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The effect of temperature on the impedimetric response of bioreceptor hosting hydrogels

Anthony Guiseppi-Elie[a,b,c,d,*], Lauren Koch[a], Stephen H. Finley[a,e], Gary E. Wnek[f]

[a] Center for Bioelectronics, Biosensors and Biochips (C3B), Advanced Materials Research Center, Clemson University, 100 Technology Drive, Anderson, SC 29625, USA
[b] Department of Chemical and Biomolecular Engineering, Clemson University, Clemson, SC 29634, USA
[c] Department of Bioengineering, Clemson University, Clemson, SC 29634, USA
[d] Department of Electrical and Computer Engineering, Clemson University, Clemson, SC 29634, USA
[e] College of Medicine, Medical University of South Carolina, Charleston, SC 29425, USA
[f] Department of Macromolecular Science, Case Western Reserve University, Cleveland, OH 44106, USA

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ABSTRACT

Biocompatible hydrogels that serve as the hosting membrane for various bioreceptors contribute to the response of impedimetric biosensors. The temperature response of poly(2-hydroxymethacrylate) [p(HEMA)]-based hydrogel networks prepared with poly(ethylene glycol) methacrylate (PEGMA) for enhanced biocompatibility and with N-[tris(hydroxymethyl)methyl] acrylamide (HMMA) was studied. Hydrogels were cross-linked with tetraethyleneglycol diacrylate (TEGDA) and synthesized by UV initiation (2 M% DMPA photoinitiator). The p(HEMA-*co*-PEGMA-*co*-HMMA) based hydrogels were fabricated as discrete gel pads (D = 2.5 mm, H = 2 mm and V = 9.82 μL) on top of 250 μm diameter cysteamine modified and acryloyl (polyethylene glycol)$_{110}$ N-hydroxy succinamide ester (acryloyl-PEG-NHS) derivatized gold microelectrodes set within 8-well (8W1E) cell culture biochips. Gel pads were fabricated with cross-link densities corresponding to 1, 3, 5, 7, 9 and 12 M% TEGDA and were studied by frequency dependent 3-electrode electrochemical impedance spectroscopy (1 mHz to 100 kHz; 50 mV p-t-p) and by temporal 2-electrode impedimetry (64 kHz; 50 mV p-t-p) over the temperature range 30–45 °C at 90% RH or in aqueous 0.1 M Tris/KCl at pH 7.2 buffer. The p(HEMA-*co*-PEGMA-*co*-HMMA) hydrogels showed an increase in the real component of impedance with increasing cross-link density and demonstrated activation energies for impedimetric transport that ranged from 15 kJ/mol (3 M%) to 20 kJ/mol (12 M%) confirming the dominance of proton migration in the impedance of the hydrogels.

1. Introduction

The highly cross linked 2-hydroxyethyl methacrylate (HEMA) hydrogels, first developed and pioneered by Wichterle and Lim in 1960 (Wichterle and Lim, 1960), have had tremendous success in wide ranging biomedical applications (Mack et al., 1988; Ottenbrite et al., 2010). Success and continued interest in these hydrogels is motivated by their inherent unique properties of; (i) ability to imbibe large quantities of water, (ii) stability toward enzymatic degradation, and (iii) hydrolytic stability under near-neutral conditions. Of contemporary interest is the molecular level control of these properties through the judicious choice of cross-link density or molecular weight between cross links (Mabilleau et al., 2006) to achieve modulus matching with native tissue (Kaji et al., 2010) and co-polymerization with other monomer to achieve molecular recognition through imprinting (Byrne and Salian, 2008) or to confer biomimetic properties (Abraham and Guiseppi-Elie, 2005) through the use of biologically inspired monomers (Lee and Schmidt, 2010). The ability to molecularly engineer additional properties into p(HEMA)-based hydrogels has resulted in their application in areas as broad as contact lenses (Austin and Kumar, 2005), biosensors (Justin et al., 2009), in-dwelling catheters, and controlled release devices (Guiseppi-Elie, 2010a).

There have been many reports that seek to control the physicochemical, transport, and electrical properties of hydrogels through judicious and controlled changes in the composition; such as by changing the cross-link density or the chemical character of pendant moieties to the network backbone. The addition of cross linkers such as TEGDA in varying mole percents (mol%) produces changes in void volume, porosity and tortuosity within the gel and thus affects its mechanical, transport and electrical characteristics. Mabilleau et al. (2006) showed a correlation between crosslink density and mechanical stiffness in hydrated hydrogels. Boztas and Guiseppi-Elie (2009) demonstrated a reduction in

* Corresponding author at: Center for Bioelectronics, Biosensors and Biochips (C3B), Advanced Materials Research Center, Clemson University, 100 Technology Drive, Anderson, SC 29625, USA. Tel.: +1 864 656 1712; fax: +1 864 656 1713.
E-mail address: guiseppi@clemson.edu (A. Guiseppi-Elie).

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the diffusion coefficient of the redox mediator ferrocene monocarboxylic acid with increased cross link density. Justin et al. (2009) demonstrated the use of a pH-responsive hydrogel coatings for an impedimetric biomedical sensor for monitoring of tissue acidosis pursuant to hyperlactatemia during hemorrhage and hemorrhagic shock. Hydrogels also show great promise as the immobilizing membrane for the bioreceptors of various enzyme-based biotransducers (Wang, 2008). These bioanalytical systems are defined by a combination of four key components: sample presentation, biotransduction, instrumentation, and actionable data output. Hydrogels provide the highly hydrated milieu of the biotransducer that supports stable bioreceptor bioactivity and results in signals that are proportional to the activity of the analyte. Hydrogels have the capability to support bioimmobilization through the attachment of biomolecules within its 3D structure while conferring biospecificity to the biotransducer.

In the design and development of implantable (intravenous or intramuscular), pH-responsive biomedical sensors to allow for the continuous monitoring of acidosis during trauma induced hemorrhage (Guiseppi-Elie, 2010), we seek stable, pH-responsive polymers that are sensitive to small changes in pH. Small amplitude AC electrical impedance (Barsoukov and Macdonald, 2005; Yang and Guiseppi-Elie, 2008) has been shown to be an appropriately sensitive technique for the capture of the pH-response of pH-responsive hydrogels (Sheppard et al., 1995; Guiseppi-Elie et al., 1998). This paper reports on the electrical impedance properties of hydrated poly(HEMA-*co*-PEGMA-*co*-HMMA) hydrogels in Tris buffer. Hydrogel precursor mixtures of reactive monomers possessing varying cross-linker concentration were cast into micro-molds that resulted in discrete gel pads that sat on chemically modified gold microelectrodes of eight-well-one-electrode (8W1E) Electric Cell Substrate Impedance Sensing (ECIS) Cultureware™ (Rahman et al., 2009). Here we report the first use of such cultureware in the study of the impedance properties of cellular mimetic hydrogels. The hydrogel gel pads were studied by frequency dependent 3-electrode electrochemical impedance spectroscopy (1 mHz to 100 kHz; 50 mV p-t-p) and by temporal 2-electrode impedimetry at 64 kHz a function of the cross-link density (1, 3, 5, 7, 9 and 12 M% cross-linker) and as a function of temperature (10, 15, 20, 25, 30, 35, 40 and 45 °C) in physiologically relevant buffer media [0.1 M Tris/0.1 M KCl (pH 7.4)].

2. Experimental

2.1. Materials

Cysteamine, (3-aminopropyl)trimethoxysilane (γ-APS, 97%), 3-mercapto-1-propanol (95%), 2-hydroxyelthyl methacrylate (HEMA), N-[tris(hydroxymethyl)methyl] acrylamide (HMMA, 93%), poly(2-hydroxyethyl methacrylate) [p(HEMA)] (viscosity modifier), tetraethylene glycol diacrylate (TEGDA) (crosslinker), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%) (photoinitiator), and ethylene glycol were obtained from Sigma–Aldrich Chemical Co. (Milwaukee, WI). Poly(ethylene glycol) (200) monomethacrylate (PEGMA) was obtained from Polysciences, Inc. (Warrington, PA). Acryloyl (polyethylene glycol)(110) N-hydroxy succinamide ester (acryloyl-PEG(110)-NHS) was obtained from Nektar Therapeutics (Huntsville, AL). All other reagents used were of analytical grade and obtained from Sigma Chemical Co. Impedance analysis was performed using 8W1E chips (Fig. 1) acquired from Applied Biophysics (Troy, New York). The 8W1E chip possess a 250 μm diameter exposed gold electrode and a 100 cm^2 common counter electrode at the bottom of each well of an 8-well cell culture dish. A solution of tris(hydroxymethyl)aminomethane (Tris, ACS reagent, 99.8+%,) (0.1 M) and potassium chloride (KCl) (0.1 M) was prepared and pH-adjusted with hydrochloric acid (ACS reagent, 37%) to obtain 0.1 M buffer with pH 7.4. Phosphate buffered saline (PBS) (0.01 M, pH7.4) was purchased from Fisher (Fair Lawn, NJ) and used as received. All solutions were prepared with deionized (DI) water (MilliQ).

Table 1
Monomer and pre-polymer constituents and their composition within the hydrogel cocktail. Here α is the variable mol% TEGDA which varies from 1 to 12 mol% and * represents mol% of repeat units for the designated pre-polymers PEGMA and poly(HEMA).

Components	Poly(HEMA) composition (mol%)
HEMA	86.0–α
TEGDA	α
PEGMA	5.0*
HMMA	5.0
Poly(HEMA)	2.0*
DMPA	1.0
Ethylene glycol	10% (wt/wt)
Water	10% (wt/wt)

2.2. Formulation and fabrication of p(HEMA)-based hydrogel gel pads

Surface chemical modification and derivatization of the exposed 8W1E gold electrodes and surrounding Novolac® resin was imperative in order to chemically bond the pH-responsive p(HEMA)-based hydrogels. Chips were rinsed with 50:50 (v/v) DI water:isopropanol and a solution of 0.01 M cysteamine prepared in 0.01 M PBS at a pH of 8.5 was pipetted into each well and incubated for 1 h. Subsequently, the Novolac® resin was modified using a freshly prepared solution of 0.1 wt% 3-aminopropyl-trimethoxysilane (γ-APS) in 10% ethanol. The primary amines of the cysteamine and γ-APS were derivatized by reaction with 10^{-3} M acryloyl-PEG(110)-NHS in 0.1 M PBS. The acryloyl-PEG(110)-NHS solution adds a distal acrylate functional group to the end of a polyether chain via coupling to the amine groups on the functionalized gold/Novolac® surfaces. After an hour at room temperature in the clean room, the solution was removed and the wells rinsed out with DI water and blown dry with N_2. Gel pad isolators and hydrogel cocktails were immediately applied to the chemically modified and derivatized 8W1E surfaces.

Hydrogel gel pads were fashioned from monomer cocktails prepared by mixing HEMA, TEGDA, PEG(200)MA, HMMA, p(HEMA) and DMPA in a typical mol% ratio of $86-\alpha$: α: 5*:5:2*:2 where α is the variable mol% TEGDA which varied from 1 mol% to 12 mol% and where (*) indicates that the pre-polymers PEG(200)MA and p(HEMA) are represented in the formulation by their mol% of repeat units as shown in Table 1. The six different monomer formulations representing cross-link densities of α = 1, 3, 5, 7, 9 and 12 M% TEGDA were prepared and produce polymers schematically illustrated in Scheme 1. Prior to formulation, each of the individual acrylate or methacrylate containing reagents was passed over an inhibitor removal column (Sigma–Aldrich) to remove the polymerization inhibitors hydroquinone and monomethyl ether hydroquinone. The mixture was then added to a 1:1 (v/v) solution of ethylene glycol:water so that the mixed solvent comprised 20 wt% of the final cocktail. Under UV-free conditions, the final hydrogel cocktail was sonicated (1.0 min), sparged with nitrogen gas (30 s) and 9.0 μL of the hydrogel cocktail was applied evenly to the surface of chemically modified and derivatized 8W1E microelectrodes. The hydrogel cocktails were restricted to the area immediately above the 250 μm gold electrode using 2.5 mm ID × 2.0 mm (deep) (volume of 9.82 μL) cylindrical silicone isolators fabricated from press-to-seal silicone sheets (JTR24R-2.5, item number 664208; Grace-Bio Labs, Bend, Oregon). A hole puncher (4.6 mm ID), cleaned