Macrophage Embedded Fibrin Gels as a Novel *In Vitro* Platform for Assessing Implantable Glucose Sensor Performance

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Introduction: Contemporary implantable glucose sensing paradigms are only approved by the FDA for up to one week. Recent research has suggested that interaction between the implanted tissue and the sensor is the main culprit in short sensor life [1]. Early interactions between the sensor and the tissue are mediated by two processes: protein adsorption and cellular adhesion. Previous research by the authors suggests that the consumption of glucose by adhered inflammatory cells proximal to the sensor surface is the main cause for limited sensor response [2]. To further investigate this proposal, an inflammatory cell embedded fibrin gel was fabricated *in vitro* as a bioartificial surrogate to the provisional matrix that forms around the sensor early after implantation. These constructs were then subsequently wrapped around otherwise functioning sensors to analyze the effects of adhered inflammatory cell presence on sensor performance. The signal decline observed in the presence of the cell embedded gels mimics sensor response in whole blood, further demonstrating the deleterious effects of inflammatory cells on sensor function.

Methods: Gels were fabricated based upon a protocol by Bian, et al. [3]. Briefly, 1 mL fibrin gel constructs were made by combining two solutions: (1) 200 µL bovine plasma fibrinogen (10 mg/mL), 100 µL matrigel and 480 µL of cells suspended in media at 10^7 cells/mL and (2) 240 µL of 2x cell media and 20 µL bovine thrombin. The inflammatory cells used for this study were RAW 264.7 murine macrophages. Once the two solutions were combined in a cylindrical mold, the gel volume was allowed to crosslink for 45 minutes at 37°C. The constructs were removed from the mold and incubated in cell media at 37°C until used. Cell viability within the gel was assessed and confirmed via Calcein AM and Hoechst 33342 staining.

Before beginning an experiment, commercially available Medtronic Minimed SofSensors were allowed to equilibrate in RAW 264.7 cell culture media with a known glucose concentration of 100 mg/dL for 4 hours until a steady signal could be maintained. Experiments were then performed comparing sensor performance for three cases: (1) a sensor surrounded by a macrophage embedded gel, (2) a sensor surrounded by an acellular fibrin gel, and (3) a bare sensor. All three were then returned to the RAW 264.7 media with 100 mg/dL glucose and allowed to gather glucose readings for 20 hours. These results were then compared to whole blood results and computational findings previously published by this group [2].

Results/Discussion: Figure 1 is a trace of normalized glucose sensor current (unitless) as a function of time for each of the three cases described in the Methods section. As sensor signals exhibit device to device variability in the same solution, each signal trace was normalized to its steady state equilibration sensor current. As predicted, the bare sensor maintained a steady signal throughout the course of the experiment.

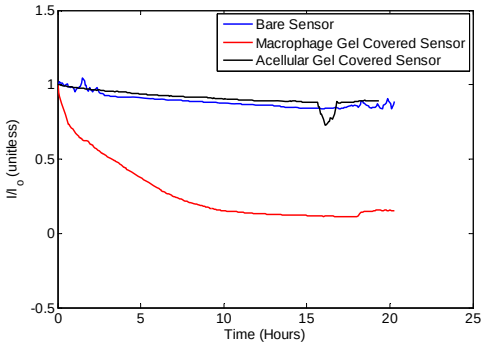

Fig. 1: Normalized sensor current as a function of time.

The rate at which the signal diminishes in the macrophage case is similar in both magnitude and shape to decreases observed in whole blood [2]. Moreover, the macrophage gel trace in Figure 1 closely mirrors computation models of glucose consumption in the provisional matrix previously reported by this group [2]. An acellular gel was found to have no deleterious effect when wrapped around a sensor, as its trace is similar to that of a bare sensor. Taken together, these data demonstrate the effect of inflammatory cell aggregation on sensor response.

Summary: Through the design of a novel *in vitro* surrogate of the provisional matrix, cellular glucose consumption was found to decrease sensor response in this early stage model of implant associated inflammation. This finding suggests that the environment around the sensor is acting as a sink for glucose, preventing the sensor from accurately sampling interstitial glucose. By understanding the modes by which sensors fail soon after implantation, sensor surfaces may be modified to account for these effects.

References: [1] Wisniewski, N. Colloids and Surfaces B. Biointerfaces 2000 18: 197-219. [2] Novak, et al. Diabetes Sci and Tech 2013 7(6): 1547-1560. [3] Bian, et al. Nature Protocols 2009 4: 1522-1534.