Hydroxyapatite-PLGA-collagen biomaterial for bone regeneration

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Introduction: Every year in excess of half a million bone grafts are performed in the United States; making bone the second most frequently transplanted material. Synthetic bone-graft materials are needed to repair bone fractures and bone defects caused by congenital disorders, traumatic injury, or surgical removal of a bone tumor. Synthetic bone graft substitutes with desired mechanical and biological properties require chemical bonding between the components and bio-activation of the polymer surface. Therefore, a novel multistep polymerization and fabrication process was developed to construct a nano hydroxyapatite (nHAP)-poly(D,L-lactide-co-glycolide)-collagen (abbreviated as nPC) biomaterial, where the components are covalently bonded together to provide suitable mechanical and biological properties for a bone graft substitute. After confirming the chemical bonding between the components of the biomaterial, the mechanical and biological properties are being characterized as a potential material for bone graft substitute.

Materials and methods: nHAP particles were used to initiate polymerization of PLGA, which were subsequently bound to collagen fibrils to obtain the nPC biomaterial. Two dimensional films were constructed from the biomaterial by melt pressing and rectangular samples with a dimension of 30-35 mm length and 5-7 mm width were cut from these films for tensile tests using dynamic mechanical analyzer (DMA). Human mesenchymal stem cells (hMSCs) were cultured on the 2-D scaffolds in osteogenic media for 5 weeks. Tensile properties of the cell cultured scaffold were obtained by cutting rectangular samples with a dimension of 10-12 mm length and 2.5-4 mm width and compared them with the scaffolds treated in the cell culture condition without seeding cells. hMSC viability, proliferation and osteogenic differentiation were characterized using live/dead, alamar blue and alkaline phosphate (ALP) activity assay respectively.

Results: Live-dead stained images verified hMSC viability on nPC after 3 days. hMSCs proliferated within 7 days on nPC (Figure 1). hMSC proliferation rate on nPC was comparable to tissue culture plastic and collagen coated plate, which were used as controls. The increase is ALP activity at week 3 (Figure 2) indicates early stage of osteogenic differentiation of hMSCs on nPC. ALP activity in week 3 for nPC was significantly higher than for controls. Subsequent decline in ALP activity in week 4 indicates mineralization. The nPC biomaterial has ultimate tensile strength of 3.4±1.1 MPa, which is close to the range of human cancellous bone 7-20 MPa and 300 times higher than the strength of pure collagen (10-12 KPa). The tensile strength of the nPC scaffold after culturing hMSCs for 5 weeks was 1.02±0.06 MPa, which was higher than the strength of scaffolds without the cells (0.69±0.08 MPa). Young's moduli for the scaffolds with cell (11.65±3.3 MPa) were 5 times higher than that of scaffolds without cells (2.14±0.71 MPa). These results indicate that the hMSCs have differentiated into osteocyte on nPC scaffolds and deposited minerals which help to increase in strength and modulus values.

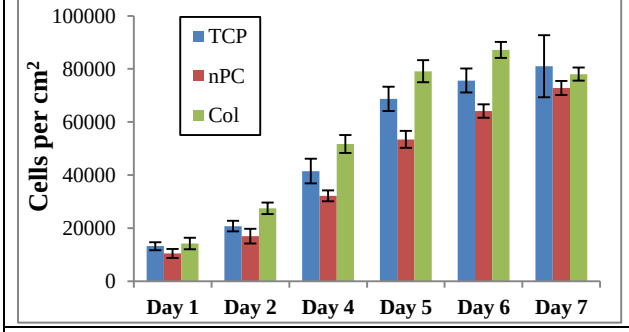

Figure 1: hMSC proliferation on tissue culture plastic (TCP), nHAP-PLGA-collagen (nPC) and collagen coated plate (Col). Data are mean±standard error of mean (n=15) for cell number as a function of time. hMSCs were seeded at a density of 9,375 cells/cm^2.

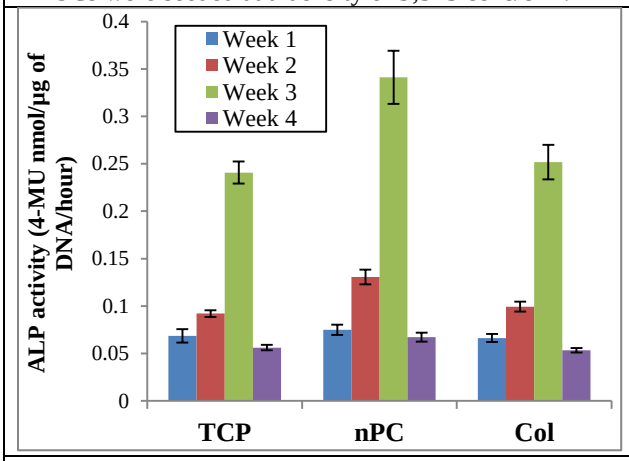

Figure 2: ALP activity of hMSCs on tissue culture plate (TCP), nHAP-PLGA-collagen (nPC) and collagen coated plate (Col) at different time points. Results show significantly higher ALP activity on nPC scaffold after 3 weeks. Error bar represents mean+/-standard error (n=9).

Conclusion: hMSCs proliferated on nPC scaffold and showed significantly higher ALP activity after 3 weeks, which indicates enhanced osteogenic differentiation. Ultimate tensile strength for nPC was comparable to human cancellous bone. Strength and modulus for cell cultured scaffolds were significantly higher than scaffolds without cells, which indicate mineral deposition from the differentiated cells.

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