Polyethylene glycol-based Microgel Coatings Reduce Leukocyte Adhesion In Vivo

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Introduction: Cell-biomaterial interactions regulate host responses to implanted devices and tissue-engineered constructs [1]. Upon implantation, synthetic materials dynamically adsorb proteins and other biomolecules that 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 non-fouling surface treatments to prevent non-specific protein adsorption, as well as systems for the delivery of anti-inflammatory agents, but these approaches have only marginally reduced 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 leukocyte adhesion and function.

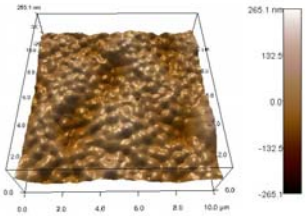
Methods: Poly(N-isopropylacrylamide) (pNIPAm) microgel particles cross-linked with 2 mol% poly(ethylene glycol) (PEG) diacrylates of 2 different chain lengths (575, 700 MW) were synthesized via free-radical precipitation polymerization [2]. We previously demonstrated that these microgel particles prevent non-specific protein adsorption [2]. Microgel particles were spin coated and covalently tethered to poly(ethylene terepthalate) (PET) substrates (8 mm dia. disks).

Biomaterial samples were washed in 70% ethanol for 4 days before use 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 hrs, and the supernatants were analyzed for cytokine secretion. In addition, samples were implanted in the intraperitoneal (IP) cavity of mice for 24 or 48 hrs to evaluate leukocyte recruitment and adhesion *in vivo* during acute inflammation. IP lavage fluid was collected and analyzed for cytokine secretion. Disks were explanted and adherent cells were stained for the macrophage marker CD68 (anti-CD68), actin (phalloidin), and DNA (Hoechst). In all experiments, unmodified PET was used as a control.

Results/Discussion: Spin coating and covalent tethering of microgels to PET resulted in uniform coatings as shown by AFM (**Fig. 1**). In addition, we have confirmed the presence of pNIPAm on the surface of PET substrates by XPS.

IC-21 macrophages adhered to and spread on PET substrates *in vitro*; in contrast, very few cells adhered to microgel-coated PET. MCP-1 cytokine levels were significantly decreased in microgel-coated samples compared to PET (**Fig. 2**).

Fig 1. Uniform deposition of PEG-based microgel coatings on PET. Scale 10μm.



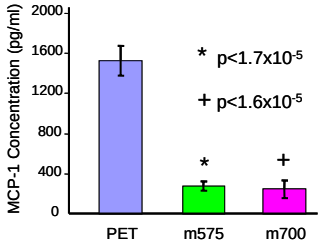


Fig 2. Relative MCP-1 levels in culture supernatant on PET (blue), microgel 575 (green), and microgel 700 (pink) determined by ELISA.

Importantly, microgel coatings also effectively reduced leukocyte adhesion and spreading to disks implanted in the intraperitoneal space (**Fig. 3**). These results demonstrate that microgel coatings render biomaterials resistant to leukocyte adhesion and spreading.

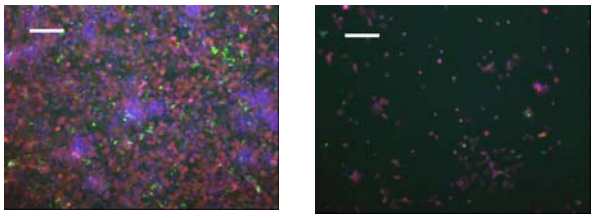


Fig 3. Leukocyte adhesion to PET (left) and microgel-coated PET (right). Explants were stained for CD68 (green), actin (red), and DNA (blue). Scale bar 100μm.

We are currently investigating the efficacy of microgel coatings *in vitro* using primary monocytes isolated from human blood. We are also evaluating the ability of microgel coatings to reduce fibrous capsule formation in a chronic inflammatory subcutaneous implantation model. In addition, we are developing microgel coatings presenting tethered bioadhesive ligands in order to provoke controlled binding of macrophages in an effort to direct their behavior.

Conclusions: Coatings of PEG-crosslinked pNIPAm microgel particles significantly reduce leukocyte adhesion on PET substrates *in vitro* and *in vivo*. This unique and versatile microgel technology has great potential to modulate the inflammatory responses in 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.

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