Osteoblast Adhesion and Proliferation on Silicate Containing Nanocomposites

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Statement of Purpose:

A variety of advanced new biomaterials, with fascinating structures and functions, have been engineered at the nanoscale. Among these are bioactive nanomaterials and polymer nanocomposites that have generated tremendous interest. Here we show bio-nanocomposite formulations with defined cellular adhesion properties can be generated by inclusion of charged silicate nanoparticles as cross-linkers to "neutral" polyethylene oxide hydrogels. The development of such formulations allows for tailoring consistent cell adhesion properties for a spectrum of potential applications ranging from bone repair that might require cell adhesion to controlled drug delivery vehicles, where adhesion is unwanted. (Figure1).

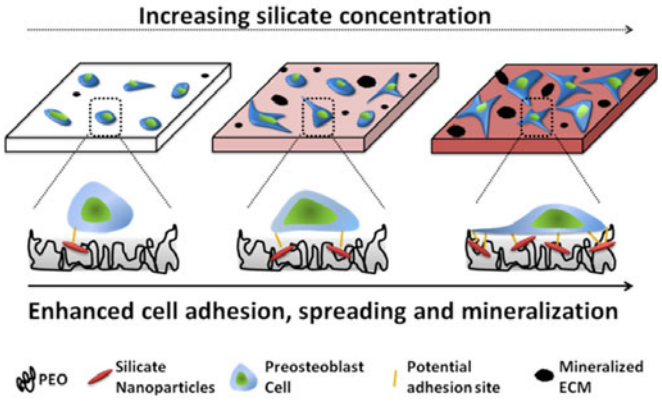

Figure 1: Schematic showing how silicate nanoparticles dispersed in a polyethylene oxide matrix enhance cell adhesion, spreading and mineralization.

Methods:

Nanocomposite hydrogels were prepared by mixing polyethylene oxide (PEO) and silicate nanoparticles (Laponite) in deionized water. An aqueous dispersion of PEO (X%) and Laponite (5-X%) was obtained. Bio-nanocomposite films were prepared by spreading, followed by solvent evaporation. The dried nanocomposite films had a final PEO-silicate ratio of 60:40, 50:50, 40:60 and 30:70. In vitro biocompatibility of the bio-nanocomposite films was evaluated by determining growth characteristics of MC3T3-E1 mouse pre-osteoblast cells. Cell adhesion and spreading were first evaluated by incubating cells on the film surface. Alkaline phosphatase activity was used as an early marker of osteoblast differentiation and von Kossa staining was used to determine the amount of extracellular calcium phosphate produced.

Results:

Osteoblast cells adhere, grow and proliferate on the nanocomposite films and mineralization is observed. The higher the silicate concentration in the nanocomposite film, the more cells adhered and spread (Figure 2). Compared to fibroblast cells, osteoblast cells proliferated better as they seem to prefer the silicate rich environment. Viability of cells was high on all film compositions, ~95%, throughout an experimental period of 2 weeks.

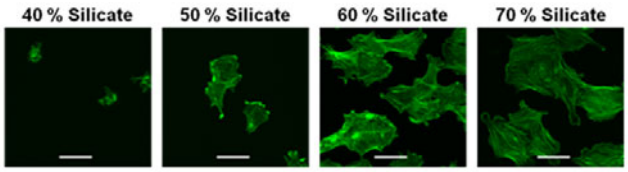

Figure 2: Cell adhesion and spreading visualized using confocal microscopy. Scale bar represent 25 microns

A significant increase in alkaline phosphatase activity (ALP) was observed suggesting that silicate plays an important role in the differentiation of preosteoblast cells (Figure 3).

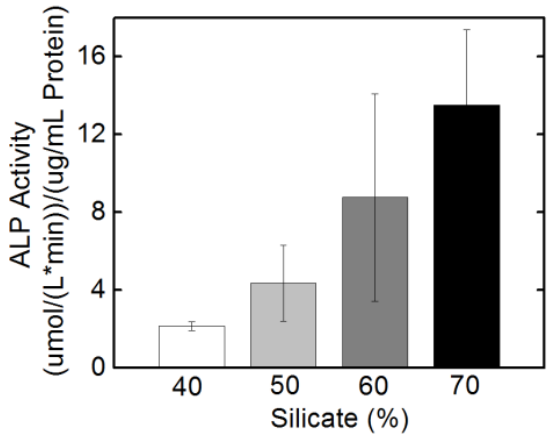

Figure 3: ALP activity of cell seeded silicate cross-linked nanocomposites after 7 days.

In order to evaluate the formation of calcium phosphate ($CaPO_4^{3-}$), nanocomposite-cell constructs were incubated with silver nitrate solution and exposed to UV (von Kossa staining). Nanocomposites containing higher silicate showed significantly higher amount of mineralized extra cellular matrix compared to nanocomposites containing lower amount of silicate.

Conclusions:

Addition of silicate improved cell adhesion and proliferation of osteoblast cells. This study demonstrates the ability of silicate to enhance the formation of mineralized extracellular matrix, thus offering new strategies for creating bioactive scaffolds for bone repair.