Development of an Angiogenesis-Promoting Biomaterial Sleeve for Subcutaneously Implanted Glucose Sensors

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Statement of Purpose: Diabetes mellitus (DM) is a disease that affects over 25 million Americans today, about 8 percent of the population [1]. An indwelling, closed-loop insulin delivery device that monitors blood glucose levels and delivers insulin accordingly would represent a significant improvement in quality of life for these individuals; however, reliable long-term glucose sensing has proven to be a major roadblock. Currently available glucose sensors survive less than a week before poor vascularization around the sensor lead results in their failure. We have previously developed a porous, dexamethasone-eluting polyurethane-based coating for the sensor lead that aims to inhibit scar tissue formation around the lead. In this project we have developed a methodology for the incorporation of bioactive peptides such as RGD, SVVYGLR, IKVAV into the existing sleeve design with the goal of promoting blood vessel formation and retention.

Methods: Textured sleeves were made using a dip-coating method using straightened copper wire as mandrels. A Tecoflex® mixture was made in 3:1 chloroform:ethanol. Next, porogen (ammonium bicarbonate crushed and sieved to 50-70 µm), drug (dexamethasone), and bioactive peptide (e.g. RGD[2], Peptite-2000®) were incorporated into it. Finally, wire mandrels were dip-coated in this mixture and dried. Porogenation process was carried out by soaking sleeves in 95°C water and then quenching in 4°C water, and drying. Drug-loaded and peptide conjugated sleeves (See Figure 1.) were characterized using scanning electron microscopy (SEM) and confocal microscopy. Additionally, the angiogenic ability of the sleeve design was assessed using a Human Umbilical Vein Endothelial Cells (HUVEC) based tubulogenesis assay. For this assay, we used sleeves with varying combinations of Tecoflex texturing, RGD, and dexamethasone with 4 replicates per condition. For each replicate, we placed a sleeve glued to a coverslip with Silicone (PDMS) in one well of a 12 well plate. Then, we seeded HUVEC in each well at a seeding density of 75,000 cells/cm^2 and observed their growth over 5 time points spread over 14 days.

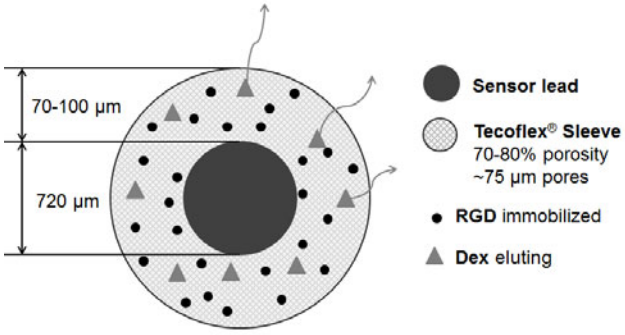

Figure 1. Sensor sleeve cross-section schematic (not to scale).

Results: High resolution SEM imaging confirmed that the inclusion of bioactive peptide did not create any significant change in Tecoflex® sleeve microstructure (see Figure 2 A and B). Labeling RGD with DyLight 488 and imaging the fluorescently-labeled RGD loaded sleeve with a confocal microscope confirmed peptide distribution in the sleeve (see Figure 2 C.).

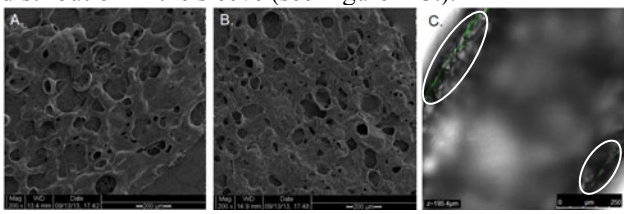

Figure 2. SEM images of textured Tecoflex® sleeve surface that is (A.) blank and (B) loaded w/ RGD; (C) Confocal z-stack imaging of sleeve annulus confirmed presence of fluorescently tagged RGD peptide (green) in sensor sleeve cross-section.

Assessing our conditions for tube formation was difficult due to the complex 3-D nature of the scaffold and low cell density. Despite a very high seeding density, the cells on top of the sleeve either migrated off or died quickly, likely because of the poor adhesive properties of Tecoflex®. However, we did see the morphology change from rounded to stretched out associated with *in vitro* tubulogenesis in some of our conditions. These morphology changes were noticeable in our positive control conditions (Tecoflex® sleeve in Matrigel®, with or without VEGF, which also exhibited tubular-network formation), and conditions containing RGD indicating improved cell-adhesion.

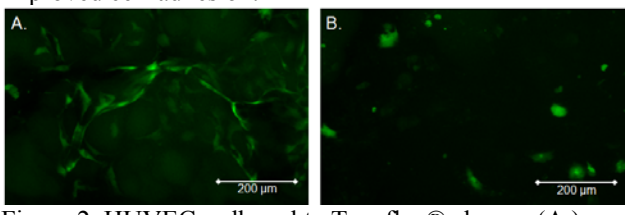

Figure 2. HUVECs adhered to Tecoflex® sleeves (A.) coated w/ Matrigel® and (B) loaded w/ RGD peptide.

Conclusions: A methodology has been developed for the presentation of bioactive peptides on textured Tecoflex® sleeves for glucose biosensor leads. Incorporation of RGD peptide improved the cell adhesiveness of the sleeve material, although optimization is needed. Ongoing experiments are investigating incorporation of additional other bioactive peptides such as SVVYGLR and IKVAV for improving cell spreading, migration, and angiogenesis outcomes on the textured sleeves.

References: [1] Centers for Disease Control and Prevention. Atlanta, GA. 2011.
[2] Craig WS, et al. Biopolymers. 1995;37(2):157-175.