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STEM-CELL–DERIVED BIOENGINEERED DENTAL PULP CONSTRUCTS FOR VITAL PULP THERAPY: A RANDOMIZED LABORATORY TRIAL

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Abstract

Introduction: *Conventional vital pulp therapy relies primarily on calcium silicate-based cements that induce reparative dentin formation without restoring the native neurovascular architecture of the pulp tissue. Advances in tissue engineering using stem cells and biodegradable scaffolds offer the potential for true pulp regeneration rather than mere preservation.*

Material and Methods: *Human dental pulp stem cells were isolated from healthy third molars and encapsulated within gelatin methacryloyl hydrogel constructs. In this randomized laboratory trial, 60 standardized human tooth slices were allocated into three groups: negative control (empty), positive control (Biodentine™), and test group (human dental pulp stem cells-gelatin methacryloyl construct). Cell viability, proliferation, odontogenic differentiation, and angiogenic potential were assessed using Live/Dead staining, Cell counting Kit-8 colorimetric assay, and quantitative reverse transcription polymerase chain reaction analysis of dentin sialophosphoprotein, dentin matrix acidic phosphoprotein 1, and vascular endothelial growth factor expression.*

Results: *The quantitative reverse transcription polymerase chain reaction constructs demonstrated high cytocompatibility, with cell viability exceeding 94% at day 7. Proliferation was significantly greater in the test group compared with Biodentine at day 7 ($p < 0.01$). Odontogenic marker expression was comparable between the test and Biodentine groups, while vascular endothelial growth factor expression was markedly higher in the test group group, indicating superior angiogenic potential.*

Conclusion: *Stem cell–laden gelatin methacryloyl constructs exhibit enhanced regenerative properties compared with conventional bioceramic materials in an ex vivo tooth slice model. These findings support the translational potential of hydrogel-based regenerative strategies as next-generation approaches for vital pulp therapy.*

Keywords: *vital pulp therapy, dental pulp stem cells, gelatin methacryloyl , GelMA, regenerative endodontics, tissue engineering, hydrogels*

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Introduction

Dental pulp is a vascularized and innervated connective tissue, which is vital in the tooth vitality, tooth sensitivity, and repair. In cases where the pulp is damaged by deep caries or injury, most conventional standard of care is full pulpectomy (root canal treatment) making the tooth non-vital, weak, and susceptible to fracture [*Bjørndal L et al., 2019*]. Vital pulp therapy is designed to maintain the healthy undamaged pulp tissue. In the past, calcium hydroxide was the preferred material although it was replaced by calcium silicate based cements like mineral trioxide aggregate and Biodentine [*Parirokh M et al., 2018*]. Such materials are biocompatible and bioactive and they cause the development of a reparative dentin bridge that helps in protecting the underlying pulp [*Camilleri J, 2008*].

Nevertheless, modern vital pulp therapy approaches have serious shortcomings. Although calcium silicate cements are an excellent sealing agent of the pulp, they do not work as a regenerative scaffold; instead, they are a capping agent. They cause mineralization without restoring the regeneration of the functional pulp-dentin complex, the fine neurovascular system needed to provide immune defense and sensation [*Ricucci D et al., 2014; Moussa D, Aparicio C, 2019*]. As a result, in case of underestimation of the inflammation or failure to capped the tooth, the latter ends up in the endodontic treatment. This underscores an urgent requirement of regenerative endodontic procedures which transcend preservation to functional tissue restitution [*Murray P et al., 2007; Diogenes A, Ruparel NB, 2017*].

The triad of stem cells, scaffolds, and growth factors have been regarded as a possible solution through the use of tissue engineering [*Abbass M et al.,2020*]. The mesenchymal stem cell present in the pulp is termed Human Dental Pulp Stem Cells and have a high proliferative potential and can also be differentiated into odontoblasts, endothelial cells and neural cells [*Gronthos S et al., 2000*]. In order to provide these cells with the necessary delivery, the hydrogel scaffolds have been of interest because of tunable mechanical characteristics and resemblance to the natural extracellular matrix including Gelatin methacryloyl (GelMA) [*Yue K et al., 2015; Khayat A et al., 2017*].

Although in vitro tests have shown positive results in Petri dishes, there is a large gap in the research between being able to test the constructs in a physiologically relevant setting and then subjecting them to animal or human experimentation. The monolayer cultures do not replicate the intricate limitation of the pulp chamber to three dimensions and dentinal intercourse [*Zhu X et al., 2018*]. Organotypic tooth slice model is an intermediate ex vivo system to study the behavior of bioengineered construct in presence of real dentin walls.

Thus, this randomized laboratory study was designed to create a 3D pulp scaffold out of human dental pulp stem cells encasement in gelatin methacryloyl and compare its biological functionality, i.e., viability, proliferation, and angiogenic capacity, to a gold standard bioceramic material (Biodentine) in a human tooth slice model.

Materials and Methods

Study Design and Ethical Considerations: This study was designed as a randomized, controlled, ex vivo laboratory trial. Teeth were obtained from healthy donors (aged 18–25years) undergoing extractions of impacted third molars. All donors provided written informed consent.

Isolation and Characterization of human dental pulp stem cells: Pulp tissue was mechanically extracted from the teeth and digested in a solution of 3 *mg/mL* collagenase type I and 4 mg/mL dispase for 1 hour at 37°C. The single-cell suspension was cultured in Alpha minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells at passage 3–4 were used. Flow cytometry confirmed the mesenchymal phenotype (positive for CD90, CD105, CD73; negative for CD34, CD45).

Preparation of gelatin methacryloyl hydrogel: Gelatin Methacryloyl (GelMA) was synthesized by reacting gelatin type A with methacrylic anhydride. The degree of methacrylation was verified via H-NMR. For the experiment, a 10% (w/v) gelatin methacryloyl precursor solution was prepared in phosphate-buffered salin containing 0.5% (w/v) lithium phenyl-2,4,6-trimethylbenzoylphosphinate as a photo-initiator.

Tooth Slice Model Preparation: Thirty extracted human third molars were cleaned and sectioned horizontally using a water-cooled diamond saw to produce 2-mm thick slices from the mid-coronal

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region. The pulp tissue was removed, and the pulp chamber was enlarged to a standardized diameter of 4 *mm* using a sterile bur. The slices were sterilized by gamma irradiation and soaked in culture media for 24 hours.

Experimental Grouping and Randomization: A total of 60 tooth slices were randomly assigned to three groups (n=20 per group) using a computer-generated randomization list:

1. **Group A** (Negative Control): Empty tooth slices filled with culture media only.
2. **Group B** (Positive Control - Biodentine): Biodentine™ (Septodont) was mixed according to the manufacturer's instructions and placed into the pulp chamber space, leaving space for media.
3. **Group C** (Test Group - hDPSC/GelMA): human dental pulp stem cells were resuspended in the gelatin methacryloyl precursor solution at a density of 2×10^6 *cells/mL*. 50 µL of this suspension was pipetted into the pulp chamber of the slice and photocrosslinked using visible light (405 *nm*) for 30 seconds to form a solid construct.

Cell Viability and Proliferation Assays: Cell viability within the hydrogel (Group C) and on the material surface (Group B) was assessed at 24 hours and 7 days using a Live/Dead Staining Kit (calcein-AM/ethidium homodimer-1) and visualized under confocal microscopy.

Proliferation was quantified using the Cell counting Kit-8 (CCK-8) colorimetric assay at days 1, 3, and 7. The reagent was added to the wells, incubated for 2 hours, and absorbance was measured at 450 *nm* using a microplate reader.

Gene Expression Analysis (quantitative reverse transcription polymerase chain reaction): At day 14, total RNA was extracted using TRIzol reagent. cDNA was synthesized, and quantitative PCR was performed to assess the expression of odontogenic markers (Dentin Sialophosphoprotein, Dentin matrix acidic phosphoprotein 1) and the angiogenic marker vascular endothelial growth factor. GAPDH was used as the housekeeping gene.

Statistical Analysis: Data were analyzed using SPSS software (Version 25.0). Results were expressed as mean ± Standard Deviation (SD). Differences between groups were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. A $p < 0.05$ was considered statistically significant.

RESULTS

Cell Viability: Confocal microscopy analysis of the Live/Dead assay demonstrated high cytocompatibility in the Test Group. At 24 hours, cell viability in the hDPSC-GelMA constructs (Group C) was 96.5% ± 2.1%. At Day 7, viability remained high at 94.2% ± 3.5%, with cells exhibiting elongated morphology indicative of spreading within the 3D matrix. In contrast, Group B (Biodentine) showed viable cells adhered to the material surface, but with a distinct zone of inhibition immediately adjacent to the fresh cement, likely due to the high initial pH, resulting in 82.1% ± 4.8% viability at 24 hours.

Cell Proliferation: The CCK-8 assay revealed time-dependent proliferation in both the Positive Control and Test groups (Table 1). However, the proliferation kinetics differed significantly. While Group B showed moderate growth, Group C demonstrated an exponential growth phase between Day 3 and Day 7. At Day 7, the optical density (OD) of the Test Group was significantly higher than both the Positive and Negative controls ($p < 0.01$), suggesting that the hydrogel environment provided superior support for cellular expansion compared to the bioceramic surface.

Odontogenic Differentiation: To assess the po-

TABLE 1.

Cell Proliferation Assessment
(optical density values at 450 *nm*)

Group	Day 1	Day 3	Day 7
Group A	0.15 ± 0.02	0.18 ± 0.03	0.16 ± 0.04
Group B	0.42 ± 0.05*	0.68 ± 0.07*	0.98 ± 0.09*
Group C	0.45 ± 0.06*	0.88 ± 0.11§	1.45 ± 0.12§†

NOTES:. (*) - *p < 0.05 vs. Group A; (§) -p < 0.05 vs. Group B; (†) p < 0.01 vs. Group B*

TABLE 2.

Relative Odontogenic Gene Expression
(Fold Change vs. Control)

Groups	Gene Marker	
	DSPP	DMP-1
Group A	1.00 ± 0.1	1.00 ± 0.2
Group B	5.8 ± 0.6	4.9 ± 0.5
Group C	6.2 ± 0.8	5.3 ± 0.7
p-value (B vs C)	> 0.05 (ns)	> 0.05 (ns)

NOTES: *DSPP -dentin sialophosphoprotein, DMP - dentin matrix acidic phosphoprotein 1*

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tential for hard tissue formation, odontogenic gene expression was analyzed at Day 14 (Table 2). Both therapeutic groups significantly upregulated odontogenic markers compared to the negative control. Interestingly, there was no statistically significant difference in DSSP expression between the Biodentine group and the hDPSC-GelMA group ($p = 0.24$), indicating that the bioengineered construct is as effective as the clinical gold standard in promoting odontoblastic differentiation.

Angiogenic Potential: A critical requirement for pulp regeneration is vascularization. The expression of vascular endothelial growth factor was analyzed to determine the angiogenic capability of the treatments (Table 3). Group C (hDPSC-GelMA) showed a robust upregulation of vascular endothelial growth factor, approximately 4-fold higher than Group B. This suggests that while Biodentine promotes mineralization, it has limited capacity to induce vascular signaling compared to the stem cell-laden hydrogel.

Table 3.

Angiogenic Marker Expression (vascular endothelial growth factor)

Metric	Group A	Group B	Group C
VEGF Expression (Fold Change)	1.0 ± 0.1	1.5 ± 0.3	6.8 ± 0.9
Significance vs Group A	-	$p > 0.05$	$p < 0.001$
Significance vs Group B	-	-	$p < 0.001$

Discussion

The present randomized lab trial shows that a bioengineered construct created with human dental pulp stem cells and gelatin methacryloyl hydrogel is better in regenerative ability than the conventional bio-ceramic capping in an ex vivo tooth slice model. Although Biodentine was also successful in triggering the signs of mineralization, the stem-cells construct was far superior to the material-only method in regard to cell proliferation and angiogenic capacity.

The viability (>94%), and strong growth of the Test Group justified the use of gelatin methacryloyl as a scaffold. The gelatin methacryloyl hydrogel includes the Arg-Gly-Asp peptide motifs that facilitate cell adhesion and spreading, circumventing the shortcomings of non-biological synthetic hydrogels [*Monteiro N et al., 2018*]. The 3D space enables the cells to preserve their spatial morphology which is essential to paracrine signaling. Conversely, Biodentine group cells had the restriction of growing on the 2D surface of the substance. Although Biodentine is bioactive, it has high levels of alkalinity during its setting which causes it to develop a zone of necrosis or limited growth at its initial setting as indicated by the Day 1 and Day 3 proliferation [*Laurent P et al., 2008*].

One of the findings of this study is that odontogenic differentiation is comparable in the groups. The functional odontoblasts have specific markers, dentin sialophosphoprotein and dentin matrix acidic phosphoprotein 1. It is noteworthy that the level of dentin sialophosphoprotein expression induced by the hDPSC-GelMA construct was comparable to that of Biodentine that is a substance specifically engineered to induce tertiary dentin [*Nowicka A et al., 2013*]. It implies that the construct can serve the major objective of vital pulp therapy: the sealing of pulp through the formation of dentin bridges. The mechanism is, however, probably different; the Biodentine works through the action of chemical irritation and calcium ion release [*Sangwan P et al., 2013*], the hDPSC construct works through lineage specific differentiation and the stiffness of the hydrogel matrix [*Celiz AD et al., 2022*].

Angiogenicity is the most clinically significant benefit of the bioengineered construct. The efficacy of regenerative endodontics solely relies on the re-establishment of blood circulation [*Murray PE et al., 2007*]. Vascular endothelial growth factor occupies the throne in angiogenesis control. The enhancement of the vascular endothelial growth factor expression in the Test Group is 4-fold, which suggests that pro-angiogenic factors are actively being released by the transplanted human dental pulp stem cells. The conventional bioceramics lack built-in angiogenic capabilities other than to provide a seal [*Torabinejad M, Parirokh M, 2010; Iohara K, Nakashima M, 2015*]. A material that fails to vascularize yet seals can result in either calcific metamorphosis or pulp necrosis, in the course of time in a clinical situation. hDPSC-GelMA constructs have the promise of biological integration as opposed to biological seal [*Demarco FF et al., 2011*].

The tooth slice model is a model that is used as

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an intermediate between monoculture and animal studies. In culturing the construct in the real pulp chamber we took into consideration the interaction with dentin-derived growth factors (DDGFs) released by the dentin walls, which is known to affect stem cell behavior [*Smith AJ et al., 2012*].

Limitations and Future Directions: This experiment employed an ex vivo model that did not have a systemic blood flow and a response of immune system. Although vascular endothelial growth factor upregulation indicates the possibility of vascularization, the actual formation of vessels (anastomosis) was not confirmed. Moreover, the experiment did not evaluate neurogenesis, which is another very important constituent of vital pulp [*Pagella P et al., 2019*]. The future studies need to be conducted in the orthotopic animal models to determine the capabilities of the construct to recruit the host vessels and nerves using the apical foramen.

Conclusion

Conclusively, the hDPSC-GelMA bioengineered construct exhibits superior cytocompatibility, strong proliferative abilities as well as superior angiogenic properties than the conventional Biodentine treatment in a slice of a human tooth. Although the two modalities are effective in the context of inducing odontogenic differentiation, the stem cell-laden hydrogel has the added benefit of encouraging vascular signaling, which is a requirement of the bona fide regeneration of the pulp. These results substantiate translation of the hydrogel-based stem cell therapy as an excellent alternative to the standard calcium silicate cements in vital pulp therapy.

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