

An implantable biochip to influence patient outcomes following trauma-induced hemorrhage

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Abstract Following hemorrhage-causing injury, lactate levels rise and correlate with the severity of injury and are a surrogate of oxygen debt. Posttraumatic injury also includes hyperglycemia, with continuously elevated glucose levels leading to extensive tissue damage, septicemia, and multiple organ dysfunction syndrome. A temporary, implantable, integrated glucose and lactate biosensor and communications biochip for physiological status monitoring during hemorrhage and for intensive care unit stays has been developed. The dual responsive, amperometric biotransducer uses the microdisc electrode array format upon which were separately immobilized glucose oxidase and lactate oxidase within biorecognition layers, 1.0–5.0 μm

thick, of 3 mol% tetraethyleneglycol diacrylate cross-linked p(HEMA-*co*-PEGMA-*co*-HMMA-*co*-SPA)-p(Py-*co*-PyBA) electroconductive hydrogels. The device was then coated with a bioactive hydrogel layer containing phosphoryl choline and polyethylene glycol pendant moieties [p(HEMA-*co*-PEGMA-*co*-HMMA-*co*-MPC)] for indwelling biocompatibility. In vitro cell proliferation and viability studies confirmed both polymers to be non-cytotoxic; however, PPy-based electroconductive hydrogels showed greater RMS 13 and PC12 proliferation compared to controls. The glucose and lactate biotransducers exhibited linear dynamic ranges of 0.10–13.0 mM glucose and 1.0–7.0 mM and response times (t_{95}) of 50 and 35–40 s, respectively. Operational stability gave 80% of the initial biosensor response after 5 days of continuous operation at 37 °C. Preliminary in vivo studies in a Sprague–Dawley hemorrhage model showed tissue lactate levels to rise more rapidly than systematic lactate. The potential for an implantable biochip that supports telemetric reporting of intramuscular lactate and glucose levels allows the refinement of resuscitation approaches for civilian and combat trauma victims.

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Introduction

Trauma-induced hemorrhage that eventuates hemorrhagic shock can lead to multiple organ dysfunction syndrome (MODS) and/or eventual death. Indeed, trauma is the most likely cause of demise for individuals who are <50 years old [1] and is implicated in 68% of warfighter battlefield fatalities [2]. These statistics will likely also emerge for our early manned inter-planetary explorations, as they already

have for the many victims of civilian highway accidents. During hemorrhage, excessive blood loss limits the transfer of vital nutrients and oxygen throughout the body. Hemorrhagic shock is often difficult to clearly ascertain [3] and may be induced by physically inflicted traumatic wounds, spontaneous internal bleeding, surgeries, and childbirth. Excessive hemorrhage is generally accompanied by peripheral vasoconstriction and results in poor peripheral perfusion, increased oxygen debt, increased tissue acidosis, elevated levels of stress cytokines, and eventual MODS [4]. There is a window of time, “the golden hour,” ranging from a few minutes to several hours, during which resuscitation and stabilization efforts must be brought to bear if they are to be effective. Resuscitation seeks, as an end point, to satisfy the tissue oxygen debt, eliminate tissue acidosis, clear all molecular biomarkers of physiologic stress, and return aerobic metabolism in all tissues [5].

During hemorrhage-induced trauma and following surgery, hemodynamics and physiology are quite delicate and can change rapidly. There is a need to initiate *immediate* and *continuous* monitoring of molecular indicators of physiologic stress and to report these in a timely manner such that they can make a difference to resuscitation approaches and hence also to survival outcomes. Currently, the principal approach is to measure global indicators of health, vital signs. Among these are core body temperature, mean arterial blood pressure, venous oxygenation, pH, and stat systematic lactate determined from drawn blood, often from an indwelling catheter placed within a major blood vessel or, in the case of reconstructive surgery of the heart, directly into the heart and exiting through the heart wall and body wall. While these gross vital signs are critically important in the evaluation and management of patient wellness, they are nonetheless subject to misinterpretation [6]. Recent work has confirmed that pre-hospital patient assessments that rely on traditional vital signs may frequently underestimate elevated lactate levels [7]. The clinical implications suggest an emphasis on outcome analyses. However, such outcomes must be better associated with bioanalytical measures of appropriate biomarkers that serve as prognostic indicators of MODS. The early work of Huckabee [8], Broder and Weil [9], and Vitek and Cowley [10] have long pointed to the importance of lactate levels as a prognosticator of hemorrhage outcomes [11–14].

Lactic acid exists in equilibrium with glucose and accumulates within the tissues under conditions of hypoxia [15]. Evidence has been compiled supporting the hypothesis that metabolites such as lactate and glucose experience widely altered levels as a result of hemorrhage [16]. Lactic acid levels in the body have been correlated with severity of hemorrhage [4, 5]. Patients receiving traumatic head injuries tend to have increased levels of lactate within the central nervous system, which has been considered a marker for a

poor clinical outcome [17]. It has been noted that traumatic brain injury may be the cause of elevated lactate levels [18]. Hypoxia-damaged brains, upon reoxygenation, will begin to use systemic lactate as an energy source to recover synaptic function, thus subsequently, it has been considered to be potentially useful for treatment in a clinical setting [19, 20].

By this evidence, it is clear that immediate and continuous measurement of lactate and glucose may serve as a “gauge” for identifying shock states. Biosensors have been developed to monitor both of these metabolites in the body in a continuous manner [21]. More in vivo studies need to be engaged to collect more useful data, the intention of which will be to find how glucose and lactate levels may effectively be correlated to the onset of hemorrhagic shock from traumatic wounds. The solution is to develop and deploy an implantable physiologic status monitoring biochip (the PSMBioChip) for the immediate and continuous monitoring of intramuscular lactate and glucose levels. We propose the development of an intramuscularly implanted sonde that will allow the continuous monitoring and real-time reporting of cumulative tissue lactate, glucose, pH, and temperature to guide resuscitation approaches, triage, and improve clinical outcomes.

Oxygen delivery ($\text{DO}_2 = \text{CO} \times \text{CaO}_2$, where CO = cardiac output and CaO_2 = oxygen content of the arterial blood), which in healthy individuals ranges from 460 to 650 $\text{ml min}^{-1} \text{m}^{-2}$, has been investigated as a function of mean arterial pressure among individuals presenting in various shock states. There was no apparent correlation between oxygen delivery and mean arterial blood pressure. Correspondingly, survival outcomes for individuals presenting with hemorrhage and hemorrhage-associated shock showed a dramatic decline when measured against increasing stat measures of systemic lactate [9]. Lactic acidosis is therefore a well-established prognosticator in hemorrhage-associated trauma [22]. Arterial lactate values of more than 5 mmol/l on admission to intensive care unit (ICU) were associated with a mortality rate exceeding 80% at 30 days.

The pathophysiology of hemorrhage and hemorrhagic shock

Hemorrhage, abnormal blood flow, may be internal and not visible or external and visible. Hemorrhage in an uncontrolled hemorrhagic shock model is accompanied by peripheral vasoconstriction. The implication is reduced peripheral blood flow with the objective of preserving core body homeostasis and function with the consequence that less oxygen-rich blood gets to peripheral tissues. Table 1 [23] lists the oxygen demand (VO_2 , 60.8 mL/min) of various tissues tabulated alongside blood flow to each tissue. Skeletal muscle is clearly shown to possess the largest oxygen demand of all the tissue types. Moreover, skeletal

Table 1 Pathophysiology of shock: oxygen supply and consumption within various organs

Organs	Blood flow (mL/min, % of CO)	Blood flow (mL/100 g)	Arterial–venous difference (vol.%)	VO ₂ (mL/min)
Heart	210 (4)	70	11.4	23.9
Brain	760 (15)	50	6.3	47.9
Kidney	1220 (24)	400	1.3	15.9
Liver	510 (10)	29	4.1	20.9
GI tract	715 (14)	35	4.1	29.3
Skeletal muscle	760 (15)	2.5	6.4	60.8
Skin	215 (4)	9.5	1.0	2.15
Other organs ^a	715 (14)	–	–	–

^a Other organs include fat, bone, and lungs. Reproduced from Jain and Fischer [23]

muscle possesses a commensurate low blood flow (2.5 mL/100 g). The combination of vasoconstriction, generalized low blood flow rate, and high oxygen demand identifies peripheral muscles as the most adversely affected tissue during hemorrhage [24]. Early detection, continuous monitoring, and swift correction of tissue hypoxia is indicated in the management of critically ill patients [25]. Oxygen delivery to the tissue bed is paramount [26]. The recent report of the International Consensus Committee on shock makes specific recommendations regarding the monitoring and management of the critically ill patient with shock [27]; among these, tissue perfusion (or reperfusion) was regarded as central to outcomes and blood lactate continues to be the gold standard by which shock states may be assessed.

The case for continuous monitoring of lactate and glucose

Following trauma and upon presentation to a first responder (medic, EMT, or remote space intervention), the ability to *immediately* and *continuously* monitor lactate levels [28], and thus cumulative oxygen debt, will allow the development of patient-specific resuscitation strategies, consistent triage, and improved allocation of scarce resources under austere situations such as in planetary exploration, battlefields, and mass casualty scenarios arising from natural or manmade disasters. Patients admitted to the surgical ICU and treated with intensive insulin therapy to approach euglycemia have shown reduced mortality (30–50%), reduced episodes of septicemia (46%), and reduced renal failure (41%) and were less likely to require prolonged use of antibiotics. Moreover, markers of inflammation were less frequently abnormal [29]. More recent studies have indicated, however, that insulin therapy to a plane corresponding to 10.0 mmol/L (conventional) rather than to 6.0 mmol/L (intensive) may have no benefit as it relates to mortality, but may have implications for morbidity [30], with conventional insulin therapy being sacrosanct [31].

We hypothesize that under conditions of poor peripheral perfusion, such as during hemorrhage, that tissue lactate levels rise and are generally discordant with systemic lactate

levels. We hypothesize further that the extent and duration of elevation of tissue lactate, taken as integral prior to return to eulactatemia, may be a better indicator of survivability in a hemorrhage model. The same may be true for glucose. Arguably, it may not be the plane to which the patient is returned, intensive or conventional insulin therapy, but rather the time spent out of plane that is implicated in patient outcomes. However, enabling real-time, continuous measurement and monitoring of tissue lactate and glucose levels has been a major technical challenge. This paper provides a general overview of our research that aims to develop a comprehensive physiological status measuring sonde that is capable of wireless reporting of critically important molecular indicators of hemorrhage-induced physiologic stress.

Goals of our research

Among the many challenges to a comprehensive physiological status monitoring sonde are: (1) design, synthesis, and characterization of soft polymeric biomaterials with *low interfacial impedance*, *fast ion transport*, *biomolecule hosting*, and *in vivo biocompatibility* to mitigate the foreign body response and allow indwelling performance of up to 6 weeks; (2) the development of implantable biotransducers suitable for continuous monitoring of interstitial glucose and lactate with retained sensitivity for up to 6 weeks possessing appropriate dynamic range, detection limit, and stability is a target; (2) development of pH-responsive smart materials for sensitive reporting of tissue pH; (4) integration of a temperature measurement device to enable absolute tissue temperature and to enable temperature correction of biospecific responses; (5) development of the mixed signal electronics to serve as the front end to such an array of sensor inputs; (6) signal processing and data fusion to empower decision making, (7) implementation of a suitable wireless reporting capability; (8) meeting power and power management needs for an implanted bioanalytical device with real time reporting requirements; and (9) eventual design of an application-specific integrated circuit (ASIC) biochip.

PSMBiochip design

Fully integrated biosensor systems and associated micro-system technologies have been the subject of scholarly research [32–36] and commercial interest [37, 38] for many years. Systems for the monitoring of glucose have been the subject of considerable research [39–41]. The PSM Biochip is a fully integrated discrete biosensor system intended for eventual implementation in a biochip (ASIC) format. In this sense, it comprises a biotransducer, associated bioinstrumentation for interrogation, capture, and processing of bioanalytical data, and a data presentation system that focuses on actionable information intended to influence patient outcomes [42]. In its discrete design, it is more appropriately described as a biosensor system. In its ASIC design, it is best described as a biochip. Figure 1 shows a schematic illustration of the discrete prototype for the PSM Biosensor System. The unit comprises a dual potentiostat, data converters, processor, RF transmitter, and battery. The multiple analyte sensing sonde, which comprises a dual-analyte biotransducer, is set distal to the encapsulated electronics.

Wireless dual potentiostat

Our vision is of a fully integrated implant biosensor system [43] fabricated as an ASIC and packaged for indwelling performance for up to 6 weeks. Such a device must: (1) manage power usage for all system components, (2) collect and condition all analog signals from the various biotransducers of the sonde, (3) digitize those analog signals, (4)

store raw or conditioned data and operational parameters, (5) wirelessly support unidirectional or bidirectional communication with a base station, and (6) be able to rapidly wake up from a low-power “sleep” for immediate data collection. Specific to the needs of implantation are: (1) a very small footprint, (2) very low power consumption, and (3) wireless transmission/reception within and through living tissue. The precursor to such an ASIC is a discrete prototype possessing all of the qualities and performance characteristics for the intended application [44]. Such a discrete dual-channel potentiostat has been developed with Pinnacle Technology, Inc. (Lawrence, Kansas, USA).

Experimental

Materials

Hydrogel constituents: poly(2-hydroxyethyl methacrylate) [p(HEMA), MW=60,000: viscosity modifier], 2-hydroxyethyl methacrylate (HEMA: principal monomer), tetraethyleneglycol diacrylate (TEGDA, technical grade: cross-linker), *N*-[tris(hydroxymethyl)methyl]-acrylamide, (HMAA, 93%: secondary monomer), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%: photoinitiator), pyrrole monomer (Py, reagent grade, 98+%: electroactive monomer), 4-(3'-pyrrolyl)butyric acid (PyBA: reagent grade, 98+%: hydrophilic electroactive monomer), 3-sulfopropyl methacrylate potassium salt (SPMA: anionic dopant monomer). Poly(ethyleneglycol)(200)monomethacrylate (PEG200MA) poly(ethyleneglycol)(400)monomethacrylate

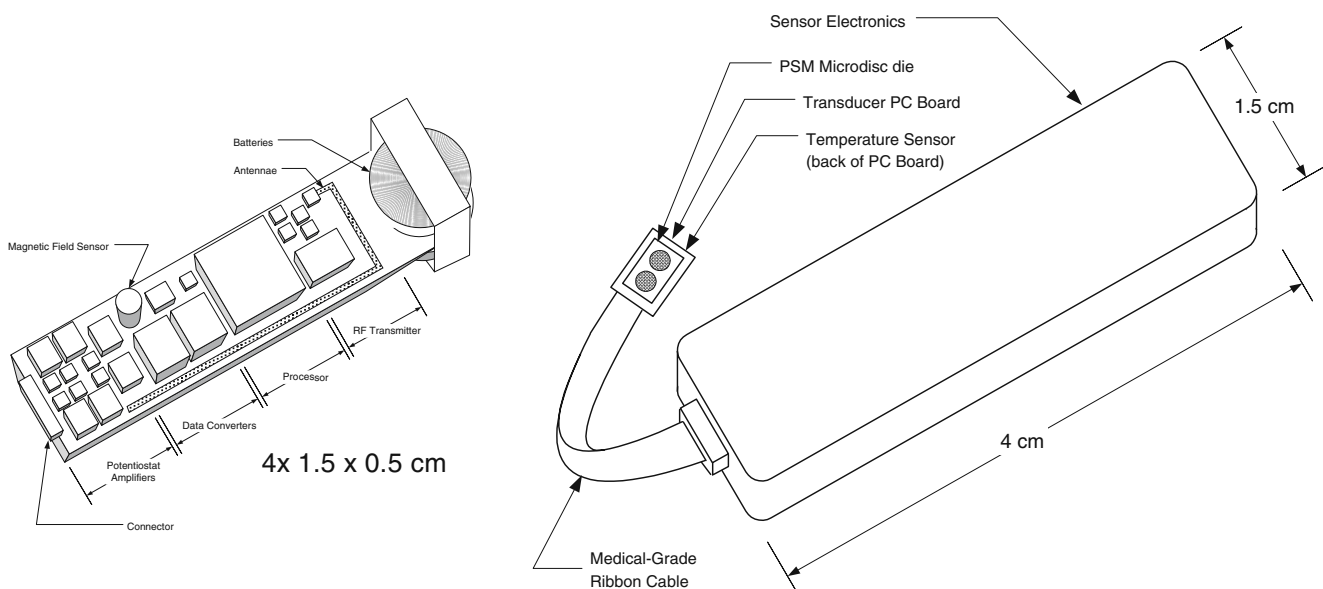


Fig. 1 Schematic illustration of the discrete prototype for the physiologic status monitoring biochip showing: the dual potentiostat, data converters, processor, RF transmitter, and battery (a) and the

physical placement of the sensing sonde and biotransducer distal to the encapsulated electronics (b)

(PEG400MA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 2-Methacryloyloxyethyl phosphorylcholine (MPC) monomer was synthesized in the laboratories of Prof. Kazuhiko Ishihara of the University of Tokyo Center for NanoBio Integration. Prior to formulation, all of the acrylate-containing reagents were passed over an inhibitor removal column (Sigma-Aldrich) to remove the polymerization inhibitors hydroquinone and monomethyl ether hydroquinone. Tris(hydroxymethyl)aminomethane (TRIS buffer, ACS reagent, 99.8+%) was pH-adjusted with hydrochloric acid (ACS reagent, 37%) to obtain 0.1 M buffer with pH 7.2. Ethanol (CHROMASOLV®) was used as received. Phosphate-buffered saline (PBS, 0.01 M, pH 7.4) was prepared in the standard way. All solutions were prepared with deionized (MilliQ DI) water. The diacrylate and methacrylate reagents were passed through an inhibitor removal column (Sigma-Aldrich) before use. Pyrrole was passed over an alumina silicate column for the removal of oligomers. The hydrogel cocktails were prepared by mixing HEMA, TEGDA, PEG (200)MA, HMMA, and DMPA in a typical ratio 86:3:5:5:1 mol% to yield a base hydrogel. Acryloyl (polyethylene glycol)₁₁₀ N-hydroxy succinimide ester (acryloyl-PEG-NHS) was obtained from Nektar Therapeutics, (Huntsville, AL, USA). All other reagents used were of analytical grade and obtained from Sigma Chemical Co.

The dual-analyte electrochemical transducer

To address the need for simultaneous monitoring of interstitial glucose and lactate, a prototype dual responsive electrochemical biotransducer has been developed [45]. Microlithographically fabricated Electrochemical Cell-on-a-Chip Microdisc Electrode Arrays (ECC MDEA 5037-Au) were developed in conjunction with ABTECH Scientific

(Richmond, VA, USA). This dual sensing electrochemical transducer possesses 37 recessed microdiscs arranged in a hexagonal array, each of $D=50\text{ }\mu\text{m}$ and total working electrode area, $\text{WEA} = 7.3 \times 10^{-4}\text{ cm}^2$ [46]. The design and microlithographic fabrication of these transducers have been described in detail elsewhere [46], and the details of the packaging of the chip or die for implantation into small vertebrate animals has similarly been described in detail elsewhere [47]. Briefly, electrochemical transducers ($0.2 \times 0.4 \times 0.05\text{ cm}$) were fabricated from magnetron sputter-deposited gold or e-gun vapor-deposited platinum (100 nm) on an adhesion promoting titanium/tungsten (Ti/W) layer (10 nm) and on an electronics grade borosilicate glass (0.5 mm thick Schott D263). The electrodes were fashioned into two separate three-electrode electrochemical cells, and these were passivated with $0.5\text{ }\mu\text{m}$ thick silicon nitride (Si_3N_4) after which the nitride layer was fluoro-plasma-etched to reveal the multiple microdiscs of the working electrode, the counter electrode ($7.3 \times 10^{-3}\text{ cm}^2$), a shared reference electrode ($7.3 \times 10^{-5}\text{ cm}^2$), and the five bonding pads. Figure 2a is an optical micrograph of the ECC MDEA 5037 electrochemical transducer.

Electropolymerization, electrochemical and electrical characterization

Electropolymerization of pyrrole and pyrrole copolymers was achieved using a PAR 283 Galvanostat/Potentiostat in chronopotentiometric mode (galvanostatically) or chronoamperometric mode (potentiostatically) using PowerSuite software. For galvanostatic electropolymerization, current was fixed at 1 mA/cm^2 and defined charge densities, typically 100 mC/cm^2 , achieved. For potentiostatic electropolymerization, voltage was fixed at $0.75\text{ V vs. Ag/AgCl}$,

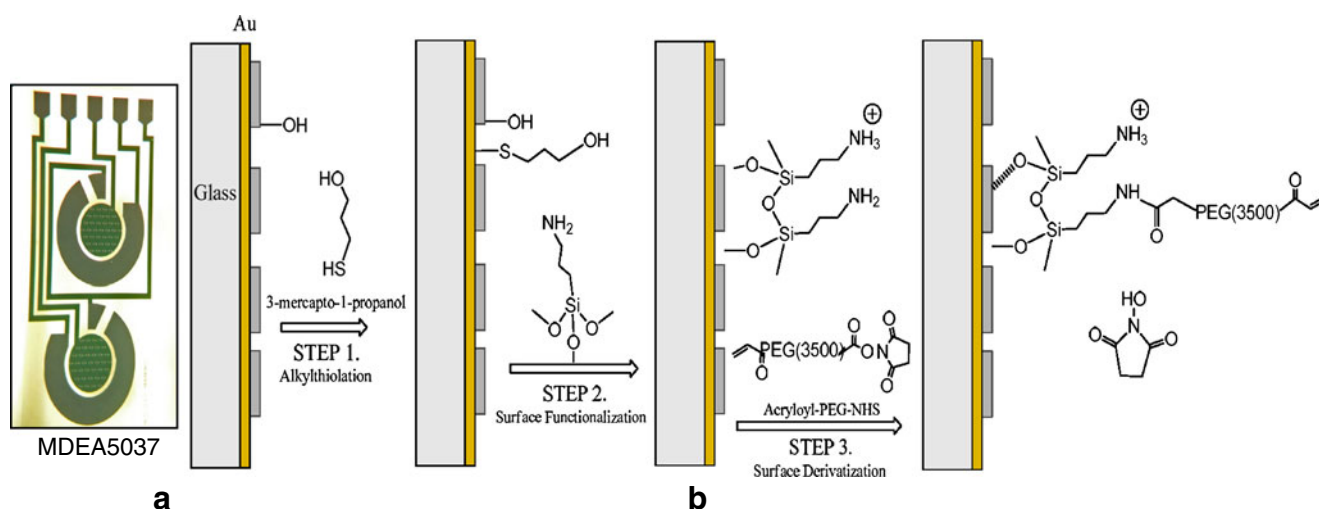


Fig. 2 **a** Optical micrograph of the MDEA 5037 electrochemical cell-on-a-chip transducer. **b** Schematic illustration of the multiple steps of surface activation, modification, and derivatization for monomer casting and bioactive electroconductive hydrogel attachment

3MCl⁻ and defined charge densities, typically 100 mC/cm², achieved. Dynamic electrochemical characterization of the electroconductive hydrogels was studied by multiple scan rate cyclic voltammetry (MSRCV) and by electrochemical impedance spectroscopy. Both were achieved using the PAR 283 Galvanostat/Potentiostat. The latter, EIS, was achieved when the PAR 283 (AMETEK, Princeton Applied Research) was interfaced to a Solartron 1260 Frequency Response Analyzer (FRA; AMETEK, Solartron Analytical, UK). Amperometric biosensor responses to glucose and/or lactate were measured at 0.70 V vs. Ag/AgCl, 3MCl⁻ in PBS 7.2 buffer at RT. Electrical characterization of the electroconductive hydrogel was done by four-point conductivity measurements or by impedance spectroscopy performed on interdigitated microsensor electrodes (IME 1050.5M-Pt-U, ABTECH Scientific, Inc.) and equivalent circuit modeling conducted within Z-View software. Three approaches were evaluated to synthesize the electroactive polymer component within the hydrogel. In the first approach, electroactive monomer and dopant anion (SPMA) were included within the hydrogel formulation prior to membrane dip casting. In these formulations, the Py and PyBA were typically 15 and 1.5 mol%, respectively, and the SPMA was 5 mol%. In the second approach, the transducers that were dip-coated with a base hydrogel and UV cross-linked were incubated in an aqueous Py and PyBA solution and the pyrrole monomers allowed to partition into the hydrogel for at least 1 h prior to electropolymerization. An 8:1 Py/PyBA (0.25:0.025 M) solution was made in DI water and its pH adjusted to 5.2 using 0.1 M Tris buffer. The third was a tandem of the forgoing two methods, and this was found to be most effective in producing uniform polypyrrole within the hydrogel.

Preparation of biotransducers

The fully assembled and packaged ECC MDEA 5037 chip [47] was developed to allow the conduct of physiologic status monitoring studies in a small vertebrate animal (Sprague–Dawley rat) hemorrhage model. To achieve this, the chip has first to be separately conferred with biospecificity to glucose (channel 1, cell A) and lactate (channel 2, cell B) through the use of molecularly engineered glucose oxidase (GOx) and lactate oxidase (LOx) to allow simultaneous measurement and monitoring of both metabolites of interest. GOx and LOx were immobilized via galvanostatic electropolymerization of Py and 4-(3-pyrrolyl)butyric acid in the presence of PEGylated-GOx or PEGylated-LOx into a p(HEMA)-based hydrogel membrane layer [48].

To accomplish this, chips bearing gold electrodes were chemically modified using an alkane thiol (overnight in 1.0 mM 3-mercapto-1-propanol or 1.0 mM cysteamine in ethanol), the silicon nitride passivation layer subsequently

modified with an organosilane (30-min immersion in 0.1 wt.% 3-aminotrimethoxysilane in ethanol, rinsed in ethanol, and cured at 120 °C for 20 min) and the terminal amines on both surfaces functionalized by immersion for 2 h in a solution of acryloyl(polyethyleneglycol)-*N*-hydroxysuccinamide (Acryloyl-PEG-NHS, MW 3,500; 1.0 mM in 0.1 M HEPES at pH 8.5) that was prepared under UV-free conditions. Figure 2b schematically illustrates these surface chemical activation, modification, and derivatization steps that serve to covalently attach the electroconductive hydrogel membrane layer to the transducer surface. The immobilized polyether provides multiple opportunities for concerted hydrogen bonding interaction between the surface and the hydrogel membrane. Following surface modification and derivatization, chips were then dip-coated by immersion and withdrawal from a monomer cocktail comprising HEMA, TEGDA, PEG(200)MA, MMA, DMPA, SPMA, Py, and PyBA in a typical ratio 62.5:3:5:5:2:1:5:15:1.5 mol% to yield an electroconductive hydrogel precursor. The coated transducers were immediately placed in a UV cross-linker and irradiated with UV light (366 nm, 2.3 W/cm², 5 min) under an inert nitrogen atmosphere. The hydrogel membrane provides a hydrated milieu for the three-dimensional bioimmobilization and hosting of the bioreceptors that confer biospecificity as well as any redox mediator that may be co-immobilized with the bioreceptor. It also serves as the reaction medium within which the electropolymerization reaction occurs.

Conferring biospecificity to the dual-analyte biotransducers

Conferring biospecificity to a multiple analyte biotransducer presents a formidable fabrication challenges. Among the several available approaches are: (1) additive and subtractive microlithography wherein a photosensitive layer containing enzyme 1 that was spun applied and lithographically developed leaving an exposed site to which another enzyme layer (enzyme 2) could be spun applied and UV cured; (2) non-contact or micro-contact printing, including ink-jet printing, drop dispensing, or spotting; and (3) electrically based techniques such as electrophoreses, dielectrophoreses, and electropolymerization. Two approaches were evaluated to confer biospecificity to the electroconductive hydrogel membrane of this work. In the first approach, the chosen oxidoreductase enzyme was included within the hydrogel formulation prior to membrane casting and so became physically entrapped as the reactive monomer became cross-linked into the hydrogel membrane and was photo-defined. In these formulations, the enzyme was typically 0.1 mg/mL. In the second approach, the MDEA 5037 with its UV cross-linked hydrogel membrane was incubated in an aqueous Py and PyBA that also contained the oxidase enzyme, and electropolymerization was used to achieve deposition of

the enzyme onto the working electrode and within the hydrogel layer. In these solutions, the enzyme was typically 1 mg/mL.

GOx and LOx were separately immobilized within the supported hydrogel membrane layer on the working electrodes of the biotransducer by electropolymerization. Electropolymerization was achieved by the application of 0.70 V vs. Ag/AgCl (potentiostatic or chronoamperometric) or at 10 mA/cm² (7.3 μ A galvanostatic or chronopotentiometric) to the working electrode immersed in a TRIS buffered (pH 5.2) aqueous solution containing an admixture of pyrrole (0.4 M), 4-(3'-pyrrolyl)butyric acid (0.04 M), and the respective enzyme (typically 1.0 mg/mL). Then, 10:1 Py/PyBA solution was made in DI water and its pH adjusted to 5.2 using 0.1 M Tris buffer. It is noteworthy that the conductive, electroactive polymer (CEP) component is allowed to initiate and grow within the immobilized hydrogel layer which serves as a multivalent macro-anion for the positively charged polypyrrole copolymer. The CEP grows from the metal|hydrogel interface and may, under poorly controlled conditions, emerge as a separate dense layer at the metal|hydrogel interface. However, the presence of the electroactive monomer and pendant dopant anions within the membrane, as well as judicious control of the reaction kinetics (current density), can result in a uniform polymer composite [49]. ECP formation also occludes the enzyme into the gel. The enzymes, possessing a net negative charge under the electropolymerization conditions, become entrapped within the hydrogel during the electropolymerization.

Following bioimmobilization (100 mC/cm², 100 s), the chips were conditioned in TRIS buffer at 4 °C and the solution changed several times to remove un-reacted monomer. The chips were then dip-coated again, this time into a second bioactive hydrogel cocktail formulation that contained 2-methacryloyloxyethyl phosphorylcholine (MPC or PCMA) and this UV cross-linked to form an additional membrane layer of 3 mol% cross-linked poly (HEMA-co-PEGMA-co-HMMA-co-PCMA) [48]. Figure 3a shows optical micrographs of the 50- μ m diameter microdiscs of the MDEA 5037 before and after electropolymerization of pyrrole, and Fig. 3b schematically illustrates the resulting biorecognition membrane layer formed from the foregoing steps as they occur on the separate working electrodes of the ECC MDEA 5037 biotransducer. Figure 4 illustrates the chemistries of the biorecognition hydrogel membrane layer that subtends the transducer and the chemistries of the bioactive device-to-tissue interface hydrogel membrane layer.

In vitro amperometric calibration of the dose–response characteristics of the biotransducer was conducted at 0.7 V in 0.1 M PBKCl 7.0 at RT in response to mutarotated glucose and sodium lactate over the range 0–20 mM.

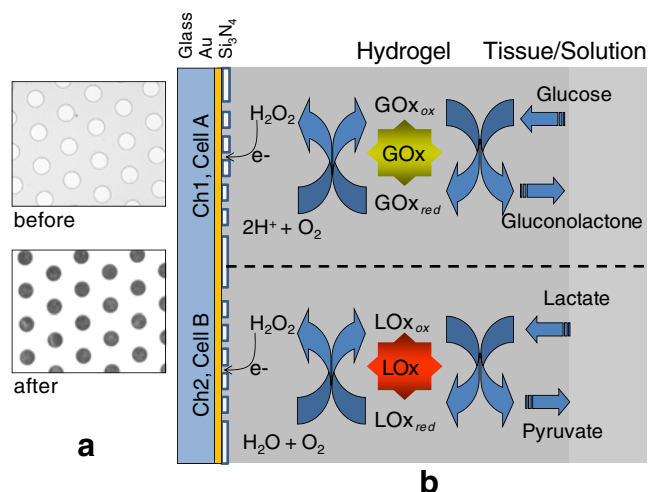
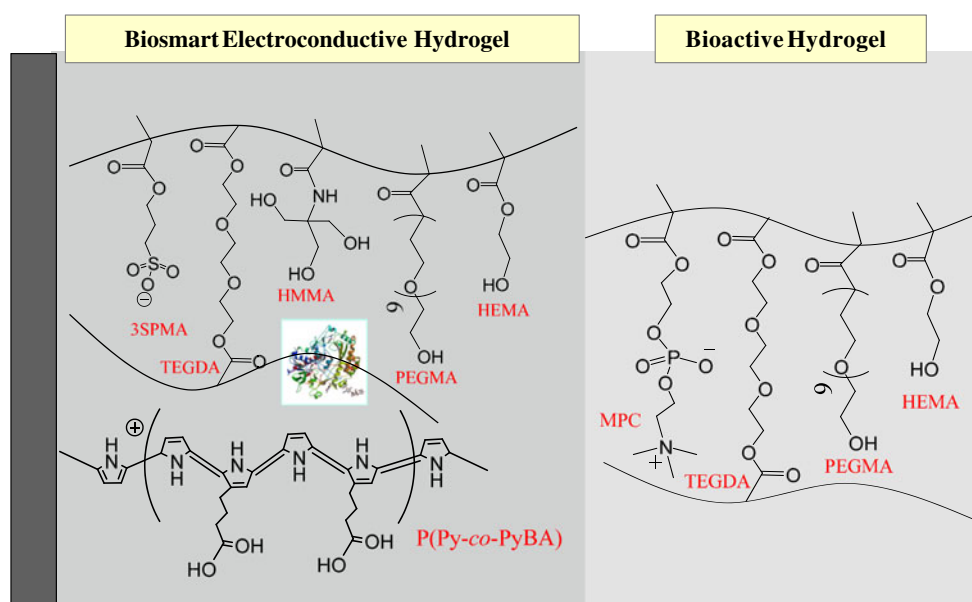


Fig. 3 **a** Microdisc array electrodes before and after electropolymerization of pyrrole. **b** Schematic illustration of conferred biospecificity by immobilization of GOx (channel 1, cell A) and LOx (channel 2, cell B) within separate hydrogel membrane layers corresponding to Ch1 and Ch2

In vitro and in vivo biocompatibility of bioactive hydrogels

To evaluate in vitro biocompatibility of our electroconductive hydrogel outer layer, rat pheochromocytoma cells (PC12 ATCC:CRL-1721, Manassas, Virginia) and human muscle fibroblasts (RMS13 ATCC:CRL-2061, Manassas, Virginia) were seeded and cultured (PC12: F-12K supplemented with 2.5% fetal bovine serum (FBS) and 15% horse serum, as well as 50 IU/mL penicillin and 50 μ g/mL streptomycin and RMS13: RPMI supplemented with FBS (10%) and 50 IU/mL penicillin and 50 μ g/mL streptomycin) on the following polymer-modified planar gold electrodes: (1) Au*, (2) Au*|Gel, (3) Au*|PPy, and (4) Au*|Gel-P (Py-co-PyBA) (where A* represents the surface-activated, modified, and derivatized surface as in Fig. 2b) and these compared to cell growth and proliferation on a reference polystyrene cell culture surface within a 24-well plate cell cultureware. Trypsinized cells were stained with Trypan blue and the final cell density determined using a hemacytometer and inverted light microscope. Cell morphology was determined following staining with rhodamine-phalloidin and 4',6-diamidino-2-phenylindole (DAPI) subsequent to fixing with 4% paraformaldehyde. To evaluate biomaterial cytotoxicity, human aortic vascular smooth muscle cells (HA-VSMC; ATCC:CRL-1999) were cultured on PEGMA and MPC containing hydrogels that formed the outer layer of the biotransducer. HA-VSMC was cultured in F-12K (ATCC, Manassas VA) supplemented with HEPES, TES, ascorbic acid (Sigma Aldrich), insulin, transferrin, sodium selenite (collectively available as ITS premix, BD Bioscience), endothelial cell growth supplement (VWR

Fig. 4 Schematic illustration of the molecular constituents of a poly(HEMA-*co*-PEGMA-*co*-HMMA-*co*-SPMA)/P(Py-*co*-PyBA) electroconductive hydrogel membrane containing an oxidoreductase enzyme and illustrated with a glucose oxidase subunit along with the bioactive hydrogel topcoat of a poly(HEMA-*co*-PEGMA-*co*-MPC) containing phosphoryl choline (MPC)



Scientific), and supplemented with 10% fetal bovine serum (Sigma Chemical Co).

To evaluate *in vivo* biocompatibility, the bioactive p(HEMA)-based hydrogels that were to form the outer device layer were tested by implantation into the trapezius muscle of Sprague–Dawley rats. All animals were anesthetized with sodium pentobarbital (35 mg/kg rat wt) administered intraperitoneally and pedal reflex used to determine the adequacy of the anesthesia. Anesthesia was then transitioned to the steroidal anesthetic Saffan (0.5 mg/kg⁻¹ min⁻¹) to maintain adequate level/duration of anesthesia for the individual rat and procedure. A preoperative subcutaneous dosage of atropine sulfate (100 µg/kg rat wt) was administered to decrease bronchial secretions and attenuate bradycardia following anaesthetization. Once unconscious, animals were placed in the ventral recumbent position on a heating pad with fore and hind limbs restrained with tape. After reaching a surgical plane of anesthesia, the animal's entire neck, back, hind limb, and abdomen was shaved and prepared with a Betadine wash and draped with a sterile towel. The test hydrogel material was inserted in the medial trapezius muscle at the level of the mid-quadriceps. The same quadracep (different area) and the opposite quadriceps area were sterilely prepped and draped to allow for placement of one to three additional hydrogel samples. This was done in an effort to minimize the number of animals needed to test tissue biocompatibility of the molecularly engineered hydrogels. Specimens were scored according to a histological grading scale composed of five categories: cell morphology, matrix staining, surface regularity, thickness of the implant material, and bonding, with a total score range from 0 to 16 (hydrogel with no foreign material accumulation being equal to 16).

Results and discussion

In vitro and *in vivo* biocompatibility of biorecognition membrane materials

Intramuscular implantation of a bioanalytical sonde must address the challenge of implant biocompatibility that arises from the eventual tissue remodeling that accompanies the trauma of implantation as well as the foreign body response. It is generally believed that the devascularized collagenous capsule that forms around the biotransducer is one important factor that compromises performance largely through its influence on the transport distances that affect substrate access [50]. In this regard, the forgoing layered structure addresses synthesis of a soft polymeric biomaterial with low interfacial impedance, facile small molecule and ion transport, the demonstrated potential for biomolecule hosting (conferred biospecificity), and the potential for *in vivo* biocompatibility. Among the multiple possible approaches to address the foreign body response are those designed to: (1) emulate the chemical character of the extracellular matrix (ECM) by achieving a form of biomimicry through the use of chemical moieties such as hyaluronic acid [51] and amino acid sequences [52] drawn from the non-receptor binding motifs of ECM proteins (biomaterials chemistry), (2) emulate the topological character of the ECM including its chemical and physical heterogeneity (nanostructure), (3) emphasize hydrogen-bonded interactions and non-equilibrium mesostructures vs. covalently bonded isotropic materials, and (4) emulate the surface character of living cells with the use of such moieties as phosphoryl choline [53–55]. The forgoing approaches pursue biomaterials design via biomimicry [56]. The

current design is an elementary approach along these lines; it seeks a highly hydrated Zwitterionic surface through the inclusion of 2-methacryloyloxyethyl phosphorylcholine to confer the biological character of the outer leaflet of cell membranes to the synthetic hydrogel [48, 57]. These approaches are not without detraction; among these are polymer degradation [58], time–temperature influences [59], and well as mechano-transduction effects [60].

To evaluate in vitro biocompatibility of the biomimetic hydrogel outer layer, the following physicochemical characteristics of a 3 mol% TEGDA cross-linked hydrogel that contained varying mole percentages of PEGMA and PCMA were studied [61]. In this way, the relative contributions of pendant PEG and PC moieties to such properties as: (1) hydration characteristics following a 5-day equilibration was established; (2) evolving dynamic contact angles, revealing both advancing and receding contact angles, over a 5-day period; (3) adsorption of the extracellular matrix proteins—collagen, fibronectin, and laminin—over a 5-day period to reveal protein adsorption isotherms; and (4) in vitro cell viability and proliferation using human muscle fibroblasts (RMS 13; ATCC:CRL-2061) and human aortic vascular smooth muscle cells (HA-VSMC; ATCC:CRL-1999) [62]. Hydrogels exhibited an increase in the percent hydration with an increase in the MPC content, a maximum of 93.8% increase with an increase in MPC of up to 10 mol%. PEGMA had a smaller influence on hydration than PCMA. Dynamic contact angles ($\theta_a = \theta_r$ for ideal surfaces) of as-cast hydrogel membranes were initially high ($\theta_a = \theta_r =$ approx. 45° , somewhat hydrophobic) and reflected homogeneity ($\theta_a - \theta_r = 1^\circ$). However, with increased pre-conditioning of p(HEMA) hydrogels in DI water, the dynamic contact angles showed considerable change after 5 days ($\theta_a = 47^\circ$, $\theta_r = 22^\circ$, becoming more hydrophobic on advancing and more hydrophilic on receding) and reflected increased heterogeneity ($\theta_a - \theta_r = 25^\circ$). However, when p(HEMA) was made to contain 10 mol% PCMA, the dynamic contact angles showed a considerable decrease ($\theta_a = 20^\circ$, $\theta_r = 17^\circ$, becoming more hydrophilic on both advancing and receding) and reflected increased homogeneity ($\theta_a - \theta_r = 3^\circ$). This temporal character, while not obtained under physiologic or even physiologic-like conditions, does suggest an area for future research in the development and use of soft condensed biomaterials, that of the temporal evolution of chemical character pursuant to molecular rearrangements of the surfaces. Adsorption of FITC dye-tagged fibronectin from solutions that were 0.0 (blank), 0.1 ng, and 1.0 ng/ml of protein at 25°C followed the Langmuir adsorption isotherm with K_d and Q_m quantitatively confirming the progressive reduction in protein adsorption when the hydrogel was pre-conditioned for varying periods (up to 5 days) in DI water and also when the MPC content was increased. A strong correlation ($R^2 = 89\%$) between the

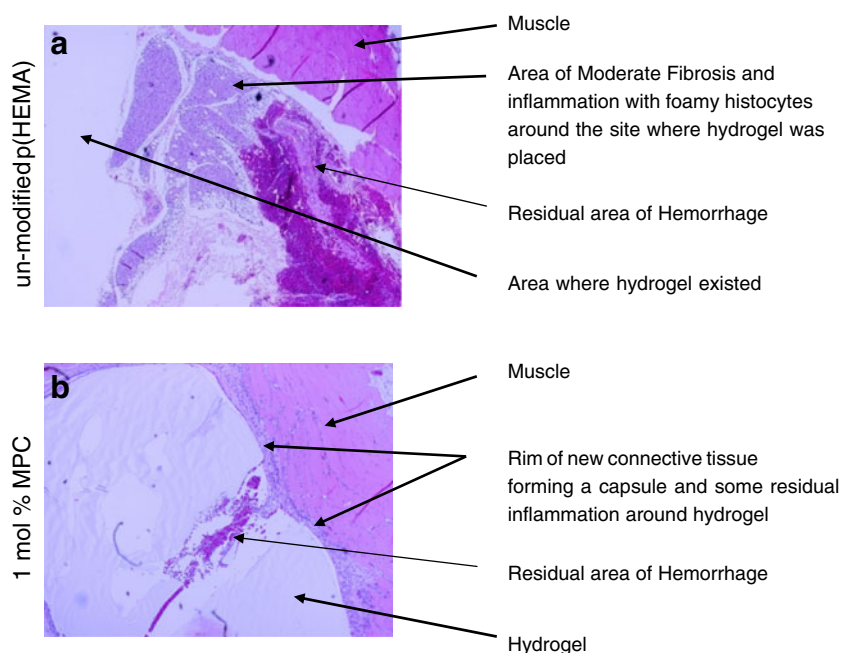
hydration levels of the hydrogels and the ability of the hydrogel to mitigate protein adsorption was found, and this was manifest through the PCMA content rather than the PGMA content. Cytotoxicity studies using human aortic vascular smooth muscle cells (HA-VSMC; ATCC; CRL-1999) produced $>80\%$ viability for all the hydrogel formulations. With RMS 13 cells, trypsinization and enumeration resulted in cell retention within the hydrogel matrix. This was studied by harvesting human muscle fibroblasts seeded on the hydrogel surfaces after 3 days of incubation using trypsin. The dsDNA of fibroblasts retained within the hydrogel matrix was stained using fluorescent DAPI and enumerated revealing a strong correlation between the MPC content and the degree of fibroblast retention within the hydrogel. Hydrogel retention of RMS 13 cells was $<1\%$ for gels containing no PEGMA or MPC, approx. 10% for hydrogels containing 0.5 mol% PEGMA and no MPC, but approx. 80% for hydrogels containing both 0.5 mol% PEGMA and 10 mol% MPC.

To evaluate in vivo biocompatibility of our biomimetic hydrogel outer layer, cylindrical test hydrogel specimens (2-mm D and 2-mm T , $n=2$ specimens per rat) were inserted into the medial trapezius muscle at the level of the mid-quadriceps of a Sprague–Dawley hemorrhage model ($n=2$ rats per specimen). Each of the hydrogel specimens ($n=4$) were found to elicit some degree of foreign body response.

Figure 5 shows the result of staining of resected hydrogels and surrounding tissue following in vivo implantation for 2 weeks in the trapezius muscle of a Sprague–Dawley hemorrhage model. Figure 5a shows the surrounding tissue and base hydrogel explants with significant encapsulation and accumulation of foreign body material, while Fig. 5b shows the modified hydrogel containing 1 mol% MPC, resulting in the formation of a thin band of encapsulation and a much reduced residual inflammation [63]. It is important to note that none of the materials exhibited a “granulomatous” type of response that is characteristic of a very vigorous foreign body response. It is also important to note that the newly deposited connective tissue did not penetrate into the body of any of the hydrogels tested, but remained on the exterior. While only preliminary and not associated with the underlying electroconductive hydrogel membrane or the transducer, this evidence indicates the potential for extended implant biocompatibility of hydrogel compositions containing phosphorylcholine.

The inner electroconductive polymer layer (Fig. 4) produces a biorecognition membrane layer that localizes molecular biorecognition (enzymes) within an electroconductive poly(HEMA)-based polypyrrole hydrogel. Conductive electroactive polymers and hydrogels have been separately shown to support aspects of in vitro and in vivo biocompatibility such as an absence of cytotoxicity and

Fig. 5 Tissue and bioactive hydrogel explants following in vivo implantation for 2 weeks in the trapezius muscle in a Sprague–Dawley hemorrhage model



excellent cell growth and proliferation. While electroconductive hydrogels have not been subjected to similar extensive investigation, they likewise promise similar, if not improved, in vitro and in vivo biocompatibility. After 4 days, cells were found to have 100% viability in all cases. Cell proliferation, however, showed a marked difference between Au*[Gel-P(Py-co-PyBA) and all other reference samples. For both RMS13 and PC12 cell lines, there was a statistically significant ($p=0.05$) increase in cell proliferation on the ECH surfaces compared to the other modified gold surfaces. There was an 81% increase in RMS13 cell density at the end of the incubation period compared to a 12% increase in PC12. The RMS13 cells on the Au*[Gel-P(Py-co-PyBA) surface demonstrated mixed morphologies (spherical and spreading), while the PC12 cells were predominantly spherical with no evidence of neurite outgrowth. Of note was the fact that the cell densities associated with the Au*[PPy and Au*[Gel samples were both dissimilar to that of the Au*[Gel-P(Py-co-PyBA) and that the ECH presented a unique property that resulted in statistically greater cell growth and proliferation and that this correlated with the extent of electropolymerization of poly(pyrrole-co-4-(3-pyrrolyl)butyric acid) within the hydrogel [64].

Electrical and electrochemical characterization of the biotransducer interface

Dynamic electrochemical and AC impedance characterization of the bioactive hydrogels and electroconductive hydrogels supported on microdisc electrode arrays has been reported [65, 66]. MSRCV and electrical (two-electrode) and electrochemical (three-electrode) impedance spectroscopy

were used to characterize the charge transfer characteristics of hydrogel layers on microdisc electrode arrays. MSRCV experiments were generally done using a PAR 283 Galvanostat/Potentiostat and, for EIS, was done when the PAR 283 was interfaced to a Solartron 1260 FRA. The conductivity of the electroconductive hydrogel varies with the extent of electropolymerization ($8 \mu\text{Scm}^{-1}$ for $0.25^\circ\text{C}/\text{cm}^2$ and $76 \mu\text{Scm}^{-1}$ for $2.0^\circ\text{C}/\text{cm}^2$) and the oxidation state, being more like the pristine hydrogel and capacitive at reducing potentials and being more polypyrrole-like and Ohmic at oxidizing potentials. The observations were reflected in the equivalent circuit parameters that describe both hydrogel coated and electroconductive hydrogel-coated electrodes [67]. Table 2 lists the impedance proprieties of various tissue types and compares these to bioactive and electroconductive hydrogels. Hydrogels closely match the impedance properties of the various tissue types and moreover may be engineered to perfectly match specific tissue types. In addition to modulating interfacial impedance, electroconductive hydrogels have also been demonstrated to contribute to interference suppression [68].

Mechanical characterization of the biotransducer interface

Mechanical matching of the implanted device to the tissue bed is extremely important in supporting long-term indwelling performance. It is believed that modulus mismatching across the device–tissue interface does, in response to micro-motions, exacerbate the foreign body response. Table 3 lists the dynamic mechanical properties (loss and storage moduli) of various tissue types and compares these to that of bioactive hydrogels. Hydrogels clearly have the potential to

Table 2 Generalized impedance properties (real and imaginary) of various tissue types compared to bioactive and electroconductive hydrogels

Organs	Frequency of interrogation (kHz)	Real component (Ω)	Imaginary component (Ω)
Heart [69] (humans, without heart failure)	50	$ Z =25.6$	
Brain [70] (piglets, cerebral impedance)	100	50	15
Kidney [71] (humans, undergoing hemodialysis)	50	480–520	30–40
Liver [72, 73] (rats, control group in liver stenosis study)	50	$ Z =200$	
Skeletal muscle [74] (bovine, healthy excised bovine tissue)	50	40	2.5
Skin [75] (human, skin at 30 °C)	50	80	120
Breast [76] (humans, healthy breast tissue)	60	130–180	–10 to –30
Lung [77] (humans, healthy group in lung cancer survey)	50	514	52
Bioactive hydrogels [65] (range of polymers: PVA, p(HEMA), alginate)	50	20–200	20–350
p(HEMA-co-PEGMA-co-HMMA) 3 mol% TEGDA [67]	50	300–500	–200.3
Electroconductive PPy hydrogel (200 mC/cm ²) [67]	50	100	–100

display a wide range of dynamic mechanical properties, but because of the potential to develop additional virtual cross-links over time (gelation), these properties may be time/temperature-dependent, may demonstrate freeze–thaw cycle rate dependency, and may change with environment (e.g., pH, divalent ions). Judicious manipulation of the cross-link density, the molecular weight between cross-link, and the time–temperature processing history provides a window into the design and control of the mechanical properties of the biorecognition membrane. A recent review emphasizing the mechanical properties of electroconductive hydrogels brings perspective to this emerging class of materials as bioactive interfaces [78].

Wireless dual potentiostat

The wireless dual potentiostat is a custom variation of their model 8151 2-channel 24-bit potentiostat shown in Fig. 6 alongside the dual responsive ECC MDEA 5037 biotransducer. The wireless dual potentiostat interfaces to the ECC

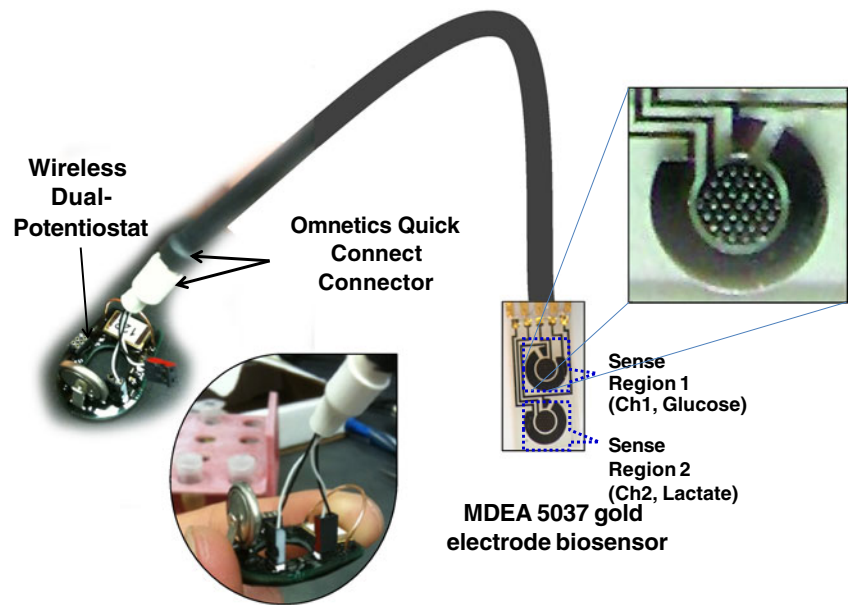
MDEA 5037 dual electrochemical biotransducer with its glucose-responsive electroconductive hydrogel region (GOx: channel 1, cell A) and LOx (channel 2, cell B) for implantation into the trapezius muscle of Sprague–Dawley rats. Shown in Fig. 7 (lower left then clockwise) are an exploded view of the head-mounting kit for the wireless transmitting dual potentiostat, the receiver base station, the Dell PC laptop, and the assembled transmitter mounted onto the head of a Sprague–Dawley rat. The discrete prototype is based on the Texas Instruments (TI) ChipCon CC1110 which features an integrated 8051 microprocessor, an 8–14-bit ADC, an RF Transceiver operating in the Medical Implant Communications Systems band 402–405 MHz, and 21 general I/O pins configured as 8 analog inputs and 13 digital I/O [92].

The bioanalytical system of dual responsive electrochemical cell-on-a-chip MDEA 5037 electrodes, wireless transmitting dual potentiostat, receiver base station, and software has been qualified for in vitro and in vivo use. The two channels of the dual potentiostat were tested with a pair

Table 3 Generalized dynamic mechanical properties (shear storage and loss moduli) of various tissue types compared to bioactive and electroconductive hydrogels

Organs	Shear storage modulus (kPa)	Shear loss modulus (kPa)
Heart [79]	~183 (human, coronary arteries)	89.6–111 (rabbit, cardiac cells) [80]
Brain [81]	2.1–16.8	0.4–18.7
Kidney [82] (pig kidney)	1.4–6.8	–
Liver [83]	0.75–3	–
GI tract	4.5–32 (rat, small intestine) [84]	8–45 (human, stomach)
Skeletal muscle [85]	22–115 (rat, soleus muscle)	–
Skin [86]	~5.0–7.0	–
Breast [87]	130	–
Lung [88]	0.25–1	0.1–1
Bioactive hydrogels [89, 90], p(HEMA) [91]	0.4–1400	0.4–1300

Fig. 6 Wireless dual potentiostat interface to the ECC MDEA 5037 dual electrochemical biotransducer with its glucose-responsive electroconductive hydrogel region (*GOx*: channel 1, cell A) and *LOx* (channel 2, cell B) for implantation into the trapezius muscle of Sprague–Dawley rats



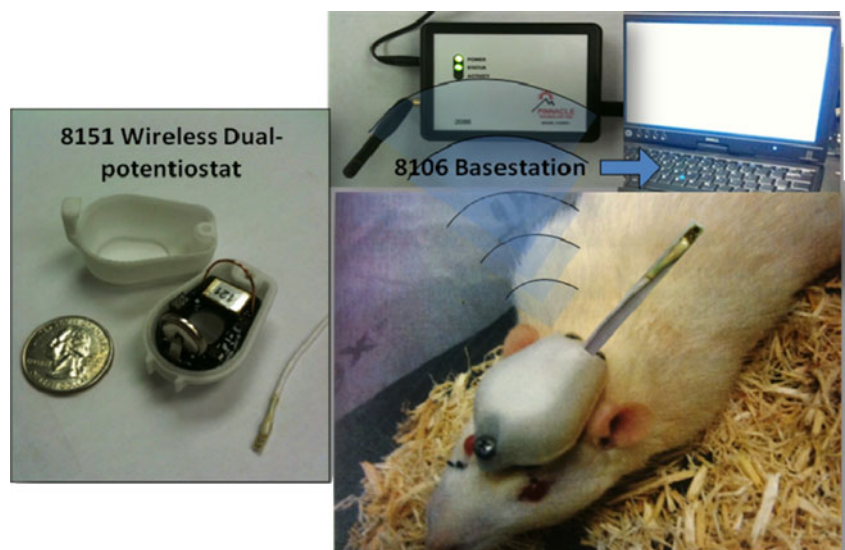
of dummy (RC) cells that comprised a parallel arrangement of a 10-M Ω resistor and a 1.0- μ F capacitor. Tested at 0.5 V, the dummy cells were switched between channels and their responses averaged to yield Ch1=51.1 $\pm$ 0.5 nA ($n=6$) and Ch2=54.4 $\pm$ 0.2 nA ($n=6$), establishing an inherent 6.5% error between the channels. When similarly tested with 10-M Ω resistors, their responses averaged to yield Ch1=49.9 $\pm$ 0.1 nA ($n=6$) and Ch2=49.6 $\pm$ 0.1 nA ($n=6$), establishing an inherent 0.6% error between the channels.

In vitro biospecific responses

The bioactive electroconductive hydrogel biosensors were tested in vitro for their response to glucose and lactate. The exemplified design uses a type 1, non-mediated biosensor configuration. Parallel designs with free and covalently

immobilized ferrocene monocarboxylic acid produced unsustained amperometric responses believed to arise from instability of the ferrocenium ion [93–95]. Figure 8 shows the amperometric dose response of the glucose- and lactate-specific biosensors to substrate challenges prepared in PBS (pH 7.4) at RT. Table 4 summarizes the key bioanalytical parameters determined for the unoptimized biosensors. The linear dynamic range for lactate measurements is inadequate to meet the needs of physiologic status monitoring during trauma-induced hemorrhage. Basal lactate levels are typically 1.0 mmol/L [96] and may be as high as 9.0 mmol/L in severely hemorrhaged patients [5]. There is clearly a need for improving the linear dynamic range for this analyte. The range of glucose concentrations for hypo- and hyperglycemic patients and diabetics (5–10 mmol/L) is adequately served by the linear dynamic range observed for the current biotrans-

Fig. 7 Sprague–Dawley rat equipped with a head-mounted wireless transmitting dual potentiostat to support intra-muscular bioanalytical measurements of lactate and glucose in the trapezius muscle. Amperometric readings were read by the potentiostat from the intramuscularly implanted MDEA 5037 biotransducer and subsequently relayed to a computer via wireless base station for analysis



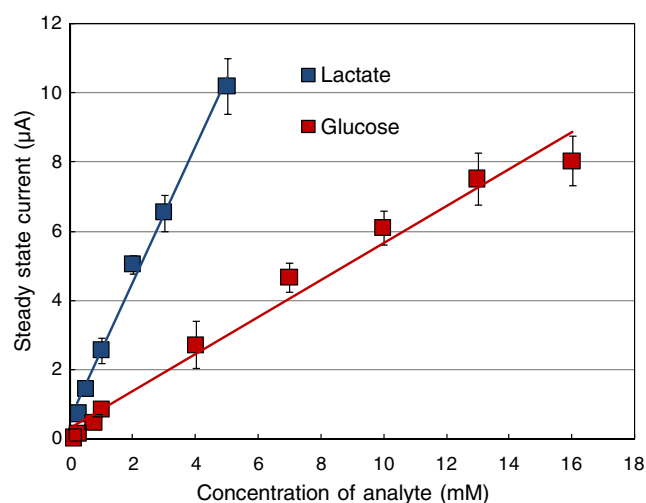


Fig. 8 In vitro response of the responsive MDEA lactate and glucose biosensor incorporating electroconductive polymer bio-smart hydrogel membrane of composition 80:10:2.5:2.5:5.0 mol% (HEMA/TEGDA/PEGMA/MPC/Py) in 0.1 M PBKCl, pH 7.0, at RT

ducers. However, among critically ill patients, particularly those subject to trauma-induced hemorrhage, insulin resistance may result in glucose levels that range from 5 to 12 mmol/L [97] and are then candidates for conventional (blood glucose >12 mmol/L) or intensive insulin therapy to achieve euglycemia [30]. Trauma patients may thus be adequately served by the observed linear dynamic range for glucose [31] in the currently configured biotransducer.

Short-term operational stability testing resulted in the glucose biotransducers producing 80% of their initial biotransducer response after 5 days of continuous storage at 37 °C and periodic testing of the biotransducer in 10 mM glucose. Exhaustive shelf-life and long-term performance testing has not yet been addressed and is the focus of current work.

In vivo biospecific responses

For preliminary in vivo bioanalytical studies, biotransducers were implanted into the trapezius muscle of a Sprague–Dawley rat hemorrhage model under IACUC-approved protocols for small vertebrate animal surgery. Rats were first anesthetized (5% then 2–3% isoflurane,

balance oxygen), prepped, and from a 2- to 5-cm-long midline abdominal incision, a 3 to 5-F Silastic catheter was surgically inserted into the inferior vena cava and tunneled subcutaneously over the ribs toward the left side of the head. The catheter was terminated in a rodent-sized vascular access port (VAP) placed in a subcutaneous pouch over the neck/scapular region. The VAP and catheter were filled with talurolidine citrate. To simulate hemorrhage and blood loss from trauma, blood was withdrawn from the rats at a rate of 2.5 mL/100 g/15 min from a femoral vein (to 40 torr) under isoflurane anesthesia. Figure 9 shows the catheterized and instrumented Sprague–Dawley rat under controlled hemorrhage conditions with intramuscularly (trapezius) implanted PSM biochip. As the controlled hemorrhage occurred, the onset of hemorrhagic shock and the changes in systemic and intramuscular glucose and lactate were observed. As expected, a total of 40–50% blood volume (approx. 6.5% of body weight) established a state of hemorrhagic shock. Systemic lactate from drawn blood was determined by electrochemical (amperometric) assay with enzyme membranes (ABL 705 Radiometer, Copenhagen, Denmark). Intramuscular lactate was determined from the implanted biotransducer. Figure 10 shows the in vivo amperometric lactate response of the intramuscularly implanted biotransducer plotted alongside the systemic blood lactate values obtained using the ABL 705 blood gas and metabolite analyzer during hemorrhage ($n=4$). Intramuscular lactate levels are shown as amperometric current rather than as lactate concentration as this would imply equivalence between the in vitro calibration condition and the in vivo test condition, which has not been established. By trend inspection, intramuscular lactate levels are clearly discordant with systemic lactate levels and rise more rapidly during the early stages of hemorrhage.

Table 4 Bioanalytical performance of the bioactive electroconductive hydrogels applied to the measurement of glucose and lactate

	Glucose	Lactate
Linear dynamic range (mM)	0.1–13.0	1.0–7.0
Sensitivity (μA/mM)	0.59	2.11
Detection limit (mM)	0.2	0.4
Response time (t_{95} , s)	50	35–40

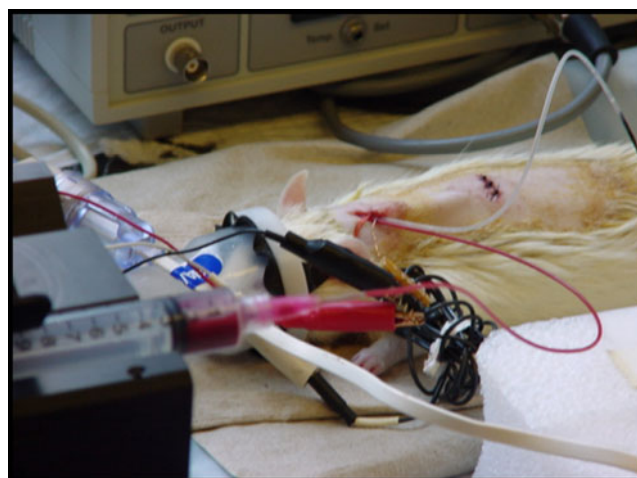


Fig. 9 Catheterized and instrumented Sprague–Dawley rat under controlled hemorrhage conditions with intramuscularly (trapezius) implanted PSM biochip

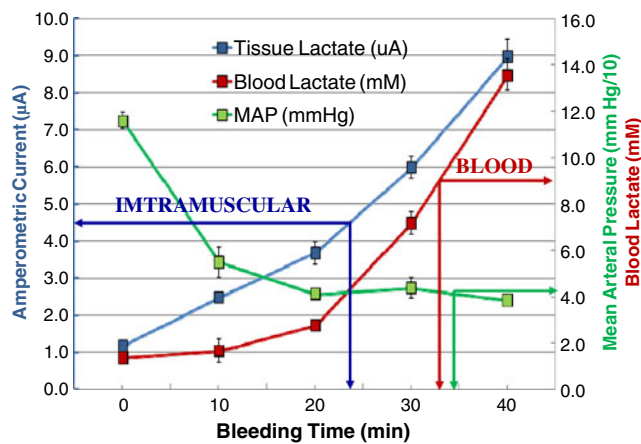


Fig. 10 In vivo amperometric response of an intramuscularly implanted lactate biosensor during hemorrhage ($n=4$), the mean arterial pressure (MAP), and the systemic blood lactate ($n=4$) obtained using a YSI Biostat Bioanalyzer

Limitations of our biochip research

The forgoing represents a status report on our progress to date toward a fully implantable biochip system for physiologic status monitoring during trauma-induced hemorrhage. There are multiple limitations and challenges to the system as described. Among these are: (1) the stability of the enzymes and their potential for denaturation within the hydrogel membrane milieu [98]. The oxidoreductase enzymes employed are globular proteins, intended by nature to work in solution environments of modest pH and ionic strength [99]. Immobilized within a hydrogel, regardless of hydration level, it provokes segmental interactions between the protein and the semi-rigid hydrogel network and provokes denaturation events [100]. One approach being pursued is the site-directed PEGylation of these proteins to create a microenvironment proximate to the protein that is more solution-like. (2) The production of peroxide that may be deleterious to membrane components, particularly to the conjugated electroactive polymer component, and to the surrounding tissues. In general, peroxide is dissipated via rapid consumption by peroxidases. However, within the biorecognition membrane, peroxide must diffuse over long distances to the electrified interface for electrochemical discharge, but also diffuses into surrounding tissues for possible dissipation by peroxidase. One approach being pursued is the co-immobilization of the oxidoreductases with a suitable peroxidase [101]. However, the potential for superoxide formation may necessitate the use of superoxide dismutase. (3) The need for co-immobilized mediator molecules or for oxidoreductase–nanoparticle conjugates to support type 2 (mediated) or type 3 (direct) biotransduction, respectively [50]. Both

approaches are being separately pursued with the covalent immobilization of pendant and flexible ferrocene moieties [95] and with hydrogel immobilization of ultrasonically processed enzyme–nanoparticle conjugates [102–105]. (4) The need for proper testing under industry standard testing protocols [106]. Proof of concept design, fabrication, and prototyping work of the forgoing must be accompanied by rigorous testing using industry standard testing techniques and protocols. (5) The power requirements for small footprint implantable devices will continue to be a challenge requiring unique power management modalities, low-profile, high-energy density batteries and alternative sources of power such as inductive and biofuel cells.

Conclusions

A dual responsive amperometric electrochemical transducer that uses the microdisc electrode array format has been designed and fabricated. This is by no means intended as a long-term indwelling device but rather as a prototype for in vitro and in vivo studies. The transducer has been rendered biospecific and biocompatible through the chemical covalent attachment of hydrogel layers that were conferred with biospecificity through the incorporation of enzymes via electropolymerization of pyrrole copolymers into the hydrogel. These electroconductive hydrogels have been shown to serve as hosting membranes for oxidoreductase enzymes, to be non-cytotoxic, and to support excellent cell viability and restricted proliferation. These “bio-smart” materials by design combine molecular biorecognition (enzymes), biocompatibility (PEG and MPC), interference shielding (PPy), and redox mediation (Fc) within p(HEMA)-based hydrogels. While the one-dimensional conductor has the potential to allow redox mediation and/or direct electron transfer between the flavin cofactors and the electrode, this phenomenon has not been explored in this work. An outer biomimetic hydrogel layer incorporating pendant phosphorylcholine moieties served to confer biocompatibility to the biotransducer. Biotransducers were integrated with a discrete, wireless dual potentiostat and tested both in vitro in buffer solution and in vivo in an instrumented Sprague–Dawley hemorrhage model. Preliminary findings point to the discordance between intramuscular lactate levels and systemic lactate levels. It is hoped that such implantable biochips will enable the immediate and continuous monitoring of glucose and lactate levels from the time of contact with the first responder through the ICU stays. In this way, the possibility for clinical studies of the relative significance of temporal excursions in both lactate and glucose as they relate to mortality and morbidity of the hemorrhaged patient will be enabled.

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