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COST STSM Reference Number: COST-STSM-MP1301-33064
Period: 2016-04-09 to 2016-04-30
COST Action: MP1301
STSM type: Regular (from Spain to Belgium)

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STSM Topic: Development of bioceramic scaffolds with directional porosity
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Development of bioceramic scaffolds with directional porosity

1. Abstract

Freeze-casting, fabrication of porous structures by removing the solvent after its solidification, have been studied for the last decade and this techniqe appears to be very interesting. The unique structure and properties of porous freeze-casted ceramics opened new opportunities in the field of cellular ceramics.
In this work, powders of the composition (Diopside - $CaMg(SiO_3)_2$: 60 wt. %, $Ca_3(PO_4)_2$ - TCP -Tricalcium Phosphate: wt. 40 %) were mixed with Water and 3 wt.% of dispersant (Dolapix CE 64) in order to prepare a slurry of 30 vol.% of powder content. A rheological study showed that the optimum viscosity was reached with 3 wt.% of dispersant (based of powder content) and present a viscosity of about 35 mPa·s at shear rate of 1000 s^{-1}. The slurry with this amount of dispersant had a zeta potential value of -23.2 mV, which assured a stable suspension (see Figure 1). The development of the porous scaffolds was achieved by freeze casting (to make the wall structure) and then drilled by laser (to make longitudinal channels). Small amounts of volume (2 mL) were employed for obtaining samples of 15 mm diameter and 8 mm of thickness. Samples were frozen using two different freezing rates. At low freezing rate, the time was about 30 minutes, while for high freezing rate the time was 3-5 minutes.
Thre is a significant difference in pore size (upper side) between the high freezing rate (length: 17.8 μm, width: 7.4 μm) and low one (length: 165 μm, width: 12 μm)
Bulk density and porosity are similar. For low freezing rate, the porosity is ≈0.54 and the bulk density is ≈1.44 g/cm^3, for high freezing rate these values are ≈ 0.49 and ≈ 1.62 g/cm^3.
Mechanical tests showed important differences between the two freezing rates (see Figure 7).
46 final samples were performed by freeze casting and drilled by laser in order to obtain multiple longitudinal channels with ≈ 2 mm of deepness, with a hole diameter ≈ 700 - 800 μm and ≈ 1 mm distance between holes (see Figures 8 and 9). The holes were performed on green bodies.

2. Introduction

The requirements for a synthetic bone substitute appear deceptively simple, that is, to supply a porous matrix with interconnecting porosity that promotes rapid bone ingrowth, yet possesses sufficient strength to prevent crushing under physiological loads during integration and healing. The ideal bone substitute is not a material that interacts as little as possible with the surrounding tissues, but one that will form a secure bond with the tissues by allowing, and even encouraging new cells to grow and penetrate. One way to achieve this is to use a material that is osteophilic and porous, so that new tissue, and ultimately new bone, can be induced to grow into the pores and help prevent loosening and movement of the implant [1].

In recent years, considerable attention has been given to the development of fabrication methods to prepare porous ceramic scaffolds for osseous tissue regeneration [2–9].
The ideal fabrication technique should produce complexshaped scaffolds with controlled pore size, shape and orientation in a reliable and economical way. However, all porous materials have a common limitation: the inherent lack of strength associated with porosity. Hence, their application tends to be limited to low-stress locations, such as broken jaws or fractured skulls. Therefore, the unresolved dilemma is how to design and create a scaffold that is both porous and strong.

Freeze casting is a simple technique to produce porous complex-shaped ceramic or polymeric parts [10]. In freeze casting, a ceramic slurry is poured into a mold and then frozen. Subsequently, the part is placed into a freeze drying to sublimate the solvent under vacuum, avoiding the drying stresses and shrinkage that may lead to cracks and warping during normal drying. After drying, the compacts are sintered in order to fabricate a porous material with improved strength, stiffness and desired porosity. The result is a scaffold with a complex and often anisotropic porous microstructure generated during unidirectional freezing. By controlling the growth direction of the ice crystals, it is possible to impose a preferential orientation for the porosity in the final material [11].
According to their microstructure, the samples can clearly be divided into three distinctive zones. In zone 1, the closest to the initial cold finger, no porosity at all is observed and the material appears dense. In the second zone, the material is characterized by a cellular morphology. Finally, in the upper zone (zone 3), the ceramic is lamellar, with long parallel pores aligned in the movement direction of the ice front. As pores obtained don´t have enough size for cell colonization, it was proposed drilling the samples by laser.

3. Materials and Methods

3.1 Materials

Diopside: $CaMg(SiO_3)_2$, which was synthesized in ICV-CSIC, Madrid.
TCP precursor: $Ca_3(PO_4)_2$, precursor of tricalcium phosphate ratio Ca / P molar 1.49, obtained by precipitation and supplied by the commercial Carlo Erba Reagents.
Distilled water: H_2O
Dolapix CE 64: Dispersant

3.2 Methods

3.2.1 Preparation of slurries

Slurries were prepared by mixing the powders (60 wt. % Diopside, 40 wt. % TCP) with water (with a constant stirring rate). A previous study showed that mixing with water was enough to desaglomerate the powders. Several amounts of dispersant were used for the rheological study and Acustosizer II. However, only 3.0 wt.% dispersant (optimal wt. %) was used to prepare the final samples.

3.2.2 Rheological study

The optimal viscosity for freeze casting was determined using the RS 1503 Rheometer viscosimeter. Slurries with 2.5 – 3.0 – 3.5 wt.% dispersant were prepared and studied.

3.2.3 Acoustosizer II

Dispersing conditions: Determination of working pH and dispersant agent content. It was done for Diopside, TCP and finally for the mixture 60 % Diopside – 40 wt.% TCP. Slurries (10 wt. % of powders) with 0.0 – 5.0 wt.% dispersant were analyzed.

3.2.4 Freeze casting

Two differents freezing rates were studied. For *low freezing rate*, a special equipment was used. The process was automatic. The freezing rate was -1.3 ºC/min. The freezing time was about 30 - 40 min. For *high freezing rate*, the process was manual. An insulated container was used and filled with liquid nitrogen. The mold was placed on a metal support and the desired amount of suspension (2 mL) was poured into it. The freezing time was about 3 - 5 min.
For preliminary tests, 10 samples were prepared. 5 samples using high freezing rate and 5 samples using low freezing rate. 1 sample of low and 1 sample of high freezing rate were used for microstructural characterization. The remaining 8 samples were used for porosity and bulk density determination and mechanical test. For the final samples, 46 samples were prepared (29 at high freezing rate and 17 at low freezing rate). Before sintering, all the samples had 17 mm of diameter and 9 mm of thickness. After sintering, they had 14-15 mm of diameter and 8 mm of thickness (because of shrinkage).

3.2.5 Microstructural study (SEM)

The microstructural characterization was done using two microscopes. One of them was for detailed analysis (placed in Mons University) and the other was for a quick overview, which was placed in INISMa – CRIBC. Both of them are property of INISMa – CRIBC.

3.2.6 Porosity and bulk density

The porosity and bulk density was determined by applying the *Archimedes method* for the 8 samples mentioned.

3.2.7 Mechanical tests

Diametral compression test was performed for the 8 samples mentioned.

3.2.8 Laser ablation

The final samples were drilled by laser ablation (before sintering), in order to obtain multiple longitudinal channels.

3.2.9 Sintering

All samples made were sintered under the following conditions:
Temperature: 1225 ºC
Time: 2 h
Heating rate: 10 ºC/min

4. Results

4.1.1 Rheological study and Acoustosizer II

Wt.% dispersant	Zeta potential (mV)	Viscosity (mPa·s) at 1000 s-1
0.0	-13.0	-
1.0	-18.1	-
2.0	-21.4	-
2.5	-	73.43
3.0	-23.2	34.64
3.5	-	67.11
4.0	-24.8	-
5.0	-25.4	-

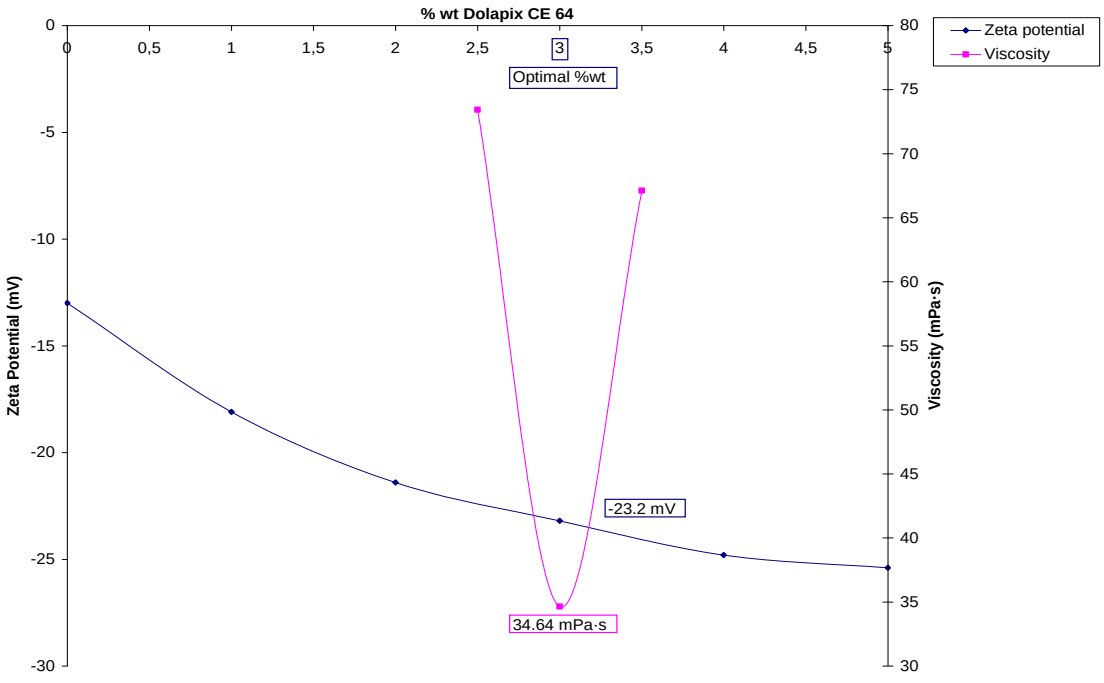

Fig 1: Zeta Potential and viscosity as a function of wt.% Dolapix CE 64

The optimal point was 3 wt.% dispersant Dolapix CE 64, which it provided a good viscosity for the slurry. Zeta potential value was also good, so it was taken for all the samples.

4.1.2 Microstructural characterization

Several photos were taken and used to determine the poro size (in the upper zone) for each freezing rate. There is a significant difference.

Parámeter/Freezing rate	High	Low
Average Width (μm)	7.4	12
Average Length (μm)	17.8	165

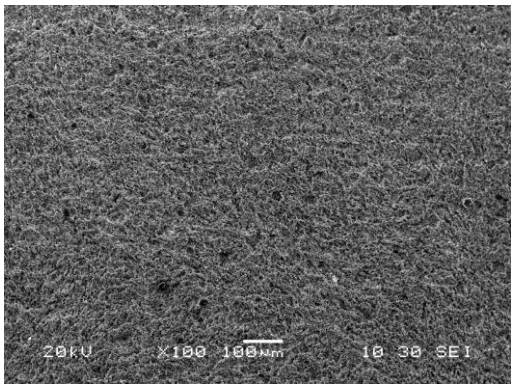

Fig 2: SEM analysis of bottom side

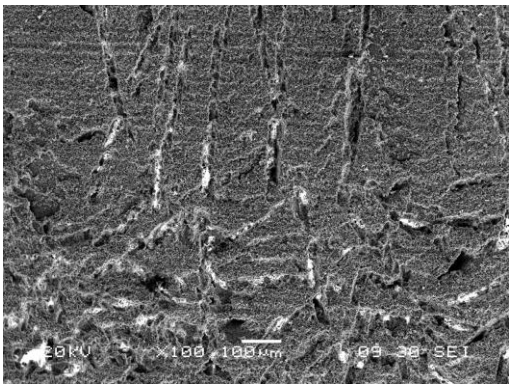

Fig 3: SEM analysis of interface

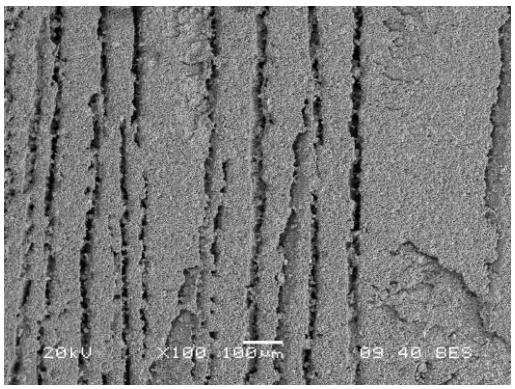

Fig 4: SEM analysis of channels

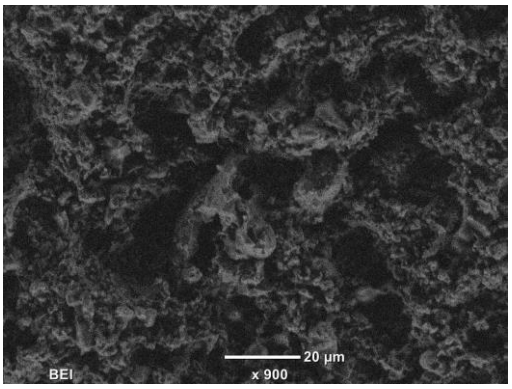

Fig 5: SEM analysis of top side for high

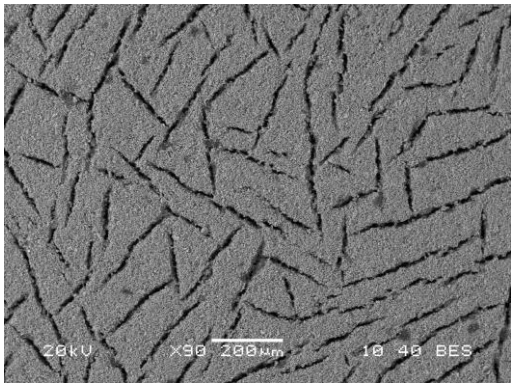

Fig 6: SEM analysis of top side for low

4.1.3 Porosity and bulk density

Freezing rate	Sample number	m_{dry} (g)	$m_{in\ water}$ (g)	$m_{with\ water}$ (g)	ρ_{bulk} (g/cm^3)	ε
Low	1	1,5652	1,0694	2,1584	1,434	0,545
	2	1,4509	0,9894	1,9693	1,478	0,529
	3	1,6015	1,0939	2,2149	1,426	0,547
	5	1,5650	1,0679	2,1614	1,428	0,545
	Average				**1,442**	**0,542**
High	1	1,7212	1,1765	2,2298	1,631	0,483
	2	1,8338	1,2530	2,3884	1,612	0,488
	3	1,4749	1,0079	1,9161	1,621	0,486
	*4**	*1,5049*	*1,0271*	*2,0522*	*1,465*	*0,534*
	Average				**1,621**	**0,486**

* The sample was removed too soon and it wasn´t frozed, so it was taken to the freezer to finish freezing. Therefore, maybe it can be considered as a low freezing rate sample. As it can be seen, there are small differences between the values of each freezing rate.

4.1.4 Mechanical tests: Diametral compression

The results in Figure 7 shows the difference between each freezing rate. The high rate of freezing allows higher loads before they reach the point of fracture. This is because it has lower porosity.

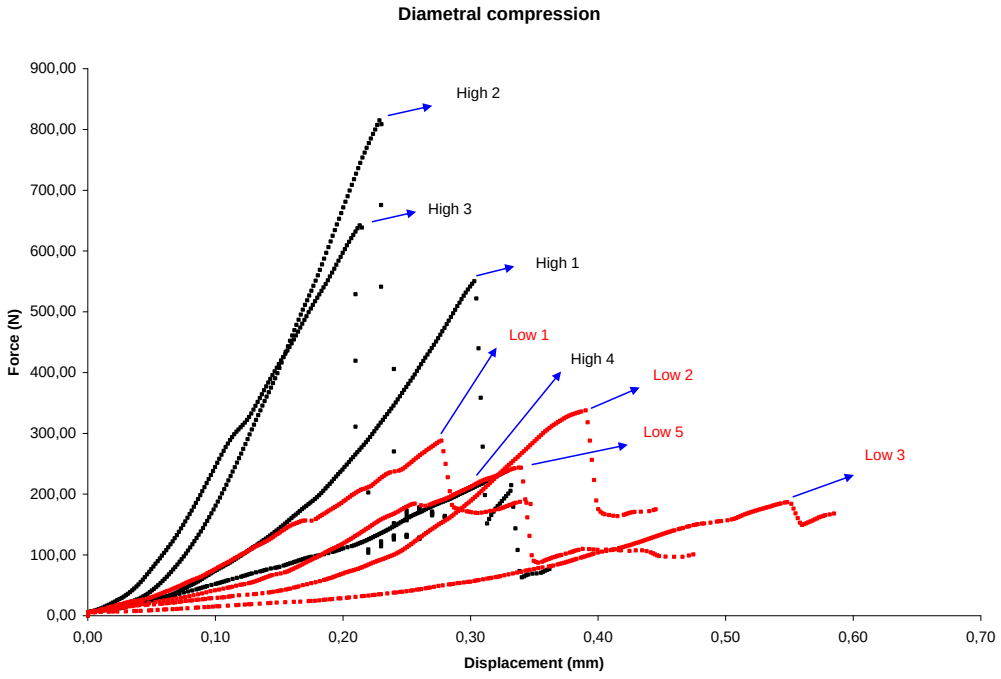

Fig 7: Diametral compression: Force applied (N) vs Displacement (mm)

* As it can be seen, High 4 sample has a similar behavior to the other low freezing rate samples. This is consistent with the fact already mentioned about its porosity and density.

4.1.5 Laser ablation and sintering

Figures 8 and 9 shows one final sample from different points of view.

Fig 8: Top view of final sample

Fig 9: Front view of final sample

5. References

[1] Suchanek W, Yoshimura M. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. J Mater Res 1998;13(1):94-117.

[2] Deville S, Saiz E, Nalla RK, Tomsia A. Freezing as a path to build complex composites. Science 2006;311:515–8.

[3] Almirall A, Larrecq G, Delgado JA, Martinez S, Planell JA, Ginebra MP. Fabrication of low temperature macroporous hydroxyapatite scaffolds by foaming and hydrolysis of an alpha-TCP paste. Biomaterials 2004;25(17): 3671–80.

[4] Charriere E, Lemaitre J, Zysset P. Hydroxyapatite cement scaffolds with controlled macroporosity: fabrication protocol and mechanical properties.
Biomaterials 2003; 24(5):809–17.

[5] del Real RP, Wolke JGC, Vallet-Regi M, Jansen JA. A new method to produce macropores in calcium phosphate cements. Biomaterials 2002;23(17):3673–80.

[6] Kawata M, Uchida H, Itatani K, Okada I, Koda S, Aizawa M. Development of porous ceramics with well-controlled porosities and pore sizes from apatite fibers and their evaluations. JMater Sci Mater Med 2004;15(7):817–23.

[7] Milosevski M, Bossert J, Milosevski D, Gruevska N. Preparation and properties of dense and porous calcium phosphate. Ceram Int 1999;25(8):693–6.

[8] Ramay HR, Zhang MQ. Preparation of porous hydroxyapatite scaffolds by combination of the gel-casting and polymer sponge methods. Biomaterials 2003;24(19):3293–302.

[9] Tian J, Tian J. Preparation of porous hydroxyapatite. J Mater Sci 2001;36(12):3061–6.

[10] Fukasawa T, Deng ZY, Ando M, Ohji T, Goto Y. Pore structure of porous ceramics synthesized from water-based slurry by freeze-dry process. J Mater Sci 2001;36(10):2523–7.

[11] Kang H-W, Tabata Y, Ikada Y. Fabrication of porous gelatin scaffolds for tissue engineering. Biomaterials 1999;20(14):1339–44.