Porous HA ceramic for bone replacement: Role of the pores and interconnections – experimental study in the rabbit

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Hydroxyapatite (HA) porous ceramics are increasingly used in biomedical applications. Their physical characteristics, such as porous volume, require perfect control of the pore shape, as well as the number and the size of their interconnections.

The aim of our study was to evaluate a new HA ceramic using polymethylmethacrylate microbeads (PMMA) as the porous agent. Four interconnection sizes (30, 60, 100 and 130 μm) with a 175–260 μm pore size and three pore sizes (175–260, 260–350 and 350–435 μm) for a 130 μm interconnection size were tested. Various HA implants were appraised by microscopic evaluation in a 4.6 × 10 mm rabbit femur cancellous bone defect 12 weeks after implantation. The best osteoconduction result was obtained in the center of the ceramic by means of a 130 μm interconnection size and a 175–260 μm mean pore size. Bone formation obtained within the pores was double that obtained in our previous study where naphtalen microbeads were used as the porous agents.

1. Introduction

The physical characteristics for development of porous ceramics as bone substitutes in biomedical applications depend on the porous volume of the biomaterial, as well as the mean pore and interconnection sizes. Moreover, creation of bone substitutes with optimal properties requires perfect control of these parameters [1, 2].

In previous studies, the influence of pore and interconnection sizes on cellular and tissular recolonization was investigated for hydroxyapatite (HA) and tricalcium phosphate β (β-TCP) ceramics with equivalent physical characteristics [3, 4]. These ceramics were obtained by mixing phosphate calcium powder with naphtalen as the porous agent. In fact, the distribution of macroporosity inside implants is not homogeneous and the average porous volume is not well controlled if the interconnection size is too small. To optimize biological efficiency in a larger range of bone applications, good control of the mean pore and interconnection sizes is required.

The aim of our study was to evaluate bone recolonization and the efficiency of the mean pore and interconnection sizes in a newly elaborated HA porous ceramic using PMMA microbeads as the porogen agent. After implantation in our experimental model, the distal cancellous bone site of the rabbit femur, four interconnection sizes, 130, 100, 60 and 30 μm with the same pore diameter size (PDS) (175–260 μm) were tested. However, to verify the quantity and quality of newly elaborated bone tissue within the pores, three pore diameter sizes (PDS) 175–260, 260–350 and 350–435 μm with one interconnection size, 130 μm, were evaluated.

2. Materials

2.1. Biomaterials: the influence of the interconnection size

Eighteen HA cylinders, 10 mm in length and 5 mm in diameter, were used. Four interconnection sizes: 30 μm (n:7); 60 μm (n:12); 100 μm (n:10); 130 μm (n:7) with the same 175–260 μm PDS were carried out.

2.2. Biomaterials: the influence of the pore size

Nine HA cylinders, 10 mm in length and 5 mm in diameter, two PDS, 175–260 μm (n:9) and 350–435 μm (n:8) with one interconnection size, 130 μm, were evaluated (Table I).

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TABLE I Microstructure of HA ceramics

Ceramic HA	Porosity (%)	Mean size (μm)		Number of Implants
		Macropores (PDS)	Interconnections (DICS)	
Constant pore size with variable interconnection size	66	175–260	130	7
	65	175–260	100	10
	64	175–260	60	12
	61	175–260	30	7
Constant interconnection size with variable pore size	66	175–260	130	7
	65	264–352	130	9
	65	352–440	130	8

2.3. Animals

Twenty-seven female adult New Zealand white rabbits with controlled sanitary status were fed with standard rabbit chow pellets and tap water *ad libitum*.

3. Methods

3.1. Surgical procedure

Under general anaesthesia, the animals were operated on, on both sides of the distal cancellous bone in the rabbit femurs. A manually-drilled cavity of 4.6 × 10 mm was created and carefully washed clean of bone debris then dried with a compress before being filled with HA cylinders [5].

3.2. Technical preparation of specimens

After sacrifice, distal femurs were harvested, cleaned of soft tissue and fixed in 10% paraformaldehyde solution pH:7.2 (Prolabo-France). The bone segments were dehydrated and embedded in methylmethacrylate without decalcification.

3.3. Histomorphometric evaluation

Two 20 μm thick medial frontal stained sections (Picro-Fuschine van Gieson) were analyzed. This coloration differentiates the mineralized bone (orange colored) from the osteoid tissue (green colored). Different histomorphometric parameters were measured by the point counting technique using an integrating eyepiece [6].

At T12 weeks after implantation:

- The occupied pore volume: OPOV in %: the pore volumes occupied by cells, fibroconnective tissue or bone tissue over the ceramic volume.
- The occupied bone tissue volume OBTV in%: represents the pore volume occupied by bone tissue (mineralized + osteoid tissue) over the total occupied pore volume.
- The mineralized bone tissue volume OMBTV in %: represents the pore volume occupied by mineralized bone tissue (without osteoid tissue) over the pore volume occupied by the bone tissue volume.
- The fibroconnective tissue volume OFTV in %: represents the pore volume occupied only by fibroconnective tissue over the total occupied pore volume.

3.4. Qualitative microscopic observation

The quantitative data was collated with microscopic observation on microradiographs, stained sections (Picro-Fuschine van Gieson), white sections under ultraviolet light but also in polarization light.

3.5. Statistical analysis

The results are expressed as the mean ± standard deviation (SD). Differences between the groups were assessed by the Mann-Whitney U test for two groups and by Krüskal-Wallis variance followed by Dunn's test for several groups. A minimum of $p < 0.05$ was required for significant difference.

4. Results

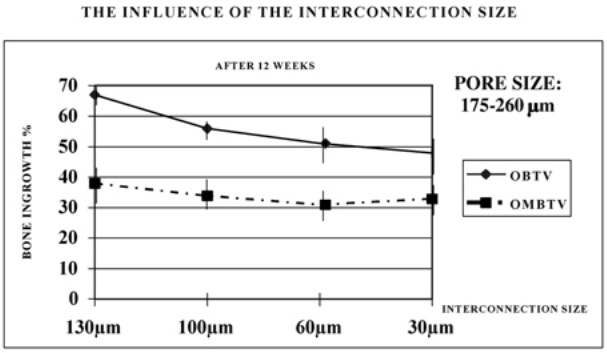
4.1. The influence of the Interconnection size

At 12 weeks, in all interconnection groups, the pores are invaded by cells and tissue. A significant difference of Krüskal-Wallis variance between groups was observed and Dunn's test shows occupied pore volume (OPOV) for the 130 μm interconnection group to be higher than the values for the 60 μm and 30 μm groups ($p < 0.05$). No difference is observed between the 130 μm and the 100 μm interconnection groups but the 100 μm interconnection group has an OPOV higher than that of the 60 μm group ($p < 0.05$). Among the pores occupied by fibroconnective tissue, the 30 μm group has a higher OPOV value than that of the 60 μm group ($p < 0.05$). Mineralized bone tissue in the 130 μm group is higher than in the 60 μm and 30 μm groups ($p < 0.05$) (Fig. 1).

4.2. The influence of pore size

Compared with the 350-435 μm pore size, the OPOV is significantly lower in the 175–260 and 260–350 μm pore groups but no difference is seen between the 175–260 and 260-350 μm groups (Fig. 2).

These results are consistent with those of our previous study where, from a biodegradable β-TCP porous ceramic, good correlation was observed at 12 weeks, with an interconnection size evaluated at 140–150 μm and mineralized bone tissue at 20.77% ($r = 0.99$; $p = 0.00$). In the present study, our result confirms that the 130 μm interconnection size promotes cellular and tissular activity inside pores, as was previously estab-



OBTV :occupied bone tissue volume inside the pores
OMBVT: occupied mineralized bone tissue volume inside the pores

Figure 1 Bone ingrowth according to the interconnection size (175–260 µm pore diameter).

lished. We must still find the pore diameter size (PDS) capacity which will carry mineralized bone tissue into the heart of the material, not only in quantity but also in quality. Our result favours a 175–260 µm mean PDS with a mineralized bone tissue of 39% and with a two-fold increase in bone tissue edification compared to that of the former study, which used naphtalen microbeads as the porous agent for the same pore and interconnection mean size (Fig. 3). Indeed, when the interconnections are insufficient because of a too-small density, in relation to pore size, fibrous connective tissue invades the heart of the ceramic without any bone tissue differentiation. This example is clearly seen in 30 µm interconnection sizes where the fibroconnective tissue rate is estimated at 14.16% (Figs 4 and 5).

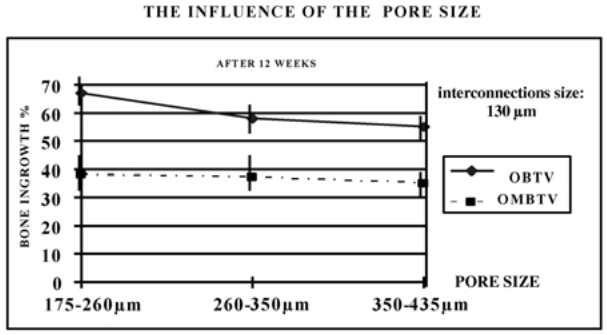


Figure 2 Bone ingrowth according to the pore size (130 µm interconnection size).

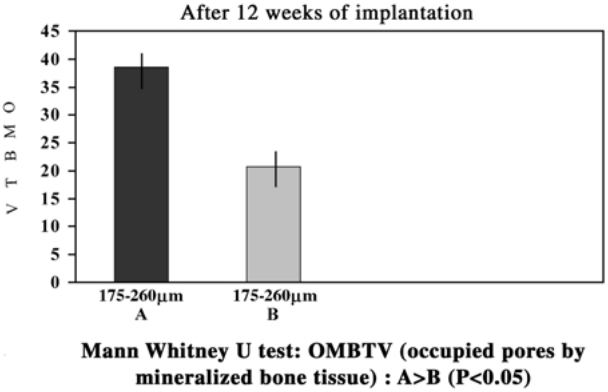


Figure 3 Well-controlled microstructure doubles bone recolonization for the same pore and interconnection size.

5. Discussion

The newly developed HA ceramic with PMMA as the porous agent improves bone tissue recolonization at the heart of the ceramic after 12 weeks of implantation. The good size of the pathways from one to several other pores has been seen to promote cellular and vascular penetration and is evaluated at 130 µm mean size after being tested under the same biological conditions. As a matter of fact, to ensure the link between pores, the density of the interconnection must be large and have a 130 µm interconnection mean size before implantation in order to immediately encourage the cells and fibroconnective tissue of the bone host to be recolonized by bone tissue as fast as possible. Under our investigative conditions, to guarantee not only the best quantity of receiving bone host but also its quality right through to the center of the material, the mean PDS has been estimated at 175–260 µm. In slowly degradable HA porous ceramics, the size of pores and interconnections must be well controlled during their fabrication because of slow modification after implantation in living bone tissue. Apart from its chemical composition, which is similar to the mineral components of bone and because of its slight degradation, the microarchitecture of HA ceramic must be well established before being implanted. The density of pores and interconnections in this material is also important because these two parameters guarantee the finality of its use as a bone substitute. It is also known that this HA ceramic material is brittle and has bad biomechanical properties so its efficiency as a bone substitute will mainly consist of its fast bone host recolonization. Another improvement in this type of bone substitute might be to increase the osteogenic potential by incorporating marrow-derived mesenchymal

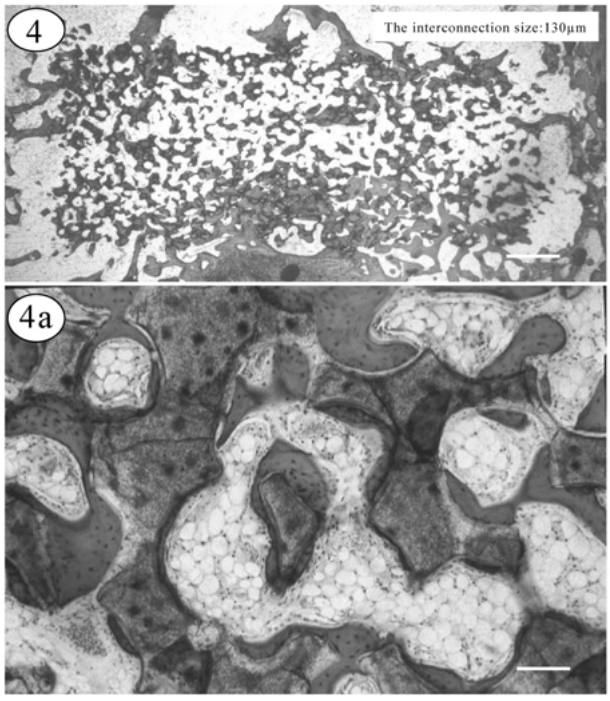


Figure 4 Stained sections (Picro–Fuschine van Gieson) after 12 weeks of implantation in a cancellous bone site: in Fig. 4, 175–260 µm pore size with 130 µm interconnection size promotes new cancellous bone at the heart of the ceramic (Fig. 4a) while in Fig. 5, the same pore size with 30 µm interconnection size shows fibrous connective tissue (Fig. 5a).

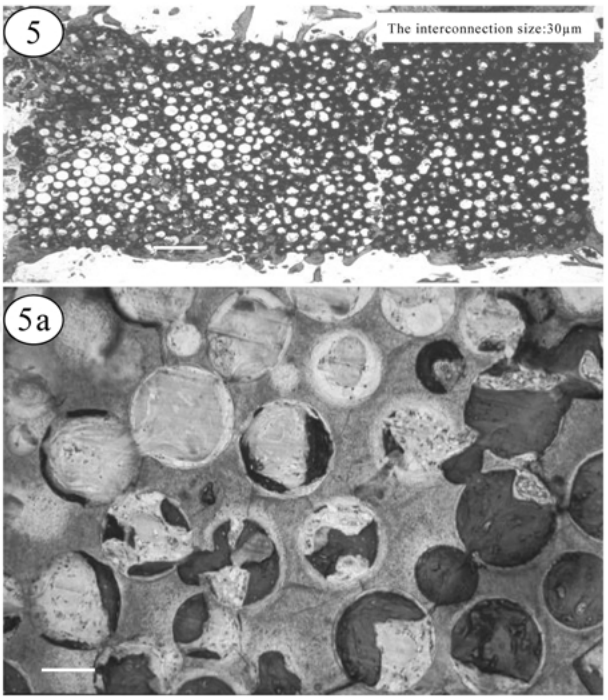


Figure 5 Stained sections (Picro-Fuschine van Gieson) after 12 weeks of implantation in a cancellous bone site: in Fig. 5, 175–260 μm pore size with 30 μm interconnection size shows fibrous connective tissue (Fig. 5a).

cells [7–9] or in associating them with demineralized bone matrix [10]. The authors highlight an enhancement of new bone formation and an increased healing rate of bone defects.

6. Conclusion

As a bone substitute for our skeleton, newly developed HA porous ceramic must have interconnection and pore sizes that are closely controlled during the fabrication process. Indeed, the density of these two parameters ensures its efficiency as a bone substitute through its speed of bone host recolonization. The best osteoconduction result in the center of the ceramic is obtained with a 130 μm mean interconnection size and a 175–260 μm PDS. A new process of fabrication with PMMA microbeads as the porous agent increases bone ingrowth inside the pores more efficiently than that of ceramics using naphtalen microbeads as the porous agent.

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