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REPAIR OF BONE DEFECTS BY BONE INDUCTIVE MATERIAL

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Experimental fibular defects in 16 rats were filled with an acid decalcified homogenous bone matrix (bone inductive material). Autogenous bone grafts in corresponding defects in the other legs of the same rats served as controls. After 3 months, 11 of the 16 defects filled with bone inductive material healed with bony union, but only 4 of the 16 defects treated with autogenous bone grafts had healed. The results suggest that bone inductive material can repair bone defects which are too large to be healed by autogenous bone grafts.

Key words: bone; bone induction; bone transplants; fractures

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Major bone defects represent a difficult problem in orthopaedic surgery. A fresh graft of autogenous cancellous bone with haemopoietic bone marrow is generally considered to be the most effective treatment in these cases. The clinical application of such grafts, however, is limited. A better method would be to use some more readily available material. Several authors have therefore studied bone induction, and promising results have been published regarding the bone inductive capacity of decalcified bone matrix (e.g. Bang 1973, Büring & Urist 1967, Simmons et al. 1974, Urist & Strates 1971). In spite of high success rates in the induction of new ectopic bone in animal experiments, decalcified bone matrix in the treatment of major bone defects has been used clinically only on a few occasions (Boyne 1973).

In this study, we compared the repair of experimental bone defects in rats by decalcified bone matrix (bone inductive material) with repair by autogenous fresh bone grafts. The results suggest that bone inductive material can repair bone defects which are too extensive to benefit from autogenous bone grafts used under experimental conditions.

MATERIAL AND METHODS

Preparation of the bone inductive material (BIM)

Long bones from adult rats of a Long Evans strain were collected, periosteum and bone marrow tissue were mechanically removed and the bone pieces were washed in distilled water. The bones were then dehydrated in three changes of absolute ethanol over a period of 3 hours, and fat was extracted by a 1 hour bath in diethyl ether. Subsequently, the bone pieces were again washed in ethanol, and transferred through an alcohol gradient down to distilled water. The washed bone tissue was then demineralized by incubating in 0.6 N HCl at +4°C for $4\frac{1}{2}$ hours, using 25 mMol HCl/g of bone tissue. After decalcification, pieces of organic matrix were washed several times in distilled water (approximately six times for 5 minutes each) until the washing water remained neutral. The material was freeze-dried and stored in sterile tubes at −20°C. This material is called bone inductive material (BIM) in this paper.

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Testing the bone inductive capacity

The bone inductive material was divided into small pieces which were compressed to form small cylinders in a device made from a tuberculin syringe. Cylinders of 0.05 cm^3 were implanted into pouches under the fascia of the latissimus dorsi muscle of adult rats. Cylinders of cellulose fibre sheets of the same size were implanted in the opposite side of the same animals as controls. Wounds were closed with silk sutures. Implants were removed after 1, 2, 3, 4, and 6 weeks, fixed in neutral formalin, decalcified if necessary, embedded in paraffin and sections of 5 μm were stained by the Weigert – van Giesson method. For histochemical demonstration frozen sections were prepared from fixed samples and stained for the demonstration of alkaline and acid phosphatase activity (Pearse 1968).

Testing the capacity to repair bone defects

Sixteen Long Evans rats (150–200 g) served as experimental animals. The operations were performed under open ether anaesthesia. The instruments were kept sterilized during the operation with ethanol and flaming, and the skin over the site of the operation was cleaned with an ethanol-ether mixture. The skin over the lateral side of the left leg was opened and the fibula freed from adjacent tissues by blunt dissection. A 2 mm section was removed, and the defect in the fibula was filled by a piece of BIM of the same size and shape. The muscles were closed with a single catgut suture and the skin with silk sutures. No additional measures were taken in order to fix the transplants. As a control, a similar operation was performed on the right legs of the same rats, but the periosteum was scraped off the pieces removed from the fibulas, and these were returned to their original positions between the cut ends of the fibulas. The wounds healed *per primam intentionem.*

Evaluation of the repair

Evaluation of the repair of the bone defects was performed in all cases by macroscopic inspection and by histological examination of microscopic preparations. In addition, an X-ray examination was performed in a group of eight rats on the 45th and 90th postoperative days. Tetracycline labelling was used in another group of eight rats as follows:

Tetracycline hydrochloride (Achromycin® Lederle) or oxytetracycline (Terramycin® Pfizer) 100 mg/kg were given i.v. on the 10th 60th and 75th days after the operation. The animals were killed on the 90th postoperative day, the bones removed and embedded in Epon by a routine method. The Epon blocks were split longitudinally with a dentist's circular saw so that the fibulas were divided into two parts. The cut surfaces were examined and photographed using an incident fluorescent outfit Ploem (Leitz Gmbh) with an HBO 200 lamp and GB 12 and K 530 filters in a Leitz Orthoplan microscope. The lines of the two tetracyclines could be distinguished from each other because of their different fluorescent tone.

RESULTS

Bone inductive capacity

The homogenous bone inductive material (BIM) implanted beneath the latissimus dorsi muscle of rats was surrounded with a fibrous connective tissue capsule, and after 1 week, small islands of hyaline cartilage could occasionally be observed between the pieces of BIM. Connective tissue cells and blood vessels proliferated and invaded the implanted material, filling the canals in the BIM. After 2 weeks hyaline cartilage was present in abundance in all samples, and islands of ossifying tissue could be observed (Figure 1). After 6 weeks, bone marrow with haemopoetic islands was seen. Alkaline phosphatase activity, estimated by histo-

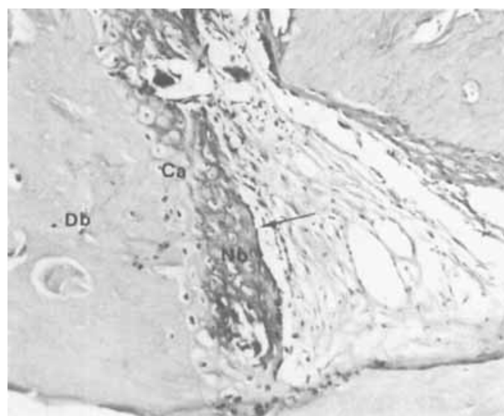

Figure 1. Newly formed cartilage (Ca) and bone (Nb) with osteoblast lining (arrow) on a decalcified bone matrix (Db) after 3 weeks in rat dorsal muscle. No signs of rejection are seen.

chemical staining reaction, increased during the first 4 weeks, and declined thereafter. The activity was observed in certain cell groups on the surface of the pieces of BIM, these cells were regarded as young osteoblasts. Acid phosphatase activity followed the same pattern, but was confined to the giant cells in the areas of bone resorption.

In some areas of the samples a mild leucocytic infiltration was observed, as well as some foreign-body giant cells, but signs of more extensive inflammatory reactions were absent and no rejection reaction occurred.

In control experiments, where cylinders of cellulose fibres were implanted, only a granulomatotic foreign-body reaction was observed. No cartilage or bone formation could be found either macroscopically or microscopically.

Repair of bone defects

The results of the experiment of the repair of fibular defects by BIM are given in Table 1 and illustrated in Figures 2–5. All but one of the 16 defects treated with BIM showed abundant callus formation, and in 11 cases bony union was present. Five cases in the experimental groups showed incomplete healing without a continuous bone bridge across the area of defect, although abundant cartilaginous and fibrous calli were present in three cases. In two cases of the experimental group the healing was unsatisfactory with only fibrous tissue in the area of the bone defect and extensive lysis of the BIM graft. In one case the healing was so complete, that the fibula was remodelled to the normal appearance, and most of the healed fibulas showed a continuous marrow cavity 90 days after the operation.

On the control side, where the defects were filled with fresh allogenous bone grafts without periosteum, four of the sixteen defects healed with a continuous bony bridge. In these cases, the calli were small in size and the bone remodelled to its normal shape. The allogeneic bone graft could still be seen with deposits of new bone on its surface. In five cases, fibrous and cartilaginous calli formed pseudoarthrotic unions even with a cavity lined with cartilage and resembling a real joint cavity in some cases. In seven cases, the allogeneic graft had undergone extensive lysis and only fibrous tissue was left in place of the bone defect.

Signs of more extensive inflammatory reactions or any signs of rejection were not observed in the defects filled with BIM or allogeneic bone grafts.

Table 1. Repair of fibular defects by bone inductive material or autogenous bone grafts

Result of experiment	Bone defect filled with bone inductive material	autogenous bone graft	Total
Healed with bony union	11	4	15
Not healed	5	12	17
Total	16	16	

Experimental fibular defects in the right legs of 16 rats were filled with acid decalcified homogenous bone matrix (bone inductive material), and corresponding defects in the left legs of the same rats were treated with autogenous bone grafts. After 3 months, a significantly higher proportion of the defects treated with the inductive material showed healing with bony union than did those treated with autogenous bone grafts ($P \leqslant 0.026$, X^2-test).

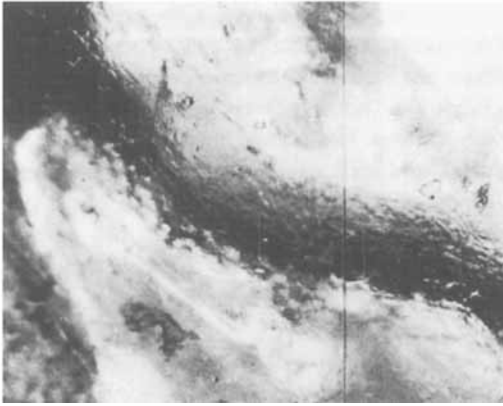

Figure 2. A pseudoarthrosis in a rat fibula repaired 3 months previously with an autogenous bone graft. The tetracycline fluorescence lines are clearly visible in the calcified tissue.

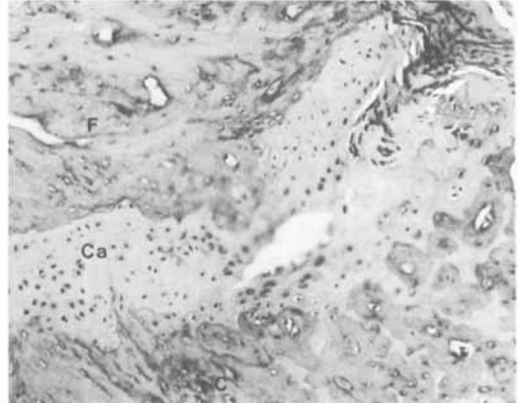

Figure 4. The same bone as in Figure 3. An incomplete cartilaginous bridge (Ca) is seen between the fibula (F) and the callus (C) on the control side (autogenous graft).

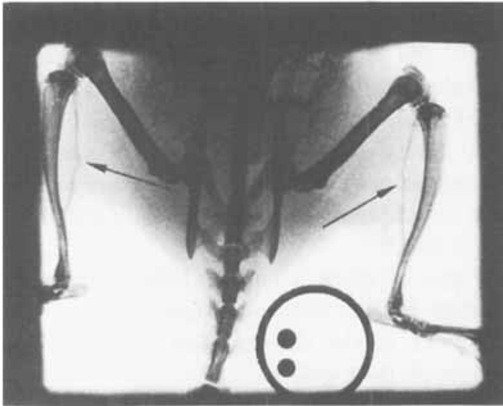

Figure 3. A X-ray of a rat 90 days after the operation. On the right (marked) side repaired with an autogenous bone graft a pseudoarthrosis is seen. On the contralateral side the callus is angulated but continuous calcification is present.

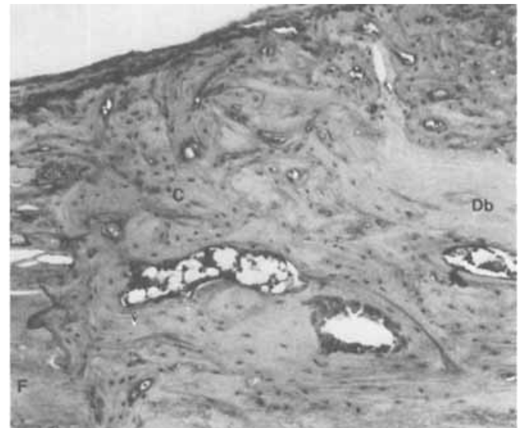

Figure 5. Callus formation in the fibula repaired with bone inductive material. Remnants of the material (Db) are still seen in the middle of the callus (C); (F) fibula.

DISCUSSION

The unanimous opinion is that fresh allogenous bone, preferably with cancellous bone and marrow tissue, is the best material for bone grafts. This has been proved both by clinical experience and in numerous experiments (e.g. Boyne 1973, Puranen 1966). Even freshly frozen material stored in deep freezers (bank bone) is less satisfactory. Two phases in the formation of new bone are observed when fresh bone grafts are used (Axhausen 1956). The early phase during the first 3 weeks may be due to the activity of surviving cells in the graft, and the late phase, beginning within 4 to 8 weeks after transplantation, is supposed to be accomplished by cells derived from the host tissue (Chalmers 1959, Puranen 1966, Elves & Pratt 1975).

When grafts of living bone are used, surviving transplant cells may participate in the formation of new bone tissue. The immune response can cut short the activity of transplanted cells if the material used is

identified as foreign. The immune response against bone tissue, however, is generally mild, and practically absent if grafts are frozen before the transplantation (Heiple et al. 1963).

The results obtained during the present experiment confirmed the osteoinductive capacity of an acid decalcified homogenous bone matrix. The method of BIM preparation destroys its antigenity, so that rejection did not occur even with heterogenous BIM transplants in a series of preliminary experiments. These observations agree with the extensive studies of Urist & Strates (1971) and Iwata & Urist (1972). The bone inductive capacity of BIM is believed to reside in the organic matrix and requires the immediate contact of host cells with the inductive material (Urist & Strates 1971). Progenitor cells which are capable of being transformed into chondroblasts and osteoblasts are blood borne or derived from the connective tissues and blood vessels of the granulation tissue which rapidly grows and invades the transplanted material (Friedenstein 1973).

Boyne (1973) obtained good results when he repaired an artificial defect in the mandibles of Rhesus monkeys with surface decalcified bone, and also reported promising results when this method was used in patients. Jonck (1975) observed that when a bone defect was filled with undecalcified bone shavings, decalcination took place with the formation of new bone. These observations together with the extensive studies of Urist & Strates (1971) suggest that the bone inductive capacity resides in the organic matrix of bone and that calcium has to be removed before the inductive material can work.

In the present experiment, BIM was more effective in repairing bone defects than autogenous fresh bone transplants. This may be because the periosteum was removed from the living bone transplants thus slowing down the first rapid phase of production of the new bone. Furthermore, the response to the BIM is species dependent and in rats BIM works efficiently (Urist & Craven 1970).

The results obtained during this investigation suggest that the problem of seeking suitable material for bone grafting still requires further experimentation. Although it is very likely that autogenous grafts are superior to all other means, it may be possible to also obtain other materials which give comparable or at least satisfactory results clinically in selected cases.

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