Osteoblast phenotype and collagen production is enhanced on composite chitosan/calcium phosphate scaffolds

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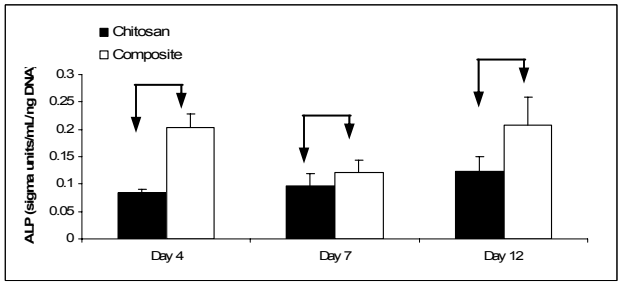
Introduction: We have developed a novel porous microsphere-based bone graft material composed of nanocrystalline calcium phosphate in a chitosan matrix to overcome issues limiting the use of autografts (limited quanitity, donor site morbidity, etc.) in bone grafting. Chitosan and calcium phosphate are both attractive bone graft materials because chitosan exhibits antimicrobial properties, promotes osteogenesis, is biodegradable, and enhances wound healing, while calcium phosphate is known to be very osteoconductive and is widely used as an implant coating[1,2]. The composite graft material has mechanical properties similar to cancellous bone and porosity and pore size sufficient to support new bone and vascular tissue ingrowth[3]. This study examines the ability of these composite scaffolds to support osteoblast phenotypic expression and matrix production *in vitro*.

Methods: Chitosan and composite scaffolds were fabricated as previously described, and sterilized by exposure to ethylene oxide gas[3]. To examine the ability of composite scaffolds to support osteoblast phenotypic expression and matrix production *in vitro*, hFOB 1.19 cells (ATCC #CRL-11372) were seeded onto composite scaffolds at a density of 10^5 cells/scaffold. Scaffolds were first placed in individual wells of a 48-well tissue culture plate, and cells were added to the scaffolds in 0.5 mL of media. Plates were continuously shaken for 4 hours, and then scaffolds were removed to new 48-well plates. Plates were cultured in complete cell culture media (1:1 mixture of DMEM:Ham's F-12 with 10% FBS, 1% antibiotic/antimycoic 0.3 mg/mL G418 sulfate, and 10 nM vitamin D3) at 39.5°C/5% CO_2 for 12 days and were maintained on a Belly Dancer (Stovall, Greensboro, NC) to promote continuous nutrient and waste transport into and out of the scaffold. On days 4, 7, and 12, cell media was removed, and cells were lysed in 0.5 mL deionized water with a sonic dismembrator (Fisher Scientific, Fair Lawn, NJ) (n=5 per scaffold per day). In preliminary studies, ALP activity and collagen production by hFOB cells grown on tissue culture plastic were highest prior to day 12. Therefore, in this study, only days 4, 7, and 12 were examined.

To estimate cell number at each time point, total dsDNA was measured (PicoGreen dsDNA detection kit, Invitrogen, Carlsbad, CA). Alkaline phosphatase activity was also measured from the cell lysate by a p-nitrophenol based method (Sigma, St Louis, MO). The amount of CICP (C-Terminal of Type I Collagen) in the media is indicative of Type I collagen production and was measured by ELISA (METRA CICP EIA kit, Quidel, San Diego, CA). The morphology of cells growing on chitosan and composite scaffolds was also examined by scanning electron microscopy (SEM).

Results: There were no differences in dsDNA between chitosan and composite scaffolds at any time point, and the total amount of DNA increased only slightly over the course of the experiment for both types of scaffolds. hFOB cells are a conditionally immortalized cell line that proliferate rapidly at a permissive temperature of 34°C. However, at 39° C, cell division is slowed and cells achieve a mature normal osteoblast phenotype.

Cells cultured on composite scaffolds exhibited increased ALP activity when compared with cells cultured on chitosan scaffolds on days 4, 7, and 12 (Fig 1). Similarly, significantly more CICP was produced by cells cultured on composite scaffolds than by cells on chitosan scaffolds on days 4 and 7, indicating that composite scaffolds induced more type I collagen production than chitosan scaffolds (Fig 2). No differences in cell morphology could be detected by SEM.



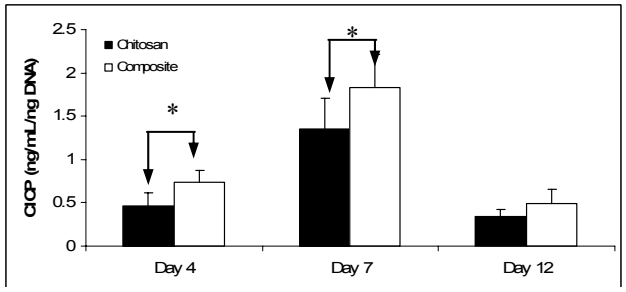


Fig 2 CICP (Type I collagen) production (n=5) by hFOB cells was significantly higher on composite scaffolds than chitosan scaffolds on days 4 and 7 ($p<0.05$)

Conclusions: Osteoblast cells cultured on composite chitosan/calcium phosphate microsphere-based scaffolds exhibited increased ALP activity and type I collagen production when compared with cells cultured on scaffolds composed of only chitosan. These results indicate that these composite scaffolds enhance osteoblast phenotypic expression and extracellular matrix *in vitro*, and, therefore, have strong potential to induce bone regeneration *in vivo*.

References:

1. Khor E. Elsevier; 2001.
2. Yang Y, et. al.. Biomaterials 2005;26(3):327-37.
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