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Molecularly engineered p(HEMA)-based hydrogels for implant biochip biocompatibility

Sheena Abraham[a], Sean Brahim[a], Kazuhiko Ishihara[d], Anthony Guiseppi-Elie[a,b,c,*]

[a]*Center for Bioelectronics, Biosensors and Biochips (C3B), Virginia Commonwealth University, P.O. Box 843038, 601 West Main Street, Richmond, Virginia 23284-3038, USA*
[b]*Department of Emergency Medicine, Virginia Commonwealth University, Richmond, Virginia 23284-3038, USA*
[c]*Department of Chemical Engineering, Virginia Commonwealth University, Richmond, Virginia 23284-3038, USA*
[d]*Department of Materials Engineering, School of Engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*

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Abstract

The strategy of phospholipid-based biomimicry has been used to molecularly engineer poly(2-hydroxyethyl methacrylate) [p(HEMA)]-based hydrogels for improved in vitro and potential in vivo biocompatibility. Two methacrylate-based monomers, poly(ethylene glycol) (200) monomethacrylate (PEGMA) and 2-methacryloyloxyethyl phosphorylcholine (MPC), were incorporated at varying mole fractions of 0.0–0.5 mol% PEGMA and 0–10 mol% MPC respectively, into 3 mol% tetraethyleneglycol diacrylate (TEGDA) cross-linked p(HEMA) networks. Upon hydration of these engineered hydrogels, a reduction in receding contact angle from $22 \pm 1.2^\circ$ for p(HEMA) to $8 \pm 2.7^\circ$ for p(HEMA) containing 0.5:10 mol% PEGMA:MPC was observed, reflecting the significant increase in surface hydrophilicity with increasing PEGMA and MPC content upon prolonged hydration. Hydrogels containing MPC showed a temporal increase in hydrophilicity following continuous immersion in DI water over 5 days. Hydrogels containing 0.5 mol% PEGMA and MPC in the range of 5–10 mol% displayed reduced protein adsorption when incubated with the common extracellular matrix proteins; fibronectin, collagen or laminin, producing up to 64% less protein adsorption compared to p(HEMA). Compositional optima for cell viability and proliferation established from two-factor Central Composite design analysis of human muscle fibroblasts cultured on these hydrogels suggest that those containing PEGMA between 0.3 and 0.5 mol% and MPC levels around 5–10 mol% exhibit desirable characteristics for implant material coatings—high viability (>80%) with low proliferation (<40%), confirming a lack of cytotoxicity.

Keywords: p(HEMA) hydrogels; Biocompatibility; Phosphorylcholine; Polyethylene glycol; Protein absorption; Human muscle fibroblasts; Wettability

1. Introduction

Cross-linked hydrogels are hydrophilic polymer networks capable of imbibing large amounts of water yet remain insoluble and preserve their three-dimensional shape. In general, such hydrogels possess good biocompatibility [1–3], as evidenced by their prolific use in a wide variety of biomedical applications such as ophthalmic and vascular prostheses [4,5], drug delivery systems [6,7], soft tissue replacement [8], and scaffolds [9]. Cells and proteins generally display a low tendency for adhesion to hydrogel surfaces because of the low interfacial free energy of the hydrogels when in contact with body fluids [3]. One of the most extensively studied hydrogels in biomedical applications is poly(2-hydroxyethyl methacrylate), [p(HEMA)], a thermoset that is

*Corresponding author. Center for Bioelectronics, Biosensors and Biochips (C3B), Virginia Commonwealth University, P.O. Box 843038, 601 West Main Street, Richmond, Virginia 23284-3038, USA. Tel.: +1 804 827 7016; fax: +1 804 827 7029.
E-mail address: guiseppi@vcu.edu (A. Guiseppi-Elie).

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not enzymatically degraded or hydrolysed by acidic or alkaline solutions [1]. Numerous studies have been conducted to modify p(HEMA) with the aim of improving its mechanical properties [10–12], its electro-responsive properties [13] and to elicit better physiologic responses [14].

Inspired by the structural biology of cellular components and sub-components, a wide range of novel biomaterials that are based on functional mimicking of naturally occurring structures have emerged [15]. This strategy, called biomimicry, aims to design molecular architectures and materials topologies that are capable of defeating the body's multiple defenses that begin with the inflammatory cascade. In this sense, such materials are biologically inspired smart or "bio-inspired" materials. Among the many possible implementations of this strategy is the inclusion or grafting of phosphorylcholine (PC) containing moieties [16,17] and polyethylene glycol (PEG) chains [18,19] pendant to the backbone of already non-cytotoxic materials such as p(HEMA) [20,21]. Phosphorylcholine is found on the extracellular surface as the terminal or head group of phosphatidylcholine and sphingomyelin [22] that comprise the outer leaflet of the lipid bilayer that forms the matrix of the plasma membrane. The in-vivo biocompatibility of polymers containing such synthetic phospholipids has been previously confirmed [23]. Similarly, synthetic PEG or its high molecular weight equivalent, poly(ethylene oxide), has been widely studied and is commonly used to confer protein-resistant characteristics to surfaces [24–29], as well as to provide a matrix for protein/biomolecule stabilization and hence promote long-term bioactivity [30] of immobilized enzymes. Because of the initiating role of protein adsorption in the inflammatory cascade, it is believed that structures that are resistant to protein adsorption may likewise make good bio-inspired in vivo candidates.

The present study focuses on the combined role of PEG and MPC in influencing the in-vitro biocompatibility of cross-linked p(HEMA) hydrogels. Cross-linked p(HEMA) of varying composition was prepared to contain 0.0–0.5 mol% PEGMA and 0–10 mol% MPC within 3 mol% TEGDA cross-linked p(HEMA) networks. The polymers were studied for their in vitro biocompatibility. In vitro biocompatibility was assessed from cell viability and cell proliferation studies performed using cultured muscle fibroblasts. The morphology adopted by the cells and their infiltration of the hydrated hydrogel were of particular note. The hydrated polymers were also studied by confocal scanning fluorescence microscopy for their ability to mitigate the adsorption of fluorescently labeled common extracellular matrix proteins, namely, fibronectin, collagen, and laminin. Additionally, polymers were cast and cross-linked onto glass microscope cover slips and were studied by dynamic contact angle measurements from which the advancing and receding contact angles were correlated with protein adsorption and cell viability and proliferation. To achieve the foregoing, glass surfaces had to be modified and functionalized to accommodate long-term immersion and the swelling and deswelling responses of the supported hydrogels.

Hydrogels of PEG content of 0.5 mol% and MPC content of 5–10 mol% exhibit the desirable characteristics for implant material coatings, namely, high viability ($>80\%$) with low proliferation ($<40\%$), confirming a lack of cytotoxicity and resistance to fibroblast proliferation. In vivo biocompatibility studies on these materials are currently in progress.

2. Materials and methods

2.1. Materials

γ-Aminopropyl trimethoxysilane (γ-APS, 97%), poly(2-hydroxyethyl methacrylate) [p(HEMA)], tetraethyleneglycol diacrylate (TEGDA) and flourescein isothiocyanate (FITC) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Poly(ethylene glycol) (200) monomethacrylate (PEGMA) was obtained from Polysciences, Inc., (Warrington, PA). 2-Methacryloyloxyethyl phosphorylcholine (MPC) monomer was synthesized as previously described elsewhere [31]. Acryloyl (polyethylene glycol)$_{110}$ *N*-hydroxy succinamide ester (Acryl-PEG-NHS) was obtained from Nektar Therapeutics, (Huntsville, AL). Photo-initiator 2, 2-dimethoxy-2-phenyl acetophenone (99%, MW ca. 256.30) was obtained from Sigma Chemical Co. (St. Louis, MO). Human muscle fibroblasts (CRL-2061) were purchased from American Type Culture Collection, (Manassas, VA). Culture medium, RPMI 1640 (Cellgro®, Mediatech, Herndon, VA) was supplemented with 10% Fetal Bovine Serum (FBS) (Sigma Chemical Co.). Murine fibronectin (lyophilized powder), murine collagen (Type IV (Miller) powder cell culture tested) and murine laminin (in Tris buffered NaCl), used in the protein adsorption study were also obtained from Sigma Chemical Co. All other reagents used were of analytical grade and obtained from Sigma Chemical Co.

2.2. Surface modification and functionalization

Microscope glass slides (5×2 cm dimensions) and microscope glass cover slips (2×2 cm dimensions) were cleaned for 3 min each in boiling trichloroethylene, boiling acetone, then ultrasonically washed in isopropyl alcohol for 1 min followed by rinsing profusely with DI water. Commercially available sterile 8-well cell culture plates (Nalgene NUNC 155383) were used as received without further cleaning. Cleaned slides and cover slips and the glass surfaces of the culture plates were each