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Review

# Development and regeneration of salivary gland toward for clinical application

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ABSTRACT

Salivary gland hypofunction, also termed as xerostomia, is caused by radiation therapy for head and neck cancer or Sjögren's syndrome, leading to a detrimental impact on oral health and quality of life. This review describes current studies on salivary gland development and the translational approaches of basic science to treating patients with dysfunction and hypofunction of the salivary gland. Here, we review the most recent studies that have offered better insight into the mechanisms of salivary gland development and regeneration. Furthermore, we highlight proposed approaches with the aim of recovering salivary gland function using both gene- and cell-based therapy. A thorough understanding of the mechanisms involved in salivary gland development is necessary in order to design effective therapies for regeneration and repair of damaged salivary glands.



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## 1. Introduction

Many papers have reported the potential of regenerative medicine. However, many scientific challenges need to be overcome. In particular, many clinicians in the field of dentistry hope to realize the regeneration of the tooth and bone. Since cultured epidermal skin was developed using tissue engineering [1], cultured dermis has also been developed and recently made available to clinics worldwide. The epithelial cells of the oral membrane are similar to skin keratinocytes. Bioengineered oral mucosae have also been developed as well as bioengineered skin. Various reports regarding the development and clinical implementation of bioengineered oral mucosa sheets have been published since 1990. Bioengineered oral mucosa sheets are expected to be a useful tool for clinical

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application, among others. Recently, tissue engineering therapy has been used to regenerate tissues such as alveolar bone, local cartilage, skin epidermis, corneal epithelium, and pancreatic islets. As fetal bovine serum and mouse-originated feeder cells are required for these culture methods, risk factors with respect to mad cow disease and/or unknown pathogens are present. To avoid these problems, a new strategy that does not involve fetal bovine serum and mouse-originated feeder cells needs to be developed.

Induced pluripotent stem (iPS) cells can be generated from fully differentiated non-pluripotent cells with similar pluripotency to that of embryonic stem (ES) cells. iPS cells can be a powerful tool in regenerative medicine. Many researchers have attempted to regenerate organs, such as salivary glands, kidneys, and lungs. However, their potential tumorigenicity poses a significant challenge to clinical use [2,3]. Conversely, ES cells and other stem cells have a self-renewing microenvironment and multi-lineage developmental potential. Recent studies have shown the potential of microenvironments to exert external control, which is involved in defining the stem cell niche. The stem cell niche may represent a significant entry point for the therapeutic modulation of stem cell behavior [4]. Although the microenvironment is known to be derived from iPS cells, the niche of iPS cells has not been studied in depth [3]. In this review, the mechanisms of development and regeneration approaches using the salivary gland are described. These analyses may provide new concepts for the functional regeneration of salivary glands using tissue engineering.

## 2. Structure and function of salivary gland

Humans and mice have three major salivary glands: submandibular glands, the parotid glands, and the sublingual glands, which are responsible for secreting approximately 90% of the saliva in the mouth [5]. In addition, hundreds of minor salivary glands are present under the mucosa in the oral cavity, which secrete the remaining 10% of the saliva together. Salivary glands produce saliva and play an important role in the homeostasis of the oral cavity such as food digestion, taste, moisturizing, and immunoreaction. Acinar cells of the salivary glands secrete the serous fluid and mucus of saliva, which contain water, salt, and protein, among others. However, duct cells absorb salt and adjust to salivary secretion accordingly. Myoepithelial cells surround the acini-interposed section; these cells are controlled by the nerve that regulates salivation by contraction. However, this phenomenon has not yet been clearly demonstrated. Salivary glands contain three types of ducts based on their morphology and histological shape: intercalated, striated, and granular. Saliva flows from the acinar units through the ductal system into the oral cavity.

## 3. Early development of salivary gland

The signals that induce the migrating neural crest cells to form a mesenchymal condensation at the appropriate location beside the oral epithelium are still unclear. The mesenchyme provides signals for the thickening of the oral epithelium and the formation of placodes at embryonic day 11 (E11). Knockout mice for *Fgf10*, *Fgfr2b*, *Pitx1*, and *p63* lack salivary glands, indicating that these genes are essential for the early development and patterning of salivary glands. In organs such as the liver and pancreas, the endothelial cells provide critical signals for organogenesis [6]. However, the role of endothelial cells in early salivary gland development has not been investigated yet. The salivary placode invaginates into the mesenchyme, which begins to condense. The epithelial bud grows into the mesenchyme forming a primary bud on a stalk. The neural crest-derived neuronal precursors coalesce to form the parasympathetic submandibular ganglion (PSG), which is wrapped around the epithelial stalk that subsequently forms the major secretory duct. The signals that initiate this interaction have not been defined [7].

## 4. Branching morphogenesis

Salivary glands are formed by the developmental process of branching morphogenesis, which involves cell proliferation, cleft formation, differentiation, cell migration, apoptosis, and reciprocal interaction between the epithelial, mesenchymal, neuronal, and endothelial cells [8]. As the end bud enlarges at E13, clefts in the epithelium delineate the first three to five buds, which correspond to major lobules of the gland; in parallel, axons from the PSG extend along the epithelium to envelop the end buds. The gland becomes highly branched by E14 and begins differentiating functionally at E15. Branching morphogenesis and differentiation continue until birth [9,10]. We review specific mechanisms involved in branching morphogenesis in the following sections.

### 4.1. Cleft formation

Cleft formation is an essential process that occurs due to two separate phenomena: cleft initiation and progression. Basement membrane (BM) dynamics are the possible driving forces for cleft formation. Fibronectin is essential for cleft initiation [8], and its accumulation rapidly induces the expression of *Btbd7* (BTB (POZ) domain containing 7), which induces the expression of *Snail2* and suppresses the level of E-cadherin, a cell–cell adhesion molecule [11]. This results in a loss of the columnar cell organization in the outer layer of the epithelial cells at the base of the forming cleft, as well as the formation of intercellular gaps for cleft progression. Other extracellular matrix (ECM) proteins in the BM accumulate at the cleft sites including the laminin chains α1 and α5 [12], perlecan, and heparanase, an endoglycosidase enzyme that cleaves heparan sulfate chains [13]. Submandibular glands (SMGs) in laminin α5 (Lamα5)-deficient mice showed delayed branching morphogenesis with delayed cleft formation. The loss of GSK3β by either pharmacological inhibition or transcriptional repression promotes cleft formation [14]. Electron microscopy analysis clearly indicated the cytoskeletal dynamics of cleft formation. Ultrastructural images showed the presence of a cytoplasmic shelf with a core of microfilaments in tissues at the base of the cleft [15]. The shelf may be a matrix attachment point to induce cleft elongation via cytoskeleton attachment, and inhibition of the actin cytoskeleton polymerization in turn inhibits cleft formation. Another study showed that cleft initiation and progression are physically and biochemically distinct. Rho-associated coiled-coil containing kinase (ROCK) biochemically regulates the transition of initiated clefts to undergo cleft progression [16].

### 4.2. Proliferation

Cleft formation is coordinated with cell proliferation during branching morphogenesis with the increase in the size of the epithelium. Rapid proliferation of the developing SMG is mainly localized at the peripheral end buds, suggesting the presence of proliferating progenitor cells. Fibroblast growth factor (FGF) signaling is essential for the proliferation and survival of salivary gland progenitors. *Fgfr2b*−/− and *Fgf10*−/− mice have no salivary glands, although epithelial buds are formed but degenerate by E12.5 [17]. Both exogenous FGF10 and FGF7 bind to Fgfr2b and increase the epithelial proliferation of SMG; however, FGF7 induces budding whereas FGF10 induces duct elongation [18]. These differences are probably due to the binding affinities of the FGFs to heparan sulfate as well as the endocytic recycling of the FGFR.