A RADIOGRAPHICAL AND BIOMECHANICAL STUDY OF DEMINERALIZED BONE MATRIX IMPLANTED INTO A BONE DEFECT OF RAT FEMURS WITH AND WITHOUT BONE MARROW

by M. GEBHART* and J. LANE**

Repair of large bone defects represents a challenge to orthopedic surgery since autogenous graft is not available in large amounts. Demineralized bone matrix (DBM) which contains bone morphogenic protein, a potent osteoinductive glycoprotein, and collagen, an osteoconductive matrix, may be an effective substitute for these graft materials. Bone marrow which contains osteoprogenitor cells could potentiate the osteoinductive and osteoconductive properties of demineralized bone matrix.

This study tested the ability of demineralized bone matrix with and without bone marrow to bridge large segmental defects, and evaluated the results both radiographically and biomechanically as compared to autogenous (isogenous) cancellous bone graft. Demineralized bone-matrix segments implanted into a plated femoral segmental defect in rats resulted in firm union in most animals. Bone marrow significantly enhanced bone formation of demineralized bone-matrix implants at an early stage but with time, differences between bone marrow-augmented and bone marrow-deprived demineralized bone implants were no longer demonstrable radiographically and biomechanically. Newly formed bone had about 50% of the strength of the contralateral control bones. Femurs implanted with cancellous bone isografts had similar evidence of absolute union rate, radiographic and mechanical properties as DBM-implanted femurs.

Keywords : demineralized bone matrix ; bone ; bone marrow.

Mots-clés : matrice osseuse déminéralisée ; os ; moëlle osseuse.

SAMENVATTING

M. GEBHART en J. LANE. Radiologisch en biomechanisch onderzoek van gedemineraliseerd botimplantaat met en zonder toevoeging van benmerg.

Het herstellen van grote botdefecten is door het onvoldoende aanwezig zijn van grote hoeveelheden autologisch bot een ingewikkeld orthopedisch probleem.

Het gedemineraliseerde botmatrix bevat enerzijds een glycoproteïne, het BMP (bone morphogenic protein), dat de botnieuwvorming veroorzaakt en anderzijds collageen dat osteoconductief is.

Dit gedemineraliseerde botmatrix zou een belangrijke bron kunnen zijn als bottransplantaat.

Het beenmerg, dat botvormende cellen bevat, zou de osteo-inductieve en osteo-conductieve eigenschappen van gedemineraliseerde bottransplantaties kunnen verbeteren.

Dit onderzoek had als doel te onderzoeken of de bottransplantatie van gedemineraliseerd bot, met of zonder beenmerg, een overbrugging van segmentdefecten van het femur bij ratten kan doen ontstaan. De resultaten werden radiologisch en biomechanisch bekeken en vergeleken met de uitslagen verkregen indien men autologische spongieuze bottransplantaties had uitgevoerd in plaats van die met gedemineraliseerd bot.

* Department of Orthopedic Surgery, Jules Bordet Institute, Université Libre de Bruxelles, Brussels (Belgium).

** Department of Orthopedic Surgery, Memorial Sloan Kettering Cancer Center, New York (U.S.A.).

Wanneer het gedemineraliseerde bot in de botdefecten van bijbenen geïmplanteerd en gefixeerd werd door een plaat, dan werd in het algemeen een overbrugging door nieuw bot en een mechanische stabiliteit geconstateerd.
Het toevoegen van beenmerg versnelde op significante wijze de nieuwvorming van bot, door de aanwezigheid van het drie weken tevoren gedemineraliseerde bot.
Maar men heeft dit verschil noch radiologisch noch biolomechanisch kunnen aantonen 6, 9 of 12 weken na de transplantatie.
Het nieuwgevormde bot had, vergeleken met het als controle dienende contralaterale dijbeen, een 50% mechanische weestand tegen een breuk.
De dijbenen met een spongieus gemineraliseerde bottransplantatie hadden eenzelfde mechanische en radiologische genezing in vergelijking met de dijbenen waarbij een gedemineraliseerde bottransplantatie was uitgevoerd.

RÉSUMÉ

M. GEBHART et J. LANE. Étude radiographique et biomécanique d'implants osseux déminéralisés avec et sans adjonction de moelle osseuse.

La réparation de larges défects osseux représente un défi pour la chirurgie orthopédique, vu la quantité insuffisante de greffes osseuses autogéniques. La matrice osseuse déminéralisée (DBM) contient une glycoprotéine, la BMP (bone morphogenic protein), induisant la néoformation osseuse, et du collagène constituant un support ostéoconductif. Cette matrice osseuse déminéralisée pourrait être une source de greffes osseuses importantes. La moelle osseuse, qui contient des cellules ostéoprogénitrices, pourrait potentialiser les propriétés ostéoinductives et ostéoconductives des greffes d'os déminéralisé.
Le but de cette étude est d'établir si l'implantation d'os déminéralisé avec ou sans moelle osseuse peut conduire à des pontages osseux de défects segmentaires d'os longs chez les rats. Les résultats ont été évalués sur le plan radiologique et biomécanique et comparés aux résultats obtenus en utilisant des greffes osseuses spongieuses isologues à la place de l'os déminéralisé. Quand l'os déminéralisé a été implanté dans des défects osseux de fémurs et maintenu par une plaque, un remplissage par un os néoformé et une stabilité mécanique ont été généralement observés. L'adjonction de moelle osseuse accélérait significativement la néoformation osseuse induite par la présence de l'os déminéralisé à trois semaines, mais ces différences n'ont plus été observées, ni radiologiquement ni sur le plan biomécanique, à six, neuf et douze semaines après l'implantation. L'os nouvellement formé avait environ 50% de résistance mécanique à la fracture comparativement aux fémurs contralatéraux de contrôle. Les fémurs ayant subi l'implantation d'os spongieux minéralisé avaient une fréquence de guérison mécanique et radiologique similaire comparativement aux fémurs ayant subi l'implantation d'os déminéralisé.

INTRODUCTION

Repair of large bone defects represents a major challenge to the orthopedic discipline. Autogenous cancellous bone graft is currently the preferred bone-graft material (18, 33, 49, 62). This transplanted bone is partially revascularized in situ, while the dead bone is substituted with new cancellous bone by the process of creeping substitution (1, 2, 10, 38, 51). Rejection of the transplanted autogenous bone is unusual. However, three major problems may arise : 1) the amount of autogenous bone available may be insufficient, especially in children and in large bone defects ; 2) postoperative morbidity at the donor site such as pain, blood loss, wound problems, cosmetic disability, infection and nerve damage are not infrequent ; 3) the shape of the transplanted bone is not always optimal to fill the defect.
These difficulties have prompted the search for materials able to substitute for the deficient bone. The ideal material should have the following characteristics : good biological acceptance, at most only minimal antigenic activity, mechanical strength, adaptability to the bone defect's configuration, relative inexpensiveness, and ease of sterilization (34). In addition, the material should be progressively replaced by normal bone to produce firm union to the host bones. Three different general categories of bone-graft substitutes deserve consideration.

1. Inorganic materials including porous carbon (28), hydroxyapatite (20, 24), porous polyethylene (45), ceramic $CaPO_2$, and plaster of Paris (36) all show good biological acceptance. However, they

have no osteoinductive potential. Bone ingrowth from the adjacent bone is the dominant mechanism of bone substitution. Since these substances have no osteoinductive capacities, the bone ingrowth is slow and inconstant (19) by the process of osteoinduction.

2. Allogenous bone transplants have the great advantage of their initial mechanical strength. The outcome of these mineralized allotransplants is moderate bone resorption and minimal bone apposition during the first 12 to 24 months. This bone resorption can be increased by immunological rejection or low-grade infection. Associated postoperative morbidity, consisting of fractures, non-union, low-grade infection and even complete bony resorption is high (> 30%) (14, 30).

3. Demineralized bone allografts and demineralized allotransplants have only recently been reconsidered as bone-graft materials of potential clinical interest (33, 39, 40, 52, 53, 58). Senn (46) in 1889 made the observation that osteomyelitic cavities of bone filled with demineralized bone (which was used as a vehicle for instillation of antiseptic solutions) demonstrated neoformation of bone. The following year Deaver (12), Miller (31) and MacKie (29) reported similar encouraging observations, but Bier (4) in 1892 and Schmitt (44) in 1883 reported negative results with this method. In 1931 Huggins (21, 22) observed bone formation when bladder epithelium was implanted into soft tissue containing muscle or fascia. In 1965, Urist (52) described bone-inductive potency of 0.6 N HCl decalcified and lyophylized bone matrix. Since then attempts have been made to define the biochemical structure of the bone-inducing principle (9, 17, 32, 42, 43, 48, 54, 55, 56) which has not yet been fully characterized. It has been shown that in soft tissues, implanted demineralized bone matrix produces appositional bone formation, rapid creeping substitution and rapid resorption of the bone matrix (6). This was mainly observed in rat and rabbit models. In larger animals like dogs, monkeys or sheep, this osteoinductive phenomenon was less intense. Studies dealing with implantation of demineralized bone matrix powder in bone defects of weight-bearing bones (5, 13, 16, 47, 50, 63) are an important step in the understanding of the behavior of graft materials with potential clinical application. Recently, demineralized bone-matrix allografts were used in humans (15, 23, 25, 28). Here again, bone formation is mainly assessed by radiographic and histological appearance, and not by tests dealing with the biomechanical quality of the newly formed bone.

Bone marrow by itself has bone progenitor potential (3, 8, 11, 35, 37, 41, 57, 59, 61). The addition of bone marrow has been reported to enhance bone formation within the demineralized bone matrix. The proposed explanation is the contribution of potential osteoprogenitor cells contained within the bone marrow (26, 27, 41, 47) which ought to be activated by BMP.

Our group has developed a bone-defect model which allowed implantation of different bone-graft materials in various forms and the assessment of their osteoinductive and osteoconductive properties. This system is reproducible in most of the cases and radiographic, histological and biomechanical studies of the implanted substances can be easily performed. The goals of the present study are :

1. To test the outcome of demineralized bone-matrix implants inserted into a segmental bone defect of a functioning, weight-bearing bone.
2. To study comparatively the radiographic and biomechanical properties of the bone defect with implanted demineralized bone matrix alone (osteoinductive/osteoconductive preparation) and augmented with bone marrow (osteoinductive/osteoconductive/osteoprogenitor preparation).
3. To study comparatively the grafting capability of demineralized bone matrix versus autogenous cancellous bone graft in healing segmental defects.

MATERIAL AND METHODS

A) Preparation of demineralized bone-matrix segments

Demineralized bone segments were prepared from the right and left femurs of Sprague-Dawley rats (350-400 gr). Each femur was carefully removed

from the rats and then mechanically cleaned of periosteum. The bones were cut on each end, and the bone marrow was removed from these diaphyseal segments using physiologic saline solution. The resulting bone segments were immediately transferred into 0.6 N HCl solution. Demineralization was then carried out over 72 hours at 4°C, with three HCl solution changes. At this point the acid was removed by washing the bones with distilled water for 8 hours at 4°C with continuous stirring. The bone-matrix segments were then defatted with 70% alcohol, and each segment was separately stored in small plastic tubes at minus 70°C while still immersed in the alcohol (72 hours). The ash weight of these bone segments was then determined after ashing at 600°C for 24 hours, and the process was shown to have completely demineralized the matrix.

B) Techniques of demineralized bone-matrix implantation

Sprague-Dawley rats (350-400 g) were premedicated with 30 mg of intramuscular Ketamine (Ketalar®). General anesthesia was then induced and maintained with mask ventilation (2L 0_2/min, 0.8 L N_2O/min, fluothane mixture). The right hip and lower extremity were then washed and shaved in a sterile fashion with 70% alcohol and betadine solution. Under sterile conditions a longitudinal skin incision was made over the lateral thigh, and carried down through the fascia lata. The vastus lateralis was then retracted anteriorly, and the biceps femoris swept posteriorly. At this point the circumference of the midshaft of the femur was exposed. An ultra-high molecular weight polyethylene plate (20 by 4 by 5 mm) was fixed to the anterior surface of the femur. This was accomplished by drilling four holes, two proximally and two distally through both the plate and the host bone. The polyethylene plate was fixed to the bone with four threaded Steinmann pins (1.2 mm diameter, Zimmer No. 540223). A 5-mm osteoperiosteal diaphyseal segment (2 × cross sectional diameter) was then excised from the midshaft of the femur with a rotating dental saw (fig. 1).

The animals were then divided up into 4 groups of 15 Sprague-Dawley rats so that the effect of replacement of the bony defect with demineralized bone matrix with and without marrow conservation could be examined, and a separate fifth group of 15 inbred Lewis rats was also examined as an autograft (isograft) control group. This latter group allowed comparison of results to a system that might most closely resemble that of an autogenous cancellous bone graft. In these animals cancellous bone was transferred from the proximal metaphyseal region of the tibia of a donor Lewis rat to the isogeneic Lewis recipient femoral defect. In this isogeneic group of animals, one animal was sacrificed at 3, 6, and 9 weeks for histolgic examination. Therefore only 12 animals were available for biomechanical and radiographic examination at the 12-week termination. In all other groups 15 animals were utilized for terminal analysis. The animals were therefore divided up as follows :

Table I

Group	Number of Animals		Demineralized Bone Matrix (DBM)	Marrow Supplementation (BM)
	Start	End		
I (DBM +) (BM +)	15	15	Yes	Yes
II (DBM +) (BM -)	15	15	Yes	No
III (DBM -) (BM +)	15	15	No	Yes
IV (DBM -) (BM -)	15	15	No	No
V Isografts	15	12	Isografts	No

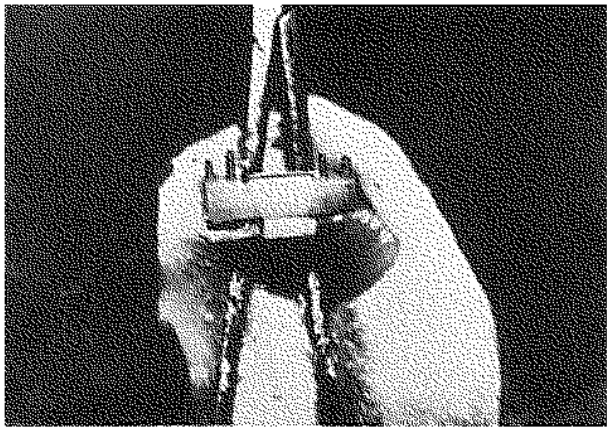

Fig. 1. — An intraoperative view of the bone defect which is implanted with a demineralized bone-matrix segment.

For the animals where conservation of bone marrow was required, the bone marrow that remained in the proximal and distal segments of the femur was supplemented with marrow removed from the resected segment. Animals used as marrow-free controls had their bone marrow removed from the proximal and distal segments of the femur by intramedullary lavage with physiologic saline solution. No supplementation of bone marrow was provided from the resected segment.

In those animals in which demineralized bone-matrix segments were implanted, the segment was fashioned to fill the bony gap. Maintenance of the implant within the defect was aided with the use of nylon sutures, helping to fix the graft to the proximal and distal host femur as well as the plate. In the Lewis rat group the graft was also molded to fill the bony defect. In all the groups the graft materials were enclosed snugly by approximating the vastus lateralis and biceps femoris over the plate-implant complex. Postoperatively the animals each received 30,000 units of penicillin G subcutaneously. The animals were held in collective plastic cages (3 animals/cage) with free access to water and standard laboratory rat food.

C) Radiographic evaluation

Radiographs of the right and left femur were obtained at 3, 6 and 9 weeks after implantation. At 12 weeks all animals were sacrificed. The right operative femur and the intact left femur along with the surrounding soft tissue attachments were carefully removed. Microradiographs (Faxitron) were taken, and the radiographs were evaluated for bone formation. Bone formation was classified into 3 categories : 1) absence of bone formation ; 2) presence of bone formation without radiographic bridging of the gap by the new bone ; 3) presence of bone formation with radiographic bridging of the gap (radiographic union).

D) Mechanical testing

At 12 weeks the femurs were freed from their soft issue attachments and evaluated for mechanical union by gentle manipulation. Those specimens that were felt to be mechanically unstable were not tested with torsional loading. In stable specimens the polyethylene plate, while still fixed to the right femur, was incised three times in its midportion to 90% of its depth. This was done in order to decrease the torsional resistance offered by the plate in our system without compromising the strength of the underlying bone. Great care was taken so as to prevent any contact with or nicking of the cortical bone. Prior experiments in our laboratory have shown that incision of the plate in this manner is a satisfactory method of evaluating the mechanical properties of interest. Right and left femurs were then potted in epoxy resin and tested for failure in torsion in a standardized torsion apparatus (7). Torque and angular displacement were recorded on an oscilloscope and then photographed. The stiffness of the bone was calculated by drawing a tangential line to the torque displacement curve. Results for maximal torque (torque recorded at specimen failure), stiffness and maximal angular displacement were expressed as a percentage of the corresponding values of the contralateral femurs.

Four femurs with radiographic evidence of pseudarthrosis and without mechanical stability, but with an intact plate were used to evaluate the torsional resistance of the incised plate. The mean failure torque (stiffness and displacement) of the plate in these 4 specimens was subtracted from the maximal failure torque (stiffness and maximal angular displacement) to determine the actual

properties of the bone. In general, the plate contributed less than 10% of the total torsion resistance offered by the bone-plate system. A modified White's fracture classification grading system (fig. 2) was used to evaluate the failure behavior of the tested bones (60). Type I fractures in this classification are defined as a non-union of the implanted bone segment and host bone. Type II fractures are defined as a fracture through the implanted bone segments of a mechanically stable bone. Type III fractures are those fractures in which the failure line involves both the implanted and host bone. Finally, a type IV fracture was one in which only host bone was involved.

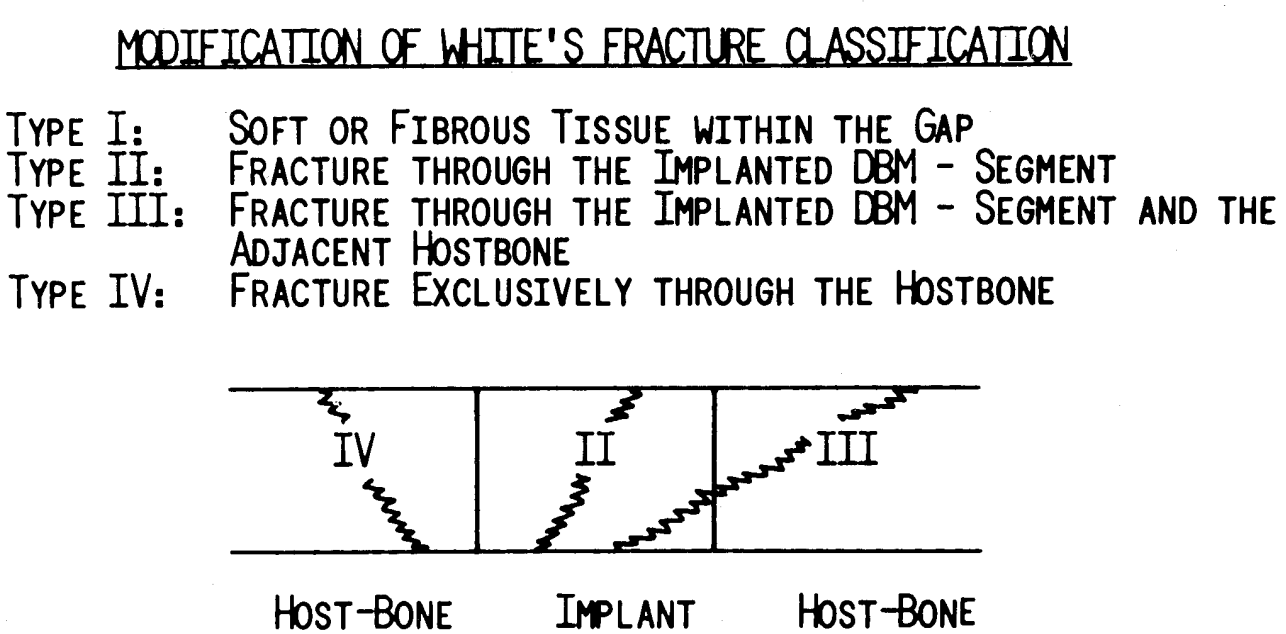

Fig. 2. — Schema illustrating the different types of failure observed during biomechanical testing.

RESULTS

No animal died during the experiment. The original procedure did not interfere with normal weight gain.

A) Radiographic results

Table II and fig. 3 detail the results of radiographic examination of the animals at 12 weeks. It is clear that the demineralized bone-matrix implanted animals both with and without bone-marrow implantation had significantly enhanced bone formation and radiographic union as compared to control and bone-marrow impregnated defects ($p < .001$). At 12 weeks 14/15 animals in both Groups I (demineralized bone matrix with bone-marrow supplementation (DBM +) (BM +)) and II (demineralized bone matrix without bone marrow (DBM +) (BM –)) had evidence of bone formation. This compared with only 3/15 animals with evidence of bone formation in Groups III (DBM –) (BM +) and IV (DBM –) (BM –). Similar results were observed if radiographic osseous bridging (fig. 4) was contrasted between both Groups I and II (12/15 and 12/15) vs. Group III and IV (2/15 and 1/15).

Table II. — Radiographic evidence of bone formation and radiographic union at twelve weeks

	Bone formation	
	Without bridging	With bridging (radiographic union)
(DBM +) (BM +)	14/15 (93%)	12/15 (80%)
(DBM +) (BM -)	14/15 (93%)	12/15 (80%)
(DBM -) (BM +)	3/15 (20%)	2/15 (13%)
(DBM -) (BM -)	3/15 (20%)	1/15 (7%)
Isograft	11/12 (92%)	10/12 (83%)

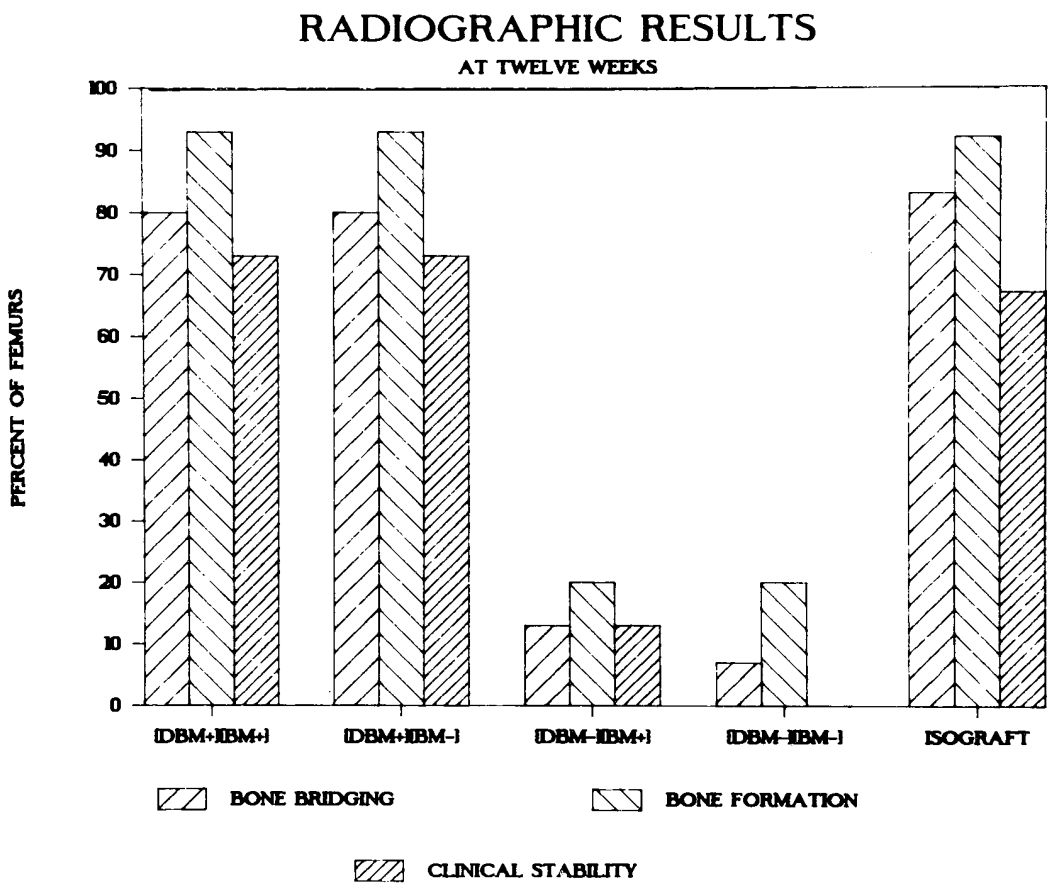

Fig. 3. — Histograms representing the percentage of animals in each group with bone formation and radiographic bridging of the bone defect, as well as clinical stability at 12 weeks.

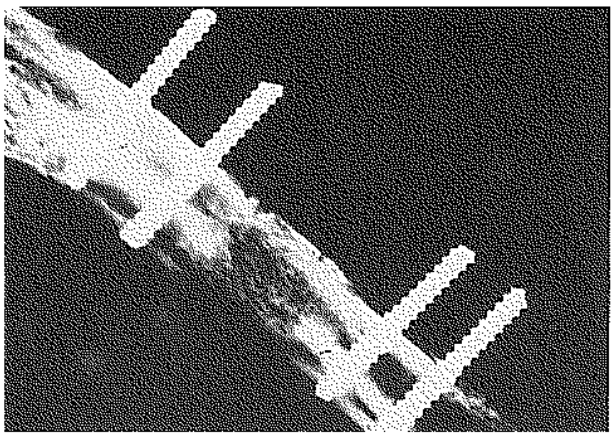

Fig. 4. — Radiographic bridging of the gap by new bone at 12 weeks in a defect filled with demineralized bone matrix.

Table III details the radiographic evidence of bone formation at various time intervals. At 3 weeks a significantly larger proportion of femurs in Group I (DBM +) (BM +)) than in Group II (DBM +) (BM –)) showed bone formation within the defect (12/15 vs. 3/15) ($p < .005$) (fig. 5). No significant difference between those specimens with and without bone marrow could be found at 6, 9, or 12 weeks (fig. 6).

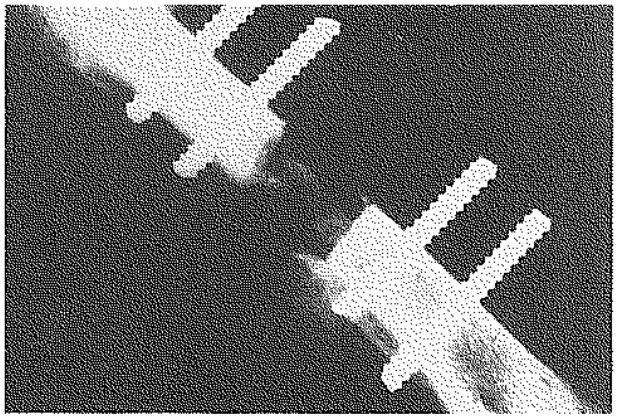

Fig. 5a

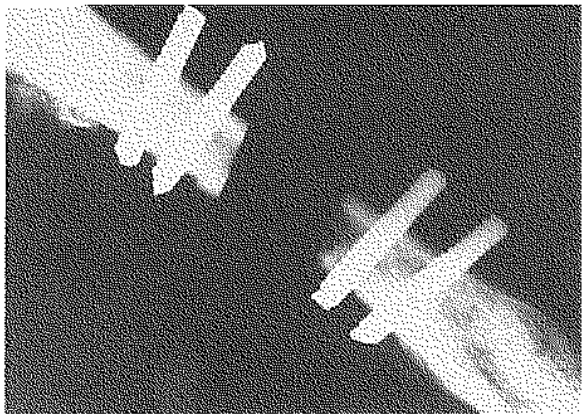

Fig. 5b

Fig. 5. — a) Bone defect in specimen implanted with demineralized bone matrix and augmented with bone marrow at 3 weeks. Bone formation is observed radiographically.
b) At 3 weeks, no bone formation is seen in a specimen implanted with only demineralized bone matrix, but without bone marrow augmentation.

Table III. — Radiographic evidence of bone formation at three-week intervals

	Number 3 weeks	% 6 weeks	9 weeks	12 weeks
(DBM +) (BM +)	12/15 (80%)	14/15 (93%)	14/15 (93%)	14/15 (93%)
(DBM +) (BM -)	3/15 (20%)	13/15 (87%)	13/15 (87%)	14/15 (93%)
(DBM –) (BM +)	0/15 (0%)	3/15 (20%)	3/15 (20%)	3/15 (20%)
(DBM –) (BM -)	0/15 (0%)	3/15 (20%)	3/15 (20%)	3/15 (20%)
Isografts	5/15 (30%)	7/14 (50%)	11/13 (85%)	11/12 (92%)
(DBM +) (BM –) vs (DBM +) (BM –)	$p < .002$	Not sig.	Not sig.	Not sig.

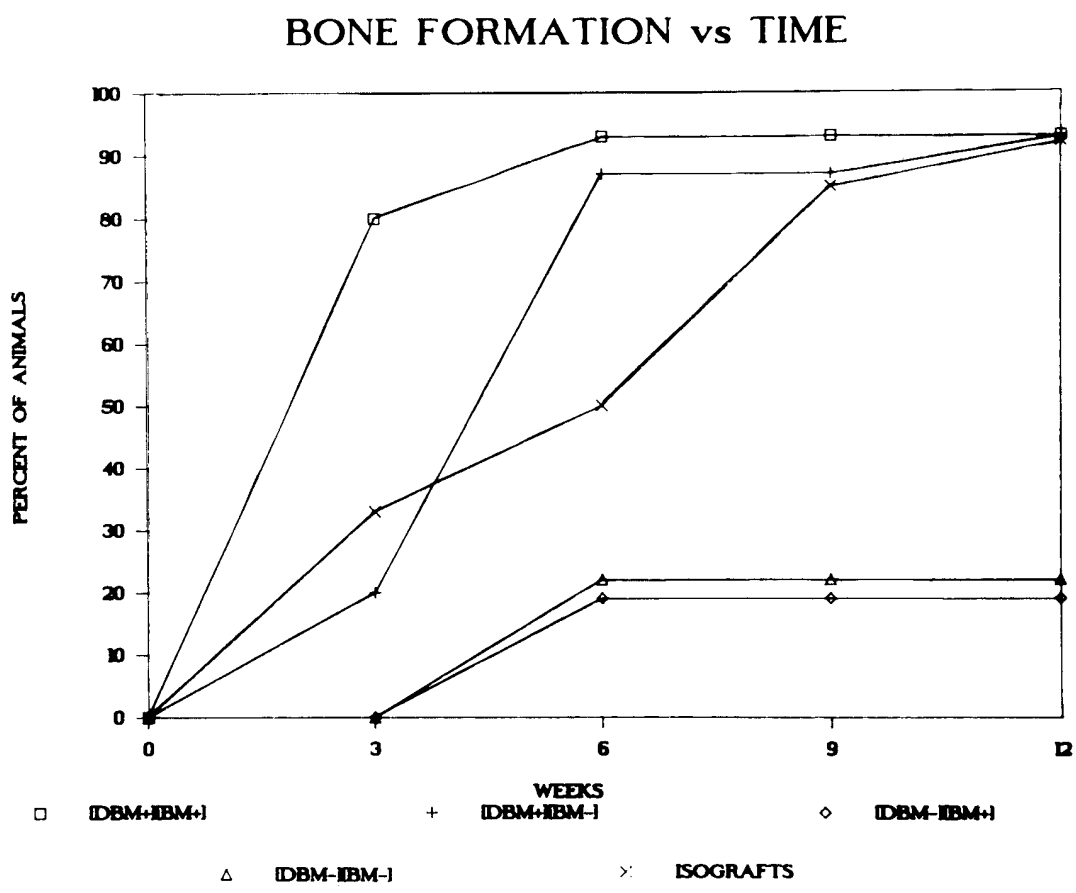

Fig. 6. — Incidence of radiographic bone formation as a function of time within bone defects filled with or without demineralized bone matrix, with or without addition of bone marrow.

In addition Tables II and III list the results obtained for the cancellous bone-graft control group. Table II shows that the number of animals exhibiting bone formation and radiographic union at 12 weeks in the isograft cancellous group as similar to the results obtained for the demineralized bone-matrix implanted group either with or without bone marrow. However in comparing the proportion of animals with bone formation over time, it appears that the isograft cancellous group took significantly longer before showing evidence of osseous development than either demineralized bone-matrix Groups I and II (3 weeks : I vs. isograft $p < .02$, II vs. isograft ns ; 6 weeks : I vs. isograft $p < .02$, II vs. isograft $p < .05$). This difference in rate had disappeared by 9 weeks.

B) Biomechanical results

Only clinically stable femurs were biomechanically tested. Only 11/15 femurs in Group I and II, 2/15 in Group III, 0/15 in Group IV and 8/12 in the Lewis isograft animals achieved clinical stability and were biomechanically tested (table IV). There was no significant difference in mechanical union rate for the two demineralized bone-matrix groups and the isogeneic cancellous group ; these three groups had a significantly increased rate compared with Group III ((DBM –) (BM +)) and Group IV ((DBM –) (BM –) ($p < .002$).

Figure 7 details the results for the biomechanical tests for Groups I, II and V. Mean values for angular displacement at maximal torque, maximal

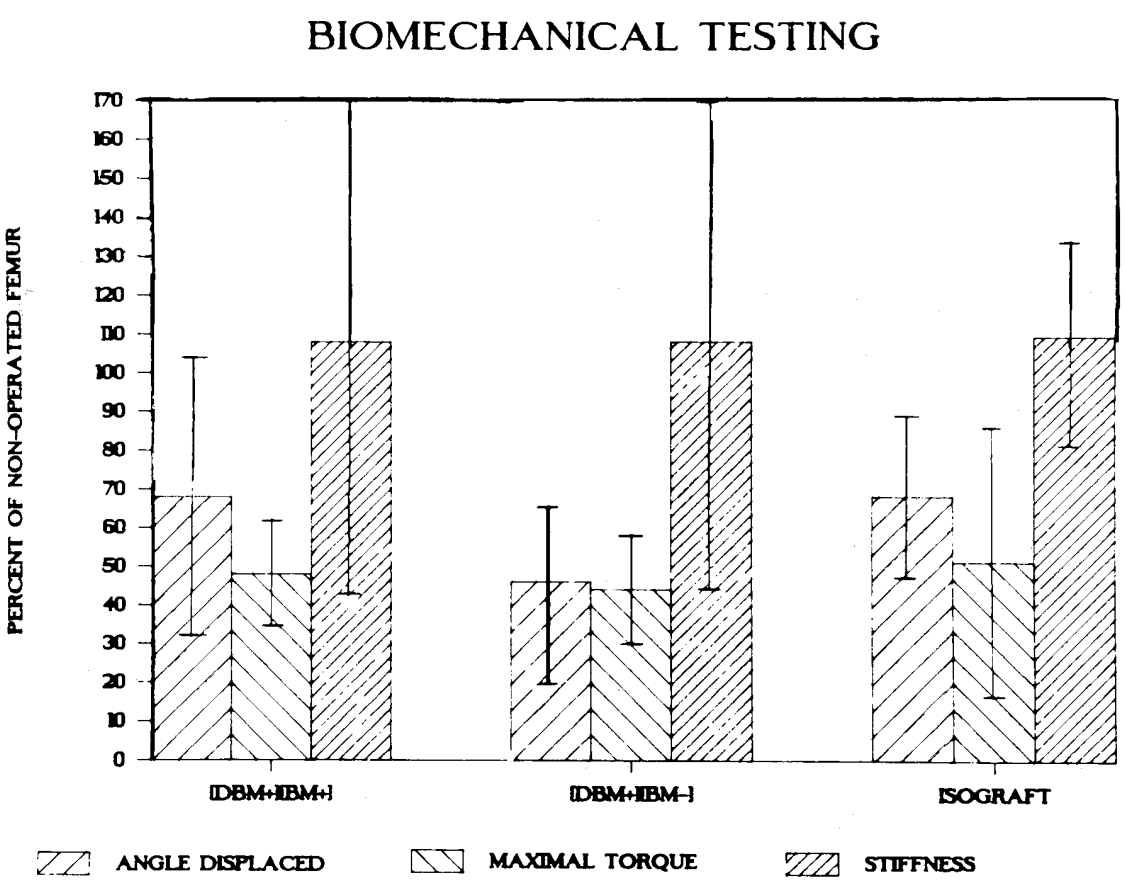

Fig. 7. — Biomechanical results for the clinically stable femurs in Groups I, II and V. The biomechanical values for the operative right femurs are expressed as a percentage of the normal contralateral left side.

Table IV. — Mechanical union

(DBM +) (BM +)	11/15	73%
(DBM +) (BM -)	11/15	73%
(DBM -) (BM +)	2/15	13%
(DBM -) (BM -)	0/15	0%
Isografts	8/12	67%

torque and stiffness were calculated for both the operative and nonoperative side. Results are listed for each variable as a percentage of the nonoperative side. The paired T-test was used to compare both sides.

For the demineralized bone-matrix implanted femurs, the average angular displacement prior tu failure was 68% (+ 36%) and 46% (+ 27%) in Group I ((DBM +) (DM +)) and Group II ((DBM +) (BM -)), respectively. Average maximal torque at failure was 48% (+ 14%), and 44% (+ 14%-), while average stiffness values were computed at 108% (+ 61%) and 108% (+ 64%), respectively. With the Student's T test for comparison between Groups I and II, no statistical difference in terms of biomechanical properties tested could be found.

The biomechanical values for Group V isograft cancellous animals were quite similar to those values detailed above. This group testing at 12 weeks revealed : a) average angular displacement at maximal torque to be 68% (+ 20%) of the nonoperative side ; b) maximal torque 51% (+ 33%) ; c) average stiffness 109% (+ 27%). When Groups I and II (separately or combined) were compared with Group V again, no statistical difference could be found.

In general, the maximal torque and angular displacement of the right operative femurs were both significantly lower than the corresponding values obtained in the contralateral control bones in Groups I, II and V. The stiffness of bone, while comparable in all three groups, did not exhibit a significant difference from the results obtained for the nonoperative control side. In the 2 animals in Group III with clinically stable femurs, results for maximal torque, angular displacement and stiffness were comparable to those already reported for the other groups.

Table V shows the distribution of the five different types of fracture patterns of failures according to White's classification during torque testing for Groups I-V.

Table V. — Types of fractures occurring during biomechanical testing

	White's classification			
	I	II	III	IV
(DBM +) (BM +)	29%	29%	36%	7%
(DBM +) (BM –)	27%	40%	33%	0%
(DBM –) (VM +)	87%	7%	7%	0%
(DBM –) (BM –)	100%	0%	0%	00%
Isograft	33%	8%	50%	8%

Striking differences can be noted : in Group III and IV the predominant failure pattern noted was that of type I with a persistent nonunion. In contrast, type II and III failures were most frequently encountered in Groups I and II (demineralized bone-matrix implant specimens with and without bone marrow), and Group V (isogeneic graft) confirming successful union of the implant to the adjacent host bone. However the rarity of type IV failure in both the demineralized bone-matrix specimens and the isogeneic graft suggested that the newly formed bone within the segmental defect still lacked mature mechanical strength 3 months after implantation.

DISCUSSION

The plated bone-defect model appears to be an excellent model to explore the efficacy of bone-graft materials. The natural history of the bone defect indicates that it will form bridging of the defect by fibrous tissue. The implantation of the demineralized bone matrix leads to increased formation of new bone, and bridging between the host and newly formed bone. This model allowed both radiographic and biomechanical testing of the implants. Few studies have dealt with the biomechanical quality of the demineralized bone-matrix induced bone, especially when implanted into a bone defect created in a functioning weight-bearing bone. In a previous study conducted in our laboratory (13), implantation of demineralized bone matrix into a segmental bone defect in the diaphysis of rat femurs was found to result in union. However in this model an external fixator stabilized the bone defect. In addition the number of animals studied was small, and a high incidence of wound infections forced removal of many of the animals from the study. The biomechanical testing in that study leads to the conclusion that energy absorption to failure was equal to that of the pinned intact cortical bones, and that deformation resulted in greater displacements prior to failure. Stiffness of the newly formed bone was markedly decreased. In addition, recent work by Bolander and Bolian (5) compared the use of plasma-coated demineralized bone matrix and autologous cancellous bone used to graft segmental defects in ulnae of rabbits. Results showed that ulnar graft of plasma-coated DBM and those grafted with autologous cancellous bone had nonsignificant differences when values for torque, energy and maximum angular deformation were compared. In addition, DBM grafts that were augmented with extracted bone proteins were significantly stronger than those without augmentation. In general, these results were similar to those found in this study, even though both a different technique and different animal model were used. In addition, Bolander and Bolian augmented their specimens with extracted bone proteins as opposed to bone marrow.

In this study we found that the newly formed bone failed at a lower angular displacement (approximately 50% of the values for the intact control femurs), and produced less torque after 12 weeks of healing. However, the slope of the torque

displacement curve for the nonoperative femurs was without significant difference as compared with the slopes for femurs with either demineralized bone matrix or isograft implants. Therefore the stiffness of the newly formed bone was comparable to the stiffness of the nonoperative intact femurs. However the newly formed bone was unable to withstand the same magnitude of torsional stress as the controls, and thus failed at a lesser maximal torque with decreased angular displacement. With more time, the segmental healing may have regained more nearly normal mechanical properties.

No significant biomechanical differences were noted if the demineralized bone-matrix implants with and without bone marrow as well as the isograft controls (representing autografts) were carefully compared at 12 weeks. Results for bone-marrow supplemented specimens showed that more rapid bone formation occurred radiographically for the first 3 weeks postoperatively. These differences were no longer statistically significant at 6, 9 or 12 weeks. The biomechanical properties and segmental union rates are comparable. Demineralized bone matrix appears to elicit earlier bone formation than cancellous graft, especially when the DBM is laced with bone marrow. Human utilization of demineralized bone matrix as a cancellous graft substitute is now under study.

CONCLUSIONS

The authors present a plated segmental bone-defect model in a functioning weight-bearing bone in rats, allowing radiographic and biomechanical testing. The model is reproducible with good animal acceptance. The demineralized bone-matrix segments implanted into this bone defect resulted in union in most of the animals. Bone marrow significantly enhanced the bone formation with demineralized bone-matrix implants at an early stage, as determined radiographically, but with time the benefit of bone-marrow augmentation disappeared. Biomechanically, newly formed bone had about half of the strength of the contralateral control bones. The demineralized bone-matrix implants had similar radiographic evidence of bone formation, biomechanical properties and absolute union rate as cancellous bone isografts (autografts).

REFERENCES

1. AXHAUSEN W. Biologische Grundlagen der freien Knochenverpflanzung. *J. Int. Chir. Bruxelles*, 1953, *13*, 341-344.
2. AXHAUSEN W. The osteogenetic phase of regeneration of bone. *J. Bone Joint Surg.*, 19956, *38-A*, 593-600.
3. ASHTON B. A., ALLEN T. D., HOWLETT C. R., EAGLESONE C. C., HATTORI A., OWEN M. Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. *Clin. Orthop.*, 1980, *151*, 294-307.
4. BIER A. Osteoplastische Nekrotomie nebst Bemerkung über die and der Kieler chirurgischen Klinik ausgefuhrten Methoden der Nekrotomie. *Arch. Klin. Chir.*, 1893, *45*, 401.
5. BOLANDER M. E., BOLLAN G. The use of demineralized bone matrix in the repair of segmental defects. Augmentation with extracted matrix proteins and a comparison with autologous grafts. *J. Bone Joint Surg.*, 1986, *68-A*, 1264-1274.
6. BOMBI J. A., RIBAS-MUJAL D., THRUETA J. An electron microscopic study of the origin of osteoblasts in implants of demineralized bone matrix. *Clin. Orthop.*, 1978, *130*, 273-284.
7. BURSTEIN A. H., FRANKEL V. H. A standard test for laboratory animal bone. *J. Biomech.*, 1971, *4*, 155-158.
8. BURWELL R. G. Studies in the transplantation of bone. The fresh composite homograft, autograft of cancellous bone. *J. Bone Joint Surg.*, 1964, *46-B*, 110-140.
9. CHALMERS J., GRAY D. H., RUSH J. Observation on the induction of bone in soft tissues. *J. Bone Joint Surg.*, 1975, *57-B*, 36-45.
10. CHASE F. W., HERNDON C. H. The fate of autogenous and homogenous bone graft. *J. Bone Joint Surg.*, 1951, *37-A*, 809-841.
11. DANIS A. Étude de l'ossification dans les greffes de moelle osseuse. *Acta Chir. Belg.*, 1957, *Suppl. 3*, 1.
12. DEAVER J. B. Secondary bone implantation by a modification of Senn's methods. *Med. News*, 1889, *55*, 714.
13. EINHORN T. A., LANE J. M., BURSTEIN A. H., KOPMAN C. R., VIGORITA V. J. The healing of segmental bone defects induced by demineralized bone matrix. *J. Bone Joint Surg.*, 1984, *66-A*, 274-279.
14. FRIEDLANDER G. Immune responses to osteochondral allografts. *Clin. Orthop.*, 1983, *174*, 58-68.
15. GLOWACKI J., MURRAY J.- E., KABAN L. B., FOLKMAN J., MULLIKEN J. B. Application of the biological principle of induced osteogenesis for craniofacial defects. *Lancet*, 1981, *1*, 959-968.

16. GUPTA D., TULI S. M. Osteoinductivity of partially decalcified alloimplants in healing of large osteoperiosteal bone defects. *Acta Orthop. Scand.*, 1982, *53*, 857-865.
17. HANAMURA H., HIGUCHI Y., NAKAGAWA M., IWATA H., NOGANI H., URIST M. R. Solubilized bone morphogenetic protein (BMP) from mouse osteosarcoma and rat demineralized bone matrix. *Clin. Orthop.*, 1980, *140*, 281-290.
18. HEIPLE K. G., CHASE S. W., HERNDON C. H. A comparative study of the healing process following different types of bone transplantation. *J. Bone Joint Surg.*, 1963, *45-A*, 1593-1616.
19. HEJNA W. F., RAY R. D. Comparative study of bone implants. *Surg. Forum*, 1963, 448-451.
20. HOLMES R. E. Bone regeneration within a coralline hydroxyapatite implant. *Plast. Reconstr. Surg.*, 1979, *63*, 626-633.
21. HUGGINS C. B. The formation of bone under the influence of epithelium of the urinary tract. *Arch. Surg.*, 1931, *22*, 377-408.
22. HUGGINS C. B., McCARROL H. R., BLACKSON B. H. Experiments of the theory of osteogenesis. *Arch. Surg.*, 1936, 915-931.
23. IWATA H., HANAMURA H., KANEKO M., YASUHARA N., TERASHIMA Y., KAJINO G., IDA K., MABUCHI Y., NAKAGAWA M. Chemosterilized autolyzed antigen extracted allogeneic (AAA) bone matrix gelatin for repair of defects from excisions of benign bone tumors. *Clin. Orthop.*, 1981, *154*, 150-155.
24. KATO JK., AOKI H., TABATA T., OGISO M. Biocompatibility of apatite ceramics in mandibles. *Biomat. Med. Devices Artif. Organs*, 1979, *7*, 291-297.
25. LIBIN B. M., WARD H. L., FISHMAN L. Decalcified lyophilized bone allograft for use in human periodontal defects. *J. Periodontol.*, 1975, *46*, 51-56.
26. LINDHOLM T. S., NILSSON O. S., LINDHOLM T. C. Extraskeletal and intraskeletal new bone formation induced by demineralized bone matrix combined with bone-marrow cells. *Clin. Orthop.*, 1982, *171*, 251-255.
27. LINDHOLM T. S., URIST M. R. A quantitative analysis of new bone formation by induction in composite grafts of bone marrow and bone matrix. *Clin. Orthop.*, 1980, *150*, 288-300.
28. LOZANO A. J., CESTERO H. J., SALYER K. E. A comparative study of the early vascularization of porous carbon and bone as a graft. *J. Biomed. Mat. Res.*, 1976, *10*, 545-548.
29. McKIE W. Clinical observation on the healing of aseptic bone cavities by Senn's method of implantation of antiseptic decalcified bone. *Med. News*, 1980, *57*, 202.
30. MANKIN H. J., DOPPELT S., TOMFORD W. Clinical experience with allograft implantation. *Clin. Orthop.*, 1983, *174*, 69-86.
31. MILLER A. G. A case of bone grafting with decalcified bone chips. *Lancet*, 1890, *2*, 618.
32. MIZUTANI H., URIST M. R. The nature of bone morphogenetic protein (BMP) fraction derived from bovine bone matrix gelatin. *Clin. Orthop.*, 1982, *171*, 213-223.
33. OIKARINEN J., KORKONEN L. K. The bone inductive capacity of various bone transplanting materials used for treatment of experimental bone defects. *Clin. Orthop.*, 1979, *140*, 208-215.
34. OIKARINEN J. Experimental spinal fusion with decalcified bone matrix and deep frozen allogeneic bone in rabbits. *Clin. Orthop.*, 1982, *162*, 210-218.
35. OWEN M. The origin of bone cells in the (postnatal) organism. *Arthr. Rheum.*, 1980, *23*, 1073-1079.
36. PELTIER L. F., JONES R. H. Treatment of unicameral bone cyst by curettage and packing with plaster of Paris pellets. *J. Bone Joint Surg.*, 1978, *60-A*, 820-822.
37. PFEIFFER C. A. Development of bone from transplanted bone marrow in mice. *Anat. Rec.*, 1948, *102*, 225.
38. PHEMISTER D. B. Treatment of ununited fractures by onlay bone graft without screw or tie fixation and without breaking down the fibrous union. *J. Bone Joint Surg.*, 1947, *29*, 946-960.
39. RAY R. D., HALLOWAY J. A. Bone implants. *J. Bone Joint Surg.*, 1957, *39-A*, 1119-1128.
40. REDDI H., HUGGINS C. Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. *Proc. Nat. Acad. Sci. USA*, 1972, *69*, 1601-1605.
41. ROSIN A., FREIBERG H., ZAJICEK G. The fate of rat bone marrow, spleen and periosteum cultivated in vivo in the diffusion chamber with special reference to bone formation. *Exp. Cell Res.*, 1963, *29*, 176-187.
42. SAMPATH T. K., REDDI A. H. Homology of bone inductive proteins from human, monkey, bovine and rat extracellular matrix. *Proc. Nat. Acad. Sci. USA*, 1983, *80*, 6591-6595.
43. SATO K., URIST M. R. Bone morphogenetic protein induced cartilage development in tissue culture. *Clin. Orthop.*, 1984, *183*, 180-187.
44. SCHMITT A. Über Osteoplastik in klinischer und experimenteller Beziehung. *Arch. Klin. Chir.*, 1893, *45*, 401.
45. SPECTOR M., HARMAN S. L., KREUTNER A. Characteristics of tissue growth into proplast and porous polyethylene implants in bone. *J. Biomed. Mat. Res.*, 1979, *13*, 677-692.
46. SENN N. On the healing of aseptic bone cavities by implantation of antiseptic decalcified bone. *Am. J. Med. Sci.*, 1889, *98*, 219-243.
47. TAKAGI K., URIST M. R. The role of bone marrow in bone morphogenetic protein induced repair of femoral massive diaphyseal defects. *Clin. Orthop.*, 1982, *171*, 224-231.
48. TAKAOKA K., OUS K., AMITANI K., KISHIMOTO R., NAKATA Y. Solubilization and concentration of a bone inducing substance from a murine osteosarcoma. *Clin. Orthop.*, 1980, *148*, 274-280.

49. TULI S. M. Bridging of bone defects by massive bone grafts in tumorous conditions and in osteomyelitis. *Clin. Orthop.*, 1972, *87*, 60-73.
50. TULI S. M., SINGH A. D. Osteoinductive property of decalcified bone matrix. An experimental study. *J. Bone Joint Surg.*. 1978, *60-B*, 116-123.
51. URIST M. R. Bone, transplants, implants, derivates and substitutes (International Course Lecture). *Am. Acad. Orthop. Surg.*, 1960, *17*, 184.
52. URIST M. R. Bone Induction. *Science*, 1965, *150*, 893-899.
53. URIST M. R., DOWELL T. A., HAY P. H., STATES B. S. Inductive substances for bone formation. *Clin. Orthop.*, 1968, *59*, 59-96.
54. URIST M. R., HUO Y. K., BROWNWELL A. G., HOHL W. M., BUYSKE J., lietz A., TEMPEST P., HUNKAPILLAR M., DELANGE K. J. Purification of bovine bone morphogenetic protein by hydroxyapatite chromatography. *Proc. Nat. Acad. Sci. USA*, 1984, 81-85.
55. URIST M. R., DELANGE R., FINERMAN G. A. M. Bone cell differentiation and growth factors. *Science*, 1983, *220*, 680-686.
56. URIST M. R. Surface decalcified allogeneic bone (SDAB) implants. *Clin. Orthop.*, 1968, *56*, 37-50.
57. URIST M. R., McLEAN F. C. Osteogenic potency and new bone formation by induction in transplants to the anterior chamber of the eye. *J. Bone Joint Surg.*, 1952, *34-A*, 443-476.
58. VAN DE PUTTE K. A., URIST M. R. Osteogenesis in the interior of intramuscular implants of decalcified bone matrix. *Clin. Orthop.*, 1965, *43*, 257-270.
59. VAUGHAN J. Osteogenesis and hematopoiesis. *Lancet*, 1981, *2*, 133-136.
60. WHITE A. A., PANJABI M. M., SOUTHWICK W. The four biomechanical stages of fracture repair. *J. Bone Joint Surg.*, 1977, *59-A*, 188-192.
61. WILLIAMS R. G. A study of bone growing from autografts of marrow in rabbits. *Anat. Rec.*, 1957, *129*, 187.
62. WILSON P. D., LANCE E. M. Surgical reconstruction of the skeleton following segmental resection of bone tumors. *J. Bone Joint Surg.*, 1965, *47-A*, 1629-1656.
63. WITTBJER J., PALMER B., ROHLIN M., THORNGREN K. G. Osteogenetic activity in composite grafts of demineralized compact bone and marrow. *Clin. Orthop.*, 1983, *173*, 229-238.

M. GEBHART
Department of Surgery
Institut Jules Bordet
Rue Héger-Bordet 1
1000 Brussels (Belgium)