Calcium Phosphate Combination Biomaterials as Human Mesenchymal Stem Cell Delivery Vehicles for Bone Repair

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Statement of Purpose: A useful scaffold for bone growth should exhibit biocompatible and biodegradable characteristics and support cell attachment, proliferation and differentiation, while being mechanically robust. Natural and synthetic forms of calcium phosphate (CPC) have been widely used in bone repair and augmentation as bone substitutes materials because of their excellent biocompatibility, bioactivity and osteoconductivity.[1] However, questions remain on how to increase cell viability in CPC materials. The objective of this work to investigate the potential of a modified injectable, nanocrystalline CPC (nCPC) to function as a delivery vehicle for osteogenic cells. The focus of this study is to prepare and evaluate, in-vitro, composite materials that combine polymeric additives with a nCPC. The effect of additives on cell viability and osteogenesis (of hMSC) are analyzed.

Methods: Cell culturing method was developed to screen nCPC (ETEX Corp.) based biomaterials with 2 carbohydrate polymers (Matrices A and B), and 1 protein polymer (Matrix C) additives (A, B and C) to identify key factors for cell support. Human bone marrow derived mesenchymal stem cells (hMSC, P3) and each type of solution mixture and the nCPC paste were injected through the mixture device. The hardened material was dislodged and then it was placed in osteogenic media. The constructs were removed for analysis at 2 and 6 weeks. The morphology was examined by SEM. Spread or gel encapsulated hMSCs were observed into the bottom of the plate from the discs. Chemical analysis and real time PCR were conducted for osteogenesis evaluation.

Results: The additives mixed groups did not show a change of gross shape, while the nCPC mixed with media showed a partially broken shape after 2 and 6 weeks. Cell migration from the nCPC materials was not observed. Although the A and C mixed nCPCs showed dispersed granular particles in the bottom of culture plates with the media mixed group, gel encapsulated cells were observed in B mixed nCPC. The A and C mixed nCPCs showed similar granular surface roughness as the media mixed group, while the B mixed nCPC had a smooth surface. In particular, proliferation and attachment of cells to the cement surface was only observed in the B mixed nCPC. The B mixed nCPC showed better DNA content, ALP activity and collagen synthesis throughout the culture period. In addition, after 6 weeks, expression of ALP and OP in the B mixed nCPC was significantly higher than in the A and C mixed nCPCs (Fig. 1).

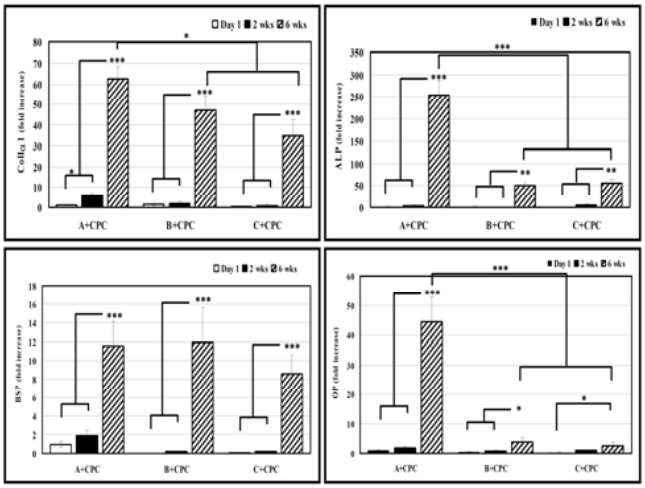

Figure 1. Transcript levels related to osteogenic differentiation markers: ColIα1, ALPase, OP and BSP.

Conclusions: The potential of the additives mixed nCPC was investigated to support osteogenesis of hMSC as an injectable cell therapy delivery system for enhanced healing of bone defects. All additives mixed nCPCs showed physical setting and hardening within 20 minutes after injection and showed good shape maintenances compared to culture media mixed nCPC. Recent work reported that cells mixed with the CPC paste had limited survival.[2] However, additives mixed nCPC in current this study showed good cell protection with increasing DNA content. In addition, this study results indicates the lineage-specific differentiation of hMSCs within the nCPC matrix. We suggest additives mixed CPC can be used as an injectable cell delivery vehicle for bone regeneration and the addition of B additive as a cell protector and a handling agent enhances bone response.

References:

1. Yang, Z. Biomaterials 1996;17(22):2131-2137.
2. Simon, CG. J Biomed Mater Res A 2004; 68(4): 628- 639.