Poly(ethylene glycol) nanogel coated surfaces display ultralow protein adsorption: Quantification by single molecule detection

Casey D. Donahoe, Lee A. Tessler, Young-Shin Jun, Donald L. Elbert, Robi D. Mitra.
Washington University in St. Louis, St. Louis, MO, USA.

Statement of Purpose: The development of surfaces that resist protein adsorption has been a challenging obstacle to the advancement of biomedical technologies. Both medical implants and bioanalytical assays require extremely low levels of protein adsorption to achieve satisfactory results. Poly(ethylene glycol) (PEG) has shown great potential for protein resistant coatings and efforts have aimed at reacting PEG with surfaces in a manner which maximizes its surface density. We previously developed a novel nanogel coating (~75nm) composed of multi-arm PEG partially crosslinked with bovine serum albumin (BSA). Initial characterization with standard protein adsorption assays showed virtually no protein adsorption.[1] However, these and other standard assays (OWLS, QCM-D, radiolabeling, surface plasmon resonance, etc.) are not sufficiently sensitive to quantify differences in protein adsorption between ultralow adsorbing surfaces. Here, we use single molecule detection (SMD) via total internal reflection microscopy (TIRF) to quantify non-specific protein adsorption to coatings that adsorb ultralow levels of protein. Resistance to cell adhesion corroborated the results seen with SMD. The nanogel coatings provided durable and ultralow resistance to protein adsorption (lower than BSA monolayers) and are especially suitable for applications that require extremely thin coatings of nanoscale thickness. SMD proved to be a powerful method in quantifying the otherwise indistinguishably low amounts of protein adsorption (picograms per cm^2) to these surfaces.

Methods: Crosslinked nanogel solutions were prepared by incubating eight arm PEG-octa-vinyl sulfone (PEG_8-VS) with BSA in PBS, pH 7.4 at 37°C until a d_{PCS} of 100 nm was reached as measured by dynamic light scattering (DLS). Diluted nanogel solutions were then reacted with either mercaptosilanated or epoxysilanated glass for 1 hr at 37°C. Unreacted vinyl sulfone groups and epoxides were capped by 37°C incubations with 50 mg/mL BSA overnight followed by 1 M Tris, pH 8.0 in PBS for 10 min. SMD was performed over 140 µm x 100 µm areas using an inverted microscope fitted for TIRF with a 640 nm, 40 mW laser for fluorescent excitation of Cy5 dyes. Surfaces examined by SMD were prepared in flow cells and loaded with 1 mL of 100 ng/mL Cy5 labeled polyclonal goat IgG for 25 min at room temperature. For some experiments, surfaces were subsequently exposed to 0.1% sodium dodecyl sulfate (SDS) for 5 min at room temperature. The IgG loading and SDS wash were repeated a second time, with images collected after every step. Cell seeding on surfaces used 3T3 fibroblasts at $2.5x10^5$ cells/cm^2 in media containing 10% fetal bovine serum (FBS). Cells were reseeded every three days with photomicrographs taken the following day until extensive cell adhesion was observed. AFM was performed in tapping mode on samples covered with PBS.

Results: SMD results are shown in Figure 1. Protein adsorption to thiol-coupled nanogel coatings were found to be 2-fold less than to PEG_8-VS alone, 4-fold less than to BSA alone, and calculated to be 1000-fold less than the limit of detection of standard protein adsorption assays. AFM of the nanogel coated surfaces revealed subtle differences between thiol-coupled and epoxy-coupled surfaces. Notably, the nanogels were seen as more of a continuous matrix on the thiol-coupled surface and as more discreet units on the epoxy coupled surface. The denser coverage on the thiol-coupled surface could explain its relatively lower levels of protein adsorption. When surfaces were washed with 0.1% SDS, the nanogel coated surfaces displayed much higher resilience to the surfactant than the BSA coated surface. While protein adsorption to the BSA coated surface increased by 410% after SDS treatment and a second round of IgG loading, protein adsorption on the thiol-coupled and epoxy-coupled nanogel surfaces increased by 170% and decreased by 20%, respectively. Cell seeding results generally matched the trends shown by SMD, with both BSA and nanogel coated surfaces resisting virtually all initial cell adhesion. However, while nanogel coated surfaces were able to resist cell adhesion for over three rounds of seeding, the BSA monolayer lost what appeared to be all its resistance after only one seeding.

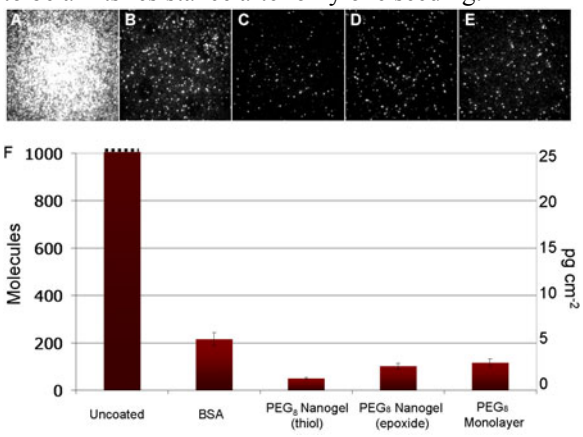

Figure 1. Single molecule detection of IgG adsorbed to (A) uncoated mercaptosilanated glass, (B) BSA coated glass, (C) thiol-coupled nanogel coated glass, (D) epoxy-coupled nanogel coated glass, and (E) PEG_8-VS coated glass. Number of molecules and surface density of adsorbed protein in the field of view are shown in (F).

Conclusions: Even at ultralow levels, protein adsorption can hinder long-term biocompatibility of implants and signal to noise ratios of bioanalytical assays. We have demonstrated the ability of SMD to distinguish ultralow levels of protein adsorption across multiple surface coatings. Thus, SMD should prove invaluable in the development of truly protein resistant biomaterials.

References:

1. Scott EA. Biomaterials. 2008;29:4481-4493.