Université de Valenciennes et du Hainaut-Cambrésis

LMCPA

Report for STSM MP1301

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Characterization of osteoblastic and endothelial cells invasion of macroporous β-TCP ceramic developed by freeze-casting method. INEB (Porto, Portugal) – LMCPA (Maubeuge, France).

1- Development of new macroporous β-TCP materials by ice-templating.

Calcium phosphate ceramics are well-known bone substitutes used in local bone surgery for filling bones defects due to their chemical composition close to bone's one. The beta calcium phosphate (β-TCP) is resorbable and can be replace by new bone tissue which is one of the major advantages of this ceramic. To have a complete bone tissue reformation, two types of cells have to colonize β-TCP materials: osteoblasts and endothelial cells and materials need to present a porous structure to let cells totally invade the materials and not only recover the surface. To this purpose several methods have been developed to mimic bone's macroporosity, including the ceramic slurry impregnation of macroporous skeletons (sponge, PMMA balls skeleton,...) which has been developed for few years in the LMCPA (Laboratoire des Matériaux Céramiques et Procédés Associés, Maubeuge, France) (Figure 1A). The cells reactions with this kind of interconnected macroporous materials have been well studied and it has been shown that osteoblast can invade the macropores.

A new casting method based on ice-templating was developed for a few years, allowing to create morphologically different porous structure. Freeze-casting produces orientated lengthen and interconnected pores that could help endothelial cells to form the tubular structures required to capillary formation (Figure 1B and C). In collaboration with the BCRC (Belgian Ceramic Research Centre, Mons, Belgium), LMCPA have developed freeze-casting for β-TCP and studied synthesis parameters enable to control pores size and volume. These studies led to the synthesis of seven β-TCP samples with pore size ranged from 50 µm to 600 µm and from 30% to 70% of porosity.

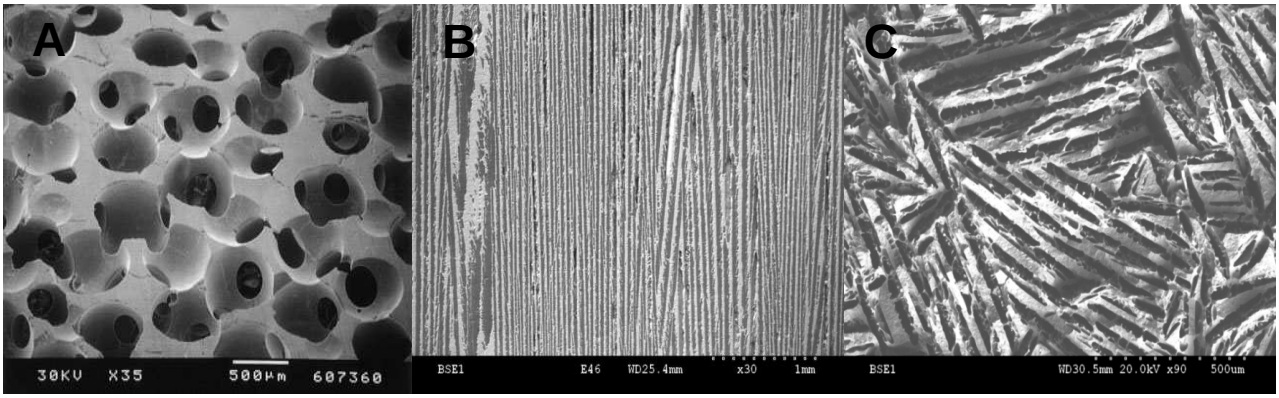

Figure 1 – SEM photography of surface of ceramic slurry infiltration of organic beads skeleton (A) and of vertical (B) and cross (C) section of β-TCP ice-templated scaffold.

The cell invasion ability of the ice-templated β-TCP samples have now to be evaluated and compared to the samples synthesized by slurry impregnation of organic beads skeleton. In this purpose, five samples with different pore sizes, closed to ice-templated sizes produced, have been synthesized. The osteoblast invasion of ice-templated samples has to be compared to the other samples and the endothelial cells invasion has to be studied for the two types of samples. In general, endothelial cells have difficulties to completely form the characteristic tubular tissue in ceramic bone substitutes and ice-templated tubular pores could enhance this tissue formation.

In collaboration with INEB, study of osteoblasts invasion have been done for the 12 samples by analysis of image at light microscope after MTT coloration of cells or at scanning electron microscope (SEM) after 1, 4 and 7 days of cells growth. Same experiments have been done for endothelial cells after 1 and 4 days of cell growth.

2- Osteoblast cells invasion.

To perform experiments, osteoblasts from human cell line have been used, the MG63 cell line. The 7 samples of ice-templating method and the 5 of the slurry impregnation method have been used as support for MG63 growth during 1, 4 and 7 days and cell invasion have been checked by light microscopy and by SEM. For the Day 1 and the Day 4, experiments have been made twice and similar results have been obtained.

Osteoblasts are able to colonise surface of the materials of all the samples whatever the way of pores synthesis, as shown in figure 2. The tests show that the porosity size larger than 100 µm of ice-tempated samples is able to let the cells invade the pores, but not the more thin pores (figure 2, right).

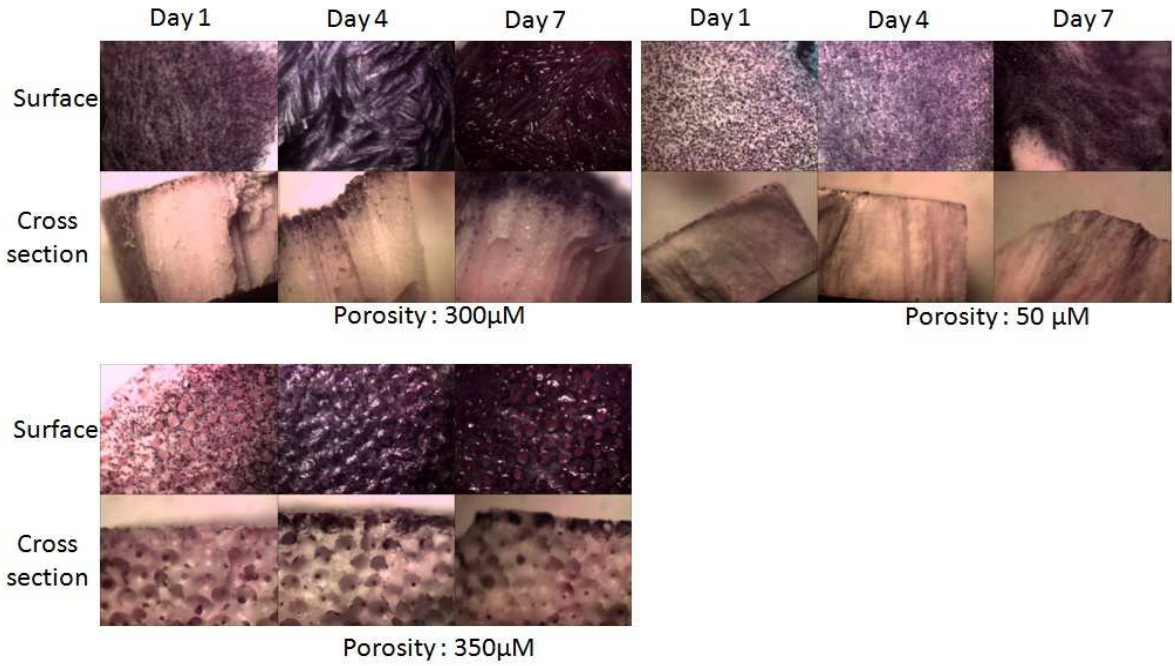

Figure 2- Light microscopy of surface and cross-section of ice template samples (top) and slurry-impregnation (bottom) samples colonized by MG63 after 1, 4 and 7 days

The Scanning Electron Microscopy (SEM) performed on these samples confirms that the results obtained by light microscopy: cells seem healthy and are able to colonize surface (figure 3, arrows) and pores, except for materials with too small pores. The most interesting information obtained by SEM is that cells are able to send cytoplasmic filaments between the walls of pores in ice-templated samples (figure 3, circle).

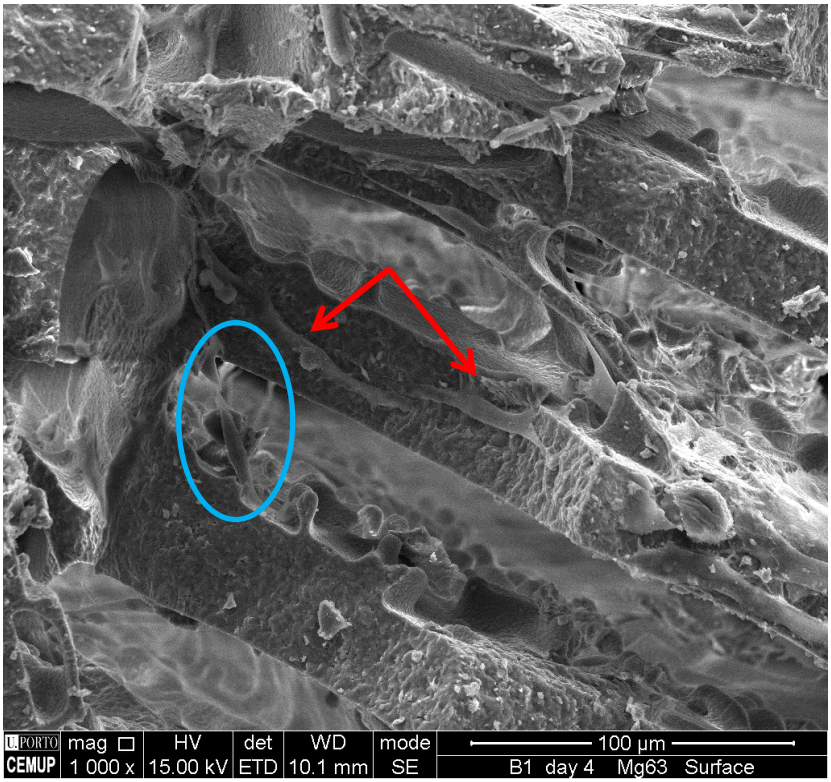

Figure 3- SEM of surface of ice template sample with cells on surface (arrows) and in pores (circle).

This phenomenon couldn't been seen in the samples prepared by slurry impregnation. The walls of pores are too distant in the slurry impregnated samples to permit this kind of cytoplasmic extensions. This means that ice-templated pores will be easier filled by a real osteoblastic tissue than the others porous structure, and then colonization of materials and mechanical properties after cell colonization should be better for the ice-templated materials.

3- Endothelial cells invasion.

Same experiments of cells invasion have been done for endothelial cells at day 1 and day 4 by using human HUVECs cell line. Results show that endothelial cells are able to colonize all samples at the first day but are died at day 4 (figure 4). Endothelial cells are really difficult to spread *in vitro* and are sensitive to changes in the culture medium. Calcium phosphate ceramics and culture medium react together, leading to chemical modifications in time, included local pH variations, which could affect endothelial cells viability. Then, it seems that endothelial cells are able to enter in the different porous materials, but *in vitro* conditions have to be modified.

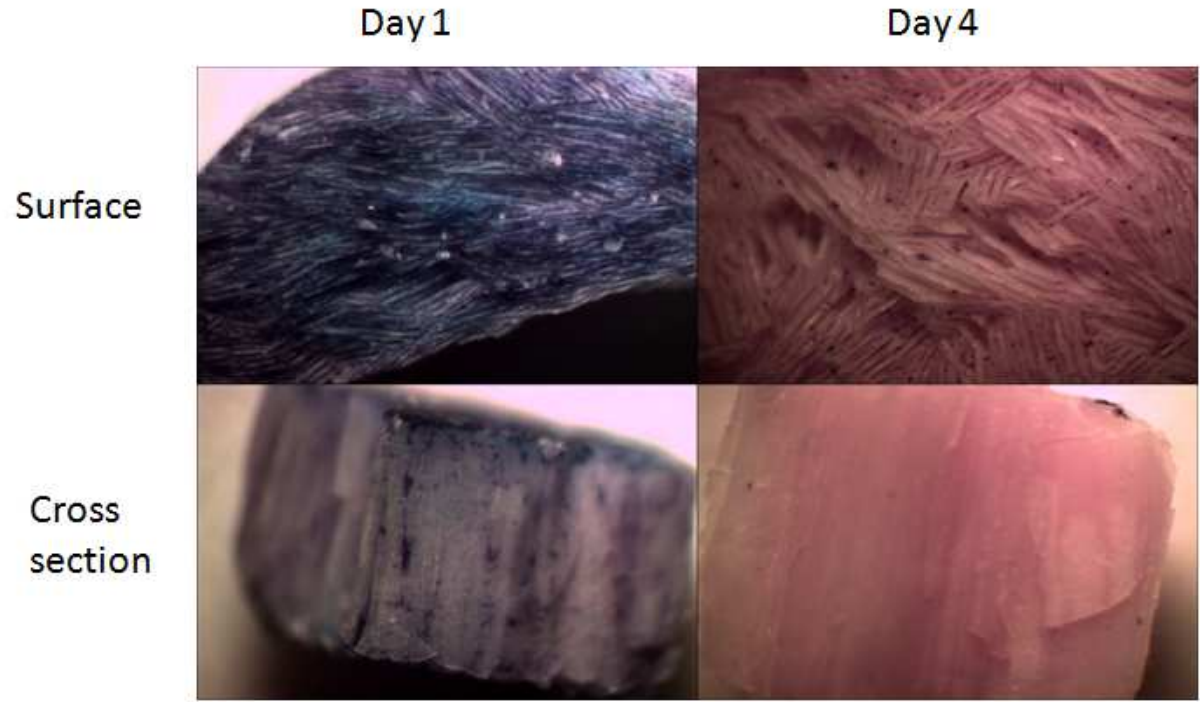

Figure 4- Light microscopy of surface and cross-section of an ice-template sample colonized by HUVEC after 1 and 4 days (2x magnification)

Conclusion and Discussion

Results obtained during this month permit to select 3 interesting porous conditions of ice-templating samples from the 7 tested. These 3 samples will be used for further investigations of cell invasion with osteoblast and endothelial cells. All experiments perform here are qualitative one's, and other tests are needed to quantify the effect of the porosity structure on cell invasion and proliferation (MTT quantification, RT-PCR analysis of genes expression,…).

For endothelial cells, experiments need to be adapted to problems with *in vitro* culture, as a flow cell culture test to avoid the pH local variations.

The quantitative tests and SEM analysis are performed by INEB on osteoblast invasion at day 7 and endothelial cells invasion at day 1 which have been prepared during the stage but not analysed. These preliminary results are really encouraging and a publication should be submitted during next year. The interesting results led LMCPA, BCRC and INEB to initiate a long term collaboration to study effect of ice-templated porosities on cell invasion. LMCPA (powder synthesis) and BCRC (ice-templating) are currently producing more samples to complete the study.