

REVIEW ARTICLE

Advances in hydrogels with biomimetic mechanical properties for dental and periodontal tissue engineering and organoid applications

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Abstract

Mechanically biomimetic hydrogels have emerged as promising extracellular matrix-mimicking platforms for dental and periodontal tissue engineering because of their capacity to reproduce the mechanical, structural, and biochemical characteristics of native tissue microenvironments. By providing controllable matrix stiffness, viscoelasticity, stress relaxation, fluid shear stress, and three-dimensional topographical cues, these materials can regulate stem cell fate, coordinate epithelial–mesenchymal interactions, guide tissue morphogenesis, and promote the functional maturation and mineralization of regenerating tissues. This review summarizes recent advances in the design and application of mechanically biomimetic hydrogels for the regeneration of dentin–pulp complexes, tooth germs, periodontal ligament, alveolar bone, and other craniofacial tissues, with particular emphasis on their emerging applications in dental and periodontal organoid engineering. Key material and design parameters, including biomimetic mineralization, mechanical tunability, three-dimensional architectural organization, and dynamic crosslinking, are systematically discussed. The mechanotransductive mechanisms by which hydrogel-derived cues regulate odontogenic, osteogenic, and periodontal differentiation are further examined. In addition, advanced fabrication strategies, including microfluidics, photocrosslinking, three-dimensional bioprinting, and decellularized extracellular matrix-based composites, are reviewed in relation to multicellular organization, vascular network formation, and reconstruction of tissue-specific interfaces. Despite substantial progress, challenges remain, including the attenuation of mechanical signals during long-term culture, insufficient spatiotemporal control of multicellular organization, limited vascularization and innervation, and discrepancies between engineered mineralized tissues and native dental hard tissues. Future developments in intelligent mechanoresponsive hydrogels, high-resolution developmental mechanobiological atlases, organoid-based multiscale validation systems, and automated biomanufacturing may facilitate the transition from empirical tissue repair toward mechanically programmed dental and periodontal regeneration.

Keywords: Hydrogel; Organoid; Dental tissue; Mechanical responsiveness; Tissue regeneration

1. Introduction

Defects of dental hard tissues, including dentin and enamel, and periodontal supporting structures, whether caused by dental caries, trauma, or developmental anomalies, are among the most common oral health conditions encountered in clinical practice. Although restorative approaches such as resin composite restorations, prosthetic crowns, and dental implants can partially restore tooth morphology and function, they primarily serve as structural substitutes rather than biologically regenerating the damaged tissues. Specifically, they fail to reconstruct a vital dentin–pulp complex, a periodontal ligament with proprioceptive capacity, and a complete tooth organ with physiological attachment to the surrounding periodontal tissues and alveolar bone. Therefore, achieving the functional regeneration of damaged dental and periodontal tissues remains a central goal of regenerative medicine.¹

Achieving this goal requires experimental platforms that not only recapitulate key developmental processes and multicellular interactions but also enable systematic investigation of the biochemical and biophysical microenvironments governing tissue formation and regeneration. In this context, organoids have emerged as a rapidly developing and highly promising technology in regenerative medicine. Through their intrinsic capacities for multicellular self-organization, lineage-specific differentiation, and partial reconstruction of tissue-specific architecture and function, organoids provide more physiologically relevant systems than conventional two-dimensional (2D) cultures. They offer versatile platforms for elucidating regenerative mechanisms, screening biomaterials and therapeutic interventions, and evaluating tissue engineering strategies. Importantly, organoids also possess translational potential as transplantable living tissue constructs. Following implantation, appropriately engineered organoids may integrate with host tissues, contribute differentiated cells, and provide paracrine and developmental signals that support tissue repair and regeneration. Their generation from patient-derived cells under controllable microenvironmental conditions also creates opportunities for personalized disease modeling, autologous transplantation, and regenerative therapy development.

Within the dental field, dental tissue organoids and organoid-like models provide valuable platforms for modeling tooth development, investigating epithelial–mesenchymal interactions, and evaluating dental and periodontal regenerative strategies. Tooth development is governed by precisely orchestrated and sequential interactions between the odontogenic epithelium and mesenchyme. Classic tissue-recombination studies established the inductive role of the dental mesenchyme

and demonstrated the temporal and positional dependence of epithelial–mesenchymal interactions during tooth-germ development.^{2,3} Subsequent studies identified bone morphogenetic protein (BMP), fibroblast growth factor, sonic hedgehog, and wingless-related integration site (Wnt) signaling as central regulators of reciprocal epithelial–mesenchymal crosstalk and tooth morphogenesis.⁴ These findings have provided the biological foundation for numerous dental tissue engineering and organoid construction strategies. By recapitulating selected aspects of native tissue organization and developmental signaling, dental tissue organoids facilitate the study of odontogenic differentiation, tissue morphogenesis, and mineralized matrix formation.⁵ Nevertheless, current systems remain limited in spatial organization, cellular diversity, vascular and neural integration, and functional maturation. These limitations arise partly because many existing systems emphasize cellular composition and biochemical induction while incompletely reproducing the dynamically evolving physical microenvironment of developing dental tissues. Thus, biochemical induction alone is insufficient to fully reproduce the dynamic tissue architecture and functional maturation observed during native tooth development.

Among the various physical cues, the mechanical properties of the extracellular matrix (ECM), including stiffness, viscoelasticity, stress relaxation, and topographical architecture, are increasingly recognized as active regulators of dental and periodontal development and regeneration.⁶ Rather than functioning merely as passive structural support, ECM mechanics modulate cell adhesion, cytoskeletal organization, nuclear deformation, lineage commitment, and matrix mineralization. Foundational work by Engler *et al.*⁷ demonstrated that mesenchymal stem cell lineage commitment is strongly influenced by substrate stiffness. Discher *et al.*⁸ subsequently synthesized broader evidence showing that growth factors, extracellular matrices, and mechanical forces jointly regulate stem cell fate. Liu *et al.*⁹ reported that substrate stiffness regulates the proliferation and osteogenic/odontogenic differentiation of human dental pulp stem cells (hDPSCs) through the Wnt signaling pathway. In dental and periodontal tissues, such extracellular cues are translated into intracellular biochemical responses through mechanotransduction pathways involving integrin β 1–focal adhesion kinase (FAK), yes-associated protein/transcriptional coactivator with PDZ binding motif (YAP/TAZ), piezo-type mechanosensitive ion channel component 1/2 (Piezo1/2), and cytoskeletal remodeling. These studies provided the conceptual basis for mechanically regulated stem cell fate determination and mechanobiology-guided tissue engineering. Accordingly, the mechanical microenvironment represents a critical regulator not only of dental and periodontal tissue regeneration but also of the organization, differentiation, and maturation of dental tissue organoids.

Hydrogels are particularly attractive platforms for reproducing these microenvironments because their hydrated polymer networks resemble important features of the native ECM. By tuning polymer composition, crosslinking density, degradability, stiffness, viscoelasticity, stress relaxation, mineralization capacity, and three-dimensional (3D) architecture, hydrogels can both provide defined yet dynamically evolving microenvironments for dental and periodontal tissue regeneration and serve as matrices for constructing and interrogating organoid and organoid-like systems. Accordingly, this review advances the central argument that functional tissue regeneration and organoid maturation are governed not solely by biochemical induction but also by the stage-specific and spatiotemporal regulation of hydrogel mechanics (Figure 1).

This review summarizes recent advances in mechanically biomimetic hydrogels for the engineering of the dentin-pulp complex, tooth germ, periodontal ligament, alveolar

bone, and related tissue interfaces, with particular emphasis on their applications in dental and periodontal organoid models. It first discusses key hydrogel design parameters, including biomimetic mineralization, mechanical tunability, 3D architectural organization, and dynamic crosslinking. It then examines the mechanotransductive pathways through which hydrogel-derived cues regulate cell differentiation, tissue morphogenesis, and mineralization. Advanced fabrication strategies and multiscale validation models are subsequently reviewed, followed by an analysis of current challenges and future directions. We propose that integrating stage-specific hydrogel mechanics with developmental mechanobiology and advanced biomanufacturing may facilitate the transition from empirical tissue repair toward mechanically programmed dental and periodontal regeneration.

Table 1 compares representative dental organoid and tissue-regeneration models with respect to their cell sources, hydrogel systems, mechanical parameters, and biological outcomes.

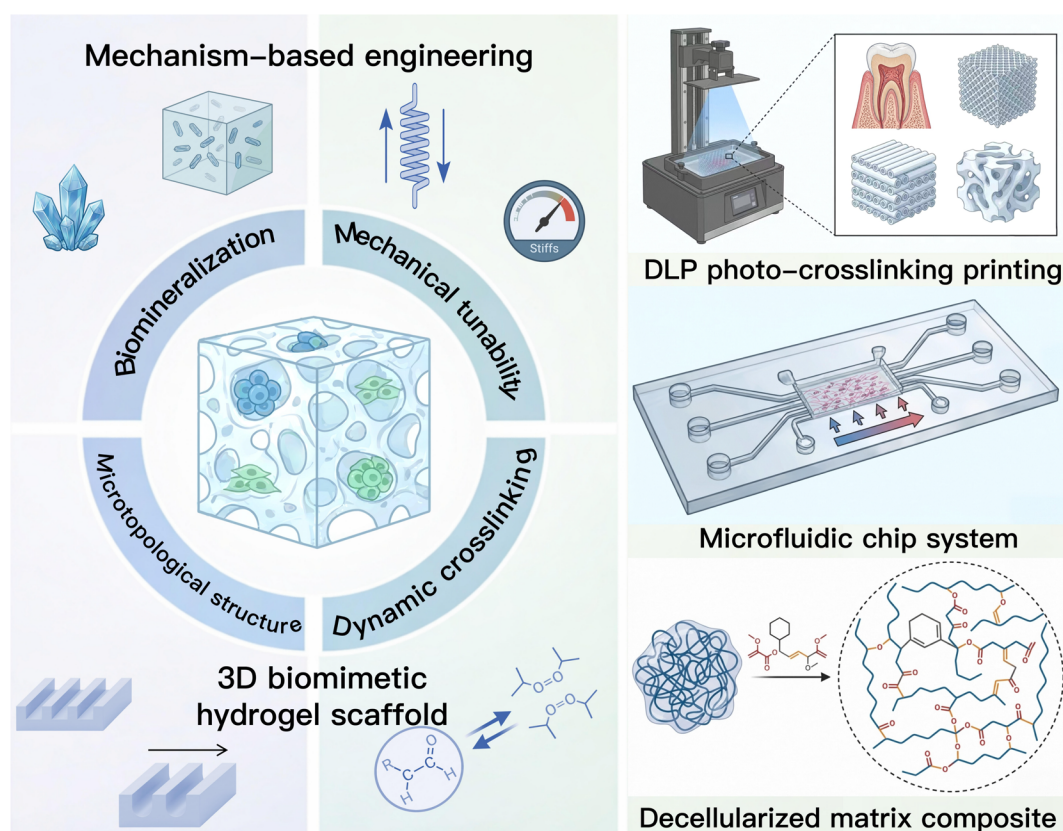


Figure 1. Schematic overview of this review. This review focuses on the design rationale and recent advances in hydrogel properties, including biomimetalization capacity, mechanical tunability, three-dimensional (3D) topographical architecture, and dynamic crosslinking. In addition, it summarizes breakthroughs in advanced biomimetic fabrication strategies such as microfluidics, photo-crosslinking, and decellularized matrix composites.

Abbreviation: DLP: Digital light processing.

Table 1. Comparison of representative dental organoid and tissue regeneration models

Model type	Cell source	Commonly used hydrogels	Key mechanical parameters	Major biological outcomes	Ref.
Tooth germ organoid	Tooth germ epithelial + mesenchymal cells	Matrigel, bioorthogonally crosslinked gelatin hydrogels	Elastic modulus 0.5–5 kPa (early stage); tunable viscoelasticity	Epithelial–mesenchymal interactions, initiation of ameloblastic and odontoblastic differentiation	10,11,12
Dental pulp organoid	DPSCs, SCAPs	GelMA, fibrin, dECM hydrogel	Elastic modulus 3–20 kPa; rapid stress relaxation	Vascular network formation, odontoblastic differentiation	13,14,15
Periodontal regeneration model	PDLSCs	F127DA (viscoelastic hydrogel), GelMA/PEGDA composite hydrogels	Elastic modulus: 29–152 kPa; $\tau_{1/2}$ = 3.17–24.69 s (tunable stress relaxation)	F127DA: Rapid stress relaxation promotes fibroblastic differentiation of PDLSCs via the integrin–FAK pathway under mechanical loading (COL-1/SCX), enhances periodontal ligament repair, and reduces root resorption and osseointegration in vivo; GelMA/PEGDA: High stiffness (48–65 kPa) promotes osteogenic differentiation of PDLSCs	16,17
Dentin mineralization model	DPSCs, SHED, DPCs	PAA–CMC–TDM composite hydrogel, GelMA mineralized scaffold	Elastic modulus 40–260 kPa; high stiffness	Upregulation of DSPP/DMP1 expression and deposition of a tubular dentin-like mineralized matrix	1,6,18
Enamel mineralization model	Dental follicle epithelial stem cells, mouse dental epithelial cells	Matrigel	Elastic modulus 1–10 kPa; surface microtopology	Ameloblast-like differentiation, upregulation of amelogenin-related	11,12,19

Abbreviations: CMC: Carboxymethyl chitosan; COL-1: Collagen type I; dECM: Decellularized extracellular matrix; DMP1: Dentin matrix protein 1; DPCs: Dental pulp cells; DPSCs: Dental pulp stem cells; DSPP: Dentin sialophosphoprotein; F127DA: Pluronic F127 diacrylate; FAK: Focal adhesion kinase; GelMA: Gelatin methacryloyl; PAA: Polyacrylic acid; PDLSCs: Periodontal ligament stem cells; PEGDA: Polyethylene glycol diacrylate; SCAPs: Stem cells from the apical papilla; SCX: Scleraxis; SHED: Stem cells from human exfoliated deciduous teeth; TDM: Treated dentin matrix.

2. Research progress on key material properties of biomimetic hydrogels

2.1. Molecular design principles of biomineralization hydrogels

The construction of mineralized models for dentin and enamel critically relies on recapitulating the ordered mineralization process of native dental hard tissues. In this context, the central principle underlying the design of biomineralization-inspired hydrogels is the establishment of a microenvironment capable of directing the nucleation and oriented growth of hydroxyapatite crystals. One effective strategy is to introduce mineralization-active sites into the hydrogel network. Huang *et al.*²⁰ identified hydrogel-forming peptides containing characteristic nucleation domains, such as α -helical conformations,

through molecular dynamics simulation and incorporated them into a gelatin methacryloyl (GelMA) matrix. By providing additional nucleation sites and strengthening organic–inorganic interfacial interactions, these peptides effectively enhanced the *in situ* mineralization capacity and compressive strength of the material. In addition, inspired by the biomineralization process, Wen *et al.*¹ used polyacrylic acid (PAA) to mimic the carboxyl-rich properties of non-collagen proteins and carboxymethyl chitosan (CMC) to mimic glycosaminoglycans, thereby constructing a dynamic network containing amorphous calcium phosphates (ACPs) through dynamic noncovalent interactions, including Ca^{2+} – COO^- coordination bonds and hydrogen bonding. Treated dentin matrix (TDM) was further incorporated into this system, yielding a PAA–CMC–TDM composite hydrogel with self-healing

capacity and injectability (Figure 2A). This system not only stabilized the gel structure and improved the mechanical properties through ACPs, but also effectively induced the odontogenic and osteogenic differentiation of dental pulp stem cells (DPSCs) by exploiting the abundance of mineralization-related proteins and cytokines within TDM.¹ These studies indicate that the precise molecular-level design of hydrogel networks, particularly the preconfiguration of mineralization templates and precursor stabilizing systems, is an important strategy for constructing scaffold-based dental hard tissue models. However, whether such mineralization strategies developed at the level of conventional tissue engineering scaffolds can be directly translated into tooth organoid systems remains uncertain. Recent studies have shown that dental epithelial organoids (DEOs) can spontaneously deposit hydroxyapatite *in vivo*, producing crystals highly similar in composition, size, and shape to those of naturally mineralized dental tissues.²¹ Likewise, dentin–pulp-like organoids derived from hDPSCs can also achieve mineral deposition in the absence of exogenous mineralization templates.²² These findings suggest that the mineralization capacity of organoids may primarily arise from cellular self-organization and directed differentiation, rather than from predesigned mineralization-active sites within the hydrogel. Nevertheless, these studies relied on Matrigel, which is characterized by substantial batch-to-batch variability and an ill-defined composition,²³ thereby limiting precise mechanistic dissection of the mineralization process. In addition, most studies have used calcium nodule formation or alkaline phosphatase (ALP) activity as criteria for mineralization, yet a fundamental distinction exists between nonspecific calcification and physiologically organized and functionally relevant mineralization within organoids, such as dentinal tubule-like structures.²⁴ How to integrate the molecular design principles of biomineralization with the stage-specific mechanical demands of tooth organoid development remains a key challenge that must be addressed in this field.

2.2. Static and dynamic mechanical regulation of biomimetic hydrogels

Natural ECM-derived materials, such as Matrigel, are compositionally complex and mechanically difficult to tune, thereby limiting precise dissection of the mechanical microenvironment in tooth organoid research.²³ By contrast, synthetic and semisynthetic hydrogels permit controlled regulation of both static and time-dependent mechanical properties, including stiffness, viscoelasticity, stress relaxation, and degradation kinetics, through polymer composition and crosslinking design. Zhang *et al.*¹⁰ developed a compositionally defined and mechanically tunable gelatin hydrogel platform based on

the bioorthogonal click reaction between tetrazine (Tz) and norbornene (Nb), enabling the *in vitro* formation of tooth germ organoids (Figure 2B). By modulating gelatin concentration and the Tz/Nb stoichiometric ratio, the elastic modulus could be precisely tuned within the range of 2–7 kPa and the storage modulus within 500–1,500 Pa. The study showed that relatively soft hydrogels successfully supported the self-organization and morphogenesis of odontogenic cells, whereas stiffer formulations suppressed cell growth. Similarly, the mechanical properties of GelMA hydrogels can be regulated by altering the weight-to-volume concentration: increasing the concentration led to a higher compressive modulus and viscosity and to reductions in pore size and swelling ratio.²⁵ Together, these findings indicate that bulk stiffness and rheological properties establish an initial mechanical window for cell proliferation, spatial organization, and lineage specification.¹⁶ Although the work by Zhang *et al.*¹⁰ demonstrated the feasibility of supporting tooth organoid formation using bioorthogonally crosslinked hydrogels, it primarily focused on the regulation of initial stiffness, while stress relaxation, a key viscoelastic parameter, and its dynamic evolution during organoid development were not systematically investigated.

In fact, viscoelasticity has been shown to exert regulatory effects on organoid development that are distinct from those of stiffness alone. Stress relaxation permits cells to remodel the matrix, whereas in elastic matrices, cell-generated tension remains constant and instead constrains dynamic matrix reconstruction.²³ This time-dependent behavior resembles that of the native ECM, whose mechanical properties continuously evolve during tissue remodeling through the reversible dissociation and reassembly of components such as collagen and glycosaminoglycans.²⁶ Dynamically crosslinked hydrogels reproduce these temporal characteristics by incorporating reversible covalent bonds or physical interactions that permit matrix remodeling, stress relaxation, and controlled degradation. For example, a double network hydrogel based on glycol chitosan achieved tunable mechanical properties through the synergistic effects of dynamic covalent bonds formed via Schiff base reactions and ionic crosslinking. Hydrogels with a moderate crosslinking density, which matched the mechanical microenvironment of native dental pulp tissue, promoted the odontoblastic differentiation and mineralization of hDPSCs in pulp-like tissue constructs *in vitro* and degraded *in vivo* in synchrony with the rate of tissue regeneration.¹³ Although this system was developed for pulp-like tissue engineering rather than tooth organoid construction, it illustrates how dynamic crosslinking can coordinate mechanical support, cell differentiation, and material degradation. The application of such dynamically evolving matrices to tooth organoids nevertheless remains

at an early stage. Liang *et al.*²⁷ developed an oxidized alginate microgel system loaded with a high density of human tooth-derived stem cells. Through Schiff base crosslinking, this system achieved a balance between initial mechanical support and rapid degradation; the microgels gradually degraded within three days, and the released cells subsequently self-aggregated to form tooth organoid-like structures. This finding suggests that transient support followed by rapid degradation may meet the mechanical requirements of early tooth-organoid development more effectively than long-term structural stability, although the precise matching between degradation kinetics and the dynamics of cellular self-assembly remains to be optimized. Rapid degradation may favor early aggregation but may not provide sufficient support for later tissue organization and mineralization. As a proof of concept from other organoid systems, Peng *et al.*²⁸ developed a dynamic matrix with stress-relaxing crosslinkers using DNA nanotechnology, enabling precise and programmable control of viscoelasticity through DNA encoding. This platform provided developmental support comparable to that of Matrigel in trophoblast organoids and induced pluripotent stem cell-derived blastoids, while exhibiting superior cell viability in mesenchymal stem cell culture. Stimuli-responsive networks may further enable pH- or temperature-dependent changes in matrix structure and mechanical behavior, providing adaptive responses to microenvironments altered by metabolic changes or inflammation.²⁴

Overall, current hydrogel designs have largely focused on achieving a specific initial stiffness, degradability, or stimulus responsiveness, while paying less attention to when mechanical transitions should occur and how rapidly they should proceed. Future studies should therefore determine how stiffness, stress relaxation, crosslinking dynamics, and degradation kinetics evolve during organoid development and how cell-mediated matrix remodeling feeds back to regulate local mechanical signaling. In particular, early morphogenesis may require soft and rapidly remodelable matrices, whereas later differentiation and mineralization may benefit from progressively enhanced mechanical support. Current evidence has not yet established the optimal sequence of these mechanical states. Matching these material transitions with the developmental chronology of tooth organoids is therefore a central challenge for mechanically programmed organoid maturation.

Because different hydrogel systems exhibit distinct mechanical properties, degradation kinetics, crosslinking mechanisms, and bioactivities, their suitability varies across dental tissue-engineering and organoid applications. Table 2 therefore summarizes the principal hydrogel systems used in current dental tissue and organoid research, together with their key mechanical characteristics, biological functions, and potential application scenarios.

Table 2. Hydrogel types and mechanical properties used in dental tissue/organoid research

Hydrogel type	Source attribute	Compatible cell types	Elastic modulus (kPa)	Stress relaxation profile	Tunable parameters	Applicable organoid models	Ref.
GelMA	Modified natural polymer	DPSCs, SCAPs	1–20	Moderate (dependent on concentration and crosslinking degree)	Photo-crosslinking density, degree of methacrylation	Dental pulp organoids, dentin mineralization models	13,14
HA	Natural	hDPSCs	0.5–5	Rapid ($\tau_{1/2} < 100$ s)	Molecular weight, crosslinking method	Dental pulp organoids, dentin regeneration research	29,30
dECM hydrogel	Natural decellularized	Multilineage dental cells	0.5–30	Tunable	Tissue source, degree of decellularization	Dental pulp organoids, tooth germ organoids	29,31
PEG-based hydrogel	Synthetic	PDLSCs	5–50	Low ($\tau_{1/2} > 1,000$ s)	Crosslinking density, molecular weight, drug loading	Periodontal regeneration models	17,32

Abbreviations: dECM: Decellularized extracellular matrix; DPSCs: Dental pulp stem cells; GelMA: Gelatin methacryloyl; HA: Hyaluronic acid; hDPSCs: Human dental pulp stem cells; PDLSCs: Periodontal ligament stem cells; PEG: Polyethylene glycol; SCAPs: Stem cells from the apical papilla.

2.3. Biomimetic microtopographical structure

Beyond bulk mechanical properties, microscale topographical features also play an important instructive role in regulating cell behavior. The emergence of patterned hydrogel technologies has created new opportunities for precisely recapitulating the microscopic morphology of tooth germ development. For example, inspired by the modulus-contrast architecture of skin, researchers embedded rigid PAA arrays into a soft polyacrylamide hydrogel to construct a heterogeneous structure with strain stiffening capability. By modulating pattern arrangement, the stress distribution across soft and stiff domains could be optimized, thereby mimicking the complex mechanical microenvironments present at interfaces such as the periodontal ligament–alveolar bone junction.³³ Microfluidic technology can likewise be used to construct organ-on-a-chip systems featuring biomimetic microgrooves or microchambers that guide the directed migration and polarity establishment of odontogenic cells (Figure 2C).³⁴ In addition, hydrogel substrates with parallel microgrooves fabricated by photolithographic patterning can induce the spontaneous alignment of dental

stem cells along the groove direction and promote their differentiation toward an odontoblast-like phenotype.³⁵ However, most studies on these topographical cues have remained at the level of 2D systems or relatively simple scaffolds, and their translation into tooth organoid systems is still at an early stage. In recent years, some studies have begun to explore the application of topographical cues in tooth organoid construction. Bektas *et al.*³⁶ seeded hDPSCs and porcine dental epithelial cells onto GelMA hydrogel microparticles with an average diameter of $158 \pm 32 \mu\text{m}$. Through gentle agitation in low-attachment culture plates, the cells underwent self-assembly and formed tooth germ organoid-like structures with an approximate volume of 50 mm^3 within 2 weeks. In this system, the curved topology of the microparticles provided an adhesive interface for the cells; however, the specific contributions of geometric parameters, including particle size, curvature, and surface roughness, to cell behavior have not yet been systematically dissected. Carvalho *et al.*³⁷ used bioprinting to fabricate GelMA scaffolds with ordered macroporous and tubular architectures and found that two distinct microstructures, grid-like and honeycomb-like, exerted markedly different

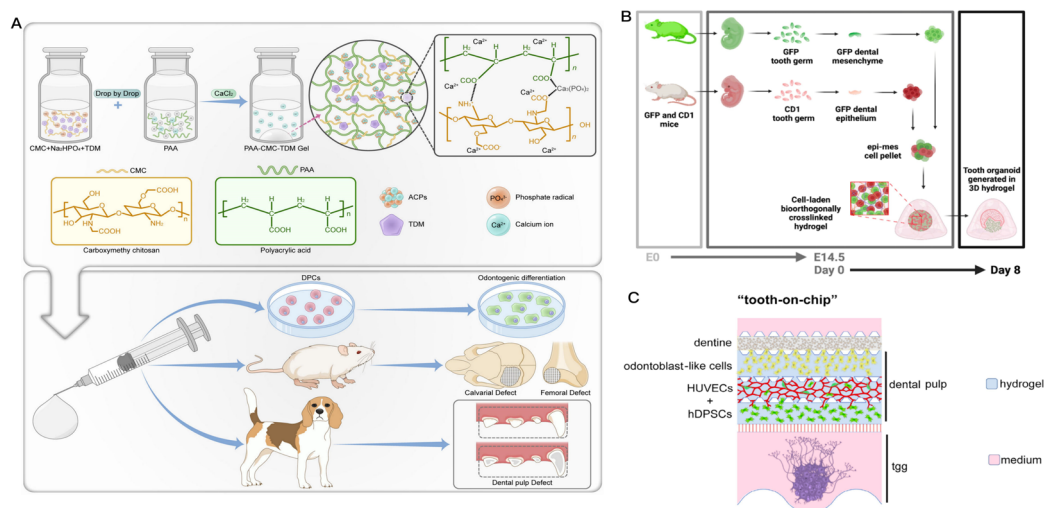


Figure 2. Biomimetic hydrogel platforms for dental tissue engineering: mineralized regeneration, tooth organoids, and microphysiological chip models. (A) Schematic illustration and experimental design of PAA–CMC–TDM mineralized hydrogel for hard tissue regeneration. Mineralized particles such as TDM or nanohydroxyapatite are uniformly dispersed in CMC/Na₂HPO₄ solution and mixed with PAA solution via dropwise addition. After complete mixing of all components, a saturated CaCl₂ solution is added to promote hydrogel formation. Reprinted from Wen *et al.*¹ (B) A gelatin hydrogel platform developed using tetrazine–norbornene bioorthogonal click chemistry successfully applied to the fabrication of three-dimensional tooth organoids: dental mesenchymal and epithelial tissues were harvested from GFP and CD-1 mouse embryos at embryonic day 14.5 (E14.5), respectively. Following enzymatic digestion into single-cell suspensions, the two cell populations were combined to form epithelial–mesenchymal cell aggregates embedded in the hydrogel (Day 0), and cultured *in vitro* to generate three-dimensional tooth organoids (Day 8). Reprinted from Zhang *et al.*¹⁰ (C) Tooth-on-a-chip model: schematic of the chip after cell seeding. The dental pulp compartment comprises a dentin layer, an odontoblast population, HUVECs, and hDPSCs; the trigeminal ganglion compartment houses trigeminal ganglion cells derived from mouse embryos. Reprinted from Cordiale *et al.*³⁴ Abbreviations: ACP: Amorphous calcium phosphate; Ca²⁺: Calcium ion; CaCl₂: Calcium chloride; CMC: Carboxymethyl chitosan; GFP: Green fluorescent protein; hDPSCs: Human dental pulp stem cells; HUVECs: Human umbilical vein endothelial cells; Na₂HPO₄: Disodium hydrogen phosphate; PAA: Polyacrylic acid; TDM: Treated dentin matrix; tgg: Trigeminal ganglion.

effects on the mineralizing differentiation of human dental pulp cells. The honeycomb-like structure promoted increased ALP activity, whereas the grid-like structure more effectively induced odontoblastic differentiation and mineralized matrix deposition. This finding suggests that topographical architecture is not merely a passive framework for morphological guidance, but rather a programmable biological cue capable of activating distinct differentiation trajectories. Nevertheless, this study still falls within the scope of conventional tissue engineering scaffolds and did not validate the regulatory effects of topographical structures on epithelial–mesenchymal interface formation or tooth germ polarity establishment in organoid systems. In addition, organ-on-a-chip technology enables dynamic fluid regulation and mechanical signal delivery through microchannel networks,⁵ thereby providing a platform for coupling topographical cues with fluid shear stress. However, current dental organ-on-a-chip models have largely focused on single tissue interfaces, such as the dentin–pulp interface, and still lack tooth germ organoid-on-a-chip systems integrating multiple cell types and complex topographical architectures. Overall, a major challenge is how to upgrade microtopographical design from contact guidance on 2D surfaces to spatial guidance of cells within organoids, and to establish a quantitative causal framework linking topographical parameters, mechanotransduction, and cell fate determination.

3. Mechanotransduction mechanisms governing the construction of dental tissue organoids

The hydrogel mechanical microenvironment contributes to dental organoid maturation not merely by providing physical support, but by activating a regulatory cascade that links mechanical input and mechanotransduction to transcriptional regulation and, ultimately, functional tissue maturation. Through interconnected signaling pathways triggered by hydrogel-derived mechanical cues, these processes ultimately determine cell fate and promote key hallmarks of maturation, including odontoblast polarization, oriented mineralization, and vascular network formation (Figure 3).

3.1. Spatiotemporal regulation of tooth germ differentiation by matrix stiffness

Matrix stiffness is not a static parameter; rather, its spatiotemporal gradients are critical for the stage-specific differentiation of tooth germ cells. During the development of the miniature pig third deciduous molar, the dental papilla exhibits a pronounced stiffness gradient along the coronal–apical axis. From embryonic day 40 to embryonic day 60, the stiffness of the coronal region increases markedly from approximately 40 kPa to 180–260 kPa, whereas that of

the apical region remains at around 30 kPa.⁶ Stiffer matrices promote paxillin maturation in dental papilla cells, thereby enhancing dentinogenic differentiation.³⁸ Pankajakshan *et al.*³⁹ further showed that DPSCs preferentially undergo endothelial differentiation on low-stiffness matrices and odontogenic differentiation on high-stiffness matrices, but this mechanosensitivity depends on sustained stimulation rather than transient exposure. However, current evidence is derived largely from 2D or simplified 3D models and primarily defines permissive initial stiffness ranges. How externally imposed stiffness interacts with endogenous cell-generated forces and evolves across developmental stages to support organoid maturation remains unclear.

3.2. Odontogenic differentiation and mineralization mediated by fluid shear stress

During tooth development and within the pulp microenvironment, cells are influenced not only by static matrix stiffness but also by fluid flow-generated shear stress. Fluid shear stress of an appropriate magnitude can markedly promote the odontogenic differentiation and mineralization of DPSCs. Lwin *et al.*⁴⁰ found that pretreatment with fluid shear stress at 0.5 Pa significantly enhanced the osteogenic differentiation capacity of hDPSCs, upregulated the expression of osteogenic marker genes and late-stage mineralization markers, and increased ALP activity and calcium nodule formation. This effect was mediated primarily through the p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway, as inhibition of p38 completely abolished shear stress-induced odontogenic differentiation. Beyond p38 MAPK signaling, mechanosensitive Piezo1/2 channels appear to act as key upstream sensors that integrate fluid shear stress with multiple downstream odontogenic pathways. Wang *et al.*⁴¹ demonstrated that under fluid shear stress stimulation, Piezo1 is activated in DPSCs and mediates extracellular calcium influx, which in turn induces the dephosphorylation and nuclear translocation of YAP through calcineurin (CaN), ultimately promoting odontogenic gene expression, including that of *DSPP* and *DMP1*, and enhancing mineralization. Pharmacological activation of Piezo1 by Yoda1 further supports the odontogenic potential of this pathway, promoting cell migration and osteogenic/odontogenic differentiation through bone morphogenetic protein 2 (BMP2) upregulation and activation of the proline-rich tyrosine kinase 2/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (PYK2/MEK/ERK) axis.⁴² Piezo1 activation may also enhance Wnt/ β -catenin signaling and the nuclear translocation of runt-related transcription factor 2, providing an additional route through which mechanical stimulation regulates odontogenic gene expression.⁴² Collectively, these findings position Piezo1 as an upstream mechanosensor that coordinates Ca^{2+} /CaN/YAP, PYK2/MEK/ERK, and Wnt/ β -catenin/Runx2 signaling

during mechanically induced odontogenic differentiation and mineralization.

Notably, the role of Piezo channels in mineralization appears to be spatiotemporally specific. Using a rat dentin injury model, Xu *et al.*⁴³ found that during the late stage of reparative dentin formation, Piezo1 and Piezo2 were broadly expressed in the odontoblast layer and dental pulp. By contrast, during the early stage of fluid shear stress loading *in vitro*, Piezo1/2 expression increased rapidly and then gradually declined, whereas the expression of mineralization-related genes, including *DSPP*, *DMP1*, and *BMP2*, increased as Piezo1/2 expression declined. Although these findings establish mechanistic links between fluid shear stress, Piezo activation, and odontogenic

mineralization, most evidence has been obtained from 2D cultures or animal models. Whether these pathways exhibit similar temporal dynamics and interact with matrix stiffness, cell–cell contact, and endogenous mechanical forces in 3D dental organoids remains unclear.

3.3. Molecular basis of cell–matrix mechanotransduction

How odontogenic stem cells sense extracellular mechanical cues and convert them into intracellular biological instructions is a central question in this field. The integrin–FAK–YAP axis in cell–matrix mechanotransduction is a common pathway in which cells sense ECM mechanics through integrins, convert force at focal adhesions into

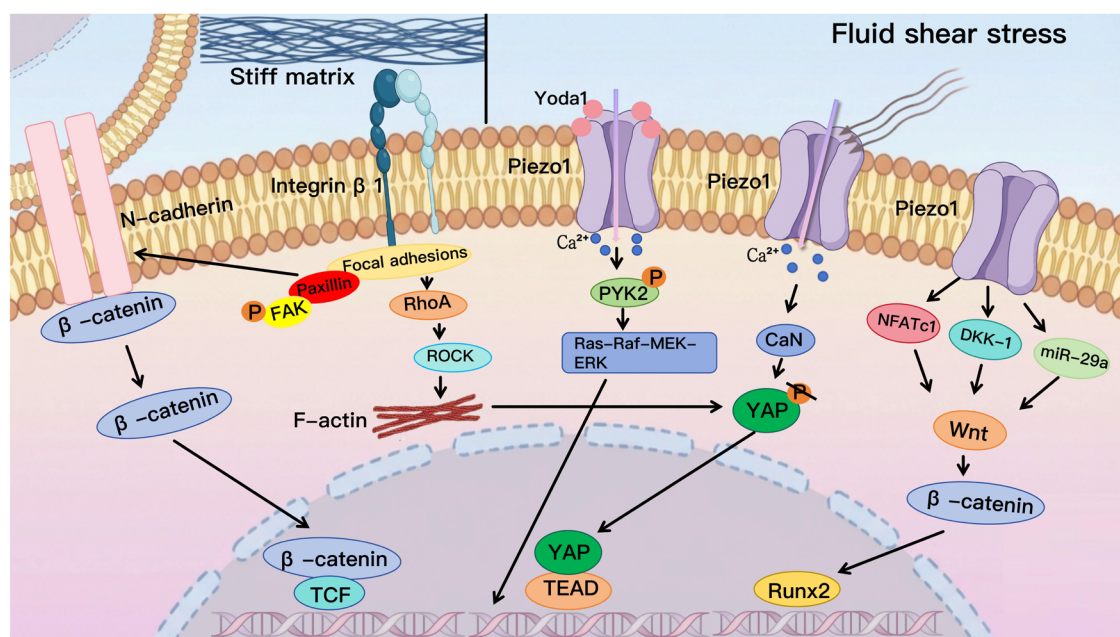


Figure 3. Schematic illustration of selected mechanotransduction pathways. From left to right: Pathway 1: On stiffer substrates, the focal-adhesion protein paxillin is significantly upregulated in dental papilla cells and forms mature punctate focal adhesions at the cell periphery. Paxillin modulates the membrane localization of N-cadherin at intercellular junctions, thereby affecting β -catenin nuclear translocation and TCF transcriptional activity, ultimately activating the expression of odontoblastic differentiation-related genes.³⁸ Pathway 2: On stiffer substrates, elevated phosphorylation levels of integrin β 1 and FAK promote focal adhesion maturation, which subsequently regulates actin cytoskeletal remodeling through the RhoA/ROCK pathway, ultimately influencing transcription factor activity.³⁸ Pathway 3: The pharmacological Piezo1 agonist Yoda1 mimics mechanical stimulation, triggering Piezo1 channel opening and Ca^{2+} influx. Upon activation, Piezo1 recruits and phosphorylates the non-receptor tyrosine kinase PYK2, which sequentially activates downstream MEK and ERK1/2, forming a complete Piezo1– Ca^{2+} –PYK2–MEK–ERK signaling cascade that efficiently transduces extracellular mechanical signals into intracellular transcriptional regulatory commands.⁴² Pathway 4: Under fluid shear stress stimulation, the mechanosensitive ion channel Piezo1 on the cell surface is activated, opening the channel and mediating substantial Ca^{2+} influx from the extracellular space, which rapidly increases the intracellular Ca^{2+} concentration. The increase in intracellular Ca^{2+} activates CaN, a phosphatase that dephosphorylates YAP, inducing conformational changes that enable YAP to escape inhibitory cytoplasmic complexes. Dephosphorylated YAP subsequently translocates into the nucleus, where it binds the transcription factor TEAD to initiate downstream transcriptional programs.⁴¹ Pathway 5: Piezo1 can also stabilize cytoplasmic β -catenin and promote its nuclear translocation, thereby activating the canonical Wnt/ β -catenin pathway and upregulating the expression and nuclear localization of the key transcription factor Runx2.⁴² Figure created by the authors with WPS Office.

Abbreviations: CaN: Calcineurin; DKK-1: Dickkopf WNT signaling pathway inhibitor 1; ERK1/2: Extracellular signal-regulated kinases 1 and 2; FAK: Focal adhesion kinase; F-actin: Filamentous actin; MEK: Mitogen-activated protein kinase kinase; miR-29a: MicroRNA-29a; NFATc1: Nuclear factor of activated T cells 1; PYK2: Proline-rich tyrosine kinase 2; RhoA: Ras homolog family member A; ROCK: Rho-associated coiled-coil-containing protein kinase; Runx2: Runt-related transcription factor 2; TCF: T-cell factor; TEAD: TEA domain transcription factor; YAP: Yes-associated protein.

biochemical signaling through FAK and associated proteins, and then regulate YAP localization and activity.^{44,45} The evidence also shows that this is not a single fixed pathway: ECM type, integrin subtype, adhesion architecture, cytoskeletal state, and force magnitude all change how strongly FAK and YAP respond.⁴⁴ YAP, a key mechanosensitive transcriptional coactivator, plays a central role in stiffness-dependent cell fate determination. In biomimetic hydrogels designed to recapitulate the high stiffness and rapid stress relaxation characteristics of young dental pulp tissue, hDPSCs exhibit marked nuclear accumulation of YAP together with reduced YAP phosphorylation. YAP knockdown attenuates the hydrogel-induced enhancement of proliferation and odontogenic differentiation. Activated YAP binds to TEA domain transcription factor 1 and upregulates downstream target genes, including *CTGF* and *CYR61*, thereby translating extracellular mechanical signals into intracellular odontogenic differentiation programs.¹⁴ In addition, the focal-adhesion protein paxillin serves as a key mechanosensory hub in matrix stiffness sensing by dental papilla cells. On stiffer matrices, paxillin expression is increased and adopts a mature punctate distribution along the cell periphery. By regulating the membrane localization of N-cadherin, paxillin influences the nuclear translocation of β -catenin and the transcriptional activity of T-cell factor, thereby activating dentinogenic differentiation-related genes.³⁸ Moreover, matrices of different stiffnesses can regulate cell fate specificity through distinct signaling networks. High stiffness promotes the expression of odontogenesis-related genes by activating Wnt signaling and regulating lipid metabolism-related genes, whereas intermediate stiffness enhances the expression of angiogenesis-related genes through enrichment of phosphoinositide 3-kinase (PI3K)–Akt and MAPK signaling pathways.¹⁸ Using a 3D self-assembled pulp organoid model generated from human dental pulp cells, Rosowski *et al.*⁴⁶ found that during the early stage of cell aggregation, traction force-mediated mechanotransduction activates transforming growth factor- β (TGF- β) signaling and downregulates Ras homolog family member A (RhoA), thereby promoting cytoskeletal remodeling and aggregate formation. Disruption of actin polymerization during this critical window completely abolished aggregation. Validation of these molecular pathways provides important guidance for tooth organoid research. The integrin–FAK–YAP axis^{14,38} offers clearly defined molecular targets for tracing how mechanical cues drive odontogenic fate in organoid systems, while the activation of distinct pathways by different stiffness ranges, such as Wnt versus PI3K–Akt,¹⁸ suggests that mechanically defined regulatory windows may exist within organoids. However, in tooth organoids containing both epithelial and mesenchymal cell populations, cell–cell interactions, such as E-cadherin-

mediated interfacial tension, may competitively regulate YAP activity. In organoids, YAP activity is likely determined by the balance between cell–matrix adhesion and cell–cell junctional tension rather than by matrix stiffness alone. This layer of regulation cannot be revealed in single-cell or single-lineage culture models and indeed constitutes a distinctive advantage of organoid systems over conventional culture platforms.

3.4. Organoid-specific validation of mechanotransduction pathways

The integrin β 1–FAK–RhoA/Rho-associated coiled-coil-containing protein kinase (ROCK) axis and YAP/TAZ nuclear translocation are currently the most extensively validated mechanosensitive pathways in 2D matrices and hydrogel culture systems. Much of the supporting evidence has been derived from monolayer or microsphere cultures of DPSCs or stem cells from the apical papilla on hydrogels with tunable stiffness.^{14,38} However, these studies have largely been based on a binary cell–material model and have not adequately considered the competitive regulation of YAP activity by cell–cell junctions in organoid systems, such as E-cadherin-mediated epithelial–mesenchymal interfacial tension. Notably, in hydrogels, matrix stiffness-induced increases in cell spreading often occur concomitantly with enhanced cell–cell contact,¹⁴ suggesting that cell–cell contact may exert a complex modulatory effect on YAP nuclear translocation. This hypothesis, however, still requires validation in organoid systems through loss-of-function and gain-of-function experiments.

Among the candidate pathways, the Piezo1/2–Ca²⁺ axis may have particular relevance for validation in dental organoids. Unlike 2D cultures, in which cells are exposed mainly to substrate-derived stimulation, cells in organoid systems are embedded within 3D matrices and may experience multidirectional compression, fluid flow, and matrix deformation.

The particular relevance of the Wnt/ β -catenin pathway in dental organoids lies in its regulation of coordinated multicellular differentiation. In single-cell culture systems, Wnt signaling primarily promotes odontogenic differentiation. By contrast, in tooth germ developmental models involving epithelial–mesenchymal interactions, Wnt gradients simultaneously regulate the fate decisions of both tissue compartments and mediate polarity establishment as well as mineralization initiation.¹⁸ This coordinated effect is a defining feature that distinguishes organoid systems from simpler biomaterial-based models and makes the Wnt-related phenotype in organoids more closely resemble the authentic state of *in vivo* tooth germ development.

Fluid shear stress-related pathways, particularly the PYK2/MEK/ERK axis, have received preliminary validation

in *in vitro* fluid-loading systems^{40,42} and may help clarify the coupling between mineralization and vascularization in 3D systems. However, in 3D organoid systems, fluid shear stress may coordinately regulate angiogenesis and mineralization, whereas this multifunctional coupling is artificially separated in conventional 2D fluid stimulation experiments. This distinction highlights the advantage of organoid models for integrative systems-level investigation.

Taken together, these pathways should be regarded as providing complementary rather than strictly hierarchical validation value. The integrin–FAK–YAP axis has the most established mechanistic foundation, Piezo1/2 is

particularly relevant to multidirectional and temporally varying mechanical stimulation, Wnt/ β -catenin is closely associated with epithelial–mesenchymal coordination, and fluid shear stress-related pathways may reveal coupling between vascularization and mineralization. Future studies should use loss-of-function and gain-of-function approaches to determine whether these pathways exhibit spatially, temporally, and lineage-specific activation patterns in multicellular dental organoids. [Table 3](#) compares the major mechanotransduction pathways in terms of their mechanical inputs, core molecular components, and downstream effects in organoid systems.

Table 3. Major mechanotransduction pathways in dental stem-cell differentiation

Signaling pathway	Mechanical activation mode	Mechanical response characteristics	Role in odontogenic differentiation	Ref.
YAP/TAZ	Matrix stiffness, stress relaxation	High stiffness + rapid stress relaxation synergistically $\rightarrow$ increased Paxillin $\rightarrow$ YAP nuclear translocation $\rightarrow$ activation of odontogenic genes; soft matrix/slow relaxation $\rightarrow$ YAP cytoplasmic retention $\rightarrow$ maintenance of undifferentiated state	Regulates DPSC fate decision, spatial patterning of dental papilla differentiation (YAP activation in high-stiffness region of the crown $\rightarrow$ odontoblast differentiation)	6,14,38
Wnt/ β -catenin	Matrix stiffness	Matrix stiffness differentially regulates Wnt activity: stiffer matrix $\rightarrow$ strong Wnt activation $\rightarrow$ osteogenic tendency; moderate stiffness $\rightarrow$ moderate Wnt activation $\rightarrow$ odontogenic differentiation; high stiffness + Wnt $\rightarrow$ regulated lipid metabolism $\rightarrow$ <i>DSPP</i> / <i>DMP1</i> expression	Regulates the osteogenic/odontogenic differentiation direction of DPSCs	9,18,19
BMP/TGF- β	ECM stiffness changes, cell–cell contact	ECM stiffness synergizes with BMP signaling to regulate odontoblastic differentiation; TGF- β signaling-dependent epithelial $\rightarrow$ ameloblastic lineage differentiation	Participates in the regulation of amelogenic/odontogenic differentiation direction in epithelial–mesenchymal interaction	6,11
Piezo1/ Ca^{2+}	Fluid shear stress, compression	Fluid shear stress opens Piezo1 $\rightarrow$ Ca^{2+} influx $\rightarrow$ p38 MAPK activation $\rightarrow$ regulation of the osteogenesis/adipogenesis balance; Piezo1 activates BMP2/ β -catenin to promote osteogenesis	Regulates mechanosensitive differentiation of periodontal ligament stem cells	40,41
Integrin–FAK	Matrix stiffness, topography	Matrix stiffness induces integrin β 1 clustering $\rightarrow$ FAK phosphorylation $\rightarrow$ ERK pathway activation $\rightarrow$ cytoskeletal remodeling $\rightarrow$ regulation of differentiation and proliferation; cumulative mechanical stimulation causes integrin β 1-mediated stem cell fatigue	Regulates DPSC responses to matrix mechanics, maintaining stemness or initiating differentiation	38,47

Abbreviations: BMP: Bone morphogenetic protein; DMP1: Dentin matrix protein 1; DPSCs: Dental pulp stem cells; DSPP: Dentin sialophosphoprotein; ECM: Extracellular matrix; ERK: Extracellular signal-regulated kinase; FAK: Focal adhesion kinase; MAPK: Mitogen-activated protein kinase; Piezo1: Piezo-type mechanosensitive ion channel component 1; TAZ: Transcriptional coactivator with PDZ-binding motif; TGF- β : Transforming growth factor-beta; Wnt: wingless-related integration site; YAP: Yes-associated protein.

4. Breakthroughs in advanced biomimetic construction technologies

4.1. Microfluidics-assisted organ-on-a-chip culture systems

Microfluidic technology provides a powerful platform for constructing physiologically relevant dynamic culture microenvironments, with its core advantage lying in the ability to recapitulate complex physical and biochemical conditions at the microscale. Such systems enable precise regulation of fluid shear stress, chemical concentration gradients, and tissue–tissue interfaces, while also supporting multicellular coculture, thereby offering a more realistic simulation of the physiological and pathological states of dental tissues. In oral tissue engineering, organ-on-a-chip technology has already shown potential as an alternative to conventional animal experiments. Oral organ-on-a-chip models now encompass multiple fields, including tooth, oral mucosa, jawbone, and salivary gland systems (Figure 4A). By reproducing perfusion-dependent fluid flow, endothelial interfaces, innervation, and biofilm–tissue interactions, these models provide a more physiologically relevant platform for investigating the responses of dental tissues to biomaterials and pathological stimuli. However, current models still fall short of fully reproducing the intrinsic complexity of oral tissues, as most incorporate only a limited number of cell types and cannot yet faithfully replicate the full mechanical and structural characteristics of native tissues.⁴⁸ To improve the physiological relevance of oral mucosal models, Phan *et al.*⁴⁹ integrated a hyaluronic acid–alginate hybrid hydrogel into a microfluidic chip to construct a full-thickness oral mucosa model. This platform enabled the stratified reconstruction of the epithelial layer, lamina propria, and endothelial network, while dynamic fluid perfusion helped maintain cell phenotype and tissue integrity. Compared with conventional Matrigel, the hybrid hydrogel more effectively promoted epithelial stratification and maintenance of fibroblast function within the chip setting. Although developed for oral mucosal modeling rather than dental organoids, this platform demonstrates how microfluidic perfusion and tissue-specific hydrogels can support stratified multicellular organization. Taken together, the synergy between microfluidic chip systems and hydrogel-based mechanical microenvironments provides an enabling platform for the transition from simple cell carriers to multifunctional microphysiological systems in the construction of pulp organoids and tooth germ organoids. Nevertheless, most current organ-on-a-chip models remain focused on 2D or quasi-3D dentin–pulp interfaces and have not yet achieved true coupling of tooth organoids with fluid shear stress. In addition, most microfluidic chips are still fabricated from materials such as polydimethylsiloxane,⁴⁸ whose mechanical properties

differ from those of native dental pulp tissue by orders of magnitude. This mismatch may interfere with cellular perception of hydrogel-derived mechanical signals.

4.2. Microscale fabrication technologies for hydrogel constructs

Microscale fabrication technologies, including microfluidic droplet generation and digital light processing (DLP), enable the construction of hydrogel-based cellular microenvironments with controlled size, geometry, and spatial organization. Using a microfluidics-assisted coaxial electrostatic droplet technique, Liang *et al.*⁵⁰ fabricated GelMA–alginate core-shell microcapsules for the 3D coculture of DPSCs and endothelial cells. This microfluidics-assisted preparation system reduced particle aggregation and the need for labor-intensive collection, both of which are commonly associated with traditional microsphere culture. Under 3D coculture conditions, endothelial cells spontaneously formed capillary-like networks while promoting the osteogenic and odontogenic differentiation of DPSCs. Photocrosslinkable hydrogels combined with advanced printing technologies such as DLP enable the fabrication of complex structures with micrometer-scale fabrication resolution while maintaining high precision. Qian *et al.*⁵¹ used a layer-by-layer DLP photocuring strategy to fabricate GelMA microspheres loaded with hDPSCs, which not only ensured efficient nutrient exchange but also significantly supported cell viability and stemness while preserving multilineage differentiation potential through their architecture. Cunha *et al.*⁵² further combined DLP-printed GelMA microgels with dentin matrix molecules, thereby enabling the sustained release of bioactive factors. Beyond hydrogel systems, Chen *et al.*⁵³ exploited DLP to fabricate personalized hydroxyapatite root scaffolds with optimized mechanical properties and nanohydroxyapatite whisker coatings that promoted DFSC osteogenic differentiation and periodontal ligament-like entheses formation *in vivo*, highlighting the broader applicability of DLP in dental hard tissue engineering. Zhu *et al.*⁵⁴ developed a DLP printing strategy based on phase separation using a cell-condensed bioink, in which dextran served as a cell-trapping agent to achieve *in situ* cell enrichment and spheroid formation within a photocrosslinked GelMA matrix, thereby markedly enhancing differentiation capacity. These studies collectively demonstrate the feasibility of DLP technology in dental tissue engineering, but its application at the level of tooth organoids remains in its infancy. Jackson *et al.*⁵⁵ found that both photocrosslinked GelMA microparticles and gelatin microspheres could support the formation of organoid-like cell clusters from hDPSCs, while a photocrosslinked GelMA composite hydrogel containing decellularized dental pulp matrix has been shown to support the formation of vascularized pulp-like tissue.⁵⁶

However, at the level of cell and microtissue construction, the endpoints of these studies have largely remained limited to differentiation markers, without systematically validating whether photocrosslinked microstructures can regulate epithelial–mesenchymal interface establishment or tooth germ polarity formation. In addition, light- and photoinitiator-associated cytotoxicity may compromise long-term organoid viability and maturation, particularly when repeated or high-dose exposure is required. Thus, further advances are still needed to move beyond the precise shaping of cell carriers toward the precise programming of developmental microenvironments for organoid formation.

4.3. Biomimetic optimization of decellularized matrix composite scaffolds

Decellularized extracellular matrix (dECM) provides tissue-specific biochemical cues that are difficult to reproduce using fully synthetic materials. Hydrogels derived from dental pulp dECM (Figure 4B) retain tissue-specific collagen components, glycosaminoglycans, and multiple growth factors, thereby supporting the viability

of DPSCs *in vitro* and promoting their odontogenic differentiation, while also facilitating *in vivo* regeneration of pulp-like tissue enriched with vascular structures and an odontoblast-like cell layer.³¹ Combining dECM with synthetic hydrogels offers a complementary strategy for engineering an optimized regenerative microenvironment. Elnawam *et al.*²⁹ extracted decellularized pulp ECM from bovine dental pulp and physically blended it with hyaluronic acid to construct a composite hydrogel. The incorporation of hyaluronic acid generated a denser hydrogel architecture, enabled the sustained release of key signaling molecules such as TGF- β 1 and fibroblast growth factor, and simultaneously preserved native matrix constituents including collagen and glycosaminoglycans. Sadeghian *et al.*⁵⁷ further integrated human dentin-derived dECM into a GelMA photocrosslinkable hydrogel containing bioactive glass. This composite system not only provided tunable mechanical strength to the scaffold but also harnessed the intrinsic bioactive signals of dECM, which acted synergistically with inorganic bioactive components to promote tissue regeneration. Although

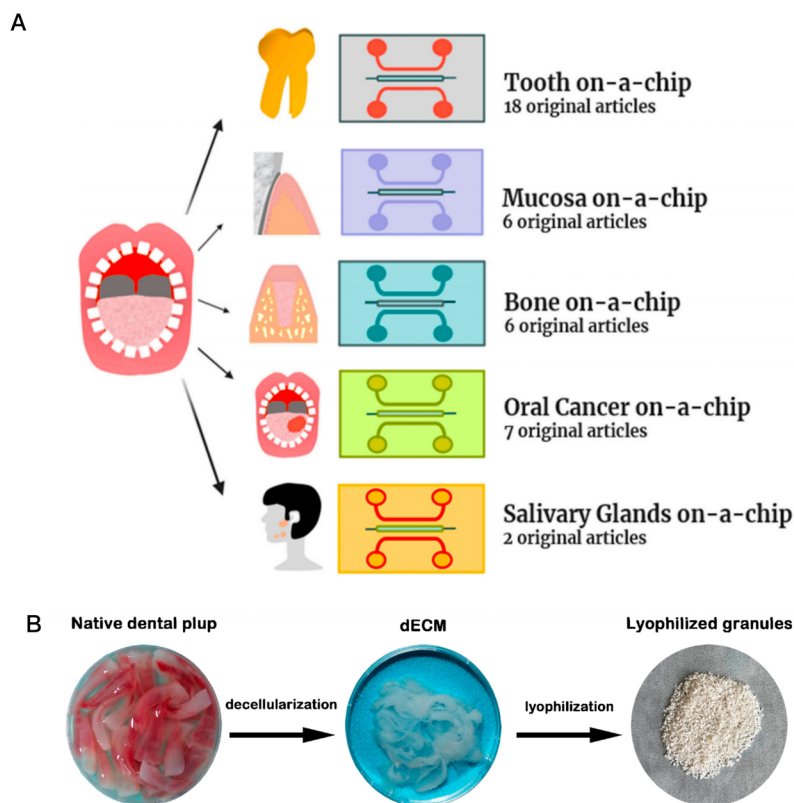


Figure 4. Representative oral tissue-derived organ-on-a-chip models and hydrogels prepared from dental pulp-derived decellularized extracellular matrix (dECM). (A) Oral tissue-derived organ-on-a-chip models encompass multiple domains, including tooth, oral mucosa, jawbone, and salivary glands. Reprinted from Pierfelice *et al.*⁴⁸ (B) Natural dental pulp tissue, dental pulp-derived dECM, and freeze-dried dECM granules. Reprinted from Yuan *et al.*³¹

the regenerative potential of dECM hydrogels in dental tissue engineering has been demonstrated in multiple studies, extending their application from conventional scaffold-based systems to organoid culture remains associated with several unresolved challenges. Xu *et al.*¹⁵ successfully generated a vascularized dental pulp organoid by coculturing human dental pulp cells and endothelial cells within a 3D matrix containing human dental pulp-derived dECM. The study directly validated the supportive role of dECM at the organoid level. However, batch-to-batch variability and the incompletely defined composition of dECM continue to limit mechanistic dissection of which specific matrix components are responsible for driving organoid vascularization. Taken together, the

integration of tissue-specific dECM with engineered hydrogel systems, including both natural polymer-based and photocrosslinkable systems, enables synergistic optimization of mechanical support, structural regulation, and bioactive induction. This hybrid strategy therefore represents a promising approach for constructing dental organoids with a more biomimetic microenvironment.

The aforementioned advanced fabrication technologies provide multidimensional technical support for the controlled fabrication of dental organoids. Table 4 compares these strategies in terms of their core principle, spatial resolution, mechanical regulation dimension, technical advantages, limitations, and potential applications in dental organoids.

Table 4. Comparison of advanced fabrication technologies for dental tissue and organoid construction

Technique	Core principle	Spatial resolution	Mechanically regulated parameter	Application in dental tissue engineering and organoid research	Technical advantages	Current limitations	Ref.
Microfluidic technology	Precise hydrodynamic control within micrometer-scale channels	10–100 μm	Tunable fluid shear stress	Vascularized dental pulp organoid modeling, multicellular coculture with dynamic perfusion, and oral mucosal model construction	Precisely simulates physiological blood flow environment; controllable real-time mechanical stimulation	Low throughput; complex chip–hydrogel interfacial integration; most platforms rely on non-biomimetic materials such as PDMS	34,48,50
DLP printing	Layer-by-layer photopolymerization using DLP	25–50 μm (printing resolution)	Controllable microsphere/microgel geometry; bulk stiffness adjustable via material composition	Construction of 3D carriers for dental pulp stem cells, sustained release of dentin matrix factors, fabrication of biomimetic tooth roots	High speed (seconds per layer); precise fabrication of complex geometries	Limited range of photoresponsive materials; potential UV-induced cell damage; high crosslinking density may compromise cell viability	51,52,53,54
dECM hydrogel conversion	Decellularization of native tissue followed by solubilization and reconstitution into hydrogel	No inherent spatial resolution (requires secondary molding techniques)	Preserves tissue-specific biochemical components and partial mechanical properties	Biomimetic microenvironment for dental pulp organoids, preservation of endogenous growth factors	High biocompatibility; retention of tissue-specific native cues	High batch-to-batch variability; limited tunability of mechanical properties; potential immunogenicity	29,31
4D bioprinting	3D printing + temporal dimension (stimulus-responsive deformation)	50–200 μm (platform-dependent)	Dynamic stiffness/shape changes (responsive to temperature, pH, light, or a magnetic field)	Dynamic morphological remodeling of tooth germ organoids (prospective application)	Temporal control over construct morphology; remote non-contact manipulation (magnetically driven)	Limited selection of responsive materials; deformation precision and controllability require improvement; lack of dental-specific evidence	58,59,60

Abbreviations: 3D: Three-dimensional; 4D: Four-dimensional; dECM: Decellularized extracellular matrix; DLP: Digital light processing; PDMS: Polydimethylsiloxane; UV: Ultraviolet.

5. Multiscale biomimetic validation models

Multiscale validation of mechanically biomimetic hydrogels requires models that examine cellular interactions, tissue-level functional integration, and cross-species conservation. Accordingly, this section discusses epithelial–mesenchymal reconstruction, vascularized organoid formation, and comparative validation across species.

5.1. *In vitro* reconstruction of dental epithelial–mesenchymal interactions

Reconstructing and validating the signaling crosstalk between dental epithelium and mesenchyme is fundamental to the successful generation of physiologically relevant tooth organoids. Hemeryck *et al.*¹¹ established an epithelial–mesenchymal composite tooth germ organoid model (Figure 5A) by coculturing human dental follicle-derived epithelial organoids with DPCs. The presence of DPCs induced the differentiation of dental epithelial stem cells toward an ameloblastic lineage, and this process was dependent on TGF- β signaling, thereby reproducing a key mesenchymal-to-epithelial inductive interaction during tooth development. Similarly, composite organoids generated using epithelial organoids derived from mouse molars and incisors together with DPCs also exhibited enhanced ameloblast-like differentiation.¹² Developmental tissue atlases provide an important *in vivo* reference for evaluating whether these organoid models reproduce physiologically relevant epithelial–mesenchymal signaling. Using single-cell transcriptomics, spatial transcriptomics, and secretomics, Zhang *et al.*¹⁹ mapped the dynamic signaling interactome between epithelial and mesenchymal cell populations during human fetal tooth development. Their study revealed that the dental epithelium acts as a major signaling source regulating mesenchymal differentiation through multiple pathways, including WNT signaling. Among these epithelial-derived signals, WNT5B was identified as a key secreted factor that promotes the differentiation of dental mesenchymal cells into odontoblasts. Although this analysis was based on developing human tooth tissues rather than organoids, the resulting signaling atlas provides a developmental benchmark for evaluating the biological fidelity of *in vitro* models. However, current composite organoids mainly validate selected lineage-induction events and have not yet fully reproduced the spatial gradients, temporal sequence, or mechanically regulated dynamics of epithelial–mesenchymal signaling. Future studies should therefore compare organoid signaling patterns with developmental reference atlases under defined mechanical conditions.

5.2. Mechanical microenvironment simulation of vascularized organoids

Functional vascularization is essential for maintaining the

viability and maturation of large dental pulp organoids, and coculture of endothelial cells with DPSCs has therefore become a widely used strategy for reconstructing the vascularized pulp microenvironment.⁶¹ Xu *et al.*¹⁵ successfully established vascularized dental pulp organoids by coculturing human dental pulp cells with endothelial cells in a 3D dECM-containing matrix, providing direct organoid-level evidence of vessel-like network formation. However, endothelial-cell inclusion alone is unlikely to generate functionally mature vasculature; perfusion-associated mechanical cues, particularly fluid shear stress, must also be reproduced.⁶² The mechanical properties of hydrogels can influence vascularization by modulating cell signaling pathways. In this context, Zhang *et al.*⁶³ developed a Notch signaling hydrogel by indirectly immobilizing the Notch ligand Jagged1 within a fibrin hydrogel possessing an appropriate mechanical modulus. In a culture environment, this bioactive matrix effectively activated the Notch signaling pathway, promoted the coordinated differentiation of DPSCs and endothelial cells, and enhanced vascular sprouting, ultimately leading to the formation of a microvascular network with lumenized structures (Figure 5B). In parallel, ephrin-B2 expressed by DPSCs has been shown to facilitate the assembly and stabilization of vascular networks through juxtacrine interactions with EphB4 receptors on endothelial cells.⁶⁴ Consistent with the importance of mechanical mimicry, Liu *et al.*⁶⁵ used 3D bioprinting to fabricate a GelMA hydrogel encapsulating stem cells from human exfoliated deciduous teeth and human umbilical vein endothelial cells, and demonstrated that its compressive elastic modulus closely matched that of native dental pulp tissue. Collectively, these studies highlight the central importance of vascularization in dental pulp regeneration. In recent years, notable progress has also been made in the generation of vascularized organoids. Liu *et al.*⁶⁶ reported that hDPSCs, when pre-induced toward an endothelial lineage under hypoxic conditions in endothelial cell-conditioned medium and then mixed with primary hDPSCs at defined ratios, were capable of self-organization and vascular network formation in 3D culture. Li *et al.*¹⁸ further engineered a triphasic mechanical architecture using GelMA hydrogels of different stiffnesses and found that high stiffness favored odontogenic differentiation, intermediate stiffness supported endothelial differentiation, and low stiffness enhanced the vascularization of periapical cells through paracrine effects. This model is particularly relevant as a multiscale validation platform because it demonstrates that spatially defined mechanical domains can coordinate odontogenic and vascular cell fates within a single construct. In addition, DPSCs under organoid culture conditions have been reported to spontaneously differentiate into CD31⁺ vascular endothelial-like cells and to form vessel-like structures within the ECM, suggesting that dental-derived cells themselves possess an intrinsic,

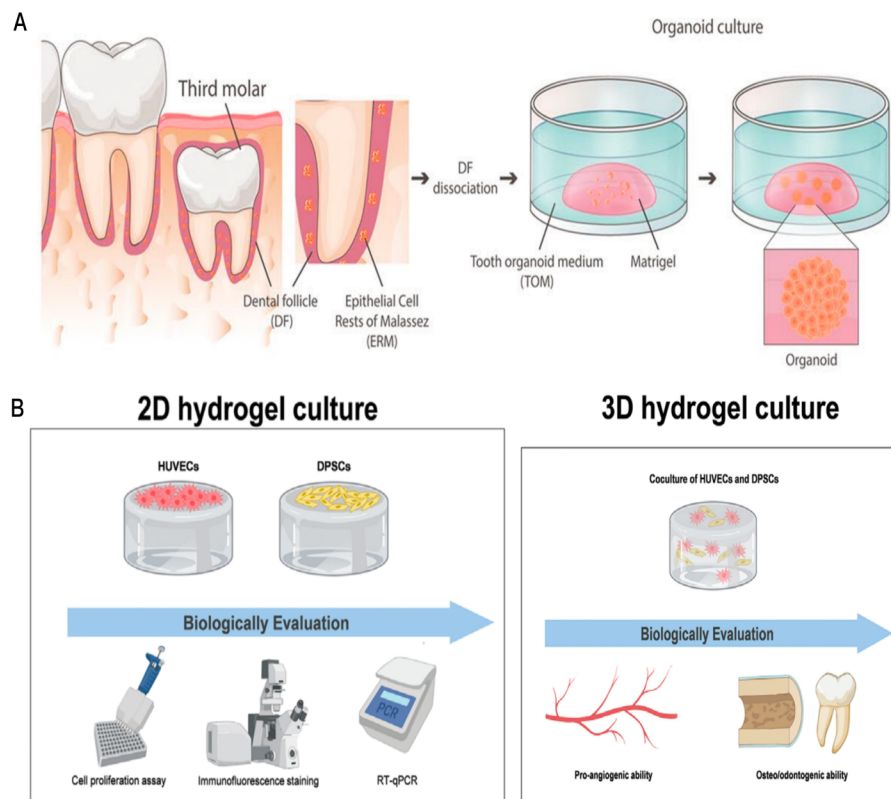


Figure 5. Multiscale biomimetic validation models comprising epithelial–mesenchymal composite tooth germ organoids, co-differentiation of dental pulp stem cells and endothelial cells, and the formation of lumenized microvascular networks via vascular sprouting. (A) Schematic of the organoid culture system. Following isolation and dissociation of dental follicle (DF) tissue, the resulting cells were seeded in tooth organoid medium (TOM) (passage 0, P0). The progressive development of organoid structures and their excellent passing capacity are shown (bright-field images at the indicated passages). Reprinted from Hemeryck *et al.*¹¹ (B) Schematics of the biological evaluation of human umbilical vein endothelial cells (HUVECs) and dental pulp stem cells (DPSCs) cultured separately on two-dimensional hydrogels and HUVECs and DPSCs cocultured within three-dimensional hydrogels. Reprinted from Zhang *et al.*⁶³

albeit limited, vasculogenic potential.⁶⁷ Despite these advances, most existing studies have used the formation of vessel-like structures as their principal endpoint, while systematic investigation of how mechanical parameters quantitatively regulate vascularization efficiency and vascular maturity is still lacking. Thus, translating the field from 2D coculture models to mechanically guided self-assembly of functional vascular networks in 3D organoids will require further breakthroughs in the quantitative design of mechanical gradients and in the long-term validation of vascular function.

5.3. Comparative studies of cross-species conserved mechanisms

The translation of insights from mouse models to human tooth regeneration depends not only on the conservation of developmental signaling pathways but also on whether

quantitative mechanical requirements are preserved across species.⁶⁸ Single-cell transcriptomic analyses have revealed substantial molecular similarity between human dental follicle-derived epithelial organoids and mouse epithelial rests of Malassez cells, with both populations expressing epithelial stem/progenitor markers, including *SOX2*, *TP63*, and *KRT5*. At the functional level, epidermal growth factor exposure similarly induces a transient proliferative activation followed by epithelial–mesenchymal transition in human DEOs, thereby recapitulating the response pattern of epithelial rests of Malassez to injury or stimulatory cues *in vivo*. Together, these findings provide molecular and functional evidence for the conservation of dental epithelial stem cells between humans and mice.¹¹ The central regulatory role of the TGF- β signaling pathway in ameloblast differentiation has likewise been validated across species models.^{69,70} However, evidence for conserved

developmental signaling does not necessarily establish that identical mechanical conditions are suitable across species. Zhang *et al.*¹⁰ demonstrated, using a bioorthogonally crosslinked gelatin hydrogel, that mouse dental cells can self-organize into tooth germ organoids in a relatively soft hydrogel environment. Equivalent responses have not yet been demonstrated in human dental cells under identical stiffness conditions. Similarly, the oxidized alginate microgel system developed by Liang *et al.*²⁷ validated the effectiveness of a temporary-support and rapid-degradation strategy in embryonic mouse dental epithelial and mesenchymal cells. Whether this strategy can be directly extended to human dental cells, however, remains to be determined.

Current evidence suggests that while dental developmental signaling is partly conserved across species, the corresponding mechanical requirements may differ. Human and mouse dental cells likely share key signaling pathways but vary in factors such as stiffness preference, responses to matrix-degradation kinetics, and developmental timing. Therefore, direct comparisons under consistent conditions are needed before applying mechanical parameters from animal models to human systems. Overall, multiscale validation should go beyond simple markers or morphology. Mechanically biomimetic dental organoids should be assessed based on their ability to replicate epithelial–mesenchymal interactions, support vascular formation, and reflect species-specific developmental features.

6. Current research controversies and technical bottlenecks

6.1. Mechanical signal attenuation during long-term culture

The long-term stability of the mechanical microenvironment is a prerequisite for the maturation and functional development of dental organoids. However, many hydrogels, particularly those based on reversible dynamic crosslinking, undergo degradation or stress relaxation during prolonged culture, resulting in attenuation of mechanical signaling.^{26,71} Although conventional chemically crosslinked hydrogels such as GelMA provide sufficient structural strength, their limited capacity for stress relaxation constrains their ability to accommodate dynamic changes in cell shape and migration, thereby restricting the self-organization and mineralization of dental pulp organoids.¹³ By contrast, dynamic hydrogels with appropriate viscoelastic properties can dissipate cell-generated traction forces through stress relaxation, thereby promoting cell spreading, matrix remodeling, and osteogenic differentiation. Yet excessively rapid mechanical decay or a mismatch between degradation kinetics and pace of tissue development may weaken their sustained regulatory influence on

organoid development.⁷² Under static culture conditions, the persistence of mechanical stimulation is inherently limited, and exogenous activation may therefore be required to compensate for signal attenuation; otherwise, organoid self-renewal and functional performance may be compromised. Accordingly, a major barrier to clinical translation lies in the design of self-stabilizing or self-reinforcing or dynamically re-crosslinking hydrogels capable of maintaining predefined mechanical properties throughout long-term culture.

6.2. Challenges in spatially precise arrangement of heterogeneous cell populations

Successful generation of tooth germ organoids requires the precise spatial positioning and ordered self-organization of epithelial, mesenchymal, endothelial, and neural cell populations within a 3D microenvironment. By pre-inducing hDPSCs toward an endothelial lineage under hypoxic conditions and in conditioned medium, and subsequently mixing them with primary DPSCs at defined ratios, investigators achieved cellular self-assembly and vascular network formation in a 3D culture system, thereby generating vascularized pulp organoids. These findings highlight the critical importance of cell-type composition and coculture conditions in governing the spatial organization of heterogeneous cell populations.^{66,73} Using single-cell RNA sequencing and spatial transcriptomics, Shi *et al.*⁷⁴ further demonstrated that dental epithelial cells can be subdivided into distinct subpopulations, including outer enamel epithelium, inner enamel epithelium, stratum intermedium, and ameloblasts, whereas dental pulp mesenchymal cells comprise multiple subtypes such as apical papilla, distal pulp, and odontoblast populations. These subpopulations exhibit highly ordered spatial distribution patterns, and the spatial proximity between epithelial and mesenchymal subsets is closely linked to their differentiation trajectories. Although biomaterial-assisted assembly strategies, including tissue engineering scaffolds and multimaterial bioprinting, have been explored to regulate cell arrangement, current approaches remain unable to fully recapitulate the complex cellular topology and regional specialization of the native tooth germ. At present, the construction of odontogenic organoids, such as tooth germ organoids, relies predominantly on scaffold materials such as Matrigel or modified hydrogels, within which epithelial and mesenchymal compartments emerge through self-organization. However, these methods provide only limited control over the spatial distribution of specific cellular subpopulations and fail to fully reproduce the fine epithelial–mesenchymal interfacial architecture characteristic of the native tooth germ.⁵ Huang *et al.*⁷⁵ similarly noted that, although organ-on-a-chip technologies can guide cell positioning to some extent through the use of parallel channels and microarchitectural features such as

grooves and micropores, existing approaches still fall short of achieving precise spatial control over cell subpopulations when constructing tooth germ-like structures with complex epithelial–mesenchymal interfaces. This limitation substantially constrains their capacity to model the fine structural organization of tooth development *in vitro*.

6.3. Mechanical property discrepancies between biomimetic mineralization and native tissues

Despite substantial progress in the development of biomimetically mineralized hydrogels, the mechanical properties of the resulting mineralized tissues remain markedly inferior to those of native dental hard tissues. First, biomimetic mineralization continues to be limited by insufficient mineralization efficiency and poor structural order, often resulting in either inadequate mineral deposition or randomly oriented crystals.⁷⁶ Consequently, these systems have difficulty reproducing the highly aligned hydroxyapatite architecture and anisotropic mechanical behavior characteristic of natural enamel.⁷⁷ Second, biomimetically mineralized hydrogels generally lack dynamic mechanical responsiveness. Although mineralization can substantially increase hydrogel stiffness and strength, this improvement is frequently achieved at the expense of toughness and viscoelasticity. For example, Wang *et al.*⁷⁸ used an enzyme-induced mineralization strategy to mineralize carboxymethyl chitosan hydrogels. While mineralization increased the storage modulus and yielded an elongation at break of 30%–35%, this tensile performance remained insufficient when compared with the viscoelastic response of native periodontal ligament tissue. Such mechanical disparity may render dentin mineralization models vulnerable to failure under physiologic masticatory loading. Both DEOs and DPSC-derived organoids are capable of spontaneously generating a hydroxyapatite-containing mineralized matrix.²¹ However, the macroscopic mechanical properties of these mineralized products, including elastic modulus and hardness, have not yet been systematically quantified. Mineralization in organoid systems also presents a unique paradox: by increasing local stiffness from the kilopascal range to the gigapascal range, mineral deposition may in turn suppress the viability and migratory capacity of undifferentiated cells.¹⁸ In conventional scaffold-based systems, mineralization is often regarded as a terminal objective; in organoid systems, however, it may instead become a disruptive factor for subsequent development. Moreover, most current studies still rely on calcium nodule formation or positive Alizarin Red staining as evidence of mineralization, without quantitative assessment of key mechanical parameters such as elastic modulus and hardness.⁸ Thus, a substantial validation gap remains between the mere appearance of mineralization and the

formation of a mechanically functional, load-bearing structure with physiological relevance.

It should be emphasized that these bottlenecks are not isolated, but are causally interconnected. To a considerable extent, the attenuation of mechanical signaling arises from cell-mediated dynamic matrix remodeling: cells degrade the original crosslinked network through matrix metalloproteinases and simultaneously deposit newly synthesized matrix, causing local stiffness to deviate from its initially designed value and thereby resulting in the temporal loss of mechanical cues.⁷⁹ However, this remodeling process is intrinsically at odds with the maintenance of spatial cell organization: if the scaffold is excessively stable, cell migration and autonomous tissue maturation are constrained; if it is overly compliant or permissive, the scaffold loses its ability to provide topographical guidance for cellular arrangement. Once the local mechanical microenvironment is shifted away from the optimal stiffness window by unintended remodeling, the mineralization front likewise loses directional guidance, leading to disordered deposition rather than the oriented mineralization pattern characteristic of native dentinal tubules.

To address this cascade of events, the design of smart responsive hydrogels offers a promising integrative solution. Programmable dynamic crosslinking systems, such as those incorporating dual dynamic boronate ester–acylhydrazone bonds, can adaptively form new crosslinks as cells degrade the pre-existing network, thereby maintaining stable cues while still permitting matrix remodeling. In parallel, strain-stiffening hydrogels combined with microtopographical patterning can locally increase stiffness in response to cell-generated traction forces and thereby maintain local alignment cues, achieving a balance between maintenance of spatial organization and dynamic tissue evolution. In this way, the mineralization front may be constrained within the optimal mechanical window for odontoblastic differentiation and, when coupled with biomimetic mineralization strategies and emerging dynamically responsive material designs, can promote directional mineral deposition. Collectively, these approaches may converge into an integrated design strategy in which dynamic crosslinking sustains mechanical signaling, strain stiffening stabilizes spatial organization, and the mechanical window directs mineralization.

Based on the above analysis, the construction of dental organoids using mechanically biomimetic hydrogels currently faces multiple technical bottlenecks. Table 5 provides a systematic summary of the key challenges and proposes corresponding solutions from three dimensions—material design, engineering strategies, and translational pathways—to guide future research.

Table 5. Current bottleneck problems and proposed solutions

Bottleneck	Specific description	Proposed solutions	Ref.
Insufficient organoid maturation	Most current dental organoids remain in the “organoid body” stage, lacking complete vascular networks, innervation, and functional mineralized tissues	(i) Combine dynamic mechanical regulation with temporal control of developmental signals; (ii) Introduce vascularized coculture strategy (endothelial cells + DPSCs); (iii) Construct epithelial–mesenchymal composite organoids to realize amelogenic/odontogenic bipotent differentiation	11,12,15,63
Bottlenecks in enamel biomineralization	(i) Difficult to reproduce the highly ordered hydroxyapatite crystal arrangement of enamel <i>in vitro</i> ; (ii) Ameloblasts undergo apoptosis immediately after secreting enamel matrix, lacking stable cell sources; (iii) Large gap between the modulus range of hydrogels (kPa–MPa) and that of natural enamel (GPa range)	(i) Establish an expandable source of ameloblast precursors using dental epithelial organoids; (ii) Identify key mineralization factors via exosomal proteomics and perform exogenous supplementation; (iii) Develop an ion-regulated hydrogel system for staged mineralization at the dentin-enamel interface, realize structural mechanical recovery mediated by amorphous calcium phosphate, and the enamel regeneration layer can achieve ultra-hard mechanical properties close to natural enamel	11,12,19,80
Challenges in scaling up and standardization	Large batch-to-batch variability in organoid size, morphology, and function; batch inconsistency and undefined composition of natural matrices (e.g., Matrigel) are core obstacles to standardization	(i) Develop high-throughput DLP printing combined with automated liquid handling platforms; (ii) Enable reproducible large-scale production using microfluidic platforms; (iii) Develop chemically defined synthetic/semi-synthetic hydrogels to replace natural matrices	50,51,54
Barriers to clinical translation	Dental organoids face challenges in safety, biomechanical matching, long-term stability, and regulatory approval from laboratory to clinic	(i) Realize minimally invasive implantation using injectable hydrogels; (ii) Ensure mechanical compatibility between implants and host tissues; (iii) Develop smart materials responsive to the oral microenvironment; (iv) Perform preclinical screening and evaluation using organ-on-a-chip; (v) Conduct safety evaluation under GLP standards	1,53,81

Abbreviations: DLP: Digital light processing; DPSCs: Dental pulp stem cells; GLP: Good laboratory practice.

7. Future technological pathways and development directions

7.1. Mechanical programming of smart responsive hydrogels

A major future direction lies in the design and fabrication of smart hydrogels capable of recapitulating the dynamic mechanical behavior of native tissues. By integrating mechanoresponsive properties with advanced manufacturing technologies such as four-dimensional (4D) printing and *in situ* bioprinting, it may become possible to generate biomimetic scaffolds whose mechanical properties evolve dynamically over time and across space, thereby more faithfully reproducing the nonlinear and

viscoelastic mechanics of soft–hard tissue interfaces such as the periodontal ligament.⁸² This includes the development of materials with programmable strain-stiffening behavior, enabling them to become mechanically stronger under load, much like native soft tissues that stiffen in response to deformation to resist damage.^{83–85} Magnetically actuated 4D bioprinted hydrogels represent another promising strategy. By incorporating magnetic nanoparticles, such systems allow remote and non-contact manipulation of living constructs, thereby offering a new tool for activating mechanosensitive signaling pathways, for example by using magnetic stimulation to accelerate endochondral ossification.^{58,59} Going a step further, molecular-level engineering strategies, such as the introduction of

mechanically regulated biocatalytic systems, may enable demand-responsive self-stiffening or self-softening of hydrogels, allowing the material to adapt to the changing mechanical requirements of different stages of tissue repair.⁸⁶ In a recent review, Yilmaz-Dagdeviren *et al.*²⁴ discussed the integration of next-generation hydrogels with organoid technologies for dental tissue regeneration, highlighting that stimulus-responsive hydrogels, including pH- and temperature-responsive systems, can provide dynamically tunable microenvironments for organoid culture through spatiotemporally controlled release and degradation. At present, however, hydrogels used in dental organoid research are still predominantly designed with fixed initial mechanical parameters,^{10,14} and thus insufficiently capture the dynamic evolution of the mechanical microenvironment during development. The central value of smart responsive hydrogels lies precisely in enabling a paradigm shift from static adaptation to dynamic programming, such that the mechanical properties of the hydrogel can actively adjust in accordance with the developmental stage of the organoid, providing a low stiffness environment during the early phase to support cell self-organization and morphogenesis, and a higher stiffness environment during later stages to direct mineralization and functional maturation.

7.2. Digital reconstruction of developmental mechanobiological atlases

Advances in digital technologies now enable the mechanical microenvironment of organ development to be analyzed and simulated with unprecedented precision, a task that requires the integration of computational modeling, advanced characterization methods, and bioprinting technologies.^{87,88} Cadamuro *et al.*⁸⁹ developed a machine learning-based predictive tool for ECM-mimetic hydrogel formulation, in which supervised learning was used to accurately forecast the rheological properties of gelatin and hyaluronic acid-based hydrogels, thereby substantially reducing the time and cost associated with conventional trial-and-error optimization. Bai *et al.*⁹⁰ further proposed the concept of an artificial intelligence (AI)-enabled virtual organoid, establishing organoid-scale digital twins in computational space. In the oral and craniofacial field, recent reviews on AI-enabled oral organoids have systematically highlighted the potential of AI to optimize culture conditions, analyze multi-omics datasets, and accelerate clinical translation.^{91,92} However, the digitalization of the mechanical microenvironment in tooth organoids is currently constrained by two major data deficiencies. First, the spatiotemporal dynamics of key mechanical parameters, such as stiffness and stress relaxation, during tooth germ development have not yet been systematically quantified. Second, high-quality datasets linking mechanical parameters to biological outcomes, essential for robust AI model training, remain

extremely limited.⁹³ In this context, the true value of digital design lies in transforming empirical trial-and-error workflows into predictive, data-driven strategies; yet such a transition ultimately depends on the prior completion of the foundational work of systematic data acquisition.

7.3. Integration of automated biomanufacturing technologies for organoids

A pivotal step in advancing organoids from laboratory research toward clinical application is overcoming the manufacturing bottlenecks associated with standardization and scalability, and the integration of automated biofabrication platforms is likely to provide a central solution. Recent studies have employed stereolithography to fabricate organ-on-chip devices for the growth of oral mucosa analogs,⁹⁴ while microfluidic tooth-on-a-chip or dentin-chip systems combined with GelMA hydrogels have been used for the screening of dental materials.⁹⁵ However, these platforms remain largely confined to 2D or quasi-3D models of single tissue interfaces. At present, the automated fabrication of dental organoids faces three major challenges. First, dental organoids comprise multiple cell types, including epithelial and mesenchymal populations, and therefore require a far greater degree of spatial precision in cell arrangement than organoids derived from a single cell type.⁷⁴ Second, the long-term culture requirements of dental organoids, often extending from several weeks to months, impose substantially higher demands on the stability and reliability of automated perfusion systems. Third, the dynamic changes in pH and calcium ion concentration that accompany mineralization must be monitored in real time and regulated through feedback control, yet current automated platforms generally lack integrated sensor modules capable of performing such functions. Consequently, the field of dental organoids still requires targeted advances in chip design, process control, and real-time monitoring before automated and standardized manufacturing can be fully realized.

8. Conclusion and key biological insights

8.1. Contributions of mechanical biomimetic strategies to dental regenerative medicine

From Engler *et al.*'s⁷ seminal discovery that matrix elasticity governs stem cell fate to the emerging application of mechanically biomimetic hydrogels in tooth organoid engineering, the field has undergone a paradigm shift from the identification of fundamental mechanobiological principles to their precise implementation in tissue engineering practice. The advent of mechanically biomimetic hydrogel strategies signifies that dental regenerative medicine is moving beyond passive repair toward active mechanical programming. By providing a precisely tunable mechanical microenvironment, bioorthogonally crosslinked hydrogels

provide well-defined 3D matrices that support tooth organoid morphogenesis.¹⁰ Biomimetic mineralized hydrogels have further demonstrated that, by recapitulating key aspects of natural mineralization, injectable materials can induce *in situ* dentin/bone regeneration while conforming to irregular defect geometries.¹ In addition, the integration of hydrogels with enabling technologies such as microfluidic encapsulation offers a feasible engineering framework for constructing dental organoids with greater structural complexity, including vascularized architectures.⁹⁶ Collectively, these advances underscore that precise recapitulation of the mechanical microenvironment is a central prerequisite for achieving functional *in situ* regeneration of dental tissues.

8.2. Methodological innovation value of cross-scale mechanistic research

Methodological advances are pivotal for moving research on the mechanical regulation of dental organoids from descriptive observation to true mechanistic dissection. In recent years, three lines of innovation have been particularly noteworthy. First, the establishment of synthetic hydrogel platforms has made it possible to independently tune mechanical parameters. Zhang *et al.*¹⁰ developed a Tz–Nb bioorthogonally crosslinked gelatin hydrogel and, for the first time, achieved *in vitro* generation of tooth germ organoids in a chemically defined synthetic matrix, thereby eliminating the confounding influence of the poorly defined components present in Matrigel. Second, progress in dynamic mechanical characterization has fundamentally revised the traditional static stiffness paradigm. Recent studies have shown that viscoelasticity exerts regulatory effects that are independent of stiffness alone: viscoelastic matrices permit stress relaxation and matrix remodeling, whereas in purely elastic matrices, cell-generated tension remains persistent and may instead constrain dynamic reorganization.^{23,97} Luo *et al.*²⁵ further demonstrated that the combination of rapid stress relaxation and appropriate stiffness promotes YAP nuclear localization and odontoblastic differentiation. This effect could not have been captured through stiffness analysis alone, underscoring the necessity of multiparametric mechanical characterization. Third, emerging multiscale mechanical characterization frameworks are reshaping evaluation standards. Platforms have begun to shift organoid mechanics from qualitative description toward quantitative analysis.⁹⁸ Nevertheless, current methodologies still face three major limitations. Most measurements remain single-time-point assessments and therefore fail to capture the dynamic evolution of the mechanical microenvironment during culture. In addition, the local stiffness jump associated with mineralization—from the kilopascal to the gigapascal range—exceeds the linear detection range of many existing methods. Finally, a standardized mapping framework linking measured

mechanical parameters to organoid design strategies has yet to be established. Thus, the field still requires sustained advances in real-time, nondestructive monitoring and in multiparametric, integrative design frameworks before mechanical characterization can evolve from a descriptive tool into a predictive guide for organoid engineering.

8.3. Feasibility analysis of clinical translation pathways

The clinical translation of dental tissue organoids will require several major bottlenecks to be addressed. Foremost among these is functional vascularization. Drawing on advances in liver organoid engineering, feasible strategies for establishing functional vascular networks include the use of 3D bioprinting to construct biomimetic lobule-like architectures and the co-encapsulation of endothelial cells with dental-derived cells.⁹⁹ In parallel, microfluidic and photocrosslinking technologies provide powerful technical support for the high-precision fabrication of biomimetic vascular structures.^{100–102} A second challenge is the long-term maintenance of mechanical signaling. To address the attenuation of mechanical cues in natural hydrogels, biomimetic fibrous hydrogels with intrinsic buffering capacity have been shown to stabilize the local microenvironment and significantly improve long-term organoid survival and function,¹⁰³ thereby offering a potential solution to the challenge of studying mineralization dynamics during prolonged culture of dental organoids. A third issue concerns automated manufacturing and personalized therapy. The successful expansion and phenotypic preservation of patient-derived tumor organoids in customized hydrogels has demonstrated that biomimetic hydrogels can serve as reliable platforms for personalized drug screening.^{104,105} Looking ahead, the integration of mechanically programmed smart responsive hydrogels with automated biofabrication technologies may enable the production of clinical-grade, standardized dental organoids and provide a stable and scalable source of regenerative products.^{24,60} Nevertheless, a critical technological gap remains between high-fidelity *in vitro* organoids and clinically implantable functional organ substitutes. First, functional integration with the host vasculature is essential. Whether the microvascular network of an implanted organoid can rapidly anastomose with the host circulation will directly determine the survival of regenerated tissue. Wimmer *et al.*¹⁰⁶ showed that 3D vascular organoids can spontaneously connect with host vessels and achieve functional perfusion after transplantation. Combined with the previously discussed strategies of 3D-printed preconstructed hierarchical vascular networks^{42,48} and 4D-printing approaches for temporally controlled factor release,⁹⁹ these advances may offer a promising route toward early vascularization of dental pulp organoids. Second, functional integration

with the host nervous system is equally critical. Pagella *et al.*¹⁰⁷ demonstrated that dental pulp tissue can guide the directional extension of trigeminal axons through axon-guidance factors such as semaphorin 3A. In the future, smart responsive hydrogels could be engineered with gradient microchannels loaded with neurotrophic factors to direct host nerve ingrowth into regenerated pulp tissue and thereby restore sensory function. Third, it is necessary to recapitulate the biomechanical buffering function of the periodontal ligament. Park *et al.*¹⁰⁸ successfully engineered a bone–ligament composite interface using fiber guiding scaffolds. In combination with patterned soft–hard domain hydrogels, strain-stiffening materials, and dynamic mechanical loading strategies, this approach may enable the preconstruction of biomimetic periodontal ligament analogs with directional mechanical buffering capacity, while gradient mineralization design could facilitate a smooth transition across soft–hard tissue interfaces. Systematic validation of such multi-tissue integration may be further enabled by organ-on-a-chip technologies, which could be used to establish an integrated “dentin–pulp–periodontal ligament–alveolar bone” multi-interface testing platform.¹⁰⁹ Such systems would allow the simultaneous *in vitro* simulation of mechanical loading and fluid perfusion, thereby serving as a critical preclinical validation platform for the functional screening of clinically relevant dental organoid constructs.

Although mechanically biomimetic hydrogels have laid an important technical foundation for the construction of dental tissue organoids, their clinical translation must satisfy regulatory, safety, and functional validation requirements that are far more stringent than those applied to *in vitro* model systems. From a regulatory perspective, hydrogel cell composite organoids possess the combined attributes of biomaterials, cell therapies, and tissue-engineered products, and are therefore generally classified as advanced therapy medicinal products, which are subject to rigorous requirements for product classification, quality control, and traceability.^{110,111} For hydrogels containing naturally derived components such as dECM, collagen, or gelatin, particular attention must be paid to residual DNA content, pathogen transmission risk, immunogenicity, and the safety of degradation products, all of which must comply with the relevant standards for biological evaluation.^{112,113} With respect to reproducibility and compatibility with good manufacturing practice (GMP), most current hydrogel systems still rely on laboratory-scale preparation and exhibit substantial batch-to-batch variability in crosslinking density, elastic modulus, stress relaxation kinetics, and degradation behavior.¹¹⁴ Future efforts should prioritize the development of compositionally defined, scalable materials suitable for aseptic manufacture under GMP conditions, for example by replacing complex animal-derived matrices

with recombinant collagen, synthetic polyethylene glycol-based systems, or chemically defined GelMA formulations. In parallel, standardized quality-control frameworks should be established to encompass rheological properties, compressive modulus, swelling ratio, endotoxin levels, and cytocompatibility.^{115,116} Immunological safety is another indispensable consideration in transplantation-based applications. Dental stem cells and epithelial–mesenchymal composite structures may elicit immune rejection or chronic inflammation after allogeneic transplantation, while residual antigens and degradation fragments from dECM hydrogels may also alter the host immune microenvironment.^{112,117} Feasible strategies to improve long-term graft survival include the use of autologous cell sources, hypoimmunogenic universal donor cells, and hydrogels with intrinsic immunomodulatory functions.¹¹⁷

Functional vascular integration and neural innervation are decisive determinants of whether dental tissue organoids can achieve true functional regeneration. Because the dentin–pulp complex is richly vascularized and innervated, rapid anastomosis between preconstructed vascular networks and host vessels after transplantation directly affects cell survival and metabolic support.^{118,119} Approaches such as microfluidic perfusion, endothelial cell coculture, and vascular endothelial growth factor gradient release may therefore be used to promote vascularization. Control of mineralization and long-term functional validation under occlusal loading constitute particularly distinctive requirements that set dental tissue organoids apart from soft-tissue organoid systems. The formation of dentin- or enamel-like structures depends not only on mineral deposition itself, but also on the spatial orientation of mineral crystals and the temporally coordinated progression of the mineralization front. Uncontrolled mineralization may lead to ectopic calcification or brittle interfacial failure; accordingly, ordered mineralization will likely require the combined use of mechanically graded hydrogels, topographically instructive architectures, and controlled release systems for mineralization precursors.¹²⁰ In addition, implanted organoids must ultimately withstand the cyclic loads generated during mastication, yet current studies remain largely confined to short-term *in vitro* culture or subcutaneous implantation in small-animal models, with little systematic evaluation of material fatigue, interfacial stability, or osseointegration under authentic occlusal conditions. Future preclinical validation should therefore incorporate dynamic mechanical loading, long-term perfusion culture, and large-animal alveolar defect models. In summary, the clinical translation of dental tissue organoids must satisfy an integrated set of requirements: they must be controllable, reproducible, manufacturable, integrable, sensory-competent, load-bearing, and capable of maintaining long-term function. Achieving this

goal will require a unified evaluation framework that incorporates materials design, GMP-compliant production, immunological safety, vascular and neural integration, spatiotemporal control of mineralization, and validation under physiologically relevant occlusal mechanics.

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Conflict of interest

The authors declare that they have no competing interests.

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