation and Comparison of Occurrence of Tooth Discoloration after the Application of Various Calcium Silicate-based Cements: An Ex Vivo Study. *J. Endod.* **42**, 140–144 (2016).
321. Madani, Z., Alvandifar, S. & Bizhani, A. Evaluation of tooth discoloration after treatment with mineral trioxide aggregate, calcium-enriched mixture, and Biodentine® in the presence and absence of blood. *Dent. Res. J. (Isfahan).* **16**, 377–383 (2019).
322. Możyńska, J., Metlerski, M., Lipski, M. & Nowicka, A. Tooth Discoloration Induced by Different Calcium Silicate–based Cements: A Systematic Review of In Vitro Studies. *J. Endod.* **43**, 1593–1601 (2017).
323. Palma, P. J. *et al.* Tooth discoloration after regenerative endodontic procedures with calcium silicate-based cements-An ex vivo study. *Appl. Sci.* **10**, (2020).
324. Vallés, M., Roig, M., Duran-Sindreu, F., Martínez, S. & Mercadé, M. Color Stability of Teeth Restored with Biodentine: A 6-month in Vitro Study. *J. Endod.* **41**, 1157–1160 (2015).
325. Adl, A., Javanmardi, S. & Abbaszadegan, A. Assessment of tooth discoloration induced by biodentine and white mineral trioxide aggregate in the presence of blood. *J. Conserv. Dent.* **22**, 164–168 (2019).
326. Al-Hiyasat, A. S., Ahmad, D. M. & Khader, Y. S. The effect of different calcium silicate-based pulp capping materials on tooth discoloration: an in vitro study. *BMC Oral Health* **21**, 1–11 (2021).
327. Koubi, G. *et al.* Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth - a prospective study. *Clin. Oral Investig.* **17**, 243–249 (2013).
328. Malkondu, Ö., Kazandağ, M. K. & Kazazoğlu, E. A review on biodentine, a contemporary dentine replacement and repair material. *Biomed Res. Int.* **2014**, (2014).

329. Kaup, M., Schäfer, E. & Dammaschke, T. An in vitro study of different material properties of Biodentine compared to ProRoot MTA. *Head Face Med.* **11**, 16 (2015).
330. Huang, X. *et al.* Clinical use of bioceramic materials. *Endod. Top.* **32**, 97–117 (2015).
331. Parirokh, M. & Torabinejad, M. Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part III: Clinical Applications, Drawbacks, and Mechanism of Action. *J. Endod.* **36**, 400–413 (2010).
332. Vivan, R. R. *et al.* Evaluation of the physical and chemical properties of two commercial and three experimental root-end filling materials. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **110**, 250–256 (2010).
333. Quintana, R. M. *et al.* Bone tissue reaction, setting time, solubility, and pH of root repair materials. *Clin. Oral Investig.* **23**, 1359–1366 (2019).
334. Pelepenko, L. E. *et al.* Dental discoloration caused by grey-mtaflow cement: Analysis of its physicochemical, biological and antimicrobial properties. *J. Appl. Oral Sci.* **28**, 1–15 (2020).
335. Brasseler. EndoSeuqence Root Repair Material: BC RRM-Fast Set PuttyTM. (2015).
336. Zamparini, F., Siboni, F., Prati, C., Taddei, P. & Gandolfi, M. G. Properties of calcium silicate-monobasic calcium phosphate materials for endodontics containing tantalum pentoxide and zirconium oxide. *Clin. Oral Investig.* **23**, 445–457 (2019).
337. Bhuva, B., Giovarruscio, M., Rahim, N., Bitter, K. & Mannocci, F. The restoration of root filled teeth: a review of the clinical literature. *International Endodontic Journal* **54**, 509–535 (2021).
338. Ng, Y.-L., Mann, V. & Gulabivala, K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. *Int. Endod. J.* **44**, 583–609 (2011).
339. Ghasemi, N., Janani, M., Razi, T. & Atharmoghaddam, F. Effect of different mixing and placement methods on the quality of MTA apical plug in simulated apexification model. *J. Clin. Exp. Dent.* **9**, e351–e355 (2017).
340. Innovative BioCeramix Inc. TotalFill Bioceramic Root Repair Material. (2018). Available at: https://www.fkg.ch/sites/default/files/201801_B_4940A_TotallFill RRM IFU_REV 1_EN_CS_DA_DE_ES_0.pdf.
341. Angelus Londrina Brazil. Bio-C Repair. (2022).
342. Tolibah, Y. A. *et al.* Evaluation of a Novel Tool for Apical Plug Formation during Apexification of Immature Teeth. *Int. J. Environ.*

Res. Public Health **19**, (2022).
343. Johnson, B. R. Considerations in the selection of a root-end filling material. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **87**, 398–404 (1999).
344. Chaniotis, A. & Ordinola-Zapata, R. Present status and future directions: Management of curved and calcified root canals. *Int. Endod. J.* **55**, 656–684 (2022).
345. Jevnikar, A. P. *et al.* Rheological Properties and Setting Kinetics of Bioceramic Hydraulic Cements: ProRoot MTA versus RS+. *Materials (Basel).* **16**, 1–14 (2023).
346. Park, J. B. & Lee, J. H. Use of mineral trioxide aggregate in the open apex of a maxillary first premolar. *J. Oral Sci.* **50**, 355–358 (2008).
347. Abedi, H. R. & Ingle, J. I. Mineral trioxide aggregate: a review of a new cement. *J. Calif. Dent. Assoc.* **23**, 36–9 (1995).
348. Srinivasan, V., Patchett, C. L. & Waterhouse, P. J. Is there life after Buckley's Formocresol? Part I - A narrative review of alternative interventions and materials. *Int. J. Paediatr. Dent.* **16**, 117–127 (2006).
349. Casas, M. J., Kenny, D. J., Judd, P. L. & Johnston, D. H. Do we still need formocresol in pediatric dentistry? *J. Can. Dent. Assoc. (Tor).* **71**, 749–751 (2005).
350. Foley, J. I. Use of mineral trioxide aggregate (MTA by postgraduates in restorative dentistry in the UK. *Eur. J. Prosthodont. Restor. Dent.* **19**, 179–183 (2011).
351. Smith, J. B. *et al.* Metrologic Evaluation of the Surface of White MTA After the Use of Two Endodontic Irrigants. *J. Endod.* **33**, 463–467 (2007).
352. Aggarwal, V., Jain, A. & Kabi, D. In vitro evaluation of effect of various endodontic solutions on selected physical properties of white mineral trioxide aggregate. *Aust. Endod. J.* **37**, 61–64 (2011).
353. Parirokh, M. & Torabinejad, M. Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part I: Chemical, Physical, and Antibacterial Properties. *Journal of Endodontics* **36**, 16–27 (2010).
354. Butt, N. & Talwar, S. In-vitro evaluation of various solvents for retrieval of mineral trioxide aggregate and their effect on microhardness of dentin. *J. Conserv. Dent.* **16**, 199–202 (2013).
355. Aggregate, T. & Mixture, C. In Vitro Evaluation of Different Solvents for Retrieval of Mineral Trioxide Aggregate and Calcium-Enriched Mixture. **11**, 223–227 (2016).
356. Mohammadi, Z. & Dummer, P. M. H. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int.*

Endod. J. **44**, 697–730 (2011).
357. Yassen, G. H. & Platt, J. A. The effect of nonsetting calcium hydroxide on root fracture and mechanical properties of radicular dentine: A systematic review. *Int. Endod. J.* **46**, 112–118 (2013).
358. Youssef, A. R. *et al.* Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. *BMC Oral Health* **19**, 1–9 (2019).
359. Hauman, C. H. J. & Love, R. M. Biocompatibility of dental materials used in contemporary endodontic therapy: A review. Part 2. Root-canal-filling materials. *Int. Endod. J.* **36**, 147–160 (2003).
360. Song, W., Li, S., Tang, Q., Chen, L. & Yuan, Z. In vitro biocompatibility and bioactivity of calcium silicate-based bioceramics in endodontics (Review). *Int. J. Mol. Med.* **48**, 1–22 (2021).
361. Goldberg, M. & Smith, A. J. Cells and extracellular matrices of dentin and pulp: A biological basis for repair and tissue engineering. *Crit. Rev. Oral Biol. Med.* **15**, 13–27 (2004).
362. Tomson, P. L. *et al.* Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. *J. Dent.* **35**, 636–642 (2007).
363. Leiendecker, A. P. *et al.* Effects of calcium silicate-based materials on collagen matrix integrity of mineralized dentin. *J. Endod.* **38**, 829–833 (2012).
364. Zancan, R. F., Di Maio, A., Tomson, P. L., Duarte, M. A. H. & Camilleri, J. The presence of smear layer affects the antimicrobial action of root canal sealers. *Int. Endod. J.* **54**, 1369–1382 (2021).
365. Viapiana, R., Guerreiro-Tanomaru, J., Tanomaru-Filho, M. & Camilleri, J. Interface of dentine to root canal sealers. *J. Dent.* **42**, 336–350 (2014).
366. Kebudi Benezra, M., Schembri Wismayer, P. & Camilleri, J. Interfacial Characteristics and Cytocompatibility of Hydraulic Sealer Cements. *J. Endod.* **44**, 1007–1017 (2018).
367. Reyes-Carmona, J. F., Felippe, M. S. & Felippe, W. T. Biomineralization Ability and Interaction of Mineral Trioxide Aggregate and White Portland Cement With Dentin in a Phosphate-containing Fluid. *J. Endod.* **35**, 731–736 (2009).
368. Donnermeyer, D., Dornseifer, P., Schäfer, E. & Dammaschke, T. The push-out bond strength of calcium silicate-based endodontic sealers. *Head Face Med.* **14**, 1–7 (2018).
369. Atmeh, A. R., Chong, E. Z., Richard, G., Festy, F. & Watson, T. F.

Dentin-cement interfacial interaction: Calcium silicates and polyalkenoates. *J. Dent. Res.* **91**, 454–459 (2012).
370. Hadis, M., Wang, J., Zhang, Z. J., Di Maio, A. & Camilleri, J. Interaction of hydraulic calcium silicate and glass ionomer cements with dentine. *Materialia* **9**, 100515 (2020).
371. Formosa, L. M., Damidot, D. & Camilleri, J. Mercury intrusion porosimetry and assessment of cement-dentin interface of anti-washout-type mineral trioxide aggregate. *J. Endod.* **40**, 958–963 (2014).
372. Li, X. *et al.* Correlative micro-Raman/EPMA analysis of the hydraulic calcium silicate cement interface with dentin. *Clin. Oral Investig.* **20**, 1663–1673 (2016).
373. Kim, J. R., Nosrat, A. & Fouad, A. F. Interfacial characteristics of Biodentine and MTA with dentine in simulated body fluid. *J. Dent.* **43**, 241–247 (2015).
374. Primus, C., Gutmann, J. L., Tay, F. R. & Fuks, A. B. Calcium silicate and calcium aluminate cements for dentistry reviewed. *J. Am. Ceram. Soc.* **105**, 1841–1863 (2022).
375. Govindaraju, L., Neelakantan, P. & Gutmann, J. L. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. *Clin. Oral Investig.* **21**, 567–571 (2017).
376. Ahmed, H. M. A. & Dummer, P. M. H. *Endodontic Advances and Evidence-based clinical guidelines*. (2022).
377. Marques, J. A., Falacho, R. I., Santos, J. M., Ramos, J. C. & Palma, P. J. Effects of endodontic irrigation solutions on structural, chemical, and mechanical properties of coronal dentin: A scoping review. *J. Esthet. Restor. Dent. Off. Publ. Am. Acad. Esthet. Dent. ... [et al.]* (2023). doi:10.1111/jerd.13135
378. Neelakantan, P., Nandagopal, M., Shemesh, H. & Wesselink, P. The effect of root dentin conditioning protocols on the push-out bond strength of three calcium silicate sealers. *Int. J. Adhes. Adhes.* **60**, 104–108 (2015).
379. Harik, R., Salameh, Z., Habchi, R. & Camilleri, J. The effect of irrigation with EDTA on calcium-based root canal sealers: a SEM-EDS and XRD study. *J. Leban. Dent. Assoc.* **49**, 12–23 (2016).
380. Donnermeyer, D., Vahdat, N., Edgar, P. & Till, S. Influence of the final irrigation solution on the push-out bond strength of calcium silicate-based , epoxy resin-based and silicone- based endodontic sealers. *Odontology* **0**, 0 (2018).
381. Lee, Y.-L. *et al.* Effects of EDTA on the hydration mechanism of

mineral trioxide aggregate. *J. Dent. Res.* **86**, 534–538 (2007).
382. Gokturk, H. & Ozkocak, I. The effect of different chelators on the dislodgement resistance of MTA Repair HP, MTA Angelus, and MTA Flow. *Odontology* **110**, 20–26 (2022).
383. Kapralos, V. *et al.* Antimicrobial and physicochemical characterization of endodontic sealers after exposure to chlorhexidine digluconate. *Dent. Mater.* **37**, 249–263 (2021).
384. Kapralos, V. *et al.* The dentine-sealer interface: Modulation of antimicrobial effects by irrigation. *Int. Endod. J.* **55**, 544–560 (2022).
385. Keskin, C., Demiryurek, E. O. & Ozyurek, T. Color stabilities of calcium silicate-based materials in contact with different irrigation solutions. *J. Endod.* **41**, 409–411 (2015).
386. F, S., A, A. & S, G. Effect of Different Irrigation Solutions on the Colour Stability of Three Calcium Silicate-Based Materials. *J. Dent. Biomater.* **4**, 373–378 (2017).
387. Kapralos, V. *et al.* Effect of chlorhexidine digluconate on antimicrobial activity, cell viability and physicochemical properties of three endodontic sealers. *Dent. Mater.* **38**, 1044–1059 (2022).
388. Alsubait, S. A. Effect of Sodium Hypochlorite on Push-out Bond Strength of Four Calcium Silicate-based Endodontic Materials when used for repairing Perforations on Human Dentin: An in vitro Evaluation. *J. Contemp. Dent. Pract.* **18**, 289–294 (2017).
389. Hong, S.-T. *et al.* Effects of root canal irrigants on the push-out strength and hydration behavior of accelerated mineral trioxide aggregate in its early setting phase. *J. Endod.* **36**, 1995–1999 (2010).
390. Chu, J. H. R., Chia, K. Y., Qui, A. L., Moule, A. & Ha, W. N. The effects of sodium hypochlorite and ethylenediaminetetraacetic acid on the microhardness of Mineral Trioxide Aggregate and TotalFill Bioceramic Putty. *Aust. Endod. J. J. Aust. Soc. Endodontology Inc* **46**, 33–39 (2020).
391. Meraji, N., Nekoofar, M. H. & Ashofteh, K. Bonding to caries affected dentine. *Dent. Mater.* 1–10 (2018). doi:10.1016/j.dental.2018.05.017
392. El-Karim, I. *et al.* European Society of Endodontology position statement: Management of deep caries and the exposed pulp. *Int. Endod. J.* (2019). doi:10.1111/iej.13080
393. Neelakantan, P., Ahmed, H. M. A., Wong, M. C. M., Matinlinna, J. P. & Cheung, G. S. P. Effect of root canal irrigation protocols on the dislocation resistance of mineral trioxide aggregate-based materials: A systematic review of laboratory studies. *Int. Endod. J.* **51**, 847–861 (2018).

394. Arias-Moliz, M. T. & Camilleri, J. The effect of the final irrigant on the antimicrobial activity of root canal sealers. *J. Dent.* **52**, 30–36 (2016).
395. Reyes-Carmona, J. F., Felippe, M. S. & Felippe, W. T. The biomineralization ability of mineral trioxide aggregate and Portland cement on dentin enhances the push-out strength. *J. Endod.* **36**, 286–291 (2010).
396. Pelozo, L. L. *et al.* Canal Drying Protocols to Use with Calcium Silicate-based Sealer: Effect on Bond Strength and Adhesive Interface. *J. Endod.* **49**, 1154–1160 (2023).
397. Gillen, B. M. *et al.* Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment: a systematic review and meta-analysis. *J. Endod.* **37**, 895–902 (2011).
398. ORSTAVIK, D. Materials used for root canal obturation: technical, biological and clinical testing. *Endod. Top.* **12**, 25–38 (2005).
399. Keleş, A. & Keskin, C. Presence of voids after warm vertical compaction and single-cone obturation in band-shaped isthmuses using micro-computed tomography: A phantom study. *Microsc. Res. Tech.* **83**, 370–374 (2020).
400. Marconi, D. F. *et al.* Influence of the root canal filling technique on the success rate of primary endodontic treatments: a systematic review. *Restor. Dent. Endod.* **47**, 1–18 (2022).
401. Tomson, R. M. E., Polycarpou, N. & Tomson, P. L. Contemporary obturation of the root canal system. *Br. Dent. J.* **216**, 315–322 (2014).
402. Drukteinis, S. Hydraulic calcium silicate-based materials for root canal obturation. *Clin. Dent. Rev.* **6**, 1–13 (2022).
403. Guinesi, A. S., Faria, G., Tanomaru-Filho, M. & Bonetti-Filho, I. Influence of sealer placement technique on the quality of root canal filling by lateral compaction or single cone. *Braz. Dent. J.* **25**, 117–122 (2014).
404. Souza, E. M. *et al.* Effect of filling technique and root canal area on the percentage of gutta-percha in laterally compacted root fillings. *Int. Endod. J.* **42**, 719–726 (2009).
405. Stamos, D. E., Gutmann, J. L. & Gettleman, B. H. In vivo evaluation of root canal sealer distribution. *J. Endod.* **21**, 177–179 (1995).
406. Holmes, S. *et al.* Volumetric Evaluation of 5 Root Canal Obturation Methods in TrueTooth 3-dimensional–Printed Tooth Replicas Using Nano–computed Tomography. *J. Endod.* **47**, 485-491.e4 (2021).
407. Penha Da Silva, P. J. *et al.* Quality of Root Canal Filling Using a Bioceramic Sealer in Oval Canals: A Three-Dimensional Analysis.

Eur. J. Dent. **15**, 475–480 (2021).
408. Aminsobhani, M., Ghorbanzadeh, A., Sharifian, M. R., Namjou, S. & Kharazifard, M. J. Comparison of Obturation Quality in Modified Continuous Wave Compaction, Continuous Wave Compaction, Lateral Compaction and Warm Vertical Compaction Techniques. *J. Dent. (Tehran).* **12**, 99–108 (2015).
409. Hommez, G. M. G., De Moor, R. J. G. & Braem, M. Endodontic treatment performed by Flemish dentists. Part 2. Canal filling and decision making for referrals and treatment of apical periodontitis. *Int. Endod. J.* **36**, 344–351 (2003).
410. Qualtrough, A. J. E., Whitworth, J. M. & Dummer, P. M. H. Preclinical endodontology: An international comparison. *Int. Endod. J.* **32**, 406–414 (1999).
411. Al Raisi, H., Dummer, P. M. H. & Vianna, M. E. How is Endodontics taught? A survey to evaluate undergraduate endodontic teaching in dental schools within the United Kingdom. *Int. Endod. J.* **52**, 1077–1085 (2019).
412. Kontakiotis, E. G., Tzanetakis, G. N. & Loizides, A. L. A l2-month longitudinal in vitro leakage study on a new silicon-based root canal filling material (Gutta-Flow). *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **103**, 854–859 (2007).
413. Hu, J., Zhu, Y., Deng, S., Wang, Z. & He, F. Outcome of root canal treatment using warm vertical compaction with bioceramic and resin-based sealers: A randomised clinical trial. *Aust. Endod. J. J. Aust. Soc. Endodontology Inc* (2022). doi:10.1111/aej.12713
414. Qu, W., Bai, W., Liang, Y.-H. & Gao, X.-J. Influence of Warm Vertical Compaction Technique on Physical Properties of Root Canal Sealers. *J. Endod.* **42**, 1829–1833 (2016).
415. Aksel, H. *et al.* Effect of heat application on the physical properties and chemical structure of calcium silicate-based sealers. *Clin. Oral Investig.* **25**, 2717–2725 (2021).
416. Schäfer, E., Schrenker, C., Zupanc, J. & Bürklein, S. Percentage of Gutta-percha Filled Areas in Canals Obturated with Cross-linked Gutta-percha Core-carrier Systems, Single-Cone and Lateral Compaction Technique. *J. Endod.* **42**, 294–298 (2016).
417. Alshehri, M., Alamri, H. M., Alshwaimi, E. & Kujan, O. Micro-computed tomographic assessment of quality of obturation in the apical third with continuous wave vertical compaction and single match taper sized cone obturation techniques. *Scanning* **38**, 352–356 (2016).

418. Wisawawatin, D., Yanpiset, K., Banomyong, D. & Jantarat, J. Gap volume and sealer penetration of C-shaped root canals obturated with cold hydraulic technique and calcium silicate sealer. *Aust. Endod. J. J. Aust. Soc. Endodontology Inc* (2022). doi:10.1111/aej.12696
419. Kaul, S. *et al.* Comparison of Sealing Ability of Bioceramic Sealer, AH Plus, and GuttaFlow in Conservatively Prepared Curved Root Canals Obturated with Single-Cone Technique: An In vitro Study. *J. Pharm. Bioallied Sci.* **13**, S857–S860 (2021).
420. Rekha, R. *et al.* Comparison of the sealing ability of bioceramic sealer against epoxy resin based sealer: A systematic review & meta-analysis. *J. Oral Biol. Craniofacial Res.* **13**, 28–35 (2023).
421. Girelli, C. F. M. *et al.* The thermoplastic techniques or single-cone technique on the quality of root canal filling with tricalcium silicate-based sealer: An integrative review. *J. Clin. Exp. Dent.* **14**, 566–572 (2022).
422. Poggio, C. *et al.* Solubility and pH of bioceramic root canal sealers: A comparative study. *J. Clin. Exp. Dent.* **9**, e1189–e1194 (2017).
423. Long, J., Kreft, J. U. & Camilleri, J. Antimicrobial and ultrastructural properties of root canal filling materials exposed to bacterial challenge. *J. Dent.* **93**, (2020).
424. Zordan-Bronzel, C. L. *et al.* Cytocompatibility, bioactive potential and antimicrobial activity of an experimental calcium silicate-based endodontic sealer. *Int. Endod. J.* **52**, 979–986 (2019).
425. Koutroulis, A., Kuehne, S. A., Cooper, P. R. & Camilleri, J. The role of calcium ion release on biocompatibility and antimicrobial properties of hydraulic cements. *Sci. Rep.* **9**, 1–10 (2019).
426. Antunes, T. B. M. *et al.* Heating stability, physical and chemical analysis of calcium silicate-based endodontic sealers. *Int. Endod. J.* **54**, 1175–1188 (2021).
427. Katakidis, A., Sidiropoulos, K., Koulaouzidou, E., Gogos, C. & Economides, N. Flow characteristics and alkalinity of novel bioceramic root canal sealers. *Restor. Dent. Endod.* **45**, (2020).
428. Al-Haddad, A. & Aziz, Z. A. C. A. Bioceramic-Based Root Canal Sealers: A Review. *Int. J. Biomater.* **2016**, (2016).
429. Eichelsdörfer, K. *et al.* Preparation and filling of small oval root canals: influence of file system and sealer. *Quintessence Int.* **53**, 656–665 (2022).
430. Cardinali, F. & Camilleri, J. A critical review of the material properties guiding the clinician's choice of root canal sealers. *Clin. Oral Investig.* **27**, 4147–4155 (2023).

431. Camilleri, J. Evaluation of Selected Properties of Mineral Trioxide Aggregate Sealer Cement. *J. Endod.* **35**, 1412–1417 (2009).
432. Al-Hiyasat, A. S., Ahmad, D. M. & Khader, Y. S. The effect of different calcium silicate-based pulp capping materials on tooth discoloration: an in vitro study. *BMC Oral Health* **21**, 1–10 (2021).
433. Bosso-Martelo, R. *et al.* Physicochemical properties of calcium silicate cements associated with microparticulate and nanoparticulate radiopacifiers. *Clin. Oral Investig.* **20**, 83–90 (2016).
434. Queiroz, M. B. *et al.* Physicochemical, biological, and antibacterial evaluation of tricalcium silicate-based reparative cements with different radiopacifiers. *Dent. Mater.* **37**, 311–320 (2021).
435. Schembri-Wismayer, P. & Camilleri, J. Why Biphasic? Assessment of the Effect on Cell Proliferation and Expression. *J. Endod.* **43**, 751–759 (2017).
436. Liu, W., Peng, W., Zhu, Y. & Chang, J. Physicochemical properties and in vitro biocompatibility of a hydraulic calcium silicate/tricalcium aluminate cement for endodontic use. *J. Biomed. Mater. Res. - Part B Appl. Biomater.* **100 B**, 1257–1263 (2012).
437. Jiménez-Sánchez, M. C., Segura-Egea, J. J. & Díaz-Cuenca, A. A microstructure insight of MTA repair HP of rapid setting capacity and bioactive response. *Materials (Basel).* **13**, (2020).
438. Almeida, L. H. *et al.* Bone tissue response to an MTA-based endodontic sealer, and the effect of the addition of calcium aluminate and silver particles. *Int. Endod. J.* **52**, 1446–1456 (2019).
439. Haji, T. H., Selivany, B. J. & Suliman, A. A. Sealing ability in vitro study and biocompatibility in vivo animal study of different bioceramic based sealers. *Clin. Exp. Dent. Res.* **8**, 1582–1590 (2022).
440. Viapiana, R. *et al.* Porosity and sealing ability of root fillings with gutta-percha and BioRoot RCS or AH Plus sealers. Evaluation by three ex vivo methods. *Int. Endod. J.* **49**, 774–782 (2016).
441. Bianco, E. *et al.* Evaluation with micro-CT of the canal seal made with two different bioceramic cements: Guttaflow bioseal and bioroot RCS. *J. Contemp. Dent. Pract.* **21**, 359–366 (2020).
442. Bilvinaite, G., Drukteinis, S., Brukiene, V. & Rajasekharan, S. Immediate and Long-Term Radiopacity and Surface Morphology of Hydraulic Calcium Silicate-Based Materials. *Materials (Basel).* **15**, 1–12 (2022).
443. Khalil, I., Naaman, A. & Camilleri, J. Properties of Tricalcium Silicate Sealers. *J. Endod.* **42**, 1529–1535 (2016).
444. Kooanantkul, C., Shelton, R. M. & Camilleri, J. Comparison of

obturation quality in natural and replica teeth root-filled using different sealers and techniques. *Clin. Oral Investig.* **27**, 2407–2417 (2023).
445. Loison-Robert, L. S. *et al.* In vitro effects of two silicate-based materials, Biodentine and BioRoot RCS, on dental pulp stem cells in models of reactionary and reparative dentinogenesis. *PLoS One* **13**, 1–19 (2018).
446. Dimitrova-Nakov, S. *et al.* In vitro bioactivity of Bioroot™ RCS, via A4 mouse pulpal stem cells. *Dent. Mater.* **31**, 1290–1297 (2015).
447. Vouzara, T., Dimosiari, G., Koulaouzidou, E. A. & Economides, N. Cytotoxicity of a New Calcium Silicate Endodontic Sealer. *J. Endod.* **44**, 849–852 (2018).
448. Ultradent Products Inc., South Jordan, UT, U. MTA Flow White Repair Cement Data Sheet. (2023).
449. Guimarães, B. M. *et al.* Chemical-physical Properties and Apatite-forming Ability of Mineral Trioxide Aggregate Flow. *J. Endod.* **43**, 1692–1696 (2017).
450. Giacomino, C. M., Wealleans, J. A., Kuhn, N. & Diogenes, A. Comparative Biocompatibility and Osteogenic Potential of Two Bioceramic Sealers. *J. Endod.* **45**, 51–56 (2019).
451. López-García, S. *et al.* Comparative cytocompatibility and mineralization potential of Bio-C sealer and totalfill BC sealer. *Materials (Basel).* **12**, 1–12 (2019).
452. Milanovic, I. *et al.* Immediate and Long-Term Porosity of Calcium Silicate–Based Sealers. *J. Endod.* **46**, 515–523 (2020).
453. Radwanski, M. *et al.* A Micro-CT Analysis of Initial and Long-Term Pores Volume and Porosity of Bioactive Endodontic Sealers. *Biomedicines* **10**, (2022).
454. Chen, B. *et al.* Cytotoxicity and the Effect of Temperature on Physical Properties and Chemical Composition of a New Calcium Silicate–based Root Canal Sealer. *J. Endod.* **46**, 531–538 (2020).
455. Yang, R. *et al.* A comparative study of dentinal tubule penetration and the retreatability of EndoSequence BC Sealer HiFlow, iRoot SP, and AH Plus with different obturation techniques. *Clin. Oral Investig.* **25**, 4163–4173 (2021).
456. Romeiro, K. *et al.* Reciproc and Reciproc Blue in the removal of bioceramic and resin-based sealers in retreatment procedures. *Clin. Oral Investig.* **24**, 405–416 (2020).
457. Kim, K., Kim, D. V., Kim, S.-Y. & Yang, S. A micro-computed tomographic study of remaining filling materials of two bioceramic sealers and epoxy resin sealer after retreatment. *Restor. Dent. Endod.*

44, 1–9 (2019).
458. Donnermeyer, D., Bürklein, S., Dammaschke, T. & Schäfer, E. Endodontic sealers based on calcium silicates: a systematic review. *Odontology* **107**, 421–436 (2019).
459. Wu, M. K., Ozok, A. R. & Wesselink, P. R. Sealer distribution in root canals obturated by three techniques. *Int. Endod. J.* **33**, 340–345 (2000).
460. Aminsobhani, M. *et al.* Coronal microleakage in root canals obturated with lateral compaction, warm vertical compaction and guttaflow system. *Iran. Endod. J.* **5**, 83–87 (2010).
461. Zhang, P., Yuan, K., Jin, Q., Zhao, F. & Huang, Z. Presence of voids after three obturation techniques in band-shaped isthmuses: a micro-computed tomography study. *BMC Oral Health* **21**, 227 (2021).
462. Hadis, M. & Camilleri, J. Characterization of heat resistant hydraulic sealer for warm vertical obturation. *Dent. Mater.* **36**, 1183–1189 (2020).
463. Donnermeyer, D., Schäfer, E. & Bürklein, S. Real-time Intracanal Temperature Measurement During Different Obturation Techniques. *J. Endod.* **44**, 1832–1836 (2018).
464. Donnermeyer, D. *et al.* Physico-chemical investigation of endodontic sealers exposed to simulated intracanal heat application: Hydraulic calcium silicate-based sealers. *Materials (Basel).* **14**, 1–11 (2021).
465. Chen, B. *et al.* Cytotoxicity and the Effect of Temperature on Physical Properties and Chemical Composition of a New Calcium Silicate–based Root Canal Sealer. *J. Endod.* **46**, 531–538 (2020).
466. Schilder, H. & Hargreaves, K. M. Filling root canals in three dimensions. *J. Endod.* **32**, 281–290 (2006).
467. Vo, K., Daniel, J., Ahn, C., Primus, C. & Komabayashi, T. Coronal and apical leakage among five endodontic sealers. *J. Oral Sci.* **64**, 95–98 (2022).
468. Hwang, J. H. *et al.* Comparison of bacterial leakage resistance of various root canal filling materials and methods: Confocal laser-scanning microscope study. *Scanning* **37**, 422–428 (2015).
469. Fransson, H. & Dawson, V. Tooth survival after endodontic treatment. *Int. Endod. J.* **56**, 140–153 (2023).
470. Peciuliene, V., Rimkuviene, J., Maneliene, R. & Ivanauskaite, D. Apical periodontitis in root filled teeth associated with the quality of root fillings. *Stomatologija* **8**, 122–126 (2006).
471. Tay, F. R. & Pashley, D. H. Monoblocks in Root Canals: A Hypothetical or a Tangible Goal. *J. Endod.* **33**, 391–398 (2007).

472. Teixeira, F. B., Teixeira, E. C. N., Thompson, J., Leinfelder, K. F. & Trope, M. Dentinal bonding reaches the root canal system. *J. Esthet. Restor. Dent.* **16**, 348–354 (2004).
473. Tay, F. R. *et al.* Effectiveness of resin-coated gutta-percha cones and a dual-cured, hydrophilic methacrylate resin-based sealer in obturating root canals. *J. Endod.* **31**, 659–664 (2005).
474. Monticelli, F. *et al.* Sealing properties of two contemporary single-cone obturation systems. *Int. Endod. J.* **40**, 374–385 (2007).
475. Ghoneim, A. G., Lutfy, R. A., Sabet, N. E. & Fayyad, D. M. Resistance to fracture of roots obturated with novel canal-filling systems. *J. Endod.* **37**, 1590–1592 (2011).
476. Eltair, M., Pitchika, V., Hickel, R., Kühnisch, J. & Diegritz, C. Evaluation of the interface between gutta-percha and two types of sealers using scanning electron microscopy (SEM). *Clin. Oral Investig.* **22**, 1631–1639 (2018).
477. Belli, S., Eraslan, O., Eskitascioglu, G. & Karbhari, V. Monoblocks in root canals: a finite elemental stress analysis study. *Int. Endod. J.* **44**, 817–826 (2011).
478. Schneider, S. W. A comparison of canal preparations in straight and curved root canals. *Oral Surg. Oral Med. Oral Pathol.* **32**, 271–275 (1971).
479. Alksne, M. *et al.* The effect of larger than cell diameter polylactic acid surface patterns on osteogenic differentiation of rat dental pulp stem cells. *J. Biomed. Mater. Res. - Part A* **107**, 174–186 (2019).
480. Darcey, J., Roudsari, R. V., Jawad, S., Taylor, C. & Hunter, M. Modern Endodontic Principles. Part 5: Obturation. *Dent. Update* **43**, 114–6, 119–20, 123–6 passim (2016).
481. Muliyar, S. *et al.* Microleakage in endodontics. *J. Int. oral Heal. JIOH* **6**, 99–104 (2014).
482. Wu, M., Dummer, P. M. H. & Wesselink, P. R. Consequences of and strategies to deal with residual post-treatment root canal infection. 343–356 (2006). doi:10.1111/j.1365-2591.2006.01092.x
483. Alves Dos Santos, G. N. *et al.* Is the quality of root canal filling obtained by cone-beam computed tomography associated with periapical lesions? A systematic review and meta-analysis. *Clin. Oral Investig.* **26**, 5105–5116 (2022).
484. Sjögren, U., Sundqvist, G. & Nair, P. N. Tissue reaction to gutta-percha particles of various sizes when implanted subcutaneously in guinea pigs. *Eur. J. Oral Sci.* **103**, 313–321 (1995).
485. de Souza Filho, F. J., Gallina, G., Gallottini, L., Russo, R. & Cumbo,

E. M. Innovations in endodontic filling materials: guttapercha vs Resilon. *Curr. Pharm. Des.* **18**, 5553–5558 (2012).

486. Araújo, V. L., Souza-Gabriel, A. E., Cruz Filho, A. M. da, Pécora, J. D. & Silva, R. G. Volume of sealer in the apical region of teeth filled by different techniques: a micro-CT analysis. *Braz. Oral Res.* **30**, (2016).
487. Suassuna, F. C. M. *et al.* Comparison of microtomography and optical coherence tomography on apical endodontic filling analysis. *Dentomaxillofac. Radiol.* **47**, 20170174 (2018).
488. Almohaimede, A. A., Almutairi, M. M., Alyousef, H. M. & Almadi, E. M. Micro - computed tomographic analysis of filling porosity of two different obturation techniques. 8–12 (2019). doi:10.4103/sjos.SJOralSci
489. Debelian, G. & Trope, M. ScienceDirect The use of premixed bioceramic materials in endodontics. *G. Ital. Endod.* **30**, 70–80 (2016).
490. Zhang, W. *et al.* Long-term porosity and retreatability of oval-shaped canals obturated using two different methods with a novel tricalcium silicate sealer. *Clin. Oral Investig.* **26**, 1045–1052 (2022).
491. Ko, S.-Y. *et al.* Main and Accessory Canal Filling Quality of a Premixed Calcium Silicate Endodontic Sealer According to Different Obturation Techniques. *Mater. (Basel, Switzerland)* **13**, (2020).
492. Kim, S. R. *et al.* Efficacy and retrievability of root canal filling using calcium silicate-based and epoxy resin-based root canal sealers with matched obturation techniques. *Aust. Endod. J.* **45**, 337–345 (2019).
493. Wang, Y., Liu, S. & Dong, Y. In vitro study of dentinal tubule penetration and filling quality of bioceramic sealer. 1–11 (2018).
494. Pedullà, E. *et al.* Root fillings with a matched-taper single cone and two calcium silicate-based sealers: an analysis of voids using micro-computed tomography. *Clin. Oral Investig.* **24**, 4487–4492 (2020).
495. Kharouf, N., Hemmerlé, J., Haikel, Y. & Mancino, D. Technical Quality of Root Canal Filling in Preclinical Training at Strasbourg University Using Two Teaching Protocols. *Eur. J. Dent.* **13**, 521–526 (2019).
496. Akbar, I. Radiographic study of the problems and failures of endodontic treatment. **9**, (2015).
497. Yamaguchi, M. *et al.* Factors that cause endodontic failures in general practices in Japan. *BMC Oral Health* **18**, 70 (2018).
498. Bardini, G. *et al.* A 12-month follow-up of primary and secondary root canal treatment in teeth obturated with a hydraulic sealer. *Clin. Oral Investig.* **25**, 2757–2764 (2021).

499. Rafeek, R. N., Smith, W. A., Mankee, M. S. & Coldero, L. G. Radiographic evaluation of the technical quality of root canal fillings performed by dental students. *Aust. Endod. J.* **38**, 64–69 (2012).
500. Moussa-Badran, S. *et al.* Technical quality of root fillings performed by dental students at the dental teaching centre in Reims, France. *Int. Endod. J.* **41**, 679–684 (2008).
501. Fong, W., Heidarifar, O., Killough, S., Lappin, M. J. & El Karim, I. A. An audit on technical quality of root fillings performed by undergraduate students. *Int. Endod. J.* **51 Suppl 3**, e197–e203 (2018).
502. Bierenkrant, D. E., Parashos, P. & Messer, H. H. The technical quality of nonsurgical root canal treatment performed by a selected cohort of Australian endodontists. *Int. Endod. J.* **41**, 561–570 (2008).
503. Sunay, H., Tanalp, J., Dikbas, I. & Bayirli, G. Cross-sectional evaluation of the periapical status and quality of root canal treatment in a selected population of urban Turkish adults. *Int. Endod. J.* **40**, 139–145 (2007).
504. Koch, M., Wolf, E., Tegelberg, Å. & Petersson, K. Effect of education intervention on the quality and long-term outcomes of root canal treatment in general practice. *Int. Endod. J.* **48**, 680–689 (2015).
505. Ramey, K., Yaccino, J. & Wealleans, J. A Retrospective, Radiographic Outcomes Assessment of 1960 Initial Posterior Root Canal Treatments Performed by Endodontists and Dentists. *J. Endod.* **43**, 1250–1254 (2017).
506. Marciano, M. A., Duarte, M. A. H. & Camilleri, J. Calcium silicate-based sealers: Assessment of physicochemical properties, porosity and hydration. *Dent. Mater.* **32**, e30-40 (2016).
507. Liang, Y.-H., Li, G., Shemesh, H., Wesselink, P. R. & Wu, M.-K. The association between complete absence of post-treatment periapical lesion and quality of root canal filling. *Clin. Oral Investig.* **16**, 1619–1626 (2012).
508. Celikten, B. *et al.* Evaluation of root canal sealer filling quality using a single-cone technique in oval shaped canals: An In vitro Micro-CT study. *Scanning* **38**, 133–140 (2016).
509. Moinzadeh, A. T., Zerbst, W., Boutsioukis, C., Shemesh, H. & Zaslansky, P. Porosity distribution in root canals filled with gutta percha and calcium silicate cement. *Dent. Mater.* **31**, 1100–1108 (2015).
510. Le, P., Fratini, E. & Chen, S.-H. Hydration-dependent dynamics of water in calcium-silicate-hydrate: A QENS study by global model. *Colloids Surf. B. Biointerfaces* **168**, 187–192 (2018).

511. Castagnola, R. *et al.* Micro-CT evaluation of two different root canal filling techniques. *Eur. Rev. Med. Pharmacol. Sci.* **22**, 4778–4783 (2018).
512. Huang, Y. *et al.* Micro-CT and nano-CT analysis of filling quality of three different endodontic sealers. *Dentomaxillofac. Radiol.* **46**, 20170223 (2017).
513. Krug, R., Krastl, G. & Jahreis, M. Technical quality of a matching-taper single-cone filling technique following rotary instrumentation compared with lateral compaction after manual preparation: a retrospective study. *Clin. Oral Investig.* **21**, 643–652 (2017).
514. Patel, S., Dawood, A., Whaites, E. & Pitt Ford, T. New dimensions in endodontic imaging: part 1. Conventional and alternative radiographic systems. *Int. Endod. J.* **42**, 447–462 (2009).
515. Jafari, F. & Jafari, S. Importance and methodologies of endodontic microleakage studies: A systematic review. *J. Clin. Exp. Dent.* **9**, e812–e819 (2017).
516. Gorni, F. G., Andreano, A., Ambrogi, F., Brambilla, E. & Gagliani, M. Patient and Clinical Characteristics Associated with Primary Healing of Iatrogenic Perforations after Root Canal Treatment: Results of a Long-term Italian Study. *J. Endod.* **42**, 211–215 (2016).
517. Ansari, I. & Maria, R. Managing curved canals. *Contemporary clinical dentistry* **3**, 237–241 (2012).
518. Sarao, S. K., Berlin-Broner, Y. & Levin, L. Occurrence and risk factors of dental root perforations: a systematic review. *Int. Dent. J.* **71**, 96–105 (2020).
519. Schäfer, E. & Dammaschke, T. Development and sequelae of canal transportation. *Endod. Top.* **15**, 75–90 (2006).
520. Abdelmotelb, M. A., Gomaa, Y. F., Khattab, N. M. A. & Elheeny, A. A. H. Premixed bioceramics versus mineral trioxide aggregate in furcal perforation repair of primary molars: in vitro and in vivo study. *Clin. Oral Investig.* **25**, 4915–4925 (2021).
521. Lopes, R. M. V. *et al.* Untouched canal areas and debris accumulation after root canal preparation with rotary and adaptive systems. *Aust. Endod. J. J. Aust. Soc. Endodontology Inc* **44**, 260–266 (2018).
522. Nagendrababu, V., Jayaraman, J., Suresh, A., Kalyanasundaram, S. & Neelakantan, P. Effectiveness of ultrasonically activated irrigation on root canal disinfection: a systematic review of in vitro studies. *Clin. Oral Investig.* **22**, 655–670 (2018).
523. Dioguardi, M. *et al.* Calcium Silicate Cements vs. Epoxy Resin Based Cements: Narrative Review. *Oral* **1**, 23–35 (2021).

524. Antonijevic, D., Zelic, K. & Djuric, M. Novel calcium silicate based dental material with the addition of biologically active soy compound. *2015 IEEE 15th Int. Conf. Bioinforma. Bioeng. BIBE 2015* (2015). doi:10.1109/BIBE.2015.7367632
525. Guerrero, F. & Berástegui, E. Porosity analysis of MTA and Biodentine cements for use in endodontics by using micro-computed tomography. *J. Clin. Exp. Dent.* **10**, e237–e240 (2018).
526. Guimarães, B. M. *et al.* Chemical-physical Properties and Apatite-forming Ability of Mineral Trioxide Aggregate Flow. *J. Endod.* **43**, 1692–1696 (2017).
527. Camilleri, J. *et al.* Porosity and root dentine to material interface assessment of calcium silicate-based root-end filling materials. *Clin. Oral Investig.* **18**, 1437–1446 (2014).
528. Li, X. *et al.* Modified tricalcium silicate cement formulations with added zirconium oxide. *Clin. Oral Investig.* **21**, 895–905 (2017).
529. Sfeir, G. *et al.* Calcium Silicate-Based Root Canal Sealers: A Narrative Review and Clinical Perspectives. *Mater. (Basel, Switzerland)* **14**, (2021).
530. Camilleri, J. *BioRoot RCS. Endo Sealer or Biological Filler?* (2019).
531. Eltair, M., Pitchika, V., Hickel, R., Kühnisch, J. & Diegritz, C. Evaluation of the interface between gutta-percha and two types of sealers using scanning electron microscopy (SEM). *Clin. Oral Investig.* **22**, 1631–1639 (2018).
532. Kalantar Motamedi, M. R., Mortaheb, A., Zare Jahromi, M. & Gilbert, B. E. Micro-CT Evaluation of Four Root Canal Obturation Techniques. *Scanning* **2021**, 6632822 (2021).
533. Acris De Carvalho, F. M. *et al.* Influence of Ultrasonic Activation on the Physicochemical Properties of Calcium Silicate-Based Cements. *Int. J. Dent.* **2021**, 6697988 (2021).
534. Yeung, P., Liewehr, F. R. & Moon, P. C. A quantitative comparison of the fill density of MTA produced by two placement techniques. *J. Endod.* **32**, 456–459 (2006).
535. Pérez-Alfayate, R., Algar-Pinilla, J., Mercade, M. & Foschi, F. Sonic activation improves bioceramic sealer's penetration into the tubular dentin of curved root canals: A confocal laser scanning microscopy investigation. *Appl. Sci.* **11**, (2021).
536. Sisli, S. N. & Ozbas, H. Comparative Micro-computed Tomographic Evaluation of the Sealing Quality of ProRoot MTA and MTA Angelus Apical Plugs Placed with Various Techniques. *J. Endod.* **43**, 147–151 (2017).

537. Lopes, F. C. *et al.* Effect of sonic and ultrasonic activation on physicochemical properties of root canal sealers. *J. Appl. Oral Sci.* **27**, 1–9 (2019).
538. Atmeh, A. R. & AlShwaimi, E. The Effect of Heating Time and Temperature on Epoxy Resin and Calcium Silicate-based Endodontic Sealers. *J. Endod.* **43**, 2112–2118 (2017).
539. Benavides García, M., Hernández Meza, E. & Reyes-Carmona, J. Ex Vivo Analysis of MTA FLOW® Biomineralization and Push-Out Strength: A Pilot Study. *Odovtos - Int. J. Dent. Sci.* **1**, 245–259 (2020).
540. An, H. J., Yoon, H., Jung, H. I., Shin, D.-H. & Song, M. Comparison of Obturation Quality after MTA Orthograde Filling with Various Obturation Techniques. *J. Clin. Med.* **10**, (2021).
541. Mori, G. G., Teixeira, L. M., de Oliveira, D. L., Jacomini, L. M. & da Silva, S. R. Biocompatibility evaluation of biodentine in subcutaneous tissue of rats. *J. Endod.* **40**, 1485–1488 (2014).
542. Escobar-garcía, D. M., Medina-rosas, M. G., González-amaro, A. M., Méndez-gonzález, V. & Flores, H. MTA-Based Cements : Biocompatibility and Effects on the Gene Expression of Collagen Type 1 and TGF- β 1. **2022**, (2022).
543. Boss, M. *et al.* Biocompatibility and Antibiofilm Properties of Calcium Silicate-Based Cements : An In Vitro Evaluation and Report of Two Clinical Cases. (2021).
544. Pedano, M. S. *et al.* Freshly-mixed and setting calcium-silicate cements stimulate human dental pulp cells. *Dent. Mater.* **34**, 797–808 (2018).
545. Ghoddusi, J., Afshari, J. T., Donyavi, Z. & Brook, A. Cytotoxic effect of a new endodontic cement and mineral trioxide aggregate on L929 line culture. **3**, 17–23 (2008).
546. Dahl, J. E., Frangou-Polyzois, M. J. & Polyzois, G. L. In vitro biocompatibility of denture relining materials. *Gerodontology* **23**, 17–22 (2006).
547. Koh, J. & Chang, H. Cytotoxicity of Newly Developed Ortho MTA Root-end Filling Materials. *J. Endod.* **38**, 1627–1630 (2012).
548. Fonseca, T. S., Silva, G. F., Jm, G. & In, C. P. S. In vivo evaluation of the inflammatory response and IL-6 immunoexpression promoted by Biodentine and MTA Angelus. 1–9 (2015). doi:10.1111/iej.12435
549. Khandelwal, A. *et al.* Review Article Periapical Healing following Root Canal Treatment Using Different Endodontic Sealers : A Systematic Review. **2022**, (2022).
550. Albakhakh, B., Al-saedi, A., Al-taee, R. & Nahidh, M. Rapid Apical

Healing with Simple Obturation Technique in Response to a Calcium Silicate-Based Filling Material. **2022**, (2022).

551. Song, M. *et al.* Chemomechanical Properties and Biocompatibility of Various Premixed Putty-type Bioactive Ceramic Cements. *J. Endod.* (2023). doi:10.1016/j.joen.2023.09.005
552. Margunato, S., Taşlı, P. N., Aydın, S., Karapınar Kazandağ, M. & Şahin, F. In Vitro Evaluation of ProRoot MTA, Biodentine, and MM-MTA on Human Alveolar Bone Marrow Stem Cells in Terms of Biocompatibility and Mineralization. *J. Endod.* **41**, 1646–1652 (2015).
553. Kim, Y., Lee, D., Song, D., Kim, H. & Kim, S. Biocompatibility and Bioactivity of Set Direct Pulp Capping Materials on Human Dental Pulp Stem Cells. 1–11 (2020).
554. Jang, G., Yu, M. & Min, K. Washout resistance of fast-setting pozzolan cement under various root canal irrigants. (2014). doi:10.5395/rde.2013.38.4.248
555. Poggio, C., Lombardini, M., Colombo, M., Beltrami, R. & Rindi, S. Solubility and pH of direct pulp capping materials: a comparative study. **13**, 181–185 (2015).
556. Kim, M., Yang, W., Kim, H. & Ko, H. Comparison of the biological properties of ProRoot MTA, OrthoMTA, and endocem MTA cements. *J. Endod.* **40**, 1649–1653 (2014).
557. Hansen, S. W., Marshall, J. G. & Sedgley, C. M. Comparison of Intracanal EndoSequence Root Repair Material and ProRoot MTA to Induce pH Changes in Simulated Root Resorption Defects over 4 Weeks in Matched Pairs of Human Teeth. *J. Endod.* **37**, 502–506 (2011).
558. (CSA), C. S. A. ISO 10993-5 in vitro cytotoxicity. *Int. Organ.* **2007**, 1–11 (2009).
559. Silva, E. J. N. L., Senna, P. M., De-Deus, G. & Zaia, A. A. Cytocompatibility of Biodentine using a three-dimensional cell culture model. *Int. Endod. J.* **49**, 574–580 (2016).
560. Kotova, A. V *et al.* Comparative Analysis of Dental Pulp and Periodontal Stem Cells: Differences in Morphology, Functionality, Osteogenic Differentiation and Proteome. *Biomedicines* **9**, (2021).
561. Sanz, J. L. *et al.* Comparative Biological Properties and Mineralization Potential of 3 Endodontic Materials for Vital Pulp Therapy: Theracal PT, Theracal LC, and Biodentine on Human Dental Pulp Stem Cells. *J. Endod.* **47**, 1896–1906 (2021).
562. Tomás-Catalá, C. J. *et al.* Comparative analysis of the biological effects of the endodontic bioactive cements MTA-Angelus, MTA

Repair HP and NeoMTA Plus on human dental pulp stem cells. *Int. Endod. J.* **50 Suppl 2**, e63–e72 (2017).
563. Paranjpe, A., Zhang, H. & Johnson, J. D. Effects of mineral trioxide aggregate on human dental pulp cells after pulp-capping procedures. *J. Endod.* **36**, 1042–1047 (2010).
564. Tomson, P. L., Lumley, P. J., Smith, A. J. & Cooper, P. R. Growth factor release from dentine matrix by pulp-capping agents promotes pulp tissue repair-associated events. *Int. Endod. J.* **50**, 281–292 (2017).
565. Wang, X. *et al.* Effect of Biodentine on Odonto / Osteogenic Differentiation of Human Dental Pulp Stem Cells. 1–13 (2023).
566. Jaroch, K. *et al.* One extraction tool for in vitro-in vivo extrapolation? SPME-based metabolomics of in vitro 2D, 3D, and in vivo mouse melanoma models. *J. Pharm. Anal.* **11**, 667–674 (2021).
567. Goldberg, M. *The Dental Pulp: Biology, Pathology, and Regenerative Therapies*. (2014). doi:10.1007/978-3-642-55160-4
568. Yu, C. & Abbott, P. V. An overview of the dental pulp : its functions and responses to injury. (2007). doi:10.1111/j.1834-7819.2007.tb00525.x
569. Ratnayake, J., Camilleri, J., Haththotuwa, T. N. & Huang, J. In Vitro Biological Testing of Dental Materials. *Methods Mol. Biol.* **2588**, 505–524 (2023).

SANTRAUKA

SANTRUMPŲ SĄRAŠAS

2D	– dvimatis
3D	– trimatis
$\%OP_{VOL}$	– procentinė atvirų porų tūrio reikšmė
$\%CP_{VOL}$	– procentinė uždarų porų tūrio reikšmė
µKT	– mikro-kompiuterinė tomografija (angl. atitinkantis trumpinys µCT)
ANOVA	– dispersinė analizė
CP_{VOL}	– uždarų porų tūris
C_{VOL}	– šaknies kanalo tūris
DI	– darbinis ilgis (angl. atitinkantis trumpinys WL)
DMSO	– dimetilsulfoksidas
EDTA	– etilendiamintetraacto rūgštis
F_{VOL}	– šaknies kanalo užpildo tūris
GE	– gydytojas endodontologas (angl. atitinkantis trumpinys ES)
GO	– gydytojas odontologas (angl. atitinkantis trumpinys GDP)
GR	– gydytojas rezidentas (angl. atitinkantis trumpinys PS)
HKSC	– hidrauliniai kalcio silikatiniai cementai (angl. atitinkantis trumpinys HCSC)
ŽDPKL	– žmogaus danties pulpos kamieninės ląstelės (angl. atitinkantis trumpinys hDPSC)
MF	– MTA Flow™
MFWhite	– MTA Flow™ White
MTA	– Mineralinis Trioksido Agregatas (angl. *Mineral Trioxide Aggregate*)
MTT bromidas	– 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolio
NaOCl	– Natrio hipochloritas
NegativeCG	– neigiama kontrolinė grupė (angl. atitinkantis trumpinys NegativeCG)
VK	– vieno kaiščio (angl. atitinkantis trumpinys SC)
OS	– odontologijos studentas (angl. atitinkantis trumpinys US)
OP_{VOL}	– atvirų porų tūris
PI	– propidžio jodias
TeigiamaKG	– teigiama kontrolinė grupė (angl. atitinkantis trumpinys PosiviteCG)

ProRootKG – kontrolinė hidraulinio kalcio silikatinio cemento ProRoot MTA grupė (angl. atitinkantis trumpinys ProRootCG)
RPM – apsisukimai per minutę
UA – ultragarsinė aktyvacija
V_{OI} – tiriamojo objekto tūris
V_{VOL} – porų tūris

ĮVADAS

Viršūninis periodontitas yra periodonto audinių uždegiminė reakcija, kurią sukelia dantų šaknų kanalų sistemoje reziduojantys mikroorganizmai ir jų veiklos produktai.[1] Dažniausiai ši patologija diagnozuojama atliekant rentgenologinius tyrimus.[2] Viršūninio periodontito paplitimas pasaulyje svyruoja nuo 16 %[3] iki 86 %.[4,5] Metaanalizių duomenimis, 52 – 58 % suaugusių žmonių visame pasaulyje turi bent vieną šios patologijos pažeistą dantį.[6,18] Nustatyta, kad viršūninis periodontitas sietinas su keliais veiksniais: gyvenamąja vieta, sisteminėmis ligomis, rentgenologinio tyrimo naudojimo dažniu diagnostikos tikslais ir dantų šaknų kanalų užpildo kokybe. [6–8]

Diagnozavus viršūninį periodontitą, reikia atlikti konservatyvų ar chirurginį endodontinį gydymą, siekiant apieviršūninių audinių gijimo, išsaugant danties funkcinį stabilumą.[9] Viršūninio periodontito gijimas po atlikto pirminio endodontinio gydymo siekia nuo 85 % iki 97 % [10,11], endodontinio pergydymo atvejais - nuo 74 % iki 82 % [12–15], o gydytų dantų funkcionalumui siekiant 91 % – 97 %.[16,17] Konservatyvaus endodontinio gydymo tikslas – atlikti chemomechaninį šaknų kanalų paruošimą ir užpildymą trimatėje erdvėje, siekiant ne tik pašalinti infekcinį užkratą, bet ir išvengti galimos reinfekcijos.[19] Tad šaknų kanalų užpildymo kokybė yra vienas iš reikšmingų veiksnių, užtikrinančių sėkmingą ilgalaikį endodontinio gydymo rezultatą.[20] Tyrimų rezultatai rodo, kad iki 60 % endodontinio gydymo nesėkmių gali būti susiję su nekokybišku šaknies kanalo užpildymu.[21] Apibūdinant šaknų kanalų užpildymo kokybę išskiriami šie vertinimo kriterijai: užpildo medžiagos tolygus homogeniškumas visu šaknies kanalo darbiniu ilgiu (DI) ir išlaikyta taisyklinga šaknies kanalo kūgio forma.[9] Užpildant dantų šaknų kanalus sukuriamas hermetiškumas turėtų tapti efektyvia apsauga nuo pakartotinio mikroorganizmų bei jų metabolizmo produktų patekimo į šaknų kanalų sistemą, taip pat užtikrinti likusių mikroorganizmų izoliavimą nuo galimų mitybos grandinių bei paskatinti apieviršūninių audinių gijimą.[22] Įrodyta, kad užpildo homogeniškumas ir sandarumas yra tiesiogiai susiję su poromis užpildo medžiagoje.[23] Todėl medžiagos porėtumas gali būti naudojamas kaip vienas iš objektyvių kriterijų vertinant šaknies kanalo užpildymo kokybę.

Siekiant užtikrinti maksimaliai sandarų dantų šaknų kanalų užpildymą siūloma didelė šaknų kanalų užpildymo metodų bei užpildų įvairovė.[24] Visgi atliktų tyrimų rezultatai rodo, kad tai negali užtikrinti nepriekaištingo trimačio šaknų kanalų sistemos užpildymo.[25,26] Lyginant užpildymo metodus ir porėtumą gaunami prieštaringi rezultatai. Vieni tyrimai rodo, jog prastesnė medžiagos adaptacija prie šaknies kanalo sienelių ir blogesnis sandarumas

sukuriamas taikant šaltos šoninės kondensacijos metodiką.[25,27] Kitų tyrimų išvados teigia, kad termoplastiniai užpildymo metodai lemia mažesnį porėtumą[28,29], tačiau vėsimo metu gutaperčiai būdingas traukimasis gali tapti porų ir tuštumų susidarymo priežastimi.[30] Be jau minėtų šių dviejų metodų aspektų, reikėtų atkreipti dėmesį ir į tai, kad šaltos šoninės kondensacijos metodika yra techniškai sudėtinga, o termoplastinėms užpildymo metodikoms būtini papildomi brangūs prietaisai.[21,24]

Hidrauliniai kalcio silikato pagrindu gaminami cementai (HKSC) arba užpildai – medžiagos, kurios naudojamos dantų šaknų kanalams užpildymti, perforacijoms uždengti ir gyvybingai pulpai išsaugoti.[31,32] Pirmajam HKSC tipui priskiriamos medžiagos turi reikšmingų trūkumų ir vienas iš jų yra ilgas kietėjimo laikas, kuris nesuteikia galimybių užbaigti gydymo per vieną apsilankymą.[33,34] Taip pat jiems būdingas mažesnis atsparumas išplovimui pradiniame kietėjimo etape[35], prastesnis pirminis stabilumas[36], dantų vainikų spalvos pokyčiai[37] bei naudojimo klinikiniame darbe sudėtingumas.[38] Nepaisant minėtų savybių, šios medžiagos yra biologiškai suderinamos, pasižymi antibakteriniu aktyvumu, mažu tirpumu, minimaliu susitraukimu ir ilgalaikiu stabilumu, dėl to jas galima naudoti kartu su pagrindiniais gutaperčios kaiščiais.[39–41] HKSC medžiagos yra vis tobulinamos, todėl IV bei V tipo užpildai pasižymi trumpesniu kietėjimo laiku, paruošimu naudoti ir pakeista konsistencija, o tai sudaro galimybę lengviau jas naudoti atliekant klinikines procedūras. Kartu išlaikomos biologinės ir bioaktyvios savybės, prilygstančios pirmo ir antro tipo HKSC užpildams. Siekiant moksliškai pagrįsto ir saugaus naujausių HKSC medžiagų naudojimo klinikinėje praktikoje, būtina ištirti *in vitro* ir *in vivo* naujus gamintojų pristatytus takius hidraulinius kalcio silikato pagrindu pagamintus cementus / užpildus, pavyzdžiui *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* (*Ultradent Products Inc*., Pietų Jordanija, UT, USA). Atlikti tyrimai suteikia reikšmingos informacijos apie naujausių tipų medžiagas, kurios gali būti rekomenduojamos naudoti klinikinėje praktikoje sėkmingai pakeisdamos I - II tipo plačiai ištirtas ir naudojamas HKSC medžiagas.

Tarp gydytojų sparčiai populiarėja vieno gutaperčios kaiščio (VK) dantų šaknų kanalų užpildymo metodika, kai dantų šaknų kanalams užpildyti naudojamas vienas gutaperčios kaištis kartu su hidrauliniu kalcio silikato pagrindu pagamintu užpildu.[42] Priešingai nei iki šiol taikyti užpildymo metodai, VK metodika yra paprasta naudoti, nereikalauja ilgo mokymosi proceso, yra patraukli gydytojams odontologams. Užpildant šaknies kanalą išvengiama vertikalaus spaudimo medžiagos kondensacijos metu, todėl teigtina, kad galėtų būti sumažinama dantų šaknų skilimų ar įtrūkimų rizika.[43] Keleto tyrimų rezultatai rodo, kad bendras užpildo porėtumas naudojant VK

metodiką yra panašus ar net mažesnis, palyginti su porėtumu taikant kitus metodus.[26,27,44] Be to, HKSC užpildai, naudojami su VK technika, pasižymi geresniu sukibimu su šaknies kanalo dentinu, negu su kitų tipų užpildais. Taip pat šie užpildai nesitraukia, tad gali lemti vienalytiškesnį ir sandaresnį dantų šaknų kanalų užpildymą.[45,46] Be to, dėl puikaus takumo, hidrofiliškumo ir mažo medžiagos dalelių dydžio galima pasiekti puikų užpildo pasiskirstymą šaknų kanalų sistemoje pritaikant tik hidraulinį gutaperčios kaiščio slėgį.[43] Naujausi klinikiniai tyrimai atskleidė gerus klinikinės sėkmės rodiklius, kai VK metodas buvo naudojamas šaknų kanalams užpildyti pirminio endodontinio gydymo arba pergydymo atvejais.[47,48]

Dantų šaknų kanalų chemomechaninio paruošimo kokybė yra reikšminga sumažinant mikroorganizmų kiekį šaknų kanalų sistemoje, o kartu turi įtakos endodontinio gydymo sėkmei.[20,21,39] Be to, įrodytas šaknų kanalų užpildymo kokybės ir gydytojo klinikinės patirties ryšys.[24,48,49] Klinikinės sėkmės rodikliai, gydymo procedūras atliekant gydytojui specialistui endodontologui, gali siekti daugiau nei 90 % arba 95 %, o tais atvejais, kai dantų šaknų kanalų gydymą atlieka gydytojai odontologai, klinikinė sėkmė mažesnė ir yra 40 – 65 %.[50–53] Sėkmės rodiklis dar mažesnis, kai endodontinį gydymą atlieka odontologijos krypties studentai.[54,55] Tyrimų rezultatai rodo, kad tik 30 – 47 % odontologijos studentų atliktų dantų šaknų kanalų gydymo procedūrų atitinka kokybės standartus, keliamus endodontiniam gydymui.[51,56] Taigi, dantų šaknų kanalų užpildymo kokybė galimai yra susijusi su maža studentų patirtimi.[51] Tikėtina, kad naujai siūloma supaprastinta vieno gutaperčios kaiščio šaknies kanalo užpildymo metodika gali būti mažiau susijusi su gydytojo klinikine patirtimi ir neturėti reikšmingos įtakos šaknų kanalų užpildymo kokybei.[26,57] Visgi, iki šiol nėra pakankamai įrodymų, kad VK užpildymo metodikos paprastumas ir klinikinis patrauklumas, nepriklausomai nuo gydytojo klinikinės patirties, užtikrins dantų šaknų kanalų užpildymo kokybę ir mažesnį porėtumą.

Daugelį metų ultragarsiniai prietaisai buvo ir yra sėkmingai naudojami atliekant endodontines gydymo procedūras, tarp jų - ir šaknų kanalų užpildymą.[58,59] Tyrimų rezultatai rodo, kad aktyvuojant dantų šaknų kanalų užpildus ultragarsu galima padidinti jų įsiskverbimą į dentino kanalėlius ir taip pagerinti skysto užpildo adaptaciją prie šaknies kanalo sienelės.[60,61] Be to, ultragarsinė energija gali turėti įtakos medžiagos dalelių pasiskirstymui ir pašalinti susidariusius oro burbulus, taip sumažindama užpildo porėtumą.[62,63] Todėl, siekiant pagerinti šaknų kanalų užpildymo kokybę, ultragarsinė užpildų aktyvacija rekomenduota kaip papildomas atliekamų procedūrų etapas.[63,64] Šias rekomendacijas reikia įvertinti papildomai, nes daugumoje ankstesnių tyrimų buvo atliekama netiesioginės ultragarsinės aktyvacijos

poveikio analizė ir nėra pakankamai duomenų apie porėtumo pasiskirstymą HKSC užpilduose, naudojamuose kartu su gutaperčios kaiščiu (VK metodika).

Galiausiai, biologinės savybės yra vienas iš pagrindinių HKSC užpildų privalumų. Šios medžiagos kietėja drėgnoje aplinkoje, esant kontaktui su aplinkiniais audiniais - susidaro hidroksiapatitas ir išsiskiria kalcio hidroksidas.[65–69] HKSC yra vienintelės medžiagos, ilgą laiką išskiriančios kalcio jonus po pirminio jų sukietėjimo susiliečiant su drėgnu dentinu ir/ar kauliniu audiniu.[70] Biologinės HKSC savybės skatina ląstelių dauginimąsi ir diferenciaciją bei mineralizuotų audinių susidarymą, pavyzdžiui, dentino tiltelio, atlikus tiesioginį pulpos padengimą[69,71], ar kietųjų audinių barjero formavimąsi po atliktos apeksofikacijos procedūros.[72,73] Daugumą HKSC galima apibūdinti kaip biologiškai suderinamus, skatinančius danties pulpos audinio gijimą net ir esant uždegiminiam procesui pulpoje arba sudarančius sąlygas gyti apieviršūniniams audiniams. Nors tyrimų metodologiniai skirtumai sunkina rezultatų palyginimą, visgi galima teigti, kad beveik visi tirti HKSC turi teigiamą poveikį žmogaus danties pulpos kamieninėms ląstelėms (ŽDPKL). Tyrimų rezultatai parodė, kad naujausių - IV ir V tipo HKSC medžiagų biologinės savybės prilygsta „aukso standartu“ laikomo I tipo *ProRoot® MTA White* savybėms.[67,74] 2018 m. atlikta sisteminė apžvalga parodė, kad visi komerciškai prieinami ir ištirti HKSC yra biologiškai suderinami, turi panašų teigiamą poveikį dantų pulpos kamieninių ląstelių diferenciacijai *in vitro* ir gali efektyviai sustiprinti dentino tiltelio susidarymą, pasireiškiant minimaliai uždegiminei reakcijai.[74] 2020 m. atliktos sisteminės apžvalgos ir metaanalizės duomenimis įvairūs HKSC gali skirtingai paveikti ŽDPKL gyvybingumą.[75] Nurodoma, kad daugybė atliktų *in vitro* tyrimų bei ilgalaikių klinikinių tyrimų leidžia teigti, kad originalus komercinis be dervos HKSC *ProRoot® MTA* yra patikimiausias pasirinkimas siekiant paskatinti kietųjų audinių barjero susidarymą po gyvybingos pulpos terapijos procedūrų.[75–77] Kita vertus, kitose apžvalgose pateikiamos išvados, kad dėl duomenų trūkumo negalima teikti prioriteto vienai iš HKSC medžiagų.[74,78–80] Atsargumas teikiant rekomendacijas patekus naujai medžiagai į rinką yra būtinas, nes net ir nedideli medžiagų sudėties pokyčiai gali turėti reikšmingą įtaką jų klinikiniam veiksmingumui. Pirmiausia, norint naudoti šiuos naujos kartos takius HKSC užpildus klinikinėje praktikoje, būtina įvertinti tokias jų biologines savybes, kaip citotoksiškumas bei įtaka ląstelių proliferacijai. Taip pat svarbu palyginti su plačiai ištirtomis I - II tipo HKSC medžiagomis, suformuojant palyginamųjų charakteristikų rekomendacijas ir teikiant patikimus duomenis ateities tyrimams *in vivo*.

TYRIMO TIKSLAS

Įvertinti takių hidraulinių kalcio silikatinių cementų fizikines ir biologines savybes bei sukuriamą dantų šaknų kanalų užpildymo kokybę *in vitro*.

TYRIMO UŽDAVINIAI

1. Įvertinti ir palyginti dantų šaknų kanalų užpildymo vieno kaiščio metodika ir takiu HKSC (*BioRoot® RCS, Septodont, Saint-Maur-des-Fosses, France*) užpildu kokybę tarp skirtingą klinikinę patirtį turinčių asmenų: odontologijos studentų, endodontologijos rezidentų, gydytojų odontologų bei gydytojų endodontologų.
2. Įvertinti lenktų perforuotų apatinio žandikaulio pirmųjų krūminių dantų šaknų kanalų užpildymo takiu HKSC (*MTA Flow™, Ultradent Products Inc., South Jordan, UT, USA*) bei vieno kaiščio metodika (*BioRoot® RCS, Septodont, Saint-Maur-des-Fosses, France*) kokybę ir jos priklausomybę nuo aktyvacijos metodų/dantų šaknų kanalų morfologijos.
3. Įvertinti takių HKSC užpildų (*MTA Flow™* ir *MTA Flow™ White* tirštos konsistencijos, *Ultradent Products Inc., South Jordan, UT, USA*) biosuderinamumą su žmogaus danties pulpos kamieninėmis ląstelėmis ir palyginti gautus rezultatus su plačiai ištirtu HKSC (*ProRoot® MTA White, Tulsa Dental Products, Tulsa, OK, USA*).

TYRIMO AKTUALUMAS IR NAUJUMAS

Šio tyrimo pagrindiniai objektai yra naujausiųjų IV tipo takių hidraulinių kalcio silikatinių cementų (HKSC) naudojimas dantų šaknų kanalams užpildyti/gyvybingai pulpai gydyti, taip pat šioms medžiagoms pritaikytų dantų šaknų kanalų užpildymo metodikų adaptacija ir pritaikomumas klinikinėje praktikoje. Tyrimas sudarytas iš trijų dalių, siekiant įvertinti uždaviniuose numatytų veiksnių įtaką endodontinėms procedūroms: įvertinama skirtingą klinikinę patirtį ir specializaciją turinčių asmenų (odontologijos studentų ir gydytojų odontologų) sukuriama dantų šaknų kanalų užpildymo kokybė, pritaikant mikrokompiuterinės tomografijos (µKT) analizę; nustatoma ultragarsinės aktyvacijos įtaka takių HKSC užpildų homogeniškumui; įvertinamas biologinis takių HKSC suderinamumas ir poveikis žmogaus danties pulpos kamieninėms ląstelėms. Šios savybės yra svarbios dantų šaknų kanalams užpildyti, siekiant sukurti vienalytį, sandarų ir biologiškai suderinamą dantų šaknų kanalų užpildą bei užtikrinant ilgalaikį užpildo stabilumą, kad ilgalaikiai klinikiniai rezultatai būtų kuo geresni. Remiantis gautais rezultatais pabrėžiama: šviežiai sumaišytų ir sukietėjusių

HKSC užpildų analizės ir rezultatų palyginimo racionalumas *in vitro* tyrimuose; takių HKSC užpildų biologinės savybės, palyginant su plačiai ištirtais HKSC; nuo klinikinės patirties nepriklausomos, lengvai įvaldomos ir atkartojamos VK užpildymo metodikos pritaikymo klinikinėje praktikoje racionalumas, naudojant takius HKSC užpildus. Naujausios kartos takūs HKSC yra analizuojami siekiant pažangių dantų šaknų kanalų užpildymo / gyvybingos pulpos gydymo protokolų ir jų pritaikymo klinikinėje praktikoje.

Moksliniuose tyrimuose dantų šaknų kanalų užpildymo įvertinimas ir lyginamoji analizė bei gauti rezultatai reikšmingai priklauso nuo tyrimui taikomų metodikų bei dantų šaknų kanalų užpildų tipo. Nepaisant to, naujų dantų šaknų kanalų užpildų sukūrimo ir panaudojimo tikslas – pagerinta dantų šaknų kanalų (per)gydymo sėkmė, medžiagų universalumas pritaikant įvairiose klinikinėse situacijose bei sutrumpinto ir lengvai įvaldomo klinikinio protokolo įdiegimas gydytojų odontologų klinikinėje praktikoje. Trūkstant mokslinių tyrimų lyginamosios analizės duomenų tarp skirtingą klinikinę patirtį turinčių gydytojų odontologų, šis tyrimas reikšmingai papildo standartizuotos analizės rezultatus, vertinant dantų šaknų kanalų užpildymo kokybę trimačiuose µKT tyrimuose priklausomai nuo odontologijos studentų ir gydytojų odontologų klinikinės patirties ir specializacijos. Taip pat šis tyrimas detaliai įvertina dantų šaknų kanalų užpildymo kiekybinius veiksnius, tokius kaip užpildo bendrąjį porėtumą, išskaidant į atviras ir uždaras užpildo poras, vertinant jų tūrį ir pasiskirstymą dantų šaknų kanalų trečdaliuose. VK metodika kartu su HKSC užpildais palyginama trimatėje µKT analizėje su užpildymo metodika, naudojant HKSC. Remiantis laboratoriniais tyriais įvertinama ultragarsinės aktyvacijos (papildomas veiksnys klinikinio protokolo etape) įtaka HKSC užpildams. Gauti rezultatai gali būti panaudoti racionalioms rekomendacijoms praplėsti tyrimais pagrįstą klinikinį protokolą gydytojams odontologams.

Naujausių HKSC medžiagų gausa rinkoje ir vis pristatomi nauji užpildai reikalauja nepriklausomų ir išsamių šių medžiagų tyrimų, kadangi net ir minimalūs sudėties pokyčiai gali reikšmingai paveikti ne tik fizikines, chemines ir biologines medžiagos savybes, tačiau ir lemti skirtingą elgseną klinikinėje praktikoje bei turėti įtakos gydymo sėkmei. Šio tyrimo metu bus analizuojamos naujos kartos IV tipo takių HKSC užpildų biologinės savybės ir poveikis žmogaus danties pulpos kamieninėms ląstelėms. Vienas iš esminių tyrimo metodikos ypatumų, lemiančių naujumą, yra laboratoriniams biologinio suderinamumo tyrimams naudojami šviežiai sumaišyti takūs HKSC užpildai bei ilgalaikės ŽDPKL proliferacijos analizė veikiant HKSC tirpalais. Pirminis šviežiai sumaišytų HKSC poveikis ŽDPKL leidžia įvertinti kietėjančių cementų citotoksinį poveikį ląstelėms. Šis modelis yra kliniškai svarbus siekiant įvertinti ne tik sukietėjusių HKSC užpildų, kurie mažiau

sąveikauja su aplinka, savybes, bet ir palyginti gautus rezultatus tarpusavyje ir įvertinti poveikį aplinkiniams audiniams ir ląstelėms. Šis aspektas yra reikšmingas, kadangi klinikinėje praktikoje naudojami šviežiai sumaišyti ir dar nesukietėję HKSC užpildai, tačiau sukietėję HKSC lieka ilgą laiką dantų šaknų kanalų sistemoje. Naujos kartos takios HKSC IV tipo medžiagos yra palyginamos su plačiai ištirta ir „auksiniu standartu“ HKSC medžiagų kategorijoje laikoma I tipo *ProRoot MTA*. Analizuojamas ir palyginamas šių medžiagų citotoksiškumas ŽDPKL tėkmės citometrijos metodu, apskaičiuojant apoptozės ir/ar nekrozės apimtas ląsteles, siekiant nustatyti ląstelių žūties būdą. Atliekama kiekybinė ir kokybinė ląstelių morfologijos ir skaičiaus, paveikus HKSC tirpalais, analizė. Šis įvairiapusiškas HKSC medžiagų įvertinimas netiesiogiai rodo sudėties įtaką užpildų biologinėms savybėms ir leidžia palyginti su kitais HKSC užpildais. Remiantis gautais rezultatais taip pat galima įvertinti, ar naujos kartos takūs HKSC gali būti rekomenduojami naudoti kasdienėje klinikinėje praktikoje.

Apibendrinant pasakytina, jog tyrimo analize siekiama įvertinti naujausiųjų IV tipo takių HKSC užpildų įtaką: dantų šaknų kanalų užpildymo kokybei tarp skirtingą klinikinę patirtį ir specializaciją turinčių asmenų (odontologijos studentų ir gydytojų odontologų), atliekant trimačių µKT analizę; HKSC užpildų homogeniškumui, priklausomai nuo tiesioginės ultragarsinės aktyvacijos; bei biologinėms savybėms, priklausomai nuo HKSC tipo. Tyrimas svariai prisideda prie reikšmingų ir patikimų naujos kartos takių HKSC analizės rezultatų, siekiant kurti tyrimais pagrįstus klinikinius protokolus gydytojams odontologams.

GINAMIEJI TEIGINIAI

1. Dantų šaknų kanalų užpildų porėtumas yra neišvengiamas, nepriklausomai nuo procedūrą atliekančio asmens ir/ar dantų šaknų kanalams užpildyti naudojamų takių HKSC bei metodikos.
2. Dantų šaknų kanalų užpildymo kokybė vieno kaiščio metodika tarp skirtingą klinikinę patirtį turinčių asmenų reikšmingai nesiskiria.
3. Dantų šaknų kanalai, užpildyti skystos konsistencijos takiu hidrauliniu kalcio silikatiniu cementu *MTA Flow*^{TM} (be gutaperčios kaiščio), pasižymi dideliu porėtumu, palyginti su vieno kaiščio metodika ir takiu HKSC.
4. Tiesioginė ultragarsinė aktyvacija turi reikšmingos įtakos takių HKSC užpildų porėtumui.
5. Takūs HKSC užpildai turi įtakos ŽDPKL gyvybingumui, proliferacijai ir morfologijai.
6. Naujos kartos takių HKSC *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* biologinis suderinamumas yra panašus į plačiai ištirto HKSC *ProRoot*$^{®}$ *MTA*.

1. MEDŽIAGOS IR METODAI

1.1. Takių HKSC užpildų µKT įvertinimas

1.1.1. Skirtingą klinikinę patirtį turinčių asmenų dantų šaknų kanalų užpildų porėtumo analizė

1.1.1.1. Mėginių paruošimas

Tiriamosios grupės imtis apskaičiuota naudojant G*Power 3.1 programinę įrangą (*Heinrich Heine, Iniversität Düsseldorf, Düsseldorf, Germany*), taikant Stjudento T testą ir dviejų nepriklausomų imčių vidurkius, kai alfa klaidos tikimybė 0,05, o imties galia (1-beta klaidos tikimybė) 0,95. Gauta dvylikos dantų šaknų kanalų minimali imtis. Tiriamuosius mėginius paruošė vienas kalibruotas tyrėjas. Iš viso tyrimui buvo paruošti 28 standartizuoti trimačiai plastikiniai viršutinių prieškrūminių dantų modeliai (*DRSK, Hassleholm, Sweden*), kurie pagaminami jau su paruoštomis endodontinėmis ertmėmis. Kiekvienas prieškrūminis dantis turi po du atskirus pirmojo tipo pagal *Weine* klasifikaciją šaknų kanalus. Danties šaknies kanalo darbinis ilgis (DI) nustatytas įvedus 10 dydžio *K-File* (angl.) (*Dentsply Maillefer, Ballaiques, Switzerland*) į šaknies kanalą, kol instrumento viršūnė tampa matoma už šaknies viršūninės angos. DI buvo nustatomas atitraukus instrumentą 1 mm nuo viršūninės angos. Dantų šaknų kanalai buvo paruošti vieno tyrėjo DI naudojant *HyFlex EDM* (*Coltene, Langenau, Germany*) mašininių instrumentų sistemą, taikant 400 apsisukimų per minutę greitį ir 2,5 Ncm jėgos momentą, *X-Smart* (*Dentsply Sirona, Ballaiques, Switzerland*) endodontiniu motoru. Mašininiai instrumentai naudoti gamintojo nurodyta seka nustatytu DI: *Glide-path* (10 dydžio/0,05 kūgio), *Preparation* (20 dydžio/0,05 kūgio), *OneFile* (25 dydžio/kintančio kūgio) ir *Finishing* (40 dydžio/0,04 kūgio) endodontiniai instrumentai.

Po kiekvieno instrumento naudojimo šaknų kanalai buvo pakartotinai plaunami 5 ml 3 % natrio hipochlorito (NaOCl) tirpalu (*Ultradent Products Inc., South Jordan, UT, USA*). Chemomechaninio paruošimo pabaigoje plovimas atliktas 5 ml 17 % etilendiamintetraacto rūgštimi (EDTA) (*Ultradent Products Inc., South Jordan, UT, USA*) 2 min. bei paskutiniu plovimo tirpalu - 5 ml distiliuoto vandens. Plovimui naudojami vienkartiniai švirkštai ir 29-G *NaviTip* irigacinės adatos (*Ultradent Products Inc., South Jordan, UT, USA*). Atlikus chemomechaninį paruošimą, dantų šaknų kanalai išsausinti 40 dydžio/0.04 kūgio paskutinį mašininį instrumentą atitinkančiais sauskaiščiais (*Coltene, Langenau, Germany*).

1.1.1.2. Dantų šaknų kanalų užpildymas

Paruošti dantų modeliai fiksuoti A tipo silikono medžiagoje (*3M Express, 3M ESPE, Seefeld, Germany*), suformuojant blokelius iki cemento-emalio ribos, siekiant sukurti sąlygas, panašesnes į klinikinę situaciją atliekant dantų šaknų kanalų užpildymą, ir apriboti dantų šaknų išorinio paviršiaus matomumą. Iš viso tyrime buvo suformuotos keturios tiriamosios grupės, tad paruošti tiriamieji mėginiai buvo atsitiktinai suskirstyti po septynis dantis į keturias grupes, priklausomai nuo asmens, atliekančio dantų šaknų kanalų užpildymą: odontologijos studentas (OS), endodontologijos srities gydytojas rezidentas (GR), gydytojas odontologas (GO) ir gydytojas endodontologas (GE). Patyręs mokslininkas ir gydytojas endodontologas prieš atliekant dantų šaknų kanalų užpildymą, visiems tyrimo dalyviams pristatė 3 valandų trukmės VK užpildymo metodikos teorinį-praktinį kursą.

Kiekvienoje grupėje užpildyta po 14 dantų šaknų kanalų naudojant *BioRoot RCS* (*Septodont, Saint-Maurdes-Fosses, France*) HKSC užpildą ir paskutinį mašininį instrumentą, atitinkantį vieną *HyFlex EDM* 40/0,04 kūgio gutaperčios kaištį (*Coltene, Langenau, Germany*). Iš viso užpildyta 56 dantų šaknų kanalai. Pritaikytas gutaperčios kaištis padengtas nedideliu kiekiu HKSC užpildo, sumaišyto pagal gamintojo rekomendacijas, ir švelniai įvestas į šaknies kanalą, kad būtų užpildyta šaknies kanalų sistema HKSC užpildu. Procedūra kartota du kartus, siekiant užtikrinti reikiamą HKSC užpildo kiekį šaknies kanale. Galiausiai, trečiąjį kartą gutaperčios kaištis pakartotinai padengtas HKSC užpildu ir švelniai įvestas visu darbiniu ilgiu (DI). Gutaperčia nukaitinta šaknies kanalo įeigos srityje karščio nešikliu. Endodontinė ertmė užpildyta laikinu užpildu *Cavit™-W (3M ESPE, Seefeld, Germany*) ir tiriamieji mėginiai pamerkti į termovonelę (*Thermo Scientific™Precision™; Fisher Scientific; Vantaa, Finland*), siekiant užtikrinti ir palaikyti 37 ° C aplinkos temperatūrą vieną savaitę ir sukurti sąlygas dantų šaknų kanalų užpildui sukietėti prieš tolesnę µKT analizę.

1.1.1.3. µKT skenavimas ir analizė

Visi mėginiai skenuoti aukštos raiškos µKT skeneriu SkyScan 1272 (*Bruker, Kontich, Belgium*). Skenavimai atlikti taikant 90 kV šaltinio įtampą, 111 µA spindulio srovę, 10 µm izotropinę rezoliuciją, 0,2 ° kampo sukimąsi ir 1350 ms išlaikymą. Tyrimui naudoti 0,5 mm aliuminio ir 0,038 mm vario filtrai, siekiant sumažinti galimus artefaktus. Gauti µKT vaizdai rekonstruoti naudojant NRecon v.1.7.1.0 programinę įrangą (*Bruker, Kontich, Belgium*),

pritaikant 3 dydžio artefaktų sumažinimo indeksą bei 20 % spindulio korekciją.

Rekonstruoti trimačiai dantų šaknų vaizdai analizuoti naudojant CTAn v.1.14.4.1 programinę įrangą (*Bruker, Kontich, Belgium*). Programinėje įrangoje pasirinkti dantų šaknų kanalų kontūrai, kontrasto ir šviesumo apimtis, siekiant išskirti dantų šaknų kanalų užpildus bei poras. Išskyrimas atliktas naudojantis tankio histograma, globalaus slenksčio (angl. *global threshold*) metodika. Atlikta palyginamoji originalių ir segmentuotų vaizdų analizė, kad būtų užtikrintas segmentacijos tikslumas. Šaknies kanalo tūris apibrėžtas kaip tiriamosios srities tūris (V_{OI}). Pagrindinė tiriamoji sritis išskaidyta į porų tūrį (V_{Vol}), užpildo tūrį (F_{Vol}), atviras poras (OP_{Vol}) ir uždaras poras (CP_{Vol}). Šie tūriai apskaičiuoti apdorojant segmentuotus vaizdus trimatėje erdvėje specialiai šiam tyrimui sukurtu apdorojimo algoritmu. Procentinė atvirų ($\%OP_{Vol}$) ir uždarų porų ($\%CP_{Vol}$) išraiška apskaičiuota remiantis šiomis formulėmis:

$$\%OP_{Vol} = OP_{Vol}/(V_{Vol} + F_{Vol}) \text{ x } 100,$$
$$\%CP_{Vol} = CP_{Vol}/(V_{Vol} + F_{Vol}) \text{ x } 100.$$

Nuskenuoti mėginiai buvo užkoduoti ir analizė atlikta vieno kalibruoto tyrėjo. Procentinė atvirų ir uždarų porų išraiška apskaičiuota visiems dantų šaknų kanalų trečdaliams atskirai: vainikiniams, viduriniams ir viršūniniams, kiekvienam šaknies trečdaliui apimant po 3 mm, bei tiriamosios srities tūrį (V_{OI}) apskaičiuojant iš 9 mm šaknies kanalo ilgio. Iš analizės pašalintas viršūninis 1 mm šaknies kanalo ilgis. Trimačiams tiriamųjų sričių vaizdams sukurti bei kokybinei jų analizei naudota CTVol v.2.2.3.0 programinė įranga (*Bruker, Kontich, Belgium*).

1.1.1.4. Statistinė analizė

Statistinė analizė atlikta naudojant *IBM SPSS Statistics* 29 versijos programinę įrangą (*IBM Corp., Armonk, N.Y., USA*). *Shapiro–Wilk* testas atskleidė, jog tyrimo duomenys neturi normaliojo skirstinio. Todėl skirtumai tarp tiriamųjų grupių analizuoti taikant neparametrinį *Kruskal–Wallis* testą. Aptikus statistiškai reikšmingas p reikšmes, taikytas *Mann–Whitney* testas porų analizei, siekiant atskleisti reikšmingus skirtumus tarp OS, GO, GR ir GE grupių. Skirtumai tarp dantų šaknų kanalų trečdalių tose pačiose grupėse analizuoti taikant neparametrinį *Friedman* testą bei *Wilcoxon* testą porų analizei. Pasirinktas reikšmingumo lygmuo, kai $p < 0,05$.

1.1.2. Ultragarsinės aktyvacijos įtakos apatinio žandikaulio pirmųjų krūminių dantų perforuotų mezialinių šaknų viršūnių užpildymo skirtingais HKSC ir metodikomis porėtumui analizė

1.1.2.1. Tiriamųjų mėginių paruošimas

Tyrimui atrinkta 40 apatinio žandikaulio pirmųjų krūminių dantų. Dantys buvo pašalinti dėl medicininių priežasčių, nesusijusių su šiuo tyrimu. Tyrimas patvirtintas VšĮ Vilniaus universiteto ligoninės Žalgirio klinikos etikos komiteto leidimu, protokolo numeris EK-2. Tyrimas atliktas vieno kalibruoto tyrėjo. Tiriamosios grupės imtis apskaičiuota naudojant G*Power 3.1 programinę įrangą (*Heinrich Heine, Iniversität Düsseldorf, Düsseldorf, Germany*), taikant Stjudento T testą ir dviejų nepriklausomų imčių vidurkius, kuomet alfa klaidos tikimybė 0,05, o imties galia (1-beta klaidos tikimybė) 0,95. Gauta šešiolikos dantų šaknų kanalų kiekvienai grupei minimali imtis. Tyrimui atrinkti apatinio žandikaulio pirmieji krūminiai dantys turintys atskirus mezialinių šaknų kanalus, visiškai susiformavusiomis šaknų viršūnėmis bei vidutiniškai lenktais dantų šaknų kanalais (10 – 20 °), įvertintais rentgenologiškai *Schneider* metodu.[478]

Atvertos ir suformuotos tiriamųjų dantų endodontinės ertmės, naudojant turbininį antgalį ir *EndoAccess* deimantinius grąžtus (*Dentsply Sirona, Ballaigues, Switzerland*), gausiai aušinant vandeniu. Du atskiri dantų šaknų kanalai buvo patvirtinti kliniškai ir rentgenologiškai, 10 dydžio rankiniais endodontiniais *K-File* (angl.) (*Dentsply Maillefer, Ballaiques, Switzerland*) instrumentais nustatytas dantų šaknų kanalų DI: į šaknies kanalą įvestas rankinis endodontinis instrumentas, kol instrumento viršūnė tapo matoma už šaknies viršūninio susiaurėjimo, naudojant 10x didinimą mikroskopu (*OPMI Pico, Carl Zeiss, Oberkochen, Germany*). Chemomechaninio paruošimo DI buvo nustatomas padidinus nustatytą darbinį ilgį 2 mm už šaknies viršūnės, siekiant atkartoti transportaciją su perforacija viršūniniame šaknies trečdalyje. Visi mezialiniai dantų šaknų kanalai buvo perforuojami 2 mm už šaknies viršūninio susiaurėjimo. Dantų šaknų kanalai pirmiausia išplatinti 15 ir 20 dydžio rankiniais endodontiniais *K-Flexofile* (*Dentsply Maillefer, Ballaiques, Switzerland*) instrumentais. Chemomechaninis šaknų kanalų paruošimas atliktas naudojant *ProTaper NEXT* (*Dentsply Maillefer, Ballaiques, Switzerland*) mašininius endodontinius instrumentus pagal gamintojo nurodytą seką: X1 (17/0,04), X2 (25/0,06), X3 (30/0,07), X4 (40/0,06) ir X5 (50/0,06), taikant 300 apsisukimų per minutę greitį ir 1 Ncm jėgos momentą, *X-Smart* (*Dentsply Sirona, Ballaiques, Switzerland*) endodontiniu motoru.

Po kiekvieno instrumento dantų šaknų kanalai plauti 5 ml 3 % NaOCl (*Ultradent Products Inc., South Jordan, UT, USA*). Atlikus chemomechaninį paruošimą, dantų šaknų kanalai plauti 5 ml 17 % EDTA rūgštimi (*Ultradent Products Inc., South Jordan, UT, USA*) 2 minutes bei 5 ml steriliu distiliuotu vandeniu. Plovimui naudota 29-G NaviTip adata (*Ultradent Products Inc., South Jordan, UT, USA*). Paruošti dantų modeliai fiksuoti A tipo silikono medžiagoje (*3M Express, 3M ESPE, Seefeld, Germany*), suformuojant blokelius iki cemento-emalio ribos, siekiant sukurti į klinikinę situaciją panašesnes sąlygas atliekant dantų šaknų kanalų užpildymą ir apriboti dantų šaknų išorinio paviršiaus matomumą.

1.1.2.2. Dantų šaknų kanalų užpildymas

Tiriamųjų mėginių atsitiktiniam paskirstymui į keturias grupes naudota atsitiktinio paskirstymo programinė įranga (www.random.org): 10 dantų/20 kanalų kiekvienoje grupėje, kurios suskirstytos pagal naudojamas dantų šaknų kanalų užpildymo medžiagas ir metodikas:

- BR/VK grupė — dantų šaknų kanalai užpildyti *BioRoot RCS* (*Septodont, Saint-Maur-des-Fosses, France*) HKSC ir vienu gutaperčios kaiščiu *ProTaper NEXT* X5 (*Dentsply Sirona, Ballaigues, Switzerland*). Gutaperčios kaiščių 4 mm viršūnės buvo nupjauti steriliu skalpeliu, siekiant sukurti gutaperčios sučiupimo efektą maždaug 2 mm nuo perforuotos šaknies viršūninės angos. *BioRoot RCS* HKSC sumaišytas pagal gamintojo instrukcijas, supakuotas į sterilų Skini švirkštą (*Ultradent Products Inc., South Jordan, UT, USA*) ir iškart sušvirkštas į šaknies kanalą, naudojant plastikinę *Capillary Tip* (*Ultradent Products Inc., South Jordan, UT, USA*) adatą. Adatos viršūnė įvesta maždaug 2 mm nuo perforuotos šaknies viršūnės, švirkšto stūmoklis švelniai spaudžiamas ir tuo pačiu metu adata atitraukiama nuo viršūnės užpildant šakies kanalą iki kanalo įeigos takiu *BioRoot RCS* HKSC. Sušvirkštus *BioRoot RCS* HKSC takų cementą, pritaikytas gutaperčios kaištis buvo padengiamas plonu HKSC sluoksniu ir švelniai įstumiamas į šaknies kanalą bei fiksuojamas maždaug 2 mm nuo perforuotos šaknies viršūninės angos.
- BR/VK-UA grupė — šaknų kanalai užpildyti naudojant takų HKSC *BioRoot RCS* ir vieną gutaperčios kaištį *ProTaper NEXT* X5 (*Dentsply Sirona, Ballaigues, Switzerland*), papildomai atliekant ultragarsinę užpildo aktyvaciją. Gutaperčios kaištis buvo parinktas ir pritaikytas bei takus HKSC sušvirkštas į šaknies kanalą identiškai BR/VK grupei. Užpildžius danties šaknies kanalą takiu HKSC, naudotas ET25

(*Ultradent Products Inc., South Jordan, UT, USA*) ultragarsinis instrumentas, pritvirtintas Ultrawave XS ultragarsiniame aparate (*Ultradent Products Inc., South Jordan, UT, USA*). Ultragarsinis instrumentas tiesiogiai įvedamas į šaknies kanalą, kuriame yra *BioRoot RCS* HKSC, maždaug 2 mm nuo viršūninės angos. Atlikta 10 s ultragarsinė aktyvacija vidutiniu stiprumu, naudojant *Reflex* (*Ultradent Products Inc., South Jordan, UT, USA*) technologiją, kuri automatiškai realiu laiku leidžia koreguoti ultragarsinio antgalio dažnį nuo 28 iki 36 kHz. Po aktyvacijos pritaikytas gutaperčios kaištis padengiamas plonu *BioRoot RCS* HKSC sluoksniu, švelniai įvedamas į šaknies kanalą ir fiksuojamas maždaug 2 mm nuo perforuotos šaknies viršūninės angos.

- MF grupė — dantų šaknų kanalai užpildyti takiu HCSC *MTA Flow*^{TM} (*Ultradent Products Inc., South Jordan, UT, USA*). Iš viso 0,19 g miltelių ir 3 lašai skysčio sumaišyti pagal gamintojo instrukcijas, siekiant gauti skystą cemento konsistenciją. Sumaišyta medžiaga supakuota į skaidrų Skini švirkštą ir medžiagos takumas patvirtintas švirkščiant per 29-G NaviTip adatą. Danties šaknų kanalai užpildyti takiu HKSC įstūmus adatos viršūnę maždaug 2 mm nuo perforuotos šaknies viršūnės, švelniai ir tolygiai spaudžiant švirkšto stūmoklį ir tuo pačiu metu atitraukiant nuo nustatyto DI, kol šaknies kanalas tolygiai užsipildė takiu HKSC.
- MF-UA grupė — dantų šaknų kanalai užpildyti takiu HKSC *MTA Flow*^{TM} (*Ultradent Products Inc., South Jordan, UT, USA*), papildomai atliekant ultragarsinę užpildo aktyvaciją. Takus HKSC įšvirkštas į šaknies kanalą identiškai MF grupei. Užpildžius šaknies kanalą takiu HKSC *Ultrawave* ET25 ultragarsinis instrumentas tiesiogiai įvedamas į šaknies kanalą, kuriame yra *MTA Flow*^{TM} takus HKSC, maždaug 2 mm nuo perforuotos šaknies viršūnės. Ultragarsinis instrumentas aktyvuotas 10 s 28–36 kHz dažniu ir galia, kaip nurodyta BR/VK-UA grupėje.

Po visų grupių tiriamųjų mėginių dantų šaknų kanalų užpildymo atliktos kontrolinės dantų šaknų rentgenogramos, siekiant įvertinti dantų šaknų kanalų užpildymo kokybę dvimatėje erdvėje. Danties šaknų kanalų užpildymo procedūra buvo kartojama, jei vertinant rentgenogramą nustatytas nehomogeniškas šaknies kanalo užpildymas, ir pakartotinai atliekamos kontrolinės dantų šaknų rentgenogramos. Gutaperčia nukaitinta šaknies kanalo įeigos srityje karščio nešikliu BR/VK ir BR/VK-UA grupėse. Visų grupių tiriamųjų mėginių endodontinės ertmės užpildytos laikinu užpildu *Cavit™-W* (*3M ESPE, Seefeld, Germany*) ir mėginiai pamerkti laikyti 37 °C

ir 100 % drėgmėje, siekiant užtikrinti ir palaikyti 37 °C aplinkos temperatūrą vieną savaitę ir sukurti sąlygas dantų šaknų kanalų užpildui sukietėti prieš tolesnę µKT analizę.

1.1.2.3. µKT skenavimas ir analizė

Tiriamieji dantys skenuoti prieš ir po šaknų kanalų užpildymo aukštos raiškos µKT skeneriu SkyScan 1272 (*Bruker, Kontich, Belgium*). Skenavimai atlikti taikant 100 kV šaltinio įtampą, 100 µA spindulio srovę, 9,9 µm izotropinę rezoliuciją, 0,4° kampo sukimąsi ir 1073 ms išlaikymą. Tyrimui naudotas 0,11 mm vario filtras, siekiant sumažinti galimus artefaktus. Gauti µKT vaizdai rekonstruoti naudojant NRecon v.1.7.1.0 programinę įrangą (*Bruker, Kontich, Belgium*), pritaikant 6 dydžio artefaktų sumažinimo indeksą bei 20 % spindulio korekciją.

CTAn v.1.14.4.1 programinė įranga (*Bruker, Kontich, Belgium*) naudota įvertinti dantų šaknų kanalų užpildymo kokybei viršūniniuose 5 mm. Programinėje įrangoje pasirinkti dantų šaknų kanalų kontūrai, kontrasto ir pilko atspalvio apimtis, siekiant išskirti dantų šaknų kanalų užpildus bei poras. Išskyrimas atliktas naudojantis tankio histograma, globalaus slenksčio metodika. Atlikta palyginamoji originalių ir segmentuotų vaizdų analizė, siekiant užtikrinti segmentacijos tikslumą. Šaknies kanalo tūris (C_{Vol}) apskaičiuotas iš µKT vaizdų, gautų prieš dantų šaknų kanalų užpildymą. Skenavimo vaizdai, gauti po dantų šaknų kanalų užpildymo, naudoti apskaičiuoti šaknies kanalo užpildo tūrį (F_{Vol}) ir uždarąsias poras (CP_{Vol}). Visas porų tūris (V_{Vol}) ir atvirų porų tūris (OP_{Vol}) apskaičiuoti remiantis šiomis formulėmis:

$$V_{Vol} = C_{Vol} - F_{Vol},$$
$$OP_{Vol} = V_{Vol} - CP_{Vol}.$$

Procentinė atvirų ($\%OP_{Vol}$) ir uždarų porų ($\%CP_{Vol}$) išraiška apskaičiuota remiantis šiomis formulėmis:

$$\%OP_{Vol} = OP_{Vol}/C_{Vol} \times 100,$$
$$\%CP_{Vol} = CP_{Vol}/C_{Vol} \times 100.$$

1.1.2.4. Statistinė analizė

Statistinė analizė atlikta naudojant *IBM SPSS Statistics* 29 versijos programinę įrangą (*IBM Corp., Armonk, N.Y., USA*). *Shapiro–Wilk* testas atskleidė, jog tyrimo duomenys neturi normaliojo skirstinio. Todėl skirtumai tarp tiriamųjų grupių analizuoti taikant neparametrinį *Kruskal–Wallis* testą.

Aptikus statistiškai reikšmingas p reikšmes, taikytas *Mann–Whitney* testas porų analizei. Pasirinktas reikšmingumo lygmuo, kai $p < 0,05$.

1.2. Takių HKSC biologinio suderinamumo analizė

Tyrimas atliktas vieno kalibruoto tyrėjo - Vilniaus universiteto Gyvybės mokslų centro laboratorijoje. Tyrimas yra registruotas ir patvirtintas Lietuvos bioetikos komiteto, protokolo nr. 158200-16-860-369, 2019-ųjų metų revizija. Tyrimui parama gauta iš Vilniaus universiteto Jaunųjų mokslininkų ir tyrėjų mokslo skatinimo fondo, protokolų numeriai: MSF-JM-4/2020 ir MSF-JM-14/2021.

1.2.1. Tiriamosios grupės ir medžiagos

Tiriamosios grupės:

- Neigiama kontrolinė grupė (NeigiamaKG) – žmogaus danties pulpos kamieninių ląstelių (ŽDPKL) kultūra, auganti augimo terpėje. Naudojama kaip kontrolinė atskaitos grupė be išorinių aplinkos veiksnių.
- Teigiama kontrolinė grupė (TeigiamaKG) – ŽDPKL ląstelių kultūra, auganti veikiama laikino užpildo (*IRM, Dentsply Tulsa Dental, USA*) ar vandenilio peroksido 250 µM ir 500 µM koncentracijų terpės filtratais. Ši grupė naudojama kaip kontrolinė grupė, kurioje sukuriamas vertinamas poveikis ŽDPKL ląstelių kultūrai – ląstelių nekrozė / apoptozė.
- Kontrolinė HKSC veikiama ŽDPKL ląstelių grupė (ProRootKG) – ŽDPKL ląstelių grupė, veikiama iš šviežiai sumaišyto *ProRoot MTA* (*Dentsply Tulsa Dental, USA*) HKSC išskirtu filtratu.
- Tiriamųjų takių HKSC veikiamų ląstelių grupės:
    - *MTA Flow*^{TM} veikiama ŽDPKL ląstelių kultūra (MF) – ŽDPKL ląstelių kultūra, veikiama filtratais, išskirtais iš šviežiai sumaišyto *MTA Flow*^{TM} (*Ultradent Products Inc., South Jordan, UT, USA*) tirštos konsistencijos cemento.
    - *MTA Flow*^{TM} *White* veikiama ŽDPKL ląstelių kultūra (MFWhite) – ŽDPKL ląstelių kultūra, veikiama filtratais, išskirtais iš šviežiai sumaišyto *MTA Flow*^{TM} *White* (*Ultradent Products Inc., South Jordan, UT, USA*) tirštos konsistencijos cemento.

TeigiamaKG, ProRootKG, MF ir MFWhite grupės išskirstytos į 100 %, 50 %, 25 % ir 12,5 % grupes, pagal naudojamą filtrato koncentraciją. Tyrime naudojamų HKSC medžiagų sudėtis nurodyta 1 lentelėje.

1 lentelė. HKSC medžiagos, naudojamos biologinio suderinamumo analizei, ir jų sudėtis.

Medžiagos tipas	***Patentinis pavadinimas***	***Miltelių sudėtis***	***Skystis***	***Gamintojas***
Portlando cementas	*ProRoot® MTA*	Portlando cementas, kalcio sulfato dihidratas, tetrakalcio aliuminoferitas, bismuto oksidas (rentgenokontrastiškumą suteikianti medžiaga)	Distiliuotas vanduo	*Dentsply Tulsa Dental*, USA
Takus HKSC	*MTA Flow™*	di- ir trikalcio silikatas, kalcio sulfatas, silicio dioksidas, bismuto oksidas (rentgenokontrastiškumą suteikianti medžiaga)	Vandenyje tirpus silikono pagrindo gelis	*Ultradent*, USA
	MTA Flow™ White	di- ir trikalcio silikatas, kalcio sulfatas, silicio dioksidas, tantalo oksidas (rentgenokontrastiškumą suteikianti medžiaga)		

1.2.2. Ląstelių kultūra

Paruošta pagal tiekėjo nurodymus ir išsėta žmogaus danties pulpos kamieninių ląstelių kultūra *Poietics™ Human Dental Pulp Stem Cells* (ŽDPKL) (katalogo nr. PT-5025, *Lonza*). Ląstelių kultūra auginta *alpha-medium essential* augimo terpėje (*αMEM,* Gibco, katalogo nr. 12561056), papildytoje 10 % fetaliniu veršelių serumu (*FBS; Gibco*, katalogo nr. A3160802) ir 1 % streptomicino/penicilino tirpalu (*Gibco*, katalogo nr. 10378016). Ląstelių kultūros augintos 37 °C, 5 % CO_2 inkubatoriuje. ŽDPKL monosluoksnis pakeliamas inkubuojant ląsteles 3 minutes 37 °C EDTA-tripsino (0,25 %) tirpale (*Gibco*, katalogo nr. 25200056). Tyrimams naudojami 3 - 5 ląstelių pasažai.

1.2.3. Tiriamųjų mėginių (filtratų) paruošimas

Takūs HKSC *MTA Flow™* ir *MTA Flow™ White* paruošti laminare, naudojant sterilius instrumentus ir vadovaujantis gamintojo instrukcijomis, siekiant gauti tirštos konsistencijos cementą: sumaišyti 2 dideli šaukšteliai

miltelių (0,26g) ir 3 lašai skysčio. Kiekvienai grupei paruoštas maždaug 2,12 g svorio / 1,3 cm^3 tūrio HKSC medžiagos kiekis ir perkeltas į 50 mm skersmens sterilią Petri lėkštelę. Takus HKSC tolygiai paskirstytas po Petri lėkštelę sterilia vata, suvilgyta augimo terpe. Iškart po takaus HKSC paskirstymo 13 ml augimo terpės perkelta į kiekvieną Petri lėkštelę. Tyrime taikytas ISO 10993-12:2021 standarte nurodytas cemento paviršiaus ir augimo terpės filtratui ruošti santykis: 1638 mm^2 paviršiaus plotas ir 13 ml augimo terpės tūris, arba 126 mm^2/ml. Takūs HKSC inkubuoti augimo terpėje 48 val. 37 ◦C temperatūroje ir 95 % drėgmėje be CO_2. Po inkubacijos filtratas nuimtas nuo takių HKSC, centrifuguota 15000 RPM (CL10 centrifūga, *Thermo Scientific*) ir filtruota steriliais 0,22 µm porų dydžio filtrais. Gavus 100 % takaus HKSC filtratą, paruošti 100 %, 50 %, 25 % ir 12.5 % filtratų skiedimai pridedant atitinkamus kiekius augimo terpės. Filtratai buvo iškart naudojami tolesniems tyrimams.

1.2.4. pH matavimai

pH matavimai atlikti 100 % filtratams iškart po inkubacijos ir nuėmimo nuo takių HKSC, naudojant pH analizės juosteles (*Johnson Test Papers Ltd, Tividale, UK*). Kiekvienos tiriamosios grupės pH matavimai atlikti tris kartus ir išvestas vidurkis.

1.2.5. HKSC poveikio ląstelių metaboliniam aktyvumui tyrimas

Tyrimui naudotos 3 – 5 pasažų ŽDPKL kultūros (5000 ląstelių/cm^2), išsėtos į 96 duobučių sterilias plokšteles ir inkubuotos 24 val. augimo terpėje. Po inkubacijos paruošti filtratai perkelti ant paruoštų ŽDPKL kultūrų ir inkubuota 2, 24, 48, 72, 96 ir 120 valandų, atnaujinant terpę kas 3 dienas ir įvertinant kiekvienos grupės ląstelių gyvybingumą kolorimetriniu MTT metodu. Nuliniame atskaitos taške bei kiekviename laiko intervale 100 µl 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolio bromido (*MTT, Sigma-Aldrich Co.*) tirpalo (0,5 mg/ml, ištirpinto augimo terpėje) įdėta į kiekvieną duobutę prieš tai pašalinus augimo terpę. Tiriamieji mėginiai inkubuoti 3 val. 37 °C temperatūroje, 5 % CO_2 aplinkoje. MTT tirpalas pašalintas ir į kiekvieną duobutę įdėta 100 µl DMSO (≥99,5 %, *BioScience Grade*). Kiekvieno tiriamojo mėginio gautas tirpalas perkeltas į matavimui skirtą sterilią plokštelę ir matuota spektrofotometru (*Varioskan® Flash, Thermo Scientific Waltham, Massachusetts, MA, USA*), naudojant 570 nm bangos ilgį. Ląstelių gyvybingumo rezultatai pateikti procentine / santykine išraiška pagal neigiamą kontrolinę grupę. Tyrimo metu augimo terpė/veikiami cementų

filtratai buvo atnaujinami kas 3 dienas, prieš tai pašalinant 50 µl duobutėje esančio tirpalo ir įdedant 100 µl šviežios augimo terpės / cemento filtrato.

1.2.6. Ląstelių žūties pobūdžio įvertinimas naudojant *Annexin V-FITC* ir tėkmės citometriją

Siekiant įvertinti, kokiu – apoptozės ar nekrozės būdu žūva ląstelės, ŽDPKL po 24 val. inkubacijos 50 % takių HKSC filtratais dažytos dviem *Annexin V* ir propidžio jodido (PI) dažais, pagal gamintojo instrukcijas naudojant *Annexin V-FITC* apoptozės nustatymo rinkinį (*Annexin V-FITC Apoptosis Detection Kit*, katalogo nr. 88-8005-72; *eBioscience™*), ir analizuotos tėkmės citometru (*BD Bioscience FacsCanto II Flow Cytometer, Franklin Lakes, New Jersey, U.S.*), naudojant *FCS Express* programinę įrangą (*De Novo Software, Los Angeles, CA*). Analizuojant HKSC užpildų filtratų įtaką ŽDPKL vertintos: gyvybingos, nekrozės ir ankstyvosios / vėlyvosios apoptozės apimtos ŽDPKL. Atlikti trys nepriklausomi tyrimai, atliekant keturis vidinius pakartojimus kiekvieno tyrimo metu ir išvestas matavimų vidurkis.

1.2.7. Ląstelių kokybinė ir kiekybinė analizė

Trečio pasažo ŽDPKL (10 000 ląstelių/cm^2) išsėtos į 96 duobučių plokšteles ir inkubuotos 24 val. Po inkubacijos ląstelės paveiktos tiriamųjų grupių filtratais ir inkubuotos 2, 24, 48, 72, 96 ir 120 val. Nuliniame atskaitos taške bei kiekviename tiriamajame laiko etape takių HKSC skirtingų koncentracijų filtratų poveikis ŽDPKL morfologiniams požymiams fiksuotas optiniu mikroskopu *Olympus IX51* (*Olympus UK Ltd.*). Įvertinti ląstelių kiekybiniai morfologiniai ypatumai, išmatuojant ląstelių ilgį, plotį, ilgio ir pločio santykį bei ląstelių skaičių plote. Vaizdinių duomenų analizei naudota *ImageJ* programinė įranga (*ImageJ* 1.8.0_172), pagal anksčiau aprašytą M. Alksnė ir kt. protokolą.[479]

1.2.8. Statistinė analizė

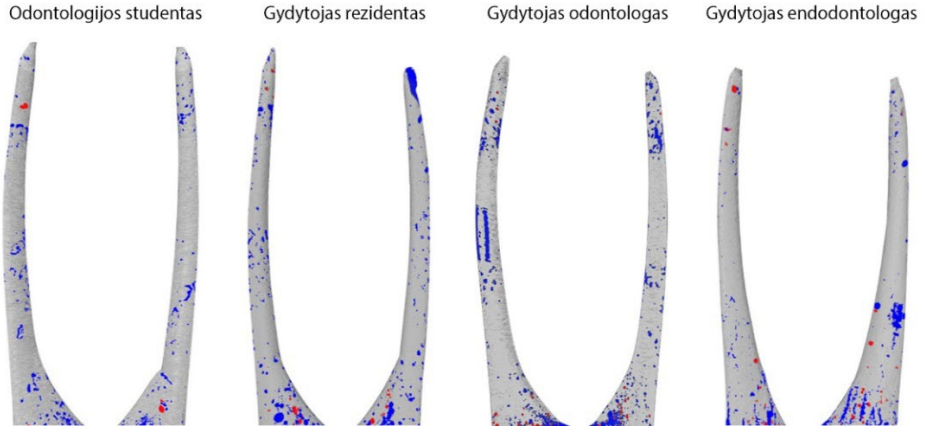
Duomenų skirstinys įvertinas taikant *Shapiro-Wilk* testą (normaliojo skirstinio sąlygos patenkintos). Dispersijų homogeniškumas įvertintas *Levene* testu. Rezultatų išskirtys identifikuotos stačiakampėmis diagramomis. Atliktos trijų krypčių ir dviejų krypčių ANOVA, siekiant įvertinti sąveiką tarp takių HKSC tipo, koncentracijų ir/ar laiko. Visi paprastieji takių HKSC koncentracijų ir laiko taškų porų palyginimai analizuoti taikant *Tukey HSD* metodiką su *Bonferroni* korekcija. Pateiktuose grafikuose stulpelio aukštis

žymi rezultato vidurkį, paklaidų juostos nurodo standartinį nuokrypį (SD). Statistiškai reikšmingi skirtumai tarp grupių nurodomi atitinkamu žymėjimu: * – $p < 0,05$, ** - $p < 0,01$ ir *** - $p < 0,001$. Kiekvieno tiriamojo etapo tyrimai pakartoti nepriklausomai tris kartus ir išvestas rezultatų vidurkis. Statistinei analizei naudotos *IBM SPSS Statistics* 29 versijos (*IBM Corp., Armonk, N.Y., USA*) ir *GraphPad Prism* 9 versijos (*GraphPad Software, San Diego, California, USA*) programinės įrangos paketai.

2. REZULTATAI

2.1. Skirtingą klinikinę patirtį turinčių asmenų sukuriamų dantų šaknų kanalų užpildų porėtumo µKT analizė

Atlikus µKT analizę, nustatytos skirtingo tūrio poros: vidinės poros - dantų šaknų kanalų užpilduose, ir išorinės poros - tarp takaus HKSC užpildo bei danties šaknies sienelės. Vertinant porų pasiskirstymą pagal tipą nustatyta, jog reikšmingai dominuoja atviros poros, nepriklausomai nuo tirtų mėginių bei šaknų kanalų trečdalių. Reprezentatyvūs atvirų ir uždarų porų vaizdai pateikti 1 paveiksle. Gutaperčios kaiščiuose porų nerasta.



1 paveikslas. Skirtingų tyrėjų grupių trimatės reprezentatyvios vieno kaiščio metodikos rekonstrukcijos, rodančios atvirų (pažymėta mėlyna spalva) ir uždarų (pažymėta raudona spalva) porų pasiskirstymą.

Dantų šaknų kanalų užpildų porėtumo tūrinės analizės rezultatai pateikiami Table lentelėje. Atvirų ir uždarų porų pasiskirstymas reikšmingai skiriasi tarp grupių vainikiniuose trečdaliuose (atitinkamai $p = 0,017$ ir $p = 0,02$). Vertinant porų lyginamąją analizę, procentinė atvirų porų išraiška reikšmingai nesiskyrė tik tarp GR ir GE grupių, o uždarų porų – tarp OS ir GR grupių ($p > 0,05$). Viduriniuose šaknų trečdaliuose visų tiriamųjų grupių atvirų porų pasiskirstymas ir kiekis išlieka panašus ($p = 0,06$). Kita vertus, uždarų porų pasiskirstymas viduriniuose šaknų trečdaliuose reikšmingai skyrėsi tarp grupių ($p = 0,014$). Nors skirtumas nebuvo statistiškai reikšmingas, GO grupėje vidutinė atvirų porų reikšmė viduriniuose šaknų trečdaliuose buvo didesnė. Porų lyginamosios analizės rezultatai rodo, kad uždarų porų kiekis reikšmingai nesiskyrė tik tarp OS ir GR grupių ($p > 0,05$). Viršūniniuose šaknų

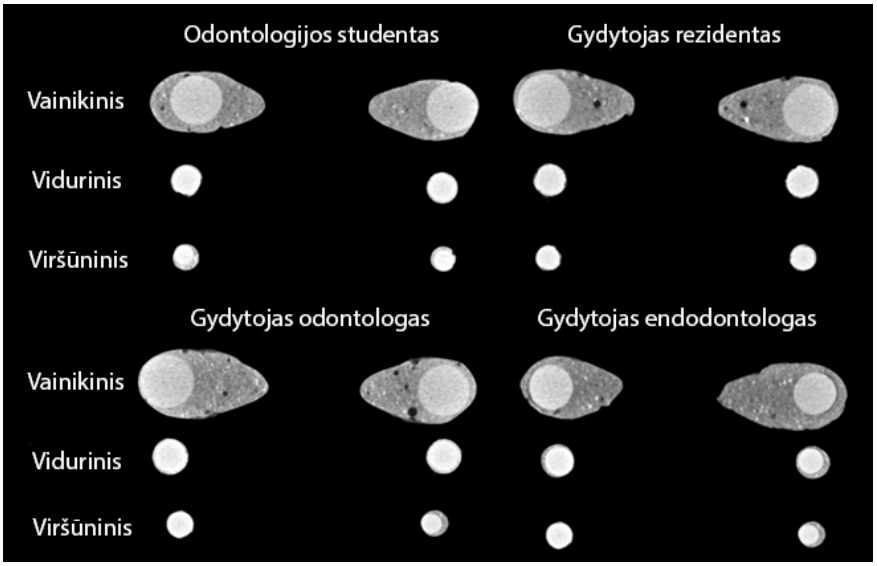
kanalų trečdaliuose reikšmingų skirtumų tarp grupių nenustatyta vertinant tiek atviras (p = 0,56), tiek uždaras (p = 0,12) poras.

2 lentelė. Skirtingų grupių atvirų ir uždarų porų vainikiniuose, viduriniuose ir viršūniniuose trečdaliuose vidutinės reikšmės (%) ir standartiniai nuokrypiai (SD).

Grupė	***N***	***Vainikinis trečdalis***		***Vidurinis trečdalis***		***Viršūninis trečdalis***	
		Atviros poros	*Uždaros poros*	*Atviros poros*	*Uždaros poros*	*Atviros poros*	*Uždaros poros*
OS	14	2,415± 3,071^{A}	0,032± 0,029^{A}	2,970± 3,361^{A}	0,001± 0,003^{A}	8,140± 6,602^{A}	0,208± 0,191^{A}
GR	14	3,567± 2,181^{B}	0,026± 0,030^{A}	5,389± 3,158^{A}	0,003± 0,008^{A}	9,778± 6,324^{A}	0,261± 0,509^{A}
GO	14	8,792± 7,973^{C}	0,369± 0,170^{B}	7,672± 7,051^{A}	0,053± 0,061^{B}	15,940± 10,001^{A}	0,169± 0,260^{A}
GE	14	3,535± 3,088^{B}	0,090± 0,094^{C}	2,592± 1,755^{A}	0,019± 0,029^{C}	10,861± 7,716^{A}	0,344± 0,378^{A}

Grupės, pažymėtos ta pačia viršutiniojo indekso raide tame pačiame stulpelyje, tarpusavyje reikšmingai nesiskiria (porų lyginamoji analizė *Mann-Whitney* testu; p > 0,05).

Skersiniai dvimačiai keturių tiriamųjų grupių reprezentatyvūs pjūviai skirtinguose šaknų kanalų trečdaliuose vaizduojami 2 paveiksle. µKT analizė parodė visų grupių dantų šaknų kanalų užpildų didžiausią porėtumą, tiek uždarų porų, tiek atvirų porų kiekį, viršūniniuose trečdaliuose. Taip pat nustatyti statistiškai reikšmingi atvirų ir uždarų porų pasiskirstymo tose pačiose grupėse skirtumai lyginant visus dantų šaknų kanalų trečdalius (p < 0,05).



2 paveikslas. Atsitiktinių OS, GR, GO ir GE tiriamųjų grupių mėginių dvimačiai skersiniai dantų šaknų kanalų pjūvių vaizdai visuose trečdaliuose, rodantys atviras ir uždaras poras dantų šaknų kanalų užpilduose.

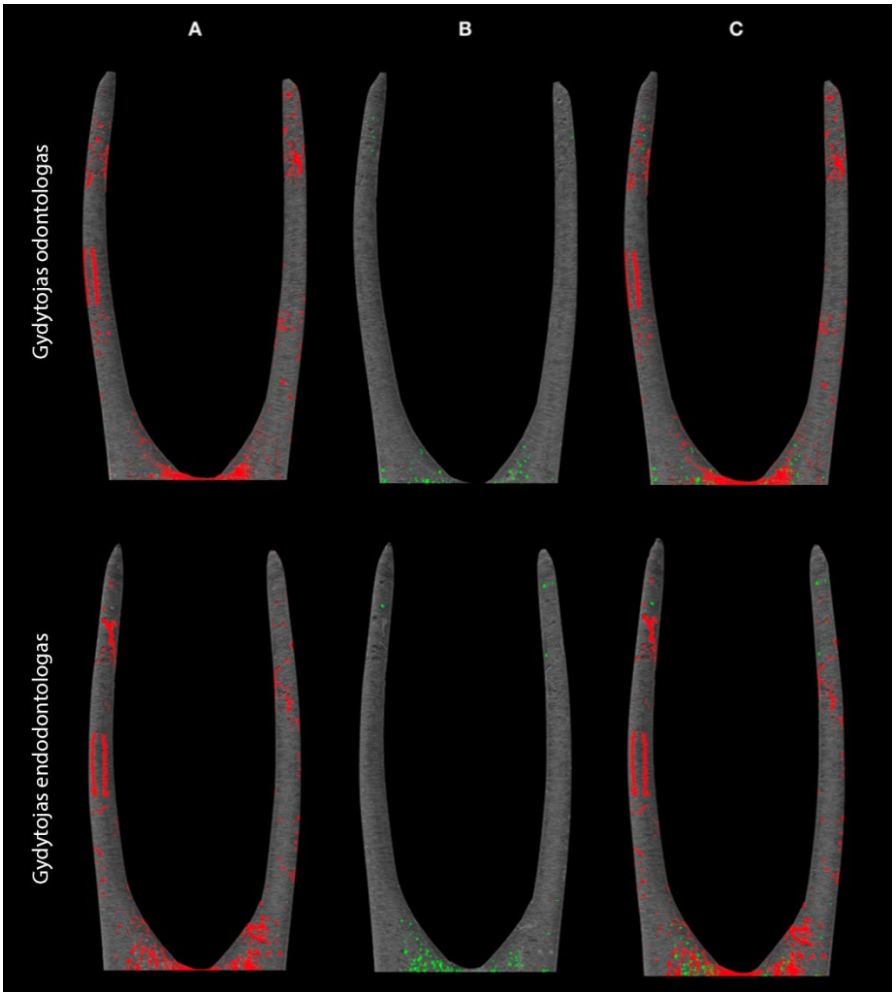
Atliekant porų lyginamąją analizę, kurios rezultatai pateikiami 3 lentelėje, statistiškai reikšmingų skirtumų nenustatyta tik tarp vainikinių ir vidurinių trečdalių atvirų porų pasiskirstymo: atvirų porų vidutinė reikšmė išliko panaši visose tiriamosiose grupėse ($p > 0,05$).

3 lentelė. Porų visuose šaknų kanalų trečdaliuose lyginamosios analizės p reikšmės.

Grupė	***Trečdaliai***	***Atviros poros***	***Uždaros poros***
OS	Vainikinis ir vidurinis	0,363	0,001*
	Vainikinis ir viršūninis	0,001*	0,004*
	Vidurinis ir viršūninis	0,001*	0,001*
GR	Vainikinis ir vidurinis	0,056	0,007*
	Vainikinis ir viršūninis	0,001*	0,024*
	Vidurinis ir viršūninis	0,006*	0,002*
GO	Vainikinis ir vidurinis	0,390	0,002*
	Vainikinis ir viršūninis	0,034*	0,041*
	Vidurinis ir viršūninis	0,010*	0,208*
GE	Vainikinis ir vidurinis	0,197	0,005*
	Vainikinis ir viršūninis	0,002*	0,001*
	Vidurinis ir viršūninis	0,001*	0,001*

*Nurodo statistiškai reikšmingą skirtumą (porų lyginamoji *Wilcoxon* testo analizė; $p < 0,05$).

Užpildytų dantų šaknų kanalų rekonstrukciniai trimačiai vaizdai, rodantys dominuojančias atviras poras GO ir GE grupėse, pavaizduoti 3 paveiksle.



3 paveikslas. GO ir GED grupių dantų šaknų kanalų užpildų trimatės rekonstrukcijos, rodančios atvirų (A, pavaizduota raudona spalva), uždarų (B, pavaizduota žalia spalva) porų pasiskirstymą ir bendrąjį porėtumą (C, perdengtos A ir B rekonstrukcijos).

2.2. Ultragarsinės aktyvacijos įtakos apatinio žandikaulio pirmųjų krūminių dantų perforuotų mezialinių šaknų viršūnių užpildo porėtumui analizė, kai naudojami skirtingi HKSC užpildai ir jų metodikos

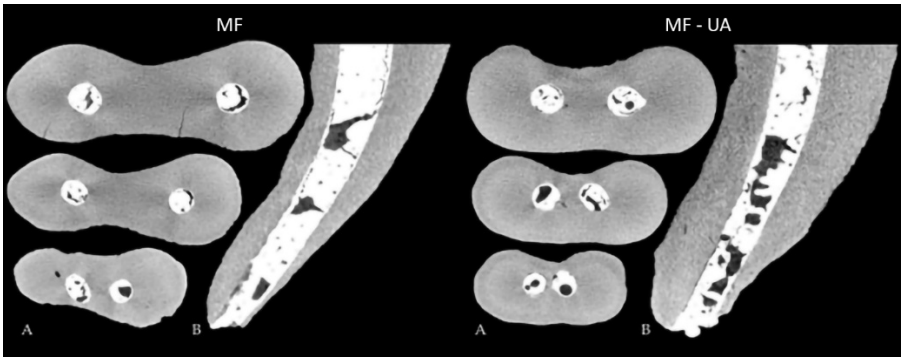
Visos taikytos dantų šaknų kanalų užpildymo metodikos lėmė užpildų porėtumą 5 mm nuo šaknies viršūnės. Porų formos ir dydžių įvairovė buvo matoma visuose viršūniniuose užpilduose, ryškiausiai vyraujant atviroms poroms. Kiekybinės tūrinės atvirų ir uždarų porų analizės rezultatai yra apibendrinti 4 lentelėje. µKT kontrolinė mėginių analizė parodė, kad dantų šaknų kanalų tūriai prieš dantų šaknų kanalų užpildymą neturėjo reikšmingų tūrinių dispersijos skirtumų ($p = 0,34$), nurodant pirminį tiriamųjų grupių tolygumą. Nepaisant to, dantų šaknų kanalų užpildų porų pasiskirstymas tarp tiriamųjų grupių reikšmingai skyrėsi ($p < 0,05$).

4 lentelė. Skirtingų tiriamųjų grupių atvirų ir uždarų porų vidutinės reikšmės (%) ir standartiniai nuokrypiai (SD).

Grupė	*N*	Atviros poros	Uždaros poros
BR/VK	20	$3,374 \pm 2,751^{A}$	$0,061 \pm 0,080^{A}$
BR/VK-UA	20	$3,390 \pm 3,428^{A}$	$0,066 \pm 0,070^{A}$
MF	20	$18,832 \pm 3,334^{B}$	$0,292 \pm 0,226^{B}$
MF-UA	20	$29,075 \pm 9,440^{C}$	$0,923 \pm 0,684^{C}$

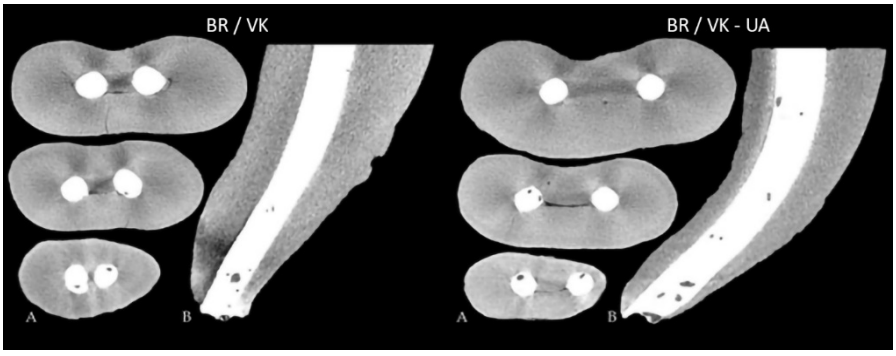
Grupės, pažymėtos ta pačia viršutiniojo indekso raide tame pačiame stulpelyje, tarpusavyje reikšmingai nesiskiria (porų lyginamoji analizė *Mann-Whitney* testu; $p > 0.05$).

Reikšmingai didesnis kiekis atvirų ir uždarų porų buvo nustatytas MF grupėse (su ultragarsine aktyvacija ir be jos), palyginti su BR/VK ir BR/VK-UA grupėmis ($p < 0,05$). Skirtumai tarp MF ir MF-UA grupių nustatyti kaip statistiškai reikšmingi ($p < 0,05$), o didžiausias užpildo porėtumas nustatytas MF-UA grupėje. Tiriamųjų grupių reprezentatyvūs vaizdai pateikti 4 paveiksle.



4 paveikslas. Reprezentatyvūs atsitiktinių tiriamųjų mėginių skersiniai pjūviai 5 mm, 3 mm ir 1 mm nuo perforuotos šaknies viršūnės (A) bei išilginiai pjūviai (B), rodantys porų pasiskirstymą MF ir MF-UA grupėse.

Statistiškai reikšmingų skirtumų tarp tiriamųjų BR/VK ir BR/VK-UA grupių mėginių nenustatyta. Šiose grupėse šaknų kanalų užpildų atvirų (p = 0,82) ir uždarų (p = 0,57) porų pasiskirstymas išliko panašus nepriklausomai nuo bendrai pastebėto mažesnio BR/VK grupės porėtumo. VK metodika ir BioRoot RCS užpildytų dantų šaknų kanalų reprezentatyvūs vaizdai pateikti 5 paveiksle.

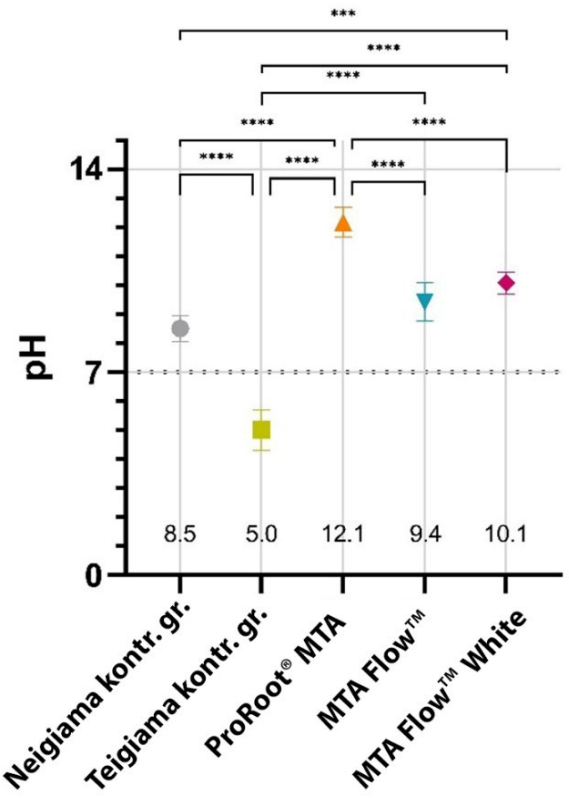


5 paveikslas. Reprezentatyvūs atsitiktinių tiriamųjų mėginių skersiniai pjūviai 5 mm, 3 mm ir 1 mm nuo perforuotos šaknies viršūnės (A) bei išilginiai pjūviai (B), rodantys dantų šaknų kanalų užpildymo kokybę ir homogeniškumą BR/VK ir BR/VK-UA grupėse.

2.3. Takių HKSC užpildų biologinio suderinamumo analizė

2.3.1. Takių HKSC tirpalų charakteristikos

Šviežiai sumaišytų MFWhite ir ProRootKG 100 % filtratų pH reikšmės statistiškai reikšmingai skyrėsi nuo kontrolinės grupės NeigiamaKG ($p < 0,05$). Kita vertus, MF nulėmė šiek tiek mažesnį pH pokytį, kuris reikšmingai nesiskyrė nuo neigiamos kontrolinės grupės ($p > 0,05$). Detali šviežiai sumaišytų tiriamųjų grupių filtratų pH analizė pateikta 6 paveiksle.



* - žymi statistiškai reikšmingus skirtumus tarp grupių (* $p < 0,05$, ** $p < 0,01$ ir *** $p < 0,001$), N = 3.

6 paveikslas. Šviežiai sumaišytų HKSC ir kontrolinių grupių 100 % filtratų vidutinės pH reikšmės.

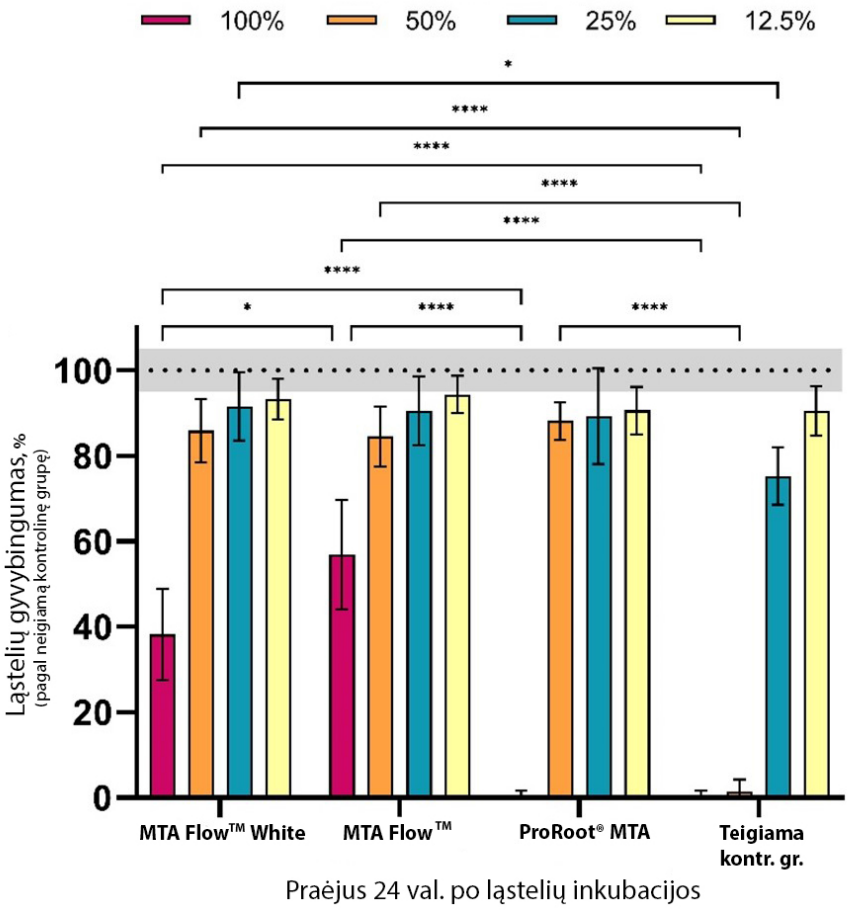
2.3.2. *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* tiriamųjų HKSC užpildų citotoksiškumo ŽDPKL analizė

ŽDPKL gyvybingumas buvo reikšmingai paveiktas šviežiai sumaišytų tiriamųjų cementų, o poveikis reikšmingai priklausė nuo veikiamo filtrato koncentracijos. Visų koncentracijų sukietėjusių takių HKSC filtratai neturėjo reikšmingos įtakos ląstelių kultūrų gyvybingumui ($p > 0,05$).

12,5 %, 25 % ir 50 % koncentracijų tiriamųjų HKSC filtratų grupėse ŽDPKL gyvybingumas išliko didesnis kaip 80 %. 100 % abiejų tiriamųjų takių HKSC MF ir MFWhite paveiktos ŽDPKL kultūros pasižymėjo reikšmingai mažesniu ląstelių gyvybingumu ($p < 0,05$), palyginti su neigiama kontroline grupe. Kita vertus, teigiamoje kontrolinėje grupėje veikiant 50 % koncentracijos filtratu gyvybingų ląstelių nebuvo aptikta, o 25 % filtratas reikšmingai sumažino ląstelių gyvybingumą, palyginti su neigiama kontroline grupe ($p < 0,05$).

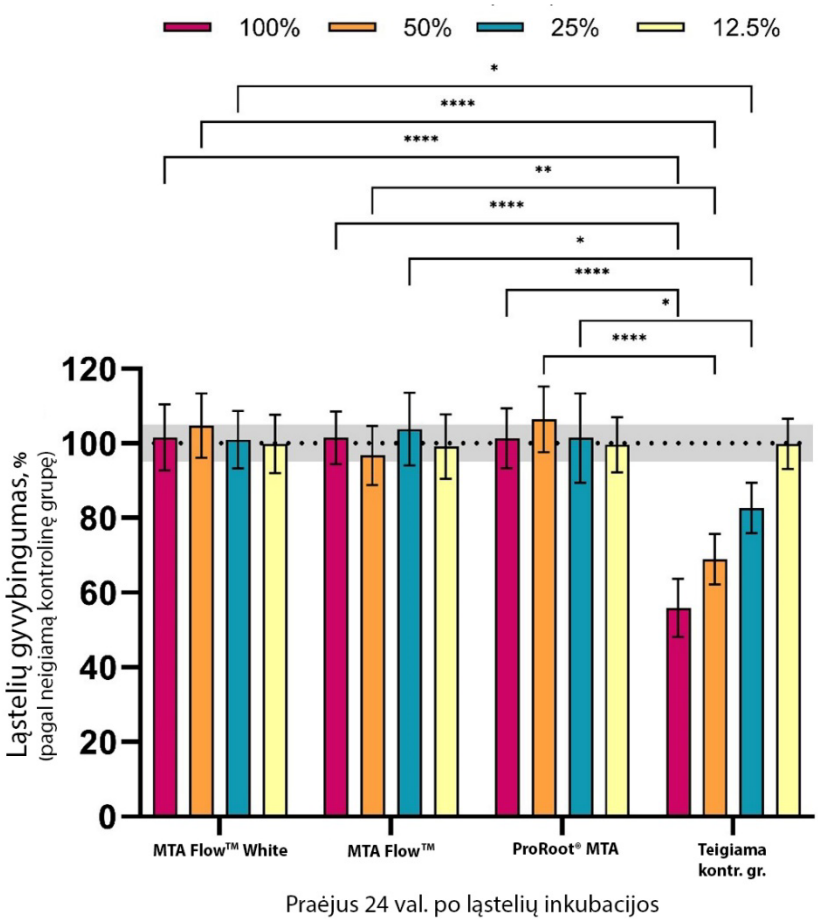
Vertinant MTT metodu po 24 val. poveikio MF ir MFWhite visų koncentracijų filtratais, reikšmingų skirtumų tarp grupių nenustatyta ($p > 0,05$). Abiejų tiriamųjų grupių cementų filtratai sukūrė panašų citotoksinį poveikį ŽDPKL ($p < 0,05$). Vis dėlto, veikiant ŽDPKL kultūras MF ir MFWhite 100 % koncentracijos filtratais, gautas reikšmingai mažesnis citotoksiškumas nei kontrolinio HKSC cemento *ProRoot MTA* grupėje ($p < 0,05$): paveiktų naujos kartos takių HKSC MF ir MFWhite 100 % koncentracijos filtratais ŽDPKL gyvybingumas buvo 38-57 %, o paveiktų kontrolinio *ProRoot MTA* filtratu ŽDPKL gyvybingumas nukrito iki 0,4-1 %. Statistiškai reikšmingų skirtumų tarp tiriamųjų grupių HKSC ir kontrolinio HKSC 50 %, 25 % ir 12,5 % koncentracijų filtratų poveikio ŽDPKL kultūroms nenustatyta. Detali šviežiai sumaišytų tiriamųjų ir kontrolinių grupių filtratų poveikio ŽDPKL gyvybingumui analizė vaizduojama 7 paveiksle.

Analizuojant filtratus, nuimtus nuo sukietėjusių tiriamųjų ir kontrolinių HKSC, statistiškai reikšmingų skirtumų nenustatyta, lyginant su neigiama kontroline grupe ($p > 0,05$). Sukietėjusių HKSC filtratų įtakos ŽDPKL rezultatai pateikti 8 paveiksle.



Rezultatai pateikti kaip procentinė išraiška nuo neigiamos kontrolinės grupės (100 % atskaitos linija ±SD pilko atspalvio sritis). * - rodo statistiškai reikšmingus skirtumus tarp grupių (* $p < 0,05$, ** $p < 0,01$ ir *** $p < 0,001$).

7 paveikslas. MTT ląstelių analizė: šviežiai sumaišytų takių HKSC ir kontrolinių grupių skirtingų koncentracijų filtratų poveikis ŽDPKL mitochondrijų fermentų aktyvumui po 24 val. inkubacijos.



Rezultatai pateikti kaip procentinė išraiška nuo neigiamos kontrolinės grupės (100 % atskaitos linija ±SD pilko atspalvio sritis). * - rodo statistiškai reikšmingus skirtumus tarp grupių (* $p < 0,05$, ** $p < 0,01$ ir *** $p < 0,001$).

8 paveikslas. MTT ląstelių analizė: sukietėjusių takių HKSC ir kontrolinių grupių skirtingų koncentracijų filtratų poveikis ŽDPKL mitochondrijų fermentų aktyvumui po 24 val. inkubacijos.

2.3.3. HKSC poveikio ŽDPKL proliferacijai analizė

Nustatyta statistiškai reikšminga trijų krypčių sąveika tarp HKSC tipo, filtrato koncentracijos ir poveikio laiko, $F(60, 360) = 188,934$; $p < 0,001$. Statistinis reikšmingumo lygmuo pasirinktas, kai $p < 0,017$, siekiant įvertinti dvikryptę sąveiką ir paprastuosius pagrindinius efektus. Nustatyta statistiškai reikšminga dvikryptė sąveika tarp tiriamojo HKSC tipo ir filtrato koncentracijos visuose analizuotuose laiko taškuose: 2 val. – $F(12, 360) =$

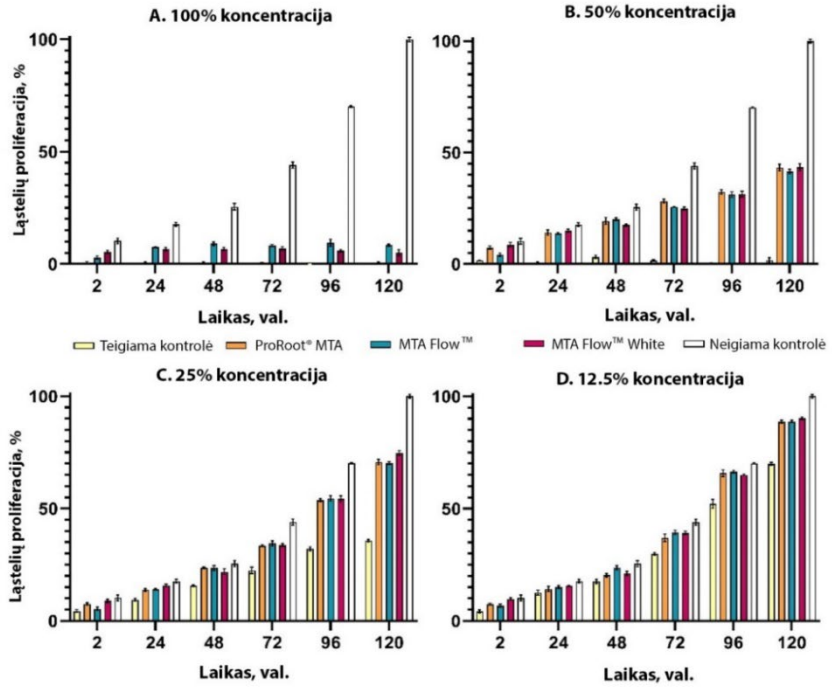
8,239; p < 0,001, 24 val. – F(12, 360) = 47,357; p < 0.001, 48 val. – F(12, 360) = 88,918; p < 0,001, 72 val. – F(12, 360) = 253,215; p < 0,001, 96 val. – F(12, 360) = 713,184; p < 0,001 ir 120 val. – F(12, 360) = 1341,966; p < 0,001. Taip pat nustatytas statistiškai reikšmingas pagrindinis tiriamojo HKSC skirtingų koncentracijų filtrato poveikis ŽDPKL proliferacijai visuose analizuotuose laiko taškuose priklausomai nuo HKSC tipo. Analizuojant HKSC filtratų koncentracijų ir laiko įtakos pagrindinių porų lyginamosios analizės rezultatus, buvo pritaikyta *Bonferroni* korekcija. ŽDPKL dauginimosi vidurkiai, patikimumo intervalai [CI] ir porų lyginamoji analizė priklausomai nuo tiriamosios grupės, filtratų koncentracijos ir poveikio laiko yra apibendrinti 9 paveiksle (A-D). Rezultatai pateikti lyginant su paskutinės dienos neigiamos kontrolinės grupės rezultatais (100 % atskaitos taškas), o porų lyginamoji analizė pateikta 5 lentelėje.

ŽDPKL proliferacija buvo reikšmingai paveikta šviežiai sumaišytų HKSC filtratų. Apskritai, tiriamųjų takių HKSC 100 % ir 50 % koncentracijų filtratai reikšmingai sumažino ŽDPKL dauginimosi intensyvumą visais laiko tarpais, palyginti su neigiama kontroline grupe (p < 0,017). Tačiau proliferaciją slopinančio poveikio stiprumas priklauso nuo filtrato koncentracijos. Teigiama kontrolinė grupė lėmė reikšmingai mažesnį ŽDPKL dauginimąsi ir/ar ląstelių žūtį, palyginti su neigiama kontroline grupe (p < 0,017).

100 % koncentracijos filtratų poveiko analizė parodė, jog dėl teigiamos kontrolinės grupės ir kontrolinio *ProRoot MTA* poveikio ŽDPKL dauginimosi tendencija išliko panaši visuose laiko taškuose. Nepaisant to, visos 100 % koncentracijos tiriamosios grupės reikšmingai paveikė ŽDPKL gyvybingumą, palyginti su neigiama kontroline grupe (p < 0,017). Nors ir abiejų tiriamųjų MF ir MFWhite 100 % koncentracijos filtratai lėmė ŽDPKL žūtį ir/ar slopino ląstelių proliferaciją, bendras gyvybingų ląstelių kiekis išliko didesnis – nuo 2,3 % iki 9,4 %, palyginti su teigiama kontroline ir kontrolinio HKSC *ProRoot MTA* grupėmis, kuriose ląstelių gyvybingumas jau po 2 val. buvo mažesnis nei 1 % (p < 0,017).

Įvertinus po 2 valandų, MF grupės tiriamojo 100 % koncentracijos filtrato slopinamasis poveikis ŽDPKL dauginimosi intensyvumui buvo dvigubai mažesnis nei MFWhite filtrato (p < 0,017). Tačiau po 24 val. MF filtratu paveiktų ŽDPKL proliferacija buvo vos 3 % didesnė nei MFWhite grupėje ar išliko panaši. Panaši tendencija nustatyta ir veikiant ląsteles 50 %, 25 % ir 12,5 % koncentracijų MF filtratais po 48 val., kuomet MFWhite grupės paveiktos ŽDPKL pasižymėjo 4,4 %, 3,5 % ir 3,1 % mažesniu dauginimosi intensyvumu nei MF grupėse (p < 0,017).

Veikiant ŽDPKL 50 %, 25 % ir 12,5 % koncentracijų tiriamųjų HKSC filtratais, gauta panaši ląstelių proliferacija visais laiko tarpais. Tačiau po 48 val. 50 % ir 12,5 % MFWhite grupės paveiktos ŽDPKL kultūros pasižymėjo reikšmingai mažesniu ląstelių dauginimosi intensyvumu: 2,6 % ir 2,5 %, palyginti su MF ($p < 0,017$). Panaši tendencija buvo matoma ir veikiant ŽDPKL kultūras 25 % koncentracijos filtratais, tačiau šiuo atveju nebuvo statistiškai reikšmingų skirtumų tarp grupių ($p > 0,017$). Po 120 valandų 25 % MF ir ProRootKG grupių paveiktos ŽDPKL kultūros pasižymėjo panašiais proliferaciniais rezultatais – apie 70 %, o 25 % MFWhite grupės paveiktų ląstelių dauginimosi intensyvumas buvo 4,8 % didesnis ($p < 0,017$). Visgi, ŽDPKL proliferacija nuo 24 val. laiko taško išliko statistiškai reikšmingai didesnė 50 % ir 25 % tiriamųjų takių HKSC filtratų paveiktose ŽDPKL grupėse nei teigiamoje kontrolinėje grupėje ($p < 0,017$).



9 paveikslas. ŽDPKL proliferacijos analizė, taikant MTT analizę skirtinguose laiko taškuose (po 2, 24, 48, 72, 96 ir 120 val.): šviežiai sumaišytų tiriamųjų HKSC ir kontrolinių grupių poveikis ŽDPKL mitochondrijų fermentų aktyvumui, veikiant skirtingų koncentracijų: A – 100 %, B – 50 % , C – 25 % ir D – 12,5 % filtratais. Kiekvienos grupės porų lyginamoji analizė detalizuota 5A – 5D lentelėse.

5A lentelė. Pagrindinių veiksnių porų lyginamoji analizė, vertinant ŽDPKL proliferaciją, kai ląstelės veikiamos 100 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais įvairiais laiko tarpais.

Koncentracija 100 %					
2 val. / 24 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		10,264* [8,36; 12,17)	9,670* [7,77; 11,57]	4,816* [2,91; 6,72]	7,367* [5,46; 9,27]
Teigiama kontrolė	17,641* [15,74; 19,54]		-0,595 [-2,5; 1,31]	-5,448* [-7,35; -3,55]	-2,897* [-4,8; -0,99]
ProRoot MTA	17,119* [15,22; 19,02]	-0,522 [-2,43; 1,38]		-4,854* [-6,76; -2,95]	-2,302* [-4,21; -0,4]
MTA Flow™ White	10,907* [9,00; 12,81]	-6,734* [-8,64; -4,83]	-6,212* [-8,11; -4,31]		2,551* [0,65; 4,45]
MTA Flow™	10,139* [8,24; 12,04]	-7,502* [-9,40; -5,60]	-6,980* [-8,88; -5,08]	-0,768 [-2,67; 1,14]	
48 val. / 72 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		25,368* [23,47; 27,27]	24,920* [23,02; 26,82]	18,720* [16,82; 20,62]	16,478* [14,58; 18,38]
Teigiama kontrolė	43,979* [42,08; 45,88]		-0,449 [-2,35; 1,45]	-6,648* [-8,55; -4,75]	-8,890* [-10,79; -6,99]
ProRoot MTA	43,420* [41,52; 45,32]	-0,559 [-2,46; 1,34]		-6,200* [-8,10; -4,30]	-8,441* [-10,34; -6,54]
MTA Flow™ White	37,003* [35,10; 38,91]	-6,976* [-8,88; -5,07]	-6,418* [-8,32; -4,51]		-2,242* [-4,14; -0,34]
MTA Flow™	35,766* [33,86; 37,67]	-8,213* [-10,12; -6,31]	-7,655* [-9,56; -5,75]	-1,237 [-3,14; 0,67]	
96 val. / 120 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		70,061* [68,16; 71,96]	69,986* [68,08; 71,89]	64,200* [62,30; 66,10]	60,696* [58,79; 62,60]
Teigiama kontrolė	99,945* [98,04; 101,85]		-0,076 [-1,98; 1,83]	-5,862* [-7,76; -3,96]	-9,366* [-11,27; -7,46]
ProRoot MTA	99,477* [97,57; 101,38]	-0,468 [-2,37; 1,44]		-5,786* [-7,69; -3,88]	-9,290* [-11,19; -7,39]
MTA Flow™ White	94,925* [93,02; 96,83]	-5,020* [-6,92; -3,12]	-4,552* [-6,46; -2,65]		-3,504* [-5,41; -1,60]
MTA Flow™	91,613* [89,71; 93,52]	-8,332* [-10,24; -6,43]	-7,864* [-9,77; -5,96]	-3,312* [-5,21; -1,41]	

Duomenys pateikti kaip vidurkiai ir patikimumo intervalai [CI]. Pastaba: *Bonferroni* korekcija pritaikyta siekiant išskirti patikimumo lygmenį, kai rezultatai laikomi statistiškai reikšmingai skirtingais: įprastas statistinio reikšmingumo lygmuo ($p < 0,05$) padalintas iš analizuojamų poveikių skaičiaus, kurių yra 3. Taigi, gautas pagrindinio veiksnio statistiškai reikšmingas skirtumas, kai $p < 0,017$ ($p < 0,05/3$).

5B lentelė. Pagrindinių veiksnių porų lyginamoji analizė, vertinant ŽDPKL proliferaciją, kai ląstelės veikiamos 50 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais įvairiais laiko tarpais.

Koncentracija 50 %					
2 val. / 24 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		8,599* [6,7; 10,5]	2,881* [0,98; 4,78]	1,651 [-0,25; 3,55]	6,038* [4,13; 7,94]
Teigiama kontrolė	17,024* [15,12; 18,93]		-5,717* [-7,62; -3,81]	-6,948* [-8,85; -5,04]	-2,561* [-4,46; -0,66]
ProRoot MTA	3,464* [1,56; 5,37]	-13,560* [-15,46; -11,66]		-1,230 [-3,13; 0,67]	3,156* [1,25; 5,06]
MTA Flow™ White	2,597* [0,69; 4,50]	-14,427* [-16,33; -12,52]	-0,867 [-2,77; 1,04]		4,387* [2,48; 6,29]
MTA Flow™	3,856* [1,95; 5,76]	-13,168* [-15,07; -11,27]	0,392 [-1,51; 2,29]	1,259 [-0,64; 3,16]	
48 val. / 72 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		22,312* [20,41; 24,22]	6,143* [4,24; 8,05]	8,057* [6,15; 9,96]	5,383* [3,48; 7,29]
Teigiama kontrolė	42,426* [40,52; 44,33]		-16,169* [-18,07; -14,27]	-14,255* [-16,16; -12,35]	-16,929* [-18,83; -15,03]
ProRoot MTA	15,740* [13,84; 17,64]	-26,686* [-28,59; -24,78]		1,913 [-0,10; 3,82]	-0,760 [-2,66; 1,14]
MTA Flow™ White	19,135* [17,23; 21,04]	-23,291* [-25,19; -21,39]	3,396* [1,49; 5,30]		-2,673* [-4,58; -0,77]
MTA Flow™	18,307* [16,40; 20,21]	-24,119* [-26,02; -22,22]	2,567* [0,66; 4,47]	-0,828 [-2,73; 1,07]	
96 val. / 120 val.	Neigiama kontrolė	Teigiama kontrolė	ProRoot MTA	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		69,845* [67,94; 71,75]	37,747* [35,84; 39,65]	38,779* [36,88; 40,68]	38,957* [37,05; 40,86]
Teigiama kontrolė	98,246* [96,34; 100,15]		-32,098* [-34,00; -30,20]	-31,066* [-32,97; -29,164]	-30,888* [-32,79; -28,99]
ProRoot MTA	56,736* [54,83; 58,64]	-41,510* [-43,41; -39,61]		1,033 [-0,87; 2,94]	1,210 [-0,69; 3,11]
MTA Flow™ White	56,642* [54,74; 58,55]	-41,604* [-43,51; -39,70]	-0,094 [-2,00; 1,81]		0,177 [-1,73; 2,08]
MTA Flow™	58,567* [56,6; 60,47]	-39,679* [-41,58; -37,78]	1,831 [-0,07; 3,73]	1,925 [-0,02; 3,83]	

Duomenys pateikti kaip vidurkiai ir patikimumo intervalai [CI]. Pastaba: *Bonferroni* korekcija pritaikyta siekiant išskirti patikimumo lygmenį, kai rezultatai laikomi statistiškai reikšmingai skirtingais: įprastas statistinio reikšmingumo lygmuo ($p < 0,05$) padalintas iš analizuojamų poveikių skaičiaus, kurių yra 3. Taigi, gautas pagrindinio veiksnio statistiškai reikšmingas skirtumas, kai $p < 0,017$ ($p < 0,05/3$).

5C lentelė. Pagrindinių veiksnių porų lyginamoji analizė, vertinant ŽDPKL proliferaciją, kai ląstelės veikiamos 25 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais įvairiais laiko tarpais.

Koncentracija 25 %					
2 val. / 24 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		5,908* [4,01; 7,81]	2,782* [0,88; 4,69]	1,393 [-0,51; 3,3]	4,890* [2,99; 6,79]
Teigiama kontrolė	8,237* [6,33; 10,14]		-3,126* [-5,03; -1,22]	-4,515* [-6,42; -2,61]	-1,018 [-2,92; 0,88]
ProRoot MTA	3,835* [1,93; 5,74]	-4,401* [-6,30; -2,50]		-1,389 [-3,29; 0,51]	2,108* [0,2; 4,01]
MTA Flow™ White	1,875 [-0,03; 3,78]	-6,362* [-8,26; -4,46]	-1,760 [-3,74; 0,06]		3,496* [1,59; 5,40]
MTA Flow™	3,592* [1,69; 5,50]	-4,644* [-6,55; -2,74]	-0,243 [-2,15; 1,66]	1,717 [-0,19; 3,62]	
48 val. / 72 val.	Neigiama Kontrolė	Teigiama Kontrolė	ProRoot MTA	MTA Flow™ White	MTA Flow™
Neigiama kontrolė		9,913* [8,01; 11,82]	1,876 [-0,03; 3,78]	3,633* [1,73; 5,54]	1,985 [-0,08; 3,89]
Teigiama kontrolė	21,513* [19,61; 23,42]		-8,037* [-9,94; -6,13]	-6,280* [-8,18; -4,38]	-7,927* [-9,83; -6,02]
ProRoot MTA	10,590* [8,69; 12,49]	-10,922* [-12,83; -9,02]		1,757 [-0,15; 3,66]	0,110 [-1,79; 2,01]
MTA Flow™ White	10,263* [8,36; 12,17]	-11,250* [-13,15; -9,35]	-0,327 [-2,23; 1,58]		-1,647 [-3,55; 0,26]
MTA Flow™	9,396* [7,49; 11,30]	-12,117* [-14,02; -10,21]	-1,195 [-3,10; 0,71]	-0,867 [-2,77; 1,04]	
96 val. / 120 val.	Neigiama Kontrolė	Teigiama Kontrolė	ProRoot MTA	MTA Flow™ White	MTA Flow™
Neigiama kontrolė		38,119* [36,22; 40,02]	16,447* [14,54; 18,35]	15,795* [13,89; 17,70]	15,614* [13,71; 17,52]
Teigiama kontrolė	64,308* [62,40; 66,21]		-21,672* [-23,57; -19,77]	-22,325* [-24,23; -20,42]	-22,506* [-24,41; -20,60]
ProRoot MTA	29,310* [27,41; 31,21]	-34,998* [-36,90; -33,09]		-0,653 [-2,74; 1,07]	-0,834 [-2,60; 0,93]
MTA Flow™ White	25,188* [23,29; 27,09]	-39,120* [-41,02; -37,22]	-4,122* [-6,03; -2,22]		-0,181 [-2,08; 1,72]
MTA Flow™	29,787* [27,88; 31,69]	-34,521* [-36,42; -32,62]	0,477 [-1,43; 2,38]	4,599* [2,70; 6,50]	

Duomenys pateikti kaip vidurkiai ir patikimumo intervalai [CI]. Pastaba: *Bonferroni* korekcija pritaikyta siekiant išskirti patikimumo lygmenį, kai rezultatai laikomi statistiškai reikšmingai skirtingais: įprastas statistinio reikšmingumo lygmuo ($p < 0,05$) padalintas iš analizuojamų poveikių skaičiaus, kurių yra 3. Taigi, gautas pagrindinio veiksnio statistiškai reikšmingas skirtumas, kai $p < 0,017$ ($p < 0,05/3$).

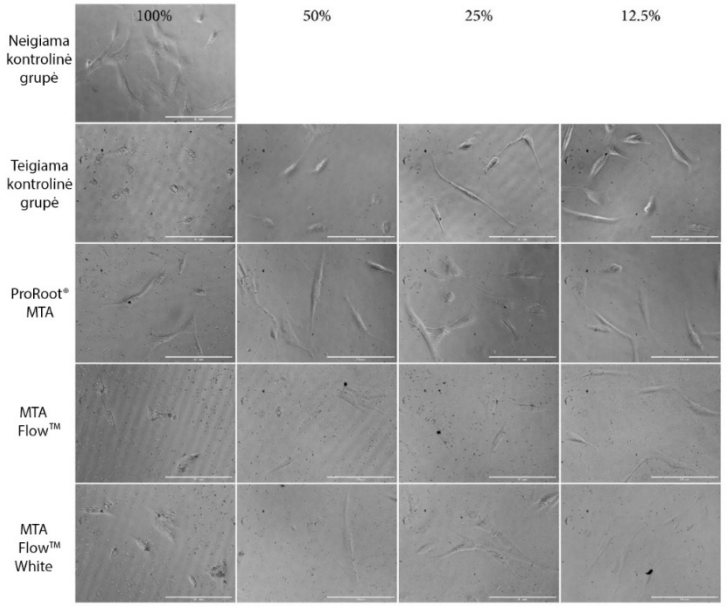
5D lentelė. Pagrindinių veiksnių porų lyginamoji analizė, vertinant ŽDPKL proliferaciją, kai ląstelės veikiamos 12,5 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais įvairiais laiko tarpais.

Koncentracija 12,5 %					
2 val. / 24 val.	Neigiama kontrolė	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė		6,037* [4,13; 7,94]	2,893* [0,99; 4,80]	0,441 [-1,46; 2,34]	3,560* [1,66; 5,46]
Teigiama kontrolė	5,090* [3,19; 6,99]		-3,143* [-5,05; -1,24]	-5,596* [-7,50; -3,69]	-2,476* [-4,38; -0,57]
ProRoot MTA	3,417* [1,51; 5,32]	-1,672 [-3,58; 0,23]		-2,452* [-4,36; -0,55]	0,667 [-1,24; 2,57]
MTA Flow Wval,ite	1,801 [-0,20; 3,70]	-2,988* [-4,89; -1,09]	-1,316 [-3,22; 0,59]		3,119* [1,22; 5,02]
MTA Flow™	2,566* [0,66; 4,47]	-2,523* [-4,43; -0,62]	-0,851 [-2,75; 1,05]	0,465 [-1,44; 2,37]	
48 val. / 72 val.	Neigiama Kontrolė	Teigiama Kontrolė	ProRoot MTA	MTA Flow™ White	MTA Flow™
Neigiama kontrolė		7,890* [5,99; 9,79]	5,142* [3,24; 7,04]	4,348* [2,45; 6,25]	1,832 [-0,07; 3,74]
Teigiama kontrolė	14,107* [12,20; 16,01]		-2,749* [-4,65; -0,85]	-3,542* [-5,45; -1,64]	-6,058* [-7,96; -4,16]
ProRoot MTA	6,972* [5,07; 8,88]	-7,135* [-9,04; -5,23]		-0,793 [-2,70; 1,11]	-3,309* [-5,21; -1,41]
MTA Flow Wval,ite	4,782* [2,88; 6,68]	-9,325* [-11,23; -7,42]	-2,190* [-4,09; -0,29]		-2,516* [-4,42; -0,61]
MTA Flow™	4,607* [2,70; 6,51]	-9,500* [-11,40; -7,60]	-2,365* [-4,27; -0,46]	-0,174 [-2,08; 1,73]	
96 val. / 120 val.	Neigiama Kontrolė	Teigiama Kontrolė	ProRoot MTA	MTA Flow™ White	MTA Flow™
Neigiama kontrolė		17,982* [16,08; 19,89]	4,325* [2,42; 6,23]	5,081* [3,18; 6,98]	3,680* [1,78; 5,58]
Teigiama kontrolė	30,086* [28,18; 31,99]		-13,657* [-15,56; -11,75]	-12,901* [-14,80; -11,00]	-14,302* [-16,21; -12,40]
ProRoot MTA	11,211* [9,31; 13,11]	-18,875* [-20,78; -16,97]		0,756 [-1,15; 2,66]	-0,645 [-2,55; 1,26]
MTA Flow Wval,ite	9,761* [7,86; 11,66]	-20,325* [-22,23; -18,42]	-1,450 [-3,35; 0,45]		-1,401 [-3,30; 0,50]
MTA Flow™	11,174* [9,27; 13,08]	-18,912* [-20,82; -17,01]	-0,037 [-1,94; 1,87]	1,413 [-0,49; 3,32]	

Duomenys pateikti kaip vidurkiai ir patikimumo intervalai [CI]. Pastaba: *Bonferroni* korekcija pritaikyta siekiant išskirti patikimumo lygmenį, kai rezultatai laikomi statistiškai reikšmingai skirtingais: įprastas statistinio reikšmingumo lygmuo ($p < 0,05$) padalintas iš analizuojamų poveikių skaičiaus, kurių yra 3. Taigi, gautas pagrindinio veiksnio statistiškai reikšmingas skirtumas, kai $p < 0,017$ ($p < 0,05/3$).

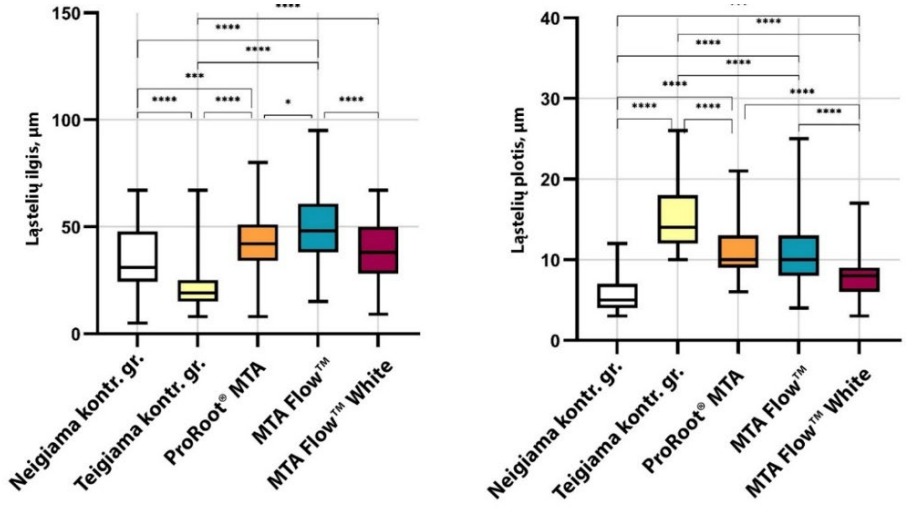
2.3.4. ŽDPKL kiekybinė ir kokybinė analizė

Tiriamųjų filtratų poveikis ŽDPKL morfologiniams pokyčiams buvo vertinamas apverstos fazės šviesiniu mikroskopu, kaip vaizduojama 10 paveiksle. Neigiamoje kontrolinėje grupėje ŽDPKL buvo verpstės formos ir pasiskirstė per visą duobutės paviršių. Taip pat pasižymėjo blyškiu, apvaliu ar ovaliu centriniu branduoliu su daugybiniais branduolėliais, kurie galimai nurodo aktyvią DNR transkripciją ir RNR sintezę. Daugybinės citoplazminės vakuolės nurodė ląstelių sekrecijos pūsleles. Priešingai, 100 % tiriamųjų HKSC grupių paveiktos ŽDPKL visuose laiko tarpuose, pasižymėjo apvalia ląstelių morfologija ir sumažėjusiu ląstelių skaičiumi. Šis ŽDPKL formos pasikeitimas, lyginant su neigiama kontroline grupe, gali būti susijęs su prasidėjusiu ląstelės žūties mechanizmu paveikus 100 % koncentracijos filtratu. Kita vertus, paveikus ląsteles 50 % filtratais, ŽDPKL ilgis ir plotis buvo reikšmingai didesnis nei neigiamos kontrolinės grupės. Mažėjant veikiančio filtrato koncentracijai, pavyzdžiui, paveikus 25 % koncentracijos tiriamojo HKSC filtratu, ląstelių morfologija tapo artima neigiamai kontrolinei grupei, nors ir ląstelių pasiskirstymas duobutėje išliko mažesnis.



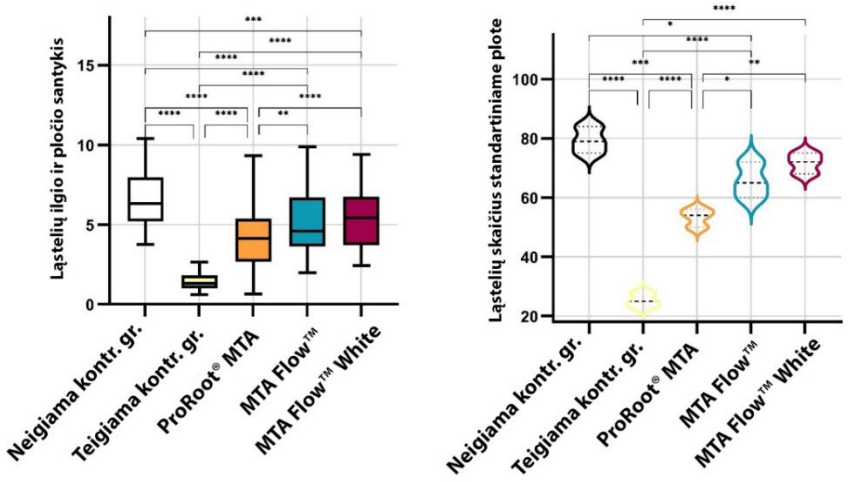
10 paveikslas. Reprezentatyvūs ŽDPKL kultūrų vaizdai, vertinant po 24 val. poveikio skirtingomis tiriamųjų ir kontrolinių grupių filtratų koncentracijomis.

Trumpiausios ir siauriausios ląstelės buvo aptiktos teigiamoje kontrolinėje grupėje (11 paveikslas). Šioje grupėje ląstelės buvo smulkiausios ir apvaliausios bei mažiausias ląstelių skaičius tiriamajame plote (12 paveikslas). ŽDPKL, paveiktos 50 % MF filtratu, pasižymėjo didesniu ilgiu nei 50 % MFWhite ar ProRootKG grupėse. Statistiškai reikšmingų skirtumų tarp 50 % MFWhite ir ProRootKG grupių paveiktų ląstelių morfologinių pokyčių nenustatyta. Siauriausios ląstelės aptiktos MFWhite grupėje, o MF ir ProRootKG paveiktų ŽDPKL plotis reikšmingai nesiskyrė. Apskritai, visų HKSC filtratų poveikis lėmė ilgesnę ir platesnę ląstelių morfologiją, tačiau kartu ir mažesnį ląstelių ilgio ir pločio santykį nei kontrolinėje grupėje. Taip pat visose tiriamosiose grupėse ląstelių skaičius buvo mažesnis nei neigiamoje kontrolinėje grupėje. Tiriamųjų takių HKSC paveiktų ląstelių ilgio ir pločio santykis reikšmingai nesiskyrė. Detali ląstelių, paveiktų 24 val. 50 % koncentracijos takių HKSC filtratais, morfologijos kiekybinė analizė vaizduojama 11 ir 12 paveiksluose.



* - žymi statistiškai reikšmingus skirtumus tarp grupių (* $p < 0,05$, ** $p < 0,01$ ir *** $p < 0,001$).

11 paveikslas. 24 val. 50 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais paveiktų ŽDPKL ilgio ir pločio analizė.



* - žymi statistiškai reikšmingus skirtumus tarp grupių (* $p < 0,05$, ** $p < 0,01$ ir *** $p < 0,001$).

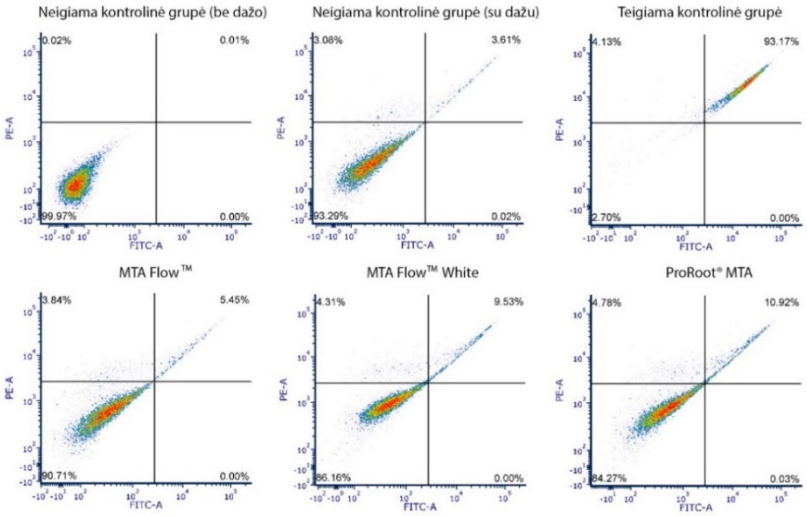
12 paveikslas. 24 val. 50 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais paveiktų ŽDPKL ilgio ir pločio santykio bei vidutinis ląstelių skaičius analizuojamame plote analizė.

2.3.5. ŽDPKL žūties pobūdžio analizė tėkmės citometrijos metodu

Paveikus ŽDPKL kultūras 50 % koncentracijos tiriamųjų HKSC MF ir MFWhite bei kontrolinių grupių filtratais, atlikta tėkmės citometrijos analizė, siekiant įvertinti, kokiu būdu žūva ląstelės. Duomenys pateikti kaip reprezentatyvios taškinės diagramos, pavaizduotos 13 paveiksle. Kairysis apatinis tėkmės citometrijos diagramos ketvirtis žymi gyvybingas ląsteles, dešinysis apatinis ketvirtis – ankstyvosios apoptozės apimtas ląsteles, viršutinis dešinysis ketvirtis – vėlyvosios apoptozės apimtas ląsteles, o kairysis viršutinis - nekrozės apimtas ląsteles. Apskaičiuoti ląstelių vidurkiai pažymėti atitinkamų taškinių diagramų ketvirčių srityse ir išreikšta procentine dalimi iš visų suskaičiuotų ląstelių.

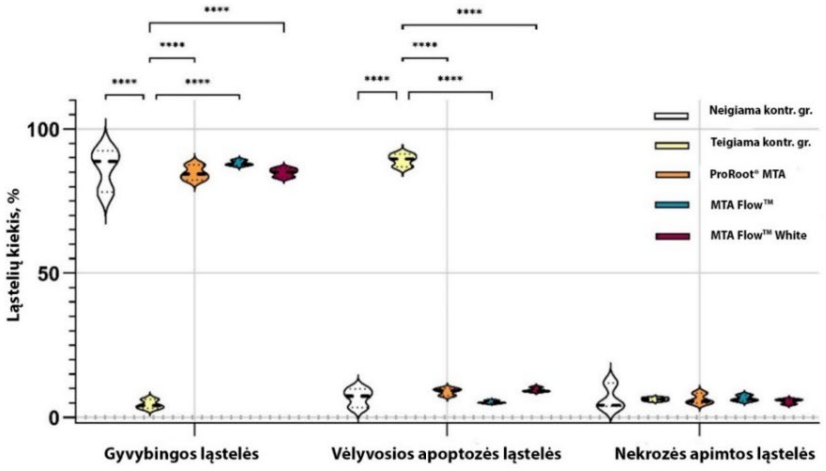
Visi tirti HKSC, taip pat ir MF bei MFWhite, ProRootKG 50 % koncentracijos filtratai lėmė ne mažesnį nei 84 % ląstelių gyvybingumą po 24 val. Priešingai, teigiama kontrolinė grupė lėmė gyvybingų ląstelių sumažėjimą iki vos 4,5 %, o gyvybingos ląstelės neigiamoje kontrolinėje grupėje sudarė daugiau nei 87 %. Vertinant ŽDPKL gyvybingas ir vėlyvosios apoptozės apimtas ląsteles, reikšmingų skirtumų tarp HKSC paveiktų ir neigiamos kontrolinės grupės ląstelių nepastebėta. Reikšmingų skirtumų nenustatyta ir vertinant nekrozės būdu žūvančias ląsteles. Detali apoptozės /

nekrozės tėkmės citometrijos analizė naudojant *Annexin V-FITC* pateikta 6 ir 7 lentelėse bei 14 paveiksle.



Taškinės diagramos kiekviename grafike atspindi gyvybingų (kairysis apatinis ketvirtis), ankstyvosios apoptozės (apatinis dešinysis ketvirtis), vėlyvosios apoptozės (viršutinis dešinysis ketvirtis) ir nekrozės (viršutinis kairysis ketvirtis) apimtų ląstelių pasiskirstymą.

13 paveikslas. Reprezentatyvios taškinės Annexin V-FITC tėkmės citometrijos analizės diagramos, analizuojant ŽDPKL kultūras, paveiktas tiriamųjų ir kontrolinių grupių 50 % koncentracijos filtratais 24 val.



*Duomenys pateikti kaip mediana, pirmasis ir trečiasis kvartiliai bei minimalios ir maksimalios reikšmės, kartu su tankio diagrama. * - žymi statistiškai reikšmingus skirtumus tarp grupių (* $p < 0,05$, ** $p < 0,01$ ir *** $p < 0,001$). Reikšmingų skirtumų vertinant nekrozės apimtų ląstelių kiekį nenustatyta.*

14 paveikslas. *Gyvybingų, vėlyvosios apoptozės ir nekrozės būdu žūstančių ląstelių medianas vaizduojantis grafikas, paveikus ŽDPKL 50 % koncentracijos tiriamųjų ir kontrolinių grupių filtratais 24 val.*

6 lentelė1. Gyvybingų, vėlyvosios apoptozės ir nekrozės būdu žūvančių ŽDPKL, paveiktų 50 % koncentracijų tiriamųjų ir kontrolinių grupių filtratais 24 val., analizė.

Grupė	*Gyvybingos ląstelės*	*Vėlyvosios apoptozės apimtos ląstelės*	*Nekrozės apimtos ląstelės*
Neigiama kontrolė (be dažo)	99,97 ± 0,03 (99,88)	0,01 (0,01)	0,1 ± 0,04 (0,11)
Neigiama kontrolė	86,46 ± 6,07 (88,75)	6,87 ± 2,69 (7,37)	6,68 ± 3,75 (4,17)
Teigiama kontrolė	4,47 ± 1,32 (4,12)	89,24 ± 1,8 (89,59)	6,29 ± 0,49 (6,29)
ProRoot MTA	84,77 ± 2,15 (84,36)	8,93 ± 1,03 (9,24)	6,30 ± 1,51 (5,64)
MTA Flow™	88,15 ± 0,79 (87,88)	5,29 ± 0,41 (5,10)	6,56 ± 0,87 (6,26)
MTA Flow™ White	84,90 ± 1,21 (85,07)	9,55 ± 0,63 (9,11)	5,56 ± 0,69 (5,83)

7 lentelė. Gyvybingų ŽDPKL tarp skirtingų tiriamųjų ir kontrolinių grupių porų lyginamoji analizė, paveikus ląsteles 50 % filtratais 24 val.

	Teigiama kontrolė	*ProRoot MTA*	*MTA Flow™ White*	*MTA Flow™*
Neigiama kontrolė	81,99* [72,70; 91,27]	1,69 [-7,60; 10,99]	1,56 [-7,72; 10,85]	-1,70 [-10,99; 7,59]
Teigiama kontrolė		-80,30* [-89,58; -71,01]	-80,42* [-89,71; -71,14]	-83,68* [-92,97; -74,39]
ProRoot MTA			-0,129 [-9,42; 9,16]	-3,385 [-12,67; 5,90]
MTA Flow™ White				-3,256 [-12,54; 6,03]

Duomenys pateikti kaip vidurkiai ir patikimumo intervalai [CI].

3. REZULTATŲ APTARTIMAS

3.1. Skirtingą klinikinę patirtį turinčių asmenų atliekamo dantų šaknų kanalų užpildymo vieno kaiščio metodika porėtumo palyginimas

Dėl fizikinių-cheminių takių HKSC užpildų savybių, užpildant šaknų kanalus šių cementų tūrio dalis šaknų kanalų užpilde gali būti didesnė nei naudojant dervinius dantų šaknų kanalų užpildus.[118] Taikant vieno kaiščio metodiką yra naudojama didesnio kūgio gutaperčia, siekiant sukurti hidraulinį slėgį šaknies kanale, pagerinti takaus HKSC pasiskirstymą ir sukurti sąlygas dantų šaknų kanalams pergydyti, jei atsirastų indikacijų.[110,489] Ankstesni tyrimai parodė, kad vieno kaiščio metodikos šaknų kanalų užpildų porėtumas yra panašus ar net mažesnis nei dantų šaknų kanalus užpildant kitomis metodikomis.[26,39,44,210,452] Nors ir yra tyrimų, rodančių, jog vieno kaiščio metodika lemia didesnį šaknies kanalo užpildo porėtumą, visgi rezultatai vertinant porų pasiskirstymą vieno kaiščio metodikos užpilduose išlieka prieštaringi.[490] Moksliniai tyrimai rodo mažesnį vieno kaiščio metodikos sukuriamą užpildų porėtumą dantų šaknų kanalų vainikiniuose ir viduriniuose trečdaliuose, lyginant su šaltos šoninės kondensacijos ar termoplastinės gutaperčios metodikomis.[26,210] Kita vertus, kiti autoriai teigia, kad vieno kaiščio metodika lemia didesnį užpildo porėtumą nei termoplastinės metodikos.[491] Nepaisant prieštaringų tyrimų rezultatų, visgi galima teigti, kad dauguma tyrėjų patvirtina vieno kaiščio metodikos panašumą su kitomis metodikomis sukuriamu dantų šaknų kanalų užpildų porėtumu.[492,493] Tyrimų rezultatų skirtumai gali būti susiję su dantų šaknų kanalų morfologija, chemomechaninio paruošimo protokolu ar fizikinėmis-cheminėmis dantų šaknų kanalų užpildų savybėmis, ribota *in vitro* testavimo modelių standartizacija, duomenų rinkimo bei analizės protokolais. Vis dėlto, remiantis naujausiųjų tyrimų duomenimis, nelieka abejonių, kad nė viena dantų šaknų kanalų užpildymo metodika negali užtikrinti idealiai homogeniško, be porų, dantų šaknų kanalų sistemos hermetizavimo.[218,494] Kita vertus, reikėtų pripažinti, kad vieno kaiščio metodika yra daug lengviau įvaldoma gydytojų odontologų, neturinčių endodontologo specializacijos[105], ir kartu lemia gerą pirminio endodontinio gydymo bei endodontinio pergydymo klinikinę sėkmę.[47,48,483]

Dauguma tyrimų, nagrinėjančių endodontinio gydymo procedūrų sėkmę, remiasi gydytojų endodontologų klinikiniais atvejais.[21,47] Tyrėjo klinikinė patirtis turi esminės įtakos endodontinių procedūrų kokybei ir klinikiniams rezultatams.[21,495] Tas pats pasakytina ir apie eksperimentinius tyrimus,

kadangi didelę patirtį turintys tyrėjai dažniausiai atlieka ir laboratorinius tyrimus.[25–27] Skirtingos kvalifikacijos gydytojų endodontinio gydymo kokybė reikšmingai skiriasi.[496,497] Ankstesniuose tyrimuose įrodyta, kad šaknų kanalų užpildymo įprastais metodais kokybė reikšmingai priklauso nuo gydytojo patirties. Pasak N. Kharouf ir kt., odontologijos studentų atliekamo šaknies kanalų užpildymo šaltos šoninės kondensacijos būdu kokybė svyruoja nuo 46,6 % iki 58,8 %.[495] Kiti tyrimai parodė panašius ar net blogesnius rezultatus, o tai reiškia, kad tik 25,2–66 % dantų šaknų kanalų užpildų yra homogeniški ir priimtinos kokybės.[499–501] Vis dėlto, šaknų kanalų užpildymo kokybė palaipsniui gerėjo baigus studijas ir įgijus klinikinės patirties.[495,498] Kita vertus, N. Kharouf ir kt. (2019) parodė, kad, odontologijos studentams atliekant dantų šaknų kanalų užpildymą vieno kaiščio metodika, užpildų porėtumas reikšmingai sumažėjo, o priimtinas homogeniškumas buvo pasiektas 84,1–90,9 % atvejų.[495] Vertinant patyrusių gydytojų endodontologų dantų šaknų kanalų plombavimą šaltos šoninės kondensacijos būdu, homogeniški užpildai sudaro 86,1–88,6 % atvejų ir pasiekiami geriausi klinikinės sėkmės rodikliai.[50,502–505] Šie tyrimai netiesiogiai rodo, kad gydytojų patirtis gali turėti tiesioginį poveikį šaknų kanalų užpildymo kokybei, kai taikomos šaltos šoninės ar karštos vertikalios kondensacijos metodikos. Tačiau reikia pabrėžti, kad atliekant visus šiuos tyrimus šaknų kanalų plombavimo kokybė buvo vertinama remiantis dvimatėmis dantų šaknų rentgeno nuotraukomis, kurios negali suteikti tikslios informacijos apie trimatį užpildų homogeniškumą.

Nedaug publikuotų tyrimų įvertino bendrąjį dantų šaknų kanalų porėtumą, užpildant vieno kaiščio metodika.[26,57,210,490] Tačiau iki šiol dar nebuvo įvertinta dantų šaknų kanalų užpildymo vieno kaiščio metodika kokybė kartu su takiu HKSC užpildu ir porėtumas tarp skirtingą klinikinę patirtį turinčių odontologijos studentų bei gydytojų odontologų. Todėl trūkstant mokslinių tyrimų, susijusių su vieno kaiščio metodikos porėtumo analize, šiuo tyrimu buvo siekiama µKT analizės būdu įvertinti ir nustatyti vieno kaiščio metodikos šaknų kanalų užpildų porėtumo tipą ir pasiskirstymą, kuriuos atliko skirtingą klinikinę patirtį turintys tiriamieji - odontologijos studentai ir gydytojai odontologai.

Bendras šaknų kanalų porėtumas tirtuose mėginiuose svyruoja nuo 2,6 % (GE grupėje, viduriniame trečdalyje atviros ir uždaros poros) iki 16,1 % (GO grupėje, viršūniniame trečdalyje atviros ir uždaros poros). B. Celikten ir bendraautorių atliktame tyrime nustatytas itin mažas vieno kaiščio metodika užpildytų šaknų kanalų užpildų porėtumas (iki 1,58 %), įskaitant atviras ir uždaras poras.[508] Šiek tiek didesni porėtumo rodikliai buvo gauti M.

Radwanski ir kt., naudojant BioRoot RCS ir VK metodiką - porėtumas siekė apie 4 %[453], o E. Pedulla ir bendraautorių atliktame tyrime – apie 6,7 %.[494] Remiantis tyrimų duomenimis, VK metodika kartu su takiu HKSC atliktų užpildų porėtumas gali vidutiniškai siekti 13±8 % viso kanalo tūrio.[218] Atliekant porėtumo analizę, ypatingas dėmesys turėtų būti skiriamas viršūniniam šaknies kanalo trečdaliui, nes jo užpildymo kokybė gali tapti lemiamu endodontinio gydymo rezultato veiksniu.[21,452] Būtent todėl šiame tyrime porėtumo pasiskirstymas VK metodikos užpilduose buvo vertinamas visuose trijuose šaknies kanalo trečdaliuose individualiai. Atlikta dantų šaknų kanalų analizė išskaidžius trečdaliais parodė, kad didžiausias porėtumas, išreikštas procentais, yra pasiskirstęs viršūniniame šaknies kanalo trečdalyje, įskaitant atviras (nuo 8,1 % iki 15,9 %) ir uždaras (nuo 0,17 % iki 0,34 %) poras visose eksperimentinėse grupėse. Nustatyti porėtumo rezultatai reikšmingai nesiskyrė, o tai rodo vienodą viršūninių trečdalių užpildymo kokybę tarp OS, GR, GO ir GE tiriamųjų grupių. Gauti rezultatai sutampa su A. Moizadeh ir bendraautorių tyrimo duomenimis - didžiausias porų tūris buvo nustatytas vieno kaiščio užpildų viršūniniuose trečdaliuose. Autoriai bendrai įvertino atvirą ir uždarą porėtumą, kuris vieno kaiščio užpilduose siekė iki 5 %.[509] Priklausomai nuo trečdalių, mažiausias porėtumas M. Radwanski ir bendraautorių tyrime buvo nustatytas vainikiniame trečdalyje (apie 0,7 %), o didžiausias – viršūniniame trečdalyje (apie 2,24 %).[453] Įrodyta, kad didesnį porėtumą viršūniniame trečdalyje gali lemti netaisyklinga šaknies kanalo skerspjūvio forma, anatominės ypatybės, užpildo takumas, kurį gali paveikti netikslus miltelių ir skysčio santykis, ir kt.[27,510] Kita vertus, R. Castagnola ir bendraautorių tyrime VK metodika pasižymėjo statistiškai reikšmingai mažesniu atvirų porų kiekiu viršūniniame šaknies trečdalyje nei vainikiniame ar viduriniame, tačiau uždarų ir mišrių porų kiekis tarp šaknų trečdalių reikšmingai nesiskyrė.[511] B. Celikten ir bendraautoriai nustatė panašų uždarų porų pasiskirstymą (iki 0,39 %), tačiau atvirų porų pasiskirstymas buvo reikšmingai didesnis (iki 1,47 %) taikant vieno kaiščio užpildymo metodiką.[508] Yan Huan ir bendraautoriai, apskaičiavę atviras ir uždaras poras visame šaknies kanalo tūryje, nustatė, jog vidinės poros sudaro apie 0,62 %, o atviros ir mišrios – apie 1,56 % šaknies kanalo tūrio.[512] Įdomu, jog B. Celikten ir kt. bei Yan Huan ir bendraautorių atlikta atskirų šaknų kanalų trečdalių analizė parodė, kad mažiausias porėtumas buvo viršūniniuose trečdaliuose ir siekė atitinkamai apie 0,21 % ir 0,3 %.[508,512] Užpildo porėtumas reikšmingai padidėjo ir vainikiniame trečdalyje, lyginant su viršūniniu, iki 0,82 % ir 0,93 %. M. Radwanski ir bendraautorių atlikta BioRoot RCS ir VK metodikos atvirų ir uždarų porų bendra bei jų pasiskirstymo šaknų kanalų

trečdaliuose µKT analizė parodė, kad naudojant BioRoot RCS porėtumas buvo reikšmingai didžiausias viršūniniame trečdalyje ir siekė apie 2,24 % viso kanalo tūrio.[453] Analizuojant bendrą šaknų kanalų porėtumą, 1,13 % sudarė atviros poros ir 2,86 % - uždaros poros. Bendrai įvertinus, šaknų viršūninių trečdalių porėtumas išlieka reikšmingu dantų šaknų kanalų užpildymo trūkumu, tad reikalingos metodikos ir/ar medžiagos, siekiant pagerinti šios šaknies kanalo dalies užpildų homogeniškumą.[509]

Vertinant atviras ir uždaras poras atskirai, viduriniame šaknies trečdalyje statistiškai reikšmingas skirtumas nustatytas tik tarp uždarų porų kiekio. Įdomu tai, jog reikšmingai mažiausias uždarų porų kiekis nustatytas OS ir GR grupėse, kuriose dantų šaknų kanalus užpildė mažiau klinikinės patirties turintys odontologijos studentas ir gydytojas rezidentas. A. Moinzadeh ir bendraautorių duomenimis, vidurinių trečdalių porėtumas, nors ir statistiškai nereikšmingai, tačiau buvo pastebimai mažesnis nei vainikinių ar viršūninių trečdalių: siekė iki 1,5 %.[509] E. Pedulla ir bendraautorių tyrime BioRoot RCS porėtumas viduriniame trečdalyje (0,78 %) nors ir panašus, tačiau buvo šiek tiek didesnis nei viršūniniame trečdalyje (0,6 %).[494] Lyginant su kitų autorių duomenimis, šiame tyrime gautos didesnės vidutinės porėtumo reikšmės, siekiančios nuo 2,6 % iki 7,7 %.

Ankstesniuose tyrimuose nustatyta, kad klinikinę reikšmę turi tik susisiekiančios porėtos ertmės šaknies kanalo užpilde (ištisinės arba atviros poros), kadangi jos sukuria porų tinklą ir yra susijusios su padidėjusia mikropralaidumo tikimybe bei prastesniais klinikiniais endodontinio gydymo rezultatais.[198,452] Pastebėta, kad, nepaisant užpildo tipo, tarpusavyje susisiekiančios arba atviros poros užpildo paviršiuje gali suformuoti spiralės formos erdvę ir veikti kaip skysčių ir mikroorganizmų difuzijos kelias.[198] Aklinos tuštumos (uždaros poros) yra įstrigusios užpildo viduje ir gali paveikti tik mechanines užpildo savybes, tačiau neturėti biologiškai reikšmingo poveikio.[26,28,198,452] Taigi, atlikus viršūninių ir vidurinių šaknų trečdalių kliniškai reikšmingų porų analizę nusatyta, kad jų pasiskirstymas tarp OS, GS, GO ir GE grupių išliko panašus.

Vainikinių trečdalių porėtumo analizė parodė statistiškai reikšmingus atvirų ir uždarų porų skirtumus. Užpildo porėtumas vainikiniame kanalo trečdalyje gali būti susijęs su didesniu šaknies kanalo tūriu šioje srityje ir atitinkamai padidėjusiu takaus HKSC, šiuo atveju *BioRoot® RCS*, tūriu.[27,371,452] Vainikinio šaknies kanalo trečdalio vieno kaiščio užpildų lyginamoji analizė atskleidė, jog atvirų porų pasiskirstymas buvo panašus GE ir GR grupėse, tačiau išliko reikšmingai didesnis nei OS grupėje. Vertinant uždarų porų pasiskirstymą vainikiniuose trečdaliuose, rezultatai GR ir OS

grupėse išliko panašūs. Be to, GO grupėje nustatytas daugiau nei dvigubai didesnis atvirų porų kiekis vainikiniuose trečdaliuose nei OS, GE ar GR grupėse. Šie rezultatai gali būti susiję su atsargesniu, švelnesniu ir lėtesniu gutaperčios kaiščio įvedimu į šaknies kanalą, kurį atlieka mažiau patyręs tiriamasis, pavyzdžiui, odontologijos studentas. Įrodyta, kad lėtas ir atsargus gutaperčios kaiščio įvedimas į šaknies kanalą užtikrina tolygesnį takaus HKSC užpildo pasiskirstymą ir galimai mažesnį užpildo porėtumą.[27,371,452] Ankstesni tyrimai atskleidė, kad užpildų porėtumas taip pat gali būti susijęs su užpildo tipu ir fizikinėmis savybėmis.[452,493,506] Buvo įrodyta, kad takūs HKSC užpildai pasižymi didesniu porėtumu iškart po šaknų kanalų užpildymo, tačiau užpildui kietėjant porų tūris ir kiekis reikšmingai sumažėja, priešingai nei dantų šaknų kanalų užpildų dervos pagrindu.[452] Siekiant sumažinti vieno kaiščio užpildų porėtumą platesniuose ir didesnio tūrio šaknų kanaluose, buvo rekomenduoti papildomi pagalbiniai gutaperčios kaiščiai, pasyviai įvedant išilgai pagrindinio gutaperčios kaiščio.[513] Dėl to šaknies kanale sukurtas didesnis hidraulinis slėgis pagerina užpildo pasiskirstymą šaknies kanale ir sumažina takaus užpildo bei porų kiekį. A. Moinzadeh ir bendraautorių atlikto tyrimo rezultatai parodė, kad bendras kanalo porėtumas vainikiniame trečdalyje siekė iki 2 %.[509] Šiek tiek mažesnis bendras porėtumas vainikiniame trečdalyje nustatytas E. Bianco ir bendraautorių tyrime - iki 1 %.[441] O didžiausią vainikinio šaknies kanalo trečdalio porėtumą nustatytė E. Pedulla ir bendraautoriai: apie 5,3 % viso kanalo tūrio.[494] Šių autorių duomenys iš dalies sutampa su atlikto tyrimo rezultatais, kadangi nustatyti vainikinių trečdalių porėtumo rezultatai svyruoja nuo 2,4 % iki 8,8 %, priklausomai nuo šaknies kanalą užpildančio asmens.

Šaknų kanalų plombavimo kokybė dažniausiai vertinama naudojant dantų šaknų rentgenogramas po šaknų kanalų gydymo, siekiant įvertinti užpildo ilgį ir homogeniškumą. Tačiau dvimatis rentgenologinis vaizdas ne visada suteikia pakankamai informacijos apie tikrąjį užpildų homogeniškumą ir sandarumą.[514] Rentgeno nuotraukose matomos užpildų poros atspindi tik dvimatę realybę ir neteikia trimatės informacijos. Todėl klinikinėje praktikoje tikslus užpildų porėtumo įvertinimas naudojant rentgenogramas yra gana ribotas, tačiau sykiu tai yra ir vienintelis gydytojams prieinamas metodas. Ankstesni tyrimai rodo, jog porų kiekis dantų šaknų kanalų užpilduose, aptinkamas atliekant tūrinę 3D µKT analizę, yra daug didesnis, nei rodo dvimačių tyrimų rezultatai.[21,137,452,496] Tačiau nėra aiškių įrodymų, leidžiančių nustatyti, koks porėtumo lygmuo yra kritinis ir gali neigiamai paveikti endodontinio gydymo rezultatus.[21,210,506] Pagrindinis µKT metodo pranašumas yra jo neinvazyvumas, atkartojamumas ir didelis tikslumas.[26,49,515] Skirtingai

nuo kitų metodų, tokių kaip bakterijų, gliukozės, radioaktyviųjų izotopų, dažų įsiskverbimo testų ar SEM tyrimų, kurie buvo naudojami užpildų porėtumui ir mikropralaidumui įvertinti, neišvengiant tam tikrų trūkumų.[515] Šiame tyrime vieno kaiščio metodikos užpildų porėtumas buvo įvertintas naudojant µKT analizę, tačiau būtina paminėti ir šio tyrimo trūkumus. Nepaisant aukštos µKT raiškos ir tikslumo, mažos poros gali būti neaptinkamos dėl medžiagų rentgenokontrastiškumo ir raiškos slenksčio nustatymų.[26,28] Be to, M. G. Gandolfi ir kt. įrodė, kad skenavimo raiška pikseliais gali turėti įtakos rezultatų tikslumui ir mažiausios poros gali būti neaptinkamos, jei pasirenkama mažesnė skiriamoji geba.[198] Visgi reikia paminėti, kad nėra vieno protokolo, kuriame būtų nurodyta optimali skenavimo raiška. Nėra patikimo mokslinio pagrindo, ar 4 µm dydžio poros turėtų didesnę ar mažesnę įtaką šaknų kanalų užpildų mikropralaidumui ir endodontinio gydymo rezultatams, nei poros, kurių skersmuo 10 µm. Todėl šiame tyrime buvo naudojama mažiau laiko reikalaujanti, tačiau vis tiek tiksli ir didelė 9,99 µm skiriamoji geba, kaip siūloma ankstesniuose tyrimuose.[26,28,40,452]

Kitas aspektas, kurį būtų galima iš dalies priskirti tyrimo trūkumams yra tai, jog naudoti standartizuoti plastikiniai 3D modeliai, siekiant optimizuoti mėginių homogeniškumą ir užtikrinti vienodą vidinę dantų šaknų kanalų anatomiją ir tūrinius parametrus visose grupėse. Nors tokiu būdu galima geriau užtikrinti minėtus tyrimo parametrus, bet tiesiogiai susieti rezultatus su klinikine praktika būtų netikslu. Dentino struktūros ypatumai, drėgmė, dantų šaknų kanalų skersmuo / tūris yra veiksniai, galintys turėti įtakos takių HKSC hidratacijai bei fizikinėms savybėms, įskaitant porėtumą.[26,371] Dėl šių priežasčių plastikiniai modeliai negali sukurti klinikinių sąlygų ir užtikrinti pakankamai drėgmės, reikalingos takių HKSC hidratacijai ir kietėjimui. Todėl hidratacijos produktų, galinčių užpildyti tarpus tarp nehidratuotų cemento dalelių ir sumažinti užpildo porėtumą, trūkumas gali neigiamai paveikti bendrą analizę.[371] Kita vertus, standartizuotų modelių naudojimo pranašumai jau buvo aptarti kituose tyrimuose.[26] Todėl, jei tomis pačiomis sąlygomis naudojami tie patys modeliai, gauti rezultatai vis tiek yra tinkami palyginti tarp eksperimentinių grupių. Be to, reikia pabrėžti, kad pagrindinis šio tyrimo tikslas buvo įvertinti odontologijos studentų, gydytojų rezidentų bei gydytojų odontologų ir endodontologų atlikto dantų šaknų kanalų užpildymo vieno kaiščio metodika 3D kokybę, naudojant klinikinės patirties veiksnį kaip pagrindinį kintamąjį.

Remiantis šio tyrimo rezultatais, galima daryti prielaidą, kad jei odontologijos studentai, bendrosios praktikos gydytojai odontologai ir endodontologai pasiekė palyginamų dantų šaknų kanalų užpildymo kokybės

rezultatų, yra tikimybė, kad apskritai gydytojai odontologai ar odontologijos studentai turėtų lengvai įvaldyti vieno kaiščio dantų šaknų kanalų užpildymo metodiką, o kokybė turėtų atitikti gydytojo specialisto endodontologo sukuriamą homogenišką šaknies kanalo užpildą. Tačiau norint patvirtinti gydytojų patirties įtaką ilgalaikiams klinikiniams rezultatams, kai naudojama vieno kaiščio metodika, būtini klinikiniai tyrimai.

3.2. Tiesioginės ultragarsinės aktyvacijos įtaka perforuotų dantų šaknų viršūnių užpildų porėtumui, naudojant skirtingus takius HKSC ir jų naudojimo metodikas

Dantų šaknų perforacijos yra viena dažniausių šiuolaikinėje endodontologijoje pasitaikančių galimų komplikacijų.[516] Nepaisant pastarųjų metų pažangos endodontologijos srityje, mechaninis lenktų šaknų kanalų paruošimas išlieka dideliu iššūkiu net ir patyrusiems gydytojams.[517] Įrodyta, jog šaknų perforacijų komplikacijų rizika stipriai koreliuoja su šaknies kanalo lenktumo laipsniu, o krūminių dantų viršūninių šaknų perforacijų paplitimas yra žymiai didesnis nei kitų dantų.[518,519] Imituojant klinikinę situaciją buvo pasirinkti pirmieji apatinio žandikaulio krūminiai dantys, turintys vidutinį mezialinių šaknų linkį.

Šaknies perforacijos gydymui kertinę reikšmę turi laikas tarp perforacijos atsiradimo ir jos hermetizavimo. Hermetizavimas yra labai svarbus etapas siekiant pagerinti pažeisto danties prognozę ir išliekamumą.[520] Tyrimai rodo, kad iki 52–79 % šaknies kanalo paviršiaus ploto gali likti nepaliesta mechaninio paruošimo metu, nepaisant naudojamų instrumentų ar instrumentavimo technikos[521], o šiandien nė vienas taikomas plovimo protokolas negali užtikrinti visiškos šaknų kanalų sistemos dezinfekcijos.[522] Todėl endodontinio gydymo užpildymo etapas turi neabejotiną reikšmę sukuriant nepalankią aplinką mikroorganizmams, likusiems po chemomechaninio dantų šaknų kanalų paruošimo ir užkertant kelią jiems prasiskverbti į apieviršūninius audinius.[22,516]

Šaknų perforacijų hermetizavimo metodikos ir medžiagos nėra standartizuotos. Dėl tyrimų gausos teigiama, kad MTA yra įvairių tipų šaknų perforacijų hermetizavimo pirmojo pasirinkimo medžiaga.[520] *MTA Flow*^{TM} yra viena iš naujausių MTA pagrindu pagamintų HKSC užpildų, kuri klinikiniu pritaikomumu pranoksta I tipo klasikinę MTA dėl įvairiapusiško klinikinio pritaikymo galimybių bei lengviau atliekamų klinikinių manipuliacijų, dėl trumpesnio kietėjimo laiko ir padidinto atsparumo galimam išplovimui sąlytyje su skysčiais kietėjimo metu.[526] Šio ir kitų tyrimų

rezultatai rodo, kad *MTA Flow*^{TM} išlaiko ar net pagerina pageidaujamas originalios MTA esmines savybes, tokias kaip biologinis suderinamumas, reikalingų perforacijų hermetizavimo medžiagoms, kontaktuojančioms su periodonto audiniais.[230] Biologinis suderinamumas ir biologinis aktyvumas priklauso nuo nuolatinio kalcio jonų išsiskyrimo ir kalcio fosfato apatito kristalų susiformavimo, kurie skatina aplinkinių kietųjų audinių regeneraciją ir remineralizaciją, kartu sumažina užpildo porėtumą.[230,452] Ankstesni tyrimai atskleidė, kad nepaisant visų modifikacijų ir pranašumų, *MTA Flow*^{TM} lemia itin porėtus viršūninius barjerus. Ši išvada atitinka šio tyrimo rezultatus - abi *MTA Flow*^{TM} grupės (su / be ultragarso aktyvacijos) pasižymėjo dideliu porėtumu. Porų atsiradimas *MTA Flow*^{TM} užpilduose gali būti siejamas su padidėjusiu skysčio ir cemento santykiu atliekant maišymo procedūrą, kad būtų pasiekta taki HKSC konsistencija. Atliktų tyrimų rezultatai rodo, kad vandens perteklius mišinyje ilgainiui išdžiūsta ir palieka poras, kurių neužpildo hidratacijos produktai. [527] Be to, bismuto oksidas, kuris yra MTA Flow™ sudėtyje kaip rentgenokontrastiškumą suteikianti medžiaga, gali neigiamai paveikti užpildo hermetiškumą, trikdydamas hidratacijos reakciją ir užpilde palikdamas didesnį nesureagavusio vandens kiekį.[186] Kai kuriose medžiagose, pavyzdžiui, *BioRoot® RCS* (*Septodont, Saint-Maur-des-Fosses, France*), bismuto oksidas keičiamas cirkonio oksidu, kuris, kaip rodo atlikti tyrimai, neturi įtakos medžiagos porėtumui.[528] Šie teiginiai gali koreliuoti su šio tyrimo rezultatais, kadangi abiejose BR/VK grupėse viršūniniai barjerai pasižymėjo reikšmingai didesniu homogeniškumu nei *MTA Flow*^{TM} grupėse.

Viršūninių šaknų perforacijų hermetizavimas *BioRoot® RCS* kartu su modifikuota vieno kaiščio metodika buvo pasiūlyta dėl paprasto naudojimo ir veiksmingumo.[48,498] Vieno kaiščio metodikos koncepcija sukuria pageidaujamas *BioRoot® RCS* fizikines ir chemines savybes[225,529], būdingas biologiškai suderinamam užpildui[530], o kūgio formos gutaperčios kaištis atlieka takaus HKSC užpildo paskirstymo ir sutankinimo funkciją.[531] Tyrimai rodo, kad įvedus gutaperčios kaištį į šaknies kanalą susidaro hidraulinis slėgis, kuris pagerina medžiagos pasiskirstymą visame šaknies kanale.[532] Todėl gutaperčios kaištis gali būti laikomas pagrindiniu veiksniu, lemiančiu reikšmingus skirtumus tarp BR/VK ir *MTA Flow*^{TM} grupių.

Tyrime buvo siekiama sumažinti porų atsiradimą BR/VK ir *MTA Flow*^{TM} grupių užpilduose naudojant ultragarsinę aktyvaciją, tačiau nė vienu iš šių metodų nesugebėta sukurti viršūninių barjerų be porų. Ultragarso taikymo poveikis daugiausia susijęs su akustinės energijos perdavimu ir kavitacijos burbuliukų susidarymu, kurie ilgainiui sprogsta, padidindami temperatūrą ir slėgį šaknies kanalo viduje.[59] Remiantis ankstesniais tyrimais, kurie nustatė

reikšmingai mažesnį užpildo porėtumą po netiesioginės ultragarsinės aktyvacijos, galima manyti, kad padidėjęs slėgis gali pašalinti įstrigusį orą, išsklaidyti sukibusias daleles, sumažinti jų paviršiaus trintį ir efektyviau įterpti užpildo daleles į organinę matricą, nepakeitus užpildo dalelių dydžio ar medžiagos sudėties.[61,63,533] Be to, ultragarsinės aktyvacijos metu susidaręs slėgis gali lemti geresnę adaptaciją tarp užpildo medžiagos ir šaknies kanalo sienelės, taip pat paskatinti intratubulinį prasiskverbimą.[59,60] Tačiau šis tikėtinas tiesioginės ultragarsinės aktyvacijos poveikis nepagerino viršūninių barjerų homogeniškumo, o priešingai - padidėjęs atvirų ir uždarų porų kiekis buvo pastebėtas tiek BR/VK-UA, tiek MF-UA grupėse.

Prastesnis ultragarsu aktyvuotų viršūninių barjerų homogeniškumas gali būti siejamas su tiesiogine ultragarsine aktyvacija, dėl kurios susidaro pernelyg didelės vibracinės jėgos. Manoma, jog per didelė ultragarsinė energija gali sukelti oro įsiskverbimą į užpildo medžiagą ir taip padidinti porėtumą.[64,534] Tačiau tiesioginės ultragarsinės aktyvacijos naudojimas neturėtų būti tiesiogiai siejamas su mažiau homogenišku šaknies kanalų užpildymu, kadangi netiesioginė ultragarsinė aktyvacija taip pat gali padidinti porėtumą.[58] Visgi, didesnis dėmesys turėtų būti skiriamas ne ultragarsinės aktyvacijos tipui, o aktyvacijos laikui, kuris gali būti tiesiogiai susijęs ir su cemento dalelių persitvarkymu, ir su šilumos išsiskyrimu.[58,535] Šiame tyrime buvo pasirinktas 10 s ultragarsinis maišymas Sisli ir kt. metodu[536], 10 s aktyvuodami ultragarsu 5 mm viršūninius barjerus jie nustatė mažesnį užpildų porėtumą. Trumpas aktyvacijos laikas gali sukelti į šoką panašų efektą, o norint pertvarkyti cemento daleles ir sumažinti porėtumą, reikia 5–10 s.[58] Kita vertus, pailgėjęs aktyvacijos laikas gali lemti temperatūros padidėjimą, o galiausiai ir takaus HKSC dehidrataciją.[60,534] Nors tyrimų, įvertinančių temperatūros sukeliamus pokyčius šaknų kanalų užpilduose, vis dar yra mažai, atlikti keli tyrimai, kurių rezultatai rodo, kad ultragarsinė aktyvacija gali padidinti temperatūrą šaknies kanalo viduje 2 °C[537], o to gali pakakti, kad suintensyvėtų vandens pasišalinimas, vykstantis net 20 °C temperatūroje.[538] Vandens netekimas gali pakeisti medžiagos reologines savybes ir padidinti kiekį porų[537,538], kurios susiformuoja dėl tarpų tarp nehidratuotų cemento dalelių.[527] Nepaisant to, galima manyti, jog netiesioginė ultragarsinė aktyvacija nelemia neigiamų temperatūrinių pokyčių, kadangi ultragarsinio instrumento sukurta energija perduodama šaknies kanalo užpildui per gutaperčios kaištį ar endodontinį instrumentą. Tai paaiškintų prieštaringus porėtumo rezultatus, gautus šiame ir ankstesniuose tyrimuose[62,536], kurie taip pat atliko ultragarsinę aktyvaciją 10 s. Tačiau šio tyrimo rezultatus sunku tiesiogiai lyginti su turima literatūra dėl

skirtingų metodikos aspektų: naudoti skirtingų tipų dantų šaknų kanalų užpildai ir užpildymo metodikos, ultragarsinės aktyvacijos tipas ir trukmė, įvertinimo metodikos ir kt.

Atliktas tyrimas rodo, jog visi viršūniniai užpildai gali turėti mikropralaidumą, neatsižvelgiant į naudojamą užpildymo metodiką, kadangi nė vienas užpildas nebuvo be porų, o atvirų porų kiekis viršijo uždarą porėtumą visose eksperimentinėse grupėse. Nepaisant to, *MTA Flow*^{TM} grupių viršūniniai barjerai (su / be ultragarsinės aktyvacijos) lėmė reikšmingai didesnį atvirų ir uždarų porų pasiskirstymą lyginant su BR/VK grupe. Todėl, patvirtinant M. Benavides-García ir kt. [539] išvadą, galima teigti, kad skystos konsistencijos *MTA Flow*^{TM} (*Ultradent Products Inc., South Jordan, UT, USA*) neturėtų būti pirmojo pasirinkimo medžiaga perforuotų dantų šaknų viršūninių barjerams sukurti. Nors vis dar nėra aiškių įrodymų, koks porėtumo lygis yra kritinis, abiejose *MTA Flow*^{TM} grupėse pastebėtas reikšmingai didesnis porų kiekis teoriškai galėtų turėti įtakos prastesniam endodontinio gydymo rezultatui.[452,540] Kita vertus, įrodyta, kad HKSC kontaktuojant su audinių skysčiais vykstant hidratacijos reakcijai porėtumas sumažėja.[527] Todėl šio tyrimo rezultatus reikėtų vertinti atsargiai, nes naudojant *in vitro* modelius neįmanoma visiškai atkurti klinikinių situacijų. Reikia atlikti tolesnius tyrimus, siekiant nustatyti klinikinį BR/VK ir *MTA Flow*^{TM} metodikų efektyvumą užpildant vidutiniškai lenktų šaknų viršūnines perforacijas bei patvirtinti neigiamą tiesioginės ultragarsinės aktyvacijos poveikį dantų šaknų kanalų užpildymo kokybei ir homogeniškumui.

3.3. Takių HKSC biologinio suderinamumo analizė

Biologinio suderinamumo *in vitro* tyrimai, atliekant ląstelių kultūrų analizę yra vienas iš pirmųjų žingsnių, kad būtų galima įvertinti HKSC medžiagų biologines savybes. Šie laboratoriniai tyrimai gali būti atliekami kontroliuojamomis sąlygomis, suteikiant reikšmingų mokslinių žinių apie galimą medžiagos citotoksinį poveikį tiriamajai ląstelių kultūrai. Biologinis dantų šaknų kanalų užpildų suderinamumas yra būtinas siekiant išvengti ryškių uždegiminių reakcijų ir įgalinti kietųjų audinių atsistatymą.[541] Todėl šiuo tyrimu buvo siekiama išanalizuoti filtratų, išskirtų iš šviežiai sumaišytų takių HKSC užpildų: „tirštos“ konsistencijos *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* (*Ultradent Products Inc., South Jordan, UT, USA*), citotoksiškumą ŽDPKL proliferacijai ir tiriamos ląstelių kultūros morfologijai.

Dauguma *in vitro* tyrimų vertina sukietėjusių HKSC medžiagų filtratų įtaką ląstelių kultūroms.[77,125,542,543] Filtratų išskyrimas iš HKSC po įvykusios

hidratacijos reakcijos yra vienas iš klasikinių metodų HKSC biologinėms savybėms ištirti. Ši sukietėjusių HKSC biologinių savybių analizė yra svarbi siekiant įvertinti ilgalaikį HKSC poveikį ląstelėms ir/ar aplinkiniams audiniams. Tačiau atliekant šaknų kanalų užpildymą naudojami tik šviežiai sumaišyti ir vis dar kietėjantys HKSC cementai / užpildai.[544] Šviežiai sumaišyti HKSC užpildai reaguoja su aplinkos skysčiais ir aplinkiniais audiniais, tokiais kaip dentinas ar apieviršūniniai audiniai, kurie paprastai dar vadinami substratu.[32,391] Tai lemia pradinį kalcio jonų išsiskyrimą, taip pat atsipalaiduoja pašaliniai ir toksiški komponentai iš šviežiai sumaišyto HKSC, padidėja pH, todėl susiformuoja stipresnis poveikis aplinkinėms ląstelėms.[32,75,314,425] Pasibaigus kietėjimo reakcijai ir sumažėjus sąveikai su aplinkiniais audiniais, bendra užpildo struktūra tampa stabilesnė ir dėl to sumažėja citotoksiškumas.[545] Šios mintys pasitvirtino atlikus bandomąjį tyrimą, kurio metu palyginti tiriamųjų šviežiai sumaišytų ir sukietėjusių takių HKSC užpildų filtratai: sukietėjusių takių HKSC filtratai neturėjo reikšmingos įtakos ŽDPKL kultūrai po 24 valandų inkubacijos. Tuo pačiu metu šviežiai sumaišytų cemento filtratų net 25 % koncentracija lėmė reikšmingai mažesnį ląstelių gyvybingumą po 24 valandų. Šios išvados sutampa su kitais tyrimais, kuriuose nustatyta, kad sukietėjusių HKSC užpildų filtratų citotoksiškumas yra mažesnis, arba reikšmingo poveikio nenustatyta.[545] Dėl to kai kuriuose naujausiuose HKSC tyrimuose buvo pakeista ar papildyta tyrimo metodika bei pradėti naudoti šviežiai sumaišyti HKSC filtratai.[260,544] Siekdami palyginti šviežiai sumaišytų ir sukietėjusių HKSC cementų poveikį ŽDPKL, šiame tyrime citotoksiškumo analizei po 24 val. naudojome filtratus, išskirtus iš abiejų tipų HKSC. Nustačius reikšmingesnį šviežiai sumaišyto HKSC poveikį ląstelėms, tolesniems tyrimams naudoti filtratai išskirti iš šviežiai sumaišytų HKSC.

Sumaišius HKSC užpildą ar sušvirkštus jį į šaknų kanalų sistemą vyksta hidratacijos reakcija, kurios šalutinis produktas - kalcio hidroksidas yra atpalaiduojamas į aplinkinius audinius ir kliniškai sukelia aplinkos šarminimą, dėl kurio kyla medžiagos sąveika su klinikine aplinka ir susidaro aplinkinis filtratas.[32] Norint įvertinti tiesioginio sąlyčio HKSC medžiagų poveikį reikia sterilizuoti tiriamuosius mėginius, o tai gali turėti įtakos medžiagos savybėms.[545] M. Pedano ir kt. aptarė galimą mėginių užteršimą ruošiant filtratus, nepaisant to, jog taršos infekcijos neaptikta.[544] Iš HKSC užpildų išskirti filtratai buvo paruošti traukos spintoje steriliais instrumentais, taip pat filtruoti per sterilius filtrus. Be to, filtratai leidžia įvertinti skirtingai nutolusių (skirtingos koncentracijos) medžiagų poveikį tiriamųjų ląstelių kultūroms. Galiausiai, skirtingos filtratų koncentracijos

sukuria galimybę išanalizuoti galimą su doze susijusį ryšį ir nustatyti idealią koncentraciją pagal tirtų ŽDPKL ląstelių jautrumą.[545,546] Iš tiesų, tik 100 % koncentracijos tiriamųjų takių HKSC filtratai lėmė kliniškai reikšmingai mažesnį ląstelių gyvybingumą po 24 valandų ir vėlesnių laiko momentų nei kontrolinėje grupėje, nors 50 %, 25 % ir 12,5 % koncentracijos filtratų ŽDPKL gyvybingumas buvo didesnis nei 70 %. Dėl to ŽDPKL morfologijos ir tėkmės citometrijos analizei buvo atrinkti 50 % koncentracijos filtratai.

MTT citotoksiškumo tyrimo rezultatai atskleidė tirtų IV tipo takių HKSC užpildų *MTA Flow™* ir *MTA Flow™ White* biologinį suderinamumą, lyginant su plačiausiai ištirtu I tipo HKSC *ProRoot MTA*. Po 24 valandų inkubacijos su 100 % HKSC filtratu ŽDPKL gyvybingumas siekė apie 40 % MFWhite ir apie 50 % MF grupėse, palyginti su neigiama kontroline grupe. HKSC sukeliamas ryškesnis pradinis ŽDPKL proliferacijos slopinimas jau analizuotas *in vitro*[544,547] ir *in vivo* tyrimuose.[541,548] Nepaisant pirminio mažesnio ląstelių gyvybingumo nei kontrolinės grupės, HKSC užpildų naudojimas *in vivo* lemia panašią arba net geresnę pirminio endodontinio gydymo klinikinę sėkmę, palyginti su kitais užpildais, ir po 36 mėnesių siekia maždaug 89 %.[549,550] Be to, po 24 valandų inkubacijos su 50 %, 25 % ir 12,5 % *MTA Flow™* ir *MTA Flow™ White* filtratais nustatytas didesnis kaip 80 % ląstelių gyvybingumas, palyginti su kontroline grupe. Statistiškai reikšmingi skirtumai nustatyti tarp *MTA Flow™* ir *ProRoot MTA* filtratų paveiktų ŽDPKL grupių: ląstelių gyvybingumas reikšmingai sumažėjo – beveik 0 %, palyginti su kontroline grupe. O tai rodo stiprų citotoksiškumą ŽDPKL ir rezultatai reikšmingai nesiskiria nuo teigiamos kontrolinės grupės. C. Maspon ir kt. tyrimo rezultatai atskleidė panašų 100 % *ProRoot MTA* filtratų citotoksiškumą po 24 valandų.[77] Nepaisant to, nustatytas ryškesnis poveikis ŽDPKL yra priimtinas, lyginant su *ProRoot MTA*, kuri daugelyje tyrimų nurodyta kaip biologiškai suderinama HKSC medžiaga su ŽDPKL.[77,551–553] Ryškesnis *ProRoot MTA* citotoksiškumas ŽDPKL gali būti susijęs su mažesniu pirminiu kietėjančio užpildo atsparumu išplovimui[554] ir dėl to ryškesniu aplinkinių audinių (ar filtratų) pH pokyčiu.[555] Šiame tyrime atlikta pH analizė taip pat parodė didesnį vidutinį *ProRoot MTA* 100 % filtratų pH po 24 valandų, kuris siekė 12. *MTA Flow™* ir *MTA Flow™ White* filtratų pH nustatytos reikšmės buvo artimesnės neigiamai kontrolinei grupei, atitinkamai siekė apie 10 ir 8,5. Šarmingesnį I tipo *ProRoot MTA* filtrato pH taip pat įrodė kitas tyrimas, kuriame *ProRoot MTA* paveikto distiliuoto vandens pH buvo apie 11,9.[556] Todėl *ProRoot MTA* mėginių pH didėja greičiau, drastiškiau, o laikui bėgant mažėja.[557] *MTA Flow™* pH analizės rezultatai yra panašūs į *B. Guimares* ir kt. tyrimo, kuriame nustatyta, kad

MTA Flow^{TM} pH po 24 valandų yra maždaug 10, o palaipsniui didėjantis ir tolygiai mažėjantis *MTA Flow*^{TM} filtratų pH yra susijęs su didesniu pirminiu stabilumu.[526] Dėl šių priežasčių, kai buvo tiriamos sukietėjusios medžiagos, ląstelių gyvybingumas po 24 valandų inkubacijos su 100 % filtratu visų trijų tirtų HKSC medžiagų citotoksiškumas nebuvo nustatytas. Mažesnė *ProRoot MTA* filtrato koncentracija sukėlė nedidelį citotoksiškumą ŽDPKL, išlaikant ląstelių gyvybingumą virš 80 %. Pagal ISO 10993-5:2009 (atnaujintą 2022 m.) standartą, užpildo citotoksiškumo riba – kai ląstelių gyvybingumas mažesnis nei 70 %.[558] Atlikta tėkmės citometrijos analizė atskleidė didesnį nei 84 % ląstelių gyvybingumą ir nebuvo nustatyta statistiškai reikšmingų skirtumų tarp grupių po 24 valandų inkubacijos, paveikus abiejų tiriamųjų takių HKSC šviežiai sumaišytų cementų 50 % filtratais. Pagal ISO 10993-5:2009 klasifikaciją, 50 % filtratai nesukėlė citotoksiškumo ŽDPKL. Vertinant atliktą ląstelių proliferacijos tyrimą reikia pastebėti, kad 100 % filtratai lėmė 40 % MFWhite ir 50 % MF paveiktų ŽDPKL mažesnę proliferaciją, lyginant su neigiama kontroline grupe. Netiesiogiai lyginant ŽDPKL proliferacijos rezultatus su gyvybingų, apoptozinių ir nekrozinių ląstelių analize, 100 % filtratų citotoksiškumas gali būti įvertintas atitinkamai kaip lengvas ir vidutinis.

ŽDPKL proliferacijai didelę įtaką turėjo šviežiai sumaišytų HKSC filtratų poveikis visose grupėse. Tačiau filtratų slopinamasis poveikis priklauso nuo jų koncentracijos. Keletas ankstesnių tyrimų analizavo MF ir MFWhite biologinį suderinamumą.[230,259,260,334] Visuose minėtuose MF tyrimuose buvo naudojamos skirtingos biologinio suderinamumo analizės metodikos. C. Bueno ir kt.[230] bei J. Mondeli ir kt.[259] analizavo HKSC atsaką žiurkių poodiniame audinyje po 7, 15, 30 ir 60 dienų. Abiejuose tyrimuose nustatyta vidutinio sunkumo uždegiminė tirštos konsistencijos MF reakcija po 7 dienų ir pastebėtas uždegiminio infiltrato sumažėjimas vėlesniais laiko momentais. Pirmajame MF analizės tyrime L. Pelepenko ir kt. išanalizavo skystos konsistencijos MF citotoksiškumą periodonto raiščių fibroblastams po 24 valandų.[334] Nors šiame tyrime buvo naudojama kliniškai reikšmingesnė trimatė ląstelių kultūra, susijusi su *in situ* šaknies kanalo užpildymo eksperimentiniu modeliu pagal anksčiau minėtą metodiką[559], eksperimente naudotas sukietėjęs HKSC. Dėl šios priežasties MF filtrato citotoksiškumo analizė naudojant apieviršūninių audinių modelį su periodonto raiščių fibroblastais neparodė citotoksiškumo tirtai ląstelių kultūrai, palyginti su neigiama kontroline grupe. Antrajame L. Pelepenko ir kt. tyrime naudotas šviežiai sumaišytas MFWhite skystos konsistencijos HKSC.[260] Kaip ir tikėtasi, išvados pasikeitė ir atskleisti statistiškai

reikšmingi skirtumai, lyginant su biologiškai suderinamu *Biodentine*. *ProRoot MTA* ir MFWhite sumažino ląstelių gyvybingumą maždaug 90–100 %, palyginti su neigiama kontroline grupe, tačiau grupėse reikšmingai nesiskyrė. Šie L. Pelepenko ir kt. tyrimo rezultatai su MFWhite skiriasi nuo šiame tyrime gautų rezultatų, kadangi taikyta kitokia metodika ir naudota ŽDPKL kultūra. Šiame tyrime nustatytas ryškesnis ir greičiau pasireiškiantis MFWhite 100 % filtrato citotoksiškumas ŽDPKL, kadangi ląstelių gyvybingumas sumažėjo iki 40 % po 24 valandų. Šiuos rezultatus būtų galima sieti su išankstiniu 100 % filtratų paruošimu ir visišku augimo terpės pakeitimu į 100 % koncentracijos filtratą po 24 valandų, o L. Pelepenko ir kt. tyrime buvo naudojamas trimatis modelis, kuomet ląstelės buvo perkeltos į augimo terpę ir tik po to mėginys su šviežiai sumaišytu HKSC buvo įterptas į terpę, todėl filtratas formuojamas laipsniškai ir tolygiai keičiasi terpės pH. Be to, literatūroje ŽDPKL apibūdinamos kaip santykinai lėtai proliferuojančios ląstelės ir greičiau nustoja augti kultūroje, o periodonto raiščio ląstelės gali daugintis greičiau ir didesnį kiekį pasažų nei ŽDPKL.[560] Todėl skirtingos ląstelių kultūros ir šviežiai sumaišyto takaus MFWhite citotoksiškumo analizės metodika gali turėti įtakos rezultatams.

Atliekant ŽDPKL proliferacijos per 5 dienas analizę, buvo pastebėtas nuo laiko priklausomas reiškinys: ląstelių proliferacija padidėjo vėlesniais laiko momentais visose tirtose HKSC 50 %, 25 % ir 12,5 % koncentracijų filtratų grupėse. Tiriamųjų MF ir MFWhite filtratų paveiktų ląstelių proliferacija buvo panaši į *ProRoot MTA* 50 %, 25 % ir 12,5 % koncentracijų ŽDPKL grupių rezultatus visais laiko momentais. Tačiau per visą tyrimo laikotarpį 100 % filtratų grupėse nepastebėta ląstelių proliferacijos padidėjimo, tik MF ir MFWhite išlaikė ląstelių gyvybingumą nuo 5 % iki 10 %, palyginti su neigiama kontroline grupe. Šio tyrimo citotoksiškumo rezultatai yra panašūs į ankstesnių tyrimų rezultatus, kuomet šviežiai sumaišytas 100 % filtratas lėmė mažiausią ląstelių gyvybingumą, todėl filtrato koncentracijos mažėjimas ir 50 % ar mažesnės koncentracijos filtratų ekspozicija ŽDPKL kultūroms lėmė didesnį ląstelių gyvybingumą.[77]

Veikiant ŽDPKL tiriamųjų cementų filtratais, ląstelių morfologinė reakcija buvo įvertinta apverstos fazės šviesiniu mikroskopu. Ląstelių fenotipas neigiamoje kontrolinėje grupėje ir tiriamosiose grupėse, paveiktose mažesnės koncentracijos filtratų, buvo panašus į anksčiau aprašytas mezenchimines kamienines ląsteles, turinčias būdingą pailgą fibroblastinę morfologiją.[561,562] Lyginant su neigiama kontroline grupe, 100 % filtratų paveikti ŽDPKL pasižymėjo apvalesne ląstelių morfologija ir sumažėjusiu ląstelių skaičiumi. Be to, pastebėtas nuo filtrato koncentracijos priklausomas

reiškinys. Sumažinus filtrato koncentraciją, morfologija buvo panašesnė į neigiamą kontrolinę grupę, išskyrus 50 % koncentracijos išimtį, kuomet morfologija tirtose MF ir MFWhite paveiktose ŽDPKL grupėse ląstelių morfologija buvo pailgesnė ir platesnė nei neigiamoje kontrolinėje grupėje. Literatūroje įrodyta, kad ŽDPKL, kultivuojamų ant MTA paviršiaus, morfologija yra labiau diferencijuota nei neigiamoje kontrolinėje grupėje.[563] Tai gali būti susiję su anksčiau minėtu HKSC bioaktyvumo potencialu.[564,565] Įdomu tai, kad 50 % MF filtrato paveiktų ŽDPKL plotis ir ilgis buvo reikšmingai didesni nei 50 % MFWhite grupėje, o ilgio ir pločio santykis tarp grupių buvo panašus. Be to, 50 % MFWhite paveiktos ląstelės buvo panašesnės formos į neigiamos kontrolinės grupės nei 50 % MF grupės ŽDPKL. Palyginimui, kiti tyrimai nustatė skirtingą ŽDPKL susitraukimo laipsnį po poveikio skirtingais HKSC filtratais[562] ir ryškesnį ląstelių pasiskirstymą.[257] Tačiau tiesioginio palyginimo negalima atlikti, kadangi jokiuose ankstesniuose tyrimuose nebuvo įvertinta ŽDPKL morfologija po poveikio MF arba MFWhite filtratais. Šio tyrimo rezultatai rodo, kad ŽDPKL indukcija su 50 % MF filtratu padidina ląstelių plotį ir ilgį, tačiau statistiškai reikšmingo ląstelių skaičiaus padidėjimo tarp tirtų takių HKSC grupių nenustatyta.

Kita vertus, atlikto tyrimo rezultatai turėtų būti vertinami kritiškai, kadangi *in vitro* rezultatai negali būti tiesiogiai pritaikomi *in vivo* situacijoms. Izoliuotos ląstelių kultūros neatspindi *in vivo* danties pulpos audinio bei šaknų kanalų sistemos. Šie dvimačiai ląstelių kultūros modeliai yra supaprastinti, kadangi *in vivo* aplinką sudaro skirtingų ląstelių kultūros ir kraujotaka, kuri gali turėti įtakos vietiniam atsakui į naudotą užpildą.[566] Taip pat, įvairios buferinės sistemos, imuninė sistema ir kiti veiksniai gali turėti įtakos ŽDPKL gyvybingumui, proliferacijai ir morfologijai.[567,568] Be to, uždegiminė danties pulpa gali keisti klinikinę eigą, palaipsniui apimti visą danties pulpos audinį ir lemti pulpos nekrozę.[567] Nepaisant to, HKSC filtratų poveikio ląstelių kultūroms analizė suteikia vertingų duomenų, kaip medžiaga gali paveikti ląsteles, o tolesnio ląstelių atsako analizė gali apibūdinti medžiagos biologinį suderinamumą, citotoksiškumą, proliferacines ir diferenciacijos reakcijas.[569] Todėl šis tyrimas atskleidė į originalios *ProRoot MTA* panašų naujos kartos takių HKSC užpildų *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* tirštos konsistencijos biologinį suderinamumą. Norint išskirti ir rekomenduoti naudoti vieną takią HKSC medžiagą prieš kitas, reikia atlikti tolesnę analizę. Tačiau lengvesnis užpildo panaudojamumas, didesnis pirminis stabilumas, greitesnė hidratacija ir dėl to stabilesni pH pokyčiai gali lemti naujos kartos takių HKSC užpildų naudojimą klinikinėje praktikoje. Be to, atsižvelgiant į panašias pilkos ir baltos

MTA Flow™ spalvos biologinio suderinamumo savybes, dėl bismuto oksido kylančią grėsmę danties vainiko spalvos pokyčiams, logiškiausia būtų rekomenduoti rinktis *MTA Flow™ White*. Vis dėlto, derinant takaus HKSC pasirinkimą ir jo naudojimo klinikinį protokolą, gali padidėti klinikinės sėkmės rodiklis, kuris turėtų būti įvertintas atliekant *in vivo* tyrimus.

IŠVADOS

Atsižvelgiant į *in vitro* tyrimo apribojimus, gali būti teigiamos šios išvados:

1. Dantų šaknų kanalų užpildai, nepriklausomai nuo gydytojo klinikinės patirties, pasižymėjo porėtumu. Atviros poros vyravo visuose dantų šaknų kanalų trečdaliuose, o reikšmingiausias porėtumas nustatytas viršūniniuose trečdaliuose.
2. Skirtingą klinikinę patirtį turinčių gydytojų odontologų atlikto dantų šaknų kanalų užpildymo kokybė ir homogeniškumas viduriniame ir viršūniniame šaknų trečdaliuose reikšmingai nesiskyrė. Statistiškai reikšmingi skirtumai nustatyti tik tarp gydytojų užpildytų tiriamųjų dantų šaknų kanalų vainikinių trečdalių.
3. Reikšmingai didesnis porėtumas nustatytas takaus *MTA Flow*^{TM} grupėje be gutaperčios nei naudojant vieno kaiščio su *BioRoot RCS* metodiką.
4. Tiesioginė ultragarsinė aktyvacija neturėjo reikšmingos įtakos vieno kaiščio metodikos užpildo porėtumui. Perforuotų dantų šaknų kanalų viršūninių trečdalių *MTA Flow*^{TM} užpildų porėtumas, atlikus tiesioginę ultragarsinę aktyvaciją, reikšmingai padidėjo.
5. Takūs HKSC *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* filtratai turėjo reikšmingos įtakos ŽDPKL ląstelių gyvybingumui, proliferacijai bei morfologijai. Poveikis ŽDPKL priklauso nuo HKSC filtrato koncentracijos. 100 % ekstraktų tirpalai lėmė mažesnį nei 70 % ląstelių gyvybingumą.
6. Naujos kartos takių HKSC *MTA Flow*^{TM} citotoksiškumo poveikis ŽDPKL yra panašus į *ProRoot MTA*.

PRAKTINĖS REKOMENDACIJOS

1. Vieno kaiščio dantų šaknų kanalų užpildymo metodika kartu su takiais HKSC užpildais yra lengvai įvaldoma skirtingą endodontinio gydymo patirtį turinčių odontologijos studentų ir gydytojų odontologų. Todėl ši metodika galėtų būti plačiai naudojama klinikinėje praktikoje dantų šaknų kanalams užpildyti.
2. Siūloma papildyti ikidiplominių studijų studentams mokomas dantų šaknų kanalų pildymo metodikas vieno kaiščio dantų šaknų kanalų užpildymo metodika.
3. Vieno kaiščio metodika kartu su takiu HKSC *BioRoot*® *RCS* užpildu turėtų būti taikoma siekiant sukurti homogenišką apatinių pirmųjų

krūminių dantų lenktų mezialinių transportuotų šaknų viršūnių su perforacijomis viršūninį barjerą.
4. Tiesioginė ultragarsinė aktyvacija neturėtų būti taikoma užpildant dantų šaknų kanalus *MTA Flow*^{TM} takiu HKSC.
5. HKSC medžiagų biologinio suderinamumo tyrimuose rekomenduojama atlikti šviežiai sumaišytų ir sukietėjusių HKSC užpildų ir/ar jų ekstraktų analizę bei gautus rezultatus palyginti tarpusavyje.
6. Naujos kartos takių HKSC užpildų *MTA Flow*^{TM} ir *MTA Flow*^{TM} *White* biologinis suderinamumas ir patogumas naudoti užtikrina jų konkurencingumą greta kitų HKSC naudojimo pagal indikacijas kasdienėje klinikinėje praktikoje.

PUBLIKACIJŲ SĄRAŠAS

Straipsniai, publikuoti tarptautinės duomenų bazės Clarivate Analytics Web of Science (CA WoS) referuojamuose leidiniuose, su citavimo indeksu:

1. Drukteinis S, Bilvinaitė G, Tušas P, Shemesh H, Pečiulienė V. Porosity Distribution in Single Cone Root Canal Fillings Performed by Operators with Different Clinical Experience: A microCT Assessment. J Clin Med. 2021 Jun 10;10(12):2569. doi: 10.3390/jcm10122569. PMID: 34200692; PMCID: PMC8230067.

2. Drukteinis S, Bilvinaitė G, Tušas P, Shemesh H, Peciuliene V. Microcomputed Tomographic Assessment of the Single Cone Root Canal Fillings Performed by Undergraduate Student, Postgraduate Student and Specialist Endodontist. J Clin Med. 2021 Mar 5;10(5):1080. doi: 10.3390/jcm10051080. PMID: 33807655; PMCID: PMC7961753.

3. Drukteinis S, Bilvinaitė G, Shemesh H, Tušas P, Pečiulienė V. The Effect of Ultrasonic Agitation on the Porosity Distribution in Apically Perforated Root Canals Filled with Different Bioceramic Materials and Techniques: A µCT Assessment. J Clin Med. 2021 Oct 27;10(21):4977. doi: 10.3390/jcm10214977. PMID: 34768498; PMCID: PMC8584978.

Publikuotos mokslinių konferencijų pranešimų santaukos:

1. P. Tušas, J. Camilleri, S. Drukteinis, V. Pečiulienė. Characterization of MTA Flow. Volume 52, Issue S1. Special Issue: European Society of Endodontology: Abstracts from the Biennial Congress 2019, 11-14 September 2019, Austria Center Vienna, Austria. https://doi.org/10.1111/iej.13172 (R075)

2. P. Tušas, M. Alksnė, J. Camilleri, S. Drukteinis, E. M. Jonaitytė, V. Bukelskienė, V. Rutkūnas, V. Pečiulienė. Cytotoxicity of Flowable Hydraulic Calcium Silicate-based Cement Leachates. The International Association for Dental Research (IADR)/CED Oral Health Research Congress. Brussels, Belgium. September 16-18, 2021. (0093). https://ced-iadr2021.com/abstract-book/

GYVENIMO APRAŠYMAS

VARDAS	Paulius
PAVARDĖ	Tušas
GIMIMO DATA	1994-08-15
GIMIMO VIETA	Panevėžys, Lithuania
El. PAŠTAS	paulius.tusas@gmail.com

MOKSLINĖS VEIKLOS INTERNETINĖ SVETAINĖ	https://www.researchgate.net/profile/Paulius-Tusas

IŠSILAVINIMAS

2009 – 2013	Panevėžio Juozo Balčikonio gimnazija, Panevėžys, Lietuva *Vidurinio išsilavinimo brandos atestatas*
2013 – 2018	Lietuvos sveikatos mokslų universiteto Odontologijos fakultetas, Kaunas, Lietuva *Odontologijos magistro vientisosios studijos*
2018 – 2021	Vilniaus universiteto, Medicinos fakulteto, Odontologijos institutas, Vilnius, Lietuva *Endodontologijos rezidentūra*

DARBINĖ PATIRTIS

2018 – 2021	Gydytojas odontologas, VšĮ Vilniaus universiteto ligoninės Žalgirio klinika
2020 – dabar	Jaunesnysis mokslo darbuotojas, Vilniaus universiteto, Odontologijos institutas
2021 – dabar	Gydytojas endodontologas, privati praktika

LICENCIJOS

2018 – dabar	Gydytojo odontologo profesinė kvalifikacija, OPL – 05355
2021 – dabar	Gydytojo endodontologo profesinė kvalifikacija, OPL – 05876

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Vilniaus universiteto leidykla
Saulėtekio al. 9, III rūmai, LT-10222 Vilnius
El. p. info@leidykla.vu.lt, www.leidykla.vu.lt
bookshop.vu.lt, journals.vu.lt
Tiražas 20 egz.