Addition of Silicate Nanoparticles to Poly(ethylene oxide) Controls Cell Adhesion

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Statement of Purpose:

Materials implanted *in vivo* are subjected to an active environment, where proteins non-specifically coat the surface of materials. The proteinaceous layer triggers the host immune system to 'wall-off' the foreign material from the rest of the organism, via the foreign body response, which can reduce the efficacy of an implanted biomedical device. One approach to reducing the natural immune response is to control protein and cellular adhesion on the surface of implanted materials. More recently, a trend of producing less complicated, more cost-effective biomaterials that still afford controlled cell adhesion has begun. Working towards this goal, we have developed a nanocomposite system, composed of poly(ethylene oxide) (PEO) and silicate nanoparticles that allows for the control of cellular adhesion.

Methods:

Silicate nanoparticle cross-linked PEO films were prepared via a well-known sol/gel exfoliation method. Gels with a specified PEO:silicate (Laponite) ratio were prepared and were then manually spread onto glass slides and dried at 25° C. NIH 3T3 mouse fibroblast cells were seeded at 7,500 cells/cm^2 in 24-well plates. Cell number was determined by incubation with CellTiter 96 AQ$_{ueous}$ One Solution Cell Proliferation Assay, 7 and 14 days after seeding. For adhesion experiments, cells were seeded at 20,000 cells/cm^2. After 3 hours, cells were fixed and fluorescently imaged with an Olympus FV1000 confocal microscope. Actin filaments were labeled with Alexa Fluor 488 phalloidin.

Results:

Cells readily adhere, grow and proliferate on nanocomposites containing higher than 40 wt. percent of silicate (**Figure 1**). Since cells do not spread on, or adhere to, pure PEO surfaces, the presence of silicate nanoparticles must be responsible for the adhesion and spreading of cells to the nanocomposite films (**Figure 2**).

Understanding the exact composition and structure of the nanocomposite films and their relation to cell adhesion is complicated by the presence of three different PEO polymer components. First, there is polymer that coats the silicate nanoparticle and whose structure is amorphous in solution as well as in the dried state. Second, the polymer bridging one or more silicate nanoparticles is referred to as the network active polymer. Finally there is the free polymer that neither coats nor attaches to any nanoparticle cross-linker, but simply builds localized regions of high PEO concentration. We hypothesize that PEO rich regions within a nanocomposite film remain nonadhesive to cells while the silicate rich regions are adhesive to cells. When submersed in PBS, regions on the silicate surfaces become exposed, due to the dynamic adsorption-desorption mechanism of the polymer and silicate, and offers cell adhesion sites. Overall, data shown in Figure 1 support our hypothesis. The more silicate nanoparticles cross-linking a nanocomposite film, the higher the cell number observed on the film and the better the cell adhesion and spreading (Figures 1 and 2).

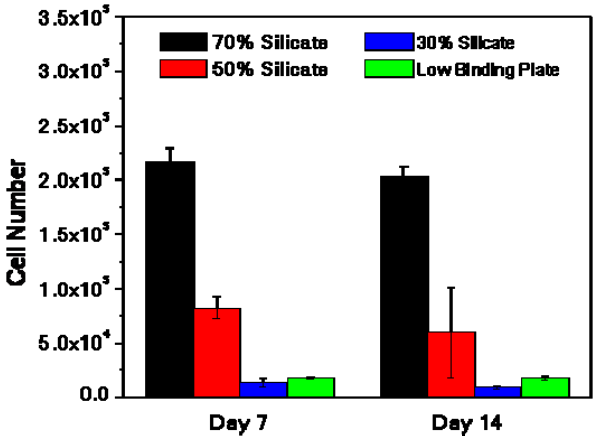

Figure 1. Fibroblast cells proliferate to a greater extent on films containing higher silicate concentrations. Increasing silicate concentration results in increased cell number. For 50-70% silicate samples, the number of cells in the plateau phase of the growth curve is proportional to the silicate concentration.

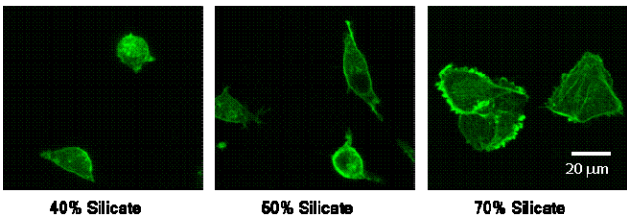

Figure 2. Representative confocal images showing an increase in cell number and cell spreading with increasing silicate concentration after 3 hours of incubation.

Conclusions:

The increase of silicate concentration within PEO based nanocomposite films results in an increase in silicate rich areas that support cell adhesion and also results in a decrease in PEO rich areas that remain nonadhesive to cells.

References:

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