# Fabrication of porous β-TCP scaffolds by combination of rapid prototyping and freeze drying technology

Liulan Lin[1], Shaohua Ju[1], Lian Cen[2], Huicun Zhang[1], Qingxi Hu[1]

[1]Rapid Manufacturing Engineering Center, Shanghai University, China
[2]Shanghai Tissue Engineering Research and Development Center, China

*Abstract* — **The scaffold with exact shape and polygradient inner structure was necessary for bone tissue engineering scaffolds to facilitate cells infiltration and proliferation. A novel method of designing and preparing bone tissue engineering scaffolds with polygradient controllable structure of both macro and micro pores was proposed in this paper. By integrating rapid prototyping and freeze drying technology, the macro and micro pores were formed respectively. The size, shape and quantity of micropores were controlled by slurry concentration. The sintered β-TCP porous scaffolds were with connective macropores of approximately 400μm and micropores of 50~300μm. The chemical composition, porosity and mechanical properties of scaffolds were measured and analyzed. The detected results indicated that the β-TCP scaffolds could fulfill the requirements of tissue engineering.**

*Keywords* — **scaffolds; Tissue engineering; FDM; Freeze drying; β-TCP**

## I. INTRODUCTION

In tissue engineering, temporary 3D bionic scaffolds are essential to guide cell proliferation and maintain native phenotypes in regenerating biologic tissues or organs[1]. The shape and inner microstructure are the two critical properties of bionic scaffolds for repairing defective bone. To satisfy tissue engineering's requirement, bionic bone scaffolds must have exact shape with the defects, polygradient porous configuration with characteristics and properties such as porosity, surface area to volume ratio, pore size, pore interconnectivity, shape, structural strength and biocompatibility. These characteristics and properties are often considered to be critical factors in their designing and fabrication [2].

Traditional methods of scaffold fabrication are mainly based on manual work and lack of corresponding designing process[3-4], so extra procedure was needed to obtain suitable shape and the microstructure wasn't able to be controlled well. To overcome the limitations of conventional techniques, rapid prototyping fabrication techniques are being explored. Using RP technologies in bionic scaffolds preparation[5-6] could fully performs the significance of designing and improves the bionic scaffolds' properties. However, it's difficult to obtain and control the micropores because of the limited manufacturing accuracy of RP technologies.

In this study, medical images processing software Mimics, 3D CAD modeling software UG NX3.0 were used in the scaffolds reconstruction and design. Bone tissue engineering scaffolds with β-TCP bioceramic as the material were prepared through the integrated method based on fused deposition modeling (FDM) and freeze drying technology. A group of scaffolds with cylinder shape were prepared and the chemical composition, microstructure, porosity and mechanical properties of these scaffolds were measured and analyzed.

## II. MATERIALS AND METHODS

### A. CAD model construction

The patient's CT data of defective skull were imported into Mimics 9.11 (The Materialise Group, Leuven, Belgium) and the 3D STL model of the skull was created through image processing and 3D reconstruction technologies. Then the restoration's 3D STL model of defect was constructed by the symmetrically repairing operation in Mimics, as shown in Fig. 1.

The models of negative mould with strut were designed in 3D CAD software UG NX 3.0(UGS PLM Solutions, USA). As shown in Fig. 2, the 3D connective channels with diameter of 0.8mm and distance of 2.5mm between adjacent channels, the X-axis pores and Y-axis pores were not in the same horizontal plane, and the strut of negative mould with the equal sizes.

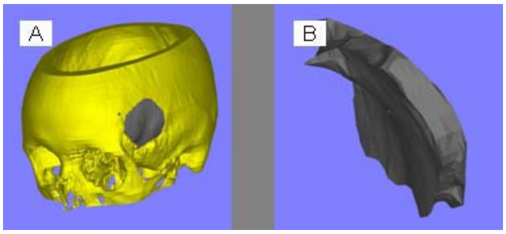

Fig. 1 Defective skull repairing (A) skull model,, (B) restoration model

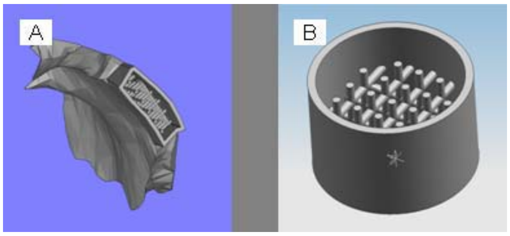

Fig. 2 Scaffold models designed (A) negative model with channel, (B) cylinder negative mould with strut

### B. Preparation of the scaffolds

The bioceramic slurry used in this work was prepared by mixing distilled water with β-TCP particulate (provided by Shanghai Tissue Engineering Center), a small amount of high-temperature binder and dispersant. The slurry was ball-milled for 12h to be homogeneous. Three kinds of slurries were prepared respectively with the β-TCP ratio was 40.0%, 50.0% and 60.0% by weight.

The negative moulds of scaffolds were fabricated through FDM (Stratasys Corp. USA), and the cylinder negative moulds were fabricated through SLA (Shanghai Union ltd. China), as shown in Fig. 3 .

There were three steps included by the whole preparation process: filling slurry into the mould, freeze drying and high temperature treatment. After filled with the slurry, the moulds were put in vacuum environment for 10 minutes to remove the gas that was interfused when filling slurry into the mould.

Freeze drying was to make the water of the slurry sublimate[7] while the slurry was kept at solid state. The moulds filled with slurry were kept at -75°C in a refrigeratory for 3 hours in order to freeze the slurry. Then the moulds were kept in a freeze drier (Anke FDC 5506, Anke Corp. China) for 24 hours. The micropores were formed by the sublimation of ice.

The negative moulds were put into an experimental stove (SX2-1013, Zufa Ltd., China) and the β-TCP ceramic was sintered. Below 550°C, the heating rate should be slow to make thermal decomposition of the ABS moulds as smoothly as possible. Above 550°C, a moderate heating with 100°C/h was adopted and kept at 1100°C for 3 hours to sinter β-TCP ceramic thoroughly and avoid grow into coarse grain.

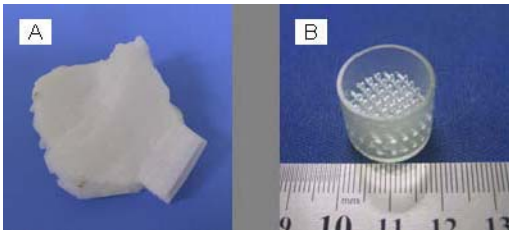

Fig. 3 Scaffolds fabricated (A) ABS part fabricated by FDM, (B) resin part fabricated by SLA

### C. Characterization of β-TCP scaffolds

Four groups of porous β-TCP scaffolds were prepared through the process based on rapid prototyping and freeze drying technology. The chemical composition, microstructure, porosity and mechanical properties of scaffolds were measured and analyzed.

- Chemical composition

Samples were analyzed using a Rigaku diffractometer with using CuKα radiation at 40kV/200mA. Scan was performed with 2θ values from 10deg to 90deg at a rate of 4.8deg/min.

- Microstructure

Microstructural characterization of the scaffolds were carried out with a scanning electron microscope (SEM, JEOL JSM-6700F) to observe the surface morphology and microstructure characterization of the scaffolds after high temperature treatment specimens.

- Porosity measurement

The Archimedes method was used to assess the porosity of the β-TCP scaffolds. Measured values were used to calculate the porosity value with Equation (1):

$$Porosity = (M_{wet} - M_{dry})/(M_{wet} - M_{sub}) \qquad (1)$$

Where $M_{wet}$ was the wet mass, $M_{dry}$ was the dry mass and $M_{sub}$ was the submerged mass.

- Mechanical properties

The compressive properties of the β-TCP scaffolds were evaluated using a Zwick BZ2.5/TS1S material testing machine (Zwick/Roell Co. Germany) with speed 0.5 mm/min.

## III. RESULTS

### A. General features

The porous β-TCP scaffolds with both macropores and micropores were prepared using slurries with β-TCP ratio 40.0wt%. The sintered scaffolds were with diameter of 9.1mm and height of 11.2mm. The horizontal linear shrinkage was about 7.1% and the vertical linear shrinkage 6.7%, corresponding to a volumetric decrease in by 19.5%. Scaffolds formed the exact shape and polygradient porous configuration with designed, as shown in Fig. 4.

### *B. Chemical composition*

Fig. 5 showed the XRD comparison of the high temperature treatment specimens and the β-TCP standard pattern. There were six highest peaks occurred at 2θ angle of 26.20°, 28.04°, 30.92°, 34.47°, 48.37°, and 53.21°, and these peaks matched with pure β-tricalcium phosphate's.

### *C. morphology*

The micro-morphology of β-TCP scaffolds with macro channels was observed with SEM, as shown in Fig. 6. The sizes of micropores were between 50µm and 300µm.The morphology of micropores was almost crack and the ceramic is lamellar, with long parallel pores aligned in the movement direction of ice front.

### *D. Influence of slurry concentration*

- Porosity

In order to evaluate the effect of slurry concentration on the porosity obtained, the porosity of the samples with different slurry concentration were assessed. The relationship between the final porosity and the initial slurry concentration was linear, provided sintering conditions was constant, as shown in Fig. 7. To these sintered β-TCP scaffolds, the porosity caused by macropores was the same as the designed model by UG NX. The porosity caused by micropores was determined by the concentration of slurry used to prepare the scaffolds.

- Compressive strength tests

The compressive strength of the samples with different slurry concentration were tested.. The compressive strength of the three kinds of scaffolds was 1.13Mpa, 0.66MPa and 0.31MPa with β-TCP ratio of 40wt%, 50wt% and 60wt%. As water ratio increasing, the compressive strength of scaffolds became lower. The porosity of scaffolds was higher and micropores connectivity was better with β-TCP ratio decreasing, that the scaffolds' mechanical properties were weakened.

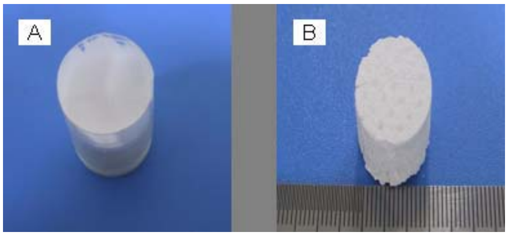

Fig. 4 Scaffold samples (A) before sintered, (B) after sintered

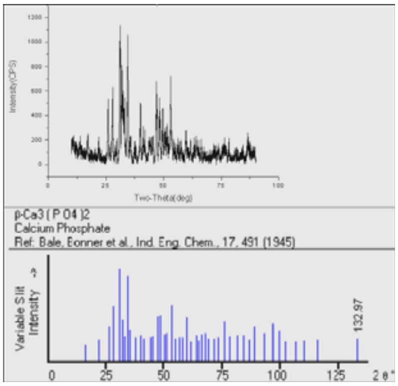

Fig. 5 The XRD spectrum compared specimens with pure β-TCP

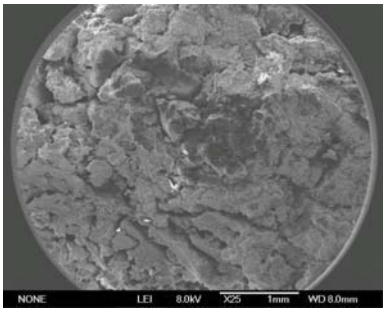

Fig. 6 SEM micrographs of β-TCP scaffolds (×25)

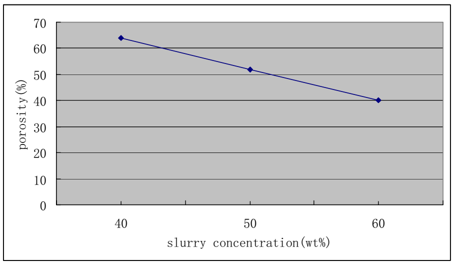

Fig. 7 Porosity of sintered scaffolds vs. slurry concentration.

## IV. CONCLUSIONS

A novel method of designing and preparing bone tissue engineering scaffolds with complex shape and controllable structure of macro and micro pores was proposed in this paper. The integration of medical image processing system and RP technologies prove highly useful for design and fabrication of complex bone defects. The application of CT and the medical image processing system make the CAD model of bone defects flexibly and accurately.

A strategy of designing scaffolds of polygradient controllable interior structure with both macro and micro pores was proposed. By integrating RP and freeze drying technology, β-TCP scaffolds of polygradient interior structure with both macro and micro pores were prepared. The micropores were formed by freeze drying. Freeze drying was to make the ice of the slurry sublimate in low temperature and pressure environment while the slurry was kept at solid state. The microstructure obtained was a replica of entangled dendrites of ice crystals. The diameter, shape and interconnectivity of the pores were regulated by the control of ice crystal growth. The relationship between the final porosity and the initial slurry concentration could be used for microstructure controlling. Next step research, the cell culture experiments will be studied to test the biology performance of scaffolds.

## ACKNOWLEDGEMENTS

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Author: Liulan Lin
Institute: Rapid Manufacturing Engineering Center
Street: 99 Shangda Rd
City: Shanghai
Country: China
Email: linliulan@staff.shu.edu.cn