A novel porous collagen scaffold around an implantable biosensor for improving biocompatibility. II. Long-term *in vitro*/*in vivo* sensitivity characteristics of sensors with NDGA- or GA-crosslinked collagen scaffolds

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Abstract: We have developed a new 3D porous and biostable collagen scaffold for implantable glucose sensors. The scaffolds were fabricated around the sensors and crosslinked using nordihydroguaiaretic acid (NDGA) or glutaraldehyde (GA) to enhance physical and biological stability. The effect of the scaffolds on sensor function and biocompatibility was examined during long-term (≥28 days) *in vitro* and *in vivo* experiments and compared with control bare sensors. To evaluate the effect of the sensor length on micromotion and sensor function, we also fabricated short and long sensors. 3D porous scaffold application around glucose sensors did not significantly affect the long-term *in vitro* sensitivity of the sensors. The scaffolds, crosslinked by either NDGA or GA, remained stable around the sensors during the 4 week *in vitro* study. In the long-term *in vivo* study, the sensitivity of the short sensors was higher than the sensitivity of long sensors presumably because of less micromotion in the subcutis of the rats. The sensors with NDGA-crosslinked scaffolds had a higher sensitivity than the sensors with GA-crosslinked scaffolds. Histological examination showed that NDGA-crosslinked scaffolds retained their physical structure with reduced inflammation when compared with the GA-crosslinked scaffolds. Therefore, the application of NDGA-crosslinked collagen scaffolds might be a good method for enhancing the function and lifetime of implantable biosensors by minimizing the *in vivo* foreign body response. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 92A: 650–658, 2010

Key words: implantable glucose sensor; collagen; porous scaffold; NDGA crosslinking; glutaraldehyde crosslinking

INTRODUCTION

To more tightly control the blood glucose levels of diabetic patients, minimally invasive and noninvasive glucose sensors have been developed.[1–3] For example, Medtronic MiniMed (Northridge, CA) developed the first enzyme-based implantable glucose sensor to be used in patients. This minimally invasive system is implanted transcutaneously. However, the sensor requires frequent calibration and has to be replaced every few days. Currently, there are no clinically available glucose sensors that can provide continuous monitoring of glucose levels for extended periods of time (months or years).

Although many strategies for continuous glucose monitoring have been developed over the past 30 years, achieving reliable and continuous glucose monitoring *in vivo* is still a very difficult task. Very often, implantable glucose sensors lose function after a relatively short period of time *in vivo* or become unreliable, despite having excellent *in vitro* performance including good selectivity, a high sensitivity, and a fast response time.[4–8] This loss of function is, in part, a consequence of protein adsorption, inflam-

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mation, fibrous encapsulation, and loss of vasculature resulting from the biofouling and the tissue trauma caused by the host response to the sensor and the surgical implantation.[9–11] Ultimately, biofouling of the biosensor membrane very much influences glucose diffusion, leading to *in vivo* sensor failures.[12,13] Overall, few successful long-term implantations of glucose sensors have been reported. Armour et al.[14] implanted 6 sensors intravascularly in dogs for up to 108 days. Three sensors still functioned with no adherent clots and with the same *in vitro* calibration curves before and after explantation. Updike et al.[15] telemetrically monitored glucose using three implanted sensors. The sensor response dropped during the initial period *in vivo* but then rose and stabilized until 42–94 days. The same group (Gilligan et al.[16]) observed a stable foreign body capsule (FBC) around the Dacron or ePTFE velour shells of their implanted sensors. The sensors eventually failed because of enzymatic degradation or biofouling of the sensor membranes. Pickup et al.[17] showed that only 50% of sensors implanted in nondiabetic subjects responded *in vivo*. Explanted sensors examined by scanning electron microscopy were coated by cells and proteins at the sensor tip. Shichiri et al.[18] and Ertefai and Gough[19] reported that the *in vivo* lag time was increased compared with the *in vitro* lag time. The increase was attributed to protein deposition and FBC tissue at the sensor tip.

To minimize biofouling and to improve sensor function, many researchers have designed new sensors with modifications to the surface of the sensor outer membrane. Moussy et al.[20,21] introduced a new sensor with a needle-type geometry and a Nafion outermost layer. Quinn et al.[22] used a photocrosslinkable copolymer containing 2-hydroxyethyl methacrylate (HEMA) and poly(ethylene glycol) (PEG) as a sensor coating material. The results showed that the copolymer-treated electrodes induced much less fibrous tissue than control electrodes due to good biological performance of the PEG material. To reproduce lipid characteristics to mimic the cell surface membranes and induce anti-thrombogenicity, Nishida et al.[23] synthesized a phosphorylcholine (PC)-containing polymer which was applied as a sensor membrane and showed excellent biocompatibility. Because of good swelling and viscoelastic properties and outstanding biocompatibility, many researchers use hydrogels such as PEG hydrogel (Quinn et al.[24]), phenylboronic acid-based hydrogel (Lei et al.[25]), and polyacrylamide hydrogel (Fernandez et al.[26]) as the outermost coating of glucose sensors. Recently, numerous strategies to control delivery of tissue response modifiers (TRM) have been reported. For example, Gifford et al.[27] used nitric oxide (NO) to downregulate mediators of the inflammatory response and Norton et al.[28] characterized vascular endothelial growth factor (VEGF) and dexamethasone (DX) delivery from sensor coatings.

We recently reported the development of new porous collagen scaffolds which were applied around implantable glucose sensors to improve their biocompatibility.[29] Collagen and its derived matrices are extensively used as natural polymers in the biomedical field because of low antigenicity, good biocompatibility, and mechanical properties. Recently, many collagen-based products have been approved by the FDA for clinical use. We fabricated porous collagen scaffolds by using a freeze-drying method followed by crosslinking using nordihydroguaiaretic acid (NDGA) or glutaraldehyde (GA).[29]

In a continuation of this study, we evaluated the sensitivity of sensors with ether NDGA- or GA-crosslinked collagen scaffolds during long-term *in vitro* and *in vivo* experiments. We also fabricated two different lengths of sensors (long and short wires) to minimize scaffold damage and compared their function *in vivo* to evaluate the effects of micromotion on the sensors.

MATERIALS AND METHODS

Materials

Type I collagen (purified from fetal bovine tendon) was a generous gift from Dr. Thomas Koob, Shriners Hospital for Children (Tampa, FL). NDGA was purchased from Cayman Chemical Co. (Ann Arbor, MI). Glucose, bovine serum albumin (BSA), and 50% (w/w) GA were obtained from Fisher Scientific (Pittsburgh, PA). Glucose oxidase (GOD) (EC 1.1.3.4., type X-S, *Aspergillus niger*, 157,500 U/g), epoxy adhesive (ATACS 5104), polyurethane (PU), and tetrahydrofuran (THF) were obtained from Sigma-Aldrich (St. Louis, MO). Dextrose injection solution (50%, w/v) and the FreeStyle™ portable glucometer were obtained from Abbott Laboratories (North Chicago, IL). Sprague-Dawley out-bred rats (male, 375–399 g) were purchased from Harlan (Dublin, VA).

Preparation of porous collagen scaffolds around implantable glucose sensors

We fabricated miniature coil-type glucose sensors loaded with crosslinked enzyme (GOD) using a platinum–iridium (Pt/Ir) wire (Teflon coated, Φ 0.125 mm, Pt:Ir = 9:1, Medwire, Sigmund Cohn Corp., Mount Vernon, NY). We applied bovine tendon type I collagen scaffolds around the sensors. Scaffolds were then crosslinked with NDGA or GA treatment as previously described[29] to minimize solubility and to improve resistance to enzymatic degradation *in vivo*. Control sensors (without scaffolds) and sensors with NDGA- or GA-crosslinked scaffolds were equilibrated in phosphate-buffered saline (PBS, pH 7.4) for

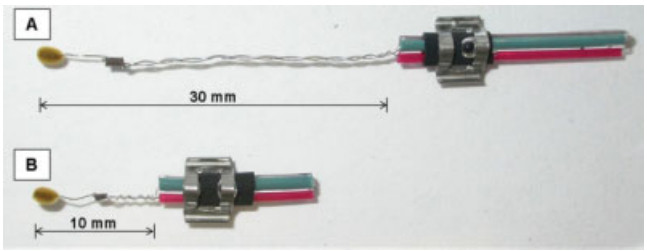

Figure 1. Photograph showing the (A) long-wire and (B) short-wire implantable glucose sensors. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

2 days at room temperature before being used *in vitro* or *in vivo*.

The initial sensitivity of all sensors was measured in 5 and 15 m*M* glucose in PBS. Amperometric measurements were performed at room temperature at 0.7 V versus Ag/AgCl. The working electrode (Pt/Ir wire) and the Ag/AgCl reference electrode of each sensor were connected to an Apollo 4000 potentiostat (World Precision Instruments, Inc., Sarasota, FL).

To investigate the effect of wire length on the sensor function, we fabricated sensors with two different lengths: 10 and 30 mm (Fig. 1). Only the wires were of different length, the sensing elements remained identical.

In vitro performance testing of sensors

To examine the long-term *in vitro* sensitivity of sensors, uncoated (control) sensors, sensors with NDGA-crosslinked collagen scaffolds, and sensors with GA-crosslinked collagen scaffolds ($n = 8$/group) were incubated in PBS at 37°C for 4 weeks. We added 0.2% (w/v) of benzoic acid to prevent bacterial contamination of the warm PBS solution. At 7, 14, 21, and 28 days, each sensor was removed and tested in glucose solution. The length of the wires for all these sensors was 30 mm. We previously observed that the difference in length (30 vs. 10 mm) did not affect the sensitivity of the sensors *in vitro*.

The sensitivity of the glucose sensors was characterized in glucose/PBS (pH 7.4) at 700 mV versus the incorporated Ag/AgCl reference electrodes. The background current was allowed to stabilize for 10 min, and the sensors were then exposed to a series of glucose solutions to examine their sensitivities and linearities. The response sensitivity (S) was repeatedly assessed by (1) measuring the response current (I_1) of a C_1 glucose solution, (2) adding a concentrated glucose solution into the measured solution to increase the glucose concentration to C_2, and (3) measuring the response current (I_2) of the resulting solution. The sensitivity was expressed as the current increase caused by a 1 m*M* glucose increase, i.e., $S = (I_2 - I_1)/(C_2 - C_1)$.

Implantation procedures

After pretesting *in vitro*, all implantable glucose sensors were disinfected using 70% ethanol and then placed in sterile PBS before implantation. During the surgical procedure, a continuous flow gas anesthesia system was used to deliver 1.5% isoflurane to the rats in 2.0 L/min oxygen flow. All protocols were approved by the University of South Florida Institutional Animal Care and Use Committee (IACUC). Forty-eight sensors (CS, eight control short sensors; CL, eight control long sensors; NS, eight NDGA-crosslinked scaffold around short sensors; NL, eight NDGA-crosslinked scaffold around long sensors; GS, eight GA-crosslinked scaffold around short sensors; and GL, eight GA-crosslinked scaffold around long sensors) were implanted subcutaneously on the back of the rats. Each rat received two of one type of sensors.

For long sensors, two 1.5-cm-long longitudinal incisions were made 1.5 cm laterally to the dorsal midline, and 3–4 cm caudally from the neck. A subcutaneous pocket was created using blunt surgical scissors. A 14 ga. I.V. catheter was inserted subcutaneously toward the incision from the 4–5 cm lower back region. The needle was withdrawn leaving the cannula in the subcutaneous tissue. The sensor wires were carefully fed into the cannula through the incision [Fig. 2(A)]. The sensor was secured to the skin by

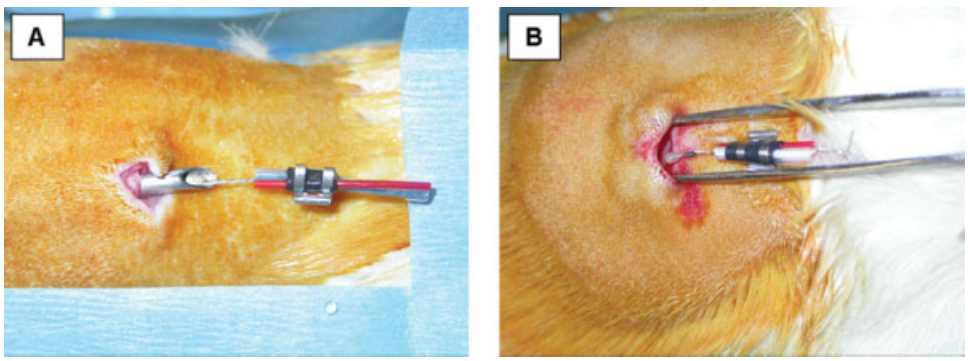

Figure 2. Surgical procedures by two different implantation techniques for (A) long-wire sensors (using a 14 ga. catheter guidance) and (B) short-wire sensors (direct implantation). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

passing a 3-0 Prolene suture through the small gap of the wound clip covering the sensor wires and incisions closed using 3-0 Prolene. The cannula was then withdrawn, leaving the sensor in the subcutaneous tissue.

For short sensors, same-sized incisions were made and a subcutaneous pocket was also created using blunt surgical scissors before implantation. However, the sensors were directly implanted through the incision without using a cannula [Fig. 2(B)]. The sensors were secured to the skin and incisions closed using the same approach utilized for the long sensors.

In addition, to evaluate the inflammatory response of the tissue around and cellular intrusion into the collagen scaffolds, we directly implanted NDGA- and GA-crosslinked scaffolds (without sensors) in the rats. At set time intervals, tissue samples containing the scaffolds were excised and embedded in paraffin. Sections (5 μm in thickness) were cut and stained with Mayers hematoxylin and eosin (H&E) stain. Stained sections were analyzed and photographed using an Olympus BX41 microscope (Olympus; Tokyo, Japan).

Long-term *in vivo* evaluation of sensors coated with scaffolds

The sensitivity of each sensor was measured every 7 days for up to 28 days or until there was no amperometric response from the implanted sensor. During each measurement period, four rats were anesthetized using isoflurane and the eight implanted sensors were continuously monitored using two Apollo 4000 potentiostats. We also considered the influence of isoflurane on glucose level. A high dose was initially used to induce rapid anesthesia followed by a low maintenance dose. During the *in vivo* study, we allowed time for the signal to stabilize and only then administered glucose to induce hyperglycemic level. After a stable signal was obtained from the sensors, 0.7 mL of sterile 50% dextrose was administered intraperitoneally using a 27 ga. needle. Following the injection, ~2–5 μL of blood samples were tested every 7 min until a stable signal was obtained by applying blood obtained from the rat tail directly to the glucose test strip. The blood glucose level was determined using the standard Freestyle™ glucometer. The amperometric response corresponding to the glycemia of the rat was recorded at the corresponding current–time intervals of each sensor. The sensor sensitivity was calculated by dividing the change in current (I) by the change in glycemia (C) between the initial (before dextrose injection) and the peak status (after dextrose injection) as follows: Sensitivity (nA/mM) = $(I_{max} - I_0)/(C_{max} - C_0)$.

Statistical analysis

All data were presented as means ± standard deviation and significance between the mean values was calculated using independent Student's t test (SPSS v.15 software, SPSS Inc., Chicago, IL). Probability values $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Preparation of implantable glucose sensors with porous crosslinked collagen scaffolds

To create a porous scaffold for implantable glucose sensors, we first fabricated coil-type glucose sensors and applied bovine tendon type I collagen scaffolds around the sensors. With a light microscope, we confirmed that the porous scaffolds thoroughly surrounded the working electrodes (Fig. 3). Both scaffolds were semitransparent in aqueous solution. GA-crosslinked scaffolds appeared white, whereas the NDGA-crosslinked scaffolds were brown. We also observed high swelling for both scaffolds around the sensors in aqueous solution. We reported previously[29] that these sponge-like matrices with porous inner structure could absorb water above 99%, thus allowing glucose to diffuse freely. The pore sizes for both NDGA- and CA-crosslinked scaffolds were identical and ranged from 20 to 100 μm in diameter (mean: ~60 μm).[29] We previously investigated the correlation between scaffold thickness and sensors sensitivity and found the optimal conditions to be three dipping/freezing cycles (in both collagen solution and liquid nitrogen) followed by freeze-drying.[29] This process ensured a consistent coating process.

Figure 4 shows a schematic of a fully assembled coil-type glucose sensor with a scaffold ready for implantation. The newly assembled sensor is composed of a two-electrode system with a glucose indicating working Pt/Ir electrode and an Ag/AgCl reference-counter electrode. We added a loop between the two electrode coils to avoid microshorting caused by the two electrodes touching each other. A surgical wound clip was applied to provide a suturing site during the implantation procedure and to prevent the sensor moving out of the skin (i.e., for anchoring).

Long-term *in vitro* evaluation of sensors with porous collagen scaffolds

The long-term *in vitro* function of the sensors was determined by tracking their sensitivity for up to 4 weeks. The preincubation sensitivity (week 0) was measured at the beginning of the *in vitro* study and the percentage of sensitivity change was calculated from the ratio of the sensitivity of the sensors at given time interval to the preincubation sensitivity. The sensitivity of all sensors was tested in 5 and 15 mM glucose/PBS. Figure 5 shows the sensitivity change of all sensors over 4 weeks. We observed a slight decrease of the sensitivity of sensors with either NDGA- or GA-crosslinked scaffolds, com-

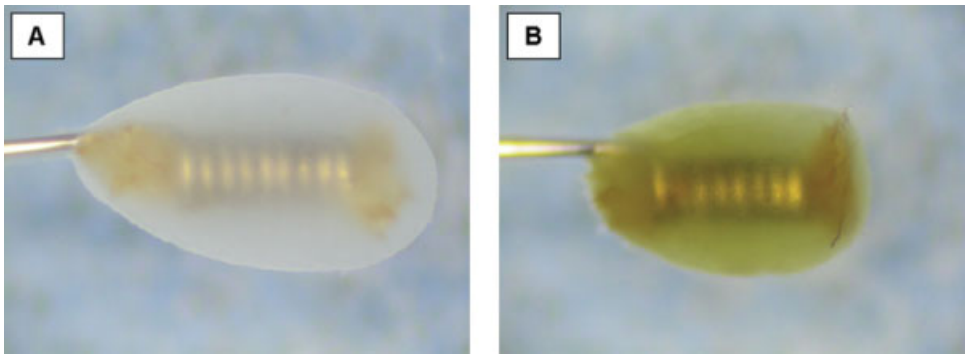

Figure 3. Photographs of implantable sensors coated with (A) GA-crosslinked porous collagen scaffold and (B) NDGA-crosslinked porous collagen scaffold. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pared with the control (no scaffold) sensors after 1 week incubation. After 2 weeks, the sensitivities of all sensors increased to a level higher than their original sensitivity, probably because of an increase in epoxy-PU membrane permeability due to progressive membrane swelling in aqueous solution. After 2 weeks, the sensitivity of the control sensors, as well as sensors with either NDGA- or GA-crosslinked scaffolds, steadily decreased; however, all sensors retained above 80% of their original sensitivity up to 4 weeks. We believe that the sensitivity decrease after 2 weeks may be caused by progressive loss of enzyme activity. Both NDGA- and GA-crosslinked scaffolds were still intact around the sensors at week 4. There was no detection of any deformation or detachment of the scaffolds from the sensor membrane surface. Although the overall trend of the sensitivity of the sensors with scaffolds was lower than with the control sensors, the application of scaffolds around sensors did not significantly affect the function of the sensors *in vitro* at week 3 and week 4 versus week 0 ($p > 0.05$).

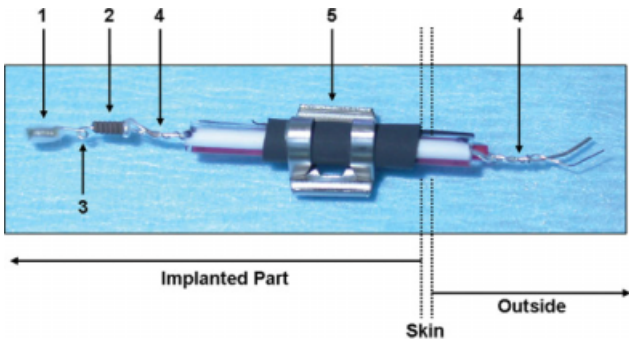

Figure 4. Short-wire implantable glucose sensor: (1) Pt/Ir working electrode coated with scaffold, (2) Ag/AgCl reference electrode, (3) loop to reduce motion and prevent microshort between electrodes, (4) wires twisted together, (5) wound clip. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Long-term *in vivo* performance of sensors with porous collagen scaffolds

In this study, short/long control sensors and sensors with NDGA- or GA-crosslinked scaffolds were implanted subcutaneously in the back of the rats. The *in vivo* sensitivity of each sensor was measured at weeks 1, 2, 3, and 4. We did not observe a difference in noise signal between the various types of sensor configurations tested. The preimplantation sensitivity of all sensors was tested using 5 and 15 m*M* glucose/PBS just before implantation.

The percentage of sensitivity change for each sensor during the 4 week study is shown in Figure 6. From this figure, a few initial observations can be made: (1) The sensitivity of all sensors dramatically

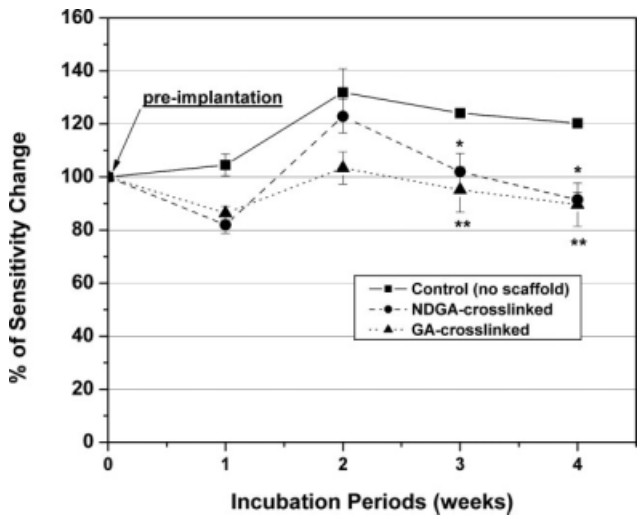

Figure 5. Long-term *in vitro* sensitivity changes of control sensors and sensors with NDGA- or GA-crosslinked collagen scaffolds. Results are shown as means ± SD. * and ** indicate *no* statistically significant differences between initial sensitivity (week 0) and sensitivity of sensors with NGDA- or GA-crosslinked scaffolds ($p > 0.05$).

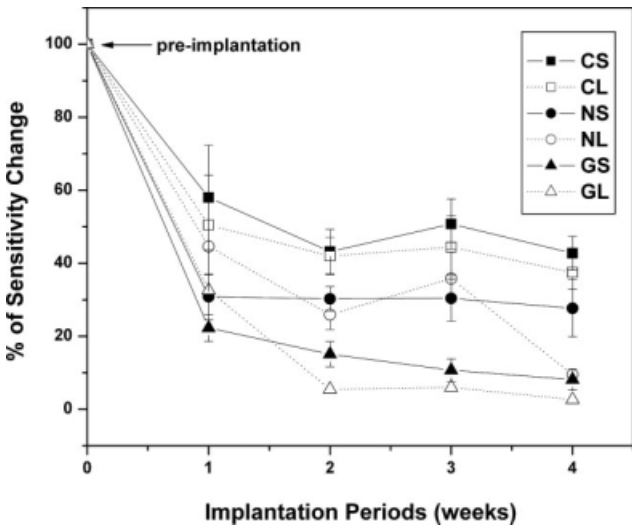

Figure 6. Long-term *in vivo* sensitivity changes of control sensors and sensors with NDGA- or GA-crosslinked collagen scaffolds. Results are shown as means ± SD. (CS, control short; CL, control long; NS, NDGA-crosslinked scaffold around short sensors; NL, NDGA-crosslinked scaffold around long sensors; GS, GA-crosslinked scaffold around short sensors; GL, GA-crosslinked scaffold around long sensors).

decreased after implantation compared with the preimplantation values ($p < 0.05$). This was probably due to tissue damage which occurred during surgical procedures and the subsequent host response including protein adsorption, blood clot, and the infiltration of inflammatory cells and other cells (e.g., fibroblasts) around the sensor tips.[30] (2) As for the *in vitro* study, the control sensors retained a higher sensitivity than the sensors with scaffolds ($p < 0.05$). The sensors with NDGA-crosslinked collagen scaffolds also had a higher sensitivity than the sensors with GA-crosslinked scaffolds ($p < 0.05$).

Table I shows the number of working sensors (used in Fig. 6) at given time intervals. Initially, 3 sensors did not work at week 1 but regained their function at week 2. Both CS and CL sensors had a higher sensor survival rate (4 out of 8, 6 out of 8, respectively) *in vivo* 4 weeks postimplantation than sensors with scaffold coatings. The use of scaffolds worsened the survival rate of the sensors. However, the short sensors had a higher survival rate than the long sensors at 4 weeks postimplantation (CL-4, NL-2, GL-1 vs. CS-6, NS-4, GS-4). We believe that this might result from the long sensors having greater range of motion when the animals move than the short sensors. The larger macromotion/micromotion may have caused more tissue and scaffold damage. This hypothesis remains to be verified. We observed scaffolds detached from the working electrode of the long NL sensors [Fig. 7(A)], whereas the NDGA-crosslinked scaffold around the short sensor NS remained in a stable position [Fig. 7(B)]. Regarding the GA-crosslinked scaffolds, we could not detect any such scaffold around both long and short sensors [Fig. 7(C,D)]. This is consistent with our previous study where we observed that the size and shape of the GA-crosslinked scaffolds were dramatically changed (degraded) after 4 weeks of implantation, whereas the NDGA-crosslinked scaffolds remained mostly intact.[29]

To evaluate inflammatory response of the tissue around and within the collagen scaffolds, we directly implanted NDGA- and GA-crosslinked scaffolds (without sensors) in the rats for up to 4 weeks ($n = 6$). The porosity in the two types of implant was identical. We used the same collagen concentration and adopted the same method to fabricate the scaffolds as used for making scaffolds around sensors. The goal of this study was to compare the tissue reactions to the two types of scaffolds. We have previously shown the inflammatory reaction to bare sensors.[31] After 2 weeks implantation, H&E staining revealed the presence of many inflammatory cells (arrows) including polymorphonuclear (PMN) cells, monocytes, and macrophages within and around the GA-crosslinked scaffolds. It showed that depth of cellular infiltration was over 300 μm from the top of the scaffold surface [Fig. 8(A)]. However, for the NDGA-crosslinked scaffolds, few inflammatory cells were observed around the scaffolds, and there was no infiltration of cells in the center region of the scaffolds (100–150 μm cellular infiltration depth) [Fig. 8(B)]. Week 4 showed infiltration of inflammatory cells and fibroblasts, along with granulation tissue deposition inside the pore of the GA-crosslinked scaffolds (over 300 μm cellular infiltration depth) [Fig. 8(C)], and again, less inflammation within and around the NDGA-crosslinked scaffolds (less than 100 μm cellular infiltration depth) [Fig. 8(D)]. This result shows that the NDGA-crosslinked collagen scaffolds are more biocompatible than the GA-crosslinked collagen scaffolds and is consistent with a report by Koob[32] showing that NDGA-crosslinked

TABLE I
Number of Sensors Working After Implantation (Out of 8 Sensors)

Scaffolds	Wire	Weeks After Implantation			
		1	2	3	4
Control (no scaffold)	Long (CL)	8	7	4	4
	Short (CS)	7	8	7	6
NDGA-crosslinked	Long (NL)	2	3	2	2
	Short (NS)	8	5	5	4
GA-crosslinked	Long (GL)	2	2	1	1
	Short (GS)	5	6	6	4

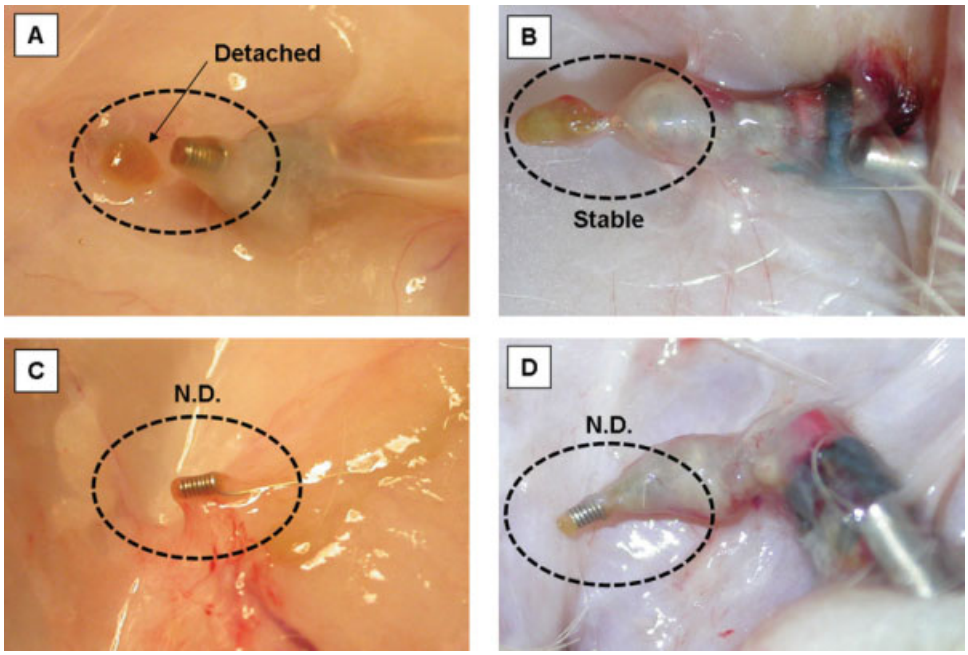

Figure 7. Representative photographs of scaffolds *in situ* after 4 weeks of implantation. (A) Long-wire sensor with NDGA-crosslinked scaffold, (B) short-wire sensor with NDGA-crosslinked scaffold, (C) long-wire sensor with GA-crosslinked scaffold, (D) short-wire sensor with GA-crosslinked scaffold. (N.D., no detection of collagen scaffold). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

collagen fibers appeared intact with little foreign body response after implantation in rabbits. The size of the GA-crosslinked scaffolds was reduced and the pore structure was deformed as the implantation time increased. We believe that fast degradation of GA-crosslinked scaffolds caused the pores to close

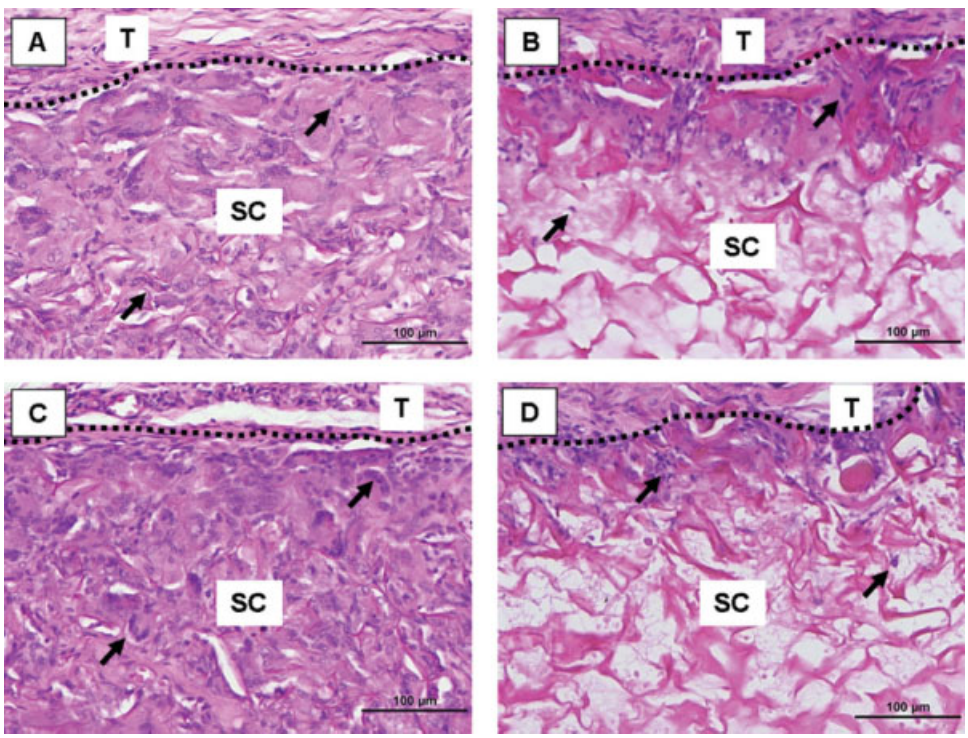

Figure 8. Hematoxylin and eosin stained sections showing tissue surrounding porous scaffolds. (A) GA- and (B) NDGA-crosslinked scaffold and after 2 weeks postimplantation and (C) GA- and (D) NDGA-crosslinked scaffold after 4 weeks postimplantation. (T, tissue surrounding scaffold, SC, scaffold, ------, tissue-scaffold margin; arrows, nuclei of some inflammatory cells). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

up due to a decrease in mechanical properties. In addition, degradation debris possibly induced more inflammation, including fibrous encapsulation around the sensor tips. NDGA scaffolds have a longer biological half-life (more resistant to enzymatic degradation *in vivo*). The potential release of neoantigens after degradation of the GA-crosslinked scaffolds could have contributed to inflammation.

CONCLUSIONS

In this study, we applied porous type I collagen scaffolds around implantable glucose sensors by a freeze-drying method and then crosslinked the scaffolds using NDGA or GA treatments. The fabricated collagen scaffolds had an open cell and interconnected pore structure. All sensors including control sensors (without scaffold) and sensors with NDGA- or GA-crosslinked scaffolds remained functional during the 4 week *in vitro* study. The application of both types of scaffolds around the sensors did not critically affect the function of these sensors *in vitro*.

In the 4 weeks *in vivo* study, the sensitivity of all sensors dramatically decreased (30–60%) after 1 week of implantation and then remained relatively stable. The sensitivity and survival rate of the short sensors were higher than the sensitivity of the long sensors possibly as a result of reduced motion within the animals. The sensors with NDGA-crosslinked scaffolds had a higher survival and sensitivity than the sensors with GA-crosslinked scaffolds. By histological examination, we confirmed that the NDGA-crosslinked scaffolds are more biocompatible than the GA-crosslinked scaffolds.

Therefore, this study shows that an NDGA-crosslinked collagen scaffold can be incorporated into the design of our implantable glucose sensor. However, the control sensors (no scaffolds) performed better than the sensors with scaffolds. The scaffolds alone did not improve the function and lifetime of our implantable glucose sensor. The data presented here go against the belief that porous coatings improve long-term sensor function as previously suggested by other studies. It is possible that our scaffolds do not have the right structure/porosity or that collagen is not the best material for this purpose. This study indicates that to use these collagen scaffolds as a way to control the local tissue environment around implanted sensors and thus improve their function and lifetime we still need to improve the scaffolds. This could potentially be achieved by using the NDGA-crosslinked collagen scaffold to also deliver various drugs (e.g., DX for anti-inflammation) and growth factors (e.g., VEGF, PDGF for angiogenesis) to modify the tissue response to the sensors. We could also evaluate other porosities.

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