Faculty and Staff Conference Proceeding:

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Citation for Article:

Foraida, Z. I., Sharikova, A., Peerzada, L. N., Khmaladze, A., Larsen, M., & Castracane, J. (2017, August 29). Double emulsion electrospun nanofibers as a growth factor delivery vehicle for salivary gland regeneration. *Proc. SPIE 10352, Biosensing and Nanomedicine X, 103520E*. doi:10.1117/12.2275489

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SPIE.

Event: SPIE Nanoscience + Engineering, 2017, San Diego, California, United States

Double emulsion electrospun nanofibers as a growth factor delivery vehicle for salivary gland regeneration

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ABSTRACT

Sustained delivery of growth factors, proteins, drugs and other biologically active molecules is necessary for tissue engineering applications. Electrospun fibers are attractive tissue engineering scaffolds as they partially mimic the topography of the extracellular matrix (ECM). However, they do not provide continuous nourishment to the tissue. In search of a biomimetic scaffold for salivary gland tissue regeneration, we previously developed a blend nanofiber scaffold composed of the protein elastin and the synthetic polymer polylactic-co-glycolic acid (PLGA). The nanofiber scaffold promoted *in vivo*-like salivary epithelial cell tissue organization and apicobasal polarization. However, in order to enhance the salivary cell proliferation and biomimetic character of the scaffold, sustained growth factor delivery is needed. The composite nanofiber scaffold was optimized to act as a growth factor delivery system using epidermal growth factor (EGF) as a model protein. The nanofiber/EGF hybrid nanofibers were synthesized by double emulsion electrospinning where EGF is emulsified within a water/oil/water (w/o/w) double emulsion system. Successful incorporation of EGF was confirmed using Raman spectroscopy. EGF release profile was characterized using enzyme-linked immunosorbent assay (ELISA) of the EGF content. Double emulsion electrospinning resulted in slower release of EGF. We demonstrated the potential of the proposed double emulsion electrospun nanofiber scaffold for the delivery of growth factors and/or drugs for tissue engineering and pharmaceutical applications.

Keywords: Tissue regeneration, nanofibers, epidermal growth factor, growth factor delivery, Raman spectroscopy.

1. INTRODUCTION

In the field of tissue regeneration, implantable scaffolds are used to direct cell organization and growth *in-vivo*. This often requires the supplementation with growth factors, proteins, vitamins, peptides, etc.¹ If administered systemically, the molecule of interest is largely metabolized by other organs and the amount reaching the target tissue may not be sufficient to induce the desired action. This may necessitate the use of larger multiple doses, which typically leads to side effects. Therefore, a scaffold that continuously delivers the molecule of interest specifically to the target tissue is promising². This is especially important for epithelial tissues because they are avascular and therefore, mainly nourished through molecules that diffuse out of the extracellular matrix¹.

The salivary glands are hollow branched epithelial structures that may be permanently damaged due to head and neck radiation therapy and autoimmunity. Damage of the salivary glands is associated with hyposalivation. Hyposalivation negatively affects speaking, swallowing as well as the oral and digestive health. Tissue regeneration of the glands utilizing biomaterial-based scaffolds is a promising long-term treatment of the condition³⁻⁶.

Electrospun fibers, which are designed to mimic the topography of the ECM, are one of the best structures for drug and growth factor delivery when compared to other carrier systems such as nanoparticles, liposomes and dendrimers. They have high loading efficiency due to their large surface area. In addition, they are very straight-forward to synthesize and their properties can be optimized in many ways to tailor the active constituent release profile⁷⁻⁸. In this study, we optimized our previously-developed elastin/polylactic-co-glycolic acid (PLGA) hybrid nanofiber scaffolds for slow delivery of growth factors using the growth factor epidermal growth factor (EGF) as a model since EGF is known to stimulate branching morphogenesis of embryonic mouse submandibular salivary glands⁹⁻¹⁰. We controlled the release of the growth factor by double-emulsion electrospinning and the use of ethyl cellulose (EC) as a release retardant and

emulsifier. Double-emulsion electrospinning is a more sophisticated method than blend electrospinning; it aims at enclosing the growth factor or molecule of interest within emulsion droplets (inner phase) which is distributed within a continuous phase which is further emulsified within another immiscible layer containing the bulk polymers of the nanofibers⁷⁻⁸. With this method, EGF is entrapped within the inner phase of a water in oil in water emulsion (w/o/w), while elastin and PLGA are in the outer phase. We hypothesize that this will decrease EGF diffusion out of the nanofibers as it must pass a longer distance and it must pass through an ethyl cellulose layer. The proposed scaffold was compared to another prepared by homogeneous blend electrospinning of EGF with elastin and PLGA as one phase. The release profile of EGF was characterized for each type of fiber.

2. MATERIALS AND METHODS

2.1. Materials

Epidermal Growth Factor from murine submaxillary gland (Cat. No. 62229-50-9), was purchased from Sigma Aldrich (St. Louis, MO). Soluble (hydrolyzed) elastin (Cat. No. ES12) was purchased from Elastin Products (Owensville, MO), poly(D-lactide-co-glycolide) (PLGA), (Cat. No. B6006-1) with a molecular weight of 95000 Da and a lactic to glycolic acid ratio of 85:15 was purchased from Birmingham Polymers (Pelham, AL). Hexafluoroisopropanol (HFIP) (Cat. No. 105228) and Ethyl cellulose (Cat. No. 45-247499), were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Preparation of elastin-PLGA nanofibers (Control)

Equal weights of 12% w/w soluble elastin in HFIP (hydrolyzed elastin from bovine neck ligament) and of 12% w/w PLGA in HFIP were mixed together to yield a solution containing 4% elastin and 4% PLGA (w/w). Sodium chloride (1% w/w) was added to the solution before electrospinning to minimize beading. The solution was pumped through a 3-mL syringe, which was connected to an automated micro-syringe pump through PTTE tubing to a (25 G) needle. The needle tip and the aluminum foil-coated collector ground were both connected to a high voltage source (14 kV). The voltage was applied to the needle tip and the collector ground while pumping the blend polymer solution at a rate of 6 $\mu\text{L}/\text{min}$ and a distance between the needle tip and the collector ground of 15 cm. The nanofibers were formed and collected on the collector ground. For cell culture experiments, the fibers were electro spun on 13 mm glass coverslips sitting on the foil surface. The glass coverslips were covered with an adhesive layer of 3% (w/w) PLGA in HFIP solution that polymerized for 45 minutes before electrospinning to prevent the delamination of the fibers when soaked for extended periods of time. The obtained nanofibers were labelled (Control).

2.3. Growth factor loading by blend electrospinning (Blend)

Blend electrospinning of growth factors and drugs is a conventional method where the loaded molecule is homogeneously distributed within the electrospinning solution and electrospun as one entity. Briefly, 100 μg of Epidermal Growth Factor from murine submaxillary gland (EGF) was reconstituted in 0.2 mL of 1% BSA in PBS to obtain a final concentration of 0.5 $\mu\text{g}/\mu\text{L}$ of EGF. A volume of 30 μL of EGF/BSA solution therefore contains 15 μg of EGF, which was added to a mixture solution of 0.75 g 12% elastin in HFIP and 0.75 g PLGA in HFIP to yield a final concentration of 4% elastin/4% PLGA and 10 ng/mg of EGF. Sodium chloride (1% w/w) was added to the solution before electrospinning to minimize beading. The solution was electrospun in a similar fashion to the control nanofibers. The obtained nanofibers were labelled (Blend).

2.4. Growth factor loading by double emulsion electrospinning (Emulsion)

Briefly, similar to blend electrospinning, we aimed at obtaining a 10 ng EGF/mg nanofiber concentration. Therefore, 30 μL of (0.5 $\mu\text{g}/\mu\text{L}$) solution of EGF in 1%BSA solution was added dropwise to 0.5 g of chloroform solution containing 2% (w/w) ethyl cellulose and mixed by vortexing for 5 minutes to yield a creamy emulsion. This emulsion was introduced dropwise to a mixture solution of 0.5 g of 12% elastin/HFIP solution and 0.5 g of 12% PLGA solution. The emulsion was further mixed by vortexing for 5 minutes. The final concentrations are 10 ng EGF/1 mg emulsion, 4% elastin (w/w) and 4% PLGA (w/w). The double emulsion system was electrospun using the same parameters as blend electrospinning. The nanofibers obtained were labelled (Emulsion).

2.5. SEM Characterization of the scaffolds

Scanning electron microscopy (SEM) imaging of the scaffolds was carried out using a Zeiss 1550 field emission scanning electron microscope (Leo Electron Microscopy Ltd., Cambridge, UK; Carl Zeiss, Jena, Germany), as described previously¹¹. Briefly, the scaffolds were mounted on 1 cm² stubs and coated with approximately 5 nm of gold-palladium to minimize sample charging. Images were captured using an in-lens detector, 1-5 kV acceleration voltage and a working distance of 2-6 mm. The microscope annotation software was used to apply scale bars, and ImageJ software was used to measure the fiber diameters from calibrated images. At least 4 scaffolds of each type were imaged and 200 nanofibers analyzed for average fiber diameter measurements.

2.6. Raman spectroscopy characterization of the nanofibers

Raman spectra of EGF, Ethyl Cellulose, and PLGA were measured using XploRA Plus confocal scanning microscope (HORIBA) with Sincerity detector. The excitation wavelength was 533 nm. The diffraction grating of 600 gr/mm was employed together with 200 μ m spectrometer slit, 40 $\times$ microscope objective, and a confocal aperture of 100 μ m. Acquisition time was 120 s times 2 accumulations for all measurements. Spike filter based on multiple accumulations was engaged.

To obtain the spectra of individual fiber components, pure droplets of each component on aluminum foil were used.

The spatial distribution measurement of blend and emulsion fibers (confocal Raman mapping) was performed using the same XploRA Plus system that was used for the collection of point spectra. The system settings were the same, except that the laser power was reduced by 50% to avoid burning the fibers, and the acquisition was 60 s times 5 accumulations per point. The confocal scanning was done either across or along the fiber. All spectra were subjected to the polynomial baseline correction routine in LabSpec software (HORIBA).

2.7. EGF-Release profile

Three known weights of each EGF-loaded nanofiber scaffold were soaked in 1 mL 1X PBS and incubated at 37 °C. At different time points, 100 μ L of the soaking PBS solution was collected and stored at -20 °C. The collected sample was replenished with fresh PBS solution to keep the volume constant. The collected samples from different time intervals were analyzed for their EGF content using EGF ELISA kit and following the manufacturer's instructions. The released amount at each time point was calculated as the average from 3 samples and represented as % cumulative release.

3. RESULTS

3.1. Morphological characterization of the nanofiber scaffolds

To assess the effect of the growth factor loading by blend and emulsion electrospinning on the nanofiber morphology as well as to choose the best formulation, we used SEM imaging to study the morphology of each scaffold type. Figure 1 shows representative SEM images of each EGF-loaded nanofiber type as compared to control nanofibers. As shown, the EGF-loaded nanofibers prepared by blend electrospinning, Figure 1b, double emulsion electrospinning, Figure 1c, are predominantly homogeneous with an average nanofiber diameter ~370 nm. This diameter is larger than the control nanofibers with an average diameter of 308 nm, Figure 1a. The increase in the nanofiber diameter may be attributed to the changes in the solution characteristics upon adding EGF.

Figure 1. SEM images of the nanofiber scaffolds. (a) Unloaded nanofibers (control), (b) Blend electrospun EGF (Blend), (c) Emulsion electrospun EGF (Emulsion). Scale bar, 2 μm .

3.2. Raman Spectroscopy Mapping of the nanofibers

In order to identify the localization of EGF within the fibers prepared by blend and emulsion electrospinning, we mapped the nanofibers using Raman spectroscopy. Figure 2a shows spectra of PLGA, EC and EGF films, which were subsequently used for fiber characterization. Pure PLGA, EC and EGF components were used as basis for Classical Least-Squares (CLS) linear regression routine in LabSpec software (HORIBA). Figure 2b shows the spectrum taken from the center of the blend nanofiber, which contains all three materials. The algorithm shows the presence of PLGA/EC/EGF, which together closely match the actual measured spectrum. It is worth noting that the strength of Raman signal is generally a function of both the amount of material within the excitation volume and the material's Raman cross-section. Therefore, the Raman peak intensity cannot be taken as a measure of the relative amount of a particular component.

Figure 3a shows the one-dimensional scan area, taken across the blend fiber. The distribution of CLS components is shown in Figure 3b, indicating no significant variations of each material within the blend. Since the size of the diffraction limited laser spot is significant comparing to the size of the fiber, even when the center of the beam is positioned just off the edge of the fiber, some part of the excitation volume still contains the fiber and thus produce Raman signals, making fiber's Raman signature to appear thicker than the actual fiber.

Next, Figure 4a shows the scan taken across the emulsion fiber, which is expected to contain the "islands" of EGF embedded in the fiber. As the scan in Figure 4b shows, while the part of the cross-section scan of the fiber (left side) contains almost no traces of EGF, the other side of the fiber contains clear traces of EGF. Finally, Figure 5a shows the scan taken along the emulsion fiber. The variation of the EGF component (Figure 5b) is a clear indication of the presence of EGF islands along the fiber.

Collectively, the findings suggest that emulsion electrospinning was successful in incorporating EGF in the form of embedded islands enclosed within ethyl cellulose farther from the nanofiber surface. On the other hand, blend electrospinning resulted in uniform distribution of EGF to both the surface and the bulk of the nanofibers.

3.3. EGF release profile

Sustained release of EGF from the nanofiber scaffolds to ensure continuous nourishment of the tissue post-implantation is one of the goals of this study. Therefore, we characterized the cumulative release behavior of EGF from the various substrates using ELISA, Figure 6. As shown, all the substrates show initial burst release of EGF followed by a slower release phase (almost plateau). As expected, the initial burst release is faster in blend electrospun EGF (Blend) where almost complete release of EGF is observed at 10 days, while it is slower in double emulsion electrospun nanofibers (Emulsion). The slower release profile in emulsion electrospun nanofibers is desirable and expected due to the inclusion of EGF within the double emulsion system and hence hindering its diffusion.

(a)

(b)

Figure 2. (a) Raman reference spectra of PLGA, EC and EGF films and (b) Spectra obtained from the center of the blend fiber, containing all three materials. The result of CLS fit of basic components of PLGA, EC and EGF is also shown.

Figure 3. (a) Optical image showing sample surface mapping recorded perpendicular to the blend fiber, (b) The distribution of CLS components of PLGA, EC and EGF, indicating a nearly uniform blend. 3-point moving average was applied to CLS components to reduce the appearance of random noise.

Figure 4. (a) Optical image showing sample surface mapping recorded perpendicular to the emulsion fiber and (b) The distribution of CLS component EGF, indicating the presence of an EGF “island” in the emulsion. The arrows indicate the location of the boundaries of the fiber (based on the EC signature, which is not shown). 3-point moving average was applied to CLS component to reduce the appearance of random noise.

Figure 5. (a) Optical image showing sample surface mapping recorded along the emulsion fiber and (b) The distribution of CLS component EGF, indicating the presence of multiple EGF “islands” in the emulsion (indicated by arrows). 3-point moving average was applied to CLS component to reduce the appearance of random noise.

Figure 6. Cumulative release profile of EGF from the various scaffolds.

4. CONCLUSIONS

A successful tissue regeneration scaffold for epithelial tissues should ideally provide sufficient nutrients and growth factors to the target tissue especially in the initial stages of regeneration. Sustained release of these factors from an implantable device is necessary to eliminate the need to systemically supplement multiple times a day. Here, we developed EGF-loaded elastin-PLGA nanofibers by blend and emulsion electrospinning. Emulsion electrospinning was successful in localizing the growth factor within a double emulsion system (w/o/w) as indicated by Raman spectroscopy studies. This resulted in favorable slower release of EGF over a duration of 10 days. The designed EGF-double emulsion electrospun nanofibers may have potential as an implantable tissue regeneration device.

ACKNOWLEDGEMENTS

This research was funded by the National Institutes of Health, National Institute of Dental and Craniofacial Research R01DE022467 to M.L and J.C., National Institutes of Health C06 RR015464 to the University at Albany, SUNY, National Science Foundation MRI award DBI0922830 to CNSE, and the University at Albany, SUNY.

REFERENCES

- [1] Ozdemir, T., Fowler, E. W., Hao, Y., Ravikrishnan, A., Harrington, D. A., Witt, R. L., Farach-Carson, M. C., Pradhan-Bhatt, S., Jia, X., "Biomaterials-based strategies for salivary gland tissue regeneration," *Biomaterials Science* 4(4), 592–604 (2016).
- [2] Lee, K., Silva, E. A., Mooney, D. J., "Growth factor delivery-based tissue engineering: general approaches and a review of recent developments," *Journal of The Royal Society Interface* 8(55), 153–170 (2010).
- [3] Eveson, J.W., "Xerostomia," *Periodontology* 2000, 48(1),85-91(2008).
- [4] Kagami, H., Wang, S., Hai, B., "Restoring the function of salivary glands," *Oral Diseases* 14(1),15-24 (2007).
- [5] Villa, A., Connell, C., Abati, S., "Diagnosis and management of xerostomia and hyposalivation," *Therapeutics and Clinical Risk Management* 11,45-51 (2015).
- [6] Yoo, C., Vines, J. B., Alexander, G., Murdock, K., Hwang, P., Jun, H.-W., "Adult stem cells and tissue engineering strategies for salivary gland regeneration: a review," *Biomaterials Research* 18(1), 9-21(2014).
- [7] Valizadeh, A., Farkhani, S. M., "Electrospinning and electrospun nanofibres," *IET Nanobiotechnology* 8(2), 83–92 (2014).
- [8] Zhang, Y., Lim, C. T., Ramakrishna, S., Huang, Z.-M., "Recent development of polymer nanofibers for biomedical and biotechnological applications," *Journal of Materials Science: Materials in Medicine* 16(10), 933–946 (2005).
- [9] Häärä, O., Koivisto, T., Miettinen, P. J., "EGF-receptor regulates salivary gland branching morphogenesis by supporting proliferation and maturation of epithelial cells and survival of mesenchymal cells," *Differentiation* 77(3), 298–306 (2009).
- [10] Dhulekar, N., Ray, S., Yuan, D., Baskaran, A., Oztan, B., Larsen, M., Yener, B., "Prediction of Growth Factor-Dependent Cleft Formation During Branching Morphogenesis Using a Dynamic Graph-Based Growth Model," *IEEE/ACM Transactions on Computational Biology and Bioinformatics* 13(2), 350–364 (2016).
- [11] Soscia, D. A., Sequeira, S. J., Schramm, R. A., Jayarathanam, K., Cantara, S. I., Larsen, M., Castracane, J., "Salivary gland cell differentiation and organization on micropatterned PLGA nanofiber craters," *Biomaterials* 34(28), 6773–6784 (2013).