Poly(ethylene glycol)-crosslinked microgel coatings reduce macrophage adhesion to biomaterials

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Statement of Purpose: Cell-biomaterial interactions regulate host responses to implanted devices and tissue-engineered constructs [1]. Upon implantation, synthetic materials dynamically adsorb proteins and other biomolecules which trigger non-specific inflammatory responses including macrophage adhesion and activation. Macrophages play central roles in the cascade of events leading to the foreign body reaction and fibrous encapsulation. These inflammatory events adversely affect the biological performance of implanted devices. Recent efforts have focused on developing non-fouling surface treatments to prevent such non-specific protein adsorption, as well as systems for the delivery of anti-inflammatory agents, but these approaches have only marginally reduced inflammation and fibrous encapsulation. By engineering surfaces to dynamically interact with biological systems, we propose to direct macrophage recruitment and thus modulate inflammatory responses. The present work focuses on engineering robust microgel coatings that prevent macrophage adhesion and function.

Methods: Thermoresponsive poly(N-isopropylmethacrylamide) (pNIPMAm) microgel particles cross-linked with 2 mol% poly(ethylene glycol) (PEG) diacrylates of 2 different chain lengths (575, 700 MW) were synthesized via free-radical precipitation polymerization [2]. We previously demonstrated that these microgel particles have narrow size distributions (500 nm) and prevent non-specific protein adsorption [2]. Microgel particles were spin coated and covalently tethered to reference biomaterial poly(ethylene terepthalate) (PET) substrates. Coatings were evaluated by contact angle and XPS analyses.

Prior to cell culture, biomaterial samples were washed in 70% ethanol for 4 days to remove endotoxin contaminants. Murine IC-21 macrophages were maintained in RPMI 1640 supplemented with 10% FBS and antibiotics. Cells were plated on samples at 67,000 cells/cm^2 in serum-containing media and cultured for 48 hr. Adherent cells were visualized via staining with calcein-AM (live cells) and ethidium homodimer (dead cells).

Results / Discussion: Microgel-based coatings represent a unique and versatile approach to surface functionalization and modification. Advantages include precise control over synthesis parameters, incorporation of multiple orthogonal functionalities, and ability to co-assemble different microgel particles to generate complex, multi-functional coatings. Spin coating and covalent tethering of microgels to PET results in uniform coatings (**Fig. 1**).

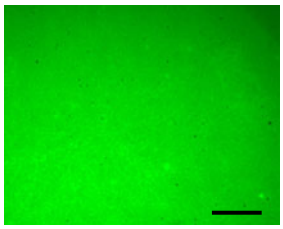

Fig. 1: Uniform deposition of microgel-based coatings on PET. Microgel particles were covalently immobilized onto PET and labeled with FITC via pendant amine groups. Scale bar 20 μm.

Macrophages adhered and spread on PET substrates (**Fig. 2**). In contrast, very few cells adhered to microgel-coated PET. The length of the PEG crosslinks had no effect on cell adhesion to these materials. No dead cells were detected on any surfaces. These results demonstrate that microgel coatings render biomaterials resistant to macrophage adhesion and spreading. Current analyses focus on IL-1β and TNF-α cytokine secretion, as well as *in vivo* leukocyte recruitment and inflammatory responses in a murine intraperitoneal implantation model.

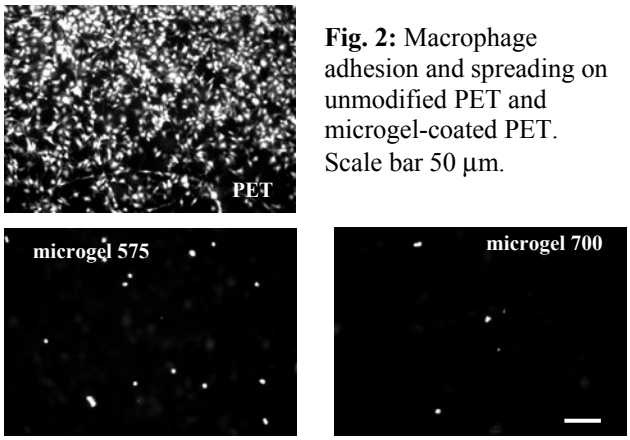

Fig. 2: Macrophage adhesion and spreading on unmodified PET and microgel-coated PET. Scale bar 50 μm.

Conclusions: Coatings of PEG-crosslinked pNIPMAm microgel particles significantly reduce macrophage adhesion and spreading on PET substrates. This technology has great potential to modulate the inflammatory response *in vivo* for biomedical and biotechnological applications.

References: [1] Anderson JM, Annu Rev Mater Res. 2001;31:81-110; [2] Nolan CM, Reyes CD, Debord JD, García AJ, Lyon LA, Biomacromolecules. 2005;6:2032-2039.

Acknowledgment: NIH R01 EB-004496, GTEC NSF ERC EEC-9731643, National Science Foundation Graduate Fellowship (AAW).