# Histological comparison of autograft, allograft-DBM, xenograft, and synthetic grafts in a trabecular bone defect: An experimental study in rabbits

**Authors' Contribution:**
**A** Study Design
**B** Data Collection
**C** Statistical Analysis
**D** Data Interpretation
**E** Manuscript Preparation
**F** Literature Search
**G** Funds Collection

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## Summary

**Background:** Different types of bone-graft substitutes have been developed and are on the market worldwide to eliminate the drawbacks of autogenous grafting. This experimental animal study was undertaken to evaluate the different histological properties of various bone graft substitutes utilized in this hospital.

**Material/Methods:** Ninety New Zealand white rabbits were divided into six groups of 15 animals. Under general anesthesia, a 4.5 mm-wide hole was drilled into both the lateral femoral condyles of each rabbit, for a total of 180 condyles for analysis. The bone defects were filled with various grafts, these being 1) autograft, 2) DBM crunch allograft (Grafton®), 3) bovine cancellous bone xenograft (Lubboc®), 4) calcium phosphate hydroxyapatite substitute (Ceraform®), 5) calcium sulfate substitute (Osteoset®), and 6) no filling (control). The animals were sacrificed at 1, 3, and 6 months after implantation and tissue samples from the implanted areas were processed for histological evaluation. A histological grading scale was designed to determine the different histological parameters of bone healing.

**Results:** The highest histological grades were achieved with the use of cancellous bone autograft. Bovine xenograft (Lubboc) was the second best in the histological scale grading. The other substitutes (Grafton, Ceraform, Osteoset) had similar scores but were inferior to both allograft and xenograft.

**Conclusions:** Bovine xenograft showed better biological response than the other bone graft substitutes; however, more clinical studies are necessary to determine its overall effectiveness.

**key words:** bone graft • autograft • allograft • xenograft • Lubboc • Grafton • Ceraform • Osteoset

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## BACKGROUND

Substances that enhance fracture healing and bone regeneration have valuable clinical application and merit future research. Advances in these technologies will enhance our ability to heal fractures, non-unions, and bone defects in a more effective and expedient manner. Bone graft is considered as the most commonly transplanted tissue.

The biology of bone grafts and their substitutes is appreciated by understanding the bone formation processes [1–5]. The term "bone graft substitute" describes a spectrum of products that have various effects on bone healing. Unfortunately, there is little information in the literature about when and where to use these devices. In general, we categorize the properties of bone graft substitutes as osteoinductive, osteoconductive, or osteogenic. Osteoinduction is a process that supports the mitogenesis of undifferentiated mesenchymal cells, leading to the formation of osteoprogenitor cells that form new bone. Osteoconduction is a property of a matrix that supports the attachment of bone-forming cells for subsequent bone formation. Osteogenesis is a relatively new term that can be defined as the generation of bone from bone-forming cells.

The first attempt at bone grafting was reported in 1668 by the Dutch surgeon Job Van Meek'ren [6]. In 1980, Lindholm and Urist were the first to try adding bone marrow to bone matrix to enhance healing in a study that quantified new bone formation [7]. New bone graft substitutes have been devised in recent decades, such as demineralized bone matrix (DBM), bone morphogenetic proteins (BMPs), calcium phosphate (CP), calcium sulfate (CS), hydroxyapatite (HA), highly purified bovine xenograft, and more, but clinical evidence of their efficacy among clinical and experimental studies. Clinical evidence for the use of currently available bone graft substitutes in humans ranges from level I to level IV studies. The Orthopaedic Trauma Association Orthobiologics Committee provided a summary of the levels of recommendation regarding various bone graft substitutes; according to their report only the osteoinductive effects of rhBMP-2 and -7 are well documented at the moment, as there is level-I evidence that supports their clinical use [4]. In contrast, there is less documentation for other bone substitutes, as they made their way into the marketplace simply by showing equivalent efficacy to a predicate medical device and have not been subjected to clinical analysis.

This experimental animal study was undertaken to evaluate the histological properties of various bone graft substitutes that are utilized in our hospital. Various histological parameters were examined, such as lamellar and woven bone formation, bone graft incorporation, the presence of the bone defect area, the progress of vascularization, and the inflammatory cellular response. These parameters were compared with a group with cancellous autograft filling, which is considered the "gold standard" of bone grafting, and an unfilled control group. The baseline hypothesis was to demonstrate if one or more of the examined grafts had histological properties similar to the cancellous bone autograft. We suggest that there are insufficient studies indicating the success presented by allografts, xenografts, and bone graft substitutes and this study was planned and conducted to compare all these common products.

## MATERIAL AND METHODS

Ninety New Zealand white rabbits were randomly divided into six groups of 15 animals. All animals were skeletally mature (approximately 14 weeks old) and weighed between 3.75 and 4.25 kg. All animal experiments were carried out according to the policies and principles established by the Hellenic Institutes of Health Guide for the Care and Use of Laboratory Animals. The design of the surgical procedures was critically reviewed and observed by an experienced veterinary surgeon. The study was approved by the ethics committee of our hospital.

In all animals the procedure was carried out under general anesthesia using an intramuscular injection of ketamine 35 mg/kg and xylazine 5 mg/kg. Using a lateral surgical approach, a 4.5-mm-wide hole was drilled to a depth of 8 mm (using a custom-made stop notch) in the lateral femoral condyle of each limb, making a total of 180 femoral condyles for analysis. The holes were irrigated with normal saline, dried with sterile gauze, and filled carefully with the various grafts as follows:

- Group I – autograft. Autograft bone was obtained from the iliac crest of each animal at the time of surgery. After blunt dissection and elevation of the periosteum, a large-bore bone-biopsy trephine was used to harvest cores of corticocancellous bone graft. Cortical bone and soft tissues were removed and the autograft bone was morselized into small cubes. These were impacted without force into the bone defect area.
- Group II – allograft DBM crunch, a demineralized bone matrix from cadaveric cortical bone (Grafton®; Osteotech Inc, NJ, USA).
- Group III – xenograft (Lubboc®; Osteocell SA, Athens, Greece), comprised of bovine trabecular bone matrix, which mainly consists of type I collagen and hydroxyapatite and is highly purified.
- Group IV – substitute calcium phosphate hydroxyapatite. $Ca_{10}(PO_4)_6(OH)_2$, a synthetic material with a structure and composition similar to bone. It is composed of 65% phosphocalcic hydroxylapatite (HAP) and 35% tricalcium phosphate (TCP) (Ceraform® bone void filler; Teknimed SA, France).
- Group V – surgical-grade substitute calcium sulfate ($CaSO_4$) (Osteoset® pellets; Wright Medical Europe SA, Amsterdam, The Netherlands).
- Group VI – control (no filling).

The soft tissues were closed in layers and the procedure was repeated on the contralateral limb. The operation sites were protected with a spica elastic bandage for a period of 48 hours. Postoperatively, the animals were allowed to walk ad libitum. The animals were managed for pain, as required, with subcutaneous injections of butorphanol tartrate (Torbutrol, 0.01 mg/kg body-weight). Intramuscular antibiotics (penicillin G procaine and benzathine, 1.5 to 2 ml per animal) were administered for two days following surgery. At the end of the study period the animals were killed with an intravenous overdose of barbiturate (Beuthanasia-D; pentobarbital, 0.45 ml/kg body-weight).

Five animals from each group were sacrificed at 1, 3, and 6 months after implantation. Overall, we had 30 rabbits

**Table 1.** Histological grading scale for the degree of healing.

| | | |
|---|---|---|
| Quality of union | Bone union | 3 |
| | Fibrous union | 2 |
| | Fibrocartilaginous union or cartilage union | 1 |
| | No sign of fibrous or other union | 0 |
| Bone defect area | No detectable | 2 |
| | Clearly observed <50% | 1 |
| | Clearly observed >50% | 0 |
| Inflammatory cells | No presence of inflammation cells | 2 |
| | Few inflammatory cells still present | 1 |
| | Increased number of inflammatory cells present | 0 |
| Neo-angiogenesis | No presence of vascular channels | 2 |
| | Few vascular channels still present | 1 |
| | Increase number of vascular channels present | 0 |
| Bone-graft incorporation | | |
| No new bone, all or most of graft visible | Graft material present, no incorporation, and no new-bone formation | 0 |
| | Graft present, some incorporation with new-bone formation, and small amount of new bone | 1 |
| | Graft present, some incorporation with new-bone formation, and moderate amount of new bone | 2 |
| Decreasing graft, increasing new bone | Graft present, some incorporation with new-bone formation continuous with host bone, and early remodeling changes in new bone | 3 |
| | Decreased amount of graft (compared with grade 3), good graft incorporation, and ample new bone | 4 |
| | Less amount of graft still visible (compared with grade 4), good incorporation of graft and new bone with host and ample new bone | 5 |
| No graft visible, extensive new bone | Difficult to differentiate graft from new bone, excellent incorporation, and advanced remodeling of new bone with graft and host | 6 |

×2 femoral condyles = 60 samples at each time period. Bone tissue samples from the implanted area of both distal femora were taken for histological evaluation. These were fixed in 10% neutral buffered formalin, decalcified with 7% $HNO_3$, and embedded in paraffin. Five slices (4–5 µm) were taken from the middle portion of both lateral femoral condyles of each animal for staining with hematoxylin-eosin (HE). The HE-stained histological sections were examined under a light microscope and evaluated by two pathologists (D.J.P. and C.D.S.) who were unaware of the original surgical procedure and the type of graft used. The specimens were evaluated with a 15-point histological grading scale to determine the quality of union, the presence of the bone defect area, the progress of vascularization, and inflammatory cell response as well as the degree of bone-graft incorporation and remodeling (Table 1). To restore the validity of the assumption of independence of the observations, the mean of the two readings (by the two pathologists) from each animal were used.

**Statistical analysis**

The histological grading results were evaluated with the Kruskal-Wallis one-way analysis of variance to examine the effects of treatment group and postoperative time and a p value of ≤0.05 was calculated to determine significance. Kruskal-Wallis multiple comparisons were performed for each graft type at each time period. An alpha level of 0.10 was chosen as the desired overall significance level. All statistical analyses were performed on a personal computer using statistical analysis software (SPSS, version 11.5, Chicago, IL, USA).

**RESULTS**

No postoperative infections or other complications were noted. One animal from the control group (VI) and three from the experimental groups (III, IV, and V) died from gastrointestinal infection during the six-month period of the study. All the other animals survived and were in good
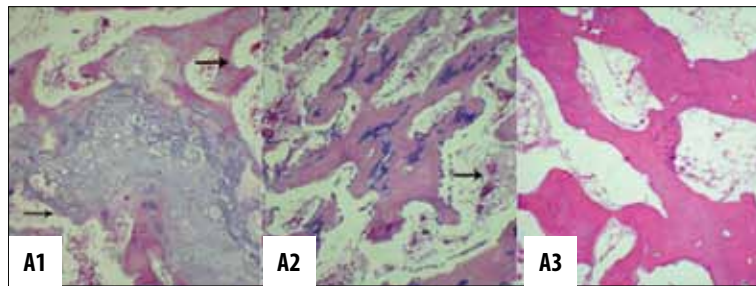
**Figure 1.** Histological characteristics of autograft (group I). (**A1**) Woven and lamellar bone can be recognized at the site of autograft implantation. Blood vessels are prominent (arrow). (**A2**) Sites of endochondral ossification are present (thin arrow). Newly formed bone is lined with activated osteoblasts (thick arrow). (**A3**) Lamellae of mature bone. No signs of inflammation.
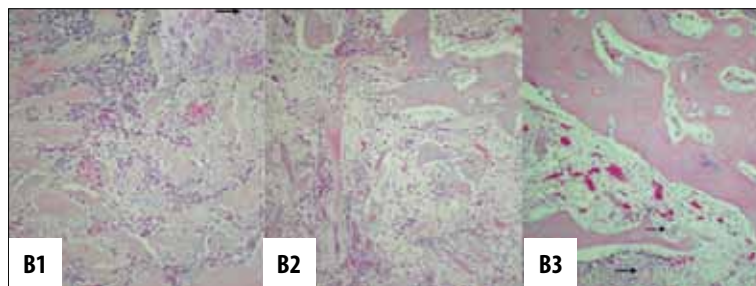


**Figure 2.** Histological characteristics of Grafton (group II). (**B1**) DBM fibers are evident, covered by activated osteoblasts. Numerous giant cells can be observed (arrow). (**B2**) Woven bone, granulation tissue, and inflammatory cells can be identified. (**B3**) New bone has filled the defect area. DBM fibers are still present (arrows).
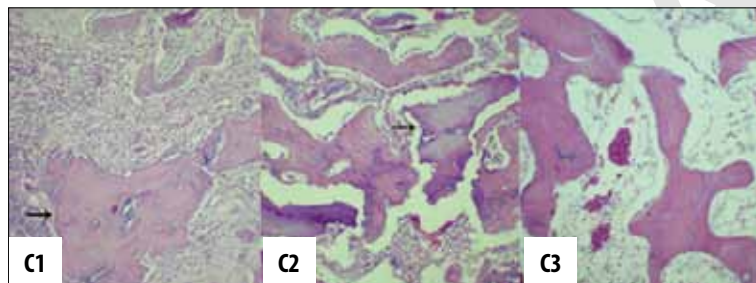


**Figure 3.** Histological characteristics of Lubboc (group III). (**C1**) Xenograft graft (arrow) surrounded by a small number of activated osteoblasts. (**C2**) Xenograft is remarkably reduced (arrow). Woven and lamellar bone has been synthesized. (**C3**) Graft material has been integrated. There is no inflammation.

health at the time of harvesting. The histological observations for each type of graft are described below.

### Group I: autograft

At one month, the area of implantation was hardly detectable and the osseous cavity was filled mainly by lamellar (mature) bone, although woven (immature) bone was also identified. Endosteal bony surfaces were lined by numerous activated plump osteoblasts as well as bone-resorbing osteoclasts. Focally, the configuration of cutting cones was marked. Between bone lamellae, blood vessels were also observed. Subperiosteal bone formation was intense. No inflammatory cells were present at the area of implantation (Figure 1-A1).

At three and six months the bone defect could not be detected at all. The lamellae of mature bone were lined by a reduced number of active osteogenic cells. Vascularization was still present, but the number of blood vessels was considerably reduced. At the subperiosteal level, the trabeculae of mature bone were observed. There were no signs of inflammation (Figure 1-A2, A3).

### Group II: demineralized bone matrix (Grafton®)

At one month, the graft fibers were clearly evident. They were surrounded by a small number of active osteoblasts. In addition, a relatively large number of multinucleated graft-

resorbing cells were present. Newly produced bone could not be observed, although active osteoblasts lined the pre-existing bone lamellae. Loose connective tissue and blood vessels occupied the bone marrow space. Numerous lymphocytes and eosinophils were present, which indicated a chronic inflammatory reaction (Figure 2-B1).

At three months, the DBM was clearly visible, although reduced in quantity. Newly produced woven bone was evident and covered the graft material. Notably, the foci of newly deposited bone that did not adhere to the graft material were identified. Moreover, granulation tissue filled the bone marrow space. Foci of endochondral osteogenesis as well as subperiosteal bone formation were observed. Lymphocytes and eosinophils were evident among bone trabeculae (Figure 2-B2).

At six months, a small number of DBM fibers were still present. Lamellar bone had been produced and woven bone was also present. New bone filled the defect area. Bony trabeculae were surrounded by active osteoblasts and osteoclasts. Moderate chronic inflammation was still present (Figure 2-B3).

### Group III: xenograft (highly purified, Lubboc®)

At one month, the graft material was evident. Lamellae of the dead bone graft spicules were covered focally by newly
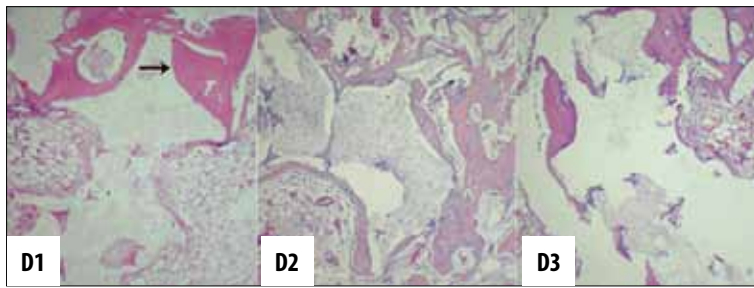
**Figure 4.** Histological characteristics of Ceraform (group IV). (**D1**) Calcium phosphate hydroxyapatite graft material and granulation tissue can be easily identified. Note the host bone spicules (arrow). (**D2**) Hydroxyapatite surrounded primarily by woven bone. (**D3**) Graft material is still evident. Lamellar and woven bone can be detected.
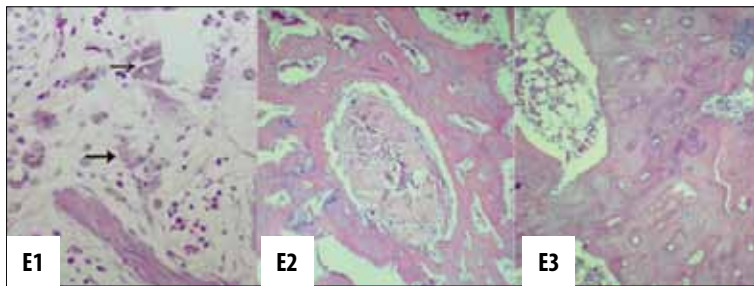


**Figure 5.** Histological characteristics of Osteoset (group V). (**E1**) Calcium sulfate particles are present. Among bony trabeculae, graft-containing macrophages can be observed (arrows). (**E2**) Calcium sulfate particles and newly formed bone are evident. (**E3**) Lamellar and woven bone can be easily identified.
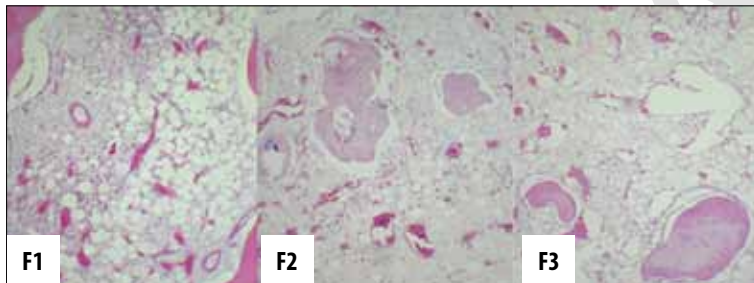


**Figure 6.** Histological characteristics of no filling (group VI). (**F1**) Bone defect, lipomatous bone marrow, and granulation tissue are evident. (**F2**) Woven bone has been produced. Mild chronic inflammation is detectable. (**F3**) Defect area is still evident, occupied mainly by granulation tissue and woven bone.

synthesized woven bone, which was rimmed by a small number of osteoblasts. Among the graft spicules, loose connective tissue and numerous blood vessels were observed. In addition, areas of mature chondrocytes around the xenograft were indicative of endochondral ossification. Subperiosteal immature bone formation was apparent. Numerous lymphocytes and eosinophils suggested the presence of moderate chronic inflammation (Figure 3-C1).

At three months, the xenograft was still evident. However, the amount of bone graft was remarkably reduced. The graft material was covered by woven and lamellar bone, which was rimmed by plump, activated osteoblasts and multinucleated bone-resorbing cells. More importantly, newly produced bone was detected at sites that were not in close proximity to the graft material. Granulation tissue was recognized among the graft spicules and vascularization was still prominent. Immature and mature bone was produced subperiosteally. Chronic inflammatory cells were present among bone lamellae (Figure 3-C2).

At six months, almost all of the xenograft material was integrated. The organization of newly formed bone displayed a lamellar pattern, although a small amount of woven bone was still evident. A few osteoblasts covered the bony surfaces. Small foci of endochondral bone formation were observed. Vascular channels were remarkably reduced. There were no inflammatory cells at the implantation sites (Figure 3-C3).

**Group IV: calcium phosphate hydroxyapatite substitute (Ceraform®)**

At one month, graft material was present. The graft particles and host bone spicules were lined by stimulated osteoblasts and sparse osteoclasts. Among the bone trabeculae, granulation tissue and newly formed vessels were identified. Moreover, subperiosteal bone synthesis was evident. Mild chronic inflammation was present (Figure 4-D1).

At three months, graft material was observed. Hydroxyapatite was surrounded mostly by woven bone. Nonetheless, mature lamellar bone was also detectable. Active osteoblasts and very few osteoclasts lined the endosteal bone surfaces. Mature cartilage cells were observed within the osteoid, indicating endochondral bone synthesis. Angiogenesis was still evident and a few inflammatory cells were identified (Figure 4-D2).

At six months, graft material was still evident. Periosteal and endosteal bone synthesis was augmented. Lamellar and woven bone was observed. Neovascularization was considerably restricted and few inflammatory cells were detected (Figure 4-D3).

**Group V: calcium sulfate substitute (Osteoset®)**

At one month, graft material particles were evident. Although the defect areas were still obvious, newly formed granu-

lation tissue and lipomatous bone marrow were present. Preexisting bone tabeculae were covered by activated osteoblasts. Among bony islands, numerous graft-containing macrophages were observed. Subperiosteal bone formation occurred around the implantation area. A few chronic inflammatory cells were evident (Figure 5-E1).

At three months, calcium sulfate particles were barely present. Lamellar and woven bone was evident, although the bone defect was not completely closed. In addition, loose fibrous tissue and neovascularization were detectable. Mild chronic inflammation was evident (Figure 5-E2).

At six months, graft material could not be identified. Complete closure of the defect had been achieved due to lamellar and woven bone synthesis. Granulation tissue and blood vessels were diminished. Areas of subperiosteal bone production were still evident. Inflammatory cells were absent (Figure 5-E3).

**Group VI: control**

At one month, the bone defect was clearly observed. The cavity was filled with lipomatous bone marrow, loose connective tissue, and vessels and there was no evidence of newly synthesized bone in the region of implant insertion. However, a small amount of woven bone was recognized at the subperiosteal level and there were no inflammatory cells (Figure 6-F1).

At three months, the bone defect was evident. Granulation tissue, woven bone, and, to a lesser extent, lamellar bone were still observed. Newly produced bone was lined by activated osteoblasts; osteoclasts appeared to be absent. At the periphery of the defect, foci of endochondral ossification were present (Figure 6-F2).

At six months, defect areas were still evident. They were composed mostly of woven bone, although regions of mature bone were also detected at the periphery of the cavity. In addition, islands of mature cartilage were observed, denoting the presence of endochondral ossification (Figure 6-F3).

**Overall results**

According to the histological grading scale, the autograft showed the best results at any given time period. Among one, third, and sixth months of implantation there were no significant differences between the various histological parameters and the overall score ($\alpha > 0.10$). All the other graft types showed significantly worse scores with respect to autograft ($p < 0.05$). Lubboc showed a significantly better score than the other three grafts (Grafton, Ceraform, and Osteoset). In contrast to autograft, there was a significant alpha level among one, third, and sixth months of implantation regarding graft incorporation and overall score in all the other graft types. The longer the time, the best integration noted. Ceraform showed the worst scores in all categories.

## Discussion

Large amounts of bone graft material are frequently utilized to elicit the healing of bone defects associated with a number of orthopedic reconstructive procedures, including joint revision, tumor resection, spinal fusion, and treatment of trauma or infection. The most common grafts used alone or in combination are autograft, allograft, demineralized bone matrix (DBM), xenograft (bovine), and substitute bone grafts (calcium sulfate and calcium phosphate hydroxyapatite). To decide which graft is the most appropriate for a given condition, an understanding of the biological function (osteogenesis, osteoinduction, osteoconduction) of each graft is necessary. Furthermore, one has always to consider that stable conditions in the host are absolutely essential for the incorporation of any graft material [8].

Although bone grafts and bone graft substitutes are widely used, there are still many unanswered questions concerning the basic processes of graft immunology, incorporation, and remodeling [2]. Any material considered for use as a bone substitute must meet the following requirements: a) it must be fully biocompatible, b) it must be able to serve as an anchoring surface for host cells, c) it must have a porosity that allows osteoconduction, and d) it must be progressively resorbed and replaced by new bone (creeping-substitution) [9].

Cancellous bone autograft remains the gold standard to which every substitute must be compared. Cancellous bone has a major advantage in that it supplies not only a bone volume, but also osteogenic cells that are capable of quickly laying down new bone [10]. In the present study, at one month after implantation the cavity was filled with mature bone. Bone trabeculae were surrounded by activated bone-producing osteoblasts as well as numerous bone-resorbing osteoclasts. These findings suggest that at one month, the autologous graft induced bone-remodeling activation. The total grade of autograft was not statistically different among one, third, and sixth months of implantation.

It is also well-known that blood vessels play a key role in bone formation as they transport growth factors and bone-related cytokines to the remodeling area [11]. Accordingly, extensive vascularization among bony trabeculae one month after implantation was observed. However, the number of blood vessels and the formation of granulation tissue were significantly reduced at six months, indicating a decline in the bone-remodeling process. Sections taken from the areas of autografting did not display any inflammatory reaction, which suggests harmonious graft incorporation. The advantage of autologous bone graft lies in its excellent incorporation properties, which are due to osteogenesis, osteoinduction, and osteoconduction and the fact that it demonstrates histocompatibility and a low risk of transmitting disease. However, there is a limited quantity of autologous bone graft and there is a 15–21% possibility of donor-site complications [12].

The histological sequence of events associated with bone allograft repair is qualitatively similar, if not identical, to that observed in autografts. The biological behavior of allografts, however, is comparatively inferior to that of autografts. Bone allograft is available in many preparations: demineralized bone matrix (DBM), morselized and cancellous chips, corticocancellous and cortical grafts, and osteochondral and whole-bone segments.

Demineralized bone matrix (DBM) acts as an osteoconductive and, possibly, an osteoinductive material, revasculariz-

es quickly, and its biological activity is ascribed to proteins and the various growth factors present in the extracellular matrix. [4,13,14]. In this study, the presence of activated osteoblasts surrounding collagen fibers of the graft material as well as host bone trabeculae observed during the first month suggest that an osteoconductive process was induced by the DBM graft. The large amount of granulation tissue, along with the woven bone and the multinucleated giant cells at the site of graft fibers, all of which were observed at the third month, indicate that bone remodeling was in progress. Interestingly, newly formed bone and cartilage islands were evident not only in areas in close proximity to graft putty, but also at more distant sites. This observation implies that in addition to osteoconductive properties, DBM also demonstrates some osteoinductive properties. This is further supported by the presence of mature bone (next to and distant from the implanted site) six months after implantation. The presence of chronic inflammatory cells throughout the six-month period is regarded as a mild nonspecific inflammatory reaction to the implanted DBM. Our results are in conformity with those of the reported clinical applications of demineralized bone matrix [15–17].

Xenograft bone grafts represent an unlimited supply of available material if it could be processed to be safe for transplantation in a human host. The main concern in products of bovine origin is the potential transmission of prion infection. However, current EEC regulations permit their surgical therapeutic use if obtained from cattle under 6 months of age, which are supposed to be not at risk of prion transmission. Lubboc is a purified trabecular bone matrix (which mainly consists of type I collagen and hydroxyapatite) that fulfils this requirement. It is manufactured in four stages, including high-pressure washing to remove the bone marrow, delipidation by extraction using organic solvents, two treatments with 8 M urea to extract proteins, and sterilization by accelerated electron beams at 25 kGy. There are few reports in the literature regarding xenograft bone substitutes. Some have shown good results in animal models [18,19] and clinical studies [20,21], while others have shown slower integration than human allograft [22] or lower union rates with persistent radiolucent lines and local complications [23].

In the present study, Lubboc induced the activation of host osteo-producing cells since bone graft endosteal surfaces were surrounded by plump/activated osteoblasts, a finding observed in the first and third months which is suggestive of an osteoconductive function. In addition, the presence of granulation tissue and the identification of chondrocytes around the graft as well as in areas far from the implantation site indicate that Lubboc also has osteoinductive properties. At 6 months, lamellar/mature bone was observed throughout the medullary cavity and the cancellous and compact/cortical bone. Although Lubboc shares approximately the same biological behavior with DBM, the absence of inflammation and the almost complete remodeling suggest that this biomaterial was more effective in the present study. Lubboc showed significantly better results in all histological parameters as well as in the final score with respect to DBM. Since type I collagen is the main component of the extracellular matrix of the bone, the addition of collagen to composites of hydroxyapatite and tricalcium phosphate significantly improves the regeneration of bone defects com-

pared with the use of minerals alone [6,24,25]. Collagen appears to provide an excellent substrate for vascular invasion, the deposition of minerals, and the initiation of bone remodeling. It may also serve as a bone-formation inducer with the implant by binding circulating proteins and a number of growth factors [26–29]. Recently, Jager et al. [30], in a comparative experimental study, found significant differences (p<0.05) between the tested biomaterials (including Grafton and Lubboc) regarding their biocompatibility and toxic effects to human bone marrow cultures. Grafton lead to a significant decrease of pH, which is considered to be a major factor for cell death. Another recent study by Keskin et al. [31] revealed that when xenografts are combined with autologous bone marrow, their incorporation into the host bed accelerates significantly.

To avoid the morbidity associated with autologous bone harvesting and the possibility of transmission of diseases with the use of allografts/xenografts, researchers have developed several artificial bone graft substitutes [32,33]. The most commonly used calcium phosphate ceramics are hydroxyapatite and tricalcium phosphate used *ex vivo* in the form of implant coatings and defect fillers [34,35]. The histological pattern of osteoid with viable osteoblasts and multinucleated giant cells is suggestive of active remodeling in the newly formed bone [36].

Ceraform bone void filler is an osteoconductive macroporous implant made of synthetic beta tricalcium phosphate (30–40%) and hydroxyapatite (60–70%). It has a multidirectional interconnected porosity structure, similar to that of human cancellous bone. The porosity is 60–85% and the pore size 150–400 microns. In the present study, during the first month, Ceraform particles were surrounded by activated bone-producing osteoblasts. Foci of endochondral ossification were detected within the osteoid. Granulation tissue and blood vessels were observed among bone trabeculae, whereas woven bone was gradually replaced by lamellar bone during the third and sixth months. Moreover, cartilage was almost completely transformed into bone tissue through the process of endochondral osteosynthesis. However, the newly formed bone was restricted to the graft area. These findings suggest that calcium phosphate hydroxyapatite substitute has osteoconductive properties. The overall score for Ceraform was very low in contrast to the other tested grafts.

Calcium sulfate (plaster of Paris) has been used since 1892 as a bone void filler. Recently, surgical-grade calcium sulfate has been used as a bone graft substitute [37–39]. Multicenter clinical studies have demonstrated that the trabecular bone filling is qualitatively similar to that seen in autografts [40]. In the present study, activated osteoblasts, mature chondrocytes, and granulation tissue were evident from the first month. These histological findings indicate that Osteoset has osteoconduction properties. No evidence of osteo-inducing activity could be detected. At three and six months, woven bone was gradually substituted by lamellar bone, while inflammatory cells were not evident. These data suggest that surgical-grade calcium sulfate is a host-environment-friendly biomaterial that induces satisfactory bone production. The histological grade score for Osteoset was similar to that of the DBM substitute and superior to the Ceraform substitute (p<0.05). A recent animal study by Fujishiro et al. [41] showed that impacted DBM preparations (AFT Bone

Void Filler) in a sheep vertebral body contained significantly more new bone at 12 weeks postoperatively than the defects treated with calcium sulfate or left empty.

## CONCLUSIONS

The present study compared the histological properties of several bone graft substitutes which are widely utilized nowadays. Autograft remains the "gold standard" of grafting, showing excellent incorporation properties. Bovine xenograft (Lubboc) induced lamellar bone synthesis more efficiently than DBM allograft. Bone substitutes (Ceraform and Osteoset) were inferior to both allografts and xenografts. Although the literature contains a large number of studies on the effects of different agents and modalities on bone repair and healing, it still is unclear how these agents work or in what circumstances they should be used. Additional multicenter prospective randomized studies are needed to define the indications, specifications, dosage, limitations, and contraindications in the treatment of bone defects. Studies are also needed to address the full clinical feasibility of the role of each modality in fracture healing and repair.

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