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REVIEW ARTICLE

Restoring the function of salivary glands

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Salivary gland destruction occurs as a result of various pathological conditions such as radiation therapy for head and neck cancer and Sjögren's syndrome. As saliva possesses self-cleaning and antibacterial capability, hyposalivation is known to deteriorate dental caries and periodontal disease. Furthermore, hyposalivation causes mastication and swallowing problems, burning sensation of the mouth and dysgeusia. Currently available treatments for dry mouth are prescription for artificial saliva, moisturizers and medications which induce salivation from the residual tissue. Unfortunately, these treatments cannot restore the acini functions. This review focuses on various efforts to restore the function of damaged salivary gland. First, the possibility of salivary gland regeneration and tissue engineering is discussed with reference to stem cells, growth factors and scaffold materials. Second, the current status of gene transfer to salivary glands is discussed.
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Introduction

Salivary gland impairment resulting in xerostomia can occur as a consequent of irradiation therapy to the head and neck cancer patients, Sjögren's syndrome (SS) as well as other medical conditions mainly the usage of xerogenic medications (Atkinson and Fox, 1992; Fox, 1998; Ship *et al*, 2002). Xerostomia is an important clinical concern in oral health and is known to induce various problems including dental caries, periodontitis, denture problems, mastication and swallowing problems, burning sensations, and dysgeusia (Atkinson *et al*, 2005). Muscarinic agonist medications such as pilocarpine and cevimeline induced salivary secretion from the residual functional tissue (Fox, 2004). However, they only provided temporary relief of symptoms and had a limited effect on the recovery of damaged tissue. Accordingly, the development of a novel treatment to restore or regenerate damaged salivary gland tissue is eagerly awaited.

Recently, concepts of regenerative medicine and tissue engineering have drawn much attention (Langer and Vacanti, 1993; Baum *et al*, 1999a; Alsberg *et al*, 2001; Kaigler and Mooney, 2001; Bücheler and Haisch, 2003). In humans, the potential for regeneration is limited except for organs such as the liver, which can regenerate from 10% of the residual tissue (reviewed by Chamuleau and Bosman, 1988; Taub, 1996, 2004; Fausto, 2000). The three fundamental components in regenerative medicine include (1) graft cell, (2) growth factors, and (3) scaffold (Cima *et al*, 1991; Reddi and Cunningham, 1991). Clinically, these concepts have been reported as successful in regenerating skin (Hefton *et al*, 1983; Gallico *et al*, 1984), corneal epithelium (Germain *et al*, 1999), cartilage (Mow *et al*, 1991; Vacanti and Vacanti, 1994), and bone (Syftestad *et al*, 1985; Caplan, 1987; Reddi and Cunningham, 1991; Crane *et al*, 1995). Although the regeneration of more complex organs is still underway, successful regeneration of the human bladder has been reported recently (Atala *et al*, 2006). Currently, considerable efforts have been made for the regeneration of pancreas (beta cells), liver, kidney, heart, tooth and even the central nervous system (CNS). A search for specific stem cells and induction to a favorable phenotype are the major goals of most of these studies. The primary purpose of this review was to consider the potential for successful salivary gland tissue regeneration and tissue engineering.

Genetic modification is another remarkable approach to restoring the function of salivary glands (Delporte *et al*, 1997; Baum *et al*, 1999b). It might be also feasible to modify the status of autoimmune diseases such as SS

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using gene transfer techniques. The concept of gene therapy has proved useful for various diseases such as severe combined immunodeficiency (SCID) (Cavazzana-Calvo *et al*, 2000). Besides, some potential side effects have recently been reported such as the development of leukemia in those patients with SCID who have been treated using retroviral-mediated gene transfer (Hacein-Bey-Abina *et al*, 2003). This fact suggests the need for further investigation to understand the basic mechanisms of gene transfer and genetic modification.

Reports of experiments on animal models have revealed that the physical and biological characteristics of salivary glands provide unique advantages favoring successful gene transfer (Baum *et al*, 2002; Zufferey and Aebischer, 2004). Considering such inherent advantages, the efficacy and safety of applying gene transfer to salivary glands is believed to have extensive clinical value. The prospects seem promising to restore the salivary gland function by gene transfer to the gland *in vivo*.

In this review, the possibility of restoring salivary gland function was discussed in relation to novel approaches including tissue engineering and gene therapies.

Salivary gland regeneration

Recently, the interdisciplinary area of science called *regenerative medicine* has attracted much attention (Lowenheim, 2003; Mironov *et al*, 2004; Brockes and Kumar, 2005). Regeneration is a physiological function of living organisms, which enables the repair of lost or damaged tissue. Regenerative capacity differs among species and organs. For example, a newt is known for its surprising ability to regenerate a complete eye or leg after resection. In contrast, humans have a much more limited ability for regeneration. The liver is known to have an amazing ability for regeneration, enabling the organ to regain its normal size after a 90% hepatectomy (reviewed by Chamuleau and Bosman, 1988; Taub, 1996, 2004; Fausto, 2000). On the other hand, most human organs including the CNS have only a limited regenerative ability, although the possibility of CNS regeneration has been reported only recently (Burgo *et al*, 2002; Vergara *et al*, 2005).

The concept of regenerative medicine based on the body's naturally existing capacity for regeneration can be deliberately enhanced by the manipulation of cells and growth factors and by providing growing space using scaffolds. This concept has been proven feasible in the tissue engineering of skin (Hefton *et al*, 1983; Gallico *et al*, 1984). Early clinical trials for tissue regeneration have also been reported in the field of dentistry. The regeneration of periodontal tissue using a barrier membrane (Nyman *et al*, 1982a,b; Gottlow *et al*, 1984) and enamel matrix-derived protein has been successfully applied clinically (Hammarstrom, 1997; Hammarstrom *et al*, 1997; Heijl, 1997).

At present, detailed knowledge of the underlying mechanisms of tissue regeneration remains scarce. Cells, especially somatic stem cells, are considered to play important roles during tissue regeneration. Various factors including growth and transcription factors are known to be expressed during tissue regeneration and are also considered essential for the regeneration process. Interestingly, the sequential expressions of those factors observed during tissue regeneration are mostly recapitulation of what happens during development. Fundamental understanding of the molecular mechanisms of development would provide us clues for a novel strategy of tissue and organ regeneration in the future. Technically, such cells and factors can be applied exogenously, such as cell therapies or growth factor therapies. However, endogenous cells and factors may also contribute to the regeneration process. The requirement for those components might differ depending on the type of tissue and the size of defect. Further studies will be necessary to better understand the fundamental mechanisms of tissue regeneration.

Stem cells are a characteristic group of cells which possess self-renewal capability and pluripotency (reviewed by Mayhall *et al*, 2004; Molofsky *et al*, 2004). Those currently available for clinical treatment are somatic stem cells, which can be found in the adult body (Garry *et al*, 2003; Horwitz, 2003), and whose use helps to avoid ethical problems as neither donors nor fertilized eggs are required. Stem cells can be found in the bone marrow, fat and possibly in most of the tissues in the human body. Mesenchymal stem cells, one of the most well-characterized somatic stem cells, are usually obtained by bone marrow aspiration, and can differentiate into various types of cells including osteoblasts, chondroblasts, and nerve cells (reviewed by Burry and Murphy, 2004; Gregory *et al*, 2005; Risbud and Shapiro, 2005). In organs such as the liver and the pancreas, the presence of tissue-specific stem cells/precursors has been suggested (reviewed by Matthews and Yeoh, 2005; Otonkoski *et al*, 2005; Soria *et al*, 2005; Theise, 2006). For example, the pancreatic and hepatic cell types have shown remarkable plasticity, which can de- and transdifferentiate into each other under appropriate conditions (Otonkoski *et al*, 2005). Major efforts have been made for the elucidation of the molecular mechanisms underlying these processes, which could lead to pancreatic islet regeneration. Similarly, human embryonic stem cells have been an emerging field of science, and efforts have been made to achieve targeted differentiation of these cells into a transplantable beta-like cell (Otonkoski *et al*, 2005).

Salivary gland stem cell and possibility of cell transplantation

To date, however, the characteristics of salivary gland stem cells have not been well understood. Research into the development of the salivary gland 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 (reviewed by Redman, 1987; Cutler, 1989). Accordingly, the stem cell population of salivary glands is considered to be present in the intercalated duct (Man *et al*, 1995, 2001). However, it is noteworthy that not only stem cells but also differentiated cells might play key roles during salivary gland tissue regeneration.

During the regeneration process following ductal ligation, cell proliferation was observed in many cell types including basal, myoepithelial, and oxiphilic cells as well as striated and excretory duct cells (Ihrler *et al*, 2002). Similarly, in chronic sialoadenitis, proliferative indices had increased significantly in mature acinar cells, intercalated ductal cells, and myoepithelial cells (Ihrler *et al*, 2004). Presumably, such regeneration processes are not antagonistic to the presence of stem cell populations in the intercalated ducts. The stem cell/precursor cell population may usually provide cells during the normal cell renewal process. When severe damage to the gland occurs, the differentiated cells may de-differentiate and proliferate to induce a rapid recovery of the gland. A similar phenomenon has been reported for the regeneration of the liver, in which various types of mature cells play major roles during tissue regeneration (Santoni-Rugiu *et al*, 2005). When the regenerative capacity of mature cells is impaired, hepatic progenitor cells are activated and expand into the liver parenchyma (Santoni-Rugiu *et al*, 2005).

Does a salivary gland-specific stem cell actually exist? If so, what is the nature of this kind of stem cell? Recently, several exciting works have been published in this field (Table 1). In the regenerating submandibular gland, the presence of a stem cell population able to differentiate into hepatic and pancreatic cell lineages has been reported (Okumura *et al*, 2003). That population of cells is positive for certain cell-surface markers such as Sca-1 and c-Kit, and is thought to be endoderm-derived (Hisatomi *et al*, 2004). More recently, the occurrence of proliferative, multipotent salivary gland stem/progenitor cells has been reported in neonatal mice (Kishi *et al*, 2006). A similar cell type was also reported in adult mice, although their pluripotency was limited (Kishi *et al*, 2006). 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. Great emphasis should be placed on understanding the nature of those stem cells to achieve salivary gland regeneration therapy.

Cell transplantation has been used in various fields of medicine. Transfusion and bone marrow transplantation, for example, are commonly accepted. Recently, the possibilities of cell transplantation have also been explored to regenerate the functions of various organs. Islet transplantation has been successfully performed on patients with diabetes (reviewed by Calne, 2005; Hatipoglu *et al*, 2005). Most remarkably, a group in Edmonton has reported excellent results from 1- and 2-year follow-ups of patients with type I diabetes, which further supports the islet transplantation concept (Shapiro *et al*, 2000). Most of the established cell transplantation (a.k.a. cell therapy) requires a donor able to provide a sufficient number of cells. A dearth of such donors and the possible immunoreaction 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.

The prospects for successful glandular tissue regeneration using cell transplantation remain uncertain. 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. Up to now, only limited information has been available about the fate of cells transplanted into salivary gland.

Our group has investigated the fate of the transplanted cultured salivary epithelial cells in the regenerating submandibular gland in rats (Sugito *et al*, 2004). Fluorescent-labeled salivary epithelial cells were injected into normal and atrophic rat submandibular glands. Our results showed that the transplanted cells could attach and remain in the regenerating gland for at least 4 weeks. However, such cells were not observed when they were transplanted to normal glands, suggesting that both cell attachment and survival are significantly affected by the environment of the host organ. More recently, the potential of mesenchymal stem cells to regenerate salivary glands was reported using a radiation-damage model (Lombaert *et al*, 2006). Interestingly, the transplanted bone marrow-derived cells were shown to improve the function of the salivary gland, while differentiation of the transplanted cells was not confirmed. The possible use of bone marrow-derived stem cells to replace oral mucosa has been reported (Tran *et al*, 2003). If the fraction of more potent stem cells can be isolated from the bone marrow, it would be feasible to restore damaged salivary gland cells using bone marrow-derived stem cells. Furthermore, as stated above, it would also be feasible to use tissue-specific stem cells when salivary gland stem/progenitor cells will become available. The incorporation of stem cells into the atrophic or damaged tissue will open up the possibility of an alternative treatment in the future.

Table 1 Potential cell sources for salivary gland regeneration and tissue engineering

Markers	*Phenotype*	*Species*	*Reported by*
ND	Duct/acinar	Rat	Horie *et al* (1996), Sugito *et al* (2004)
ND	Duct/acinar	Human	Bücheler *et al* (2002)
$Sca\text{-}1^{+}/C\text{-}kit^{+}$	Liver/pancreas	Mouse	Okumura *et al* (2003)
ND	Duct	Human	Tran *et al* (2005)
ND	Acinar	Mini-pig	Sun *et al* (2006)
ND	Duct/acinar/myoepithelial	Rat	Kishi *et al* (2006)

ND, not determined.

Factors which affect salivary gland tissue regeneration
Growth factors usually act as strong mitogens for most of the cells in various tissues including the salivary gland. In both human- and rat-cultured submandibular gland epithelial cells, basic fibroblast growth factor accelerated cell proliferation (Hiramatsu *et al*, 2000). Ohlsson *et al* (1997) reported the effect of systemic administration of epidermal growth factor (EGF) on the pancreas and salivary glands. It was concluded that EGF increased the labeling index of serous and ductal cells in the parotid gland.

On the other hand, tissue regeneration is an enormously complex process involving multiple growth factors/transcription factors and their sequential (and coordinated) expression. The molecular mechanisms underlying the regenerating process for the salivary gland are largely unknown. During skin wound healing, a serial activation of growth factors together with the recruitment of inflammatory cells occurs in the regenerating area. It is unclear whether or not a specific signal is required for the regeneration of salivary gland. For example, hepatocyte growth factor is a well-known protein which promotes the regeneration of liver and even protects tissue from damage (Nakamura *et al*, 1986, 1989; Kinoshita *et al*, 1991; Ishiki *et al*, 1992). It would be significant to determine the specific factors for salivary gland regeneration. We have examined the gene-expression profile in a regenerating submandibular gland after ductal ligation and removal. Total RNA was extracted from the gland, and the gene-expression profile was compared with 12 h to 6 days and also 36 h to 6 days. Gene-expression profiles were independently analyzed using DNA microarray and fluorescent differential display (FDD) technique. From a preliminary analysis using FDD, 16 clones have been identified (Sugito *et al* 2004). Using the microarray analysis, genes related to inflammation, regeneration, and adhesion molecules were mainly detected (Sugito *et al* 2004). More precise study of the roles of those genes during regeneration may lead to improving our understanding of their possible mechanisms.

Tissue engineering of salivary gland

If the gland damage is severe and the residual tissue can no longer be restored, an alternative approach is required. One of the most interesting interdisciplinary approaches for this purpose is tissue engineering, which utilizes cells, biodegradable scaffolds, and signals to regenerate tissues. Historically, the concept of tissue engineering has been regarded as almost identical to, or a distinct area of, regenerative medicine. However, in this review, we focused on this topic aside from salivary gland regeneration, as the ultimate goal of studies in this field is to generate neo-salivary glands.

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. Furthermore, the appropriate cell culture conditions must be established. Possible cell sources can be divided into (1) progenitor/stem cells from salivary glands and (2) pluripotent stem cells from other tissues (such as bone marrow or even embryonic stem cells). Although the embryonic stem cell has a significant potential to generate various tissues, it is difficult at present to apply it to salivary gland tissue engineering out of ethical and safety concerns.

In early studies of artificial salivary glands, a human salivary cell line, known as HSG, was used (Wang *et al*, 1999; Aframian *et al*, 2000). 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 (Aframian *et al*, 2002).

A pioneer work on culturing salivary gland epithelial cells was reported by Brown (1974). Since then, several culture procedures have been published, initially by use of feeder cells (Horie *et al*, 1996; Aframian *et al*, 2004), and more recently using a serum-free medium for epithelial cells (Joraku *et al*, 2005; Tran *et al*, 2005). The most important recent development involves the discovery of a multipotent stem cell population in adult salivary glands (Okumura *et al*, 2003; Hisatomi *et al*, 2004; Kishi *et al*, 2006). The potential of these cells for engineering salivary gland tissue has not been proved. However, accumulating knowledge about the stem cell population in adult salivary gland may provide a more realistic possibility for the development of artificial tissue-engineered salivary glands in the future.

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 (Wang *et al*, 1999; Aframian *et al*, 2000, 2002; Bücheler *et al*, 2002; Chen *et al*, 2005; Joraku *et al*, 2005; Sun *et al*, 2006). The materials consisted of a denuded rat tracheal preparation (Wang *et al*, 1999), poly-L-lactic acid (PLLA), polyglycolic acid (PGA) and PGA/PLLA (Aframian *et al*, 2000; Joraku *et al*, 2005), chitosan (Chen *et al*, 2005) and poly (ethylene glycol)-terephthalate (PEFT)/poly (butylene terephthalate (PBT) (Sun *et al*, 2006). Importantly, most of the polymers must be precoated with matrix proteins such as fibronectin and collagen I (Aframian *et al*, 2000; Chen *et al*, 2005).

Current status and potential of salivary gland tissue engineering
The results of our recent study using miniature pig parotid gland-derived cells showed that the cells adhere and grow on biocompatible materials, maintaining an acinar cell phenotype and showing α-amylase activity (Sun *et al*, 2006). The initial trials to generate an artificial salivary gland by use of cultured salivary gland cells and biodegradable scaffolds have demonstrated the potential of salivary gland tissue engineering. However,

most of these studies could show only a limited capability of the transplanted cells to regenerate salivary gland tissue as a living organ. It would be a realistic step to generate an artificially made ductal structure with an epithelial cell lining, which though not identical to, could partially compensate for, the function of the damaged gland. Furthermore, the availability of stem cell populations from salivary glands might enable the true regeneration of functional organs using a tissue engineering approach.

Recently, a simple tissue engineering approach using isolated cells and scaffold has been proved feasible to generate more complex structures such as tooth germ (Young *et al*, 2002). The analysis of the regeneration process showed the importance of the epithelial–mesenchymal interaction, which recapitulates the natural developmental process of tooth germ (Honda *et al*, 2005). The epithelial–mesenchymal interaction has been well studied using salivary gland primordium as well as tooth germ, and previous studies have shown similarities between these two organs. Although the potential of epithelial–mesenchymal interaction using adult salivary gland-derived cells has not yet been reported, the discovery of potent stem cells could be sufficient to generate a neo-salivary gland.

Gene therapy and therapeutics in salivary glands

Salivary glands are connected to the oral cavity via ducts. This anatomic structure enables easy access to the gland per-orally (O'Connell *et al*, 1995, 1996; Kagami *et al*, 1996). Conventional cannulation techniques can be applied to introduce viral or non-viral vectors into the gland. Furthermore, cannulation-mediated gene transfer to the gland is beneficial in limiting the extension of the vectors systemically compared with that using drip infusion to a vein (Kagami *et al*, 1996; Delporte *et al*, 1998).

Gene therapy for irradiation-induced hyposalivation

A study demonstrated the potential of gene therapy to correct irradiation-induced salivary hypofunction (Delporte *et al*, 1997). An adenovirus-mediated water channel (aquaporin-1, AQP1) gene transfer into irradiated submandibular glands showed increased saliva flow in a rat model (Delporte *et al*, 1997). A study evaluated the efficacy of a single administration of AdhAQP1 to the parotid glands of adult rhesus monkeys. In this study, a single parotid gland of rhesus monkeys was irradiated with a single dose of 10 Gy and AdhAQP1 was administered intraductally at 19 weeks postirradiation and salivary secretion examined 3, 7, and 14 days later. The results, however, were inconsistent, and only two of the four AdhAQP1-treated monkeys displayed increased salivary flow rates compared with the animal administered an irrelevant virus (O'Connell *et al*, 1999).

Rats and mice are the most frequently used animal models in the studies of salivary gland gene transfer. Recently, the miniature pig has been increasingly used as a large animal model in a variety of biomedical studies (Hainsworth *et al*, 2002; Screaton *et al*, 2003). The parotid glands of miniature pigs are almost identical to those of humans in terms of their volume and morphology (Wang *et al*, 1998). Luciferase and β-galactosidase genes were administered to miniature pig parotid glands by a recombinant adenoviral vector. Luciferase assays indicated that gene transfer to miniature pig salivary glands could be readily accomplished using rAd5 vectors. The results from X-Gal staining have shown that the β-galactosidase expression was observed in both acinar and ductal cells. Thus, the results of salivary gland gene transfer from rodent studies can be extended to a larger animal model, and support the value of using miniature pigs for preclinical applications of gene transfer to these tissues (Li *et al*, 2004).

The effects of a solitary mega-dose protocol of ionizing radiation (IR) on the structure and function of miniature pig parotid glands was evaluated by our group. Our results showed that the structural changes induced by single, regional mega-doses of IR were generally identical to those induced by the fractionated radiation dose protocol, and similar to those found in humans. At the 16-week time point, the salivary flow rates had decreased approximately 60% in the 15-Gy group and by around 80% in the 20-Gy group. These findings indicated that the parotid glands of miniature pigs locally irradiated with a single dose 20 Gy may be useful as a large animal model for the studies of gene transfer into irradiation-damaged salivary gland (Li *et al*, 2005).

A study was performed to evaluate whether Ad-hAQP1 would be effective in improving the salivary secretion of irradiated miniature pig salivary glands, which are ~100-fold larger than those of rats. Subsequent administration of the AdhAQP1 vector resulted in a dose-dependent increase in parotid salivary flow (Shan *et al*, 2005). Three days following administration of the highest dose used herein, 2.5×10^5 pfu AdhAQP1/μl infusate (10^9 pfu total/gland), a marked increase in parotid salivary secretion was observed, reaching on average ~80% of pre-IR levels. Conversely, administration of the same dose of control Ad vector encoding luciferase showed no significant effect on salivary flow. The effective dose of AdhAQP1 was comparable to that confirmed in the reporter transgene expression analysis in both murine and miniature pig salivary glands. Importantly, this effective dose in miniature pig was only 20% of that required to be effective in irradiated rats (Shan *et al*, 2005) (Table 2). Localized delivery of AdhAQP1 to IR-damaged salivary glands is useful in transiently increasing salivary secretion in both small and large animal models with no significant risk of general adverse effects. Based on these results, Baum *et al* (2005) have developed a clinical trial to determine whether the hAQP1 cDNA transfer strategy will be clinically effective in increasing salivary flow in patients with IR-induced parotid hypofunction.

Gene therapy for Sjögren's syndrome impaired salivary gland function

At present, although the exact pathogenesis of SS is unclear, several possible immunologic mechanisms have

Table 2 Effect of AdhAQP1 on salivary flow in irradiated animals

Species	*Vector*	*Dose*	*Salivary flow (% control[a])*
Rat[b]	AdhAQP1	5 × 10e9 pfu/gland	83.6
	Addl312	5 × 10e9 pfu/gland	36.1
Mini-pig[b]	AdhAQP1	10e9 pfu/gland	81.0
	AdCMVLuc	10e9 pfu/gland	30.0

[a]Control data in rat experiments derived from animals unirradiated but infected with the same vector. Control data in mini-pig experiments derived from preirradiation salivary flow rates in same animals. [b]Data from previous report by Delporte *et al* (1997) and Shan *et al* (2005). For rat experiments, animals received 21 Gy, while mini-pigs received 20 Gy, each in a single dose. Data shown are average percentage control results seen 3 days following vector delivery. 100% is considered equivalent to normal salivary flow.

been proposed which might play roles in the tissue destruction of salivary glands (Delaleu *et al*, 2004; Hjelmervik *et al*, 2005). Potential target genes in gene therapy for SS-damaged hyposalivation include inflammatory mediators, cytokine inhibitors, apoptotic molecules, cell–cell interaction, or intracellular molecules.

Interleukin 10 (IL-10) is a homodimeric protein with a wide spectrum of immune activities. One study showed that vector-encoded hIL-10 was biologically active *in vivo* by challenging rAAVhIL10-treated IL-10 knockout mice with lipopolysaccharide to induce endotoxic shock 8 weeks after systemic delivery (Yamano *et al*, 2002). A recombinant AAVhIL10 vector was administered to the salivary glands of non-obese diabetic (NOD) mice and its effects on the stimulated salivary flow rate were measured (Kok *et al*, 2003). The animals receiving the rAAVhIL10 showed markedly higher salivary flow rates than those observed in the sham group of animals. In addition to the effects on salivary function, rAAVhIL-10 administration led to marked improvements in histologically assessed inflammatory changes in the submandibular glands.

Vasoactive intestinal peptide (VIP), initially discovered as a gastrointestinal hormone, exhibits abundant functions, ranging from neurotransmitter, vasodilator, and bronchodilator effects to acting as a trophic agent, secretagogue, and immunomodulator (Said, 1986; Delgado *et al*, 2002; Voice *et al*, 2002; Gozes and Furman, 2003). A recombinant serotype 2 adeno-associated virus encoding the human VIP transgene (rAAV2hVIP) was administered into the submandibular gland of a female NOD mice to examine its ability to alter the progressive SS-like dysfunction in NOD mice. While it led to higher salivary flow rates, there were no differences in focus scores or apoptotic rates. In the experimental group, increased expression of VIP in submandibular gland and serum, and a reduction in cytokines IL2, IL10, IL12 (p70), and tumor necrosis factor-α in submandibular gland extracts were observed compared with the control vector results. The results indicated that local delivery of rAAV2hVIP can have disease-modifying and immunosuppressive effects in submandibular gland of the NOD mouse model of SS (Lodde *et al*, 2006).

Furthermore, a key study reported that the treatment of acute and chronic sialadenitis in B6-gld/gld mice with local fasL gene transfer resulted in a significant reduction in the number of inflammatory foci and in the level of tissue destruction in salivary glands (Fleck *et al*, 2001).

Gene transfer to salivary glands

Many reports hypothesize that a gene transfer to salivary glands can lead to stable long-term secretion of a therapeutic protein into the bloodstream or the saliva for therapeutic purposes. Investigations clearly demonstrated the potential of salivary glands as a systemic gene therapeutic target. It was shown that rat salivary glands, after being administered the rAd5 vector encoding human α-1-antitrypsin (hα1-AT), were able to secrete the transgene protein into the bloodstream (Kagami *et al*, 1996). This potential was extended in subsequent studies using another rAd5 vector encoding human growth hormone (hGH), also administered to rat salivary glands. These results provided the first demonstration of systemic biologic activity from an endocrine transgene product secreted into the bloodstream from salivary glands (He *et al*, 1998). Following rAAV2 vector encoding human erythropoietin (hEPO) gene transfer to mouse salivary glands, the concentration of hEPO in serum was stable throughout the experiment from 10 to 54 weeks. Furthermore, the transgene-encoded hEPO was functional, because the hematocrit levels in all infected animals followed a similar pattern and remained elevated throughout the experiment (Voutetakis *et al*, 2004).

Most recently, an adenoviral serotype 5 (Ad5) vector encoding hEPO cDNA or an adeno-associated virus serotype 2 (AAV2) vector encoding either the hEPO or hGH cDNA was administered to individual submandibular salivary glands of Balb/c mice (Voutetakis *et al*, 2005). AAV2 vectors led to a stable gene transfer, unlike the results with the Ad5 vectors. Indeed, hEPO production in one mouse was observed for a period of 2 years after administration of AAVhEPO to the salivary glands. hEPO, which is a constitutive pathway secretory protein, was readily secreted into the bloodstream from the salivary glands, yielding therapeutically adequate serum levels. Conversely, hGH, a regulated secretory pathway protein, was preferentially secreted into saliva. Salivary glands may be an attractive candidate target tissue for gene therapeutics of some monogenetic endocrine deficiency disorders. At present, AAV2 vectors seem particularly useful for such applications, and transgenes encoding constitutive secretory pathway hormones are more suitable for this application with salivary glands than those encoding regulated secretory pathway hormones (Baum *et al*, 1999a; Voutetakis *et al*, 2005). These studies demonstrated that gene delivery to salivary glands might not be limited to the treatment of salivary gland disorders, but may also be an attractive approach to cure certain cases of major systemic diseases such as hemophilia and diabetes.

Salivary glands normally produce and secrete into the saliva a variety of beneficial proteins that play important roles in maintaining the oral cavity and upper gastrointestinal tract tissue homeostasis and integrity. Investigations have demonstrated that transgenic proteins can be effectively secreted into saliva for therapeutic purposes. The cDNA for histatin 3, an anti-candidal peptide normally found in the saliva of Old World primates and humans, was expressed in rat salivary glands using a rAd5 vector (O'Connell *et al*, 1996). The transgenic histatin 3 produced in rat saliva was highly effective for killing azole-resistant *Candida albicans*. Moreover, many other naturally occurring antimicrobial peptides such as defensins and magainins have been identified and those peptides might be clinically useful against resistant microorganisms. The therapeutic potential of antimicrobial peptides appears to be in their effectiveness as target genes for gene therapeutics in salivary glands (O'Connell *et al*, 1996). Another valuable potential application of local salivary gland gene therapeutics is to deliver growth factors or cytokines, such as EGF, keratinocyte growth factor, and IL-11, to promote mucosal wound healing (Palomino *et al*, 2000; Sonis *et al*, 2000; Dorr *et al*, 2001; Baum *et al*, 2004). In clinical or preclinical protein therapeutic studies, the above mentioned substances have shown considerable potential. Transient local expression of these genes after salivary gland gene transfer might be more effective and less expensive in promoting mucosal wound healing in patients with delayed wound healing such as diabetics.

Conclusion and future prospects

The replacement of damaged or lost tissue is a fascinating challenge, especially if the replacement can be achieved using autologous graft cells, requiring no special considerations such as mechanical degradation or immunologic reaction. Regenerative medicine 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. Furthermore, understanding of the detailed mechanisms of salivary gland development is critical for the exploitation of salivary gland regeneration therapy. Initial clinical trials (i.e., a phase I/II, dose escalation studies) using adenoviral vector encoding hAQP1 gene in patients with IR-induced parotid gland hypofunction can test the safety and efficacy of this strategy. Should this strategy prove useful, a long-lived vector with a persistent expression of hAQP1, e.g., a serotype 2 or 5 adeno-associated viral vector, may be used in the future for long-term correction of a salivary gland hypofunction induced by irradiation. Moreover, this strategy may be easily expanded to the treatment of SS and for both systemic and local (upper gastrointestinal tract) gene therapeutics.

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