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ORIGINAL ARTICLE

Effect of Dexamethasone-Loaded Poly(Lactic-Co-Glycolic Acid) Microsphere/Poly(Vinyl Alcohol) Hydrogel Composite Coatings on the Basic Characteristics of Implantable Glucose Sensors

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Abstract

Background:

Hydrogels alone and in combination with microsphere drug delivery systems are being considered as biocompatible coatings for implantable glucose biosensors to prevent/minimize the foreign body response. Previously, our group has demonstrated that continuous release of dexamethasone from poly(lactic-co-glycolic acid) (PLGA) microsphere/poly(vinyl alcohol) (PVA) hydrogel composites can successfully prevent foreign body response at the implantation site. The objective of this study was to investigate the effect of this composite coating on sensor functionality.

Methods:

The PLGA microsphere/PVA hydrogel coatings were prepared and applied to glucose biosensors. The swelling properties of the composite coatings and their diffusivity to glucose were evaluated as a function of microsphere loading. Sensor linearity, response time, and sensitivity were also evaluated as a function of coating composition.

Results:

The PLGA microsphere/PVA hydrogel composite coating did not compromise sensor linearity (sensors were linear up to 30 mM), which is well beyond the physiological glucose range (2 to 22 mM). The sensor response time did increase in the presence of the coating (from 10 to 19 s); however, this response time was still less than the average reported values. Although the sensitivity of the sensors decreased from 73 to 62 nA/mM glucose when the PLGA microsphere loading in the PVA hydrogel changed from 0 to 100 mg/ml, this reduced sensitivity is acceptable for sensor functionality. The changes in sensor response time and sensitivity were due to changes in glucose permeability as a result of the coatings. The embedded PLGA microspheres reduced the fraction of bulk water present in the hydrogel matrix and consequently reduced glucose diffusion.

continued →

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Abbreviations: (GOx) glucose oxidase, (MW) molecular weight, (PBS) phosphate-buffered saline, (PLGA) poly(lactic-co-glycolic acid), (Pt) platinum, (PU) polyurethane, (PVA) poly(vinyl alcohol)

Keywords: glucose biosensor, hydrogel, linearity, microsphere, response time, sensitivity

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Abstract cont.

Conclusions:

This study demonstrates that the PLGA microsphere/PVA hydrogel composite coatings allow sufficient glucose diffusion and sensor functionality and therefore may be utilized as a smart coating for implantable glucose biosensors to enhance their *in vivo* functionality.

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Introduction

Significant progress has been made toward development of biosensors for real-time monitoring of various metabolic analytes to aid in treatment and management of diseases such as diabetes.[1–3] Continuous monitoring, for example of glucose, provides information on not only the glucose level at a specific time point, but also the pattern of change with time.[1,4] It is very important for diabetes patients to master the trend of their glucose levels on a daily basis and to understand how it changes with different activities. Such continuous monitoring of glucose will allow early detection of hyperglycemia/hypoglycemia to prevent various complications associated with diabetes. Moreover, continuous monitoring will allow the realization of the "artificial pancreas" concept to ensure tight control of glucose through optimal insulin dosing.[1,4]

Various glucose sensors based on electrochemical, optical, and piezoelectric transduction mechanisms are under development, with a common goal of achieving stable and reliable performance.[1,2] However, owing to their simplicity in design and ease of miniaturization, electrochemical biosensors based on Clark-type glucose sensors are the most common. First-generation Clark-type glucose sensors employ the flavoenzyme glucose oxidase (GO_x) immobilized on top of a working electrode.[3] The flavin adenine dinucleotide, *FAD*, redox cofactor of GO_x catalyzes the oxidation of glucose to glucarolactone, as shown in **Equations (1)** and **(2)**:

$$Glucose + GO_x(FAD) \rightarrow Gluconolactone + GO_x(FADH_2), \quad (1)$$

$$GO_x(FADH_2) + O_2 \rightarrow GO_x(FAD) + H_2O_2 \quad (2)$$

The generated H_2O_2 (hydrogen peroxide) is amperometrically assessed on the surface of the working electrode via **Equation (3),** which relates current to glucose concentration as shown in **Figure 1**:

$$H_2O_2 \xrightarrow{0.7V} O_2 + 2H^+ + 2e^- \quad (3)$$

Despite considerable efforts, no fully implantable biosensor is currently commercially available for the continuous monitoring of glucose. Although promising results have been obtained during *in vitro* experiments with various implantable glucose biosensors, these devices progressively lose their function *in vivo* following implantation. It has become evident that the tissue reaction to the implanted sensors, especially the interactions at the tissue–sensor interface, such as inflammation and fibrous encapsulation, plays a critical role in this loss of sensor functionality.[5]

Biocompatible hydrogel coatings in combination with tissue modifier delivery systems have been investigated as a means to overcome the foreign body response to implanted devices such as biosensors.[5–10] Previously, our group has developed a composite coating consisting of poly(vinyl alcohol) (PVA) hydrogel and poly(lactic-co-glycolic acid) (PLGA) microspheres.[6,8,10] It has been demonstrated that this composite coating is able to counter the foreign body response in a rat model for up to 3 months via controlled release of the anti-inflammatory dexamethasone drug.[11] There are several unique advantages of this composite coating: (1) PVA can be easily physically cross-linked by freeze–thaw cycling, which avoids the risk of destabilizing the sensing element during processing caused by the introduction of toxic agents; (2) PVA hydrogels can be produced that posses mechanical strength similar to human soft tissue, and these properties can be tuned by modifying the freeze–

thaw cycling process; and (3) PLGA is biodegradable, programmable for temporal controlled drug delivery, and, importantly, it has been used in Food and Drug Administration-approved products. Moreover, the dosage of dexamethasone needed for complete suppression of foreign body response was below the threshold needed to cause any systemic effects.[8,12] However, in order to apply the PLGA microsphere/PVA hydrogel composites as implantable biosensor outer coatings, it is critical to understand their influence on the basic characteristics of sensors, including linearity, sensitivity, and response time.

In the present study, PLGA microsphere/PVA hydrogel composite films with various microsphere loadings were prepared, and their swelling properties and glucose diffusivities were determined. The impact of the composite coatings on sensor linearity, response time, as well as sensitivity was investigated as a function of coating composition. An attempt was made to compare change in coating physical properties to change in the basic characteristics of coated sensors. Knowledge gained from the present work will be useful in future development of implantable glucose sensors as well as composite coatings aimed at facilitating application of other implantable devices.

Materials and Experimental Methods

Materials

Glucose oxidase enzyme (E.C. 1.1.3.4, 157, 500 U/g, *Aspergillus niger*), glutaraldehyde (25% w/v solution in water), phenol, bovine serum albumin, glutaraldehyde (50% w/v), and D-glucose (reagent grade) were purchased from Sigma. The PVA [99% hydrolyzed, molecular weight (MW) 133 KDa] was obtained from Polysciences Inc. (Warrington, PA). Platinum (Pt) and silver wire were purchased from World Precision Instruments. The PLGA Resomer RG503H 50:50 (MW 25 KDa, carboxylic acid end uncapped) were gifts from Boehringer-Ingelheim. Methylene chloride was obtained from Fisher Scientific (Pittsburgh, PA). Poly(vinyl alcohol) (MW 30 to 70 KDa) and dexamethasone were obtained from Sigma (St. Louis, MO). Selectophore (catalog number 81367; Tecoflex™) polyurethane (PU) was purchased from Fluka and used as is.

Experimental Methods

Preparation of Poly(Vinyl Alcohol) Hydrogel/Poly(Lactic-Co-Glycolic Acid) Microsphere Composite Films

Preparation of Poly(Vinyl Alcohol) Solutions. A PVA (99% hydrolyzed, MW 133 KDa) aqueous solution (5% w/v) was prepared by mixing polymer powder (1 g) with distilled water (20 ml). The mixture was heated above 80 °C for approximately 45 min, while stirring slowly. When the mixture became clear, the solution was cooled back to room temperature and kept overnight to remove any air bubbles and allow the dissolved polymer to reach equilibrium.

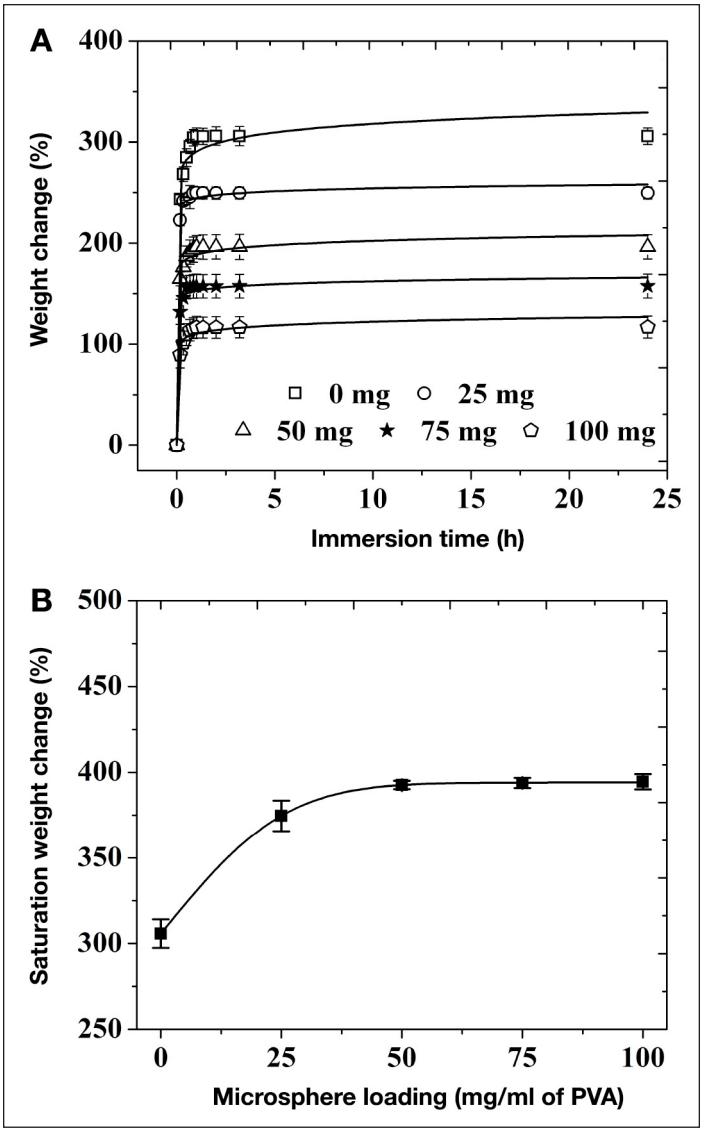

Figure 1. (A) Percentage of weight change of PVA hydrogels loaded with various amounts of dexamethasone–PLGA microspheres immersed in PBS buffer (pH 7.4) at 37 °C as a function of immersion time. **(B)** Equilibrium water content of PVA hydrogels as a function of dexamethasone–PLGA microsphere loading.

Preparation of Poly(Lactic-Co-Glycolic Acid) Microspheres. Dexamethasone-loaded/blank PLGA microspheres were prepared using an oil-in-water emulsion solvent extraction/evaporation technique as described previously.[13] Briefly, 2 g PLGA were dissolved in 8 ml methylene chloride. For dexamethasone-loaded microspheres, 200 mg of dexamethasone were dispersed in this solution. This

organic phase was emulsified in 40 ml of a 1% (w/w) PVA (average MW 30 to 70 KDa) solution and homogenized at 10,000 rpm for 2.5 min using a Power Gen 700D Homogenizer (Fisher Scientific). The resultant emulsion was poured into 500 ml of a 0.1% (w/w) PVA (average MW 30 to 70 KDa) solution and stirred under vacuum to achieve rapid evaporation of methylene chloride. The hardened microspheres were washed three times with deionized water and collected via filtration (0.45 μm). The prepared microspheres were kept under vacuum overnight and later stored at 4 °C until further use.

Preparation of Poly(Lactic-Co-Glycolic Acid) Microsphere/Poly(Vinyl Alcohol) Hydrogel Composite Films. The PLGA microsphere/PVA hydrogel composite films were prepared as described previously.[10,14] Briefly, PLGA microspheres were weighted and dispersed into PVA hydrogel (5% w/v) solution, then this suspension was filled into a premade mold and subjected to three freeze–thaw cycles of 2 h freezing at -20 °C followed by 1 h thawing at 25 °C. The thickness of the film in its dry state is approximately 100 μm.

Characterization of Composite Films

Equilibrium Swelling Studies. The equilibrium water content (H) was experimentally determined using **Equation (4)**, where W_s is the equilibrium swollen weight of PLGA/PVA composite films, W_d is the dry weight of the films, and $W_{d(PVA)}$ is the dry weight of PVA present in the films:

$$H = (W_s - W_d) / W_{d(PVA)} \times 100\%. \quad (4)$$

The PLGA/PVA composite films were dried in a vacuum desiccator at room temperature until virtually no change in weight was observed. To measure W_s, the swollen films were removed from the phosphate-buffered saline (PBS) buffer solution (pH 7.4, 0.1 M, 37 ± 0.5 °C) and gently wiped with a lint-free tissue prior to weighing.

In Vitro Glucose Diffusivity Studies. The PLGA/PVA composite films were incubated in PBS buffer (pH 7.4) at 37 °C for 3 h to ensure equilibrium swelling prior to conducting the *in vitro* diffusion studies. Franz cell apparatus were used. The receptor and donor chambers were filled with 12 ml of pure PBS buffer solution and 3 ml PBS buffer solution containing 300 mg/dl glucose, respectively. The composite films were fixed between the donor and the receptor chambers. Every 10 min, 100 μl samples were withdrawn from the receptor chamber and replaced with fresh PBS to maintain a constant volume in the receptor chamber. The amount of glucose was analyzed using high-performance liquid chromatography (mobile phase, water; column temperature, 85 °C; flow rate, 0.6 ml/min).

Characterization of Glucose Biosensors with Composite Coatings

Fabrication of Working Electrode. Miniaturized working electrodes (sensors) were fabricated by coiling a 50 μm Pt wire on a thicker (100 μm diameter) Pt line, which served as the backbone. The total surface area of the working electrode was 3 mm^2. The sensors were electrochemically cleaned in a 0.5 M sulfuric acid solution via cycling the potential between -0.21 and 1.25 V until a stable background was reached.[15] Next, a film of polyphenol was electropolymerized on the Pt working electrode from a 40 mM phenol solution in aqueous acetate buffer by scanning the applied potential between 0 and 1 V versus standard calomel electrode 51 times at a scan rate of 0.05 V/s. This polyphenol layer (approximately 10 nm thick) was shown to block oxidation of other endogenous species (e.g., ascorbic acid, uric acid, and acetaminophen), which are likely to oxidize at the sensor operating potential.[16–18] The GO_x enzyme was subsequently immobilized by dip coating the Pt/poly(p-phenylenediamine) electrode in a solution of 140 mg/ml GO_x, 56 mg/ml bovine serum albumin, and 25% w/v glutaraldehyde, the latter of which enables enzyme cross linking. After incubation overnight, these sensors were soaked in PBS buffer to allow uncross-linked enzyme to leach out.[19]

The sensors were subsequently coated with a flux-limiting membrane (1–3 μm in thickness) based on PU by dip coating the GO_x-modified working electrodes with a 3% (w/w) PU solution in 98% tetrahydrofuran/2% dimethylformamide (w/w). These sensors were referred to as device U. At this point, all devices U were tested for their sensitivity, and only those that yielded similar sensitivity (within 5% standard deviation) were used for future studies.

Deposition of Polyurethane/Poly(Vinyl Alcohol) Hydrogel Stacked Membranes. Following PU deposition, device U sensors were coated with the as-prepared PVA solution (with and without dexamethasone-loaded microspheres) and were immediately gelled by three freeze–thaw cycles (as described earlier) to physically crosslink the PVA and yield devices UP (without microspheres) and device UP_{Dex} (with dexamethasone-loaded microspheres). Here it should be noted that an 18 G needle-based mold was used to enable the composite hydrogel coating to avoid pin holes and to achieve reproducibility. The thickness of the PVA coating (with or without the PLGA microspheres) in its dry state is approximately 100 μm, which is similar to the thickness of the films used for permeability studies.

In Vitro Amperometric Experiments. The tests were performed in stirred PBS solution (pH 7.4) maintained at 37 °C.

A potential of 0.7 V was applied between the Pt working electrode and an Ag/AgCl reference electrode using a CH Instruments (Model CHI1010A) electrochemical analyzer. The sensor response current versus various glucose concentrations were determined by raising the glucose levels in the test cell by 2 mM every 100 s up to 30 mM, following an initial background stabilization period of ~8 min.

Results

Swelling Property of the Poly(Lactic-Co-Glycolic Acid) Microsphere/Poly(Vinyl Alcohol) Hydrogel Composites

As shown in **Figure 1A**, regardless of the amount of microspheres present, all composite films reached an equilibrium swollen state within 1 h after soaking in PBS buffer solution at 37 °C. The equilibrium water content of the PLGA microsphere/PVA hydrogel composite films increased from approximately 305% to approximately 390%, with incremental increase in microsphere loading between 0 and 100 mg microsphere/ml PVA hydrogel as shown in **Figure 1B**.

Influence of Poly(Lactic-Co-Glycolic Acid) Microsphere Loading and Degradation on Glucose Diffusivity of the Composite Hydrogel Films

The initial glucose diffusivity of the PLGA/PVA composite films was measured as a function of microsphere loading. The glucose flux decreased with increase in microsphere loading (**Figure 2**). The PVA hydrogel films without any PLGA microspheres exhibited the highest glucose flux of approximately 21 mM/(min × cm^2). Incorporation of PLGA microspheres within the PVA matrix decreased the initial glucose flux by approximately 2 to 2.3 mM/(min × cm^2) for every 25 mg increment in microsphere loading per milliliter of PVA hydrogel solution up to 100 mg/ml.

Performance of Glucose Biosensors with Poly(Lactic-Co-Glycolic Acid) Microsphere/Poly(Vinyl Alcohol) Hydrogel Composites

The basic characteristics of the glucose sensors, including linearity, response time, and sensitivity, were determined for sensors with various coatings. Sensor response time is defined as the time taken to reach 90% of the saturation amperometric current following an aliquot addition of glucose. Sensor sensitivity is defined as the slope of the current response versus the glucose concentration plot over the entire physiological glucose range.

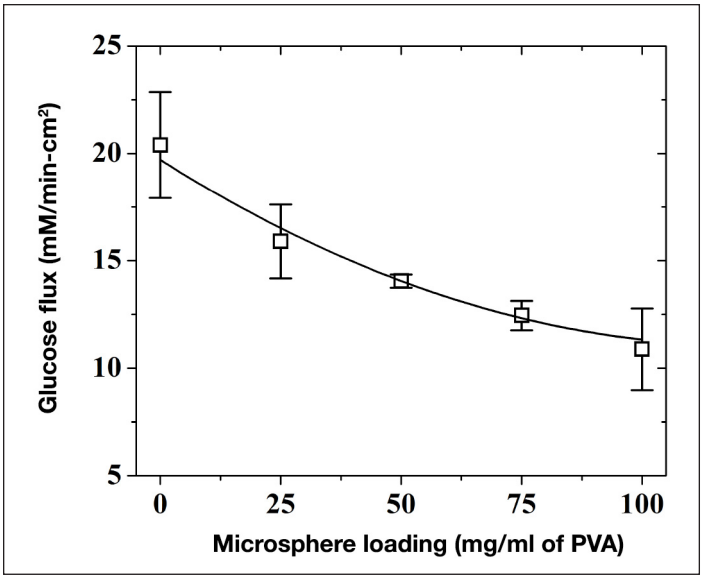

Figure 2. Glucose flux through PLGA microsphere/PVA hydrogel composite films as a function of microsphere loading. All composite films were incubated in PBS buffer (pH 7.4) at 37 °C for 1 h prior to testing, and all experiments were conducted at 37 °C using PBS buffer (pH 7.4).

The amperometric responses/linearity of the glucose sensors coated with blank PVA hydrogel and PVA hydrogel loaded with PLGA microspheres (75 mg microsphere/ml PVA solution) is illustrated in **Figure 3**. As a control, the response of bare glucose sensors coated with only the flux-limiting PU membrane (device U) is also shown. Similar to the devices U, both the PVA-coated devices (devices UP) and PVA/PLGA-coated

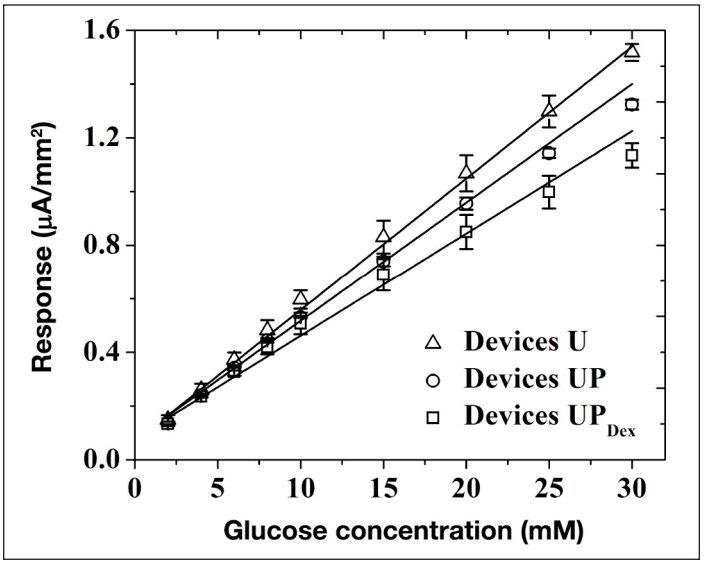

Figure 3. Saturation amperometric current versus glucose concentration for device U (no PVA coating), device UP (PVA hydrogel coating), and device UP_{Dex} (75 mg/ml dexamethasone-loaded PLGA microsphere/PVA hydrogel composite coating). All experiments were conducted at 37 °C in PBS buffer solution (pH 7.4).

devices (devices UP_{Dex}) showed a linear increase in amperometric response for glucose concentrations as high as 30 mM, which is well beyond the physiological range (2 to 22 mM). However, the addition of a PVA hydrogel coating on top of the PU layer lowered the amperometric response by approximately 20%. The presence of the PLGA microspheres in the PVA hydrogel coatings further decreased sensor sensitivity to approximately 70% of the value obtained with sensors coated with only PU (devices U).

Figure 4 illustrates the response time of the sensors tested in **Figure 3**. The addition of a PVA hydrogel coating on top of the PU layer increased the response time by approximately two-fold. The presence of the PLGA microspheres in the PVA hydrogel coatings further increased the response time by approximately three-fold. **Figure 5** shows the variation in sensor response time as a function of PLGA microsphere loading. Sensor response time increased with increasing amounts of microspheres before saturating around 50 mg of microspheres per milliliter of hydrogel.

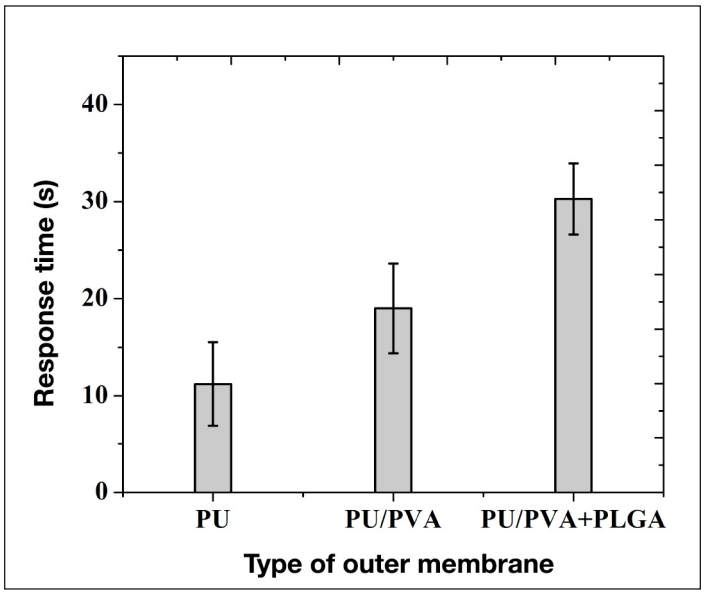

Figure 4. Response time of device U (no PVA coating), device UP (PVA hydrogel coating), and device UP_{Dex} (75 mg/ml dexamethasone-loaded PLGA microsphere/PVA hydrogel composite coating). All experiments were conducted at 37 °C in PBS buffer solution (pH 7.4).

Figure 6 shows the variation in sensor sensitivity as a function of PLGA microsphere loading. A reverse relationship was observed between sensor sensitivity and microsphere loading. Sensor sensitivity decreased from 73 to 62 nA/mM glucose as microsphere loading increased from 0 to 100 mg microsphere/ml PVA hydrogel.

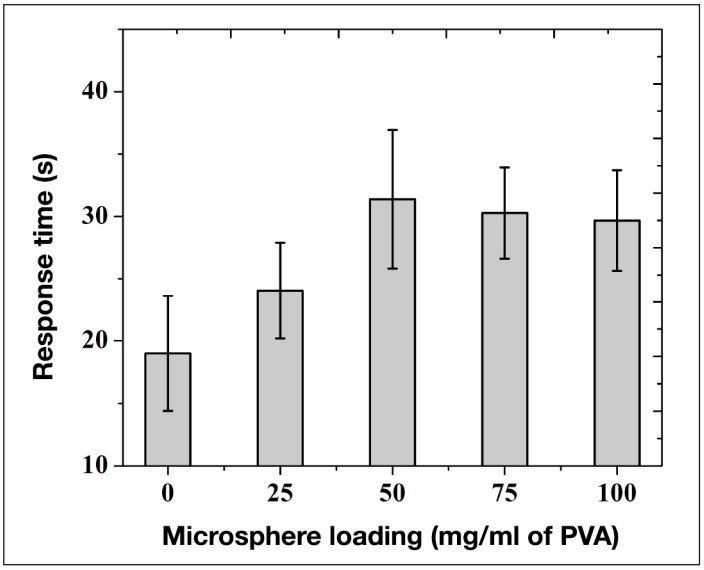

Figure 5. Response time of device U when coated PVA/PLGA composite coatings with various amounts of microsphere loadings (0, 25, 50, 75, or 100 mg microsphere/ml PVA hydrogel). All experiments were conducted at 37 °C in PBS buffer solution (pH 7.4).

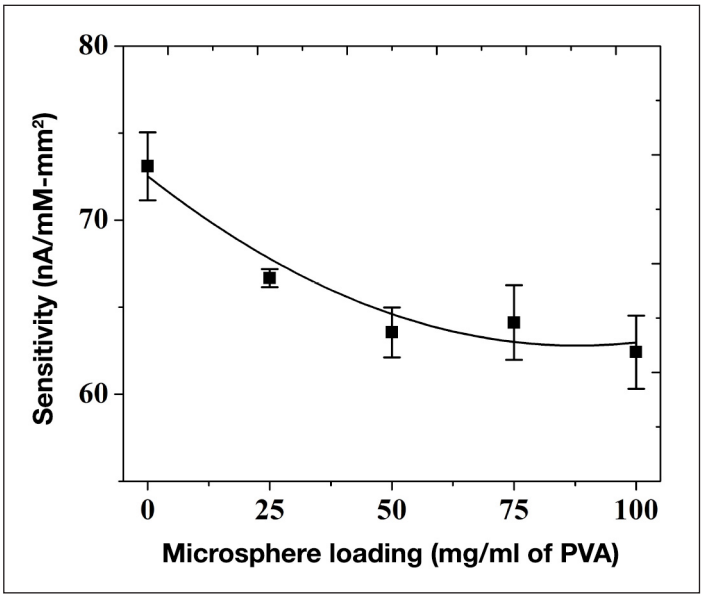

Figure 6. Sensitivity of glucose sensors coated with PVA/PLGA composite coatings with various amounts of microsphere loadings (0, 25, 50, 75, or 100 mg microspheres/ml PVA hydrogel). All experiments were conducted at 37 °C in PBS buffer solution (pH 7.4).

Discussion

Swelling Properties of the Poly(Lactic-Co-Glycolic Acid) Microsphere/Poly(Vinyl Alcohol) Hydrogel Composite Coatings

The degree of swelling of the PLGA microsphere/PVA composite coatings depends mainly on the properties of the PVA hydrogel. The embedded PLGA microspheres

are hydrophobic in nature and absorbed a negligible amount of water compared with the PVA hydrogel. In general, the crosslinking density and the extent of amorphous regions in the hydrogel network determine the swelling behavior. The lower the crosslinking density and the higher the amorphous region, the higher the hydrogel swelling ratio. For PVA hydrogels formed by freeze–thaw cycling, the initial polymer concentration and the duration and temperature of the freeze–thaw cycling play important roles in the final crosslinking density. The degree of swelling decreases when the PVA concentration increases. Higher PVA concentrations result in greater degrees of chain entanglement, more intermolecular hydrogen bonding, larger crystalline structures, and less distance between PVA crystallites. Consequently, there is a reduced "pore" size for water to occupy and less free -OH groups in the PVA chains available to form hydrogen bonds with water. This all leads to higher crosslinking density and reduced swelling. In the present study, the total number of PVA molecules per unit volume was the same in all groups, and theoretically, the saturation–swelling ratio should also be the same for all groups. However, it was observed that the presence of microspheres increased the swelling ratios of the PVA hydrogels. This indicated that the crosslinking densities of the composite films containing microspheres were less in comparison with those without microspheres. This observation is in accordance with our previous results,[9] which showed that the Young's modulus of PVA hydrogel films was higher compared with that of PLGA microsphere/PVA hydrogel composites. The PLGA microspheres apparently presented physical obstacles to the formation of PVA crystals. Consequently, less intermolecular hydrogen bonds were formed between polymer molecules, which resulted in lower crosslinking densities and higher swelling ratios.

Glucose Diffusivity of the Poly(Lactic-Co-Glycolic Acid) Microsphere/Poly(Vinyl Alcohol) Composite Films

The diffusivity within the PVA hydrogels formed by freeze–thaw cycling is governed by the "pore" or "free volume" theory. According to this theory, analyte diffusion only occurs through the water phase of hydrogels. More specifically, it occurs only in bulk and not bound water. This is due to the unfavorable entropy change associated with the transfer of (glucose) molecules from bulk to bound water. In the current study, increased microsphere loading in the PVA hydrogel matrix resulted in a higher swelling ratio but lower initial glucose diffusivity. This observation indicates that the higher the microsphere loading, the more bound water and the less bulk water. The presence of PLGA microspheres affects the water state in two ways: (1) the presence of the PLGA microspheres presents a physical obstacle to the formation of ice crystals during the freezing process, thus suppressing formation of large ice crystals, and this, in turn, results in smaller "pores" in the final hydrogel matrix (which are later occupied by water upon hydration) and (2) the microspheres occupy some of the "free volume" of the system, which further decreases the space available for bulk water.[20] The nonlinear decrease in glucose flux with increasing microsphere loading suggests that, apart from reduced bulk water, factors such as increased diffusion tortuosity and microstructure also affect glucose permeability through the hydrogel composites. It is worth noting that, because PLGA microspheres are degradable, the glucose diffusivity is expected to increase with time as the microspheres gradually degrade.

Effect of the Composite Coating on the Basic Characteristics of Glucose Sensors

The glucose biosensor our group is developing is a miniaturized (0.5 × 0.5 × 5 mm) implantable sensor aimed for long-term continuous "real-time" monitoring (1 month or longer). The sensor is so small that it can be injected using a hypodermal needle. The basic characteristics of this type of biosensor include sensor linearity, response time, and sensor sensitivity. The PLGA microsphere/PVA hydrogel composite coatings for the implantable biosensors are capable of controlling the local tissue reaction to the biosensors post-implantation. It has been demonstrated in our previous work that this composite coating is able to fully suppress foreign body response for up to 3 months.[10] Here, it was observed that the incorporation of a blank PVA hydrogel on top of the PU-coated sensors resulted in a 20% decrease in sensor sensitivity and a two-fold increase in response time. This indicates that the PVA layer acts as an additional diffusional barrier to glucose. The addition of the microspheres to the PVA hydrogel further decreased glucose flux and therefore further reduced sensor sensitivity. This effect saturated at high microsphere loadings. Even though sensor sensitivity decreased in the presence of PVA hydrogel or PLGA/PVA composite coatings, the addition of glucose resulted in a linear increase in the amperometric response for glucose concentrations up to 30 mM. This linearity is attributed to the presence of the inner PU membrane that decreases glucose diffusion without affecting oxygen diffusion. The fact that the presence of the PLGA microsphere/PVA hydrogel does not affect linearity implies that this coating does not impose any resistance to oxygen diffusion. Although the PVA matrix is hydrophilic in nature (opposite to the

hydrophobic nature of oxygen), due to the small molecular size of oxygen and the microporous structure of the PVA matrix, oxygen is able to diffuse rapidly through the composite coating. This is a very significant finding because it is important, yet usually difficult, to obtain a linear response in the physiological range of glucose (2 to 22 mM).[3] The presence of the composite coating increased sensor response by approximately three-fold (from 10 to 30 s). It should be noted that this 30 s increase is negligible compared with the 11–13 min lag time observed between blood and interstitial fluid and will not affect the performance of the biosensors. Future studies are focused on understanding the role of the PU membrane during sensor operation in low-oxygen environments as well as developing a means to deposit PU in a controlled manner to yield high sensor-to-sensor reproducibility.

Conclusions

The current study has demonstrated the feasibility of using a PLGA microsphere/PVA hydrogel composite system as an outer coating for implantable glucose biosensors. This coating not only provides sustained release of dexamethasone over an extended period of time to prevent foreign body response, but also allows sufficient diffusion of glucose into the sensing element for sensor functionality. Most notably, the PLGA microsphere/PVA hydrogel composite coating did not compromise sensor linearity within and beyond the physiological range of glucose. Even though the presence of composite coatings increased sensor response time by approximately two-fold (since it acted as an additional diffusional barrier to the glucose), the response time was still below average reported values.[21] The presence of composite coatings also reduced sensor sensitivity compared with sensors without coatings; however, the sensitivity was still within the functional range. These observations were related to changes in glucose permeability upon addition of composite coatings. The presence of microspheres in the PVA hydrogel affected the pore size of the hydrogel and occupied "free space," resulting in less bulk water and thus lower glucose permeation. The knowledge gained from the current study will be utilized to obtain a better understanding of sensor performance *in vivo*.

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Disclosure:

Fotios Papadimitrakopoulos and Santhisagar Vaddiraju disclose a competing financial interest with Biorasis Inc. Fotios Papadimitrakopoulos is one of its two founders, and Santhisagar Vaddiraju is partially employed by this company.

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