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Selective functionalization of nanofiber scaffolds to regulate salivary gland epithelial cell proliferation and polarity

Shraddha I. Cantara [a], David A. Soscia [b], Sharon J. Sequeira [a], Riffard P. Jean-Gilles [a], James Castracane [b], Melinda Larsen [a,*]

[a] Dept. of Biological Sciences, University at Albany, State University of New York, 1400 Washington Avenue, Life Sciences Bldg., Albany, NY 12222, USA
[b] College of Nanoscale Science and Engineering, University at Albany, State University of New York, 257 Fuller Road, Albany, NY 12203, USA

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ABSTRACT

Epithelial cell types typically lose apicobasal polarity when cultured on 2D substrates, but apicobasal polarity is required for directional secretion by secretory cells, such as salivary gland acinar cells. We cultured salivary gland epithelial cells on poly(lactic-co-glycolic acid) (PLGA) nanofiber scaffolds that mimic the basement membrane, a specialized extracellular matrix, and examined cell proliferation and apicobasal polarization. Although cells proliferated on nanofibers, chitosan-coated nanofiber scaffolds stimulated proliferation of salivary gland epithelial cells. Although apicobasal cell polarity was promoted by the nanofiber scaffolds relative to flat surfaces, as determined by the apical localization of ZO-1, it was antagonized by the presence of chitosan. Neither salivary gland acinar nor ductal cells fully polarized on the nanofiber scaffolds, as determined by the homogenous membrane distribution of the mature tight junction marker, occludin. However, nanofiber scaffolds chemically functionalized with the basement membrane protein, laminin-111, promoted more mature tight junctions, as determined by apical localization of occludin, but did not affect cell proliferation. To emulate the multifunctional capabilities of the basement membrane, bifunctional PLGA nanofibers were generated. Both acinar and ductal cell lines responded to signals provided by bifunctional scaffolds coupled to chitosan and laminin-111, demonstrating the applicability of such scaffolds for epithelial cell types.

1. Introduction

The salivary glands are required for producing saliva to lubricate the oral cavity, maintain the oral tissues, initiate the process of digestion and initiate an immune response against invaders that enter through the oral cavity [1]. The submandibular salivary gland contains two major epithelial cell types that produce saliva: acinar cells and ductal cells. Acinar cells produce saliva while ductal cells modify the saliva composition and transport the saliva into the oral cavity [2]. Both cell types are required for production of normal saliva. Both acinar and ductal epithelial cells are apicobasally polarized, and maintenance of this structure is critical for directional secretory function [3]. In the adult salivary gland, most epithelial cells have a basement membrane located on the basal side and a lumen on the apical side. Apicobasal polarity of such cells is maintained primarily by tight junctions (TJ) with some contribution by adherens junctions (AJs). TJs are complexes of proteins including the transmembrane proteins, claudins and occludin, and the cytoplasmic scaffolding protein, ZO-1, that are associated with the apical region of the lateral membrane of polarized epithelial cells [4–6].

In vivo, epithelial cells rest upon a fibrous network of secreted proteins, carbohydrates, and other molecules, known as the basement membrane [7] that is largely assembled by these cells. The components of the basement membrane provide signals to cells to induce and maintain apicobasal polarity [7,8]. Laminin proteins, primary components of the basement membrane, have been shown to be required for establishment and maintenance of apicobasal polarity in a variety of epithelial cell types, including renal and dental epithelia [9].

Several approaches to engineering salivary gland tissue have been proposed, and one approach is to culture cells on a scaffold, which provides signals to cells from the basal side. Salivary gland cell proliferative responses to films composed of polyvinyl alcohol (PVA), poly (ethylene-co-vinyl alcohol) (EVAL) or polyvinylidene fluoride (PVDF) have been tested [10]; however, this study did not examine cell polarity, which is important for cell function. Although culture of salivary gland cells on Matrigel, an exogenous basement

* Corresponding author. Fax: +518 442 4767.
E-mail address: mlarsen@albany.edu (M. Larsen).

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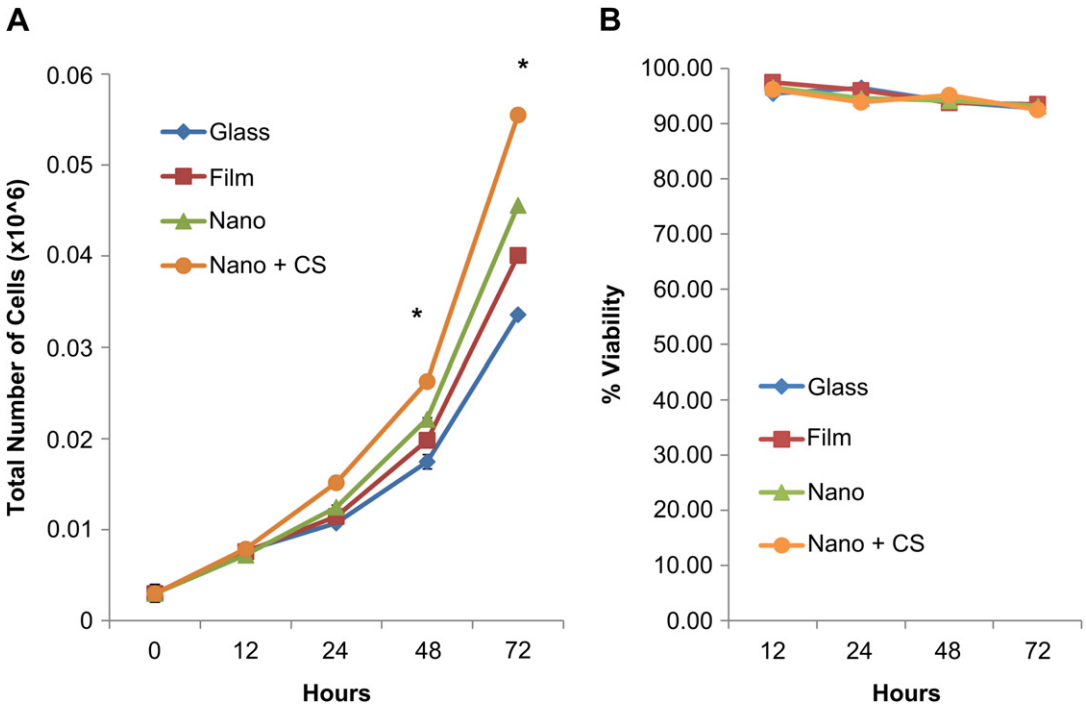

Fig. 1. Chitosan modification of nanofibers increases SIMS cell proliferation but does not affect cell viability. (A) Increased cell proliferation was observed for cells cultured on chitosan-modified scaffolds as compared with cells cultured on unmodified nanofibers and flat substrate controls for 12–72 h. Mean ± SEM of 2 experiments. One-way ANOVA with Bonferroni post tests indicate a significant difference ($^*p < 0.05$) between cells grown on chitosan-modified nanofibers and unmodified nanofibers at 48 and 72 h (B) % cell viability is not adversely affected for cells cultured on any substrates at any time point. Mean ± SEM of 2 experiments. One-way ANOVA with Bonferroni post-tests results indicate no significant difference ($p > 0.05$) between cells on any substrate type at any time point.

membrane derived from a murine tumor [11], demonstrated that Matrigel stimulates apicobasal polarity in salivary gland cells [8], this material is impractical for *in vivo* implantation in humans. Scaffolds that can direct epithelial cell behavior will be necessary for engineering a functional salivary gland.

Many polymeric and natural materials have been engineered to create scaffolds having nanoscale features that replicate structures in natural cell environments. While nanofiber scaffolds have been used extensively as scaffolds for mesenchymal cells to mimic the extracellular matrix, few studies have investigated their utility for epithelial cells. We previously generated poly-lactic-co-glycolic acid (PLGA) scaffolds to mimic the fibrillar characteristics of the basement membrane and showed that SIMS cells, a salivary gland ductal epithelial cell line, can attach and proliferate on these PLGA nanofiber scaffolds [12]. Additionally, the morphology of SIMS cells and PARC10 cells, salivary gland cell lines, cultured on the nanofibers more closely resembled that of adult salivary gland cells *in vivo*. These cells self-organized into clusters and exhibited less cell spreading, as expected of epithelial cells *in vivo*. Additionally, the epithelial cells produced fewer organized focal adhesions, which are typically produced by mesenchymal cells and also by cells cultured on artificial flat surfaces [13]. Thus, the PLGA nanofiber scaffolds appeared to support epithelial cell morphology; however the effect on apicobasal polarity was not determined.

In this study, we covalently attached molecules to the nanofiber scaffolds to "functionalize" the scaffolds to better replicate the properties of native basement membrane. We attached two molecules to the scaffolds, both individually and in combination, to modulate cell proliferation and cell polarity independently: chitosan, a deactylated form of the crustacean exoskeleton molecule, chitin, and the basement membrane protein, laminin-111. We examined the responses of salivary gland acinar and ductal cells to the functionalized scaffolds by examining both cell proliferation and apicobasal polarity.

2. Materials and methods

2.1. Cell culture

Two immortalized salivary gland cell lines were used: SIMS cells, an immortalized mouse ductal submandibular epithelial cell line [14] and SMGC10 cells, an immortalized rat submandibular acinar epithelial cell line [15]. SIMS cells were cultured in Dulbecco's High Glucose Modified Eagle Medium (DMEM) (Invitrogen Cat No. 11995) supplemented with 10% Heat-Inactivated Fetal Bovine Serum (HI FBS) (Invitrogen Cat No. 10082) and Penicillin–Streptomycin (1×) (Invitrogen Cat. No. 15140), as previously reported [13]. SMGC10 cells were cultured as previously published [15] with minor modifications in Dulbecco's Modified Eagle Medium (DMEM)/F-12 with Phenol Red, 15 mM HEPES, and L-Glutamine (Invitrogen Cat No. 11330) supplemented with 5 μg/ml insulin (Sigma Cat No. 16634), 0.1 μM retinoic acid (Sigma Cat No. 2625), 2 nM L-3,5,3′-triiodothyronine (T_3) (Sigma Cat No. T-5516), 1 μM hydrocortisone (Sigma Cat No. H-0135), 4 μg/ml transferrin (Invitrogen Cat No. 11105-021), 50 ηg/ml epithelial growth factor (EGF), (Peprotech Cat. No. AF-100-15), 2 mM glutamine (Invitrogen Cat No. 11330), trace element mix (MP Biomedicals Cat No. 091676649), 50 μg/ml gentamicin (Invitrogen Cat No. 15710-064), and 2.5% HI FBS. Both cell types were maintained in 10 cm polystyrene tissue culture plates (Corning Cat. No. 430167) at 37 °C with 5% CO_2 in a humidified incubator and passaged at 80–90% confluence.

2.2. Making of PLGA fiber scaffolds and film

PLGA fiber scaffolds were generated by electrospinning using a design of experiment (DOE) approach, as previously described [12,13]. Briefly, PLGA polymer containing a lactic to glycolic acid ratio of 85:15 and a molecular weight of 95,000 Da (Birmingham Polymers) was dissolved in hexafluoroisopropanol (HFIP) with 1% NaCl (*w*/*v*); an 8% (*w*/w) solution was used for nanofibers while an 18% solution was used for microfibers. The polymer solutions were ejected out of a syringe using an automated syringe pump at a flow rate of 3 μl/min for nanofibers and 10 μl/min for microfibers. Constant voltage was provided by attaching a wire from the power supply to the needle. Voltage for nanofibers was 10 kV while voltage for microfibers was 12 kV. The needle was suspended vertically over a grounded aluminum collector plate at a distance of 15 cm. Pre-cleaned 12 mm diameter glass coverslips