In Vivo Performance of Complex 3D Calcium Phosphate Cement Scaffolds

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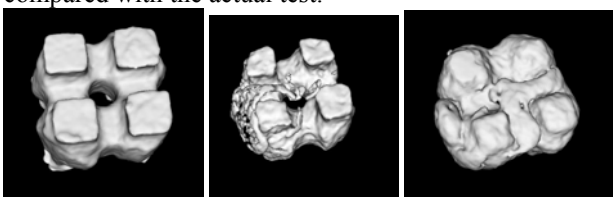
Introduction :
Qualitative analysis of the new bone formed within ceramic scaffold is relatively easy by conventional histology. On the other hand, quantitative data are difficult to obtain. In this work, micro-computed tomography was used as a possible technique to obtain quantitative data on the three-dimensional structure of newly formed bone and of scaffold degradation after four- and eight- weeks *in vivo*. Porous calcium phosphate cement scaffold with interconnected pores were computationally designed by an image-based approach and fabricated by indirect solid freeform fabrication (ISFF). Scaffolds were then implanted subcutaneously to demonstrate tissue in-growth. The work done illustrates the possibility of non-destructive quantitative analysis of bone formation in bioceramic scaffolds.

Materials and Methods:
Calcium phosphate monobasic, monohydrate 99% (MCPM) from Strem Chemicals and beta-tri calcium phosphate (β-TCP) from Plasma Biotel Ltd. were used as received. A dicalcium phosphate dehydrate (DCPD) cement slip was prepared from a 1:1 molar ratio of MCPM and β-TCP with distilled water added, P/L =1. The slip was cast into a complex orthogonal designed mold and the mold was removed by ethanol. Scaffolds were then seeded with bone morphogenetic protein-7 (BMP-7) transduced fibroblasts and implanted subcutaneously in five- to eight- week old immunocompromised mice to demonstrated tissue in-growth. Four specimens from each experimental group (four and eight weeks timepoints) were scanned in water using a MS-130 high resolution micro-CT scanner (GE Medical Systems, Toronto, Canada) at 15 micron voxel resolution, at 100 kV and 110 mA. Micro-CT images of scaffolds were conducted before and after four- and eight-weeks of implantation to determine the newly formed bone and the remaining scaffold in implants. Software 'Analyze' (Maya Clinic, MN) was used for the quantitative analysis of the new bone formed. Histological staining is used to confirm the presence of bone.

Results / Discussion:
Isosurface images of the μ-CT reconstructed data illustrate the newly formed bone that has grown onto and within an orthogonal pore DCPD scaffold after 4 weeks of implantation. Because the similar phases in of the bone and scaffold from CT scan, Analyze software provides a quantitative map of the scaffold. Image processing is used to 'subtract' scaffold from the CT scan in order to allow a more accurate observation of the bone formed. The volume percent of newly formed bone were 4.81±3.8 and 12.77±3.17 mm^3 after 4 and 8 weeks implantation, respectively. The newly formed bone was not found in the DCPD scaffold without cells nor the DCPD pellets (no macropores). The histology results confirmed the presence of the bone. The scaffolds were then mechanically tested. The results (Fig. 2) showed that the presence of more bone in-growth invasion of bone to the pores, and implant envelopment results in an increase of the moduli, despite the degradation of the scaffolds. The prediction of the stiffness from finite-element will be compared with the actual test.



(a) **(b)** **(c)**

Fig. 1 Isosurface view from micro-CT data of the scaffold (a) before-, (b) after 4 weeks, and (c) after 8 weeks of implantation.

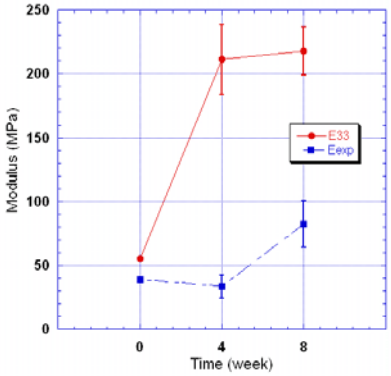


Fig. 2 Correlation between the computational and the experimental modulus at 0, 4, and 8 weeks.

Conclusions:
We have shown that, the DCPD scaffolds with macropores show promising results of bone formation and sufficient mechanical strength for bone repair. The micro-CT technique associated with Analyze software offers the possibility to quantify the newly formed bone and of the degraded DCPD scaffold.

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References:
[1] Friedman CD, et al. *J. Biomed. Mater. Res. (Appl. Biomater.) 1998;43:428-32.*
[2] Yoshikawa T, et al. *Biomed. Mater. Eng.*1996;6:345-51.
[3] Schek RM, et al. *Biomaterials* 2005; in press.
[4] Hollister SJ, *Biomaterials* 2002;23(20):4095-103.