Nonfouling and Functionalizable Hydrogels based on Polyampholyte Chemistries

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Statement of Purpose: There is a significant interest in tissue engineering for polymeric scaffolds that can be controlled to have the desired properties for a wide range of tissue types. Properties that are essential to control include the physical properties, resistance to nonspecific protein adsorption, and incorporation of tissue specific biological cues. However, there are few materials that present all three of these features. Our lab has recently demonstrated that polyampholyte polymer brush coatings have both a resistance to nonspecific protein adsorption and protein conjugation capacities.[1] Polyampholyte materials are made from mixtures of positively charged and negatively charged monomer subunits. In this work, we demonstrate multi-functional hydrogel materials based on this approach. It is also possible to tailor the physical properties of the hydrogels based on monomer selection criteria. These materials present a unique platform for tissue engineering applications.

Methods: Hydrogels were formed from mixtures of positively charged [2-(acryloyloxy) ethyl] trimethyl ammonium chloride (TMA) and negatively charged 2-carboxy ethyl acrylate (CAA) monomers using a range of triethylene glycol dimethacrylate (TEGDMA) cross-linker concentrations. The nonfouling properties were demonstrated with fibrinogen (FBG) and lysozyme (LYZ) using enzyme linked immunosorbent assays (ELISA) with antibodies specific to each protein. FBG conjugation to the TMA:CAA hydrogels was also completed using EDC/NHS conjugation chemistry and confirmed using ELISA. The biocompatibility of these hydrogels in the presence and absence of conjugated FBG were confirmed using MC3T3-E1 cell adhesion assays.

Results: Nonspecific protein adsorption to TMA:CAA hydrogel substrates with varying concentrations of TEGDMA cross-linker densities were determined for both FBG and LYZ using ELISA. The results, shown in Figure 1, show that the amount of nonspecific protein adsorption from both proteins is less than 10% of that seen on the positive fouling control, tissue culture polystyrene. Additionally, there are no differences in the amount of nonspecific protein adsorption between the TMA:CAA samples and a known nonfouling control composed of sulfobetaine methacrylate (SBMA). The results suggest that the TMA:CAA hydrogels are nonfouling.

The ability of TMA:CAA hydrogels to covalently attach proteins using EDC/NHS conjugation chemistry was also investigated and the results are demonstrated in Figure 2. In this Figure, it can be seen that the activated TMA:CAA hydrogels have a greater than three times level of FBG present as compared to the nonfouling (not activated) control. The inset images in Figure 2 also show the drastic differences in the amount of protein present on these substrates. The sample with conjugated protein (activated) is completely coated with protein, while the control sample only has protein on the outer edges. The nonfouling control has no visible adsorption.

Biocompatibility assays have been conducted both in the presence and absence of conjugated proteins. The results show an improvement in the level of adherent cells in the presence of conjugated FBG. Finally, the physical properties of the TMA:CAA hydrogels were quantified using standard techniques. The results of these characterizations will be presented and compared to another polyampholyte hydrogel chemistry to demonstrate that it is possible to tailor additional material properties based on monomer selection criteria.[2]

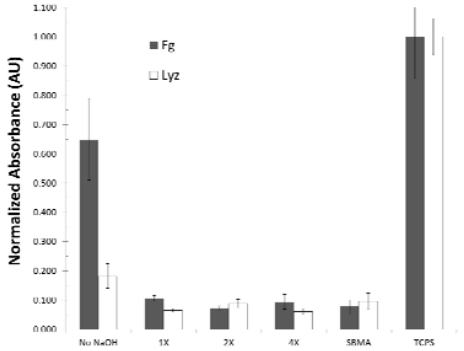

Figure 1. Mean ± error of the nonspecific protein adsorption to TMA:CAA hydrogels. The results are presented as the absorbance following 30 minutes of exposure and normalized to the results for TCPS.

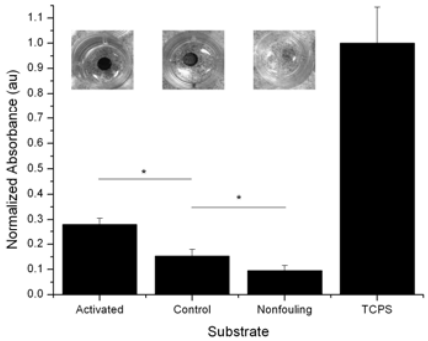

Figure 2. Mean ± error of the FBG conjugation levels on TMA:CAA hydrogels. The results are presented as the absorbance measured following 30 minutes of exposure and normalized to the results for TCPS.

Conclusions: The results of this study clearly demonstrate that polyampholyte hydrogels have resistance to nonspecific protein adsorption. Furthermore, by selecting different monomer subunits it is possible to tailor additional hydrogel properties and provide protein conjugation capacity. The hydrogels are biocompatible and represent a promising platform for tissue engineering. Our on-going work is focused on controlling the location of conjugated proteins within the TMA:CAA hydrogels.

References: 1. Tah T et al. Col Surf B. 2012: 93: 195-201. 2. Dobbins, SC et al. JPCB. Accepted 2012.