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ORIGINAL STUDY

Bone ingrowth into two porous ceramics with different pore sizes : An experimental study

Laurent Galois, Didier Mainard

From the University Hospital of Nancy, France

Many properties of porous calcium phosphate ceramics have been described, but how pore size influences bony integration of various porous ceramics remains unclear. This study was performed to quantify the bony ingrowth and biodegradability of two porous calcium phosphate ceramics with four different pore size ranges (45-80 µm, 80-140 µm, 140-200 µm, and 200-250 µm). Hydroxyapatite (HA) and β-tricalcium phosphate (TCP) cylinders were implanted into the femoral condyles of rabbits and were left *in situ* for up to 12 months. The percentage of bone ingrowth and the depth of ingrowth within the pores were determined. Biodegradability of the implants was also evaluated.

Bone ingrowth occurred at a higher rate into the TCP than into the HA ceramics with the same pore size ranges. The amount of newly formed bone was statistically smaller ($p < 0.05$) into ceramics with 45-80 µm pore size than with larger pore size, whatever the implantation time for HA and until four months for TCP. No statistical difference was noted between the three highest pore size ranges. No implant degradation was noted up to four months. Our results suggest that a pore size above 80 µm improves bony ingrowth in both HA and TCP ceramics. Bone formation was higher in the TCP than in the HA implants.

INTRODUCTION

Bone graft substitutes, especially calcium phosphate ceramics, are a useful alternative to biologic materials such as autografts (*23*), allografts (*22*) and xenografts, and natural materials such as coral (*6*) for filling bone defects in orthopaedic surgery (*10, 12, 14*). *In vitro* and *in vivo* studies (*2, 3*) have shown calcium phosphate ceramics to be fully biocompatible. The physical and chemical properties of these substances make them bioactive in that they are able to induce specific biologic reactions (*5*). In addition, calcium phosphate ceramics are osteoconductive and resorbable (*9, 20*) : they provide a scaffolding for new bone formation and their macroporosity allows cells to invade and bone to grow into the ceramic (*1, 3, 7*). The two major calcium phosphate ceramics, hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), have been investigated in animal studies (*15, 17-19, 26*) where they have been shown to be well tolerated and readily incorporated. However, there are fewer studies

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■ Laurent Galois, MD, Orthopaedic Surgeon.

■ Didier Mainard, MD, Orthopaedic Surgeon, Professor of Orthopaedics.

Department of Orthopaedic Surgery, University Hospital of Nancy, France.

Correspondence : Didier Mainard, Department of Orthopaedic Surgery, University Hospital of Nancy, 29 avenue Maréchal de Lattre de Tassigny, C.O. n° 34, F-54035 Nancy Cedex, France. E-Mail : lgalois@free.fr
didier.mainard@ciril.fr.

describing the influence of porosity on osseointegration (*1, 3, 7, 11, 24, 26*). We present an experimental study concerning the osseointegration of two porous calcium phosphate ceramics (HA and β-TCP) with four different pore size ranges. This study was performed to quantitate the bone ingrowth and biodegradability of the ceramics according to their pore size.

MATERIALS AND METHODS

Sixty New Zealand type rabbits weighing 3 kg with controlled sanitary status were fed with standard rabbit chow pellets and tap water *ad libitum*. The rabbits were divided into 2 groups (HA and β-TCP) for each interval time. Rabbits were killed after 2 weeks, 1 month, 2 months, 4 months, 6 months and 12 months. Cylinders 6 × 10 mm of HA or β-TCP were implanted into bone defects created with a drill in the femoral condyle under general anaesthesia. After shaving the knee joint, under aseptic conditions, a parapatellar skin incision was made on the medial side of the joint. An incision on the medial side of the patellar tendon provided access to the joint space and the patella was dislocated laterally. The knee was placed in full flexion and a hole 6 mm in diameter was drilled in the femoral condyle and carefully washed clean of bone debris before being filled with a ceramic cylinder (fig 1). Postoperatively, the animals were permitted cage activity without immobilisation. The animals were closely monitored for infection and other complications. All procedures were approved by the animal welfare committee. Hydroxyapatite (Synatite®) and b-tricalcium phosphate (Biosorb®) ceramics (S.B.M., Lourdes, France) were cast in cylinder form 6 mm in diameter and 10 mm in length. The porosity was 45% +/- 5%. Four pore size ranges were studied : 45-80 µm, 80-140 µm, 140-200 µm and 200-250 µm. All implants were sterilised by gamma radiation. Rabbits were killed after 2 weeks, 1 month, 2 months, 4 months, 6 months and 12 months. After the animal was sacrificed by injection, the femoral condyle was harvested and fixed in a 4% formaldehyde solution, then dehydrated successively with graded ethanol and toluene. Nondecalcified techniques were used for bone tissue preparation (*13*). Nondecalcified bone specimens were embedded in polymethylmethacrylate (PMMA) obtained by mixing methylmethacrylate (Osi, Saint-Quentin Fallavier, France), benzoyle peroxide (Merck, Darmstadt, Germany) and dibutylphthalate (Merck, Darmstadt, Germany). Nondecalcified 8-mm thick sections were made perpendicular to the long axis of the ceramic cylinder, using a Leica apparatus (Nuβloch, Germany) equipped with a tungsten knife. Solochrome-cyanine R and Masson’s Trichrome were then applied. Stained sections were studied under a light microscope (Olympus, Tokyo, Japan). Measurements were taken over the entire implant surface for histomorphometry. The magnification used for the measurements was × 20. Three parameters were studied : the depth of bone ingrowth into the ceramic (average of ten measurements), the surface of bone ingrowth, the relative surface of bone ingrowth (bone surface in the implant section/total implant section surface) and the rate of ceramic resorption. These parameters were studied for each pore size range, each interval time and each biomaterial. In each case, three sections were analysed and the average value determined. The results are expressed as the mean +/- standard deviation. Differences between groups were assessed by the Mann-Whitney *U* test for two groups and by Krüskal-Wallis variance for several groups. A minimum of $p < 0.05$ was required for a significant difference.

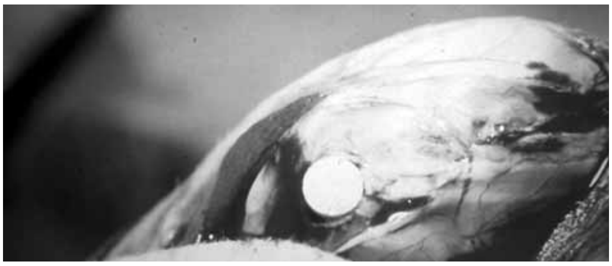

Fig. 1. — A hole 6 mm in diameter is drilled carefully in the femoral condyle and the ceramic cylinder is implanted.

RESULTS

No fibrosis or adverse inflammation was observed at any implantation time. No clinical problems occurred during the implantation period, except for one animal at one month, which developed an infection of the knee and was eliminated from the study. All other animals survived the observation period and did not show any signs of illness or adverse reaction to the implant material. Histological observation was performed for both biomaterials.

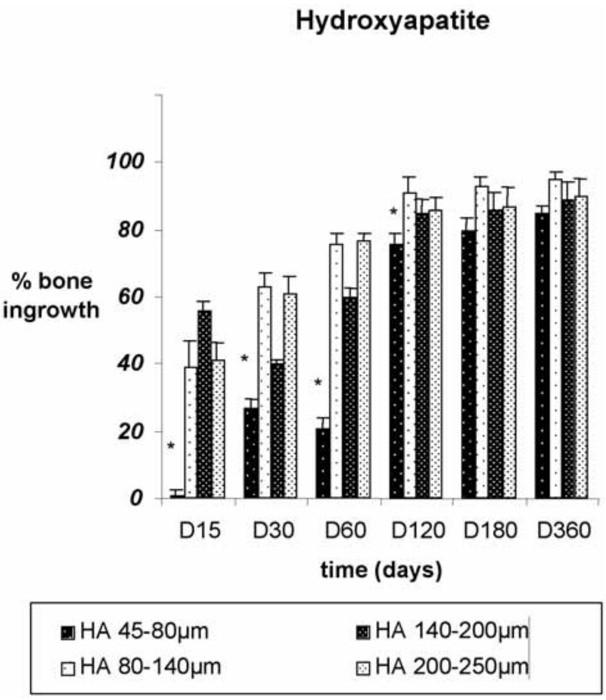

Fig. 2. — Percentage of bone ingrowth into HA implant

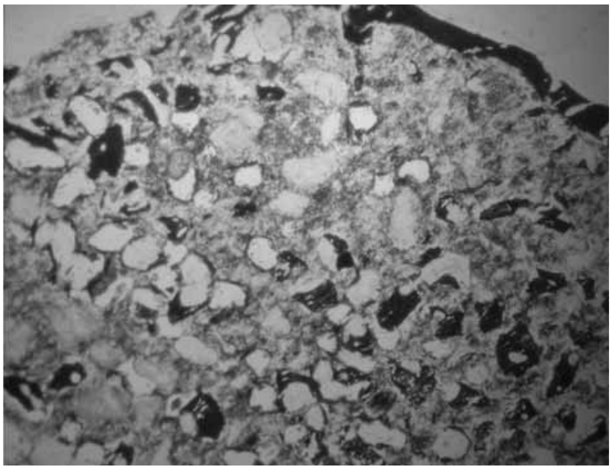

Fig. 3. — Stained section (Solochrome-cyanine R) after 12 months of implantation of the HA ceramic. The surface of the ceramic is almost completely colonised by newly formed bone.

Hydroxyapatite (fig 2 and 3)

After two weeks, newly formed bone was found around and in the periphery of the implants, except those with a pore size range of 45-80 µm. Tissue ingrowth into pores throughout the implants was seen from 80-140 µm pore size range with 39% rehabitation. There was a statistically significant difference ($p < 0.05$) regarding bone ingrowth between the smaller pore size range and the others. One month following implantation, bone formation seen within pores was slightly higher and trabecular bone was thicker and deeper than at two weeks, especially in ceramics with a pore size range of 80-140 and 200-250 µm. Morphometric analysis showed respectively 63% and 61% of bone ingrowth and a centripetal bony ingrowth of 1.12 and 1 mm in depth. Incipient bone formation was seen in implants with a pore size range of 45-80 µm. In the control hole, only fibrous tissue could be seen at one month.

At two months, the new bone tissue seen in the pores increased for each pore size range, except for the smallest. From 80-140 µm, bone ingrowth was clearly visible. Bone ingrowth reached 77% in the implants with 200-250 µm pore size.

At four months, there was still a statistically higher percentage of bone ingrowth into the three highest pore size ranges compared with the smallest. Nevertheless, significant new bone tissue was formed inside the 45-80 µm ceramic (76%). The depth of bone ingrowth into the cylinders was respectively 1.4 mm for the 45-80 pore size and 1.59 mm for the 200-250 µm pore size.

After six months, new bone tissue reached the centre of the implants with the three highest pore size intervals. Degradation of the ceramic was about 5%.

At 12 months, the surface of the ceramic was almost completely colonised by the newly formed bone. There was still a statistically ($p < 0.05$) lower bone ingrowth in the 45-80 µm pore size in comparison with the others. The percentage of ceramic resorption was limited to 10%. In the control hole, fibrous tissue was still visible in its centre.

β-Tricalcium phosphate (fig 4 and 5)

At two weeks, implants of β-TCP were already colonised by the newly formed bone, even those with the smallest pore size (21%).

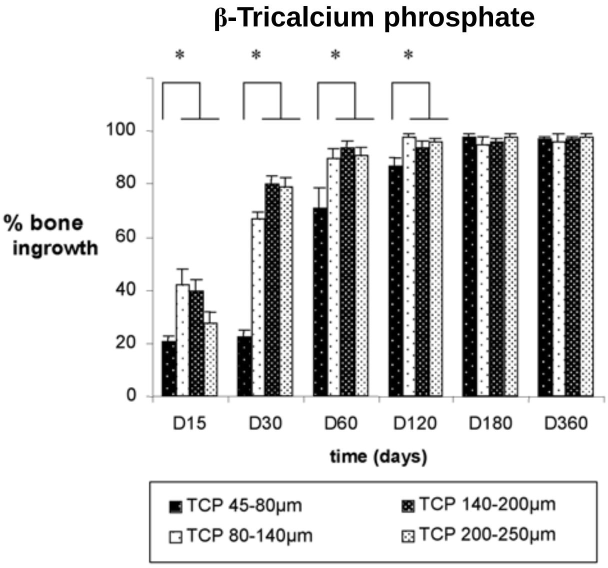

Fig. 4. — Percentage of bone ingrowth into the TCP implant

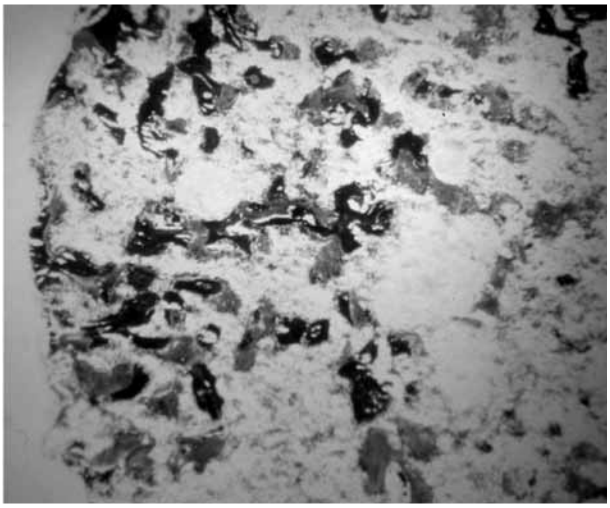

Fig. 5. — Stained section (Solochrome-cyanine R) after 12 months of implantation of the TCP ceramic. Note partial resorption of the TCP and bone ingrowth on the surface.

One month following implantation, the percentage of bone ingrowth increased with the pore size (23%, 67%, 80% and 79% respectively). All values were significantly higher in the three highest pore size ranges compared with the smallest ($p < 0.05$). Nevertheless, there were no statistical differences between the 80-140, 150-200 and 200-250 μm pore size ranges. The evolution of the depth of new bone formation was similar to the percentages with values superior to 1 mm for the three highest pore size ranges. Fibrous tissue was present within the control cavity, without any bone formation.

At two and four months, the trend was similar. Bone ingrowth reached more than 90%.

At six months, no difference was noted regarding bone ingrowth for all pore size ranges. The whole surface of the implant was colonised by newly formed bone. Implant resorption was about 30%.

At 12 months, the amount of newly formed bone was unchanged. No statistical difference could be noted between the various pore size ranges. The percentage of bone ingrowth was 96-98%. The degradation of the ceramic was about 60%.

Comparison of HA and β-TCP

The β-TCP was colonised faster and at a higher rate than the HA. Whereas a statistical difference in bone ingrowth continued to be noted for the HA implants between the 45-80 μm pore size and the three higher pore sizes whatever the implantation time, no such difference could be noted beyond four months for the β-TCP. In fact, a pore size superior to 80 μm seems to promote bone and tissue ingrowth at the beginning.

Data related to the depth of bone ingrowth paralleled those related to the percentage of bony ingrowth.

Ceramic resorption reached 60% for β-TCP and only 10% for HA at 12 months.

DISCUSSION

During the past ten years there has been an increased use of bone substitutes with numerous products available in orthopaedic surgery (*4*). One of the most common synthetic alternatives to autologous bone graft, the “gold standard,” is calcium phosphate ceramics, such as HA or β-TCP, the most widely used materials, because of their absence of toxicity, their biocompatibility, and their osteoconductivity permitting the "creeping substitution" process. The success of calcium phosphate ceramics depends on the formation of healing bone tissue that will bring about the incorporation of the implant. The important feature in the physical structure of a synthetic ceramic is its porosity. Pore size, volume of porosity and interconnections between the pores are three crucial parameters (*8*). Pore structure is of great importance for osteoconduction (*9*). Cell colonisation and bone ingrowth apparently occur for a minimal macropore size, not quite defined yet, and a reduction in macroporosity may have negative results for osseointegration. Many studies have shown the relationship between bone ingrowth and pore parameters, particularly the percentage of porosity, the pore size and the degree of pore interconnectivity (*16, 23, 27*), but these studies also report confusing data on porosity, so that optimal macroporosity parameters have not yet been defined. Cell ingrowth into porous ceramic is, in fact, not well understood. It is generally accepted that 80-100 µm is the minimal pore size for osteoconduction (*3, 24, 26*). Nevertheless, intervals mentioned in the literature are very variable. Bobyn *et al* (*1*) have shown that a pore size of 150-400 µm was recommended to provide bone ingrowth into metallic implants. Hulbert *et al* (*16*) showed optimal bone ingrowth into 150-200 µm ceramic, but also demonstrated that interconnection between pores was a more crucial parameter than the pore size itself. Flautre *et al* (*8*) demonstrated the important role of both interconnection size and pore size ; the best osteoconduction in HA was obtained with a 130-µm interconnection size and a 175-to 260-µm mean pore size. Uchida *et al* (*26*) showed better osteointegration with pore size greater than 200 µm. Finally, in the literature, a pore size below 80 µm seems to be critical for osteoprogenitor cells to penetrate into the pores. Nevertheless, in our study bone ingrowth into ceramic with 45-80 µm pore size was possible, but it was delayed compared to the higher pore size ranges. Another study conducted by Eggli *et al* (*7*) had already reported notable bone ingrowth for pores smaller than 100 µm. An explanation for their results was the number of interconnections, greater in small pores than in large pores. Our results indicate that a pore size above 80 µm is recommended to accelerate and to improve the bony ingrowth within the ceramic. Nevertheless, we did not find any statistical difference in osseointegration between the three higher pore size ranges : 80-140, 140-200 and 200-250 µm. The optimum pore size could be defined as the interval 80-250 µm, according to our results. Pores of more than 500 µm diameter seem to be too large, and seem to discourage bone ingrowth (*21*). The nature of the ceramic also influences bone formation. We found that bony ingrowth occurred quicker and at a higher rate within β-TCP implants than within HA implants. The high resorption potential of β-TCP, compared to the poor resorption of HA, will leave larger space for the newly formed bone (*20*). This different resorption rate, correlated with the Ca/P ratio, can explain the difference in bony ingrowth into both ceramics. Moreover, the pore size itself also seems to play an important role in the bone ingrowth process, as demonstrated by Gauthier *et al* (*11*).

Whether or not a calcium phosphate ceramic used as a bone substitute will succeed depends on the formation of healing bone tissue that will bring about the incorporation of the implant. In the biological, biochemical and physiochemical parameters involved, the pore size is a crucial parameter. The level of porosity, pore size distribution and degree of pore interconnectivity significantly influence the extent of bone ingrowth. Macroporous calcium phosphates, in particular HA and β-TCP, have achieved considerable success as bone graft substitute materials. Our findings suggest that β-TCP with an optimum pore size of 80-250 µm is the most suitable ceramic for filling bone defects.

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