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## The Relevance of Physical Characterisation in the Evaluation of the Performance of Bone Allografts

F. Judas<sup>1</sup>, A. Proença<sup>1</sup>, M. Moura<sup>2</sup>, A. Piedade<sup>2</sup> and M. Figueiredo<sup>3</sup>

<sup>1</sup> Serviço de Ortopedia do Hospital da Universidade de Coimbra (HUC), Coimbra, Portugal

<sup>2</sup> Instituto Pedro Nunes, Rua Pedro Nunes, PT-3030-199 Coimbra, Portugal

<sup>3</sup> Universidade de Coimbra, Departamento de Engenharia Química,  
Faculdade de Ciências e Tecnologia, Pinhal de Marrocos,  
PT-3030-290 Coimbra, Portugal

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**Abstract.** The present work compares bone allografts with synthetic hydroxyapatite regarding density, porosity, pore size distribution and phase identification. The measurement of these parameters enables a more complete knowledge of the grafts structural properties, which necessarily leads to a better selection of the most appropriate bone substitutes.

### Introduction

Orthopaedic surgeons are frequently confronted with bone defects originated by congenital traumatic and tumoral processes. These defects need to be reconstructed through an appropriate filling which enables good bone consolidation and good function recuperation.

For this purpose autogenous bone is currently regarded as the optimal material. Autografts are, however, limited in the availability of material and may result in the donor site morbidity. Allografts, in turn, are available in different forms, types and dimensions and thus constitute a good alternative. Bone grafts prepared from natural and synthetic materials have also been used to treat small defects; however, in cases that require structural or biomechanical support their clinical use is limited [1].

Although allografts are currently used, very little is known about their physical properties, in contrast to the vast information available in the literature for biomaterials [2,3].

This motivated the present work which presents a comparative study between different allografts and a synthetic material (hydroxyapatite), in terms of density, porosity, pore size distribution and phase identification.

### Experimental

Three kinds of freeze-dried femoral allografts – morcellised cancellous and undecalcified and decalcified cortical bones – provided by HUC bone bank were analysed. The reason for this selection was to study samples with quite different characteristics: very porous, like the cancellous bone, and very compact, like the cortical segments. The decalcification process of the latter consisted in immersing the undecalcified bone in 0.6 M HCl aqueous solution for 72 hours (this procedure also minimises potential risks of disease transmission).

A calcium phosphate ceramic – hydroxyapatite (*Ceros*<sup>R</sup>) – was chosen as a synthetic bone substitute. This was supplied in granular form and characterised in terms of particle size by image analysis (*CUE2/Olympus*), the mean equivalent spherical diameter (number weighted) being 2 mm. These materials are illustrated in Fig. 1 and 2.

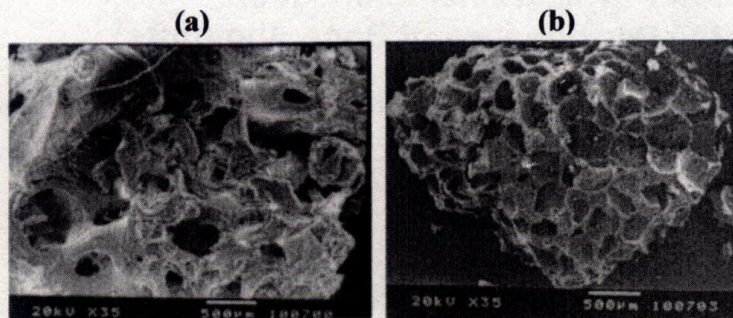


Fig. 1 – Scanning electron micrographs (SEM) of cancellous bone (a) and hydroxyapatite (b).

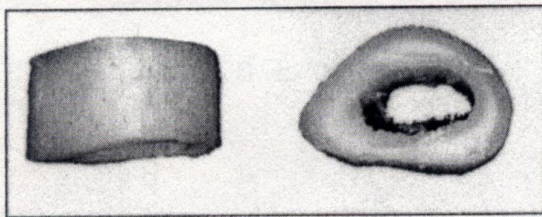


Fig. 2 – Segment of cortical bone (longitudinal and cross section views).

The following techniques were used in the physical characterisation:

- helium pycnometry (*Accupyc / Micromeritics*) to determine the materials absolute density (mass of material divided by the solid volume, irrespective of interparticle spaces and pores) [4];
- mercury porosimetry (*Poresizer 9320 / Micromeritics*) to measure the bulk density (which includes all the voids), porosity and pore size distribution, in the range of 1 to 1000  $\mu\text{m}$  [4];
- X-ray diffraction (*Philips X'Pert* diffractometer,  $5^\circ < 2\theta < 120^\circ$ ) to provide a qualitative phase identification [5].

## Results and Discussion

Table 1 shows the values obtained for the absolute and bulk density as well as for the porosity.

Table 1 – Absolute density, bulk density and porosity of the samples studied.

SAMPLES	Absolute Density ( $\text{g}/\text{cm}^3$ )	Bulk Density ( $\text{g}/\text{cm}^3$ )	Porosity (1-1000 $\mu\text{m}$ ) (%)
Undecalcified cortical	1.96	1.88	3
Morcellised cancellous	1.76	0.97	41
Decalcified cortical	1.36	0.83	29
Hydroxyapatite	2.93	1.02	49

These results lead to the subsequent conclusions:

- regarding the absolute density, hydroxyapatite presents the highest value, considerably larger than the allografts. This is most probably related not only to differences in chemical composition but mainly to distinct molecular arrangements (as discussed below);
- with respect to the bulk density, the undecalcified cortical bone exhibits the largest value (approximately double from the other samples);
- the differences between the above densities are obviously reflected on the porosity of each sample. The maximum values of this parameter were obtained for hydroxyapatite and cancellous bone, and the minimum for undecalcified cortical bone. The considerable increase in porosity observed after decalcification of this bone ( $\approx 10$  fold) is evident in the SEM of Fig. 3.

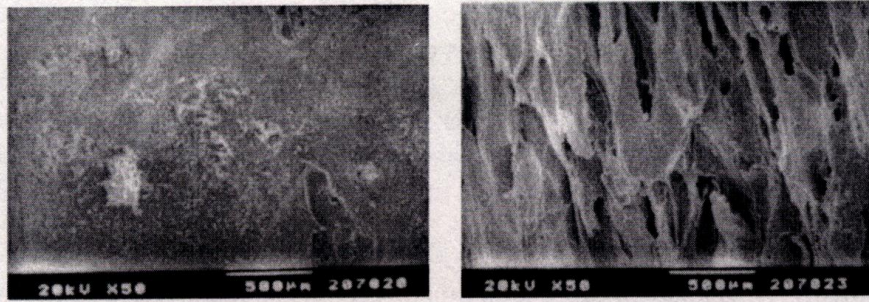


Fig. 3 - SEM of undecalcified and decalcified cortical bone.

The analysis of the various X-ray diffraction patterns (Fig. 4) reveals that although the allografts present identical phase constitution as that of the hydroxyapatite, their peaks are broader in consequence of the lower degree of structural order. In the decalcified bone the peaks corresponding to the calcium phosphate were not detected.

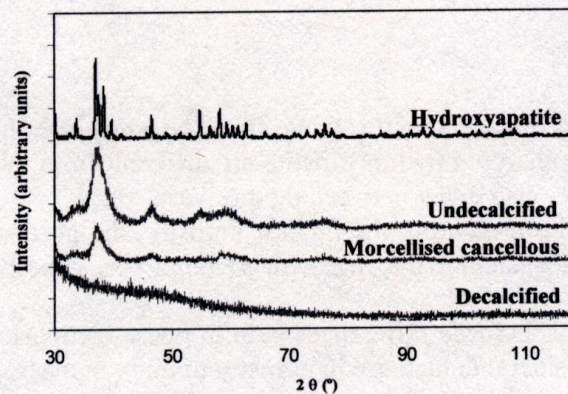


Fig. 4 – X-ray diffractograms of the various samples.

Frequently, more important than knowing the porosity of the material, it is to know its pore structure. The pore size distribution curves are displayed in Fig. 5 for all samples, with the exception of undecalcified cortical bone since this presented a very low porosity (undetectable in the graph).

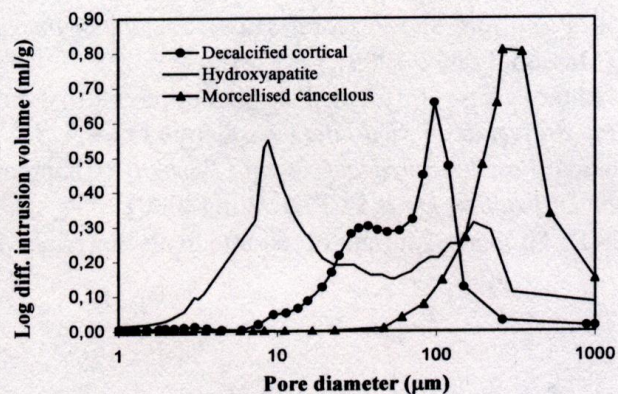


Fig. 5 - Pore size distribution curves for the various samples.

As it can be seen, different materials possess pores in different size ranges, some being bimodal. This type of information is quite relevant, as it is often mentioned that pores smaller about 100  $\mu\text{m}$  are not effective for osteointegration [6]. Table 2 summarises the results of mercury intrusion in terms of total porosity, percentage of pores larger than the quoted minimum and modes of the pore size distribution curves. Despite the fact that hydroxyapatite exhibits the largest porosity of all samples, the percentage of pores larger than 100  $\mu\text{m}$  is only about 30 %, whereas for the cancellous allograft that percentage is higher than 90 %.

Table 2 – Porosity, percentage of pores larger than 100  $\mu\text{m}$  and modes of pore size distribution.

SAMPLES	Total Porosity (1-1000 $\mu\text{m}$ ) (%)	% Pores > 100 $\mu\text{m}$	Distribution Modes ( $\mu\text{m}$ )
Morcellised cancellous	41	93	250
Decalcified cortical	29	30	100
Hydroxyapatite	49	32	8.5

### Conclusions

From the physical parameters evaluated in this work, it can be concluded that:

- the characteristics of the analysed hydroxyapatite are different from the allografts, although the characteristics of the latter also differ between themselves;
- besides the hydroxyapatite presenting the highest porosity, the pore size distribution does not seem to be the most adequate for the ingrowth of bone (i.e. penetration of cells and living tissue);
- the decalcification process induced differences both in phase composition and in the bone pore structure, denoting a considerable increase in porosity (mainly in macroporosity).

Although the biological and chemical properties are crucial in the search of a grafting material that could closely mimic natural bone, physical properties, as those measured in this work, also play an important role. Furthermore, their knowledge is most relevant to fully interpret the mechanisms of bone regeneration, as well as to evaluate the results of experimental surgery.

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