Key Engineering Materials Vol. 587 (2014) pp 63-68 © (2014) Trans Tech Publications, Switzerland
doi:10.4028/www.scientific.net/KEM.587.63

Comparative critical study of commercial calcium phosphate bone substitutes in terms of physico-chemical properties

Thomas Miramond[1,2a], Pascal Borget[2,b], Serge Baroth[2,c], Guy Daculsi[1,d]

[1]Inserm Nantes University, Dental Faculty, 1 place A.Ricordeau 44042 Nantes, France
[2]Biomatlante SA, 5 rue Edouard Belin, 44360 Vigneux-De-Bretagne, France

[a]thomas.miramond@univ-nantes.fr, [b]pascalborget@biomatlante.com,
[c]sergebaroth@biomatlante.com, [d]guy.daculsi@univ-nantes.fr

Keywords: calcium phosphate, microstructure, pore distribution, specific surface area, bone substitutes

Abstract. Physico-chemical characteristics impact directly or indirectly the bioactive properties of biomaterials, it is then essential to correlate it with their effect *in vivo*. A panel of biomaterials available on the market, based on Hydroxyapatite (HA) and Tricalcium phosphate (β-TCP) is studied in terms of surface area, hydrophilicity, porosity, zeta potential, crystalline phases and density. This study highlights the disparity of commercial calcium phosphates (CaP) properties, and demonstrates how the quality criteria required for such bone substitute based on biomimicry concept, whose pores distribution is certainly the more relevant, are often incompletely or not respected according to literature.

Introduction

Since the advent of synthetic apatitic biomaterials from academic research in the last five decades, many bone substitutes have been developed either in single crystalline phase such as Hydroxyapatite or β-Tricalcium phosphate (β-TCP), biphasic calcium phosphate HA/ β-TCP (BCP) or even multiphasic with presence of alpha-TCP or bioglass in addition of classical HA/ β-TCP [1]. Although intended to the same use and therefore the same purpose, that is to say bone regrowth, the characteristics claimed differ significantly from one manufacturer to another, from one product to another. That is why this characterization study was conducted to assess the fundamental differences in terms of physicochemical properties correlated to biological implication *in vivo* forward from experts in the field through published data. As the most commonly used crystalline phases are HA and β-TCP, we focused on commercial samples made of these phases exclusively. 4 main properties were then assessed according to literature; the first one was the specific surface area (SSA) which is linked to proteins adsorption involving specific cell response [2]. The second one was the ratio HA/TCP implying bioactivity and absorption rate change *in vivo* since HA is less resorbable than β-TCP. Moreover the calcium release is also strongly dependent of this ratio for the same reason [3]. Absorption rate is a combination of chemical dissolution and resorption by osteoclastic cells and/or macrophages [4]. The third one was the porosity; this is considered as a fundamental parameter and certainly the most important of structural properties. Foremost the macroporosity, as commonly defined between 100 and 500 microns [5-6], involves cell invasion in the concavities of scaffold and thus homogeneous bone regrowth in its volume. The biomaterial will chemically dissolve in biological fluids especially thanks to micropores therein. This second class of porosity is about to set it in a range between 100 nm and 5µm [5-6]. The micropores does not affect the cell invasion, because it is too narrow, but the penetration of body fluids containing proteins, growth factors and bioactive adsorbable biomolecules. It is the combination of a controlled macroporosity with homogeneous microporosity that offers the best configuration to optimize bone regrowth, vascularization and subsequent absorption of biomaterial by host environment [7].

Materials and Methods

Twenty-four commercial calcium phosphate samples were collected to be analyzed. All these scaffolds are intended to be used as human bone substitute. Description and provenance are listed in Table 1.

X-ray diffraction was performed using Copper Kα radiation (λ=1.5418 Â) with a scan rate of 2s per step and a step size of 0.02° (2θ) on a PW 1830 X-ray generator (Philips).

The samples were sputter coated with gold-palladium before being visualized using SEM (LEO 1450VP) to assess granule and grain size and morphology. An SEM apparatus equipped with an energy dispersive X-ray microanalysis system (EDX Inca x-sight, Oxford Instruments) was used on all samples to determine trace elements and ascertain purity.

SSA was measured using the BET method by nitrogen adsorption at 77 K (ASAP 2010, Micromeritics).

Pore distributions were assessed by mercury intrusion porosimetry (Autopore IV 9500, Micromeritics). Prior to analysis, the samples were degassed to remove physisorbed gases. A low pressure test was first performed, followed by a high pressure test (varying from 0 to 30000 psi). Bulk density was measured after low pressure cycle.

Skeletal density was evaluated by helium pycnometry using 5 measurment for each sample (200mg) (AccuPyc 1330, Micromeritics).

Being limited in terms of scaffold quantity, destructive mercury intrusion porosimetry was only performed on five representative samples: MBCP, MBCP+, Interpore200, Ceraform and Ceraform Revolution.

Table 1: Panel of analyzed commercial bone substitutes

	MBCP	MBCP+	Ceraform	Interpore200	Cross-Bone	Bongros
Manufacturer	Biomatlante	Biomatlante	Teknimed	InterPore	Biotech International	BioA
Morphology	granules	granules	granules	granules	granules	granules
Size	0,5-1 mm	0,5-1 mm	3 mm	0,425-1 mm	0,5-1 mm	5-6 mm

	Bicalphos	Syncera	BoneMedik	Granulado	Calciresorb 35	Ceraform Revolution
Manufacturer	Medtronic	Oscotec	Meta Biomed	Keramat	Ceraver	Teknimed
Morphology	granules	granules	granules	granules	granules	granules
Size	0,5-1 mm	0,4-1mm	0,5-1 mm	0,5-1,4mm	2-3 mm	5-10mm

Results

Macro-microstructure visualization by SEM

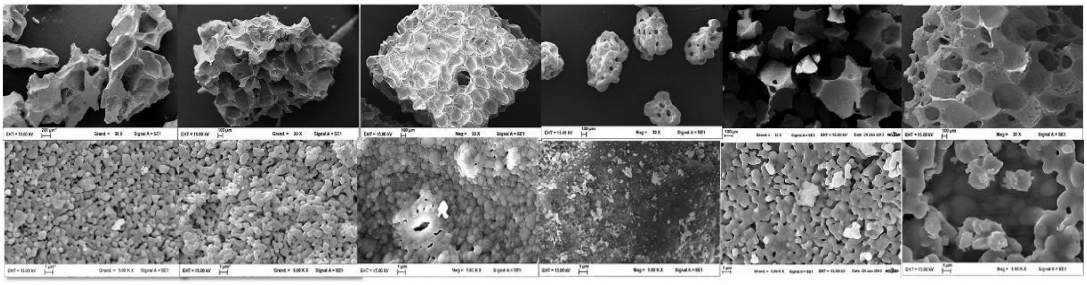
It obviously appeared macro and microstructures vary a lot from one scaffold to another. Some granules are round shape (Syncera), others cubic (Ceraform), coral-like (BoneMedik) or without particular morphology. Crystallographic grain size were very different, as shown in Figure 1, from 0,5 to more than 10µm. Presence of both micropores and macropores was observed only in 4 of the 12 total analyzed scaffolds: MBCP, MBCP+, Cross-Bone and BCP Bicalphos.

Specific Surface Area (SSA) evaluation by BET

The specific surface areas measured range from a few square meters per gram of material as noticed in Figure 1. Biomaterials having smaller crystallographic grains and high macro-micro porosity are

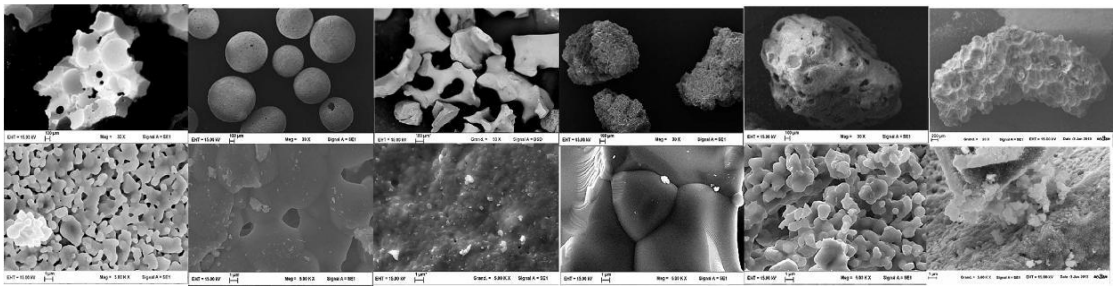
those having a higher specific surface area such as MBCP, MBCP+, Bicalphos and Cross-Bone. Ceraform Revolution scaffold was found to be constituted by a nanoscale crystals layer which provided highest specific surface are but no microporosity was observed by SEM.

A



A	MBCP	MBCP+	Ceraform	Interpore200	Cross-Bone	Bongros
Composition announced	HA 60 TCP 40	HA 20 TCP 80	HA 65 TCP 35	HA > 90 TCP < 10	HA 60 TCP 40	HA 100 TCP 0
Ratio HA/TCP measured [%]	HA 63 TCP 37	HA 21 TCP 79	HA 64 TCP 36	HA 100 TCP 0	HA 64 TCP 36	HA 100 TCP 0
Grain size [µm]	0,5 - 1	0,5 - 1	0,5 -1	hetero-geneous	0,5-1	1,5-3
SSA [m^2/g]	5	6	6	4	3	2
Type of porosity	Macro-Micro	Macro-Micro	Macro	Meso-Nano	Macro-Micro	Macro

B



B	Bicalphos	Syncera	BoneMedik	Granulado	Calciresorb 35	Ceraform Revolution
Composition announced	HA 60 TCP 40	HA 0 TCP 100	HA 60 TCP 40	HA 0 TCP 100	HA 65 TCP 35	HA 65 TCP 35
Ratio HA/TCP measured [%]	HA 62 TCP 38	HA 0 TCP 100	HA 39 TCP 61	HA 0 TCP 100	HA 89 TCP 11	HA 64 TCP 36
Grain size [µm]	1,5-3	1-5	0,5-1	5-20	0,5-2,5	Hetero-geneous
SSA [m^2/g]	4	3	< 1	< 1	2,5	8
Type of porosity	Macro-Micro	Micro	none	none	Meso-Micro	Macro-Nano

Figure 1: XRD, BET and SEM analysis of commercial scaffolds

Density and pore distribution by mercury intrusion porosimetry and pycnometry

The pore distributions obtained by mercury porosimetry highlighted the clear differences between the bone substitutes. The proportion of macropores superior to 100 micrometers may vary from 15% for Ceraform to 65% for Interpore and the proportion of micropores between 100 nanometers and 1 micrometer ranging from 10% for Interpore to almost 70% for MBCP. It should be also noted that some biomaterials are mesoporous and nanoporous such as Ceraform and Intepore, but none of them possess micropores between 5 and 1 micron as seen in Figure 2.

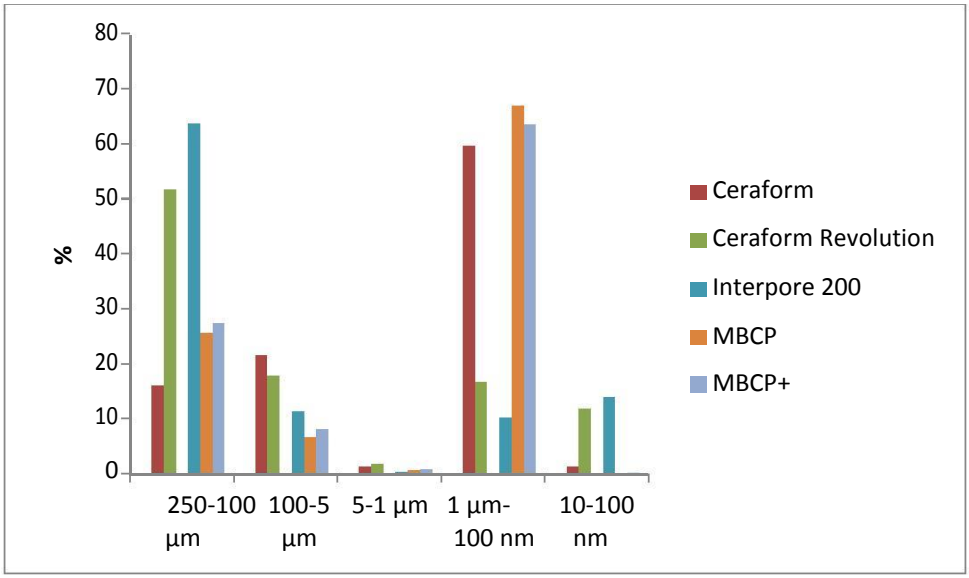


Figure 2: Pore distribution of 5 bone substitutes by mercury porosimetry

Skeletal density measurements by helium pycnometer coupled with bulk density measurements by mercury intrusion porosimétrie were used to calculate the total porosity of 5 scaffolds as described in Table 2. These scaffolds have very different total porosity ranging from 37% for Ceraform Revolution to almost 75% for MBCP+.

Table 2: Density measurements and total porosity calculation of 5 bone substitutes

	Bulk density [g/cm^3]	Skeletal density [g/cm^3]	Total Porosity [%]
Ceraform	1,89	3,01	37,2
Ceraform Revolution	1.52	3,15	51,7
Interpore 200	1.05	3,13	66,4
MBCP	0.79	3,05	74.1
MBCP+	0.77	3,05	74.7

Discussion

The results are coherent towards correlations between observations by electron microscopy, specific surface area measurements and porosimetry. The importance of macroporosity and microporosity is widely argued in the literature regarding the properties of cell invasion and angiogenesis [6-8]. Some authors believe that concavities macropores play a direct role in osteoinduction [9]. It appears that some scaffolds are non-apatitic, while the mineral phase of bone is formed of organic non-stoichiometric apatite [3]. We can thus discern biomaterials using a biomimetic approach by the presence of hydroxyapatite crystalline phase, alone or combined with TCP, of those who do not have hydroxyapatite at all. The highly bioactive apatitic dissolution-precipitation phenomena described in the literature [10] intervene especially with a balanced biphasic composition HA/TCP. Indeed, according to their kinetics of dissolution HA is more stable as apatite phase and TCP has a faster absorption rate. HA/TCP ratio were often verified as just but sometimes measurement

significantly differed from announced composition. The controlled HA / TCP ratio is nevertheless fundamental to influence and predict *in vivo* behavior [11]. Homogeneity is also a fundamental factor as it improves the reproducibility and repeatability, some were showing microstructural heterogeneities with high variation of crystallographic grains size and heterogeneous pore distribution. Sometimes some granules presented mesopores instead of macropores and nanopores instead of micropores, and then are responding only partially to the quality criteria necessary for optimal bone growth as described in the literature [6]. Moreover, the distribution of pores can be relevant only if the volume of pores is high, that is to say, if the bulk density is low. Hence, some scaffolds have adequate pore distribution but a low overall pore volume. Bioceramics that best meet these criteria of porosity and controlled bioactive composition in this panel are then MBCP, MBCP+, Cross-Bone and Bicalphos.

Conclusion

It emerges from this study that there is high variability in the properties of bone substitutes available to surgeons on the market. These differences are very important since they have a direct impact on the response of host tissues. In particular, it is apparent that the dual macro-micro porosity, essential to the kinetic balance between invasive bone growth and the resulting scaffold absorption, is rarely observed in these implantable medical devices. This is because of oversized crystallographic grains closing intrinsic microporosity or the total or partial absence of macropores from process engineering. Some biomaterials also have a heterogeneous microstructure which may involve non-repeatable and non-reproducible results, though it is a predominant factor for clinical use. Finally, the measured surface charges also vary greatly and require further studies to predict protein adsorption behavior of biological fluids and so cell response. It is then necessary for clinicians to have enough critical insight and scientific information regarding the offers of bone substitutes to optimize the effectiveness of their interventions.

Acknowledgements

The research leading to these results has received funding from the European Union's 7th Framework Programme under grant agreement no FP7-HEALTH-2009-241879 (REBORNE) Authors would like to thank Paul Pilet, Hélène Terrisse, Stéphane Grolleau, Nicolas Stéphant and Eric Chevrel for their help to this work.

References

[1] S. Dorozhkin, Biphasic, triphasic and multiphasic calcium orthophosphates. Acta Biomaterialia. (2012) 963-977

[2] O. Malard, H. Gautier , G. Daculsi, In Vivo Demonstration of 2 Types of Microporosity on the Kinetic of Bone Ingrowth and Biphasic Calcium Phosphate Bioceramics Resorption Key Engineering Materials.1233(2007) 361-363.

[3] R.Z. LeGeros, S. Lin, R. Rohanizadeh, D. Mijares, J.P. LeGeros. Biphasic calcium phosphate bioceramics: preparation, properties and applications. Journal of Materials Science: Materials in Medicine. 14(3) (2003) 201-209.

[4] S. Yamada, D. Heymann, J.M. Bouler, G. Daculsi, Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatite/beta-tricalcium phosphate ratios. Biomaterials. 18(15) (1997)1037-1041

[5] A. Uchida, S. Nade, et al. Bone ingrowth into three different porous ceramics implanted into the tibia of rats and rabbits. Journal of Orthopaedic Research. 3(1) (1985) 65-77.

[6] G. Daculsi, S. Baroth, et al. 20 years of Biphasic Calcium Phosphate Bioceramics Development and Applications. Advances in Bioceramics and Porous Ceramics II. (2009) 45-58

[7] P. Ducheyne, S. Radin, L. King, The effect of calcium phosphate ceramic composition and structure on in vitro behavior. I. Dissolution. Journal of Biomedical Materials Research 27(1) (1993) 25-34.

[8] M.C. Von Doernberg, B. Von Rechenberg, M. Bohner, S. Grünenfelder, G.H. van Lenthe, R. Müller , et al, In vivo behavior of calcium phosphate scaffolds with four different pore sizes. Biomaterials. 27 (2006) 5186-98.

[9] P. Habibovic, K. de Groot, Osteoinductive biomaterials—properties and relevance in bone repair. J Tissue Eng Regen Med. 1 (2007)25-32.

[10] G. Daculsi, R.Z. LeGeros, E. Nery, K. Lynch, B. Kerebel, Transformation of biphasic calcium phosphate ceramics in vivo: ultrastructural and physicochemical characterization. Journal of biomedical materials research 23, (1989) 883.

[11] A.M. Barradas, H. Yuan, C.A. van Blitterswijk, P. Habibovic, Osteoinductive biomaterials: current knowledge of properties, experimental models and biological mechanisms. European cells & materials 21 (2011) 407.