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Emulsion-based synthesis of polycaprolactone/bioactive glass 45S5 microparticles for bone regeneration applications

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Supplementary material for this article is available [online](#)

Abstract

Polycaprolactone (PCL) and bioactive glass 45S5 (BG) are widely employed within bone tissue engineering due to their mutually complementary properties: PCL contributes structural integrity and biodegradability, whereas BG imparts osteoinductive properties to the composite. While patient-specific bulk scaffolds can restore structural defects, their fabrication requires individualized imaging, design, and manufacturing, increasing both time and cost. Hydrogels offer better conformity to irregular geometries but often lack sufficient mechanical stability and usually degrade rapidly. Microparticle-based systems represent a shape-adaptive alternative that can conform to complex defect geometries without customization, while maintaining structural integrity. In this study, PCL/BG microparticles (MPs) were fabricated using the emulsion solvent evaporation method and evaluated *in vitro*. The microparticles supported adhesion and spreading, maintaining cell-cell and cell-particle interactions for over a month. Periosteum mesenchymal stem cells cultured on the MPs were responsive to adipogenic and osteogenic induction, as evidenced by increased expression of lineage-specific genes under both conditions. Overall, these findings suggest that PCL/BG microparticles are biocompatible and capable of guiding stem cell differentiation, supporting their potential use in bone regeneration strategies.

1. Introduction

Bone is a highly dynamic tissue that remodels itself throughout life through a coordinated remodeling process, in which osteoclasts resorb old or damaged bone matrix and osteoblasts deposit newly mineralized bone [1]. This balance between bone resorption and formation maintains skeletal homeostasis, adapts bone architecture to mechanical demands, and contributes to the repair of microdamage [1, 2]. However, the endogenous regenerative capacity of bone is limited when critical-size defects (>2.5 cm in humans) occur due to trauma, tumor removal, infection or metabolic disorders [3, 4]. Autologous bone grafting remains the gold standard for treating such defects, as it supplies an osteoconductive matrix, viable osteogenic cells and osteoinductive growth factors [5]. Nevertheless, autografts are limited in

quantity, require more surgical time and result in additional blood loss [6]. Moreover, harvesting autologous grafts introduces donor-site morbidity: pain at the donor site is the most frequent complication, and other problems include nerve injury, haematoma, infection and even fracture at the harvest site [5, 6]. These limitations have stimulated the development of biomaterial-based strategies for bone regeneration, aiming to provide mechanical support and create an environment that promotes new bone formation.

Bioactive glass 45S5 (BG) is a second-generation biomaterial that interacts with the biological environment through controlled ion release, promoting tissue bonding while being gradually resorbed and replaced as new tissue forms [7]. In the context of bone regeneration, BG is highly bioactive and osteoconductive as it rapidly forms a hydroxycarbonate apatite layer that bonds strongly to bone and its

ionic dissolution products stimulate both osteogenesis and angiogenesis [7]. Nonetheless, BG on its own is brittle, dissolves quickly and has poor handling properties, limiting its use as a standalone graft [8]. Combining BG with a degradable polymer has the potential to overcome these issues by providing mechanical integrity while retaining bioactivity [9].

Polycaprolactone (PCL) is an aliphatic, semi-crystalline polyester that is biocompatible, biodegradable and easily processable [10]. At physiological temperature, PCL exists in a rubbery state, providing toughness and flexibility [11]. Owing to its availability, cost and FDA-approved status, PCL has already been incorporated into multiple medical devices, highlighting its clinical relevance and translational potential [12–15]. On the other hand, pure PCL has drawbacks: the polymer's hydrophobicity limits cell adhesion, its slow degradation rate contributes to prolonged inflammatory responses, delays tissue remodeling and its mechanical stiffness is insufficient for load-bearing bone applications [16–18]. Incorporating inorganic particles such as BG into the PCL matrix improves mechanical strength and allows modulation of composite biodegradation and bioactivity.

PCL/BG composites have therefore been widely explored for bone regeneration, particularly as electrospun membranes, porous scaffolds, and additively manufactured constructs [19–21]. These studies generally demonstrate that BG can improve the bioactivity of PCL-based materials by promoting apatite formation, enhancing osteogenic responses, and modifying degradation behavior [19–21]. However, most reported PCL/BG systems are fabricated as continuous structures with predefined geometries, which may limit their adaptability to irregular defects without patient-specific design or additional shaping.

Rather than fabricating large, continuous scaffolds, many groups now assemble bone-regeneration constructs from free-packed granular systems, as they adapt to irregular defects and naturally create interconnected pores that support vascular and cellular infiltration [22–25]. The biological response to these particles depends strongly on their size and porosity. Studies show that very small ($<100\text{ }\mu\text{m}$) traditional allograft bone particles have reduced osteogenic capacity, whereas very large fragments ($>1\text{ mm}$) may reduce available surface area and hinder new bone formation [26]. Importantly, the optimal balance is not universal: craniofacial applications often employ smaller granules to conform to delicate anatomical sites [25], dental ridge augmentations demand particles that maintain graft volume while still providing interparticle spaces sufficient for vascular infiltration [22], and sinus-lift procedures rely on larger granules that maintain open spaces under the lifted membrane while still allowing vascularized bone to infiltrate the grafted site [27].

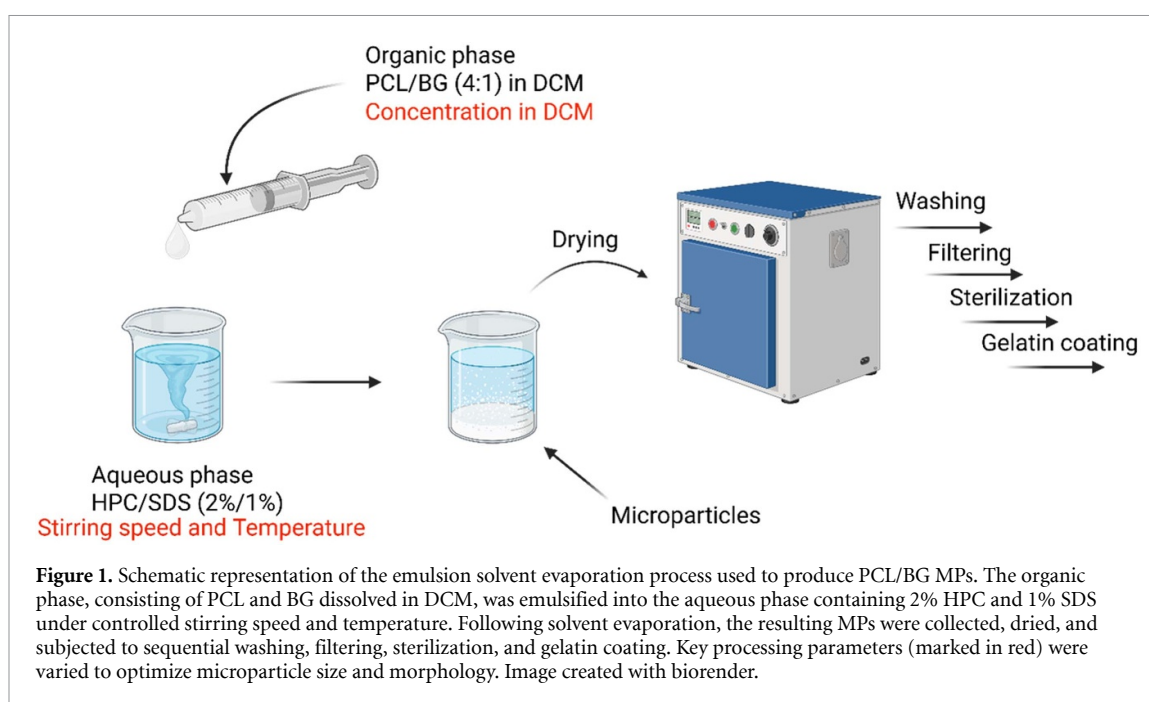
This diversity of requirements underscores the need for careful optimization of both size and porosity according to clinical context. Particle size also determines, from the cell's perspective, the surface topology it encounters and thus influences the cytoskeletal tension and the intracellular signaling pathways that drive differentiation [28]. Restricting cell spreading or reducing cytoskeletal tension has been shown to block bone morphogenetic protein-induced Smad signaling and osteogenic differentiation, whereas surfaces that permit cell spreading and generate RhoA-mediated tension promote osteogenesis [28]. Within this landscape, particles in the $100\text{--}500\text{ }\mu\text{m}$ range have emerged as a particularly promising window, offering high surface area for osteogenic cells, interparticle gaps wide enough for vascularization and sufficient bulk for mechanical integrity, making them a rational starting point for further application-specific refinements [29, 30].

Here, we report the fabrication of PCL/BG microparticles (MPs) using the emulsion solvent evaporation method. We optimized the synthesis parameters to obtain MPs within the $100\text{--}500\text{ }\mu\text{m}$ size range, characterized their morphology and surface porosity, and evaluated their biocompatibility *in vitro*. We further assessed whether the MPs support mesenchymal stem cell differentiation toward adipogenic and osteogenic lineages, to evaluate their influence on lineage commitment and multipotency. By combining the structural properties of PCL with the osteoinductive nature of BG in a granular format, this work aims to develop a versatile biomaterial suitable for bone regeneration strategies.

2. Methods

2.1. Preparation of PCL/BG MPs

PCL (M_w 80 000 Da, Rimless Industry Co., Ltd, Changchun, China)/BG (particle size— $10\text{ }\mu\text{m}$, MO-Sci Corporation, Rolla, MO, USA) MPs were fabricated using the emulsion solvent evaporation technique. Briefly, PCL and BG were combined in a mass ratio of 4:1 and dissolved in dichloromethane (DCM) (Honeywell International Inc., Charlotte, NC, USA), with the total mass of solids comprising 20% of the organic solvent volume (if not noted otherwise). 1 ml of this organic phase was added dropwise into 20 ml of aqueous phase consisting of 1% sodium dodecyl sulfate (SDS) (Merck KGaA, Darmstadt, Germany) and 2% hydroxypropyl cellulose (HPC) (M_w 100 000 Da, Merck KGaA), under continuous stirring at 500 rpm (if not noted otherwise) and maintained for an additional minute to ensure uniform particle formation. The fabrication was performed in a 25 ml Schott Duran® glass beaker (DWK Life Sciences GmbH, Wertheim, Germany) with a magnetic stir bar of 25 mm length and 8 mm diameter



(Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The whole fabrication process is depicted in figure 1.

The resulting emulsion was dried at 40 °C overnight to evaporate both water and DCM. After drying, MPs were filtered (excluding particle size experiments) using 500 µm pore-size sieves (Merck KGaA) and washed continuously with deionized water for 20 min using a 90 µm pore-size sieve (Merck KGaA). The filtered particles were then incubated in 5% Decon 90 (Decon Laboratories Ltd, East Sussex, UK) solution overnight for surface decontamination. After thorough rinsing with MilliQ water, the particles were transferred to a sterile chamber containing 70% ethanol, which was allowed to evaporate completely. Final sterilization was performed using UV-C light for 20 min prior to use. Before cell seeding, PCL/BG MPs were incubated in 0.1% sterile gelatin (PAN-Biotech GmbH, Aidenbach, Germany) overnight at 4 °C to provide cell-adhesive motifs and improve cell attachment. After coating, the MPs were washed with sterile PBS and transferred to culture plates.

2.2. PCL/BG MPs size measurement

Following fabrication, the particle size distribution of PCL/BG MPs was evaluated by optical imaging. The entire 90 mm radius Petri dish, filled with MPs fabricated from 0.5 ml of organic phase, was captured with a CCD camera (EXi Blue™—QImaging, Surrey, Canada) attached to an inverted phase-contrast microscope (Olympus IX51—Olympus Co., Tokyo, Japan). The diameter of individual particles was measured manually using ImageJ software (version 153 c, Java 1.8.0_172), and the resulting dataset was used to evaluate the size distribution.

2.3. pH measurement

Material-induced changes in the pH of the Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific Inc.) were tracked over time using an Orion 320 pH meter (Thermo Fisher Scientific Inc.). The meter was calibrated immediately prior to measurements using certified pH buffer standards following the manufacturer's instructions. Materials ($n = 3$ per group) were weighed using an analytical balance and placed directly into individual wells of a 24-well plate (TPP Techno Plastic Products AG, Trasadingen, Switzerland). MPs and PCL were weighed at 0.2 g per well, while BG was weighed at 0.04 g per well which corresponds to 20% of the MP mass. Processed BG is defined as BG that underwent the same preparation steps as the MPs—washing, sterilization, and gelatin coating—prior to pH measurement. After the material placement, 2 ml of serum and antibiotic-free DMEM was added to each well and incubated under static conditions at RT.

At each time point (0, 5, 10, 30, 60, 180, 360 min, and 3 d), the pH electrode was rinsed with deionized water and gently blotted dry, then immersed into the respective wells and allowed to equilibrate until the reading stabilized before recording the pH value.

To account for baseline pH variation of the medium, DMEM without added materials was used as the control. The data are presented as ΔpH versus control, calculated at each time point as:

$$\Delta\text{pH} = \text{pH}_{\text{group}} - \text{pH}_{\text{control}},$$

where pH_{group} represents the measured value for BG, processed BG, or PCL and $\text{pH}_{\text{control}}$ corresponds to DMEM incubated without material at a respective time point.

2.4. Ion leaching evaluation

The ion release profile of the MPs was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using Optima 7000 DV (PerkinElmer Inc., Waltham, MA, USA). Gelatin-coated PCL/BG MPs (3.5 g) and BG (0.7 g, corresponding to 20% of the MP mass) were dispersed in 50 ml of MilliQ water and maintained under static conditions at 37 °C. At each time point (1 h, 6 h, 24 h, 72 h, and 168 h), the solution was shaken, and 1 ml of supernatant was withdrawn using a sterile syringe, without replenishing the solution. Collected samples were immediately centrifuged at 2000 g for 3 min and decanted, leaving 100 µm of supernatant and making sure no MPs or BG was collected. All samples were analyzed in triplicate.

The supernatants were diluted with 5% nitric acid (Rotipuran® Supra 69%, Carl Roth), and element concentrations (Ca, Na, P, and Si) were quantified using calibration curves produced by an appropriate dilution of standard solutions (single-element ICP standards, 1000 mg l⁻¹, Carl Roth). Calibration accuracy was verified by periodic measurement of standard solutions throughout the analytical sequence. Three technical replicates were measured for each element.

2.5. SEM imaging

A scanning electron microscope (TM-1000; Hitachi high-technologies Corp., Tokyo, Japan) was used to analyze the surface morphology of the MPs. Surface porosity was quantified from SEM micrographs using ImageJ software (153 c, Java 1.8.0_172). Images were first processed by applying a Gaussian blur filter with a sigma value of 2.00 to reduce noise. The images were then inverted to enhance the contrast between the MPs and the background.

To standardize the measurement area and minimize edge effects caused by MPs' curvature, a circular region of interest (ROI) was defined at the center of a particle, with a diameter equal to the calculated radius of the whole particle. This ensured that surface porosity was measured in a relatively flat region of the MPs' surface. Surface porosity within this ROI was calculated using the '3D Objects Counter' tool, with manual threshold adjustment to accurately segment pore regions. The porosity percentage was defined as the ratio of total pore area to ROI area.

2.6. Cell culture isolation and cultivation

Rat-derived Periosteum mesenchymal stem cell (PMSCs), dental pulp stem cells (DPSCs), and pulmonary trunk endothelial cells (PTECs) were used in the experiments. DPSCs and PTECs were isolated, characterized and modified previously, as described by Alksnė [31]. The PMSCs were isolated in the present study and the procedure was approved by the licenses of the state food and veterinary Service

of Lithuania, No. G2-250, 2023–06-06, and was performed in accordance with the Lithuanian Law on Welfare and Protection of Animals and Directive 2010/63/EU on the protection of animals used for scientific purposes.

For the isolation of PMSCs, rat periosteum fragments were excised and immediately transferred into ice-cold serum-free culture medium with 300 U ml⁻¹ penicillin and 300 µg ml⁻¹ streptomycin (Thermo Fisher Scientific Inc., Waltham, MA, USA). In a laminar flow cabinet, the tissue was washed for 5 min in 5 ml HBSS supplemented with Ca²⁺ and Mg²⁺, 300 U ml⁻¹ penicillin and streptomycin 300 µg ml⁻¹. The washed tissue was then minced using sterile instruments and incubated in 4–5 ml of sterile-filtered (0.22 µm) digestion solution containing serum-free DMEM supplemented with antibiotics (100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin) and collagenase I (Merck KGaA) (final concentration 1 mg ml⁻¹). Digestion was carried out on a heated shaker at 37 °C and 300 rpm overnight. After digestion, samples were centrifuged at 400 × g for 10 min, and the cell pellet was resuspended in growth medium. Cells were seeded into 24-well or 6-well plates (TPP Techno Plastic Products AG), depending on the initial tissue amount, and cultured until 70%–80% confluence. Early passages were replated into new flasks to facilitate fibroblast separation.

All cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin 100 U ml⁻¹, streptomycin 100 µg ml⁻¹). All cell cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were passaged into new flasks upon reaching 70%–80% confluence. For detachment, cells were treated with 0.05% trypsin-EDTA solution (trypsin from Thermo Fisher Scientific Inc.; EDTA from Merck KGaA) in PBS.

2.7. Lentiviral production and transduction

Lentivirus was produced using the HEK293T cell line (American Type Culture Collection (ATCC), Manassas, VA, USA) using the packaging (pCMV-dR8.91), envelope (pCMV-VSV-G), and transfer vectors (pLJM1-mCherry or pLJM1-EGFP) (All vectors from Addgene, Watertown, MA, USA). HEK293T cells (7 × 10⁴ cells cm⁻²) were seeded and grown overnight to 70%–80% confluence, then transfected with Lipofectamine 3000 (Thermo Fisher Scientific Inc.) according to the manufacturer's protocol using a plasmid mass ratio of 2:1.2:0.8 (pLJM1-mCherry/pLJM1-EGFP:pCMV-dR8.91:pCMV-VSV-G). After 18 h, the medium was replaced with growth medium containing 20% FBS, and viral supernatant was collected 24 h later, supplemented with polybrene (8 µg ml⁻¹) (Merck KGaA), filtered (0.22 µm), and stored at –80 °C. For transduction, PTECs and DPSCs were plated at 10⁴ cells cm⁻², and the next day, the lentiviral suspension was added at a

1:2 volume ratio (virus-polybrene mixture: growth medium). After 24 h, the medium was replaced with standard growth medium for an additional 24 h, followed by puromycin selection for 3–5 d to obtain red-fluorescent PTEC-mCherry and green-fluorescent DPSC-EGFP cultures.

2.8. Cell immortalization

Primary PMSCs were immortalized using a pCW-SV40 (all vectors from Addgene) lentiviral system. Lentiviral particles were produced in HEK293T cells cultured in DMEM supplemented with 10% FBS and 1% antibiotics. PMSCs were seeded in 6-well plates and transfected at 70%–90% confluence using Lipofectamine 3000 in Opti-MEM (Thermo Fisher Scientific Inc.) with a plasmid mass ratio of pCW-SV40: packaging pCMV-dR8.91: envelope pCMV-VSV-G = 5:5:1 (total DNA 2.5 µg per well). After ~18 h, the medium was replaced with DMEM containing 20% FBS, and viral supernatant was collected 24 h later, supplemented with polybrene (final 8 µg ml⁻¹), filtered through a 0.45 µm filter, and used immediately or stored at -80 °C. For transduction, PMSCs were seeded 1 d prior in multi-well plates and incubated overnight with virus-containing supernatant diluted 1:1 in PMSC growth medium (DMEM + 10% FBS + 1% antibiotics). The next day, medium was replaced with fresh growth medium, and blasticidin (Thermo Fisher Scientific Inc.) selection (2 µg ml⁻¹) was applied for 3–5 d to obtain stable immortalized PMSC cultures for expansion and downstream experiments.

2.9. Cell proliferation

PMSCs were seeded at a density of 1.3×10^5 cells per well in an 8-well chamber slide (Thermo Fisher Scientific Inc.) onto two different substrates: gelatin-coated PCL/BG MPs and standard tissue culture plastic (TCP). Cell proliferation was assessed at 1, 3, and 7 d post-seeding using the Click-iT™ EdU Imaging Kit (Thermo Fisher Scientific Inc.), with a modified protocol. At each time point, EdU was added directly to the culture medium at a final concentration of 10 µM and incubated with the cells for 24 h at 37 °C. Following incubation, cells were fixed with 4% paraformaldehyde (Merck KGaA) for 15 min at room temperature under sterile conditions, then washed twice with PBS (Thermo Fisher Scientific Inc.). After fixation, samples were stored in PBS at 4 °C until staining. EdU incorporation was detected via fluorescent azide conjugation according to the kit instructions, and nuclei were counterstained with DAPI (Thermo Fisher Scientific Inc.). Fluorescent images were acquired using an inverted fluorescence microscope (ECLIPSE Ti2-E; Nikon Instruments Inc., Tokyo, Japan), and proliferation was quantified by calculating the percentage of EdU-positive nuclei relative to the total number of DAPI-stained nuclei.

2.10. Assessment of PMSC differentiation potential

PMSCs were seeded directly onto gelatin-coated PCL/BG MPs or TCP surface in 12-well plates (TPP Techno Plastic Products AG), and then were exposed to adipogenic and osteogenic culture conditions noted below (the numbers of cells used for each type of differentiation and time point are in table S1. Culture media were fully replaced every 2–3 d throughout the induction period. After the induction period, total RNA was extracted using Direct-zol RNA Kit (Zymo Research Corp., Irvine, CA, USA) and complementary DNA was synthesized from 1500 ng of total RNA using the ZymoScript™ RT PreMix (Zymo Research Corp.), both according to the manufacturer's instructions. Gene expression analysis was performed by quantitative PCR (qPCR) to assess lineage-specific markers corresponding to each differentiation type. qPCR reactions were run on the QuantStudio™ 3 (Thermo Fisher Scientific Inc.) using SYBR™ Green Universal Master Mix (Thermo Fisher Scientific Inc.) with the following cycling conditions: initial polymerase activation at 95 °C for 10 min and 35 cycles of 95 °C for 15 s, 64 °C for 15 s, and 72 °C for 1 min. Melt-curve analysis was performed as follows: 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s, with a ramp rate of 0.15 °C s⁻¹ between 60 °C and 95 °C. Presented relative analyzed genes' expression levels were calculated using 2^{-ΔΔCt} method. Ct values were normalized to the expression of the *Gapdh* gene and fold changes were calculated relative to the expression of the same target gene in undifferentiated cells seeded on TCP at the initial time point. The primer sequences are listed in supplementary table S2.

For adipogenic differentiation, cells were maintained for 7 d in DMEM (4.5 g l⁻¹ of glucose) (Thermo Fisher Scientific Inc.) supplemented with 10% FBS, 1% penicillin-streptomycin, 1% glutamine, 1 µM dexamethasone, 200 µM indomethacin, 1 µM 3-isobutyl-1-methylxanthine, and 10 µg/ml recombinant human insulin (all from Merck KGaA). Following induction, expression of adipogenic markers *Pparg* and *Fabp4* was evaluated by qPCR.

To induce osteogenic differentiation, cells were cultured for 7 d in DMEM (1 g l⁻¹ of glucose) supplemented with 10% FBS, 1% penicillin-streptomycin, 10 mM β-glycerophosphate (Merck KGaA), 25 µg ml ascorbic acid (Merck KGaA), and 1 µM dexamethasone. Osteogenic differentiation was evaluated by measuring the expression of *Runx2*, *Osc*, and *Osp*.

2.11. Alkaline phosphatase (ALP) staining

ALP activity was assessed to evaluate early osteogenic differentiation of PMSCs cultured on PCL/BG MPs and TCP. Immortalized rat PMSCs were seeded in 24-well plates at densities corresponding to the differentiation experiments (figure S1), with an additional day 14 time point (10,000 cells/well on MPs and 1,000 cells/well on TCP). MPs were applied

as a single-layer coating per well prior to seeding. Cells were cultured in DMEM (1 g l⁻¹ glucose) supplemented with 10% FBS and 1% penicillin-streptomycin. After an initial attachment period (3–4 h), the medium was replaced with either growth medium (control) or osteogenic differentiation medium, which is described above. Media were refreshed every 2–3 d.

At days 1, 3, 7, and 14, ALP staining was performed at room temperature. Samples were washed twice with Tris-buffered saline (TBS; pH 7.6) and fixed with 4% paraformaldehyde for 90 s, followed by 2–3 additional washes with TBS. For MP samples, particles were transferred to new wells using cut pipette tips to minimize cell detachment and exclude cells attached to TCP. ALP activity was detected using an NBT/BCIP substrate solution (Thermo Fisher Scientific Inc.) for 60 min in the dark. The reaction was stopped by washing twice with TBS. Samples were imaged immediately using bright-field microscopy at 4× magnification.

2.12. Statistical analysis

All statistical analyses were performed using RStudio (R version 4.5.1). Data are presented as either median ± interquartile range (IQR) or mean ± standard deviation (SD), based on at least three independent experiments, each with a minimum of three samples per group. Normality of the data was assessed using the Shapiro-Wilk test when the sample size was five or more ($n \geq 5$). For smaller sample sizes ($n < 5$), a normal distribution was assumed. Since all datasets met the assumptions for normality, for comparisons involving three or more groups, one-way analysis of variance was used, followed by Tukey's post hoc test to identify specific group differences. Differences were considered statistically significant at $p < 0.05$. Graphs were prepared using the ggplot2 package in R. Statistically significant differences in figures are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$) or by compact letter display, where groups sharing the same letter are not significantly different. If alternative symbols were used, corresponding explanations are provided in the figure legends.

3. Results

3.1. Synthesis of PCL/BG MPs

Considering that the size of the MPs mainly depends on the concentration of the organic phase and the stirring speed of the aqueous phase, we initiated our fabrication experiments with particles fabricated using 20% w/v PCL/BG (4:1 mass ratio) in DCM and a stirring speed of 500 rpm at 20 °C (figure 2). Concentrations below 20% resulted in an overly dilute organic phase, which appeared watery

and produced unstable emulsions, while concentrations above 20% led to excessive viscosity, occasionally hindering droplet breakup during emulsification. Subsequent optimization, informed by particle size distribution analyses, showed that stirring speeds in the range of 300–500 rpm did not result in efficient microparticle formation. Based on these observations, higher stirring speed, 700 rpm, was later adopted to improve the yield of particles in the 100–500 µm range. Microparticles produced under these conditions (20% w/v in DCM, 700 rpm and 20 °C) were used for *in vitro* experiments.

The physicochemical properties of the microparticles were further evaluated, particularly their effects on local pH and ionic composition, which are known to influence cellular behavior.

3.2. Influence of PCL/BG microparticles on medium pH and ionic environment

To assess how the different materials influence the surrounding environment, temporal pH changes in culture medium were monitored and analyzed alongside PCL/BG MP-derived leachates over time (figure 3). Unprocessed BG induced a rapid and sustained alkalization of the medium, with ΔpH reaching approximately +1.5 after 3 d (corresponding to an increase from ~7.4 to ~8.9; figure 3(A)), consistent with ion exchange and dissolution processes at the glass surface. This was paralleled by substantial accumulation of dissolution products in the surrounding medium (figure 3(B)), indicating active glass-solution interaction and progressive leaching. Processed BG (washed, sterilized, and gelatin-coated) produced a similar but attenuated response (ΔpH ≈ 0.8–0.9). In contrast, PCL/BG microparticles exhibited markedly moderated leaching behavior, with only minor pH changes (ΔpH ≈ 0.1), maintaining near-physiological conditions. The ΔpH values remained considerably lower than those of BG alone throughout the experiment (figure 3(A)), and the corresponding leachate profiles showed reduced and slower accumulation of glass-derived species (figure 3(B)). This indicates that embedding BG within the PCL matrix restricts direct exposure of the glass surface to the aqueous phase, thereby modulating dissolution kinetics and buffering the immediate chemical impact on the medium. The PCL-only condition showed minimal changes in pH, remaining comparable to control levels.

At early time points, BG-derived samples showed essentially no measurable phosphorus in solution, whereas detectable levels were observed in the MD-derived samples. The temporal trends of calcium and phosphorus followed an expected inverse relationship: increases in calcium concentration were accompanied by decreases in phosphorus levels, consistent with precipitation or re-deposition processes occurring in the medium.

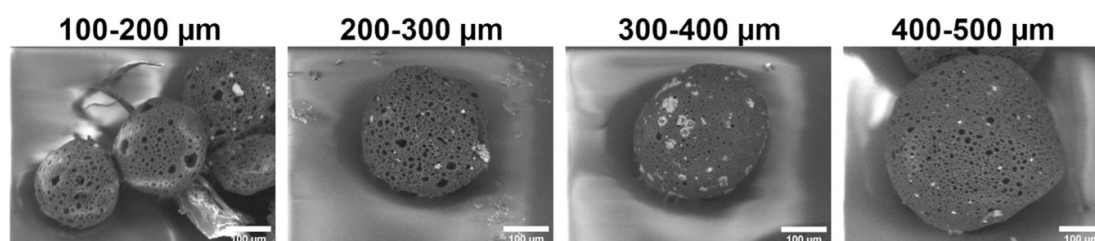


Figure 2. Representative SEM images of PCL/BG MPs, sorted into four size ranges: 100–200 μm , 200–300 μm , 300–400 μm , and 400–500 μm . These MPs were fabricated using 20% w/v in DCM and a stirring speed of 500 rpm at 20 $^{\circ}\text{C}$. Scale bars = 100 μm .

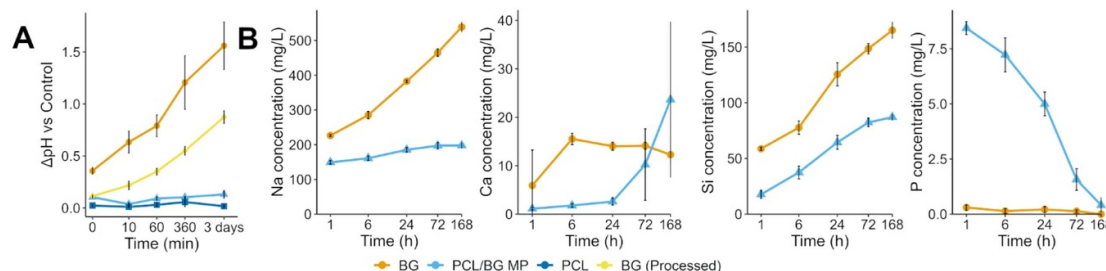


Figure 3. Material-driven alterations in the chemical environment over time. (A) Changes in medium pH in the presence of BG, processed BG, PCL/BG MPs, and PCL. (B) Concentration profiles of material-derived leachates.

Next, we focused on optimizing the yield of MPs within the 100–500 μm size range relevant for bone regeneration applications. This was achieved by adjusting three key parameters: stirring speed, organic phase concentration, and aqueous phase temperature (see methods, figure 1).

3.3. Effect of stirring speed on particle size

Since the particle size in the emulsion solvent evaporation method is strongly governed by the stirring speed of the aqueous phase, we varied the stirring speed from 300 to 900 rpm at a fixed organic phase concentration of 20% (w/v) at 20 $^{\circ}\text{C}$ (figure 4(A)). The widest size distribution and largest particles were observed at lower speeds (300–400 rpm). As stirring speed increased, particularly beyond 600 rpm, the particle distribution narrowed, and the mean particle size shifted downwards, with 900 rpm producing the smallest particles. Nonetheless, stirring speed over 700 rpm resulted in more clumps of particles (figure S1), therefore, 700 rpm is optimal for producing the smallest particles. This trend is also confirmed by size distribution histograms (figure 4(B)). At lower speeds, a greater proportion of particles fell within the 300–500 μm range, while speeds over 700 rpm resulted in more than 40% of particles falling in the 100–200 μm range.

3.4. Effect of polymer concentration on particle size

It is well established that the concentration of the organic phase can influence microparticle formation by altering solution viscosity and droplet

breakup dynamics, thereby affecting particle size and morphology. To evaluate its role in our system, PCL/BG solutions were prepared at concentrations of 20%, 22.5%, 25%, 27.5%, and 30% (w/v), while the aqueous phase temperature and stirring speed were kept fixed at 20 $^{\circ}\text{C}$ and 500 rpm, respectively (figure 5(A)). However, within the investigated range, increasing the concentration of the organic phase did not have an explicit effect on average particle size (figure 5(A)), but more notably, it affected the consistency of particle formation. Higher polymer concentrations substantially increased solution viscosity, rendering the organic phase too thick for stable and reproducible droplet formation, which resulted in a broader size distribution with a greater fraction of particles exceeding 500 μm as well as an increased proportion below 100 μm (figure 5(B)). The particles were so large in some cases that they could not be captured using the microscope.

3.5. Effect of temperature on particle size

Knowing that emulsion temperature modulates droplet breakup and thus could impact the microparticle size distribution, we prepared emulsions at 20, 25, 30, 35 and 40 $^{\circ}\text{C}$, while keeping the stirring speed and organic phase concentration fixed at 500 rpm and 20% (w/v), respectively (figure 6). At the lowest temperature (20 $^{\circ}\text{C}$), particles were larger and exhibited the widest size distribution, with a considerable fraction exceeding 300 μm in size. As the temperature increased to 25 $^{\circ}\text{C}$ –30 $^{\circ}\text{C}$, the particle

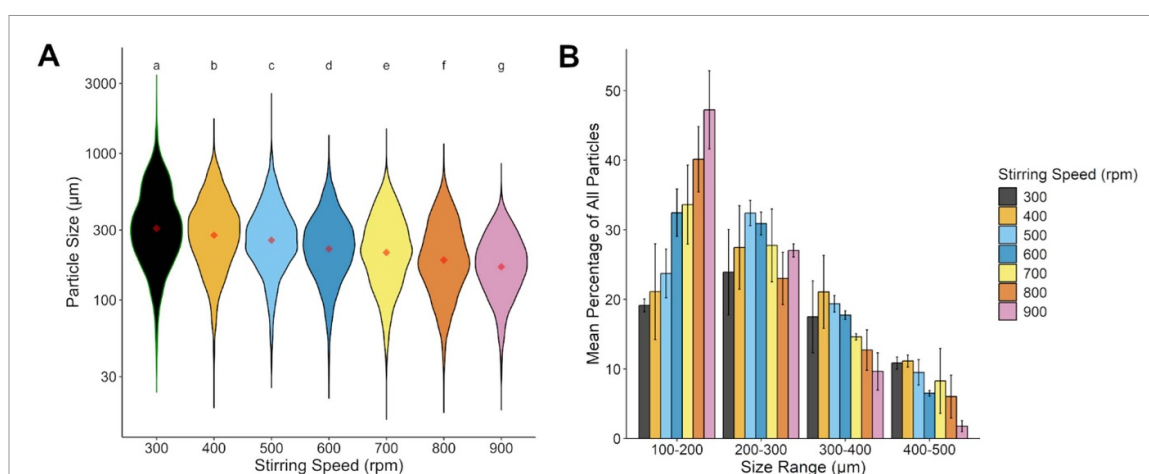


Figure 4. Effect of stirring speed on MP size distribution. (A) Particle size distributions at different stirring speeds of the aqueous phase during fabrication ($n = 2554\text{--}3392$). Red diamonds indicate mean particle sizes, and statistically different groups are marked with distinct letters ($p < 0.05$). (B) Mean percentage of particles within defined size ranges across different stirring speeds.

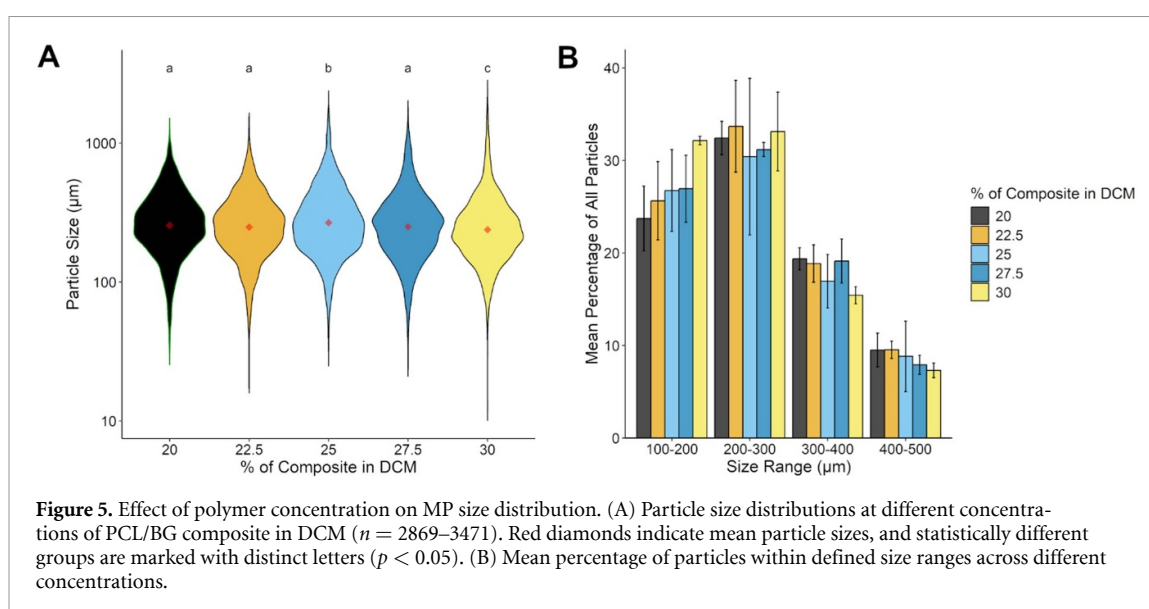
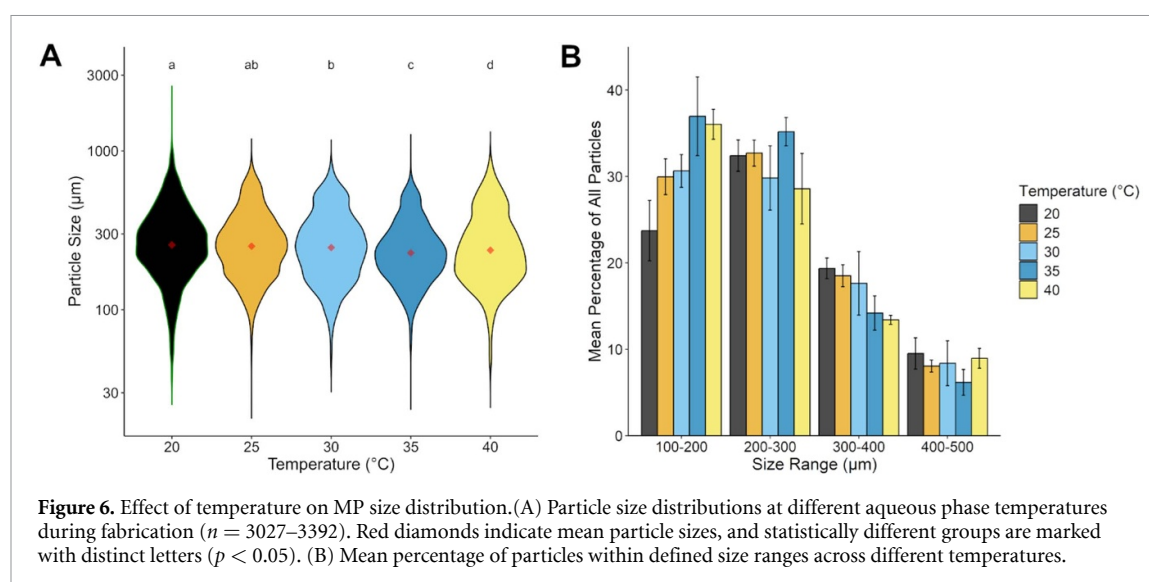


Figure 5. Effect of polymer concentration on MP size distribution. (A) Particle size distributions at different concentrations of PCL/BG composite in DCM ($n = 2869\text{--}3471$). Red diamonds indicate mean particle sizes, and statistically different groups are marked with distinct letters ($p < 0.05$). (B) Mean percentage of particles within defined size ranges across different concentrations.

distribution shifted downward, with a greater proportion of particles in the 100–200 μm range and fewer in the 300–400 μm range. At 35 $^{\circ}\text{C}$, particles were smallest and most uniform, with nearly 40% in the 100–200 μm range and only $\sim 15\%$ between 300 and 400 μm . At the highest temperature of 40 $^{\circ}\text{C}$, the distribution remained skewed toward smaller particles. These trends were also confirmed by size distribution histograms (figure 6(B)), which showed broad distributions with large outliers at 20 $^{\circ}\text{C}$, compared to narrower distributions centered around 260–280 μm at 25 $^{\circ}\text{C}$ –40 $^{\circ}\text{C}$. Despite differences in distribution width, the mean particle size varied only modestly, with 20 $^{\circ}\text{C}$ emulsions yielding slightly larger averages and 35 $^{\circ}\text{C}$ emulsions producing the smallest means. Nevertheless, the fabrication was the most stable at 20 $^{\circ}\text{C}$, therefore, it was used for the synthesis of MPs for subsequent experiments.

3.6. Surface porosity of MPs

The surface porosity of the resulting MPs was calculated for each condition to evaluate how stirring speed, concentration and temperature could influence the surface (figure 7). Porosity tended to decrease with increasing stirring speed, suggesting that faster agitation might produce denser particles with fewer surface voids (figure 7(A)). Representative SEM images further illustrate these trends, showing more open and porous surface at lower stirring speeds (300 rpm) and denser, less porous morphology at higher speeds (900 rpm) (figure S2). In the concentration series, no clear relationship between microparticle radius and surface porosity was observed (figure 7(B)). In contrast, the temperature series showed a positive association between radius and surface porosity (figure 7(C)), although this relationship was evaluated under fixed stirring speed and organic-phase concentration. Therefore,



these results should be interpreted as trends observed under fixed experimental conditions, rather than as combined effects between different fabrication parameters.

3.7. PMSC biocompatibility with PCL/BG MPs

To establish the suitability of PCL/BG MPs for bone regeneration applications, it was first necessary to determine whether they could support cell attachment, survival, and proliferation over time. The opaque nature of MPs posed a challenge to evaluate these characteristics by conventional phase-contrast imaging, therefore, fluorescently labeled cells were used to directly track their behavior on the MPs' surfaces.

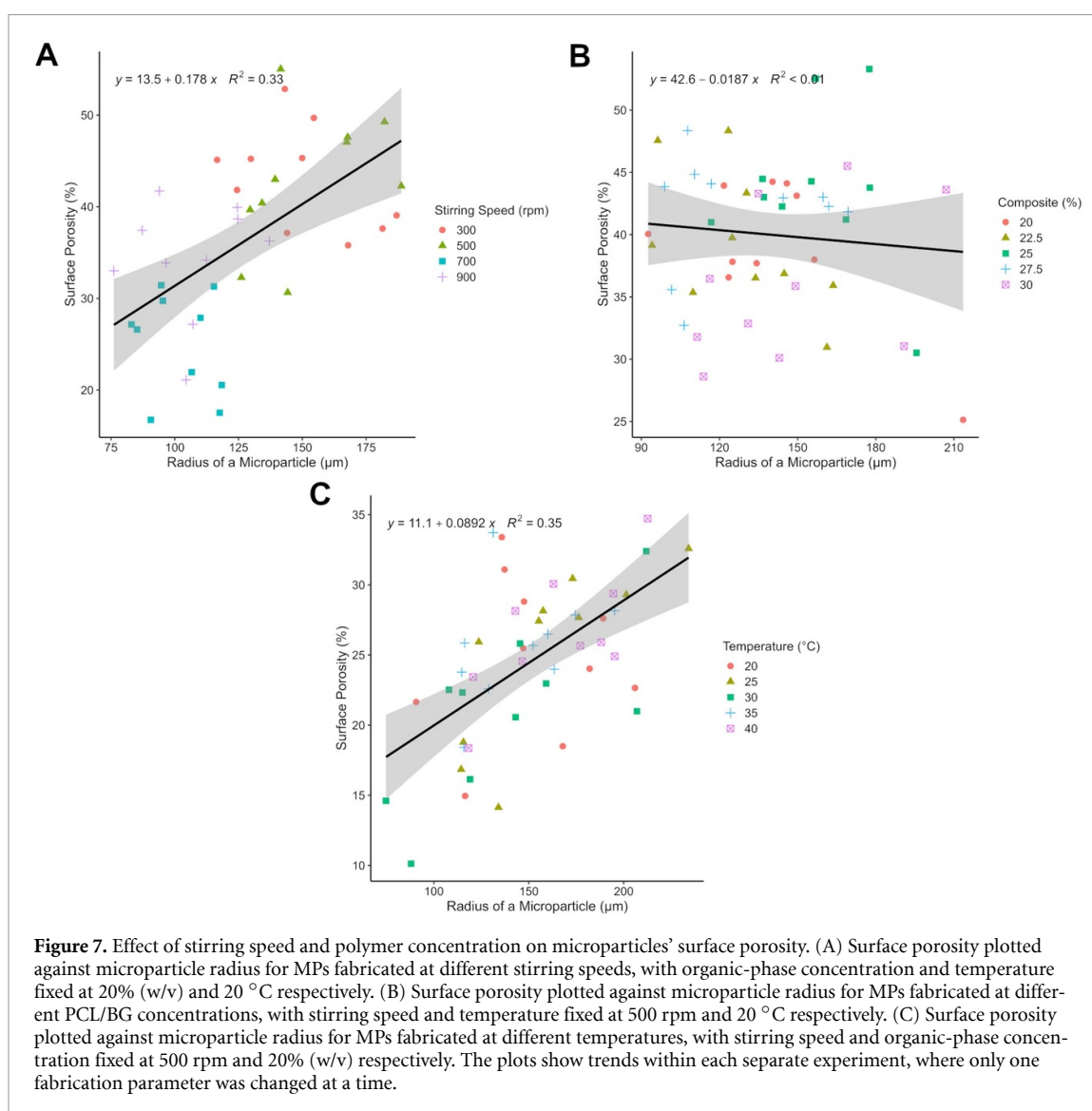
Fluorescent DPSCs and PTECs were selected as representative stromal and epithelial populations. Both readily adhered to the microparticles, remained viable, and spread across the surface topography, frequently bridging adjacent particles to form multicellular connections (figure 8(A)). These observations indicated that the microparticles provided a favorable substrate for initial adhesion and network formation.

To further assess proliferative capacity, PMSCs were used, since these cells were intended for subsequent differentiation studies. Proliferation was quantified with the EdU incorporation assay since formazan-based viability assays, such as MTT, were incompatible with our MPs due to the influence of BG on cellular metabolism and pH of the medium. On TCP, PMSCs initially showed robust proliferation (90.2% EdU-positive at day 1, decreasing to 53.5% at day 3). By contrast, PMSCs seeded onto MPs displayed a reduced proliferative response, with only 31.7% EdU-positive cells on day 1, falling to 2.3% by day 7.

3.8. PMSC differentiation on PCL/BG MPs

Reduced proliferation typically indicates ongoing differentiation. Consequently, we examined the main differentiation pathways that counteract one another—osteogenic and adipogenic (figure 9). Under osteogenic induction, the overall transcriptional profiles observed on MPs were similar to those of on TCP, indicating that the MP system does not compromise the cells' responsiveness to osteogenic cues. *Runx2* expression peaked at early time points and declined thereafter in both groups, consistent with its role as an early transcriptional regulator. However, *Col1a1* expression was consistently higher on MPs at day 1 (approximately 2–3-fold compared to TCP), regardless of induction, suggesting an early enhancement of matrix-related gene expression in the microparticle environment. Among the analyzed markers, *Opn* showed a pronounced late-stage increase, particularly on TCP under induced conditions. Importantly, despite minor differences in magnitude, MPs did not attenuate osteogenic gene expression under induction, but rather supported a progression that is qualitatively equivalent to the standard TCP condition. In the absence of osteogenic stimuli (figure 9(B)), gene expression remained lower and generally decreased over time. *Runx2* and *Col1a1* followed similar declining trends on both substrates, while *Opn* levels remained modest, with slightly higher values on TCP at later time points. Crucially, the comparable expression dynamics observed under induced conditions suggest that MPs provide a permissive environment for osteogenic differentiation while introducing a three-dimensional architecture absent on TCP.

Adipogenic marker expression demonstrated a clear substrate-dependent effect (figure S3), with both *Ppar γ* and *Fabp4* consistently reduced on MPs compared to TCP under both spontaneous and



induced conditions, particularly at early time points. These findings indicate that MPs suppress adipogenic commitment. Immunotypic characterization of the PMSCs used for the differentiation experiment is provided in figure S4.

3.9. ALP activity of PMSCs on PCL/BG MPs

To further assess osteogenic progression at the functional level, ALP staining was evaluated at days 1, 3, 7, and 14 (figure 10). At day 1, ALP staining was minimal in all conditions, with only occasional positive cells detected. By day 3, ALP signal increased, particularly on TCP, where staining became more apparent and evenly distributed, while MP samples showed only sparse and weak staining localized to individual particles. At day 7, TCP cultures exhibited extensive and dense ALP staining covering large continuous areas. In contrast, MP cultures displayed more discrete staining patterns, with ALP-positive regions confined to specific areas on particle surfaces. These regions frequently corresponded to cell accumulations bridging adjacent particles. By

day 14, strong and widespread ALP staining persisted on TCP. In MP samples, staining remained less extensive but was clearly associated with localized cell clusters distributed across the particle network. Higher-magnification insets further highlight that ALP-positive cells on MPs are organized around particle surfaces and interparticle contact points.

Together, these observations indicate that while TCP supports more extensive and uniform ALP staining, the MPs promote localized and organized osteogenic activity within a three-dimensional environment, consistent with physiologically common cell clustering during osteogenic differentiation.

4. Discussion

Several processing routes can produce solid MPs suitable for bone regeneration. Conventional single emulsification, as the one used in this study, mixes a polymer solution with a large aqueous phase under stirring and, after solvent evaporation, solid

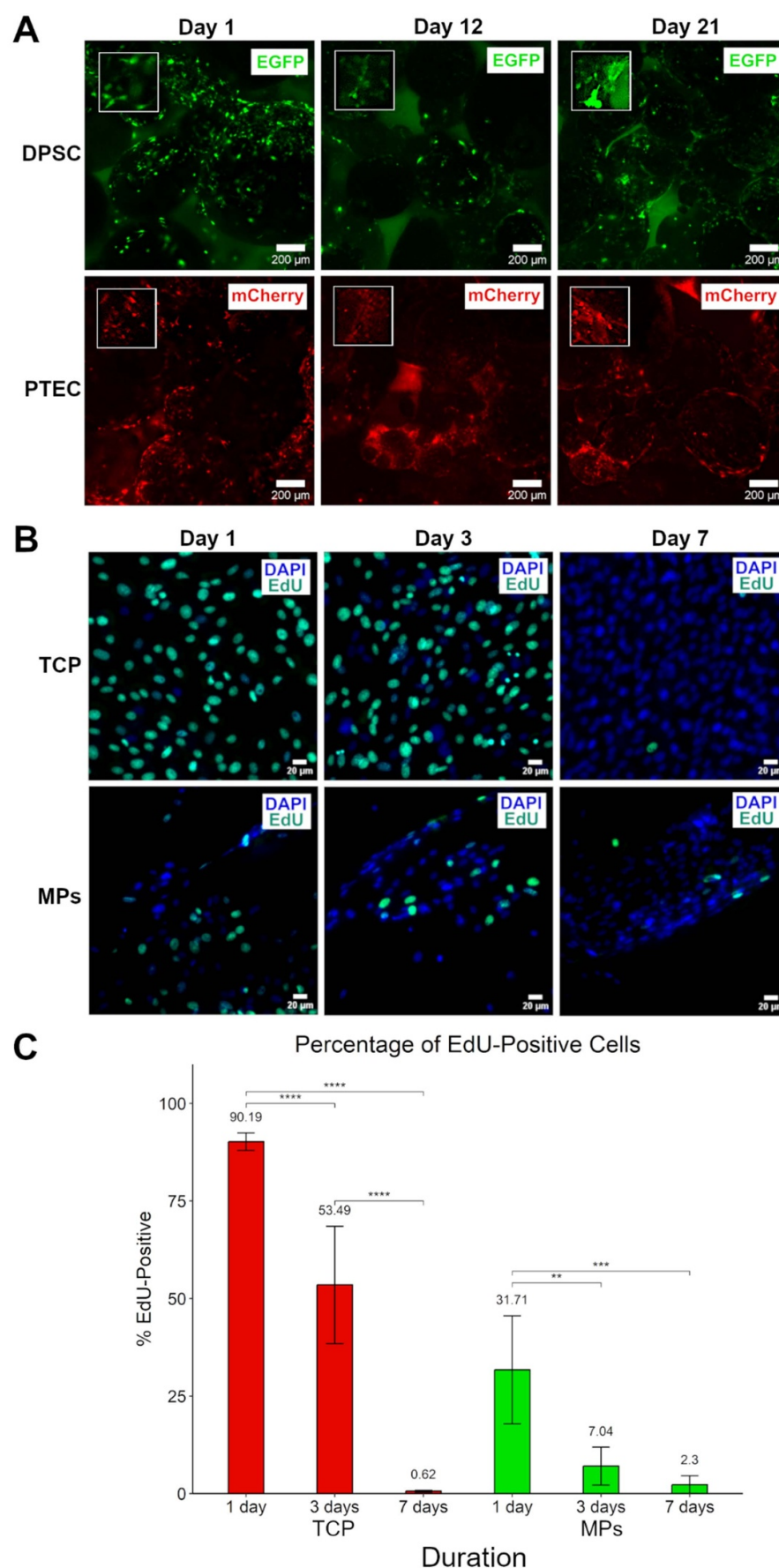


Figure 8. (A) Representative fluorescence images of EGFP-expressing DPSCs and mCherry-expressing PTECs cultured on PCL/BG MPs over a 21-day period. Scale bars = 200 μ m. (B) Representative fluorescence images of EdU and DAPI staining of PMSCs cultured on PCL/BG MPs at days 1, 3, and 7. Scale bars = 20 μ m. (C) Quantification of EdU-positive cells on MPs and standard TCP at days 1, 3, and 7 ($n = 3$). Data are shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

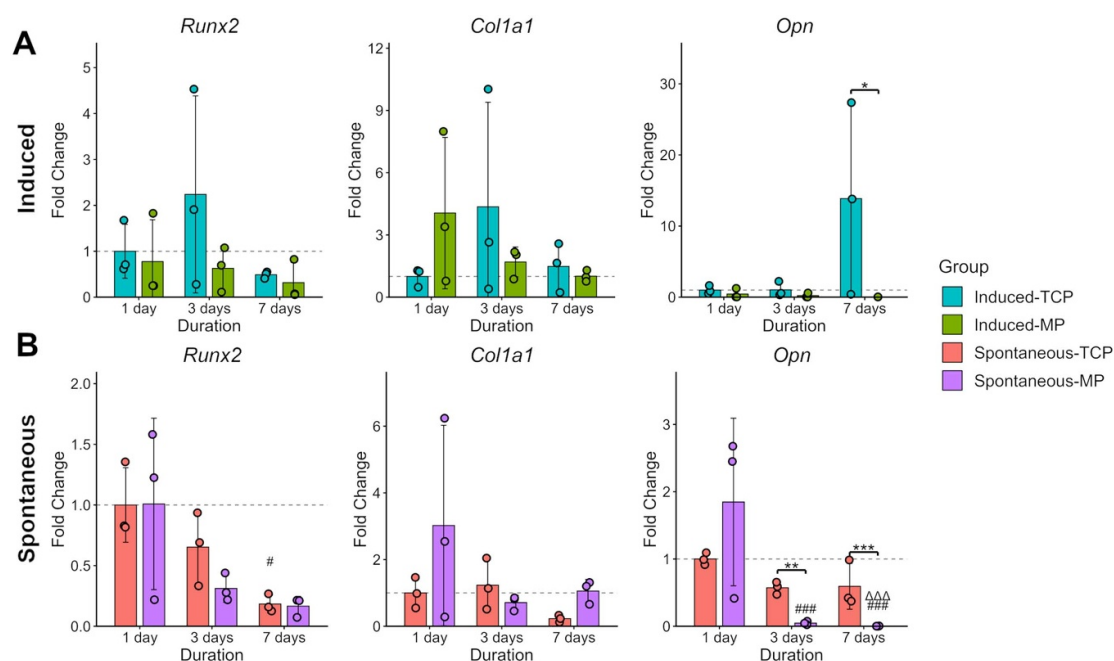


Figure 9. Temporal expression of osteogenic markers on TCP and MP surfaces. Gene expression profiles of *Runx2*, *Col1a1*, and *Opn* are shown under (A) induced and (B) spontaneous culture conditions at 1, 3, and 7 days. Bars represent mean fold change, points show individual replicates and error bars indicate $\pm$ SD. Statistically significant differences between different conditions at the same time point are marked with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The symbol # shows statistically significant differences compared to day 1 within the same condition group (# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$), while Δ demonstrates statistically significant differences compared to day 7 within the same condition group ($\Delta p < 0.05$; $\Delta\Delta p < 0.01$; $\Delta\Delta\Delta p < 0.001$).

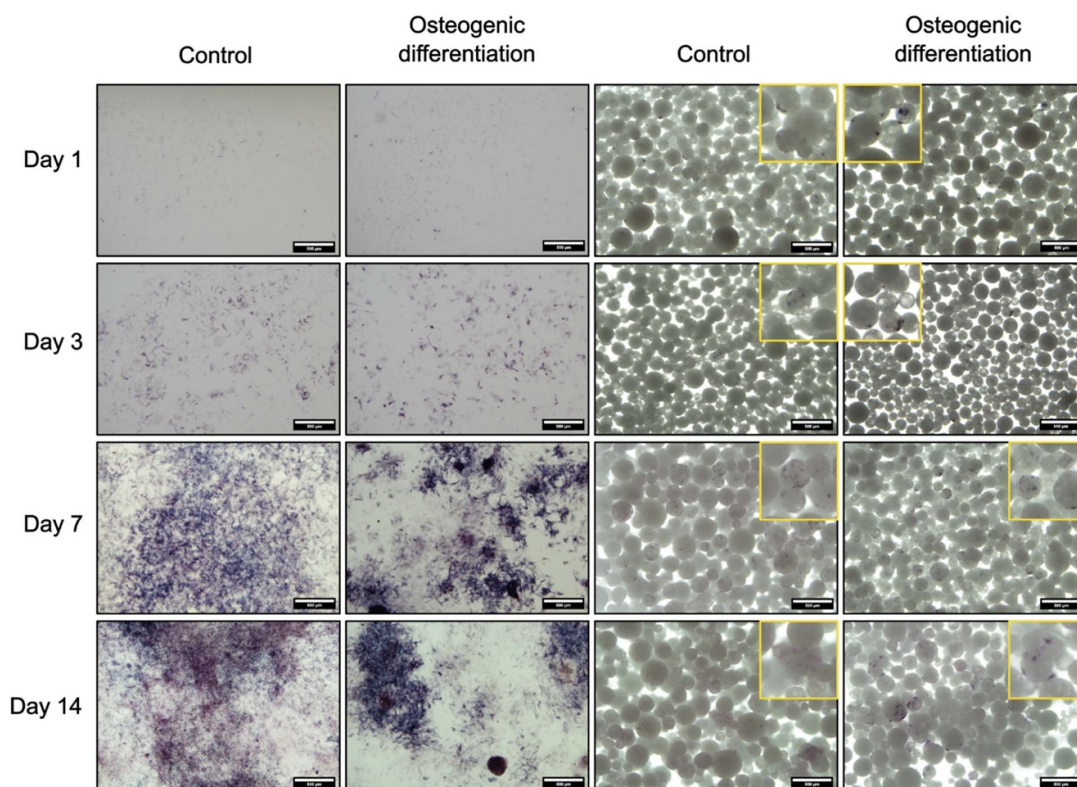


Figure 10. ALP staining in cells undergoing osteogenic differentiation on TCP and MP surfaces. Representative bright-field images of ALP-stained cells cultured under control or osteogenic differentiation conditions on TCP and MP at days 1, 3, 7, and 14. Scale bars = 500 μ m.

particles are washed, dried and collected [32]. It is cost-effective and simple, but shear forces are not uniform, leading to broad size distributions and limited control over porosity and surface topography [32]. Membrane emulsification improves on this by pressing the polymer solution through microporous membranes at controlled rates to produce monodisperse droplets with tunable diameters [33]. Monodisperse particles are considered a drawback for defect filling, as a dispersed particle population can fill a defect more compactly than a uniform one, though overly tight packing could impede cell infiltration. Notably, the maximum theoretical packing density for equally-sized spheres is $\sim 74\%$, whereas a range of sphere sizes can exceed this by filling interstices [34]. Double emulsification creates core-shell structures: a water-in-oil emulsion is re-emulsified in water, allowing incorporation of hydrophilic cores and functional additives such as hydroxyapatite or antibacterial nanoparticles [35, 36]. Nevertheless, this method suffers from structural fragility, faster degradation due to high surface area, and greater process complexity compared to the single emulsion technique [32]. Pickering emulsions use particles like hydroxyapatite or chitosan to stabilize droplets, avoiding surfactant-related cytotoxicity while simultaneously improving biocompatibility and mechanical properties [37]. The aqueous reactivity of BG used in this system is a bigger liability for the use of Pickering emulsions, as substantial pH and ion concentration fluctuations would be expected [38]. Additionally, our particles do not rely on a fragile shell structure for stability, so they are less prone to collapsing [37]. Spray drying atomises polymer solutions into a hot gas stream, rapidly generating solid particles with controllable size and shape [39]. The process offers high throughput and scalability but is incompatible with the BG powder used in this study [39]. Compared with these methods, our single-emulsion approach yields solid, sponge-like PCL/BG MPs that are structurally robust and cost-efficient, albeit with a broader size distribution.

In order to increase the amount of particles in the size range of 100–500 μm that is suitable for most bone regeneration applications [22, 25–27, 29, 30, 40, 41], we have optimized the fabrication process by varying the stirring speed and temperature of the aqueous phase, as well as the concentration of the organic phase. The stirring speed had a pronounced impact on droplet break-up and, therefore, on particle size: lower speeds (300–400 rpm) produced large, heterogeneous MPs, whereas higher speeds (< 700 rpm) yielded smaller, more uniform particles. 700 rpm speed was considered optimal because particles fabricated using higher speeds formed clumps (figure S1). Increasing the polymer concentration from 20% to 30% (w/v) broadened the size distribution and generated more particles outside the desired range, indicating that a more dilute solution

promotes uniform droplet break-up. Using the thickest organic phase (30%), in some cases, the fabricated particles were too large to fit within the microscope field of view. Emulsion temperature also modulated droplet break-up: at 20 $^{\circ}\text{C}$ the MPs were larger and exhibited the broadest size distribution, but as temperature increased to 25 $^{\circ}\text{C}$ and 30 $^{\circ}\text{C}$ the distribution shifted downward, with a greater proportion of particles in the 100–200 μm range and fewer in the 300–400 μm band. The reduction of size due to increased temperature is a result of faster solvent evaporation, reduced viscosity of the aqueous phase and surface tension of the droplets [42].

Scanning electron microscopy confirmed that the PCL/BG MPs produced via single emulsification were sponge-like with overlapping surface pores. Increasing the stirring speed reduced both particle size and surface porosity, indicating that more vigorous agitation yields denser MPs. This trend also underscores the well-known trade-off between porosity and mechanical strength: larger pores support vascularisation and osteogenesis but compromise mechanical integrity and hasten degradation [43]. Previously mentioned double-emulsion and Pickering methods can produce highly porous or even hollow MPs with large internal surface areas, leading to rapid mass loss and fragile shell structures that collapse readily [32]. In contrast, our MPs have modest surface porosity and sponge-like cores (figure S5), so they should degrade more gradually and maintain scaffold support as new tissue forms. A slower degradation profile sustains the stability of the local microenvironment as byproducts accumulate at lower concentrations, pH shifts are less abrupt, and inflammatory responses are minimised, nonetheless, the resorption must not be too slow [44]. By carefully tuning particle size, porosity, and composition, it is possible to balance mechanical support with degradation kinetics to control the microenvironment and match the pace of tissue regeneration. PCL/BG composites have previously been investigated using a range of bioactive glass compositions, including 45S5, 13–93, borate or borosilicate glasses, mesoporous bioactive glasses, and ion-doped systems [21, 45–47]. These studies established the general value of combining PCL with BG, as the polymer provides a degradable and processable matrix, while the BG phase improves the PCL's limited bioactivity by promoting apatite formation, supporting cell attachment, and enhancing osteogenic differentiation [21, 45–47]. However, the cellular response to these composites depends not only on BG content and distribution, but also on glass composition [21, 45–47]. BGs with different network structures and modifier ions differ in dissolution rate, pH response, and released ionic species, which can shift the balance between proliferation, stress response, migration, and differentiation [21, 45–48]. For example,

highly reactive BGs can provide stronger ionic stimulation but may also induce greater alkalization or osmotic stress, whereas slower-dissolving or ion-doped glasses may produce more gradual biological effects [47, 48]. Therefore, results obtained with one PCL/BG system cannot be directly generalized to another, particularly when comparing highly reactive BG 45S5 with alternative glass compositions.

In this context, the present study was designed to evaluate whether 45S5 BG, despite its high reactivity, could be incorporated into a PCL-based free-packed microparticle system while maintaining cell compatibility and osteogenic responsiveness. Most previous PCL/BG composites have been developed as predefined continuous constructs, such as porous scaffolds, electrospun fibrous membranes, or additively manufactured scaffolds [19–21]. These architectures are useful for controlled scaffold design, but they do not fully address situations where irregular defects require adaptable filling without patient-specific shaping. A free-packed system also creates a different biological environment from a continuous scaffold, since cells must attach to individual particles, bridge interparticle spaces, and form local cell-particle-cell networks [49]. In our system, embedding 45S5 BG within the PCL matrix moderated the abrupt pH shift observed for BG alone, while the MPs supported long-term cell attachment, spreading, and interparticle bridging. The reduced EdU incorporation compared with TCP should therefore not be interpreted simply as poor cytocompatibility, but as a response to a curved, discontinuous, BG-containing microenvironment in which cells appear to shift from rapid proliferation toward migration, aggregation, and early lineage commitment.

Although both EGFP-expressing DPSCs and mCherry-expressing PTECs adhered to and spread across the PCL/BG MP surface, the culture environment provided by the MPs differed substantially from TCP. Such a response is not unexpected for BG-containing substrates, as BG dissolution products can influence the balance between proliferation and differentiation depending on glass composition [47]. For example, human mesenchymal stem cells exposed to increasing amounts of 45S5 or 1393 BG MPs show progressively lower proliferation and metabolic activity, with much stronger suppression in the high-sodium 45S5 BG [47]. Moreover, extracts from BGs upregulate osteogenic differentiation-related gene expression such as *Runx2*, showing a shift toward differentiation [50, 51]. On our PCL/BG MPs, these cues likely combine with three-dimensional curvature to promote cell migration, aggregation, and early lineage commitment. Supporting this, human mesenchymal stem cells exhibited significantly higher F-actin intensity and osteocalcin levels compared to cells on flat surfaces, indicating that convex curvature promotes early osteogenic differentiation [52].

Observations that cells on convex spherical substrates adopt a snail-like configuration and must remodel a larger contact area for movement are also seen in our cells that were cultured on the MPs [53] (figures 7(A) and S6). Together, these factors shift cells from proliferation toward migration and differentiation, explaining the decline in EdU-positive cells on PCL/BG MPs. The coexistence of mesenchymal stem cells and endothelial cells on our PCL/BG particles for over 21 d demonstrates that they can support complex cell-cell interactions required for tissue regeneration (figure S7).

Interestingly, the osteogenic medium did not markedly enhance *Runx2* expression in PMSCs, which already exhibited high baseline levels on day 1 (mean $\Delta Cq_{(Runx2-Gapdh)}$ of 8.278 on day 1), suggesting an intrinsic osteogenic predisposition. Nevertheless, they still demonstrated the ability to differentiate into adipocytes, as evidenced by the gene expressions of *Pparg* and *Fabp4*. The MPs did not impair osteogenic differentiation and modestly attenuated early adipogenic gene expression under adipogenic conditions. This was further supported at the functional level by the presence of ALP-positive regions at later time points. In contrast to TCP, where ALP activity was broadly distributed, staining on MPs was spatially confined to discrete regions associated with cell accumulations. This pattern likely reflects the three-dimensional and discontinuous nature of the microparticle substrate, which promotes localized cell-cell interactions and restricts cell spreading. This behavior may be further explained by enhanced cell migration on the MPs, potentially driven by BG-derived ionic cues, followed by the formation of multicellular aggregates spanning multiple particles. Within these clusters, cells likely experience increased cell-cell interactions and localized microenvironments that promote matrix deposition and spatially confined differentiation. Rather than indicating reduced osteogenic potential, this suggests that differentiation occurs in a spatially organized manner, with osteogenic activity concentrated at sites of higher cell density, consistent with the localized microenvironments observed during early bone formation [54].

There are, however, several limitations. While the single-emulsion method enables reproducible fabrication of PCL/BG microparticles in a relatively simple and scalable manner, it inherently provides limited control over particle uniformity and internal architecture. As droplet formation during emulsification is governed by shear forces, the resulting MPs exhibit a relatively broad size distribution and predominantly dense cores with modest surface porosity. Surface modification with gelatin was therefore applied to enhance hydrophilicity and improve cell compatibility, partially mitigating the intrinsic hydrophobicity of PCL [55, 56]. Increasing the

organic-phase concentration or lowering the stirring speed produced oversized particles ($>500\text{ }\mu\text{m}$) and heterogeneous MPs, while very high stirring speeds caused clumping, illustrating the difficulty of achieving a narrow size distribution with this method. Compared with more advanced fabrication techniques, such as double-emulsion strategies or microfluidic approaches, single emulsification offers less precise control over pore architecture and structural homogeneity. Future optimization using such techniques may enable improved architectural uniformity, enhanced mass transport properties, and more controlled three-dimensional microenvironments. Nonetheless, within the scope of the present study, the fabricated MPs provided a suitable and biologically supportive platform for long-term culture and differentiation. Experiments despite these limitations, the results suggest that PCL/BG MPs synthesized by emulsion solvent evaporation method are a promising tool for bone regeneration. Producing solid MPs in the $100\text{--}500\text{ }\mu\text{m}$ range provides a favorable balance of surface area and mechanical persistence: the particles offer a durable, osteoconductive framework that can be tightly packed but still permits cell attachment and eventual vascular or cellular penetration. This combination of attributes is an advantage of our emulsion-based approach, even if the initial void fraction is lower than in highly porous or monodisperse systems. Future optimizations to increase cell infiltration without sacrificing structural benefits, to tune composition and surface characteristics, and to integrate the particles into modular tissue constructs, coupled with thorough *in vivo* evaluation, will be key steps towards translating this technology into clinical applications.

5. Conclusion

The present study demonstrates that emulsion-synthesized PCL/BG MPs can be reliably produced and tailored to the $100\text{--}500\text{ }\mu\text{m}$ size range by adjusting emulsion parameters. Scanning electron microscopy confirmed their round morphology and interconnected surface pores. *In vitro* evaluation showed that the MPs supported adhesion and responsiveness to lineage-specific induction. Although proliferative activity on the MPs was limited, their ability to promote directed differentiation highlights their promise for regenerative applications. Notably, the MPs appeared to attenuate early adipogenic commitment, while osteogenic differentiation responses were preserved and comparable to the control surface under osteogenic induction. Future research should explore strategies to further enhance cellular response, such as adjusting surface chemistry to improve adhesion or incorporating doped bioactive glass for controlled ion release.

Assessing mechanical performance in aggregated constructs and undertaking *in vivo* studies using bone defect models will be essential to translating this technology into clinically viable bone regeneration therapies.

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Data availability statement

The data cannot be made publicly available upon publication because no suitable repository exists for hosting data in this field of study. The data that support the findings of this study are available upon reasonable request from the authors.

Supplementary data 1 available at <https://doi.org/10.1088/1748-605X/ae834e/data1>.

Conflict of interest

The authors declare no competing interests.

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