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Irma
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Evaluation of enamel microcracks' characteristics before and after removal of metal and ceramic brackets for teeth from younger- and older-age groups: an *in vitro* study

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VILNIAUS UNIVERSITETAS

Irma
DUMBRYTĖ

Emalio mikroįtrūkimų charakteristikų vertinimas prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio ir vyresnio amžiaus grupėse: *in vitro* tyrimas

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Contents

List of Abbreviations

2D	Two-dimensional
3D	Three-dimensional
$2r$	Diameter
ANOVA	ANalysis Of VAriance
Calif.	California
CI	Confidence Interval
COP	Confocal Optical Profilometry/Profilometer
d	Effect size
DEJ	Dentin-Enamel Junction
EMC	Enamel MicroCrack
F	Fisher's exact test
FEM	Finite Element Model
h	Height
HAP	HydroxyAPatite
Ili.	Ilinois
Ind.	Indiana
ITF	Incomplete Tooth Fracture
l	Length
LODI	Lift-Off Debonding Instrument
MA	Measurement Area
Max	Maximum (highest) value in the data set
Min	Minimum (lowest) value in the data set
n	Number of observations or sample size
N.C.	North Carolina
NS	Non-Significant
OCT	Optical Coherence Tomography
OR	Odds Ratio
P_{EMC}	Pronounced Enamel MicroCrack
Q_1	First (lower) quartile (25th percentiles)
Q_2	Second quartile (median, 50th percentiles)
Q_3	Third (upper) quartile (75th percentiles)

r	Pearson's product moment correlation coefficient
RC (%)	Relative percentage Change
rho	Spearman's rank correlation coefficient
SD	Standard Deviation
SEM	Scanning Electron Microscopy/Microscope
U.K.	United Kingdom
U.S.	United States
W_{EMC}	Weak Enamel MicroCrack
x	Measurement step
$\bar{x}$	Sample mean
$\tilde{x}$	Median
$\hat{x}$	Mode
χ^2	Chi-squared test

1. Introduction

1.1. Relevance of the Study

With the highly skilled orthodontists and rapidly evolving technologies, almost every patient can be rewarded with a smile - an aspiration for life. Many more questions concern the possible undesirable changes in the tooth structure during treatment with brackets, especially following the debonding procedure. [1] Several studies have demonstrated that the bracket removal leads to irreversible alterations in the enamel irrespective of the debonding techniques and residual adhesive removal methods used. [2–6] Due to the forces generated during debonding, enamel microcracks (EMCs), a form of teeth damage, may develop and morphological changes of their parameters may appear. [4, 7–10] EMCs, quite often visible by the naked eye both by the patients and the dentists, may compromise the integrity of the enamel, cause stain, and plaque accumulation on the rough fractured surface, thus increasing susceptibility to carious lesions and damaging the appearance of the teeth. [4,11–13] Furthermore, the question about the effect of EMCs on the sensitivity of the teeth during the debonding procedure has been already raised. [4, 14]

As orthodontic treatment is on the margins of pathology alleviation and aesthetic improvement, it is important due to the principles of ethical provision of medical care that a clear benefit-to-harm relationship exists. [4] Understanding this basic principle has led to the necessity of publishing scientific reports dealing with the effect of the brackets' removal procedure on EMCs. [4] Over the last two decades an increasing number of studies have been presented, analyzing topics ranging from distribution of frequency of cracks [7, 15, 16] and their increased numbers and lengths, [17] or changes in frequency and severity of EMCs [18] to the evaluation of specific EMCs' characteristics (e.g. number, direction, length). [4, 8–10, 19–21] However, attention has not been paid to the width parameter that best describes the extent of enamel damage. Although the ageing-related changes in enamel microstructure and its mechanical properties are known, this issue has not been taken into consideration during EMCs examination either. [22, 23]

Progress in laboratory techniques introduced methods for EMCs detection, such as staining, transillumination, ultrasound, or optical coherence tomography (OCT). [4,15,18,24–27] However, several techniques (scanning electron microscopy (SEM), stereomicroscopy, confocal optical profilometry (COP), three-dimensional (3D) scanning methods) have been proved to be appropriate not only for visualization of EMCs, but also for measuring volumetric enamel loss, actual depth of the removed enamel, or performing spot or line measurements of EMCs' parameters. [1, 2, 4, 8–10, 17, 24–26, 28] No method for precise detection of the same EMC before and after debonding, and direct quantitative analysis of its characteristics under laboratory conditions has been presented in the relevant literature.

Nowadays, patients have high esthetic demands and pay more attention to the possible enamel damage that takes the form of EMCs following brackets' removal. [7,8,29] Enamel irregularities and visible EMCs are often observed by patients at the beginning of orthodontic treatment and thus questions arrise whether it is advisable to bond brackets on such teeth. [29] Using ceramic brackets causes more concern, because the physical properties of ceramics, such as hardness, high bond strength, and low fracture toughness or brittleness, have led to many reports of irreversible enamel surface damage during the debonding procedure. [18, 29–31] Thus, as patients' awareness is growing and documentation of EMCs is difficult, it is important to develop an understanding of the effect of metal and ceramic brackets' removal on visible EMCs and those EMCs that can be visualized only under SEM. [29]

1.2. The Aim of the Study

To evaluate and compare qualitative and quantitative enamel microcracks' (EMCs) characteristics before and after removal of metal and ceramic brackets for the teeth from the younger- and older-age groups.

1.3. Objectives of the Research

I To present a method for direct quantitative evaluation of an individual EMC employing SEM before and after debonding metal and ceramic brackets.

II To evaluate and compare severity, direction, location, length, and width of EMCs before and after metal and ceramic brackets' removal for the teeth from the younger-age group.

III To evaluate and compare severity, direction, location, length, and width of EMCs before and after debonding metal and ceramic brackets for the teeth from the older-age group.

IV To compare length and width of EMCs before and after metal and ceramic brackets' removal for the teeth from the younger- and older-age groups.

V To ascertain whether there is a correlation between the original dimensions of EMCs and the likelihood of them increasing during debonding.

VI To evaluate and compare the characteristics (length and width) of EMCs having varying degrees of severity (i.e. visible EMCs and those EMCs that can be seen only under SEM) before and after debonding metal and ceramic brackets.

VII To determine whether EMCs that can be visualized only employing SEM might progress to visible ones after brackets' removal, and to identify whether EMCs visibility, taken alone, is of any prognostic value.

VIII To determine if the predictions of irreversible changes in the tooth structure during debonding could be made from a set of the EMCs' parameters, age group, and type of the bracket used at the beginning of the treatment.

1.4. Significance of the Study

The findings of this study will complement the existing literature on the knowledge of the changes of qualitative and quantitative EMCs' characteristics during debonding of metal and ceramic brackets on teeth from two different age groups. The results will disclose whether orthodontic treatment with fixed appliances might result in greater enamel damage for the teeth from the older-age group. Data gathered will provide orthodontists with information on whether selection of ceramic brackets should be regarded as a contraindication for older patients due to the higher risk of enamel surface damage during debonding. Thorough examination of the EMCs width parameter in the occlusal, middle, and cervical thirds of the buccal tooth surface will enable clinicians to identify areas that are more prone to cracking, and thus need special attention during brackets' removal in order to minimize possible enamel damage.

Analysis of the teeth with visible EMCs before the bonding procedure will provide both the orthodontists and the patients with information on whether such teeth are more prone to greater enamel surface damage following debonding. Because of the laboratory devices used for EMCs' parameters evaluation and due to the obtained measurements of quantitative EMCs' characteristics, the reasons for the EMC visibility could be revealed, too.

Data gathered will also help to determine guidelines for the predictions about the higher risk of greater enamel surface damage during debonding from a set of the EMCs' characteristics, age group, and type of the bracket used at the beginning of the treatment. Thus, new teeth evaluation protocol that includes EMCs analysis during intraoral examination could be developed.

Finally, the presented novel method for EMCs examination, employing SEM and derived formulas, will help to detect and measure precisely with micrometer resolution the same EMC before bonding and after metal and ceramic brackets' removal for the teeth from both age groups. Such innovation could help to come up with strategies for *in vivo* analysis of EMCs by creating a fiber-optic microscope for measuring EMCs' parameters or for evaluating other enamel structure defects intraorally, thereby helping to diagnose and plan treatment for teeth with enamel irregularities.

1.5. Approbation of the Research

Publications (Web of Science)

1. Dumbryte, I., Vebriene, J., Linkeviciene, L., and Malinauskas, M., "Enamel microcracks in the form of tooth damage during orthodontic debonding: a systematic review and meta-analysis of *in vitro* studies," *Eur. J. Orthod.* **40**(6), 636-648 (2018).

2. Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Enamel microcracks in terms of orthodontic treatment: A novel method for their detection and evaluation," *Dent. Mater. J.* **36**(4), 438-446 (2017).

3. Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Does orthodontic debonding lead to tooth sensitivity? Comparison of teeth with and without visible enamel microcracks," *Am. J. Orthod. Dentofacial Orthop.* **151**(2), 284-291 (2017).

4. Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "The prognostic value of visually assessing enamel microcracks: *Do debonding and adhesive removal contribute to their increase?*," *Angle Orthod.* **86**(3), 437-447 (2016).

5. Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "Enamel cracks evaluation – A method to predict tooth surface damage during the debonding," *Dent. Mater. J.* **34**(6), 828-834 (2015).

6. Dumbryte, I., Linkeviciene, L., Malinauskas, M., Linkevicius, T., Peciuliene, V., and Tikuisis, K., "Evaluation of enamel micro-cracks characteristics after removal of metal brackets in adult patients," *Eur. J. Orthod.* **35**(3), 317-322 (2013).

Presentations (poster and oral)

1. Dumbryte I, Linkeviciene L, Linkevicius T, Malinauskas M. "Enamel microcracks following debonding: can tooth damage risk be predictable?" **Poster presentation**. CED-IADR/NOF Oral Health Research Congress. Madrid, Spain, 2019. **Participation in the Hatton Competition**.

2. Dumbryte I, Linkeviciene L, Linkevicius T, Malinauskas M. "What enamel microcracks characteristics lead to greater risk of tooth damage during orthodontic debonding?" **Oral presentation**. 10th Congress of the Baltic Orthodontic Association. Vilnius, Lithuania, 2018.

3. Dumbryte I, Vebriene J, Linkeviciene L, Malinauskas M. "Enamel microcracks as the form of teeth damage during orthodontic debonding: a systematic review and meta-analysis of *in vitro* studies" **Poster presentation**. 94th Congress of the European Orthodontic Society. Edinburgh, Scotland, 2018.

4. Dumbryte I, Linkeviciene L, Linkevicius T, Malinauskas M. "Enamel microcracks in terms of orthodontic treatment: a novel method for their detection and evaluation" **Poster presentation**. 93th Congress of the European Orthodontic Society. Montreux, Switzerland, 2017.

5. Dumbryte I, Linkeviciene L, Linkevicius T, Malinauskas M. "The prognostic value of visually assessing enamel microcracks during orthodontic debonding: the correlation between *in vitro* and *in vivo* results" **Poster presentation**. 92th Congress of the European Orthodontic Society. Stockholm, Sweden, 2016.

6. Dumbryte I, Linkeviciene L, Linkevicius T, Malinauskas M. "The prognostic values of visually assessing enamel microcracks during orthodontic debonding: the correlation between *in vitro* and *in vivo* results" **Poster presentation**. 9th Congress of the Baltic Orthodontic Association. Riga, Latvia, 2016.

7. Dumbryte I, Jonavicius T, Linkeviciene L, Linkevicius T, Malinauskas M. "Do existing enamel cracks always lead to tooth surface damage following the debonding?" **Poster presentation**. 8th International Orthodontic Congress. London, United Kingdom, 2015.

8. Dumbryte I, Jonavicius T, Linkeviciene L, Linkevicius T, Malinauskas M. "Pronounced enamel cracks - greater risk of enamel surface damage following debonding metal brackets?" **Poster presentation**. 90th Congress of the European Orthodontic Society. Warsaw, Poland, 2014.

9. Dumbryte I, Jonavicius T, Linkeviciene L, Linkevicius T, Malinauskas M. "Influence of patient age on the changes of enamel cracks characteristics after removal of metal brackets" **Poster presentation**. 90th Congress of the European Orthodontic Society. Warsaw, Poland, 2014.

10. Dumbryte I, Linkeviciene L, Malinauskas M, Linkevicius T, Jonavicius T, Peciuliene V. "Evaluation of enamel microcracks after removal of metal and ceramic brackets" **Oral presentation**. Lithuanian Chamber of Orthodontists Conference. Kaunas, Lithuania, 2013.

11. Dumbryte I, Linkeviciene L, Malinauskas M, Linkevicius T, Jonavicius T. "Comparison of enamel cracks characteristics after removal of metal and ceramic brackets" **Poster presentation**. 89th Congress of the European Orthodontic Society. Reykjavik, Iceland, 2013.

12. Dumbryte I, Linkeviciene L, Malinauskas M, Linkevicius T, Peciuliene V, Jonavicius T. "Comparison of enamel cracks characteristics after removal of metal and ceramic brackets: an *in-vitro* study" **Poster presentation**. 8th Congress of the Baltic Orthodontic Association. Tallinn, Estonia, 2013.

13. Dumbryte I, Linkeviciene L, Malinauskas M, Linkevicius T, Peciuliene V, Jonavicius T. "Comparison of enamel cracks characteristics after removal of metal and ceramic brackets in young patients" **Poster presentation**. 4th Baltic Scientific Conference in Dentistry. Tartu, Estonia, 2012. **The best poster presentation certificate**.

14. Dumbryte I, Linkeviciene L, Linkevicius T, Gibaviciute I, Malinauskas M. "Evaluation of enamel cracks characteristics after removal of metal brackets in adult patients" **Poster presentation**. 87th Congress of the European Orthodontic Society. Istanbul, Turkey, 2011.

15. Dumbryte I, Linkeviciene L, Linkevicius T, Gibaviciute I, Malinauskas M. "Evaluation of enamel cracks characteristics after removal of metal brackets in adult patients" **Poster presentation**. 7th Congress of the Baltic Orthodontic Association. Vilnius, Lithuania, 2011. **The best poster presentation certificate**.

2. Literature Review

2.1. Enamel Microstructure and Its Mechanical Properties

Enamel, possessing a complex microstructure that differs depending on distance from the tooth's outer surface, plays an important role in the protection of the dentin and the pulp. It is the most highly mineralized tissue of the human body and consists of 96.0 % mineral, 1.0 % protein, and 3.0 % water by weight. [32] For the molar teeth, enamel thickness is largest near the cusps (up to ≈ 2.5 mm), and thinnest in the cervical region (≈ 0.5 mm). [23, 32] On the microstructural level the inorganic part is comprised of crystal rods (sometimes called prisms, 4 –8 µm in diameter) that extend from the dentin-enamel junction (DEJ) to ≈ 6 –12 µm below the tooth surface. [33–35] Each single enamel rod is composed of bundles of nanometer-scale carbonated hydroxyapatite (HAP) crystals (≈ 25 nm thick, ≈ 100 nm wide, and ≥ 100 nm long) that are covered by an ≈ 1 nm thick organic layer. [33, 34, 36, 37] Every rod is separated by a very thin (≤ 1 µm) layer of protein-based organic matrix. [23, 38–40] The rods extend ≈ perpendicularly from the DEJ to the tooth's surface. [23] However, looking in more detail, the arrangement of the rods varies depending on their location: in the enamel closest to the tooth's surface the rods extend in a nearly parallel manner, whereas in the enamel near the DEJ the rods extend within groups that are obliquely oriented to one another. [34] Such microstructure of the enamel, the arrangement of mineral and organic components lead to its specific mechanical properties (i.e. contribute to high fracture toughness and the mechanisms of crack growth resistance). [34]

Three main mechanical properties of the enamel can be distinguished: hardness, elasticity, and fracture behavior. [41] It has been discovered that enamel is a type of anisotropic material and its mechanical properties are related to the location, chemical components, and arrangement patterns of the enamel rods. [23, 42, 43] Studies have found that the hardness and elastic modulus of enamel, some of the elastic property indices, increase with distance from the DEJ: enamel near the tooth's surface exhibits the highest hardness and elastic modulus. [23] When the hardness and elastic modulus were evaluated in terms of absolute distance from the DEJ, the highest gradient in these mechanical properties was found within the cervical region. [23] There is no agreement in the literature regarding the exact values of the aforementioned mechanical properties: the hardness ranges $\approx 3-6$ GPa, the elastic modulus - $70-120$ GPa. [23, 36, 44] Whereas in other studies the maximum hardness of 3.5 GPa at the tooth's surface has been determined with gradual decrease from the surface of the enamel to the DEJ. [45] At a distance of $100-600$ µm from the DEJ the hardness of enamel remained stable ($2.0-2.5$ GPa). [45] In addition to the importance of the location, the chemical components and degree of mineralization also play an important role in the mechanical properties of the enamel. [23, 42, 46] Studies have shown that both hardness and elastic modulus are positively correlated with calcium content. [41, 42] In cases of hypomineralized enamel, statistically significant reductions in the hardness and elastic modulus were observed with relatively minor decrease in mineral content. [47, 48] It has been estimated that a 3 GPa reduction in the elastic modulus could be expected with a 1.0 % reduction in volume concentration of HAP. [49] Finally, the mechanical properties of the enamel vary depending on the rod orientation, arrangement of the HAP crystals within each rod, and different position on the same rod. [23, 41, 50] Statistically significant differences in the mechanical properties of the enamel were discovered parallel and perpendicular to the rod, with the lowest hardness and elastic modulus values perpendicular to the enamel rod axis. [23, 36] Thus, for the cracks parallel to the rods less energy was required to induce fracture (i.e. in terms of fracture enamel which is parallel to the rods is very weak) compared with those oriented perpendicularly to the rods (i.e. enamel which is perpendicular to the rods is much more resistant to fracture). [51–53]

With regard to fracture properties of the enamel, it has been found that the internal enamel demonstrates strong resistance to fracture and that crack growth resistance increases from outside to inside. [41, 51, 54] The rise in crack growth resistance within the inner enamel can be explained by several mechanisms of toughening including crack bridging, crack deflection, and microcracking (i.e. capability of the enamel microstructure to promote guided crack growth and arrest, resulting in substantial toughening). [51] The following average fracture toughness values have been calculated: outer enamel (at the tooth's surface) (0.67 ± 0.12) MPa $m^{0.5}$, inner enamel (2.62 ± 1.39) MPa $m^{0.5}$/mm (growth toughness increases with proximity to the DEJ), at fracture (2.07 ± 0.22) MPa $m^{0.5}$. [51]

It has been explained that the material's brittleness is proportional to the hardness and elastic modulus, and inversely proportional to the square of the fracture toughness. [55] Thus, greater values of hardness and elastic modulus near the tooth's surface could lead to higher brittleness of the outer enamel in comparison with the inner enamel. [23, 55]

Since it has been determined that the mechanical properties of the enamel depend on the rod orientation and arrangement of the HAP crystals, values of the mechanical properties should only be presented together with information on microstructural orientation. [43]

2.2. Changes in the Mechanical Properties of the Enamel With Aging

With aging there are natural changes in the mineral content of the enamel. [23] First of all, the reduction in the proteinaceous matrix that resides along the rod boundaries takes place because of natural maturation of the enamel and intake of substances lowering oral pH. [32] Secondly, prolonged exposure of dental enamel to mineral iones and fluoride inside the oral cavity could stimulate replacement of the matrix with fluoro-apatites, thus leading to higher enamel density (increase in mineral content, particularly at the tooth's surface) and lower permeability. [23,56–58] One study has shown that the enamel of older-age patients (≥ 55 age) possesses 16.0 % higher elastic modulus and 12.0 % greater hardness compared with the younger-age enamel at the tooth's surface. [23] As it has already been demonstrated, these mechanical properties of the enamel might correlate with the fracture toughness and brittleness. [23, 59] Therefore, due to the natural changes in the enamel microstructure with age there may be a decrease in the fracture toughness and an increase in brittleness at the tooth's surface. [23] This might lead to easier crack initiation in the outer enamel compared with the younger-age patients. Whereas no age-dependent differences in hardness and elastic modulus have been observed near the DEJ. [23]

2.3. Enamel Microcracks

2.3.1. Formation of Enamel Microcracks

Careful evaluation of teeth with an intense light source during routine dental examination can often reveal microcracks in the enamel. They can be described as EMCs that usually do not cross the DEJ and have no loss or visible separation of tooth structure (typical images of EMCs demonstrated in Fig. 2.1). [60–64] These EMCs have been also categorized as incomplete tooth fractures (ITFs) and the following definition has been proposed: "*a fracture plane of unknown depth and direction passing through tooth structure that, if not already involving, may progress to communicate with the pulp and/or periodontal ligament*". [64] The formation of EMCs has generally been attributed to the abnormalities in the maturation process, occlusal forces (e.g. generated during dental attrition and abrasion), traumatic injuries (e.g. provoked due to tooth extraction procedure), temperature variations, and restorative processes. [13, 62, 65–67] As previously published studies have demonstrated, EMCs can be noticed following debonding at the end of the orthodontic treatment. [4, 7, 8, 13, 17, 68]

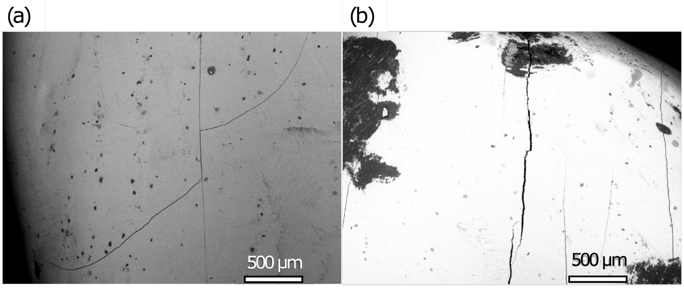

Figure 2.1: SEM micrographs of EMCs on the buccal tooth surface.

Understanding that the bracket removal procedure leads to the formation of EMCs poses the question, why does enamel get fractured during debonding? According to the recommendations presented in a review article published in 1975, an estimated bond strength of 5.9 – 7.9 MPa was found to be adequate for most clinical orthodontic cases. [69] Nevertheless, the usage of this "clinically acceptable" value has been criticized, as it has never actually been tested whether it is a sufficient *in vitro* bond strength for clinical usage. [70, 71] The mean tensile strength of enamel is (10.3 ± 2.6) MPa. [72] When comparing numbers of those two groups, it seems that every time during debonding we should be "on the safe side". However, the mean bond strength for the different types of brackets (metal or ceramic with various bracket base design and size), adhesives, and enamel conditioners (type of etching material, acid etch technique) combinations might range between 5.6 – 17.0 MPa. [73–77] Even higher bond strength values of 20.2 – 30.7 MPa could be found in the literature. [15, 17] Thus, when the debonding force exceeds the tensile strength of the enamel, fracture of the enamel surface occurs. [78]

Still, sometimes enamel fractures can be found with even lower bond strength values (e.g. 9.7 MPa). [79] However, no enamel damage has been discovered at bond strengths ≤ 8.2 MPa. [76, 79] The magnitude of the debonding force plays an important role in the enamel damage formation. [76] It has been demonstrated that the risk of enamel damage amounted to only 5.0 % at bond strengths ≤ 11.9 MPa. [76] When the bond strength was > 11.9 MPa, the risk of enamel damage reached 21.0 %, while for bond strengths > 14.7 MPa, the risk of enamel damage was ≈ 50.0 %. [76]

Although small EMCs may not result in tooth fracture, over prolonged periods their growth can be detrimental as they may pose a risk of multiple pathological and undesired consequences that can render the tooth unsavable later on. [80] The pathological consequences may vary from dentinal cracks, carious lesions, severely undermined enamel often contributing to microleakage around restorations, pulpal and periodontal involvement to complete tooth fracture. [27, 62, 80–87] The enamel cracks have been shown to be a permeable pathway that allows caries-producing bacteria access to the DEJ. [81] Thus, carious lesions within the tooth can be identified without visible decay signs at the surface. [81] In previously published literature, asymptomatic crack lines were considered as precursors to the symptomatic *cracked tooth syndrome*. [62, 80] Due to structural changes in the enamel the appearance of the teeth might be compromised as well.

However, as enamel cracks do not always penetrate into dentin, the existence of enamel cracks, even dramatic ones, does not necessarily show the presence of an incomplete coronal fracture or *cracked tooth syndrome*. [27] On the contrary, significant enamel cracks may also exist without dentinal cracks. [27] Thus, while assessing the prognosis of teeth with EMCs, additional factors, such as the age of the patient, the existance and location of wear facets, parafunctional activity patterns, and qualitative EMCs' characteristics (severity, direction, and location as it relates to occlusal loading and restorations), must be included in the diagnostic process. [27, 66]

Classification system of enamel cracks based on visual examination at ×16 has already been presented in the literature. [27] It has been suggested that type I cracks (i.e. craze lines; vertical cracks not related to restorations and without pigment accumulation; cracks following natural anatomic grooves; with superficial stain penetration; resulting from shrinkage of the composite material) have little or no risk of underlying pathology. [27] Meanwhile, moderate risk of tooth damage is inherent in type II cracks (i.e. wedge-shaped enamel ditching arising from a loss of tooth structure with no prior (or with an adjoining) restoration, often associated with a wear facet and localized occlusal loads centered over an otherwise benign crack; cracks that deviate or do not follow anatomic grooves). [27] Finally, the risk of underlying pathology is high in type III cracks (i.e. diagonal cracks branching off from a vertical crack (often indicating late-stage oblique incomplete fracture); horizontal or diagonal cracks usually rising from the corner of a restoration and narrowing as they extend gingivally (typically nonlinear); cracks housing debris, with or without previous restorations; pairs of cracks bordering the discolored enamel area (cusps or marginal ridge), with a high risk of dentin cracking and future complete fracture; cracks surrounded by brown, gray, or white shadow). [27] Clinically, it is important to be able to identify those EMCs that posses a moderate or high risk of underlying pathology, and consider appropriate treatment modalities.

2.3.2. Characteristics of Enamel Microcracks

The effect of the debonding procedure on the enamel damage has been extensively analyzed in the orthodontic literature. [10, 11, 16–18, 73, 88–92] Some of the studies [11, 73, 88, 90] evaluated the enamel surface only after brackets' removal, and no attention was paid to how enamel looked before bonding. However, most of the studies included tooth surface analysis both before the bonding procedure and after brackets' removal, therefore undesirable changes in the enamel structure due to debonding could be assessed. [10, 17, 18]

Systematic review of the studies published between January 2000 and July 2017 (inclusive) [4] demonstrated rising scientific interest in EMCs in relation to brackets' removal ranging from distribution of frequency of cracks [7, 15] and increased crack numbers and lengths, [17] or changes in frequency and severity of EMCs [18] to the evaluation of specific EMCs' characteristics both qualitatively and quantitatively. [8–10, 19–21] Length [8–10, 17, 19–21] and number [8–10, 17–21] of EMCs were the most frequently examined parameters, followed by direction evaluation. [4, 8–10, 18, 20]

One of the first attempts to measure the length of EMCs was published in 1978. [67] Calculation of the length parameter was carried out by superimposing a grid of known size with the resulting photographic slide at various time intervals for the teeth subjected to the thermal cycling procedure. [67] However, the details of the grid size used and the reference points selected were not included in the study. Subsequent published studies employed a variety of image processing software (e.g. Adobe Photoshop CS software (Adobe Systems Incorporated, San Jose, Calif., U.S.), [8–10, 20] Stereolith (Version 1, Shiraz, Iran) [19]) for the assessment of EMCs length before and after debonding. Some studies highlighted the ability to perform linear measurements for EMCs length analysis with the technique utilized. [93] It has been also demonstrated that EMCs length calculation could be carried out using a ruler in the center of the microscope lens before and after brackets' removal. [94] Nevertheless, guidelines on how to determine initial and final length measurement points were not provided, and it was not explained how to find exactly the same EMC before and after the debonding procedure. [8–10, 19, 20] This is especially relevant in those cases when EMC changes its direction while extending through the buccal tooth surface. Detailed description of the methodology is necessary in order to replicate the study as well.

The literature review has revealed that the number of EMCs was usually determined from the magnified images of the buccal tooth surface obtained employing laboratory devices, such as stereomicroscope equipped with a camera [8–10, 17, 19–21, 93] or transillumination with a fiber optic light head, [18] and image processing software. The same evaluation procedure was repeated before and after debonding, so the changes in the number of EMCs could be assessed.

Analysis of the direction parameter was carried out from the magnified images of the buccal tooth surface. [8–10, 20] Despite the slight differences, all the presented classifications of the direction characteristic in already published studies were based on the EMC's position in relation to the longitudinal axis of the tooth's crown. The following classification of the direction parameter was proposed: vertical (0 –30° to the long axis of the crown), oblique (31 –45° to the long axis of the crown), horizontal (46 –90° to the long axis of the crown), and mixed (when EMC changed direction). [10] Whereas in another study the direction characteristic was defined as: vertical (0 –30° to the long axis of the tooth), oblique (30 –60° to the long axis of the tooth), and horizontal (60 –90° to the long axis of the tooth). [9] Sometimes only rough classification of the direction parameter was provided (e.g. vertical, horizontal, and oblique) without a detailed description in which cases EMC could be considered as vertical, horizontal, or oblique. [18] However, all the above mentioned studies lack information on how the same EMC was identified before and after debonding.

One of the first classifications of the severity parameter was presented in a study published nearly 40 years ago. [13] In the latter study differentiation between weak EMCs (W_{EMCs}, those which are not apparent under normal room illumination but could be detected utilizing the fiber optic light source) and pronounced EMCs (P_{EMCs}, the ones that could be seen with the naked eye under normal room illumination without the use of diagnostic aids) was proposed. [13] The aforementioned criterion for P_{EMCs} was used in order to avoid a range of borderline cases when extra light was employed. [13] The same classification of the severity characteristic or description of P_{EMCs} was widely applied in the subsequent published studies. [9, 10, 18, 20]

2.3.3. Methods for Enamel Microcracks Evaluation

Various methods, such as staining, transillumination, ultrasound, or OCT have been presented in the literature for EMCs detection. [1, 24–27] Some of these techniques (e.g. staining and transillumination) can be applied directly intraorally for the visual evaluation and diagnosis of EMCs. [1, 27] Historically, methylene blue dye, caries indicator, transillumination, and alternative hydration and dehydration of tooth structure methods have been used for the visualization of EMCs. [27] Although previously transillumination was the most common modality for EMCs diagnosis, several drawbacks were detected when using it without magnification. [27] First of all, transillumination dramatizes all EMCs and irrespective of the severity they appear as structural cracks. [27] Secondly, delicate color changes are rendered invisible. [27] Thus, in order to avoid the aforementioned drawbacks, the majority of studies that employed the latter technique for EMCs analysis used transillumination with a fiber optic light head. [13, 15, 18] It is interesting to note that already in the 1980s, transillumination with fiber optic light was recommended for a more accurate assessment of EMCs. [13]

However, it is known that certain changes of EMCs' parameters occur during force application procedures in the course of orthodontic treatment. [1] The tendency of the development of greater enamel damage during debonding requires a detailed quantitative analysis of EMCs. Replication technique that combines *in vivo* and *in vitro* measurement has been introduced as a reliable method for crack morphological studies. [95] It has also been used as an alternative technique for non-carious cervical lesions analysis, [96] and tooth surface loss evaluation. [2,6,28,97] However, accuracy of indirect measurements is always introducing additional errors (example of a scanned EMC replica is shown in Fig. 2.2).

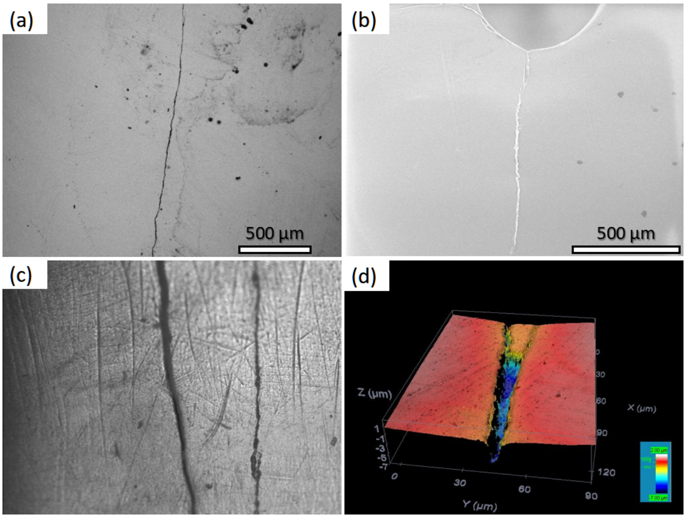

Figure 2.2: Images of EMCs employing: (a,b) SEM, (c,d) COP; (a,c) direct images, (b) replica of EMC scanned with SEM, (d) profile of EMC by COP.

There are several laboratory techniques (SEM, stereomicroscopy, COP, 3D scanning methods) that can be applied for measuring volumetric enamel loss, or the actual depth of the removed enamel, or for performing spot or line measurements of EMCs' parameters. [1, 2, 4, 8–10, 17, 24–26, 28] The methods which are most commonly used and demonstrated in the literature, their advantages and disadvantages in terms of the examination of the quantitative parameters of EMCs are presented in Table 2.1 and images of EMCs using different techniques are shown in Fig. 2.2. [1, 24–26, 98–102]

In the majority of studies evaluating EMCs in relation to the debonding procedure, stereomicroscopy technique was chosen for the visualization and analysis of cracks, [7–10, 17, 19–21] followed by transillumination with a fiber

Table 2.1: Comparison of methods for EMCs' characteristics evaluation.

Method; device	Working principle	EMCs' characteristics evaluated	Advantages	Disadvantages
Stereomicroscopy; stereomicroscope (equipped with a camera)	Optical microscopy	Number Direction Length Location	Direct evaluation of EMCs. No necessity for specific sample preparation. Non-destructive technique.	Lower resolution compared to SEM, useful magnification only up to 1000-2000 times. Due to limited magnification no possibility to measure width parameter. Lateral characterization of a tooth surface (2D), no possibility to measure depth of EMCs. Allows only a few measurements per tooth surface. Difficult to analyze non-flat surfaces. Time-consuming procedure. *In vitro* measurements.
SEM; SEM	Electron microscopy	Number Direction Length Location Width	Direct evaluation and measurement of EMCs. Higher resolution (higher than 1 nm) and ability of a higher magnification (up to 2 million times) compared to stereomicroscopy; visualization of structures that would not normally be visible via optical microscopy. Possibility to view the 3D external shape of an object. Non-destructive, highly sensitive technique.	Requirements for sample preparation (not obligatory to coat the teeth with a conductive layer for EMCs evaluation). Lateral characterization of a surface (2D), no possibility to measure depth of EMCs. Allows only a few measurements per tooth surface. Difficult to analyze non-flat surfaces. Time-consuming procedure. *In vitro* measurements.
COP; confocal optical profilometer	Optical microscopy	Number Direction Length Location Width Depth	Direct evaluation and measurement of EMCs. Lateral and axial characterization (3D) of a surface. No necessity for specific sample preparation. Non-destructive, highly sensitive technique.	Allows only a few measurements per tooth surface. More sensitive to non-flat surfaces than SEM. Time-consuming procedure. *In vitro* measurements.
OCT; optical coherence tomographer	Low-coherence interferometry	Number Direction Length Location Enamel loss (volumetric) Depth	Real-time image. High spatial resolution (spatial axial resolution of a few µm). Allows accurate and reproducible measurement of many points (can measure up to 50.000 points) of tooth surfaces and performs volumetric calculations of the total loss of substance (3D imaging). Non-destructive, non-radiative technique. Time efficient procedure.	Indirect measurement (through replication procedure) because tooth surface causes scattering of the laser beam and consequent loss of resolution. Limited penetration depth and scanning range. *In vivo* and *in vitro* measurements.
Ultrasound	High frequency sound waves	Number Direction Length Location Enamel loss (volumetric) Depth	Real-time image. High resolution. Accurate method. Non-destructive, non-radiative technique. Time efficient procedure.	Sample preparation (selection of appropriate coupling media for teeth enamel). Sensitive technique to non-flat surfaces. *In vivo* and *in vitro* measurements.

optic light head method. [15, 18]

Although SEM technique is routinely utilized for subjective observation of surfaces (e.g. surface roughness evaluation, detection of EMCs or other tooth structure irregularities) following brackets' removal, it has certain advantages over stereomicroscopy, COP and 3D scanning methods, such as OCT or ultrasound, in terms of EMCs evaluation (the advantages of SEM technique are listed in Table 2.1). [1]

2.4. Bonding Procedure

Systematic literature review on *in vitro* studies examining EMCs' characteristics before and after debonding revealed that different types of brackets were selected for enamel damage analysis. [4] The distribution of metal and ceramic brackets among the selected studies was as follows: [4] out of ten studies metal brackets were used in six of them, [8,10,15,19–21] ceramic brackets were chosen in three studies, [7,9,18] and both metal and ceramic brackets were selected in one study. [17]

Regarding bonding procedure two basic types of techniques can be distinguished: conventional, i.e. covering etching and rinsing procedures, and self-etch, i.e. combining etching and priming steps into one, eliminating the need for rinsing. [73,76] It has been demonstrated that lower but still clinically adequate bond strength can be obtained with self-etching primers [73,76] while resulting in less enamel damage, thus being more conservative, compared with the conventional system. [76,103,104]

2.4.1. Bonding Metal Brackets

Typically, metal brackets are bonded to enamel through mechanical retention. This type of retention can be achieved by welding mesh wires of different diameters to the bracket base, incorporating various designs in the mesh itself, [68, 105, 106] inserting milled undercuts in the bracket bases or manufacturing sandblasted, chemically etched, sintered with porous metal powder bracket bases. [107, 108] Employment of laser-structured bases is another innovative method to improve brackets' retention. [68, 109] Since the most appropriate type of bond - mechanical retention - for metal brackets has already been established, studies analyzing EMCs following debonding are more concentrated on other variables such as different bonding materials, [8, 15, 19–21] various instruments and techniques for metal brackets' removal [10, 19] rather than comparing different bracket base designs or retention protocols.

2.4.2. Bonding Ceramic Brackets

On the contrary, bonding ceramic brackets causes more concern. First of all, studies have shown that due to the physical properties of ceramics undesirable and irreversible changes in the tooth structure might occur during the debonding procedure. [18, 29–31] Secondly, proper type of bond selection (e.g. ceramic brackets with chemical or mechanical retention) is a critical issue in order to overcome or at least minimize the potential risk of EMCs during brackets' removal. [7, 17] Thus, knowledge of the structure of ceramics and physical properties of ceramic brackets could help understand why there is a greater risk of enamel damage following debonding this type of bracket and how to avoid or diminish it.

All the ceramic brackets which are currently available in the market are mainly composed of aluminium oxides (Al_2O_3). [78, 110, 111] Such composition has good aesthetics, is biocompatible, resistant to temperature and chemical changes, and possesses good bond strength. [111–115] Due to their differences during manufacturing process, two types of ceramic brackets can be distinguished, i.e. monocrystalline and polycrystalline. [78, 92, 114] The most evident difference between these two types of ceramic brackets is their optical clarity, with monocrystalline brackets being noticeably clearer and more translucent. [78]

The physical properties of ceramics (as a result of their atomic bonding) such as hardness, tensile strength, and fracture toughness or brittleness [78, 110] are closely related to the changes in the enamel structure during debonding. Extremely high hardness of aluminium oxide is a very significant physical property of ceramic brackets, an advantage over stainless steel brackets. [78, 92, 110] It is important to emphasize that ceramic brackets are nine times harder than stainless steel brackets or enamel. [112] Thus, in case of contacts between teeth and ceramic brackets, severe enamel abrasion might occur very quickly. [78, 116, 117]

The ability to resist structural failure is called tensile strength, [110] and it is much higher in monocrystalline alumina than in polycrystalline alumina, whereas in polycrystalline alumina it is higher than in stainless steel. [92, 112, 118] This characteristic of ceramics depends on the condition of the ceramic surface. [112, 119, 120] Thus, even the smallest surface imperfections or cracks can significantly reduce the load required for ceramic bracket fracture. [18, 78, 110] The latter physical property leads to low degree of deformation ($< 1.0\,\%$) of ceramic brackets making them more brittle. Meanwhile, metal brackets deform 20.0 % under stress condition before fracturing. [78, 112, 119, 120]

The third physical property of ceramics which is important to orthodontics, fracture toughness (the measure of a material's ability to resist fracture), is 20-40 times lower than that of stainless steel. [112, 120] For this reason it is much easier to fracture a ceramic bracket compared to a metal one, and ceramic brackets are more likely to shatter during the debonding procedure. Among ceramic brackets, polycrystalline ceramic brackets possess higher fracture toughness than monocrystalline ones. [121, 122] Whereas fracture toughness of enamel is lower than that of ceramic brackets [78] and bonded to brittle, rigid enamel ceramic brackets have little ability to absorb stress. [112] Thus, a lot of studies done on enamel damage assessment following ceramic brackets' removal confirm that this is a relevant issue to clinicians. [18, 89, 92]

Another clinically important aspect with regard to ceramic brackets - the most appropriate type of bond selection. Three different ceramic bracket bonding mechanisms can be distinguished: chemical retention, mechanical retention, and a combination of the two. [18, 111, 123] Chemical retention is achieved through silane-treated chemically retentive bases where a silane coupler works as a chemical mediator between the bracket base and the adhesive resin. [17, 111, 124] Mechanical retention can be provided by undercuts, grooves or indentations in the bracket base which allow mechanical interlocking with the adhesive. [7, 17, 123] Manufacturers are introducing into the market various ceramic bracket base designs (e.g. buttons, mechanical balls, micro-crystalline, polymeric bases where bonding occurs between the enamel and the polymer instead of the enamel and the ceramics) for mechanical retention. [7, 111]

Studies have demonstrated that chemically retained ceramic brackets possessed high bond strength that might lead to enamel damage during debonding. [7, 9, 123, 125, 126] The difference in bond strength between mechanically and chemically retained ceramic brackets could be explained by the way that stress concentration is distributed over the bonding surfaces. [78] In case of mechanical retention, there are high localized stress concentrations around the sharp edges of the grooves or other retention points incorporated in the base of the bracket resulting in brittle failure of the adhesive. [78, 114] Whereas for chemical retention, a much greater distribution of stress over the whole adhesive interface occurs without the presence of any localized stress areas. [78, 114] Thus, the shear bond must be much greater in order to cause debonding and pure adhesive failure. [78, 114] When comparing the effect of different ceramic bracket bonding mechanisms on the enamel surface (including the effect on possible EMCs increase or new EMCs formation), no statistically significant differences in enamel damage were noticed between the mechanical retention and polymer base brackets groups. [7] However, the debonding procedure of chemically retained ceramic brackets resulted in statistically significant enamel damage. [7] The results of a subsequently published study also indicated a considerable risk of enamel damage (greatest increase in number and length of EMCs) when debonding chemically retained ceramic brackets with pliers. [9] In contrast, sources can be found demonstrating no statistically significant difference in the number or length of EMCs after debonding metal brackets, ceramic brackets with mechanical retention, and even ceramic brackets with chemical retention (using a sharp-edged pliers for all brackets' removal), thereby leading to the conclusion that removal of ceramic brackets did not result in greater risk of enamel damage compared to metal ones. [17] Systematic review of *in vitro* studies on EMCs' parameters evaluation before and after debonding revealed a variety of variables (e.g. different types of retention, debonding methods) that could lead to the difference in the aforementioned results. [4]

2.5. Debonding Procedure

On the basis of *in vitro* literature review on EMCs, two main bracket removal techniques can be distinguished: conventional, i.e. with the use of appropriate pliers by hand, [7, 9, 10, 18, 19] and mechanical, i.e. with the help of a testing machine. [8, 15, 17, 20, 21]

2.5.1. Conventional Debonding

Although with the conventional bracket removal method it is almost impossible to control and standardize the actual debonding forces, it is likely that such a debonding pattern would help to avoid forces larger than those which occur in clinical practice when brackets are removed by hand. [11] Removal of brackets with the appropriate pliers by hand applies a bilateral force at the bracket base-adhesive interface and most of the adhesive should remain on the enamel surface. It is suggested that such a debonding pattern has the advantage of protecting the enamel surface. [73, 127] The selection of pliers depends on the type of bracket (e.g. metal or ceramic), bracket base design, and type of retention (e.g. chemical, mechanical retention, or a combination of both).

Regarding metal brackets, there are no strict specific instructions what kind of pliers should be used. Systematic literature review [4] of *in vitro* studies on EMCs evaluation during debonding demonstrated that metal brackets were removed using medium ligature cutters (peeling force; Dentaurum, Pforzheim, Germany), single-blade bracket remover (peeling force; Dentaurum), two-blade bracket remover (shear force; Dentaurum), [10] lift-off debonding instrument (shear force; LODI, 3M Unitek, Monrovia, Calif., U.S.), and bracket removing pliers (pressure force; Dentaurum). [19]

In contrast, manufacturers of ceramic brackets routinely provide recommendations on how to debond their brackets properly (i.e. information about the selection and position of pliers, and the mode of force application). Thus, the majority of *in vitro* studies on EMCs following ceramic brackets' removal were performed in accordance with these references: Weingart pliers were used for chemical (torsional rotation force; Fascination, Fascination 2; Dentaurum, Inspringen, Germany) [7, 9] or mechanical (squeezing force; APC Plus Clarity; 3M Unitek) retention brackets, [18] Howe pliers were selected for mechanical retention brackets (squeezing force; Clarity; 3M Unitek), [7] orthodontic wire cutter for mechanical retention with a polymer base brackets (squeezing force; InVu; TP Orthodontics, LaPorte, Ind., U.S.), [7] specific plastic debonding pliers were applied for mechanical retention brackets (peeling force; Inspire Ice; Ormco, Orange, Calif., U.S.), [9] and Debonding instrument was chosen for mechanical retention brackets (squeezing force; Clarity; 3M Unitek). [18] Recently almost each new release of aesthetic brackets comes along with new debonding pliers. [128–130]

2.5.2. Mechanical Debonding

Mechanical debonding enables more standardized procedures because all the specimens can be stressed in a direction which can be established precisely, and with a specific crosshead speed. However, this bracket removal technique is abrupt and unilateral in nature. [73] Therefore, it is often criticized for not representing a clinical stress situation realistically. [131] Based on force application during debonding with the testing machine, the following modes of load application can be distinguished: shear, tension, and torsion. [70] Studies where finite element model (FEM) analysis was used demonstrated that the mode of force application has large effect on the degree of the bond strength values and the location of the enamel damage. [11, 76] However, there is no consensus in the literature as to which of the debonding forces could detach the bracket more easily and result in a lower risk of enamel damage. [11,70,76] It has been concluded that in a clinical situation the stresses applied during bracket removal are a combination of tensile, shear, and torsion forces. [76]

2.5.3. Other Debonding Techniques

In addition to the conventional debonding, several other techniques have been suggested for ceramic bracket removal in order to reduce the rate of enamel damage: the ultrasonic method which requires the use of special tips, the electrothermal method which involves transmission of heat to the adhesive through the bracket, [18, 89, 132] and lasers. During the application of the electrothermal method, softening of the adhesive resin at a temperature above 150 –200° occurs and this enables debonding at a significantly reduced force level. [9,133] However, some studies have revealed signs of pulp damage (slight inflammation and odontoblastic disruption; localized damage of the pulp with slight infiltration of inflammatory cells) thus reducing the popularity of this technique. [9,134,135]

When a laser is used for the debonding procedure, its light initiates a photothermal interaction that leads to thermal softening of the composite. [133,136] Different laser wavelengths have been used for removing ceramic brackets in various studies. A systematic review of EMCs' characteristics analyses [4] revealed one study in which laser-assisted debonding was performed. [9] In the latter research, following carbon dioxide laser (peak power 188 W, frequency 400 Hz, pulse duration 500 µm with a wavelength of 10.6 µm) application, ceramic brackets were removed using specific pliers by hand, according to the manufacturer's recommendations. [9] The authors concluded that the laser-assisted debonding which they had used could result in ceramic bracket removal with minimal damage to the tooth tissues. [9]

Although the above mentioned methods have been applied more or less successfully for debonding, at least until now bracket removal with pliers remains the most popular and perhaps the most convenient approach used in daily clinical practice. [18] In general, efforts should be made to select a bracket removal method that would be sufficiently safe, widely used among other practitioners, and simulate more closely the debonding forces applied in actual clinical situations. [59]

2.5.4. Residual Adhesive Removal

During debonding the following failure patterns can be distinguished: cohesive bracket fracture (i.e. within the bracket); cohesive resin fracture (i.e. within the bonding material); cohesive enamel fracture (i.e. within the enamel); adhesive fracture at the bracket-resin interface; and adhesive fracture at the resin-enamel interface. [88, 126] If the bond failure site is the enamel-adhesive interface, the enamel surface looks clean (i.e. without adhesive), but there is a greater risk of undesirable changes in the enamel structure. [19] Whereas when a greater amount of resin remains on the tooth surface (bond failure at the bracket-adhesive interface), more finishing procedures are required for residual adhesive removal. [19] It is important to emphasize that the debonding pattern when much adhesive is left on the enamel has the advantage of protecting the enamel surface. [18, 127] However, attention should be paid on removing the residual adhesive carefully.

Routinely, in clinical practice the debonding procedure is followed by residual adhesive removal for cleaning-up the enamel surface and restoring the aesthetic appearance of the teeth. [137–140]

On the contrary, *in vitro* studies analyzing EMCs' characteristics in relation to debonding do not follow one standardized protocol regarding the residual adhesive removal. Systematic review of *in vitro* studies [4] revealed that from ten selected studies examining EMCs, in five of them residual adhesive was removed after debonding, [7, 9, 17, 18, 21] while four trials analyzed EMCs with adhesive remnants left on the enamel surface. [8, 10, 15, 19] In one study EMCs' parameters were measured before and after residual adhesive removal. [20]

For a long time studies have been looking for the best way to remove the residual adhesive without damaging the enamel surface. It was demonstrated that the use of the slow-speed tungsten carbide bur for the adhesive remnants' removal resulted in the least enamel loss. [103,141,142] Whereas, later published literature found that different clean-up methods had no statistically significant effect on enamel surface alterations, and that it was difficult to achieve adequate clean-up without enamel loss. [2] Therefore, in the studies evaluating EMCs after debonding, tungsten carbide bur (with specific blade configuration) operated in a slow-speed or high-speed handpiece was the most frequently selected instrument for the clean-up of the enamel surface. [4, 7, 9, 20, 21] Only a few studies emphasized application of water coolant in the course of the residual adhesive removal. [20, 21]

Previously published studies discovered that with the use of fiber optic light source enamel cracks could still be seen without difficulty through the adhesive. [90] The adhesive remnants were not removed in order to avoid altering the enamel surface by any rotary instruments, [90] or obliterating shallow cracks and filling them with debris, thus leading to difficulties in distinguishing cracks from the surrounding intact enamel. [10] Such approach has an impact on the methodological diversity of *in vitro* studies.

3. Materials and Methods

3.1. Enamel Microcracks' Characteristics Before and After Debonding Metal and Ceramic Brackets for the Teeth from the Younger- and Older-Age Groups

3.1.1. Teeth Selection

Extracted human maxillary premolars were included in the study. The teeth were extracted for orthodontic reasons or periodontal pathology from two groups of patients: a younger-age group (age range, 18-34 years), and an older-age group (age range, 35–54 years). [23, 143, 144] The primary criteria for the teeth selection were as follows: (1) intact buccal enamel with no white spots, signs of dental fluorosis or enamel hypoplasia; (2) no pre-treatment with any chemical agents (such as hydrogen peroxide); (3) no previous orthodontic, endodontic or restorative treatment; (4) specimens correctly stored following extraction. The secondary criteria for the teeth selection were: buccal enamel surface with EMCs or without them. Date, age of the patient, and the reason for extraction have been marked. The teeth were excluded if they did not meet the inclusion criteria and additionally due to the following reasons: (1) enamel structure defects (such as enamel tear outs) due to the extraction procedure; (2) wedge defects in the cervical region of the buccal tooth surface.

Before commencing the study, a power analysis was carried out in order to calculate the sample size. [145] Since there is no standardized method for sample size estimation of *in vitro* studies, the sample size was calculated using the following formula adapted from a previous study: [146]

$$Sample\ size = 2\ SD^2\ (Z^{\alpha/2} + Z^{\beta})^2/d^2 \qquad (3.1)$$

Where:

standard deviation (SD) = from a previously published study, [59]

$Z^{\alpha/2} = Z_{0.05/2} = Z_{0.025} = 1.96$ (from Z table) at type 1 error of

5.0 %, [147, 148]

Z^{β} = Z $_{0.20}$ = 0.842 (from Z table) at 80.0 % power, [148]

d = effect size = minimum difference of mean width values between two groups (from the first study evaluating width parameter of EMCs). [59]

It was determined that at the level of significance $\alpha = 0.05$ and at the power of the test of 0.80, the sample size should yield 80 teeth with EMCs (Group 1) and 80 teeth without EMCs (Group 2), both for the younger- and older-age groups. Taking into account the methodology and the need to form three subgroups having equal numbers of teeth (Subgroup 1, 2, and 3) in each group (Group 1 and 2), the number of teeth in each of the latter groups was increased to 90. This procedure was carried out both for the younger- and older-age groups separately. Thus, the final sample size included 360 teeth, half of them were extracted from patients who were 18-34 years old (mean ($\bar{x}$) = 27.99±5.19 years, median ($\tilde{x}$) = 29 years, mode ($\hat{x}$) = 34 years) and the rest 180 teeth were collected from subjects who were 35-54 years old ($\bar{x}$ = 42.36±7.05 years, $\tilde{x}$ = 40 years, $\hat{x}$ = 35 years). [149]

3.1.2. Sample Preparation

The teeth were prepared in accordance with the guidelines of the International Organization for Standardization (ISO/TS 11405; 2003). [150] The extracted teeth were decontaminated in 0.5 % chloramine-T solution and then stored in specimen tubes containing distilled water that was changed weekly before preparation and testing (example of the collection and storage of samples is presented in Appendix).

Prior to examination, the root of each tooth was embedded in a silicone matrix of the following diamensions: diameter ($2r$) = 26.5 mm, height (h_s) = 8.0 mm, so that the line passing through the most convex point of the buccal tooth surface would be parallel to the ground ($h_{s1} = h_{s2}$, as demonstrated in Fig. 3.1). The time lapse from extraction to testing was up to 12 months.

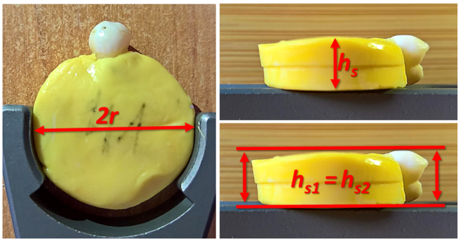

Figure 3.1: Fixing samples in a silicone matrix for the examination with SEM: left - top view (diameter, $2r$), right - side views (height, h_s).

3.1.3. Initial Examination of the Enamel Surface Employing a Scanning Electron Microscope

The research was conducted in line with the protocol demonstrated in Fig 3.2.

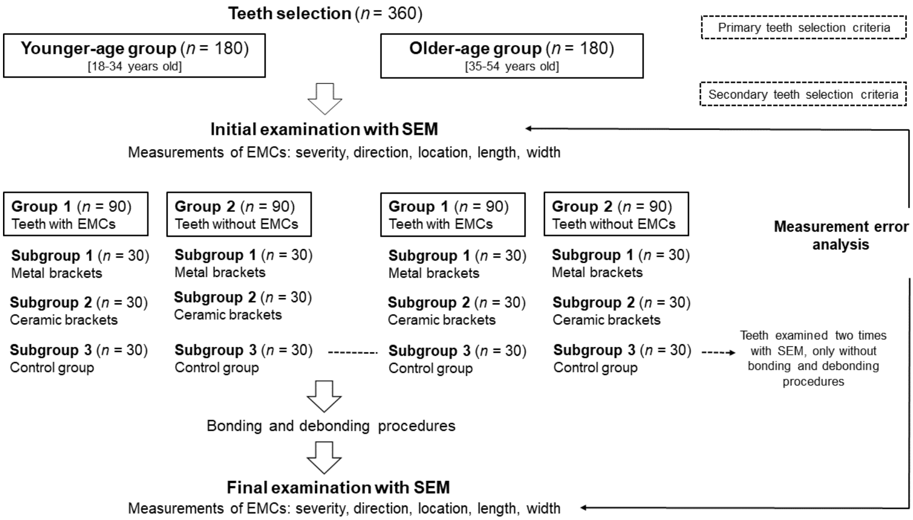

Figure 3.2: Graphical representation of the study protocol.

The presented methodology for EMCs assessment is innovative and for the first time introduced in the literature. The buccal enamel surfaces of all the teeth included in the study (both from the younger- and older-age groups) were examined with a SEM (Hitachi Tabletop Microscope (TM-1000), Tokyo, Japan) that was connected to a computer for image capturing (SEM used in the study depicted in Fig. 3.3). The SEM was operated at 15 kV, $< 5 \times 10^{-2}$ Pa (electron gun vacuum) and at $\approx 30-50$ Pa (specimen chamber vacuum). Appropriate and standardized position of each tooth for the scanning procedure was determined. Highest resolution and contrast images were obtained when the distance to the SEM detector was optimal, which corresponded to the 1 mm below the top of the entrance to the sample observation chamber (Fig. 3.4).

The teeth were not coated with a conductive layer prior to SEM examination. The initial evaluation of EMCs was performed at ×50–100. The selection of optimal magnification for each tooth depended on the height of the tooth‘s crown and enamel surface morphology. For the teeth with larger crowns and having visible by the naked eye EMCs ×50-60 was selected. On the contrary, for the teeth with smaller crowns and not so expressive morphology of the buccal tooth surface ≥ ×70-100 was chosen. This adaptation of the magnification magnitude enabled to reconstruct the buccal tooth surface from similar number of micrographs for all the teeth. If sometimes it was not clear whether there was an EMC, only the site which raised doubts was examined at higher

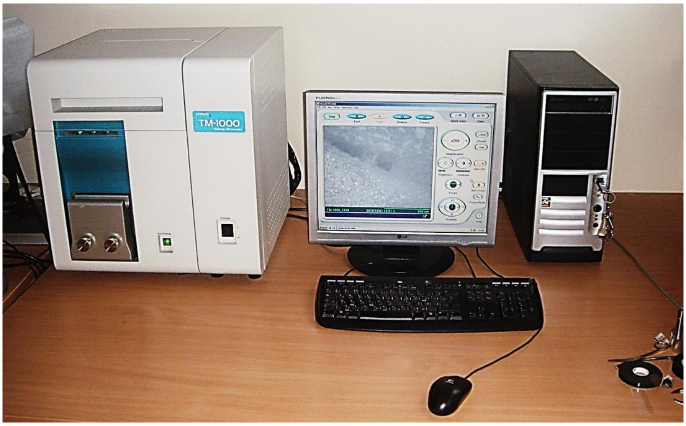

Figure 3.3: A SEM (Hitachi Tabletop Microscope (TM-1000), Tokyo, Japan) connected to a computer for image capturing.

magnification for confirmation or rejection of the existence of the EMC. However, if any EMCs or at least indistinct areas at ×50–100 were not noticed, those teeth were considered to be without EMCs and were not analyzed at higher magnification (an explanation regarding the chosen magnification will be presented in Discussion). Scanning procedure of all the teeth was performed by experienced operators according to the standardized protocol (as described above). Due to the large sample size scanning of the teeth from the younger- and older-age groups was carried out by two different operators under identical working conditions.

An evaluation of the buccal tooth surface and further detailed examination of EMCs is presented in Fig. 3.5. The SEM micrographs of the buccal enamel surfaces of all the teeth were taken. In order to reconstruct images of some larger crowns, stitching of high resolution SEM micrographs was performed using digital image processing software. For every tooth guiding anatomical

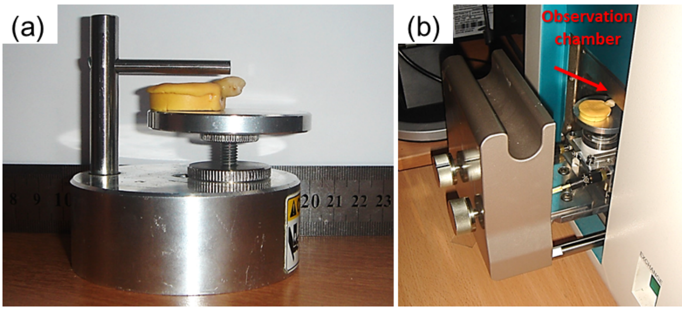

Figure 3.4: Tooth positioning for the scanning procedure: (a) scale of sample height determination for the scanning procedure, (b) sample prepared for putting (1 mm below the top of the entrance) to the sample observation chamber.

landmarks were chosen that helped to reconstruct the buccal enamel surface with EMC and identify the same EMC before and after debonding. The number of micrographs depended on the size of the buccal tooth surface and position of EMC. From these digital SEM micrographs, vertical height (h, the distance between two tangents passing through the highest and the lowest points of the buccal tooth surface) of every tooth's crown was measured. For detailed mapping of EMCs, the buccal enamel surface was divided into three zones of equal height: 1st zone - cervical third, 2nd zone - middle third, and 3rd zone - occlusal third (Fig. 3.5). [8,11,18,91]

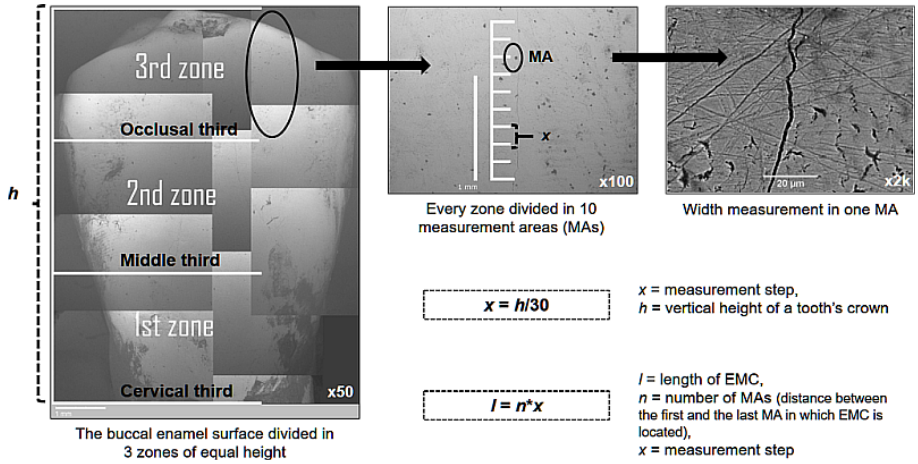

Figure 3.5: Evaluation of the buccal enamel surface with SEM. A measurement step (x, the distance between two measurement areas [MAs]) and length (l) of EMC were quantified utilizing formulas. For l analysis, the number (n) of MAs, that is, the distance between the first and the last MA in which an EMC was located, was calculated.

Following an initial examination utilizing SEM, the teeth from the younger- and older-age groups were divided into two groups of 90: Group 1, teeth having EMCs, Group 2, teeth without EMCs (Fig 3.2). The presence of EMC was the main criterion for grouping teeth. The teeth from Groups 1 and 2 were randomly assigned to one of the three subgroups, using the lottery method. Each tooth was assigned a unique number. The numbers were put in a bowl and thoroughly mixed. Without looking, the examiner selected thirty numbers for Subgroup 1, thirty numbers for Subgroup 2, and the rest were assigned to Subgroup 3. The teeth that were assigned those numbers were then included in the sample. The procedure was performed for Group 1, teeth with EMCs, and Group 2, teeth without EMCs, separately. This was done for the teeth both from the younger- and older-age groups.

Using a digitally sketched ruler, every zone was divided into 10 measurement areas (MAs); a total of 30 MAs of each tooth was obtained (Fig. 3.5).

With a help of derived formula, a measurement step (x, the distance between two MAs) was calculated. One EMC of every tooth was analyzed in detail. In cases of several visualized EMCs, the longest one was chosen. For all the teeth in Subgroups 1 and 2 (from Group 1, teeth with EMCs, and Group 2, teeth without EMCs, both from the younger- and older-age groups), qualitative and quantitative characteristics of the longest EMC were examined before and after debonding: severity, direction, location, length, and width.

Based on the visibility of the EMCs, they were classified into P_{EMCs} (visible under direct inspection with the naked eye using normal room illumination) and W_{EMCs} (not apparent under normal room illumination but visible with the help of SEM, Fig 3.6). [13, 18] Assessing the visibility of the EMCs was carried out repeatedly by the same examiner (I.D.) three times every other day. For the standardization, the same location, time of day, and tooth position were chosen.

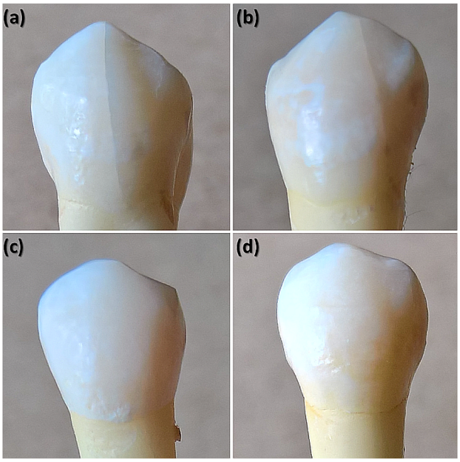

Figure 3.6: An example of teeth with P_{EMCs} (a,b) and W_{EMCs} (c,d).

The direction of EMC was classified as: vertical (0 –30° to the longitudinal axis of the crown), oblique (31 –45° to the longitudinal axis of the crown), horizontal (46 –90° to the longitudinal axis of the crown), and mixed (when EMC changes its direction while extending through the buccal tooth surface, Fig. 3.7). [10]

The location was specified as occlusal, middle, and cervical third of the buccal tooth surface depending on the position of the EMC (as demonstrated in Fig. 3.5). Longer EMCs were located in more than one third of the buccal tooth surface, e.g. cervical and middle third, middle and occlusal third or extended throughout cervical, middle, and occlusal thirds of the tooth surface.

The length of the longest EMC was calculated and the width was measured in each zone where the EMC was located (10 MAs of the width could be registered in every zone; formulas for length calculation presented in Fig. 3.5).

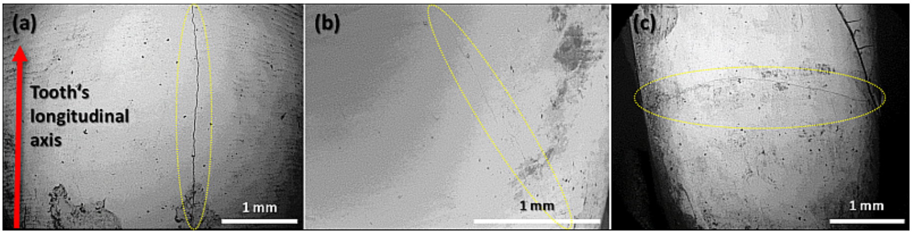

Figure 3.7: Classification of the direction of EMCs: (a) vertical, (b) oblique, (c) horizontal. It is not possible to depict mixed direction EMC from one micrograph using single magnification.

In Subgroups 3 (from Group 1, teeth with EMCs, and Group 2, teeth without EMCs, both from the younger- and older-age groups), the teeth were subjected to the same analysis but without bonding. All the teeth from Subgroups 3 were examined twice by SEM, as were the other specimens after the same time and means of storage. These teeth served as a control in order to study the effect of dehydration on existing EMCs or formation of new ones. All the evaluations and measurements of EMCs' parameters were performed by the same examiner (I.D.).

3.1.4. Bonding Procedure

3.1.4.1. Metal Brackets

In Subgroups 1, both for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the younger- and older-age groups, teeth were bonded with mechanically retained maxillary premolar metal brackets (Discovery; Dentaurum, Ispringen, Germany) with 0.22″ slots. The average surface area of the bracket base was measured and recorded as 11.9 mm^2 (Fig. 3.8).

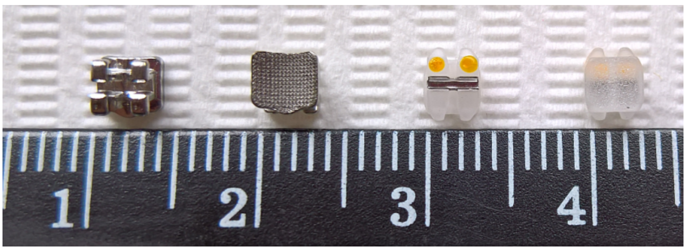

Figure 3.8: Metal (Discovery; Dentaurum, Ispringen, Germany) and ceramic (Clarity; 3M Unitek, Monrovia, Calif., U.S.) brackets for the bonding procedure.

All the teeth were prepared in accordance with the standardized requirements for the bonding procedure. The buccal surface of each tooth was cleaned (fluoride free paste using a rubber prophylaxis cup attached to a slow handpiece for 10 s, washed with air/water spray for 15 s, and dried with a stream of oil-free compressed air), etched with 34.5 % phosphoric acid gel (Vococid; Voco, Cuxhaven, Germany) for 30 s, rinsed with water for 20 s, and then dried with oil-free compressed air for 10 s (the surface of the etched enamel had a frosty appearance). After etching, a thin uniform coat of primer (Contex Primer; Dentaurum) was applied and cured with light for 10 s. The bonding base of the bracket was applied with a similar amount of resin adhesive (Transbond XT; 3M Unitek, Monrovia, Calif., U.S.). An example of bonding materials used is presented in Appendix. Then, the bracket was firmly positioned on the enamel surface with the help of a bracket-holding tweezer. The accurate bracket position was determined using a gauge, the excess adhesive was removed from around the base of the bracket with an explorer, this way ensuring uniform resin adhesive thickness. The light-cure adhesive was polymerized for 20 s (10 s from each proximal side) using a halogen light (Mini LED; Satelec, Cambridgeshire, U.K.).

3.1.4.2. Ceramic Brackets

In Subgroups 2, both for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the younger- and older-age groups, teeth were bonded with uncoated maxillary premolar ceramic brackets (Clarity; 3M Unitek) with mechanical bases and 0.22 ″ metal slots. The average base area was 11.83 mm^2 (Fig. 3.8). Bonding procedure for ceramic brackets was the same as for metal ones. After bonding, all the teeth were placed in distilled water at 37 °C and stored for 24 h prior to further testing. [150]

3.1.5. Debonding Procedure

3.1.5.1. Removal of Metal Brackets

Debonding of metal brackets was carried out with the conventional Utility/Weingart (Dentaurum) pliers by hand (debonding instrument demonstrated in Fig. 3.9(a)). The mesio–distal edges of the bracket wings were squeezed gently until the bracket was removed. [18]

3.1.5.2. Removal of Ceramic Brackets

Ceramic brackets were removed with the help of a Debonding instrument (3M Unitek) on the basis of the manufacturers' recommendations (debonding pliers shown in Fig. 3.9(b)). The pliers were placed against the mesial and distal sides of the bracket and were positioned symmetrically against the buccal surface of the bracket to optimize contact surface area. The instrument was gently squeezed until the bracket collapsed, then was gently rocked in the mesial-distal direction until the bracket became completely separated from the enamel.

Figure 3.9: Debonding instruments for (a) metal and (b) ceramic brackets' removal, (c) carbide-finishing bur for residual adhesive removal.

All the bonding and deboning procedures were performed by the same examiner (I.D.) in accordance with the aforementioned protocol.

3.1.5.3. Residual Adhesive Removal

After debonding both metal and ceramic brackets, all visible residual adhesive was carefully removed from the surface of the teeth with a slow-speed handpiece and a carbide-finishing bur, under normal clinical conditions (example of a carbide-finishing bur used in Fig. 3.9(c)). [103] Light movements of the bur were used in order not to scratch the enamel. Water cooling was not employed when the last remnants were removed, and polishing instruments were not utilized. The removal of residual adhesive was considered complete when the buccal enamel surface seemed smooth and free of composite to the naked eye, under the dental operatory light. [141] Polishing was not included in the methodology, because of difficulties in standardizing pressure and duration of this procedure in the clinical situation. [141]

3.1.6. Final Examination of the Enamel Surface with a Scanning Electron Microscope

Following the removal of brackets, the enamel surface was reevaluated with the SEM as described in Subsection 3.1.3. Qualitative and quantitative characteristics of the same EMC (as before the bonding procedure) were examined and measured: severity, direction, location, length, and width. The width of the longest EMC (determined during the initial examination) was evaluated in the same segment before and after debonding regardless of the changes in the EMC's length. Assessment of the teeth for new EMCs using SEM was performed repeatedly three times every other day by the same investigator (I.D.).

3.2. Effect of Specific Enamel Microcracks' Characteristics, Age Group, and Type of the Bracket Used on the Enamel Surface Damage During Removal of Metal and Ceramic Brackets

Graphical representation of the study protocol is demonstrated in Fig. 3.10. After initial evaluation of the enamel surface employing SEM (as presented in Subsection 3.1.3) and assignment of teeth from Group 1 (teeth having EMCs, both from the younger- and older-age groups) to Subgroups 1 (bonded metal brackets) and 2 (bonded ceramic brackets), qualitative and quantitative EMCs' characteristics were analyzed: severity, direction, location, and length before and after debonding. Methodology for EMCs evaluation is demonstrated in Subsection 3.1.3. A control group was not included in this study since there was no statistical comparability.

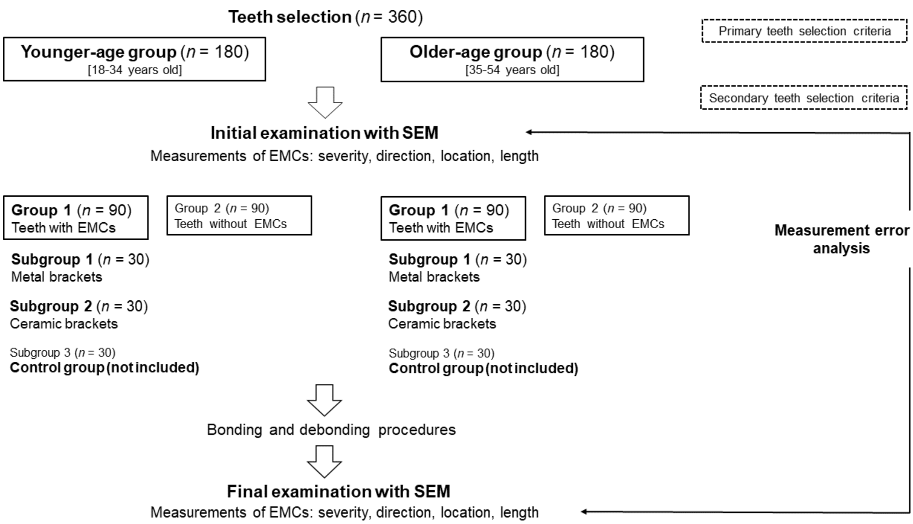

Figure 3.10: Graphical representation of the study protocol.

3.3. Pronounced and Weak Enamel Microcracks' Characteristics Before and After Debonding Metal and Ceramic Brackets

As one of the objectives was to evaluate and compare characteristics of EMCs having varying degrees of severity (EMCs visible to the naked eye (P_{EMCs}) and those EMCs that can be seen only under SEM (W_{EMCs})), the distribution of P_{EMCs} and W_{EMCs} among previously formed Subgroups 1 (bonded metal brackets) and 2 (bonded ceramic brackets) of Group 1 (teeth with EMCs), both from the younger- and older-age groups was calculated. Due to insufficient number of teeth with W_{EMCs} and uneven distribution of P_{EMCs} and W_{EMCs} among the subgroups, additional 90 extracted human maxillary premolars were selected for the present study. The proper number of teeth had been confirmed by estimating the sample size. [29] The teeth extracted for orthodontic reasons were collected based on the primary (as presented in Subsection 3.1.1) and secondary (i.e. P_{EMCs} or W_{EMCs} on the buccal enamel surface) teeth selection criteria. In view of the low distribution of W_{EMCs} among the teeth from the older-age group, all the samples were selected from the patients who were 18-34 years old ($\bar{x}$ = 26.72±4.79 years, $\tilde{x}$ = 26.50 years, $\hat{x}$ = 24 years). Preparation of the selected teeth for further analysis was carried out in the same manner as described previously (in Subsection 3.1.2).

The study was performed in line with the protocol presented in Fig. 3.11.

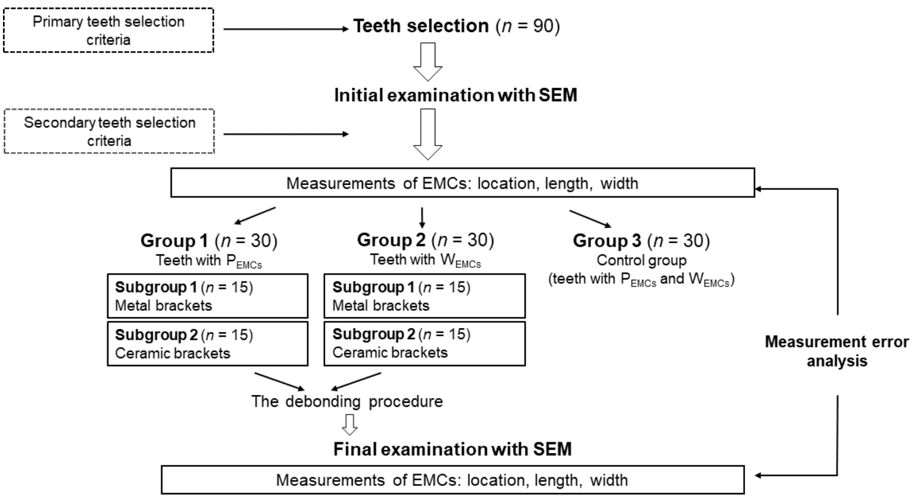

Figure 3.11: Graphical representation of the study protocol.

After a direct inspection with the naked eye under normal room illumination and initial examination with the SEM (as explained in Subsection 3.1.3), the teeth were divided into three groups of 30: Group 1, teeth having P_{EMCs}, Group 2, teeth showing W_{EMCs}, and Group 3, a control group (comprised of an equal number of teeth with P_{EMCs} and W_{EMCs}). EMCs were classified into P_{EMCs} and W_{EMCs} based on their visibility. Parameters (location, length, and width) of each individual EMC were evaluated before and after debonding. In Group 3 (control group), the bonding procedure was not carried out. However, the teeth were analysed two times by SEM, as were the other specimens from Groups 1 and 2 after the same time and means of storage. Methods for examining and measuring EMCs were explained in detail in Subsection 3.1.3. All the evaluations and measurements of EMCs' characteristics were carried out by the same examiner (I.D.).

The teeth from Groups 1 and 2 were randomly assigned to one of the two subgroups, using the lottery method (as described in Subsection 3.1.3). In Subgroup 1, 15 teeth, both from Groups 1 and 2, were bonded with maxillary premolar metal brackets (Discovery; Dentaurum). In Subgroup 2, 15 teeth, both from Groups 1 and 2, were bonded with maxillary premolar ceramic brackets (Clarity; 3M Unitek). Brackets' characteristics, bonding, and debonding methods were presented in Subsection 3.1.4 and Subsection 3.1.5.

All the bonding and debonding procedures were performed by the same examiner (I.D.) in line with the aforementioned protocol. After brackets' removal, the enamel surfaces of all the teeth were reevaluated with the SEM as explained in Subsection 3.1.6.

3.4. Measurement Error Analysis

Measurement errors were analyzed using a method suggested by Bland and Altman. [151, 152] Measurements of already scanned images were repeated for 30 teeth, both from the younger- and older-age groups (teeth selected from the study presented in Section 3.1). Thus, in total 60 teeth were remeasured. There was 100.0 % agreement between the two length measurements of EMCs. The mean of the differences between the two width measurements was -0.02 µm, while the limits of agreement were -0.42 and 0.38, indicating that 95.0 % of the differences between these two measures, excepting the measures crossing the dashed lines in Bland-Altman plot, were within this range (as depicted in Fig. 3.12).

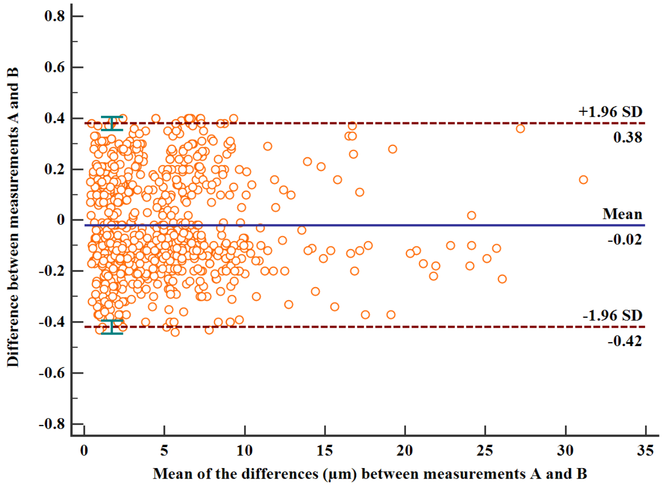

Figure 3.12: Bland-Altman plot. The solid line shows the mean of the differences (µm) between the two width measurements. Dashed lines show the upper (mean + 1.96 standard deviation, SD) and lower limits (mean - 1.96 SD) of the 95.0 % confidence interval (CI) of agreement.

3.5. Statistical Analysis

Statistical analysis was performed using the Statistical Package SPSS 17.0 (SPSS Inc., Chicago, Ili., U.S.). Before starting the statistical evaluation, tests were carried out in order to examine data distribution. This procedure was done for continuous random variables. When the results of the Kolmogorov-Smirnov test demonstrated that the variables (length measurements, length and width measurements of different severity EMCs) fulfilled a normal distribution

($P > 0.05$), parametric statistics for further analysis was applied. Examples of data distribution for EMCs length measurements for the teeth from the younger- and older-age groups are demonstrated in Appendix. The mean ($\bar{x}$), standard deviation (SD), maximum (Max), and minimum (Min) values were calculated for each variable. Relative percentage change (RC (%)) was assessed using the following formula: [153]

$$Relative\ percentage\ change = (y - x)/|x| \times 100\% \tag{3.2}$$

Where:

x = mean value before bonding,

y = mean value after debonding,

|x| = absolute value of x without regard to sign.

Analysis of variance (ANOVA) with Fisher's exact test (F) was carried out to evaluate differences among the three different subgroups. A paired samples *t*-test was performed to determine differences between the measurements before and after debonding. An independent samples *t*-test was applied to compare values between two unrelated groups (e.g. younger- and older-age groups, metal and ceramic brackets, P_{EMCs} and W_{EMCs}) on the same continuous, dependent variable.

For the normally distributed data, Pearson's product moment correlation coefficient (Pearson's r) was selected to assess the strength and direction of the linear relationship between pairs of variables. [154] The following guide ("Rule of thumb") can be used for interpreting the size of the correlation coefficients: 0.90 to 1.00 (-0.90 to -1.00) - very high/very strong positive (negative) correlation, 0.70 to 0.90 (-0.70 to -0.90) - high/strong positive (negative) correlation, 0.50 to 0.70 (-0.50 to -0.70) - moderate positive (negative) correlation, 0.30 to 0.50 (-0.30 to -0.50) - low/weak positive (negative) correlation, 0.00 to 0.30 (0.00 to -0.30) - negligible correlation. [154, 155]

In those cases when the continuous variable did not follow normal distribution (Kolmogorov-Smirnov test, $P < 0.05$), non-parametric statistics was applied (width measurements). In order to allow a detailed data interpretation, Max, Min, Q_1 (first (lower) quartile, 25th percentiles), Q_2 (second quartile, median, 50th percentiles), and Q_3 (third (upper) quartile, 75th percentiles) values were given in respective tables. The Wilcoxon signed-rank test was used to assess differences before and after debonding (significance demonstrated as Z and P values). The Mann-Whitney U test was applied to examine differences between two unrelated groups (e.g. younger- and older-age groups, metal and ceramic brackets, P_{EMCs} and W_{EMCs}) on the same continuous, dependent variable (significance demonstrated as U and P values). Since the width measurements were not normally distributed, non-parametric Spearman's rank correlation coefficient (Spearman's rho) was used to determine a linear relationship between two variables (width values of EMCs before bonding and after brackets' removal). [154, 155]

Severity and direction parameters of EMCs were reported as percentages of the total number of teeth in separate subgroups. In order to study the probability of independence of variables, Pearson's chi-squared (χ^2) test was used.

Binary logistic multivariable regression was applied to predict the extent of tooth damage following the debonding procedure from a set of the EMCs' characteristics, age group, and type of the bracket used. Calculated length values (before and after debonding) of EMCs (Group 1, teeth with EMCs, both from the younger- and older-age groups) represented enamel surface damage during brackets' removal. A dependent (response) variable was the length of the EMC, which was converted from continuous scale to nominal, using variance analysis of continuous values [(0.24 thru 3.00 = 1) (3.01 thru 10.15 = 2); 1 - small/low, 2 - large/high]. Five independent (explanatory) variables (severity, direction, location of EMC, age group, and bracket type) were included in the model. For the purposes of statistical analysis, vertical and oblique direction (EMC inclination $< 46°$), as well as horizontal and mixed (inclination $\geq 46°$ or EMC changing its direction) were combined. The same procedure was carried out for the location parameter, thus creating two groups (EMC located in the occlusal, or middle, or occlusal and middle third of the buccal tooth surface; cervical, or middle and cervical, or in all three thirds of the buccal tooth surface) for statistical data evaluation.

For graphical presentation of normally distributed variables, error bars with 95.0 % CI were shown. In case of overlapping of those intervals between two comparison groups, it was stated that there was no statistical difference between them. In other cases, with no overlapping CI, the statistical significance of the differences was evident with 95.0 % probability. Scatterplots were created for the visualization of correlation. To visualize differences between two qualitative variables, Mosaic plots were applied using JMP statistical software (SAS Institute Inc., Cary, N.C., U.S.). [156] Significance for all statistical tests was based on P values ($P = 0.05$) or 95.0 % CI.

4. Results

4.1. Enamel Microcracks' Characteristics Before and After Debonding Metal and Ceramic Brackets for the Teeth from the Younger- and Older-Age Groups

4.1.1. Enamel Microcracks' Characteristics Before and After Removal of Metal and Ceramic Brackets for the Teeth from the Younger-Age Group

Following repeated teeth evaluations for new EMCs using SEM, no discrepancies between results were observed. After repeated EMCs visibility assessments, no discrepancies between findings were recorded either.

Group 1 (Teeth with EMCs)
Distribution of P_{EMCs} and W_{EMCs} before and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) is presented in Table 4.1. Results from the statistical analysis indicated that there was no statistically significant difference ($\chi^2 = 0.739$, $P = 0.390$) between bracket type (metal or ceramic) and severity of EMC (P_{EMC} or W_{EMC}) after debonding (the difference between variables is demonstrated in Fig. 4.1). There were no changes in EMCs severity after metal brackets' removal. Whereas three EMCs, invisible to the naked eye, progressed to visible ones following ceramic brackets' debonding.

Distribution of the direction parameter before and after removal of metal and ceramic brackets is shown in Table 4.2. The majority of EMCs (73.3 % in Subgroup 1 and 93.3 % in Subgroup 2) had vertical direction, followed by oblique (16.7 %) in Subgroup 1 or mixed (6.7 %) in Subgroup 2. The results revealed that there were no changes in the direction of EMCs after debonding of either metal or ceramic brackets.

The mean length values of EMCs for Group 1 before and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) are presented in

Table 4.1: Distribution of P_{EMCs} and W_{EMCs} for Group 1 (teeth with EMCs) from the younger-age group before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2)

		Distribution of P_{EMCs} and W_{EMCs}					
		Before bonding			After removal		
	Bracket	P_{EMCs}	W_{EMCs}	Total	P_{EMCs}	W_{EMCs}	Total
Metal (Subgroup 1)	*n*	20	10	30	20	10	30
	% Within bracket	66.7	33.3	100.0	66.7	33.3	100.0
Ceramic (Subgroup 2)	*n*	20	10	30	23	7	30
	% Within bracket	66.7	33.3	100.0	76.7	23.3	100.0
Total	*n*	40	20	60	43	17	60
	% Within bracket	66.7	33.3	100.0	71.7	28.3	100.0

Table 4.2: Distribution of direction for Group 1 (teeth with EMCs) from the younger-age group before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2)

		Distribution of direction				
		Before bonding				
	Bracket	Vertical	Oblique	Horizontal	Mixed	Total
Metal (Subgroup 1)	*n*	22	5	1	2	30
	% Within bracket	73.3	16.7	3.3	6.7	100.0
Ceramic (Subgroup 2)	*n*	28	0	0	2	30
	% Within bracket	93.3	0	0	6.7	100.0
Total	*n*	50	5	1	4	60
	% Within bracket	83.3	8.3	1.7	6.7	100.0
		After removal				
	Bracket	Vertical	Oblique	Horizontal	Mixed	Total
Metal (Subgroup 1)	*n*	22	5	1	2	30
	% Within bracket	73.3	16.7	3.3	6.7	100.0
Ceramic (Subgroup 2)	*n*	28	0	0	2	30
	% Within bracket	93.3	0	0	6.7	100.0
Total	*n*	50	5	1	4	60
	% Within bracket	83.3	8.3	1.7	6.7	100.0

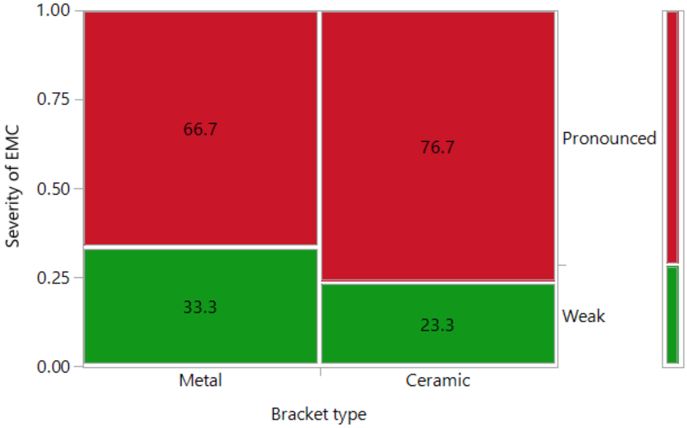

Figure 4.1: A Mosaic plot. The difference between the severity of EMC and bracket type after debonding (χ^2 = 0.739, P = 0.390). Numerical values represent the percentages of P_{EMCs} and W_{EMCs} within bracket type.

Table 4.3. Measurements of EMCs characteristics enabled comparisons between two subgroups: EMCs from the ceramic brackets subgroup possessed higher mean overall length values both before (P = 0.092) and after debonding (P = 0.059; Fig. 4.2). However, the changes in the length parameter during brackets' removal were not statistically significant for both subgroups.

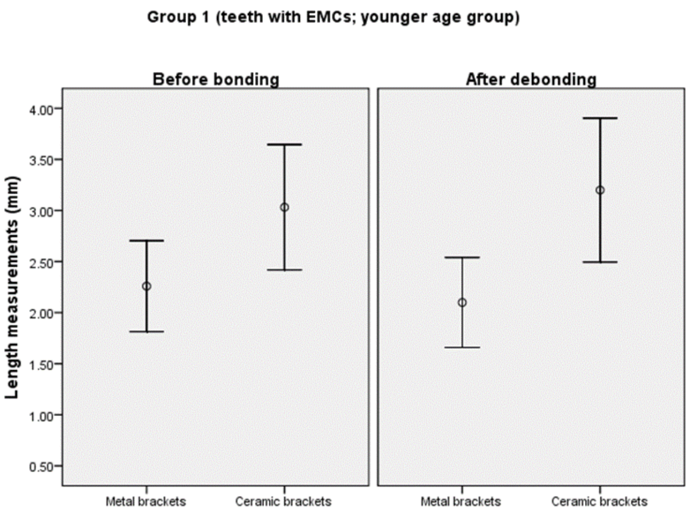

Figure 4.2: EMCs length mean values (mm) *with* 95.0 % *CI for Group 1 (teeth with EMCs) from the younger-age group before bonding and after removal of metal (Subgroup 1, n = 30) and ceramic brackets (Subgroup 2, n = 30).*

Descriptive parameters for width measurements of EMCs and comparison between width values before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) are provided in Table 4.4. Re-

Table 4.3: Length of EMCs for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the younger-age group before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2), and for Subgroup 3 (control group, teeth with EMCs)[a]

Group 1 (teeth with EMCs)										
Length of EMCs (mm) before and after removal of metal brackets (Subgroup 1)										
	Before bonding (n = 30)				After removal (n = 30)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P****
Overall length	2.26	1.19	5.72	0.56	2.10	1.18	5.28	0.24	-7.08	0.252
Length of EMCs (mm) before and after removal of ceramic brackets (Subgroup 2)										
	Before bonding (n = 30)				After removal (n = 30)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P****
Overall length	3.03	1.64	6.82	0.80	3.20	1.89	7.13	0.80	5.61	0.087
Length of EMCs (mm) before initial and after final measurements (Subgroup 3)										
	Before bonding (n = 30)				After removal (n = 30)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P****
Overall length	3.07	1.75	7.59	0.76	3.09	1.78	7.59	0.76	0.65	0.558
	($F_{(1,88)}$=1.471, P = 0.228)**				($F_{(1,88)}$=1.327, P = 0.252)**					
Group 2 (teeth without EMCs)										
Length of EMCs (mm) before and after removal of metal brackets (Subgroup 1)										
	Before bonding				After removal (n = 7)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	-[b]	-	-	-	1.21	0.42	1.80	0.69		-[c]
Length of EMCs (mm) before and after removal of ceramic brackets (Subgroup 2)										
	Before bonding				After removal (n = 5)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	-[b]	-	-	-	1.15	0.52	1.92	0.72		-[c]

[a] x̄, mean; Max, maximum; Min, minimum; SD, standard deviation; RC (%), relative percentage change.
[b] Absence of EMCs in Group 2 before bonding.
[c] No statistics could be computed because of absence data before bonding.
P = 0.05 shows statistically significant difference. *Significance values based on paired samples *t*-test. **Significance values based on ANOVA with Fisher's exact test. Statistically significant values bolded.

sults of Wilcoxon signed-rank test demonstrated that debonding both metal (Z = -5.750, P = 0.000) and ceramic brackets (Z = -6.098, P = 0.000) led to statistically significant increase in EMCs' overall width. The median scores for the overall width were higher after metal (1.70 µm) and ceramic brackets' (2.34 µm) removal then before bonding (1.48 µm for Subgroup 1 and 1.98 µm for Subgroup 2). There was a tendency for width increase nearly in every zone after brackets' removal (Table 4.4).

However, no statistically significant differences in width measurements were calculated between separate zones after debonding both types of brackets (as demonstrated in Table 4.5).

Results of Mann-Whitney U test indicated a statistically significant difference between width values both before bonding metal and ceramic brackets (U = 34593.000, P = 0.000) and after their removal (U = 24522.500, P = 0.000; shown in Table 4.6) with higher mean rank values observed in ceramic brackets subgroup.

A very strong positive correlation was found between EMCs' length and their increase during the debonding of metal and ceramic brackets (Pearson's r = 0.905, P = 0.000; Fig. 4.3). This shows that the length of EMCs following brackets' removal increases with the increase in length values before bonding. A moderate positive correlation was observed between the width of EMCs and their progress after brackets' removal (Spearman's rho = 0.596, P = 0.000; Fig. 4.4). This result demonstrates that with the increase in width before bonding there is an increase in width of EMCs following brackets' removal.

Group 2 (Teeth without EMCs)
In Group 2, new EMCs were reported in 7 out of 30 teeth (23.3 %) after debonding metal brackets (Subgroup 1) and in 5 out of 30 teeth (16.7 %) following removal of ceramic brackets (Subgroup 2). In Subgroup 1, the majority of new EMCs (5 teeth, 71.4 %) were not visible to the naked eye and only 2 teeth (28.6 %) possessed P_{EMCs}. In the ceramic brackets subgroup, all of these newly formed EMCs were W_{EMCs}.

Distribution of the direction characteristic was as follows: in Subgroup 1, 6 teeth (85.7 %) possessed vertical EMCs, in 1 tooth (14.3 %) EMC demonstrated a mixed direction; in Subgroup 2, the majority of EMCs (4 teeth, 80.0 %) ran vertically, followed by oblique direction (1 tooth, 20.0 %).

The mean length values of these new EMCs are given in Table 4.3. Newly formed EMCs demonstrated from 1.74 (metal brackets) to 2.78 times (ceramic brackets) lower length values compared with the parameters of EMCs in Subgroup 1 (P = 0.044) and Subgroup 2 (Group 1, teeth with EMCs) after the debonding procedure (P = 0.000; Fig. 4.5).

Descriptive parameters for width measurements of new EMCs are presented in Table 4.4.

Table 4.4: Descriptive parameters for width of EMCs for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the younger-age group. Comparison between width before bonding and after metal (Subgroup 1) and ceramic brackets' (Subgroup 2) removal[a]

Group 1 (teeth with EMCs)								
Width of EMCs (μm) before and after removal of metal brackets (Subgroup 1)								
			Max	Min	Quartiles			Significance
					Q1	Q2	Q3	
First zone	Before bonding	(n = 30)	10.49	0.28	1.00	1.75	2.31	**(Z = -2.576, P = 0.010)**
	After removal	(n = 30)	13.45	0.31	1.23	1.71	2.62	
Second zone	Before bonding	(n = 30)	10.49	0.37	0.92	1.48	2.09	**(Z = -5.127, P = 0.000)**
	After removal	(n = 30)	5.88	0.31	1.26	1.70	2.37	
Third zone	Before bonding	(n = 30)	3.12	0.28	0.55	1.07	1.47	(Z = -1.590, P = 0.112)
	After removal	(n = 30)	3.03	0.58	1.01	1.76	2.15	
Overall width	Before bonding	(n = 30)	10.49	0.28	0.92	1.48	2.13	**(Z = -5.750, P = 0.000)**
	After removal	(n = 30)	13.45	0.31	1.23	1.70	2.47	
Width of EMCs (μm) before and after removal of ceramic brackets (Subgroup 2)								
			Max	Min	Quartiles			Significance
					Q1	Q2	Q3	
First zone	Before bonding	(n = 30)	5.92	0.42	1.13	1.97	2.87	**(Z = -3.599, P = 0.000)**
	After removal	(n = 30)	6.98	0.55	1.70	2.39	3.38	
Second zone	Before bonding	(n = 30)	16.01	0.28	1.27	2.12	3.38	**(Z = -3.798, P = 0.000)**
	After removal	(n = 30)	18.72	0.55	1.69	2.39	3.58	
Third zone	Before bonding	(n = 30)	9.40	0.40	0.76	1.40	3.19	**(Z = -3.258, P = 0.001)**
	After removal	(n = 30)	11.30	0.56	1.20	2.13	3.31	
Overall width	Before bonding	(n = 30)	16.01	0.28	1.13	1.98	3.18	**(Z = -6.098, P = 0.000)**
	After removal	(n = 30)	18.72	0.55	1.62	2.34	3.40	
Group 2 (teeth without EMCs)								
Width of EMCs (μm) before and after removal of metal brackets (Subgroup 1)								
			Max	Min	Quartiles			Significance
					Q1	Q2	Q3	
First zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	2.16	0.35	0.62	0.93	1.23	
Second zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	3.12	0.46	0.98	2.16	2.82	
Third zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	7.06	4.05	4.05	5.26	7.06	
Overall width	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	7.06	0.35	0.77	1.20	2.31	
Width of EMCs (μm) before and after removal of ceramic brackets (Subgroup 2)								
			Max	Min	Quartiles			Significance
					Q1	Q2	Q3	
First zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 5)	4.32	1.01	1.10	2.49	2.91	
Second zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 5)	5.21	0.49	1.14	2.35	2.74	
Third zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 5)	-[c]	-	-	-	-	
Overall width	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 5)	5.21	0.49	1.16	2.37	2.73	

[a] Max, maximum; Min, minimum; Q1, lower quartile (25th percentiles); Q2, second quartile (median, 50th percentiles); Q3, upper quartile (75th percentiles). [b] Absence of EMCs in Group 2 before bonding. [c] Absence of EMCs in third zone of Group 2 after debonding ceramic brackets. [d] No statistics could be computed because of absence data before bonding. **P** = 0.05 shows statistically significant difference. Significance values based on Wilcoxon signed-rank test. Statistically significant values bolded.

Table 4.5: Width measurements differences between width in separate zones before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the younger-age group

Group 1 (teeth with EMCs)				
Width measurements (µm) differences before and after metal brackets' removal (Subgroup 1)				
	Before bonding (n = 30)		After removal (n = 30)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	133.35	11468.00	103.46	8484.00
Second zone	120.60	19657.00	100.16	12019.00
	(U = 6291.000,	P = 0.184)	(U = 4759.000,	P = 0.693)
First zone	62.05	5336.50	50.54	4144.00
Third zone	38.13	991.50	44.19	707.00
	(U = 640.500,	**P = 0.001)**	(U = 571.000,	P = 0.414)
Second zone	99.58	16231.00	69.56	8347.50
Third zone	66.31	1724.00	60.53	968.50
	(U = 1373.000,	**P = 0.004)**	(U = 832.500,	P = 0.389)
Width measurements (µm) differences before and after ceramic brackets' removal (Subgroup 2)				
	Before bonding (n = 30)		After removal (n = 30)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	128.00	13312.50	130.30	13160.50
Second zone	139.41	23002.50	132.25	21293.00
	(U = 7852.500,	P = 0.242)	(U = 8009.000,	P = 0.839)
First zone	87.52	9102.00	83.79	8462.50
Third zone	76.76	4759.00	76.31	4578.50
	(U = 2806.000,	P = 0.163)	(U = 2748.500,	P = 0.325)
Second zone	119.43	19705.50	113.90	18338.00
Third zone	99.56	6172.50	103.22	6193.00
	(U = 4219.500,	**P = 0.042)**	(U = 4363.000,	P = 0.269)
Group 2 (teeth without EMCs)				
Width measurements (µm) differences after metal brackets' removal (Subgroup 1)				
	Before bonding		After removal (n = 7)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	-[a]	-	11.85	201.50
Second zone	-[a]	-	20.27	263.50
			(U = 48.500,	**P = 0.008)**
First zone	-[a]	-	19.00	57.00
Third zone	-[a]	-	9.00	153.00
			(U = 0.000,	**P = 0.007)**
Second zone	-[a]	-	7.00	91.00
Third zone	-[a]	-	15.00	45.00
			(U = 0.000,	**P = 0.009)**
Width measurements (µm) differences after ceramic brackets' removal (Subgroup 2)				
	Before bonding		After removal (n = 5)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	-[a]	-	15.89	322.00
Second zone	-[a]	-	15.33	143.00
			(U = 91.000,	P = 0.894)
First zone	-[a]	-	5.00	45.00
Third zone	-[a]	-	-[b]	-
			-[c]	
Second zone	-[a]	-	11.00	231.00
Third zone	-[a]	-	-[b]	-
			-[c]	

[a] Absence of EMCs in Group 2 before bonding. [b] Absence of EMCs in third zone of Group 2 after debonding ceramic brackets. [c] Due to insufficient number of valid cases Mann-Whitney U test could not be performed. P = 0.05 shows statistically significant difference. Significance values based on Mann-Whitney U test. Statistically significant values bolded.

Table 4.6: Width measurements differences between width values before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs), and between width values after debonding for Group 1 and Group 2 from the younger-age group

	Group 1 (teeth with EMCs) Width measurements (µm) differences before and after metal and ceramic brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Metal brackets (Subgroup 1, n = 30)	263.79	72543.00	221.99	48393.50
Ceramic brackets (Subgroup 2, n = 30)	336.49	111378.00	303.34	97676.50
	(U = 34593.000,	**P = 0.000)**	**(U = 24522.500,**	**P = 0.000)**
	Group 2 (teeth without EMCs) Width measurements (µm) differences after metal and ceramic brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Metal brackets (Subgroup 1, n = 7)	-[a]	-	27.32	901.50
Ceramic brackets (Subgroup 2, n = 5)	-[a]	-	37.15	1114.50
			(U = 340.500,	**P = 0.033)**
	Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) Width measurements (µm) differences after metal brackets' removal			
			After removal	
			Mean Rank	Sum of Ranks
Group 1 (teeth with EMCs, n = 30)			130.58	28467.00
Group 2 (teeth without EMCs, n = 7)			95.73	3159.00
			(U = 2598.000,	**P = 0.010)**
	Width measurements (µm) differences after ceramic brackets' removal			
			After removal	
			Mean Rank	Sum of Ranks
Group 1 (teeth with EMCs, n = 30)			178.98	57630.50
Group 2 (teeth without EMCs, n = 5)			149.92	4497.50
			(U = 4032.500,	P = 0.135)

[a] Absence of EMCs in Group 2 before bonding.
P = 0.05 shows statistically significant difference. Significance values based on Mann-Whitney U test. Statistically significant values bolded.

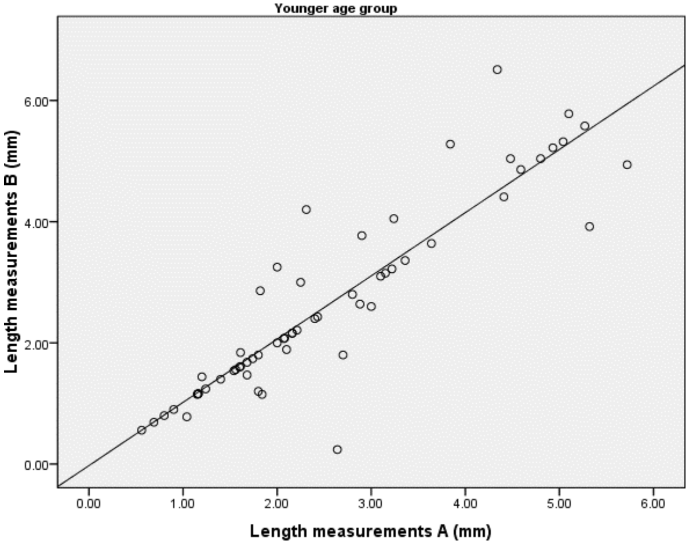

Figure 4.3: Correlation between EMCs' length values before bonding metal and ceramic brackets (Length measurements A, mm*) and their increase during the debonding (Length measurements B,* mm*) for Group 1 (teeth with EMCs) from the younger-age group (Pearson's r = 0.905; P = 0.000).*

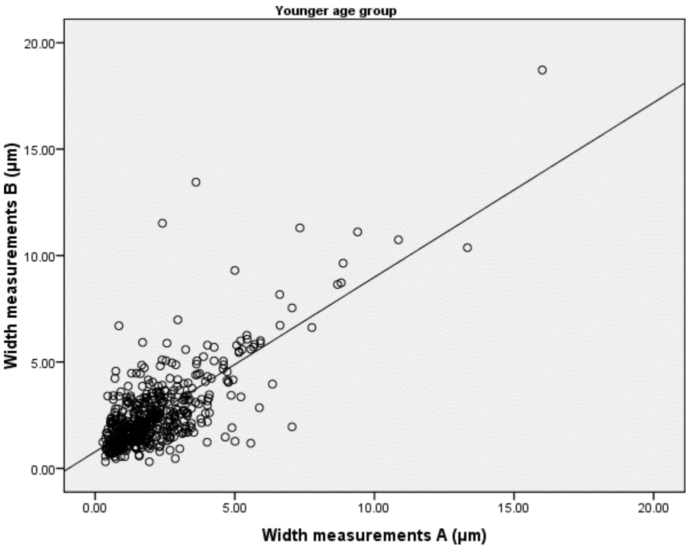

Figure 4.4: Correlation between EMCs' width values before bonding metal and ceramic brackets (Width measurements A, µm*) and their increase during the debonding (Width measurements B,* µm*) for Group 1 (teeth with EMCs) from the younger-age group (Spearman's rho = 0.596; P = 0.000).*

Results of the Mann-Whitney U test revealed that there was a statisti-

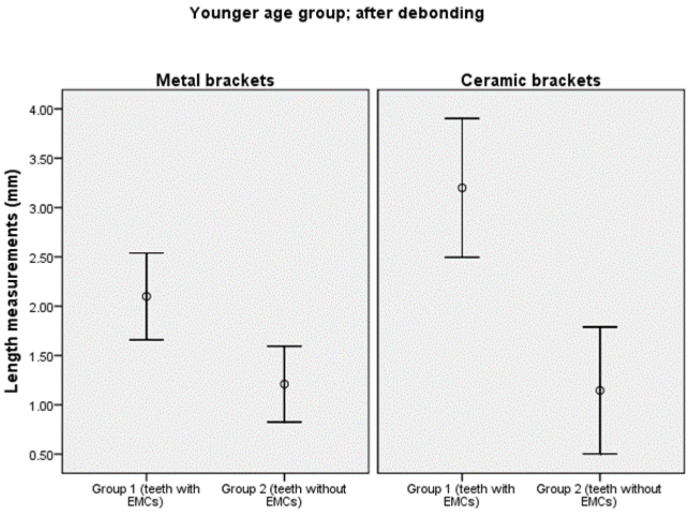

*Figure 4.5: EMCs' length mean values (*mm*) with* 95.0 % *CI for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the younger-age group after removal of metal (Group 1, Subgroup 1, n = 30; Group 2, Subgroup 1, n = 7) and ceramic brackets (Group 1, Subgroup 2, n = 30; Group 2, Subgroup 2, n = 5).*

cally significant difference between the measured width after debonding metal brackets in Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs, U = 2598.000, P = 0.010; Table 4.6) with lower mean rank values of newly formed EMCs. The differences in measurements between width values in separate zones after debonding both types of brackets are given in Table 4.5. Finally, further analysis demonstrated a statistically significant difference in measurements between width values after metal and ceramic brackets' removal (U = 340.500, P = 0.033; Table 4.6).

Control group (Teeth with and without EMCs)

Descriptive parameters and changes in length and width measurements of EMCs in Subgroup 3 (Group 1, teeth with EMCs) are presented in Table 4.3 and Table 4.7.

The percentage of EMCs visible and invisible to the naked eye did not differ between initial and final measurements: 73.3 % (22 teeth) of EMCs were P_{EMCs} and 26.7 % (8 teeth) were W_{EMCs}. The changes in the direction parameter were not observed either: 86.7 % (26 teeth) of EMCs ran verticaly, 13.3 % (4 teeth) EMCs were of mixed direction. New EMCs were not registered in Subgroup 3 (Group 2, teeth without EMCs).

Table 4.7: Descriptive parameters for width of EMCs for Group 1 (teeth with EMCs) from the younger- and older-age groups. Comparison between width after initial and final measurements (Subgroup 3)[a]

					Quartiles			
Younger-age group — Width of EMCs (µm) before initial and after final measurements (Subgroup 3)			Max	Min	Q1	Q2	Q3	Significance
First zone	Initial measurements	(n = 30)	10.73	0.46	1.48	2.20	3.39	(Z = -1.157, P = 0.247)
	Final measurements	(n = 30)	10.73	0.71	1.56	2.30	3.28	
Second zone	Initial measurements	(n = 30)	9.26	0.31	1.38	2.23	3.60	(Z = -1.490, P = 0.136)
	Final measurements	(n = 30)	9.89	0.33	1.36	2.17	3.43	
Third zone	Initial measurements	(n = 30)	7.16	0.29	0.70	1.34	2.86	(Z = -1.132, P = 0.258)
	Final measurements	(n = 30)	8.49	0.31	0.74	1.44	2.65	
Overall width	Initial measurements	(n = 30)	10.73	0.29	1.23	2.04	3.38	**(Z = -2.161, P = 0.031)**
	Final measurements	(n = 30)	10.73	0.31	1.34	2.14	3.23	
Older-age group — Width of EMCs (µm) before initial and after final measurements (Subgroup 3)			Max	Min	Q1	Q2	Q3	Significance
First zone	Initial measurements	(n = 30)	11.59	0.46	1.47	2.46	4.62	(Z = -1.051, P = 0.293)
	Final measurements	(n = 30)	11.59	0.59	1.61	2.55	4.81	
Second zone	Initial measurements	(n = 30)	10.90	0.31	1.44	2.75	4.30	(Z = -1.658, P = 0.097)
	Final measurements	(n = 30)	10.79	0.28	1.49	2.63	4.15	
Third zone	Initial measurements	(n = 30)	9.11	0.25	0.78	2.02	3.82	(Z = -1.473, P = 0.141)
	Final measurements	(n = 30)	9.11	0.25	0.97	2.31	3.56	
Overall width	Initial measurements	(n = 30)	11.59	0.25	1.37	2.47	4.23	**(Z = -2.455 P = 0.014)**
	Final measurements	(n = 30)	11.59	0.25	1.41	2.53	4.15	

[a] Max, maximum; Min, minimum; Q1, lower quartile (25th percentiles); Q2, second quartile (median, 50th percentiles); Q3, upper quartile (75th percentiles).
P = 0.05 shows statistically significant difference. Significance values based on Wilcoxon signed-rank test. Statistically significant values bolded.

4.1.2. Enamel Microcracks' Characteristics Before and After Removal of Metal and Ceramic Brackets for the Teeth from the Older-Age Group

Following repeated assessment of the teeth for new EMCs employing SEM and EMCs visibility evaluation, no discrepancies between results were observed.

Group 1 (Teeth with EMCs)
Distribution of P_{EMCs} and W_{EMCs} before and after debonding metal (Subgroup 1) and ceramic brackets (Subgroup 2) is reported in Table 4.8. There were identical changes in EMCs severity after metal and ceramic brackets' removal: in both subgroups, one EMC which used to be invisible to the naked eye progressed to a visible one.

The distribution of the direction parameter before and after metal and ceramic brackets' removal is described in Table 4.9. The majority of EMCs (96.7 % in Subgroup 1 and 80.0 % in Subgroup 2) ran vertically, followed by mixed direction (3.3 %) in Subgroup 1 or oblique (10.0 %) and mixed (10.0 %) in Subgroup 2. The results indicated that there were no changes in the direction of EMCs in either of the subgroups following the debonding procedure.

The mean length values of EMCs in Group 1 before and after debonding metal (Subgroup 1) and ceramic brackets (Subgroup 2) are given in Table 4.10. The analysis of EMCs' parameters revealed that EMCs from the metal brackets subgroup possessed higher mean overall length values, both before (P = 0.223) and after debonding (P = 0.185; Fig. 4.6).

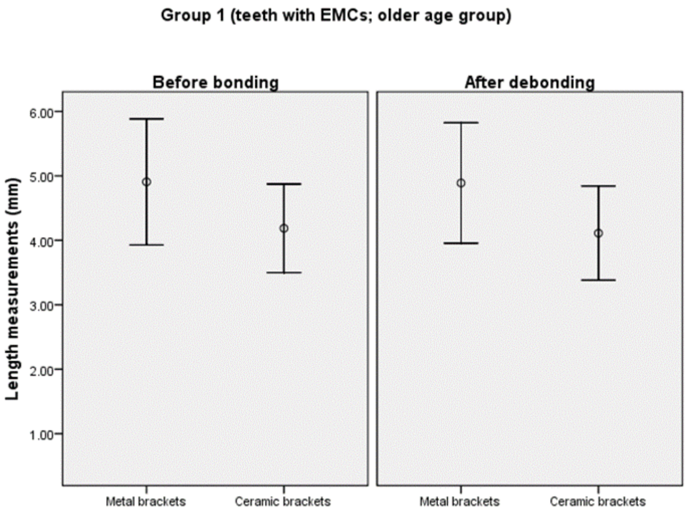

*Figure 4.6: EMCs length mean values (*mm*) with* 95.0 % *CI for Group 1 (teeth with EMCs) from the older-age group before bonding and after removal of metal (Subgroup 1, n = 30) and ceramic brackets (Subgroup 2, n = 30).*

Table 4.8: Distribution of P_{EMCs} and W_{EMCs} for Group 1 (teeth with EMCs) from the older-age group before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2)

		Distribution of P_{EMCs} and W_{EMCs}					
		Before bonding			After removal		
	Bracket	P_{EMCs}	W_{EMCs}	Total	P_{EMCs}	W_{EMCs}	Total
Metal (Subgroup 1)	*n*	27	3	30	28	2	30
	% Within bracket	90.0	10.0	100.0	93.3	6.7	100.0
Ceramic (Subgroup 2)	*n*	27	3	30	28	2	30
	% Within bracket	90.0	10.0	100.0	93.3	6.7	100.0
Total	*n*	54	6	60	56	4	60
	% Within bracket	90.0	10.0	100.0	93.3	6.7	100.0

Table 4.9: Distribution of direction for Group 1 (teeth with EMCs) from the older-age group before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2)

		Distribution of direction				
		Before bonding				
	Bracket	Vertical	Oblique	Horizontal	Mixed	Total
Metal (Subgroup 1)	*n*	29	0	0	1	30
	% Within bracket	96.7	0	0	3.3	100.0
Ceramic (Subgroup 2)	*n*	24	3	0	3	30
	% Within bracket	80.0	10.0	0	10.0	100.0
Total	*n*	53	3	0	4	60
	% Within bracket	88.3	5.0	0	6.7	100.0
		After removal				
	Bracket	Vertical	Oblique	Horizontal	Mixed	Total
Metal (Subgroup 1)	*n*	29	0	0	1	30
	% Within bracket	96.7	0	0	3.3	100.0
Ceramic (Subgroup 2)	*n*	24	3	0	3	30
	% Within bracket	80.0	10.0	0	10.0	100.0
Total	*n*	53	3	0	4	60
	% Within bracket	88.3	5.0	0	6.7	100.0

Table 4.10: Length of EMCs for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the older-age group before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2), and for Subgroup 3 (control group, teeth with EMCs)[a]

Group 1 (teeth with EMCs)										
Length of EMCs (mm) before and after removal of metal brackets (Subgroup 1)										
	Before bonding (n = 30)				After removal (n = 30)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P****
Overall length	4.91	2.62	10.15	1.75	4.89	2.50	10.15	1.60	-0.41	0.892
Length of EMCs (mm) before and after removal of ceramic brackets (Subgroup 2)										
	Before bonding (n = 30)				After removal (n = 30)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P****
Overall length	4.19	1.84	7.29	1.10	4.11	1.96	7.02	0.92	-1.91	0.458
Length of EMCs (mm) before initial and after final measurements (Subgroup 3)										
	Before bonding (n = 30)				After removal (n = 30)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P****
Overall length	3.29	1.75	7.59	0.76	3.31	1.76	7.59	0.58	0.61	0.757
	($F_{(1,88)}$=7.022, P = 0.010)**				**($F_{(1,88)}$=6.423, P = 0.013)****					
Group 2 (teeth without EMCs)										
Length of EMCs (mm) before and after removal of metal brackets (Subgroup 1)										
	Before bonding				After removal (n = 7)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	-[b]	-	-	-	1.90	1.20	4.18	0.72		-[c]
Length of EMCs (mm) before and after removal of ceramic brackets (Subgroup 2)										
	Before bonding				After removal (n = 7)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	-[b]	-	-	-	1.24	0.55	2.04	0.75		-[c]

[a] x̄, mean; Max, maximum; Min, minimum; SD, standard deviation; RC (%), relative percentage change.
[b] Absence of EMCs in Group 2 before bonding.
[c] No statistics could be computed because of absence data before bonding.
P = 0.05 shows statistically significant difference. *Significance values based on paired samples t-test. **Significance values based on ANOVA with Fisher's exact test. Statistically significant values bolded.

Descriptive parameters for width measurements of EMCs and comparison between width values before and after debonding metal (Subgroup 1) and ceramic brackets (Subgroup 2) are presented in Table 4.11. A Wilcoxon signed-rank test revealed that debonding both types of brackets resulted in a statistically significant increase in EMCs' overall width. Statistically significant changes were observed in each zone of the buccal tooth surface in both Subgroup 1 and Subgroup 2 (as demonstrated in Table 4.11).

A further examination of the results indicated a statistically significant difference in width between the first zone (cervical third, mean rank = 195.32) and the second zone (middle third, mean rank = 171.87) after metal brackets' removal with higher mean rank values calculated for the cervical third (U = 14302.500, P = 0.034). Whereas following debonding ceramic brackets a statistically significant difference was observed between the first zone (mean rank = 123.27) and the third zone (occlusal third, mean rank = 142.16, U = 7461.000, P = 0.044; as shown in Table 4.12).

The analysis of EMCs' width parameter in relation to the bracket type revealed a statistically significant difference in width between Subgroup 1 (metal brackets, mean rank = 561.35) and Subgroup 2 (ceramic brackets, mean rank = 520.73) before the bonding procedure with higher mean rank values in Subgroup 1 (U = 135312.000, P = 0.033; Table 4.13). However, after debonding the difference in width measurements between the two subgroups was not statistically significant (P = 0.751).

A very strong positive correlation was identified between EMCs length and their increase during removal of metal and ceramic brackets (Pearson's r = 0.969, P = 0.000; Fig. 4.7). This finding reveals that the length of EMCs following brackets' removal increases with the increase in length values before bonding. A strong positive correlation was found between the width of EMCs and their progress related to debonding (Spearman's rho = 0.727, P = 0.000; Fig. 4.8). This shows that with the increase in width before bonding there is also an increase in width of EMCs following brackets' removal.

Group 2 (Teeth without EMCs)

Following debonding of both metal (Subgroup 1) and ceramic brackets (Subgroup 2), 76.7 % of analyzed teeth did not show new EMCs. EMCs were recorded in 7 out of 30 (23.3 %) teeth in both subgroups. In the metal brackets subgroup, 2 teeth (28.6 %) possessed P_{EMCs} and 5 teeth (71.4 %) showed W_{EMCs}. In the ceramic brackets subgroup, the distribution of different severity EMCs was as follows: in 4 teeth (57.1 %) EMCs were visible to the naked eye, while the remaining 3 teeth (42.9 %) demonstrated W_{EMCs}.

Regarding the distribution of the direction characteristic, all newly formed EMCs ran vertically, both in Subgroup 1 and Subgroup 2.

The mean length values of new EMCs are presented in Table 4.10. Newly

Table 4.11: Descriptive parameters for width of EMCs for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the older-age group. Comparison between width before bonding and after metal (Subgroup 1) and ceramic brackets' (Subgroup 2) removal[a]

Group 1 (teeth with EMCs)								
Width of EMCs (µm) before and after removal of metal brackets (Subgroup 1)								
			Max	Min	Quartiles Q1	Q2	Q3	Significance
First zone	Before bonding	(n = 30)	29.75	0.55	3.60	6.15	9.22	**(Z = -4.621, P = 0.000)**
	After removal	(n = 30)	30.39	0.99	4.56	6.94	12.78	
Second zone	Before bonding	(n = 30)	25.92	0.37	3.44	6.74	10.42	**(Z = -3.405, P = 0.001)**
	After removal	(n = 30)	30.58	0.77	3.24	6.26	11.67	
Third zone	Before bonding	(n = 30)	22.12	0.47	3.70	6.75	10.19	**(Z = -2.885, P = 0.004)**
	After removal	(n = 30)	30.73	0.46	3.13	8.26	11.69	
Overall width	Before bonding	(n = 30)	29.75	0.37	3.60	6.36	10.17	**Z = -6.248, P = 0.000)**
	After removal	(n = 30)	30.73	0.46	3.60	6.99	11.89	
Width of EMCs (µm) before and after removal of ceramic brackets (Subgroup 2)								
			Max	Min	Quartiles Q1	Q2	Q3	Significance
First zone	Before bonding	(n = 30)	27.35	0.52	2.98	5.37	8.76	**(Z = -5.756, P = 0.000)**
	After removal	(n = 30)	16.94	0.73	3.77	7.29	9.68	
Second zone	Before bonding	(n = 30)	25.62	0.46	3.87	6.02	8.54	**(Z = -4.191, P = 0.000)**
	After removal	(n = 30)	25.94	0.83	4.20	6.87	10.25	
Third zone	Before bonding	(n = 30)	25.76	0.37	2.95	6.15	10.02	**(Z = -5.812, P = 0.000)**
	After removal	(n = 30)	35.04	0.67	3.78	7.51	10.10	
Overall width	Before bonding	(n = 30)	27.35	0.37	3.40	5.85	9.04	**(Z = -8.898, P = 0.000)**
	After removal	(n = 30)	35.04	0.67	3.96	7.12	10.92	
Group 2 (teeth without EMCs)								
Width of EMCs (µm) before and after removal of metal brackets (Subgroup 1)								
			Max	Min	Quartiles Q1	Q2	Q3	Significance
First zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	5.43	0.44	0.94	1.85	3.25	
Second zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	5.36	0.43	1.06	2.17	3.05	
Third zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	7.42	0.37	1.05	1.21	5.02	
Overall width	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	7.42	0.37	1.03	1.82	3.05	
Width of EMCs (µm) before and after removal of ceramic brackets (Subgroup 2)								
			Max	Min	Quartiles Q1	Q2	Q3	Significance
First zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	5.26	0.46	1.30	2.47	3.53	
Second zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	4.57	0.54	1.33	2.19	3.00	
Third zone	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	-[c]	-	-	-	-	
Overall width	Before bonding		-[b]	-	-	-	-	-[d]
	After removal	(n = 7)	5.26	0.46	1.33	2.19	3.40	

[a] Max, maximum; Min, minimum; Q1, lower quartile (25th percentiles); Q2, second quartile (median, 50th percentiles); Q3, upper quartile (75th percentiles). [b] Absence of EMCs in Group 2 before bonding. [c] Absence of EMCs in third zone of Group 2 after debonding ceramic brackets. [d] No statistics could be computed because of absence data before bonding. P = 0.05 shows statistically significant difference. Significance values based on Wilcoxon signed-rank test. Statistically significant values bolded.

Table 4.12: Width measurements differences between width in separate zones before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the older-age group

Group 1 (teeth with EMCs)				
Width measurements (µm) differences before and after metal brackets' removal (Subgroup 1)				
	Before bonding (n = 30)		After removal (n = 30)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	201.51	38286.00	195.32	32227.50
Second zone	212.58	47619.00	171.87	34202.50
	(U = 20141.000,	P = 0.348)	**(U = 14302.500,**	**P = 0.034)**
First zone	167.78	31879.00	147.17	24283.00
Third zone	177.24	27117.00	139.71	17045.00
	(U = 13734.000,	P = 0.380)	(U = 9542.000,	P = 0.452)
Second zone	189.21	42382.50	157.94	31429.50
Third zone	188.70	28870.50	166.00	20251.50
	(U = 17089.500,	P = 0.964)	(U = 11529.500,	P = 0.450)
Width measurements (µm) differences before and after ceramic brackets' removal (Subgroup 2)				
	Before bonding (n = 30)		After removal (n = 30)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	182.19	28421.00	170.85	23064.50
Second zone	198.74	45115.00	176.81	37661.50
	(U = 16175.000,	P = 0.150)	(U = 13884.500,	P = 0.590)
First zone	137.94	21518.50	123.27	16641.00
Third zone	153.28	20386.50	142.16	18339.00
	(U = 9272.500,	P = 0.120)	**(U = 7461.000,**	**P = 0.044)**
Second zone	178.78	40582.00	165.08	35162.50
Third zone	183.44	24398.00	182.10	23490.50
	(U = 14704.000,	P = 0.681)	(U = 12371.500,	P = 0.123)
Group 2 (teeth without EMCs)				
Width measurements (µm) differences after metal brackets' removal (Subgroup 1)				
	Before bonding		After removal (n = 7)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	-[a]	-	25.56	613.50
Second zone	-[a]	-	27.30	764.50
			(U = 313.500,	P = 0.680)
First zone	-[a]	-	18.96	455.00
Third zone	-[a]	-	17.58	211.00
			(U = 133.000,	P = 0.712)
Second zone	-[a]	-	21.30	596.50
Third zone	-[a]	-	18.63	223.50
			(U = 145.500,	P = 0.500)
Width measurements (µm) differences after ceramic brackets' removal (Subgroup 2)				
	Before bonding		After removal (n = 7)	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
First zone	-[a]	-	20.70	414.00
Second zone	-[a]	-	19.26	366.00
			(U = 176.000,	P = 0.694)
First zone	-[a]	-	10.50	210.00
Third zone	-[a]	-	-[b]	-
			-[c]	
Second zone	-[a]	-	10.00	190.00
Third zone	-[a]	-	-[b]	-
			-[c]	

[a] Absence of EMCs in Group 2 before bonding. [b] Absence of EMCs in third zone of Group 2 after debonding ceramic brackets. [c] Due to insufficient number of valid cases Mann-Whitney U test could not be performed. P = 0.05 shows statistically significant difference. Significance values based on Mann-Whitney U test. Statistically significant values bolded.

Table 4.13: Width measurements differences between width values before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs), and between width values after debonding for Group 1 and Group 2 from the older-age group

	Group 1 (teeth with EMCs)			
	Width measurements (µm) differences before and after metal and ceramic brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Metal brackets (Subgroup 1, n = 30)	561.35	318288.00	484.82	235623.00
Ceramic brackets (Subgroup 2, n = 30)	520.73	268698.00	479.13	228543.00
	(U = 135312.000,	**P = 0.033)**	(U = 114540.000,	P = 0.751)
	Group 2 (teeth without EMCs)			
	Width measurements (µm) differences after metal and ceramic brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Metal brackets (Subgroup 1, n = 7)	-[a]	-	49.62	3175.50
Ceramic brackets (Subgroup 2, n = 7)	-[a]	-	55.91	2180.50
			(U = 1095.500,	P = 0.300)
	Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs)			
	Width measurements (µm) differences after metal brackets' removal			
			After removal	
			Mean Rank	Sum of Ranks
Group 1 (teeth with EMCs, n = 30)			299.20	145412.50
Group 2 (teeth without EMCs, n = 7)			95.51	6112.50
			(U = 4032.500,	**P = 0.000)**
	Width measurements (µm) differences after ceramic brackets' removal			
			After removal	
			Mean Rank	Sum of Ranks
Group 1 (teeth with EMCs, n = 30)			273.79	130599.00
Group 2 (teeth without EMCs, n = 7)			71.46	2787.00
			(U = 2007.000,	**P = 0.000)**

[a] Absence of EMCs in Group 2 before bonding.
P = 0.05 shows statistically significant difference. Significance values based on Mann-Whitney U test. Statistically significant values bolded.

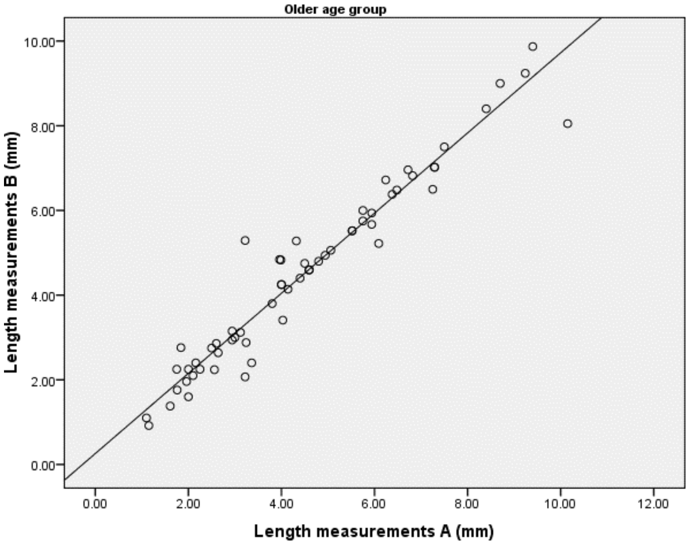

Figure 4.7: Correlation between EMCs length values before bonding metal and ceramic brackets (Length measurements A, mm*) and their increase during debonding (Length measurements B,* mm*) for Group 1 (teeth with EMCs) from the older-age group (Pearson's r = 0.969; P = 0.000).*

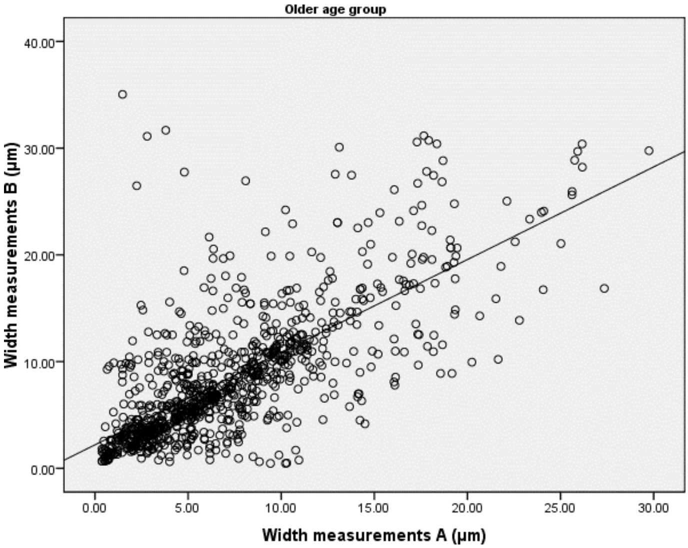

Figure 4.8: Correlation between EMCs width values before bonding metal and ceramic brackets (Width measurements A, µm*) and their increase during debonding (Width measurements B,* µm*) for Group 1 (teeth with EMCs) from the older-age group (Spearman's rho = 0.727; P = 0.000).*

formed EMCs demonstrated from 2.57 (metal brackets, $P = 0.000$) to 3.31 times (ceramic brackets, P = 0.000) lower mean overall length values compared with the parameters of EMCs in Subgroup 1 and Subgroup 2 (Group 1, teeth with EMCs) after brackets' removal (Fig. 4.9).

Descriptive parameters for width measurements are given in Table 4.11.

The width characteristic of new EMCs followed the same pattern as the length parameter. There was a statistically significant difference between width measurements of EMCs in Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) after debonding both metal (U = 4032.500, P = 0.000) and ceramic brackets (U = 2007.000, P = 0.000) with higher mean rank values found in Group 1 (as shown in Table 4.13). The differences in width measurements between separate zones where EMCs were located after removal of metal and ceramic brackets can be found in Table 4.12. Following debonding, no statistically significant difference in width measurements between the two types of brackets was identified (Table 4.13).

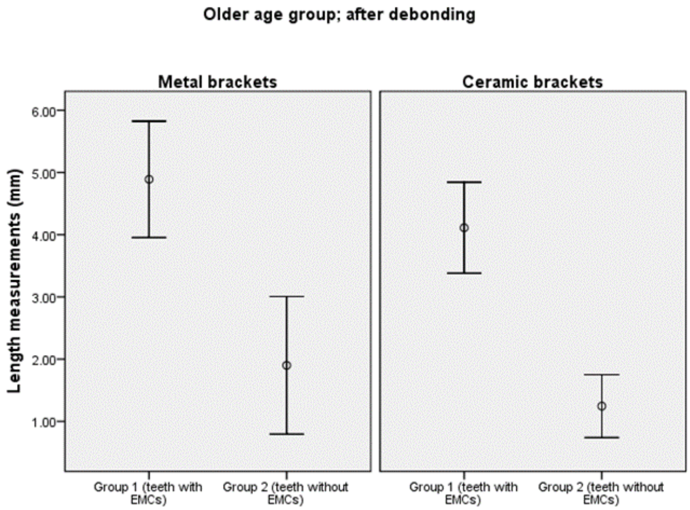

*Figure 4.9: EMCs length mean values (*mm*) with* 95.0 % *CI for Group 1 (teeth with EMCs) and Group 2 (teeth without EMCs) from the older-age group after removal of metal (Group 1, Subgroup 1, n = 30; Group 2, Subgroup 1, n = 7) and ceramic brackets (Group 1, Subgroup 2, n = 30; Group 2, Subgroup 2, n = 7).*

Control group (Teeth with and without EMCs)

The changes in length measurements of EMCs in Subgroup 3 (Group 1, teeth with EMCs) are given in Table 4.10. The differences in measurements between width values after initial and final measurements are presented in Table 4.7.

The percentage of P_{EMCS} and W_{EMCs} did not differ between the initial and final measurements: 93.3 % (28 teeth) of EMCs were visible and 6.7 % (2 teeth) of EMCs were not visible to the naked eye. The changes in the direction characteristic were not recorded, either: 83.3 % (25 teeth) of EMCs ran in the vertical direction and 16.7 % (5 teeth) were of mixed direction. New EMCs were not observed in Subgroup 3 (Group 2, teeth without EMCs).

4.1.3. Comparison of Enamel Microcracks' Characteristics Before and After Debonding Metal and Ceramic Brackets for the Teeth from Two Different Age Groups: Younger and Older

Group 1 (Teeth with EMCs)

Results from the statistical analysis indicated that there was a statistically significant difference between the age groups (younger or older) and the severity of EMCs (P_{EMCs} or W_{EMCs}) before bonding metal (χ^2 = 4.812, P = 0.028; Fig. 4.10) and ceramic brackets (identical distribution of P_{EMCs} and W_{EMCs} as for metal brackets, χ^2 = 4.812, P = 0.028; Fig. 4.10), and after metal brackets' removal (χ^2 = 6.667, P = 0.010; Fig. 4.11). However, no statistically significant difference (χ^2 = 3.268, P = 0.071; Fig. 4.12) between the aforementioned variables was observed after debonding ceramic brackets.

Due to the absence of oblique and horizontal direction EMCs in certain cases, differences between age groups (younger or older) and direction of EMCs (vertical, oblique, horizontal, or mixed) before and after debonding could not be evaluated.

EMCs from the older-age group demonstrated higher mean length values. Before the bonding procedure the difference in mean overall length between the two age groups was as follows (demonstrated in Fig. 4.13): Subgroup 1 (metal brackets), 2.65 mm, P = 0.000; Subgroup 2 (ceramic brackets), 1.16 mm, P = 0.063. The tendency of longer EMCs for the teeth from the older-age group was observed following debonding, too: Subgroup 1 (metal brackets), 2.79 mm, P = 0.000; Subgroup 2 (ceramic brackets), 0.91 mm, P = 0.071 (as depicted in Fig. 4.13).

Results of the Mann-Whitney U test showed that there was a statistically significant difference between the width values for the teeth from the older- and younger-age groups, both before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2), with higher mean rank values for the teeth from the older-age group (calculations presented in Table 4.14).

Group 2 (Teeth without EMCs)

Following debonding, no new EMCs were recorded in more than 76.0 % of the teeth from both age groups. Newly formed EMCs showed higher mean overall length values in the older-age group after removal of metal (P = 0.175) and ceramic brackets (P = 0.761; as demonstrated in Fig. 4.14). Regarding the width parameter, EMCs from the older-age group showed greater width values,

Table 4.14: Width measurements differences between width values for the teeth from the younger- and older-age groups before bonding and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2)

	Group 1 (teeth with EMCs)			
	Width measurements (µm) differences before and after metal brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Younger-age group (n = 30)	184.87	50840.00	158.38	34526.50
Older-age group (n = 30)	536.27	304063.00	439.58	213633.50
	(U = 12890.000,	***P* = 0.000)**	**(U = 10655.500,**	***P* = 0.000)**
	Width measurements (µm) differences before and after ceramic brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Younger-age group (n = 30)	249.02	82426.50	229.82	74002.50
Older-age group (n = 30)	536.24	276701.50	514.88	245597.50
	(U = 27480.500,	***P* = 0.000)**	**(U = 21999.500,**	***P* = 0.000)**
	Group 2 (teeth without EMCs)			
	Width measurements (µm) differences before and after metal brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Younger-age group (n = 7)	-[a]	-	41.71	1376.50
Older-age group (n = 7)	-[a]	-	52.76	3376.50
			(U = 815.500,	*P* = 0.067)
	Width measurements (µm) differences before and after ceramic brackets' removal			
	Before bonding		After removal	
	Mean Rank	Sum of Ranks	Mean Rank	Sum of Ranks
Younger-age group (n = 5)	-[a]	-	33.05	991.50
Older-age group (n = 7)	-[a]	-	36.50	1423.50
			(U = 526.500,	*P* = 0.479)

[a] Absence of EMCs in Group 2 before bonding.
P = 0.05 shows statistically significant difference. Significance values based on Mann-Whitney U test. Statistically significant values bolded.

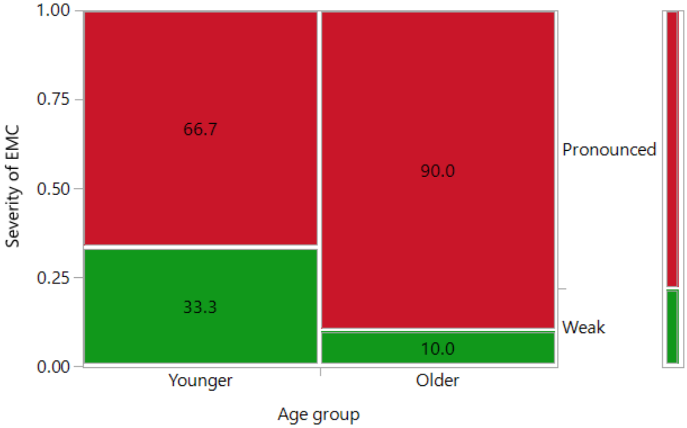

Figure 4.10: A Mosaic plot. The difference between the severity of EMC and the age group before bonding metal brackets (χ^2 = 4.812, P = 0.028). For ceramic brackets the difference identical. Numerical values represent the percentages of P_{EMCs} and W_{EMCs} within each age group.

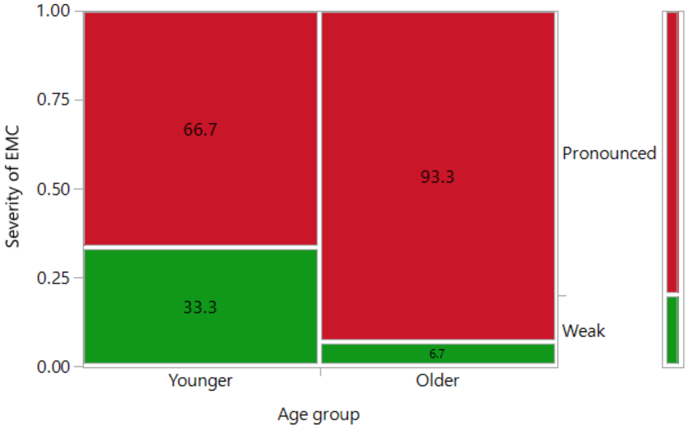

Figure 4.11: A Mosaic plot. The difference between the severity of EMC and the age group after debonding metal brackets (χ^2 = 6.667, P = 0.010). Numerical values represent the percentages of P_{EMCs} and W_{EMCs} within each age group.

too. However, the difference in width between the two age groups was not statistically significant after debonding either types of brackets (Table 4.14).

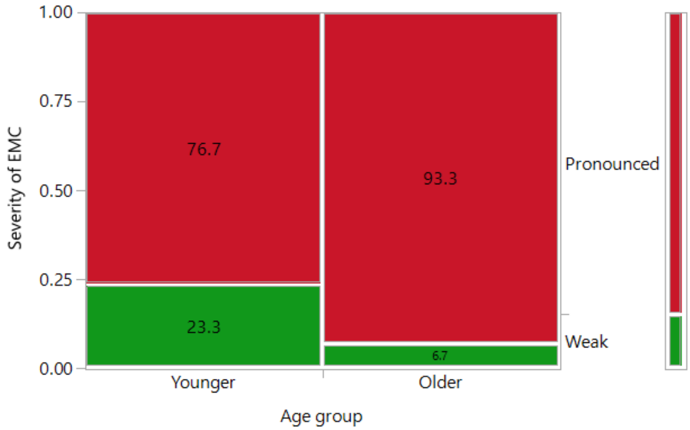

Figure 4.12: A Mosaic plot. The difference between the severity of EMC and the age group after debonding ceramic brackets (χ^2 = 3.268, P = 0.071). Numerical values represent the percentages of P_{EMCs} and W_{EMCs} within each age group.

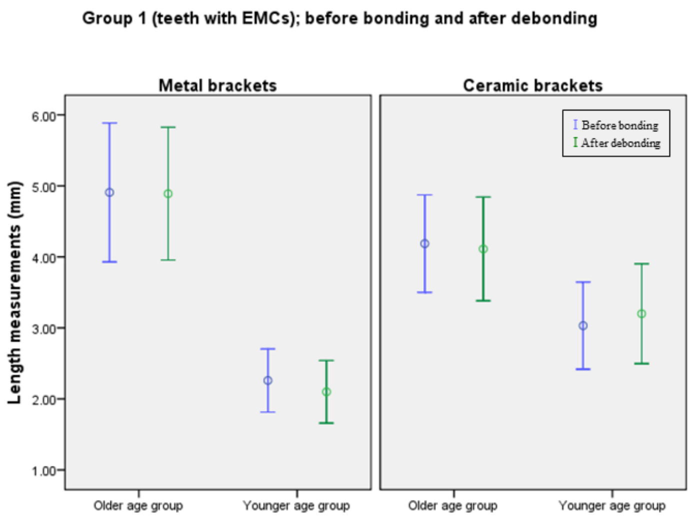

Figure 4.13: EMCs length mean values (mm) *with* 95.0 % *CI for Group 1 (teeth with EMCs) from the older- and younger-age groups before bonding and after removal of metal (Subgroup 1, n = 30) and ceramic brackets (Subgroup 2, n = 30).*

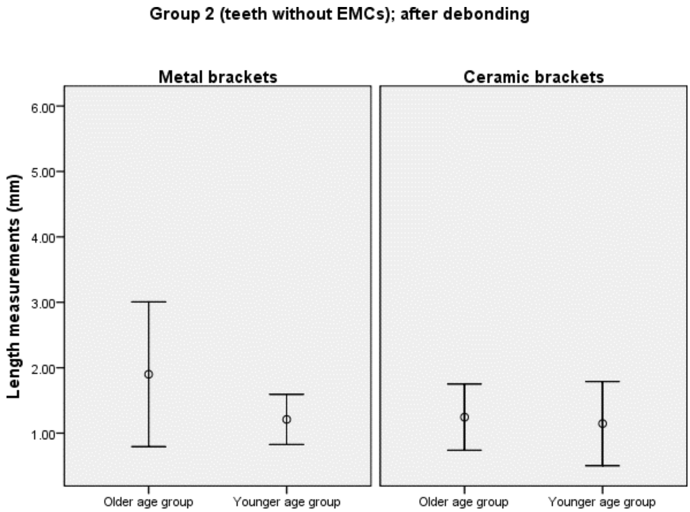

Figure 4.14: EMCs length mean values (mm) *with* 95.0 % *for Group 2 (teeth without EMCs) from the older- and younger-age groups after removal of metal (older-age group, Group 2, Subgroup 1, n = 7; younger-age group, Group 2, Subgroup 1, n = 7) and ceramic brackets (older-age group Group 2, Subgroup 2, n = 7; younger-age group, Group 2, Subgroup 2, n = 5).*

4.2. Effect of Specific Enamel Microcracks' Characteristics, Age Group, and Type of the Bracket Used on the Enamel Surface Damage During Removal of Metal and Ceramic Brackets

Impact of five different factors (severity, direction, location of EMC, age group, and bracket type) on the extent of enamel surface damage (calculated length values (before and after debonding) of EMCs represented enamel surface damage) during brackets' removal is demonstrated in Table 4.15.

Table 4.15: Impact of five different factors (severity, direction, location of EMC, age group, and bracket type) on the extent of enamel surface damage during debonding (dependent variable - length value). P values, odds ratio (OR), and 95.0 % *CI from binary logistic modeling*

Independent variables	*P*	OR*	95.0% CI for OR Lower	95.0% CI for OR Upper
Severity of EMC***	0.023	2.633	1.145	6.057
Direction of EMC**	0.538	0.715	0.245	2.085
Location of EMC**	0.189	0.659	0.354	1.228
Age group***	0.000	3.083	1.674	5.679
Bracket type****	0.064	1.695	0.970	2.959

*OR values: 1 = independent variable has no impact; < 1 = lowers the risk; > 1 = increases the risk of greater extent enamel surface damage during debonding.
**OR < 1 and its' Upper 95.0 % CI value > 1, variable statistically insignificantly lowers the risk of greater extent tooth damage formation.
***OR > 1 and its' Lower 95.0 % CI value > 1, variable statistically significantly increases the risk of greater amount enamel surface defects.
****OR > 1 its' Lower 95.0 % CI value < 1, variable statistically insignificantly increases the risk of greater extent enamel damage formation during debonding.

The severity of EMC (i.e. P_{EMC} or W_{EMC}) had a statistically significant effect on the extent of tooth damage during debonding (OR = 2.633, $P = 0.023$). For given direction, location parameters, age group, and bracket type, the odds of the undesirable changes in the enamel structure were higher by a factor of 2.633 for P_{EMCs} thus, 2.63 times increased the risk of greater amount enamel damage during brackets' removal. 23.3 % of W_{EMCs} and 55.3 % of P_{EMCs} led to the increase in the risk of greater formation of tooth surface defects (the difference between variables is demonstrated in Fig. 4.15).

The direction parameter did not lead to statistically significant changes in the enamel structure during debonding (OR = 0.715, $P = 0.538$). A more detailed evaluation demonstrated that 50.0 % of EMCs with inclination < 46° to the longitudinal axis of the tooth crown and 44.4 % of EMCs having inclination ≥ 46° or changing their direction during extension through the buccal tooth surface were predisposed to the higher risk of greater extent tooth surface damage during brackets' removal (the difference between variables is presented

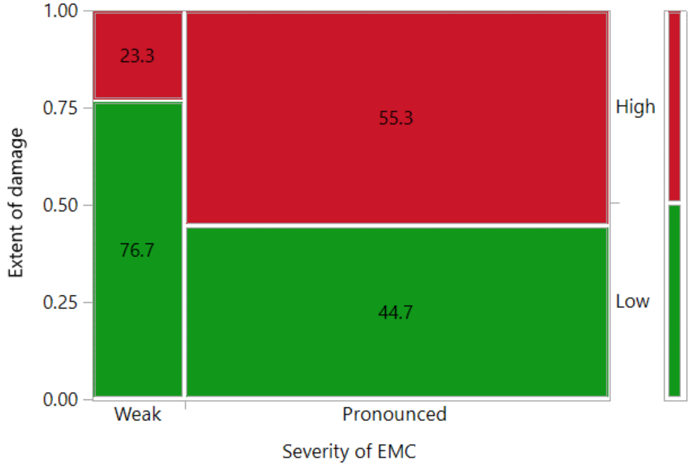

Figure 4.15: A Mosaic plot. The difference between the extent of enamel damage during debonding and severity of EMC (χ^2 = 14.525, P = 0.000). Numerical values represent the percentages of P_{EMCs} and W_{EMCs} within each group.

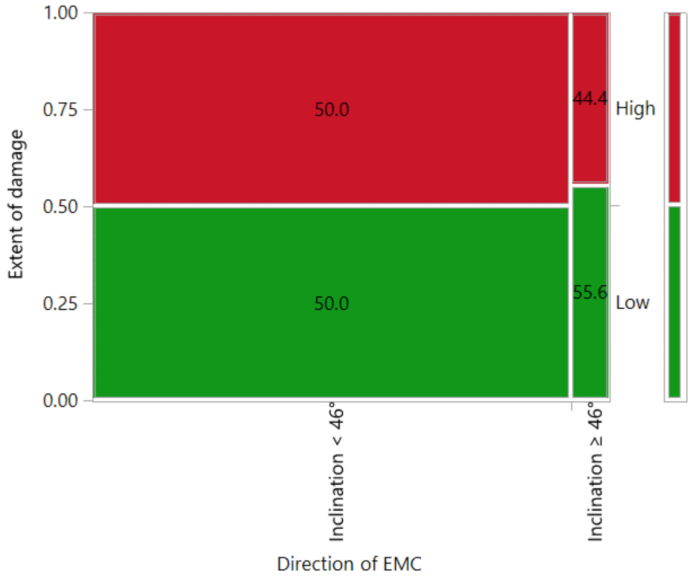

Figure 4.16: A Mosaic plot. The difference between the extent of enamel damage during debonding and direction of EMC (χ^2 = 0.206, P = 0.650). Numerical values represent the percentages of different inclination EMCs within each group.

in Fig. 4.16). For given severity, location parameters, age group, and bracket type, for an increase of 1 degree in EMCs inclination the odds of greater undesirable changes in the enamel structure decreased by a factor of 1.40 (0.715 of initial value).

Location of EMC on the buccal tooth surface did not reveal statistically significant effect on the extent of tooth damage during the debonding procedure (OR = 0.659, P = 0.189). For given severity, direction parameters, age group, and bracket type, for an additional millimeter in the distance (i.e. from the occlusal towards cervical part of the tooth), the odds of greater enamel damage were lower by a factor of 1.52 (0.659 of initial value). 63.6 % of EMCs occupying occlusal, or middle, or occlusal and middle third of the buccal tooth surface and 36.1 % of EMCs that were located in the cervical, or middle and cervical, or extended through the whole buccal tooth surface led to the increase in the risk of greater amount tooth damage during debonding (the difference between variables is shown in Fig. 4.17).

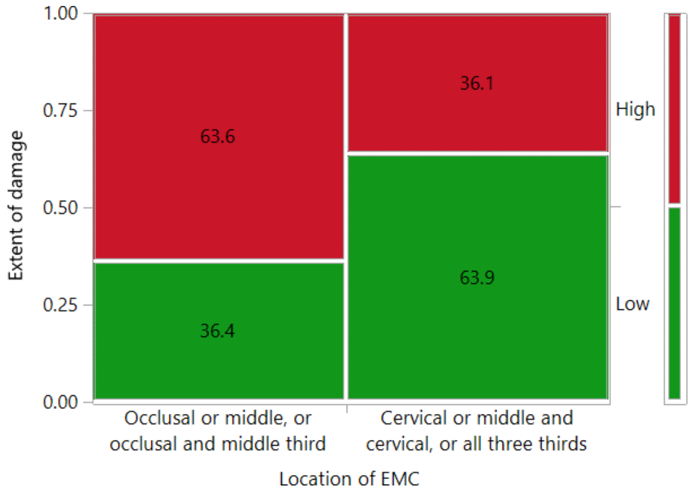

Figure 4.17: A Mosaic plot. The difference between the extent of enamel damage during debonding and location of EMC (χ^2 = 18.138, P = 0.000). Numerical values represent the percentages of different location EMCs within each group.

Age group showed a statistically significant effect on the amount of enamel damage during brackets' removal (OR = 3.083, P = 0.000). For given severity, direction, location parameters, and bracket type, the odds of the undesirable changes in the enamel structure for the teeth from the older-age group were 3.08 times that from the younger-age group. 66.7 % of teeth from the older- and 32.5 % of teeth from the younger-age group led to the increase in the risk of greater amount tooth surface damage formation (the difference between variables is depicted in Fig. 4.18).

The effect of bracket type on the extent of tooth damage during debonding was also evident, however, not statistically significant (OR = 1.695, P = 0.064). For given severity, direction, location parameters, and age group, the odds of having greater extent tooth structure defects in the ceramic brackets group were 1.70 times that in the metal brackets group. 43.3 % of the teeth bonded

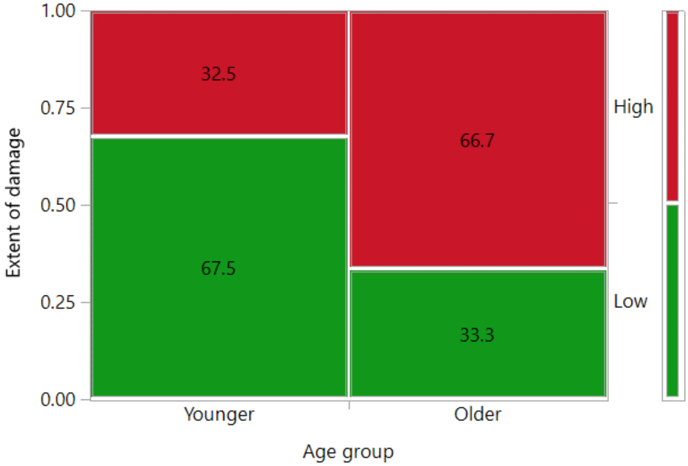

Figure 4.18: A Mosaic plot. The difference between the extent of enamel damage during debonding and age group (χ^2 = 28.019, P = 0.000). Numerical values represent the percentages of different age EMCs within each group.

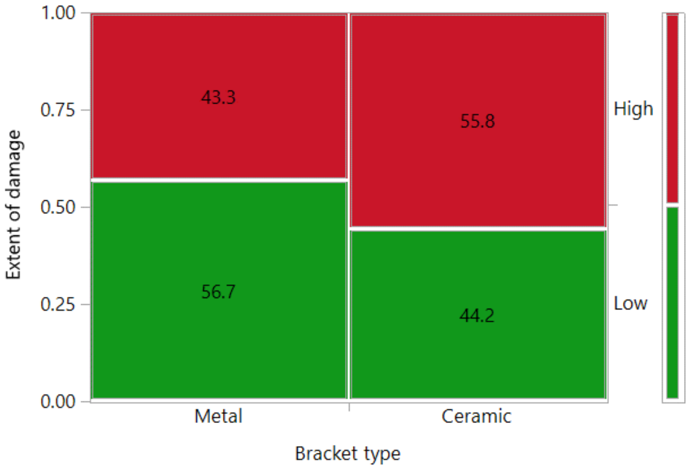

Figure 4.19: A Mosaic plot. The difference between the extent of enamel damage during debonding and bracket type (χ^2 = 3.750, P = 0.053). Numerical values represent the percentages of different bracket type within each group.

with metal brackets and 55.8 % of those bonded with ceramic brackets let to the increase in the risk of greater amount enamel surface defects during the removal procedure (the difference between variables is demonstrated in Fig. 4.19).

The coefficient of determination (Nagelkerke R^2 = 0.215) showed that all five independent variables had an impact on the extent of enamel damage during debonding by 21.5 %.

4.3. Pronounced and Weak Enamel Microcracks' Characteristics Before and After Debonding Metal and Ceramic Brackets

Descriptive statistics of the width and length measurements of P_{EMCs} and W_{EMCs} before and after metal brackets' debonding are demonstrated in Table 4.16. Visible EMCs showed higher mean overall length values before (P = 0.943) and after brackets' removal (P = 0.520; Fig. 4.20) compared with invisible EMCs. Mean overall width was greater for P_{EMCs} only after debonding (P = 0.972; as depicted in Fig. 4.21). Further analysis revealed that width values increased for both types of EMCs after metal brackets' removal, although a statistically significant result was noticed only in the P_{EMCs} group (increase in mean overall width for P_{EMCs}, 0.57 µm, P = 0.005; for W_{EMCs}, 0.32 µm, P = 0.067). The greatest increase in width for visible EMCs was observed in the first zone (cervical third, 0.72 µm, P = 0.199), followed by the third zone (occlusal third, 0.58 µm, P = 0.027; Table 4.16). In the invisible EMCs group, the extent of width increase did not differ between zones. After metal brackets' removal, none of the invisible EMCs progressed to visible ones.

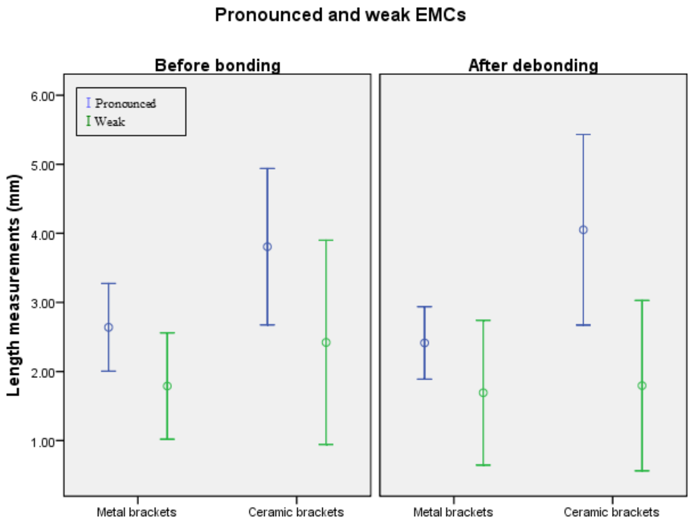

Figure 4.20: P_{EMCs} and W_{EMCs} length mean values (mm) *with* 95.0 % *CI before and after removal of metal (n = 15) and ceramic brackets (n = 15).*

The mean width and length values of P_{EMCs} and W_{EMCs} before and after ceramic brackets' removal are provided in Table 4.17. Visible EMCs showed greater mean overall length (before bonding, P = 0.782; after debonding, P = 0.095; Fig. 4.20) and width values (before bonding, P = 0.003; after debonding, P = 0.042; Fig. 4.21). However, the same amount of increase in

Table 4.16: Width and length of P_{EMCs} and W_{EMCs} before bonding and after removal of metal brackets[a]

	Width of P_{EMCs} (µm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
First zone	1.74	0.96	4.01	0.28	2.46	2.64	13.45	0.31	41.38	0.199
Second zone	1.53	0.83	4.60	0.46	2.00	1.08	5.88	0.31	30.72	**0.000**
Third zone	1.14	0.71	2.59	0.28	1.72	0.77	3.03	0.58	50.88	**0.027**
Overall width	1.53	0.86	4.60	0.28	2.10	1.64	13.45	0.31	37.25	**0.005**
	Length of P_{EMCs} (mm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	2.64	1.24	5.72	1.60	2.41	1.02	4.94	1.15	-8.71	0.181
	Width of W_{EMCs} (µm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
First zone	1.71	0.76	3.21	0.38	2.02	0.76	3.67	0.82	18.13	0.057
Second zone	1.58	0.72	3.43	0.37	1.89	0.64	3.12	0.83	19.62	0.079
Third zone	-[b]	-	-	-	-[b]	-	-	-	-	-[c]
Overall width	1.65	0.74	3.43	0.37	1.97	0.71	3.67	0.82	19.39	0.067
	Length of W_{EMCs} (mm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	1.79	1.08	3.84	0.56	1.69	1.46	5.28	0.24	-5.59	0.768

[a] x̄, mean; Max, maximum; Min, minimum; SD, standard deviation; RC (%), relative percentage change.
[b] Absence of W_{EMCs} in third zone before bonding and after metal brackets' removal.
[c] No statistics could be computed because of absence data before and after debonding.
P = 0.05 shows statistically significant difference. Significance values based on paired samples t-test. Statistically significant values bolded.

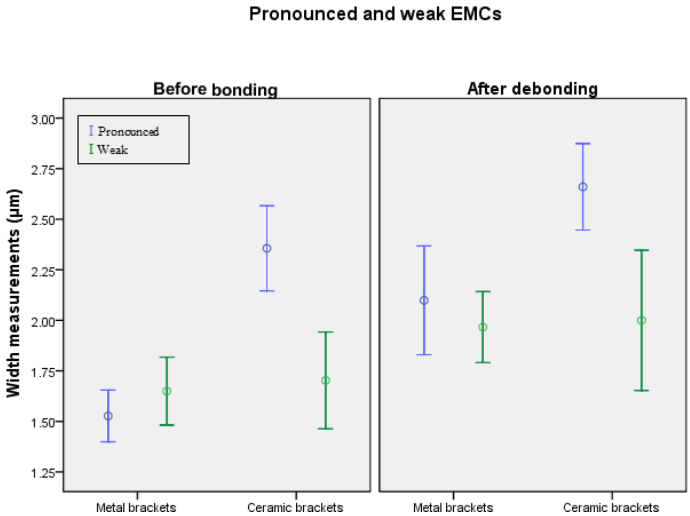

Figure 4.21: P_{EMCs} and W_{EMCs} width mean values (µm) with 95.0 % CI before and after removal of metal (n = 15) and ceramic brackets (n = 15).

mean overall width was calculated for both types of EMCs after removal of ceramic brackets (0.30 µm, P = 0.058 for P_{EMCs} and P = 0.095 for W_{EMCs}). Following debonding, the difference in width was greatest in the third zone (occlusal third) for P_{EMCs} (0.57 µm, P = 0.043) and the first zone (cervical third) for W_{EMCs} (0.59 µm, P = 0.017; Table 4.17). After ceramic brackets' removal, four (26.7 %) invisible EMCs progressed to visible EMCs.

Both types of EMCs demonstrated higher mean overall length (before bonding, P = 0.120 for P_{EMCs} and P = 0.208 for W_{EMCs}; after debonding, P = 0.072 for P_{EMCs} and P = 0.674 for W_{EMCs}) and width (before bonding, P = 0.000 for P_{EMCs} and P = 0.608 for W_{EMCs}; after debonding, P = 0.032 for P_{EMCs} and P = 0.689 for W_{EMCs}) values before and after ceramic brackets' removal compared with metal ones (as presented in Fig. 4.20 and Fig. 4.21).

Changes in width and length values for the control group (Group 3) are presented in Table 4.18. Differences in mean overall width and length for both types of EMCs were quite small and not statistically significant.

Table 4.17: Width and length of P_{EMCs} and W_{EMCs} before bonding and after removal of ceramic brackets[a]

	Width of P_{EMCs} (µm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
First zone	2.68	1.38	5.92	0.42	2.83	1.49	6.98	0.85	5.60	0.614
Second zone	2.41	1.43	5.92	0.28	2.74	1.36	5.92	0.71	13.69	0.109
Third zone	1.62	1.11	5.57	0.45	2.19	0.96	3.53	0.56	35.19	**0.043**
Overall width	2.36	1.40	5.92	0.28	2.66	1.34	6.98	0.56	12.71	0.058
	Length of P_{EMCs} (mm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	3.81	2.05	9.16	0.90	4.05	2.39	9.16	0.90	6.30	0.265
	Width of W_{EMCs} (µm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
First zone	1.68	0.94	4.02	0.49	2.27	1.03	4.39	0.55	35.12	**0.017**
Second zone	1.93	1.10	4.58	0.51	1.52	0.86	3.76	0.55	-21.24	0.903
Third zone	0.53	0.14	0.78	0.42	-[b]	-	-	-	-	-[c]
Overall width	1.70	1.02	4.58	0.42	2.00	1.03	4.39	0.55	17.65	0.095
	Length of W_{EMCs} (mm)									
	Before bonding (n = 15)				After removal (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	2.42	1.77	5.51	0.80	1.80	0.99	3.22	0.80	-25.62	0.374

[a] x̄, mean; Max, maximum; Min, minimum; SD, standard deviation; RC (%), relative percentage change.
[b] Absence of W_{EMCs} in third zone after ceramic brackets' removal.
[c] No statistics could be computed because of absence data after debonding.
P = 0.05 shows statistically significant difference. Significance values based on paired samples *t*-test. Statistically significant values bolded.

Table 4.18: Width and length of P_{EMCs} and W_{EMCs} before initial and after final measurements (control group)[a]

	Width of P_{EMCs} (µm)									
	Initial measurements (n = 15)				Final measurements (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
First zone	3.15	1.87	9.88	0.74	2.96	1.51	6.94	0.93	-6.03	0.173
Second zone	3.30	2.03	9.26	0.31	3.29	2.24	9.89	0.33	-0.30	0.846
Third zone	2.16	1.64	7.16	0.29	2.26	1.64	8.49	0.31	4.63	0.076
Overall width	2.97	1.95	9.88	0.29	2.95	1.98	9.89	0.31	-0.67	0.786
	Length of P_{EMCs} (mm)									
	Initial measurements (n = 15)				Final measurements (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	3.70	1.43	5.94	0.90	3.83	1.40	5.94	0.90	3.51	0.448
	Width of W_{EMCs} (µm)									
	Initial measurements (n = 15)				Final measurements (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
First zone	1.83	0.83	3.40	0.46	2.02	0.64	3.40	0.76	10.38	0.162
Second zone	1.17	0.47	1.79	0.56	1.36	0.72	2.61	0.51	16.24	0.127
Third zone	0.63	0.09	0.72	0.51	0.73	0.17	0.96	0.57	15.87	0.229
Overall width	1.55	0.80	3.40	0.46	1.72	0.76	3.40	0.51	10.97	0.289
	Length of W_{EMCs} (mm)									
	Initial measurements (n = 15)				Final measurements (n = 15)					
	x̄	SD	Max	Min	x̄	SD	Max	Min	RC (%)	***P***
Overall length	2.22	1.56	5.22	0.96	2.20	1.62	5.22	0.87	-0.90	0.892

[a] x̄, mean; Max, maximum; Min, minimum; SD, standard deviation; RC (%), relative percentage change.
P = 0.05 shows statistically significant difference. Significance values based on paired samples *t*-test. Statistically significant values bolded.

5. Discussion

The presented work is the first and only large-scale, standardized, *in vitro* experimental study carried out in Lithuania that examines possible effects of metal and ceramic brackets' debonding on enamel for the teeth from the younger- and older-age groups. The main objective of the study was to evaluate and compare EMCs' characteristics (severity, direction, location, length, and width) before and after removal of metal and ceramic brackets from teeth from two different age groups. The selected model of an *in vitro* research allowed us to make direct precise measurements of the quantitative EMCs' parameters. Although an *in vitro* study design was chosen, before the start of the research the opportunities to carry out an *in vivo* analysis of EMCs, e.g. by using a fiber-optic microscope, were also assessed. [1] It is possible to detect the EMC intraorally with such equipment, however, at the moment, measurements of the quantitative EMC parameters ensuring micrometer resolution needed for precise description of an individual EMC can not be made directly clinically. [1] Alternatively, replication of the buccal tooth surface is another technique combining *in vivo* and *in vitro* measurement, e.g. OCT requires replication procedure because tooth surface causes scattering of the laser beam and consequent loss of resolution. [28] However, the use of indirect measurements always introduces additional errors. [1] Furthermore, in all cases, the standardization of the experiment is crucial for the precise evaluation (detection of the same measurement sites) of the EMCs before and after bracket removal in order to make a comparative analysis. [1] On the other hand, in laboratory (*in vitro*) placing a marker on the buccal tooth surface (or using guiding anatomical landmarks) can be employed as a simple and reliable method for locating EMCs. [1] *Vice versa*, the detection of the same place of the EMC intraorally after two years of treatment with brackets is technically restricted. [1] Thus, precise examination of enamel damage under laboratory conditions remains the most important source of information about the changes of EMCs' characteristics during orthodontic treatment. Finally, the results of an *in vitro* study will always serve as a control for future clinical trials on the enamel damage evaluation. [1]

The results of the present study demonstrated that it was possible to evaluate, measure, and compare severity, direction, location, length, and width characteristics of EMCs before and after debonding with the help of SEM. Additionally, the obtained results revealed the versatility of this method by applying the same analysis for the teeth from various age groups (younger- and older-age groups having enamel with different mechanical properties). [22, 23, 157] The latter device has higher resolution and the capacity for a greater magnification compared to optical microscope (e.g. stereomicroscope), and is less sensitive to non-flat surfaces than COP. [1] This is relevant when sample size is composed of premolars having convex vestibular surface.

Since literature review showed only a few studies where examination of enamel surface employing SEM was described in detail, [11, 91] our own protocol for the evaluation of EMCs was created. Therefore, a group of teeth was experimentally examined before starting to analyze the study sample with SEM. [59] Performed trials demonstrated that it was possible to detect EMCs at ×50–100. [59] Thus, during the initial examination ≥ ×100 was not chosen. [59] This was done for several reasons. First of all, it was difficult and not always possible to measure width and length parameters of such small EMCs that were apparent only at ≥ ×100 precisely with the SEM used in the current study. Secondly, ≈ two-thirds of the evaluated teeth had P_{EMCs}, so there were no problems in detecting EMCs at such magnification. Finally, the same range of magnification used for all the teeth brought more accuracy and uniformity to the research. A more detailed quantitative analysis of the selected EMCs was carried out at ≤ ×2k magnification (morphological diversity of EMCs is shown in Fig. 5.1).

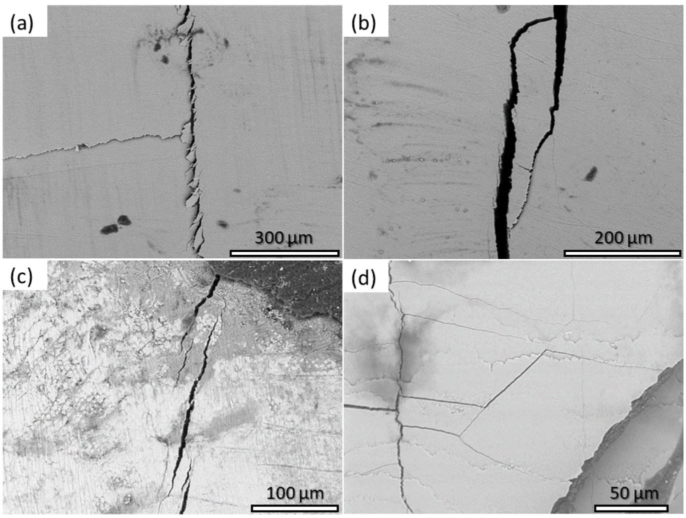

Figure 5.1: A closer look to EMCs from SEM micrographs at various magnifications.

After bonding, all the teeth were placed in distilled water at 37 °C and stored for 24 h prior to further testing in accordance with the guidelines of the

International Organization for Standardization (ISO/TS 11405; 2003). [150] It has been demonstrated that 24 h from bonding to bracket removal is sufficient time to achieve adequate bond strength. [158] In addition, it is technically easy to remove brackets 24 h after bonding. The latter period is widely used in published *in vitro* studies, which also facilitates comparison of the results obtained. [71] It is important to note that extended storage of samples in distilled water prior to debonding (comparative periods: 10 min, 24 h, 1 week, and 4 weeks) did not result in statistically significant changes in mean bond strength. [159] However, when the teeth were left in distilled water for 30 days, bond strength decreased. [160] It could be explained firstly by hydrolysis that leads to degradation of the adhesive interface components. [161] Secondly, water may also infiltrate and weaken the mechanical properties of the polymer matrix. [161]

The teeth selected for the study were extracted from two groups of patients: younger-age group (age range, 18-34 years), and older-age group (age range, 35-54 years) based on the described mechanical properties of the enamel and dentin with aging and already presented classifications in the literature. [23, 143, 144] The findings of the present study indicated that irrespective of the bracket type, teeth from the older-age group showed higher overall width values before (median value 2.95 - 4.30 times greater) and after debonding (median value 3.04 - 4.11 times greater) compared with the younger-age group. Length of EMCs was 2.17 times greater before bonding metal brackets (P = 0.000) and 1.38 times greater before bonding ceramic brackets (P = 0.063) for the teeth from the older-age group, too. After the removal procedure, 2.33 times longer EMCs were observed in Subgroup 1 (metal brackets, P = 0.000) and 1.28 times longer - in Subgroup 2 (ceramic brackets, P = 0.071) compared with the younger-age group. It was not the aim of the current study to compare age-specific EMCs' characteristics. However, for the teeth from the older-age group, the risk of greater extent tooth structure defects during debonding increased 3.08 times. Aging and the related changes in the mechanical properties of human enamel (i.e. increase in hardness, elastic modulus, brittleness, and decrease in fracture toughness with age, as presented in more detail in Section 2.2) could explain the aforementioned difference. [1, 22, 23, 157] The tendency that wider and longer EMCs are more characteristic for the teeth from the older-age group was already found in a previously published study. [1] It is important to note that the current study, which was conducted with the double sample size, confirmed the effect of age on quantitative EMCs' parameters.

When evaluating EMCs *in vivo*, especially for the teeth from the older-age group, it is relevant to consider possible tooth wear, i.e. physiological or pathological, and evaluate variables, such as the location of wear facets, or the amount of tooth tissue lost. Because enamel is a type of anisotropic material (as explained in Section 2.1), the expression of EMCs could depend on the degree of tooth wear and the type of pathological tooth wear (classified by etiological factor). It has been demonstrated that when mandibular second premolars are unworn or moderately worn, i.e. occlusal contact areas are mainly located in the steeply inclined slopes of the buccal cusp, the occlusal forces create tensile stresses in the buccal cervical region of the tooth and in the root. [162] This could lead to the formation of multiple EMCs in the cervical area of the tooth because enamel is the thinnest in the latter zone. When the tooth wear increases, i.e. the buccal cusp becomes flatter and the contact areas with the antagonistic teeth increase, the directions of the occlusal forces change from oblique to nearly parallel to the longitudinal axis of the tooth crown. [162,163] As a consequence, tensile stresses reduce and shift from the buccal side (in unworn and slightly worn teeth) to the mesial and distal sides (in teeth with advanced wear) [162] where enamel could be more resistant to cracking compared to the buccal cervical region. Although no direct relationship has been found between EMCs and non-carious cervical lesions, it is believed that enamel surface areas with EMCs may reduce the resistance of the latter zones to pathological dental wear. [162] Each type of pathological tooth wear (abfraction, attrition, abrasion, erosion, as classified by etiological factor) has specific location of wear facets with different enamel resistance to cracking. [164] It can be assumed that the longer the tooth is exposed to a particular traumatic factor, the greater the risk of damage to certain areas (depending on the nature of the traumatic factor) of the enamel and formation of EMCs. According to published literature, it can also be argued that in the case of tooth wear, the expression of EMCs would be more dependent on the posteruptive age of the tooth with deciduous enamel (characteristic numerous, extensive, deep, branched EMCs, notable differences between enamel surface and subsurface EMCs' parameters) showing the lowest resistant to wear as compared to permanent teeth enamel (features polished, shiny wear zones rather than craters, smaller number, length, depth values for both surface and subsurface EMCs, minimal cracks bifurcation). [165] It is important to note that enamel samples with longer, deeper, and more numerous EMCs were considered more prone to wear. [165]

A further analysis of the study results revealed that debonding led to the changes in the width of EMCs, i.e. after metal and ceramic brackets' removal, the width of all EMCs increased. Measured overall width values were greater after debonding both for the teeth from the younger- and older-age groups. The phenomenon of widening EMCs during brackets' removal is related to high bond strength of brackets and the existing discrepancy between the tensile strength of enamel and the debonding force (as described in Subsection 2.3.1). However, it should be emphasized that bond strength *in vitro* is higher than *in vivo* (because of the oral humidity (e.g. saliva, acid), patient abuse, and masticatory forces), thus the obtained increase in EMCs may be greater than in a clinical situation. [1, 29, 166]

The applied technique enabled the evaluation of the EMC morphology in the occlusal, middle, and cervical thirds of the buccal tooth surface, and localization of the same measurement site before and after debonding. [1] During the examination of the width parameter in every zone the greatest changes in the width of EMCs were noticed in the occlusal and cervical parts. This suggests that forces during debonding are more concentrated on these zones of the buccal tooth surface rather than the middle one. [16,91,142] This finding is supported by a previously published study demonstrating that the most affected area was not the middle of the buccal surface, where the bracket had been fixed, but rather the approximal and cervical borders. [91] Different enamel quality in distinct zones, especially for the teeth from the older-age group, might be the reason why the location of EMC on the buccal tooth surface has an effect on its characteristics. [29] Thus, a greater EMCs increase in the cervical area after debonding may be due to a thinner enamel layer. [23, 29] Because of the thin enamel in the latter region, not only is the immediate transient stress large, but it also remains high during the environmental temperature changes. [67] Whereas, the increase in the enamel brittleness with distance from the DEJ to the occlusal surface might explain greater EMCs increase in the third zone (occlusal part) following brackets' removal. [1, 22]

The perception that EMCs might progress to the deeper enamel layers raises the question whether there is any relation between the width of EMC and its depth? Experimental evaluation of the depth parameter of a group of teeth with different width values using COP (Sensofar PLU 2300, Barcelona, Spain) did not show statistically significant differences in depth values between the EMCs with higher and lower width parameters. Thus, the results obtained can not confirm either the ratio between the width of EMC and its depth, or that the depth of EMC can be assessed on the basis of width values.

Regarding length characteristic, no statistically significant changes were observed after debonding on the teeth from either the younger- or the older-age group. The increase in the mean overall length was calculated only in Subgroup 2 (ceramic brackets) for the younger-age group (0.17 mm, $P = 0.087$). Meanwhile, lower mean overall length values were recorded following debonding metal brackets for the teeth from both age groups and after ceramic brackets' removal for the teeth from the older-age group. This is similar to the outcome of a previously published study indicating that most teeth (82.0 %) experienced no increase in EMCs after removal of brackets using a universal testing machine. [90] Further support for this argument can be found in another study reporting that 63.9 % of evaluated teeth did not show an increase in length of EMCs after the debonding procedure. [17] In contrast to the above mentioned findings, some studies reported an increase of length measurement after removal of metal and ceramic brackets. [9,10,19,20]

Difference in the results could be attributed to the methodological disparities among the studies. First of all, different debonding techniques (i.e. conventional or mechanical, as already explained in Section 2.5) can be distinguished while analyzing *in vitro* studies dealing with EMCs. It must be pointed out that in the present study all brackets were removed with appropriate pliers by hand. Although it was not possible to control or standardize the actual debonding forces, it is likely that removal of brackets by hand using pliers helped to avoid larger than the hand debonding loads used in clinical practice. [11] Furthermore, mechanical testing is often criticized for not representing a clinical stress situation realistically. [131] Thus, efforts were made to choose a debonding method that would be safe enough and simulate more closely the debonding forces applied in actual clinical situations. [59] Secondly, a lack of standardized protocol regarding the residual adhesive removal procedure following debonding should be pointed out. In accordance with the results of recent systematic review of EMCs during orthodontic debonding, in two out of seven studies included in the meta-analysis, evaluation of the buccal enamel surface was carried out without adhesive remnants' elimination after brackets' removal, [10,19] and in one trial EMCs' characteristics were measured before and after residual adhesive removal. [4,20] Meanwhile in the current research all visible residual adhesive was eliminated with the use of a slow-speed handpiece and a carbide-finishing bur. It must be emphasized, that efforts were made to minimize the undesirable effect of rotary instruments on tooth enamel and evaluation of EMCs. Light movements of the bur were used in order to avoid scratching the enamel. Water cooling was not employed when the last remnants were removed because water lessens the contrast with enamel. [59] Furthermore, according to previously performed research, the least enamel loss was seen after the use of this method of the residual adhesive removal. [103,141] Finally, different laboratory techniques employed for EMCs analysis and a lack of a detailed description of how the length was measured could explain inconsistency between different studies. It is important to note that following debonding and adhesive removal it is more difficult to measure EMC as part of it may still be filled with residual adhesive. So when the changes in the length parameter are small after brackets' removal, it is possible to calculate even lower mean overall values after debonding.

SEM can be utilized for detecting new EMCs by comparing the reconstructed images of the buccal tooth surface before and after brackets' removal. [1] Analyzing the teeth without EMCs before the bonding procedure, new EMCs were recorded in 7 out of 30 (23.3 %) teeth both for the younger- and older-age groups after debonding metal brackets, 5 out of 30 (16.7 %, younger-age group) teeth, and 7 out of 30 (23.3 %, older-age group) teeth after ceramic brackets' removal. Thus, the majority of the examined teeth did not show new EMCs. Small enough percentage of teeth with new EMCs can be attributed to the selected debonding method. Removal of brackets with pliers by hand applies a bilateral force at the bracket base – adhesive interface. It has been suggested that this debonding pattern has the advantage of protecting the enamel surface, [73, 127] and thus debonding by hand employing appropriate pliers for specific bracket type has been widely used in other studies. [7, 10, 18, 19, 59, 73] Nonetheless, there is no agreement in the literature regarding the effect of debonding on the enamel, and the results of different studies vary from no enamel damage after brackets' removal [5] to 25.0 % of teeth with new EMCs after debonding metal brackets [17] and ≈ 20.0 –34.0 % - after debonding ceramic brackets. [17, 18] The measured width and length parameters of new EMCs were lower compared to the corresponding values of the teeth with EMCs before bonding. The latter difference suggests that it is not the bracket removal procedure but the enamel morphology and its structural changes that plays a greater role in the enamel damage formation. [1] Furthermore, it has been revealed that the stress required to propagate an existing EMC is much lower than that required to initiate a new one. [67]

In the present study EMCs evaluation was carried out on teeth with intact buccal enamel and no previous endodontic, orthodontic or restorative treatment (as described in Subsection 3.1.1). One can only assume that restorations (direct and indirect), irregular dental contacts, and parafunctions (such as bruxism) could affect the formation of EMCs during orthodontic treatment *in vivo*. Restorations destroy the integrity of the tooth structure and weaken tooth resistance to occlusal force. [167] As a result, the increased frequency of EMCs could be observed following debonding. It has been demonstrated that cracks resulting from polymerization shrinkage of composites show little to no risk of an underlying pathology. [27] Meanwhile, horizontal or diagonal cracks that normally propagate from the corner of a restoration and sometimes are filled with debris have a high risk of an underlying pathology. [27] It can be hypothesized that irregular dental contacts could lead to occlusal overload, concentration of tensile stresses in the weakest areas of the tooth crown (usually with the thinnest enamel layer), and that might result in the formation of multiple EMCs. For parafunctions, particularly severe bruxism, the magnitude (higher bite forces), duration (hours rather than minutes), direction (lateral rather than vertical), type (shear rather than compression), and magnification (4 to 7 times higher than normal) of normal masticatory forces change. [168] This increases the load on the teeth during orthodontic treatment leading to the higher risk of developing EMCs and other enamel lesions (due to occlusive overload).

There is evidence in the literature of correlation between the tissue dehydration and the dynamic dimensional changes within dentin and enamel, [169] as well as between dehydration and the fatigue crack growth resistance. [143] In order to analyze the possible effect of dehydration on existing EMCs or formation of new ones, a control group was included in the study. [59] The teeth in the control group were subjected to the same analysis, only without bonding and debonding procedures. Results revealed that new EMCs were not recorded. Differences in the mean overall length (0.02 mm) were not statistically significant in both age groups. When evaluating the width parameter of EMCs (i.e. overall width and width in each zone), statistically significant difference was noticed only for overall width both for the younger- and older-age groups. However, the recorded maximum and minimum values of the overall width were almost identical after the initial and the final measurements for both age groups. Furthermore, the differences of median values between these two measurements were very small (0.10 µm, younger-age group; 0.06 µm, older-age group). Thus, it would not be appropriate to consider these results as clinically significant. Therefore, the findings of the present research did not demonstrate dependence of the width and length of EMCs on dehydration which occurs when specimens are prepared for SEM scanning and observation. [59]

One of the objectives of the research was to observe if the predictions about the irreversible changes in the tooth structure during debonding could be made from a set of the EMCs' characteristics, age group, and type of the bracket used at the beginning of the treatment.

Severity of the EMC (P_{EMC} or W_{EMC}) and age group (teeth from the younger- or older-age group) were two independent variables that showed a statistically significant effect on the amount of enamel damage during brackets' removal. The odds of greater enamel damage were higher for P_{EMCs} and for the teeth from the older-age group. Despite the fact that nowadays an ever-increasing number of patients start noticing EMCs and wondering about their effect on the tooth structure, the characteristics of P_{EMCs} have been poorly described in the literature so far. Just a few facts were known about the changes in the number parameter after debonding. [9] Results of the present study revealed that for more visible EMCs, 2.63 times increased the risk of greater amount tooth structure defects during brackets' removal. Therefore, the effect of these EMCs on the enamel damage during debonding should not be neglected and further studies are necessary for a more comprehensive evaluation of P_{EMCs} characteristics. A detailed explanation about the changes in the mechanical and optical properties of the enamel with aging presented in Section 2.2.

The effect of bracket type on the extent of tooth damage during brackets' removal was also evident, however, it was not statistically significant. When using ceramic brackets, the risk of having greater extent tooth structure defects during debonding increased 1.70 times. As already discussed in the paragraph about formation of new EMCs (in Section 5), so far there has been no one common conclusion in the literature regarding the effect of debonding metal and ceramic brackets on the enamel. In the present study uncoated maxillary premolar ceramic brackets (Clarity; 3M Unitek) with mechanical bases were selected as being one of the most common and widely used ceramic brackets both in clinical practice and in scientific research. This allowed the results to be compared with other studies. To date, no study assessing the effect of different aesthetic brackets on EMCs has been conducted. However, based on the physical properties of ceramics (as presented in more detail in Subsection 2.4.2) it can be assumed that the choice of sapphire brackets instead of ceramic ones could have led to a higher degree of enamel damage.

Direction and location parameters of EMCs did not lead to statistically significant changes in the enamel structure during debonding. A more detailed analysis revealed that 50.0 % of EMCs with inclination $< 46°$ to the longitudinal axis of the tooth crown and 44.4 % of EMCs having inclination $\geq 46°$ or changing their direction during extension through the buccal tooth surface led to a higher risk of greater extent tooth damage during the removal of brackets. Previously published studies demonstrated that horizontal EMCs showed a high risk of an underlying pathology. [27] Thus, it was interesting to notice that in the present study this type of direction was not related to a high possibility of a greater amount of enamel defects. The difference in the results can be explained by several reasons. First of all, during SEM analysis of the teeth (before and after removal of metal (Subgroup 1) and ceramic brackets (Subgroup 2) for Group 1 (teeth with EMCs), both from the younger- and older-age groups) just one horizontal EMC was found and included in further investigation; thus, sample size was too small for the examination of horizontal EMCs' parameters. A low percentage (3.3 %, 1 tooth of 30) of horizontal EMCs corresponded with previously published results indicating that this type of EMC was more common to the maxillary and mandibular incisors rather than premolars. [13] Secondly, in other cases, where several EMCs of different directions were observed, horizontal EMCs were left aside because they were not the longest ones. Vertical EMCs demonstrated the highest length values and were selected for more detailed quantitative analysis. [66]

Finally, the study aimed to evaluate and compare characteristics of EMCs having varying degrees of severity (visible EMCs and those EMCs that can be visualized only under SEM) before and after debonding metal and ceramic brackets.

The obtained findings demonstrated that P_{EMCs} possessed higher mean overall length before and after removal of both types of brackets. Mean overall width was greater for P_{EMCs} only before and after debonding ceramic brackets. Although a literature search did not reveal any studies addressed to this specific subject, i.e. dealing with the parameters of different severity EMCs, calculated overall length and width values of P_{EMCs} and W_{EMCs} were lower than those found in other studies (evaluating the dimensions of EMCs without considering their severity). [9, 10, 29, 59]

Following brackets' removal, the mean overall width of all EMCs increased.

Changes for P_{EMCs} were 0.57 µm with metal brackets and 0.30 µm with ceramic brackets; for W_{EMCs}, - 0.32 µm with metal brackets and 0.30 µm with ceramic brackets. The apparent tendency of the increase in the width parameter after debonding is consistent with the already mentioned findings. [1, 29, 59]

The lack of statistically significant differences in width and length between P_{EMCs} and W_{EMCs} before bonding poses the question, what makes EMCs visible? [29] Experimental evaluation of the depth parameter of these two groups of EMCs using COP (Sensofar PLU 2300, Barcelona, Spain) did not show statistically significant differences, either. [29] Thus, such findings suggest that it is not the morphology of an EMC (length, width, or depth) that determines its visibility, but the instrument used for the evaluation (SEM, COP, or human eye). [29] SEM is a device that performs lateral characterization of a tooth's surface (2D view). Its data registration is based on electric conductivity (back scattered electrons) of the sample surface. Employing SEM, we can analyze the length, width, geometry of an EMC, but not the depth parameter. Meanwhile, COP gives us lateral and axial characterization of a tooth's surface (3D view) based on sample surface optical reflectivity. This means that besides length, width, and geometry evaluation, we are able to measure the depth of an EMC. Finally, the human eye possesses all the features of the above-listed devices - although having a much lower spatial resolution. [29] It is important to note, that the human eye has the ability to see the depth (penetrate into deeper subsurface layers in case of a transparent object, like tooth enamel). Thus, it offers a chance to see abyssal EMC which is in subsurface. Additionally, the "human eye" cognitively averages the whole view, and this determines whether an EMC will be visible as a crack, or appear hidden. [29] Therefore, solid and unbroken EMCs were visible in contrast to fragmented or branched ones. [29]

6. Statements to Defend

I The debonding procedure leads to changes in EMCs' characteristics (severity, direction, location, length, and width) and formation of new EMCs.

• Dumbryte, I., Linkeviciene, L., Malinauskas, M., Linkevicius, T., Peciuliene, V., and Tikuisis, K., "Evaluation of enamel micro-cracks characteristics after removal of metal brackets in adult patients," *Eur. J. Orthod.* **35**(3), 317-322 (2013).

II There is a difference in EMCs' parameters (severity, direction, location, length, and width) before and after debonding for the teeth from the younger- and older-age groups.

• Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Enamel microcracks in terms of orthodontic treatment: A novel method for their detection and evaluation," *Dent. Mater. J.* **36**(4), 438-446 (2017).

III Visibility of EMCs before bonding, taken alone, is of low prognostic value for predicting EMCs increase after brackets' removal.

• Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "The prognostic value of visually assessing enamel microcracks: *Do debonding and adhesive removal contribute to their increase?*," *Angle Orthod.* **86**(3), 437-447 (2016).

IV Evaluation of EMCs' characteristics at the beginning of the orthodontic treatment can be used to predict a higher risk of greater tooth surface damage during brackets' removal.

• Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "Enamel cracks evaluation – A method to predict tooth surface damage during the debonding," *Dent. Mater. J.* **34**(6), 828-834 (2015).

7. Conclusions

I The proposed method, combining SEM and derived formulas, enabled a precise detection of the same EMC (showing length range, 0.24 – 10.15 mm; width range, 0.25 – 35.04 µm) before and after orthodontic debonding, and a quantitative examination of its characteristics (length and width).

II Irrespective of the bracket type, teeth from the older-age group demonstrated a higher mean overall length and width before and after debonding compared with the younger-age group.

III In both age groups, the debonding procedure led to the increase in the width parameter of EMCs. However, no statistically significant changes in the severity, direction, and length were observed. Regarding the location, the highest increase in the width appeared in the occlusal and cervical thirds of the buccal tooth surface.

IV Debonding of ceramic brackets resulted in wider EMCs compared with metal ones with regard to the teeth from the younger-age group. No statistically significant difference of the bracket type on the width of EMCs was observed in the older-age group, nor on the length characteristic in either age group.

V There was a positive correlation (from moderate to very strong) between length and width values of EMCs before bonding and their increase during metal and ceramic brackets' removal in both age groups.

VI Although in the majority of cases teeth with visible EMCs showed higher mean overall length and width values compared to those with invisible EMCs, they were not predisposed to a greater EMCs increase after debonding.

VII Invisible EMCs can progress to visible ones after debonding, especially when ceramic brackets are used. Thus, the visibility of EMCs before bonding, taken alone, is of low prognostic value for predicting EMCs increase after brackets' removal.

VIII EMCs showing a set of specified characteristics (i.e. visible EMCs of teeth from the older-age group with vertical or oblique inclination, and located closer to the occlusal surface) at the beginning of treatment, together with the use of ceramic brackets, might lead to a 21.5 % higher risk of greater enamel damage during debonding.

8. Practical Recommendations

I The proposed technique for examination of EMCs - employing SEM and derived formulas - proved to be versatile and could be applied for all teeth, both from the younger- and older-age groups, having enamel with different mechanical properties.

II EMCs increase following metal and ceramic brackets' removal could be regarded as an unavoidable consequence of debonding rather than a failure of orthodontic treatment for both age groups. Patients should be fully informed of this issue (e.g. orally and in writing) before initiating orthodontic treatment.

III Evaluation of EMCs (i.e. determination of severity, direction, location parameters) could be included in the standard protocol for intraoral examination. When EMCs are visible to the naked eye before bonding, taking pictures of such teeth for documentation should be suggested.

IV It is recommended to take into account the condition of the enamel when choosing fixed appliances. It is important to know which patients will benefit from traditional brackets systems as well as which individuals with EMCs before the bonding procedure may require alternative brackets systems, e.g. lingual brackets, or different treatment modalities.

V If following intraoral examination the patient is detected to be at a higher risk of enamel damage after debonding (i.e. there are recorded visible EMCs, especially in teeth other than the maxillary central incisors and canines, of vertical or oblique direction, and located closer to the occlusal surface) and pre-treatment EMCs have already caused aesthetic complaints on the patient's part, alternative treatment methods may be suggested, e.g. orthodontic therapy using aligners.

Bibliography

[1] Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Enamel microcracks in terms of orthodontic treatment: A novel method for their detection and evaluation," *Dent. Mater. J.* **36**(4), 438–446 (2017).

[2] Ryf, S., Flury, S., Palaniappan, S., Lussi, A., van Meerbeek, B., and Zimmerli, B., "Enamel loss and adhesive remnants following bracket removal and various clean-up procedures *in vitro*," *Eur. J. Orthod.* **34**(1), 25–32 (2012).

[3] Arhun, N. and Arman, A., "Effects of orthodontic mechanics on tooth enamel: a review," *Semin. Orthod.* **13**(4), 281–291 (2007).

[4] Dumbryte, I., Vebriene, J., Linkeviciene, L., and Malinauskas, M., "Enamel microcracks in the form of tooth damage during orthodontic debonding: a systematic review and meta-analysis of *in vitro* studies," *Eur. J. Orthod.* **40**(6), 636–648 (2018).

[5] Alessandri Bonetti, G., Zanarini, M., Incerti Parenti, S., Lattuca, M., Marchionni, S., and Gatto, M. R., "Evaluation of enamel surfaces after bracket debonding: an *in-vivo* study with scanning electron microscopy," *Am. J. Orthod. Dentofacial Orthop.* **140**(5), 696–702 (2011).

[6] Pont, H. B., Özcan, M., Bagis, B., and Ren, Y., "Loss of surface enamel after bracket debonding: An *in-vivo* and *ex-vivo* evaluation," *Am. J. Orthod. Dentofacial Orthop.* **138**(4), 387.e1–387.e9 (2010).

[7] Kitahara-Céia, F. M. F., Mucha, J. N., and dos Santos, P. A. M., "Assessment of enamel damage after removal of ceramic brackets," *Am. J. Orthod. Dentofacial Orthop.* **134**(4), 548–555 (2008).

[8] Shahabi, M., Heravi, F., Mokhber, N., Karamad, R., and Bishara, S. E., "Effects on shear bond strength and the enamel surface with an enamel bonding agent," *Am. J. Orthod. Dentofacial Orthop.* **137**(3), 375–378 (2010).

[9] Ahrari, F., Heravi, F., Fekrazad, R., Farzanegan, F., and Nakhaei, S., "Does ultra-pulse CO_2 laser reduce the risk of enamel damage during debonding of ceramic brackets?," *Lasers Med. Sci.* **27**(3), 567–574 (2012).

[10] Heravi, F., Rashed, R., and Raziee, L., "The effects of bracket removal on enamel," *Aust. Orthod. J.* **24**(2), 110–115 (2008).

[11] Chen, C. S., Hsu, M. L., Chang, K. D., Kuang, S. H., Chen, P. T., and Gung, Y. W., "Failure analysis: enamel fracture after debonding orthodontic brackets," *Angle Orthod.* **78**(6), 1071–1077 (2008).

[12] Zachrisson, B. U. and Buyukyilmaz, T., "Bonding in orthodontics," in [*Orthodontics: current principles and techniques*], Graber, T. M., Vanarsdall, R. L., Vig, K. W. L., ed., 612–619, St Louis: Elsevier-Mosby, 4th ed. (2005).

[13] Zachrisson, B. U., Skogan, O., and Höymyhr, S., "Enamel cracks in debonded, debanded, and orthodontically untreated teeth," *Am. J. Orthod.* **77**(3), 307–319 (1980).

[14] Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Does orthodontic debonding lead to tooth sensitivity? Comparison of teeth with and without visible enamel microcracks," *Am. J. Orthod. Dentofacial Orthop.* **151**(2), 284–291 (2017).

[15] Rix, D., Foley, T. F., and Mamandras, A., "Comparison of bond strength of three adhesives: composite resin, hybrid GIC, and glass-filled GIC," *Am. J. Orthod. Dentofacial Orthop.* **119**(1), 36–42 (2001).

[16] Tecco S., Tetè S., D'Attilio M., and Festa, F., "Enamel surface after debracketing of orthodontic brackets bonded with flowable orthodontic composite. A comparison with a traditional orthodontic composite resin," *Minerva Stomatol.* **57**(3), 81–94 (2008).

[17] Habibi, M., Nik, T. H., and Hooshmand, T., "Comparison of debonding characteristics of metal and ceramic orthodontic brackets to enamel: an *in-vitro* study," *Am. J. Orthod. Dentofacial Orthop.* **132**(5), 675–679 (2007).

[18] Bishara, S. E., Ostby, A. W., Laffoon, J., and Warren, J. J., "Enamel cracks and ceramic bracket failure during debonding *in vitro*," *Angle Orthod.* **78**(6), 1078–1083 (2008).

[19] Salehi, P., Pakshir, H., Naseri, N., and Baherimoghaddam, T., "The effects of composite resin types and debonding pliers on the amount of adhesive remnants and enamel damages: a stereomicroscopic evaluation," *J. Dent. Res. Dent. Clin. Dent. Prospects* **7**(4), 199–205 (2013).

[20] Heravi, F., Shafaee, H., Abdollahi, M., and Rashed, R., "How is the enamel affected by different orthodontic bonding agents and polishing techniques?," *J. Dent. (Tehran, Iran)* **12**(3), 188–194 (2015).

[21] Baherimoghadam, T., Akbarian, S., Rasouli, R., and Naseri, N., "Evaluation of enamel damages following orthodontic bracket debonding in fluorosed teeth bonded with adhesion promoter," *Eur. J. Dent.* **10**(2), 193–198 (2016).

[22] Park, S., Quinn, J. B., Romberg, E., and Arola, D., "On the brittleness of enamel and selected dental materials," *Dent. Mater.* **24**(11), 1477–1485 (2008).

[23] Park, S., Wang, D. H., Zhang, D., Romberg, E., and Arola, D., "Mechanical properties of human enamel as a function of age and location in the tooth," *J. Mater. Sci. Mater. Med.* **19**(6), 2317–2324 (2008).

[24] Culjat, M. O., Singh, R. S., Brown, E. R., Neurgaonkar, R. R., Yoon, D. C., and White, S. N., "Ultrasound crack detection in a simulated human tooth," *Dentomaxillofac. Radiol.* **34**(2), 80–85 (2005).

[25] Leão Filho, J. C. B., Braz, A. K. S., de Araujo, R. E., Tanaka, O. M., and Pithon, M. M., "Enamel quality after debonding: evaluation by optical coherence tomography," *Braz. Dent. J.* **26**(4), 384–389 (2015).

[26] Hsieh, Y. S., Ho, Y. C., Lee, S. Y., Chuang, C. C., Tsai, J. C., Lin, K. F., and Sun, C. W., "Dental optical coherence tomography," *Sensors (Basel)* **13**(7), 8928–8949 (2013).

[27] Clark, D. J., Sheets, C. G., and Paquette, J. M., "Definitive diagnosis of early enamel and dentin cracks based on microscopic evaluation," *J. Esthet. Restor. Dent.* **15**(7), 391–401; discussion 401 (2003).

[28] Al Shamsi, A. H., Cunningham, J. L., Lamey, P. J., and Lynch, E., "Three-dimensional measurement of residual adhesive and enamel loss on teeth after debonding of orthodontic brackets: an *in-vitro* study," *Am. J. Orthod. Dentofacial Orthop.* **131**(3), 301.e9–15 (2007).

[29] Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "The prognostic value of visually assessing enamel microcracks: *Do debonding and adhesive removal contribute to their increase?*," *Angle Orthod.* **86**(3), 437–447 (2016).

[30] Bishara, S. E., "Ceramic brackets and the need to develop national standards," *Am. J. Orthod. Dentofacial Orthop.* **117**(5), 595–597 (2000).

[31] Özcan, M., Finnema, K., and Ybema, A., "Evaluation of failure characteristics and bond strength after ceramic and polycarbonate bracket debonding: effect of bracket base silanization," *Eur. J. Orthod.* **30**(2), 176–182 (2008).

[32] Ten Cate, A. R., [*Oral histology: development, structure, and function*], St. Louis: Mosby, 5th ed. (1998).

[33] Robinson, C., Kirkham, J., and Shore, R. C., [*Dental enamel: formation to destruction*], Boca Raton, FL: CRC Press, 1st ed. (1995).

[34] Bajaj, D., Park, S., Quinn, G. D., and Arola, D., "Fracture processes and mechanisms of crack growth resistance in human enamel," *JOM* **62**(7), 76–82 (2010).

[35] Gray, H., Williams, P. L., and Bannister, L. H., [*Gray's anatomy: the anatomical basis of medicine and surgery*], New York: Churchill Livingstone, 38th ed. (1995).

[36] Habelitz, S., Marshall, S. J., Marshall, G. W. Jr., and Balooch, M., "Mechanical properties of human dental enamel on the nanometre scale," *Arch. Oral Biol.* **46**(2), 173–183 (2001).

[37] Frazier, P. D., "Adult human enamel: an electron microscopic study of crystallite size and morphology," *J. Ultrastruct. Res.* **22**(1), 1–11 (1968).

[38] Weber, D. F., "Sheath configurations in human cuspal enamel," *J. Morphol.* **141**(4), 479–489 (1973).

[39] Berkovitz, B. K. B., Boyde, A., Frank, R. M., Höhling, H. J., Moxham, B. J., Nalbandian, J., and Tonge, C. H., [*Teeth (part of the Handbook of microscopic anatomy book series)*], Springer Berlin Heidelberg (1989).

[40] Krause, W. J., [*Krause's essential human histology for medical students*], Universal Publishers, 3rd ed. (2005).

[41] Zhang, Y. R., Du, W., Zhou, X. D., and Yu, H. Y., "Review of research on the mechanical properties of the human tooth," *Int. J. Oral Sci.* **6**(2), 61–69 (2014).

[42] Cuy, J. L., Mann, A. B., Livi, K. J., Teaford, M. F., and Weihs, T. P., "Nanoindentation mapping of the mechanical properties of human molar tooth enamel," *Arch. Oral Biol.* **47**(4), 281–291 (2002).

[43] Xu, H. H., Smith, D. T., Jahanmir, S., Romberg, E., Kelly, J. R., Thompson, V. P., and Rekow, E. D., "Indentation damage and mechanical properties of human enamel and dentin," *J. Dent. Res.* **77**(3), 472–480 (1998).

[44] Mann, A. B. and Dickinson, M. E., "Nanomechanics, chemistry and structure at the enamel surface," in [*The Teeth and Their Environment. Monogr Oral Sci.*], Duckworth, R. M., ed., 105–131, Basel, Karger (2006).

[45] Roy, S. and Basu, B., "Mechanical and tribological characterization of human tooth," *Mater. Charact.* **59**(6), 747–756 (2008).

[46] Braly, A., Darnell, L. A., Mann, A. B., Teaford, M. F., and Weihs, T. P., "The effect of prism orientation on the indentation testing of human molar enamel," *Arch. Oral Biol.* **52**(9), 856–860 (2007).

[47] Mahoney, E. K., Rohanizadeh, R., Ismail, F. S. M., Kilpatrick, N. M., and Swain, M. V., "Mechanical properties and microstructure of hypomineralised enamel of permanent teeth," *Biomaterials* **25**(20), 5091–5100 (2004).

[48] Mahoney, E. K., Ismail, F. S. M., Kilpatrick, N. M., and Swain, M. V., "Mechanical properties across hypomineralized/hypoplastic enamel of first permanent molar teeth," *Eur. J. Oral Sci.* **112**(6), 497–502 (2004).

[49] Staines, M., Robinson, W. H., and Hood, J. A. A., "Spherical indentation of tooth enamel," *J. Mater. Sci.* **16**(9), 2551–2556 (1981).

[50] Jeng, Y. R., Lin, T. T., Hsu, H. M., Chang, H. J., and Shieh, D. B., "Human enamel rod presents anisotropic nanotribological properties," *J. Mech. Behav. Biomed. Mater.* **4**(4), 515–522 (2011).

[51] Bajaj, D. and Arola, D. D., "On the R-curve behavior of human tooth enamel," *Biomaterials* **30**(23-24), 4037–4046 (2009).

[52] Rasmussen, S. T., Patchin, R. E., Scott, D. B., and Heuer, A. H., "Fracture properties of human enamel and dentin," *J. Dent. Res.* **55**(1), 154–164 (1976).

[53] Rasmussen, S. T. and Patchin, R. E., "Fracture properties of human enamel and dentin in an aqueous environment," *J. Dent. Res.* **63**(12), 1362–1368 (1984).

[54] Bajaj, D. and Arola, D., "Role of prism decussation on fatigue crack growth and fracture of human enamel," *Acta Biomater.* **5**(8), 3045–3056 (2009).

[55] Quinn, J. B. and Quinn, G. D., "Indentation brittleness of ceramics: a fresh approach," *J. Mater. Sci.* **32**(16), 4331–4346 (1997).

[56] Robinson, C., Kirkham, J., Brookes, S. J., and Shore, R. C., "Chemistry of mature enamel," in [*Dental enamel: formation to destruction*], Robinson, C., Kirkham, J., Shore, R. C., ed., 167–191, Boca Raton, FL: CRC Press, 1st ed. (1995).

[57] Bertacci, A., Chersoni, S., Davidson, C. L., and Prati, C., "*In vivo* enamel fluid movement," *Eur. J. Oral Sci.* **115**(3), 169–173 (2007).

[58] Lester, K. S. and Boyde, A., "Relating developing surface to adult ultrastructure in Chiropteran enamel by SEM," *Adv. Dent. Res.* **1**(2), 181–190 (1987).

[59] Dumbryte, I., Linkeviciene, L., Malinauskas, M., Linkevicius, T., Peciuliene, V., and Tikuisis, K., "Evaluation of enamel micro-cracks characteristics after removal of metal brackets in adult patients," *Eur. J. Orthod.* **35**(3), 317–322 (2013).

[60] Sutton, P. R. N., "Transverse crack lines in permanent incisors of Polynesians," *Aust. Dent. J.* **6**(3), 144–150 (1961).

[61] Maxwell, E. H. and Braly, B. V., "Incomplete tooth fracture. Prediction and prevention," *J. Calif. Dent. Assoc.* **5**(6), 51–55 (1977).

[62] Abou-Rass, M., "Crack lines: the precursors of tooth fractures - their diagnosis and treatment," *Quintessence Int. Dent. Dig.* **14**(4), 437–447 (1983).

[63] Luebke, R. G., "Vertical crown-root fractures in posterior teeth," *Dent. Clin. North Am.* **28**(4), 883–894 (1984).

[64] Ellis, S. G., “Incomplete tooth fracture–proposal for a new definition,” *Br. Dent. J.* **190**(8), 424–428 (2001).

[65] Xu, H. H. K., Kelly, J. R., Jahanmir, S., Thompson, V. P., and Rekow, E. D., “Enamel subsurface damage due to tooth preparation with diamonds,” *J. Dent. Res.* **76**(10), 1698–1706 (1997).

[66] Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., “Enamel cracks evaluation – A method to predict tooth surface damage during the debonding,” *Dent. Mater. J.* **34**(6), 828–834 (2015).

[67] Lloyd, B. A., McGinley, M. B., and Brown, W. S., “Thermal stress in teeth,” *J. Dent. Res.* **57**(4), 571–582 (1978).

[68] Sorel, O., El Alam, R., Chagneau, F., and Cathelineau, G., “Comparison of bond strength between simple foil mesh and laser-structured base retention brackets,” *Am. J. Orthod. Dentofacial Orthop.* **122**(3), 260–266 (2002).

[69] Reynolds, I. R., “A review of direct orthodontic bonding,” *Br. J. Orthod.* **2**(3), 171–178 (1975).

[70] Eliades, T. and Brantley, W. A., “The inappropriateness of conventional orthodontic bond strength assessment protocols,” *Eur. J. Orthod.* **22**(1), 13–23 (2000).

[71] Finnema, K. J., Ozcan, M., Post, W. J., Ren, Y., and Dijkstra, P. U., “*In-vitro* orthodontic bond strength testing: a systematic review and meta-analysis,” *Am. J. Orthod. Dentofacial Orthop.* **137**(5), 615–622.e3 (2010).

[72] Bowen, R. L. and Rodriguez, M. S., “Tensile strength and modulus of elasticity of tooth structure and several restorative materials,” *J. Am. Dent. Assoc.* **64**, 378–387 (1962).

[73] Elekdag-Turk, S., Isci, D., Ozkalayci, N., and Turk, T., “Debonding characteristics of a polymer mesh base ceramic bracket bonded with two different conditioning methods,” *Eur. J. Orthod.* **31**(1), 84–89 (2009).

[74] Arici, S. and Minors, C., “The force levels required to mechanically debond ceramic brackets: an *in vitro* comparative study,” *Eur. J. Orthod.* **22**(3), 327–334 (2000).

[75] Üsümez, S., Orhan, M., and Üsümez, A., “Laser etching of enamel for direct bonding with an Er,Cr:YSGG hydrokinetic laser system,” *Am. J. Orthod. Dentofacial Orthop.* **122**(6), 649–656 (2002).

[76] Lamper, T., Ilie, N., Huth, K. C., Rudzki, I., Wichelhaus, A., and Paschos, E., “Self-etch adhesives for the bonding of orthodontic brackets: faster, stronger, safer?,” *Clin. Oral Investig.* **18**(1), 313–319 (2014).

[77] Kim, Y. K., Park, H. S., Kim, K. H., and Kwon, T. Y., “Effect of adhesive resin flexibility on enamel fracture during metal bracket debonding: an *ex vivo* study,” *Eur. J. Orthod.* **37**(5), 550–555 (2015).

[78] Jena, A. K., Duggal, R., and Mehrotra, A. K., “Physical properties and clinical characteristics of ceramic brackets: a comprehensive review,” *Trends Biomater. Artif. Organs* **20**(2), 101–115 (2007).

[79] Retief, D. H., “Failure at the dental adhesive–etched enamel interface,” *J. Oral Rehabil.* **1**(3), 265–284 (1974).

[80] Alassaad SS., “Approaches to managing asymptomatic enamel and dentin cracks,” *Gen. Dent.* **62**(6), 58–62 (2014).

[81] Walker, B. N., Makinson, O. F., and Peters, M. C. R. B., "Enamel cracks. The role of enamel lamellae in caries initiation," *Aust. Dent. J.* **43**(2), 110–116 (1998).

[82] Agar, J. R. and Weller, R. N., "Occlusal adjustment for initial treatment and prevention of the cracked tooth syndrome," *J. Prosthet. Dent.* **60**(2), 145–147 (1988).

[83] Krell, K. V. and Rivera, E. M., "A six year evaluation of cracked teeth diagnosed with reversible pulpitis: treatment and prognosis," *J. Endod.* **33**(12), 1405–1407 (2007).

[84] Pitts, D. L. and Natkin, E., "Diagnosis and treatment of vertical root fractures," *J. Endod.* **9**(8), 338–346 (1983).

[85] Bader, J. D., Shugars, D. A., and Martin, J. A., "Risk indicators for posterior tooth fracture," *J. Am. Dent. Assoc.* **135**(7), 883–892 (2004).

[86] Ailor, J. E., "Managing incomplete tooth fractures," *J. Am. Dent. Assoc.* **131**(8), 1168–1174 (2000).

[87] Mamoun, J. S. and Napoletano, D., "Cracked tooth diagnosis and treatment: An alternative paradigm," *Eur. J. Dent.* **9**(2), 293–303 (2015).

[88] Chen, H. Y., Su, M. Z., Chang, H. F. F., Chen, Y. J., Lan, W. H., and Lin, C. P., "Effects of different debonding techniques on the debonding forces and failure modes of ceramic brackets in simulated clinical set-ups," *Am. J. Orthod. Dentofacial Orthop.* **132**(5), 680–686 (2007).

[89] Bishara, S. E. and Trulove, T. S., "Comparisons of different debonding techniques for ceramic brackets: an *in vitro* study. Part I. Background and methods," *Am. J. Orthod. Dentofacial Orthop.* **98**(2), 145–153 (1990).

[90] Bishara, S. E., Fonseca, J. M., and Boyer, D. B., "The use of debonding pliers in the removal of ceramic brackets: force levels and enamel cracks," *Am. J. Orthod. Dentofacial Orthop.* **108**(3), 242–248 (1995).

[91] Schuler, F. S. and van Waes, H., "SEM-evaluation of enamel surfaces after removal of fixed orthodontic appliances," *Am. J. Dent.* **16**(6), 390–394 (2003).

[92] Birnie, D., "Ceramic brackets," *Br. J. Orthod.* **17**(1), 71–74 (1990).

[93] Ahangar Atashi, M. H., Sadr Haghighi, A. H., Nastarin, P., and Ahangar Atashi, S., "Variations in enamel damage after debonding of two different bracket base designs: An *in vitro* study," *J. Dent. Res. Dent. Clin. Dent. Prospects* **12**(1), 56–62 (2018).

[94] Ahangar Atashi, M. H. and Kachoei, M., "Does mechanical locking-base ceramic brackets reduce cracks at debonding?," *J. Clin. Exp. Dent.* **4**(5), e266–270 (2012).

[95] Despain, R. R., Lloyd, B. A., and Brown, W. S., "Scanning electron microscope investigation of cracks in teeth through replication," *J. Am. Dent. Assoc.* **88**(3), 580–584 (1974).

[96] Nguyen, C., Ranjitkar, S., Kaidonis, J. A., and Townsend, G. C., "A qualitative assessment of non-carious cervical lesions in extracted human teeth," *Aust. Dent. J.* **53**(1), 46–51 (2008).

[97] Tantbirojn, D., Pintado, M. R., Versluis, A., Dunn, C., and Delong, R., "Quantitative analysis of tooth surface loss associated with gastroesophageal reflux disease: a longitudinal clinical study," *J. Am. Dent. Assoc.* **143**(3), 278–285 (2012).

[98] MicrobeHunter Microscopy Magazine, "Electron microscopes vs optical (light) microscopes." `http://www.microbehunter.com/electron-microscopes-vs-optical-light-microscopes/` (n.d.). Accessed: 2018-08-07.

[99] Yamada, M. K., Uo, M., Ohkawa, S., Akasaka, T., and Watari, F., "Non-contact surface morphology analysis of CO_2 laser-irradiated teeth by scanning electron microscope and confocal laser scanning microscope," *Mater. Trans.* **45**(4), 1033–1040 (2004).

[100] Imai, K., Shimada, Y., Sadr, A., Sumi, Y., and Tagami, J., "Noninvasive cross-sectional visualization of enamel cracks by optical coherence tomography *in vitro*," *J. Endod.* **38**(9), 1269–1274 (2012).

[101] Passos, V. F. and Santiago, S. L., "Methodologies to analyze the micromorphological alterations of enamel subjected to abrasion/erosion," *Dent.* **4**(9), 255 (2014).

[102] Cross, S. E., Kreth, J., Wali, R. P., Sullivan, R., Shi, W., and Gimzewski, J. K., "Evaluation of bacteria-induced enamel demineralization using optical profilometry," *Dent. Mater.* **25**(12), 1517–1526 (2009).

[103] Hosein, I., Sherriff, M., and Ireland, A. J., "Enamel loss during bonding, debonding, and cleanup with use of a self-etching primer," *Am. J. Orthod. Dentofacial Orthop.* **126**(6), 717–724 (2004).

[104] Horiuch, S., Kaneko, K., Mori, H., Kawakami, E., Tsukahara, T., Yamamoto, K., Hamada, K., Asaoka, K., and Tanaka, E., "Enamel bonding of self-etching and phosphoric acid-etching orthodontic adhesives in simulated clinical conditions: debonding force and enamel surface," *Dent. Mater. J.* **28**(4), 419–425 (2009).

[105] Matasa, C. G., "Direct bonding metallic brackets: Where are they heading?," *Am. J. Orthod. Dentofacial Orthop.* **102**(6), 552–560 (1992).

[106] Trakyali, G. and Sinmazisik, G., "A comparative study of shear bond strength of three different bracket bases bonded to porcelain surfaces," *Marmara Dent. J.* **1**(1), 24–28 (2013).

[107] Knox, J., Hubsch, P., Jones, M. L., and Middleton, J., "The influence of bracket base design on the strength of the bracket-cement interface," *J. Orthod.* **27**(3), 249–254 (2000).

[108] Siomka, L. V. and Powers, J. M., "*In vitro* bond strength of treated direct-bonding metal bases," *Am. J. Orthod.* **88**(2), 133–136 (1985).

[109] Sernetz, F. and Binder, F., "Improvement of bond strength of orthodontic titanium brackets and tubes by laser structuring," in [*Proceedings of the 5th International Conference on Joining Ceramics, Glass and Metal*], 82–85, DVS-Berichte, Band 184, Jena, Germany (1997).

[110] Karamouzos, A., Athanasiou, A. E., and Papadopoulos, M. A., "Clinical characteristics and properties of ceramic brackets: A comprehensive review," *Am. J. Orthod. Dentofacial Orthop.* **112**(1), 34–40 (1997).

[111] Ansari, M. Y., Agarwal, D. K., Gupta, A., Bhattacharya, P., Ansar, J., and Bhandari, R., "Shear bond strength of ceramic brackets with different base designs: comparative *in-vitro* study," *J. Clin. Diagn. Res.* **10**(11), ZC64–ZC68 (2016).

[112] Swartz, M. L., "Ceramic brackets," *J. Clin. Orthod.* **22**(2), 82–88 (1988).

[113] Odegaard, J. and Segner, D., "Shear bond strength of metal brackets compared with a new ceramic bracket," *Am. J. Orthod. Dentofacial Orthop.* **94**(3), 201–206 (1988).

[114] Viazis, A. D., Cavanaugh, G., and Bevis, R. R., "Bond strength of ceramic brackets under shear stress: an *in vitro* report," *Am. J. Orthod. Dentofacial Orthop.* **98**(3), 214–221 (1990).

[115] Flores, D. A., Caruso, J. M., Scott, G. E., and Jeiroudi, M. T., "The fracture strength of ceramic brackets: a comparative study," *Angle Orthod.* **60**(4), 269–276 (1990).

[116] Viazis, A. D., DeLong, R., Bevis, R. R., Douglas, W. H., and Speidel, T. M., "Enamel surface abrasion from ceramic orthodontic brackets: a special case report," *Am. J. Orthod. Dentofacial Orthop.* **96**(6), 514–518 (1989).

[117] Viazis, A. D., DeLong, R., Bevis, R. R., Rudney, J. D., and Pintado, M. R., "Enamel abrasion from ceramic orthodontic brackets under an artificial oral environment," *Am. J. Orthod. Dentofacial Orthop.* **98**(2), 103–109 (1990).

[118] Metals and Ceramics Information Center, [*Engineering Property Data on Selected Ceramics Volume III, Single Oxides*], Metals and Ceramics Information Center (1981).

[119] Viazis, A. D., Chabot, K. A., and Kucheria, C. S., "Scanning electron microscope (SEM) evaluation of clinical failures of single crystal ceramic brackets," *Am. J. Orthod. Dentofacial Orthop.* **103**(6), 537–544 (1993).

[120] Scott, G. E. Jr., "Fracture toughness and surface cracks - the key to understanding ceramic brackets," *Angle Orthod.* **58**(1), 5–8 (1988).

[121] Hertzberg, R. W., [*Deformation and fracture mechanics of engineering materials*], John Wiley & Sons, 2nd ed. (1983).

[122] Iwasa, M. and Brandt, R. C., "Fracture toughness of single crystal alumina," in [*Structure and Properties of MgO and* AL_2O_3*. Advances in Ceramics*], Kingery, W. D., ed., **10**, 767–779, Columbus, OH: American Ceramic Society (1986).

[123] Bishara, S. E., Fehr, D. E., and Jakobsen, J. R., "A comparative study of the debonding strengths of different ceramic brackets, enamel conditioners, and adhesives," *Am. J. Orthod. Dentofacial Orthop.* **104**(2), 170–179 (1993).

[124] Wang, W. N., Meng, C. L., and Tarng, T. H., "Bond strength: a comparison between chemical coated and mechanical interlock bases of ceramic and metal brackets," *Am. J. Orthod. Dentofacial Orthop.* **111**(4), 374–381 (1997).

[125] Bishara, S. E., Olsen, M. E., and Von Wald, L., "Evaluation of debonding characteristics of a new collapsible ceramic bracket," *Am. J. Orthod. Dentofacial Orthop.* **112**(5), 552–559 (1997).

[126] Eliades, T., Viazis, A. D., and Lekka, M., "Failure mode analysis of ceramic brackets bonded to enamel," *Am. J. Orthod. Dentofacial Orthop.* **104**(1), 21–26 (1993).

[127] Bishara, S. E., Olsen, M. E., VonWald, L., and Jakobsen, J. R., "Comparison of the debonding characteristics of two innovative ceramic bracket designs," *Am. J. Orthod. Dentofacial Orthop.* **116**(1), 86–92 (1999).

[128] Ormco, "Damon Clear Debonding Demo." `https://youtu.be/ewlnxBFShbc` (2010-09-29). Accessed: 2018-08-27.

[129] American Orthodontics, "Radiance Plus Cosmetic Bracket Debonding." `https://youtu.be/xreHQ8i-Ztc` (2017-04-24). Accessed: 2018-08-27.

[130] 3M Orthodontics, "Debonding Clarity ADVANCED brackets off the archwire." http://3mortholearning.com/member/classroom.asp?x_classID=1175 (2012-03-13). Accessed: 2018-08-27.

[131] Eliades, G., "Clinical relevance of the formulation and testing of dentine bonding systems," *J. Dent.* **22**(2), 73–81 (1994).

[132] Bishara, S. E. and Trulove, T. S., "Comparisons of different debonding techniques for ceramic brackets: an *in vitro* study. Part II. Findings and clinical implications," *Am. J. Orthod. Dentofacial Orthop.* **98**(3), 263–273 (1990).

[133] Strobl, K., Bahns, T. L., Willham, L., Bishara, S. E., and Stwalley, W. C., "Laser-aided debonding of orthodontic ceramic brackets," *Am. J. Orthod. Dentofacial Orthop.* **101**(2), 152–158 (1992).

[134] Dovgan, J. S., Walton, R. E., and Bishara, S. E., "Electrothermal debracketing: patient acceptance and effects on the dental pulp," *Am. J. Orthod. Dentofacial Orthop.* **108**(3), 249–255 (1995).

[135] Jost-Brinkmann, P. G., Stein, H., Miethke, R. R., and Nakata, M., "Histologic investigation of the human pulp after thermodebonding of metal and ceramic brackets," *Am. J. Orthod. Dentofacial Orthop.* **102**(5), 410–417 (1992).

[136] Oztoprak, M. O., Nalbantgil, D., Erdem, A. S., Tozlu, M., and Arun, T., "Debonding of ceramic brackets by a new scanning laser method," *Am. J. Orthod. Dentofacial Orthop.* **138**(2), 195–200 (2010).

[137] Faria-Júnior, E. M., Guiraldo, R. D., Berger, S. B., Correr, A. B., Correr-Sobrinho, L., Contreras, E. F. R., and Lopes, M. B., "*In-vivo* evaluation of the surface roughness and morphology of enamel after bracket removal and polishing by different techniques," *Am. J. Orthod. Dentofacial Orthop.* **147**(3), 324–329 (2015).

[138] Zarrinnia, K., Eid, N. M., and Kehoe, M. J., "The effect of different debonding techniques on the enamel surface: an *in vitro* qualitative study," *Am. J. Orthod. Dentofacial Orthop.* **108**(3), 284–293 (1995).

[139] Brudevold, F., Gardner, D. E., and Smith, F. A., "The distribution of fluoride in human enamel," *J. Dent. Res.* **35**(3), 420–429 (1956).

[140] Coups-Smith, K. S., Rossouw, P. E., and Titley, K. C., "Glass ionomer cements as luting agents for orthodontic brackets," *Angle Orthod.* **73**(4), 436–444 (2003).

[141] van Waes, H., Matter, T., and Krejci, I., "Three-dimensional measurement of enamel loss caused by bonding and debonding of orthodontic brackets," *Am. J. Orthod. Dentofacial Orthop.* **112**(6), 666–669 (1997).

[142] Tüfekci, E., Merrill, T. E., Pintado, M. R., Beyer, J. P., and Brantley, W. A., "Enamel loss associated with orthodontic adhesive removal on teeth with white spot lesions: an *in vitro* study," *Am. J. Orthod. Dentofacial Orthop.* **125**(6), 733–739 (2004).

[143] Bajaj, D., Sundaram, N., Nazari, A., and Arola, D., "Age, dehydration and fatigue crack growth in dentin," *Biomaterials* **27**(11), 2507–2517 (2006).

[144] Nazari, A., Bajaj, D., Zhang, D., Romberg, E., and Arola, D., "Aging and the reduction in fracture toughness of human dentin," *J. Mech. Behav. Biomed. Mater.* **2**(5), 550–559 (2009).

[145] Festing, M. F. W. and Altman, D. G., "Guidelines for the design and statistical analysis of experiments using laboratory animals," *ILAR J.* **43**(4), 244–258 (2002).

[146] Charan, J. and Kantharia, N. D., "How to calculate sample size in animal studies?," *J. Pharmacol. Pharmacother.* **4**(4), 303–306 (2013).

[147] Statistics How To, "Z Alpha/2 (za/2): What is it, How to Find it." `http://www.statisticshowto.com/z-alpha2-za2/` (2014-11-26). Accessed: 2018-09-03.

[148] Dhulkhed, V. K., Dhorigol, M. G., Mane, R., Gogate, V., and Dhulkhed, P., "Basic statistical concepts for sample size estimation," *Indian J. Anaesth.* **52**(6), 788–793 (2008).

[149] Camden County College, "List of abbreviations and standard notation used in statistics." `https://www.coursehero.com/file/21426192/notation-statistics-complete-LIST/` (n.d.). Accessed: 2019-01-27.

[150] ISO Central Secretary, "Dental materials: testing of adhesion to tooth structure," Standard ISO/TS 11405:2003, International Organization for Standardization, Geneva, CH (2003).

[151] Bland, J. M. and Altman, D. G., "Statistical methods for assessing agreement between two methods of clinical measurement," *Lancet* **327**(8476), 307–310 (1986).

[152] Giavarina, D., "Understanding Bland Altman analysis," *Biochem. Med. (Zagreb)* **25**(2), 141–151 (2015).

[153] percentages.calculators.ro, "Relative percentage change (relative percentage increase or decrease)." `http://percentages.calculators.ro/15-percentage-increase-from-original-number-to-new-value.php` (n.d.). Accessed: 2019-01-27.

[154] Mukaka, M. M., "Statistics corner: A guide to appropriate use of correlation coefficient in medical research," *Malawi Med. J.* **24**(3), 69–71 (2012).

[155] Hinkle, D. E., Wiersma, W., and Jurs, S. G., [*Applied Statistics for the Behavioral Sciences*], Boston, Mass.: Houghton Mifflin, 5th ed. (2002).

[156] Theus, M. and Urbanek, S., [*Interactive Graphics for Data Analysis: Principles and Examples*], London: Chapman & Hall/CRC, 1st ed. (2008).

[157] Zheng, Q., Xu, H., Song, F., Zhang, L., Zhou, X., Shao, Y., and Huang, D., "Spatial distribution of the human enamel fracture toughness with aging," *J. Mech. Behav. Biomed. Mater.* **26**, 148–154 (2013).

[158] Yamamoto, A., Yoshida, T., Tsubota, K., Takamizawa, T., Kurokawa, H., and Miyazaki, M., "Orthodontic bracket bonding: enamel bond strength vs time," *Am. J. Orthod. Dentofacial Orthop.* **130**(4), 435.e1–435.e6 (2006).

[159] Hajrassie, M. K. and Khier, S. E., "In-vivo and in-vitro comparison of bond strengths of orthodontic brackets bonded to enamel and debonded at various times," *Am. J. Orthod. Dentofacial Orthop.* **131**(3), 384–390 (2007).

[160] Trites, B., Foley, T. F., and Banting, D., "Bond strength comparison of 2 self-etching primers over a 3-month storage period," *Am. J. Orthod. Dentofacial Orthop.* **126**(6), 709–716 (2004).

[161] Sena, L. M. F., Barbosa, H. A. M., Caldas, S. G. F. R., Ozcan, M., and Souza, R. O. A., "Effect of different bonding protocols on degree of monomer conversion and bond strength between orthodontic brackets and enamel," *Brazi. Oral Res.* **32**(0), e58 (2018). doi: `10.1590/1807-3107bor-2018.vol32.0058`.

[162] Benazzi, S., Nguyen, H. N., Schulz, D., Grosse, I. R., Gruppioni, G., Hublin, J. J., and Kullmer, O., "The evolutionary paradox of tooth wear: simply destruction or inevitable adaptation?," *PLoS One* **8**(4), e62263 (2013).

[163] Aubry, M., Mafart, B., Donat, B., and Brau, J. J., "Brief communication: Study of noncarious cervical tooth lesions in samples of prehistoric, historic, and modern populations from the south of France," *Am. J. Phys. Anthropol.* **121**(1), 10–14 (2003).

[164] Levrini, L., Di Benedetto, G., and Raspanti, M., "Dental wear: A scanning electron microscope study," *BioMed Res. Int.* **2014**, 1–7 (2014).

[165] Ijbara, M., Wada, K., Tabata, M. J., Wada, J., Inoue, G., and Miyashin, M., "Enamel microcracks induced by simulated occlusal wear in mature, immature, and deciduous teeth," *Biomed Res. Int.* **2018**, 1–9 (2018).

[166] Pickett, K. L., Sadowsky, P. L., Jacobson, A., and Lacefield, W., "Orthodontic *in vivo* bond strength: comparison with *in vitro* results," *Angle Orthod.* **71**(2), 141–148 (2001).

[167] Pottmaier, L. F., Linhares, L. d., Baratieri, L. N., and Vieira, L. C., "Evaluation of the fracture resistance of premolars with extensive and medium cavity preparations restored with direct restoring systems," *Indian J. Dent. Res.* **29**(4), 465–469 (2018).

[168] Samera Singh, D., Singh, D. P., and Nitya, D., "Bruxism: its multiple causes and its effects on dental implants: a review," *J. Oral Health Craniofac. Sci.* **2**, 57–63 (2017).

[169] Zhang, D., Mao, S., Lu, C., Romberg, E., and Arola, D., "Dehydration and the dynamic dimensional changes within dentin and enamel," *Dent. Mater.* **25**(7), 937–945 (2009).

Appendix

Materials and Methods

Example of samples collection and storage is presented in Fig. 1.

Figure 1: Samples collection and storage in specimen tubes containing distilled water.

Bonding materials used are depicted in Fig. 2.

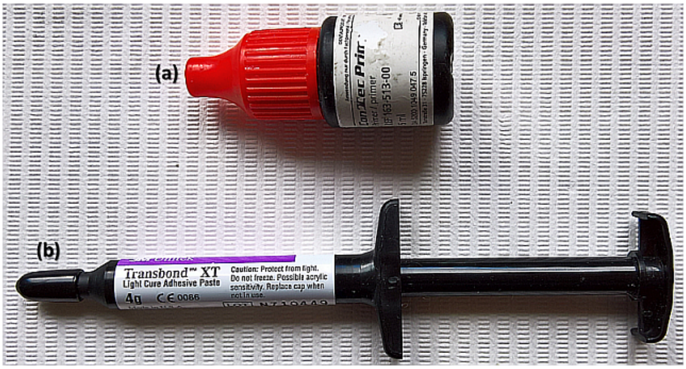

Figure 2: Bonding materials: (a) primer (Contex Primer; Dentaurum) and (b) resin adhesive (Transbond XT; 3M Unitek).

Statistical Analysis

Distribution of data for EMCs length measurements for Group 1 (teeth with EMCs; Subgroup 1, 2, and 3) and Group 2 (teeth without EMCs; Subgroup 1 and 2) from the younger-age group is given in Fig. 3, Fig. 4, Fig. 5, and Fig. 6.

Distribution of data for EMCs length measurements for Group 1 (teeth with EMCs; Subgroup 1, 2, and 3) and Group 2 (teeth without EMCs; Subgroup 1 and 2) from the older-age group is demonstrated in Fig. 7, Fig. 8, Fig. 9, and Fig. 10.

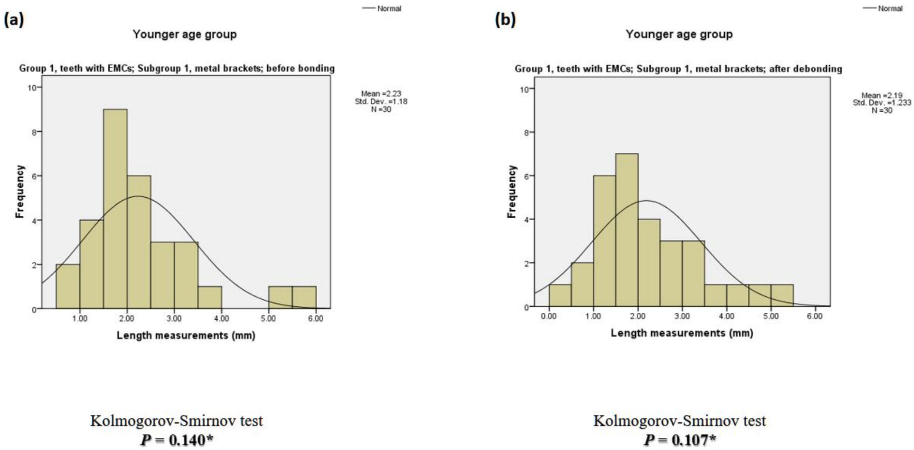

Figure 3: Distribution of data for EMCs length measurements. Two-modal distribution: (a) before bonding, 1st peak – at 1.50 mm*, 2nd peak – at* 2.00 mm *when the mean* 2.23 mm*; (b) after debonding, 1st peak – at* 1.00 mm*, 2nd peak – at* 1.50 mm *when the mean* 2.19 mm*. *P > 0.05, data normally distributed.*

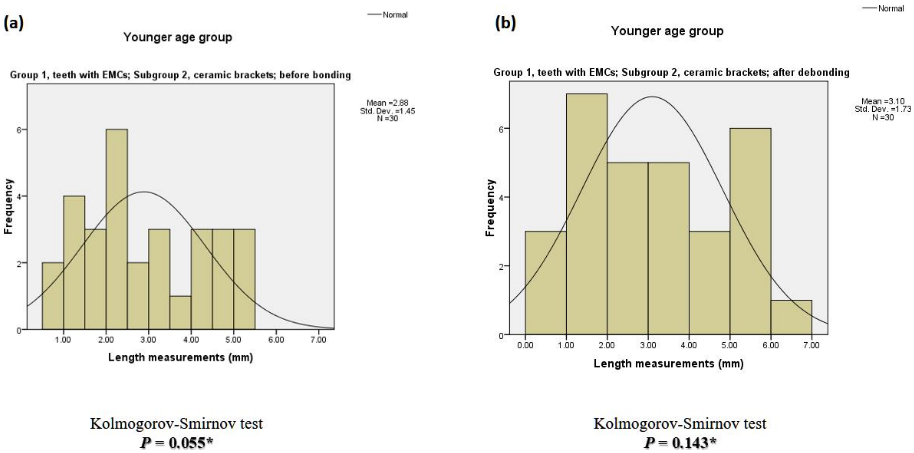

Figure 4: Distribution of data for EMCs length measurements. Two-modal distribution: (a) before bonding, 1st peak – at 1.00 mm*, 2nd peak – at* 2.00 mm *when the mean* 2.88 mm*; (b) after debonding, 1st peak – at* 1.00 mm*, 2nd peak – at* 5.00 mm *when the mean* 1.00 mm*. *P > 0.05, data normally distributed.*

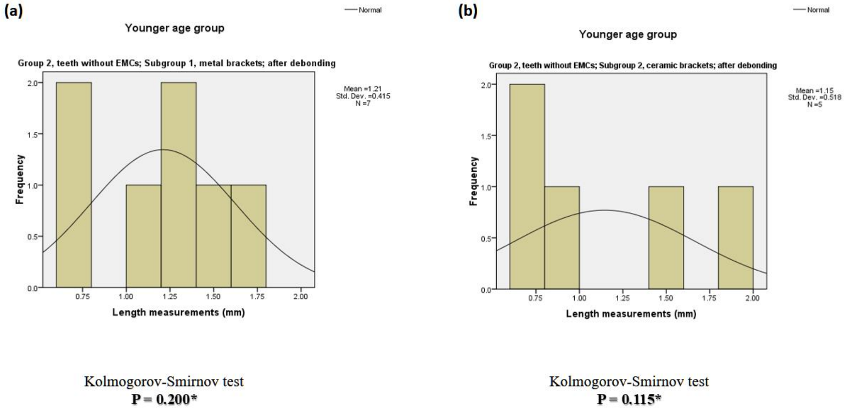

Figure 5: Distribution of data for EMCs length measurements. Two-modal distribution: (a) Subgroup 1, 1st peak – at 0.60 mm*, 2nd peak – at* 1.20 mm *when the mean* 1.21 mm*. One-modal distribution: (b) Subgroup 2, peak – at* 0.60 mm *when the mean* 1.15 mm*. *P > 0.05, data normally distributed.*

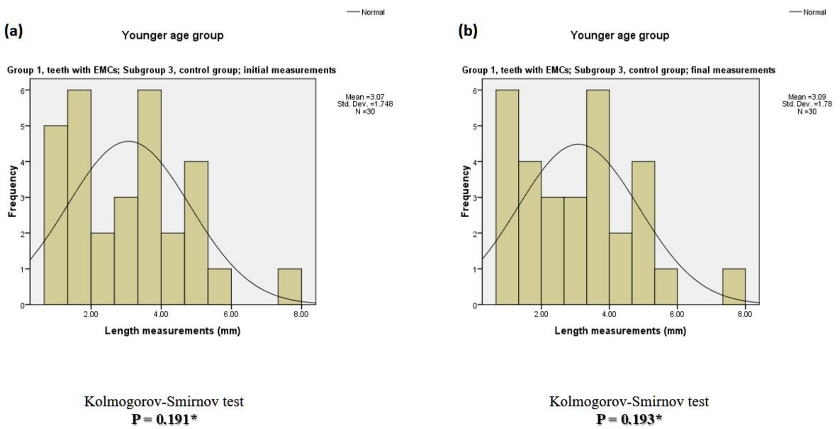

Figure 6: Distribution of data for EMCs length measurements. Four-modal distribution: (a) initial measurements, 1st peak – at 0.75 mm*, 2nd peak – at* 1.25 mm*, 3rd peak – at* 3.25 mm*, 4rd peak – at* 4.75 mm *when the mean* 3.07 mm*; three-modal distribution: (b) final measurements, 1st peak – at* 0.75 mm*, 2nd peak – at* 3.25 mm*, 3rd peak – at* 4.75 mm *when the mean* 3.09 mm*. *P > 0.05, data normally distributed.*

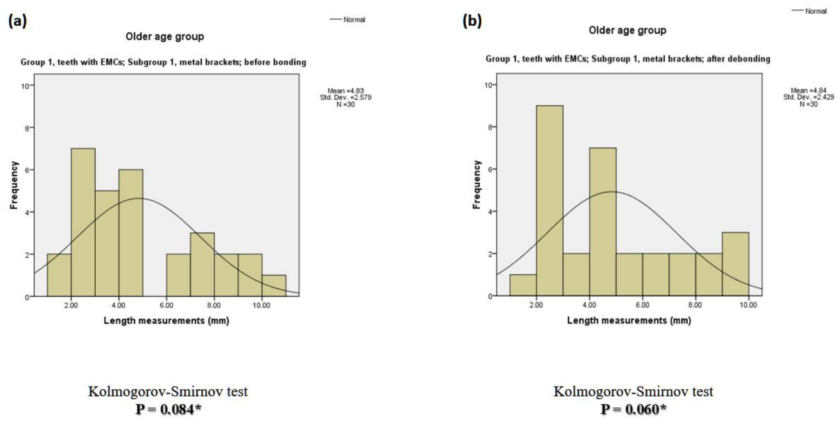

Figure 7: Distribution of data for EMCs length measurements. Three-modal distribution: (a) before bonding, 1st peak – at 2.00 mm*, 2nd peak – at* 3.00 mm*, 3rd peak – at* 4.00 mm *when the mean* 4.83 mm*; (b) after debonding, 1st peak – at* 2.00 mm*, 2nd peak – at* 4.00 mm*, 3rd peak – at* 9.00 mm *when the mean* 4.84 mm*. *P > 0.05, data normally distributed.*

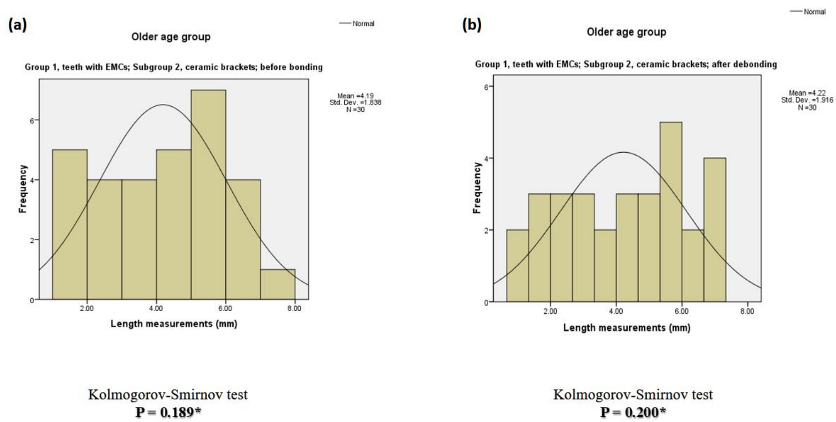

Figure 8: Distribution of data for EMCs length measurements. Two-modal distribution: (a) before bonding, 1st peak – at 1.00 mm*, 2nd peak – at* 5.00 mm *when the mean* 4.19 mm*; (b) after debonding, 1st peak – at* 5.25 mm*, 2nd peak – at* 6.75 mm *when the mean* 4.22 mm*. *P > 0.05, data normally distributed.*

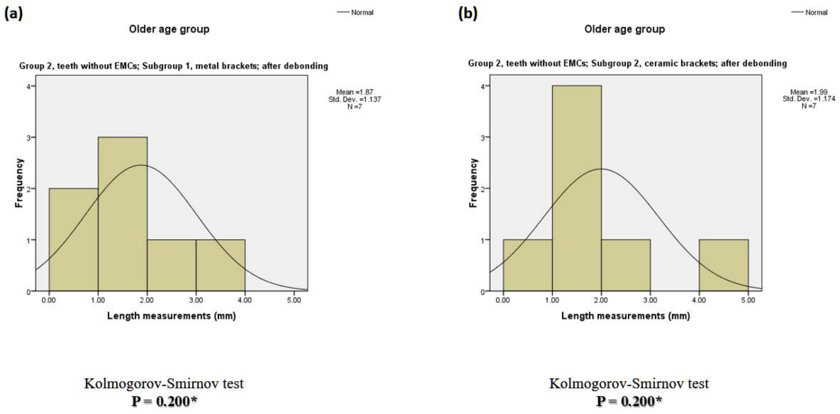

Figure 9: Distribution of data for EMCs length measurements. Two-modal distribution: (a) Subgroup 1, 1st peak – at 0.20 mm*, 2nd peak – at* 1.00 mm *when the mean* 1.87 mm*; (b) Subgroup 2, 1st peak – at* 1.00 mm*, 2nd peak – at* 4.00 mm *when the mean* 1.99 mm*. *P > 0.05, data normally distributed.*

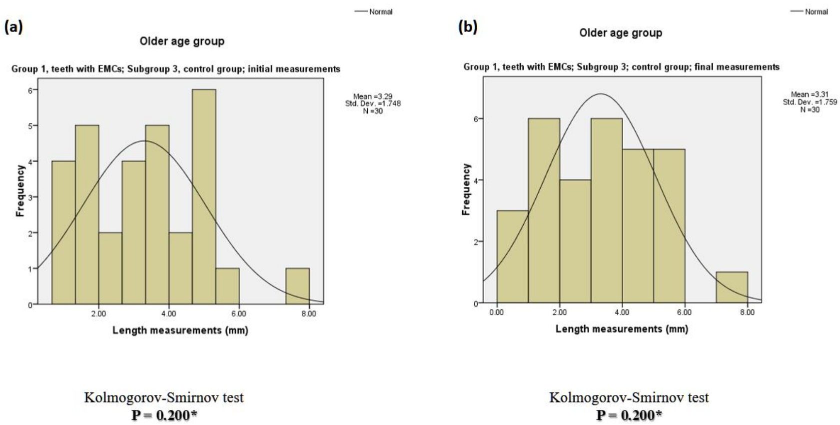

Figure 10: Distribution of data for EMCs length measurements. Four-modal distribution: (a) initial measurements, 1st peak – at 0.75 mm*, 2nd peak – at* 1.25 mm*, 3rd peak – at* 3.25 mm*, 4rd peak – at* 4.75 mm *when the mean* 3.29 mm*; three-modal distribution: (b) final measurements, 1st peak – at* 0.125 mm*, 2nd peak – at* 1.00 mm*, 3rd peak – at* 5.00 mm *when the mean* 3.31 mm*. *P > 0.05, data normally distributed.*

SANTRAUKA

Sutrumpinimai

3D	Trimačiai (angl. *three-dimensional*)
EMC	Emalio mikroįtrūkimas (angl. *enamel microcrack*)
h	Aukštis
JAV	Jungtinės Amerikos Valstijos
KOP	Konfokalinė optinė profilometrija
l	Ilgis
MA	Matavimo sritis (angl. *measurement area*)
n	Stebėjimų skaičius arba imties dydis
OKT	Optinė koherentinė tomografija
OR	Šansų santykis (angl. *odds ratio*)
P_{EMC}	Daugiau pastebimas emalio mikroįtrūkimas (angl. *pronounced enamel microcrack*)
PI	Pasikliautinasis intervalas
r	Pirsono tiesinės koreliacijos koeficientas
rho	Spirmeno ranginės koreliacijos koeficientas
SN	Standartinis nuokrypis
SEM	Skenavimo elektronų mikroskopija/mikroskopas
W_{EMC}	Mažiau pastebimas emalio mikroįtrūkimas (angl. *weak enamel microcrack*)
x	Matavimo žingsnis
$\bar{x}$	Imties vidurkis
$\tilde{x}$	Mediana
$\hat{x}$	Moda
χ^2	Chi kvadratu kriterijus

Įvadas

Tiriamoji problema ir jos aktualumas

Aukštos kvalifikacijos gydytojų ortodontų ir sparčiai tobulėjančių technologijų dėka pasiekti ortodontinio gydymo rezultatai (t.y. atkurta taisyklinga dantų padėtis bei sukandimas) džiugina tiek pacientą, tiek ir gydytoją ortodontą. Net ir esant sudėtingai klinikinei situacijai besikreipiančiam žmogui galima pasiūlyti keletą alternatyvių gydymo metodų. Šiandien vis daugiau klausimų pacientams kelia galimi nepageidaujami pokyčiai danties emalio struktūroje ortodontinio gydymo, ypač breketų nuėmimo procedūros metu. [1] Keletas atliktų tyrimų atskleidė, kad breketų nuėmimas sąlygoja negrįžtamus emalio pakitimus nepriklausomai nuo to, kokia breketų nuėmimo ir klijavimo medžiagos likučių pašalinimo metodika naudojama. [2–6] Dėl breketų nuėmimo procedūros metu sukuriamų jėgų gali formuotis emalio mikroįtrūkimai (EMCs), viena iš dantų pažeidimo formų, ir įvykti tam tikrų EMCs charakteristikų morfologiniai pokyčiai. [4, 7–10] EMCs, pakankamai dažnai pastebimi tiek paciento, tiek ir gydytojo ortodonto, gali suardyti danties struktūros vientisumą, sąlygoti dėmių ir apnašo kaupimąsi ant šiurkštaus įskilusio paviršiaus, taip padidindami ėduonies pažeidimų atsiradimo riziką ir pablogindami dantų estetinį vaizdą. [4, 11–13] Be to, jau buvo iškeltas klausimas dėl EMCs įtakos dantų jautrumui atliekant breketų nuėmimo procedūrą. [4, 14]

Kadangi ortodontinis gydymas balansuoja tarp dantų padėčių bei sukandimo anomalijų korekcijos ir estetinio tobulumo ribos, dėl medicinos etikos principų yra svarbu, kad būtų išlaikytas aiškus naudos ir žalos santykis. [4] Šio pagrindinio principo suvokimas sudarė sąlygas mokslinių publikacijų, tiriančių breketų nuėmimo poveikį EMCs, paskelbimui. [4] Per pastaruosius du dešimtmečius publikuota daug tyrimų, analizuojančių temas pradedant EMCs dažnio pasiskirstymu, [7, 15, 16] skaičiaus ir ilgio padidėjimu, [17] dažnio bei matomumo pokyčiais [18] ir baigiant konkrečių EMCs charakteristikų įvertinimu (pvz. skaičiaus, krypties, ilgio). [4, 8–10, 19–21] Tačiau dėmesys nebuvo atkreiptas į EMCs pločio parametrą, kuris geriausiai apibūdina emalio pažeidimo mastą. Nors yra žinoma apie amžinius emalio struktūros ir jo mechaninių savybių pokyčius, į šį klausimą taip pat nebuvo atsižvelgta atliekant EMCs tyrimus. [22,23]

Dėl nuolatinio technologijų progreso literatūroje buvo pristatyti šie metodai: transiliuminacija, ultragarsas, optinė koherentinė tomografija (OKT), padedantys surasti danties paviršiuje esančius EMCs. [4,15,18,24–27] Įrodyta, kad tam tikri laboratoriniai metodai (skenavimo elektronų mikroskopija (SEM), stereomikroskopija, konfokalinė optinė profilometrija (KOP), trimačiai (3D) skenavimo metodai) tinkami ne tik EMCs vizualizacijai, bet ir tūrinio emalio netekimo, pašalinto emalio gylio, taškinių ar linijinių EMCs parametrų matavimui. [1,2,4,8–10,17,24–26,28] Vis dėlto iki šiol nėra sukurtas metodas, sudarantis galimybę laboratorinėmis sąlygomis tiksliai nustatyti tą patį EMCs prieš ir po breketų nuėmimo procedūros ir tiesiogiai išmatuoti jo kiekybines charakteristikas.

Šiais laikais pacientai kelia aukštus estetinius reikalavimus ir daugiau dėmesio kreipia į galimą emalio pažeidimą, kuris pasireiškia EMCs pavidalu po breketų nuėmimo. [7,8,29] Ortodontinio gydymo pradžioje pacientai dažnai pastebi emalio paviršiaus nelygumus ir EMCs, todėl yra keliami klausimai gydytojui ortodontui, ar galima klijuoti breketus ant tokių dantų. [29] Dėl fizikinių keramikos savybių, tokių kaip kietumas, didelis ryšio stipris, trapumas ar mažas atsparumas lūžiams, keramikinių breketų naudojimas sąlygoja dar didesnį susirūpinimą. Literatūros šaltiniuose pateikti duomenys apie negrįžtamus emalio paviršiaus pažeidimus šio tipo breketų nuėmimo metu. [18,29–31] Taigi, augant pacientų informuotumui ir esant sudėtingai EMCs dokumentacijai, svarbu išsiaiškinti, koks yra metalinių ir keramikinių breketų nuėmimo poveikis daugiau pastebimiems EMCs ir tiems, kurie nėra matomi plika akimi tiesioginės apžiūros metu, o stebimi naudojant skenavimo elektronų mikroskopą (SEM). [29]

Darbo tikslas

Įvertinti ir palyginti kokybines ir kiekybines emalio mikroįtrūkimų charakteristikas prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio ir vyresnio amžiaus grupėse.

Darbo uždaviniai

I Sukurti metodą tiesioginiam kiekybiniam individualaus EMC įvertinimui naudojant SEM prieš metalinių bei keramikinių breketų nuėmimą ir po jo.

II Įvertinti ir palyginti EMCs matomumą, kryptį, lokaciją, ilgį ir plotį prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio amžiaus grupėje.

III Įvertinti ir palyginti EMCs matomumą, kryptį, lokaciją, ilgį ir plotį prieš metalinių bei keramikinių breketų nuėmimą ir po jo vyresnio amžiaus grupėje.

IV Palyginti EMCs ilgį ir plotį prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio ir vyresnio amžiaus grupėse.

V Nustatyti, ar yra koreliacija tarp pirminių EMCs ilgio ir pločio matmenų ir jų didėjimo breketų nuėmimo metu tikimybės.

VI Įvertinti ir palyginti skirtingo matomumo (t.y. daugiau pastebimų ir mažiau pastebimų) EMCs charakteristikas (ilgį ir plotį) prieš metalinių bei keramikinių breketų nuėmimą ir po jo.

VII Apibrėžti, ar mažiau pastebimi EMCs gali progresuoti į daugiau pastebimus po breketų nuėmimo; nustatyti, ar vien tik EMCs matomumas turi prognozinę vertę.

VIII Nustatyti, ar apie negrįžtamus danties struktūros pokyčius galima prognozuoti pagal tam tikrus EMCs parametrus, amžiaus grupę ir breketo tipą ortodontinio gydymo pradžioje.

Mokslinis naujumas ir aktualumas

Šio tyrimo išvados papildys literatūroje paskelbtą medžiagą apie kokybinių ir kiekybinių EMCs charakteristikų pokyčius metalinių ir keramikinių breketų nuėmimo metu skirtingo amžiaus grupėse. Rezultatai padės atskleisti, ar ortodontinis gydymas breketų sistemomis gali sąlygoti didesnį emalio pažeidimą dantims, priklausantiems vyresnio amžiaus grupei lyginant su jaunesnio amžiaus grupe. Turimi duomenys suteiks gydytojams ortodontams informacijos apie tai, ar keramikinių breketų naudojimas vyresnio amžiaus grupėje turėtų būti laikomas kontraindikacija dėl didesnės emalio pažeidimo rizikos breketų nuėmimo procedūros metu. Detalus EMC pločio parametro tyrimas kakleliniame, viduriniame ir okliuziniame trečdaliuose leis gydytojams lengviau identifikuoti tas danties vestibulinio paviršiaus sritis, kuriose yra didesnė EMCs formavimosi tikimybė. Taigi į šias sritis turėtų būti atkreipiamas dėmesys breketų nuėmimo metu.

Dantų, turinčių daugiau pastebimus EMCs prieš breketų klijavimo procedūrą, tyrimas suteiks žinių tiek gydytojams ortodontams tiek ir pacientams apie tai, ar tokie dantys yra labiau linkę į emalio paviršiaus pažeidimą po breketų nuėmimo. EMCs parametrų analizei naudojamos metodikos ir gautų duomenų apie kiekybines charakteristikas dėka, tikėtina, kad EMCs matomumo priežastys taip pat galės būti atskleistos.

Gauti rezultatai padės nustatyti, ar apie negrįžtamus danties struktūros pokyčius galima prognozuoti pagal tam tikrus EMCs parametrus, amžiaus grupę ir breketo tipą ortodontinio gydymo pradžioje. Taigi, būtų sukurtas EMCs įvertinimo protokolas, kuris galėtų būti įtrauktas į standartinį klinikinį paciento ištyrimą.

Galiausiai, sukurta inovatyvi EMCs tyrimo metodika naudojant SEM ir išvestas formules padės tiksliai nustatyti ir išmatuoti mikrometrų skiriamąja geba tą patį EMC prieš ir po metalinių bei keramikinių breketų nuėmimo dantims, priklausantiems jaunesnio ir vyresnio amžiaus grupėms. Tai galėtų būti panaudojama rengiant EMCs *in vivo* analizės strategijas, pvz. sukuriant optinio pluošto mikroskopą klinikiniam EMCs parametrų matavimui ar kitų emalio struktūros defektų įvertinimui, tokiu būdu padedant diagnozuoti ir planuoti gydymą dantų, turinčių emalio pažeidimus.

Tyrimo medžiaga ir metodai

Tyrimas sudarytas iš trijų dalių. Pirmojoje dalyje *in vitro* matuojamos EMCs charakteristikos prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio ir vyresnio amžiaus grupėse. Antrojoje - atliekama EMCs parametrų, amžiaus grupės ir breketo tipo poveikio analizė emalio pažeidimams formuotis breketų nuėmimo metu. Trečioji dalis skirta daugiau pastebimų ir mažiau pastebimų EMCs charakteristikų tyrimui prieš metalinių bei keramikinių breketų nuėmimą ir po jo. Pirminiai dantų įtraukimo kriterijai, bandinių ruošimas, emalio paviršiaus tyrimas, breketų klijavimo bei nuėmimo procedūros identiškos. Toliau pateikiamas tik pirmosios tyrimo dalies metodikos aprašymas.

Emalio mikroįtrūkimų charakteristikų prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio ir vyresnio amžiaus grupėse *in vitro* matavimas

Dantų atranka ir bandinių ruošimas

Į tyrimą buvo įtraukta 360 pašalintų intaktinių viršutinio žandikaulio prieškrūminių dantų, kurie atitiko pirminius ir antrinius dantų įtraukimo kriterijus. Imties dydžio apskaičiavimui atlikta galios analizė. [145] Šimtas aštuoniasdešimt dantų pašalinta jaunesnio amžiaus (amžiaus riba, 18-34 metai, vidurkis ($\bar{x}$) = 27.99±5.19 metai, mediana ($\tilde{x}$) = 29 metai, moda ($\hat{x}$) = 34 metai) ir 180 dantų - vyresnio amžiaus pacientams (amžiaus riba, 35-54 metai, $\bar{x}$ = 42.36±7.05 metai, $\tilde{x}$ = 40 metų, $\hat{x}$ = 35 metai). [23, 143, 144, 149] Išrauti dantys buvo dezinfekuojami 0.5 % chloramino-T tirpale ir laikomi distiliuotame vandenyje specialiuose konteineriuose iki ištyrimo pradžios (Tarptautinės standartizacijos organizacijos rekomendacijos, ISO/TS 11405; 2003). [150] Prieš pat pradedant tolimesnį tyrimą visi bandiniai buvo įtvirtinti standartizuotų parametrų silikono matricoje taip, kad linija, einanti per labiausiai išgaubtą vestibulinio paviršiaus tašką, būtų lygiagreti grindims.

Pradinis emalio paviršiaus tyrimas naudojant skenavimo elektronų mikroskopą

Naudojamas detalaus EMCs įvertinimo metodas yra inovatyvus ir pirmą kartą paskelbtas literatūroje. Visų paruoštų bandinių vestibuliniai paviršiai buvo tiriami naudojant SEM (Hitachi stalinis mikroskopas (TM-1000), Japonija), kuris buvo sujungtas su kompiuteriu vaizdų užfiksavimui. Pirminiam įvertinimui, ar tiriamasis bandinys turi EMCs, pasirinktas x50–100. Jeigu esant tokiam padidinimui EMCs nebuvo stebimi, laikoma, kad šis dantis prieš breketų klijavimą EMCs neturėjo. SEM ir programinės įrangos, skirtos skaitmeninių vaizdų apdorojimui, pagalba buvo atkurtas kiekvieno bandinio vestibulinio paviršiaus vaizdas. Pasirinkti anatominiai orientyrai (t.y. charakteringos danties vestibulinio paviršiaus sritys) sudarė galimybę ne tik tiksliai atkartoti danties paviršių, bet ir identifikuoti tą patį EMCs prieš ir po breketų nuėmimo procedūros.

Remiantis SEM mikrografomis buvo išmatuotas kiekvieno tiriamojo bandinio vainiko vertikalus aukštis (h) ir vestibulinis paviršius padalintas į 3 vienodo aukščio zonas: pirma zona - kaklelinis, antra zona - vidurinis ir trečia zona - okliuzinis trečdalis. [8, 11, 18, 91]

Atlikus pradinį emalio paviršiaus ištyrimą, visi bandiniai, tiek priklausantys jaunesnio, tiek ir vyresnio amžiaus grupėms, buvo suskirstyti į dvi tiriamąsias grupes po 90 dantų: Grupė 1 - dantys, turintys EMCs, Grupė 2 - dantys be EMCs. EMC buvimas - tai pagrindinis kriterijus grupuojant dantis. Vėliau naudojant loterijos metodą, bandiniai, priklausantys Grupei 1 ir Grupei 2, atsitiktine tvarka buvo paskirstyti į vieną iš trijų pogrupių: Pogrupis 1 - klijuojami metaliniai breketai, Pogrupis 2 - klijuojami keramikiniai breketai ir Pogrupis 3 - kontrolinė grupė.

Kiekvieno danties detaliai analizuojamas tik vienas EMC. Jeigu bandinys turėjo keletą EMCs, buvo pasirenkamas ilgiausias jų. Visų dantų, priklausančių Pogrupiui 1 ir Pogrupiui 2 (Grupė 1 - dantys su EMCs ir Grupė 2 - dantys be EMCs, tiek jaunesnio, tiek vyresnio amžiaus grupės), įvertintos ir išmatuotos kokybinės bei kiekybinės ilgiausio EMC charakteristikos prieš ir po breketų nuėmimo: matomumas, kryptis, lokacija, ilgis ir plotis.

Remiantis matomumu EMCs buvo klasifikuojami į daugiau pastebimus EMCs (P$_{EMCs}$) - matomi plika akimi tiesioginės apžiūros metu esant įprastam kambario apšvietimui ir mažiau pastebimus (W$_{EMCs}$) - nėra matomi esant įprastam kambario apšvietimui, tačiau stebimi naudojant SEM. [13, 18]

Priklausomai nuo EMC palinkimo danties išilginės ašies atžvilgiu kryptis klasifikuojama į vertikalią ($0-30°$ išilginei danties vainiko ašiai), įstrižą ($31-45°$ išilginei danties vainiko ašiai), horizontalią ($46-90°$ išilginei danties vainiko ašiai) ir įvairią (kai EMCs keičia savo kryptį besitęsdamas vestibuliniu danties paviršiumi). [10]

Lokacija nurodoma kaip kaklelinis, vidurinis ir okliuzinis vestibulinio paviršiaus trečdalis priklausomai nuo EMC padėties. Ilgesni EMCs gali lokalizuotis daugiau negu viename trečdalyje arba tęstis per visą vestibulinį danties paviršių.

Ilgiausio EMC ilgis ir plotis apskaičiuojami remiantis formulėmis.

Dantys, priklausantys kontrolinei grupei, du kartus buvo tiriami SEM tokiu pačiu metodu, tačiau šiems bandiniams nebuvo atliekamos breketų klijavimo ir nuėmimo procedūros. Kontrolinė grupė buvo suformuota norint įvertinti dehidracijos poveikį esamų EMCs charakteristikų pokyčiams ir naujų EMCs formavimuisi. Visų EMCs parametrų matavimus atliko tas pats tyrėjas (I.D.).

Breketų klijavimo ir nuėmimo procedūros

Tyrime naudojami metaliniai (Discovery; Dentaurum, Vokietija) ir keramikiniai breketai (Clarity; 3M Unitek, JAV). Visi bandiniai buvo paruošti laikantis breketų klijavimo procedūros reikalavimų.

Užklijavus breketus bandiniai buvo laikomi 37 °C temperatūros distiliuotame vandenyje 24 h prieš tolimesnį jų tyrimą.

Metalinių breketų nuėmimui naudotos Utility/Weingart (Dentaurum) replės, keramikinių - specialus, šio tipo breketams sukurtas nuėmimo instrumentas (3M Unitek). Nuėmus abiejų tipų breketus klijavimo medžiagų likučiams pašalinti pasirinktas volframo-karbido grąžtas.

Remdamasis nurodytu protokolu visas breketų klijavimo ir nuėmimo procedūras atliko tas pats tyrėjas (I.D.).

Galutinis emalio paviršiaus tyrimas naudojant skenavimo elektronų mikroskopą

Po breketų nuėmimo procedūros visų bandinių vestibuliniai paviršiai buvo pakartotinai tiriami naudojant SEM tokia pačia metodika kaip ir prieš breketų klijavimą. Vertinamos ir matuojamos kokybinės ir kiekybinės to paties EMC charakteristikos: matomumas, kryptis, lokacija, ilgis ir plotis.

Matavimo paklaidų įvertinimas

Matavimo paklaidų įvertinimas atliktas naudojant Blando ir Altmano pasiūlytą metodą. [151, 152] Matavimai pakartoti 60 dantų, pusė jų priklausė jaunesnio ir likusi dalis - vyresnio amžiaus grupei. Nustatytas 100.0 % atitikimas tarp dviejų EMC ilgio matavimų. Skirtumo tarp dviejų EMC pločio matavimų vidurkis -0.02 µm.

Statistinė duomenų analizė

Statistinė duomenų analizė atlikta naudojant duomenų kaupimo ir analizės SPSS 17.0 programinį paketą (JAV). Tolydžių kintamųjų pasiskirstymui pagal normalųjį dėsnį įvertinti taikytas Kolmogorovo-Smirnovo testas bei grafinis kintamųjų pasiskirstymo vaizdavimas histogramomis.

Kai kintamieji buvo pasiskirstę pagal normalųjį dėsnį, jų statistinei analizei taikyta vienfaktorinė dispersinė analizė, porinis Stjudento t kriterijus priklausomoms imtims ir neporinis Stjudento t kriterijus nepriklausomoms imtims. Kitais atvejais taikyti neparametriniai kriterijai: Vilkoksono kriterijus dviejų priklausomų imčių lyginimui ir Mano, Vitnio ir Vilkoksono kriterijus dviejų nepriklausomų imčių lyginimui.

Pagal normalųjį dėsnį pasiskirsčiusiems tolydiems kintamiesiems skaičiuotas Pirsono tiesinės koreliacijos (Pirsono r) koeficientas. Duomenims, kurių skirstiniai neatitiko normaliojo skirstinio kreivės, skaičiuotas Spirmeno ranginės koreliacijos (Spirmeno *rho*) koeficientas.

Prognozei atlikti taikyta binarinė logistinė regresinė analizė. Skirtumai tarp grupių laikyti statistiškai reikšmingais, kai P reikšmė lygi 0.05 arba nepersikloja 95.0 % PI.

Rezultatai

Pristatomi pagrindiniai kiekvienos tyrimo dalies rezultatai.

Emalio mikroįtrūkimų charakteristikų prieš metalinių bei keramikinių breketų nuėmimą ir po jo jaunesnio ir vyresnio amžiaus grupėse *in vitro* matavimas

EMCs matomumo ir krypties charakteristikų pasiskirstymas prieš metalinių ir keramikinių breketų fiksaciją bei po breketų nuėmimo jaunesnio ir vyresnio amžiaus grupėse pateiktas 1 lentelėje.

1 lentelė: EMCs matomumo ir krypties parametrų pasiskirstymas prieš breketų fiksaciją ir po breketų nuėmimo jaunesnio ir vyresnio amžiaus grupėse[a]

EMCs parametrai	**Jaunesnio amžiaus grupė (18-34 metai)**	**Vyresnio amžiaus grupė (35-54 metai)**
MATOMUMAS	**Prieš breketų fiksaciją** 66.7 % P_{EMCs} 33.3 % W_{EMCs} (MB ir KB)	90.0 % P_{EMCs} 10.0 % W_{EMCs} (MB ir KB)
	Po breketų nuėmimo 66.7 % P_{EMCs} 33.3 % W_{EMCs} (MB) 76.7 % P_{EMCs} 23.3 % W_{EMCs} (KB)	93.3 % P_{EMCs} 6.7 % W_{EMCs} (MB ir KB)
KRYPTIS	**Prieš breketų fiksaciją ir po nuėmimo** 73.3 % vertikalūs 16.7 % įstriži 6.7 % įvairios krypties 3.3 % horizontalūs (MB) 93.3 % vertikalūs 6.7 % įvairios krypties (KB)	96.7 % vertikalūs 3.3 % įvairios krypties (MB) 80.0 % vertikalūs 10.0 % įstriži 10.0 % įvairios krypties (KB)

[a] MB, metaliniai breketai; KB, keramikiniai breketai.

EMCs lokacijos, pločio ir ilgio charakteristikų pokyčiai po metalinių ir keramikinių breketų nuėmimo jaunesnio ir vyresnio amžiaus grupėse pateikti 2 lentelėje.

Nustatytas statistiškai reikšmingas EMCs pločių skirtumas ($P = 0.000$) tarp dantų, priklausančių jaunesnio ir vyresnio amžiaus grupėms (tiek prieš, tiek po abiejų tipų breketų nuėmimo). Didesni vidutiniai rangai apskaičiuoti vyresnio amžiaus grupėje. Vyresnio amžiaus grupei priklausantiems dantims taip pat būdingos didesnės vidutinės ilgio vertės (tiek prieš, tiek ir po metalinių bei keramikinių breketų nuėmimo).

Naujai susiformavę EMCs yra siauresni lyginant su prieš breketų fiksaciją buvusių EMCs pločiais po nuėmimo. Mažesni vidutiniai rangai apskaičiuoti po metalinių (jaunesnio amžiaus grupė, $P = 0.010$; vyresnio amžiaus grupė, $P = 0.000$) ir keramikinių breketų nuėmimo (jaunesnio amžiaus grupė, $P = 0.135$; vyresnio amžiaus grupė, $P = 0.000$).

Naujai susiformavusių EMCs bendrasis ilgis mažesnis (jaunesnio amžiaus grupė, 1.7 (metaliniai breketai, $P = 0.044$) - 2.8 kartus (keramikiniai breketai, $P = 0.000$); vyresnio amžiaus grupė, 2.6 (metaliniai breketai, $P = 0.000$) - 3.3 kartus (keramikiniai breketai, $P = 0.000$)) lyginant su prieš breketų fiksaciją buvusių EMCs ilgiais po nuėmimo.

Vyresnio amžiaus grupėje naujai susiformavusių EMCs pločių ir ilgių vertės didesnės lyginant su jaunesnio amžiaus grupe.

Emalio mikroįtrūkimų parametrų, amžiaus grupės ir breketo tipo poveikio analizė emalio pažeidimams formuotis metalinių ir keramikinių breketų nuėmimo metu

Apskaičiuotas penkių skirtingų nepriklausomų kintamųjų (matomumo, krypties, lokacijos, amžiaus grupės ir breketo tipo) poveikis emalio pažeidimams (EMCs ilgio vertės atspindėjo emalio pažeidimą) atsirasti breketų nuėmimo metu (3 lentelė).

Rezultatai atskleidė, kad EMCs matomumas (P_{EMCs} ar W_{EMCs}) ir amžiaus grupė turi statistiškai reikšmingą įtaką emalio pažeidimams formuotis. Dantims, turintiems daugiau pastebimų EMCs, 2.63 kartus padidėjo rizika emalio pažeidimams atsirasti breketų nuėmimo metu. Dantims, priklausantiems vyresnio amžiaus grupei, 3.08 karto padidėjo rizika nepageidaujamiems pokyčiams emalio struktūroje atsirasti breketų nuėmimo procedūros metu.

Nustatyta, jog keramikinio breketo tipas turi didesnę įtaką emalio pažeidimams formuotis, tačiau rezultatas nėra statistiškai reikšmingas. Keramikinių breketų grupėje rizika emalio pažeidimams atsirasti buvo 1.70 karto didesnė

2 lentelė: EMCs parametrų (lokacijos, pločio ir ilgio) pokyčiai po metalinių ir keramikinių breketų nuėmimo jaunesnio ir vyresnio amžiaus grupėse[a]

EMCs parametrai	**Jaunesnio amžiaus grupė (18-34 metai)**	**Vyresnio amžiaus grupė (35-54 metai)**
PLOTIS	Po MB ir KB nuėmimo bendrasis EMCs plotis padidėjo (**$P = 0.000$**).	
LOKACIJA	Didesnis EMCs plotis (didesnės medianos) beveik visose zonose po breketų nuėmimo.	
	Statistiškai reikšmingas skirtumas tarp zonų po abiejų tipų breketų nuėmimo nenustatytas.	Statistiškai reikšmingas skirtumas tarp 1 zonos ir 2 zonos po MB (didesni vidutiniai rangai 1 zonoje, **$P = 0.034$**) bei tarp 1 zonos ir 3 zonos po KB nuėmimo (didesni vidutiniai rangai 3 zonoje, **$P = 0.044$**).
	Platesni EMCs (didesni vidutiniai rangai) prieš ir po KB nuėmimo (**$P = 0.000$**).	Prieš klijavimą platesni EMCs (didesni vidutiniai rangai) MB pogrupyje (**$P = 0.033$**), po nuėmimo skirtumas tarp abiejų tipų breketų statistiškai nereikšmingas ($P = 0.751$).
	Spirmeno $rho = 0.596$, **$P = 0.000$**	Spirmeno $rho = 0.727$, **$P = 0.000$**
ILGIS	Statistiškai nereikšmingi ilgio parametro pokyčiai po MB ir KB nuėmimo.	
	Ilgio skirtumas tarp MB ir KB statistiškai nereikšmingas (tiek prieš, tiek po nuėmimo).	
	Pirsono $r = 0.905$, **$P = 0.000$**	Pirsono $r = 0.969$, **$P = 0.000$**

[a]MB, metaliniai breketai; KB, keramikiniai breketai.
$P = 0.05$, skirtumas tarp grupių statistiškai reikšmingas. Statistiškai reikšmingas skirtumas paryškintas.

3 lentelė: Penkių skirtingų nepriklausomų kintamųjų (matomumo, krypties, lokacijos, amžiaus grupės ir breketo tipo) poveikis emalio pažeidimams atsirasti breketų nuėmimo metu (priklausomas kintamasis - EMCs ilgis). P *reikšmės, šansų santykis (OR) ir* 95.0 % *pasikliautinasis intervalas (PI) apskaičiuoti pritaikius binarinę logistinę regresinę analizę*

Nepriklausomi kintamieji	P	OR*	OR 95.0 % PI Apatinis	OR 95.0 % PI Viršutinis
Matomumas EMC***	0.023	2.633	1.145	6.057
Kryptis EMC**	0.538	0.715	0.245	2.085
Lokacija EMC**	0.189	0.659	0.354	1.228
Amžiaus grupė***	0.000	3.083	1.674	5.679
Breketo tipas****	0.064	1.695	0.970	2.959

*OR reikšmė: 1 = nepriklausomas kintamasis įtakos neturi; < 1 = mažina riziką; > 1 = didina riziką emalio pažeidimams atsirasti breketų nuėmimo metu.
**OR < 1 ir višutiniojo 95.0 % PI reikšmė > 1, kintamasis statistiškai nereikšmingai mažina riziką emalio pažeidimams atsirasti.
***OR > 1 ir apatiniojo 95.0 % PI reikšmė > 1, kintamasis statistiškai reikšmingai didina riziką emalio pažeidimams atsirasti.
****OR > 1 ir apatiniojo 95.0 % PI reikšmė < 1, kintamasis statistiškai nereikšmingai didina riziką emalio pažeidimams atsirasti.

negu metalinių breketų grupėje.

Nustatyta statistiškai nereikšminga EMC krypties ir lokacijos įtaka emalio pažeidimams formuotis. Apskaičiuota, kad padidėjus EMCs palinkimo kampui 1 laipsniu danties išilginės ašies atžvilgiu rizika emalio pažeidimams atsirasti sumažėjo 1.40 karto (0.715 pradinės vertės). Atstumui padidėjus 1 milimetru (t.y. nuo okliuzinio kaklelinio trečdalio link) rizika emalio pažeidimams atsirasti sumažėjo 1.52 karto (0.659 pradinės vertės).

Determinacijos koeficientas (Nagelkerke R^2 = 0.215) atskleidė, kad visų penkių nepriklausomų kintamųjų (EMC matomumo, krypties, lokacijos, amžiaus grupės ir breketo tipo) įtaka emalio pažeidimo apimčiai breketų nuėmimo metu siekė 21.5 %.

Daugiau pastebimų ir mažiau pastebimų emalio mikroįtrūkimų charakteristikų tyrimas prieš metalinių bei keramikinių breketų nuėmimą ir po jo

Didesnės vidutinės bendrojo ilgio ir pločio vertės būdingos daugiau pastebimiems EMCs.

Po breketų nuėmimo vidutinis bendrasis EMCs plotis padidėjo, tačiau išplatėjimo apimtis buvo panaši tiek P_{EMCs} (metaliniai breketai, 0.57 µm, P = 0.005; keramikiniai breketai, 0.30 µm, P = 0.058), tiek W_{EMCs} tarpe (metaliniai breketai, 0.32 µm, $P = 0.067$; keramikiniai breketai, 0.30 µm, $P = 0.095$).

Didesnės vidutinės bendrojo EMCs ilgio ir pločio vertės užfiksuotos prieš ir po keramikinių breketų nuėmimo. Prieš breketų klijavimo procedūrą mažiau pastebimų EMCs progresija į daugiau pastebimus EMCs nustatyta tik keramikinių breketų nuėmimo metu (keturi (26.67 %) W_{EMCs} tapo P_{EMCs}).

Ginamieji teiginiai

I Breketų nuėmimo procedūra turi įtakos EMCs charakteristikų (matomumo, krypties, lokacijos, ilgio ir pločio) pokyčiams ir naujų formavimuisi.

• Dumbryte, I., Linkeviciene, L., Malinauskas, M., Linkevicius, T., Peciuliene, V., and Tikuisis, K., "Evaluation of enamel micro-cracks characteristics after removal of metal brackets in adult patients," *Eur. J. Orthod.* **35**(3), 317-322 (2013).

II Dantys, priklausantys jaunesnio ir vyresnio amžiaus grupėms, pasižymi skirtingais EMCs parametrais (matomumas, kryptis, lokacija, ilgis ir plotis) prieš breketų nuėmimą ir po jo.

• Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Enamel microcracks in terms of orthodontic treatment: A novel method for their detection and evaluation," *Dent. Mater. J.* **36**(4), 438-446 (2017).

III Vien tik EMCs matomumas prieš breketų klijavimo procedūrą turi mažą prognozinę vertę dėl emalio pažeidimų atsiradimo po breketų nuėmimo.

• Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "The prognostic value of visually assessing enamel microcracks: *Do debonding and adhesive removal contribute to their increase?*," *Angle Orthod.* **86**(3), 437-447 (2016).

IV EMCs charakteristikų įvertinimas ortodontinio gydymo pradžioje gali būti naudojamas kaip metodas, galintis numatyti didesnę dantų pažeidimo riziką breketų nuėmimo procedūros metu.

• Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "Enamel cracks evaluation – A method to predict tooth surface damage during the debonding," *Dent. Mater. J.* **34**(6), 828-834 (2015).

Išvados

I Sukurtas metodas naudojant SEM ir išvestas formules sudarė sąlygas to paties EMC (būdingos ilgio ribos, 0.24 –10.15 mm; pločio ribos, 0.25 –35.04 µm) nustatymui prieš breketų nuėmimą ir po jo bei kiekybiniam EMC parametrų (ilgio ir pločio) įvertinimui.

II Dantys, priklausantys vyresnio amžiaus grupei, pasižymėjo didesnėmis vidutinėmis bendrojo EMCs ilgio ir pločio vertėmis prieš metalinių ir keramikinių breketų nuėmimą ir po jo lyginant su jaunesnio amžiaus grupe.

III Abiejose amžiaus grupėse breketų nuėmimo procedūra lėmė EMCs pločio didėjimą, bet nebuvo stebimi statistiškai reikšmingi EMCs matomumo, krypties ir ilgio pokyčiai. Vertinant EMCs lokacijos charakteristiką didžiausias pločio padidėjimas nustatytas okliuziniame ir kakleliniame trečdaliuose.

IV Keramikinių breketų nuėmimas nulėmė platesnius EMCs lyginant su metaliniais breketais dantims, priklausantiems jaunesnio amžiaus grupei. Statistiškai reikšmingas breketo tipo poveikis EMCs pločiui vyresnio amžiaus grupėje ir ilgio parametrui abiejose amžiaus grupėse nepastebėtas.

V Nustatyta teigiama koreliacija (nuo vidutinio stiprumo iki stiprios) tarp EMCs ilgio ir pločio matmenų prieš breketų klijavimą bei jų didėjimo metalinių ir keramikinių breketų nuėmimo metu abiejose amžiaus grupėse.

VI Nors daugeliu atvejų dantys, turintys daugiau pastebimus EMCs, pasižymėjo didesnėmis vidutinėmis bendrojo EMCs ilgio ir pločio vertėmis lyginant su mažiau pastebimais EMCs, tačiau ilgio ir pločio padidėjimo apimtis po breketų nuėmimo tarp šių dviejų grupių buvo panaši.

VII Mažiau pastebimi EMCs gali progresuoti į daugiau pastebimus po breketų nuėmimo, ypač naudojant keramikinius breketus. Vien tik EMCs matomumas prieš breketų klijavimo procedūrą turi mažą prognozinę vertę dėl emalio pažeidimų atsiradimo.

VIII Dantys su EMCs, pasižyminčiais nurodytų charakteristikų deriniu (t.y. daugiau pastebimi EMCs vyresnio amžiaus grupėje, vertikalios ar įstrižos krypties, išsidėstę arčiau okliuzinio paviršiaus) ortodontinio gydymo pradžioje ir ant kurių klijuojami keramikiniai breketai, linkę į didesnę emalio pažeidimo riziką breketų nuėmimo metu iki 21.5 %.

Praktinės rekomendacijos

I Sukurta EMCs tyrimo metodika naudojant SEM ir išvestas formules yra universali ir gali būti taikoma tiek jaunesnio, tiek vyresnio amžiaus grupėms priklausančių dantų, kurių emalis pasižymi skirtingomis mechaninėmis ir optinėmis savybėmis, įvertinimui.

II Abiejose amžiaus grupėse EMCs padidėjimas po metalinių ir keramikinių breketų nuėmimo gali būti laikomas neišvengiama breketų nuėmimo procedūros pasekme, tačiau ne ortodontinio gydymo komplikacija. Apie tai pacientai turėtų būti įspėjami, pvz., žodžiu ir raštu, prieš pradedant ortodontinį gydymą.

III EMCs (t.y. matomumo, krypties, lokacijos parametrų) įvertinimas galėtų būti įtrauktas į standartinį klinikinį paciento ištyrimą. Tokiais atvejais, kai EMCs yra aiškiai matomi prieš breketų klijavimo procedūrą, rekomenduojama šiuos dantis nufotografuoti.

IV Planuojant ortodontinį gydymą ir renkantis breketų sistemą rekomenduojama atsižvelgti į dantų emalio būklę. Svarbu žinoti, kuriems pacientams bus naudingas gydymas tradicinėmis breketų sistemomis, taip pat kuriems asmenims, turintiems EMCs ortodontinio gydymo pradžioje, galima rekomenduoti alternatyvias breketų sistemas, pvz., vidinius breketus, ar gydymo metodus.

V Jeigu atlikus klinikinį ištyrimą pacientui nustatoma didesnė emalio pažeidimo rizika po breketų nuėmimo (t.y. užfiksuojami daugiau pastebimi EMCs, ypač kituose dantyse negu viršutinio žandikaulio centriniai kandžiai ir iltiniai dantys, vertikalios ar įstrižos krypties, išsidėstę arčiau okliuzinio paviršiaus) ir jau prieš gydymą esantys EMCs sukelia didelius estetinius nusiskundimus, pacientui galėtų būti siūlomi alternatyvūs ortodontinio gydymo metodai, pvz., dantų tiesinimas kapomis.

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List of Publications

Publications (Web of Science)

1. Dumbryte, I., Linkeviciene, L., Malinauskas, M., Linkevicius, T., Peciuliene, V., and Tikuisis, K., "Evaluation of enamel micro-cracks characteristics after removal of metal brackets in adult patients," *Eur. J. Orthod.* **35**(3), 317-322 (2013).

2. Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "Enamel cracks evaluation – A method to predict tooth surface damage during the debonding," *Dent. Mater. J.* **34**(6), 828-834 (2015).

3. Dumbryte, I., Jonavicius, T., Linkeviciene, L., Linkevicius, T., Peciuliene, V., and Malinauskas, M., "The prognostic value of visually assessing enamel microcracks: *Do debonding and adhesive removal contribute to their increase?*," *Angle Orthod.* **86**(3), 437-447 (2016).

4. Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Does orthodontic debonding lead to tooth sensitivity? Comparison of teeth with and without visible enamel microcracks," *Am. J. Orthod. Dentofacial Orthop.* **151**(2), 284-291 (2017).

5. Dumbryte, I., Linkeviciene, L., Linkevicius, T., and Malinauskas, M., "Enamel microcracks in terms of orthodontic treatment: A novel method for their detection and evaluation," *Dent. Mater. J.* **36**(4), 438-446 (2017).

6. Dumbryte, I., Vebriene, J., Linkeviciene, L., and Malinauskas, M., "Enamel microcracks in the form of tooth damage during orthodontic debonding: a systematic review and meta-analysis of *in vitro* studies," *Eur. J. Orthod.* **40**(6), 636-648 (2018).

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