

# Surface elasticity and charge concentration-dependent endothelial cell attachment to copolymer polyelectrolyte hydrogel

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## Abstract

The surface micromechanical properties of 2-hydroxyethyl methacrylate (HEMA) and 2-methacryloxyethyl trimethyl ammonium chloride (MAETAC) copolymer hydrogels are probed using atomic force microscopy. HEMA–MAETAC polyelectrolyte hydrogels with increasing positive charge concentrations ranging from 0 to 400 mM in increments of 40 mM, are fabricated using different proportions of HEMA and MAETAC monomers. Increasing proportions of positively charged MAETAC monomers produce hydrogels with increasingly swollen states and correspondingly decreasing measures of stiffness, or Young's modulus. Increasing the relative proportion of charged monomers also increases the hysteresis in the approaching and retracting components of the force spectroscopy curves. When these hydrogels are equilibrated in cell-culture media without fetal bovine serum and a pH-controlled CO<sub>2</sub> environment, precipitation reactions increase the variability of the Young's modulus estimates. Adding a buffer, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, maintains physiological pH without the use of a CO<sub>2</sub> environment, and thus reduces salt precipitation reactions and the variability of the Young's modulus. The attachment of porcine pulmonary artery endothelial cells increases with increasing prepared hydrogel charge concentration and decreasing elasticity.

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## 1. Introduction

The development of atomic force microscopy (AFM) [1] and the subsequent development of atomic force spectroscopy (AFS) [2] have made it possible to probe the surface and mechanical properties of materials at a submicron scale. This is potentially significant because the surface micromechanical properties of natural and synthetic hydrogels play a critical role in their use as biomaterials. As a result, the application of AFM technology to soft hydrogel biomaterials has a number of important applications in medicine and biomedical engineering.

Hydrogel substrate compliance has been identified as one of the key physical factors in the response of many cell types [3]. For example, both fibroblast cell locomotion and

focal adhesions have been shown to be regulated by substrate compliance [4]. Aortic smooth muscle cells have been found to spread and organize their cytoskeletons and focal adhesions to a greater extent on stiff gels than on soft gels [5]. In addition, vascular smooth-muscle-cell migration has been shown to be controlled and directed by gradients in substrate compliance [6]. Further, morphotype and the expression of the focal adhesion plaque proteins of endothelial cells are also modulated by substrate elasticity [7]. Even native mesenchymal stem cells have been shown to specify lineage and commit to phenotypes with extreme sensitivity according to substrate elasticity [8]. Thus, the surface mechanical properties of synthetic hydrogels are emerging as an important factor in the adhesion and biological response of cells that are in contact with these materials.

The mechanical properties of synthetic hydrogels reflect the complicated combination of the neutral and charged

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monomer concentrations, the solvent quality, the chemical dissociation effects and many other factors [9–16]. These competing and nonlinear effects make it challenging to predict and understand the mechanical properties of these materials. In particular, the incorporation of charged monomers into an otherwise neutral and inert background has been exploited in a number of studies to promote cell adhesion and proliferation [17–20].

Atomic force microscopy is becoming an important tool for testing these complex materials, having been used both to image the surface nanotopography and to measure the surface elasticity of hydrogels in liquid solution [21–26]. The vast majority of studies that have used AFM to probe hydrogels and other biomaterials, however, have only considered isolated and random samples. By fabricating a sequence of hydrogels with systematically varying charged monomer compositions, the mechanical phase states can be more easily appreciated and understood.

This study examines the surface elasticity and charge effects on endothelial cell seeding of a sequence copolymer polyelectrolyte hydrogels. A neutral monomer, 2-hydroxyethyl methacrylate (HEMA), was copolymerized with varying proportions of the basic monomer, 2-methacryloxyethyl trimethyl ammonium chloride (MAETAC). Hydrogels were prepared with increasing positive charge concentrations, ranging from 0 to 400 mM. The equilibrium swelling behavior was measured as a function of the monomer composition or the equivalently positive charge concentration, and was compared with AFM measurements of the surface elasticity. The effect of these parameters on endothelial cell attachment was demonstrated by seeding these hydrogels with porcine pulmonary artery endothelial cells (PPAECs).

## 2. Materials and methods

### 2.1. Hydrogel preparation

A series of copolymer hydrogels, with increasing proportions of positively charged monomers, was synthesized using the neutral monomer HEMA and the basic monomer MAETAC. Specifically, 1.54 M HEMA (Polysciences) and 1.14 M HEMA/0.4 M MAETAC (Polysciences) monomer stock solutions containing 40% ethylene glycol (solvent) and 20.2 mM ethyleneglycol dimethacrylate (Aldrich) were initially prepared. By mixing relative proportions of the neutral HEMA and basic MAETAC stock solutions together, pre-gel solutions containing a total 1.54 M monomer concentration, but with MAETAC concentrations ranging from 0 to 400 mM, were prepared at 40 mM increments. Stock solutions of 4 wt.% initiator ammonium persulfate (APS) (Aldrich) and 15 wt.% accelerator sodium metabisulfite (SMBS) (Aldrich) were also prepared. To start the polymerization reaction, 200  $\mu$ l of the 4 wt.% APS initiator stock solutions and 200  $\mu$ l of the 15 wt.% SMBS accelerator stock solutions were added to 10 ml of the pre-gel monomer mixtures. After the monomer

solutions were mixed, they were filtered with a 0.2  $\mu$ m filter and injected between two glass plates separated by a 400  $\mu$ m Teflon spacer and allowed to polymerize for 24 h at room temperature. After polymerization, the copolymer hydrogel membranes were removed from their molds and allowed to equilibrate in 154 mM NaCl solutions for several days. The NaCl bath solutions were replaced everyday to ensure equilibration.

### 2.2. Hydrogel shrinking/swelling experimental measurements

After polymerization and washing, the copolymer hydrogels were cut into circular discs for use in the AFM measurements. For the measurements of hydrogel equilibrium swelling behavior measurements, the hydrogels polymerized in their molds were cut out using a cork borer with 16.46 mm inner diameter,  $D_0$ , and washed in a 154 mM NaCl solution for several days. The shrinking/swelling behavior was recorded by measuring the hydrogel disc diameters,  $D$ , using an electronic digital caliper. The relative shrinking/swelling ratio,  $(D - D_0)/D_0$ , was evaluated after equilibration in solution relative to the inner diameter,  $D_0$ , of the borer that was used to cut the hydrogel.

### 2.3. AFM measurements

Surface imaging and force spectroscopy measurements were performed using an MFP-3D-BIO™ atomic force microscope (Asylum Research Inc., Santa Barbara, CA). All non-contact mode surface imaging was performed with an Olympus BioLever A cantilever (Olympus, Japan) in 154 mM NaCl solution. The scanning area was  $10 \times 10 \mu$ m. In order to provide a physiological temperature environment, a closed fluid cell was used with the BioHeater™ System (Asylum Research Inc., Santa Barbara, CA). Force spectroscopy measurements were performed with a 6  $\mu$ m polystyrene microsphere affixed to a V-shaped silicon nitride cantilever (Bioforce Nanoscience, Ames, IA), which allowed force–displacement curve measurements under indentation without puncturing the soft gel surface. The nominal spring constant of the cantilever was  $0.06 \text{ N m}^{-1}$ . All measurements were made at  $37 \pm 0.1 \text{ }^\circ\text{C}$ .

The position-sensitive detector signal of the AFM was calibrated by taking the slope of the deflection voltage vs. the  $z$  displacement curve measured using a reference glass substrate surface in saline solution. Ten force–displacement curve measurements at each of five different randomly selected locations were acquired with a probing speed of  $2 \mu\text{m s}^{-1}$  and an approach–retraction frequency in the ranging of 0.2 and 1 Hz. The indentation depth,  $\delta$ , was controlled in the range of 60–600 nm, by changing the trigger voltage of the force–displacement curve measurements in order to satisfy the basic assumption of the spherical Hertz model [24,26]. It will be recalled that the slope of a force–displacement curve describes the elastic properties of a sample qualitatively. The more elastic a sample, the steeper the slope of the force–displacement

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