Clickable PEG nanogel coatings compared to PEG/BSA nanogels: synergy between PEG and BSA contributes to ultralow protein adsorption as assessed by single molecule fluorescence

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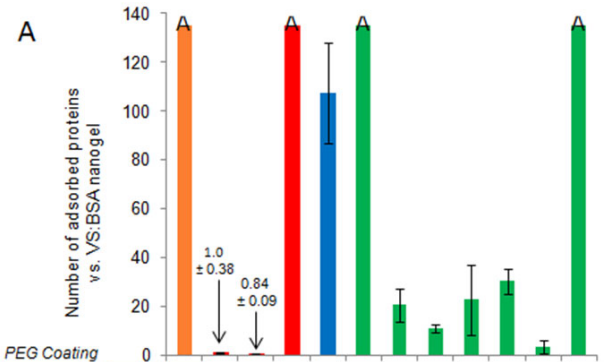
Statement of Purpose: A wide variety of hydrophilic surface coatings have been developed that strongly resist protein adsorption. Techniques such as OWLS, SPR, QCM and radiolabeling may detect low levels of protein adsorption, but generally fail to detect protein adsorption below 1 ng/cm^2. Such low levels of protein adsorption may not promote cell adhesion in the short term, but surfaces that exhibit amounts of protein adsorption that are too low to adequately measure with common techniques may fail when cultured with cells for extended periods of time or when exposed to high concentrations of serum. Cell adhesion is a useful and clinically relevant measure of biocompatibility but is difficult to standardize and may require weeks before cell adhesion commences. We have developed a method to quantify the adsorption of low amounts of adsorbed protein using single molecule fluorescence techniques.[1] Using total internal reflectance fluorescence microscopy and multiply labeled proteins, adsorption of individual protein molecules may be detected. This bottom-up approach allows the measurement of sub pg/cm^2 amounts of protein adsorption. The method is reproducible enough to distinguish between surface coatings with similar chemistries but differences in resistance to cell spreading. In this study, we compare the performance of coatings formed from clickable PEG nanogels to PEG/bovine serum albumin (BSA) nanogels, as well as the well-studied PLL-g-PEG surface coating.

Methods: All nanogel coatings were attached to mercaptosilanated glass for both cell adhesion and TIRF. Clickable nanogels were synthesized from four-arm PEG-azide and four-arm PEG-alkyne. PEG solutions were mixed at equimolar ratios of azides to alkynes and copper (II) sulfate (10 mg/mL in water) was added at 0.004 equivalents per azide/alkyne. The mixture was rotated at 40 rpm at room temperature until a z-averaged size of 100 nm was measured by dynamic light scattering (DLS). The reaction was halted by adding EDTA. Clickable nanogel solutions were mixed with the photoinitiator Irgacure-2959 0.15 mg/mL in water) and either PBS or 1.5 M sodium sulfate in PBS. Nanogel mixtures were incubated with mercaptosilanated glass surfaces and illuminated with a UV flood lamp (365 nm, 100 W) for 20 min to attach nanogels by a thiol-yne reaction. Nanogels crosslinked via Michael addition chemistry were synthesized from PEG-VS and either BSA or PEG-amine as described before and diluted to 100 mg/mL before freezing.[1] TIRF microscopy was performed as previously described with Cy-5 labeled fibrinogen.[1]

Results: PLL-g-PEG has been studied as a non-adhesive substrate in over 150 publications. Nonetheless, some stability issues have been noted and resistance to cell adhesion is not zero. However, by TIRF microscopy, protein adsorption is too great to measure. This is contrasted with measurements by OWLS that suggest protein adsorption on PLL-g-PEG coatings is nearly undetectable. Using this as a starting point, we sought to develop substrates that outperformed PLL-g-PEG by TIRF. Two competing systems were tested. One consisted of partially polymerized solutions of multiarm PEG-azide and multiarm PEG-alkyne. In the presence of copper, a gel will form in minutes to hours, depending on the copper concentration. However, if the copper is chelated prior to gelation, size exclusion chromatography revealed that >30% of the mass of the PEG was contained in molecules > 100,000 Da. These partially polymerized solutions contained some nanogels with sizes > 10-100 nm by DLS. Using a thiol-yne chemistry, the PEG nanogel-containing solutions were attached to mercaptosilanated glass. AFM confirmed that larger structures with sizes 50-100 nm decorated the surface. By TIRF, protein adsorption was too high to measure, unless the reaction was performed in the phase-separated state in a sodium sulfate solution. Doing so allowed the protein adsorption to approach, but not equal, the low amounts observed with PEG/BSA nanogels. When BSA was replaced with PEG-octaamine in these nanogels, protein adsorption was 100x higher. Thus, the all PEG systems seem to have less intrinsic resistance to protein adsorption, an observation that is supported by long-term resistance to fibroblast adhesion.



Conclusions: Nanogel coatings are ultralow protein adsorbing, but PEG/BSA systems appear to be superior to all PEG systems.

References: 1) Tessler et al., *J. R. Soc. Interface,* 2011, 8:1400