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From Stem Cell to Smile: Organoid Technology and the Future of Biological Tooth Replacement: A Review.

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ABSTRACT :

Background: Organoids are self-organising, three-dimensional (3D) multicellular structures derived from stem or progenitor cells that recapitulate the architecture and selected functions of their tissue of origin. Since the first intestinal organoids were grown from single Lgr5+ stem cells in 2009, the technology has extended to the tooth, dental pulp, periodontium, salivary glands, oral mucosa, taste epithelium and oral cancers.

Aim: To define dental organoids, classify their types, describe their construction, and appraise their significance across dental disciplines, with particular reference to prosthodontics.

Methods: Narrative review of literature indexed in PubMed (2007–2026) using the terms "organoid", "tooth", "dental epithelium", "ameloblast", "dental pulp", "periodontal", "salivary gland", "oral mucosa", "taste" and "oral squamous cell carcinoma", supplemented by landmark developmental-engineering studies.

Key findings: Dental organoids can be classified by cell source (adult stem cell, pluripotent stem cell, embryonic tooth germ, patient tumour, reprogrammed somatic cell), by tissue modelled (tooth epithelium, dentin–pulp, tooth root/periodontium, salivary gland, oral mucosa/tongue, tumour) and by complexity (single-lineage organoid, assembloid, toothoid, vascularised construct, organoid-on-chip). Human tooth organoids from the dental follicle recapitulate the epithelial-cell-rest stemness phenotype and ameloblast differentiation; iPSC-derived ameloblast organoids lay down an enamel-like matrix; dentin–pulp and tooth-root organoids mineralize; salivary organoids restore saliva in irradiated animals; and oral cancer organoids predict individual drug and radiation sensitivities.

Conclusion: Dental organoids bridge two-dimensional culture and animal models and are reshaping research on odontogenesis, disease modelling, biomaterial testing and personalised oncology. Lack of vascularization, innervation, and immune components, reliance on animal-derived matrices, and poor standardization still prevent their chairside use; however, they represent the most credible current route towards a biological tooth replacement—the ultimate prosthesis.

Keywords: Organoids; Assembloids; Tooth regeneration; Ameloblasts; Tumouroids; Bio-tooth.

Introduction

For most of the last century, oral biology was studied through two imperfect windows: flat (two-dimensional, 2D) monolayer cultures of a single cell type, and animal models whose dentition, salivary physiology and immune responses differ substantially from those of humans. Monolayer cells lose polarity, cell–matrix signalling and the cell–cell crosstalk that governs tissue behaviour, whereas animal models are costly, ethically burdened and often poorly predictive of human outcomes [4]. The past decade has added a third window—the organoid—which grows in three dimensions, self-organises, and retains much of the cellular diversity and architecture of the native organ [1,2].

The tooth is an appealing but challenging focus for organoid technology. Tooth development relies on stepwise, back-and-forth signaling between oral epithelium and neural-crest-derived mesenchyme. After eruption, enamel-forming ameloblasts are lost, so enamel cannot naturally regenerate. The tooth itself is made up of enamel, dentin, pulp, cementum, periodontal ligament (PDL), and alveolar bone [11]. Organoids make it possible to study each of these parts, and increasingly, how they interact, in a laboratory setting [6].

For prosthodontists, this topic is more than just academic. Every fixed, removable, or implant prosthesis is a mechanical replacement for a lost biological organ. Advances in developmental engineering, from bioengineered tooth germs that erupted and worked in a mouse jaw [7,8] to human tooth organoids and assembloids [9,10], suggest a future where replacements could be biological. This review explains what dental organoids are, describes their types, outlines how they are made, and discusses their importance in dentistry.

Historical Evolution

The concept rests on century-old observations that dissociated embryonic cells can re-aggregate and rebuild organ-like structures. The modern era began when Sato and colleagues showed that a single Lgr5+ intestinal stem cell, embedded in a laminin-rich matrix with defined niche factors, could build crypt–villus units without a mesenchymal niche [2]. Dentistry followed two parallel tracks: the developmental-engineering track (bioengineered tooth germs) and the stem-cell organoid track (expandable epithelial and mesenchymal organoids).

Table 1. Milestones in organoid research relevant to dentistry

2007	Bioengineered organ-germ method (Nakao et al.)	Reconstituted dental epithelial and mesenchymal cells formed a structurally correct tooth with blood vessels and nerves	7
2009	Lgr5+ intestinal organoids (Sato et al.)	Defined the adult-stem-cell organoid paradigm—matrix + niche factors + self-organisation	2
2009	Fully functional bioengineered tooth (Ikeda et al.)	Bioengineered tooth germ transplanted into adult mouse alveolar bone erupted and functioned	8
2014	Taste-bud organoids from Lgr5+/Lgr6+ cells (Ren et al.)	First oral sensory-epithelium organoids	24
2014	"Organogenesis in a dish" (Lancaster & Knoblich)	Widely adopted definition and conceptual framework of organoids	1
2016	Human salivary gland organoids restore saliva (Pringle et al.)	Organoid-derived cells rescued irradiated glands in vivo	19
2019	Oral mucosal and HNSCC organoid biobank (Driehuis et al.)	31 tumour organoid lines; personalised drug and radiation testing	25
2020	Dentin–pulp-like organoids from hDPSCs (Jeong et al.)	First mesenchymal dentin–pulp organoid; responded to Biodentine	16
2022	First human tooth organoids (Hemeryck et al.)	Dental follicle-derived organoids with epithelial-cell-rest stemness and ameloblast potential	9
2022	hiPSC-derived salivary glands (Tanaka et al.)	Modelled SARS-CoV-2 infection; engrafted orthotopically	22
2023	Mouse molar/incisor tooth organoids and assembloids	Tooth-type-specific organoids; co-differentiation into odontoblast/cementoblast-like cells	10
2023–24	hiPSC ameloblast organoids; toothoids; tooth-root organoids	Enamel-like matrix in vitro; chemically defined whole-tooth reconstitution; self-assembled root	12,15,18

Definition and Core Concepts

Lancaster and Knoblich described an organoid as a collection of organ-specific cell types that arises from stem cells or organ progenitors and self-organises—through cell sorting and spatially restricted lineage commitment—in a manner that resembles in vivo organ development [1]. Consensus work by expert groups has since refined this into a set of minimum criteria: a 3D structure, derived from stem/progenitor cells (or tissue containing them), comprising more than one cell type of the target organ, organised in a manner resembling the native tissue, and displaying at least some organ-specific function [3].

- A **dental organoid** is a self-organising, three-dimensional, stem-cell-derived micro-tissue that reproduces the cellular composition, spatial organisation and at least one specialised function (e.g., enamel-matrix secretion, dentin mineralisation, saliva production, taste transduction) of a tooth component or oral tissue.

- The broader term **oral organoid** includes salivary gland, oral mucosa, tongue/taste and oral tumour organoids.

Essential hallmarks: **self-renewal** (long-term expansion), **self-organisation**, **multi-lineage differentiation**, **tissue fidelity** and **function**.

Related terminology

- **Spheroid** – a simple aggregate of cells (often a single line) that lacks stem-cell-driven self-organisation and lineage hierarchy.
- **Assembloid** – a composite of two or more organoids or cell populations (e.g., dental epithelial organoid + pulp stem cells) that recreates tissue interactions such as epithelial–mesenchymal signalling [9,10].
- **Toothoid** – a reconstituted tooth germ grown under chemically defined conditions that continues normal tooth development in vitro [15].
- **Tumouroid** (tumour organoid / patient-derived organoid, PDO) – an organoid grown from a patient's tumour that preserves its genetic and phenotypic features [25].
- **Organ-on-a-chip** – a microfluidic device in which cells or organoids are perfused and mechanically stimulated to mimic physiology [30,31].

Table 2. Organoids compared with other experimental models

Architecture	Flat; no polarity	Simple 3D aggregate	Tissue-like 3D, polarised	Native
Cell diversity	Usually one type	Limited	Multiple lineages	Complete
Human relevance	Moderate	Moderate	High (patient-specific)	Species differences
Long-term expansion	Senescence/drift	Limited	Yes; can be biobanked	Not applicable
Vasculature / immunity / nerves	Absent	Absent	Usually absent	Present
Throughput and cost	High / low	High / low	Medium / medium	Low / high
Ethical burden	Low	Low	Low (consent issues)	High

Biological Basis and Generation of Dental Organoids

Organoid formation uses the natural ability of stem cells to rebuild tissue when given a supportive 3D environment. Four main components are needed (Figure 1): a cell source with stem or progenitor potential, a 3D extracellular matrix, soluble factors that support self-renewal, and signals that guide cells to become specialized [1,5].

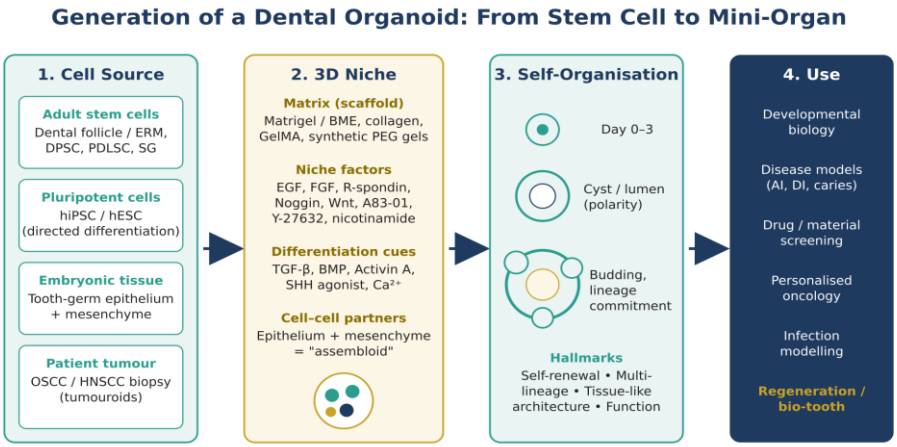


Figure 1. Steps in the generation of a dental organoid. ERM, epithelial cell rests of Malassez; DPSC/PDLSC, dental pulp/periodontal ligament stem cells; SG, salivary gland; AI/DI, amelogenesis/dentinogenesis imperfecta.

1 Cell sources

•Adult (tissue-resident) stem cells – the dental follicle of unerupted third molars, whose epithelial cell rests of Malassez (ERM) contain dental epithelial stem cells [9]; dental pulp stem cells (DPSCs) [16]; PDL stem cells [18]; salivary gland stem/progenitor cells [19]; Lgr5+/Lgr6+ taste progenitors [24].

•Pluripotent stem cells – human induced pluripotent stem cells (hiPSCs) or embryonic stem cells, directed through oral-epithelial intermediates toward ameloblast or salivary fates [12,13,22].

•Embryonic tooth-germ cells – dissociated dental epithelium and mesenchyme that re-aggregate into bioengineered tooth germs or toothoids [7,14,15].

•Tumour tissue – biopsies of oral/head-and-neck squamous cell carcinoma [25].

•Reprogrammed somatic cells – e.g., oral keratinocytes reprogrammed with TGF-β1 to an enamel-organ-like phenotype [26].

2 Matrix and niche

Most epithelial organoids are grown in a basement membrane extract (Matrigel/BME), which provides laminin, collagen IV, and growth factors. The culture media usually include EGF, FGF, R-spondin, and Noggin to activate Wnt signaling and block BMP signaling, a TGF-β receptor inhibitor (A83-01), nicotinamide, and a ROCK inhibitor to help single cells survive. New hydrogels such as gelatin methacrylate (GelMA) and polyethylene glycol (PEG) are being used to improve consistency across batches [11,17].

3 Signalling pathways that drive differentiation

Organoids follow the same developmental pathways as natural tooth formation, so the same signals guide their growth. TGF-β signaling is crucial for enamel formation; in human tooth organoids, TGF-β increased ameloblast differentiation, whereas blocking its receptor halted this process [9]. BMP activation led to strong enamel formation in late-stage toothoids. Using Activin A and a Hedgehog agonist together helped start tooth development [15]. EGF induced transient cell growth, followed by a shift toward epithelial-mesenchymal transition, similar to ERM behavior in the body [9]. Most importantly, the interaction between epithelial and mesenchymal cells is key: adding pulp stem cells to epithelial organoids (assembloids) was enough to start ameloblast differentiation [9,10].

Types of Dental and Oral Organoids

Dental organoids can be classified in three ways: by cell source, by the tissue they model, and by their level of complexity (Figure 2). The most clinically useful way is by tissue type, which is used in the following sections. Table 3 lists the main types.

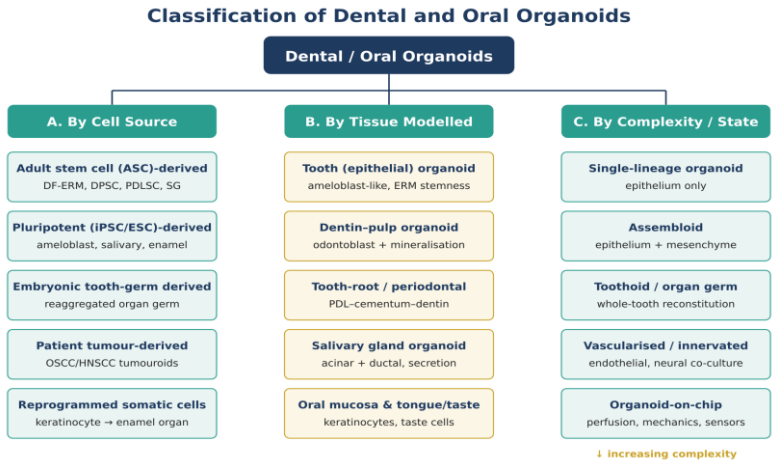


Figure 2. A three-axis classification of dental and oral organoids. DF-ERM, dental follicle–epithelial cell rests of Malassez; OSCC/HNSCC, oral/head and neck squamous cell carcinoma.

1 Tooth (dental epithelial) organoids

Hemeryck and colleagues generated the first human tooth organoids from dental follicle tissue of unerupted wisdom teeth. These were long-term expandable and displayed a stemness phenotype matching the ERM; single-cell transcriptomics confirmed the congruence and revealed stem-cell features mirroring those in mouse. The organoids could undergo ameloblast differentiation, enhanced by TGF-β, and in assembloids with pulp stem cells, ameloblast differentiation was triggered by the mesenchymal partner [9]. Corresponding mouse organoids from early-postnatal molars and incisors were tooth-type-specific, expressed dental epithelial stem-cell markers, and, in assembloids, co-differentiated into junctional-epithelium-like and odontoblast/cementoblast-like cells; they also survived and differentiated in vivo [10]. A standardised protocol is now available [32].

2 Pluripotent stem cell-derived ameloblast organoids

Because adult humans have no ameloblasts, hiPSCs are an important alternative source. hiPSC-derived human ameloblast organoids (hAOs) formed enamel in response to calcium, mineralised on interaction with dental mesenchyme, showed both osteogenic and odontogenic potential, and supported tooth formation when combined with mouse dental mesenchyme [12]. Serum-free, growth-factor-defined protocols now direct hiPSCs through oral epithelium to suspended "induced early ameloblast" organoids—models directly relevant to amelogenesis imperfecta [13]. A simplified reaggregation strategy mixing human keratinocyte stem cells or hiPSCs with mouse dental mesenchymal cells achieved tooth formation in more than 90% and ameloblastic differentiation in 100% of chimeric germs at optimal ratios [14]. Hermans et al. have reviewed the progression from iPSC models through organoids to bioprinting of dental epithelium [11].

3 Tooth-germ organoids, bioengineered organ germs and toothoids

The organ-germ method of Tsuji's group compartmentalised dissociated epithelial and mesenchymal cells at high density in collagen; the resulting tooth germ produced a structurally correct tooth with penetrating vessels and nerves [7]. When transplanted into the alveolar bone of adult mice, the bioengineered germ erupted and became a fully functioning tooth [8]—proof of principle for biological tooth replacement. More recently, a chemically defined culture ("toothoid") supported reconstitution and continued development with normal anatomy; reconstituted cells resumed development from the stage at which they had been isolated rather than resetting their developmental clock [15].

4 Dentin–pulp organoids

Jeong et al. produced dentin–pulp-like organoids by embedding human DPSCs in Matrigel and exposing them to odontogenic medium; organoids differentiated for 11 days best balanced stemness and odontoblast features, mineralised on micro-CT, could be dissociated and re-formed, and responded biologically to Biodentine [16]. Pulp organoid research is still early; vascularisation, innervation and integration with the root canal environment are the major unmet goals [17].

5 Tooth-root and periodontal organoids

Using scaffold-free self-assembly, mixtures of human DPSCs and PDLSCs organised into constructs resembling the full tooth root—dentin–pulp internally and PDL–cementum externally [18]. Such models are directly relevant to periodontal regeneration and to the biology of the tooth–bone interface that implants cannot reproduce.

6 Salivary gland organoids

Human salivary stem/progenitor cells self-renew and form multilineage organoids (salispheres); transplanted organoid-derived cells restored saliva production and improved regeneration of irradiated glands, with c-Kit+ selection enriching regenerative potency [19]. Murine parotid organoids have been used to measure stem-cell radiation response in vitro [20], and human salivary organoids from patients taking β-blockers revealed Notch-mediated progenitor control underlying drug-induced hyposalivation [21]. hiPSC-derived salivary glands resemble embryonic glands, can be infected by SARS-CoV-2 and engraft orthotopically in mice [22]. These advances are summarised in reviews of salivary regeneration [23].

7 Oral mucosa, tongue and taste organoids

Single Lgr5+ or Lgr6+ cells from taste tissue generated continuously expanding organoids that contained differentiated taste cell types [24]. Long-term culture conditions for human oral mucosal organoids have been established and are used both as normal controls and as a substrate for infection and toxicity studies [6,25].

8 Oral cancer organoids (tumouroids)

Driehuis et al. derived 31 organoid lines from head and neck squamous cell carcinoma; these recapitulated known genetic features, remained tumorigenic upon xenotransplantation, and showed patient-specific responses to cisplatin, carboplatin, cetuximab, and radiotherapy, positioning them as a platform for personalised therapy [25].

9 Bone and enamel-like constructs

Bone organoids—callus, woven and trabecular bone and marrow models—are relevant to alveolar ridge biology and osseointegration; a recent consensus statement proposes standards for their construction and evaluation [28]. In a different strategy, TGF-β1-reprogrammed oral keratinocytes formed organoids that secreted an amelogenin-rich matrix in the presence of serotonin; after mineralisation and densification, this yielded an enamel-like material with prism-like crystal alignment and enamel-comparable properties [26].

Table 3. Principal types of dental and oral organoids

Human tooth organoid	Dental follicle (ERM) of unerupted third molars	Long-term expansion; ERM stemness; TGF-β-driven ameloblast differentiation; assembloids with pulp cells	9
Mouse tooth organoid	Postnatal molar and incisor epithelium	Tooth-type specificity; in vivo survival; odontoblast/cementoblast-like co-differentiation	10,32
Ameloblast organoid	hiPSC	Enamel-matrix proteins; calcium-responsive mineralisation; tooth formation with mesenchyme	12,13
Reaggregated tooth germ / toothoid	Embryonic dental epithelium + mesenchyme; hKSC/hiPSC chimeras	Whole-tooth morphogenesis; enamel induction by BMP; >90% tooth formation	7,14,15
Dentin–pulp organoid	hDPSC in Matrigel	Odontoblast markers; mineralisation; response to Biodentine	16,17
Tooth-root organoid	hDPSC + hPDLSC (scaffold-free)	Root-like dentin–pulp core with PDL–cementum surface	18
Salivary gland organoid	Adult SG stem cells; hiPSC	Acinar/ductal lineages; saliva restoration after irradiation; viral infection	19–22
Taste / oral mucosa organoid	Lgr5+/Lgr6+ taste cells; mucosal keratinocytes	Taste-cell lineages; stratified epithelium	24,25
Oral cancer tumouroid	OSCC/HNSCC biopsies	Retain tumour genetics; predict drug and radiation response	25

Methods of Construction

Table 4. Techniques used to construct dental organoids

Matrix-embedded (dome) culture	Cells in BME/Matrigel domes with niche medium	Tooth, salivary, mucosal organoids [9,19,25]	Robust and expandable Animal-derived, variable matrix
Suspension / low-attachment culture	Cells aggregate without adhesion	Induced early ameloblast organoids [13]	Simple, scalable Limited size, necrotic core
Reaggregation / organ-germ method	High-density epithelial + mesenchymal recombination	Bioengineered tooth germ, toothoid [7,14,15]	Whole-tooth morphogenesis Requires embryonic odontogenic mesenchyme
Scaffold-free self-assembly	Cells secrete their own matrix and fold into tissue	Tooth-root organoid [18]	No exogenous scaffold Mechanical weakness
3D bioprinting	Layer-wise deposition of cell-laden bio-inks	Dental epithelium/ameloblast constructs [11]	Spatial control, patient-specific shape Cell viability, resolution
Organoid-on-chip	Microfluidic perfusion and mechanical cues	Emerging pulp and periodontal chips [30,31]	Vascular flow, sensors Complex, costly

Significance of Organoids in Dentistry

Dental organoids are important in four main areas: understanding development, modeling disease, testing drugs and materials, and regenerating tissues. Each of these areas connects to different clinical fields (Figure 3; Table 5).

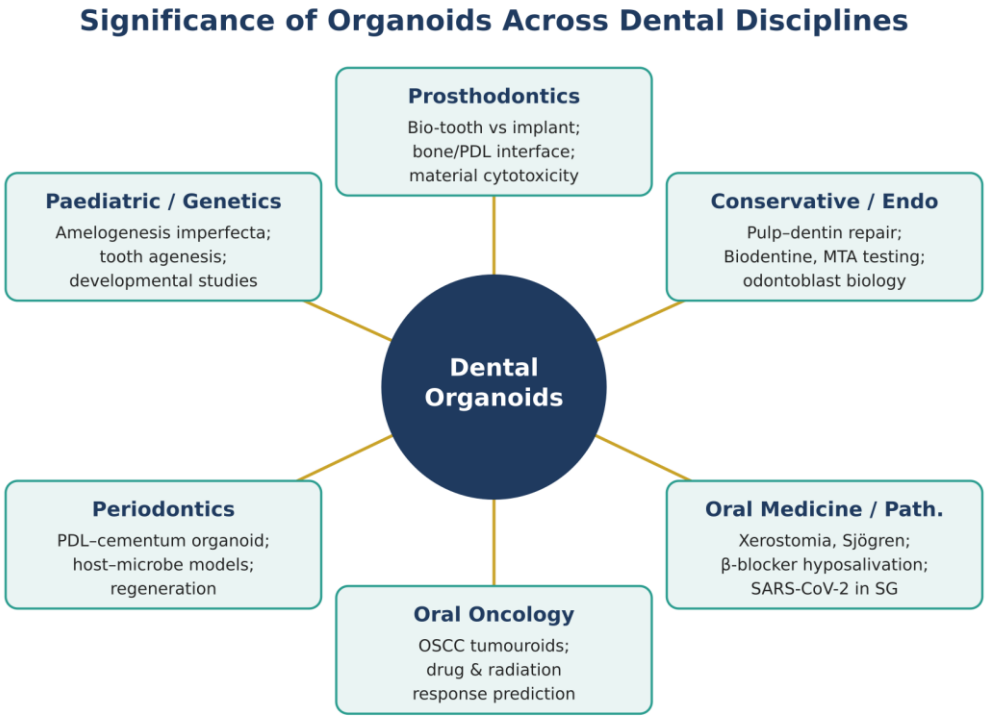


Figure 3. Significance of organoids across dental disciplines. PDL, periodontal ligament; SG, salivary gland; OSCC, oral squamous cell carcinoma.

1 Understanding odontogenesis and stem-cell biology

Human tooth epithelial stem cells were previously almost inaccessible before the advent of organoids. Tooth organoids now allow the ERM stem-cell compartment, its response to growth factors and its interplay with dental mesenchyme to be studied in human tissue [9], and toothoids allow stage-specific dissection of the signalling that sustains tooth fate and enamel formation [15].

2 Disease modelling

•Developmental anomalies – hiPSC-derived ameloblast organoids from affected patients serve as models of amelogenesis imperfecta and related enamel defects [11,13].

•Salivary dysfunction – organoids have modelled radiation-induced xerostomia [19,20], β-blocker-associated hyposalivation [21] and viral infection of salivary tissue [22].

•Infection and inflammation – oral mucosal and tongue organoids are being used to examine host–microbe interactions relevant to periodontitis and mucosal disease [6].

3 Drug and biomaterial testing

Organoids provide a human, 3D, multi-lineage substrate for testing pulp-capping agents (e.g., Biodentine) [16], regenerative growth factors and, potentially, the cytotoxicity of restorative and prosthodontic materials such as resin cements, CAD-CAM polymers and 3D-printed denture-base resins—

tests currently performed largely on monolayers.

4 Personalised oral oncology

Tumouroids from oral cancer biopsies can be screened against chemotherapeutic agents, targeted drugs and radiation within weeks, enabling individualised treatment selection and the discovery of agents not normally used for the disease [25].

5 Regenerative dentistry

Organoids and assembloids are building blocks for regenerating enamel [12,26], dentin–pulp [16,17], root and periodontium [18], salivary glands [19,22] and ultimately whole teeth [8,15,27]. Clinical precedent exists outside dentistry: transplantation of colonic organoids formed the basis of a first-in-human trial for intractable ulcerative colitis [29].

6 Relevance to prosthodontics

Prosthodontics is the discipline most directly challenged—and potentially transformed—by organoid research:

The bio-tooth as the ultimate prosthesis. Dental implants osseointegrate without a PDL, so they lack physiological mobility, proprioception and the capacity to adapt to orthodontic or occlusal forces. The bioengineered tooth erupted into function within the jaw [8], and tooth-root organoids with a PDL–cementum surface [18] point towards replacements that restore the periodontal mechanoreceptive interface.

Alveolar ridge and implant site biology. Bone organoids [28] offer human models for osseointegration, peri-implant bone remodelling and the influence of implant surfaces and loading.

Biocompatibility of prosthodontic materials. Gingival, mucosal, and pulp organoids enable 3D testing of monomer release, cytotoxicity, and inflammatory responses to denture-base resins, temporary crown materials, and luting agents.

Xerostomia and removable prostheses. Saliva is essential for denture retention, comfort and mucosal health. Salivary organoid therapy for radiation-induced or drug-induced hyposalivation [19,21] could directly improve the prosthodontic prognosis of head-and-neck cancer survivors and elderly edentulous patients.

Maxillofacial rehabilitation. Organoid-derived tissues may eventually complement obturators and facial prostheses by regenerating the mucosa, glands, and bone at resected sites.

Table 5. Significance of organoids by dental discipline

Prosthodontics & implantology	Biological tooth replacement; PDL-bearing roots; bone-organoid models of osseointegration; biocompatibility of prosthetic materials; management of xerostomia
Conservative dentistry & endodontics	Dentin–pulp regeneration; testing of pulp-capping and endodontic materials; odontoblast biology
Periodontics	PDL–cementum regeneration; host–pathogen interaction models; drug testing
Paediatric dentistry & genetics	Models of amelogenesis imperfecta, tooth agenesis and other developmental anomalies
Oral medicine & pathology	Xerostomia, Sjögren-like dysfunction, drug-induced hyposalivation, viral tropism of salivary glands
Oral & maxillofacial surgery / oncology	Personalised drug and radiation testing for OSCC; regeneration of resected tissues
Dental education & research	Human-relevant 3D models that reduce animal use (3Rs)

Limitations and Challenges

Table 6. Challenges of dental organoids and emerging solutions

Absent vasculature	Size limit; necrotic core; poor graft survival	Endothelial co-culture, vascularised assembloids, perfusion chips [17,30]
Absent innervation and immune cells	Incomplete physiology; no pain/defence modelling	Neural-crest and immune-cell co-culture
Incomplete maturation	Fetal-like phenotype; thin, immature enamel	Stage-specific cues (BMP, TGF-β), longer culture, in vivo maturation [9,15]
Dependence on Matrigel	Batch variability; xenogeneic; not GMP-compatible	Synthetic PEG/GelMA hydrogels; chemically defined media [11,15]
Embryonic odontogenic mesenchyme required	Human whole-tooth formation still relies on mouse mesenchyme	Induction of odontogenic competence in human iPSC/DPSC mesenchyme [12,14]
Heterogeneity and poor standardisation	Low reproducibility between laboratories	Consensus definitions and benchmarking [3,28]
Size, shape and colour control of a bio-tooth	Functional and aesthetic mismatch with neighbouring teeth	Bioprinting, scaffold templates, digital design [11,27]
Cost, time, regulation and ethics	Barrier to clinical translation	GMP manufacturing, donor-consent frameworks, regulatory pathways

It is important to be clear: no dental organoid has been used in clinical practice yet. Most successful tooth regeneration has occurred in rodents using embryonic cells, and human models so far reproduce only parts of the tooth, not the whole organ [27].

Future Directions

1. 1.Vascularised and innervated assembloids combining epithelial, mesenchymal, endothelial and neural components.
2. 2.Organoid-on-chip platforms incorporating occlusal loading, fluid shear, and biosensors to model the tooth under functional conditions [30,31].
3. 3.3D bioprinting with digital design, using CBCT/intraoral-scan data to template patient-specific crown and root morphology [11].
4. 4.Gene editing (CRISPR) of patient iPSC organoids to study and correct inherited dental disorders.
5. 5.Living biobanks of oral tumour and salivary organoids for precision medicine [25].
6. 6.Artificial intelligence and single-cell omics to benchmark organoid fidelity against native tissue atlases.
7. 7.First-in-human dental trials, most plausibly salivary gland organoid transplantation for radiation-induced xerostomia, following the precedent of colonic organoid therapy [19,29].

Conclusion

Dental organoids are 3D microtissues that self-organize and mimic the cells, structure, and some functions of teeth and oral tissues. Organoids have now been made for dental epithelium, enamel-forming ameloblasts, dentin-pulp, tooth root and periodontium, salivary glands, oral mucosa, taste epithelium, and oral cancers. They are also being combined into more complex assembloids and toothoids. Dental organoids are important for studying tooth development, modeling disease, testing materials and drugs, personalized cancer treatment, and regenerative dentistry. In prosthodontics, they could shift the field from mechanical replacements to biological ones. Challenges such as securing blood supply, nerves, full development, standardization, and regulatory approval still need to be addressed before organoids can be used in patients. However, the progress from the first intestinal organoid in 2009 to human tooth organoids in just a decade shows that biological tooth replacement is becoming a real possibility.

REFERENCES

1. 1.Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. Science. 2014;345(6194):1247125. doi:10.1126/science.1247125
2. 2.Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459(7244):262-5. doi:10.1038/nature07935
3. 3.Marsee A, Roos FJM, Verstegen MMA; HPB Organoid Consortium; Gehart H, de Koning E, et al. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. Cell Stem Cell. 2021;28(5):816-32. doi:10.1016/j.stem.2021.04.005
4. 4.Jensen C, Teng Y. Is it time to start transitioning from 2D to 3D cell culture? Front Mol Biosci. 2020;7:33. doi:10.3389/fmolb.2020.00033
5. 5.Iwasawa K, Takebe T. Organogenesis in vitro. Curr Opin Cell Biol. 2021;73:84-91. doi:10.1016/j.ceb.2021.06.007

6. 6.Sano T, Renn TY, Kanematsu T, Hsieh MS, Hsu CC, Chang WJ. Organoid in dentistry: models for oral biology and disease. J Dent Sci. 2025;20(3):1816-23. doi:10.1016/j.jds.2025.05.002

7. 7.Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, et al. The development of a bioengineered organ germ method. Nat Methods. 2007;4(3):227-30. doi:10.1038/nmeth1012

8. 8.Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci U S A. 2009;106(32):13475-80. doi:10.1073/pnas.0902944106

9. 9.Hemeryck L, Hermans F, Chappell J, Kobayashi H, Lambrechts D, Lambrichts I, et al. Organoids from human tooth showing epithelial stemness phenotype and differentiation potential. Cell Mol Life Sci. 2022;79(3):153. doi:10.1007/s00018-022-04183-8

10. 10.Hermans F, Hemeryck L, Bueds C, Torres Pereiro M, Hasevoets S, Kobayashi H, et al. Organoids from mouse molar and incisor as new tools to study tooth-specific biology and development. Stem Cell Reports. 2023;18(5):1166-81. doi:10.1016/j.stemcr.2023.03.011

11. 11.Hermans F, Hasevoets S, Vankelecom H, Bronckaers A, Lambrichts I. From pluripotent stem cells to organoids and bioprinting: recent advances in dental epithelium and ameloblast models to study tooth biology and regeneration. Stem Cell Rev Rep. 2024;20(5):1184-99. doi:10.1007/s12015-024-10702-w

12. 12.Kim KH, Kim EJ, Kim HY, Li S, Jung HS. Fabrication of functional ameloblasts from hiPSCs for dental application. Front Cell Dev Biol. 2023;11:1164811. doi:10.3389/fcell.2023.1164811

13. 13.Alghadeer A, Patni AP, Li Z, Lim YC, Mathieu J, Ruohola-Baker H. Protocol for generating three-dimensional induced early ameloblasts using serum-free media and growth factors. STAR Protoc. 2024;5(2):103100. doi:10.1016/j.xpro.2024.103100

14. 14.Lin C, Liu S, Huang M, Zhang Y, Hu X. Induction of human stem cells into ameloblasts by reaggregation strategy. Stem Cell Res Ther. 2024;15(1):332. doi:10.1186/s13287-024-03948-1

15. 15.Zhang Z, Hu H, Xu Z, Shan C, Chen H, Xie K, et al. A chemically defined culture for tooth reconstitution. Adv Sci (Weinh). 2025;12(3):e2404345. doi:10.1002/advs.202404345

16. 16.Jeong SY, Lee S, Choi WH, Jee JH, Kim HR, Yoo J. Fabrication of dentin-pulp-like organoids using dental-pulp stem cells. Cells. 2020;9(3):642. doi:10.3390/cells9030642

17. 17.Gao XH, Li XL, Fan B, Fan W. Engineering organoids for dental pulp tissue regeneration and functional reconstruction. Regen Med. 2025;20(5):203-20. doi:10.1080/17460751.2025.2514899

18. 18.Calabrese TC, Rothermund K, Gabe CM, Beniash E, Davidson LA, Syed-Picard FN. Self-assembly of tooth root organoid from postnatal human dental stem cells. Tissue Eng Part A. 2024;30(9-10):404-14. doi:10.1089/ten.TEA.2023.0219

19. 19.Pringle S, Maimets M, van der Zwaag M, Stokman MA, van Gosliga D, Zwart E, et al. Human salivary gland stem cells functionally restore radiation damaged salivary glands. Stem Cells. 2016;34(3):640-52. doi:10.1002/stem.2278

20. 20.Serrano Martinez P, Cinat D, van Luijk P, Baanstra M, de Haan G, Pringle S, et al. Mouse parotid salivary gland organoids for the in vitro study of stem cell radiation response. Oral Dis. 2021;27(1):52-63. doi:10.1111/odi.13475

21. 21.Wang X, Serrano Martinez P, Terpstra JH, Shaalan A, Proctor GB, Spijkervet FKL, et al. β-Adrenergic signaling induces Notch-mediated salivary gland progenitor cell control. Stem Cell Reports. 2021;16(11):2813-24. doi:10.1016/j.stemcr.2021.09.015

22. 22.Tanaka J, Senpuku H, Ogawa M, Yasuhara R, Ohnuma S, Takamatsu K, et al. Human induced pluripotent stem cell-derived salivary gland organoids model SARS-CoV-2 infection and replication. Nat Cell Biol. 2022;24(11):1595-605. doi:10.1038/s41556-022-01007-6

23. 23.Lombaert I, Movahednia MM, Adine C, Ferreira JN. Concise review: salivary gland regeneration: therapeutic approaches from stem cells to tissue organoids. Stem Cells. 2017;35(1):97-105. doi:10.1002/stem.2455

24. 24.Ren W, Lewandowski BC, Watson J, Aihara E, Iwatsuki K, Bachmanov AA, et al. Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. Proc Natl Acad Sci U S A. 2014;111(46):16401-6. doi:10.1073/pnas.1409064111

25. 25.Driehuis E, Kolders S, Spelier S, Lõhmussaar K, Willems SM, Devriese LA, et al. Oral mucosal organoids as a potential platform for personalized cancer therapy. Cancer Discov. 2019;9(7):852-71. doi:10.1158/2159-8290.CD-18-1522

26. 26.Bazina F, Brouxhon SM, Graham UM, Kyrkanides S. Serotonin contributes to the in vitro production of a biomimetic enamel-like material from reprogrammed oral epithelial keratinocytes. Orthod Craniofac Res. 2021;24(4):494-501. doi:10.1111/ocr.12475

27. 27.Jin Z, Yang B, Zhang S, Liu Z, Wang Y, Lin A, et al. Stem cell-driven biomedical technologies for tooth regeneration: engineering scaffolds, organoid models, and molecular targeted strategies. Stem Cell Res Ther. 2026;17(1). doi:10.1186/s13287-026-05044-y

28. 28.Wang J, Chen X, Li R, Wang S, Geng Z, Shi Z, et al. Standardization and consensus in the development and application of bone organoids. Theranostics. 2025;15(2):682-706. doi:10.7150/thno.105840

29. 29.Watanabe S, Kobayashi S, Ogasawara N, Okamoto R, Nakamura T, Watanabe M, et al. Transplantation of intestinal organoids into a mouse model of colitis. Nat Protoc. 2022;17(3):649-71. doi:10.1038/s41596-021-00658-3

30. 30.Low LA, Sutherland M, Lumelsky N, Selimovic S, Lundberg MS, Tagle DA. Organs-on-a-chip. Adv Exp Med Biol. 2020;1230:27-42. doi:10.1007/978-3-030-36588-2_3

31. 31.Cao UMN, Zhang Y, Chen J, Sayson D, Pillai S, Tran SD. Microfluidic organ-on-a-chip: a guide to biomaterial choice and fabrication. Int J Mol Sci. 2023;24(4):3232. doi:10.3390/ijms24043232

32. 32.Hermans F, Vankelecom H, Bronckaers A, Lambrichts I. Establishment of 3D tooth organoid culture from early-postnatal mouse molar and incisor. Methods Mol Biol. 2025. doi:10.1007/7651_2025_623