Characterization of chitosan / nanocrystalline hydroxyapatite composite scaffolds for bone tissue engineering

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Introduction: Chitosan, a natural polysaccharide, is an attractive biomaterial because of its biocompatibility, enhancement of wound healing, promotion of cell adhesion and migration, and antimicrobial properties[1]. Calcium phosphate (CaP) is widely used as an orthopedic biomaterial because of its osteoconductive properties[2]. In natural bone tissue, nanocrystalline calcium phosphate is dispersed in a natural polymer matrix[3]. We have developed a novel porous scaffold for use in bone tissue engineering that is composed of nanocrystalline CaP in a chitosan matrix[4,5]. In this study, we characterize the physical properties of this chitosan/CaP scaffold and examine its ability to promote osteoblast growth.

Methods: Chitosan microspheres were made by dripping a 3.5% chitosan (92.3% DDA) solution in 2% acetic acid into a NaOH/methanol solution. Chitosan/ CaP composite microspheres were made in a similar manner using a solution of 3.5% chitosan, 100 mM $CaCl_2$, and 60 mM NaH_2PO_4. The chitosan/CaP microspheres were left in the NaOH/methanol solution for 24 hours to allow the initial amorphous CaP to develop into crystalline hydroxyapatite[4]. Then, both types of microspheres were washed with water until they reached a neutral pH. The microspheres were quickly washed with 1% acetic acid, packed into 13 mm diameter tubes, and dried at room temperature. The acetic acid dissolved the surface of the microspheres slightly so that they were able to stick together to form a porous scaffold, but still retain their spherical geometry. The scaffolds were viewed with a scanning electron microscope (SEM) equipped with an energy dispersive spectrophotometer (EDS). X-ray diffraction (XRD) was used to characterize the crystallinity and crystallite size of the CaP nanocrystals[4]. The water content, swelling ratio, and density were measured, and density was used to calculate the porosity of the scaffolds. Dissolution of the scaffolds was measured both with and without lysosyme. Weight loss was measured at days 1, 4, 7 and 14 and Ca release were measured at days 2 ,4 ,6 ,8 ,10 ,12, and 14 (Calcium reagent kit, Pointe Scientific). To measure cell attachment and growth, scaffolds were placed in a 48 well plate, and HEPM cells (ATCC) were seeded at 10^5 cells/scaffold and gently shaken for 2 hrs. Then, the scaffolds were removed to a new 48 well plate, and the remaining cells were counted. At 3, 5, and 7 days, cell growth was measured by dsDNA analysis (PicoGreen dsDNA Quantification kit, Invitrogen). On Day 7, scaffolds were stained using a Live/Dead cell viability kit (Molecular Probes), and viewed with a fluorescent microscope.

Results: SEM images of the scaffolds showed that both the chitosan and chitosan/CaP scaffolds consisted of microspheres approximately 1 mm in diameter with pore sizes between 100 and 600 μm. The composite scaffolds had a much rougher surface than the chitosan scaffolds. EDS revealed that the Ca:P ratio of the composite scaffolds was 2.0±0.1, and elemental mapping showed that Ca and P were evenly distributed throughout the scaffold. XRD showed that the composite scaffolds contained hyroxyapatite crystals with an average size of 198±55 nm and a crysallinity index of 16.7±6.8%. The water content of the chitosan and composite scaffolds was similar (17.2±0.5% vs. 17.1±0.2%), but the swelling ratio was significantly lower for the composite scaffolds (176.0±7.9% vs. 160.3±5.5%). Both types of microspheres had approximately the same density (1.9 g\cm^3) and porosity (35.5±6.7). Neither type of scaffold showed any significant weight loss after 2 weeks in PBS with or without lysozyme. For chitosan/CaP scaffolds, less than 2 μg calcium per mg of scaffold dry weight was released over two weeks, and no detectable calcium was released from chitosan scaffolds. 60% of HPEM cells attached to both the chitosan scaffolds and the chitosan/CaP scaffolds in two hours. However, as shown by dsDNA analysis, cell growth was significantly increased on the composite scaffolds (Fig. 1). This was confirmed by Live/Dead staining, where many more cells were seen on composite scaffolds than on chitosan scaffolds. On the composite scaffolds, cells had begun to grow into the interior pores of the scaffold.

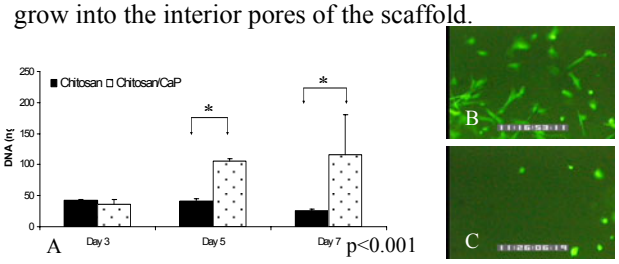

Figure 1 (A) DNA from HPEM cells grown on chitosan and chitosan/CaP scaffolds (B) HEPM cells on chitosan/CaP scaffold (Day 7). (C) HEPM cells on chitosan scaffold (Day 7)

Conclusions: This scaffold has an interconnected porous structure with pore sizes that can facilitate bone ingrowth[3]. The scaffold is composed entirely of biocompatible, biodegradable materials, but does not degrade quickly even in the presence of lysozyme. Osteoblast cells were able to attach and grow well on the composite scaffold, and had begun to grow into the interior pores after 7 days. These results clearly demonstrate that this composite scaffold has the potential to be used in bone regeneration.

References:

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