Bone formation in coralline hydroxyapatite

Effects of pore size studied in rabbits

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We analyzed osseous reactions in the rabbit femoral condyle to coralline hydroxyapatite bone substitutes of various pore sizes by radiology and histology. The results were compared to bone repair of empty cavities and to integration of allografts.

Spontaneous bone repair of the empty cavities took approximately 12 weeks, while integration of the cryopreserved allografts occurred after 9 weeks. However, no signs of new bone formation were found with the 200 μm pore size hydroxyapatite. In contrast, there was substantial production of bone within the 500 μm pore size implants at 12 and 26 weeks. Our results indicate that the pore size of the coralline hydroxyapatite influenced the development of bone in the implants in the cancellous bone bed of the rabbit femoral condyle. The results also show that spontaneous bone repair should be taken into consideration when the integration of implants is evaluated.

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When a bone substitute with some mechanical strength is needed, hydroxyapatite appears most appropriate, as it represents the natural mineral in human bone and approximates the natural structure of cancellous bone (Cooke 1992). Some hydroxyapatite bone substitutes are made from bovine bone, others are produced synthetically. The coral-derived material investigated in our study is characterized by a particularly high interconnectivity of the pores to facilitate ingrowth of osteogenic tissue. In a cancellous bone defect of the rabbit femoral condyle, we have compared bone formation in hydroxyapatite implants of two pore sizes with spontaneous bone repair and with the response to cryopreserved bone allografts.

Material and methods

16 Chinchilla rabbits weighing 3–4 kg were kept in cages, fed pelleted Alma® rabbit standard diet (Botzenhardt KG, W-8960 Kempten, FRG), and received tap water ad lib.

Implants

The hydroxyapatite implants were derived from 2 genera of reef-building corals. Those with an average pore size of 200 μm (HA 200) were produced by hydrothermal conversion of the calcium carbonate structure of the exoskeleton of Porites (Interpore 200®). The bone substitute with an average pore size of 500 μm (HA 500) and pore interconnections of about 260 μm diameter was converted from the exoskeleton of Goniopora (Interpore 500®). The structure of this material resembles the architecture of cancellous bone (solid volume 25–35 percent) (White and Shors 1986, Holmes et al. 1986, Ripamonti 1991) vs. 25 percent in human cancellous bone (Frisch and Bartl 1990)). Cylindrical implants of 6 mm diameter and 15 mm length were specially prepared by the manufacturer (Interpore International, Irvine, CA, U.S.A.).

Allografts were harvested from femoral condyles of Chinchilla rabbits with a 6-mm hollow drill, double foil packed under sterile conditions and then stored at –70 °C.

Implantation

The rabbits received an intramuscular injection of 1 mL methylparaben (Combelen®; 10 mg/mL) 1 hour before the operation as premedication. Anesthesia was then induced by intramuscular administration of 1 mL ketamine hydrochloride (Ketavet®; 50mg/mL), approximating a dose of 15 mg/kg body weight and 1 mL xylazine hydrochloride (Rompun®; 20 mg/mL), i.e., approximately 5 mg/kg body weight. In

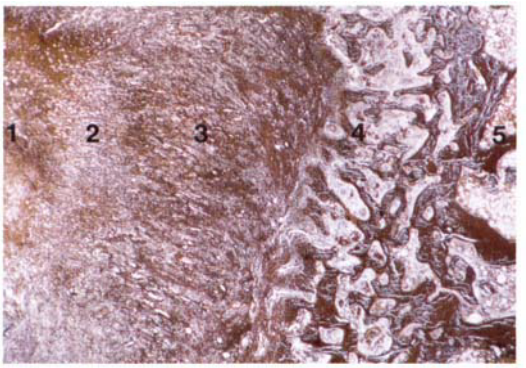


Figure 1. Bone repair in empty cavity. 2 weeks postoperatively. Central blood clot, partial reorganization of the defect by invasion of mesenchymal cells and fibroblasts from the edge toward the center. Zones of repair: 1) central blood clot, 2) mesenchymal cell reaction, 3) fibrotic reaction, 4) woven bone, and 5) original cancellous bone bed. Gomori, ×32.

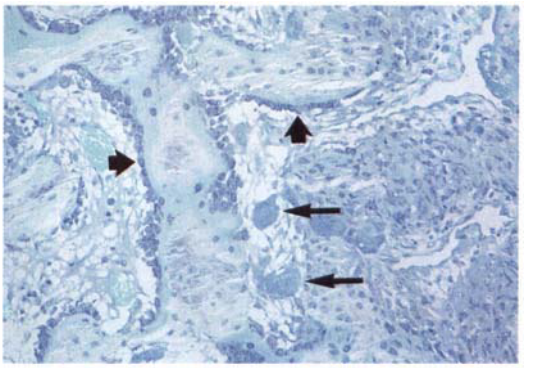


Figure 2. 6 weeks postoperatively. Bone remodeling showing osteoclasts (thin arrows) and seams of osteoblasts producing osteoid (thick arrows). Connective tissue with mesenchymal cells at right. Note highly vascularized area adjadent to the trabeculae at left. Giemsa, ×100.

some animals, 0.5 to 1 mL of the ketamine/xylazine combination was also given intravenously. After shaving, disinfection, and draping, a straight 3 cm incision was made over the lateral femoral condyle. The fascia was split, and a 6 mm hole was drilled into the condyle. After rinsing with 0.9% NaCl, the HA 200 or HA 500 bone substitute or the bone allograft was implanted, or the hole was left empty as a control. Both lateral condyles of each rabbit were operated on. The fascia was closed by 3–0 atraumatic Vicryl® sutures, skin closure was performed by stapling. At the end of each observation period, the rabbits were killed by intravenous administration of 5 mL pentobarbital (Nembutal®; 60 mg/mL).

32 operations were performed. HA 200 and HA 500 were each implanted into 8 femoral condyles, observation periods being 2, 6, 12, and 26 weeks. Allografts were implanted 8 times, and 8 empty holes served as controls. Observation periods for these 2 groups were 2, 6, 9, and 12 weeks.

Evaluation

Anteroposterior and lateral digiscan macroradiographs of the distal femora were taken at the end of the observation periods.

Specimens were then fixed in Schaffer's solution for 3 days, dehydrated and embedded in methylmetacrylate. Serial 3-mm sections were cut. Giemsa, Gomori, and Ladewig stains were used for assessment of cellular details, fibrosis and mineralization, respectively (Frisch and Bartl 1990). Special attention was paid to the cellular reactions at the edges of the implants, to bone remodeling, to osteoid formation, and to the development of bone bridges between host and allograft.

Results

Empty cavities

The bone cavities were initially filled with blood clot, followed by rapid invasion of mesenchymal cells from the edges of the defects. After 2 weeks, numerous osteoblasts were found in the cavities, as well as fibroblasts (Figure 1). At 6 weeks, there was massive formation of osteoid, and of trabeculae from the edges inwards, leaving empty only a small central area of the former cavity (Figure 2). At 12 weeks, all specimens showed almost complete osseous reorganization, i.e., the cavities were filled with new cancellous bone. Osteocytes and osteoblasts appeared normal, osteoclasts were rare, and fibroblasts were no longer detected.

Bone allografts

At 2 weeks postoperatively, osteoclasts and fibroblasts were found at the host-graft interface, where the drilling had caused mechanical damage. In some instances, there was massive lymphocytic infiltration. Numerous mesenchymal cells had invaded the graft, as well as cells with the appearance of osteoblasts. At 6 and 9 weeks, osteoblasts dominated, broad osteoid seams were present and direct bridging had occurred between host bone and the trabeculae of the allograft (Figure 3). At 12 weeks, there was complete integration of the allograft in all cases. The bone allograft could hardly be distinguished from the surrounding original cancellous network of the femoral condyle.

Hydroxyapatite implants HA 200

At 2 weeks, the HA 200 implants showed no inva-

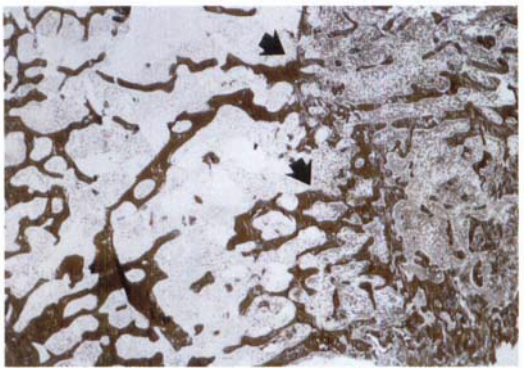


Figure 3. Implantation of cryopreserved allograft, 9 weeks. Bone bridges (arrows) between host bone (left) and allograft (right). Gomori, ×38.

sion of mesenchymal cells from the surrounding tissue. Even after 6 weeks, only minimal cellular invasion was detected, with no signs of cellular differentiation. At 12 weeks, no osteoclasts or osteoblasts were found inside the bone substitute. At 26 weeks, the implants were not integrated but remained sharply delineated from the surrounding host bone, without any signs of new bone formation and remodeling (Figure 4).

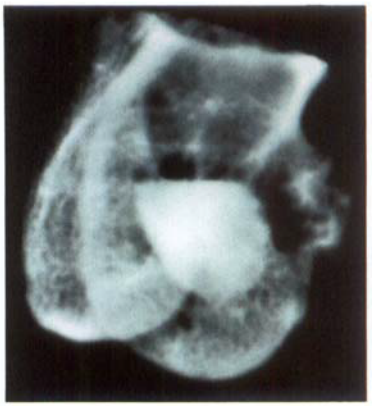
Hydroxyapatite implants HA 500

At 2 weeks, the pores were filled with mesenchymal cells. At 6 weeks, fibroblasts and osteoblasts had entered the implant, with substantial osteoid production. At 12 weeks, there was intense interdigitation between host bone and bone substitute with massive penetration of the hydroxyapatite pores by osteoblastic cells. In the 26-week specimens, newly formed mineralized bone had developed in the pores of the entire implant (Figure 5).

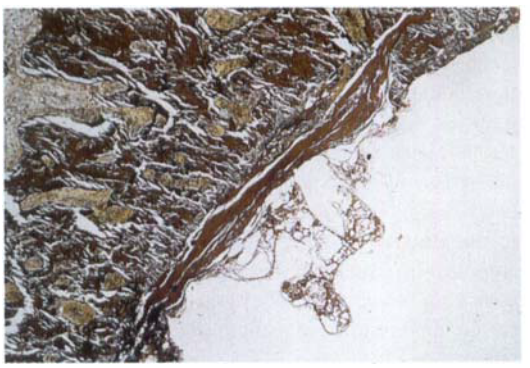
The observations for each group examined are summarized in the Table 1.

Figure 4. Osseous reaction to the 200 μm pore size hydroxyapatite bone substitute Interpore 200®.

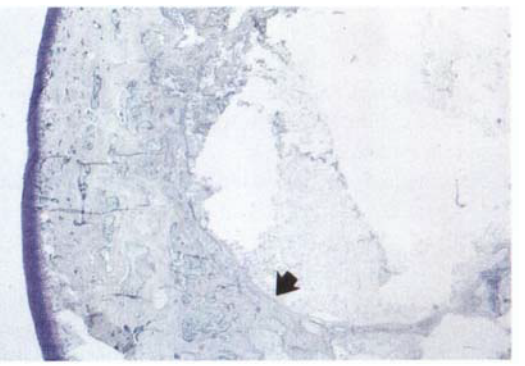
Macrophotography, 12 weeks. Only superficial attachment to host bone, no penetration inside the material. ×10.



Radiograph, 12 weeks. Sharp delineation between hydroxyapatite and surrounding bone, with occasional cystic reaction.

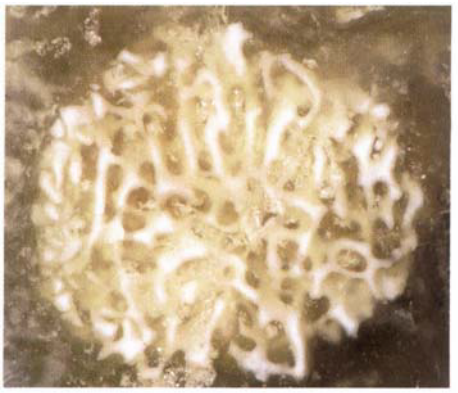


Histology, 6 weeks. Only minimal invasion of tissue at the rim of the bone substitute (the hydroxyapatite material is brittle, it gets lost during the cutting process and does not show in the microscopic views). Gomori, ×150.

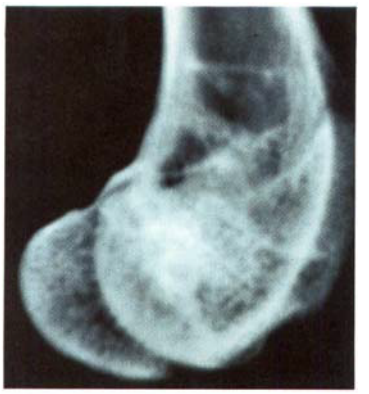


Histology, 26 weeks. No integration of the bone substitute. Note circular bony demarcation at the interface (arrow). Giemsa, ×20.

Figure 5. Osseous reaction to the 500 µm pore size hydroxyapatite bone substitute Interpore 500®.

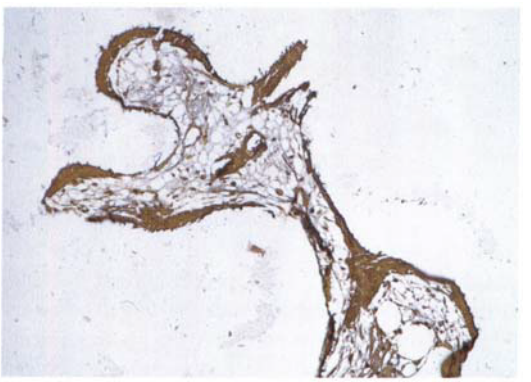


Macrophotography, 12 weeks. Massive interdigitation of implant with surrounding bone. ×13.



Radiograph, 12 weeks. No delineation between implant material and bone.

Histology, 2 weeks. Invasion of mesenchymal tissue inside the wide bone substitute pores. Gomori, ×12.



Histology, 6 weeks. Detail illustrating osteoblastic activity with production of small osteoid seams, directly attached to the hydroxyapatite material. Gomori, ×150

Discussion

There are some reports on successful integration of HA 200 in dental surgery (Holmes 1979, Finn et al. 1980, Piecuch et al. 1983, Boyne 1986) and, less frequently, on the use of HA 200 in orthopedics (Sartoris et al. 1986a). Radiographic and biomechanical assessments of HA 500 in a canine metaphyseal defect model were performed by Sartoris et al. (1986b) who claimed that this bone substitute was even better than autogenous bone grafts as regards the radiographic and mechanical parameters. In another study of HA 500 in canine metaphyseal defects, Holmes et al. (1986) reported that at 12 months two thirds of the HA 500 surface was covered with appositional bone. Martin et al. (1989) reported rapid bony integration of HA 500 in a canine cancellous bone defect, but they found the material less suitable in cortical defects, due to its low mechanical strength.

Bucholz et al. (1989) performed a prospective randomized comparison of HA 500 and autogenous bone grafts in 40 tibial plateau fractures. Since there were no differences in the outcome between the two groups, the authors suggested that this porous hydroxyapatite is as effective as an autograft in these specific circumstances.

Ripamonti et al. (1991) found new bone formation after heterotopic implantation of HA 500 in adult primates and concluded that this bone substitute represents a biological alternative to autogenous bone grafts for the controlled initiation of bone formation in humans.

However, our results contradict the findings of Eggli et al. (1988) that bone developed in implants with 50–100 µm pore sizes while only little bone was found in implants with 200–400 µm pores. In their synthetic hydroxyapatite, numerous pore intercon-

Table 1. Synopsis of bone repair in empty cavity and osseous reactions to bone allograft and to hydroxyapatite implants. 8 femoral condyles investigated in each group

Weeks postop.	Empty bone cavity	Bone allograft	Hydroxyapatite 200 µm pores	Hydroxyapatite 500 µm pores
2	Central blood clot, mesenchymal cell invasion, osteoblasts, fibroblasts (Figure 1)	Invasion of mesenchymal cells into allograft	No invasion of mesenchymal cells, nor of any other tissue cells	Extensive mesenchymal cell invasion
6	Formation of osteoid trabeculae from the edges inwards	Osteoblast activity, osteoid seams, occasional bone bridges	Only minimal invasion of undifferentiated cells (Figure 4)	Fibroblast and osteoblast activity, production of osteoid (Figure 5)
9	Only small residual central defect, new trabeculae, beginning	Numerous bone bridges with seams of active osteoblasts. Production of cancellous bone (Figure 3)		
12	Cavity almost completely cellous bone and hematopoiesis	Complete integration of restitution of bone marrow stroma and hematopoiesis	Still no cellular activity inside the bone substitute. Partial demarcation at the edge of the implant	Massive penetration of pores with osteoblastic cells, mineralization of osteoid
26			No signs of cellular invasion, no signs of new bone formation or remodeling (Figure 4)	Newly formed mineralized bone and hematopoiesis throughout the entire implant

nections were found only in the smaller pore size material, while there were practically no interconnections between the pores of the larger pore size substance. The authors considered the pore interconnections existing prior to implantation to be the decisive precondition for ingrowth of bone.

Predecki et al. (1972) studied the kinetics of bone growth into cylindrical channels of different diameters in aluminum oxide and titanium. They found ingrowth of bone into channels with a diameter of 200 µm, more into 300 µm channels and again less when the diameter was 500 µm. The influence of different pore size on tissue ingrowth was also studied by Uchida et al. (1984), who implanted synthetic hydroxyapatite into rat and rabbit skulls. They reported that ingrowth of connective tissue was denser when the hydroxyapatite with 210-300 µm pores was used than when the 150-210 µm pore size material was used.

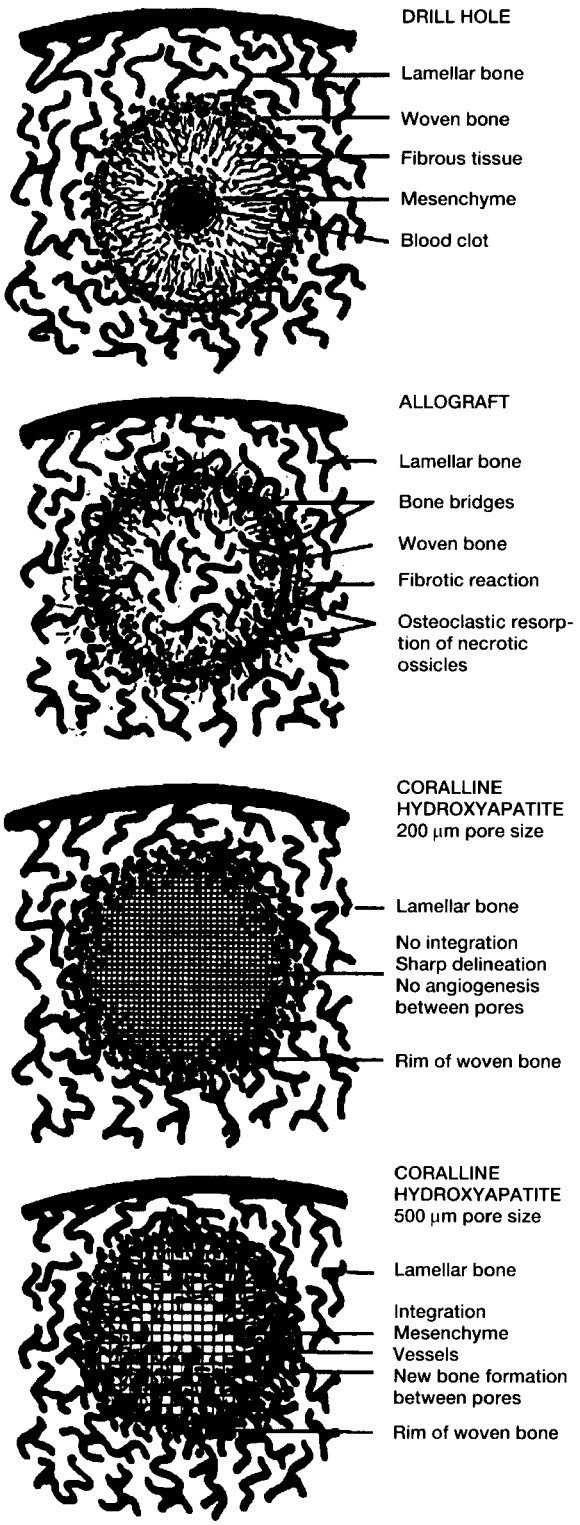
Shimazaki and Mooney (1985) implanted cylindrical coral-derived hydroxyapatite bone substitutes of two pore sizes into rabbit tibiae. Using this material with multiple pore interconnections, the authors found that the larger pore size (260–600 µm) seemed to be more associated with greater bone ingrowth than the smaller pore size (100–300 µm) was.

Apparently, particular attention must be paid not only to the size of the pores, but to their interconnections. While a thin layer of a 200 µm pore size hydroxyapatite may be useful for coating a metal endoprosthesis to improve its attachment to the surrounding bone bed (Cameron et al. 1976, Bobyn et al. 1980, Cook et al. 1985), a larger porosity seems essential when the hydroxyapatite is used as a bone substitute. Klawitter and Hulbert (1971) stressed that a ceramic with pore sizes of about 100 µm would be of little value for bone ingrowth, if the interconnecting pore size was only a few microns. The HA 500 bone substitute, featuring an average interconnecting pore size of 260 µm (Holmes et al. 1986), apparently has distinct advantages over the HA 200 material when development of new cancellous bone within the hydroxyapatite is required.

The mechanisms of bone repair in an empty cavity, allograft integration and osseous reactions to the 200 and 500 µm hydroxyapatite implants are summarized in Figure 6.

Allograft integration occurred after an initial immunologic response, with formation of new bone inside the allograft, as well as with direct bone bridging. A different type of bone remodeling took place in the empty cavities, where the initial blood clot was reorganized by mesenchymal cells, followed by ingrowth of fibroblasts and osteoblasts, with spontaneous osteoneogenesis from the edges to the center of the cavities. The latter finding is in contrast to results published by Katthagen (1987), who found that 6 mm drill holes in the rabbit femoral condyle

Figure 6. Overview of bone repair in empty bony cavity, cryopreserved bone allograft and in coralline hydroxyapatite with two pore sizes.



remained empty, with no regeneration of bone for months. On the other hand, even 10 mm circular metaphyseal defects in dogs were reported to be filled with spontaneously regenerated bone within 3 weeks (Schweiberer 1970). Chigira et al. (1992) report successful spontaneous bone repair of large osseous defects in 17 cases, after resection of benign lesions in humans.

We concluded that the 500 µm pore size hydroxypatite investigated in our study may be suitable as a bone substitute in metaphyseal defects. Further studies should be conducted to allow comparison of this coral-derived hydroxyapatite to other bone substitutes, possibly in combination with an osteogenic protein, as recently proposed (Miller et al. 1991, Ripamonti et al. 1992).

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