Hydrothermal Conversion of Marine Skeletons to Calcium Phosphates

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Statement of Purpose:

Marine $CaCO_3$ skeletons have the hierarchical and optimally designed architectures created by nature. In our experiment, seashells were hydrothermally converted to hydroxyapatite [HAP, $Ca_{10}(PO_4)_6(OH)_2$], and sea urchin spines were converted to Mg–substituted tricalcium phosphate [β-TCMP, $(Ca,Mg)_3(PO_4)_2$], while maintaining their original structures. After implantation of converted shell and spine samples in rat femoral defects for 6 weeks, there was new bone growth up to and around the implants. Some new bone was found to migrate through the pores of converted spine samples and grow inward. These results show good bioactivity and osteoconductivity of the implants, indicating the converted shell and spine samples can be used as bone defect filler.

Methods:

Strombus gigas (conch) shell, *Tridacna gigas* (Giant clam) shell and spines of the echinoid *Heterocentrotus trigonarius* and *Heterocentrotus mammillatus* were cut into small pieces for hydrothermal conversion with $(NH_4)_2HPO_4$ solution in an autoclave at different temperatures for different durations.

The samples were characterized by XRD, SEM, EDS and FT-IR spectra. Quasi-static compression tests of converted shell and spine samples were conducted on a servohydraulic test frame at a stain rate of $\sim 10^{-3}$/s.

For in vivo tests, 15 rats were used for converted clam, conch and spine samples (5 each). A 2.0mm cylinder sample was inserted into a femoral defect, laterally. The other leg defect was left empty as an untreated control. The eXplore RS micro CT scanner was used for high resolution, in vivo, three-dimensional femur imaging. For histological study, the specimens were cut and milled into approximately 50-75 microns sagittal sections and stained with a Sanderson Rapid bone stain and counter stained using Acid Fuchsin.

Results:

Dense HAP structures were created from the shells throughout the majority of the samples at relative low temperature ~200℃. Spines were converted to bioresorbable β-TCMP by the hydrothermal reaction at 180℃, while maintaining the three-dimensional interconnected porous structures. Partially converted shell samples with dense HAP layers on the surface growing inward and original shell structures inside have an average fracture stress about 137-218MPa, which is close to the compressive strength of compact human bone (Fig. 1). The average compressive strength of urchin spines and converted spines (β-TCMP) are about 42 and 23MPa, respectively. Shells and spines can also be machined into different shapes for particular applications before the hydrothermal conversion.

The micro-CT images at six weeks show that the implants did not move, and untreated control defects remain empty with no evidence of a spontaneous fusion. Histological study (Fig. 2) reveals that there is newly formed bone growing up to and around the implants. There is no evidence of fibrosis tissue ring around the implants, also indicating that there is no loosing of implants. In contrast, the untreated controls remain empty with some evidence of a fibrosis ring around the defect hole.

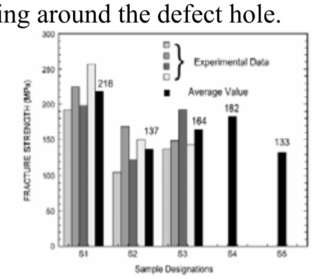

Fig. 1. Quasi-static compression results of conch converted at 180□ for 4 days (S1), 8 days (S2), 20 days (S3) and average compression strength of compact human bone loaded parallel to the bone axis (S4) and loaded normal to the bone axis (S5) [1].

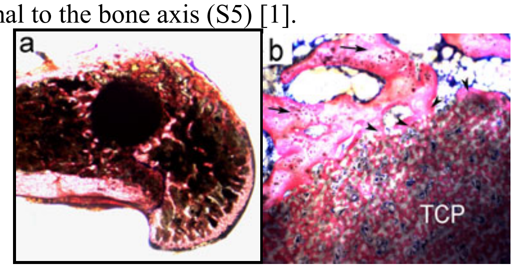

Fig. 2. Histological sections after 6 weeks implantation: (a) β-TCMP implant in the femoral defect and (b) high magnification of β-TCMP implant (arrow heads show the newly formed bone and black arrows indicate adjacent host bone).

Conclusions:

Seashells were hydrothermally converted to dense HAP, and spines were converted to porous β-TCMP at low temperatures 180-200℃, while maintaining their original structures. Partially converted shell samples have an average fracture stress about 137-218MPa, close to that of compact human bone. After 6 weeks implantation in rat femoral defects, new bone was found to grow up to and around the implants, indicating good bioactivity.

References:

[1] Suchanek W. J. Mater. Res. 1998;13:94-117.