Specific Calcium Phosphate Ceramics Facilitate Cell Proliferation and Adhesion on Ostochondral Allograft

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Introduction: The treatments of choice for articular cartilage damage are: tissue regeneration strategies for younger patients with small areas of damage and total joint replacement for patients with widespread tissue damage. Tissue regeneration strategies have not provided consistent results, and total joint replacement requires extensive bone resection and loss of proprioception. A new cartilage resurfacing strategy, which utilizes stem cells collected from patients and grown into tissues on allografts, can provide a useful alternative. To succeed, understanding cell proliferation and tissue formation on allograft surfaces is essential. The purpose of this study was to evaluate the potential of calcium phosphate ceramic (CPC) particles as surface coatings on allografts to facilitate stem cell proliferation and differentiation.

Methods: Tissue culture wells were prepared by the application of an implantable epoxy to each well bottom, followed by a coating of one of four types of calcium phosphate ceramic (CPC) particles. Three previously tested CPCs, which showed promise in facilitating bone, cartilage and endothelial cell adhesion, and one previously untested CPC formulation were used. Human fat tissues collected in accordance with an IRB approved protocol were utilized to extract adipose derived stem cell (ADSC) for culture from the cells of a single human donor. This was done to eliminate genetic variation. Well surfaces were seeded at a density of 10,000 cells/well. Adhesion and proliferation were analyzed at 2 hours, 24 hours, 48 hours and 7 days by removing the media suspension, rinsing each coated well with phosphate buffered saline twice, and then counting the cells with a hemocytometer after trypsinizing.

Following the evaluation of results of the first experiment, bone plugs from the condyles of dog carcasses and canine adipose tissue were collected following IACUC approved protocols. ADSCs were isolated from the adipose tissue of a single dog and cultured. Cultures were expanded to allow 50,000 cells/plug. Cell adhesion was analyzed at 48 hours by counting cells with a hemocytometer after trypsinizing.

In both experiments, culture medium was DMEM/F12 supplemented with 10% FBS, 1% penicillin-streptomycin-fungizone, 0.25 ng/ml TGF- beta 1, 5 ng/ml EGF and 1 ng/ml ßFGF.

Results: Cultures in the first experiment showed a wide variation in proliferation responses to the four different CPC surfaces for up to 48 hours (Figure 1). By day seven, the variation in response was much smaller. In agreement with previous experiments utilizing bone, cartilage and endothelial cells, the stem cell proliferation increased slightly in epoxy-coated control wells. CPC 19, a crystalline, large particle sized hydroxyapatite facilitated rapid stem cell growth early in the experiment and cell growth stayed consistently higher than on other CPC particle surfaces. By comparison, cells grown on CPCs 3 and 6, which had not previously been noted to facilitate cell proliferation, did show a modest response. Cells grown on the CPC 2 surfaces, which previously facilitated cartilage cell proliferation, did not show a consistent response in this experiment.

As CPC 19 showed a promising response in flat culture, canine bone plugs were coated with CPC 19 in the second experiment. Coated and non-coated bone plugs were compared, with canine ADSCs showing increased proliferation on the coated bone plugs (Figure 2).

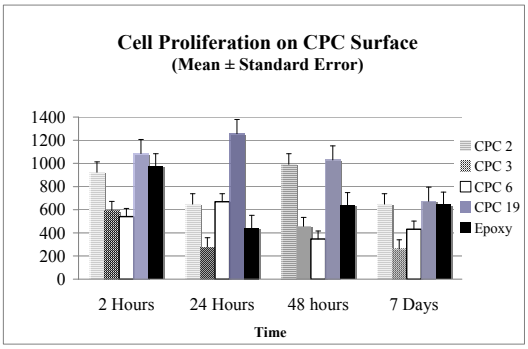

Figure 1: Cell numbers after incubation on CPC coatings

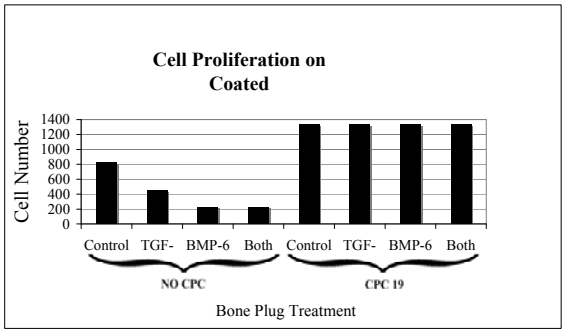

Figure 2: Cell numbers on coated and uncoated bone plugs after 48 h

Conclusions: Previously published studies showed that CPC particles of various sizes, shapes, and chemical compositions affected osteoblast, chondrocyte and endothelial cell proliferation and attachment on scaffolds. In those studies, cartilage and bone cells showed the best response to CPC surfaces with a high degree of crystallinity. Microscopy showed a secondary effect due to the size of imbedded CPC particles. Particle size greater than 100 µm suppressed metabolism. Endothelial cell response to the CPC coatings was strongly affected by particle size. Metabolic increases coincided with particle size increases.

The results of this current study demonstrated that CPC structure and shape affect adipose derived stem cell proliferation. In addition, canine adipose derived stem cells responded to one particular CPC coated surface with increased proliferation and adhesion on the particles on allograft surfaces.

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