Repair of bone defects with marrow cells and porous ceramic

Experiments in rats

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We studied the role of bone-marrow reconstituted porous ceramics in enhancing healing of a 5-mm femoral diaphyseal defect fixed with a rigid polyethylene plate in rats. Osseous repair was evaluated by histologic scoring. When blocks of porous calcium phosphate ceramics alone were introduced into the defects, most cases showed fibrous tissue interposition at the host bone-ceramic junction 1 month after implantation, and only four of 12 defects developed osseous or osteochondral union at both the proximal and distal junctions 2 months after surgery. However, when the ceramic was combined with syngeneic viable marrow cells, new bone formation occurred in isolated pore regions of the ceramic at 1 month, and extensive bone formation was seen in most pore regions 2 months after implantation. Out of 12 implants, complete bone union was seen in eight, and one showed osseous or osteochondral union at both junctions 2 months after surgery. Our results indicated that composite grafts of porous calcium phosphate ceramics and marrow cells may be clinically applicable to enhance osteogenesis and osteoconduction.

In the research for synthetic implants to bridge massive bone defects, calcium phosphate ceramics demonstrate biocompatible properties—no toxicity or immunologic response (Cameron et al. 1977, Jarcho 1981, Kato et al. 1979). Also, the porous form of ceramics is resorbable and osteoconductive (Cameron et al. 1983, deGroot 1980). However, this osteoconduction is usually observed in only restricted areas that are adjacent to the preexisting host bone (Cameron et al. 1977), whereas the ceramic itself has no osteogenic ability (Nade et al. 1983, Ohgushi et al. 1989, MacDavid et al., 1979). Thus, ceramics alone would not appear to be useful for the repair of massive bony defects.

Our previous experiments (Ohgushi et al. 1989) in subcutaneous or intramuscular sites showed that marrow cells in such porous ceramics resulted in osteogenesis in the interstices of the ceramic. However, whether or not this ceramic formatted for osteogenesis functions in orthotopic sites to stimulate bone healing (Carter and Hayes 1979) cannot be addressed by ectopic implantations. We investigated the healing potential of the ceramic composite with syngeneic marrow cells in a rat femoral-bone defect that usually does not heal without the addition of osteo-enhancing materials.

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Materials and methods

The (4 × 4 × 23 mm) bone plates used were fabricated from high-density polyethylene with holes for Kirschner wire insertion. The ceramic was a porous calcium phosphate (60 percent hydroxyapatite and 40 percent B-tricalcium phosphate, mean pore size 400 μm, supplied by Zimmer Corp., Warsaw, Indiana), and was cut to a standard size of 3 × 3 × 5 mm.

Collection of marrow cells

The femora from 12, 220-gram syngeneic male Fisher rats were removed, freed of soft tissue and periosteum, and each diaphyseal portion was removed by cutting the metaphysis with a water-cooled burr. The marrow plugs from the diaphysis were hydrostatically forced into 0.4–0.6-mL heparinized syngeneic rat serum. The marrow was disaggregated by sequential passage

through 18- and 20-gauge needles to obtain a single-cell suspension of $5–10 \times 10^8$ cells/mL of nucleated cells as estimated using a hemocytometer. The viability was over 90 percent as estimated by the trypan-blue dye exclusion test.

Surgical procedure

Syngeneic male Fisher rats (101 rats, mean body weight 253 g) were anesthetized by intramuscular injection of Nembutal following light ether inhalation. The right femurs were exposed by a lateral incision, and the polyethylene plate was applied anterolaterally and fixed with two threaded (1.1 mm) Kirschner wires and two cerclage wires. A 5-mm extraperiosteal diaphyseal defect was made by cutting the femur with a Sugairtome with saline irrigation. A ceramic block, either soaked in rat serum as a control or in the marrow-cell suspension, was inserted in the defect and fixed with cerclage wire over the plate and the ceramic (Figure 1). We estimated by cell counting that $1–2 \times 10^7$ cells/implant were delivered with each piece of ceramic. The fascia and skin were sutured, and 0.1 mm of penicillin G (3×10^5 units/mm) was injected i.m.

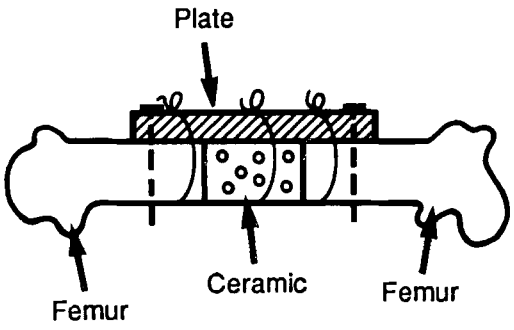

Figure 1. Schematic representation of ceramic implantation in a segmental rat-femur defect

There were 24 rats in the control group in which the defect was made, but not repaired; in 21 the defect was repaired with the ceramic without marrow cells, whereas in 28 rats the ceramic-marrow cell composite was used. Rats were killed after 1 and 2 months for histologic evaluation. Radiographs were taken at 1-month intervals and correlated with histologic data. There were no intraoperative deaths or infections.

Histologic evaluation

The entire femur was dissected from each animal, fixed in 10 percent buffered formalin, and decalcified in RDO (Rapid Bone Decalcifier, Dupage Kinetics Laboratories, Inc.). The plates and wires were removed, and a longitudinal section through the middle portion of the femur (perpendicular to the plated surface) was made with a blade. The segment was embedded in paraffin with the cut surface facing the blade of the microtome, cut into 5-μm sections, and stained with Mallory-Heidenhein stain (Humason 1972).

Histologic scoring of bone healing was made by assigning points according to type of union between the host bone and ceramic implant (Table 1), with one point for osteochondral union and two points for osseous union.When continuous bone formation between proximal and distal femur was observed, one point was added; also when isolated bone formation was observed within the pore region of a ceramic implant, one point was added for a maximum score of six points. Sections that showed only one junction of the ceramic femoral interface were excluded because both proximal and distal junctions were necessary for histologic evaluation. Three rats were also excluded be-

Table 1. Evaluation of union

Histologic scoring system[a]	Points	Radiographic scoring system
Nonunion[b]	0	Nonunion[b]
Osteochondral union[b]	1	Possible union[b]
Osseous union[b]	2	Radiographic union[c]
Bone bridge between proximal and distal end	1	Continuous radiodense area over implant in the defect
Bone formation in pore regions of the ceramic[c]	1	Radiodense appearance in the implant (ceramic)
Maximum score	6	Maximum score

[a] Details are described in Methods. Points derived from corresponding histologic or radiographic observations are represented in the center.
[b] Proximal and distal junctions were evaluated separately.
[c] Bone formation in isolated pore regions (not related to the bone formed by osteoconduction) or massive bone formation in the pore regions was included.

cause of failure of fixation. Corresponding radiographic evaluation was also done with a maximum score of 6 (Table 1). The scoring was performed independently without knowledge of the study group by two of us and one orthopedic surgeon who was not associated with this study. Therefore, each specimen had three scores, and a majority or a median of the score was chosen as representative for each specimen. The Wilcoxon rank-sum test for tied data was used to analyze the distribution difference of each study group.

Results

Controlled defects that were not repaired with either ceramic or ceramic composites seldom healed or demonstrated bone formation in any animal. Histologic features showed that none of the defects had spontaneous osseous or osteochondral continuity 1 month after surgery, and only two of the 12 defects had this continuity 2 months after surgery. When blocks of ceramics alone were introduced into the defects, four of the 12 defects showed osseous or osteochondral union at both the proximal and distal junctions 2 months after surgery. Thus, this material alone did not contribute significantly to repair. However, when the ceramic was combined with marrow cells, complete bone union was seen in eight of 12 implants, and one showed osseous or osteochondral union at both junctions 2 months after surgery.

Histologic and radiographic analysis indicated that there were increasing differences ($P < 0.01$) between ceramic composites with marrow and without marrow in samples at and 2 months after surgery (Table 2). Radiographic evaluation was somewhat more difficult because the femur was rather small and the ceramic itself radiopaque (Figure 6). However, at 1 month the score of the ceramic with marrow was higher than that without ($P < 0.01$), and all the radiographic scoring values showed the same tendency as the histologic evaluation (Figures 2–5).

Discussion

Our previous studies (Ohgushi et al. 1989) and those of others (MacDavid et al. 1979, Nade et al. 1983) have shown that ceramic itself is not osteogenic or osteoinductive in ectopic sites. However, when combined with a fresh marrow cell suspension, we have demonstrated (Ohgushi et al. 1988) that osteogenesis occurs in the pore region of the ceramic and bone formed was in direct contact with the ceramic surface and that the incidence of osteogenesis surpassed that of chondrogenesis. The data presented here indicate that such a marrow-cell reconstituted ceramic also supported osteogenic activity in an orthotopic site.

Others have reported that a composite graft of granular calcium phosphate ceramics with collagen (Grundel et al. 1987) or with marrow (Sandhu et al. 1987) showed better results than controls in an experimental segmental defect. However, the block form of the ceramic as used in this study did not exhibit healing potential unless it was combined with marrow. In this regard, we have observed that the details of such a reconstituted composite graft of marrow cells and ceramic were important for the success of the implant. Specifically, cell viability and total cell count in the ceramic are extremely important in inducing osteogenesis (Ohgushi et al. 1989). A viable cell load of more than 5×10^6 cell ceramic blocks of $3 \times 3 \times 4$ mm showed consistent osteogenesis in ectopic sites, whereas less than 5×10^5 per block gave only marginal osteogenesis (Ohgushi et al. 1989). The present method used to con-

Table 2. Healing potential of ceramic with (+) or without marrow (–) in segmental rat-femur defect

		Sacrificed at month	Score*							Median score	Wilcoxon rank sum test
			0	1	2	3	4	5	6		
Histologic evaluation	–	1	3	4	2	–	–	–	–	1	$P < 0.01$
	+	1	–	3	1	2	3	3	-	3.5	$P < 0.01$
	–	2	1	1	6	-	4	-	–	2	$P < 0.01$
	+	2	–	1	–	2	1	4	4	5	$P < 0.01$
Radiologic evaluation	–	1	1	4	8	5	3	-	–	2	$P < 0.01$
	+	1	1	1	5	7	9	2	–	3	$P < 0.01$
	–	2	–	2	3	1	2	3	1	3.5	NS
	+	2	–	–	2	1	4	4	2	4	NS

* The scoring system is described under Methods and in Table 1. The values that appear in the histologic and radiographic evaluations are the number of specimens that showed these scores.

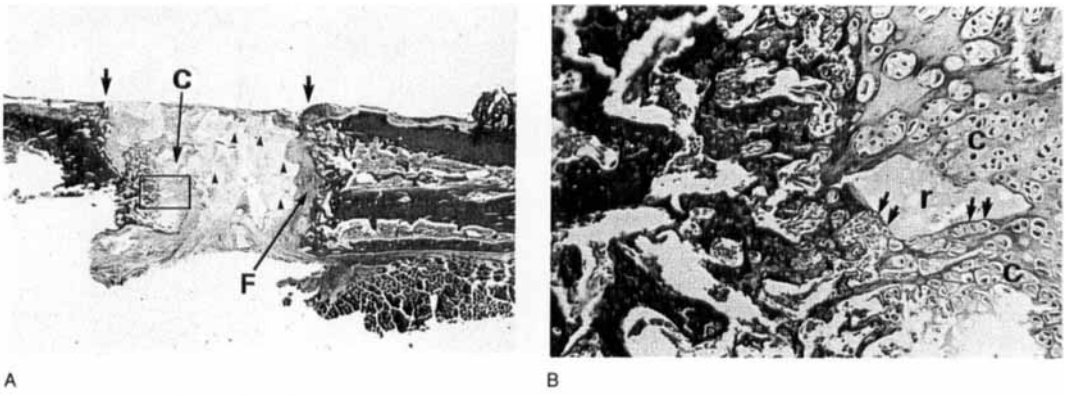

Figure 2. Ceramic without marrow in rat-femur defect 1 month after surgery.
A. Arrows indicate junction between ceramic and femur. Remnants of ceramic that remain following histologic decalcification are indicated by (▲). Osteochondral union (C) and fibrous tissue intervening (F) between the ceramic and femur are seen (total histologic score is 1, Mallory-Heidenhein stain, x18).
B. Higher magnification of the rectangular area. Cartilage is present around the ceramic in direct contact with the remnant of ceramic (r). Arrows indicate ceramic-cartilage interface. Endochondral ossification is clearly seen on left side (x44).

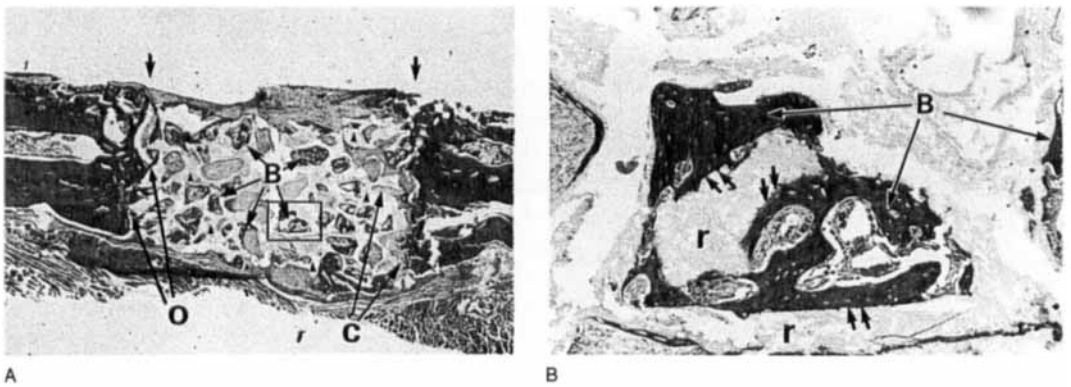

Figure 3. Ceramic with marrow in a rat-femur defect 1 month after surgery.
A. Arrows indicate junction between the ceramic and femur. The remnant of ceramic (▲), osseous union (O), and osteochondral union (C) are indicated. Note the induced bone (B) in the pore region of the ceramic (total histologic score is 4, Mallory-Heidenhein stain, x18).
B. Higher magnification of the rectangular area. Induced bone (B) is in direct contact with the ceramic remnant (r), with arrows indicating the ceramic-bone interface, x44.

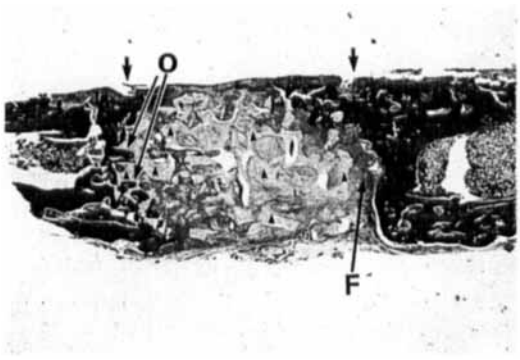

Figure 4. Ceramic without marrow in rat gap 2 months after surgery. Arrows indicate junction between ceramic and femur. The remnant of ceramic (▲), osseous union (O), and fibrous tissue interposition (F) are indicated (total histologic score is 2, Mallory-Heidenhein stain, x18).

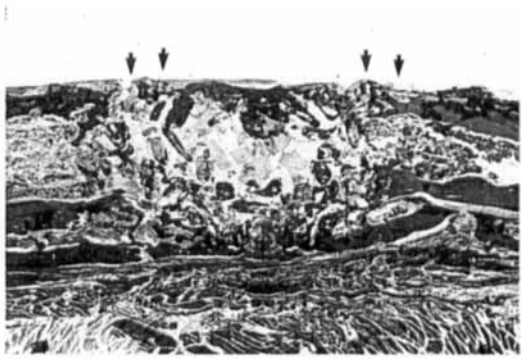

Figure 5. Ceramic without marrow in rat-femur gap 2 months after surgery. Arrows indicate junction between ceramic and femur. Extensive bone formation is seen around the junction and in the pore region of the ceramic; also continuous bone formation over the ceramic is seen (total histologic score is 6, Mallory-Heidenhein stain, x18).

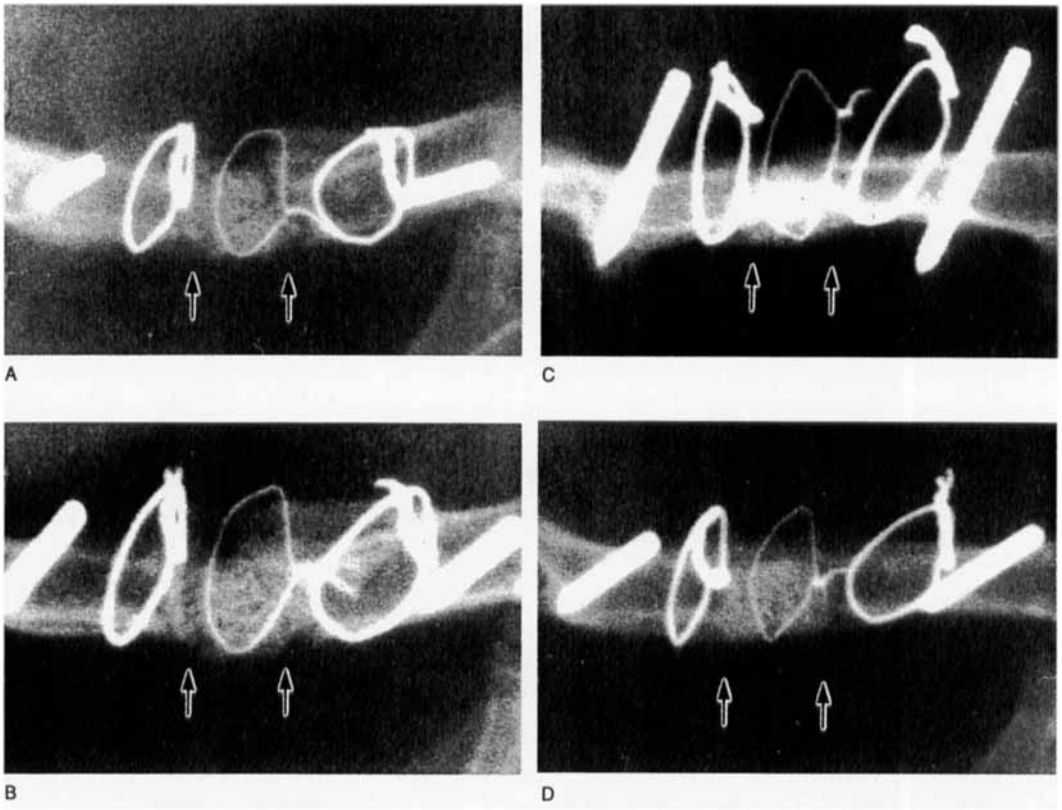

Figure 6. Radiographs of rat-femur gap 1 month (A and C) and 2 months (B and D) after surgery.

struct these composite grafts for the orthotopic site was essentially the same as the previous method used in the ectopic studies that showed consistent osteogenesis. This ectopic system is helpful as a rapid screening technique for forecasting the success of different methods to induce osteogenesis in an orthotopic site.

Bone morphogenetic protein (Takagi and Urist,1982, Nilson et al. 1986) or other materials such as demineralized bone matrix (Bolander and Balian 1986) have been reported to reconstitute segmental bone loss by osteoinduction. It is possible that composites of bone marrow and ceramics are also acting in an osteoinductive role; the bone marrow cells may themselves secrete a soluble factor that induces surrounding mesenchymal cells to migrate into the area and differentiate toward osteoblastic lines of cells. Further, undifferentiated bone marrow cells themselves in the appropriate environment may be pushed toward osteoblastic lines. Either of these mechanisms could be important in providing the appropriate potential for repair of osseous defects.

For the present experiments, we used a porous calcium phosphate ceramic consisting of a 60 percent hydroxyapatite and 40 percent B-tricalcium phosphate. However, other preliminary studies have indicated that ceramics of hydroxyapatite made from calcium carbonate exoskeleton of the coral implant with a mean pore size of 200 and 500 μm (Interpor Inc., Irvine, CA) also can show osteogenesis when combined with marrow cells in ectopic sites (Ohgushi et al. 1989). Therefore, various types of calcium phosphate ceramics may function as osteogenic implants in the presence of marrow cells (Grundel et al. 1987, Ohgushi et al. 1988, Sandhu et al. 1987). Our data indicate that a composite graft of ceramics (Holmes et al. 1984) and autogenous fresh marrow cells may have clinical applications in the repair of osseous defects.

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