Chitosan enhances adhesion and proliferation of osteoblast cells on nanocomposite films

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Statement of Purpose:

Chitosan has attracted much attention as a biomedical material due to its unique characteristics such as biocompatibility, degradation, antimicrobial and wound healing properties. Synthetic and natural polymers such as chitosan can be used to synergistically combine the advantageous chemical, physical and biological properties of the individual components to produce superior biomaterials. Our approach is predicated upon the development of silicate cross-linked polyethylene oxide (PEO) hydrogel films containing chitosan. While pure chitosan hydrogels often have poor mechanical properties and suffer from batch to batch variations, the bio-nanocomposite PEO-silicate-chitosan gels discussed below overcome some of these troubles because only low concentrations of chitosan are used. Chitosan thus adds all the advantageous properties mentioned above without hampering the mechanical strength of the hydrogel. In addition, the ability of silicate based materials such as bioglass have been found to enhance osteoblast proliferation and gene expression, thus offering inspiration for creating new biomaterials for bone repair. Here, we investigate the effect of chitosan on the adhesion and growth of osteoblast cells on bio-nanocomposite hydrogel films.

Methods:

Nanocomposite hydrogels were prepared by mixing PEO and silicate nanoparticles (Laponite) in deionized water. Chitosan solution was added drop wise to the hydrogel until the desired concentration was reached. Bio-nanocomposite films were prepared by spreading followed by solvent evaporation and drying. The PEO to silicate ratio was kept constant (e.g. ca. 60% silicate and ca. 40% PEO) and the amount of chitosan was increased from 0 to 11 %.In vitro biocompatibility of the bio-nanocomposite films was evaluated by determining growth characteristic of MC3T3-E1 mouse preosteoblast 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 done to determine the amount of extracellular calcium phosphate present.

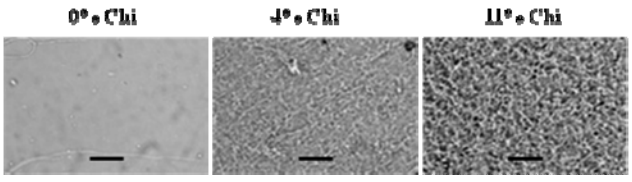

Figure 1: Optical microscopic images showing formation of aggregates due to addition of chitosan. Scale bar represents 50 micron.

Results:

Addition of chitosan solution results in the formation of chitosan containing aggregates within the silicate cross-linked PEO network (Figure 1). These micron sized aggregates reinforce the nanocomposite and scatter visible light as can be seen by optical microscopy. Overall, addition of chitosan results in enhanced adhesion and spreading of preosteoblast cells. Complex organization of actin filaments was observed after cells attached to the surfaces. All of the nanocomposite films support proliferation and high cell viability was observed during in vitro culture.

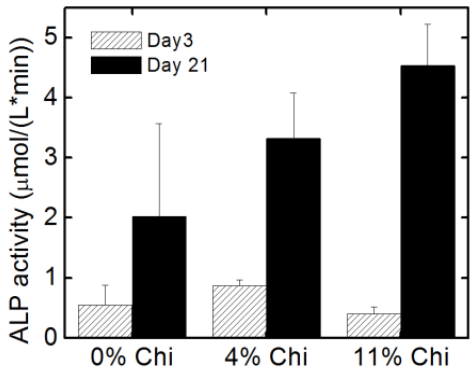

Figure 2: Alkaline phosphatase activity of preosteoblast cell on nanocomposites film.

When compared to nanocomposite films without chitosan, chitosan was found to significantly enhance alkaline phosphatase (ALP) production and support formation of mineralized extracellular matrix (Figure 2). With addition of chitosan, an increase in ALP activity of MC3T3-E1 cells was observed, which is associated with the progressive differentiation of preosteoblast cells. The significant increase in ALP activity due to addition of chitosan suggests that chitosan plays an important role in differentiation of preosteoblast cells. In order to evaluate the formation of calcium phosphate, nanocomposite-cell constructs were stained by von Kossa after 28 days of culture. All the nanocomposite films stained positive for calcium phosphate in a chitosan concentration dependant manner and displayed a significantly higher amount of mineralized matrix compared to the positive control (TCPS). An increase in chitosan concentration thus enhances production of mineralized matrix.

Conclusion:

Our study indicates that the multi-component system consisting of chitosan/PEO/ and silicate nanoparticles supports the adhesion and growth of osteoblasts as well as the mineralization of the nanocomposite surfaces which might prove useful in developing strategies for bone repair.