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In Vitro and In Vivo Evaluation of a Novel Polymer-Ceramic Composite Scaffold for Bone Tissue Engineering

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INTRODUCTION

Currently available bone grafts present certain limitations such as donor-site morbidity for autografts and risk of disease transmission for allografts. These limitations suggest a need for alternative strategies. The use of polymer/ceramic composites as scaffolds for trabecular bone tissue engineering capitalizes on the benefits of both materials. Polymers are easily formed and shaped and add structural rigidity while calcium phosphates impart osteoconductivity and osteointegration. We have developed a biodegradable, microsphere-based scaffold for bone tissue engineering based on poly(lactide-co-glycolide)/calcium phosphate composite microspheres in which a nanocrystalline hydroxyapatite is synthesized within the forming microspheres.

MATERIALS AND METHODS

Briefly, poly(lactide-co-glycolide) (PLAGA) was mixed with methylene chloride and separate aqueous solutions of calcium nitrate tetrahydrate ($Ca(NO_3)_2$-$4H_2O$) and ammonium hydrogenphosphate (NH_4HPO_4). Initial molar ratios of Ca/P were 1.67. The final composition of microspheres formed two groups of composite microspheres; low polymer/ceramic ratio (27% calcium phosphate content after mixing) and high polymer/ceramic ratio (17% calcium phosphate content after mixing). Scaffolds were formed from each group of microspheres by heating the microspheres above the glass transition temperature of the polymer (~52°C) in a stainless steel mold for 90 minutes. Scaffolds formed from both the low and high polymer/ceramic ratio microspheres were seeded with mouse pre-osteoblast cells (MC3T3-E1) and evaluated for cell proliferation and alkaline phosphatase expression after 3, 7, 14, and 21 days in culture. Cell culture was maintained with minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 3mM β-glycerophosphate and 5mg/ml ascorbic acid and was changed every other day. In vivo evaluation of pure polymer, low, and high polymer/ceramic microsphere scaffolds was conducted by forming a 10mm segmental defect in the ulnae of male New Zealand white rabbits. Cylindrical scaffolds 5mm in diameter x 10mm in length were placed in defects representing the three different experimental groups. Sample size was n=6 rabbits per group. Healing was monitored via radiographs after 4 and 8 weeks at which time animals were sacrificed and implants isolated for histology. Radiographs were evaluated for optical density within the defect site and compared between time points using a Student's T-test ($p<0.05$). Scaffolds and undecalcified surrounding tissue were evaluated histologically by sectioning and staining scaffolds with Goldner's Trichrome stain for osteoid and mineralized matrix, and Von Kossa for newly mineralized tissue within and adjacent to the scaffold.

RESULTS and DISCUSSION

Results of cell studies indicated statistically significant proliferation between each time point over the 21 day incubation for cells seeded on scaffolds formed from both the low and high polymer/ceramic ratio composite microspheres, while alkaline phosphatase expression per cell was enhanced at the earliest time points with both the low and high polymer/ceramic ratio as compared to cells grown on tissue culture polystyrene. The formation of a nanocrystalline hydroxyapatite within the microspheres allowed for the dissolution of calcium ions from the hydroxyapatite more readily than a more crystalline form of hydroxyapatite. This dissolution may serve to enhance the osteointegration of the scaffold to pre-existing bone and the formation of an apatite layer on the scaffold in vivo by providing a site-specific delivery of calcium ions. The addition of nanocrystalline hydroxyapatite also appeared to have no inhibitory effect on cell proliferation while it enhanced alkaline phosphatase expression as compared to cells on tissue culture polystyrene.

Radiographs of in vivo studies indicate areas of radiodensity within the defect after 8 weeks of healing suggesting the formation of mineralized tissue within the defect site. Radiodensity is evident along the radial border, in the forming callus, and at the ends of the defect site in each of the composite scaffolds (low and middle) as well as in the pure polymeric scaffold (PLAGA). Analysis of the optical density of the radiographs within the defect site shows a statistically significant increase in radiodensity in the low polymer/ceramic ratio scaffold, but also shows trends toward an increase in radiodensity in both the high polymer/ceramic ratio scaffold and the pure polymeric scaffold as well. This data suggests that despite the large variations seen within each groups in degree of radiodensity, the overall trend is toward increased mineralization from 4 to 8 weeks. Histological analysis of areas adjacent to and within the composite scaffolds shows cellular infiltration throughout the microsphere structure. There is also evidence of both osteoid formation and mineralized bone formation along the margins of the scaffold structure and between microspheres from the scaffold itself. Further, osteoclasts are noted along mineralized tissue adjacent to the

scaffold, with resorption lacunae evident at the border of newly mineralized tissue, suggesting bone remodeling. Results of cell culture experiments suggest that calcium phosphate incorporation may enhance healing over pure polymer scaffolds. In vivo data supports these claims with enhanced mineralization in the composite structures.

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