Re-engineering the Functions of a Terminally Differentiated Epithelial Cell *in Vivo*

BRUCE J. BAUM,[a,d] SONGLIN WANG,[a] EDNA CUKIERMAN,[b]
CHRISTINE DELPORTE,[a] HIDEAKI KAGAMI,[a] YITZHAK MARMARY,[a]
PHILIP C. FOX,[a] DAVID J. MOONEY,[c] AND KENNETH M. YAMADA[b]

[a]*Gene Therapy and Therapeutics Branch and* [b]*Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland 20892, USA*
[c]*Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan 48109–2136, USA*

ABSTRACT: Because of their easy access, and important role in oral homeostasis, mammalian salivary glands provide a unique site for addressing key issues and problems in tissue engineering. This manuscript reviews studies by us in three major directions involving re-engineering functions of salivary epithelial cells. Using adenoviral-mediated gene transfer *in vivo*, we show approaches to i) repair damaged, hypofunctional glands and ii) redesign secretory functions to include endocrine as well as exocrine pathways. The third series of studies show our general approach to develop an artificial salivary gland for clinical situations in which all glandular tissue has been lost.

INTRODUCTION

An age-old clinical axiom states that the mouth is the gateway to the body. Indeed, many systemic diseases present with clinical signs easily recognizable in the open mouth.[1] Analogously, the mouth provides several advantages for the applied scientist or clinician interested in tissue engineering, but none is more important than ease of access.

For many years, we have been studying mechanisms by which salivary glands produce their secretions.[2,3] Salivary glands have provided a valuable experimental model for generations of scientists interested in neurofunctional controls, from Claude Bernard and Ivan Pavlov to the present day. They are highly responsive epithelial tissues whose function can be readily and non-invasively measured.[4,5]

In humans there are three major pairs of salivary glands (parotid, submandibular, and sublingual). These glands consist almost entirely of well-differentiated epithelial cells that exist as a monolayer bordering on an extensively arborized lumen.[6] Each of these major glands has a direct exit into the mouth through a single main excretory duct. Thus, from an open mouth the cannulation of the duct orifice, a procedure which in humans requires no anesthesia, affords direct access to the luminal membrane of virtually every epithelial cell in this secretory tissue. It is our belief that

[d]Author for correspondence: GTTB, NIDCR, NIH, 10 Center Drive, MSC 1190, Building 10, Room 1N113, Bethesda, MD 20892; 301-496-1363 (voice); 301-402-1228 (fax); bruce_j_baum@nih.gov (e-mail).

this situation provides a valuable model for addressing several important issues in tissue engineering. In this manuscript we will review results from two distinct areas of our tissue-engineering studies: i) the *in vivo* repair of salivary glands considered to be irreversibly damaged owing to irradiation and ii) the *in vivo* redesign of salivary glands to function in an endocrine capacity. Additionally, we will describe very new efforts in a third direction, the replacement of destroyed glands with a "first generation" artificial salivary gland.

GENERAL APPROACH

Our studies directed at *in vivo* re-engineering of salivary epithelial cells (i.e., for repair or redesign of function) have utilized replication-deficient recombinant adenoviruses to transfer genes into the target cells. After introduction of recombinant adenoviruses to the glands via intraductal delivery through the excretory duct orifice, these vectors readily infect both ductal and acinar cells in rodent glands, typically resulting in ~20–30% of the cells transduced.[7,8] While adenoviral vectors are extremely efficient at transferring genes to, and thus changing the phenotype of, a salivary cell *in vivo*, they are not without negative features. The two most significant drawbacks of recombinant adenoviral vectors are i) their inability to integrate their DNA into the host cell chromosome and ii) their induction of a potent immune response involving innate, cellular and humoral immunity.[9,10] As in other parenchymal tissues, the consequence of these drawbacks in salivary glands is the quite transient expression of the transgene.[11,12] Nonetheless, recombinant adenoviral vectors are extremely useful for proof of principle experiments.

Our studies directed at the replacement of destroyed gland tissue utilize more traditional tissue-engineering concepts.[13] Our goal is to create a functional new tissue using allogeneic graft cells grown and appropriately organized on a suitable biocompatible substratum.

IN VIVO GLAND REPAIR

Each year in the U.S. ~30,000 individuals undergo therapeutic ionizing radiation to their salivary glands during radiation therapy for head and neck maligancies.[14] Acinar cells, the fluid-, salt- and protein-secretory cell type in the glands, are most sensitive to radiation and are readily destroyed.[15] Patients whose salivary glands are thus rendered hypofunctional suffer from rampant dental caries (decay), frequent mucosal infections (such as oral Candidiasis), dysphagia (swallowing difficulties), as well as considerable pain and discomfort. At present, there is no conventional effective therapy for this condition.

Several years ago, we began examining a strategy which sought to convert the irradiation-surviving absorptive ductal epithelial cells into a secretory, water-permeable phenotype. We hypothesized that remaining ductal epithelial cells would be capable of generating a $KHCO_3$-rich fluid, in the absence of acinar cells, if they are transduced with (and express) a gene encoding a facilitated water permeability pathway, a water channel.[16] We chose to test this hypothesis by constructing a recombi-

TABLE 1. Effect of AdhAQP1 on fluid secretion from irradiated rat submandibular glands[a]

Treatment	Saliva Flow (μl/100g body weight in 15 min) ± SEM
Sham IR + Addl312	36.6 ± 6.8 (4)
IR + Addl312	12.2 ± 3.7 (6)
IR + AdhAQP1	30.6 ± 3.5 (9)

[a]Data modified from presentation in Delporte *et al.*[16] IR = 21 Gy irradiation. Addl312 is a control virus encoding no transgene while AdhAQP1 encodes human AQP1. Numbers in parentheses = number of animals studied. Irradiation (or sham treatment) was performed and 4 months later the indicated virus was administered to the submandibular glands. Saliva was collected 3 days later.

nant, type 5 adenovirus encoding aquaporin-1 (AQP1).[16] AQP1 is the archetypal mammalian water channel and generally exists in a non-polarized distribution about the plasma membrane.[17] The pivotal experiment tested the ability of the recombinant virus, termed AdhAQP1, to enhance fluid secretion from rat submandibular glands which had been exposed 4 months earlier to 21 Gy X-irradiation (TABLE 1). Animals receiving a control virus showed ~65% reduction in salivary flow rates when compared to animals that had been sham-irradiated. However, animals receiving AdhAQP1 after irradiation secreted saliva at control levels.[16] Furthermore, the saliva secreted was significantly higher in $[K^+]$ than control saliva, consistent with our hypothesized secretory mechanism.

These results provide considerable encouragement towards the possible utility of this approach for the effective clinical treatment of patients with radiation-induced salivary hypofunction. Further, the data are suggestive of the possible re-engineering of a cellular phenotype *in vivo* from a non-fluid secreting to a fluid secreting phenotype. Our recent work suggests that near maximal, osmotically obligated transepithelial water movement can be realized at low levels of cellular transduction, at least in an *in vitro* model system.[18] While this general approach, utilizing adenoviral-mediated gene transfer, is not yet ready for clinical use (especially because of the transient expression as described above), we believe it represents a significant achievement. Currently, we are examining the safety and efficacy of the strategy in a non-human primate model.

IN VIVO GLAND REDESIGN

For much of this century, there have been reports suggesting that salivary glands were capable of secreting in an endocrine (directly to the bloodstream) as well as exocrine (saliva to the mouth) manner.[19,20] While this is not a widely accepted view, physiologically, if true it would offer many significant therapeutic opportunities using gene transfer.[21] We directly tested the ability of rat salivary glands to secrete a foreign transgene product, human α1-antitrypsin (hα1AT), a secretory protein made in the liver, into the bloodstream. We administered a recombinant adenovirus, AdMLPhα1AT, to adult rats and measured hα1AT levels in serum, saliva, and gland extracts.[22] We detected hα1AT in all compartments for 4–7 days. On day 4, peak lev-

TABLE 2. Levels of hα1AT in serum from rat submandibular gland arterial and venous blood after administration of AdMLPhα1AT[a]

Sample	hα1AT (ng/ml)
Carotid Artery	19.4 ± 10.6
Submandibular Vein	67.9 ± 27.2

[a]Data modified from presentation in Kagami *et al*.[22] These represent results (mean ± SEM) obtained with 7 animals whose right carotid arteries and submandibular veins were sampled 24 hours after admistration of AdMLPhα1AT (5×10^9 pfu) to the right submandibular gland. The range of hα1AT levels in sera from carotid arteries was 0–70.7 ng/ml, while for sera from submandibular veins the levels were 5.5–223.4 ng/ml. In each of the 7 animals, the venous level of hα1AT was greater than the arterial level.

els achieved were ~5 ng/ml (serum), ~70 ng/ml (saliva), and ~10 ng/mg protein (gland extract). Furthermore, we were able to show that the concentration of hα1AT in venous blood exiting the gland was consistently higher than that in arterial blood entering the gland (TABLE 2). These studies unequivocally demonstrated that hα1AT could be secreted in an endocrine manner from rat salivary glands.[22]

The hα1AT studies did not, however, demonstrate that the transgene product secreted from the glands was functional systemically. Recently, we showed that this was possible using a different recombinant virus, AdCMVhGH, encoding human growth hormone (hGH).[21] Effective therapeutic levels of GH are ~5 ng/ml in serum, comparable to what was achieved with hα1AT. Importantly, hGH can bind to, and activate, rodent GH receptors. Forty-eight hours after intraductal administration of AdCMVhGH to rat submandibular glands, serum hGH levels were ~16 ng/ml versus background levels of ~1 ng/ml seen in control rats. We also observed a concomitant increase in serum insulin-like growth factor levels (~33%), serum triglycerides (~twofold), and the serum BUN/creatinine ratio (~35%) indicating the hGH secreted from the salivary glands was physiologically functional and systemically active.

These aggregate results strongly support the notion that salivary glands may provide a useful target site for the therapeutic delivery of transgene products for systemic use. This view also has been supported by the recent studies of Goldfine *et al*.[23] Following the retrograde instillation of plasmid DNA into salivary glands, they observed measurable levels of endocrine hormones in rat serum. Although not yet demonstrated clearly, there appears to be a constitutive secretory routing pathway to the bloodstream in glandular epithelial cells. However, our studies with hα1AT show that a transgene product can be secreted simultaneously by both the exocrine (saliva) and endocrine (serum) pathways. Currently, we are attempting to determine if there are protein-based sorting signals which we can utilize to direct a transgene product preferentially into one or the other pathway.

AN ARTIFICIAL SALIVARY GLAND?

There are many patients who effectively have lost all functional salivary epithelium, both acinar and ductal, and experience severe salivary hypofunction. These include many irradiated patients (above) as well as individuals with Primary Sjögren's

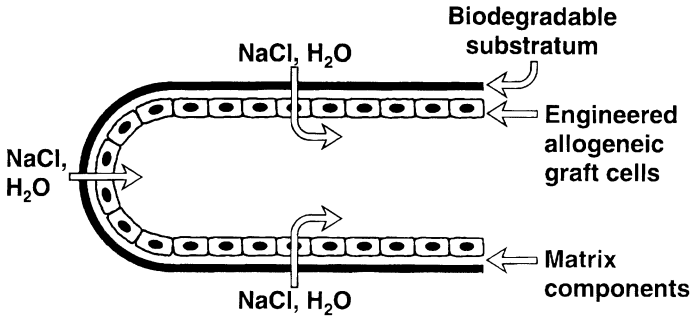

FIGURE 1. Schematic depiction of a first generation artificial salivary gland constructed as a blind-end tube. The design requirements for such a device include i) a porous, slowly biodegradable substratum; ii) matrix components (peptides or macromolecules) that promote formation of a polarized epithelial cell monolayer; iii) allogeneic epithelial cells genetically engineered to secrete water and salt unidirectionally; and iv) an overall form easily implantable in the buccal mucosal with an "exit" to the oral cavity. See text for additional detail.

syndrome, an autoimmune exocrinopathy. In the absence of any glandular epithelial tissue to manipulate by gene transfer, an alternative patient management strategy is needed. Although it is possible to transplant mammalian salivary glands,[24] this option would likely prove to be clinically inadequate because of an insufficient donor supply, the continuous need for immunosuppression, as well as the surgical difficulty. Using well-accepted tissue-engineering principles,[13] we are exploring the development of an orally implantable, artificial fluid-secreting device, a "first-generation" artificial salivary gland (FIG. 1).

The initial device which we have envisioned would consist of a blind-end tube made of a salt and water-permeable, somewhat porous, slowly biodegradable substrate. Previously, while investigating an approach to engineer intestinal tissue, Mooney *et al*. reported the construction of a tubular and biodegradable substrate which promoted vascularization following implantation.[25] This type of substrate could be engineered to contain suitable matrix components necessary to promote the polarization, growth and organizational behavior of allogeneic graft cells. The graft cells would be genetically engineered to be capable of unidirectional salt and water movement in response to autonomic neurotransmitter stimulation.[3]

Since native salivary epithelia exist essentially as a monolayer lining a lumen,[6] the substrate ideally should induce the polarized cells to form a monolayer affording complete coverage of its internal surface. Such a device also should be small enough to implant conveniently into the buccal mucosa with a maintained intra-oral exit.

Much of our initial effort has focused on the allogeneic graft cell. We have chosen to use a human submandibular gland cell line, termed HSG.[26] The phenotype of these cells is known to be highly responsive to different extracellular matrix components.[27–29] Further, they are useful targets for gene transfer methods[7] and can utilize established salivary gland cell-specific promoter elements.[30] Clearly, much additional work is needed before such an artificial fluid-secreting device can be realized, but we believe that it is conceptually feasible.

SUMMARY

This manuscript has provided an overview of three directions for our tissue-engineering studies using salivary glands as a model tissue. While salivary glands are not often considered in tissue-engineering efforts, they present an inviting target because of their easy access and organizational structure. Additionally, salivary glands suffer significant clinical disease for which there is no effective conventional therapy available. We believe that the spectrum of studies reviewed herein demonstrate salivary glands are valuable models to address key issues and problems in tissue engineering.

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