Design, Selective Laser Sintering, Properties and *In Vitro* Biological Evaluation of Osteoconductive Nanocomposite Scaffolds for Bone Tissue Engineering

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Introduction: Rapid prototyping technologies including selective laser sintering (SLS), which are widely used in the traditional manufacturing industries, have attracted great attention in recent years for constructing tissue engineering scaffolds [1]. One of the appealing aspects of using these technologies is that the scaffold architecture (pore size, pore shape, porosity, etc.) can be designed and that scaffolds of the specific architecture and of consistent and good quality can then be made according to the design. For bone tissue engineering, scaffolds made of polymer-based composites containing osteoconductive nanoparticles promote bone cell attachment and proliferation and bone tissue formation [2]. In this study, totally biodegradable composite scaffolds based on osteoconductive and biodegradable calcium phosphate (Ca-P) nanoparticles and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) were designed and fabricated via SLS. Their structure, properties and *in vitro* biological performance were studied using various techniques.

Materials and Methods: Ca-P nanoparticles and Ca-P/PHBV nanocomposite microspheres were produced following established procedures [3] and the Ca-P/PHBV microspheres were used in SLS to form scaffolds. Three tetragonal porous scaffold models with the same 3D orthogonal periodic porous architecture but of different pore sizes were designed using SolidWorks®. The respective design was exported into an STL format and transferred to a modified Sinterstation® 2000 system for producing scaffolds via SLS. The structure and properties of scaffolds formed were studied. For *in vitro* evaluation, human osteoblast-like cells (SaOS-2 cells) were seeded onto Ca-P/PHBV nanocomposite scaffolds and also PHBV scaffolds. The cell viability and proliferation were determined through live and dead viability/cytotoxicity kit and MTT assay. The cell morphology and ALP activity on different scaffolds were observed and measured.

Results and Discussion: Ca-P particles had sizes of 10-30 nm and a Ca:P molar ratio of around 1.5. Ca-P/PHBV nanocomposite microspheres contained 12.9 wt% of Ca-P. The average diameter for Ca-P/PHBV microspheres was 46.3 μm (53.2 μm for PHBV), which made them suitable for SLS. 3D scaffolds were successfully fabricated via SLS (Fig.1), with pore sizes ranging from 600 μm to 1 mm. With a decrease in pore size, the porosity of sintered scaffolds decreased from 80.7±0.7% to 36.9±1.4% while mechanical properties increased. SEM examination revealed that the porous structure of each layer for scaffolds with pore size 1.0 mm or 0.8 mm was well formed by SLS and the pores were clearly identifiable and comparable to the designs (Fig.2). However, for scaffolds with the designed pore size of 0.6 mm, loose Ca-P/PHBV microspheres were seen on pores and hence affected the pore size. The quality of sintered scaffolds was highly affected by the designed pore size, the size of Ca-P/PHBV microspheres and the scan resolution of the SLS machine. To demonstrate the feasibility of using SLS to form clinically usable scaffolds, a 3D human proximal femoral condyle model was built from CT scans and then processed to a porous model. This model was scaled down by 60% for scaffold production and a Ca-P/PHBV scaffold was successfully fabricated via SLS with the pore size of 0.8 mm and the strut size of 1.0 mm (Fig.3).

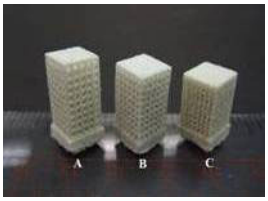

Fig.1 Sintered Ca-P/PHBV scaffolds with different pore sizes and strut sizes

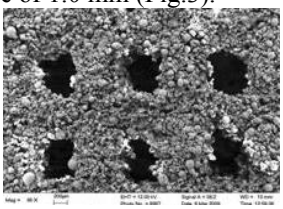

Fig.2 A Ca-P/PHBV scaffold with the designed pore size of 0.8 mm

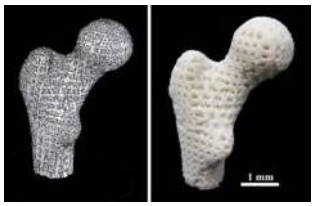

Fig.3 A model of proximal femoral condyle (left) and sintered scaffold (right)

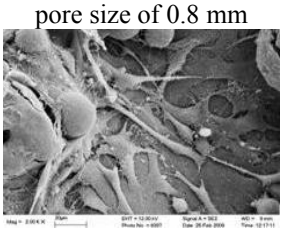

Fig.4 SaOS-2 cells seated on Ca-P/PHBV scaffold and cultured for 7 days

For *in vitro* biological evaluation, scaffolds with the designed pore size of 0.8 mm were used. SaOS-2 cells cultured on scaffolds exhibited high cell viability on both PHBV and Ca-P/PHBV scaffolds. For Ca-P/PHBV nanocomposite scaffolds, the incorporation of Ca-P nanoparticles in scaffolds enhanced the proliferation and ALP expression of SaOS-2 cells. After 7 day culture, SaOS-2 cells attached well and spread over the strut surface and interacted favorably with the scaffolds (Fig.4).

Conclusions: 3D scaffolds with controllable scaffold architecture can be designed and manufactured using SLS. The sintered Ca-P/PHBV nanocomposite scaffolds have interconnecting porous structures and their mechanical properties depend on the designed scaffold architecture. It is feasible to use clinical imaging data to create custom-made scaffolds for patients. The incorporation of osteoconductive Ca-P nanoparticles in scaffolds enhances osteoblastic cell attachment and proliferation.

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References:

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