



## Emerging organoid-based platforms to study salivary gland hypofunction

Yoojin Seo<sup>1</sup>, Hyung-Sik Kim<sup>1,2,3</sup>

<sup>1</sup>Department of Oral Biochemistry, Dental and Life Science Institute, School of Dentistry, Pusan National University, Yangsan, Korea

<sup>2</sup>Department of Life Science in Dentistry, School of Dentistry, Pusan National University, Yangsan, Korea

<sup>3</sup>Education and Research Team for Life Science in Dentistry, Pusan National University, Yangsan, Korea

**Received:** December 12, 2021

**Revised:** February 8, 2022

**Accepted:** February 15, 2022

**Correspondence to:**

Hyung-Sik Kim, PhD

Department of Oral Biochemistry,  
School of Dentistry, Pusan National  
University, 49, Busandaehak-ro,  
Mulgeum-eup, Yangsan 50612, Korea  
E-mail: hskimcell@pusan.ac.kr

Xerostomia is a pathologic condition of hyposalivation due to salivary gland (SG) dysfunction. Although xerostomia significantly affects the quality of patients' life, there is no satisfactory treatment for this disease. Importantly, the senior population is more susceptible to xerostomia than younger individuals and the prevalence of the disease is much higher in elderly women than in men. However, the mechanisms underlying these clinical correlations have not yet been elucidated and further studies are required. Given that cell lines exhibiting saliva-producing abilities are not available, the generation and maturation of salivary gland organoids (SGOs) have been spotlighted as a modeling system to investigate the homeostasis of SG stem cells, as well as the pathophysiology of SGs in disease. In this review article, we will review the latest reports dealing with the generation and maturation of SGOs by defining the stem cells in SGs. We will also discuss the recent literature proposing strategies to model disease and regenerate damaged tissues.

**Keywords:** Organoids; Salivary glands; Xerostomia; Acinar cells; Salivary duct; Stem cells

### Introduction

Saliva is one of the most indispensable substances for maintaining oropharyngeal health. The various components of saliva, including anti-microbial peptides, mucus, and amylase, can protect the soft and hard oral tissues from invaders, promote tissue recovery, and help digestion [1–4].

Xerostomia, also known as dry mouth syndrome, is a pathologic condition of hyposalivation due to salivary gland (SG) dysfunction. This condition causes great discomfort in speaking, chewing, swallowing, and wearing dentures, thereby significantly lowering the quality of life of affected patients. Several autoimmune diseases (e.g., Sjögren syndrome [SS]) and medical interventions (e.g., radiotherapy for head and neck cancer) often

lead to xerostomia; however, its etiology has not been fully elucidated [5–7]. Up to 10% of the general population experiences dry mouth, while the senior population seems to be more susceptible to xerostomia. Histologically, the number of acinar cells decreases while the amount of fat and fibrous tissue increases in the SG with aging. The prevalence of the disease is much higher in women than in men. Moreover, according to recent reports, dry mouth syndrome is frequently diagnosed in menopausal women, implying a correlation between salivary function and sex hormones, such as estrogen [8,9]. It is well known that the decrease in estrogen levels after menopause correlates with various complications such as facial flushing, headache, skin changes, osteoporosis, and xerostomia. Importantly, more than 25% of elderly women complain of discomfort due to xerostomia after

menopause [10–12]. However, there is no cure available and the current treatment of xerostomia is limited to non-specific allopathic medicine with several side effects. Therefore, safe and effective therapeutic approaches should be developed.

Recent advances in 3-dimensional (3D) culture technology allow pluripotent and multipotent stem cells to exhibit their remarkable self-organizing properties, and the resulting organoids reflect key structural and functional properties of mature organs such as the kidney, lung and gut. Organoid technology can therefore be used to model human organ development “in a dish” with properties that closely resemble the real organ in its structure and function [13,14]. In addition, since organoids preserve tissue heterogeneity compared to single-cell cultures, various pathologic conditions and disease features can be simulated more efficiently than conventional approaches [15]. In addition, mature organoids can be used as transplantable tissue in the field of cell-based regenerative medicine [16,17]. In these aspects, understanding and treating xerostomia will necessitate the optimization of the culture method for salivary gland organoids (SGOs) and utilization of SGOs as a disease-modeling system for xerostomia. Therefore, this review summarized the cutting-edge technologies for the generation and maturation of organoids using SG stem cells, as well as their application for understanding the development of SG and modeling SG hypofunction to treat the disease.

**Ethics statement:** This study was a literature review of previously published studies and was therefore exempt from institutional review board approval.

## Establishment of SSp and SGOs

In the field of developmental biology, an *ex vivo* organotypic slice culture method has been widely applied to study SG organogenesis [18–21]. In this format, SG epithelial tissue, mesenchyme, and extracellular matrix (ECM) reconstitute 3D structure that reflects several aspects of developmental hallmarks including branching morphogenesis and acinar cell maturation as observed *in vivo*. With the combination of various chemical treatments and mesenchyme-free culture conditions, organotypic slice culture is useful to define essential signaling pathways and regulatory niche factors contributing to SG development; however, long-term maintenance and subculture, as well as experimental manipulation, are excessively limited because this technique requires naïve tissue slices. Organotypic slice culture is also generally optimized with embryonic- and neonate-de-

rived samples, while culturing adult tissue is still challenging. For these reasons, SGOs can be an excellent alternative to solve the current limitations of the organotypic 3D culture method [22]. Several attempts to culture salispheres (SSp) and SGOs have been conducted since the mid-2000s. SGOs can be derived from adult stem/progenitor cells (ASPCs), embryonic SG germ tissue, and pluripotent stem cells (PSCs) [23].

### 1. SGOs derived from SG tissue containing ASPCs

Most SGOs established to date are generated from freshly dissociated SG tissue-derived cells. In 2008, the Coppes group provided the first evidence of the regenerative capacity of SS-isolated salivary gland stem cells (SGSCs) for restoring the impaired SG function [24]. In this paper, dissociated murine submandibular gland (SMG) cells were prepared by mechanical dissection and collagenase/hyaluronidase treatment, and then applied to a floating culture system similar to other epithelial tissue-derived cells. Within a few days, this system generated SSp with proliferative progenitors (>9,000 SSp from 2 to  $3 \times 10^6$  cells) but prolonged culture over 10 days resulted in a loss of self-renewal ability due to spontaneous differentiation. Indeed, the expression level of putative stem cell markers Sca-1, c-kit, and Musashi-1 within the SSp gradually increased by days 3 to 5 then declined; in contrast, periodic acid–Schiff-positive mucin-producing cells and SG duct-like cells were detected in long-term cultured SSp, implying that SSp with differentiation capacity can be derived from SGSCs *in vitro*.

After the successful generation of human SSp from the parotid gland (PG) and SMG using the same protocol applied to murine samples [25], researchers have tried to optimize culture conditions for long-term maintenance and functional maturation of SGOs. In general, floating culture and embedding into ECM material such as Matrigel are the most frequently utilized methods to generate SGOs and different culture conditions are required for SGSC expansion and induction of differentiation/maturation (Table 1) [24–32]. Of note, Maimets et al. [29] applied well-defined culture conditions for intestinal epithelial organoids to establish SGOs and revealed that the canonical Wnt/ $\beta$ -catenin pathway is required for the maintenance of SGSC self-renewal *in vitro*. The addition of R-spondin1 and Wnt3a to the traditional medium significantly improved sphere-forming efficiency with increased population doubling level, while disruption of Wnt signaling using chemical antagonists impaired SGO formation and SGSC expansion. Furthermore, the transplantation of Wnt-induced SGOs into irradiated SG led to functional recovery accompanied with morphological restoration partially by the incorporation of SGOs into recipient tissue, im-

plying that Wnt activation is necessary for the long-term SGSC maintenance *in vitro*.

Meanwhile, not only freshly dissociated SG cells but also long-term cultured cells can be applied to SGO formation. In a recent study conducted by Sui et al. [30], the authors isolated epithelial stem/progenitor cells from human SMGs (hSMGepiS/PCs) and applied them in an ECM-free monolayer culture condition before the induction of SGO formation with Fgf10 treatment. Of note, SGOs derived from long-term cultured hSMGepiS/PCs could generate SG-like structures both *in vitro* and *in vivo*, implying that the stemness of SGSCs also can be maintained with a traditional 2-dimensional culture system for several passages. Since commercial media specialized for keratinocyte expansion was used in this study, it would be interesting to investigate the optimized combination of growth factors and supplements for the culture of hSMGepiS/PCs that contributes to SGSC maintenance during the monolayer culture.

Considering that dissociated cells from the SG consist of differentiated mature cells as well as SGSCs and progenitors, many attempts have been reported to distinguish the SGSC population for SGO establishment. Based on the previous findings in other glandular- and epithelial tissue biology, several candidates such as c-kit, CD49f, CD24, CD29, CD133, and CD326 (EpCAM) have been suggested as SGSC markers (Table 1) [24–32]. Nanduri et al. [27] classified the potential

SGSC population among primary SS-dissociated cells into 4 groups according to their pattern of CD24/CD29 expression. Interestingly, each population displayed a distinctive difference in sphere-forming efficiency, and CD24<sup>hi</sup>/CD29<sup>hi</sup> cells exhibited the highest secondary-sphere formation capacity. They also could generate both lobular and ductal-type organoids upon induction of differentiation, while CD24<sup>hi</sup>/CD29<sup>lo</sup> and CD24<sup>lo</sup>/CD29<sup>hi</sup> groups predominantly differentiated into lobular organoids. In a similar study by Xiao and colleagues, the lineage marker (CD31/CD45)-negative, CD24<sup>high</sup>-c-Kit<sup>+</sup>Sca1<sup>+</sup>-enriched population was suggested as putative stem cells in SMG since their stemness and differentiation capacity are much higher than other groups [33]. In addition, the pan-epithelial marker EpCAM can be utilized as an SGSC marker [29]. Although EpCAM is stably expressed throughout the SG epithelium, researchers have focused on regional differences in patterns of EpCAM expression. The protein level of EpCAM is relatively high and low in the SGSC-residing ductal compartment and fully matured acinus, respectively. Interestingly, most EpCAM<sup>+</sup> ductal cells co-express  $\beta$ -catenin, a signal transducer in the Wnt signaling pathway, suggesting that EpCAM<sup>+</sup> cells might represent Wnt-responsive SGSCs. Indeed, sorted EpCAM<sup>high</sup> cells are capable of self-renewal and generating differentiated SGOs with the lumen and secretory component.

**Table 1.** Representative studies of SGO establishment

Cell origin	Main culture method	Suggested SGSC markers	Key findings	Reference
M: SMG cells	Ex: floating Df: embedding in collagen type I	c-kit	The first proof of the potential use of SGSC transplantation to rescue SG injury	[24]
H, M: PG and SMG cells	Ex: floating & embedding in Matrigel Df: embedding in collagen type I	c-kit	Human SGs contain a similar putative stem cell population as rodents	[25]
M: SMG cells	Ex: floating	c-kit CD24 CD29 CD49f CD133	Different stem cell-associated markers are expressed in mouse SGSCs	[26]
M: SMG cells	Ex: embedding in Matrigel	c-kit CD24 CD49f	Organoid-derived SGSCs contribute to tissue homeostasis	[31]
M: SMG cells	Ex: embedding in Matrigel Df: embedding in Matrigel: collagen type I	CD24 CD29	Long-term functional activity of SGO-derived single CD24 <sup>hi</sup> CD29 <sup>hi</sup> SGSC	[27]
H: SMG cells	Ex: embedding in Matrigel Df: embedding in Matrigel: collagen type I	c-kit	The first data exploring the potential of human SGSCs on <i>in vivo</i> engraftment and functionality	[28]
M: Epcam <sup>high</sup> SMG cells	Ex & Df: embedding in Matrigel	Epcam <sup>high</sup>	Wnt signaling is essential for SGSC maintenance <i>in vitro</i>	[29]
M: PG, SMG and SLG cells	Ex: floating	c-kit CK5 CK14 Sca-1 CD24 CD29 CD49f	SGOs can be derived from different anatomic origins	[32]
H: epiS/PCs isolated from SMG	Ex & Df: embedding in Matrigel	K5 CD49f Ascl3	SGOs can be generated from 2D-cultured SG epithelial cells	[30]

SGO, salivary gland organoid; SGSC, salivary gland stem cell; M, mouse; SMG, submandibular gland; Ex, expansion; Df, differentiation; SG, salivary gland; H, human; PG, parotid gland; SLG, sublingual gland; CK, cytokeratin.

## 2. SGOs generated from PSCs

In contrast to ASCs, which possess lineage-restricted potential, PSCs including embryonic stem cells (ESCs) and induced PSCs can be differentiated into multiple tissue-specific cells upon appropriate manipulations of gene expression and signaling pathways that mimic the developmental procedure [34]. Recently, Tanaka et al. [4] successfully generated orthotopically functional SG from ESCs by regulating morphogen signaling and transcriptional networks. To elucidate the key pathways contributing to SG organogenesis, the authors conducted RNA sequencing analysis with developing embryonic SMG tissues and found that 2 transcription factors, Sox9 and Foxc1, were crucial for the initiation of differentiation in ESCs into SG rudiments. ESCs were applied to the consecutive induction of oral epithelium, and then developed as SG-like tissue called induced SGs (iSGs) in the presence of Fgf7/Fgf10 co-treatment. *In vitro* characterization revealed that iSGs express several functional markers of SGs and exhibit similar gene expression patterns with developing SMGs rather than post-natal SMGs. Of note, a reconstituted structure consisting of iSGs and supportive mesenchyme could be integrated and differentiated into mature SGs after *in vivo* transplantation, providing important preclinical proof-of-concept evidence regarding organ replacement therapy using organoids.

## Advanced techniques for improving the present SGO culture methods

In general, the self-renewal of SGSCs should be maintained during long-term passaging and the organoid forming efficiency, doubling time and the proliferative capacity of SGOs are investigated to evaluate the SGSC population. In addition, cellular complexity and structural similarity to the naïve organ are important to determine the organoid quality, especially for differentiated organoids. Therefore, matured SGOs should contain both polarity-organized ductal and functional acinar cells, as observed in the epithelial region of SGs. Various functional tests, including a calcium ( $\text{Ca}^{2+}$ ) flux assay, amylase secretion assay, and lumen swelling assay, followed by cholinergic stimulation, have been applied to assess the functionality of mature SGOs.

Several attempts have been conducted to fulfill these criteria (Table 2) [29,35–43]. Specifically, the regulation of signaling pathways associated with SG organogenesis is one of the common strategies to improve basic culture conditions. For instance, sustained activation of the Wnt- $\beta$ -catenin axis significantly consolidates the self-renewal and organoid-forming efficiency of SGSCs [29]. Treatment of the Rho kinase inhibitor, Y27632, can increase the population of c-kit<sup>+</sup> and CK5<sup>+</sup> SGSC-like cells in SGOs under expansion conditions, while it accelerates the differentiation of pro-acinar Mist<sup>+</sup> cells into aquaporin 5-posi-

**Table 2.** Various strategies for SGO improvement

Cell origin	Modification	Advanced features	Reference
M: Epcam <sup>high</sup> SMG cells	Wnt signaling activation	Extensive <i>in vitro</i> expansion	[29]
H: PG, SMG and SLG cells	Alk inhibition by LDN193189 and SB431542 treatment	Budding and/or branching morphology was significantly increased	[36]
M: SMG cells	Rho kinase inhibition by Y27632 treatment	Increased CK5 basal progenitor marker Increased acinar cell differentiation	[35]
M: SMG cells	RA treatment	Increased lumen formation in branching duct-like structures	[38]
H: PG epithelial cells	Cultured in bioactive basement membrane peptide-modified HA 3D hydrogel systems	Higher mRNA transcript levels encoding the progenitor markers Increased acinar cell differentiation	[40]
M: SMG cells	Cultured with chitosan-fabricated biomaterials	Stimulated cellular organization to maintain primitive spheroid structure	[41]
H: SG epithelial cells	Cultured with hydrogel-micropatterned nanofibrous microwells	Stimulated acinar-like spheroid organization	[42]
H: single clonal SGSCs	Cultured with hydrogel-micropatterned nanofibrous microwells	Increased stemness of SGSCs	[43]
Rat: SG stem/progenitor cells	Cultured with decellularized ECM hydrogels	Increased SG functional differentiation with $\alpha$ -amylase enzyme activity	[39]
M: SMG cells	Embedded in neururin-containing laminin-111 ECM	Epithelial innervation and branching morphogenesis <i>in vivo</i>	[37]

SGO, salivary gland organoid; M, mouse; SMG, submandibular gland; PG, parotid gland; SLG, sublingual gland; Alk, anaplastic lymphoma kinase; RA, retinoic acid; H, human; HA, hyaluronic acid; SG, salivary gland; SGSC, salivary gland stem cell; ECM, extracellular matrix.

tive (AQP5<sup>+</sup>) acinar cells during the Fgf2-mediated maturation process [35]. In addition, chemical inhibition of activin receptor-like kinase signaling or treatment of morphogenic proteins such as the Fgf family can induce the development of acini-containing budding structure in SGOs [30,36,37]. In addition to the branching morphogenesis, Kim et al. [38] focused on recapitulating the lumen formation process during the maturation of SGOs. According to that study, the Fgf2/Fgf10 combination initiates the budding and branching elongation process, and the addition of retinoic acid (RA) enhances lumen formation within this duct-like compartment. The authors further revealed that activation of RA signaling upregulates the vasoactive intestinal protein pathway, one of the essential mediators in ductal growth and contiguous lumen formation during the development, and directly stimulates CK7<sup>+</sup> luminal cell differentiation.

Early studies have documented the importance of the ECM in regulating the morphogenesis of developing SGs [44]. Thus, the ECM and ECM-mimicking substances in SGO culture not only function as a physical scaffold, but also provide biological interactions facilitating the generation and maturation of SGOs. Matrigel is the most commonly used hydrogel at present, but potential benefits of other natural- and synthetic biomaterials have been explored for organoid culture [38,44]. The natural ECM obtained from naïve SG tissue can be utilized as hydrogel or scaffold for SG-derived cell culture after decellularization [39,45]. Srinivasan et al. [40] fabricated hyaluronic acid (HA) hydrogels with the basement membrane peptides from laminin and perlecan to provide *in vivo*-like enriched cues and applied them in SGO culture. They found that SGOs embedded in peptide-modified HA hydrogel showed enhanced progenitor potential with increased differentiation capacity towards acinar cell lineage compared to those cultured with naïve HA. In another study, researchers generated various concentrations of chitosan in the form of soluble or coating material and investigated whether chitosan-based biomaterials would be applicable to SGO culture [41]. It was noted that both types of chitosan affected the morphology of SGOs significantly by suppressing spontaneous cavitation in a dose-dependent manner. Chitosan seems to affect the cellular adhesion and polarity establishment during the SGO organization process, contributing to the maintenance of SGOs in the primitive state.

Meanwhile, bioengineering techniques have been applied to aid the 3D organization of dissociated SG cells. Lim and colleagues have studied the benefits of hydrogel-micropatterned nanofibrous microwells in the self-assembly of human PG-derived epithelial cells (hPECs). They developed polyethylene glycol hydrogel-micropatterned polycaprolactone nanofibrous

microwells to culture hPECs and found that microwell-cultured SG aggregates exhibit a higher level of acini phenotypic markers such as AQP5 and  $\alpha$ -amylase with enhanced tight junction and structural protein expression [42]. This system is applicable to culture clonally expanded SGSCs, in which it contributes to the 3D organization of SGSCs followed by the generation of functional SGOs [43]. Considering the unstable quality due to batch-to-batch variation and xenogenic features of Matrigel, well-defined synthetic biomaterials would be an excellent alternative to Matrigel for the therapeutic application of SGOs in regenerative medicine.

## Application of SGOs for understanding development, modeling disease, and regenerating tissues

Increasingly many attempts have recently been conducted to apply organoid technology as an alternative for the conventional modeling systems represented by cell line culture and animal models in the field of biomedical research. Since the derivation of SGOs from SGSCs recapitulates several key aspects of the developmental process, including self-organization, budding formation, and duct elongation, SGOs have emerged as a model system to understand SG development and homeostasis. Several studies have been conducted to elucidate the putative SGSC population by evaluating the organoid forming capacity of SGSC candidates expressing single- or multiple SC markers as described above. Researchers also take advantage of SGOs to verify hypotheses extrapolated from organotypic cell culture and animal studies. For instance, the importance of the fibroblast growth factor family members in acini cell development and the role of the RA signaling pathway on lumen formation have been confirmed in the process of SGO differentiation. Orhon et al. [46] indicated the role of autophagy in regulating the self-renewal and regenerative response of SGSCs upon injury signals using SGOs. They treated SGOs with the autophagy blockers bafilomycin A1 and chloroquine and found that the organoid-forming capacity of SGSCs dropped significantly due to the impaired autophagy. This phenomenon was reproduced in an Atg5-deficient SGO line established from Atg5 knockout model mice. Of note, when the endogenous autophagy flux was activated in SGSCs by Tat-beclin 1 peptide treatment, the self-renewal efficiency of SGOs improved without affecting differentiation capacity. Based on these findings, the authors revealed that basal autophagy levels in SGSCs might be regulated depending on their status (quiescence or active) and suggested the autophagy pathway as a key modulator in homeostasis and

regeneration of slowly self-renewing organs.

SGOs possess excellent potential for modeling SG-associated diseases and elucidating their pathological mechanisms. Since xerostomia is the most common condition associated with the SG hypofunction, researchers have tried to recapitulate this condition in SGOs from different perspectives. A recent study demonstrated the modeling of radiotherapy-associated xerostomia by evaluating the radiation response of SGOs [47]. Maimets et al. [48] compared the characteristics of young and old mouse-derived SGOs mainly focusing on the self-renewal of SGSCs. Although aged SMGs contain a higher number of CD24<sup>hi</sup>/CD29<sup>hi</sup> cells than young SMGs, SGSCs from old mice showed a lower organoid-forming capacity than that of young cells, indicating that stemness and self-renewal ability of SGSCs might be declined with aging. Meanwhile, hyposalivation is one of the major symptoms of the autoimmune disorder SS, although the causal relationship remains unclear. To explore the characteristics of SGSCs in primary SS, SGOs were established from PG biopsy samples of SS-affected patients and compared with healthy SGOs [49]. It was noted that both SGO generation efficiency and the yield of EpCAM<sup>high</sup> SGSCs were significantly lower in SS samples compared to the control. RNA sequencing analysis of SGOs revealed that gene sets associated with cell cycle progression, cell division, and senescence phenotype were significantly upregulated in SS-SGOs; indeed, the average telomere length in control SGSCs was greater than that of SS-derived SGSCs, implying that SS-SGSCs might be subjected to replicative senescence-induced injuries. The authors further found that a combination of pro-inflammatory cytokines (interferon- $\alpha$ , tumor necrosis factor, and interleukin-6) induced an accelerated proliferation in normal SGOs, leading to premature aging. Since the organoid system is more physiologically relevant to naïve tissue than to established cell lines, SGOs can be applied to study cancer biology and Takeda et al. [50] reported the first approach utilizing SGOs as a salivary adenoid cystic carcinoma (ACC) model. In this study, several ACC organoid lines were generated then orthotopically transplanted into NGS mice to obtain a patient-derived tumor xenograft. Interestingly, cancer SGOs maintain the genetic and histological phenotypes of their original tumor tissues and their reactivity to chemotherapeutic agents can be evaluated by *in vitro* drug sensitivity assays. Therefore, these studies suggest the value of SGOs not only for investigating the mechanism of incurable SG-associated disorders, but also for establishing a novel therapeutic strategy.

Finally, organoids are regarded as promising tools in the field of cell therapeutics and regenerative medicine [22,51].

Since tissue-specific stem cells are stably maintained and expanded within organoids, continuously cultured SGOs can provide SGSCs with high potential, and transplantation of these SGO-derived stem cells can improve radiation- or ligation-mediated SG damage *in vivo* [24,25,28,31]. Furthermore, not only isolated SGSCs but also SGOs themselves are capable of tissue restoration. Several attempts have been conducted to test the therapeutic impact of reconstituted SGO-mesenchyme-biomaterial complex [4,32,52]. After the orthotopic engraftment of bioengineered SG structures *in vivo*, they were integrated into the recipient tissue properly with a differentiated structure. Notably, transplanted artificial SGs could produce saliva, leading to the amelioration of dry mouth symptoms of the SG-deficient mice model, demonstrating the possibility of organ replacement therapy through the combination of biotechnology and organoid technology.

## Conclusion and limitations of current technology

Despite the remarkable achievements in the establishment of SGOs, current technology has several limitations. Compared to other well-defined epithelial organoid systems, culture conditions for SGOs have not been optimized yet [14]. Of note, although the present expansion media can support SGSC enrichment *in vitro*, the organoid differentiation protocol for the induction of both duct and acini compartments in SGOs still needs to be improved. Naïve SG tissue consists of an organized ductal system and produces multiple secretory proteins through the cooperative action of exocrine acini cells and contractile myoepithelial cells [53]. Furthermore, humans have 3 major SGs—the PG, SMG, and sublingual gland—with multiple minor SGs, and each gland has distinctive histological characteristics and secretory profiles. Similar to other exocrine organs, the production of saliva is tightly regulated by the autonomous nervous system via interactive crosstalk between receptor signaling [54–56]. In these aspects, Lee et al. [32] successfully generated region-specific SGOs with unique cellular composition, although their secretory functions were not determined. Therefore, it would be important to check the expression pattern of receptors (cholinergic and purinergic receptors) and to test the reactivity of differentiated SGOs to various stimuli for functional evaluation. In addition, current organoids mainly consist of epithelium-derived cells and ECM; thus, the establishment of a co-culture system with other components including mesenchyme, vascular structure, immune cells, and microbiota would provide an advanced SG platform to study the mechanisms un-

derlying SG organogenesis, regeneration, and disease development. Taken together, improved SGOs that overcome current limitations might critically contribute to the development of stem cell-based therapeutics for disease modeling and regeneration.

## Notes

### Conflict of interest

No potential conflict of interest relevant to this article was reported.

### Funding

This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. NRF-2019R1A2C2085876 and NRF-2021R1I1A1A01055654).

### ORCID

Yoojin Seo, <https://orcid.org/0000-0002-7133-0838>

Hyung-Sik Kim, <https://orcid.org/0000-0002-7588-7393>

### Data availability

Please contact the corresponding author for data availability.

## References

- Mandel ID. The functions of saliva. *J Dent Res* 1987;66 Spec No:623–7.
- Pringle S, Van Os R, Coppes RP. Concise review: Adult salivary gland stem cells and a potential therapy for xerostomia. *Stem Cells* 2013;31:613–9.
- Tabak LA, Levine MJ, Mandel ID, Ellison SA. Role of salivary mucins in the protection of the oral cavity. *J Oral Pathol* 1982;11:1–17.
- Tanaka J, Ogawa M, Hojo H, Kawashima Y, Mabuchi Y, Hata K, et al. Generation of orthotopically functional salivary gland from embryonic stem cells. *Nat Commun* 2018;9:4216.
- Napeñas JJ, Brennan MT, Fox PC. Diagnosis and treatment of xerostomia (dry mouth). *Odontology* 2009;97:76–83.
- Nederfors T. Xerostomia and hyposalivation. *Adv Dent Res* 2000;14:48–56.
- Ritter AV. Xerostomia (dry mouth). *J Esthet Restor Dent* 2006;18:306.
- Foglio-Bonda PL, Rocchetti V, Nardella A, Fantinato M, Sandhu K, Foglio-Bonda A. Salivary pH and flow rate in menopausal women. *Eur Rev Med Pharmacol Sci* 2019;23:918–22.
- Dalal PK, Agarwal M. Postmenopausal syndrome. *Indian J Psychiatry* 2015;57(Suppl 2):S222–32.
- Rukmini JN, Sachan R, Sibi N, Meghana A, Malar CI. Effect of Menopause on saliva and dental health. *J Int Soc Prev Community Dent* 2018;8:529–33.
- Orellana MF, Lagravère MO, Boychuk DG, Major PW, Flores-Mir C. Prevalence of xerostomia in population-based samples: a systematic review. *J Public Health Dent* 2006;66:152–8.
- Van Palenstein Helderman WH, Beijin W. A case report of high caries activity due to the drug therapy in Parkinson's disease: the problem of dry mouth: xerostomia. *Ned Tijdschr Tandheelkd* 1983;90:545–50.
- Yin X, Mead BE, Safaee H, Langer R, Karp JM, Levy O. Engineering stem cell organoids. *Cell Stem Cell* 2016;18:25–38.
- Kim J, Koo BK, Knoblich JA. Human organoids: model systems for human biology and medicine. *Nat Rev Mol Cell Biol* 2020;21:571–84.
- Fujii M, Sato T. Somatic cell-derived organoids as prototypes of human epithelial tissues and diseases. *Nat Mater* 2021;20:156–69.
- Huch M, Koo BK. Modeling mouse and human development using organoid cultures. *Development* 2015;142:3113–25.
- Grassi L, Alfonsi R, Francescangeli F, Signore M, De Angelis ML, Addario A, et al. Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. *Cell Death Dis* 2019;10:201.
- Patel N, Sharpe PT, Miletich I. Coordination of epithelial branching and salivary gland lumen formation by Wnt and FGF signals. *Dev Biol* 2011;358:156–67.
- Hoffman MP, Kidder BL, Steinberg ZL, Lakhani S, Ho S, Kleinman HK, et al. Gene expression profiles of mouse submandibular gland development: FGFR1 regulates branching morphogenesis in vitro through BMP- and FGF-dependent mechanisms. *Development* 2002;129:5767–78.
- Knox SM, Lombaert IM, Reed X, Vitale-Cross L, Gutkind JS, Hoffman MP. Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis. *Science* 2010;329:1645–7.
- Nedvetsky PI, Emmerson E, Finley JK, Ettinger A, Cruz-Pacheco N, Prochazka J, et al. Parasympathetic innervation regulates tubulogenesis in the developing salivary gland. *Dev Cell* 2014;30:449–62.
- Lombaert I, Movahednia MM, Adine C, Ferreira JN. Concise Review: Salivary gland regeneration: therapeutic

- approaches from stem cells to tissue organoids. *Stem Cells* 2017;35:97–105.
23. Gao X, Wu Y, Liao L, Tian W. Oral organoids: progress and challenges. *J Dent Res* 2021;100:454–63.
  24. Lombaert IM, Brunsting JF, Wierenga PK, Faber H, Stokman MA, Kok T, et al. Rescue of salivary gland function after stem cell transplantation in irradiated glands. *PLoS One* 2008;3:e2063.
  25. Feng J, van der Zwaag M, Stokman MA, van Os R, Coppes RP. Isolation and characterization of human salivary gland cells for stem cell transplantation to reduce radiation-induced hyposalivation. *Radiother Oncol* 2009;92:466–71.
  26. Nanduri LS, Maimets M, Pringle SA, van der Zwaag M, van Os RP, Coppes RP. Regeneration of irradiated salivary glands with stem cell marker expressing cells. *Radiother Oncol* 2011;99:367–72.
  27. Nanduri LS, Baanstra M, Faber H, Rocchi C, Zwart E, de Haan G, et al. Purification and ex vivo expansion of fully functional salivary gland stem cells. *Stem Cell Reports* 2014;3:957–64.
  28. Pringle S, Maimets M, van der Zwaag M, Stokman MA, van Gosliga D, Zwart E, et al. Human salivary gland stem cells functionally restore radiation damaged salivary glands. *Stem Cells* 2016;34:640–52.
  29. Maimets M, Rocchi C, Bron R, Pringle S, Kuipers J, Giepmans BN, et al. Long-term in vitro expansion of salivary gland stem cells driven by wnt signals. *Stem Cell Reports* 2016;6:150–62.
  30. Sui Y, Zhang S, Li Y, Zhang X, Hu W, Feng Y, et al. Generation of functional salivary gland tissue from human submandibular gland stem/progenitor cells. *Stem Cell Res Ther* 2020;11:127.
  31. Nanduri LS, Lombaert IM, van der Zwaag M, Faber H, Brunsting JF, van Os RP, et al. Salisphere derived c-Kit+ cell transplantation restores tissue homeostasis in irradiated salivary gland. *Radiother Oncol* 2013;108:458–63.
  32. Lee HW, Hsiao YC, Chen YC, Young TH, Yang TL. Salispheres from different major salivary glands for glandular regeneration. *J Dent Res* 2019;98:786–94.
  33. Xiao N, Lin Y, Cao H, Sirjani D, Giaccia AJ, Koong AC, et al. Neurotrophic factor GDNF promotes survival of salivary stem cells. *J Clin Invest* 2014;124:3364–77.
  34. McCauley HA, Wells JM. Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. *Development* 2017;144:958–62.
  35. Koslow M, O'Keefe KJ, Hosseini ZF, Nelson DA, Larsen M. ROCK inhibitor increases proacinar cells in adult salivary gland organoids. *Stem Cell Res* 2019;41:101608.
  36. Yoshimoto S, Yoshizumi J, Anzai H, Morishita K, Okamura K, Hiraki A, et al. Inhibition of Alk signaling promotes the induction of human salivary-gland-derived organoids. *Dis Model Mech* 2020;13:dmm045054.
  37. Vining KH, Lombaert IM, Patel VN, Kibbey SE, Pradhan-Bhatt S, Witt RL, et al. Neurturin-containing laminin matrices support innervated branching epithelium from adult epithelial salispheres. *Biomaterials* 2019;216:119245.
  38. Kim D, Yoon YJ, Choi D, Kim J, Lim JY. 3D organoid culture from adult salivary gland tissues as an ex vivo modeling of salivary gland morphogenesis. *Front Cell Dev Biol* 2021;9:698292.
  39. Shin K, Koo KH, Jeong J, Park SJ, Choi DJ, Ko YG, et al. Three-dimensional culture of salivary gland stem cell in orthotropic decellularized extracellular matrix hydrogels. *Tissue Eng Part A* 2019;25:1396–403.
  40. Srinivasan PP, Patel VN, Liu S, Harrington DA, Hoffman MP, Jia X, et al. Primary salivary human stem/progenitor cells undergo microenvironment-driven acinar-like differentiation in hyaluronate hydrogel culture. *Stem Cells Transl Med* 2017;6:110–20.
  41. Lee HW, Hsiao YC, Young TH, Yang TL. Maintenance of the spheroid organization and properties of glandular progenitor cells by fabricated chitosan based biomaterials. *Biomater Sci* 2018;6:1445–56.
  42. Shin HS, Kook YM, Hong HJ, Kim YM, Koh WG, Lim JY. Functional spheroid organization of human salivary gland cells cultured on hydrogel-micropatterned nanofibrous microwells. *Acta Biomater* 2016;45:121–32.
  43. Shin HS, Hong HJ, Koh WG, Lim JY. Organotypic 3D culture in nanoscaffold microwells supports salivary gland stem-cell-based organization. *ACS Biomater Sci Eng* 2018;4:4311–20.
  44. Sequeira SJ, Larsen M, DeVine T. Extracellular matrix and growth factors in salivary gland development. *Front Oral Biol* 2010;14:48–77.
  45. Lilliu MA, Seo YJ, Isola M, Charbonneau AM, Zeitouni A, El-Hakim M, et al. Natural extracellular matrix scaffolds recycled from human salivary digests: a morphometric study. *Oral Dis* 2016;22:313–23.
  46. Orhon I, Rocchi C, Villarejo-Zori B, Serrano Martinez P, Baanstra M, Brouwer U, et al. Autophagy induction during stem cell activation plays a key role in salivary gland self-renewal. *Autophagy* 2021 May 19 [Epub]. <https://doi.org/10.1080/15548627.2021.1924036>.
  47. Serrano Martinez P, Cinat D, van Luijk P, Baanstra M, de Haan G, Pringle S, et al. Mouse parotid salivary gland organ-

- oids for the in vitro study of stem cell radiation response. *Oral Dis* 2021;27:52–63.
48. Maimets M, Bron R, de Haan G, van Os R, Coppes RP. Similar ex vivo expansion and post-irradiation regenerative potential of juvenile and aged salivary gland stem cells. *Radiother Oncol* 2015;116:443–8.
49. Pringle S, Wang X, Verstappen GM, Terpstra JH, Zhang CK, He A, et al. Salivary gland stem cells age prematurely in primary Sjögren's syndrome. *Arthritis Rheumatol* 2019;71:133–42.
50. Takada K, Aizawa Y, Sano D, Okuda R, Sekine K, Ueno Y, et al. Establishment of PDX-derived salivary adenoid cystic carcinoma cell lines using organoid culture method. *Int J Cancer* 2021;148:193–202.
51. Almansoori AA, Kim B, Lee JH, Tran SD. Tissue engineering of oral mucosa and salivary gland: disease modeling and clinical applications. *Micromachines (Basel)* 2020;11:1066.
52. Ogawa M, Oshima M, Imamura A, Sekine Y, Ishida K, Yamashita K, et al. Functional salivary gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 2013;4:2498.
53. Proctor GB. The physiology of salivary secretion. *Periodontol* 2000 2016;70:11–25.
54. Nakamura T, Matsui M, Uchida K, Futatsugi A, Kusakawa S, Matsumoto N, et al. M(3) muscarinic acetylcholine receptor plays a critical role in parasympathetic control of salivation in mice. *J Physiol* 2004;558(Pt 2):561–75.
55. Nakamoto T, Brown DA, Catalán MA, Gonzalez-Begne M, Romanenko VG, Melvin JE. Purinergic P2X7 receptors mediate ATP-induced saliva secretion by the mouse submandibular gland. *J Biol Chem* 2009;284:4815–22.
56. Gautam D, Heard TS, Cui Y, Miller G, Bloodworth L, Wess J. Cholinergic stimulation of salivary secretion studied with M1 and M3 muscarinic receptor single- and double-knock-out mice. *Mol Pharmacol* 2004;66:260–7.