A Novel PCL/Chitosan/TCP Composite Porous Scaffold for Bone Tissue Engineering

INTRODUCTION:
Tissue engineering strategies that combine porous biomaterial scaffolds with cells capable of osteogenesis have been shown promising as effective bone graft substitutes (1). However, one challenge is to design a porous structure, providing adequate mechanical support, allowing cell migration and nutrients diffusion, as well as mimicking the natural biophysical and biochemical properties for cell ingrowth and mineralization (2). The present study aimed to produce an osteopromotive porous composite scaffold by incorporating chitosan/tricalcium phosphate into a hierarchical polycaprolactone scaffold made by fused deposition method.

METHODS:
Polycaprolactone (PCL) scaffolds were plotted using rapid prototyping providing a uniform and ordered micro architecture throughout a cylindrical scaffold with Ø = 10 mm and h = 5 mm. The chitosan/tricalcium phosphate (TCP) sponge was incorporated into the PCL scaffold by freeze-drying.

Telomere-elongated human bone marrow mesenchymal stem cells (hMSCs-TERT) were seeded into the PCL/chitosan/TCP scaffolds (1×10⁶ cells/scaffold) and cultured in spinner flask with osteogenic stimulation medium for 21 days.

Cell/scaffold constructs were harvested at day 1, day 7, day 14 and day 21. Cell viability, DNA amount, ALP activity, calcium contents, and histology within the scaffolds were determined accordingly.

RESULTS SECTION:
Cells and extracellular matrix deposition on the scaffold was observed by scanning electron microscope. (Fig.1)
Cell viability showed that the composite scaffold is biocompatible. Cells grewed into the macro and micro pores of the scaffold. Cell density in the scaffold increased with time. (Fig.2)
DNA quantification and ALP activity: DNA amount increased during the culture period. ALP activity revealed decreased amounts of enzyme activity from day 7 and increased again from day 14. (Fig. 3)
Mineralization: Von Kossa staining and calcium contents showed that the scaffold was osteopromotive. (Fig.4) The quantification data of calcium contents increased from day 7 to day 21. The amount was about 3 fold higher than PCL based scaffold on day 21.
Histology: A highly cellular penetration depth (> 2 mm) was observed on day 7. A homogeneous cellular distribution in the composite scaffold on day 21 was observed with H&E, Toluidine blue and Hoechst staining. (Fig.5)

DISCUSSION:
In conclusion, the PCL/chitosan/TCP scaffold had a favorable environment for cell attachment, proliferation and osteogenic differentiation of hMSCs. These novel composite porous scaffold materials have the potential for a wide range of bone tissue engineering.

REFERENCES:

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