

THE EFFECT OF A BIPHASIC CERAMIC ON CALVARIAL BONE REGENERATION IN RATS

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KEY WORDS

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Bioactive ceramics (calcium phosphate ceramics, hydroxyapatite ceramics) are now extensively used in oral surgery. The purpose of this study was to assess the effect of a new biphasic ceramic (Ceraform) on the osteogenesis in a rat calvarial defect model. Fifteen Wistar rats were used in this study. Two symmetrical 3-mm wide defects were created in the skull of each rat. The left defect was left empty as a control and the right defect was filled with the ceramic. The rats were sacrificed at day 30, and the calvarial specimens were processed for qualitative and quantitative histological examinations. The material exhibited no adverse effects, but no bone healing was noted either. No statistical difference regarding bone regeneration was observed between the 2 defects ($P > .05$). This study showed that Ceraform did not elicit any inflammatory reaction; however, it had no effect on bone regeneration, and this material seems suitable only as a space-maintaining material.

INTRODUCTION

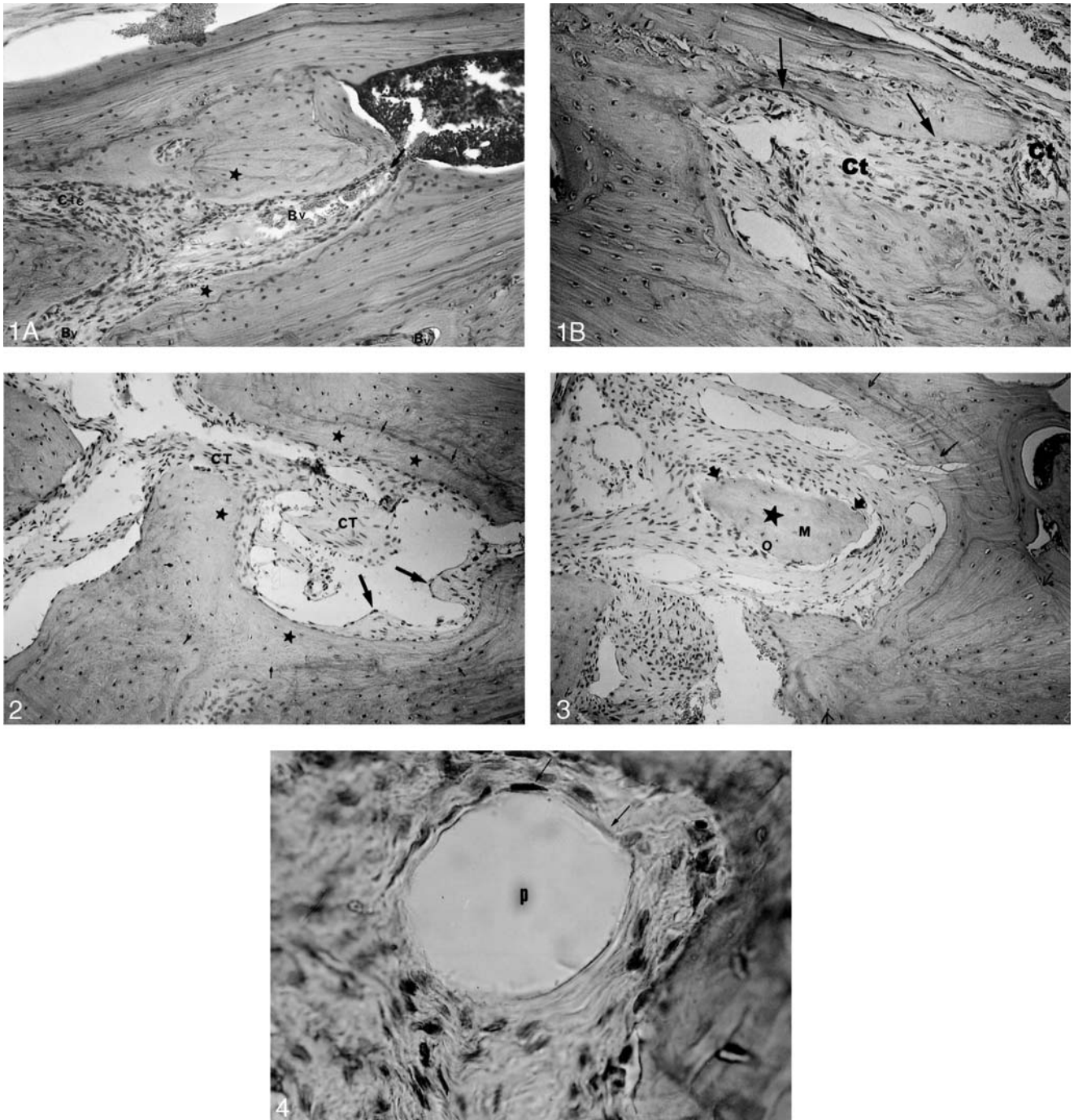
Hydroxyapatite (HA) and other calcium phosphate (CaP) ceramics have been widely studied over the past decade in the field of oral surgery.¹ They are also currently used as a coating for uncemented metallic implants to improve osteointegration.² The porous structure of the ceramics is claimed to enhance bone deposition and implant stabilization in the recipient bone. The optimal pore size, which varies between 50 and 565 μm according to some authors,^{3,4} is still under discussion. However, porosity of the material is inversely proportional

to the mechanical stability of these CaP-based ceramics.⁵ This loss of stability is often cited as a limitation in the use of CaP-based ceramics in clinical practice. A convenient compromise to overcome this problem is to use a biphasic ceramic that maintains its mechanical resistance until the resorption is achieved.⁴ The purpose of the present study was to assess the effect of a biphasic ceramic material (Ceraform) on the bone healing in experimentally created calvarial defects in rats.

MATERIAL AND METHODS

Material

Ceraform is a commercially available ceramic manufactured by



FIGURES 1–4. FIGURE 1. (a) Control defect: connective tissue (Ctc) invading the bone defect. The migration of bone marrow cells (arrow) into newly formed bone area (*) from the neighboring bone part in the control group. Connective tissue cells in the connective tissue (Ctc) of fibrillar matrix of closure area. Blood vessels (Bv) seem hyperemic in this region (hematoxylin-eosin, original magnification $\times 10$). (b) Control defect: the defect area includes connective tissue (Ct). Arrows indicate bone defect margins (hematoxylin-eosin, original magnification $\times 20$). FIGURE 2. Experimental defect: connective tissue (CT) invading the defect between the particles. Only marginal bone formation (*) noted at the defect margin. The particles are surrounded by a single layer of fibroblasts (thick arrows). Thin arrows indicate bone defect margins (hematoxylin-eosin, original magnification $\times 40$). FIGURE 3. Experimental defect: the specimen shows a new bone island (*) in the defect area, which is developed without periosteal activity. Osteocytes (O) and matrix (M) are present with the bone island. This new bone island is surrounded by osteoblast-like cells (thick arrows). Thin arrows indicate bone defect margins (hematoxylin-eosin, original magnification $\times 40$). FIGURE 4. Experimental defect: the particles (P) are surrounded by a single layer of fibroblasts (thick arrows) within the connective tissue (hematoxylin-eosin, original magnification $\times 100$).

Teknimed SA (Vic-en-Bigorre, France). This material is a synthetic biphasic ceramic made of HA 65% and tricalcium phosphate 35%. Porosity is between 60% and 80%. Pore-size diameters are between 100 and 300 μm . Mechanical strength is comprised between 20 and 70 mPa. The material is available as a block or in granular form and sterilized by gamma radiation.

Methods

This study was carried out on 15 Wistar rats weighing between 200 and 300 g. They were anesthetized with an intramuscular combination of Ketamin hydrochloride (Ketalar, Parke Davis, Berlin, Germany) and Xylazin chloride (Rompun, BayerAG, Leverkusen, Germany). A mid-line incision was carried out on the head, and periosteal flaps were reflected laterally. Then, 2 symmetrical 3-mm diameter circular wide full-thickness (0.6 mm in depth) bone defects were created in both parietal bones with a surgical trephine under saline irrigation. Experimental defects on the right were filled loosely with 10 to 15 Ceraform particles (mean granular diameter between 900 and 1200 μm) mixed with blood harvested from the rat. The left symmetric defects were left empty for control.

The skin flaps were repositioned and sutured with nonresorbable suture material. After 10 days, the sutures were removed and the rats were carefully followed up. The rats were sacrificed at day 30 to assess the early phase of bone healing. The skin was removed from the calvaria and the skulls were harvested for histological examination. Tissue samples were fixed in 10% buffered neutral formalin for 24 hours and decalcified in a mixture of

formic acid and hydrochloric acid for 24 hours. The specimens were rinsed with tap water and dehydrated by a series of increasing concentrations of ethanol. The specimens were then embedded in paraffin, and 5- to 7- μm thick sections were prepared in the transverse plane and stained with hematoxylin-eosin. Specimens were evaluated under a light microscope (Jenamed 2, Carl Zeiss, Jena, Germany). A semiquantitative method was used to quantify bone regeneration⁶: grade 0 = no or limited marginal bone formation, grade 1 = partial bone formation, and grade 2 = complete bone formation bridging the defect.

Statistical analysis

Fisher exact test was used for statistical analysis, and $P < .05$ was chosen as statistically significant.

RESULTS

In all rats the healing process was uneventful until the end of the experiments.

Histological findings

Control Defects

No pathological reaction, such as mononuclear cell infiltration, was in the defect or the subcutaneous tissues. No bone healing was observed (grade 0) in any of the control sites. A periosteum-derived vascular connective tissue was invading the defect from the external surface of the skull area, and the defects were filled only with vascular connective tissue (Figure 1).

Implanted Defects

In 13 of the 15 study specimens, there were no signs of inflamma-

tory reaction surrounding the foreign body particles, and no giant cells were observed in the spaces between the particles. In the 2 other specimens, a dense inflammatory reaction was noticed in the subcutaneous tissue. In all specimens except 1, no bone healing was evidenced at the defect site (Figure 2). In 1 specimen was a bony island in the defect with osteoblast-like cells (Figure 3). High-powered magnification showed that a single layer of fibroblast-like cells surrounded the particles (Figure 4). The quantitative results are summarized in the Table. No statistical difference was noted between the healing of the 2 differently approached defects ($P > .05$).

DISCUSSION

Assessment of the effect of new bone implants remains difficult because of the lack of standardization of the experimental models.⁷ The defect location and size seem to be the most critical factors for bone healing. Calvarial defects were considered in this study because this new biphasic ceramic is more particularly dedicated to oral surgery. A cranial defect was used in this study because the membranous bone is similar to that encountered in periodontal defects. This defect was not a critical size defect, but the lack of bone healing at the control sites confirmed the validity of the model. A short observation period was considered because the initial period is critical for bone healing.

The results at the experimental sites were disappointing, for no bone healing was evidenced in 14 of 15 cases. However, the lack of bone regeneration was expected because of the granular structure of the implants. A survey of the

TABLE

Histological classification of bone healing at day 30

Site	No Closure	Partial Closure	Complete Closure
Control site	15	0	0
Experimental site*	14	1	0

* $P > .05$.

literature showed that almost none of the natural or synthetic granular ceramics are either osteoconductive or osteoinductive in mandibular or cranial defects.⁸⁻¹⁰ Nevertheless, granular ceramics have gained wide clinical acceptance because they have a predictable resorption rate and are easy to handle. The main problem encountered with that form is postoperative migration of the particles. The dispersion of the particles can be avoided in several ways, with the most useful one being mixing the particles with the patients blood¹¹ or a plastic material such as plaster of paris.¹² The rationale of engineering the biphasic form was to benefit from the complementary effects of the 2 ceramics. The biological activity of an implant is influenced by 2 variables: the implant composition and granulometry. Tricalcium phosphate ceramics are generally claimed to undergo more rapid resorption than HA and to be more effective on early bone healing.^{13,14} However, they display poor mechanical properties, and some authors advocate the combination with HA for this reason. The size of the particles used in this study is also debatable. Malard et al¹⁵ have shown that a micronized particular form is more suitable for bone healing because more bone factors are released from the operative site. However, this form is less easy to handle and not suitable for load-bearing sites. The granular material used in this study might be useful as space filler in guided

bone regeneration to prevent the collapse of the membrane.¹⁶

In conclusion, this study indicates that the utilization of biphasic ceramic (Ceraform) did not cause any effect on the bone healing response in experimentally created calvarial defects in rats.

NOTE

This study was presented at Europerio Congress 4, June 19–21, 2003, Berlin, Germany.

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REFERENCES

1. LeGeros RZ. Properties of osteoconductive biomaterials: calcium phosphates. *Clin Orthop*. 2002;395:81–98.
2. Geurs NC, Jeffcoat RL, McGlumphy EA, Reddy MS, Jeffcoat MK. Influence of implant geometry and surface characteristics on progressive osseointegration. *Int J Oral Maxillofac Implants*. 2002;17:811–815.
3. Chang BS, Lee CK, Hong KS, et al. Osteoconduction at porous hydroxyapatite with various pore configurations. *Biomaterials*. 2000;21:1291–1298.
4. Gauthier O, Bouler JM, Aguado E, Pilet P, Daculsi G. Macroporous biphasic calcium phosphate ceramics: influence of macropore diameter and macroporosity percentage on bone ingrowth. *Biomaterials*. 1998;19:133–139.
5. Le Huec JC, Schaefferbeke T, Clement D, Faber J, Le Rebellier A.

Influence of porosity on the mechanical resistance of hydroxyapatite ceramics under compressive stress. *Biomaterials*. 1995;16:113–118.

6. Bosch C, Melsen B, Vargervik K. Importance of the critical size bone defect in testing bone regenerating materials. *J Craniofac Surg*. 1998;9:310–316.

7. Aaboe M, Pinholt EM, Hjorting-Hansen E. Healing of experimentally created defects. *Br J Oral Maxillofac Surg*. 1995;33:312–318.

8. Cancian DC, Hochuli-Vieira E, Marcantonio RA, Marcantonio E Jr. Use of BioGran and Calcitite in bone defects: histologic study in monkeys (*Cebus apella*). *Int J Oral Maxillofac Implants*. 1999;14:859–864.

9. Dupoirieux L, Pourquier D, Souyris F. Powdered eggshell: a pilot study on a new bone substitute for use in maxillofacial surgery. *J Craniomaxillofac Surg*. 1995;23:187–189.

10. Handschel J, Wiesmann HP, Stratmann U, Kleinheinz J, Meyer U, Joos U. TCP is hardly resorbed and not osteoconductive in a non loading calvarial model. *Biomaterials*. 2002;23:1689–1695.

11. Byrd HS, Hobar PC, Shewmake K. Augmentation of the craniofacial skeleton with porous hydroxyapatite granules. *Plast Reconstr Surg*. 1993;91:15–22.

12. Su-Gwan K, Hak-Kyun K, Sung-Chul L. Combined implantation of particulate dentine, plaster of Paris, and a bone xenograft (Bio-Oss) for bone regeneration in rats. *J Craniomaxillofac Surg*. 2001;29:282–288.

13. Egli PS, Muller W, Schenk RK. Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbit. A comparative histomorphometric and histologic study of bony ingrowth and implant substitution. *Clin Orthop*. 1988;232:127–138.

14. Nagahara K, Isogai M, Shibata K, Meenaghan MA. Osteogenesis of hydroxyapatite and tricalcium phosphate used as a bone substitute. *Int J Oral Maxillofac Implants*. 1999;7:72–79.

15. Malard O, Bouler JM, Guicheux J, et al. Influence of biphasic calcium phosphate granulometry on bone ingrowth, ceramic resorption, and inflammatory reactions: preliminary in vitro and in vivo study. *J Biomed Mater Res*. 1999;46:103–111.

16. Ignatus AA, Ohnmacht M, Claes LE, Kreidler J, Palm F. A composite polymer/tricalcium phosphate membrane for guided bone regeneration in maxillofacial surgery. *J Biomed Mater Res*. 2001;58:564–569.