Resistance of Self-interpenetrating Poly(Sulfobetaine) Hydrogels to Fibrinogen Adsorption and Platelet Adhesion

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Statement of Purpose: In our previous studies, sulfobetaine methacrylate (SBMA) was grafted onto gold surfaces covered with atom transfer radical polymerization (ATRP) initiators. The surface was shown to be highly resistant to protein adsorption and cell adhesion[1]. In this study, hydrogels based on poly(SBMA) were prepared and their protein adsorption and platelet adhesion were compared to poly(HEMA) hydrogels. A second network of poly(SBMA) was introduced to increase the mechanical properties of the hydrogels.

Methods: The hydrogels were prepared by adding SBMA or HEMA monomers into tetraethylene glycol dimethacrylate (TEGDMA). Free radical polymerization was initiated by sodium metabisulfite and ammonium persulfate in a mixed solution. The reaction was carried out between a pair of glass substrates, separated with a PTFE spacer of 0.4 mm at 37ºC for 12 h. The second network was introduced using SBMA with TEGDMA as a cross-linker and potassium persulfate as an initiator. The hydrogels were immersed in the solution for 24 hours, and then put into a pair of glass substrates at 60ºC for 6 h. After polymerization, the hydrogels were immersed in a large amount of DI water for one week and the water was changed every day to remove residual chemicals. The gel was then equilibrated in sterilized PBS solution, which was changed every day for another week. The hydrogels were punched into disks with a diameter of 10 mm and stored in sterilized buffer solution until use.

An enzyme-linked immunosorbent assay (ELISA) was used to measure protein adsorption. The hydrogel disks were put into a 24-well plate and 0.5 mL of 1mg/mL fibrinogen (Fg) from human plasma was added to the disks at 37°C for 90 min. Next a BSA solution (1mg/mL, 0.5 mL) was added to block non-specific binding to the surface for 90 min. Anti-human Fg was used at a concentration of 5.5 µg/mL to measure the amount of adsorbed Fg. The disks were incubated with anti-human Fg (HRP, 1mg/mL, 0.5 mL) and reacted with chromogenic substrate o-phenylenediamine. The optical density was determined with a microplate reader.

Whole blood was isolated from fresh blood of healthy human donors by centrifugation. Platelets were suspended into platelet suspension buffer with a concentration of $5x10^7$ platelets/mL. Poly(HEMA) and poly(SBMA) hydrogel disks (diameter 1 cm) were pretreated with 10% plasma (24-well plate, 500 µl/ well, incubated at 37 °C for 1.5 h), then blocked with 4 mg/ml BSA at 37°C for 1.5 h (800µl/well). Poly(HEMA) and poly(SBMA) hydrogels were incubated in platelet solution in a 24-well polystyrene plate for 1 h at 37°C. The adherent platelet number was determined by a lactate dehydrogenase (LDH) assay kit after lysis with 1% Triton X-100 for 30 min[2].

Results / Discussion: Four transparent hydrogels, poly(SBMA), poly (HEMA), poly(SBMA) penetrated with a poly(SBMA) network, and poly(HEMA) penetrated with a poly(SBMA) network were prepared. The first poly(SBMA) hydrogel has little mechanical strength and was easily broken while handling. After the second poly(SBMA) network was introduced, the mechanical strength and toughness of the hydrogel was increased.

Figure 1 compares Fg adsorption on the four hydrogels. Fg adsorption on the poly(SBMA) hydrogel is less than that on the poly(HEMA) hydrogel. The penetration of a second poly(SBMA) network into both poly(SBMA) and poly(HEMA) gels does not affect the Fg adsorption. Furthermore, results in Figure 2 show that the poly(SBMA) hydrogel is better than the poly(HEMA) for resisting platelet adhesion.

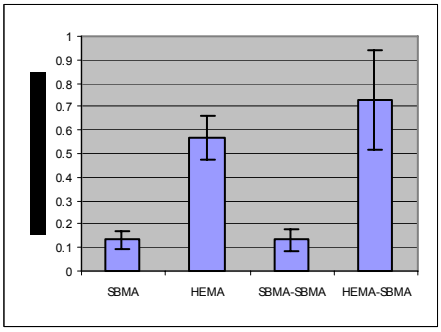

Figure 1. Fg adsorption on four different hydrogels.

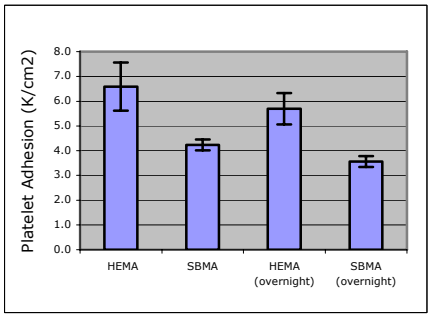

Figure 2. Static platelet adhesion on poly(HEMA) and poly(SBMA) hydrogels. After cell adhesion, these samples were soaked in PBS buffer for two hours or overnight before measurements were taken.

Conclusions: Poly(SBMA) hydrogels have less Fg adsorption and platelet adhesion than poly(HEMA) hydrogels. The introduction of a second network of poly(SBMA) increases the mechanical properties of the poly(SBMA) hydrogel while maintaining its protein-resistant properties.

References: 1. Zhang, Z.; Chen, S.; Chang, Y.; Jiang, S., 230th ACS National Meeting, Washington, DC, 2005. 2. Kwak, D.; Wu, Y.; Horbett, T. A., J Biomed Mater Res 74A, 69–83, 2005.