AN EXPERIMENTAL COMPARISON OF THE EFFECTS OF CALCIUM SULFATE PARTICLES AND β-TRICALCMIUM PHOSPHATE/HYDROXYAPATITE GRANULES ON OSTEOGENESIS IN INTERNAL BONE CAVITIES

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ABSTRACT
This experimental study was carried out to investigate the effect of medical grade calcium sulphate and β-tricalcium phosphate/hydroxyapatite on new bone formation. Additionally, the study compared these materials for infection, resorption, biocompatibility, immune reaction, fibrotic encapsulation, foreign body reaction and physical attachment.
Forty, five-month-old female Wistar Albino rats were used. The 40 rats in the study were divided into 2 groups. Medical grade calcium sulphate particles (Surgiplaster®, Bio-Lok International Company) were applied to the rats in group 1 and β-tricalcium phosphate/hydroxyapatite granules (Camceram® Cam Implants by an osteotech, Inc. Company) to those in group 2. On days 10, 21, 30 and 60 postoperatively the femurs were sacrificed and investigated histopathologically. The Mann-Whitney U test was applied to the data obtained as a result of the histopathological analysis of the specimens.
No statistically significant differences were observed between the 2 groups.
In conclusion, it was determined that both materials resulted in similar fibrous tissue and inflammation responses, that their biocompatibilities were very good and that they did not cause foreign body reaction. Osteogenesis also was observed in the 2 groups after day 21. The effects of calcium sulphate on bone formation were faster than those of β-tricalcium phosphate/hydroxyapatite. Osteogenesis was not completed to the same extent in the calcium sulphate group as in the β-tricalcium phosphate/hydroxyapatite group.

Keywords: Medical grade calcium sulphate particles, β-tricalcium phosphate/hydroxyapatite granular, osteogenesis, bone defects, experimental.

Introduction
As in other regions of the skeletal system, bone grafts are also needed in the reconstruction of congenital and acquired bone deformities caused by traumatic, degenerative, infectious, cystic and neoplastic lesions in the oral and maxillofacial region. All substances that may assist in the overcoming of deficiencies occurring in the living organism as a result of these agents, and in restoring function, or that assist in the ordered and rapid completion of such deficiencies by the organism are known as “biomaterials” (1, 17, 22).

The principle features sought in graft and implant materials used in the filling of bone defects are; no antigenic property, resistance to infection, minimum risk of postoperative complication, revascularisation, the facilitation of osteoinduction, osteoconduction and osteogenesis, provision of defect stability, easy shaping, the provision of a radiolucent appearance and long-term preservation (3, 12, 17, 23).

The use of alloplastic materials developed as an alternative to bone grafts has increased considerably in recent years. The aim behind the use of these materials is the provision of a matrix formation that permits bone growth into the implant (15).

The aim in this study was an experimental and statistical comparison of the effects on osteogenesis of medical grade calcium sulphate particles and β-tricalcium phosphate/hydroxyapatite granules that have entered used in the field of oral and maxillofacial surgery in recent years.

Materials and Methods
The study was submitted as a project to the Dicle University Experimental Animal Ethical Committee, and the committee’s approval was obtained. Forty, four-month-old Wistar Albino type female rats weighing 200-240 grams were used as experimental subjects. Operations were performed at the Dicle University Health Sciences and Research and Application Centre Experimental Animal Operating Theatre, and histopathological investigations at the Dicle University Medical Faculty Pathology Department.

Medical grade calcium sulphate hemihydrate particles (Surgiplaster, Bio-Lok International Co.) and β-tricalcium phosphate/hydroxyapatite granules (Camceram, Cam Implants Osteotech Inc.) were used in the study.

The experimental animals were anaesthetised by intramuscular injection of 0.1 ml xylozin hydrochloride (Rompun, Bayer, Turkey) and 0.2 ml ketamin (Ketalar, Eczacıbaşı, Turkey). The femur was exposed using sharp dissection (Figs. 1, 2).

A 10 mm long, 3 mm deep and 2 mm wide bone cavity was opened in the femur using a no. 0.14 round bur (Komet, Germany) under serum physiologic irrigation (Fig. 3).
Medical grade calcium sulphate particles (Surgiplaster, Bio-Lok International Co.) were applied to the first group of 20 experimental animals, and β-tricalcium phosphate/hydroxyapatite granules (Cameram, Cam Implants Osteotach Inc.) to the second group of 20 animals. The periost and subcutaneous tissues were then closed primarily.

For the purpose of prophylaxis, a single dose of antibiotic (gentamicin 0.05 ml/kg) was injected immediately after surgery. Experimental animals were housed in separate cages to prevent them harming one another. Five experimental subjects from each group were sacrificed at the end of follow-ups on days 10, 21, 30 and 60 postoperatively by intraperitoneal injection of an anticoagulant.
overdose of sodium thiopentone (Pental Sodium, I.E. Ulugay İlaç San. TAŞ), and the femurs were extracted.

The specimens were taken for histopathological examination at the Dicle University Medical Faculty Pathology Laboratory. Preparates were examined and scored under a light microscope by a pathologist with no knowledge of the study (Table 1). Osteoblastic activity, foreign tissue reaction, infection, osteogenesis, fibrotic tissue growth, physical attachment, biocompatibility and resorption in the graft criteria were reviewed at histopathological examination. The values obtained were statistically analysed using the Mann-Whitney U-test.

**Table 1**

Histopathological scoring table used for statistical analysis

<table>
<thead>
<tr>
<th>No osteogenesis</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak osteogenesis</td>
<td>2</td>
</tr>
<tr>
<td>Medium osteogenesis</td>
<td>3</td>
</tr>
<tr>
<td>Good osteogenesis</td>
<td>4</td>
</tr>
<tr>
<td>Perfect osteogenesis</td>
<td>5</td>
</tr>
</tbody>
</table>

**Results and Discussion**

All operation regions healed normally in both groups and no complication of any kind was encountered in the postoperative period.

**A—medical grade calcium sulphate hemihydrate group**

**Day 10 findings.** Natural inflammatory changes, mesenchymal cell and capillaryed granulation tissue migration were observed around the graft material on day 10. No foreign body reaction or graft resorption was observed. There was no osteogenesis around the grafts in specimens taken in this period (Fig. 7).

**Day 21 findings.** Generally, a weak osteogenesis potential was observed during the third week. In addition, physical attachment, graft material surrounded by fibrous tissues and fibroblast-like cells were observed, and it was determined that these tissues were well vascularised. It was also determined in specimens taken during this period that new bone formation and resorption in the graft had begun, that inflammation was declining (Fig. 8).

**Day 30 findings.** Graft resorption in the defects in which the graft material was implanted and osteogenesis around it were observed to have increased further compared to the day 21 results. There was no foreign body reaction and the graft biocompatibility was good. Generally, the bone forming in this period showed high osteoblastic activity, and fibrous tissue and inflammation had decreased, being replaced by new bone trabecules around the graft particles (Fig. 9).

**Day 60 findings.** It was observed that the greater part of the medical grade calcium sulphate had been resorbed, and that the small part remaining had dispersed into the intertrabecular area and was mainly included in the new bone. Generally, newly formed bone and bone marrow and high osteoblastic activity were observed in this period (Fig. 10).
Bone healing scores in the group administered β-tricalcium phosphate/hydroxyapatite

Day 10 findings. Natural inflammatory changes around the β-TCP/HA material, mesenchymal cells on the edge of porous sections and capillary loose granulation tissue were observed on day 10. No foreign body reaction or graft resorption were seen in the specimens taken. Graft material physical attachment and tissue compatibility were perfect. There was as yet no osteogenesis in the porous parts of the graft in this period.

Day 21 findings. In the third week osteogenesis potential and physical attachment were determined to be weaker compared to the medical grade calcium sulphate group. In addition, it was determined that in this period fibrous tissue development and vascularisation were moderate, inflammation had declined, new bone foci had begun to form and no foreign body reaction was observed.

Day 30 findings. In this period it was observed that osteogenesis between grafts was at a medium level and that most newly formed bone was localised in the centre of the graft roof-type structure. It was determined that there was very little resorption in the graft, that medium level osteoblastic activity had begun, and that grafts’ attachment to each other and compact bone had increased.

Day 60 findings. On day 60 it was seen that osteogenesis in the bone defect was good and had formed at more or less the same level compared to the other group. A very low level of resorption was observed in the graft, that medium level osteoblastic activity had begun, and that grafts’ attachment to each other and compact bone had increased.

Group I (Calcium sulphate) and II (β-TCP/HA) bone healing scores on days 10, 21, 30 and 60 were analysed by constructing a table (Tables 2, 3).

TABLE 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day 10</th>
<th>Day 21</th>
<th>Day 30</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Subject 2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Subject 3</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Subject 4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Subject 5</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Bone healing scores in the group administered medical grade calcium sulphate hemihydrate particles (Group I).

TABLE 3

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day 10</th>
<th>Day 21</th>
<th>Day 30</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Subject 2</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Subject 3</td>
<td>2</td>
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<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Subject 4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Subject 5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Bone healing scores in the group administered β-tricalcium phosphate/hydroxyapatite granules (Group 2).

Tables 2, 3

Data were analysed using the Mann-Whitney U-test in order to determine which groups there was a statistical difference between in terms of bone healing scores.

Mann-Whitney U-test results

<table>
<thead>
<tr>
<th>Mann-Whitney U</th>
<th>Group 1 Median</th>
<th>Group 2 Median</th>
<th>U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10</td>
<td>1.00</td>
<td>1.00</td>
<td>10</td>
<td>0.513 (NS)</td>
</tr>
<tr>
<td>Day 21</td>
<td>3.00</td>
<td>2.00</td>
<td>7.5</td>
<td>0.314 (NS)</td>
</tr>
<tr>
<td>Day 30</td>
<td>4.00</td>
<td>3.00</td>
<td>7.5</td>
<td>0.221 (NS)</td>
</tr>
<tr>
<td>Day 60</td>
<td>4.00</td>
<td>4.00</td>
<td>10.5</td>
<td>0.606 (NS)</td>
</tr>
</tbody>
</table>

Since variables were discontinuous, the Mann-Whitney U-test was selected for statistical analysis, with two-way variance and a P<0.05 level of significance. The SPSS 12.0 package program was used for statistical analyses.

As a result of the analyses it was concluded that there was no significant difference between the groups in terms of osteogenesis values on days 10, 21, 30 and 60.

Bone graft is frequently indicated in bone deformities that arise for acquired or congenital reasons and in areas of osteotomy performed due to dentofacial deformities. If such defect areas are left to heal spontaneously mature fibrous tissue formation begins in the region following fibrotic structure migration. Complications such as non-union and encapsulation may also arise together with fibrotic healing of this kind. In order to avoid these complications reconstruction with graft materials is required for the purpose of providing regeneration of bone cells in the region (18, 21, 25).

Another unchanging subject of debate has been the graft material in bone defects being compatible with the tissue in which it is implanted and at the same time contributing to new tissue formation, in other words having ideal graft characteristics (18, 21, 25). Many materials have been and still are being tested for the purpose of finding the ideal bone graft, but no graft material establishing 100% of the desired characteristics has yet been found. Bone is the most transplanted tissue. More than 2.2 million bone grafts are used worldwide every year (15). Autogenous bone graft is noteworthy as being the most frequently selected graft for these procedures (5). Autogenous bone contains bone minerals, collagen, growth factors and osteoprogenitor cells required for osteoconduction and osteoinduction. However, alternatives to autogenous graft have been sought due to such disadvantages as the requirement for a second operation site, the length of surgery, limited level of acquisition, donor area morbidity and complications, and additional blood loss (14).

Allografts have been the most preferred alternative to autografts in recent times. However, the use of allografts has
also been limited because the processes applied in order to eliminate risks such as disease transfer risk and foreign body reaction weaken osteogenic properties (6, 13).

Major advances have been made in synthetic materials in research carried out with the aim of finding an alternative. Before this research synthetic materials were seldom preferred over autografts and allografts. The use of synthetic grafts was limited to a level of 10% of all grafts world-wide (15). The reasons for this limited use were unpredictable resorption periods, difficulty in shaping, foreign body reaction and insufficient clinical and experimental studies. As studies regarding synthetic increased, their superiority compared to autografts and allografts emerged and their use spread (4, 7).

Calcium sulphate containing grafts, part of this group and produced as an alternative to other materials, are a synthetic material that have proved themselves due to their having been used for some 100 years (8, 17). The calcium sulphate content we used in our study was medical grade calcium sulphate hemihydrate (Surgiplast, Bio-Lok International Co.). Calcium sulphate, also known as “plaster of Paris,” is obtained from gypsum and has the formulation CaSO4 2(H2O). In his study, Fowler reported that calcium sulphate was used by Dreesman and Peltier and could comfortably be used as a bone graft (10, 20). Peltier et al. (20) and Turner et al. (24) reported that calcium sulphate was an osteoconductive material that permitted osteogenic cell and blood vessel growth and that new bone developed in the cavity emerging as calcium sulphate dissolved. Today, medical grade calcium sulphate is frequently used in various types of bone defect. It can also be monitored radiologically due to the fact it is radio-opaque (16).

In our study it was determined at histopathological examination that calcium sulphate was resorbed from the periphery towards the centre. In addition, all the three-wall defects produced were seen to heal, and it was concluded that with this property calcium sulphate was more successful in defects of this kind.

In the light of the findings obtained in our study and our clinical experience we concluded that calcium sulphate was tissue-compatible and quickly initiated osteogenesis. In addition, calcium sulphate does not lead to foreign body reaction and can produce a good degree of physical attachment between the bone and the graft. It undergoes rapid resorption and is of an osteoconductive nature. Although calcium sulphate is easily shaped, it is brittle in contact with water. For that reason, it is useful for the region to which it is applied to be kept dry until the graft material hardens. Bearing these properties in mind, it was concluded that calcium sulphate can be used as a graft material with the aim of reconstruction in oral and maxillofacial surgery.

The other synthetic material used in this study was β-TCP/HA, biphasic calcium phosphate (Camceram). The graft material consists of 60% HA, 40% consisting of β -TCP. The HA has a 100% crystal structure, while the TCP is entirely made up of beta tricalcium. β-TCP/HA has an osteoconductive nature and encourages bone growth with the 100-500 μm pores in its structure. β-TCP/HA is biologically resorbed and performs optimal bone growth and shaping. Since the content is of a character close to that of natural bone, it has exceedingly good biocompatibility. The graft material can be used on its own, or combined with autografts or bone marrow (9).

In a pediatric study, Passuti et al. applied a β-TCP/HA combination to 12 patients with severe scoliosis and combined this with autogenous graft, carrying out internal fixation and fusion. At the end of an average of 15 months of follow-up, they reported that successful fusion had been obtained in both the group administered the autograft combination and in that administered β-TCP/HA (11). In another study, Bucholz et al. applied HA and cancellous autograft in a series of 40 metaphasic defects and reported that no radiological or clinical difference in terms of osteogenesis was determined at follow-up (19).

Recent studies recommend the use of graft materials prepared for use in bone defects either in combination within themselves or with autogenous graft or growth factors. The reason for these combinations is the disadvantages that emerge when graft materials are used on their own and the wish to achieve an ideal graft tolerated by other graft materials used in combination. The β-TCP/HA material we used in our study is a combination produced in compatibility with that aim. Both graft materials used in this combination have been used many times on their own and positive results obtained. However, the fact that β-TCP in particular exhibits a brittle characteristic in the defect in which it is implanted, is unable to resist forces and that HA is not resorbed and remains for a long time in the defect region has led to various complications. In order to eradicate these complications, the two graft materials were combined and began being used in bone defects (2, 17).

Conclusions
As a result of the experimental and statistical comparison of the effects on osteogenesis of medical grade calcium sulphate particles and β-tricalcium/hydroxyapatite granules, which have recently entered the field of oral and maxillofacial surgery;
1) Although there was no very apparent difference between them, they differed from one another in terms of osteogenesis time and resorption duration. While calcium sulphate particles initiated faster osteogenesis they were more quickly resorbed, the β-TCP/HA granules began osteogenesis later in the defects to which they were applied and resorption took place in a later period.
2) No foreign body reaction or infections were observed in either graft material, and inflammation at more or less the same level was observed throughout the wound healing procedure.
3) While no biocompatibility problem was observed, it was determined that β-TCP/HA was more biocompatible than calcium sulphate.
4) When their effects on bone were examined; osteogenesis was observed in both groups after day 21, but it was
determined that calcium sulphate carried out osteogenesis in a faster manner, but that the emerging bone volume was not as ideal as that obtained with β-TCP/HA. Statistically, no significant difference was observed between the two groups.

5) No problem was determined in the physical attachment that both graft materials established with healthy bone in the defect region. In addition, more powerful physical attachment was observed in the subject group in which β-TCP/HA was used.

6) The graft materials used in both groups prevented fibrotic tissue progressing to the defect region, and microscopic examination determined negligible fibrotic growth.

7) Both graft materials gave an almost perfect result in the three-walled defects formed.

In the light of these findings, it was concluded that medical grade calcium sulphate hemihydrate particles and β-TCP/HA granules had similar healing processes in three-walled defects up until day 10, and that in the days that followed calcium sulphate dissolved rapidly and initiated fast osteogenesis, whereas β-TCP/HA was more ideal in terms of the newly forming bone volume. Statistically, it was concluded that there was no significant difference in terms of osteogenesis in the two groups, and that the combination of these synthetic graft materials with other graft materials needs to be supported by new experimental and clinical studies.

REFERENCES