Fabrication of Biodegradable β-TCP Scaffolds Using Natural Biogenic Magnesian Calcite Skeletons

Xing Zhang[1], Lina Yang[1], Lei Cao[1], Wei Cui[1], Zheng Guo[2], Yun Bai[1], Rui Yang[1]
[1]Institute of Metal Research, Chinese Academy of Sciences, Shenyang, Liaoning, 110016, China
[2]Department of Orthopedics, Xijing Hospital, The Fourth Military Medical University, Xi'an, 710199, China

Statement of Purpose: Beta-tricalcium phosphate (β-TCP) scaffolds have been widely studied and used as bone substitutes due to their good biocompatibility, osteoconductivity and biodegradability. However, β-TCP powders were primarily synthesized by calcination of precursor materials [1] or solid-state reactions [2] at high temperatures. In our study, conversion from natural biogenic magnesian calcite to β-TCP by an ion-exchange reaction at relatively low temperatures was explored. Beta-TCP scaffolds were then prepared from spines of sea urchin and granules from starfishes mainly comprising of calcite with high-content magnesium. Biocompatibility, ability for new bone formation and biodegradability of the β-TCP scaffolds derived from sea urchin spines were evaluated using *in vitro* and *in vivo* experiments.

Methods: Dried sea urchin spines and starfishes were bought from a marine product store in China. For the hydrothermal reactions, sea urchin spine samples (granule and cylindrical-shape samples) were boiled in deionized water for an hour and subsequently soaked in 10wt.% sodium hydrochloride solution for 30 min, and then thoroughly rinsed with deionized water to remove contaminants and organic components in the skeleton. The cleaned samples were then reacted with a $(NH_4)_2HPO_4$ solution at different temperatures for various periods to produce β-TCP scaffolds. X-ray diffraction, FT-IR and EDX spectra, and SEM images were employed to characterize the chemical composition and structural morphology of the samples. The porous β-TCP scaffolds prepared from sea urchin spines were soaked in simulated body fluid (SBF) to investigate formation of bone mineral like apatite particles on these scaffolds, and culture of mouse MC3T3-E1 cells (a pre-osteoblast cell line) on the scaffolds was performed to evaluate cytotoxicity. Granule and cylindrical-shape β-TCP scaffolds were implanted in the rabbit femoral condyle defects to investigate new bone formation *in vivo*, whereas β-TCP scaffolds were used to fill the Ti6Al4V spinal fusion cages to study their degradability and effects on bone regeneration during spinal fixation. Micro-CT images of the implants and Van-Gieson staining of histological sections were obtained to study new bone formation and degradation of the β-TCP scaffolds.

Results: XRD, FTIR and EDX results confirmed the conversion of magnesian calcite from sea urchin spines and starfishes to β-TCP scaffolds, while preserving their original three-dimensional (3D) porous structures shown by SEM images. Bone-mineral like apatite particles formed on the resulting β-TCP scaffolds after soaking in SBF for one week, whereas an increasing number of apatite particles assembled after soaking for two weeks and three weeks, indicating that these scaffolds have great potential of bone-bonding. SEM and Van-Gieson staining images showed that mouse MC3T3-E1 cells can adhere to and spread on the β-TCP scaffolds, suggesting non-cytotoxicity of the scaffolds. Micro-CT results (Fig. 1) revealed bone regeneration initiated around the β-TCP scaffolds after implantation in rabbit femoral critical-size defects for one month. After implantation for three months, ingrowth of the newly formed bone into the porous structures of the implants was clearly observed and the whole defect areas were fully filled, while the defects in the control group remained unfilled although a small amount of regenerated bony tissues was also found. Van-Gieson staining of histological sections of the implants displayed that the volume of newly formed bone increased with the increase of implantation time, which is consistent with the micro-CT results. Most parts of the porous structures of the implanted β-TCP scaffolds were filled with the newly formed bone after three months post-operation, and tight interfaces between the newly formed bone and the scaffold, as well as new bone cells, were found in the histological sections. SEM images and EDX data from the implant sections further confirmed the above results. Moreover, specially designed β-TCP scaffolds using sea urchin spines were well aligned to the center of Ti6Al4V spinal fusion cages that were then used for spinal fixation of beagles. Micro-CT results showed clearly degradation of the β-TCP scaffolds, and new bony tissue formed and bound tightly to the remaining β-TCP scaffolds after implantation for seven months.

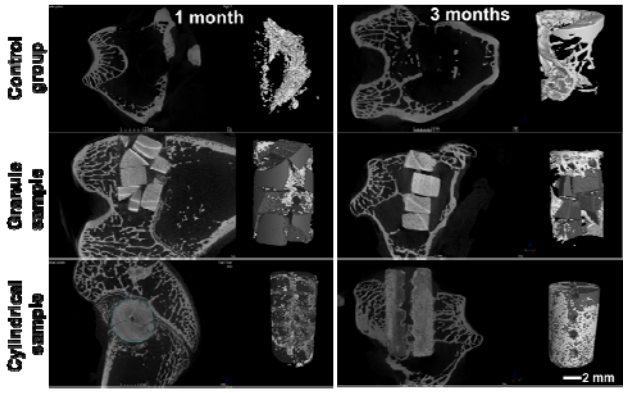

Figure 1. Micro-CT images of β-TCP scaffolds converted from sea urchin spines after implantation in the rabbit femoral condyle defects for one and three months. The unfilled defects were used as the control group.

Conclusions: Natural biogenic magnesian calcite skeletons can be converted to β-TCP scaffolds by a hydrothermal reaction at relatively low temperature while preserving their original 3D porous structures. Moreover, β-TCP scaffolds prepared from sea urchin spines showed good biocompatibility and bone bonding ability, which supported ingrowth of new bone into their porous structures and can be degraded *in vivo*, indicating great potential for applications in bone defect repair.

References: [1] Zhang X. J Mater Sci Mater Med. 2008;19:3063-3070. [2] Zhang X. Mater Sci Eng C. 2009;29:2003-2010.