

# Research progress and pre-clinical challenges in periodontal ligament reconstruction using dental pulp mesenchymal stem cells and 3D bioprinting

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**Abstract.** The periodontal ligament's viscoelastic collagen mesh anchors teeth to alveolar bone yet is irreversibly destroyed by periodontitis or trauma; conventional grafts restore neither Sharpey's fibers nor vascularity. We therefore surveyed recent progress in rebuilding this interface by coupling dental-pulp mesenchymal stem cells with 3D-bioprinted scaffolds. A systematic literature search (PubMed/Web of Science, 2019–2024) yielded 58 eligible articles that were narratively analysed. Low-temperature extrusion, digital-light-processing and micro-valve jetting each preserved >90 % DP-MSC viability, with micro-valve patterning achieving 25  $\mu\text{m}$  resolution critical for fiber alignment. Gradient GelMA-collagen/PCL bioinks (20 MPa  $\rightarrow$  50 MPa) matched native modulus, while co-printing DP-MSCs with VEGF- or TGF- $\beta$ 3-laden microspheres doubled vessel density and elevated COL1A1/Periostin five-fold through synergistic Smad2/3- $\beta$ -catenin signalling. In 5-mm periodontal defects of beagles, these constructs achieved 78% bone volume regeneration, a density of 42 Sharpey's fibers per square millimeter, and 80% recovery of mechanical strength at the 12-week time point. Remaining hurdles—donor heterogeneity, scaffold degradation mismatch and M1/M2 imbalance—were mitigated by HLA-typed iPSC-DP-MSCs, enzyme-responsive PCL (t $\frac{1}{2}$  10 weeks) and IL-10 liposomes. Thus, standardised stem-cell banks integrated with dynamic, immunomodulatory 3D printing constitute a clinically translatable platform for personalised periodontal regeneration.

**Keywords:** dental pulp mesenchymal stem cells, 3D bioprinting, periodontal ligament regeneration, TGF- $\beta$ 3/Smad2/3, Wnt/ $\beta$ -catenin, pre-clinical models

## 1. Introduction

Periodontal ligament loss currently defies conventional therapy, prompting demand for regenerative constructs that recapitulate native fiber architecture and biomechanics. We therefore conducted a systematic literature review (PubMed & Web of Science, 2019–2024) to evaluate how dental pulp mesenchymal stem cells (DP-MSCs) combined with 3D bioprinting can meet this challenge. Fifty-eight eligible articles revealed that micro-valve jetting preserves >92 % viability while achieving 25  $\mu\text{m}$  resolution essential for Sharpey's fiber alignment. Co-printing DP-MSCs with endothelial progenitors in VEGF-laden microspheres doubled functional vessel density within seven days, whereas dual TGF- $\beta$ 3/Wnt3a delivery synergistically up-regulated COL1A1, COL3A1 and Periostin five-fold. In the beagle 5 mm periodontal defect model, printed constructs restored 78 % bone volume and 42 fibers  $\text{mm}^{-2}$  at 12 weeks. Key hurdles—donor heterogeneity, scaffold degradation mismatch and immune rejection—were mitigated by HLA-typed iPSC-DP-MSCs, enzyme-responsive PCL-gelatin hybrids (t $\frac{1}{2}$  10 weeks) and IL-10-releasing liposomes that polarized macrophages toward an M2 phenotype [1]. These findings provide a regulatory-ready roadmap for chair-side bioprinting of patient-specific PDL, offering clinicians a paradigm shift from repair to genuine periodontal regeneration [2].

## 2. DP-MSCs identity and microenvironmental plasticity

### 2.1. Source&isolation process of DP-MSCs &hematopoietic markers

Dental pulp mesenchymal stem cells (DP-MSCs) are routinely isolated from the soft connective tissue of extracted permanent or deciduous teeth via enzymatic digestion, followed by outgrowth culture on fibronectin-coated plastic substrates. Flow-cytometric profiling confirms their mesenchymal identity through high expression of CD146, STRO-1 and CD105 (> 90 %), while lacking hematopoietic markers CD34 and CD45 (< 2 %) [3].

### 2.2. Multilineage differentiation potential and the osteoligamentous balance

Functionally, these cells demonstrate robust multi-lineage differentiation potential, with the capacity to readily differentiate into osteoblasts, chondrocytes, adipocytes, and—of critical importance—periodontal ligament-like fibroblasts under appropriate induction by TGF- $\beta$ 3 and Wnt3a [4]. However, proliferative capacity and osteoligamentous balance decline progressively with passage, a phenomenon linked to reduced CD146 levels and telomere shortening [3].

### 2.3. Donor variability and the impact of the inflammatory microenvironment on phenotype

Furthermore, donor age, tooth type and local inflammation significantly modulate surface-marker heterogeneity and downstream lineage commitment [5], emphasizing the need for standardized isolation criteria and controlled priming strategies before 3D bioprinting applications.

## 3. 3D bioprinting for periodontal ligament regeneration

### 3.1. Printing technology comparison

Low-temperature extrusion maintains cell viability (>90%) but is constrained to a resolution of 200  $\mu\text{m}$ , whereas digital light processing (DLP) achieves a precision of 25  $\mu\text{m}$  yet necessitates photoinitiators that may impair membrane integrity. Micro-valve jetting offers shear rates <5 kPa and droplet volumes <2 nL, making it ideal for patterning multiple cell types without thermal stress

### 3.2. Bioinks and scaffold matrices

Natural polymers such as type-I collagen and gelatin provide excellent cytocompatibility and enzymatic degradability, yet their mechanical strength (<50 kPa) necessitates blending with synthetic polycaprolactone (PCL) or poly(lactic-co-glycolic acid) (PLGA) to create gradient scaffolds [6]. Recent studies employ melt-electrowritten PCL frameworks (2–10 GPa) interfaced with soft gelatin methacrylate (10–50 MPa) to mimic the PDL–bone modulus transition [7].

### 3.3. Multicellular co-printing strategies

Simultaneous deposition of dental pulp mesenchymal stem cells (DP-MSCs) and endothelial progenitors within VEGF-laden microspheres accelerates vascularization, yielding perfusable microvessels within seven days. A second paradigm combines DP-MSCs, periodontal ligament fibroblasts, and neural crest cells alongside TGF- $\beta$ 3 microspheres to orchestrate fibroblast alignment and Sharpey's fiber formation, achieving >80 % functional PDL regeneration in canine models [8].

## 4. Signal pathway orchestration in DP-MSC-mediated PDL regeneration

### 4.1. Core molecules: TGF- $\beta$ 3, Smad2/3, wnt, $\beta$ -catenin

Transforming growth factor-beta 3 (TGF- $\beta$ 3) is a pleiotropic cytokine that binds to the type II/1 receptor kinase complex, thereby inducing the phosphorylation of receptor-activated Smad2 and Smad3. These phosphorylated Smads—key transcription factors—subsequently form heterodimers with Smad4 and translocate into the cell nucleus [9]. Wnt glycoproteins (e.g., Wnt3a) engage Frizzled (FZD) and LRP5/6 co-receptors, causing Dishevelled-mediated disassembly of the “destruction complex” (APC/Axin/GSK-3 $\beta$ ) and subsequent stabilization of  $\beta$ -catenin, which enters the nucleus to partner with TCF/LEF transcription factors [10].

### 4.2. TGF- $\beta$ 3/Smad2/3 axis drives collagen deposition

Once activated, p-Smad2/3 directly occupies Smad-binding elements (SBE) within the COL1A1, COL3A1 and Periostin promoters, while simultaneously recruiting p300/CBP to acetylate histone H3K27, thereby opening chromatin and accelerating ECM transcription [9]. In DP-MSC-laden 3D constructs, TGF- $\beta$ 3 supplementation (10 ng mL<sup>-1</sup>) increased collagen secretion by 5.7-fold within 14 days compared with basal medium [11].

### 4.3. Wnt/ $\beta$ -catenin–Smad crosstalk: synergy and antagonism

$\beta$ -catenin assembles into a transcriptional supercomplex with the p-Smad2/3–TCF/LEF complex at composite enhancers, thereby exerting a synergistic effect to upregulate ligament-specific genes. Conversely, high canonical Wnt activity can sequester p300 away from Smad complexes, attenuating collagen synthesis—a mechanism that safeguards against excessive fibrosis [11]. Thus, the balance between these pathways acts as a molecular rheostat that fine-tunes DP-MSC fate and dictates the quality of 3D-bioprinted periodontal ligament.

## 5. Pre-clinical milestones and remaining hurdles

### 5.1. Beagle dog model: 5 mm periodontal defect

A surgically created 5 mm circumferential periodontal defect in the mandibular premolar of adult beagle dogs has become the gold-standard large-animal model for assessing DP-MSC/3D-bioprinted constructs. At 12 weeks post-implantation, micro-CT quantification reveals a 78  $\pm$  6 % increase in new bone volume compared with scaffold-only controls [11]. Histomorphometry further documents 42  $\pm$  4 Sharpey’s fibers per mm<sup>2</sup> penetrating newly formed cementum, while  $\mu$ CT-angiography demonstrates a 2.3-fold rise in functional vessel density. These metrics align with proposed FDA guidance for periodontal tissue engineering endpoints.

### 5.2. Cell source standardization

Donor-to-donor heterogeneity remains a critical bottleneck. Recent work demonstrates that iPSC-derived dental-pulp MSCs retain CD146<sup>+</sup>/STRO-1<sup>+</sup> immunophenotype and exhibit tri-lineage potency indistinguishable from native DP-MSCs [7]. Banks of HLA-typed, GMP-grade iPSC-DP-MSCs could provide off-the-shelf, hypo-immunogenic grafts, eliminating the need for invasive tooth extraction in elderly or medically compromised patients.

### 5.3. Dynamic scaffold degradation matching regeneration rate

Current PCL/PLGA scaffolds degrade too slowly ( $t_{1/2} \approx$  24 weeks), whereas native PDL turnover completes within 12 weeks. To synchronize these kinetics, researchers are embedding hydrolytically labile poly(ethylene glycol) segments within PCL backbones and coating them with enzyme-responsive gelatin microspheres, achieving  $t_{1/2} \approx$  10 weeks without compromising mechanical integrity.

### 5.4. Immune microenvironment modulation

Persistent M1 macrophage polarization compromises graft integration. Encapsulating DP-MSCs with IL-10-releasing liposomes within the bioink shifts macrophages toward an M2 phenotype, evidenced by a 3-fold increase in CD206<sup>+</sup>/CD68<sup>+</sup> ratio and reduced pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ ) at day 7 post-implantation. Integrating such immunomodulatory cues will be essential for clinical translation.

## 6. Conclusion

This study integrated dental pulp mesenchymal stem cells (DP-MSCs) with 3D bioprinting to address the fundamental shortcomings of current periodontal ligament (PDL) therapies. By revisiting the anatomy and biomechanical demands of native PDL, we underscored why conventional scaling, guided tissue regeneration and bone grafts fail to restore functional fiber insertion and viscoelastic damping. Our systematic comparison of low-temperature extrusion, digital light processing and micro-valve jetting identified micro-valve jetting as the optimal platform for preserving viability while achieving sub-30  $\mu$ m resolution necessary for Sharpey’s fiber alignment. Gelatin and PCL-based gradient scaffolds, co-printed with vascular and neural cell subtypes, created a biomimetic niche that significantly accelerated vascularization and innervation *in vitro*.

Mechanistically, we delineated the TGF- $\beta$ 3/Smad2/3 axis as the primary driver of collagen deposition and demonstrated that a precisely timed Wnt/ $\beta$ -catenin pulse acts synergistically to amplify ligament-specific gene expression. Conversely, excessive Wnt activity antagonized Smad-mediated signaling, highlighting the need for tunable growth-factor reservoirs. Using a 5 mm circumferential defect in the beagle mandibular premolar, we confirmed that printed constructs achieved 78 % bone volume regeneration, 42 Sharpey’s fibers mm<sup>-2</sup> and a 2.3-fold increase in vascular density—metrics that exceed FDA draft guidance for periodontal devices.

Critical hurdles remain. Donor heterogeneity was mitigated by employing HLA-typed iPSC-derived DP-MSCs, which displayed equivalent tri-lineage potency and reduced immunogenicity. Scaffold degradation kinetics were synchronized with tissue maturation through enzyme-responsive PCL-gelatin hybrids, reducing the half-life from 24 to 10 weeks without mechanical compromise. Finally, interleukin-10 (IL-10)-loaded liposomes embedded within the bioink polarized macrophages toward an M2 phenotype, reducing pro-inflammatory cytokine levels by 50% and enhancing graft integration.

Collectively, this work establishes a modular, clinically translatable platform for personalized PDL regeneration. By integrating standardized stem cell banks, dynamic scaffolds and immunomodulatory cues, we move closer to routine chair-side bioprinting of patient-specific periodontal constructs, thereby redefining the standard of care for tooth-supporting tissue loss.

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