

Modulating the macrophage foreign body response through controlled adhesion to surfaces using fibrinogen-derived peptides

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Statement of Purpose: Host immune response to an implant affects both efficacy and performance of the device when implanted. Macrophages adhere to the implant due to the array of proteins that differentially adsorb to its surface over the course of the inflammatory response [1]. By modulating the adhesive signals presented to on a surface, we hypothesize that we can modulate macrophage cell adhesion, thereby also creating a novel system for studying macrophage activity.

Methods: Poly(*N*-isopropylacrylamide) (pNIPAm) microgel particles (100 mM total monomer concentration) were synthesized with 2 mol % poly (ethylene glycol) (PEG) diacrylate (MW 575) by a free radical precipitation polymerization method [2]. For incorporating functional groups that can be later modified, the microgel particles were synthesized with 10 mol % acrylic acid as a co-monomer. Particles were then conjugated with either RGD (GRGDSPK) or fibrinogen-derived peptide P2 (YSMKKTTMKIIPFNRLTIG) using EDC-NHS chemistry. PET discs were cut to a standard size using an 8 gauge biopsy punch. Activated PET samples were then used as a platform on which to graft the microgels. IC-21 macrophage cells were obtained from ATCC and cultured using RPMI-1640 media, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin (PS). All microgel-coated and control PETs were sterilized overnight in 70% EtOH, washed 3x in sterile PBS, and then equilibrated in PBS for at least 1 hour before seeding with cells. Each PET was seeded with 1 mL of media per 24-well, containing 40,000 IC-21 cells.

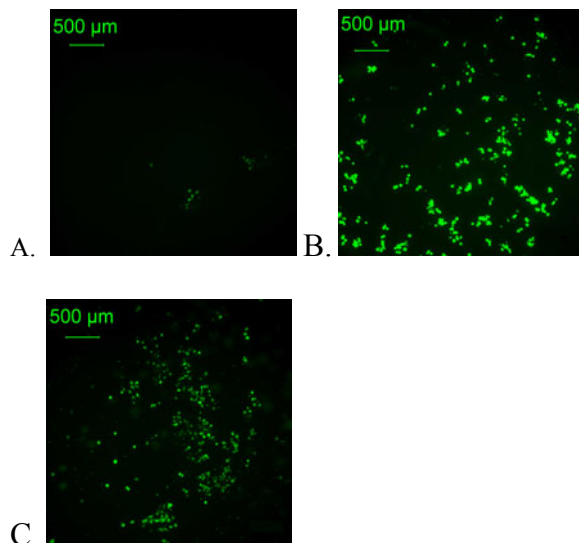


Figure 1. A. Base microgels B. Microgels with 2% P2 and IC-21 cells C. Microgels with 2% RGD and IC-21 cells.

After 4 hours, the samples were transferred to fresh wells, 1mL of complete media was added to each well, and then incubated overnight. The following day, each sample was imaged using calcein-AM and fluorescent microscopy.

Results: We demonstrate that PEG-NIPAAm microgels can be used to present ligands for controlled cell adhesion while maintaining a low background signal. IC-21 cells showed significant adhesion to the P2-peptide-conjugated particles when seeded for 4 hours. Similarly, they adhered well to the control RGD-tethered particles. Adherent cell numbers were significantly higher on microgel particles presenting either RGD or P2 compared to unfunctionalized microgel surfaces, which supported low levels of cell adhesion.

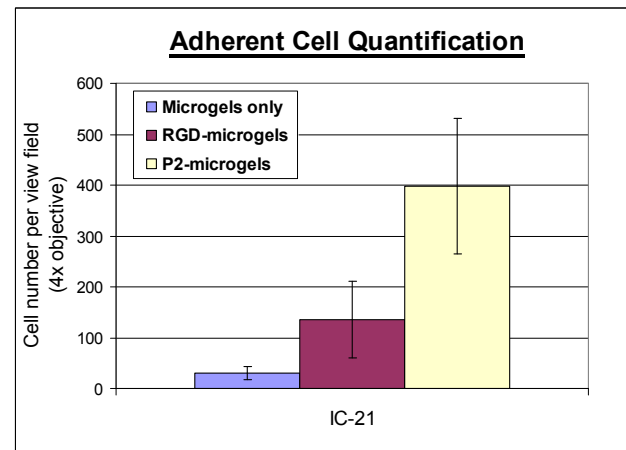


Figure 2: Quantification of adhered cells to each microgel surface. Each group has N=3-4

Conclusions: PEG-NIPAAm microgels can be successfully tethered with peptides in order to study macrophage signaling and adhesion in a controlled manner. Our results show that the base microgel provides a minimally adhesive background signal on which we can add specific peptide signals to create a controlled system for studying macrophage response to surfaces. Using the peptide-conjugated microgels grafted on PET, we showed that we can control IC-21 macrophage adhesion to biomaterial surfaces. Based on the modular nature of this system we can use this technology to further explore macrophage responses to biomaterials using appropriate signaling elements tethered to these microgels.

References:

1. Anderson, JM et al, Semin. Immunol 2007
2. Singh, N. et al Biomacromolecules 2007

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