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Advancement From Stem Cells to Oral Tissue Organoids: A Step Towards Tissue Regeneration

[1] Dr. Tummanepally Sai Manasa, [2] Dr. Jammula Surya Prasanna

[1] PG Student, Department of Periodontics and Implantology, Panineeya Mahavidyalaya Institute of Dental Sciences and Research Centre, Kamala Nagar, Dilsukhnagar, Hyderabad, Telangana, India

[2] MDS, Professor, Department of Periodontics and Implantology, Panineeya Mahavidyalaya Institute of Dental Sciences and Research Centre, Kamala Nagar, Dilsukhnagar, Hyderabad, Telangana, India

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Corresponding Author: **Dr. Tummanepally Sai Manasa**

Abstract

The primary goal of periodontal therapy has been to facilitate the complete restoration and regeneration of the compromised periodontal tissues, aiming to return them to their original form and function. Recent advances in the field of stem cell biology and regenerative medicine have led to various opportunities for tissue engineering and gene-based approaches in periodontal therapy. Stem cells, characterized by their capacity of renewal and differentiation into various specialized cells, have revolutionized regenerative medicine and played a vital role in the emergence of tissue engineering-based treatments. Three-dimensional (3D) cell culture systems have emerged as a significant breakthrough. These systems enable the cultivation of complex structures known as organoids and spheroids, closely mirroring the in-vivo conditions, and facilitating cellular growth and interaction within their microenvironment. This has greatly enhanced our understanding of the spatiotemporal dynamics of organogenesis and organ function. In this review, we describe the recent technological developments in the field of regenerative therapy.

Keywords: 3D Cell Cultures, Organogenesis, Organoids, Regenerative Medicine, Spheroids, Stem Cells

1. Introduction

In general, human cells and tissues have a limited regeneration potential. However, recent advancement in stem cell research and tissue engineering promises novel techniques for tissue regeneration in dental practice in the near future [1]. Latest research on embryology, stem cells, and tissue engineering have propelled significant advancements in the field of regenerative medicine, leading to remarkable progress in biotechnology.

Regenerative medicine is dedicated to the replacement, or regeneration of injured, diseased, or dysfunctional tissues. Periodontal regeneration aims at restoring the lost periodontium by enhancing the periodontal attachment, reducing pocket depth (PD), and limiting gingival recession. Thus, periodontal regeneration is considered the gold standard of periodontal treatment [2].

Tissue engineering is a multidisciplinary field integrating the principles and methods of engineering and life sciences to aid in the development of biological substitutes for the restoration and maintenance of damaged tissues and organs. Langer (1993) put forward tissue engineering as a potential technique for regenerating lost periodontal tissues. The tissue engineering approach to bone and periodontal regeneration involves the synergistic combination of three key elements: progenitor cells, a scaffold or supporting matrix, and signalling molecules. A critical but sometimes overlooked prerequisite is the need to ensure vascularization to the newly formed tissues.

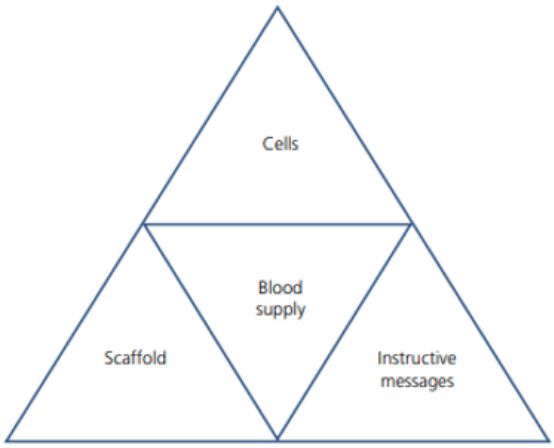

Fig 1: The key components of a tissue-engineered construct: cells, scaffold, instructive messages, and blood supply

Stem cells

The identification and manipulation of stem cells has played a significant role in advancing regenerative medicine and facilitating the development of clinical therapies based on tissue engineering [3]. Stem cells (SCs) have the ability to self-renew and differentiate into more specialised cells. Depending on their differentiation potential, SCs can be categorized as totipotent, pluripotent, or multipotent cells. Totipotent cells are those having the capacity to differentiate into both embryonic and extraembryonic tissues. Pluripotent stem cells (PSCs) differentiate into the three primary embryonic germ layers, namely ectoderm, endoderm, and mesoderm. Only a limited range of specialised cells can be formed from multipotent stem cells [4].

In periodontal regeneration, mesenchymal stem cells (MSCs) displayed promising results in both *in vitro* and human studies. MSCs can be extracted from dental and non-dental tissues. Dental tissue-derived MSCs include dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle precursor cells (DFPCs), and stem cells derived from apical papilla (SCAPs). Various non-dental stem cells are bone marrow MSCs (BMMSCs), adipose tissue derived stem cells (ASCs), embryonic stem cells (ESCs), and induced-pluripotent stem cells (iPSCs). These have also been extensively investigated for their potential role in periodontal regeneration [2].

Dental pulp stem cells (DPSCs)

DPSCs are a mesenchymal type of stem cells located within the dental pulp. They can differentiate in-vitro into dentin and in-vivo into dentin-pulp-like complexes, and they have the potential to become osteogenic and chondrogenic. Recently, immature dental pulp stem cells have been identified, which are a pluripotent sub-population of DPSCs generated using dental pulp organ culture, providing a promising avenue for further research and potential applications in regenerative medicine.

Stem cells from human exfoliated deciduous teeth (SHEDs)

These are a specific type of dental pulp stem cells obtained from exfoliated deciduous teeth. They were first discovered by Dr. Song Tao Shi in 2003. The primary role of these cells seems to be the formation of mineralized tissue, useful for enhancing orofacial bone regeneration. One notable characteristic of SHEDs is their high proliferation rate and immuno-modulatory properties. They can differentiate into osteoblasts and could express an immuno-regulatory potential on T cells, macrophages, and dendritic cells. Nakamura *et al*. in a study compared the "stemness" of SHEDs to DPSCs and BMMSCs and observed that SHEDs showed a higher proliferation rate than DPSCs and BMMSCs and higher gene expression for cell proliferation and extracellular matrix elements [4].

Periodontal ligament stem cells (PDLSCs)

These are the cells isolated from the perivascular wall of the periodontal ligament, which is harvested from the extracted tooth roots. These stem cells are recognized for their potential to differentiate into osteogenic (bone-forming), chondrogenic (cartilage-forming), and adipogenic (fat-forming) cell lineages. Like bone marrow MSCs (BMMSCs) and DPSCs, PDLSCs also exhibit immunosuppressive characteristics. They also possess the ability to form a cementum/PL complex-like structure.

PDLSCs are also isolated from cryopreserved periodontal ligaments. In this process they retain their stem cell characteristics, the ability of single-colony strain generation, cementum/periodontal ligament-like tissue formation, MSC surface markers expression, and multipotential differentiation. This makes cryopreserved PDLSCs a convenient and readily available source of MSCs. Trubiani *et al*. showed that PDLSCs had regenerative potential when seeded onto a biocompatible scaffold, thus increasing their use in graft biomaterials for bone tissue engineering [12].

Stem cells from apical papilla (SCAP)

These are a population of MSCs that are found in the apical papilla of permanent teeth with immature roots. They were initially identified by Sonoyama *et al*. The development of dentin in-vivo is carried out by odontoblast-like cells, which are differentiated from these specialised MSCs. Due to their proximity to the periapical tissue vasculature, SCAPs can also survive in cases of pulp necrosis. Hence, these cells can generate primary odontoblasts even after endodontic disinfection, which complete root formation under the influence of the surviving Hertwig's epithelial root sheath.

Non-dental stem cells

Bone marrow MSCs (BMMSCs), being the first MSCs identified, have shown osteogenic, adipogenic, chondrogenic, and myogenic differentiation. BMMSCs have the potential of differentiating into ameloblast-like cells and periodontal tissue cells. BMMSCs can upregulate the expression of odontogenic genes and contribute to new tooth formation on recombining with embryonic oral epithelium.

Adipose-derived stem cells (ASCs) are abundant cells derived from adipose tissues. ASCs have undergone osteogenic, chondrogenic, adipogenic, and neurogenic differentiation and are capable of *in vitro* growth. ASCs can be extracted in large quantities from subcutaneous or lipo-suctioned adipose tissue pieces. These have been extensively used in regenerative medicine.

Embryonic stem cells (ESCs), pluripotent type of stem cells, are found in human blastocysts. They show extraordinary potential for differentiation due to their ability to develop into almost all cell lineages It has been demonstrated that when co-cultured with PDLSCs or embryonic oral epithelial cells, ESCs can develop into the odontogenic and

periodontal cell lineages.

Since its initial discovery in 2006, induced pluripotent stem cells (iPSCs) have attracted a lot of attention in the field of regenerative medicine. They can be produced from a somatic cell and are a subtype of pluripotent stem cell. They can continuously divide and produce all other types of cells in the body.

Scaffold or supporting matrix

Scaffolds and biomaterials are critical components in tissue regeneration. Their function is to mimic the physiological environment necessary for cellular growth, expansion, and differentiation. Many biodegradable and biocompatible biomaterials have been developed throughout the years to aid in the regenerating process. Biomaterials are available as natural or synthetic. Natural biomaterials, namely natural polymers (collagen, laminin, elastin, chitosan, silk, platelet-rich plasma, bone sialoprotein), are typically less toxic, more environmentally friendly, and less expensive. They are chosen for cell adhesion, cell-responsive degradation, appropriate cell signalling, and fast breakdown that does not result in immunological rejection. Tooth tissue engineering also employs synthetic materials that are often more flexible and elastic than natural ones, such as hydroxyapatite, polylactic acid, polyglycolic acid, polycaprolactone, and polylactic-co-glycolic acid.

The major roles of the supporting matrices are I) Structural reinforcement- to prevent the collapse of the surrounding tissue into the wound site. II) Barrier -to restrict cellular migration in a selective manner III) Scaffold- for cellular migration and proliferation IV) Serve as a time- release mechanism for signalling molecules [5].

ECM Scaffolds Derived from Decellularized Tissues - As tissue decellularization techniques progress, acellular tissue-derived scaffolds are being presented as a viable alternative to other scaffolds. After transplantation, the addition of DE cells and DPSCs to acellular scaffolds resulted in the development of enamel and dentine.

Signaling molecules

Signalling molecules are proteins that can act locally or systemically to influence cell growth and function in a variety of ways. The two types of signalling molecules that have attracted the most attention are growth factors and morphogens, which function by changing the cell phenotype, causing stem cells to differentiate into bone making cells. These cytokines have a variety of actions, including mitogenic (proliferative), chemotactic (stimulate directed cell migration), and angiogenic (stimulate new blood vessel creation). Platelet rich plasma (PRP), enamel matrix derivatives (EMD), and other signalling molecules are examples.

Growth factors are peptides that transmit signals between cells, modulating their activity. They enhance regeneration by stimulating cell migration, adhesion and subsequent spreading, cellular proliferation, differentiation, and matrix formation through a variety of cell-tissue interactions.

Bone morphogenetic proteins (BMPs) are regulatory glycoproteins belonging to the TGF-β superfamily. These molecules predominantly drive mesenchymal stem cell development into chondroblasts and osteoblasts. BMPs have been identified from both bovine and human sources. Much of the scientific interest in periodontal regeneration has been focused on BMP-2, BMP-3 (osteogenin), and BMP-7.

Platelet Rich Plasma (PRP) is an autologous platelet concentrate containing numerous essential growth factors including PGDF, TGF, IGF, EGF, and VEGF. Additionally, PRP contains proteins (such as fibrin, fibronectin, and vitronectin) that act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue, and epithelial migration [6].

Enamel Matrix Derivatives (EMD) are acidic extracts that contain a hydrophobic protein assembly of amelogenins and can regenerate all periodontal tissues. This is derived from developing porcine teeth and has been proven to contain TGF and BMP to promote bone growth. Enamel matrix proteins are made up of numerous proteins, including amelogenin (which comes in several sizes), amelin (ameloblastin/sheathlin), enamelin, tuft proteins, and proteases. The most prevalent component, amelogenin, accounts for more than 90% of the matrix [6].

Cell sheet engineering

This technique outperforms the conventional method since it involves the separation of grown cells without the use of an enzymatic approach. The use of temperature responsive dishes for cell sheet engineering gives a novel technique for producing tissues that do not require a specific scaffold. The cell sheets that result retain their original extracellular matrix and cell-cell interaction. To modulate cell-surface adhesion, Okano *et al* (1995) used temperature changes in cell culture and a surface-grafted temperature-responsive polymer called poly N-isopropylacylamide (PIPAAm) [6].

Oral organoids

The study and production of gastruloids, spheroids, and organoids to imitate the physiological features and tissue architecture of embryonic stages, tissues, and organs has made remarkable progress in the previous decade [4]. The utilisation of tissue-derived stem cells and pluripotent stem cells based on stem cell biology and tissue engineering technologies has revolutionised organoid approaches for regenerative medicine [7]. Organoid systems are one of the most promising platforms for stem cell harnessing because they can mimic many critical aspects of a stem cell niche and its resultant tissue.

An organoid is a complex three-dimensional (3D) structure formed from somatic cells, adult stem cells/progenitor cells, pluripotent stem cells (PSCs), or cell lines that has architectures and capabilities like *in vivo* organs (Liu *et al*. 2019) [8].

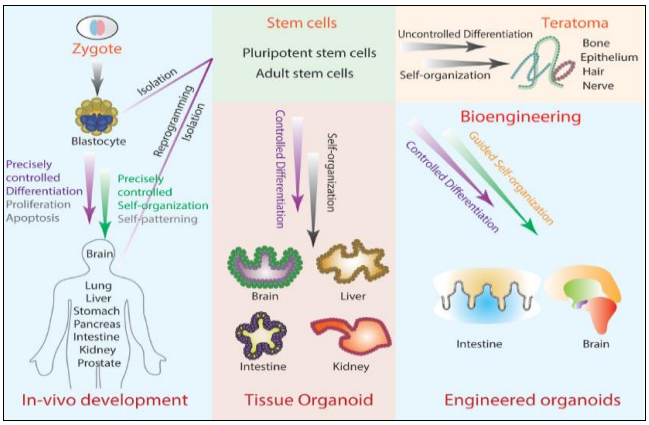

Fig 2: Organoid Development [9]

Oral organoids constitute the complex 3D structures that arise from stem cells or organ-specific progenitor cells through a process of self-organization, re-creating the architectures and functionalities comparable to those found in the oral and maxillofacial region [8]. Cells, scaffold, and construction techniques are three critical components in the production of oral organoids. When cells are placed in a 3D environment with certain growth factors and tiny chemicals, they have an inherent ability to assemble into complex structures.

Tooth germ organoids

To achieve functional tooth organ regeneration, the bioengineered tooth must develop into a tooth loss region following orthotopic transplantation of the bioengineered tooth germ and restore physiological tooth functions, as well as afferent responsiveness to noxious stimulation. A tooth germ-like structure was generated in standard tooth germ culture medium by combining dissociated dental ectodermal (DE) and mesodermal (DM) cells from mandibular tooth germs at the cap stage in ED14.5 mice with a collagen gel droplet (Nakao *et al*. 2007). Following transplanting, the reconstituted tooth germ produced a fully bioengineered tooth in the tooth cavity. This study was the first to show that a whole tooth germ organoid could be successfully reconstituted [8].

Scaffold-free construction techniques have also been developed. A dense aggregate of DPSCs was cultivated in a low attachment plate to mimic mesenchymal condensation, which results in intercellular connections and a dense aggregate. The DPSCs condensate and cells of epithelial origin were subsequently co-cultured in a medium suitable for both cell types. Tooth germ organoids with an epithelial sheath structure around mesenchymal cells emerge after 4 weeks of 3D culture (Rosowski *et al*. 2019). The designed tooth requires not just the hardness and elastic modulus of hard tissue, but also the blood supply and nerve regeneration of soft tissue. Cells having angiogenic capacity can be added to the cell mixture to aid in the vascularization of organoids. Human umbilical vein endothelial cells (HUVECs) were incubated along with DE and DM cells to create vascularized tooth germ organoids (Smith *et al*. 2017). Notably, the tooth germ organoid generated mineralized tissues that closely resembled the original structures in size and shape and displayed tooth markers.

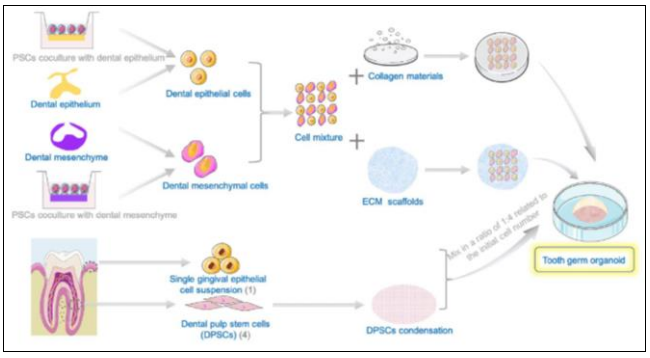

Fig 3: Schematic diagram of the construction of tooth germ organoid [8].

Salivary gland organoids

Salivary gland organoids may be produced from pluripotent stem cells, non-salivary epithelial stem cells, such as dental follicle stem cells, or stem cells derived from salivary glands. The restoration of salivary gland duct structure and secretory activity is crucial for the construction of salivary gland (SG) organoids.

According to recent research, salivary gland stem/progenitor cells can be grown and differentiated to produce organoids that can restore gland function *in vivo*. According to the expression of differentiation markers, the construction of structures, and the response to neurotransmitters *in vitro*, the artificial niche stimulated the growth of salivary gland organoids. The lack of ongoing stimulating stimuli, however, causes a decline in the secretory function (Sui *et al*. 2020). In studies on the development of salivary gland organoids, two primary approaches have been identified.

PSC-derived SG organoids*:* Tanaka and colleagues (2018) induced the cells at the outer layer of ESC aggregates to differentiate into oral ectoderm by using several cytokines and small molecules, such as BMP4, SB-431542 (inhibitor of transforming growth factor [TGF-β]), LDN-193189 (inhibitor of BMP), and FGF2. The aggregates were then infected with recombinant adenoviruses that encoded Foxc1 and Sox9. To encourage salivary gland formation from oral ectoderm, FGF7 and FGF10 were grown in the infected outer layers that had been isolated. The generated SG rudiment displayed mature SG features, after orthotopic transplantation into mice with excised SGs, including saliva secretion [8].

ASC-derived SG organoids: ASC-derived SG organoids can be created using 3D bioprinting. Using magnet dots, 3D structures were created by spatially arranging KIT+ DPSCs that had magnetic nanoparticle tags. The cells were then treated with FGF10 to stimulate SG epithelial cell differentiation by mimicking SG epithelial morphogenesis and neurogenesis. The technique, which relies on the inclusion of magnetic 3D bioprinting, has produced SG organoids with neuronal compartments, secretory activity, and epithelial polarity but with restricted vascularization. A vascularized salivary gland organoid has not yet been created using a specific method. The creation of an SG organoid that can last and work in the host for a long time should be the subject of further research.

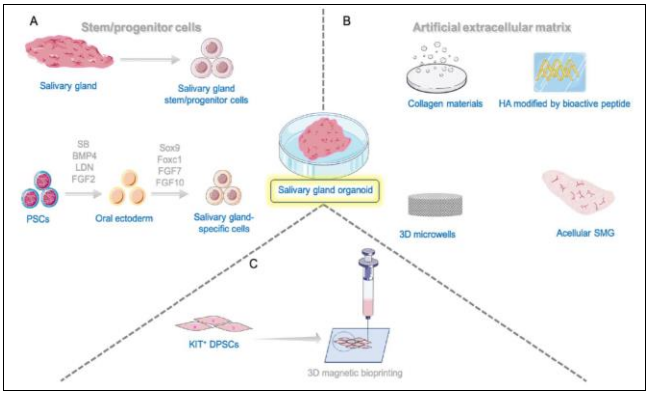

Fig 4: Schematic diagram of the construction of salivary gland organoid [8]

Lingual epithelium organoids

Understanding the maintenance of lingual epithelial tissue, the origin of tongue cancer, is certainly significant as tongue cancer is one of the most common malignant diseases in the

world [10]. Lingual epithelium is constantly replaced in animals, and the turnover rate of mouse lingual epithelium, at 6-7 days, is 4-5-fold higher than that of dorsal skin, indicating the presence of stem cells in the papillae.

Previous research on tongue development has shown that the tongue epithelium stays histologically immature until the 13th day of gestation, with pre-papilla placodes and fungiform papillae appearing on the 14th and 15th days, respectively [10]. The development of lingual epithelium was studied using organ culture of rat embryonic tongues. On the 13th day of gestation, whole tongues were extracted from rat embryos and incubated at the air/liquid interface in the presence of media containing foetal bovine serum (FBS) and B-27. Fungiform papillae were seen in the epithelium after two days of culture, a stage of development similar to the tongue of an intact embryo on the 15th day of gestation.

Lingual epithelial stem cells (LESCs) are cultivated in Matrigel supplemented with cytokines such as noggin, and R-spondin. Under these conditions, three forms of organoids are formed: round-shaped organoids with concentric cell configurations, rugged- and round-shaped organoids with reticulated cell arrangements, and rugged- and round-shaped organoids with reticulated cell arrangements [11]. The round-shaped organoids with concentric cell configurations, which are typical of filiform papillae, have a multilayer keratinized epithelium and a stratum corneum. Immature organoids extracted after 3 days of development in recipient mice tongues established a concentrically organised stratum corneum, indicating a potential role in regenerative medicine.

Taste bud organoids

Taste buds are independent organs on the tongue, and the loss of taste affects quality of life. Lgr5+ or Lgr6+ taste bud stem cells or circumvallate papilla tissues can be used to create taste bud organoids. Lgr5+ or Lgr6+ cells are isolated from taste buds, and are cultured in DMEM/F12 using R-spondin 1, Noggin, Jagged 1, N-acetylcysteine, EGF, N2, and B27. Sorted cells in media are combined with equal volumes of cold Matrigel before being seeded on organoid culture plates (Ren *et al*. 2014). These taste bud organoids exhibit phenotypic traits comparable to original tissues, including multiple layers of epithelial cells in the outer layer containing stem/progenitor cells and taste cells in the inner layer.

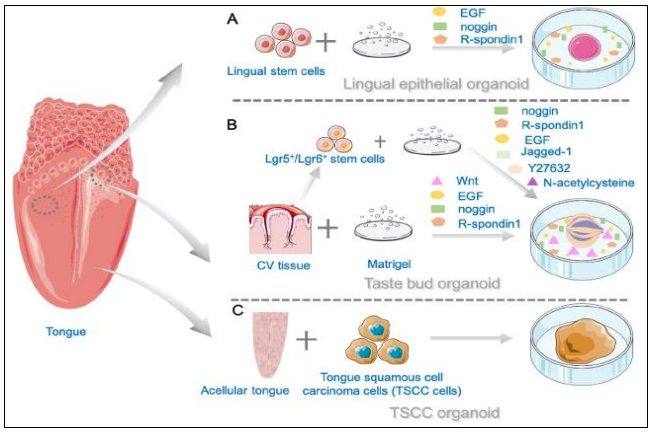

Fig 5: Schematic representation of the construction of tongue-derived organoid [8]

Applications of oral organoids

Oral organoids are excellent models for *in vitro* and *in vivo* research, as well as for the development of personalised medicine for disease therapy. Oral organoids are a prospective source of therapeutic tissues and functional cell types.

Oral Organoids as Models of Development

Organoids mimic natural organogenesis to some extent and provide a more in-depth understanding of the process while also being more experimentally accessible than animal models. Tooth germ organoids, for example, exhibit odontogenic markers and are capable of epithelial invagination into the condensed mesenchyme, simulating reciprocal tissue interactions in human tooth development.

Oral Organoids for Disease Modelling

The use of organoids in tumour research demonstrates especially notable benefits in the targeted therapy of oral malignancies. Organoids, in contrast to monolayer cell lines, are genetically stable throughout long-term cultivation. One of the most common malignant diseases in the maxillofacial region is tongue cancer. By seeding the decellularized tongue extracellular matrix (TEM) with the TSCC cell line CAL27, tongue squamous cell carcinoma (TSCC) organoids have been produced. It will be possible to explore the viability of drug testing and screening applications since TSCC organoids can simulate tumour pathologies (Zhao *et al*. 2017).

Conclusion

Oral organoids serve as a bridge linking 2D cell cultures and *in vivo* animal models. They accurately mimic the 3D structure of the organ being studied while being relatively stable and accessible. Oral organoids may serve as an alternative for oral organ transplantation and help us understand the underlying mechanism of human oral development and disease progression. Currently, methods such as 3D bioprinting and fabricated polymer scaffolds are being used for organoid construction. However, difficulties persist in accurately controlling the self-organization of organoids.

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