Surface Modification of Selective Laser Sintered Nanocomposite Scaffolds and Osteogenic Differentiation of Mesenchymal Stem Cells on the Scaffolds

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Statement of Purpose: In orthopedics and plastic and reconstructive surgery, bone tissue engineering is explored as a promising alternative to autologous or allogenic bone grafting. One of the effective strategies in bone tissue engineering is to combine a biodegradable scaffold with growth factors or other bioactive molecules to regulate the osteogenic differentiation of stem cells, stimulating tissue formation and accelerating healing. The precisely controlled architecture (pore size, shape, interconnectivity, etc.) of tissue engineering scaffolds and the temporal and spatial delivery of bioactive molecules in a sustained manner are of vital significance. In this study, 3D porous scaffolds based on Ca-P/PHBV nanocomposite were fabricated via selective laser sintering (SLS). The surface modification of Ca-P/PHBV nanocomposite scaffolds was conducted firstly through the physical entrapment of gelatin on the strut surface of scaffolds. Heparin was then immobilized on the surface of gelatin-modified scaffolds through covalent conjugation. The proliferation and osteogenic differentiation of human umbilical cord derived mesenchymal stem cells (hUC-MSCs) on Ca-P/PHBV nanocomposite scaffolds before and after surface modification were investigated.

Methods: 3D Ca-P/PHBV scaffolds were selective laser sintered using Ca-P/PHBV nanocomposite microspheres [1]. The surface modification of Ca-P/PHBV scaffolds (of a two-layer structure, ~70 mg/scaffold) by gelatin was achieved through the physical entrapment of gelatin on the scaffold struts. Heparin was immobilized on gelatin-modified scaffolds by conjugating carboxylic groups in heparin to amine groups in gelatin via EDC/NHS chemistry. The content of conjugated heparin was analyzed using the toluidine blue colorimetric method. hUC-MSCs were seeded onto Ca-P/PHBV scaffolds with and without surface modification. The morphology and proliferation of hUC-MSCs on scaffolds were studied. Stem cells alone and cell-scaffold constructs were cultured using a osteogenic medium and differentiated up to 21 days. The stem cells induced by osteogenic medium were treated with alkaline phosphate staining and von Kossa staining, and the ALP activity of different samples was measured to study the differentiation of hUC-MSCs towards osteogenic lineage.

Results: The designed cylindrical porous scaffold model had solid pillar with diameter of 1 mm and interval of 0.8 mm. Well-sintered Ca-P/PHBV scaffolds (Fig.1) were obtained. The porosity of sintered scaffolds was 61.75±1.24%, which is higher than the theoretic value (53.49%) of the designed scaffold model. The surface modification significantly increased the hydrophilicity of Ca-P/PHBV scaffolds without remarkably affecting their surface morphology and mechanical properties. During cell culture, an increase in absorbance from day 1 to day 7 was observed in MTT assay, indicating a trend of hUC-MSC cell proliferation on all scaffolds. Compared to non-modified nanocomposite scaffolds, heparin-immobilized scaffolds exhibited higher cell proliferation rates at the early stage. After 21 days of culture, hUC-MSCs became confluence and interacted favorably with all scaffolds, showing normal morphology and phenotype.

Fig.1 A photo of a sintered scaffold

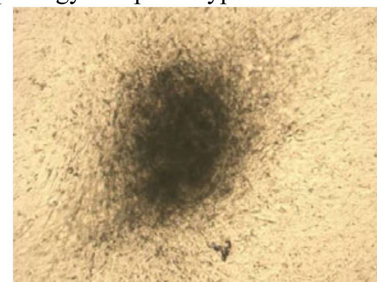
Fig.2 von Kossa staining of hUC-MSCs

For the osteogenic differentiation of hUC-MSCs, ALP stain was evidently observed after 3-week treatment with osteogenic supplementation. And von Kossa staining for calcium accumulation revealed a positive calcium deposition (Fig.2). The ALP activity on the scaffolds with and without surface modification increased with increasing osteogenic differentiation time. However, no significant difference was observed between scaffolds before and after surface modification for all cell culture times. The heparin-immobilized scaffolds should provide good binding affinity with bone formation and growth-related growth factors such as the bone morphogenetic proteins (BMPs) and thus capable of blocking the degradation of protein and prolonging its release time [2]. The controlled release of BMPs may further regulate the osteogenic differentiation of hUC-MSCs. This investigation is currently under way.

Conclusions: Good-quality 3D nanocomposite scaffolds with were fabricated via SLS using Ca-P/PHBV microspheres. The sintered scaffolds could be further surface modified with heparin through an intermediate gelatin layer. In the osteogenic medium, hUC-MSCs seeded on Ca-P/PHBV scaffolds with or without surface modification proliferated and differentiated into osteogenic lineage. No major difference was observed for hUC-MSCs on Ca-P/PHBV scaffolds with or without surface modification during the cell culture time. It is expected that when BMP is attached to heparin-immobilized scaffolds, its controlled release will enhance osteogenic differentiation of hUC-MSCs.

References

[1] Duan B, Wang M, Zhou WY, Cheung WL. *Proc. 8th World Biomaterials Congress*, Amsterdam, 2008.

[2] Freeman I, Cohen S. (2009) *Biomaterials*, **30**(11):2122-2131.