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Porous, Dexamethasone-Loaded Polyurethane Coatings Extend Performance Window of Implantable Glucose Sensors *in vivo*

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Abstract

Continuous glucose sensors offer the promise of tight glycemic control for insulin dependent diabetics; however, utilization of such systems has been hindered by issues of tissue compatibility. Here we report on the *in vivo* performance of implanted glucose sensors coated with Dexamethasone-loaded (Dex-loaded) porous coatings employed to mediate the tissue-sensor interface. Two animal studies were conducted to 1) characterize the tissue modifying effects of the porous Dex-loaded coatings deployed on sensor surrogate implants, and 2) investigate the effects of the same coatings on the *in vivo* performance of Medtronic MiniMed SOF-SENSOR™ glucose sensors. The tissue response to implants was evaluated by quantifying macrophage infiltration, blood vessel formation, and collagen density around implants. Sensor function was assessed by measuring changes in sensor sensitivity and time lag, calculating the Mean Absolute Relative Difference (MARD) for each sensor treatment, and performing functional glucose challenge test at relevant time points. Implants treated with porous Dex-loaded coatings diminished inflammation and enhanced vascularization of the tissue surrounding the implants. Functional sensors with Dex-loaded porous coatings showed enhanced sensor sensitivity over a 21-day period when compared to controls. Enhanced sensor sensitivity was accompanied with an increase in sensor signal lag and MARD score. These results indicate that Dex-loaded porous coatings were able to elicit an attenuated tissue response, and that such tissue microenvironment could be conducive towards extending the performance window of glucose sensors *in vivo*.

Graphical Abstract

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Introduction

It is well-established that tight regulation of blood glucose levels is the single most important factor in preventing complications from diabetes [1]. Researchers have been developing closed-loop artificial pancreas (AP) systems where real-time changes in glucose are used to trigger an insulin pump to deliver the appropriate dosages. In recent years, AP development has been stalled due to the unreliability and untimely failure of implantable glucose sensors [2].

Classical continuous glucose sensors are amperometric and percutaneous systems that rely on glucose oxidase [3–5]. Due to the short *in vivo* life of these sensors, new fully subcutaneous systems that rely on novel biomaterial platforms, unconventional enzymes, and novel optical technologies for glucose detection are in various stages of development [6–8]. However, the complexities of development and regulatory approval of these novel platforms makes their availability uncertain. Therefore, in addition to developing new approaches to sensing, it is imperative to also improve upon classical glucose sensing systems for technological advances to reach the public in acceptable timeframes.

Upon implantation, a glucose sensor is presented with a dynamic microenvironment that results from the foreign body response (FBR) [9, 10]. Within seconds of implantation, proteins biofoul the sensor surface followed soon by the arrival of immune cells that attempt to degrade and consume the sensor. Since the sensor cannot be eliminated by the host, this response often culminates in the formation of an avascular fibrous capsule that surrounds the implanted sensor. As a result, blood glucose measurements from the sensor may be delayed, erratic, attenuated, and/or unreliable [6, 9–11].

Initial biomaterial strategies to extend glucose sensor functionality have focused on preventing protein adsorption through the incorporation of hydrogel coatings. Although anti-biofouling strategies proved to be well suited for acute sensor applications, they appear to be insufficient to achieve reliable long-term glucose sensor performance [12–14]. Most recently, modeling and experimental studies have shown that inflammation and tissue encapsulation are critical stages affecting sensor function *in vivo* [15, 16].

As summarized in Table 1 *in vivo* strategies for modulating the tissue response to implanted sensors have employed either the release of “tissue response modifiers”, topographical cues, or biomimicry. Strategies for the attenuation of inflammation and the promotion of tissue vascularization have employed the release of growth factors (VEGF, PDGF), nitric oxide

[17, 18], or anti-inflammatory glucocorticoids [19–21]. Topographical approaches have exploited the application of porous polymer coatings, whereas biomimicry approaches have used tissue-like overlayers of collagen and/or cells [11, 22–24]. To date, combined strategies to improve sensor biocompatibility have involved either dual agent release or antifouling gels merged with localized drug delivery.

Previously, we reported on the characterization of porous polyurethane coatings with a controllable microstructure, tunable drug loading and bioactive release of Dexamethasone (Dex) [25]. However, questions regarding the effects of coatings on the FBR and subsequent effects on glucose sensor function still remained. The current article presents two animal studies that assessed the effects of porous Dex-loaded coatings on Medtronic MiniMed SOF-SENSOR™ glucose sensors implanted in rat dorsal subcutis for up to 21 days. The first study examined whether Dex-loaded porous coatings deployed on fully subcutaneously-implanted Tygon® tubes both diminished inflammation and promoted vascularity of the tissue surrounding the implants. The second study examined whether Dex-loaded porous coatings deployed on functional percutaneous sensors resulted in improved sensor performance *in vivo*. Results of both studies indicate that Dex-loaded porous coatings are capable of (1) eliciting an attenuated tissue response and (2) extending the performance window of implanted functional glucose sensors.

Methods

Porous Coatings

Coating preparation—As detailed elsewhere [25], porous coatings were fabricated by gas-foaming/salt-leaching technique. Briefly, a polyurethane solution of 6.5% w/w was prepared by dissolving Tecoflex® 93A pellets (Lubrizol, Technologies) in a solution of 75:25 chloroform to ethanol ratio. Forty-five milligrams of pharmaceutical grade Dexamethasone (Sigma, D9184) were added to the polymer solution to obtain a desired weight ratio of Dexamethasone/polyurethane solution of 0.8% v/v. The solution was then stirred until clear. One and a half grams of sieved ammonium bicarbonate salt particulates (50–75 µm) were added and homogeneously mixed. Concentration of salt particles was varied in ratios to obtain scaffolds of desired porosities. Copper wire mandrels (Belden, 20 AWG) were used in place of glucose sensors to protect glucose oxidase bioactivity. Mandrels were allowed to dry overnight under vacuum. Finally, coatings were cut to a length of 1.5 cm to match Medtronic MiniMed SOF-SENSOR™ tip dimensions.

Coating Sterilization—Dex-free and Dex-loaded porous coatings were slid onto Medical Grade Tygon® tubing mandrels (formulation S-54-HL Saint-Gobain, Courbevoie, France) to prevent deformation during sterilization and handling. Coatings were ethylene oxide sterilized and allowed to out-gas for at least seven days prior to implantation.

Tygon® Tubing Implantation Study

Specimen preparation—Tygon® tubing was selected for testing of the foreign body response. Tygon® implants were of similar dimensions (1.5 cm long and 0.8 cm outer diameter) and mechanical properties to the tips of Medtronic MiniMed SOF-SENSOR™. As

shown in Figure 1a, Bare, Dex-free porous and Dex-loaded porous coated Tygon® implants were prepared for implantation. The coatings fitted snugly over the Tygon® tubing and did not need further affixing. Bare and coated implants were ethylene oxide sterilized as described above. Bare Tygon® tubing was used as a positive control because Tygon® is a silicone known to undergo fibrous encapsulation when subcutaneously implanted [26].

Specimen implantation—All National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication no. 85–23 Rev. 1985) were observed. Approval for these studies was granted by the Institutional Animal Care and Use Committee at Duke University prior to initiation of these studies. Sprague-Dawley rats (male; 200–300 g; CD-type, Charles River Labs, Raleigh, NC) were anesthetized with 3% isoflurane in oxygen, the dorsal areas were shaved and the skin prepped with chlorhexidine and alcohol three times. The rats were placed in the prone position and a sterile field was created over the dorsum. Bare, Dex-free and Dex-loaded porous coated Tygon® implants were introduced into the dorsal subcutis of rats (n = 28). All implants were inserted via a trocar introducer. A sterile 12-gauge needle was used to access the subcutaneous plane; implants were inserted into the needle and advanced into the rat subcutis using a plunger. The needle barrel was drawn back while the plunger simultaneously remained stationary, leaving the implant in the desired site. The minimal cutaneous wound did not require primary closure. A visual representation of this procedure can be found in the supplemental Figure 1. Tygon tubing implants were randomly rotated through the positions outlined in Figure 1b to minimize positional artifacts.

Specimen Explantation—Implants were explanted after 3 and 7 days to monitor the early stages FBR and 14 and 21 days to monitor the late stages FBR (n=7 for each time point). Following sacrifice, the implants and surrounding tissue were surgically excised and flash frozen over liquid nitrogen.

Functional Sensor Study

Sensor Implants Preparation—Deployment of coatings over the tips of Medtronic MiniMed SOF-SENSOR™ glucose sensor was performed under a laminar flow hood. One day prior to surgery, coatings were removed from sterile packaging deployed over the tips of functional glucose sensors (Fig. 1c). Coatings were further affixed by securing coatings to the external plastic housing of the sensors using USP Class VI medical grade EP42HT epoxy. The epoxy was allowed to cure for 1 hour under the sterile hood. After curing, sensors with and without coatings were placed in sterile packaging and stored in the dark at 4 °C.

Sensor Implantation—All National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication no. 85–23 Rev. 1985) were observed. Approval for these studies was granted by the Institutional Animal Care and Use Committee at Duke University prior to initiation of these studies. Sprague-Dawley rats (male; 300–350 g; CD-type, Charles River Labs, Raleigh, NC) were shaved and prepped prior to surgery (n=21). Larger rats than those employed for the non-functional Tygon® Tubing implantation study were used because these animals needed to accommodate three Medtronic MiniMed SOF-SENSOR™ glucose sensors per subject. These sensors were either treated with Dex-loaded

porous coatings or Dex-free porous coatings, or were bare with no treatment. Sensors were placed in a circular arrangement on the dorsum of rats (Fig. 1d) and implanted as described below.

Aseptic technique was followed for all surgical procedures. Briefly, after anesthesia induction, the dorsal areas of the rat were shaved and the skin prepped with chlorhexidine/ alcohol three times. The rat was then placed in the prone position and a sterile field was created over the dorsum. A 12-gauge needle was used to puncture the dorsal skin. The sensing tip of each sensor with or without coatings was then gently inserted through the needle wound into the subcutaneous plane. The standard external plastic housing was then secured to the dorsum using braided nylon sutures (3–0). This procedure was repeated to implant the remaining two sensors. Antibiotic ointment was administered around the sutured areas. Implanted sensors were then connected to their respective MiniLink™ external recorders. Sensors and transmitters were further secured to the rat dorsum by wrapping them with Vetrap® wound dressing. The Vetrap® wound dressing was affixed using sutures. After implantation, one drop of blood was collected from the tail via a needle stick for a glucometer measurement. The rats were then allowed to recover and returned to their cages.

Continuous Glucose Monitoring—Blood glucose concentrations were monitored continuously using the implanted Medtronic MiniMed SOF-SENSOR™ glucose sensors until failure of the devices or experiment termination (3, 7 or 21 days post implantation). The glucose sensor continuously monitored glucose levels within each rat. When in contact with interstitial glucose, subcutaneously implanted sensor tips produced an electrical current that was stored in the MiniLink™ external recorder. The sensor system reported an average current measurement (nA) every 5 minutes that represented the blood glucose concentration during that time. Additionally, date and time information for each average current measurement were appended to the recorded data. These average current measurements were downloaded from the MiniLink™ recorders utilizing a Medtronic MiniMed iPro™2 Dock and the SMuRF Utility software package. Data were further analyzed using Microsoft Excel and GraphPad Prism software.

Blood Glucose Sampling—Blood glucose levels were measured using OneTouch Ultra test strips and a hand-held monitor (OneTouch, Milpitas, CA). During each measurement, one drop of blood was collected via a single needle stick to the tail vein. Date and time of each of these measurements was recorded. These measurements were collected every 12 hours until failure of the implanted continuous glucose sensors or experiment termination (days 3, 7 or 21 post implantation). Glucose sampling data were used to calculate sensor sensitivity values.

Glucose Bolus Tests—Tests to examine the functionality of the sensor were performed at days 1, 3, 7, 14 and 21 post-implantation. Prior to anesthesia induction a tail-vein blood glucose measurement was recorded to establish a glucose baseline and be used to construct a one-point calibration curve. Tail vein blood glucose measurements were performed as described above. After anesthesia induction, each rat was be injected with 5 units/Kg of insulin intraperitoneally. Glucose levels were monitored via tail vein needle sticks and a hand-held meter every 5–10 min until glucose levels were under 100 mg/dL and sustained

for 15–20 min. Rats were put under anesthesia induction again and a dosage of 2 g/Kg of 50% dextrose solution was delivered intraperitoneally. Tail vein blood measurements were performed and recorded every 10–15 minutes until baseline glucose levels were reached and sustained for a period of 30 min. In order to test sensor function, glucose readings obtained from a handheld meter were compared to current outputs (nA) from continuous glucose sensors. Data from Glucose Bolus Tests were used to calculate changes in sensor lag time and functional disparity between sensors (MARD).

Sensor Explantation—Sensors were harvested at days 3, 7 and 21 post-implantation (n=7 for each time point). Following sacrifice, surgical scissors were used to remove external sutures and sever sensor tips from the external plastic housing. Sensor tips and surrounding tissue were surgically excised and flash frozen over liquid nitrogen.

Histology

Serial cryosections of 10 μ m thickness were cut from frozen explanted specimens. Slides were kept at -80°C until staining and mounting was performed.

Masson's Trichrome—A Masson's trichrome stain kit (Sigma-Aldrich, St. Louis, MO) was used to assess collagen deposition around implants. Frozen slides were first placed in Bouin's solution overnight at 4°C , then rinsed with deionized water and dried in a fume hood for 30 minutes. Slides were then placed in Weigert's Hematoxylin for 5 min and rinsed with running tap water for 5 minutes. Afterwards, slides were transferred to 100% Biebrich Scarlet-Acid Fuchsin solution for 1 minute and rinsed with DI water. Slides were then transferred to a Phosphomolybdic and Phosphotungstic Acid Solution for 5 minutes, followed immediately by 10% Aniline Blue solution for 1 minute. The slides were rinsed three times in fresh solutions of 1% acetic acid and allowing them to sit for 2 min. After rinsing in acetic acid, the slides were then dehydrated consecutively through a series of 75%–100% ethanol solutions. Slides were rinsed and placed in Citrisolv for 2 minutes. Slides were then dipped in 100% ethanol and allowed to dry for 1–2 minutes before mounting. Slides were mounted and stored in a dry environment until imaging.

CD31 Immunohistochemistry—Donkey serum and secondary antibody (donkey anti-mouse coupled with Dy Light-488) were purchased from Jackson ImmunoResearch (West Grove, PA). Primary antibody (mouse monoclonal anti-Rat CD31) was purchased from BD Biosciences (San Jose, CA). Hoechst 33342 was purchased from Sigma-Aldrich (St. Louis, MO). FluorSave™ Reagent was purchased from EMD Millipore (Darmstadt, Germany). Frozen slides were first fixed in acetone for 20 minutes at 4°C . Slides were then removed from acetone and allowed to dry under a fume hood for 30 min at RT. A liquid blocking pen was used to encircle tissue samples. After 1 hour, the samples were washed 1x with 1x PBS (–/–) for 5 minutes. Slides were blocked for 30 minutes at RT in a blocking solution of 5% donkey serum. The buffer was then replaced with the primary antibody (diluted 1:200 in blocking solution) for 1 hour. Tissue samples were rinsed 3 times for 5 minutes with 1x PBS (–/–). Secondary antibody solution (diluted 1:100 in blocking solution) was then applied to the tissue samples and allowed to incubate for 30 minutes in the dark. The slides were then rinsed with 1x PBS (–/–) in the dark for 5 minutes. Hoechst solution (1 mg Hoechst 33342

in 4 ml DI water, diluted 1:100 in 1x PBS (–/–) was applied to the samples for 5 minutes in the dark. The slides were again washed for 5 minutes with 1x PBS (–/–) in the dark. Slides were mounted using FluorSave™ Reagent, and stored protected from light at 4 °C until imaged.

CD68 Immunohistochemistry—Donkey serum and secondary antibody (donkey anti-mouse coupled with Dy Light-488) were purchased from Jackson ImmunoResearch (West Grove, PA). Primary antibody (mouse monoclonal anti-rat CD68, MCA341R) was purchased from AbD Serotech (Raleigh, NC). Hoechst 33342 was purchased from Sigma-Aldrich (St. Louis, MO). Frozen slides were first fixed in acetone for 20 minutes in a 4 °C refrigerator. Slides were then removed from acetone and allowed to dry under a fume hood for 30 minutes. A liquid blocking pen was used to encircle the tissue samples. After 30 minutes, the samples were washed 1x TBS (–/–) for 5 minutes. Slides were blocked for 1 hour at RT in a blocking solution of 5% donkey serum. The buffer was then replaced with the primary antibody (diluted 1:100 in blocking solution) for 1 hour. Tissue samples were rinsed 3 times for 5 minutes with 1x PBS (–/–). Secondary antibody solution (diluted 1:100 in blocking solution) was then applied to the tissue samples and allowed to incubate for 1 hour in the dark. The slides were then rinsed with 1x TBS (–/–) in the dark for 5 minutes. Hoechst solution (1 mg Hoechst 33342 in 4 ml DI water diluted 1:100 in 1x PBS (–/–)) was applied to the samples for 5 minutes. The slides were again washed for 5 minutes with 1x TBS (–/–) in the dark. Slides were mounted using FluorSave™ Reagent, and stored protected from light at 4 °C until imaged.

Image Analysis

Image Collection—Low magnification Images of Masson's trichrome stained samples were collected using the 10x objective on a Nikon Eclipse TE2000-U with a Nikon Digital Sight DS-2Mv Digital Camera (Nikon Inc., Melville, New York). A 6 X 6 array of higher magnification images was taken by stitching individual 20x fields together. Images of CD31 and CD68 stained samples were collected with the 20x objective on a Nikon Eclipse TE2000-U with a Nikon Digital Sight DS-2Mv Digital Camera (Nikon Inc., Melville, New York). Images were taken by stitching individual 20x images to generate 6x10 large-image fields. Illumination at 488 nm was used to image CD68 and CD31 receptors by capturing the 525 nm emission from the counterstained cell nuclei (Hoechst Dye 33342 stain).

Foreign Body Capsule Collagen Density—The density of the collagen in the capsule that formed around sensor and Tygon® tubing implants was calculated by averaging four 50 × 50 μm² fields per tissue sample using a previously developed MATLAB (The MathWorks, Natick, MA) program (Fig. 2a). This program determines the percent collagen from images of Masson's Trichrome stained tissue sections [11]. In short, the program defines collagen from trichrome-stained images by the characteristic blue-green color, divides the number of collagen positive pixels by the total pixels in the image. This ratio was then multiplied by 100 to obtain the percent collagen coverage for that field.

Microvessel and Macrophage Density—The number of microvessels around implants was determined from CD31 stained sections (Fig. 2b), while the number of macrophages

around implants was determined from CD68 stained sections (Fig. 2c). For each image, a 100 µm wide band beginning at the edge of the implant and moving outward was defined. Using ImageJ (National Institutes of Health, Bethesda, MD) this zone was selected and overlaid onto thresholded CD31 or CD68 images. Using the ‘analyze particles’ function the number of structures (count number) and the size of the selected zone in µm² were obtained. These measurements were then used to calculate the number of microvessels (CD31) or macrophages (CD68) per mm².

Glucose Sensor Performance

Sensitivity Measurements—Data from glucose sampling procedure were used to calculate sensor sensitivity over the duration of the study. Sensitivity for each sensor treatment was calculated based on the following formula [11]:

$$Sensitivity = \frac{I_i}{RGM_i}$$

Where *RGM* is the glucose reading from a conventional glucose meter (mg/dL) and *I* is the current measurement from the continuous glucose sensor (nA).

Mean Average Relative Difference (MARD)—Glucose Bolus data was analyzed using the Mean Absolute Relative Difference (MARD) calculation that measures the average variance between the sensor and the reference measurements blood glucose concentration. The lower the MARD, the more accurate the device is considered. The MARD for a given glucose bolus test was calculated as follows [27]:

$$MARD = \frac{1}{n} * \left(\sum_i^n \frac{|SGM_i - RGM_i|}{RGM_i} \right)$$

Where *n* is the number of measurements during the glucose bolus, *SGM* the sensor glucose measurement (implanted Medtronic MiniMed SOF-SENSOR™), and *RGM* is the reference glucose measurement (OneTouch Ultra test strips). Both measurements were expressed in units of mg/dL.

Continuous glucose sensor current measurements (nA) were converted to units of mg/dL by referencing a one-point calibration curve. This calibration curve was constructed by performing a glucose measurement using the OneTouch Ultra meter prior to the start of the glucose bolus test.

Sensor Lag Time—Sensor Lag Time is the difference between the time that glucose concentration peaks in the blood and the time that the sensor measures a peak concentration. Sensor lag time was calculated as follows [11, 15].

$$Sensor\ Lag\ Time = SGM_{time} - RGM_{time}$$

SGM_{time} is the time of the peak sensor glucose measurement (implanted Medtronic MiniMed SOF-SENSOR™) and RGM_{time} is the time of the peak reference glucose measurement (OneTouch Ultra test strips).

Statistical Analysis

All data are presented as mean $\pm$ standard error of the mean. Data were tested for normality via Shapiro-Wilk tests, and quantile-quantile plots. Because the data were not normally distributed, they were analyzed using the Wilcoxon Each Pair test. The Bonferroni procedure was applied to control the familywise error rate and to determine significance. Statistical significance was determined as $p < 0.05$.

Results

Histological Analysis of Tygon® Tubing Implantation Study

Tygon® tubing was selected that had dimensions and compliance similar to the recording tip of Medtronic MiniMed SOF-SENSOR™ glucose sensors. A total of 84 Tygon® tubing specimens were fully implanted in the rat dorsal subcutis for 3, 7, 14 or 21 days. Specimens were either bare tubing or tubing deployed with either a Dex-free or Dex-loaded porous coatings.

Figure 3 plots the percent collagen coverage (Fig. 3a), the macrophage density (Fig. 3b), and the microvessel density (Fig. 3c) in the tissue surrounding Tygon® tubing subcutaneously implanted in rats. See Supplemental Figures 2–4 for representative histological images.

The percent of collagen that occupied the tissue around bare implants appeared to be relatively consistent over time with bare implants at days 14 and 21 appearing elevated albeit not significantly (Fig. 3a).

Dex-loaded implants had significantly lower macrophage densities within 100 μ m of the implant at days 3, 7, and 14; however, at day 21 there was no significant difference in the density of macrophages regardless of implant type (Fig. 3b).

There was no difference in the density of blood vessels within 100 μ m of the implant at days 3 and 7 regardless of implant type; however at day 14 the vessel density of Dex-free porous coatings was significantly higher with respect to bare Tygon® tubing and Dex-loaded porous coatings, and at day 21 both Dex-free and Dex-loaded porous coatings had significantly higher vessel densities than the bare implants (Fig. 3c).

The Tygon® tubing study showed that Dex-loaded implants attenuated the inflammatory response, while porous coatings regardless of Dex-loading increased vascularity around implants.

Histological Analysis of Functional Sensor Implantation Study

A total of 63 functional sensors were percutaneously inserted in the dorsal subcutis of rats for 3, 7 or 21 days. The day 14 time point was eliminated because the tubing results at day 7 and 14 were similar (Fig. 3) and access to functional glucose sensors was limited.

Specimens were either bare sensors or sensors deployed with Dex-free and Dex-loaded porous coatings.

Figure 4 plots the percent of collagen coverage (Fig. 4a), the macrophage density (Fig. 4b), and the microvessel density (Fig. 4c) in the tissue surrounding percutaneous sensor implants. See Supplemental Figures 5–7 for representative histological images.

There was no difference in the percent of collagen that occupied the tissue around the bare or the two treated sensors at 3 and 7 days post-implantation; however, at day 21 the mean collagen density around the bare sensors was about 2-fold greater than the two treated sensors (Fig. 4a). The spread resulted in no significant differences.

Dex-loaded porous coatings had significantly lower macrophage densities within 100 μ m of the sensor at day 3 and 7 post-implantation when compared to the Dex-free porous and bare controls; however, at day 21 the number of macrophages around Dex-loaded implants increased, but no significant differences between the bare and treated sensors were observed at day 21.

The density of microvessels within 100 μ m of sensors was essentially the same at day 3 and 7 for the bare and treated sensors, but at day 21 the treated sensors both had significantly higher vessel densities compared to the bare sensors. There was no significant difference between the Dex-free and Dex-loaded porous coated treatments at day 21 (Fig. 4c).

The functional sensor study also showed that Dex-loaded implants attenuated the inflammatory response, while porous coatings regardless of Dex-loading increased vascularity around implants.

It should be noted that a total of six sensors failed in this functional sensor study (9.5%). For the day 3 group, one uncoated sensor failed prematurely due to dislodging, while one Dex-free coated sensor failed due to electrical malfunction (though the sensor probe remained in place). All sensors in the day 7 group remained functional and attached to the backs of rats. At day 21 two bare sensors, one Dex-free and one Dex-loaded coated sensor failed prematurely due to extrusion from the rat subcutis.

Glucose Sensor Performance

Sensitivity—Bare sensors and sensors coated with Dex-free and Dex-loaded porous coatings were inserted in dorsal subcutis of rats for continuous, real-time glucose monitoring over 21 days. At least five out of seven sensors for each treatment group remained implanted until experiment termination. Figure 5 plots the average sensor sensitivities for all treatment groups recorded over the 21 day implantation period. All sensors were similarly stable for the first 10–12 days of the study. However, by 15 days the sensitivity of the bare and Dex-free porous coated sensors had decreased significantly compared the Dex-loaded sensors.

Sensor Lag Time—Figure 6 contains responses of the OneTouch control, bare sensors and sensors coated with Dex-free and Dex loaded porous coatings to intraperitoneal glucose bolus injections at 1 day post-implantation. The sensor lag time for each treatment was

determined as the difference between the peak blood glucose measurement of the OneTouch and the peak interstitial glucose measurements of the implanted sensors.

Table 2 lists the sensor lag times for the different sensor treatments after 1, 3, 7, 14 and 21 days implantation. In general sensor lag times increased with implantation time for all sensor treatments. The bare sensors exhibited shorter average lag times on days 1, 3, 7 and 14 compared to Dex-loaded and Dex-free porous coated sensors; however the spread resulted in no significant differences. In contrast, at day 21 the Dex-loaded treatment had a lag time of ~20 min while the bare and Dex-free treatments both failed to respond to glucose challenges (lag times of >60min).

MARD—Table 3 lists the MARD values for all sensor treatments after 1, 3, 7, 14 and 21 days implantation. The bare sensors exhibited shorter average MARD values on days 1, 3, and 7 than did the Dex-loaded and Dex-free porous coated sensors. By day 21, Dex-free porous coated and bare sensors failed to respond glucose challenges, while Dex-loaded porous coated sensors responded to challenges but displayed a high MARD value of ~ 63 %. The trend of the MARD values was similar to that seen with the sensor lag times, including no significance due to spread in the data.

Discussion

The commercial success of implantable glucose sensors has been hindered by their premature failure that has been attributed largely to the deleterious tissue effects such as biofouling, the immune response, and fibrous encapsulation [7, 9, 10, 12]. Bungay et al. described quantitatively how various aspects of the foreign body response impairs mass transport to a sensor; specifically, the quantitative hindrance by molecular biofouling, structural changes, and consumption by the invading cells [28]. More recently, *in vitro* testing and modeling studies have identified the consumption of glucose by inflammatory cells and lack of vascularity around implanted sensor tips as key factors that lead to untimely failure [11, 15, 16]. A desirable intervention would therefore be one that both curbs inflammation while promoting vascularization of the tissue surrounding the implanted sensor.

Dexamethasone (Dex) loaded porous polyurethane coatings that combined angiogenic texturing with local delivery of the anti-inflammatory agent were developed in an effort to achieve the dual effect of curbing inflammation, and promoting the vascularization around indwelling sensors. Dex is a potent glucocorticoid associated with diminished activation of immune cells and up-regulation of anti-inflammatory cytokines [21, 29, 30], yet localized delivery of Dex is often accompanied by decreased vascularity at the sensor-tissue interface [20, 21, 30–32]. It is also well established that surface texturing with feature sizes on the order of cellular dimensions can induce blood vessel formation around medical implants [11, 25, 33–35].

Previously we reported on the *in vitro* testing physical characterization, and initial *in vivo* testing of the Dex-loaded porous polyurethane coatings [25]. These coatings fit snugly on the surface of Medtronic MiniMed SOF-SENSOR™ glucose sensors, were highly porous

(~85 %), and had a controlled thickness (~ 81 μm) and pore size (~76 μm). Moreover, Dex-loaded porous coatings minimally interfered with sensor performance *in vitro*, and released ~ 270 μg of Dex over 14 days. Preliminary dosing studies also showed that this Dex payload from coating elicited no systemic effects. However, questions remained regarding 1) the effects of coatings on the pathology of the surrounding tissue bed, and 2) concomitant effects on glucose sensor function *in vivo*.

The first part of the current study was the histological characterization of Dex-loaded porous coatings deployed on sensor-sized Tygon® tubing implants that were implanted subcutaneously in the rat dorsum, and on fully functional Medtronic MiniMed SOF-SENSOR™ glucose sensors that were percutaneously inserted in the rat dorsum.

In the Tygon® tubing study Dex-loaded and Dex-free porous coatings were able to disrupt collagen deposition around implants (Fig 3a). By day 21, the average collagen density around Dex-loaded and Dex-free porous coatings was almost 2-fold less than that of bare implants. Also, Dex-loaded porous coatings showed significantly less inflammatory macrophages around the implants than bare or Dex-free porous controls by days 3 and 7 (Fig 3b). By day 21, however, there was no difference in the number of inflammatory cells surrounding the implants. Dex-loaded porous coatings had equivalent levels of vascularization to that of Dex-free controls and almost four-times more vessels than bare Tygon® controls by day 21 (Fig 3c).

In the fully functional sensor histology study again Dex-loaded and Dex-free porous coated sensors had, on average, a lower collagen density than did bare implants; however these differences were not statistically significant (Fig 4a). Dex-loaded coatings showed a significantly lower number of macrophages in the tissue surrounding implanted sensors during early timepoints, when compared to Dex-free and bare controls (Fig 4b); yet, there was no difference by day 21. Also, coated sensors regardless of Dex-loading significant higher levels of vascularity when compared to bare sensors by day 21 post-implantation (Fig 4c).

The observation that both the Tygon® tubing and functional sensor studies showed similar histology suggests that Dex-loaded porous coatings were capable of attenuating inflammation, decreasing collagen density of the foreign body capsule, and promoting capillary formation in the tissue adjacent to implants. Tissue changes in the form of an attenuated inflammatory response can be attributed to the timed and bioactive release of Dex from the coating surface. It has been previously reported that Dex-loaded porous coatings have a daily release of ~ 30 $\mu\text{g}/\text{day}$ of bioactive Dex during the first 7 days, and a release daily release of ~7 μg from days 7–14 [25]. This amounted to almost a full deployment of Dex within the first 14 days, which is also the window when the greatest anti-inflammatory action was observed in the tissue surrounding Dex-loaded coatings (Fig 2b and 3b). Similar results were found in reports by Klueh et al. where the inflammation around implanted sensor probes was diminished when Dex was locally delivered [20]. Moreover, several studies have reported on a decrease in macrophage density as a function of Dex release from indwelling microdialysis probes and osmotic pumps [36–38].

Once the Dex payload was fully delivered from the coatings, the textured surface from Dex-loaded porous coatings became the main mediator of the response. The increased vascularity and decreased collagen density of the tissues surrounding Dex-loaded porous coatings by day 21 post-implantation (Figs. 3a, 3c, 4a and 4c) can be attributed to the effects of controlled coating microstructure. As previously reported, Dex-loaded porous coatings had a porosity of ~ 85% and a pore size of ~76 μm [25], features which have been shown to enhance tissue vascularization and disrupt collagen capsule formation around implanted sensor leads [11, 22]. Moreover, several studies have reported on the angiogenic effects of texturing to increase vascularity around medical implants [11, 25, 33–35]. Though the exact mechanism by which topographical cues promote angiogenesis and tissue integration have yet to be elucidated, it is believed that pores within the 20–100 μm provide an optimal environment for transport of cellular by-products and migration of endothelial cells [39]. Also, studies comparing collagen density around solid implants to porous implants of (PVA and ePTFE) noted that the density of the capsule that developed around the porous materials was much lower than that of solid materials [35]. Furthermore, Braber et al. showed that specific features in the size, shape of topographical cues alter the way fibroblasts orient and attach to substrates [40].

The second portion of the current study was the evaluation of glucose sensor performance, where Dex-loaded porous coated sensors maintained significantly higher sensitivity levels when compared Dex-free porous coated and bare sensors from days 15 – 21 post-implantation (Fig. 5). The extended performance window of Dex-loaded porous coated sensors can be attributed to both the significant depletion of macrophages and increase in vascularity of the tissue surrounding the implants. Dex-loaded porous coatings showed significantly less inflammatory macrophages around the implants than bare or Dex-free porous controls by days 3–14 (Figs. 3b and 4b). Recent reports have suggested that macrophages not only contribute to premature sensor failure by enzymatic degradation and phagocytosis but rather impair sensor function via direct surface fouling, the secretion of cytokines that induce fouling by neighboring cells, and by maintaining a metabolic state of high glucose consumption [16, 41]. Therefore, decreasing the density of macrophages around implanted sensor leads could translate into improved performance.

Modeling studies have also shown that tissue vascularization is the most important predicting sensor signal sensitivity [42]. Dex-loaded porous coated sensors had significant higher levels of vascularity when compared to bare sensors by day 21 post-implantation (Fig 4c). A more extensive vascular network would allow for less impeded transport of glucose to the sensor surface, thus allowing sensors to be more sensitive through the 21 day study.

It is important to note that even though sensors deployed with Dex-free porous coatings resulted in a more vascularized microenvironment, this response did not translate into an extension of sensor functionality (Fig 5). Similarly, studies using porous PLLA coatings that were Dex-free found that although the implantation of coated sensors resulted in a well vascularized microenvironment, this response also did not translate into an extension of sensor functionality *in vivo* [11, 22]. Therefore, not only a vascularized tissue network is necessary to yield adequate long-term performance but also a mediation inflammation is required. These results highlight the importance of mediating both the inflammatory and

repair stages of FBR to create a tissue microenvironment conducive for long-term performance of glucose sensors *in vivo*.

Finally, the general trend of higher sensor lag times and MARDs scores for sensors with porous coatings and longer implantation times may be attributed to the increased transport pathlength imposed by the coatings and temporal changes in the tissue-sensor interface (Fig 6). Studies have shown that increasing the distance that glucose has to migrate to reach the sensor surface is the greatest determinant at increasing glucose sensor lag [42]. Moreover, *in vitro* functional sensor studies by Koshwanez et.al. [11] have similarly showed that sensors deployed with thick porous coatings (>200 μm) had elevated lag times when compared to thinner coatings (<100 μm).

Conclusion

The development of coatings that can extend the functional life of implantable glucose sensors should address key aspects of the foreign body reaction such as inflammation and capsule vascularity. This study evaluated the effectiveness of porous Dex-loaded coatings at modifying the tissue response to glucose sensor implantation and the subsequent effects on glucose sensor function *in vivo*. Dex release from porous coatings attenuated inflammation induced by subcutaneous Tygon® tubing and sensor implants, while topographical cues presented by coatings promoted the vascularization of the near-by tissue. The dual effect of diminished inflammation and increased vascularization of the tissue adjacent to sensors treated with Dex-loaded porous coatings allowed sensors to remain highly sensitive throughout the 21 day study when compared to bare and porous controls. However, due to the increase in the glucose's diffusion path, porous coated sensors regardless of Dex-loading experienced an increase in sensor lag and MARDs. Future work should focus on developing approaches that not only hinder macrophage migration and promote vascularity around sensor implants, but attempt to glucose sensor lag.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Schiffrin A, Belmonte M. Multiple daily self-glucose monitoring: its essential role in long-term glucose control in insulin-dependent diabetic patients treated with pump and multiple subcutaneous injections. *Diabetes Care*. 1982; 5:479–484. [PubMed: 6765224]
2. Jaremko J, Rorstad O. Advances toward the implantable artificial pancreas for treatment of diabetes. *Diabetes care*. 1998; 21:444–450. [PubMed: 9540030]

3. Gross TM, Bode BW, Einhorn D, Kayne DM, Reed JH, White NH, Mastrototaro JJ. Performance evaluation of the MiniMed® continuous glucose monitoring system during patient home use. *Diabetes Technology & Therapeutics*. 2000; 2:49–56. [PubMed: 11467320]
4. Mastrototaro JJ. The MiniMed continuous glucose monitoring system. *Diabetes technology & therapeutics*. 2000; 2:13–18.
5. Sachedina N, Pickup J. Performance assessment of the Medtronic-MiniMed Continuous Glucose Monitoring System and its use for measurement of glycaemic control in Type 1 diabetic subjects. *Diabetic medicine*. 2003; 20:1012–1015. [PubMed: 14632702]
6. Heo YJ, Takeuchi S. Towards Smart Tattoos: Implantable Biosensors for Continuous Glucose Monitoring. *Advanced Healthcare Materials*. 2013; 2:43–56. [PubMed: 23184404]
7. Oliver N, Toumazou C, Cass A, Johnston D. Glucose sensors: a review of current and emerging technology. *Diabetic Medicine*. 2009; 26:197–210. [PubMed: 19317813]
8. Wisniewski NA, Helton K, McMillan WA. Tissue-integrating sensors. Google Patents. 2011
9. Wisniewski N, Reichert M. Methods for reducing biosensor membrane biofouling. *Colloids and Surfaces B: Biointerfaces*. 2000; 18:197–219. [PubMed: 10915944]
10. Nichols SP, Koh A, Storm WL, Shin JH, Schoenfisch MH. Biocompatible materials for continuous glucose monitoring devices. *Chemical reviews*. 2013; 113:2528–2549. [PubMed: 23387395]
11. Koschwanetz H, Yap F, Klitzman B, Reichert W. In vitro and in vivo characterization of porous poly-L-lactic acid coatings for subcutaneously implanted glucose sensors. *Journal of Biomedical Materials Research Part A*. 2008; 87:792–807. [PubMed: 18200540]
12. Wisniewski N, Moussy F, Reichert W. Characterization of implantable biosensor membrane biofouling. *Fresenius' journal of analytical chemistry*. 2000; 366:611–621. [PubMed: 11225773]
13. Yang Y, Zhang SF, Kingston MA, Jones G, Wright G, Spencer SA. Glucose sensor with improved haemocompatibility. *Biosensors and Bioelectronics*. 2000; 15:221–227. [PubMed: 11219733]
14. Quinn CA, Connor RE, Heller A. Biocompatible, glucose-permeable hydrogel for *in situ* coating of implantable biosensors. *Biomaterials*. 1997; 18:1665–1670. [PubMed: 9613815]
15. Novak MT, Yuan F, Reichert WM. Modeling the relative impact of capsular tissue effects on implanted glucose sensor time lag and signal attenuation. *Analytical and bioanalytical chemistry*. 2010; 398:1695–1705. [PubMed: 20803006]
16. Novak MT, Yuan F, Reichert WM. Macrophage embedded fibrin gels: An in vitro platform for assessing inflammation effects on implantable glucose sensors. *Biomaterials*. 2014; 35:9563–9572. [PubMed: 25175597]
17. Koh A, Lu Y, Schoenfisch MH. Fabrication of Nitric Oxide-Releasing Porous Polyurethane Membranes Coated Needle-Type Implantable Glucose Biosensors. *Analytical chemistry*. 2013
18. Nichols SP, Koh A, Brown NL, Rose MB, Sun B, Slomberg DL, Riccio DA, Klitzman B, Schoenfisch MH. The effect of nitric oxide surface flux on the foreign body response to subcutaneous implants. *Biomaterials*. 2012
19. Norton L, Tegnell E, Toporek S, Reichert W. In vitro characterization of vascular endothelial growth factor and dexamethasone releasing hydrogels for implantable probe coatings. *Biomaterials*. 2005; 26:3285–3297. [PubMed: 15603824]
20. Klueh U, Kaur M, Montrose DC, Kreutzer DL. Inflammation and glucose sensors: use of dexamethasone to extend glucose sensor function and life span in vivo. *Journal of diabetes science and technology (Online)*. 2007; 1:496.
21. Hickey T, Kreutzer D, Burgess D, Moussy F. In vivo evaluation of a dexamethasone/PLGA microsphere system designed to suppress the inflammatory tissue response to implantable medical devices. *Journal of biomedical materials research*. 2002; 61:180–187. [PubMed: 12007197]
22. Koschwanetz H, Reichert W, Klitzman B. Intravital microscopy evaluation of angiogenesis and its effects on glucose sensor performance. *Journal of Biomedical Materials Research Part A*. 2010; 93:1348–1357. [PubMed: 19911378]
23. Ju YM, Yu B, West L, Moussy Y, Moussy F. 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. *Journal of Biomedical Materials Research Part A*. 2010; 92:650–658. [PubMed: 19235209]

24. Prichard H, Schroeder T, Reichert W, Klitzman B. Bioluminescence imaging of glucose in tissue surrounding polyurethane and glucose sensor implants. *Journal of diabetes science and technology*. 2010; 4:1055. [PubMed: 20920425]
25. Vallejo-Heligon SG, Klitzman B, Reichert WM. Characterization of porous, dexamethasone-releasing polyurethane coatings for glucose sensors. *Acta biomaterialia*. 2014; 10:4629–4638. [PubMed: 25065548]
26. James SJ, Pogribna M, Miller BJ, Bolon B, Muskhelishvili L. Characterization of cellular response to silicone implants in rats: implications for foreign-body carcinogenesis. *Biomaterials*. 1997; 18:667–675. [PubMed: 9151998]
27. Noujaim SE, Horwitz D, Sharma M, Marhoul J. Accuracy Requirements for a Hypoglycemia Detector: An Analytical Model to Evaluate the Effects of Bias, Precision, and Rate of Glucose Change. *Journal of diabetes science and technology (Online)*. 2007; 1:652–668.
28. Bungay PM, Morrison PF, Dedrick RL. Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life sciences*. 1990; 46:105–119. [PubMed: 2299972]
29. Norton L, Koschwanez H, Wisniewski N, Klitzman B, Reichert W. Vascular endothelial growth factor and dexamethasone release from nonfouling sensor coatings affect the foreign body response. *Journal of Biomedical Materials Research Part A*. 2007; 81:858–869. [PubMed: 17236219]
30. Cato AC, Wade E. Molecular mechanisms of anti-inflammatory action of glucocorticoids. *Bioessays*. 1996; 18:371–378. [PubMed: 8639160]
31. Hickey T, Kreutzer D, Burgess DJ, Moussy F. In vivo evaluation of a dexamethasone/PLGA microsphere system designed to suppress the inflammatory tissue response to implantable medical devices. *Journal of Biomedical Materials Research*. 2002; 61:180–187. [PubMed: 12007197]
32. Patil SD, Papadimitrakopoulos F, Burgess DJ. Concurrent delivery of dexamethasone and VEGF for localized inflammation control and angiogenesis. *Journal of controlled release*. 2007; 117:68–79. [PubMed: 17169457]
33. Vaddiraju, S.; Wang, Y.; Qiang, L.; Burgess, DJ.; Papadimitrakopoulos, F. Macroscopic porosity generation in outer hydrogel membranes to offset sensitivity loss in implantable glucose sensors. *Body Sensor Networks (BSN), 2013 IEEE International Conference on, IEEE; 2013; p. 1-5.*
34. Updike SJ, Shults MC, Gilligan BJ, Rhodes RK. A subcutaneous glucose sensor with improved longevity, dynamic range, and stability of calibration. *Diabetes Care*. 2000; 23:208–214. [PubMed: 10868833]
35. Ward WK, Slobodzian EP, Tiekotter KL, Wood MD. The effect of microgeometry, implant thickness and polyurethane chemistry on the foreign body response to subcutaneous implants. *Biomaterials*. 2002; 23:4185–4192. [PubMed: 12194521]
36. Ward WK, Hansen JC, Massoud RG, Engle JM, Takeno MM, Hauch KD. Controlled release of dexamethasone from subcutaneously-implanted biosensors in pigs: Localized anti-inflammatory benefit without systemic effects. *Journal of Biomedical Materials Research Part A*. 2010; 94A: 280–287. [PubMed: 20186727]
37. Keeler GD, Durdik JM, Stenken JA. Localized delivery of dexamethasone-21-phosphate via microdialysis implants in rat induces M (GC) macrophage polarization and alters CCL2 concentrations. *Acta biomaterialia*. 2015; 12:11–20. [PubMed: 25449921]
38. Keeler GD, Durdik JM, Stenken JA. Effects of Delayed Delivery of Dexamethasone-21-Phosphate via Subcutaneous Microdialysis Implants on Macrophage Activation in Rats. *Acta biomaterialia*. 2015
39. Bryers JD, Giachelli CM, Ratner BD. Engineering biomaterials to integrate and heal: the biocompatibility paradigm shifts. *Biotechnology and bioengineering*. 2012; 109:1898–1911. [PubMed: 22592568]
40. den Braber ET, de Ruijter JE, Ginsel LA, von Recum AF, Jansen JA. Quantitative analysis of fibroblast morphology on microgrooved surfaces with various groove and ridge dimensions. *Biomaterials*. 1996; 17:2037–2044. [PubMed: 8902235]
41. Klueh U, Frailey JT, Qiao Y, Antar O, Kreutzer DL. Cell based metabolic barriers to glucose diffusion: Macrophages and continuous glucose monitoring. *Biomaterials*. 2014

42. Novak, MT. Modeling the tissue effects of the Foreign Body Response on Glucose Sensor Function. Duke University; Durham, NC: 2010.

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Figure 1.

Porous dexamethasone-releasing coatings were implanted in the dorsum of rats. (a) Picture of Tygon® tubing implants with and without porous, Dex-releasing coatings (b) Configuration of subcutaneous implants in the dorsum of rats (c) Picture of Medtronic MiniMed glucose Sensor™ with and without highly porous Dex-releasing coatings (d) Configuration of percutaneous Medtronic MiniMed Sensors in the dorsum of rats. Implants were randomly rotated through the positions shown above.

Figure 2.

Photomicrographs of sensor implants coated with Dex-loaded porous coatings after 21 days in rats. a) Masson's trichrome images were used to compute the collagen density of the foreign body capsule. Four random $50 \times 50 \mu\text{m}^2$ fields (red squares) were analyzed per image. CD68 (b) and CD31(c) images were used to calculate the density of macrophages and microvessels respectively around implants. For each image, a "ring-like" $100 \mu\text{m}$ wide zone beginning from the edge of the implant (white dotted line) and moving outward (red dotted line) was defined and analyzed. Scale bars = $200 \mu\text{m}$ for (a) and $400 \mu\text{m}$ for (b) & (c).

Figure 3.

Collagen, macrophage and blood vessel density of the tissue surrounding Tygon® tubing subcutaneous implants in rats; a) Collagen Density of bare implants increased over time; b) Tissues surrounding Dex-loaded implant had significantly less macrophages when compared to the tissue surrounding Dex-free and Bare implants at days 3,7 and 14; c) The tissues surrounding implants showed no differences in vascularity at days 3 and 7. On day 14, vascularity around Dex-free porous implants significantly increased. By day 21, vascularity for all porous implants regardless of Dex-loading had significantly increased compared to controls. * denotes $p<0.05$ or less compared to Bare tubing; . # denotes $p<0.05$ or less compared to Dex-free porous coated implants; .(n=7)

Figure 4.

Collagen, macrophage and blood vessel density of the tissue surrounding implanted Medtronic MiniMed SOF-Sensor glucose sensors in rats; a) Collagen Density of bare implants increased over time; b) The tissues surrounding Dex-loaded porous coatings had significantly less macrophages than tissues surrounding Dex-free porous and Bare Sensor implants at days 3 and 7; c) Tissues surrounding implants showed no differences in vascularity at days 3 and 7. By day 21, vascularity for all porous implants regardless of Dex-loading had significantly increased when compared to controls; # denotes $p < 0.05$ or less compared to Dex-free porous coated implants; .(n=5 or greater)

Figure 5.
Plot of sensor sensitivities of implanted Medtronic MiniMed SOF-Sensor glucose sensors. Dex-releasing porous coated sensors retained high sensitivity levels through the 21 day period when compared to Dex-free porous coated and bare controls. Sensitivity of Dex-free porous and bare controls decreased after day 12.

Figure 6. Representative Glucose Bolus Test of implanted Medtronic MiniMed SOF-Sensor glucose sensors after 1 day post-implantation. All coated sensors regardless of Dex-loading had increased sensor lag when compared to controls.

Table 1

Recent strategies used to regulate tissue response to implanted glucose sensors

Strategy	Material and Platform	Active Agent (Delivery Window)	Test subject/implant site/implant type	Length of Study	Ref
Attenuate inflammation	Rapid Release of small molecule donors	Nitric Oxide (1–3 days)	Rat/percutaneous/microdialysis probes	2 weeks	[18]
Anti-fouling, attenuate inflammation	PVA Hydrogel and drug loaded PLGA microspheres	Dex (1–3 months)	Rat/subcutaneous/stainless steel needle	4 weeks	[21]
Attenuate inflammation, promote vascularization	pHema-PEG hydrogel and PLGA microspheres	Dex and VEGF (3 days for Dex, 2 months for VEGF)	Rat/percutaneous/microdialysis probes	6 weeks	[19, 25]
Tissue biomimicry	Adipose Derived Stem Cells (ASCs) coatings	N/A	Rat, percutaneous, microdialysis probes	8 weeks	[24]
Tissue biomimicry	Porous Collagen Coatings	N/A	Rat/percutaneous/glucose sensors	4 weeks	[23]
Promote tissue integration, vessel growth	Porous pHEMA and silicone scaffolds	N/A	Mouse/percutaneous cylindrical scaffolds	6 months	[26]
Promote tissue integration, vessel growth	Porous ePTE coatings	N/A	Dog/percutaneous/glucose sensors	3–6 months	[27]
Promote tissue integration, vessel growth	Porous PLLA coatings	N/A	Rat/ sub- and percutaneous/glucose sensors	3 weeks	[11]

Table 2

Sensor Lag Time during glucose bolus test for Normal Rats (n=5 or greater).

	Time Lag (Min)		
Time Point	Sensor Treatment (Mean $\pm$ SEM)		
	Bare	Dex-Free Porous Coating	Dex-Loaded Porous Coating
Day 1	3 $\pm$ 3	13 $\pm$ 7	12 $\pm$ 6
Day 3	3 $\pm$ 3	12 $\pm$ 6*	11 $\pm$ 4*
Day 7	10 $\pm$ 6	18 $\pm$ 7	19 $\pm$ 8
Day 14	12 $\pm$ 8	22 $\pm$ 9*	17 $\pm$ 6
Day 21	> 60 ^a	> 60 ^a	20 $\pm$ 8*

^a Sensors failed before test

* denotes p<0.05 or less compared to Bare Sensor

Table 3

Mean Average Relative Deviation during glucose bolus test for Normal R0ats (n=5 or greater).

	MARD (%)		
Time Point	Sensor Treatment		
	Bare	Dex-Free Porous Coating	Dex-Loaded Porous Coating
Day 1	27.0 ± 7.6	32.4 ± 8.5	30.9 ± 9.0
Day 3	18.9 ± 3.2	38.8 ± 11.1	32.7 ± 4.6
Day 7	29.7 ± 4.1	34.4 ± 16.9	31.4 ± 9.8
Day 14	52.5 ± 10.8	61.1 ± 14.4	48.2 ± 13.7
Day 21	> 100 ^a	>100 ^a	63.1 ± 12.7

^aDid not respond to glucose challenge