In vivo Evaluation of Porous Dexamethasone Releasing Coatings for Glucose Biosensors in Diabetic Rat Animal Model

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Statement of Purpose: Type I diabetes affects millions of patients in the US each year. Due to poor glucose control, patients with diabetes experience impaired wound healing, poor circulation, ketoacidosis, and an increased incidence of blindness. One of the most promising glucose management techniques, is the use of implantable biosensors for continuous monitoring. Unfortunately, the long-term use of such devices has been limited due to the Foreign Body Response (FBR). In order to improve biocompatibility of glucose sensors, we have developed porous polyurethane coatings that release dexamethasone (DX). By combining drug delivery and texturing techniques, we have created coatings capable of curving inflammation and promoting vascularization.

For the present study, we characterized the porous coatings' morphology, drug release, effect on sensor response, and drug bioactivity *in vitro*. To evaluate the effects of coatings on the FBR, we subcutaneously implanted coated non-functional glucose sensors in normal and diabetic rats. We then analyzed and compared the FBR in both models over the short (3-7 days) and long-term (14-21 days).

Methods: Porous Tecoflex® 93A dexamethasone releasing coatings were fabricated using the salt-leaching/gas foaming technique. Porosity, pore size, and thickness were evaluated employing MicroCT. Dexamethasone release was monitored over a period of 15 days via HPLC. *In vitro* sensor calibration and glucose challenges were performed on Medtronic MiniMed Sof-Sensor ™. Bioactivity assessment of the coatings was performed by measuring apoptosis of peripheral blood derived human monocytes. Apoptosis was determined via Annexin V staining and quantified by flow cytometry. Non-functional glucose sensors with and without drug releasing coatings were subcutaneously implanted in normal and diabetic rat animal models. One month pre-implantation, diabetes was induced in normal rats by delivering 40mg/Kg of streptozotocin daily via i.p. injection for 3 days. Coatings were implanted directly into the dorsum of rats and retrieved 3, 7, 14, and 21 days post-implantation. Histological evaluation of implants allowed for the study of the FBR. Immune cell infiltration was assessed via H&E and CD68 macrophage marker staining. Collagen deposition was evaluated via Masson's Trichrome stain and vascularization was detected via CD31 immunohistochemistry.

Results: Porous-DX releasing coatings of controlled microstructure were successfully fabricated. MicroCT evaluation of coatings showed an average pore size of 78.5 ± 6.75 µm, coating thickness of 100.5 ± 13.45 µm, and porosity of 81 ± 9.4 % (Figure 1a). Dexamethasone release studies showed a burst release, with 83% being deployed within the first 5 days and slower release kinetics over subsequent days (Figure 1b). Porous coatings did not significantly affect sensor response time, signal linearity, or sensitivity (Figure 1c). Dexamethasone released from our coatings proved to maintain its bioactivity by significantly enhancing apoptosis of human derived peripheral blood monocytes (Figure 1d).

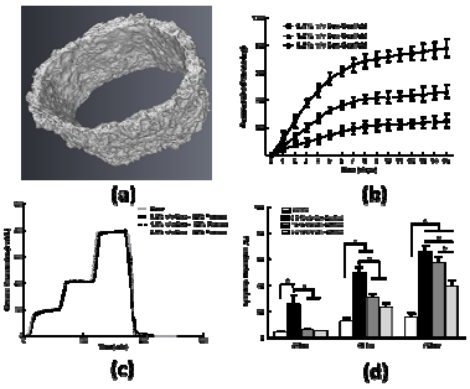

Figure 1. Characterization and testing of DX-Tecoflex® 93 A coatings for Medtronic glucose sensors,(a) MicroCT reconstruction of porous coatings (b) coatings exhibit controlled DX release over 14 days, (c) Sensor performance is not negatively affected by porous coatings and (d) dexamethasone released form porous coatings induces apoptosis in human monocytes.

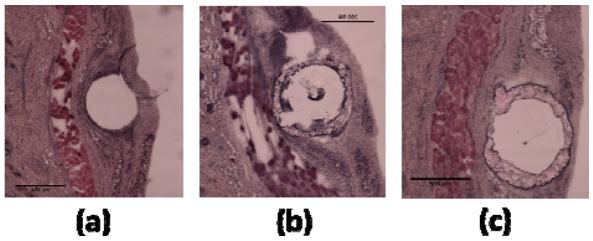

Figure 2. Dexamethasone releasing coatings decrease local inflammation and immune cell infiltration in diabetic rats at day 3 post-implantation. (a) Bare Sensor, (b) Porous Coating – no DX, (c) Porous Coating – DX.

Subcutaneous rat studies of non-functional glucose sensors showed that porous-DX releasing coatings effectively decreased tissue inflammation within the initial 3 day period in normal and diabetic rats (Figure 2). Preliminary results also show that macrophage infiltration was curved in diabetic rats when compared to normal rats. This suggests that diabetic rats mount a less aggressive immune response to sensor implantation. Finally, porous-DX releasing coatings appeared to reduced collagen deposition, and increase CD31 expression (vascularity) for 14 and 21-day implants in both groups.

Conclusions: We were able to successfully fabricate and characterize porous Tecoflex® 93A dexamethasone releasing coatings. This coatings proved to be effective at modulating inflammation and improving tissue remodeling to glucose sensor implantation in normal and diabetic animal models.

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