Bioactive Electroconductive Hydrogels Yield Novel Biotransducers for Glucose

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Summary: A class of bioactive, stimuli-responsive co-joined interpenetrating networks of inherently conductive polymers and highly hydrated hydrogels are being developed for implantable biodevice interfaces and for electric field induced release of elutable drugs. A novel microfabricated multidisc electrode array biotransducer intended for trauma management has been coated with poly(HEMA)-polypyrrole and characterized by cyclic voltammetry and chronoamperometry using ferrocene monocarboxylic acid (FcCOOH) as a probe molecule. Electrodeposition of polypyrrole (700 mV *vs.* Ag/AgCl) to 100 mC/cm^2 onto the hydrogel coated microdisc electrode array resulted in large and unstable background currents relative to uncoated electrodes. Overoxidation of polypyrrole (0–1.2 V *vs.* Ag/AgCl, 20 cylces, 100 mV/s) eliminates background current. Dose-response curves with FcCOOH showed that the transducer has good reproducibility with molecules of facile electrochemical properties. Polypyrrole provides interference screening of endogenous interferents in biosensor applications with a 12:1 rejection ratio. GOx was immobilized via electropolymerization of polypyrrole into hydrogel coated MDEA 5037s to yield biotransducers with sensitivity of 0.045 μA mM^{-1} cm^{-2}. Changes to improve biotransducer sensitivity are proposed.

Keywords: biosensors; conductive polymers; electrodes; enzymes; hydrogels; microarrays

Introduction

Simple Clark style amperometric biosensors[1] continue to be highly investigated because of their many potential applications in the field of clinical chemistry, bioprocess monitoring, biotechnology, and in the food industry.[2] Such devices integrate the molecular recognition of enzymes such as glucose oxidase and lactate oxidase with solid state electrodes that may be coinage metals, carbonaceous materials or semiconductors. Recently, another new application of glucose and lactate biosensors was developed for the management of hemorrhagic shock in critically injured patients.[3] Hemorrhage brought on by trauma can lead to multiple organ dysfunction syndrome (MODS) and/or eventual death.[4] To increase the survival rate of the injured who may be at risk for hemorrhagic shock, it is imperative that we develop and deploy a simple, rapid, sensitive, continuous and selective on-site platform that can quantify glucose and lactate levels in a suitable tissue bed.[5] Both glucose and lactate have been identified as trauma-associated biomarkers. The continuous monitoring of glucose and lactate levels in injured patients becomes a significant parameter in evaluating the severity of injury and understanding the progress

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toward hemorrhagic shock. Both are intended to aid the clinician in optimizing resuscitation and reanimation.

Stability of enzymes is highly dependent on their mode of immobilization. The immobilization technique to localize different enzymes at the surface of multi-analyte electrodes is an engineering challenge in the fabrication of glucose and lactate biotransducers. This challenge is particularly relevant in the fabrication of fully implantable multi-analyte biosensors when the electrode sites are micron dimensions and are adjacent to each other such as in a CMOS device.[6] Various immobilization procedures have been developed for macro-electrodes or single microelectrodes. Among these are physical adsorption,[7] enzyme entrapment and encapsulation within polymer membranes with or without covalent tethering that may be spun applied, dipped-coated, sprayed, covalently immobilized using homo- or hetero-bifunctional conjugation agents,[8–11] or adsorbed via cross-linking with bifunctional cross-linkers such as glutaraldehyde.[12] None of these additive techniques are however appropriate for closely spaced microelectrodes on a CMOS device.

The additive placement and entrapment of enzymes on such devices may be achieved by electropolymerization. Electropolymerization produces controllable thin films of inherently conductive electroactive polymers (CEP) deposited at electrode sites.[13] During formation such polymers entrap counter anions that serve as ''dopants''. In the presence of enzymes, which are often negatively charged, electropolymerization leads to formation of an enzyme enriched membrane layer deposited directly on the electrode.[14] These innovations offer the advantage of high efficiency of enzyme loading, ease of preparation, and stability of the enzyme/CEP/hydrogel membrane. Polypyrrole (PPy) is also known to impart interference suppression for amperometric biosensors[15] and enables control of the hybrid polymer membrane properties. In this work the effectiveness of polypyrrole to act as a screen for interferents is tested with a novel transducer intended for use as a trauma management platform, the Electrochemical-Cell-on-a-Chip Multidisc Electrode Array (MDEA 5037).[16] Our goal was to investigate if PPy would have any deleterious influence on an amperometric electrochemical reaction of ferrocene ($Fc\text{-}Fc^{+}$), a surrogate redox couple, for what would otherwise be a mediator in a mediated enzyme biosensor reaction. The design of a glucose amperometric biotransducer fabricated via entrapment of glucose oxidase by polypyrrole into hydrogel membranes applied as a bioactive layer on a MDEA 5037 (transducer) has been characterized.

Experimental Part

MDEA 5037 Transducers

Fully packaged and assembled platinum MDEA 5037-Pt transducers were acquired (Abtech Scientific Inc. Richmond, Virginia). The MDEA 5037, available in Au and Pt, is a single transducer (chip) with two independently addressable electrochemical cells on the chip. Transducers are fabricated using microlithographic techniques.[17,18] There are a total of five on-board electrodes per chip, two working electrodes (WE), two counter electrodes (CE), and one shared reference electrode (RE). Working electrodes, consist of 37 platinum discs ($d = 50\,\mu m$, working electrode area $= 7.3 \times 10^{-4}$ cm^{-2}) exposed through a $0.5\,\mu m$ thick layer of silicon nitride (Si_3N_4) in a hexagonal packed array with a center-to-center distance of $100\,\mu m$.[18] Microdisc working electrodes promote hemispherical diffusion of analytes to their surface having consequences on limits of detection, sensitivity, dynamic range, and response rate.[19] The MDEA 5037 design was selected based on observed bioanalytical performance of lactate biotransducers tested as a function of microdisc feature size.[20]

Cleaning Procedures of MDEA 5037s

Transducers were successively ultrasonicated in ethanol, isopropyl alcohol (IPA),

and deionized (DI) water for 5 minutes each followed by drying with ultra-high purity (UHP) nitrogen. Ultraviolet/ozone cleaning then took place in a standard UV-Ozone cleaner (Boekel Industries) for 10 minutes followed by ultrasonication in IPA for 1 minute before drying again with UHP nitrogen. Electrochemical cleaning was performed in 0.5 M H_2SO_4 by cyclic voltammetry from −0.2–1.5 V *vs.* Ag/AgCl (3 M KCl) for 20 cycles at 100 mV/s. Transducers were finally thoroughly rinsed in flowing DI-water before use.

Electrodeposition of PPy and Characterization of Electrodeposited PPy on MDEA 5037s

All reagents were acquired from Sigma-Aldrich. A solution of 0.4 M pyrrole (Py) was prepared in DI-water with 0.01 M dodecylbenzene sulfonic acid (DBSA) as a dopant anion. A charge density of 100 mC/cm^2 was applied to working electrodes as previously described.[20] All electropolymerization and overoxidation techniques throughout this body of work were performed with a PAR 283 potentiostat (Princeton Applied Research) system. Briefly, electropolymerization was carried out potentiostatically at the working electrodes of the MDEA 50 37s at 700 mV *vs.* Ag/AgCl. Approximately 10 seconds was needed to achieve the desired charge density (Figure 1). This produced a black, oxidized polypyrrole film onto the individual discs of the MDEA 5037 working electrodes (Figure 2). Overoxidation of polypyrrole was performed in 0.1 M sodium phosphate buffer (PBS) at pH = 7.2 using cyclic voltammetry from 0–1.2 V *vs.* Ag/AgCl for 20 cycles at 100 mV/s. Only two to three cycles were necessary to reach the stated overoxidation (Figure 3 & 4).

A wireless dual potentiostat (Model 8151, Pinnacle Technologies) intended for use with the implantable dual responsive MDEA 5037 transducer has been previously described for use in small vertebrate animal studies.[20] This system was used to measure background currents of: i) cleaned electrodes, ii) freshly electropolymerized polypyrrole (PPy) electrodes, and iii) overoxidized polypyrrole (OO-PPy) electrodes. The Model 8151 wireless dual potentiostat is a two-electrode system that applies a programmable bias potential to the working electrode and the on-board counter electrode of the MDEA 5037 which serves as both counter and pseudo-reference electrodes. The 8151 potentiostat was used to measure the chronoamperometric response of the various modified MDEA 50 37 s to ferrocene monocarboxylic acid (FcCOOH).

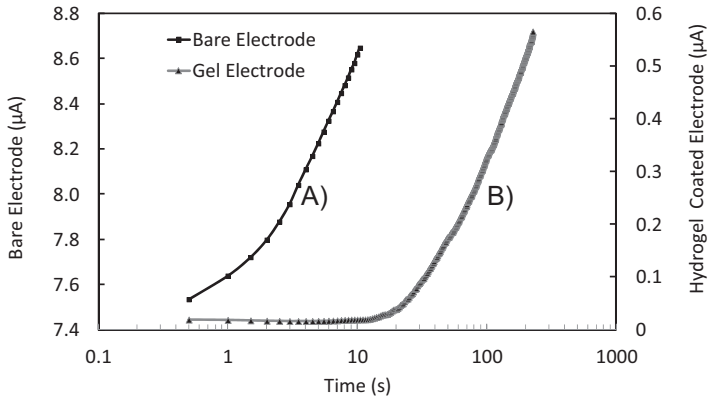

Figure 1.
Kinetics of potentiostatic electropolymerization of Py at MDEA 5037-Pt electrodes: (A) Bare at 700 mV *vs.* Ag/AgCl in 0.4/0.01 M Py/DBSA (B) Hydrogel coated at 1,000 mV *vs.* Ag/AgCl in DI-water, 0.4 M Py, 0.2 mg/mL GOx to 100 mC/cm^2.

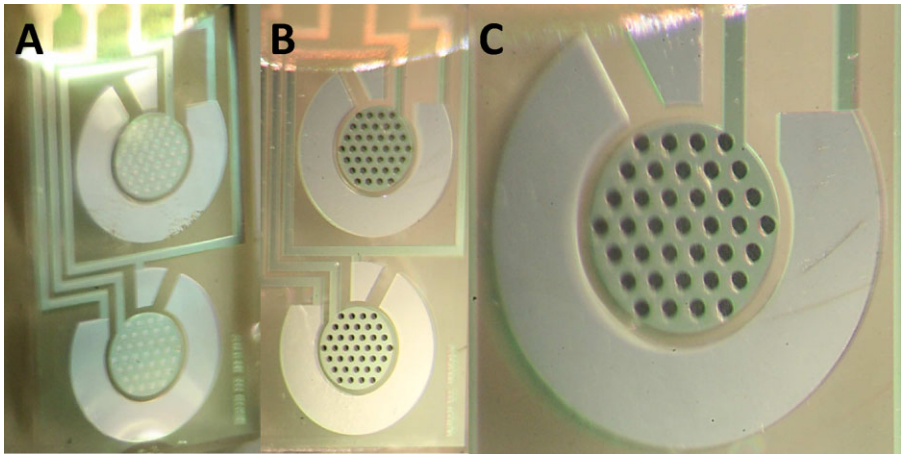

Figure 2.
(A) A cleaned and bare MDEA 5037-Pt before electropolymerization showing the working electrodes of both channels being clean. (B) Dark, oxidized polypyrrole on the MDEA 5037 working electrodes of both channels. (C) Close-up of the polypyrrole layer on the microdisc array of the working electrode.

MDEA 5037 Biotransducer Fabrication and Characterization

A cleaned MDEA 5037 transducer was surface modified to covalently link to a poly(2-hydroxyethyl methacrylate) based hydrogel using a previously published surface modification protocol.[20] Briefly, the cleaned transducer was incubated in 0.1 wt% 3-aminopropyltrimethoxysilane in anhydrous toluene for 30 minutes followed by ultrasonication in toluene, 1:1 toluene:ethanol, ethanol, and DI-water for 5 minutes each. Subsequent curing took place in a 0.22 μm convection oven at 110 °C for 20 minutes. Resulting terminal amine functional groups were derivatized by immersion in acryloyl(polyethyleneglycol-N-hydroxysuccinimide) at 1.0 mM in 0.1 M HEPES at pH 8.5 for 2 hours. The transducer was subsequently dried and dipped into a 3 mol% TEGDA cross-linked poly(HEMA)-based hydrogel cocktail that was prepared by mixing 2-hydroxyethyl methacrylate (HEMA),

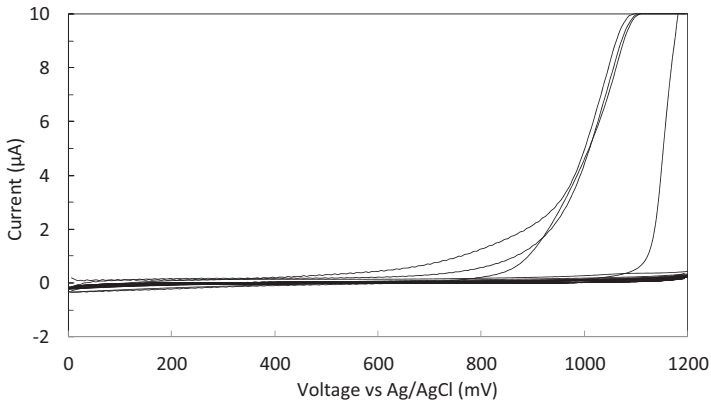

Figure 3.
Cyclic voltammogram of the overoxidation of polypyrrole at the working electrode of an MDEA 5037 (20 cycles at 100 mV/s, PBS buffer, pH = 7.2).

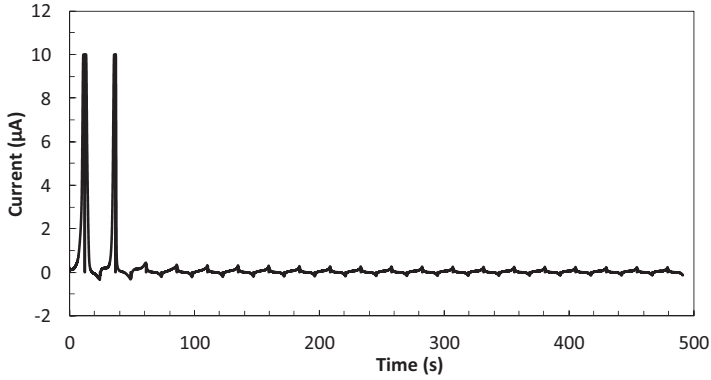

Figure 4.
A total of 20 cycles of overoxidation was applied to working electrodes and the current response shows that polypyrrole oxidation peaks diminish greatly after only two cycles.

tetra(ethylene glycol) diacrylate (TEGDA, technical grade), poly(ethylene glycol) (400)monomethacrylate (PEGMA), *N*-[Tris(hydroxymethyl)methyl]acrylamide (HMMA, 93%), polyvinylpyrrolidone (pNVP), 2-(Dimethylamino) ethyl methacrylate (DMAEMA, 98%), 2-(Methacryloyloxy)ethyl 2-(Trimethylammonio) ethyl phosphate (MPC) purchased from Tokyo Chemical Industry CO., LTD. and the photo-initiator 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99 + %) in typical ratio 78:3:5:1:5:2:5:1 mol% and 1:1 (v/v) solution of ethylene glycol/water corresponding to 20% of the volume of the monomer cocktail.[20] The biosmart hydrogel layer incorporates previously characterized chemistries necessary for biocompatibility and protection of enzymes within a well hydrated milieu.[20] The film was then crosslinked with UV light for 5 min (CX-2000 Crosslinker, UVP, Upland, CA) under an inert nitrogen atmosphere. The hydrogel was allowed to hydrate overnight in PBS buffer at 37 °C. For making the transducer bioactive, a solution of 0.4 M pyrrole monomer (Py, reagent grade 98 + %) and 0.2 mg/mL of glucose oxidase (GOx, E.C. 1.1.3.4 from Aspergillus niger) was prepared in DI-water. The hydrated hydrogel was allowed to equilibrate for one hour in this solution. Electropolymerization was then carried out potentiostatically at 1000 mV *vs.* Ag/AgCl. A charge density of 100 mC/cm^2 was applied to each of the separate working electrodes of the hydrogel modified MDEA 5037. Polypyrrole was subsequently overoxidized with cyclic voltammetry as discussed earlier. The bioactive MDEA 5037 was placed in PBS buffer and stored at 4 °C. The biotransducer was then placed in pH 7.2 PBS at room temperature at under gentle stirring for interrogation with β-D(+)-glucose from 0–40 mM. Interrogation was performed in three electrode mode using a BAS 100 Electrochemical Analyzer (Bioanalytical Systems, Inc.) with a working electrode array of the MDEA 5037 at 400 mV *vs.* Ag/AgCl and with a platinum mesh as the counter electrode. Cleaned/bare electrodes were also interrogated in the same fashion as a control. Sensitivity, linear dynamic range, response time, detection limit, the apparent Michaelis-Menten constant, and maximum current were determined for the biotransducer.

Results and Discussion

The PAR Model 283 Potentiostat and a three electrode configuration were used for electropolymerizations. Figure 1 shows the kinetics of potentiostatic electropolymerization of Py at a bare MDEA 5037-Pt

electrode using 700 mV *vs.* Ag/AgCl in 0.4/0.01 M Py/DBSA aqueous solution (A). The figure also shows the kinetics of potentiostatic electropolymerization of Py at a hydrogel coated electrode using 1,000 mV *vs.* Ag/AgCl in 0.4 M Py, 0.2 mg/mL GOx in DI-water (B). Electropolymerization within the hydrogel requires a higher potential (1,000 vs. 700 mV), is accompanied by a longer induction period because the nucleation kinetics of polypyrrole at the surface of the electrode is slower, and is associated with an order of magnitude less current. The hydrogel layer presented a transport barrier to facile eletropolymerization as pyrrole monomer must first diffuse to the electrode-hydrogel interface to initiate polymerization. Once synthesized, the background current of modified electrodes was examined using the Model 8151 wireless dual potentiostat.

The Model 8151 dual potentiostat uses a two electrode configuration and +700 mV was separately applied to the two working electrodes of the MDEA 5037 with respect to their on-board counter electrodes (Figure 2). Cleaned electrodes had no observable background current (0.0 nA) in gently stirred PBS buffer. However, polypyrrole-hydrogel electrodes had a large and unstable background current averaging 59.8 ($\pm$30) nA (n = 2). Overoxidation of the polypyrrole using cyclic voltammetry brought the background current back to non-detect or zero.

Electroconductive hydrogels synthesized from polypyrrole within poly-(HEMA) networks have been shown to decrease limits of detection and increase sensitivity to analytes at the cost of narrowing the linear dynamic range of biotransducers.[20,21] This is believed to be the result of the electrical impedance between the electrode surface and the solution being lowered by the presence of polypyrrole. Furthermore, the upper limits of analyte detection are lost due to exceedingly high background current from oxidation of polypyrrole networks.[21] For the relatively high charge density (100 mC/cm^2) used in this work, the overoxidation process was necessary in order to provide and maintain a low and stable background current above which glucose responses could be measured. The magnitude of background current as a function of charge density was not investigated. The amount and technique by which polypyrrole was incorporated into the hydrogel is of paramount concern for effectively balancing enzyme immobilization, signal stability, and sensitivity of the biosensor system.[22]

Interrogation of MDEA 50 37 s with Ferrocene Monocarboxylic Acid

Ferrocene monocarboxylic acid, a well-known reversible redox probe molecule, was used to interrogate cleaned and OO-PPy MDEA 5037 s. The pKa value for FcCOOH is 4.2, the same as that of ascorbic acid which is a well-known in vivo biosensor interferent.[23–25] Interrogation was performed in PBS at pH = 7.2 with a concentration range of 0.0–0.4 mM FcCOOH. Each electrochemical cell of the MDEA 5037 was interrogated independently and was analyzed based on 4 repeats. A potential of 700 mV was applied to the working electrode with respect to the on-board counter electrode using the wireless 8151 dual potentiostat. The dose response curves of the cleaned bare and OO-PPy transducers were observed (Figure 5).

The dose response curve of freshly cleaned, bare platinum transducers to FcCOOH showed an expected linear response. The sensitivity of bare transducers averaged approximately 350 $\pm$ 25 nA/mM and $R^2 = 0.9969$ (Figure 5). The performance of Cell A (top cell, average sensitivity = 348 $\pm$ 20 nA/mM) and Cell B (bottom cell, sensitivity = 361 $\pm$ 36 nA/mM) were not significantly different based on a two-tailed t-test (p = 0.702). We have concluded that for very facile electrochemical reactions (large heterogeneous rate constants), where the device under test behaves as a resistor, the wireless potentiostat appears quite capable of delivering desired reproducibility. However, for real electrode reactions that may be quasi-

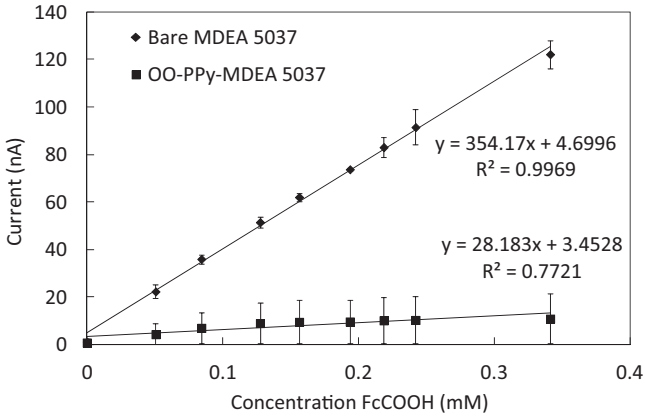

Figure 5.
The dose response of cleaned/bare and OO-PPy MDEA 5037 electrochemical cells (for both populations $n = 4$) interrogated in two electrode mode using a 700 mV bias potential of working versus counter electrodes; Error bars portray 95% confidence intervals.

reversible and have low heterogeneous rate constants, the potentiostat is unable to respond uniformly between the two electrochemical cells of the MDEA 5037 transducers. We propose to take up these issues with our collaborating manufacturer, Pinnacle Technologies, Inc. In previously published work we have shown that when resistors distributed with the 8151 were tested, the unit returns matching current responses. However, when true dummy cells comprising parallel resistor-capacitor pairs were tested, the results show statistically significant differences between the two channels.[20]

The dose-response of the transducer modified with OO-PPy showed a decreased average sensitivity to FcCOOH with 28 ± 31 nA/mM, a reduction of 92%. This was significantly different ($p = 10^{-6}$) when compared to cleaned/bare electrodes. The drastic loss of current response is likely due to anion exclusion of the negatively charged $FcCOO^-$ (as the anionic form of FcCOOH, $pKa = 4.2$)[26] is electrostatically repulsed by dopant anions within the positively charged OO-PPy film. When comparing the sensitivities of the bare electrode response to the polypyrrole coated electrode response, this ratio is approximately 12:1. This represents effective interference shielding (a rejection ratio of 12:1) of anionic interferents, such as ascorbic acid, that plague amperometric biosensors. These results can be confirmed through amperometric response of OO-PPy transducers to FcCOOH as a function of buffer pH that result in shifts of anionic to neutral forms of FcCOOH.

Interrogation and Characterization of the MDEA 5037 Biotransducer

Characterization of the biotransducer was performed using one of the cells (lower cell) of the MDEA 5037. Electropolymerization of polypyrrole into a hydrogel coated MDEA 5037 transducer's working electrode required 227 seconds to reach a charge density of 100 mC/cm^2 (Figure 1). Electropolymerization of hydrogel coated transducers at 700 mV was unsuccessful. A larger overpotential of 1,000 mV was necessary in order to achieve a successful polymerization. This may be due to lack of efficient dopant anion and/or an effect caused by the hydrogel coating. The observed overoxidation of polypyrrole in the biotransducer was similar to that observed with cleaned transducers. Dopant anions were purposefully excluded to allow negatively charged GOx to act as a counter anion for positively charged polypyrrole in

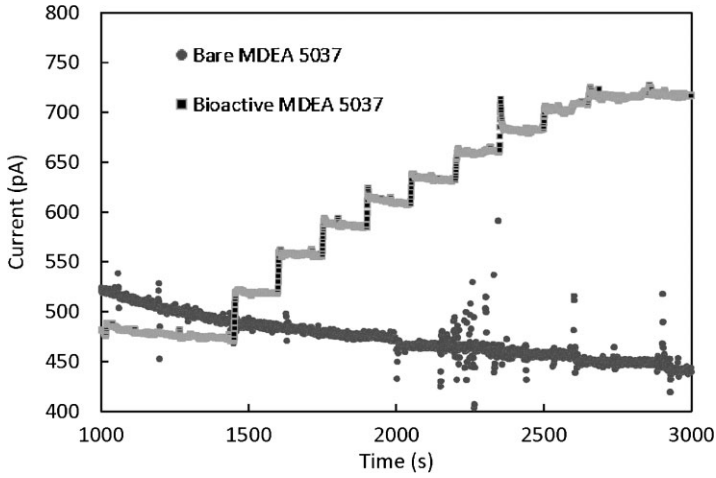

Figure 6.
Amperometric response of bare and bioactive MDEA 5037 transducers to glucose infusion every 150 seconds (BAS 100 analyzer, Working electrode array at 400 mV *vs.* Ag/AgCl, CE: Platinum mesh).

hopes of increasing enzyme immobilization yields.

Amperometric response of the biotransducer and a cleaned transducer to glucose interrogation was observed (Figure 6). No current response was noted with bare electrodes. The biotransducer showed an increase in oxidative current with increasing glucose concentration. The steady state current measured after each glucose infusion was measured to generate a dose response curve for the biotransducer (Figure 7). Lineweaver-Burke analysis was used to determine the enzyme kinetic parameters (Table 1). Two regions of linear dynamic range of amperometric response were noted based on concentration range: i) low concentrations ranging from 1–5 mM glucose with a sensitivity of 33 pA/mM and ii) high concentrations ranging from 8–33 mM glucose with a sensitivity of 4 pA/mM. The break between ranges was variable. The observed response time ranged from 2–8 seconds. The limits of

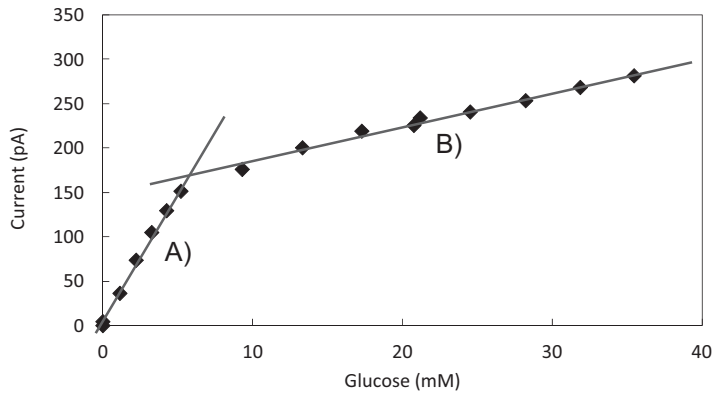

Figure 7.
Dose response of biotransducer to glucose infusions (BAS 100 analyzer, Working electrode array at 400 mV *vs.* Ag/AgCl, CE: Platinum mesh).

Table 1.
Enzyme kinetic parameters of the MDEA 5037 biotransducer.

Parameter of interest	(A) Low concentrations (1 to 5 mM)	(B) High concentrations (8–33 mM)
Sensitivity (pA/mM)	33	4
Linear Dynamic Range (mM)	1 to 5	8 to 33
Response Time (s)	2–8	2–8
Detection Limit (mM)	0.02	n/a
K_M (mM)	0.57	2.97
I_{max} (pA)	422	553

detection were found to be 0.02 mM of glucose, a resolution adequate for clinically relevant changes in glucose. The observed affinity for glucose was high with apparent Michaelis constants of 0.57 (low concentration regime) and 2.97 (high concentration regime) of glucose respectively. The overall maximum current was determined to be 553 pA.

Design Challenges in the Development of Electroconductive Hydrogel Based Biotransducers

The overall current response of the MDEA 5037 biotransducer was in the range of picoamperes, corresponding to a sensitivity of 33 and 4 pA/mM for regions A and B respectively. The wireless Model 8151 dual potentiostat was not designed to accommodate picoampere level of resolution and therefore the BAS 100 system was used to characterize the biotransducer. A three electrode mode, with Ag/AgCl as a reference, was employed in order to ensure a stable reference potential during characterization. These initial characterizations show that it is possible to generate bioactive MDEA 5037 biotransducers using biosmart hydrogels[27] as a bioactive layer with polypyrrole as a means of selective enzyme immobilization. There are four major points of concern that must be addressed: i) the optimized electropolymerization of polypyrrole into the hydrogel membrane, ii) the amount of enzyme immobilized by electropolymerization within the membrane, iii) the instantaneous and temporally retained bioactivity of the immobilized enzyme, and iv) the sensitivity of the resulting biotransducer to glucose.

The required overpotential (1,000 mV) for initiating electropolymerization of pyrrole in DI-water in the presence of GOx as the only supporting electrolyte (GOx is negatively charged and may bring with it certain buffer ions) may offset additional yields in immobilized enzymes due to denaturation by electrophoresis of enzyme through polypyrrole and hydrogel. This may be addressed by including pyrrole monomer and pyrrolyl-methacrylate monomer components directly into the hydrogel cocktail to better facilitate electropolymerization.[21] The inclusion of pyrrole and 4-(3-pyrrolyl)butyric acid components within the hydrogel enhances electropolymerization kinetics and provides for compatibility with the immobilized enzyme.[28]

The observed sensitivity of the amperometric response to hydrogen peroxide produced by the entrapped GOx enzyme in our system was shown to be limited. While the MDEA 5037-Pt is an optimized chip based on previously tested design criteria for micro electrode arrays,[29,30] the current findings suggest that the techniques established for conferring bioactivity to the transducers [6,31] must be modified and optimized for the MDEA 5037-Pt transducer. Higher current densities are necessary if the MDEA 5037-Pt biotransducer is to be used with the commercially available, Model 8151 wireless dual potentiostat. The sensitivity of the MDEA 5037 biotransducer in terms of current density was 45 and 5.5 nA mM^{-1} cm^2 for the low (<5 mM) and high concentration ranges of glucose respectively. Current densities of 0.7 μA mM^{-1} cm^{-2} have been reported for planar, low

roughness platinum electrodes modified with GOx-PPy using ca. 96 mC/cm^2 applied charge density.[32] These were however, not hydrogel coated electrodes and are therefore expected to suffer temporal loss of bioactivity.[27] The evidence strongly suggests that insufficient enzyme is being incorporated into the hydrogel membrane during electropolymerization or that immobilized enzyme is experiencing a loss of bioactivity upon immobilization. Since electropolymerization is not known to adversely compromise GOx bioactivity, we must infer that it is the former influence.

Increasing the effective working surface area through increased roughness of the platinum electrodes has been shown to increase sensitivity to a current density from 0.7 to 103 μA mM^{-1} cm^{-2}.[32] Thus, under-potentially deposited Pt to confer a nanotopography and electrodeposition of platinum black to increase surface area are possible options for increasing the sensitivity of our system. However, increasing enzyme entrapment will also increase sensitivity. At sensitivities an order of magnitude higher than reported, the MDEA 5037 would provide sufficient current density to be matched with the wireless PT Model 8151 dual potentiostat.

Conclusion

The MDEA 5037 biotransducer was shown to be highly reproducible with analyzing ferrocene monocarboxylic acid, (FcCOOH), a facile redox probe. Significant unstable background current is generated at electrodes with freshly polymerized polypyrrole when interrogating in two electrode mode using onboard counter electrode as a quasi-reference electrode. Overoxidation of 100 mC/cm^2 polypyrrole is achieved rapidly (1 minute) using cyclic voltammetry in phosphate buffer. When modified with over-oxidized polypyrrole (OO-PPy), the transducer was shown to reject access of the negatively charged FcCOOH, analogous to interference shielding of ascorbic acid. A biotransducer fabricated with the MDEA 5037 using previously established protocols was successful. An amperometric response to glucose was observed for the MDEA 5037 biotransducer but the sensitivity was not adequate for use with the 8151 wireless potentiostat. Modifications to the current strategies for conferring biospecificity have been proposed for improving biotransducer sensitivity.

Acknowledgements: The authors acknowledge support from the US Department of Defense (DoDPRMRP) grant PR023081/DAMD17-03-1-0172 and the Consortium of the Clemson University Center for Bioelectronics, Biosensors and Biochips (C3B).

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