NDGA-reinforced 3D Porous Collagen Scaffold for Implantable Glucose Sensor

Young Min Ju[1], Bazhang Yu[2], Yvonne Moussy[3] and Francis Moussy[1,2]

[1]Biomedical Engineering Program, [2]Department of Chemical Engineering, [3]Department of Mechanical Engineering, University of South Florida, 4202 E. Fowler Avenue, ENB 118, Tampa, FL 33620-5350, USA

Statement of Purpose: Although many new technologies for glucose sensing have been developed over the past 30 years, achieving reliable continuous glucose monitoring is still a very difficult task. Very often, implantable glucose sensors will lose function after a relatively short period of time *in vivo* or become unreliable. This loss of function is in part a consequence of inflammation and fibrosis resulting from the tissue trauma caused by the sensor implantation and by reactions within the tissue[1].

The goal of this study is to develop a new non-degradable 3D porous collagen scaffold around implantable glucose sensors to improve their biocompatibility by minimizing tissue reactions while stimulating angiogenesis. We fabricated 3D porous scaffolds by using a freeze-drying method and reinforced them using Nordihydroguaiaretic Acid (NDGA), a plant compound with antioxidant properties[2]. We also applied NDGA-reinforced collagen scaffolds to the coil-type implantable glucose sensors to evaluate their *in vitro* and *in vivo* sensitivity behaviors.

Methods: The collagen scaffolds were prepared by a freeze-drying method. Collagen purified from pepsin-solubilized type I fetal bovine tendon was dissolved in 3% acetic acid solution to prepare a 1% (w/v) solution. The solution was applied to a cylinder-shaped mold and then freeze-dried to remove solvent so that a cylindrical 3D porous scaffold was fabricated. The fabricated 3D porous scaffolds were crosslinked by using NDGA solution as follows; dried 3D porous collagen scaffold was hydrated in 1M of NaCl solution for 30 min. Scaffold was then treated with NDGA in phosphate buffered saline. NDGA suspended in 0.4 N NaOH solution was added directly to PBS in which the scaffold was suspended. The scaffold was agitated in the NDGA solution overnight. The scaffold was removed, rinsed with di-water for 18h at room temperature and dried. For comparative analyses on the effectiveness of the NDGA cross-linking protocol, non-crosslinked scaffolds were crosslinked for 2h with 0.5 % glutaraldehyde (GA) in ethanol. The physical stability of the scaffold was tested in rat. We also fabricated coil-type amperometric glucose sensors (0.7 mm diam.) using Pt/Ir wire, crosslinked Glucose Oxidase and an external Epoxy-PU membrane. A collagen scaffold was fabricated around the sensor, by dip-coating the sensor in a 1% (w/v) collagen solution followed by freeze-drying. The 3D porous scaffold around the glucose sensor was reinforced with NDGA to minimize water solubility and to resist enzymatic degradation. The glucose sensor was characterized *in vitro* and *in vivo* (Rat Model, 1- 4 weeks) at 700mV versus an incorporated Ag/AgCl reference electrode.

Results / Discussion: We observed that hydrated NDGA-reinforced collagen scaffolds had significantly higher form stability than uncrosslinked and GA-crosslinked collagen scaffolds. The water uptake behavior of crosslinked scaffolds showed no significant differences with each crosslinked scaffolds (above 99%). The NDGA-crosslinked scaffolds were much more stable then GA-crosslinked scaffolds *in vivo* during the 4 week study after subcutaneous implantation in rats.

In addition, GA- and NDGA-crosslinked collagen scaffolds were then applied to our glucose sensors. The sensitivity of the implantable glucose sensor with and without collagen scaffold was evaluated long-term *in vitro* and i*n vivo.* We confirmed that the scaffold application caused only a very small change in sensor sensitivity and response time *in vitro*. The amperometric response corresponding to the glycemia of the rat was obtained at the corresponding current time intervals of the sensors by injecting of 0.5 - 0.7cc of 50% glucose solution intraperitoneally (Fig. 1).

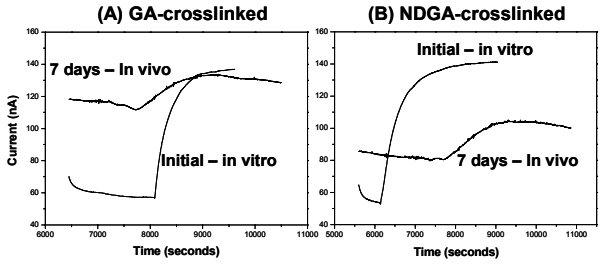

Figure 1. Amperometric response curves of the glucose sensors with GA- and NDGA-crosslinked scaffold 7days post-implantation.

Conclusions: In this study, type I collagen scaffolds were crosslinked by GA and/or NDGA. NDGA-reinforced scaffolds were much more stable *in vivo* then GA-crosslinked scaffold. Also, 3D porous scaffold application around glucose sensors did not significantly affect the sensors sensitivity *in vitro*. *In vivo* evaluation of the sensor coated with scaffold is currently underway.

References:

1. Reichert WM, et al., Chap. 28 Biosensors. In: Von Recum A, editor. Handbook of biomaterials evaluation. 1999. p 439-460.

2. Koob TJ, et al., Comp. Biochem. Physiol. 2002; Part A(133), p1171-92.

Acknowledgments:

This research was supported by grant R01 EB00164 from the NIH