Bone Morphogenetic Protein Incorporated Nanocomposite Scaffolds and Induction of Osteogenic Differentiation of Mesenchymal Stem Cells

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Introduction
Due to its distinctive advantages, selective laser sintering (SLS), which is a mature rapid prototyping technology, is being investigated for constructing nanocomposite bone tissue engineering (BTE) scaffolds [1]. One of the effective strategies in BTE is to combine a TE scaffold with bioactive molecules for regulating the osteogenic differentiation of stem cells, stimulating tissue formation and accelerating healing. Engineering functional cell-scaffold complexes with desired scaffold architecture, hierarchical structure, and temporal and spatial delivery capability of bioactive molecules in a controlled manner is still a scientific and technological challenge [2]. In this study, 3D SLS sintered Ca-P/PHBV scaffolds were surface modified for incorporating recombinant bone morphogenetic protein-2 (rhBMP-2). The behaviour of human umbilical cord derived mesenchymal stem cells (hUC-MSCs) on the scaffolds was then investigated.

Materials and Methods
3D BTE scaffolds were designed and Ca-P/PHBV nanocomposite scaffolds were made via SLS using Ca-P/PHBV nanocomposite microspheres (Fig.1). For the surface modification of Ca-P/PHBV scaffolds, gelatin was firstly physically entrapment on the scaffold struts. Heparin was then immobilized on gelatin-modified scaffolds by conjugating carboxylic groups in heparin to amine groups in gelatin via EDC/NHS chemistry. rhBMP-2 was finally attached to heparin immobilized Ca-P/PHBV scaffolds. For the in vitro study, hUC-MSCs were seeded onto Ca-P/PHBV scaffolds with or without surface modification (and hence the incorporation of rhBMP-2). hUC-MSCs alone and cell-scaffold constructs were cultured using an osteogenic medium up to 21 days. The cell morphology, proliferation and differentiation of hUC-MSCs on scaffolds were investigated.

Results and Discussion
The surface modification by gelatin/heparin significantly improved the hydrophilicity of Ca-P/PHBV scaffolds without affecting their surface morphology and mechanical properties, thus improving the cell proliferation rate at the early stage of cell culture. After 21 days of culture, hUC-MSCs became confluence and interacted favorably with all scaffolds, showing normal morphology and phenotype.

![Fig.1. Design (left) and sintered scaffold (right).](image)

![Fig.2. ALP staining of hUC-MSCs after osteogenic induction.](image)

For the osteogenic differentiation of hUC-MSCs, ALP stain was evidently observed (Fig.2) after 14 day culture of scaffolds with an rhBMP-2 concentration of 1000 ng/ml in the osteogenic medium. Alizarin Red staining for calcium deposition. By providing good binding affinity with rhBMP-2, the heparin in surface modified Ca-P/PHBV scaffolds were capable of blocking the degradation of rhBMP-2 and prolonging its release time and hence improved the osteogenic differentiation of hUC-MSCs on these scaffolds.

Conclusions
Surface modification of Ca-P/PHBV scaffolds by gelatin/heparin was successful. The incorporation of rhBMP-2 on the scaffolds facilitated osteogenic differentiation of hUC-MSCs. Multifunctional scaffolds with customized structure and controlled delivery capability for bioactive agents have promising potential for bone regeneration applications.

References