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Review

Engineered organoids in oral and maxillofacial regeneration

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SUMMARY

Oral and maxillofacial organoids, as three-dimensional study models of organs, have attracted increasing attention in tissue regeneration and disease modeling. However, traditional strategies for organoid construction still fail to precisely recapitulate the key characteristics of real organs, due to the difficulty in controlling the self-organization of cells *in vitro*. This review aims to summarize the recent progress of novel approaches to engineering oral and maxillofacial organoids. First, we introduced the necessary components and their roles in forming oral and maxillofacial organoids. Besides, we discussed cutting-edge technology in advancing the architecture and function of organoids, especially focusing on oral and maxillofacial tissue regeneration via novel strategy with designed cell-signal scaffold compounds. Finally, current limitations and future prospects of oral and maxillofacial organoids were represented to provide guidance for further disciplinary progression and clinical application to achieve organ regeneration.

INTRODUCTION

The treatment and repair of oral and maxillofacial defects remain challenging since it is difficult to regenerate tissues and restore their function and aesthetics.[1] Typically, current approaches to restore maxillofacial defects caused by cancer, trauma, and infection, primarily involve artificial prosthesis[2] and autograft.[3] However, considering the drawbacks of current ways in the morphological similarity, morbidity, and availability,[4] a more eminent and biocompatible strategy is highly desirable for further optimizing the treatment of oral and maxillofacial regeneration, which is a promising alternative in clinical application.

Organoids, three-dimensional (3D) structures generated from self-organizing stem cells, including pluripotent stem cells (PSCs), adult stem cells (ASCs), and other specific cell lines, have high similarity to native organs in architectural and functional complexity.[5,6] Compared with a two-dimensional (2D) cell culture system, the more sophisticated 3D organoids model can effectively simulate the structure and properties of original organs, particularly in their gene and protein expression patterns, cell-matrix interactions, and metabolic functions.[7] Moreover, the self-renewable ability and accessibility of genetic manipulation of organoids made them appropriate for studying organ development and pathophysiology *in vitro*. Therefore, organoids have been applied in modeling organogenesis[8] and developmental disorders,[9] diagnosing diseases,[10] and repairing defects.[11]

Recently, widely used organoids have been employed in the structure-complicated and disease-diverse oral and maxillofacial system, including tooth germ organoids,[12] salivary gland organoids,[13] oral cancer organoids,[14] taste bud organoids,[15] lingual epithelial organoids,[16] and temporomandibular joint organoids.[17] As shown in Figure 1, the development of oral and maxillofacial organoids mainly involves stem cells such as ASCs, PSCs, and the microenvironments (or niches) of different stem cells during organogenesis. With the supplement of culture components such as extracellular matrix (ECM) and molecules, ASCs and PSCs can reprogram and differentiate into different types of cells thereby forming various organoids.[18] The developed organoids serve as an excellent model for treating various oral and maxillofacial diseases including oral cancer,[19] teeth regeneration[20] and salivary gland repair.[21]

However, the stochastic and uncertain nature of the self-organization of stem cells during organoid development *in vitro* gives rise to immature morphology and incomplete functions. These limitations associated with conventional oral and maxillofacial organoids should be addressed prior to their clinical applications.[22] Efforts have been made to engineer organoids to ensure their morphology and functions similar

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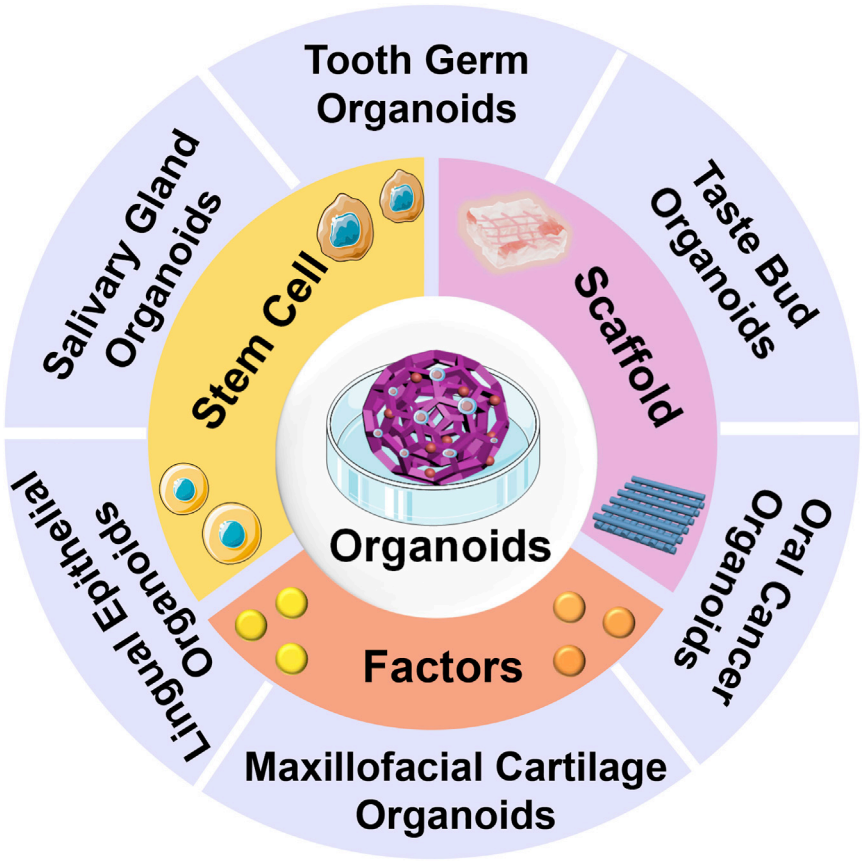

Figure 1. Components and categories of maxillofacial and oral organoids

to native organs. The advancements made in organoid culturing strategies could be attributed to genetic editing,[23–25] optimizing the composition of the ECM,[26–28] and developing new scaffold materials.[29–31] These techniques aid in regulating cell proliferation and differentiation. Remarkable progression has been made in the bio-engineered organoids which have enhanced the therapeutic potential and clinical value of oral and maxillofacial organoids.

Therefore, in this study, we have summarized the current techniques used for oral and maxillofacial organoid development. We have also discussed the challenges associated with organoid development. First, we shed light on the conventional methodology and the fundamental components required for organoid development. In the second section, we have discussed and listed various strategies used to engineer organoids. In the third section, we focus on specific bioengineering methods and advancements in oral and maxillofacial organoid development. Finally, we have highlighted the challenges associated with organoid development and the future prospects of oral and maxillofacial organoids.

Stem cells, scaffolds, and growth factors are essential components for generating oral and maxillofacial organoids, including tooth germ organoids, salivary gland organoids, lingual epithelia organoids, taste bud organoids, oral cancer organoids, maxillofacial cartilage organoids.

CONVENTIONAL ORGANOIDS DESIGN

The development of organoids primarily involves the self-organization of different types of cells, which are regulated by basic signal pathways. Therefore, three factors, including cell types, supporting structure, and endogenous and exogenous signals, are fundamental requirements during the process of organoid construction. Any changes in these factors may impact organoid development and its functions.

Cells

Cells for generating organoids derive from ASCs,[10] PSCs,[32] and somatic cells.[33] Since the restriction of somatic cells on differentiation, these somatic cells are less extensively applied in organoid construction versus stem cells, and will not be the focus here. Stem cells including PSCs and ASCs have the intrinsic capability to assemble into complex structures with the presence of suitable exogenous factors and appropriate supporting structure, due to their multipotent differentiation and self-renewal capability. In general, the behavior and fate of cells in the process of organoid formation involve proliferation, differentiation, and self-organization, which contains cell sorting and architectural rearrangement.[34] Therefore, the characteristics of organoids partly rely on the starting cell type.

ASCs, known as tissue stem cells, are undifferentiated cells derived from specific tissues. Their sources and self-renewal abilities enable them to differentiate into tissue-specific cell types.[35] Tissue-specific ASCs can spontaneously form corresponding organoids, which mitigate the uncertainty of cell differentiation when cultured in the microenvironment, simulating the development of actual *in vivo* organs. Therefore, ASC-derived organoids can be utilized to study tissue biology by efficiently imitating the homeostatic or regenerative conditions of corresponding original tissues.[36] For example, dental pulp stem cells, as ASCs derived from dental pulp tissues have outstanding odontogenic ability, which can generate tooth germ organoids by simulating dental mesenchyme.[37] However, the method to directly reconstruct organoids using ASCs may be hard because of their unavailable source from some special tissues such as the heart and brain.

PSCs exhibit more excellent self-renewal and multipotent differentiation capability.[38] Most PSC-derived organoids are in the immature stage since the development toward mature cells needs a continuous culture period and intricate interaction with other co-culture cells.[39] These procedures may be hard to control in most PSCs due to the difficulty in reconstructing embryonic developmental processes *in vitro*. Nevertheless, tissue-specific organoids including teeth,[40] brain,[41] and liver[42] can still be generated if the differentiated PSCs are cultured in the suitable culture medium and supporting scaffold. Additional studies are required to standardize the procedure for developing PSC-derived organoids prior to further clinical application. Besides, this feature also allows the PSC-derived organoids to be widely used to explore cell-cell interaction, organogenesis, and developmental defects.

Although both of ASCs and PSCs retain the genetic information from their primitive cells, ASC-derived organoids tend to sustain original tissue phenotype, so *in vitro* culturing of ASC-derived organoids is easier compared with PSC-derived organoids. In contrast, even though the culturing conditions of PSC-derived organoids are complex and time-consuming, the superior potential for PSCs to differentiate into various types of cells enables them to generate unavailable tissues, which overcome the shortcomings of ASCs.

Culturing conditions

In vitro conditions for culturing and generating organoids should be similar to their *in vivo* microenvironment to regulate the behavior and fate of cells.[43] These conditions are mainly composed of supporting structures in the culture system and endogenous and exogenous signals including different growth factors. Multiple strategies have been designed to create appropriate niches for the development of organoids.

Supporting structure

ECM, the basic component which contributes to the growth and adhesion of cells during organogenesis, plays an imperative role in providing 3D structural support during organoid morphogensis.[44] Various natural and synthetic materials are used as scaffolds, providing structural support for organoid generation.

The scaffolds made of natural materials possess bioactive motifs such as Arg-Gly-Asp (RGD) and are used for culturing organoids since they mimic conditions similar to *in vivo* microenvironments, which are required for culturing stem cells. Matrigel, a natural source scaffold derived from Engelbreth-Holm-Swarm mouse sarcoma, can efficiently mimic the natural basement membrane.[45] It has been widely applied in creating organoids such as intestinal,[46] tooth,[12] and gland[47] organoids due to their excellent biocompatibility, bioactivity, and biodegradability. However, there are disadvantages to solely using natural scaffolds to advance the development of organoids. First, their insufficient mechanical stiffness limits the natural scaffolds to be extensively applied in organoid development. Further, natural scaffolds have a low resistance to changes in physical conditions such as pH and temperature. Hence, it is challenging to precisely modulate the culturing conditions *in vitro* and modify natural scaffolds.[48]

Compared with natural materials, synthetic scaffolds are characterized by their superior mechanical stiffness and better resistance to changes in physical conditions. Polyethylene glycol (PEG), poly (lactic-co-glycolic acid) (PLGA), and polyurethane (PU)-modified hydrogels have been reported as more prominent 3D scaffolds for their improved biocompatibility and mechanical properties to induce organoids.[49] However, synthetic scaffolds have an uncontrolled degradation rate and lack bioactive motifs such as RGD, which restricts their use in organoid generation. Therefore, it is necessary to design techniques to modify synthetic scaffolds for organoid culture.

Apart from the scaffold, various studies have used other techniques to provide a microenvironment conducive to organoid development. Takasato et al. used an air-liquid interface method to create kidney organoids by generating a cell pellet on a thin and microporous membrane.[50] The cell pellet developed into a multi-layered structure similar to the architecture of the actual kidney *in vivo,* despite the lack of scaffolds for support. Besides, 3D suspension culture induces the transformation of cell aggregation to organoids with spherical shape to generate cerebral,[51] cerebellar,[52] and optic cup organoids.[53] Although some low concentrations of Matrigel have the capacity to efficiently generate polarized epithelial structures, scaffold materials are not necessary for cell embedding owing to specific and controlled self-organization in this suspension culture system.[53]

Various strategies and techniques are used to recapitulate *in vivo* microenvironments for generating and culturing organoids. On the contrary, these techniques have not yet to be optimized to promote organoid development. So, it is still necessary to make efforts to modify the scaffold and investigate a more suitable culture system.

Endogenous and exogenous signals

Organoid formation is usually regulated by endogenous and exogenous signals including growth factors and molecules, which play important roles in the self-renewal and differentiation of stem cells. All signaling molecules and factors required for the self-organization of stem cells should be present in the culture medium for the successful development of organoids. Therefore, in order to ensure proper self-organization, missing signals should be exogenously supplied.

Regarding oral and maxillofacial organoids, signal molecules such as bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) and signal pathways including Wnt/β-catenin play an important role in inducing orderly self-organization of stem cells.[54] For example, epithelial cells supplemented with alternative exogenous growth factors including FGF2 and laminin-111 could form salivary gland organoids with normal secretory function, compared with those treated with epidermal growth factor (EGF).[55] Besides, PSC-derived organoids were developed primarily relying on the exposure to exogenous signals at specific times. McCracken et al. treated PSCs with the bioactive molecule FGF4 to induce the formation of posterior foregut spheroids. Exogenous signaling molecules such as EGF and retinoic acid were added after 3 days of culturing PSCs to differentiate posterior foregut spheroids into gastric antrum organoids.[38] On the contrary, few organoids' derivations only depend on endogenous signals. Eiraku et al. reported that mouse PSCs were cultured in a serum-free medium with a low concentration of growth factors which promoted the generation of the neuroepithelium and subsequently developed into optic cup organoids without additional exogenous signals.[53] Hence, for meeting the need for spatial and temporal cues of self-organization of organoids, studies should focus on exploring tissue-specific developmental mechanisms to determine the exact exogenous factors to be applied.

Even though these factors are necessary for regulating the differentiation and organization of stem cells, cell-autonomous self-organization, as the critical part of organoids generation, remains difficult to manage.[8] Therefore, approaches to bioengineering organoids have attracted increasing attention in order to pursue well-organized organoids, and the specific strategies developed in recent years are described as follows.

METHODS FOR ENGINEERING ORGANOIDS

Various strategies for engineering organoids include advancing the biochemical and biophysical properties of scaffolds, improving the self-organization of stem cells, and optimizing the components of the culture niches. The aim of engineering organoids is to narrow the gaps between *in vitro* and *in vivo* model systems and recapitulate the important characteristics of actual organs. The generation of 3D organoids

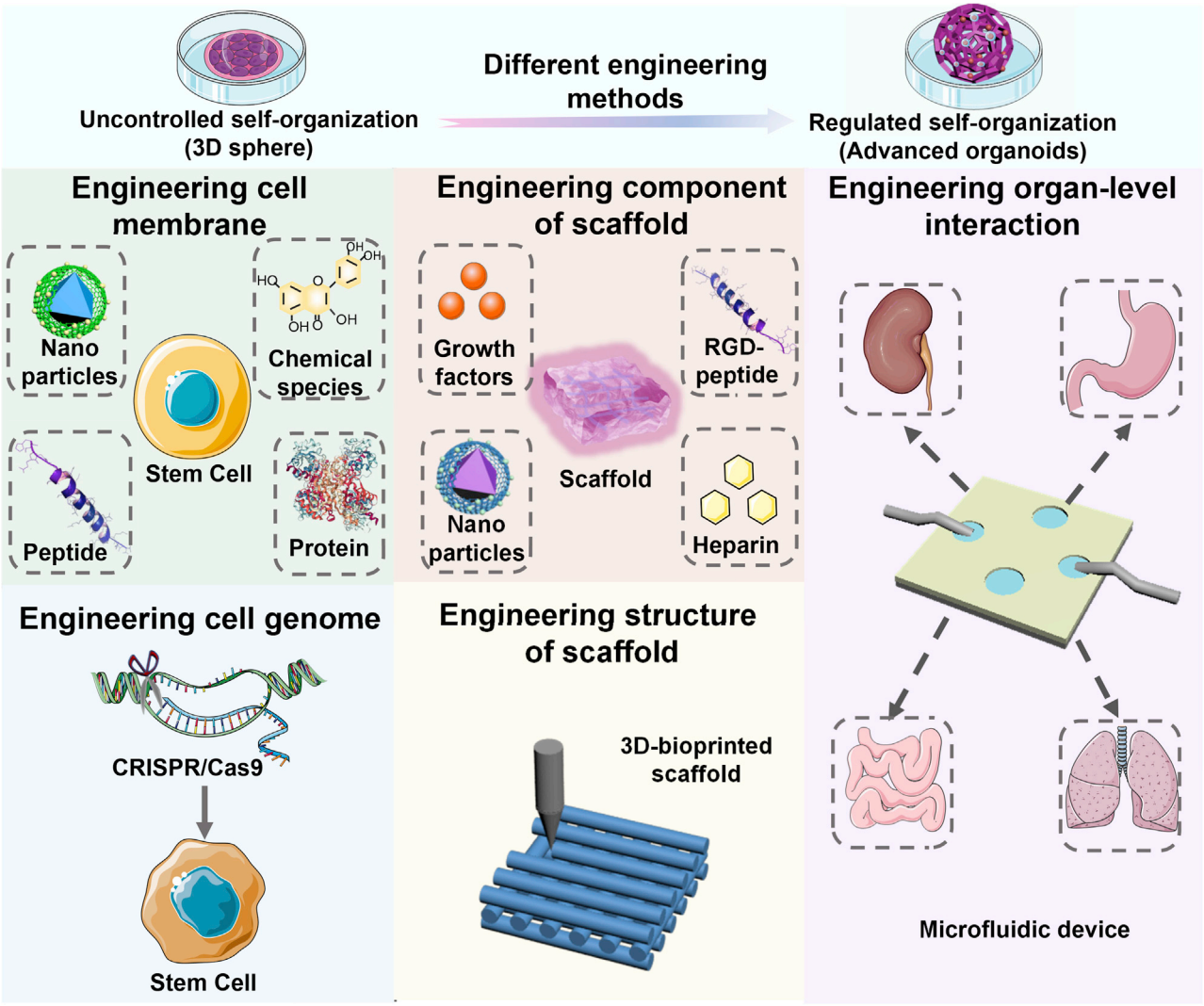

Figure 2. Methods to engineer organoids

involves the self-renewal and differentiation of cells and the process of self-organization.[56] These behaviors are mainly controlled by the components in the niches. Therefore, in order to create a spatially and temporally coordinated environment thereby realizing precise self-organization to form organoids, tremendous efforts have been made to bioengineer the cells and culturing conditions used to generate organoids (Figure 2).

Engineering the cell

Organoids are composed of different cell populations arranged in 3D structures. Therefore, to improve the organoids' robustness, promote cell aggregation, and shorten the incubation period, it is necessary to develop strategies that can modify the cells and change their inherent characteristics including advancing cell surface and genome.

Engineering cell surface

The shape and size of the starting cell aggregation are significant determinants for the successful formation of organoids, which can be achieved via modifying the cell surface.[57] Appropriate cell aggregation necessary for differentiation can be achieved via modifying the cell membrane to improve cell-cell adhesion. Several proteins,[58] peptides,[59] nanoparticles,[60] and bio-orthogonal chemical species[61] have been applied as functional molecules in the engineering cell membrane. Rogozhnikov et al. developed oxyamine and

ketone moieties-loaded liposomes to deliver chemical functionality to the cardiac cell surface. This newly developed liposome enabled the modified cardiac cells to aggregate and arrange among different cell types to form 3D structures without any scaffolds.[61] Fayol et al. reported that combined cell membrane with magnetic nanoparticles and utilized external magnetic fields to manipulate cell aggregation and re-arrangement thereby forming cartilage tissues.[62] Besides, the program of cell-cell adhesion could be changed and modified by binding the synthetic 3D DNA fragments into the cell surfaces.[63]

Genetic engineering

Apart from modifying cell surface, heritable genome editing is another effective approach for constructing more sophisticated organoids by controlling the fate and behavior of cells.[64] Various studies have successfully used genetic engineering to edit genomes for altering stem cell differentiation and self-organization capability, as well as for the treatment of several genetic diseases.

The inherent response of cells generating organoids can be modified with the application of targeted gene editing technology, Clustered Regularly Interspaced Short Palindromic Repeats-associated 9 system (CRISPR-Cas9). The modified genes trigger the expression of some substances beneficial for cell terminal differentiation and promote organoids maturation. Velazquez et al. used a targeted CRISPR-Cas9-based technique for the transcriptional activation of an enzyme superfamily to reprogram a tissue-regulated network and promote PSC-derived organoid maturation.[65] This gene modification was able to reprogram a tissue-regulated network and improve the cell maturation in PSC-derived organoids. However, the use of targeted genetic editing tools to control the self-organization of organoids is restricted by our limited understanding of cell behavior and regulatory networks. Further efforts to study how to precisely control the development of organoids via gene editing remain important.

Besides, gene editing technology can also be employed in the treatment of single gene mutation-induced diseases and explore different signals function in disease models. For example, CRISPR-Cas9 was used to correct the mutation in the retinitis pigmentosa GTPase regulator gene, which could effectively repair photoreceptor structure and restore the electrophysiological properties in retinal organoids.[66] The expression of the adenomatous polyposis coli was knockdown by gene editing technology in Barrett epithelial organoids to explore the role of WNT signal pathway in the neoplastic transformation of Barrett epithelial cells. The result confirmed that the successful activation of the WNT signal pathway could induce neoplastic phenotype.[67]

Engineering the culturing conditions

Although cell aggregation is required for organoid formation, the behavior of cells is highly dependent on the culturing conditions which influence the spatiotemporal self-organization of organoids.[68] In order to engineer components of the culture conditions, various state-of-the-art approaches are applied to modify the components and structure of the scaffolds. These will allow researchers to precisely control cell proliferation and differentiation for its application.[69–71]

Engineering components of scaffolds

Conventional scaffolds such as animals-derived Matrigel are used in organoid generation with limited applications due to the risk of immunogenicity. Besides, their composition is heterogeneous, which made it difficult to customize and design organoids-based experiments.[72–74] Therefore, a number of polymer-based synthetic hydrogels such as PLGA[75] and PEG-based[76] hydrogels have been developed to produce designer ECM and customize niche structures. Several nanoparticles such as silica nanoparticles,[77] metal, and metal oxide nanoparticles[78] have been incorporated to improve mechanical stiffness and strength and enhance the magnetic property for engineering cell niches *in vitro*. However, the above-mentioned synthetic hydrogels cannot control and manipulate the self-organization of stem cells, which only solve fundamental problems during the culture microenvironments. Hence, the purpose of engineering components of scaffolds is to optimize the components of niches and promote the aggregation of stem cells in scaffolds.

In recent years, various approaches to emulating *in vivo* microenvironments by modifying the developed hydrogels have drawn increasing attention. In order to supply more exogenous signals to facilitate the self-organization of stem cells, polymers-based synthetic hydrogels are decorated with different types of bioactive molecules. PEG-based hydrogels modified with RGD peptides are developed to support the

expansion and differentiation of intestinal stem cells. The susceptibility of soft RGD-functionalized synthetic hydrogels toward matrix metalloproteinases renders them more physiological by degradation over time to generate intestinal organoids.[79] Besides, compared with natural basement membrane extract, the synthetic fibrin-based hydrogels exhibited similar physical characterization to support stem cells as a 3D scaffold. For instance, the laminin-111-functionalized fibrin-based hydrogels possessed more robust properties to form and expand intestinal organoids since the laminin contained the necessary chemical signals for cell self-organization.[80]

Growth factors, which are sequestered or immobilized in ECM macromolecules *in vivo*, can also be immobilized and delivered by scaffolds instead of diffusible in a culture medium. Compared with free diffusible growth factors, hydrogel-delivered growth factors possess a prolonged and stable presentation due to the prevention of enzymatic growth factor degradation. Heparin, as an anionic polymer, can make electrostatic interactions with different kinds of growth factors and protect them from losing bioactivity.[81] Zieris et al. created heparin-functionalized PEG hydrogels to immobilize the fibroblast growth factor and vascular endothelial growth factor and regulate cell microenvironment thereby controlling the proliferation, growth, and differentiation of endothelial cells.[82] Wylie et al. used orthogonal chemistry of peptide binding pairs to incorporate and fixed stem cell differentiation factors and ciliary neurotrophic factor to the different locations of agarose hydrogels. This chemical scaffold had the capacity to realize spatially controlled organoids development via regulating cell growth and differentiation.[83]

Engineering structure of scaffolds

Given the present deficiency of mechanical stiffness and morphology of natural scaffold materials, the construction of organoids with designed structures remains difficult. To circumvent this limitation, a novel type of scaffold called hybrid scaffolds was designed. Hybrid scaffolds, which are made of two or more scaffolds, contain natural and synthetic scaffolds, thus combining the advantages of both systems, such as enhanced biocompatibility and ease of modification.[84] Therefore, hybrid scaffolds have bilayers and possess superior mechanical and biological properties. Using suitable growth factors and cells, these hybrid scaffolds can promote the aggregation and differentiation of stem cells.[76] Recently, hybrid scaffolds produced by 3D printing technology have been utilized to engineer organoids since they can induce stem cell aggregation with predefined shapes. Li et al. printed tubular composite scaffolds with defined shapes that were capable of guiding the growth, proliferation, and differentiation of stem cells to generate bile duct organoids.[85] Superparamagnetic iron oxide nanoparticles loaded-gelatin methacryloyl hydrogel was fabricated and covered the abovementioned printed scaffolds to ensure the biocompatibility and stiffness of hybrid scaffolds for the requirement of organoid formation. Besides 3D printing, laser cutting is a material reduction process that can provide artificial boundaries to the scaffold, thereby allowing the formation of morphologically controlled organoids. Nikolaev et al. used a laser-shaped metric to form intestinal organoids to predefined tubular architecture, a crypt-villus-like patterning. More importantly, the external pumping system continuously removes the dead cells generated over time, which extended the lifespan of cells in organoids and enabled the interactions between tubes and niches. The crypt and villus regions control the growth and self-organization of stem cells, thus indicating that scaffold geometry can provide morphogenic cues required for organoid development.[86]

Engineering organism-level environments

Organogenesis and disease progression involve more than one specific organ and requires interaction with other cells, tissues, and organs. Therefore, methods to engineer organism-level environments are important during the process of organoid formation and application for tissue regeneration. Vascularization of organoids and mimicking organism-level interaction via microfluidic devices can be realized by advancing organism-level context.

Vascularization

Organoid maturation is limited by the lack of functional vascularization, which fails to meet the increased metabolic demand of the culturing organoids, thus causing necrosis in the core of organoids. Hence, various methods to form functional vascular attract more attention in order to promote organoid maturation. The achievements mainly focus on developing pre-vascularizing organoids and functional capillary beds for perfusable organoid platforms.

In recent years, several researchers have reported that seeding endothelial cells with organoids can form vasculature. Rossen et al. co-cultured endothelial cells with mesenchymal stem cells to generate blood-vessel

organoids with controllable size and structure by sacrificial hydrogels technology.[87] In addition to endothelial cells, mesodermal progenitor cells were also confirmed to promote organoid vascularization when co-cultured stem cells, since the differentiation of mesoderm-derived angioblasts initiated the development of the vascular system.[88] Besides, mechanical factors such as flow and shear forces, which affected tissue homeostasis and morphogenesis thereby promoting organoid vascularization, could also be adjusted by microfabricated devices.[89] For instance, the maturation of endothelial cells, which contributed to the formation of a vascular network, could be triggered by blood-flow-induced shear stresses. Homan et al. employed the controllable fluid flow in the microfluidic device to advance the maturation of kidney organoids with vascular networks by the formation of perfusable lumens.[90]

The interaction between internal vasculature and external functional vessels in ECM is the key to forming integrated perfusion structures in organoids to mimic organs *in vivo*. Hence, in addition to the generation of pre-vascularizing organoids, methods to develop functional capillary beds also play an important role in the vascularization of organoids and *in vivo* perfused vasculature.[91] Chen et al. fabricated a microfluidic device with three parallel fluidic channels and implanted human umbilical vein endothelial cells (HUVECs) to fibrin gels in the middle region of the device to co-culture with human lung fibroblasts (HLFs).[92] The capillary beds could be generated after 3 days because endothelial cells in gels could simulate *in vivo* niches to form vessels, which was confirmed by the perfusion of fluorescent beads. Besides, Nashimoto et al. confirmed the capillary beds were able to integrate with vessel-like structures in large spheroids, which were made from HLFs and HUVECs.[93] Driven by factors secreted by HLFs, endothelial cells on the side walls could reach the spheroid thereby integrating vessels between capillary beds and spheroids. However, functional capillary beds have not yet established a stable connection with stem cell-derived organoids via an entire perfusion platform to provide sustained nutrient supplements for organoid construction. Therefore, more efforts remain required to form a better perfusion platform with organoids.

Engineering interactions at organism-level

The advent of microfluidic devices has created a powerful platform for mediating dynamic tissue-tissue interaction and organ-level interaction in organoids. Some advantages provided by microfabricated devices include: (1) They can dynamically simulate *in vivo* microenvironment by screening tissue and cellular components using biosensors.[94] (2) The devices make it convenient to co-culture organoids with other cells such as endothelial cells in order to vascularize and neutralize organoids via cell-organoid interaction.[95] (3) A holistic understanding of physiology and organogenesis can be achieved by recapitulating physiological interactions with multiple organoids via the microfabricated device.[96] For example, Jin et al. co-cultured liver, intestinal and stomach organoids in microfluidic devices to study the crosstalk among different organoids during organogenesis.[97] The flow of different culture media across the culture chambers enables communication among these three organoids. The liver organoids were incubated with the primary bile acid chenodeoxycholic acid, and the expression of the bile acid synthesis enzyme was evaluated to study the influence of interaction between liver and intestinal organoids on bile acid homeostasis. A decrease in bile acid synthesis enzyme expression was observed in liver organoids supplemented with bile acid chenodeoxycholic acid. This could be attributed to paracrine factors secreted by the intestinal organoid, which indicates the crosstalk between the liver and intestinal organoids. Despite the advancement in the development of oral and maxillofacial organoids, studies on the interactions among different oral organoids have not been reported yet. Increasing attention has been paid to exploring the crosstalk of multiple organoids for understanding the onset of diseases and organogenesis. Zhao et al. generated oral cancer organoids from patients with oral squamous cell carcinoma (OSCC) and co-cultured them with cancer-associated fibroblasts (CAF) to study the interaction between CAF and oral cancer organoids.[14] The result showed that CAF secreted lactate to promote the growth of oral cancer organoids, suggesting that inhibiting lactate production by CAF could prevent the progression of oral cancer.

Compared with the limitation of traditional organoids, including central necrosis, malformation and dysfunction, different methods to engineer organoids are able to regulate self-organization to form organoids with vascularization, normal function, and morphological similar to the counterparts *in vivo*. Three main strategies for developing bioengineered organoids include engineering stem cells, engineering scaffolds, and engineering organ-level environments. First, stem cells can be engineered by genetic editing and cell surface engineering. Genetic editing can be performed using the CRISPR/Cas9 technique to knock out and overexpress genes, which can alter the fate and properties of stem cells. Cell surface engineering uses nanoparticles, peptides, proteins, and chemical moieties to modify cell surfaces and facilitate cell

Table 1. Summary of various construction strategies for maxillofacial and oral organoids

Organoids types	Cell types	Main scaffolds	Culturing niches	Advantages of current strategies	Limitations of current strategies
Tooth germ organoids	Dental mesenchymal cells; Hental epithelial cells; iPSCs; HUVECs	Collagen; GelMA; PLGA hydrogel	DMEM; FCS	Teeth with entire structure and vascularization have been formed	Difficult to maintain and control different shapes of teeth
Salivary gland organoids	iPSCs; Human epithelial cells	PEG hydrogel; PLGA Nanofibers-modified elastin; Mat rigel;	BMP4; FGF7; FGF10; FGF2	Structure-entire and function-normal salivary glands have been generated	Lack of vascularization; the size of the organoid generated was small
Oral cancer organoids	Cells from patients with OSCC	Matrigel; Silica fiber scaffold; Decellularized ECM	bFGF	The migratory and invasive ability of cells is similar to OSCC	The inability of organoids to precisely recapitulate the tumor microenvironment
Taste bud organoids	$Lgr5^{+}$cells	Matrigel; Decellularized ECM	DMEM/F12	Organoids can recapitulate the taste epithelium homeostasis	The inability of organoids to dynamically analyze taste signal transmission
Lingual epithelial organoids	Lingual epithelial cells	Matrigel	EGF; Noggin; R-spondin	Growing and maturating in muscular layer of tongue instead of only in epithelial layer	Lack of components that mimic *in vivo* niche and control self-organization to form a specific morphology
Maxillofacial cartilage organoids	Neural crest stem cells	No scaffolds	FGF8; Ascorbic acid	Organoids successfully express cartilage differentiation factors	Methods to regeneration maxillofacial cartilage with entire structure and stiffness still unexplored

iPSCs, induced pluripotent stem cells; PLGA, poly(lactic-co-glycolic acid); HUVECs, human umbilical vein endothelial cells; GelMA, gelatin methacrylate; OSCC, oral squamous cell carcinoma; FGF, fibroblast growth factor ; EGF, epidermal growth factor; BMP, bone morphogenetic protein; ECM, extracellular matrix; PEG, polyethylene glycol; DMEM, dulbecco's modified eagle medium.

aggregation and differentiation. Second, scaffold engineering can be performed by engineering the components and the structure of the scaffolds. Growth factors and RGD peptides are incorporated in the scaffolds to recapitulate the *in vivo* microenvironment of their counterparts. Furthermore, 3D printing technology has been used to create a scaffold for generating organoids with specific and defined morphology. Third, the use of microfluidic devices guarantees a more sophisticated microenvironment for multi-organoids development by mimicking the interactions between different organs.

STRATEGIES FOR ENGINEERING ORAL AND MAXILLOFACIAL ORGANOIDS

The generation of oral and maxillofacial organoids is highly dependent on aggregation, differentiation, and self-organization of stem cells with the supplement and guidance of scaffolds and various growth factors. Current progress made in forming oral and maxillofacial organoids for hard and soft tissues has been listed as follows (Table 1).

Oral and maxillofacial hard tissues organoids

Tooth germ organoids

Tooth, as a complex tissue, is composed of hard tissues including enamel, dentin and cementum, and soft tissues including dental pulp. These structures are formed under the control of the epithelial-mesenchymal interactions and related signal molecules. Hence, the formation of tooth organoids is highly dependent on the spatial and temporal interactions between epithelial and mesenchymal to form the complex structure. In recent years, the generation of tooth germ organoids has raised more and more attention, which can be used to repair tissue damage and make progress in tooth regeneration (Figure 3).

The first evidence of tooth germ organoid formation was reported by Tsuji et al. through co-culturing dissociated embryonic tooth germ-derived dental epithelial (DE) and dental mesenchymal (DM) cells in a collagen gel.[20] The developed tooth germ organoids could generate entirely bioengineered tooth after transplantation in alveolar fossa. The identical method has been used to successfully form tooth germ

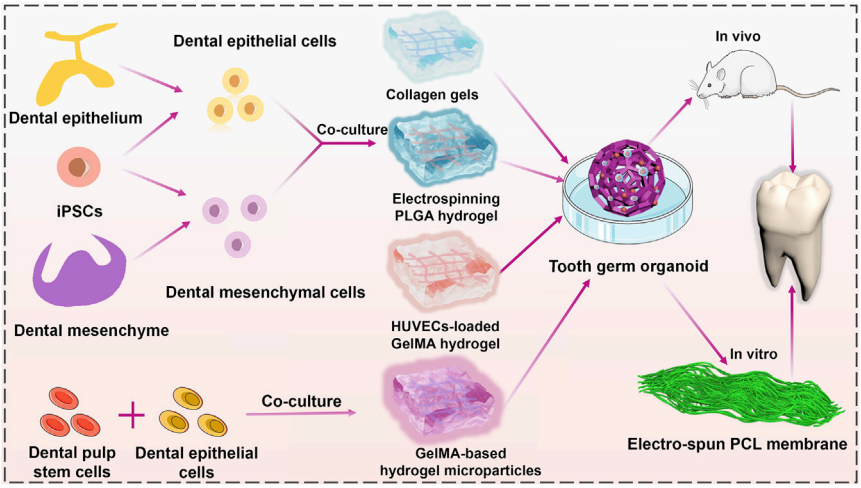

Figure 3. Schematic diagram of generation of engineered tooth germ organoids and tooth regeneration

The construction of tooth germ organoids mainly involves dental mesenchymal cells and dental epithelial cells co-culturing in collagen scaffolds and realizing self-organization with the stimulation of growth factors. In order to overcome the limited source of stem cells, PSCs were induced by feeder cells and differentiated into mesenchymal and epithelial cells. Besides, to further narrow the gap between actual tooth and tooth generated by organoids, various bioengineering scaffolds including electrospinning PLGA hydrogel, HUVECs-based GelMA hydrogel, and GelMA-based hydrogel microparticles have been utilized to advance tooth germ organoids. An entire tooth can be gained via implanting organoids into animal alveolar pockets or developing with the electrospinning PCL membrane *in vitro*. iPSCs, induced pluripotent stem cells; PLGA, poly(lactic-co-glycolic acid); HUVECs, human umbilical vein endothelial cells; GelMA, gelatin methacrylate; PCL, polycaprolactone.

organoids for whole tooth regeneration in canine[98] and porcine[99] model. However, given that the autogenous tooth germs are source-limited and most of them are derived from third molars, the generation of anterior teeth may be impeded due to differences in genetic lineage. This prompted the use of PSCs in developing the tooth-germ organoids. Under the co-culture condition of dulbecco's modified eagle medium (DMEM) and the guidance of mouse dental mesenchymal cells as feeder cells, the PSCs can differentiate into DM and DE cells and thereby forming tooth germ organoids.[40] After transplantation in alveolar fossa, the tooth germ organoids developed into a tooth-like structure with stiffness and elastic modulus similar to the actual teeth. Numerous studies have paid attention to bio-engineering tooth germ organoids to regenerate well-structured tooth.

Several efforts and progress have been made in bio-engineering tooth germ organoids by modifying scaffolds. Nano-hydroxyapatite-contained electrospinning PLGA scaffolds were developed to study the effect of novelty-engineering scaffolds on generating tooth germ organoids and structure-distinct teeth *in vitro*. After seeding DM and DE cells, the fiber in the scaffolds guided the cell aggregation with planned orientation, and nano-hydroxyapatite promoted stem cell differentiation and ECM production. Although the effects of hybrid scaffolds in tooth generation have not been studied *in vivo*, it provides a valuable strategy to form spatially controlled tooth germ organoids.[100]

Gelatin methacrylate (GelMA)-based hydrogel, a photo-crosslinking hydrogel with outstanding porosity, degradation rates, and suitable stiffness has been applied in organoid development, compared with polymer-based hydrogels. The RGD sequence contained in GelMA, a natural cell-binding motif, also contributes to cell adhesion and matrix metalloproteinase secretion. This bioactive motif plays a significant role in natural ECM structure and organogenesis.[101] Nevertheless, the teeth formed in GelMA hydrogels were small and diffused in the pores of scaffolds. So, HUVECs-loaded GelMA hydrogels were fabricated. Compared with GelMA hydrogels, HUVEC-encapsulated hydrogels enabled DE and DM cells to form tooth germs with predictable sizes and shapes and promote the functional vascularization of tooth germ organoids.[102] To further improve this model, various modifications were made to the GelMA scaffolds, like optimizing cell density, cell culture duration, and culture medium to achieve a bioengineered tooth bud.[103] However, the distinctive enamel and dentin structure remain unavailable, which means the GelMA method still requires more progression and modification. Therefore, in order to further improve this model, Bektas

et al. fabricated GelMA-derived hydrogel microparticles to provide superior surface area for cell attachment and cell-cell interaction, which were beneficial for self-organization. The human dental pulp stem cells and porcine dental epithelial cells were seeded into the engineering scaffold and co-cultured to form tooth germ organoids *in vitro*.[104]

Moreover, Zhang et al. fabricated decellularized tooth bud scaffold which retained more components and architecture of natural ECM. After the reseeding of DE cells, DM cells, and HUVECs, the newly developed tooth germ organoids were able to generate bio-engineered entire teeth with specific sizes and shapes. The bioengineered teeth contained correct enamel, pulp, dentin, and tooth roots under the guidance of decellularized scaffold.[12]

In addition to different approaches to modifying scaffolds, the culture microenvironment also plays a crucial role in the development of organoids. Eap et al. created a polycaprolactone (PCL) membrane by electrospinning and functionalized it with various growth factors to closely mimic *in vivo* microenvironment of the actual organ. The tooth-germ organoids were coated with this newly developed membrane, which secreted appropriate signals and simulated the microenvironment during organogenesis to generate a vascularized tooth both *in vitro* and *in vivo*.[105]

In conclusion, the above-mentioned progress represents the current advanced methods to perfect the whole structure and function of teeth generated by tooth germ organoids. Although the application of PSCs may mitigate the problem in a limited number of embryonic tooth cells, it is necessary to explore more efficient and effective approaches to bio-engineer niches, which will contribute to the generation of the entire structures of teeth.

Oral and maxillofacial soft tissues organoids

Salivary gland organoids

The development of salivary glands is dependent on epithelium and mesenchyme interaction. The key to structuring the salivary gland organoids with the secretary function is to mimic the microenvironment of organogenesis, particularly the endogenous and exogenous signals.[106] Compared with other oral and maxillofacial organoids, research related to the bio-engineered salivary gland organoids is relatively more sophisticated. Thus, numerous efforts have been made to accelerate the pace of clinical application (Figure 4).

In the former study, entire 3D salivary gland organoids were successfully structured *in vitro* by seeding human salivary gland cells in the Matrigel scaffold.[107] Besides, Tanaka et al. found the indispensable role of sex-determining region Y box 9 (Sox9) and forkhead box protein C1 (Foxc1) in the formation of salivary gland organoids. The PSCs were seeded into Matrigel and incubated with various molecules including BMP4 and FGF2 to differentiate into oral ectoderm. Subsequently, after the infection of recombinant adenovirus encoding Sox9 and Foxc1, the outer layer of the oral ectoderm was further cultured with FGF10 and FGF7 to develop into salivary gland organoids.[13] After transplantation in mice, the induced salivary gland organoids exhibited not only similar morphology and gene expression with normal organs, but also possessed the saliva secretion ability. Nevertheless, the nonhuman-derived scaffolds limited the clinical application to repair and regenerate salivary glands in humans. Hence, these scaffolds could only be applied to study the protein expression pattern of the salivary glands.[74]

Alternatively, complex hydrogel scaffolds have been created for the generation of salivary gland organoids for clinical application. The bio-engineered salivary gland organoids were developed by a niche-independent method with the guidance of PEG hydrogel-incorporated PCL nanofibrous microwell scaffolds.[108] The generation process involved two steps: priming and differentiation. After seeding on the scaffolds and forming aggregation, single clonal salivary gland stem cells further differentiated to generate salivary gland organoids, whereas the size of the 3D structure is relatively limited. Moreover, membrane protein-combined hydrogels also raise much more attention. Elastin, as an ECM protein beneficial for cell adhesion and stem cell differentiation, was modified with PLGA nanofibers via electrospinning.[109] The hybrid scaffolds could advance the nanofibers in elasticity, wettability, and chemical nature and mimic the niches giving rise to better epithelial cell line self-organization. However, prior to clinical application, several studies have reported immunogenicity and tumorigenesis concerns about these protein-fabricated hybrid hydrogels as scaffolds to form salivary gland organoids.[110,111] Besides, the byproducts generated by degrading these scaffolds could alter the pH and increase the temperature of niche, which could have serious adverse effects on the body.[112]

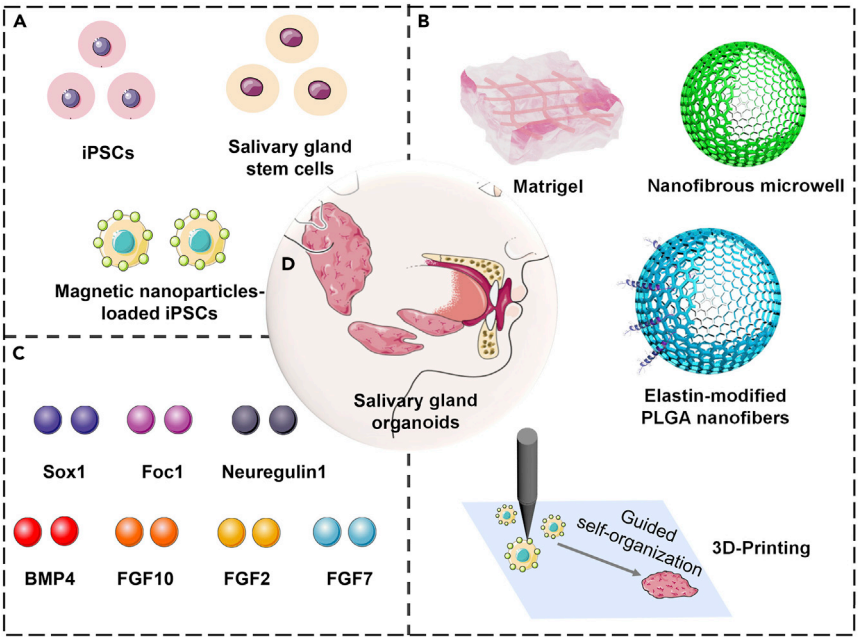

Figure 4. Schematic diagram of generation of engineered salivary gland organoids

(A) Stem cells source to generate salivary gland organoids containing PSCs, salivary gland cells and, magnetic nanoparticles modified iPSCs.

(B) Matrigel, 3D microwell, and elastin-loaded PLGA nanofibers are needed to structure salivary gland organoids. Moreover, instead of the utilization of scaffolds, a 3D bioprinting platform is also able to organize magnetic nanoparticle-modified PSCs to form specific morphology of organoids.

(C) In culture systems, different growth factors are of necessity for cell self-organization to form organoids.

(D) Salivary gland organoids can be generated by seeding the stem cells onto scaffold with the supplement of appropriate growth factors. iPSCs, induced pluripotent stem cells; PLGA, poly (lactic-co-glycolic acid).

To overcome this shortcoming, a scaffold-free strategy is developed to culture the salivary gland organoids by the modification of the cell surface. Cell-based bioprinting has emerged as a promising platform to generate salivary gland organoids. Adine et al. fabricated a magnetic 3D bioprinting platform to spatially control the self-organization and formed bio-engineered innervated salivary gland organoids with bio-functional secretory epithelia.[113] The surface membrane of human dental pulp stem cells (hDPSCs) was tagged with magnetic nanoparticles and subsequently seeded in 96-well plates to form cell aggregation. After the incubation with FGF10, the 3D spheroids grew into 3D salivary gland organoids with better neuronal compartments and excellent secretory function; however, these salivary gland organoids had limited vascularization. The same method was also applied in studying the role of exosomes derived from salivary gland organoids. The salivary gland organoids were developed by stable magnetic 3D bioprinting platforms, which provided a promising alternative to repair and regenerate the damaged salivary gland.[114]

Besides, glandular diversity and heterogeneous cell types would be successfully maintained in developed salivary gland organoids via modulating culture niches. Yoon et al. reported that compared to conventional culture components such as EGF and WNT signals, neuregulin 1 had the capacity to promote stem cell differentiation and avoid single-cell dissociation. This culture component could effectively develop organoids with stable and recapitulative cell diversity as opposed to generating excessive keratinized core.[115] The advanced research for alternative components of the microenvironments may be promising in generating cell-type-specific organoids for salivary gland regeneration.

Despite the advancement in bioengineering salivary gland organoids, the vascularization of salivary gland organoids and the identification of essential and crucial signals for the successful generation of organoids are a few concerns that should be addressed. Therefore, in order to repair and regenerate damaged salivary glands for clinical application, future studies are supposed to concentrate on optimizing the size and function of organoids.

Oral cancer organoids

Oral cancer has been the sixth most malignant cancer worldwide due to the rising incidence, severity and prognosis.[116] Mainstream therapy of oral cancer mainly contains surgery, radiotherapy, and chemotherapy, which may fail to completely treat refractory oral cancer due to relatively limited research on oral cancer mechanisms. However, oral cancer organoids, which mimic *in vivo* tumor microenvironments, have been developed, which could be used to study the underlying mechanism of tumorigenesis. This could provide a suitable platform for developing therapeutic strategies, specifically personalized treatment.

Tanaka et al. successfully generated head and neck cancer organoids by modifying protocols to generate tumor-derived spheroids. The tissues of patients with cancers were cultured in DMEM and transferred to Matrigel supplemented with bFGF to generate 3D spheroids. Versus the original tumor, head and neck cancer organoids possessed similar biochemical and biophysical characteristics, particularly the tumor protein p53 mutational status.[13] Further, cancer stem cells harvested from patients and cultured in Matrigel could generate oral cancer organoids, which exhibit histopathology and cellular heterogeneity of the tumor sample. However, cancer stem cell properties such as enrichment and expansion may be hard to maintain *in vitro*. In order to solve the present problem and prolong the lifespan of cancer organoids, Zhao et al. bioengineered the niches of organoids by co-culturing cancer-associated fibroblast with oral cancer organoids to explore the function of fibroblast in tumorigenesis.[14] Results demonstrated that the co-culture of cancer-associated fibroblast with organoids successfully promoted stem cell properties and improved the organoids-forming ability by secreting lactate.

Although there are few advantages of using conventional methods to generate oral cancer organoids, several methods have been developed to bioengineer oral cancer organoids by simulating *in vivo* tumor microenvironments. Decellularized tongue extracellular matrix is fabricated as a scaffold to form tongue squamous cell carcinoma (TSCC) organoids by seeding TSCC cells.[117] After decellularization, ECM still retained components such as laminin and collagen, which simulates the TSCC microenvironment and promote the formation of TSCC organoids, retaining the migratory and invasive properties of the original cells. Moreover, Noi et al. fabricated a non-woven silica fiber sheet as a novel scaffold to support human oral squamous cell-3 cells for the generation of oral cancer organoids.[118] The silica fiber scaffold structure was similar to the loose connective tissue structure *in vivo,* which would not inhibit cell growth and migration. Compared with animal-derived Matrigel, silica fiber scaffold is easy to be modified.

The present progress achieved in oral cancer organoids development is still inadequate. The inability of current oral cancer organoids to precisely recapitulate the tumor microenvironment still plagues the application in personalized cancer therapy and drug screening. More advanced methods to engineer organoids deserve to be studied in the future.

Taste bud organoids

Taste buds, as effective taste receptors existing in fungiform, foliate, and circumvallate papillae, are important for maintaining the quality of life. Taste bud organoids are able to explore the underlying mechanism of taste function and contribute to taste bud regeneration *in vitro*. Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) cells are ASCs in posterior tongue. Ren et al. seeded $Lgr5^{+}$ cells on the Matrigel to generate taste bud organoids with mature taste receptor cells.[119]

However, taste bud organoids constructed by conventional methods are unable to form accessible localization of taste receptor cells. These cells are enclosed inside the organoid and play an imperative role in evaluating taste response via the application of calcium imaging. So, Adpaikar et al. utilized a suspension combined Matrigel method to culture taste bud organoids, which could recapitulate the taste epithelium homeostasis.[120] The epithelium of circumvallate and foliate papillae-derived cell pellets were cultured in DMEM/F12 medium with the supplement of various epidermal growth factors and guidance of Matrigel as a scaffold. After 4 days of culture, 3D spherical structure was re-suspended in a culture medium without Matrigel to develop into engineered taste bud organoids. An increased number of stem cells and taste receptor cells were observed in the newly developed taste bud organoids and can benefit regenerative medicine. Besides, Lee et al. used decellularized tongue matrix as scaffolds on microfluidic devices to fabricate an artificial tongue device, which could efficiently mimic *in vivo* taste bud microenvironment and serve as an efficient and sensitive taste sensor system.[121] After seeding appropriate stem cells, the developed device could be used to generate taste bud organoids similar to taste buds *in vivo*. The advantage of this device

was to yield stable substance exchange and apply decellularized tongue-derived matrix promoting cell adhesion and providing growth factors necessary for organogenesis. More efficient and functional sensors for taste detection may also be realized by the microfluidic system via dynamically analyzing signal transmission. Even though taste bud organoids have not been generated by this method yet, this device indeed constructs a strategy for precisely mimicking niches *in vivo* and applicating in taste tissue regeneration.

Overall, current methods to generate advanced taste bud organoids with increased taste receptor cells still lack more sensitive taste sensor platforms to verify the effectiveness of taste sensors of organoids compared with their *in vivo* counterparts. Therefore, in order to achieve clinical taste bud regeneration, microfluidic devices, which can provide a more stable system to standardly monitor the taste sensation of taste bud organoids, are supposed to be applied in the development of taste bud organoids.

Lingual epithelial organoids

Due to the epidemic of tongue cancer around the world, researchers studying the mechanism of lingual epithelial renewal and regeneration through organoids draw more and more attention. Hisha et al. generated lingual epithelial organoids using B cell-specific Moloney murine leukemia virus insertion site 1 positive lingual epithelial cells, which were seeded in Matrigel and supplemented with growth factors such as EGF, noggin, and R-spondin1. These organoids were round in shape and had concentric cell arrangement.[122] After transplantation, the grafted organoids grew and maturated in the muscular layer of the tongue instead of only in the epithelial layer, indicating the potential application in lingual regeneration. However, the limitation of this method is the difficulty of organoids to form specific morphology even though culture conditions are identical. This may attribute to insufficient culture components to mimic niches *in vivo* and uncontrollable self-organization to generate organoids with similar shapes and sizes.

Although current lingual epithelial organoids have repaired tongues in the muscular layer rather than only in the epithelial layer, there is an urgent need to develop innovative approaches to bio-engineer organoids. Studies should focus on controlling the self-organization of stem cells in organoids by evaluating complex niches and using shape-controlled devices to develop fully functional lingual epithelial organoids with morphology similar to actual organs for clinical regeneration.

Maxillofacial cartilage organoids

Maxillofacial cartilage is comprised of hyaline cartilage of the nose, developing bones of the head, and fibrocartilage of temporomandibular joints. In order to generate hyaline cartilage organoids, Crispim et al. used spinner flasks to culture bovine chondrocytes with the supplement of notochordal cell-derived matrix, which was a decellularized biologic matrix similar to cartilage in composition.[17] Subsequently, the newly developed hyaline cartilage organoids were encapsulated in hydrogel alginate with suitable elastic property which could enhance the proliferation of cells and simultaneously sustain the expression of sex-determining region Y-box 9, indicating the excellent chondrocytes activity after *in vitro* culture for 24 days. This research guaranteed the mass production of cartilage organoids with appropriate chondrocyte activity, showing the potential for large-scale cartilage regeneration. However, whether this method is suitable for human cartilage regeneration requires to be verified by using human chondrocytes to construct cartilage organoids.

Currently, even though advanced cartilage organoids with superior chondrogenic activity and potential for mass production, the clinical application of organoids for maxillofacial cartilage regeneration still faces challenges including source-limited human embryonic stem cells and xenogeneic immune rejection. Therefore, more research should be encouraged to employ PSCs and ASCs derived from humans as cell sources in developing maxillofacial cartilage organoids before clinical therapy.

LIMITATIONS OF CURRENT ORAL AND MAXILLOFACIAL ORGANOIDS DESIGN AND PROSPECTS

Although oral and maxillofacial organoids have been successfully used for tissue regeneration in past decades, functional and morphological differences exist in organoids formed *in vitro* and actual organs. Embryonic stem cells are widely used to develop mature organoids; however, their source is limited and has ethical concerns, thereby restricting their clinical application.

Apart from the source of cells, there is still uncertainty regarding the underlying mechanisms of organogenesis and components required for culturing the organoids, which play an important role in regulating the self-organization of stem cells. These factors pose a major challenge in organoid culturing and development. As an alternative to embryonic stem cells, PSCs are utilized in generating various organoids. However, PSCs have diverse differentiation potential, resulting in immature organoid formation. It is difficult to precisely control the self-organizing ability of PSCs *in vitro* unless the cells are cultured in optimized conditions in the presence of supporting niche and suitable growth factors, which can effectively mimic *in vivo* microenvironment. Therefore, different technologies for developing organoids by simulating the chemical and physical characteristics of the culture microenvironment and allowing spatiotemporal modulation of bioactive factors become an emerging strategy. Tremendous advancements have been made in modifying intestinal[123] and neural[124] organoids by bioengineering organoids. However, only a few studies have focused on improving the functions and morphology of oral and maxillofacial organoids. Thus, considering various methods to engineer other organoids and specific properties of oral and maxillofacial organoids, several feasible approaches deserving to be implemented for improving oral and maxillofacial organoids are listed as follows.

Most current methods to modify oral and maxillofacial organoids rely on simulating the stiffness and architecture of ECM through 3D bioprinting and novel biomaterials as scaffolds. However, precise spatiotemporal regulation and control of self-organization need to be attained by utilizing microfluidic devices and further engineered scaffolds. For instance, multiple growth factors facilitating stem cell differentiation can be spatially immobilized in modified hydrogels.[83] The scaffolds developed using photochemistry could be used to promote hydrogel degradation on light stimulation in a time-dependent manner. This fabricated scaffold could mimic the degradation of the enamel matrix for regulating the architecture of tooth germ organoids for tooth regeneration.[125] Besides, the stable and sufficient cues provided by microfluidic devices can mimic *in vivo* microenvironment, which can control the self-organizing ability of organoids for the long term and prolong their lifespan of the organoids. In addition to mimicking the *in vivo* microenvironment, the intricated microfluidic devices can also simulate the interaction between various organs by culturing different organoids. This would aid in understanding the influence of multi-organ diseases on the functions and morphology of oral organoids, which would be impossible to study using single organoids.[126] Moreover, using microfluidic systems to high-throughput screening and detection niches provides a cost-effective platform to explore the most optimized niches of oral organoids and enables the study of organoids development and clinical applications.[121]

Further, more sophisticated oral organoids can be generated via gene editing. The utilization of CRISPR-Cas9 technology can further elucidate the mechanism of organogenesis and disease progression to alleviate the limited understanding of oral and maxillofacial systems. After acquiring complex knowledge of the signal pathway of organogenesis, genes contributing to oral and maxillofacial organoids generation may be knocked in via genome editing.[127] This technology is able to effectively elevate the expression of various signal pathways thereby forming mature organoids. Besides, with the combination of gene editing, optogenetic technology may also become another effective approach to precisely regulate the self-organization of stem cells in a spatiotemporal manner.[128] It was reported that optogenetics combined with CRISPR-Cas9 could precisely control the activation of WNT/β-catenin signal pathway in PSCs thereby triggering transcriptional changes and mesoderm differentiation of PSCs.[129] This research indicates that optogenetics may also be a promising tool for regulating various signal pathways to spatiotemporally control the fate of stem cells for designing oral and maxillofacial organoids.

The above-mentioned approaches may guide the progression of oral organoids in the future by controlling microenvironment components as well as reprogramming genomes to manipulate the self-organization of cells. The construction of engineered oral and maxillofacial organoids is gaining momentum, which is bound to prosper regeneration and development of oral and maxillofacial systems and achieve more breakthroughs in the global healthcare and dental field.

Conclusion

Oral and maxillofacial organoids break through the bottleneck of conventional 2D culture since they effectively recapitulate the architecture and function of tissues and cells *in vitro*. These characteristics enable them to be widely applied in tissue regeneration and disease treatment. To overcome the shortcomings of the construction of oral and maxillofacial organoids, various advanced methods to engineer oral and

maxillofacial organoids make it possible to generate organoids that are similar in structures and functions to their *in vivo* counterparts. Various approaches to engineering organoids have been represented, through which structure and function similar to the actual organs could be promised for organoids produced *in vitro*. Currently, various methods, such as 3D bioprinting and fabricated polymer scaffolds, can effectively mimick niches *in vivo* for organoid construction. However, challenge especially in precisely regulating the self-organization of organoids still exist. More efforts in a multidisciplinary manner to further bioengineer oral and maxillofacial organoids are required for oral and maxillofacial regeneration.

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AUTHOR CONTRIBUTIONS

Yu Wang contributed to the conception, design, and drafted the article. Yao Sun contributed to the conception and design and critically revised the article. All authors gave final approval and agree to be accountable for all aspects of the work.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Liu, G., Guo, Y., Zhang, L., Wang, X., Liu, R., Huang, P., Xiao, Y., Chen, Z., and Chen, Z. (2019). A standardized rat burr hole defect model to study maxillofacial bone regeneration. Acta Biomater. *86*, 450–464. https://doi.org/10.1016/j.actbio.2018.12.049.

2. Robb, G.L., Marunick, M.T., Martin, J.W., and Zlotolow, I.M. (2001). Midface reconstruction: surgical reconstruction versus prosthesis. Head Neck *23*, 48–58. https://doi.org/10.1002/1097-0347(200101)23:1<48::AID-HED8>3.0.CO;2-H.

3. Chen, Q., Abasi, K., Gong, Z., Ling, B., Liu, H., and Shao, B. (2018). Inferiorly pedicled nasolabial flap for reconstruction of anterior maxilla defects class I and II. J. Craniofac. Surg. *29*, 457–459. https://doi.org/10.1097/scs.0000000000004203.

4. Chen, X., Bai, S., Li, B., Liu, H., Wu, G., Liu, S., and Zhao, Y. (2016). Fabrication of gelatin methacrylate/nanohydroxyapatite microgel arrays for periodontal tissue regeneration. Int. J. Nanomed. *11*, 4707–4718. https://doi.org/10.2147/ijn.S111701.

5. Fatehullah, A., Tan, S.H., and Barker, N. (2016). Organoids as an in vitro model of human development and disease. Nat. Cell Biol. *18*, 246–254. https://doi.org/10.1038/ncb3312.

6. Rossi, G., Manfrin, A., and Lutolf, M.P. (2018). Progress and potential in organoid research. Nat. Rev. Genet. *19*, 671–687. https://doi.org/10.1038/s41576-018-0051-9.

7. Yin, X., Mead, B.E., Safaee, H., Langer, R., Karp, J.M., and Levy, O. (2016). Engineering stem cell organoids. Cell Stem Cell *18*, 25–38. https://doi.org/10.1016/j.stem.2015.12.005.

8. Lancaster, M.A., and Knoblich, J.A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. Science *345*, 1247125. https://doi.org/10.1126/science.1247125.

9. Zhang, D.Y., Song, H., and Ming, G.L. (2021). Modeling neurological disorders using brain organoids. Semin. Cell Dev. Biol. *111*, 4–14. https://doi.org/10.1016/j.semcdb.2020.05.026.

10. Dotti, I., and Salas, A. (2018). Potential use of human stem cell-derived intestinal organoids to study inflammatory bowel diseases. Inflamm. Bowel Dis. *24*, 2501–2509. https://doi.org/10.1093/ibd/izy275.

11. Schwank, G., Koo, B.K., Sasselli, V., Dekkers, J.F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C.K., et al. (2013). Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell *13*, 653–658. https://doi.org/10.1016/j.stem.2013.11.002.

12. Zhang, W., Vazquez, B., Oreadi, D., and Yelick, P.C. (2017). Decellularized tooth bud scaffolds for tooth regeneration. J. Dent. Res. *96*, 516–523. https://doi.org/10.1177/0022034516689082.

13. Tanaka, N., Osman, A.A., Takahashi, Y., Lindemann, A., Patel, A.A., Zhao, M., Takahashi, H., and Myers, J.N. (2018). Head and neck cancer organoids established by modification of the CTOS method can be used to predict in vivo drug sensitivity. Oral Oncol. *87*, 49–57. https://doi.org/10.1016/j.oraloncology.2018.10.018.

14. Zhao, H., Jiang, E., and Shang, Z. (2021). 3D co-culture of cancer-associated fibroblast with oral cancer organoids. J. Dent. Res. *100*, 201–208. https://doi.org/10.1177/0022034520956614.

15. Liu, S., Zhu, P., Tian, Y., Chen, Y., Liu, Y., Chen, W., Du, L., and Wu, C. (2022). Preparation and application of taste bud organoids in biomedicine towards chemical sensation mechanisms. Biotechnol. Bioeng. *119*, 2015–2030. https://doi.org/10.1002/bit.28109.

16. Hisha, H., and Ueno, H. (2019). Organoid culture of lingual epithelial cells in a three-dimensional matrix. Methods Mol. Biol. *1576*, 93–99. https://doi.org/10.1007/7651_2016_3.

17. Crispim, J.F., and Ito, K. (2021). De novo neo-hyaline-cartilage from bovine organoids in viscoelastic hydrogels. Acta Biomater. *128*, 236–249. https://doi.org/10.1016/j.actbio.2021.04.008.

18. Li, L., and Xie, T. (2005). Stem cell niche: structure and function. Annu. Rev. Cell Dev. Biol. *21*, 605–631. https://doi.org/10.1146/annurev.cellbio.21.012704.131525.

19. Driehuis, E., Kolders, S., Spelier, S., Lõhmussaar, K., Willems, S.M., Devriese, L.A., de Bree, R., de Ruiter, E.J., Korving, J.,

Begthel, H., et al. (2019). Oral mucosal organoids as a potential platform for personalized cancer therapy. Cancer Discov. *9*, 852–871. https://doi.org/10.1158/2159-8290.Cd-18-1522.

20. Nakao, K., Morita, R., Saji, Y., Ishida, K., Tomita, Y., Ogawa, M., Saitoh, M., Tomooka, Y., and Tsuji, T. (2007). The development of a bioengineered organ germ method. Nat. Methods *4*, 227–230. https://doi.org/10.1038/nmeth1012.

21. Koslow, M., O'Keefe, K.J., Hosseini, Z.F., Nelson, D.A., and Larsen, M. (2019). ROCK inhibitor increases proacinar cells in adult salivary gland organoids. Stem Cell Res. *41*, 101608. https://doi.org/10.1016/j.scr.2019.101608.

22. Randriamanantsoa, S., Papargyriou, A., Maurer, H.C., Peschke, K., Schuster, M., Zecchin, G., Steiger, K., Öllinger, R., Saur, D., Scheel, C., et al. (2022). Spatiotemporal dynamics of self-organized branching in pancreas-derived organoids. Nat. Commun. *13*, 5219. https://doi.org/10.1038/s41467-022-32806-y.

23. Roper, J., Tammela, T., Akkad, A., Almeqdadi, M., Santos, S.B., Jacks, T., and Yilmaz, Ö.H. (2018). Colonoscopy-based colorectal cancer modeling in mice with CRISPR-Cas9 genome editing and organoid transplantation. Nat. Protoc. *13*, 217–234. https://doi.org/10.1038/nprot.2017.136.

24. Sun, Y., and Ding, Q. (2017). Genome engineering of stem cell organoids for disease modeling. Protein Cell *8*, 315–327. https://doi.org/10.1007/s13238-016-0368-0.

25. Teriyapirom, I., Batista-Rocha, A.S., and Koo, B.K. (2021). Genetic engineering in organoids. J. Mol. Med. *99*, 555–568. https://doi.org/10.1007/s00109-020-02029-z.

26. Cho, A.N., Jin, Y., An, Y., Kim, J., Choi, Y.S., Lee, J.S., Kim, J., Choi, W.Y., Koo, D.J., Yu, W., et al. (2021). Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. Nat. Commun. *12*, 4730. https://doi.org/10.1038/s41467-021-24775-5.

27. Heo, J.H., Kang, D., Seo, S.J., and Jin, Y. (2022). Engineering the extracellular matrix for organoid culture. Int. J. Stem Cells *15*, 60–69. https://doi.org/10.15283/ijsc21190.

28. Lee, H.J., Mun, S., Pham, D.M., and Kim, P. (2021). Extracellular matrix-based hydrogels to tailoring tumor organoids. ACS Biomater. Sci. Eng. *7*, 4128–4135. https://doi.org/10.1021/acsbiomaterials.0c01801.

29. Abdollahi, S. (2021). Extracellular vesicles from organoids and 3D culture systems. Biotechnol. Bioeng. *118*, 1029–1049. https://doi.org/10.1002/bit.27606.

30. Oksdath, M., Perrin, S.L., Bardy, C., Hilder, E.F., DeForest, C.A., Arrua, R.D., and Gomez, G.A. (2018). Review: synthetic scaffolds to control the biochemical, mechanical, and geometrical environment of stem cell-derived brain organoids. APL Bioeng. *2*, 041501. https://doi.org/10.1063/1.5045124.

31. Wu, D.T., Munguia-Lopez, J.G., Cho, Y.W., Ma, X., Song, V., Zhu, Z., and Tran, S.D. (2021). Polymeric scaffolds for dental, oral, and craniofacial regenerative medicine. Molecules *26*, 7043. https://doi.org/10.3390/molecules26227043.

32. Low, J.H., Li, P., Chew, E.G.Y., Zhou, B., Suzuki, K., Zhang, T., Lian, M.M., Liu, M., Aizawa, E., Rodriguez Esteban, C., et al. (2019). Generation of human PSC-derived kidney organoids with patterned nephron segments and a de novo vascular network. Cell Stem Cell *25*, 373–387.e9. https://doi.org/10.1016/j.stem.2019.06.009.

33. Fujii, M., and Sato, T. (2021). Somatic cell-derived organoids as prototypes of human epithelial tissues and diseases. Nat. Mater. *20*, 156–169. https://doi.org/10.1038/s41563-020-0754-0.

34. Lei, M., Schumacher, L.J., Lai, Y.C., Juan, W.T., Yeh, C.Y., Wu, P., Jiang, T.X., Baker, R.E., Widelitz, R.B., Yang, L., and Chuong, C.M. (2017). Self-organization process in newborn skin organoid formation inspires strategy to restore hair regeneration of adult cells. Proc. Natl. Acad. Sci. USA *114*, E7101–E7110. https://doi.org/10.1073/pnas.1700475114.

35. Henon, P.R. (2003). Human embryonic or adult stem cells: an overview on ethics and perspectives for tissue engineering. Adv. Exp. Med. Biol. *534*, 27–45. https://doi.org/10.1007/978-1-4615-0063-6_3.

36. Clevers, H. (2016). Modeling development and disease with organoids. Cell *165*, 1586–1597. https://doi.org/10.1016/j.cell.2016.05.082.

37. Sharpe, P.T. (2016). Dental mesenchymal stem cells. Development *143*, 2273–2280. https://doi.org/10.1242/dev.134189.

38. McCracken, K.W., Catá, E.M., Crawford, C.M., Sinagoga, K.L., Schumacher, M., Rockich, B.E., Tsai, Y.H., Mayhew, C.N., Spence, J.R., Zavros, Y., and Wells, J.M. (2014). Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature *516*, 400–404. https://doi.org/10.1038/nature13863.

39. Xinaris, C., Brizi, V., and Remuzzi, G. (2015). Organoid models and applications in biomedical research. Nephron *130*, 191–199. https://doi.org/10.1159/000433566.

40. Kim, E.J., Yoon, K.S., Arakaki, M., Otsu, K., Fukumoto, S., Harada, H., Green, D.W., Lee, J.M., and Jung, H.S. (2019). Effective differentiation of induced pluripotent stem cells into dental cells. Dev. Dynam. *248*, 129–139. https://doi.org/10.1002/dvdy.24663.

41. Xu, R., Boreland, A.J., Li, X., Erickson, C., Jin, M., Atkins, C., Pang, Z.P., Daniels, B.P., and Jiang, P. (2021). Developing human pluripotent stem cell-based cerebral organoids with a controllable microglia ratio for modeling brain development and pathology. Stem Cell Rep. *16*, 1923–1937. https://doi.org/10.1016/j.stemcr.2021.06.011.

42. Messina, A., Luce, E., Hussein, M., and Dubart-Kupperschmitt, A. (2020). Pluripotent-stem-cell-derived hepatic cells: hepatocytes and organoids for liver therapy and regeneration. Cells *9*, 420. https://doi.org/10.3390/cells9020420.

43. Sasai, Y. (2013). Cytosystems dynamics in self-organization of tissue architecture. Nature *493*, 318–326. https://doi.org/10.1038/nature11859.

44. Wang, D., Guo, Y., Zhu, J., Liu, F., Xue, Y., Huang, Y., Zhu, B., Wu, D., Pan, H., Gong, T., et al. (2022). Hyaluronic acid methacrylate/pancreatic extracellular matrix as a potential 3D printing bioink for constructing islet organoids. Acta Biomater. https://doi.org/10.1016/j.actbio.2022.06.036.

45. Pampaloni, F., and Stelzer, E. (2010). Three-dimensional cell cultures in toxicology. Biotechnol. Genet. Eng. Rev. *26*, 117–138. https://doi.org/10.5661/bger-26-117.

46. Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., and Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature *459*, 262–265. https://doi.org/10.1038/nature07935.

47. Gao, Z., Wu, T., Xu, J., Liu, G., Xie, Y., Zhang, C., Wang, J., and Wang, S. (2014). Generation of bioartificial salivary gland using whole-organ decellularized bioscaffold. Cells Tissues Organs *200*, 171–180. https://doi.org/10.1159/000371873.

48. Nicoletti, A., Fiorini, M., Paolillo, J., Dolcini, L., Sandri, M., and Pressato, D. (2013). Effects of different crosslinking conditions on the chemical-physical properties of a novel bio-inspired composite scaffold stabilised with 1, 4-butanediol diglycidyl ether (BDDGE). J. Mater. Sci. Mater. Med. *24*, 17–35. https://doi.org/10.1007/s10856-012-4782-4.

49. Liu, H., Wang, Y., Cui, K., Guo, Y., Zhang, X., and Qin, J. (2019). Advances in hydrogels in organoids and organs-on-a-chip. Adv. Mater. *31*, e1902042. https://doi.org/10.1002/adma.201902042.

50. Takasato, M., Er, P.X., Becroft, M., Vanslambrouck, J.M., Stanley, E.G., Elefanty, A.G., and Little, M.H. (2014). Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. Nat. Cell Biol. *16*, 118–126. https://doi.org/10.1038/ncb2894.

51. Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K., and Sasai, Y. (2008). Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell *3*, 519–532. https://doi.org/10.1016/j.stem.2008.09.002.

52. Muguruma, K., Nishiyama, A., Kawakami, H., Hashimoto, K., and Sasai, Y. (2015). Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. Cell Rep. *10*, 537–550. https://doi.org/10.1016/j.celrep.2014.12.051.

53. Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature *472*, 51–56. https://doi.org/10.1038/nature09941.

54. Tanaka, J., Ogawa, M., Hojo, H., Kawashima, Y., Mabuchi, Y., Hata, K., Nakamura, S., Yasuhara, R., Takamatsu, K., Irié, T., et al. (2018). Generation of orthotopically functional salivary gland from embryonic stem cells. Nat. Commun. *9*, 4216. https://doi.org/10.1038/s41467-018-06469-7.

55. Hosseini, Z.F., Nelson, D.A., Moskwa, N., Sfakis, L.M., Castracane, J., and Larsen, M. (2018). FGF2-dependent mesenchyme and laminin-111 are niche factors in salivary gland organoids. J. Cell Sci. *131*, jcs208728. https://doi.org/10.1242/jcs.208728.

56. Lewis, A., Keshara, R., Kim, Y.H., and Grapin-Botton, A. (2021). Self-organization of organoids from endoderm-derived cells. J. Mol. Med. *99*, 449–462. https://doi.org/10.1007/s00109-020-02010-w.

57. Amaral, A.J.R., and Pasparakis, G. (2019). Cell membrane engineering with synthetic materials: applications in cell spheroids, cellular glues and microtissue formation. Acta Biomater. *90*, 21–36. https://doi.org/10.1016/j.actbio.2019.04.013.

58. Krishnamurthy, S., Muthukumaran, P., Jayakumar, M.K.G., Lisse, D., Masurkar, N.D., Xu, C., Chan, J.M., and Drum, C.L. (2019). Surface protein engineering increases the circulation time of a cell membrane-based nanotherapeutic. Nanomedicine *18*, 169–178. https://doi.org/10.1016/j.nano.2019.02.024.

59. Cheng, H., Byrska-Bishop, M., Zhang, C.T., Kastrup, C.J., Hwang, N.S., Tai, A.K., Lee, W.W., Xu, X., Nahrendorf, M., Langer, R., and Anderson, D.G. (2012). Stem cell membrane engineering for cell rolling using peptide conjugation and tuning of cell-selectin interaction kinetics. Biomaterials *33*, 5004–5012. https://doi.org/10.1016/j.biomaterials.2012.03.065.

60. Stephan, M.T., and Irvine, D.J. (2011). Enhancing cell therapies from the outside in: cell surface engineering using synthetic nanomaterials. Nano Today *6*, 309–325. https://doi.org/10.1016/j.nantod.2011.04.001.

61. Rogozhnikov, D., O'Brien, P.J., Elahipanah, S., and Yousaf, M.N. (2016). Scaffold free bio-orthogonal assembly of 3-dimensional cardiac tissue via cell surface engineering. Sci. Rep. *6*, 39806. https://doi.org/10.1038/srep39806.

62. Fayol, D., Frasca, G., Le Visage, C., Gazeau, F., Luciani, N., and Wilhelm, C. (2013). Use of magnetic forces to promote stem cell aggregation during differentiation, and cartilage tissue modeling. Adv. Mater. *25*, 2611–2616. https://doi.org/10.1002/adma.201300342.

63. Akbari, E., Mollica, M.Y., Lucas, C.R., Bushman, S.M., Patton, R.A., Shahhosseini, M., Song, J.W., and Castro, C.E. (2017). Engineering cell surface function with DNA origami. Adv. Mater. *29*. https://doi.org/10.1002/adma.201703632.

64. Nie, J., and Hashino, E. (2017). Organoid technologies meet genome engineering. EMBO Rep. *18*, 367–376. https://doi.org/10.15252/embr.201643732.

65. Velazquez, J.J., LeGraw, R., Moghadam, F., Tan, Y., Kilbourne, J., Maggiore, J.C., Hislop, J., Liu, S., Cats, D., Chuva de Sousa Lopes, S.M., et al. (2021). Gene regulatory network analysis and engineering directs development and vascularization of multilineage human liver organoids. Cell Syst. *12*, 41–55.e11. https://doi.org/10.1016/j.cels.2020.11.002.

66. Deng, W.L., Gao, M.L., Lei, X.L., Lv, J.N., Zhao, H., He, K.W., Xia, X.X., Li, L.Y., Chen, Y.C., Li, Y.P., et al. (2018). Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. Stem Cell Rep. *10*, 1267–1281. https://doi.org/10.1016/j.stemcr.2018.02.003.

67. Liu, X., Cheng, Y., Abraham, J.M., Wang, Z., Wang, Z., Ke, X., Yan, R., Shin, E.J., Ngamruengphong, S., Khashab, M.A., et al. (2018). Modeling Wnt signaling by CRISPR-Cas9 genome editing recapitulates neoplasia in human Barrett epithelial organoids. Cancer Lett. *436*, 109–118. https://doi.org/10.1016/j.canlet.2018.08.017.

68. Sthijns, M.M.J.P.E., LaPointe, V.L.S., and van Blitterswijk, C.A. (2019). Building complex life through self-organization. Tissue Eng. Part A *25*, 1341–1346. https://doi.org/10.1089/ten.TEA.2019.0208.

69. Hofer, M., and Lutolf, M.P. (2021). Engineering organoids. Nat. Rev. Mater. *6*, 402–420. https://doi.org/10.1038/s41578-021-00279-y.

70. Kretzschmar, K., and Clevers, H. (2016). Organoids: modeling development and the stem cell niche in a dish. Dev. Cell *38*, 590–600. https://doi.org/10.1016/j.devcel.2016.08.014.

71. Murrow, L.M., Weber, R.J., and Gartner, Z.J. (2017). Dissecting the stem cell niche with organoid models: an engineering-based approach. Development *144*, 998–1007. https://doi.org/10.1242/dev.140905.

72. Aisenbrey, E.A., and Murphy, W.L. (2020). Synthetic alternatives to Matrigel. Nat. Rev. Mater. *5*, 539–551. https://doi.org/10.1038/s41578-020-0199-8.

73. Klotz, B.J., Oosterhoff, L.A., Utomo, L., Lim, K.S., Vallmajo-Martin, Q., Clevers, H., Woodfield, T.B.F., Rosenberg, A.J.W.P., Malda, J., Ehrbar, M., et al. (2019). A versatile biosynthetic hydrogel platform for engineering of tissue analogues. Adv. Healthc. Mater. *8*, e1900979. https://doi.org/10.1002/adhm.201900979.

74. Kozlowski, M.T., Crook, C.J., and Ku, H.T. (2021). Towards organoid culture without Matrigel. Commun. Biol. *4*, 1387. https://doi.org/10.1038/s42003-021-02910-8.

75. Davoudi, Z., Peroutka-Bigus, N., Bellaire, B., Jergens, A., Wannemuehler, M., and Wang, Q. (2021). Gut organoid as a new platform to study alginate and chitosan mediated PLGA nanoparticles for drug delivery. Mar. Drugs *19*, 282. https://doi.org/10.3390/md19050282.

76. Vallmajo-Martin, Q., Broguiere, N., Millan, C., Zenobi-Wong, M., and Ehrbar, M. (2020). PEG/HA hybrid hydrogels for biologically and mechanically tailorable bone marrow organoids. Adv. Funct. Mater. *30*, 1910282. https://doi.org/10.1002/adfm.201910282.

77. Heinemann, S., Heinemann, C., Wenisch, S., Alt, V., Worch, H., and Hanke, T. (2013). Calcium phosphate phases integrated in silica/collagen nanocomposite xerogels enhance the bioactivity and ultimately manipulate the osteoblast/osteoclast ratio in a human co-culture model. Acta Biomater. *9*, 4878–4888. https://doi.org/10.1016/j.actbio.2012.10.010.

78. Shi, J., Yu, X., Wang, L., Liu, Y., Gao, J., Zhang, J., Ma, R., Liu, R., and Zhang, Z. (2013). PEGylated fullerene/iron oxide nanocomposites for photodynamic therapy, targeted drug delivery and MR imaging. Biomaterials *34*, 9666–9677. https://doi.org/10.1016/j.biomaterials.2013.08.049.

79. Gjorevski, N., Sachs, N., Manfrin, A., Giger, S., Bragina, M.E., Ordóñez-Morán, P., Clevers, H., and Lutolf, M.P. (2016). Designer matrices for intestinal stem cell and organoid culture. Nature *539*, 560–564. https://doi.org/10.1038/nature20168.

80. Broguiere, N., Isenmann, L., Hirt, C., Ringel, T., Placzek, S., Cavalli, E., Ringnalda, F., Villiger, L., Züllig, R., Lehmann, R., et al. (2018). Growth of epithelial organoids in a defined hydrogel. Adv. Mater. *30*, e1801621. https://doi.org/10.1002/adma.201801621.

81. Mammadov, R., Mammadov, B., Guler, M.O., and Tekinay, A.B. (2012). Growth factor binding on heparin mimetic peptide nanofibers. Biomacromolecules *13*, 3311–3319. https://doi.org/10.1021/bm3010897.

82. Zieris, A., Prokoph, S., Levental, K.R., Welzel, P.B., Grimmer, M., Freudenberg, U., and Werner, C. (2010). FGF-2 and VEGF functionalization of starPEG-heparin hydrogels to modulate biomolecular and physical cues of angiogenesis. Biomaterials *31*, 7985–7994. https://doi.org/10.1016/j.biomaterials.2010.07.021.

83. Wylie, R.G., Ahsan, S., Aizawa, Y., Maxwell, K.L., Morshead, C.M., and Shoichet, M.S. (2011). Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. Nat. Mater. *10*, 799–806. https://doi.org/10.1038/nmat3101.

84. Zhou, K., Yu, P., Shi, X., Ling, T., Zeng, W., Chen, A., Yang, W., and Zhou, Z. (2019). Hierarchically porous hydroxyapatite hybrid scaffold incorporated with reduced graphene oxide for rapid bone ingrowth and repair. ACS Nano *13*, 9595–9606. https://doi.org/10.1021/acsnano.9b04723.

85. Li, H., Yin, Y., Xiang, Y., Liu, H., and Guo, R. (2020). A novel 3D printing PCL/GelMA scaffold containing USPIO for MRI-guided bile duct repair. Biomed. Mater. *15*, 045004. https://doi.org/10.1088/1748-605X/ab797a.

86. Nikolaev, M., Mitrofanova, O., Broguiere, N., Geraldo, S., Dutta, D., Tabata, Y., Elci, B., Brandenberg, N., Kolotuev, I., Gjorevski, N., et al. (2020). Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature *585*, 574–578. https://doi.org/10.1038/s41586-020-2724-8.

87. Rossen, N.S., Anandakumaran, P.N., Zur Nieden, R., Lo, K., Luo, W., Park, C., Huyan, C., Fu, Q., Song, Z., Singh-Moon, R.P., et al. (2020). Injectable therapeutic organoids using sacrificial hydrogels. iScience *23*, 101052. https://doi.org/10.1016/j.isci.2020.101052.

88. Wörsdörfer, P., Rockel, A., Alt, Y., Kern, A., and Ergün, S. (2020). Generation of vascularized neural organoids by co-culturing with mesodermal progenitor cells. STAR Protoc. *1*, 100041. https://doi.org/10.1016/j.xpro.2020.100041.

89. Lu, D., and Kassab, G.S. (2011). Role of shear stress and stretch in vascular mechanobiology. J. R. Soc. Interface *8*, 1379–1385. https://doi.org/10.1098/rsif.2011.0177.

90. Homan, K.A., Gupta, N., Kroll, K.T., Kolesky, D.B., Skylar-Scott, M., Miyoshi, T., Mau, D., Valerius, M.T., Ferrante, T., Bonventre, J.V., et al. (2019). Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat. Methods *16*, 255–262. https://doi.org/10.1038/s41592-019-0325-y.

91. Mirabella, T., MacArthur, J.W., Cheng, D., Ozaki, C.K., Woo, Y.J., Yang, M., and Chen, C.S. (2017). 3D-printed vascular networks direct therapeutic angiogenesis in ischaemia. Nat. Biomed. Eng. *1*, 0083. https://doi.org/10.1038/s41551-017-0083.

92. Chen, M.B., Whisler, J.A., Jeon, J.S., and Kamm, R.D. (2013). Mechanisms of tumor cell extravasation in an in vitro microvascular network platform. Integr. Biol. *5*, 1262–1271. https://doi.org/10.1039/c3ib40149a.

93. Nashimoto, Y., Hayashi, T., Kunita, I., Nakamasu, A., Torisawa, Y.S., Nakayama, M., Takigawa-Imamura, H., Kotera, H., Nishiyama, K., Miura, T., and Yokokawa, R. (2017). Integrating perfusable vascular networks with a three-dimensional tissue in a microfluidic device. Integr. Biol. *9*, 506–518. https://doi.org/10.1039/c7ib00024c.

94. Zhang, Y.S., Aleman, J., Shin, S.R., Kilic, T., Kim, D., Mousavi Shaegh, S.A., Massa, S., Riahi, R., Chae, S., Hu, N., et al. (2017). Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc. Natl. Acad. Sci. USA *114*, E2293–E2302. https://doi.org/10.1073/pnas.1612906114.

95. Pham, M.T., Pollock, K.M., Rose, M.D., Cary, W.A., Stewart, H.R., Zhou, P., Nolta, J.A., and Waldau, B. (2018). Generation of human vascularized brain organoids. Neuroreport *29*, 588–593. https://doi.org/10.1097/wnr.0000000000001014.

96. Sequeira, R.C., Criswell, T., Atala, A., and Yoo, J.J. (2020). Microfluidic systems for assisted reproductive technologies: advantages and potential applications. Tissue Eng. Regen. Med. *17*, 787–800. https://doi.org/10.1007/s13770-020-00311-2.

97. Jin, Y., Kim, J., Lee, J.S., Min, S., Kim, S., Ahn, D.H., Kim, Y.G., and Cho, S.W. (2018). Vascularized liver organoids generated using induced hepatic tissue and dynamic liver-specific microenvironment as a drug testing platform. Adv. Funct. Mater. *28*, 1801954. https://doi.org/10.1002/adfm.201801954.

98. Ono, M., Oshima, M., Ogawa, M., Sonoyama, W., Hara, E.S., Oida, Y., Shinkawa, S., Nakajima, R., Mine, A., Hayano, S., et al. (2017). Practical whole-tooth restoration utilizing autologous bioengineered tooth germ transplantation in a postnatal canine model. Sci. Rep. *7*, 44522. https://doi.org/10.1038/srep44522.

99. Wang, F., Wu, Z., Fan, Z., Wu, T., Wang, J., Zhang, C., and Wang, S. (2018). The cell re-association-based whole-tooth regeneration strategies in large animal, Sus scrofa. Cell Prolif. *51*, e12479. https://doi.org/10.1111/cpr.12479.

100. van Manen, E.H.C., Zhang, W., Walboomers, X.F., Vazquez, B., Yang, F., Ji, W., Yu, N., Spear, D.J., Jansen, J.A., and Yelick, P.C. (2014). The influence of electrospun fibre scaffold orientation and nano-hydroxyapatite content on the development of tooth bud stem cells in vitro. Odontology *102*, 14–21. https://doi.org/10.1007/s10266-012-0087-9.

101. Smith, E.E., and Yelick, P.C. (2019). Bioengineering tooth bud constructs using GelMA hydrogel. Methods Mol. Biol. *1922*, 139–150. https://doi.org/10.1007/978-1-4939-9012-2_14.

102. Smith, E.E., Zhang, W., Schiele, N.R., Khademhosseini, A., Kuo, C.K., and Yelick, P.C. (2017). Developing a biomimetic tooth bud model. J. Tissue Eng. Regen. Med. *11*, 3326–3336. https://doi.org/10.1002/term.2246.

103. Smith, E.E., Angstadt, S., Monteiro, N., Zhang, W., Khademhosseini, A., and Yelick, P.C. (2018). Bioengineered tooth buds exhibit features of natural tooth buds. J. Dent. Res. *97*, 1144–1151. https://doi.org/10.1177/0022034518779075.

104. Kilic Bektas, C., Zhang, W., Mao, Y., Wu, X., Kohn, J., and Yelick, P.C. (2022). Self-assembled hydrogel microparticle-based tooth-germ organoids. Bioengineering *9*, 215. https://doi.org/10.3390/bioengineering9050215.

105. Eap, S., Bécavin, T., Keller, L., Kökten, T., Fioretti, F., Weickert, J.L., Deveaux, E., Benkirane-Jessel, N., and Kuchler-Bopp, S. (2014). Nanofibers implant functionalized by neural growth factor as a strategy to innervate a bioengineered tooth. Adv. Healthc. Mater. *3*, 386–391. https://doi.org/10.1002/adhm.201300281.

106. Yoshimoto, S., Yoshizumi, J., Anzai, H., Morishita, K., Okamura, K., Hiraki, A., and Hashimoto, S. (2020). Inhibition of Alk signaling promotes the induction of human salivary-gland-derived organoids. Dis. Model. Mech. *13*, dmm045054. https://doi.org/10.1242/dmm.045054.

107. Maria, O.M., Zeitouni, A., Gologan, O., and Tran, S.D. (2011). Matrigel improves functional properties of primary human salivary gland cells. Tissue engineering. Tissue Eng. Part A *17*, 1229–1238. https://doi.org/10.1089/ten.TEA.2010.0297.

108. Shin, H.S., Hong, H.J., Koh, W.G., and Lim, J.Y. (2018). Organotypic 3D culture in nanoscaffold microwells supports salivary gland stem-cell-based organization. ACS Biomater. Sci. Eng. *4*, 4311–4320. https://doi.org/10.1021/acsbiomaterials.8b00894.

109. Foraida, Z.I., Kamaldinov, T., Nelson, D.A., Larsen, M., and Castracane, J. (2017). Elastin-PLGA hybrid electrospun nanofiber scaffolds for salivary epithelial cell self-organization and polarization. Acta Biomater. *62*, 116–127. https://doi.org/10.1016/j.actbio.2017.08.009.

110. Delamarre, E., Taboubi, S., Mathieu, S., Bérenguer, C., Rigot, V., Lissitzky, J.C., Figarella-Branger, D., Ouafik, L., and Luis, J. (2009). Expression of integrin alpha6beta1 enhances tumorigenesis in glioma cells. Am. J. Pathol. *175*, 844–855. https://doi.org/10.2353/ajpath.2009.080920.

111. Nam, K., Maruyama, C.L., Wang, C.S., Trump, B.G., Lei, P., Andreadis, S.T., and Baker, O.J. (2017). Laminin-111-derived peptide conjugated fibrin hydrogel restores salivary gland function. PLoS One *12*, e0187069. https://doi.org/10.1371/journal.pone.0187069.

112. da Silva, D., Kaduri, M., Poley, M., Adir, O., Krinsky, N., Shainsky-Roitman, J., and Schroeder, A. (2018). Biocompatibility, biodegradation and excretion of polylactic acid (PLA) in medical implants and theranostic systems. Chem. Eng. J. *340*, 9–14. https://doi.org/10.1016/j.cej.2018.01.010.

113. Adine, C., Ng, K.K., Rungarunlert, S., Souza, G.R., and Ferreira, J.N. (2018). Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands. Biomaterials *180*, 52–66. https://doi.org/10.1016/j.biomaterials.2018.06.011.

114. Chansaenroj, A., Adine, C., Charoenlappanit, S., Roytrakul, S., Sariya, L., Osathanon, T., Rungarunlert, S., Urkasemsin, G., Chaisuparat, R., Yodmuang,

S., et al. (2022). Magnetic bioassembly platforms towards the generation of extracellular vesicles from human salivary gland functional organoids for epithelial repair. Bioact. Mater. *18*, 151–163. https://doi.org/10.1016/j.bioactmat.2022.02.007.

115. Yoon, Y.J., Kim, D., Tak, K.Y., Hwang, S., Kim, J., Sim, N.S., Cho, J.M., Choi, D., Ji, Y., Hur, J.K., et al. (2022). Salivary gland organoid culture maintains distinct glandular properties of murine and human major salivary glands. Nat. Commun. *13*, 3291. https://doi.org/10.1038/s41467-022-30934-z.

116. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., and Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer *136*, E359–E386. https://doi.org/10.1002/ijc.29210.

117. Zhao, L., Huang, L., Yu, S., Zheng, J., Wang, H., and Zhang, Y. (2017). Decellularized tongue tissue as an in vitro model for studying tongue cancer and tongue regeneration. Acta Biomater. *58*, 122–135. https://doi.org/10.1016/j.actbio.2017.05.062.

118. Noi, M., Mukaisho, K.I., Yoshida, S., Murakami, S., Koshinuma, S., Adachi, T., Machida, Y., Yamori, M., Nakayama, T., Yamamoto, G., and Sugihara, H. (2018). ERK phosphorylation functions in invadopodia formation in tongue cancer cells in a novel silicate fibre-based 3D cell culture system. Int. J. Oral Sci. *10*, 30. https://doi.org/10.1038/s41368-018-0033-y.

119. Ren, W., Lewandowski, B.C., Watson, J., Aihara, E., Iwatsuki, K., Bachmanov, A.A., Margolskee, R.F., and Jiang, P. (2014). Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. Proc. Natl. Acad. Sci. USA *111*, 16401–16406. https://doi.org/10.1073/pnas.1409064111.

120. Adpaikar, A.A., Zhang, S., Kim, H.Y., Kim, K.W., Moon, S.J., Lee, J.M., and Jung, H.S. (2022). Fine-tuning of epithelial taste bud organoid to promote functional recapitulation of taste reactivity. Cell. Mol. Life Sci. *79*, 211. https://doi.org/10.1007/s00018-022-04242-0.

121. Lee, J.S., Cho, A.N., Jin, Y., Kim, J., Kim, S., and Cho, S.W. (2018). Bio-artificial tongue with tongue extracellular matrix and primary taste cells. Biomaterials *151*, 24–37. https://doi.org/10.1016/j.biomaterials.2017.10.019.

122. Hisha, H., Tanaka, T., Kanno, S., Tokuyama, Y., Komai, Y., Ohe, S., Yanai, H., Omachi, T., and Ueno, H. (2013). Establishment of a novel lingual organoid culture system: generation of organoids having mature keratinized epithelium from adult epithelial stem cells. Sci. Rep. *3*, 3224. https://doi.org/10.1038/srep03224.

123. Kim, G.A., Spence, J.R., and Takayama, S. (2017). Bioengineering for intestinal organoid cultures. Curr. Opin. Biotechnol. *47*, 51–58. https://doi.org/10.1016/j.copbio.2017.05.006.

124. Fedorchak, N.J., Iyer, N., and Ashton, R.S. (2021). Bioengineering tissue morphogenesis and function in human neural organoids. Semin. Cell Dev. Biol. *111*, 52–59. https://doi.org/10.1016/j.semcdb.2020.05.025.

125. Yavitt, F.M., Brown, T.E., Hushka, E.A., Brown, M.E., Gjorevski, N., Dempsey, P.J., Lutolf, M.P., and Anseth, K.S. (2020). The effect of thiol structure on allyl sulfide photodegradable hydrogels and their application as a degradable scaffold for organoid passaging. Adv. Mater. *32*, e1905366. https://doi.org/10.1002/adma.201905366.

126. Jalili-Firoozinezhad, S., Miranda, C.C., and Cabral, J.M.S. (2021). Modeling the human body on microfluidic chips. Trends Biotechnol. *39*, 838–852. https://doi.org/10.1016/j.tibtech.2021.01.004.

127. Artegiani, B., Hendriks, D., Beumer, J., Kok, R., Zheng, X., Joore, I., Chuva de Sousa Lopes, S., van Zon, J., Tans, S., and Clevers, H. (2020). Fast and efficient generation of knock-in human organoids using homology-independent CRISPR-Cas9 precision genome editing. Nat. Cell Biol. *22*, 321–331. https://doi.org/10.1038/s41556-020-0472-5.

128. Revah, O., Gore, F., Kelley, K.W., Andersen, J., Sakai, N., Chen, X., Li, M.Y., Birey, F., Yang, X., Saw, N.L., et al. (2022). Maturation and circuit integration of transplanted human cortical organoids. Nature *610*, 319–326. https://doi.org/10.1038/s41586-022-05277-w.

129. Hellwarth, P.B., Chang, Y., Das, A., Liang, P., Lian, X., Repina, N.A., and Bao, X. (2021). Optogenetic-mediated cardiovascular differentiation and patterning of human pluripotent stem cells. Adv. Genet. *2*, e202100011. https://doi.org/10.1002/ggn2.202100011.