Scilight

Regenerative Medicine and Dentistry
https://www.sciltp.com/journals/rmd

Review

Organoids in Dentistry and Oral Medicine: From Disease Models to Regenerative Medicine

Shangyan Li [1,†], Hang Ruan [1,†], Jiqing Huang [1,†], Linyuan Yu [1], Boyang Zhang [1], Ruijia Chen [1], Kecheng Chen [1], Lan Yang [1,*], Janak Lal Pathak [1,*] and Qing Zhang [1,2,3,*]

[1] School and Hospital of Stomatology, Guangdong Engineering Research Center of Oral Restoration and Reconstruction, Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medicine, Guangzhou Medical University, Guangzhou 510182, China
[2] School of Medicine and Dentistry & Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, QLD 4222, Australia
[3] Laboratory for Myology, Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, 1081 BT Amsterdam, The Netherlands
* Correspondence: yangl1900@163.com (L.Y.); j.pathak@gzhmu.edu.cn; (J.L.P.); zhangqing@gzhmu.edu.cn (Q.Z.)
† These authors contributed equally to this work.

How To Cite: Li, S.; Ruan, H.; Huang, J.; et al. Organoids in Dentistry and Oral Medicine: From Disease Models to Regenerative Medicine. *Regenerative Medicine and Dentistry* **2025**, *2*(3), 14. https://doi.org/10.53941/rmd.2025.100014

Received: 23 July 2025
Revised: 25 August 2025
Accepted: 19 September 2025
Published: 24 September 2025

Abstract: Organoids have emerged as powerful three-dimensional models that recapitulate the structure and function of dental and oral tissues through the self-organization of stem or progenitor cells. Recent advances in organoid technology have enabled the generation of tooth, salivary gland, and oral mucosal constructs, offering unprecedented opportunities in disease modeling, drug testing, and regenerative therapies. Despite rapid progress, research in this field remains fragmented, necessitating a comprehensive synthesis of current knowledge. This review systematically examines the latest strategies for constructing organoids and their transformative applications in dentistry and oral medicine, including pathogenesis studies, high-throughput drug screening, tissue engineering, and host-microbiome interactions. Furthermore, we critically evaluate the advantages and limitations of existing methodologies while outlining future directions for innovation. By consolidating key insights, this work aims to accelerate the standardization and clinical translation of organoid-based approaches, ultimately advancing precision medicine in dentistry and oral healthcare.

Keywords: organoids; oral medicine; culture technique; regeneration; stem cells; disease models

1. Introduction

Dental and oral diseases, including caries, periodontal disease, and oral cancers, represent a major global health challenge, with profound impacts on patients' quality of life and socioeconomic well-being [1,2]. While regenerative and precision medicine offer promising therapeutic avenues [3–9], their development is hampered by the absence of preclinical models that faithfully mimic human oral pathophysiology. Traditional approaches, such as animal studies and 2D cell cultures, suffer from critical limitations: interspecies anatomical and immunological disparities reduce the translational relevance of animal data [10], while monolayer cell systems fail to capture the complex 3D microenvironment and multicellular interactions of oral tissues [11].

To bridge this gap, organoid technology has emerged as a transformative tool. Organoids enable more precise disease modeling and support the development of innovative regenerative therapies in dentistry and oral medicine. These self-organizing, three-dimensional structures, derived from stem cells or patient-derived tissues, recapitulate the architectural and functional complexity of native oral tissues, including their cellular diversity and

microenvironmental interactions [12]. By bridging the gap between traditional models and human physiology, organoids provide unparalleled insights into disease mechanisms, regenerative processes, and therapeutic responses [13–15].

This paper systematically examines the development and applications of oral/dental organoids, from their construction strategies to their transformative potential in disease models, drug discovery, and regenerative therapies (Figure 1). We critically evaluate both the advantages and limitations of current methodologies and discuss emerging opportunities in microbiome research and personalized medicine. Finally, we highlight key challenges and future directions, underscoring how organoid technology is poised to redefine research paradigms and clinical translation in oral health.

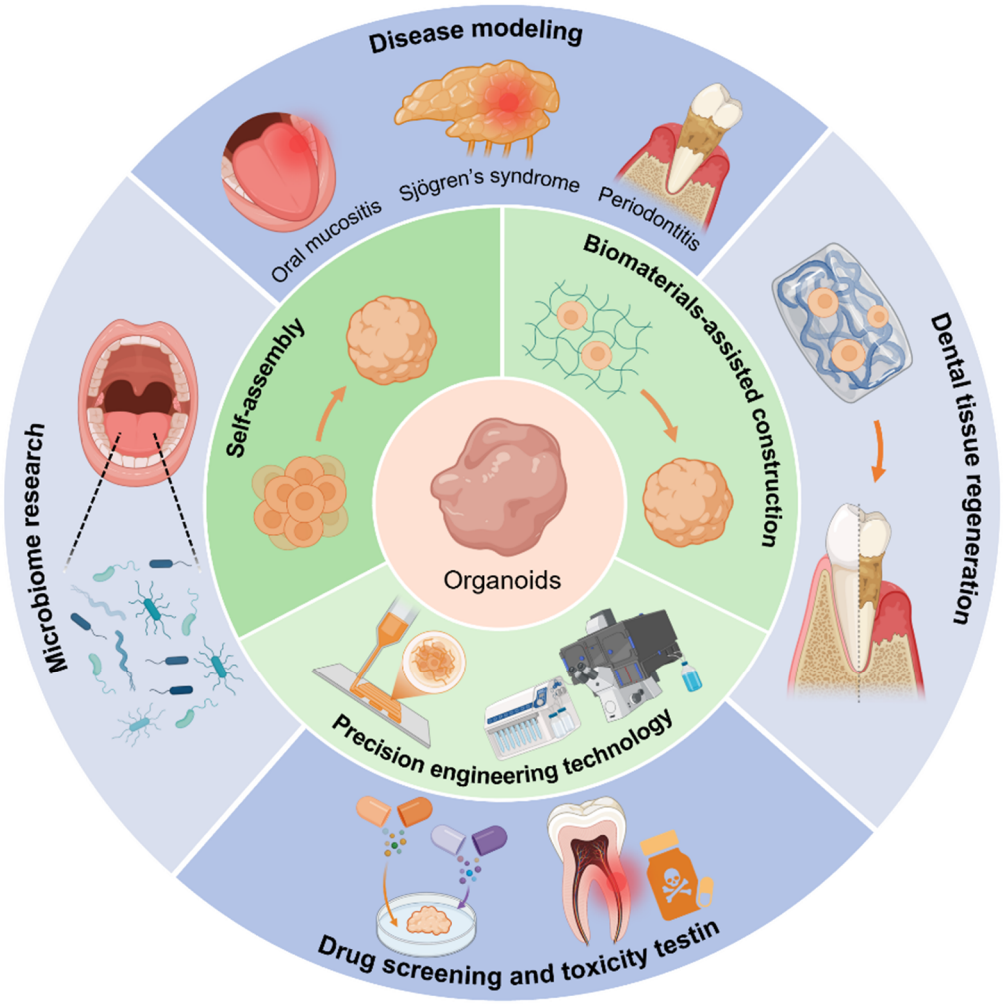

Figure 1. Schematic overview of oral/dental organoid construction strategies and their biomedical applications.

2. Construction Strategies

The development of physiologically relevant oral and dental organoids requires precise recapitulation of the native tissue microenvironment. This process hinges on guiding stem cell differentiation and 3D self-organization through bioengineered approaches. Current construction strategies in this field primarily encompass the following methods and technologies: (1) spontaneous self-assembly of stem cells; (2) biomaterials-assisted construction; and (3) precision engineering technology. Each approach exhibits close connections in reproducing the complex structures and functions of oral tissues (from tooth buds to salivary glands), demonstrating a clear trend toward complementarity and convergence (Table 1).

2.1. Self-Assembly

Spheroids are relatively simple structures formed by the aggregation of cells [16], often lacking the complex multicellular organization and functional differentiation found in organoids [17]. Organoids, on the other hand, are more complex, comprising multiple cell types that better mimic the structure and function of natural tissues.

They exhibit a higher level of self-organization and functional maturity [18]. Self-assembly techniques harness the innate capacity of cells to organize into three-dimensional structures, mirroring the processes observed during embryonic development [19]. By leveraging cell adhesion molecules and extracellular matrix interactions, this scaffold-free approach enables the spontaneous formation of complex tissue architectures [20]. This endows three-dimensional cell spheroids with structures and functions resembling those of primitive tissues and organs, thereby generating organoids [21]. In dental research, self-assembly has emerged as a powerful method for generating organoids that faithfully replicate odontogenic tissues such as dental pulp and dentin [22].

Dental pulp stem cells (DPSCs) demonstrate remarkable self-organization capabilities, forming spheroids of 200–500 μm diameter within seven days when cultured under low-adhesion conditions [19]. This process is mediated by cadherin-dependent cell aggregation, which can be further enhanced through pretreatment with proanthocyanidins [20]. Such interventions improve collagen crosslinking, resulting in a greater than 40% increase in mechanical stability and producing organoids with properties more closely resembling native tissues [20]. The potential of coordinated self-assembly becomes particularly evident in the generation of complex tissue interfaces, such as tooth root-like structures [22]. When DPSCs are co-cultured with periodontal ligament stem cells (PDLSCs) in specific ratios, they spontaneously organize into bilayered organoids featuring distinct tissue compartments [22]. The inner core develops into tubular dentin-like tissue, while the outer layer expresses characteristic markers like cementum protein 1 (CEMP1) and forms Sharpey's fiber-like structures, effectively recreating the natural tooth root interface [22]. Salivary gland organoids constructed using self-assembly principles exhibit significant physiological function, with α-amylase secretion levels reaching 68% of those in natural glands [23].

The self-assembly of organoids is influenced by multiple signaling pathways and intercellular interactions. Among these, Wnt/β-catenin and TGF-β/BMP signaling pathways play particularly prominent roles in the self-assembly of oral organoids [24]. Research indicates that Wnt signaling serves as the key driver for the self-assembly of salivary gland stem cells during long-term culture of salivary gland organoids [25]. Meanwhile, during the process of generating organoids from human gingival-derived stem cells, TGF-β/BMP signaling pathways suggest that they promote the proliferation of dental epithelial cells and maintain cellular viability in regulating tooth development [26,27]. Furthermore, within the co-culture system, the adhesion and self-assembly of DPSCs with endothelial cells (ECs) provide a pathway for establishing vascularized dental pulp organoids. Studies confirm that FOXO1 and FGF2 participate in regulating angiogenesis, while vascular endothelial growth factor A (VEGFA) emerges as a key signaling pathway governing the differentiation of vascular endothelial cells within dental pulp tissue [28]. In co-cultures of osteoblasts and angiogenic cells, interactions between actin cytoskeletons, gap junctions, and the secretion of target cell-targeting factors also provide essential pathways for cellular self-assembly [29].

Various methods for manufacturing cell spheroids include suspension droplets, microporous membranes, microfluidics, magnetic separation, and bioreactors [30–32]. However, challenges remain in terms of cost-effectiveness, high spheroid loss rates during production, and cumbersome medium exchange processes [32]. Recent technological innovations are addressing the challenges of scalability and standardization in organoid production [33–35]. Microfluidic platforms incorporating photoresponsive pyroelectric materials now enable precise control over droplet arrays, allowing simultaneous generation of hundreds of uniform spheroids while facilitating real-time observation of cellular dynamics [33]. Automated systems employing magnetic levitation and robotic integration have dramatically increased production capacity, achieving outputs of up to 2,000 organoids per hour [34]. Using a novel scaffold-free/substrate-free culture system called Magnetic 3D Levitation (M3DL) to assemble primary salivary gland-derived cells (SGDCs) enables these cells to produce extracellular matrix. Compared to traditional culture systems and cell spheroids, these organoids consistently exhibit spheroids with higher cell viability and mitogenic capacity [36]. Computational modeling approaches further refine the process by optimizing critical parameters such as initial cell density, with predictions suggesting that approximately 50,000 cells per spheroid can maintain optimal oxygen gradients and reduce central necrosis while preserving high cell viability [35]. Additionally, the development of columnar/poured-plate platforms enhances flow rates during pouring, promotes 3D cell growth and high-throughput production, reduces cell death in the core zone, and offers high flexibility and user-friendliness [37]. These advancements collectively represent significant progress toward the reliable, large-scale production of physiologically relevant oral organoids for both research and clinical applications [33–35].

2.2. Biomaterials-Assisted Construction

Scaffold materials play a pivotal role in providing a three-dimensional growth microenvironment that guides tissue formation [22]. Natural polymers such as collagen, gelatin, and alginate are widely used due to their inherent biocompatibility, with decellularized dental pulp matrix (dDPM) hydrogel standing out for its ability to retain native ECM proteins and enhance DPSC migration and mineralization [38]. Gelatin methacrylate (GelMA) hydrogels have emerged as a key biomaterial for achieving dental pulp regeneration due to their injectability, rapid gelation, and excellent biocompatibility [39]. Polylactic acid (PLA), polycaprolactone (PCL), and other materials have been utilized to fabricate suitable hydrogels or nanofiber-based scaffolds. These scaffolds promote chondrogenic differentiation of dental pulp stem cells and organoid tissue construction due to their physical properties [40]. Research has demonstrated that a customized gelatin cryogel incorporating hyaluronic acid (HA) and hydroxyapatite (HYP) successfully induces chondrogenic and osteogenic differentiation in mesenchymal stem cells (MSCs), while also exhibiting the ability to self-assemble into osteochondral organoids [41]. Additionally, synthetic polymers, including poly (lactic-co-glycolic acid) (PLGA) and PCL, offer advantages in tunable degradation rates and mechanical properties, exemplified by thermosensitive PNIPAM hydrogels that undergo gelation at body temperature, making them suitable for minimally invasive applications [42]. Despite the numerous advantages of both natural and synthetic scaffold materials, they also present challenges such as batch-to-batch variability and processing sterilization [43]. When selecting biomaterials, a balance should be sought across multiple aspects including biocompatibility, mechanical properties, and processability.

Advances in biomaterials and engineering have enabled the development of dental organoids. The application of various cross-linking techniques-including those triggered by light, specific enzymes, or temperature-and the integration of diverse polymeric materials have opened new avenues for organoid development [44,45]. A study utilized a photopolymerizable biomaterial combining GelMA hydrogel with decellularized bone matrix (BMdc) and deproteinized bovine bone matrix (BMdp). Following seeding with human dental pulp cells (HDPC), this material enhanced odontoblast differentiation and mineral deposition, effectively promoting dentin regeneration [46]. Egg white (EW) and gelatin hydrogels can be prepared into three-dimensional hydrogel scaffolds through sequential temperature-dependent gelation. Chemical crosslinking enhances mechanical strength and stability, exhibiting tunable mechanical properties, high affinity for water, and excellent cell proliferation capacity, offering strong potential for organoid growth [47]. Additionally, composite scaffolds that combine natural and synthetic materials address the limitations of single-component systems—for instance, hybrid scaffolds integrating PLGA with decellularized salivary gland matrix have proven effective in supporting functional salivary gland regeneration [48]. The utilization of these biomaterial-based approaches will actively advance organoid construction in biomedical and clinical translation. Future biomaterials can be engineered to deliver dynamic molecules that facilitate real-time modulation of organoid phenotypes and functions, thereby enhancing organoid development [45].

2.3. Precision Engineering Technology

The development of organoids such as dental pulp and periodontal tissues requires the selection of appropriate construction techniques, with each technique playing a unique role in accurately reproducing the natural structure and physiological functions of these tissues.

2.3.1. 3D Bioprinting for Precision Tissue Fabrication

Currently, 3D bioprinting technology is emerging as a novel approach at the intersection of tissue engineering and translational regenerative medicine. This strategy may open new possibilities for achieving patient-specific, defect-site-specific constructs [49]. 3D bioprinting enables the biomimetic fabrication of complex oral tissues through computer-assisted spatial positioning and layer-by-layer deposition of cell-laden bioinks [50]. This technology has demonstrated significant potential in dental pulp regeneration, periodontal interface reconstruction, and jawbone repair, with key advancements focusing on bioink innovation and multiscale structural control [51–56]. Bioinks must balance bioactivity and printability, leading to the development of hybrid systems such as gelatin methacryloyl/decellularized extracellular matrix (GelMA/dECM), which combine the biological cues of natural materials with the tunable rheology of synthetic polymers [57]. In terms of enhancing post-printing cell viability, the in situ spheroid formation method-where DPSCs self-assemble into spheroids within printed structures-preserves stem cell potential and multipotent differentiation capacity, thereby supporting the formation of more complex organoids [53].The printed structure also influences cell viability. A study printing dentine-pulp guiding structures with instructive niches found that the embedded DPSCs exhibited enhanced viability, migration, and proliferation capabilities [58].

The true strength of 3D bioprinting lies in its ability to replicate the hierarchical structure and heterogeneous interfaces of native tissues [55,57]. Multimaterial printing techniques allow for the simultaneous or sequential deposition of bioinks with distinct compositions, enabling precise mimicry of intricate tissue boundaries [59]. For example, dual-channel systems co-printing DPSC-laden GelMA with mineralized alginate have successfully reconstructed pulp-dentin complexes featuring biomimetic dentinal tubules (Figure 2B) [54]. Similarly, gradient scaffold designs address the mechanical and biological demands of the periodontal ligament-alveolar bone interface, where a stiff β-TCP/PEGDA composite mimics bone's load-bearing capacity while a molybdate-enriched porous GelMA layer guides functional ligament fiber regeneration (Figure 2C) [55].

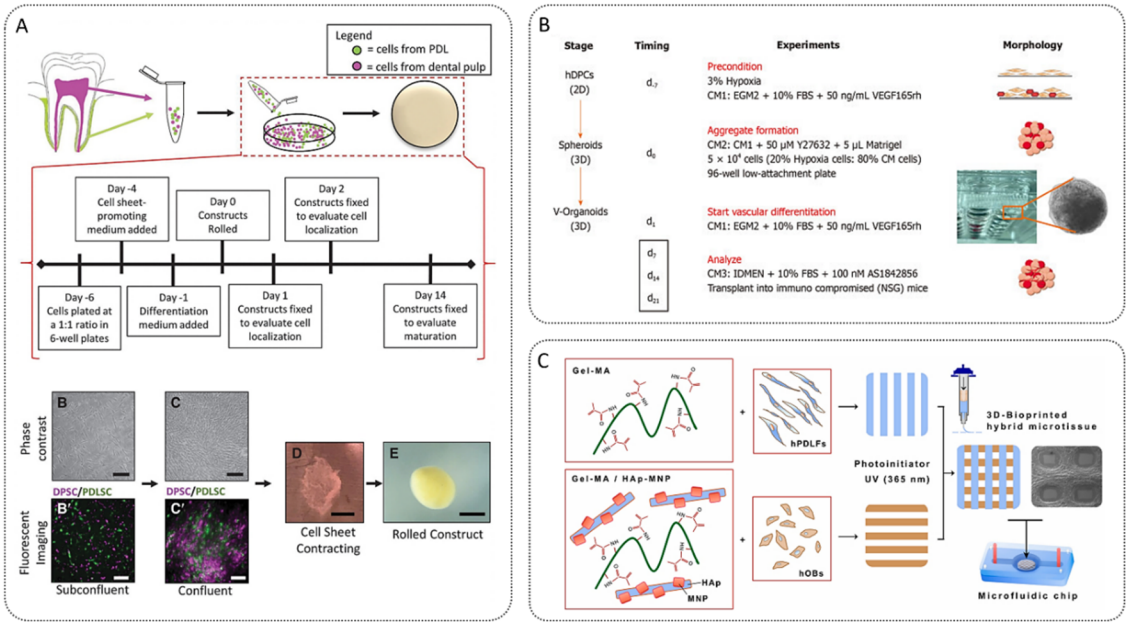

Figure 2. Biomimetic construction strategies for oral tissue engineering. (**A**) Scaffold-free organoid formation using DPSCs and PDLSCs, demonstrating self-assembly capabilities, Copyright 2023, Calabrese [22]; (**B**) Schematic diagram of the timeline for generating Vorganoids from human dental pulp stem cells, Copyright 2024, Liu [28]. (**C**) Microfluidic-integrated dual-material bioprinting platform for generating functional periodontal microtissues, Copyright 2020, Vurat [55].

2.3.2. Microfluidic Technology

Microfluidic technology has revolutionized oral tissue engineering by enabling precise control of fluid shear stress, nutrient gradients, and cellular spatial organization through microscale channel networks [60,61]. Unlike static culture systems, microfluidic platforms overcome the limitations of traditional methods by accurately simulating dynamic oral environments, including salivary flow and masticatory forces [62]. Under the biological principles of organoid self-assembly, microfluidic technology provides a highly biomimetic and controllable in vitro culture and testing platform for organoids, demonstrating significant potential in the fields of dental pulp regeneration, salivary gland modeling, and periodontal tissue engineering.

The key advantage of microfluidic systems lies in their ability to spatially coordinate interactions among multiple cells or structures. For instance, a gingival sulcus-on-a-chip model compartmentalizes keratinocytes, fibroblasts, and endothelial cells under continuous perfusion, successfully replicating the stratified epithelial barrier and enabling studies of inflammatory responses and wound repair [63]. Similarly, the "tooth-on-a-chip" model, comprising dentin discs and DPSCs, was employed to investigate dentin permeability and the extent of DPSC damage under the action of silver diamine fluoride [64]. More advanced "implant-on-a-chip" systems integrate titanium surfaces with gingival cocultures, modeling the complex 3D interplay between host tissue, biomaterials, and oral microbiota to study peri-implantitis pathogenesis and implant biocompatibility [65].

Microfluidic platforms also excel in simulating physiological shear stress, a critical factor in tissue development and function. Studies show that periodontal ligament stem cells exposed to shear stresses of 5–15 dyn/cm^2 activate the Piezo1-Ca^{2+}-AMOT-YAP mechanotransduction pathway, enhancing osteogenic and cementogenic differentiation [66]. In pulp regeneration, fluid shear stress upregulates METTL3-mediated m6A methylation, increasing PDGF-BB secretion and accelerating reparative dentin formation via the p38-MAPK signaling cascade [67].

Additionally, salivary gland-on-a-chip models quantify how flow dynamics influence cariogenic biofilm formation, providing new insights into hydrodynamic factors in caries development [68].

Recent advancements in integrated microfluidics are expanding applications in diagnostics and therapeutics. A continuous-flow PCR array chip enables rapid detection of three periodontal pathogens within just eight minutes [69], while isothermal amplification chambers facilitate chairside caries risk assessment [70]. Drug screening platforms now replicate periodontal pocket fluid dynamics to optimize localized antimicrobial and regenerative therapies [71]. Cutting-edge wireless implantable micropumps further represent a breakthrough in precision medicine, offering on-demand antibiotic and growth factor release for peri-implantitis management [72].

The development of oral/dental organoid construction strategies exhibits a trend toward multi-technology integration and multi-level synergy. Self-assembly approaches capitalize on the innate capacity of cells for self-organization, facilitating multicellular structure formation and functional differentiation under controlled signaling cues. Biomaterials provide essential structural support and modulate biological activity, while precision engineering technologies-such as 3D bioprinting and microfluidics-enable high-fidelity biomimetic replication of organoids with respect to spatial architecture, mechanical properties, and biochemical microenvironments. Future advancements will focus on deeper interdisciplinary convergence to enhance the physiological relevance of organoids, thereby supporting dynamic models for studying oral development and disease mechanisms. These efforts will promote standardized, scalable, and clinically applicable organoid systems while facilitating progress toward personalized regenerative therapies.

3. Applications in Research and Medicine

Oral and dental organoids represent a transformative advancement in biomedical research, serving as three-dimensional, self-assembled culture systems that faithfully replicate the complex architecture, cellular diversity, and functional properties of native oral tissues. Derived from either pluripotent or adult stem cells, these sophisticated models integrate innovations across tissue engineering, developmental biology, and materials science. Their remarkable biological fidelity has established them as powerful platforms for multiple applications, including disease pathogenesis studies, therapeutic development, regenerative medicine approaches, and investigations of host-microbial dynamics. By bridging the gap between conventional cell culture and in vivo systems, oral organoids are revolutionizing our understanding of oral and maxillofacial disorders while accelerating translational discoveries.

3.1. Disease Models and Pathological Recapitulation

The complex nature of oral and maxillofacial diseases, which frequently involve coordinated pathological changes across diverse tissue types, including dental, periodontal, mucosal, and glandular components [73], presents unique challenges for traditional research models. Organoid technology addresses these limitations through its capacity for self-organization, enabling the generation of patient-specific or genetically engineered disease models that maintain tissue-specific pathological features. This innovative approach has emerged as a particularly valuable tool for studying disease mechanisms, with growing applications in both fundamental research and regenerative medicine. By preserving the genetic and phenotypic characteristics of donor tissues, organoid models provide unprecedented opportunities to investigate disease progression, test therapeutic interventions, and develop personalized treatment strategies for various oral pathologies.

Table 1. Strategies for constructing oral/dental organoid disease models.

Oral disease	Cell type	Scaffold	Culture System	Outcome	Applications	Reference
OSCC	CSCs and CAFs	Matrigel	Co-culture of CSCs and CAFs organoids	Primary tumor organoid was established	Mechanism of tumor-matrix interaction and specific drug screening	[74]
	Tumor cells, CAFs, and PFs	Matrigel	Fibroblast adherent organoid culture	The crucial role of the Notch pathway in CAFs/PFs was elaborated.	Role of Notch signaling and screening of Notch inhibitors	[75]
Oral Mucositis	Human TSCC cell line SCC15 and mouse fibroblast cell line 3T3	Collagen Type I Matrix	L-glutamine and hydrocortisone-added culture system with transwell insert	Pathological features of oral mucositis were established	Mechanism of chemotherapy-induced mucosal damage and role of commensal microorganisms in mucositis	[76]
	Mouse CVP $Lgr5^+$ taste bud stem cells	Matrigel	Stem cell factors such as EGF and Noggin, N2, B27, and NAC were added.	Radiation and SIRT1 inhibitors doses were tested.	Mechanism of radiotherapy-damaged taste buds and the role of stem cells in mucosal repair	[77]
Sjögren's Syndrome	Human salivary gland epithelial cells	GelMA	Growth factors FGF2 and FGF10 and small molecule inhibitors were used.	Functional salivary gland organoids were obtained.	Screening of Tofacitinib to treat radiation-induced xerostomia	[78]
	Human salivary gland stem cells, C57BL/6N mouse submandibular gland stem cells	Type 1 atelocollagen and Matrigel	Human cells were cultured in AdDMEM/F12 medium, while mouse cells were cultured in DMEM/F12+WRN conditioned medium	Organoids with acinar and ductal cells were successfully implanted into mice salivary glands.	Developing protocol for a clinical-grade organoid culture	[79]
Dental enamel damage and loss	Epithelial cells derived from human dental follicles (DF) and DPSCs	Matrigel	Cultured in the mineralization-inducing medium.	Human dental epithelial organoid was established.	Screening of small molecule inhibitors targeting the TGF-β pathway on enamel regeneration	[80]
	Mouse and human-derived ameloblast-like cells and odontoblast-like cells	PLGA	Cells were co-cultured with PLGA microscaffolds	Organoid with epithelial-mesenchymal interaction	Mechanism of enamel damage	[81]
Periodontal disease	hPDLFs and hOBs	Gel-MA/HAp-MNPs	Microfluidic dynamic culture in PDMS material dual-channel chips	The double-layered stable structure	Simulation of PDL-AB interface inflammatory response	[55]
	DFCs and PDL-hTERT immortalized cell line (derived from periodontal ligament)	No scaffold	The cells were cultured in low/high glucose medium	DFC type organ cells arranged in an orderly manner	Construction of functional PDL organoids with periodontitis microenvironment	[82]

3.1.1. Oral Squamous Cell Carcinoma (OSCC) Organoid Models: Advancing Precision Oncology

OSCC, the most common malignant tumor of the oral cavity, has been increasingly studied using advanced organoid models that faithfully recapitulate the complex tumor microenvironment (TME). Researchers have developed innovative three-dimensional co-culture systems by combining patient-derived tumor organoids with cancer-associated fibroblasts (CAFs), enabling detailed investigation of critical tumor-stroma interactions [74]. These models have proven particularly valuable for studying contact-dependent cellular crosstalk, revealing how direct tumor cell contact triggers phenotypic transitions in adjacent fibroblasts [75]. A significant advancement in this field involves the identification of stromal nicotinamide N-methyltransferase (NNMT) as a key regulator of tumor behavior. Using fibroblast-attached organoid (FAO) models, studies have demonstrated that NNMT expression in the tumor stroma promotes both organoid self-assembly [83] and sustains angiogenic potential in OSCC organoids [84], establishing it as a promising therapeutic target for interventions targeting extracellular matrix remodeling and anti-angiogenic strategies.

Particular progress has been made in modeling tongue squamous cell carcinoma (TSCC), the most prevalent OSCC subtype [85]. Researchers have achieved enhanced pathological fidelity by culturing TSCC cells on decellularized tongue extracellular matrix (TEM), resulting in organoids that better preserve tumor heterogeneity and histopathological characteristics [86]. These TEM-based models now serve as superior platforms for both TSCC research and regenerative medicine applications, offering more clinically relevant systems for studying tumor biology and testing therapeutic interventions.

The evolution of OSCC organoid systems has progressed remarkably from initial "epithelium-only" models to current integrated multicellular platforms capable of dissecting diverse TME interaction mechanisms. These advanced models provide a comprehensive framework for investigating cancer cell-stroma crosstalk while enabling the development of precision-targeted therapies against key molecular drivers. By maintaining the biological complexity of native tumors while incorporating patient-specific genomic data, modern organoid systems support multidimensional exploration of personalized treatment approaches. This technological advancement is particularly transformative for drug sensitivity screening and chemoresistance studies, bridging the gap between basic research and clinical application. Ultimately, these developments are accelerating the implementation of precision medicine paradigms in oral oncology, offering new opportunities for understanding tumor biology and improving therapeutic outcomes [74,75,83–86].

3.1.2. Modeling Oral Mucositis Using Organoid Technology

Oral mucositis (OM) represents a frequent and debilitating complication of cancer therapy, severely compromising patients' oral function, nutritional intake, and overall quality of life while often limiting treatment tolerance [87]. Recent advances in organoid technology have enabled the development of sophisticated models to study this complex condition. Researchers have established an innovative chemotherapy-induced mucositis model utilizing oral mucosal organoids derived from human epithelial cell lines cultured within fibroblast-embedded collagen matrices, followed by exposure to 5-fluorouracil (5-FU) [76]. This biomimetic system successfully reproduces hallmark pathological features of clinical OM, including suppressed DNA synthesis, characteristic apoptotic patterns, and distinctive cytoplasmic vacuolization, thereby providing an unprecedented experimental platform for investigating disease pathogenesis.

Beyond chemotherapy-induced injury models, organoid systems have also been adapted to study radiation-induced damage, particularly in evaluating radiation responses of epithelial stem cells [77]. These models offer crucial mechanistic insights into the cellular processes underlying radiation-associated complications such as taste dysfunction and mucosal ulceration, facilitating the development of targeted protective strategies. The evolution of OM organoid technology represents a significant advancement in mucosal pathobiology research, as it enables comprehensive simulation of disease progression throughout various cancer treatment modalities. By bridging the gap between basic research and clinical application, these organoid systems accelerate the translation of mechanistic discoveries into effective therapeutic interventions, ultimately aiming to enhance treatment tolerance and restore patients' quality of life during oncologic care [76,77,87].

3.1.3. Sjögren's Syndrome and Salivary Gland Organoid Models

Sjögren's syndrome (SS) is a chronic autoimmune disorder that primarily targets exocrine glands [88], leading to progressive lymphocytic infiltration, glandular tissue destruction, and subsequent salivary dysfunction that manifests as debilitating xerostomia. Recent advances in organoid technology have enabled the development of more physiologically relevant disease models through the innovative use of patient-derived salivary gland epithelial cells (SGECs) obtained from xerostomia patients and labial salivary gland biopsy (LSGB) specimens [78]. These patient-

specific organoid systems accurately recapitulate SS-associated pathological damage, providing an unprecedented experimental platform for investigating disease mechanisms and screening potential therapeutic compounds.

Tissue engineering approaches have significantly enhanced salivary gland organoid development through the use of three-dimensional biomaterial scaffolds. Studies demonstrate that collagen-based matrices substantially improve organoid construction efficiency while maintaining tissue-specific functionality [89]. By culturing human salivary gland cells within these bioactive scaffolds, researchers have not only optimized organoid formation but also pioneered novel stem cell-based regenerative strategies. Particularly promising are collagen-cultured organoids derived from human salivary gland stem cells, which exhibit strong potential for allogeneic transplantation applications and represent a critical step toward clinical translation [79].

Current investigations focus on deciphering the microenvironmental regulation of epithelial stem and progenitor cells during salivary gland development and applying these insights to xerostomia models [90]. These organoid-based systems have proven instrumental in elucidating the central role of stem cells in glandular regeneration while simultaneously advancing the therapeutic potential of stem cell-derived treatments [91]. By bridging fundamental research with clinical applications, salivary gland organoid technology offers new regenerative medicine approaches for restoring secretory function and addressing refractory conditions like SS-associated xerostomia, marking a significant advancement in the management of autoimmune exocrinopathies.

3.1.4. Modeling Enamel Defects Through Organoid Technology

The intricate process of tooth development fundamentally relies on precisely coordinated epithelial-mesenchymal interactions [92], with any disruption in the enamel-dentin complex potentially leading to structural defects or complete enamel loss. Recent breakthroughs in organoid technology have successfully replicated these critical developmental mechanisms in vitro, opening new avenues for dental research. A pioneering mesenchymal-epithelial organoid model derived from dental follicle tissue has demonstrated the capacity to drive epithelial stem cell differentiation into functional ameloblasts [80]. This innovative system provides an invaluable platform for investigating the multifaceted causes of enamel defects, including hereditary conditions such as amelogenesis imperfecta, bacterial infections, and various genetic mutations.

Building upon these foundational discoveries, researchers have developed an advanced three-dimensional bilayer organoid system that combines dental pulp-derived ameloblast-like cells with odontoblasts [81]. This sophisticated model more accurately recapitulates the complex reciprocal signaling between epithelial and mesenchymal components that characterizes the bell stage of odontogenesis. These organoid advancements collectively offer novel perspectives for studying both normal tooth morphogenesis and pathological conditions, serving as powerful tools that bridge basic research and clinical applications. The models enable detailed mechanistic studies of enamel and dentin formation while providing diagnostic capabilities for rare dental disorders including amelogenesis imperfecta and dentin dysplasia.

These technological innovations establish a continuous research framework that maintains fidelity to the core principle of epithelial-mesenchymal crosstalk while connecting fundamental developmental biology with pathological processes. By faithfully recreating the dynamic interactions that govern tooth formation, these organoid systems not only deepen our understanding of dental development but also pave the way for innovative approaches in regenerative dentistry and the treatment of structural dental anomalies [80,81,92]. The integration of developmental biology with organoid technology represents a significant stride forward in addressing longstanding challenges in dental research and clinical practice.

3.1.5. Advances in Periodontal Disease Models Using Organoid Technology

Periodontitis, a chronic inflammatory condition affecting more than one billion people worldwide [93], remains the leading cause of adult tooth loss. This destructive disease manifests through characteristic pathological changes including periodontal ligament (PDL) degradation and progressive alveolar bone resorption. Recent innovations in three-dimensional microfluidic tissue engineering have revolutionized our ability to study periodontal pathology through the development of sophisticated bioink-based models that accurately simulate the periodontal microenvironment [93]. These advanced systems successfully recreate the composite anatomical structure of the periodontal ligament and adjacent alveolar bone, providing unprecedented opportunities for mechanistic investigations.

Significant progress has been made in biomaterial development for periodontal models, with composite materials combining gelatin methacryloyl hydrogels and hydroxyapatite-magnetic iron oxide nanoparticles demonstrating exceptional biocompatibility and mechanical properties that closely mimic native periodontal tissues. In parallel, scaffold-free organoid approaches utilizing immortalized periodontal ligament fibroblast cell

lines have enabled precise quantification of dental follicle cell differentiation potential [82], establishing reliable in vitro platforms for studying functional PDL regeneration. These technological advances have created new possibilities for both basic research and clinical translation in periodontology.

Contemporary research efforts are increasingly focused on deciphering the regulatory mechanisms that maintain periodontal ligament homeostasis. Modern organoid systems that integrate cellular components, biologically active scaffolds, and physiologically relevant mechanical stimuli are providing novel insights into the molecular pathways driving periodontal tissue destruction. These comprehensive models serve as a transformative research framework that bridges fundamental pathological studies with functional tissue reconstruction approaches. By combining multiple technological platforms, this collaborative research strategy is accelerating the development of innovative periodontal repair therapies, while simultaneously providing the theoretical foundation and technical support needed to achieve both structural and functional regeneration in clinical practice [82,93]. The integration of these advanced organoid systems represents a significant step forward in our understanding and treatment of periodontal diseases.

3.2. Drug Screening and Toxicity Testing

Oral organoid technology is transforming pharmaceutical development by providing physiologically relevant human models for therapeutic screening and safety assessment. These advanced three-dimensional systems offer unprecedented opportunities to evaluate mucosal anti-inflammatory compounds, anticancer agents, and dental biomaterials while addressing the critical need for ethical and precise drug development approaches [94,95]. Building upon the proven success of organoid models in other organ systems, oral organoids demonstrate particular promise for accelerating clinical translation through their ability to recapitulate disease-specific pathophysiology and drug responses.

The predictive power of oral organoids is exemplified by their application in oncology research. Head and neck squamous cell carcinoma (HNSCC) organoid biobanks have proven effective for validating responses to conventional chemotherapeutics (cisplatin, carboplatin) and targeted agents (cetuximab), successfully correlating genomic profiles with phenotypic drug sensitivity [96]. This approach reaches its full potential in personalized medicine, where patient-derived OSCC organoids cultured from surgical specimens maintain original tumor genomics while revealing individual therapeutic vulnerabilities through drug sensitivity testing [97]. The integration of artificial intelligence with these organoid systems further enhances screening accuracy and efficiency, highlighting the need to establish comprehensive oral disease-specific biobanks to support large-scale precision medicine initiatives [97].

For toxicity evaluation, dental pulp organoids co-cultured with endothelial cells provide human-relevant alternatives to animal testing, offering ethical platforms for assessing dental material biocompatibility and pulp regeneration strategies while reducing clinical trial uncertainties [98]. Additionally, organoid cultures derived from circulating tumor cells (CTCs) in head and neck cancer (HNC) patients offer numerous advantages, including stable gene expression, patient-specific origin, and high-throughput screening capabilities. These cultures can serve as a central platform for real-time assessment and prediction of treatment response and drug resistance in clinical settings, thereby advancing personalized medicine for HNC patients [99]. Advanced organoid systems incorporating stem cells and growth factors enable detailed analysis of drug-induced cellular behaviors, providing mechanistic insights into therapeutic effects [100]. Molecular-level discoveries are also emerging, as demonstrated by periodontal ligament organoids that identify specific receptor domains as potential antiviral targets through analysis of compound-viral protein interactions [101].

These applications collectively position oral organoids as indispensable tools for modern drug development, offering human-relevant pharmacological data while reducing animal testing dependence. As the field progresses, key challenges include standardizing organoid biobanking protocols, optimizing AI-integrated screening platforms, and accelerating the clinical translation of organoid-guided therapies. By bridging the gap between bench research and clinical application, oral organoid technology is reshaping therapeutic development pipelines and paving the way for more effective, personalized treatment strategies [94–97,100,101].

3.3. Tissue Regeneration

The three-dimensional architecture of oral organoids has emerged as a powerful platform for regenerative medicine by faithfully recreating the intricate cell-ECM interactions that govern native tissue development and function. These sophisticated models are driving innovations across multiple fronts of oral tissue engineering, from stem cell biology to vascular and neural integration, offering transformative potential for clinical applications.

Stem Cell-Driven Regeneration: Recent advances have identified several potent stem cell populations within dental tissues that serve as the foundation for organoid-based regeneration. DPSCs, PDLSCs and dental follicle cells demonstrate remarkable regenerative capabilities when cultured under appropriate conditions. Studies show that DPSCs cultured in odontogenic differentiation media (ODM) with supportive matrices significantly upregulate dentin-specific markers DSPP and DMP-1 [102], highlighting their potential for dentin-pulp complex regeneration. The accessibility, high proliferative capacity, and multilineage differentiation potential of these cells [103] make them particularly valuable for orofacial reconstruction and dental tissue engineering applications.

Biomaterial Innovation: The success of in vitro tooth regeneration depends critically on advanced biomaterials that can guide three-dimensional tissue development. Cutting-edge bioprinting technologies now enable precise spatial control over cellular organization and matrix composition. The integration of GelMA with decellularized extracellular matrix bioinks has opened new possibilities for periodontal tissue regeneration [51], while bioorthogonally crosslinked hydrogels have proven essential for directing tooth organoid morphogenesis [104]. These engineered biomaterial systems provide the necessary structural and biochemical cues to support the formation of complex tissues.

Vascular Network Development: Establishing functional vascularization remains a key challenge in tissue engineering. Recent coculture strategies have made significant progress, with vascularized dental pulp organoids successfully generated through DPSC-endothelial cell cocultures [98]. Enhanced VEGF expression in periodontal ligament stem cell-endothelial cell systems promotes robust angiogenesis [104], addressing the critical need for nutrient delivery and waste removal in engineered tissues [105]. These vascularized models represent a major advancement in creating clinically viable tissue constructs.

Neural Integration: The field has achieved notable breakthroughs in neural integration, a critical requirement for functional oral tissue regeneration. Researchers have successfully generated neuronal-like cells from dental stem cells and incorporated them into three-dimensional midbrain-like organoids [106]. These models not only provide platforms for studying neurodegenerative processes but also represent significant progress toward achieving functional reinnervation in regenerated oral tissues.

By synergistically combining stem cell biology, advanced biomaterials, and engineered vascular/neural components, oral organoid technology is transitioning from basic research models to functional tissue replacements. This comprehensive approach addresses the full spectrum of challenges in oral tissue regeneration, from fundamental mechanistic understanding to clinical implementation, marking a new era in regenerative dentistry. The integration of these technologies offers promising solutions for reconstructing complex orofacial defects and represents a paradigm shift in dental therapeutics.

3.4. Advances in Oral Microbiome Research Using Organoid Models

The oral cavity represents a highly complex microbial ecosystem where diverse bacterial communities maintain delicate balances between health and disease states [107]. Three-dimensional oral organoids have emerged as transformative tools for investigating these host-microbiome interactions under precisely controlled conditions, enabling researchers to dissect the mechanisms by which specific microbial species either maintain oral health or contribute to pathologies including dental caries, periodontitis, and OSCC. These models provide critical insights into oral-systemic disease connections that have long eluded conventional research approaches.

Modern organoid systems now successfully recreate key aspects of oral micro-ecology, permitting detailed examination of epithelial-microbial interaction [108]. Innovative asymmetric gas co-culture platforms maintain physiological oxygen gradients that preserve the viability of anaerobic bacteria while supporting gingival epithelial barrier function, offering unprecedented opportunities to study bacterial invasion mechanisms and epithelial immune responses [108]. More advanced bioreactor-integrated gingival organoids incorporate dynamic fluid flow, ionic composition, and mechanical forces that mimic natural oral conditions. These systems not only sustain complex microbial communities but also reproduce the homeostatic regulatory mechanisms of periodontal pockets, including epithelial barrier maintenance and microbial population balance [109,110]. Such technological advances provide essential experimental evidence for understanding both healthy microbial homeostasis and dysbiotic shifts during disease progression.

The oral microbiome's role in systemic health is becoming increasingly apparent through organoid research. Periodontitis-associated dysbiosis, particularly involving *Porphyromonas gingivalis* (*Pg*), has been precisely modeled using dual-chamber systems that reveal how *Pg* signal molecules induce metabolic changes in Fusobacterium nucleatum to accelerate pathogenic biofilm formation [111]. These findings highlight the model's potential for identifying novel therapeutic targets to prevent or reverse dysbiosis. Equally significant are discoveries about oral microbial translocation, where oral bacteria colonizing gastric and intestinal sites

demonstrate site-specific composition changes that may drive distinct carcinogenic processes [112]. Furthermore, organoid studies confirm that severe periodontitis can trigger systemic inflammation with potential cardiovascular consequences [113,114], while emerging research implicates oral microbial imbalances in diabetes and neurodegenerative conditions like Alzheimer's disease [115].

Oral microbiome research has thus evolved from studying single pathogens to investigating complex microbial ecosystems and their systemic impacts. Organoid technology stands at the forefront of this paradigm shift, serving as an indispensable bridge between basic biological discoveries and clinical applications. These advanced models not only enable early detection and precise diagnosis of oral diseases but also pave the way for developing personalized treatments and regenerative solutions. By elucidating the fundamental mechanisms governing oral microbial homeostasis, organoid systems are transforming our approach to preventing and treating both oral and systemic diseases [107–116].

4. Advantages and Challenges

As innovative three-dimensional models that faithfully recapitulate tissue-specific genetic characteristics and cellular heterogeneity, organoids have emerged as powerful tools in oral research with tremendous potential for both basic science and clinical applications. However, their widespread adoption and translation to clinical practice still present significant challenges that must be addressed.

4.1. High-Fidelity Physiological Models

Oral/dental organoids excel in reproducing the intricate structural and functional complexity of native tissues, maintaining authentic cellular diversity, physiological activities, and molecular expression profiles [117–119]. These models have become indispensable for studying tissue regeneration and disease pathogenesis, with their sophisticated 3D architecture enabling accurate simulation of critical cell-cell interactions and extracellular matrix (ECM) microenvironments—essential factors for understanding complex disease mechanisms [118–120]. In dental development research, tooth germ organoids have proven particularly valuable by successfully modeling the epithelial-mesenchymal crosstalk that drives human odontogenesis, providing unprecedented insights into natural tooth formation processes [118–120].

4.2. Enhanced Translation and Cost Efficiency

Organoid technology addresses several limitations of traditional animal models, which are often hampered by species-specific differences, prolonged experimental timelines, and high costs [121]. By enabling direct observation of human biological processes in vitro, organoids offer a more physiologically relevant and cost-effective alternative, particularly for pharmaceutical research where animal studies frequently yield inconclusive results [122]. Organoids derived from human immortalized cell lines are crucial for simulating disease types and preserving disease-specific characteristics, making them valuable for fundamental molecular biology and translational research such as drug discovery and testing [123]. Additionally, organoids better preserve the histological structure and molecular characteristics of the primary tumor site compared to 2D cell cultures. Organoids derived from salivary gland carcinoma (SGC) patients reproduce tumor heterogeneity and clonal selection during cultivation, making them an ideal platform for drug screening, targeted therapy validation, and combination therapy research [124]. These human-derived systems significantly improve experimental reproducibility and throughput while reducing ethical concerns associated with animal testing, ultimately accelerating drug development pipelines and providing researchers with more reliable investigative platforms [125–127]. The enhanced predictive capability of organoid models promises to bridge the longstanding gap between preclinical studies and clinical outcomes in oral health research.

4.3. Current Limitations

4.3.1. Structural and Functional Constraints

Despite significant advances, contemporary oral/dental organoid systems still face critical limitations in replicating the full complexity of native tissues. Current models struggle with inadequate vascularization and innervation, incomplete hard tissue biomimicry, and insufficient immune microenvironment representation [118,120]. Dental pulp organoids, while capable of generating dentin-like matrices, fail to reproduce the functional neurovascular networks essential for studying pain pathways and inflammatory responses. Similarly, enamel-producing organoids demonstrate markedly inferior mineralization density and mechanical properties compared

to natural tooth structures. Perhaps most notably, the absence of integrated immune components in current models severely limits their utility for investigating immune-mediated oral diseases [118,128,129]. These functional gaps underscore the need for more sophisticated co-culture systems that can better approximate tissue complexity.

4.3.2. Technical and Economic Barriers

The field currently lacks standardized protocols and consistent characterization metrics for organoid cultivation and application [130]. Significant variability in culture conditions across research groups leads to substantial batch-to-batch inconsistencies, undermining experimental reproducibility and data comparability [131,132]. For instance, in applying organoids to achieve dental pulp regeneration, it is essential to determine the optimal stem cell density and organoid quantity. Organoids must be mass-produced in standardized and uniform sizes, and tissues with similar biological characteristics must be constructed for clinical trial applications [133]. Furthermore, limited nutrient availability, inefficient metabolic waste clearance, and complex internal anatomy collectively contribute to the size limitations observed in current pulp organoid engineering approaches [134]. Certain purified recombinant protein factors exhibit poor solubility and insufficient long-term storage stability, limiting the accurate modeling of specific tumor niches and reducing the reproducibility of tumor organoid clinical translation [135]. While organoid-based screening costs approximately 5% of equivalent animal studies, the technology faces substantial economic hurdles. Long-term maintenance requires costly growth factors and specialized media, while large-scale implementation necessitates expensive automation infrastructure. Conventional manual culture methods remain prohibitively labor-intensive for industrial-scale applications, particularly in high-throughput drug discovery or clinical settings [136,137]. Although emerging bioreactor and automation technologies show potential for scaling production, achieving cost-effective manufacturing while maintaining biosafety and functional integrity requires further technological innovation [127]. Additionally, due to conflicts between time and throughput, optimizing workflows and shortening the turnaround time for organoid generation and drug sensitivity testing (DST) is essential for drug screening in tumor patients at different stages [138]. These challenges highlight the need for collaborative efforts to establish quality standards and develop more sustainable cultivation systems.

4.3.3. Ethical Considerations and Biomaterial Limitations

The field of oral/dental organoid research continues to face significant ethical and material challenges that must be addressed for successful clinical translation. The persistent ethical concerns surrounding embryonic stem cell (ESC) usage [132]. have prompted increased focus on induced pluripotent stem cells (iPSCs) as an alternative. However, patient-derived iPSC organoids introduce their own complexities, including potential immune rejection risks and biosafety considerations that require thorough evaluation.

Current biomaterial options present substantial limitations for clinical applications. The widely utilized Matrigel, sourced from animal tumors, suffers from multiple drawbacks including an undefined composition, significant batch-to-batch variability, and potential tumorigenic risks, all of which create barriers to regulatory approval and clinical adoption [139]. These challenges have driven urgent research efforts to develop fully characterized synthetic or decellularized matrix alternatives with improved biocompatibility and reproducibility.

An additional concern involves the genomic stability of long-term organoid cultures. Extended in vitro maintenance may lead to genetic alterations that could affect phenotypic accuracy, potentially compromising their reliability for disease models and drug screening applications [132]. This stability issue highlights the need for improved culture protocols and rigorous quality control measures to ensure the biological relevance of organoid models throughout extended experimental timelines. Additionally, in drug screening tests, organoid DST must be integrated into the regulatory framework of clinical practice. Guidelines addressing quality control and ethical considerations related to organoid DST should be established to ensure safe and reliable drug use [138]. Addressing these multifaceted challenges will be crucial for advancing organoid technology toward clinical implementation while maintaining ethical standards and scientific rigor.

5. Future Perspectives

The field of oral/dental organoid technology is undergoing transformative advancements, with multiple breakthroughs emerging at the forefront of biomedical research. Oral/dental organoids represent a paradigm shift in in vitro modeling, offering unprecedented capabilities to reconstruct the three-dimensional microenvironment of human oral tissues. These innovative platforms demonstrate significant potential in drug development, disease mechanism research, and personalized treatment approaches (Figure 3).

5.1. Matrix Materials for Organoid Construction

Matrix materials play a central role in supporting organoid growth and differentiation during their construction. Thus, selecting appropriate matrix materials is one of the critical steps in organoid development. However, current matrix materials still face challenges such as poor mechanical stability, low strength, and rapid degradation rates [140]. Developing advanced matrix materials that accurately mimic the native extracellular matrix environment represents a major direction for future research. Several innovative strategies have been implemented, including optimizing component ratios [141], employing strategic chemical modifications [142], applying advanced cross-linking techniques [143], and designing composite matrices incorporating other biopolymers or synthetic components [144,145]. The ultimate objective is to engineer tunable biomimetic matrices with enhanced structural stability, controllable degradation profiles, and tissue-specific mechanical properties tailored for diverse organoid applications.

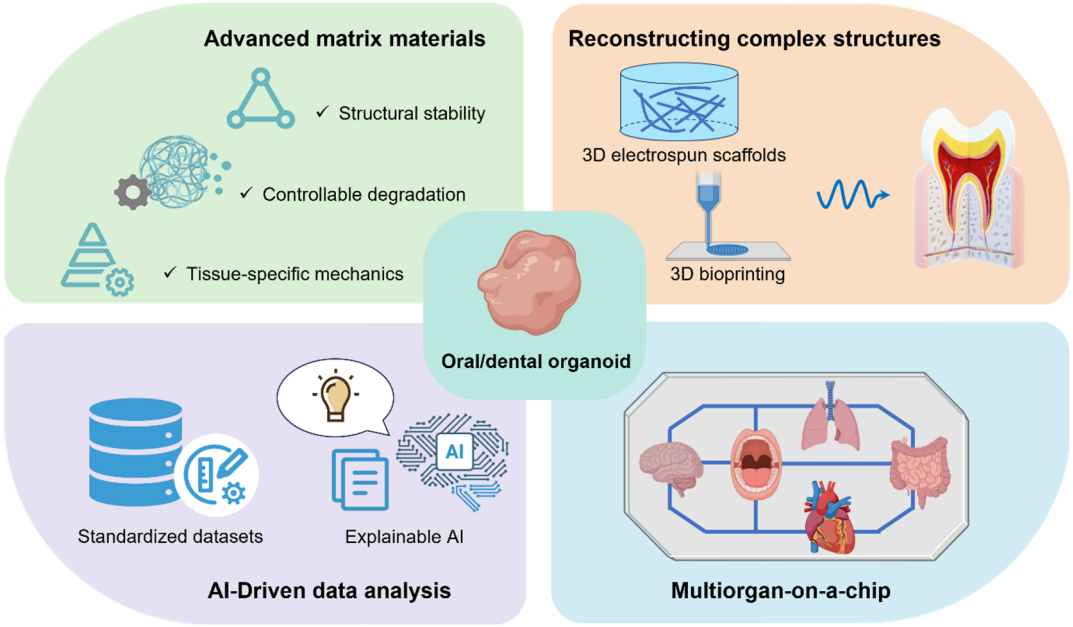

Figure 3. Future perspectives in oral/dental organoid technology.

5.2. Reconstructing Complex Oral Structures

Organs such as teeth and the tongue exhibit highly complex structures, which current organoid models struggle to recapitulate. Notably, bioengineering tools enable the orchestrated assembly of multiple tissue components, offering a promising solution to this challenge. Biomimetic scaffolds can facilitate the simulation of various tissues and their interactions; for instance, 3D electrospun scaffolds help establish appropriate mesenchymal-epithelial interactions [146]. Beyond biomimetic scaffolds, 3D bioprinting has also been utilized to enhance structural complexity. Laser-assisted bioprinting has been applied to fabricate capillary networks [147]. Moreover, magnetic 3D bioprinting has enabled precise control over cell positioning to generate organoids featuring multiple structures, including epithelium, ducts, and neurons [148]. The integration of bioengineering tools to construct organoids with more sophisticated architectures remains an important research direction.

5.3. AI-Driven Organoid Data Analysis

The integration of organoid technology with artificial intelligence (AI) holds significant promise for accelerating data analysis in organoid applications [149,150]. Deep learning has already demonstrated considerable potential in organoid segmentation and analysis [151]. Despite the substantial promise of AI and machine learning in organoid research, several key challenges remain. The lack of standardized datasets in current studies results in poor model generalizability across different laboratories and organoid types. Furthermore, model interpretability remains a major obstacle for clinical translation. Developing explainable AI is essential to clarify decision-making processes and build clinical trust. Leveraging AI in oral/dental organoid research can greatly enhance the efficiency of data analysis. The establishment of standardized datasets and interpretable models constitutes a promising future research direction.

5.4. Multiorgan-on-a-Chip

Current single-organoid systems are inadequate for modeling the complex interactions among different organs. A potential future direction involves interconnecting multiple organs from the organ level to the systemic level. Such integrated systems would allow comprehensive investigation of oral-systemic connectivity and provide a foundation for studying how oral pathologies affect distant organ functions. Technically, this can be achieved by integrating organoids with microfluidic technologies to develop organ-on-a-chip devices and linking multiple chips [152]. Modular microfluidic systems, which enable fluidic coupling through interconnected designs or testboards, are particularly promising due to their operational flexibility and experimental versatility [152,153]. These advances will not only improve our understanding of oral–systemic disease relationships but also serve as foundational components in the development of comprehensive "human-on-a-chip" platforms [154].

6. Conclusions

Oral/dental organoids represent a transformative breakthrough in dental and craniofacial research, establishing unprecedented capabilities for modeling human diseases, evaluating therapeutic responses, and developing regenerative strategies. These sophisticated three-dimensional models have overcome fundamental limitations of conventional cell cultures and animal studies by faithfully recreating the structural and functional complexity of native oral tissues, providing novel insights into diverse pathologies ranging from oral malignancies to salivary gland dysfunction and periodontal disorders. While significant progress has been made, key challenges persist in achieving complete physiological relevance, particularly regarding functional vascularization and the recreation of intricate tissue microenvironments. Current innovations in biofabrication techniques, microfluidic integration, and automated culture systems are actively addressing these limitations, complemented by emerging computational methods that enhance experimental scalability and analytical precision.

The clinical potential of oral/dental organoids continues to expand, with two particularly promising applications leading the way: personalized medicine approaches leveraging patient-derived models for tailored therapy development, and high-throughput pharmaceutical screening platforms that accelerate therapeutic discovery. As the field progresses, the integration of multi-modal organoid systems with advanced imaging and machine learning technologies, coupled with rigorous clinical validation, will be critical for translating these research tools into clinical practice. Looking forward, the continued refinement of oral/dental organoid technology promises to revolutionize precision dentistry, offering new paradigms for disease prevention, diagnosis, and treatment that will ultimately transform patient care. These advancements position organoids as indispensable tools that will bridge the gap between basic research and clinical application in oral health sciences, driving innovation across both academic and therapeutic domains.

Author Contributions

S.L., H.R. and J.H.: writing—review & editing, writing—original draft, visualization, software, methodology, formal analysis, data curation; L.Y. and B.Z.: conceptualization and review; R.C. and K.C.: data collection, writing—original draft, visualization; L.Y., J.L.P. and Q.Z.: writing—review & editing, supervision, resources, project administration, funding acquisition, conceptualization. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the National Natural Science Foundation of China (32301129), the Science and Technology Planning Projects of Guangzhou City, China (No. 202201020203), and the Undergraduate Teaching Quality and Teaching Reform Engineering Projects of Guangzhou Medical University (No. 2023ZLGC080, 2022-124-1).

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

No new data were generated in this study.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Peres, M.A.; Macpherson, L.M.D.; Weyant, R.J.; et al. Oral diseases: A global public health challenge. *Lancet* **2019**, *394*, 249–260. https://doi.org/10.1016/s0140-6736(19)31146-8.
2. Ferrari, A.J.; Santomauro, D.F.; Aali, A.; et al. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990–2021: A systematic analysis for the Global Burden of Disease Study 2021. *Lancet* **2024**, *403*, 2133–2161. https://doi.org/10.1016/s0140-6736(24)00757-8.
3. Ju, R.; Gao, X.; Zhang, C.; et al. A Versatile Immune Protective Armor to Enhance the Regenerative Potential of Exogenous Stem Cells. *ACS Appl. Mater. Interfaces* **2025**, *17*, 23600–23612. https://doi.org/10.1021/acsami.5c00253.
4. Tang, Z.; Chen, X.; Hong, X.; et al. 3D printing personalized orally disintegrating tablets with complex structures for the treatment of special populations. *Int. J. Pharm.* **2025**, *673*, 125371. https://doi.org/10.1016/j.ijpharm.2025.125371.
5. Xiong, W.; Liu, Y.; Zhou, H.; et al. Human dental pulp stem cells mitigate the neuropathology and cognitive decline via AKT-GSK3β-Nrf2 pathways in Alzheimer's disease. *Int. J. Oral Sci.* **2024**, *16*, 40. https://doi.org/10.1038/s41368-024-00300-4.
6. Yu, F.; Zhong, Y.; Zhang, B.; et al. A New Theranostic Platform Against Gram-Positive Bacteria Based on Near-Infrared-Emissive Aggregation-Induced Emission Nanoparticles. *Small* **2024**, *20*, e2308071. https://doi.org/10.1002/smll.202308071.
7. Chen, C.; Xie, Z.; Yang, S.; et al. Machine Learning Approach to Investigating Macrophage Polarization on Various Titanium Surface Characteristics. *BME Front.* **2025**, *6*, 0100. https://doi.org/10.34133/bmef.0100.
8. Miao, X.; Wu, X.; You, W.; et al. Tailoring of apoptotic bodies for diagnostic and therapeutic applications:advances, challenges, and prospects. *J. Transl. Med.* **2024**, *22*, 810. https://doi.org/10.1186/s12967-024-05451-w.
9. Li, J.; Zhao, Y.; Chen, S.; et al. Research Hotspots and Trends of Bone Xenograft in Clinical Procedures: A Bibliometric and Visual Analysis of the Past Decade. *Bioengineering* **2023**, *10*, 929. https://doi.org/10.3390/bioengineering10080929.
10. Hajishengallis, G. Illuminating the oral microbiome and its host interactions: Animal models of disease. *FEMS Microbiol. Rev.* **2023**, *47*, fuad018. https://doi.org/10.1093/femsre/fuad018.
11. Fitzgerald, K.A.; Malhotra, M.; Curtin, C.M.; et al. Life in 3D is never flat: 3D models to optimise drug delivery. *J. Control. Release* **2015**, *215*, 39–54. https://doi.org/10.1016/j.jconrel.2015.07.020.
12. Rossi, G.; Manfrin, A.; Lutolf, M.P. Progress and potential in organoid research. *Nat. Rev. Genet.* **2018**, *19*, 671–687. https://doi.org/10.1038/s41576-018-0051-9.
13. Drost, J.; Clevers, H. Organoids in cancer research. *Nat. Rev. Cancer* **2018**, *18*, 407–418. https://doi.org/10.1038/s41568-018-0007-6.
14. Mansour, A.A.; Gonçalves, J.T.; Bloyd, C.W.; et al. An in vivo model of functional and vascularized human brain organoids. *Nat. Biotechnol.* **2018**, *36*, 432–441. https://doi.org/10.1038/nbt.4127.
15. Schafer, S.T.; Mansour, A.A.; Schlachetzki, J.C.M.; et al. An in vivo neuroimmune organoid model to study human microglia phenotypes. *Cell* **2023**, *186*, 2111–2126.e2120. https://doi.org/10.1016/j.cell.2023.04.022.
16. Abuwatfa, W.H.; Pitt, W.G.; Husseini, G.A. Scaffold-based 3D cell culture models in cancer research. *J. Biomed. Sci.* **2024**, *31*, 7. https://doi.org/10.1186/s12929-024-00994-y.
17. Urzì, O.; Gasparro, R.; Costanzo, E.; et al. Three-Dimensional Cell Cultures: The Bridge between In Vitro and In Vivo Models. *Int. J. Mol. Sci.* **2023**, *24*, 12046. https://doi.org/10.3390/ijms241512046.
18. Goldrick, C.; Guri, I.; Herrera-Oropeza, G.; et al. 3D multicellular systems in disease modelling: From organoids to organ-on-chip. *Front. Cell Dev. Biol.* **2023**, *11*, 1083175.
19. Rosowski, J.; Bräunig, J.; Amler, A.K.; et al. Emulating the early phases of human tooth development in vitro. *Sci. Rep.* **2019**, *9*, 7057. https://doi.org/10.1038/s41598-019-43468-0.
20. Yang, S.; Leung, A.Y.P.; Wang, Z.; et al. Proanthocyanidin surface preconditioning of dental pulp stem cell spheroids enhances dimensional stability and biomineralization in vitro. *Int. Endod. J.* **2024**, *57*, 1639–1654. https://doi.org/10.1111/iej.14126.
21. Marsee, A.; Roos, F.J.M.; Verstegen, M.M.A.; et al. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. *Cell Stem Cell* **2021**, *28*, 816–832. https://doi.org/10.1016/j.stem.2021.04.005.
22. Calabrese, T.C.; Rothermund, K.; Gabe, C.M.; et al. Self-Assembly of Tooth Root Organoid from Postnatal Human Dental Stem Cells. *Tissue Eng. Part A* **2024**, *30*, 404–414. https://doi.org/10.1089/ten.TEA.2023.0219.
23. Adine, C.; Ng, K.K.; Rungarunlert, S.; et al. Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands. *Biomaterials* **2018**, *180*, 52–66. https://doi.org/10.1016/j.biomaterials.2018.06.011.

24. Sano, T.; Renn, T.Y.; Kanematsu, T.; et al. Organoid in dentistry: Models for oral biology and disease. *J. Dent. Sci.* **2025**, *20*, 1816–1823. https://doi.org/10.1016/j.jds.2025.05.002.
25. Maimets, M.; Rocchi, C.; Bron, R.; et al. Long-Term In Vitro Expansion of Salivary Gland Stem Cells Driven by Wnt Signals. *Stem Cell Rep.* **2016**, *6*, 150–162. https://doi.org/10.1016/j.stemcr.2015.11.009.
26. Lee, Y.S.; Park, Y.H.; Seo, Y.M.; et al. Tubular dentin formation by TGF-β/BMP signaling in dental epithelial cells. *Oral Dis.* **2023**, *29*, 1644–1656. https://doi.org/10.1111/odi.14170.
27. Song, Y.M.; Na, K.H.; Lee, H.J.; et al. The Effects of Transforming Growth Factor-β1 on the Differentiation of Cell Organoids Composed of Gingiva-Derived Stem Cells. *Biomed. Res. Int.* **2022**, *2022*, 9818299. https://doi.org/10.1155/2022/9818299.
28. Liu, F.; Xiao, J.; Chen, L.H.; et al. Self-assembly of differentiated dental pulp stem cells facilitates spheroid human dental organoid formation and prevascularization. *World J. Stem. Cells* **2024**, *16*, 287–304. https://doi.org/10.4252/wjsc.v16.i3.287.
29. Bi, M.; Yang, K.; Yu, T.; et al. Cell-based mechanisms and strategies of co-culture system both in vivo and vitro for bone tissue engineering. *Biomed. Pharmacother.* **2023**, *169*, 115907. https://doi.org/10.1016/j.biopha.2023.115907.
30. Licata, J.P.; Schwab, K.H.; Har-El, Y.E.; et al. Bioreactor Technologies for Enhanced Organoid Culture. *Int. J. Mol. Sci.* **2023**, *24*, 11427. https://doi.org/10.3390/ijms241411427.
31. Seo, J.Y.; Park, S.B.; Kim, S.Y.; et al. Acoustic and Magnetic Stimuli-Based Three-Dimensional Cell Culture Platform for Tissue Engineering. *Tissue Eng. Regen. Med.* **2023**, *20*, 563–580. https://doi.org/10.1007/s13770-023-00539-8.
32. Lu, C.; Jin, A.; Gao, C.; et al. Synergistic Approach of High-Precision 3D Printing and Low Cell Adhesion for Enhanced Self-Assembled Spheroid Formation. *Biosensors* **2024**, *15*, 7. https://doi.org/10.3390/bios15010007.
33. Zhou, S.; Yang, J.; Li, R.; et al. Live Imaging of 3D Hanging Drop Arrays through Manipulation of Light-Responsive Pyroelectric Slippery Surface and Chip Adhesion. *Nano Lett.* **2023**, *23*, 10710–10718. https://doi.org/10.1021/acs.nanolett.3c02570.
34. Baillargeon, P.; Shumate, J.; Hou, S.; et al. Automating a Magnetic 3D Spheroid Model Technology for High-Throughput Screening. *SLAS Technol.* **2019**, *24*, 420–428. https://doi.org/10.1177/2472630319854337.
35. Han, X.; Tang, S.; Wang, L.; et al. Multicellular Spheroids Formation on Hydrogel Enhances Osteogenic/Odontogenic Differentiation of Dental Pulp Stem Cells Under Magnetic Nanoparticles Induction. *Int. J. Nanomed.* **2021**, *16*, 5101–5115. https://doi.org/10.2147/ijn.S318991.
36. Ferreira, J.N.; Hasan, R.; Urkasemsin, G.; et al. A magnetic three-dimensional levitated primary cell culture system for the development of secretory salivary gland-like organoids. *J. Tissue Eng. Regen. Med.* **2019**, *13*, 495–508. https://doi.org/10.1002/term.2809.
37. Lekkala, V.K.R.; Kang, S.Y.; Liu, J.; et al. A Pillar/Perfusion Plate Enhances Cell Growth, Reproducibility, Throughput, and User Friendliness in Dynamic 3D Cell Culture. *ACS Biomater. Sci. Eng.* **2024**, *10*, 3478–3488. https://doi.org/10.1021/acsbiomaterials.4c00179.
38. Liang, L.; Wang, S.; Zhang, X.; et al. Multi-site enhancement of osteogenesis: Peptide-functionalized GelMA hydrogels with three-dimensional cultures of human dental pulp stem cells. *Regen. Biomater.* **2024**, *11*, rbae090. https://doi.org/10.1093/rb/rbae090.
39. Huang, L.; Chen, X.; Yang, X.; et al. GelMA-based hydrogel biomaterial scaffold: A versatile platform for regenerative endodontics. *J. Biomed. Mater. Res. B Appl. Biomater.* **2024**, *112*, e35412. https://doi.org/10.1002/jbm.b.35412.
40. Salvador-Clavell, R.; Martín de Llano, J.J.; Milián, L.; et al. Chondrogenic Potential of Human Dental Pulp Stem Cells Cultured as Microtissues. *Stem. Cells Int.* **2021**, *2021*, 7843798. https://doi.org/10.1155/2021/7843798.
41. Yang, Z.; Wang, B.; Liu, W.; et al. In situ self-assembled organoid for osteochondral tissue regeneration with dual functional units. *Bioact. Mater.* **2023**, *27*, 200–215. https://doi.org/10.1016/j.bioactmat.2023.04.002.
42. Yuan, S.; Yang, X.; Wang, X.; et al. Injectable Xenogeneic Dental Pulp Decellularized Extracellular Matrix Hydrogel Promotes Functional Dental Pulp Regeneration. *Int. J. Mol. Sci.* **2023**, *24*, 17483. https://doi.org/10.3390/ijms242417483.
43. Sui, Y.; Zhou, Z.; Zhang, S.; et al. The comprehensive progress of tooth regeneration from the tooth development to tissue engineering and clinical application. *Cell Regen.* **2025**, *14*, 33. https://doi.org/10.1186/s13619-025-00249-7.
44. Huang, S.; Wu, Y.; Zhao, H.; et al. Advancements in bone organoids: Perspectives on construction methodologies and application strategies. *J. Adv. Res.* **2025**, in press. https://doi.org/10.1016/j.jare.2025.06.011.
45. Musah, S.; Arzaghi, H. Unleashing the power of biomaterials to enhance organoid differentiation and function. *Nat. Methods* **2024**, *21*, 1575–1577. https://doi.org/10.1038/s41592-024-02393-5.
46. da Silva, I.S.P.; Bordini, E.A.F.; Bronze-Uhle, E.S.; et al. Photo-crosslinkable hydrogel incorporated with bone matrix particles for advancements in dentin tissue engineering. *J. Biomed. Mater. Res. A* **2024**, *112*, 2273–2288. https://doi.org/10.1002/jbm.a.37777.
47. Pele, K.G.; Amaveda, H.; Mora, M.; et al. Hydrocolloids of Egg White and Gelatin as a Platform for Hydrogel-Based Tissue Engineering. *Gels* **2023**, *9*, 505. https://doi.org/10.3390/gels9060505.

48. Pouraghaei, S.; Moztarzadeh, F.; Chen, C.; et al. Microenvironment Can Induce Development of Auditory Progenitor Cells from Human Gingival Mesenchymal Stem Cells. *ACS Biomater. Sci. Eng.* **2020**, *6*, 2263–2273. https://doi.org/10.1021/acsbiomaterials.9b01795.
49. Zhang, X.; Contessi Negrini, N.; Correia, R.; et al. Generating Tooth Organoids Using Defined Bioorthogonally Cross-Linked Hydrogels. *ACS Macro Lett.* **2024**, *13*, 1620–1626. https://doi.org/10.1021/acsmacrolett.4c00520.
50. Ding, Z.; Huang, J.; Ren, Y.; et al. 3D bioprinted advanced cartilage organoids with engineered magnetic nanoparticles polarized-BMSCs/alginate/gelatin for cartilage tissue regeneration. *Nano Res.* **2025**, *18*, https://doi.org/10.26599/NR.2025.94907084
51. Yang, X.; Ma, Y.; Wang, X.; et al. A 3D-Bioprinted Functional Module Based on Decellularized Extracellular Matrix Bioink for Periodontal Regeneration. *Adv. Sci.* **2023**, *10*, e2205041. https://doi.org/10.1002/advs.202205041.
52. Park, J.H.; Gillispie, G.J.; Copus, J.S.; et al. The effect of BMP-mimetic peptide tethering bioinks on the differentiation of dental pulp stem cells (DPSCs) in 3D bioprinted dental constructs. *Biofabrication* **2020**, *12*, 035029. https://doi.org/10.1088/1758-5090/ab9492.
53. Zhu, S.; Liao, X.; Xu, Y.; et al. 3D bioprinting of high-performance hydrogel with in-situ birth of stem cell spheroids. *Bioact. Mater.* **2025**, *43*, 392–405. https://doi.org/10.1016/j.bioactmat.2024.09.033.
54. Han, J.; Kim, D.S.; Jang, H.; et al. Bioprinting of three-dimensional dentin-pulp complex with local differentiation of human dental pulp stem cells. *J. Tissue Eng.* **2019**, *10*. https://doi.org/10.1177/2041731419845849.
55. Vurat, M.T.; Şeker, Ş.; Lalegül-Ülker, Ö.; et al. Development of a multicellular 3D-bioprinted microtissue model of human periodontal ligament-alveolar bone biointerface: Towards a pre-clinical model of periodontal diseases and personalized periodontal tissue engineering. *Genes Dis.* **2022**, *9*, 1008–1023. https://doi.org/10.1016/j.gendis.2020.11.011.
56. Miao, G.; Liang, L.; Li, W.; et al. 3D Bioprinting of a Bioactive Composite Scaffold for Cell Delivery in Periodontal Tissue Regeneration. *Biomolecules* **2023**, *13*, 1062. https://doi.org/10.3390/biom13071062.
57. Das, S.; Jegadeesan, J.T.; Basu, B. Gelatin Methacryloyl (GelMA)-Based Biomaterial Inks: Process Science for 3D/4D Printing and Current Status. *Biomacromolecules* **2024**, *25*, 2156–2221. https://doi.org/10.1021/acs.biomac.3c01271.
58. Zhou, N.; Zhu, S.; Wei, X.; et al. 3D-bioprinted hydrogels with instructive niches for dental pulp regeneration. *Int. J. Bioprinting* **2024**, *10*, 1790.
59. Tavafoghi, M.; Darabi, M.A.; Mahmoodi, M.; et al. Multimaterial bioprinting and combination of processing techniques towards the fabrication of biomimetic tissues and organs. *Biofabrication* **2021**, *13*, 042002. https://doi.org/10.1088/1758-5090/ac0b9a.
60. Quintard, C.; Tubbs, E.; Jonsson, G.; et al. A microfluidic platform integrating functional vascularized organoids-on-chip. *Nat. Commun.* **2024**, *15*, 1452. https://doi.org/10.1038/s41467-024-45710-4.
61. Liu, S.; Chen, Y.; Chen, Y.; et al. Long-term culture and morphological maturation of taste organoids enhance taste discrimination in a biomimetic biosensor. *Microsyst. Nanoeng.* **2025**, *11*, 120. https://doi.org/10.1038/s41378-025-00978-4.
62. Machla, F.; Monou, P.K.; Artemiou, P.; et al. Design, additive manufacturing, and characterization of an organ-on-chip microfluidic device for oral mucosa analogue growth. *J. Mech. Behav. Biomed. Mater.* **2025**, *163*, 106877. https://doi.org/10.1016/j.jmbbm.2024.106877.
63. Gard, A.L.; Luu, R.J.; Maloney, R.; et al. A high-throughput, 28-day, microfluidic model of gingival tissue inflammation and recovery. *Commun. Biol.* **2023**, *6*, 92. https://doi.org/10.1038/s42003-023-04434-9.
64. Hu, S.; Muniraj, G.; Mishra, A.; et al. Characterization of silver diamine fluoride cytotoxicity using microfluidic tooth-on-a-chip and gingival equivalents. *Dent.Mater.* **2022**, *38*, 1385–1394. https://doi.org/10.1016/j.dental.2022.06.025.
65. Dhall, A.; Tan, J.Y.; Oh, M.J.; et al. A dental implant-on-a-chip for 3D modeling of host-material-pathogen interactions and therapeutic testing platforms. *Lab Chip* **2022**, *22*, 4905–4916. https://doi.org/10.1039/d2lc00774f.
66. Shi, Q.; Zheng, L.; Na, J.; et al. Fluid shear stress promotes periodontal ligament cells proliferation via p38-AMOT-YAP. *Cell. Mol. Life Sci.* **2022**, *79*, 551. https://doi.org/10.1007/s00018-022-04591-w.
67. Wang, M.; Sasaki, Y.; Sakagami, R.; et al. Perfluoropolyether-Based Gut-Liver-on-a-Chip for the Evaluation of First-Pass Metabolism and Oral Bioavailability of Drugs. *ACS Biomater. Sci. Eng.* **2024**, *10*, 4635–4644. https://doi.org/10.1021/acsbiomaterials.4c00605.
68. Kristensen, M.F.; Lund, M.B.; Schramm, A.; et al. Determinants of Microscale pH in In Situ-Grown Dental Biofilms. *J. Dent. Res.* **2023**, *102*, 1348–1355. https://doi.org/10.1177/00220345231190563.
69. Yang, B.; Wang, P.; Li, Z.; et al. A continuous flow PCR array microfluidic chip applied for simultaneous amplification of target genes of periodontal pathogens. *Lab Chip* **2022**, *22*, 733–737. https://doi.org/10.1039/d1lc00814e.
70. Wang, J.; Wang, J.; Chang, X.; et al. Rapid Detection of Streptococcus mutans Using an Integrated Microfluidic System with Loop-Mediated Isothermal Amplification. *J. Microbiol. Biotechnol.* **2023**, *33*, 1101–1110. https://doi.org/10.4014/jmb.2304.04026.
71. Oates, W.A.; Anastasiou, A.D. A novel microfluidic tool for the evaluation of local drug delivery systems in simulated in vivo conditions. *Lab Chip* **2024**, *24*, 3840–3849. https://doi.org/10.1039/d4lc00181h.

72. Xu, X.; Guo, Y.; Liu, P.; et al. Piezo Mediates the Mechanosensation and Injury-Repair of Pulpo-Dentinal Complex. *Int. Dent. J.* **2024**, *74*, 71–80. https://doi.org/10.1016/j.identj.2023.07.002.
73. Wu, Y.; Li, X.; Liu, H.; et al. Organoids in the oral and maxillofacial region: Present and future. *Int. J. Oral Sci.* **2024**, *16*, 61. https://doi.org/10.1038/s41368-024-00324-w.
74. Zhao, H.; Jiang, E.; Shang, Z. 3D Co-culture of Cancer-Associated Fibroblast with Oral Cancer Organoids. *J. Dent. Res.* **2021**, *100*, 201–208. https://doi.org/10.1177/0022034520956614.
75. Chen, X.; Li, R.; Zhao, H.; et al. Phenotype transition of fibroblasts incorporated into patient-derived oral carcinoma organoids. *Oral Dis.* **2023**, *29*, 913–922. https://doi.org/10.1111/odi.14071.
76. Sobue, T.; Bertolini, M.; Thompson, A.; et al. Chemotherapy-induced oral mucositis and associated infections in a novel organotypic model. *Mol. Oral Microbiol.* **2018**, *33*, 212–223. https://doi.org/10.1111/omi.12214.
77. Guo, Q.; Chen, S.; Rao, X.; et al. Inhibition of SIRT1 promotes taste bud stem cell survival and mitigates radiation-induced oral mucositis in mice. *Am. J. Transl. Res.* **2019**, *11*, 4789–4799.
78. Meudec, L.; Goudarzi, N.; Silva-Saffar, S.E.; et al. Development of salivary gland organoids derived from patient biopsies: A functional model of Sjögren's disease. *Ann. Rheum. Dis.* **2025**, *84*, 1195–1206. https://doi.org/10.1016/j.ard.2025.04.020.
79. Jeon, S.G.; Lee, J.; Lee, S.J.; et al. Salivary gland organoid transplantation as a therapeutic option for radiation-induced xerostomia. *Stem. Cell Res. Ther.* **2024**, *15*, 265. https://doi.org/10.1186/s13287-024-03833-x.
80. Hemeryck, L.; Hermans, F.; Chappell, J.; et al. Organoids from human tooth showing epithelial stemness phenotype and differentiation potential. *Cell. Mol. Life Sci.* **2022**, *79*, 153. https://doi.org/10.1007/s00018-022-04183-8.
81. Jerbaka, F.; Gribova, V.; Rey, T.; et al. Organotypic 3D Cellular Models Mimicking the Epithelio-Ectomesenchymal Bilayer During Odontogenesis. *Tissue Eng. Part A* **2025**, *31*, 471–488. https://doi.org/10.1089/ten.TEA.2024.0118.
82. Chu, J.; Pieles, O.; Pfeifer, C.G.; et al. Dental follicle cell differentiation towards periodontal ligament-like tissue in a self-assembly three-dimensional organoid model. *Eur. Cells Mater.* **2021**, *42*, 20–33. https://doi.org/10.22203/eCM.v042a02.
83. Zhao, H.; Li, R.; Chen, Y.; et al. Stromal nicotinamide N-methyltransferase orchestrates the crosstalk between fibroblasts and tumour cells in oral squamous cell carcinoma: Evidence from patient-derived assembled organoids. *Oncogene* **2023**, *42*, 1166–1180. https://doi.org/10.1038/s41388-023-02642-5.
84. Holkom, M.; Yang, X.; Li, R.; et al. Fibroblast regulates angiogenesis in assembled oral cancer organoid: A possible role of NNMT. *Oral Dis.* **2024**, *30*, 4982–4992. https://doi.org/10.1111/odi.14945.
85. Khalesi, S.; Abbasi, A.; Razavi, S.M. Evaluating the Clinicopathologic Parameters of Tongue Squamous Cell Carcinoma based on its Local Distribution. *Adv. Biomed. Res.* **2023**, *12*, 71. https://doi.org/10.4103/abr.abr_197_21.
86. Zhao, L.; Huang, L.; Yu, S.; et al. Decellularized tongue tissue as an in vitro model for studying tongue cancer and tongue regeneration. *Acta Biomater.* **2017**, *58*, 122–135. https://doi.org/10.1016/j.actbio.2017.05.062.
87. Elad, S.; Yarom, N.; Zadik, Y.; et al. The broadening scope of oral mucositis and oral ulcerative mucosal toxicities of anticancer therapies. *CA Cancer J. Clin.* **2022**, *72*, 57–77. https://doi.org/10.3322/caac.21704.
88. Kepple, D.D.; Thornburg, T.E.; Beckman, M.F.; et al. Elucidating Regulatory Mechanisms of Genes Involved in Pathobiology of Sjögren's Disease: Immunostimulation Using a Cell Culture Model. *Int. J. Mol. Sci.* **2025**, *26*, 5881. https://doi.org/10.3390/ijms26125881.
89. Lombaert, I.; Movahednia, M.M.; Adine, C.; et al. Concise Review: Salivary Gland Regeneration: Therapeutic Approaches from Stem Cells to Tissue Organoids. *Stem Cells* **2017**, *35*, 97–105. https://doi.org/10.1002/stem.2455.
90. Urkasemsin, G.; Ferreira, J.N. Unveiling Stem Cell Heterogeneity Toward the Development of Salivary Gland Regenerative Strategies. *Adv. Exp. Med. Biol.* **2019**, *1123*, 151–164. https://doi.org/10.1007/978-3-030-11096-3_9.
91. Tanaka, J.; Mishima, K. Application of regenerative medicine to salivary gland hypofunction. *JPN Dent. Sci. Rev.* **2021**, *57*, 54–59. https://doi.org/10.1016/j.jdsr.2021.03.002.
92. Yu, T.; Klein, O.D. Molecular and cellular mechanisms of tooth development, homeostasis and repair. *Development* **2020**, *147*, dev184754. https://doi.org/10.1242/dev.184754.
93. Hashim, N.T.; Babiker, R.; Padmanabhan, V.; et al. The Global Burden of Periodontal Disease: A Narrative Review on Unveiling Socioeconomic and Health Challenges. *Int. J. Environ. Res. Public Health* **2025**, *22*. https://doi.org/10.3390/ijerph22040624.
94. Arani, R.M.; Yousefi, N.; Hamidieh, A.A.; et al. Tumor Organoid as a Drug Screening Platform for Cancer Research. *Curr. Stem Cell Res. Ther.* **2024**, *19*, 1210–1250. https://doi.org/10.2174/011574888x268366230922080423.
95. Yan, H.H.N.; Siu, H.C.; Law, S.; et al. A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. *Cell Stem Cell* **2018**, *23*, 882–897.e811. https://doi.org/10.1016/j.stem.2018.09.016.
96. Driehuis, E.; Kolders, S.; Spelier, S.; et al. Oral Mucosal Organoids as a Potential Platform for Personalized Cancer Therapy. *Cancer Discov.* **2019**, *9*, 852–871. https://doi.org/10.1158/2159-8290.Cd-18-1522.
97. Zhang, X.Y.; Sui, Y.; Shan, X.F.; et al. Construction of oral squamous cell carcinoma organoids in vitro 3D-culture for drug screening. *Oral Dis.* **2025**, *31*, 99–109. https://doi.org/10.1111/odi.15044.

98. Xu, X.; Li, Z.; Ai, X.; et al. Human three-dimensional dental pulp organoid model for toxicity screening of dental materials on dental pulp cells and tissue. *Int. Endod. J.* **2022**, *55*, 79–88. https://doi.org/10.1111/iej.13641.
99. Lin, K.C.; Ting, L.L.; Chang, C.L.; et al. Ex Vivo Expanded Circulating Tumor Cells for Clinical Anti-Cancer Drug Prediction in Patients with Head and Neck Cancer. *Cancers* **2021**, *13*, 6076. https://doi.org/10.3390/cancers13236076.
100. Farshbaf, A.; Mottaghi, M.; Mohammadi, M.; et al. Regenerative application of oral and maxillofacial 3D organoids based on dental pulp stem cell. *Tissue Cell* **2024**, *89*, 102451. https://doi.org/10.1016/j.tice.2024.102451.
101. Hsieh, M.S.; Chen, M.Y.; Chang, Y.S.; et al. Targeting the Neuropilin-1 receptor with Ovatodiolide and progress in using periodontal ligament organoids for COVID-19 research and therapy. *Life Sci.* **2024**, *351*, 122764. https://doi.org/10.1016/j.lfs.2024.122764.
102. Jeong, S.Y.; Lee, S.; Choi, W.H.; et al. Fabrication of Dentin-Pulp-Like Organoids Using Dental-Pulp Stem Cells. *Cells* **2020**, *9*, 642. https://doi.org/10.3390/cells9030642.
103. Cen, X.; Pan, X.; Zhang, B.; et al. miR-20a-5p contributes to osteogenic differentiation of human dental pulp stem cells by regulating BAMBI and activating the phosphorylation of Smad5 and p38. *Stem Cell Res. Ther.* **2021**, *12*, 421. https://doi.org/10.1186/s13287-021-02501-8.
104. Sano, K.; Usui, M.; Moritani, Y.; et al. Co-cultured spheroids of human periodontal ligament mesenchymal stem cells and vascular endothelial cells enhance periodontal tissue regeneration. *Regen. Ther.* **2020**, *14*, 59–71. https://doi.org/10.1016/j.reth.2019.12.008.
105. Lee, J.H.; Um, S.; Jang, J.H.; et al. Effects of VEGF and FGF-2 on proliferation and differentiation of human periodontal ligament stem cells. *Cell Tissue Res.* **2012**, *348*, 475–484. https://doi.org/10.1007/s00441-012-1392-x.
106. Tatullo, M.; Cocco, T.; Ferretta, A.; et al. Unveiling the Neurodegenerative Alterations through Oral Stem Cells. *J. Dent. Res.* **2024**, *103*, 1100–1108. https://doi.org/10.1177/00220345241265661.
107. Morrison, A.G.; Sarkar, S.; Umar, S.; et al. The Contribution of the Human Oral Microbiome to Oral Disease: A Review. *Microorganisms* **2023**, *11*, 318. https://doi.org/10.3390/microorganisms11020318.
108. Pang, Z.; Cady, N.M.; Cen, L.; et al. Physiologically relevant coculture model for oral microbial-host interactions. *Int. J. Oral Sci.* **2025**, *17*, 42. https://doi.org/10.1038/s41368-025-00365-9.
109. Adelfio, M.; Callen, G.E.; Diaz, A.R.; et al. Underscoring long-term host-microbiome interactions in a physiologically relevant gingival tissue model. *NPJ Biofilms Microbiomes* **2025**, *11*, 9. https://doi.org/10.1038/s41522-024-00641-2.
110. Adelfio, M.; Bonzanni, M.; Callen, G.E.; et al. A physiologically relevant culture platform for long-term studies of in vitro gingival tissue. *Acta Biomater.* **2023**, *167*, 321–334. https://doi.org/10.1016/j.actbio.2023.06.008.
111. Rajasekaran, J.J.; Krishnamurthy, H.K.; Bosco, J.; et al. Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. *Microorganisms* **2024**, *12*, 1797. https://doi.org/10.3390/microorganisms12091797.
112. Chen, Y.; Lei, L.; Xia, M.; et al. The association between oral microbiome and gastric precancerous lesions. *Msystems* **2025**, *10*, e0132224. https://doi.org/10.1128/msystems.01322-24.
113. Plachokova, A.S.; Andreu-Sánchez, S.; Noz, M.P.; et al. Oral Microbiome in Relation to Periodontitis Severity and Systemic Inflammation. *Int. J. Mol. Sci.* **2021**, *22*, 5876. https://doi.org/10.3390/ijms22115876.
114. Lai, Y.; Liu, S.; Song, C.; et al. An update on the role and mechanisms of periodontitis in cardiovascular diseases. *Cell. Signal.* **2025**, *132*, 111770. https://doi.org/10.1016/j.cellsig.2025.111770.
115. Peng, X.; Cheng, L.; You, Y.; et al. Oral microbiota in human systematic diseases. *Int. J. Oral Sci.* **2022**, *14*, 14. https://doi.org/10.1038/s41368-022-00163-7.
116. Yamaguchi-Kuroda, Y.; Kikuchi, Y.; Kokubu, E.; et al. Porphyromonas gingivalis diffusible signaling molecules enhance Fusobacterium nucleatum biofilm formation via gene expression modulation. *J. Oral Microbiol.* **2023**, *15*, 2165001. https://doi.org/10.1080/20002297.2023.2165001.
117. Sato, T.; Vries, R.G.; Snippert, H.J.; et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **2009**, *459*, 262–265. https://doi.org/10.1038/nature07935.
118. Ou, M.; Li, Q.; Ling, X.; et al. Cocktail Formula and Application Prospects for Oral and Maxillofacial Organoids. *Tissue Eng. Regen. Med.* **2022**, *19*, 913–925. https://doi.org/10.1007/s13770-022-00455-3.
119. Yang, S.; Hu, H.; Kung, H.; et al. Organoids: The current status and biomedical applications. *MedComm* **2023**, *4*, e274. https://doi.org/10.1002/mco2.274.
120. Wang, Y.; Sun, Y. Engineered organoids in oral and maxillofacial regeneration. *Iscience* **2023**, *26*, 105757. https://doi.org/10.1016/j.isci.2022.105757.
121. Corsini, N.S.; Knoblich, J.A. Human organoids: New strategies and methods for analyzing human development and disease. *Cell* **2022**, *185*, 2756–2769. https://doi.org/10.1016/j.cell.2022.06.051.
122. Clevers, H. Modeling Development and Disease with Organoids. *Cell* **2016**, *165*, 1586–1597. https://doi.org/10.1016/j.cell.2016.05.082.
123. Mirabelli, P.; Coppola, L.; Salvatore, M. Cancer Cell Lines Are Useful Model Systems for Medical Research. *Cancers* **2019**, *11*, 1098. https://doi.org/10.3390/cancers11081098.

124. Ishikawa, T.; Ogawa, T.; Shiihara, M.; et al. Salivary gland cancer organoids are valid for preclinical genotype-oriented medical precision trials. *Iscience* **2023**, *26*, 106695. https://doi.org/10.1016/j.isci.2023.106695.
125. Zuo, J.; Fang, Y.; Wang, R.; et al. High-throughput solutions in tumor organoids: From culture to drug screening. *Stem Cells* **2025**, *43*, sxae070. https://doi.org/10.1093/stmcls/sxae070.
126. Hofer, M.; Lutolf, M.P. Engineering organoids. *Nat. Rev. Mater.* **2021**, *6*, 402–420. https://doi.org/10.1038/s41578-021-00279-y.
127. Schäffers, O.J.M.; Gribnau, J.; van Rijn, B.B.; et al. Ethical considerations for advancing research using organoid models derived from the placenta. *Hum. Reprod. Update* **2025**, *31*, 392–401. https://doi.org/10.1093/humupd/dmaf007.
128. Kim, J.; Koo, B.K.; Knoblich, J.A. Human organoids: Model systems for human biology and medicine. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 571–584. https://doi.org/10.1038/s41580-020-0259-3.
129. Papamichail, L.; Koch, L.S.; Veerman, D.; et al. Organoids-on-a-chip: Microfluidic technology enables culture of organoids with enhanced tissue function and potential for disease modeling. *Front. Bioeng. Biotechnol.* **2025**, *13*, 1515340. https://doi.org/10.3389/fbioe.2025.1515340.
130. Ren, X.; Chen, W.; Yang, Q.; et al. Patient-derived cancer organoids for drug screening: Basic technology and clinical application. *J. Gastroenterol. Hepatol.* **2022**, *37*, 1446–1454. https://doi.org/10.1111/jgh.15930.
131. Andrews, M.G.; Kriegstein, A.R. Challenges of Organoid Research. *Annu. Rev. Neurosci.* **2022**, *45*, 23–39. https://doi.org/10.1146/annurev-neuro-111020-090812.
132. Lee, H.; Yang, S.; Lee, K.J.; et al. Standardization and quality assessment for human intestinal organoids. *Front. Cell Dev. Biol.* **2024**, *12*, 1383893. https://doi.org/10.3389/fcell.2024.1383893.
133. Kim, W.; Gwon, Y.; Park, S.; et al. Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration. *Bioact. Mater.* **2023**, *19*, 50–74. https://doi.org/10.1016/j.bioactmat.2022.03.039.
134. Gao, X.H.; Li, X.L.; Fan, B.; et al. Engineering organoids for dental pulp tissue regeneration and functional reconstruction. *Regen. Med* .**2025**, *20*, 203–220. https://doi.org/10.1080/17460751.2025.2514899.
135. Zhou, C.; Wu, Y.; Wang, Z.; et al. Standardization of organoid culture in cancer research. *Cancer Med.* **2023**, *12*, 14375–14386. https://doi.org/10.1002/cam4.5943.
136. Yu, B.; Zhou, D.; Wang, F.; et al. Organoids for tissue repair and regeneration. *Mater. Today BIO* **2025**, *33*, 102013. https://doi.org/10.1016/j.mtbio.2025.102013.
137. Lancia, L.; Mauro, A.; Mattei, V.; et al. Protocol for the generation and analysis of organoids from Dental pulp stem cells (DPSCs). *Tissue Cell* **2025**, *96*, 103010. https://doi.org/10.1016/j.tice.2025.103010.
138. Veninga, V.; Voest, E.E. Tumor organoids: Opportunities and challenges to guide precision medicine. *Cancer Cell* **2021**, *39*, 1190–1201. https://doi.org/10.1016/j.ccell.2021.07.020.
139. Feng, Y.; He, D.; An, X. Hydrogel innovations for 3D organoid culture. *Biomed. Mater.* **2025**, *20*, 042001. https://doi.org/10.1088/1748-605X/add82d.
140. Strunk, T.; Joshi, A.; Moeinkhah, M.; et al. Structure, Properties and Degradation of Self-Assembled Fibrinogen Nanofiber Scaffolds. *ACS Appl. BIO Mater.* **2024**, *7*, 6186–6200. https://doi.org/10.1021/acsabm.4c00761.
141. López-León, C.F.; Planet, R.; Soriano, J. Preparation and Mechano-Functional Characterization of PEGylated Fibrin Hydrogels: Impact of Thrombin Concentration. *Gels* **2024**, *10*, 116. https://doi.org/10.3390/gels10020116.
142. Gila-Vilchez, C.; Mañas-Torres, M.C.; García-García, Ó.D.; et al. Biocompatible Short-Peptides Fibrin Co-assembled Hydrogels. *ACS Appl. Polym. Mater.* **2023**, *5*, 2154–2165. https://doi.org/10.1021/acsapm.2c02164.
143. Bindi, B.; Perioli, A.; Melo, P.; et al. Bioinspired Collagen/Hyaluronic Acid/Fibrin-Based Hydrogels for Soft Tissue Engineering: Design, Synthesis, and In Vitro Characterization. *J. Funct. Biomater.* **2023**, *14*, 495. https://doi.org/10.3390/jfb14100495.
144. Bhusal, A.; Dogan, E.; Nguyen, H.A.; et al. Multi-material digital light processing bioprinting of hydrogel-based microfluidic chips. *Biofabrication* **2021**, *14*, 014103. https://doi.org/10.1088/1758-5090/ac2d78.
145. Hakim Khalili, M.; Zhang, R.; Wilson, S.; et al. Additive Manufacturing and Physicomechanical Characteristics of PEGDA Hydrogels: Recent Advances and Perspective for Tissue Engineering. *Polymers* **2023**, *15*, 2341. https://doi.org/10.3390/polym15102341.
146. Kwon, S.; Ryu, J.H.; Kim, J.; et al. Biomimetic Catechol-Incorporated Polyacrylonitrile Nanofiber Scaffolds for Tissue Engineering of Functional Salivary Glands. *Biomater. Res.* **2025**, *29*, 0226. https://doi.org/10.34133/bmr.0226.
147. Salvadori, A.; Watanabe, M.; Markovic, M.; et al. Controlled microvasculature for organ-on-a-chip applications produced by high-definition laser patterning. *Biofabrication* **2025**, *17*, 035011. https://doi.org/10.1088/1758-5090/add37e.
148. Xu, Z.; Lei, Z.; Cheng, Q.; et al. Biomedical applications of organoids derived from the digestive system. *Front. Cell Dev. Biol.* **2025**, *13*, 1599384. https://doi.org/10.3389/fcell.2025.1599384.
149. Bai, L.; Wu, Y.; Li, G.; et al. AI-enabled organoids: Construction, analysis, and application. *Bioact. Mater.* **2024**, *31*, 525–548. https://doi.org/10.1016/j.bioactmat.2023.09.005.

150. Gao, W.; Wang, C.; Li, Q.; et al. Application of medical imaging methods and artificial intelligence in tissue engineering and organ-on-a-chip. *Front. Bioeng. Biotechnol.* **2022**, *10*, 985692. https://doi.org/10.3389/fbioe.2022.985692.
151. Abdul, L.; Xu, J.; Sotra, A.; et al. D-CryptO: Deep learning-based analysis of colon organoid morphology from brightfield images. *Lab Chip* **2022**, *22*, 4118–4128. https://doi.org/10.1039/d2lc00596d.
152. Carvalho, D.J.; Kip, A.M.; Tegel, A.; et al. A Modular Microfluidic Organoid Platform Using LEGO-Like Bricks. *Adv. Healthc. Mater.* **2024**, *13*, e2303444. https://doi.org/10.1002/adhm.202303444.
153. Zhang, H.-W.; Tamura, C.; Ahmadianyazdi, A.; et al. Advancing modular microfluidics: Stereolithographic 3D printing of reconfigurable connectors for bioanalytical applications. *Int. J. Bioprinting* **2025**, *11*, 516–531.
154. Wang, J.; Zhang, H.; Qu, Y.; et al. An eighteen-organ microphysiological system coupling a vascular network and excretion system for drug discovery. *Microsyst. Nanoeng.* **2025**, *11*, 89. https://doi.org/10.1038/s41378-025-00933-3.