Preparation of 3D Porous Collagen Scaffold around Implantable Biosensor for Improving Biocompatibility

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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[1-2].
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 cross-linked them using different concentration of glutaraldehyde (GA) and/or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) solution. The stability of the scaffolds was evaluated *in vitro* and *in vivo*. We also fabricated scaffolds around coil-type glucose sensors and measured their sensitivity.

Methods: The collagen scaffolds were prepared by a freeze-drying method. Collagen isolated from rat tail 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 different concentrations of GA solution and/or EDAC solution and then used for *in vitr*o and *in vivo* biodegradation tests. 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 cross-linked to minimize water solubility and to resist enzymatic degradation. The morphology of the sensors was observed using an optical microscope and SEM. The glucose sensor was characterized in pH 7.4 phosphate buffer at 700mV versus an incorporated Ag/AgCl reference electrode. The working electrode (Pt/Ir wire) and Ag/AgCl reference electrode were attached to a Apollo 4000 potentiostat (WPI, Inc.). The background current was allowed to stabilize for 10min, and the sensors were then exposed to a series of glucose solutions in order to examine their sensitivities and linearities.

Results / Discussion: We observed that the obtained scaffolds have interconnected open pores with 10 to 100 μm pore size through scanning electron microscopy (SEM) observation. Crosslinked collagen scaffolds had significantly higher form stability than uncrosslinked collagen scaffolds. Also, the water swelling behavior of crosslinked scaffolds showed no significant differences with the two crosslinking agents and the different solution concentrations (GA - 0.25/0.5%, EDAC - 0.25/0.5mM). The water absorption of the crosslinked scaffolds was above 98%. The non-crosslinked scaffold samples were completely degraded in collagenase solution after 1hr while for the GA or EDAC crosslinked scaffolds no degradation was observed during the 4 week *in vitro* study. The crosslinked scaffolds also remained stable *in vivo* during the 4 week study after subcutaneous implantation in rats. In addition, new vasculature inside the scaffolds was found 30 days post implantation. GA crosslinked collagen scaffolds were then applied to our glucose sensors. By microscopic viewing, we confirmed that our method produces porous scaffolds tightly wrapped around the sensor tips (Figure 1). The amperometric response curves of the glucose sensor before and after scaffolding were obtained by varying the glucose concentration. The results showed that the scaffold application caused only a very small change in sensor sensitivity and response time.

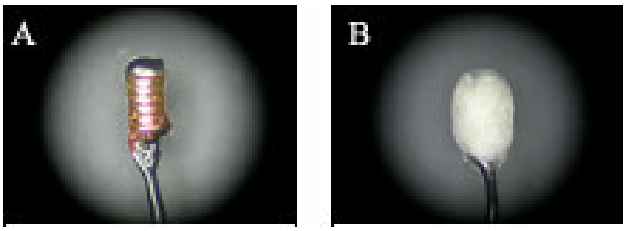

Figure 1. Optical microscopic picture of glucose sensor (0.7 mm diam.) with scaffold (B) and without scaffold (A).

Conclusions: In this study, type I collagen scaffolds were crosslinked by GA and/or EDCA. These crosslinked scaffolds are stable both *in vitro* and *in vivo* for at least 4 weeks. Also, scaffold application around glucose sensors did not significantly affect the sensors sensitivity. Our next step will be to use the scaffolds to deliver anti-inflammatory drug and angiogenic growth factors (e.g. VEGF, PDGF) in order to create a controlled local tissue environment around sensors with minimum inflammation and fibrosis but with increased blood vessel density.

References:

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