

Artificial Salivary Glands

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ABSTRACT

Head and neck cancer is the fifth most common malignancy and accounts for 3% of all new cancer cases each year. Treatment for most patients with head and neck cancers includes ionizing radiation. A consequence of this treatment is irreversible damage to salivary glands which is accompanied by a loss of fluid-secreting acinar-cells and a considerable decrease of saliva output. Despite recent improvements in treating xerostomia (e.g. saliva stimulants, saliva substitutes, and gene therapy), there is a need of more scientific advancements that can be clinically applied toward restoration of compromised salivary gland function. Here we provide a summary of the current salivary cell models that have been used to advance restorative treatments via development of an artificial salivary gland. Although these models are not fully characterized, their improvement may lead to the construction of an artificial salivary gland that is in high demand for improving the quality of life of many patients suffering from salivary secretory dysfunction.

Keywords: Artificial salivary glands, Regeneration, Cell therapy, Stem cells, Tissue engineering, Gene therapy.

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INTRODUCTION

In normal salivary glands (SGs), the ductal system that includes excretory, striated, and intercalated ducts biochemically modifies and transports saliva, produced by acinar cells, into the oral cavity. This ductal system also contains the tissue stem/progenitor cells.^{1,2} Proliferation and differentiation of these primitive cells within the ducts maintains

homeostasis of the acinar cells. Saliva plays a major role in maintaining oral health. This becomes apparent when the amount and quality of saliva is significantly reduced by medications, salivary gland neoplasms, and especially ionizing radiation (IR) therapy for tumors of the head and neck.

Head and neck cancer is the fifth most common malignancy and accounts for 3% of all new cancer cases each year.³ Radiotherapy, either alone or in combination with surgery and chemotherapy, is often applied as a treatment for head and neck cancer patients. The radiation dose by which the tumor can be treated is limited by the sensitivity of surrounding normal tissues within the field of radiation. The delayed loss of gland function after radiation is thought to be due to a loss of stem cells that are no longer able to replenish aged saliva-producing acinar cells.⁴

Current treatments for hyposalivation are limited to (i) patient education, diet and lifestyle modifications, (ii) prevention of dental and oral mucosal diseases, (iii) management of symptoms, (iv) sialogogues or salivary gland stimulants (e.g. pilocarpine and cevimeline) that induce saliva secretion from residual acinar cells and (v) use of artificial saliva. However, given that these therapies target surface-level symptoms and provide only temporary relief, development of an alternative treatment to provide a more permanent effect is essential. This review focuses on various efforts to preserve and restore the function of damaged salivary glands.

Preservation of Salivary Glands from Ionizing Radiation

Loss of acini is the principal cause of the reduced salivary function after IR. Shields can be useful if the cancer is unilateral and external to the oral cavity, as in the case of a tumor of a parotid gland. The use of the three-dimensional planning technique (3-DTP) and conformational dose delivery techniques have been shown to reduce the radiation damage to the SGs. These techniques use computerized radiation dose programs guided by computed tomography to fit more precisely the radiation doses to the site(s) of the cancer and its metastases while minimizing the IR affecting the SGs, oral mucosa, and other adjacent structures.⁵ Alternative method is to stimulate the acinar cells to secrete just prior to each dose of IR by the production of free radicals by interactions between IR and heavier metals, such as calcium and magnesium, in secretory granules of serous acini reportedly augments the damage to these cells. Even administration of pilocarpine or bethanechol prior to each dose of IR has

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resulted in significantly less loss of salivary flow rates, especially from the parotid gland.⁶

Even the use of protective agents such as amifostine (WR2721; Ethyol), heat shock proteins, tempol, in head and neck cancer patients has shown promising results with glands receiving low to moderate doses. Tempol protected the normal bone surrounding the tumor and the salivary gland acini and convoluted granular tubules. Injecting these agents in SGs before each of the fractionated IR doses with head and neck cancer, however, risks complications such as infection and increasingly rapid destruction of the injected agents by an immune response.

Other method is to transplant the main body of a salivary gland away from the main field of radiation. After transplantation, one can either leave the gland at its new location or return it to its original site after completion of IR. For the transplanted gland to be useful, its connection to the main duct must be maintained or re-established.⁷

Repair and Regeneration of Salivary Glands

Regeneration is a physiological function of living organisms, which enables the repair of lost or damaged tissue. The concept of regenerative medicine based on the body's naturally existing capacity for regeneration can be deliberately enhanced by the manipulation of cells (cell lineages or cell lines or primary cells) and growth factors and by providing growing space using scaffolds.⁸ The prospect of re-engineering SGs by repaired, redesigned, or regenerated was proposed 10 years ago³ and there has been a clear progress toward gene therapy,⁹ stem cell therapy¹⁰ and tissue engineering.¹¹ After the completion of IR, one can induce the residual salivary gland cells to regenerate acini and other parenchymal elements either by duct ligation method or directly via gene transfer or indirectly by local infiltration of factors that are involved in normal salivary gland growth, development and maintenance.

Cell Lineages

Salivary gland cells have the capacity not only to make more cells of like type, e.g. acinar cells dividing to make more acinar cells, but also to serve as progenitors for other cell types, e.g. intercalated duct cells redifferentiating into acinar or striated duct cells.¹² This is of critical importance to the choice of cells to be injected into glands to effect restoration of acini and other structures that have been destroyed by IR, or to use in creating an artificial salivary gland.¹³ Another approach in assessing cell lineages in salivary glands is to follow the cells involved in regeneration after injury. Regeneration of lobules seems to occur mainly by dedifferentiation, proliferation, and budding of cells of the larger ducts, which then differentiate into acini and all types

of ducts. At present, there are no proven ways to expand the progenitor cell population along different cell lineages. Cell lines have been developed as a tool to define the conditions required for proper organ formation *in vitro*.¹⁴

Cell Lines

The ideal salivary cell would be isolated from autologous tissue in the form of primary or stem cells. Autologous salivary cells would be accepted by the host with no risk of rejection. However, these cells have not been grown efficiently *in vitro*.¹⁵ The human submandibular gland (HSG) can be induced to differentiate from intercalated duct-like to acinar phenotype by cell-substrate interaction with a gel containing laminin-1 or matrigel or into myoepithelial cells when grown on plastic with sodium butyrate added to the medium.^{13,15,16} HSG cells were useful in evaluating the characteristics of the biomaterials used as a scaffold for an artificial salivary gland. As HSG cells lack tight junctions essential for the formation of polarized epithelial monolayers and unidirectional liquid-salt secretion, the application value of this cell line is limited. Currently, no cell line fully recapitulates the morphological and functional features of the native salivary acinar cells. Furthermore, cell lines are potentially tumorigenic. To overcome this, insertion of a suicide gene that responds to a certain agonist may be one possible way to modulate these tumorigenic cells for use *in vivo*.¹⁵

Primary Cells

Primary culture of salivary gland cells have the potential to serve as depots for replenishing cells in injured glands. Currently, the use of primary cells represents the best option for creation of an artificial salivary gland because they more closely resemble native tissue (as compared to cell lines). Furthermore, they can be manipulated *in vitro* and then transplanted to the organism in which they were derived. However, there are critical problems associated with the ability of primary cells to achieve acinar formation *in vitro*.¹⁷ Additionally, they are apoptotic when dissociated into single cell suspensions. Therefore, new approaches are necessary to maintain high viability of primary salivary cells in culture by modulation of apoptosis. Alternatively, remove a small piece of the patient's salivary gland prior to IR, from it grow acinar and/or other cells in primary culture, and inject them into the gland after IR has been completed.

Duct Ligation-Deligation Method

Salivary glands have an excellent capacity to regenerate after partial extirpation or duct obstruction, so they have been used to characterize and isolate stem/progenitor cells. During ligation of the salivary gland duct, apoptosis was

proportionately greater among acinar than duct cells and both duct and acinar cells were in stages of de-differentiation and redifferentiation in the gland. In addition, few myoepithelial cells in the ligated glands underwent apoptosis or atrophy; most collected around the dedifferentiated, duct-like structures. Interestingly, the emerging acini initially were surrounded by myoepithelial cells. As the acini became better differentiated, the myoepithelial cells translocated to the intercalated ducts. Deligation of the main ducts induces a rapid regeneration process in which the number of acinar cells increases. In general, it seems that first residual acini proliferate followed by the formation of new acini, reattachment of parasympathetic nerves and recovery of normal saliva composition and gland function.^{13,18-20}

Gene Therapy

Genetic modification is another remarkable approach to restore the function of salivary glands. Localized delivery of adenovirus mediated water channel AdhAQP1 (Aquaporin-1, AQP1) to IR-damaged salivary glands is useful in transiently increasing salivary secretion with no significant risk of general adverse effects. Salivary glands are connected to the oral cavity via ducts.^{21,22} Conventional cannulation techniques can be applied to introduce viral or nonviral vectors into the gland *in vivo*. Furthermore, cannulation-mediated gene transfer to the gland is beneficial in limiting the extension of the vectors systemically.²³ Another valuable potential application of local salivary gland gene therapeutics is to deliver growth factors or cytokines, such as epidermal growth factor (EGF), keratinocyte growth factor (KGF), and Interleukin-11, to promote mucosal wound healing.⁹

Stem Cells

Stem cells or progenitor cells are a characteristic group of cells which possess self renewal capability and pluripotency.^{24,25} Basically, three stem cell types are currently being investigated for their potential use in stem cell therapy: embryonic stem cells, induced pluripotent stem cells and adult stem cells. Neonatal (Embryonic) progenitor cells provide an attractive model for constructing an artificial salivary gland as they have been found to be involved in the regeneration of acinar, ductal, and myoepithelial salivary cells *in vitro*. Isolating progenitor cells prior to radiation therapy, expanding the cells *in vitro*, and then transplanting the cells into the patient is an ideal therapeutic approach for treating patients whose salivary glands may potentially be damaged by irradiation.²

Adult (somatic or tissue-derived) stem cells are generally organ restricted and only form cell lineages of the organ from which they originate (unipotent). Adult stem cells are able to self-renew and can differentiate to yield all specialized

cell types. Formation, maintenance, and repair of the tissue in which they reside are the primary roles of the adult stem cell. Although adult stem cells may not be as powerful or diverse as embryonic stem cells, they offer many advantages for the development of cellular therapeutics including lack of ethical problems, the possibility to using autologous cells, accessibility, stable phenotype and tissue type compatibility.¹⁵

Currently, stem cells from two different organs have been investigated: (a) from the salivary gland² or (b) from the bone marrow.^{26,27} Expansion of the salivary gland stem/progenitor cell population may prevent acinar cell depletion and subsequent gland dysfunction after radiation. Accordingly, the stem cell population of salivary glands is considered to be present in the intercalated ducts.²⁸ Research into the development of the salivary gland also has revealed that cells in the duct close to the acini are believed to provide all the cell types required for the formation of acini and ducts. However, it is noteworthy that not only stem cells but also differentiated cells might play key roles during salivary gland tissue regeneration. The potential of these particular stem cells to regenerate salivary gland tissue has yet to be proved, and there is no available stem cell source for the regeneration of salivary gland. A method had been developed to isolate and culture submandibular gland cells as salispheres which showed to contain progenitor/stem cells. The obtained salispheres were derived from putative stem cells of ductal origin and contained cells expressing stem cell markers Sca-1, c-Kit and Msi-1. These spheres were able to form functional duct and acinar-like cells *in vitro*. Strikingly, when transplanted as single cells into irradiated glands they were able to restore submandibular gland morphology and function. Most of the established cell transplantation requires a donor able to provide a sufficient number of cells. A dearth of such donors and the possible immune-reaction against the allogeneic cells are the major limitations of the therapy. If the cultured cells from autologous tissue can be used for cell therapy, these shortcomings could be overcome.²

Bone marrow-derived cells (BMDCs) have been suggested as an easy accessible source for multipotent stem cells that could potentially transdifferentiate and/or repair other nonhematopoietic organs, including salivary glands. Mesenchymal stem cells are usually obtained by bone marrow aspiration, and can differentiate into various types of cells. Specifically for the salivary glands, granulocyte colony-stimulating factor treatment induced mobilization of a large number of BMDCs to injured salivary glands to participate in repair processes that improved gland's function and morphology.^{26,29-31} Other method is to selectively retrieve hematopoietic stem cells from a sample of the patient's blood and inject these into the gland, or induce them to differentiate into salivary gland cells *in vitro*, and inject these into the damaged gland. Advantages of this approach are that there is

no delay in radiation treatment while a donor gland recovers from surgery and that the stem cell-derived cells may have greater proliferative potential than gland-derived cells.³²

For cell transplantation to be feasible, the transplanted cells would have to attach and survive in the transplanted damaged/atrophic region. Furthermore, transplanted cells would have to be integrated into the native structure and be able to differentiate into a salivary gland cell lineage. It is also possible that transplanted cells might temporally reside in residual tissue to accelerate its regeneration. Upto now, only limited information has been available about the fate of cells transplanted into salivary gland.

Factors Which affect Salivary Gland Tissue Regeneration

A whole range of growth factors, cytokines, extracellular matrix proteins and antiapoptotic proteins are involved in the development of the salivary glands. Growth factors usually act as strong mitogens for most of the cells in various tissues including the salivary gland. In human submandibular gland epithelial cells, basic fibroblast growth factor (FGF) accelerated cell proliferation. When provided at the appropriate time, FGF7, FGF8 and tumor necrosis factor- α increase budding and branching is induced by FGF10, EGF, vascular growth factors, transforming growth factor- β , bone morphogenetic protein 7, ectodysplasin and sonic hedgehog homolog expression.²³ In the salivary gland cytokines like EGF, insulin growth factor increased the labelling index of serous and ductal cells in gland also inhibit apoptosis and/or enhance proliferation. It was found that N23-KGF administration before and after irradiation of salivary glands induced a long-term expansion of duct stem/progenitor cells, which elicited short- and long-term maintenance of the acinar cell compartment, resulting in permanent preservation of the salivary gland function after irradiation.^{14,33}

Also various biomarkers have been identified for the development of salivary glands. A transcription factor $AsCl_3$ (which is essential for the determination of cell fate, development, and differentiation of numerous tissues) and a structural protein K^{5+} (a cytokeratin that forms cytoskeleton intermediate filaments) have been established as markers of progenitor cells. Furthermore, $AsCl_3$ was found to be expressed in ductal neonatal progenitor cells provide an attractive model for constructing an artificial salivary gland as they have been found to be involved in the regeneration of acinar, ductal, and myoepithelial salivary cells *in vitro*.^{34,35} At present, the only progenitor cell population that has been used to regenerate salivary glands after irradiation was isolated based on the cell surface marker c-Kit. Remarkably, postirradiation stem cell treatment with c-Kit⁺ adult salivary gland stem cells restored radiation-induced dysfunction.²

Other groups have used cell surface markers, such as Sca-1, CD133, CD24 and CD49f to enrich the epithelial progenitor cells in the salivary glands but a combination of definitive cell markers for salivary progenitor cells remain to be determined.^{14,36,37}

Tissue Engineering

Tissue engineering utilizes cells, biodegradable scaffolds, and signals to regenerate tissues. Recently, it was feasible to culture salivary epithelial cells for their eventual use in a prototype artificial salivary gland. However, this strategy can generate only one portion of the salivary parenchymal tissue (the ductal cells), and it was found difficult to regenerate fully functional salivary tissue (both ductal and acinar cells).^{38,39} An intriguing idea is to obtain cells from donor glands and to use these to grow new glands *in vitro*, or construct artificial salivary glands by lining tubular scaffolds of biodegradable or biocompatible material with the cells and transplant these into the patient's oral mucosa.

Potential Cell Sources for Salivary Gland Tissue Engineering

One of the critical issues for the salivary gland tissue engineering is the serial cultivation of the cells, as the concept of tissue engineering requires expansion from a small number of cells cell lines. Possible cell sources can be divided into (1) progenitor/stem cells from salivary glands and (2) pluripotent stem cells from other tissues.

Scaffold Materials for Salivary Gland Tissue Engineering

Another important factor in salivary gland tissue engineering is the usage of appropriate scaffold material. So far, a simple combination of cultured salivary gland epithelial cells and biodegradable materials has been used. The materials consisted of a denuded rat tracheal preparation, poly-L-lactic acid (PLLA), polyglycolic acid (PGA) and PGA/PLLA, chitosan and poly (ethylene glycol)-terephthalate/poly (butylene terephthalate). Importantly, most of the polymers must be precoated with matrix proteins, such as fibronectin and collagen I.^{40,41} Recently, an artificial salivary gland was fabricated based on the principles of tissue engineering. The device consists of a blind-end tube fabricated from porous, slowly biodegradable substratum, coated with matrix components on the inner surface of tube providing unidirectional fluid secretion and is surgically implanted in the buccal mucosa with an opening in the oral cavity. Tissue compatibility, graft cell safety and timing of implantation of these devices play an important role in their application, but still it has not been proved clinically in orofacial region.^{3,42,43}

CONCLUSION

The replacement of damaged or lost salivary gland tissue is a fascinating challenge, especially, if the regeneration can be achieved using autologous graft cells, requiring no special considerations such as mechanical degradation or immunologic reaction. Stem cell therapy and tissue engineering may thus provide new treatment modalities for atrophic salivary gland. However, such efforts are still in a very early stage, and a more basic understanding of salivary gland tissue regeneration and stem cells is required.

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