The Effect of a Biphasic Ceramic on Calvarial Bone Regeneration in Rats

Hakan Develioglu, DDS, PhD
Emel Koptagel, ProfDr
Rıştı Gedik, DDS, PhD
Laurent Dupoirieux, MD, DMD, PhD

KEY WORDS

Biphasic
Calcium phosphate ceramic
Skull defects
Rat

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. They are also currently used as a coating for uncemented metallic implants to improve osteointegration. 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, is still under discussion. However, porosity of the material is inversely proportional to the mechanical stability of these CaP-based ceramics. 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. 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 ×10). (b) Control defect: the defect area includes connective tissue (Ct). Arrows indicate bone defect margins (hematoxylin-eosin, original magnification ×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 ×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 ×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 ×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 midline 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 regeneration: 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 inflammatory 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. 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
BIPHASIC CERAMIC ON CALVARIAL BONE REGENERATION

<table>
<thead>
<tr>
<th>Site</th>
<th>No Closure</th>
<th>Partial Closure</th>
<th>Complete Closure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control site</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Experimental site*</td>
<td>14</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*P > .05.


