

# Membrane-guided bone regeneration

## Segmental radius defects studied in the rabbit

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We tested the principle of guided tissue regeneration (GTR) for healing segmental long-bone defects. 7 mm (3 animals) or 10 mm (5 animals) long segmental defects were created in the diaphyses of both radii in 8 rabbits. The defect on one side was covered with a barrier membrane of expanded polytetrafluoroethylene membrane shaped as a tube, while the contralateral side with no membrane served as the control. Healing was followed with radiographs obtained repeatedly during a 13- (n 3) or 27- (n 5) week period. Thereafter, the animals were killed and ground sections of the defect sites were prepared for histologic examination.

Radiographically, the control sites showed some early subperiosteal callus formation and nonunion

of the defects after 6 weeks. The bone ends were rounded off and sealed with cortical bone. No major changes were noted after 6 weeks.

At the test sites, subperiosteal bone formation at the bone ends was first observed radiographically at 2 weeks. At 9 weeks, a thin cortical bone bridged the defect along the inner surface of the membrane. Histologically, an interrupted line of thin, cortical bone was observed along the inner surface of the barrier membrane. Fatty bone marrow occupied the central and largest volume of the defect.

We conclude that it seems possible to use the principle of GTR to accomplish bone union of segmental long-bone defects.

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Guided tissue regeneration (GTR) (for review, see Karring et al. 1993) has been successful on jaw bone defects in several experimental and clinical studies (for review, see Nyman and Lang 1994). The technique is based on the hypothesis that the different types of tissues, located adjacent to a wound area, are formed at different rates and thereby have various abilities to occupy the wound area during healing. By the placement of a membrane barrier, a mechanical hindrance to undesirable tissue cells can be achieved, thereby giving preference to those particular cells to repopulate the defect, which have the capacity to regenerate the desired type of tissue.

We studied the possibility of using the biological principle of GTR to promote healing of segmental long-bone defects in rabbits.

### Animals and methods

8 female, New Zealand white rabbits, weighing 3–4 kg, were used. Radiographs of the forelegs showed

closed growth plates in all animals.

The rabbits were anesthetized with Hypnorm Vet (fluanxonum 10 mg and fentanyl citras 0.315 mg/mL) 1–1.5 mL subcutaneously and midazolam 4–5 mg intravenously. The operative sites were shaved and disinfected with 0.2% chlorhexidine solution. The experimental sites were infiltrated with 1% lidocain. Prophylactic cloxacillin natrium (100 mg) was administered intravenously prior to surgery.

### Surgery

Defects, 7 mm (3 rabbits) or 10 mm (5 rabbits) long, were created in the diaphysis of both radii in each rabbit. The partly calcified interosseous membrane between radius and ulna was intersected at the site of the defect. A 1.5 cm wide metal band was placed between the bones for protection of the ulna during cutting. The osteotomies were performed with a small drill during continuous irrigation with saline, and the intermediate bone cylinder was removed. In the 3 rabbits with 7 mm defects, the periosteum covering the bone ends was removed, while in the other

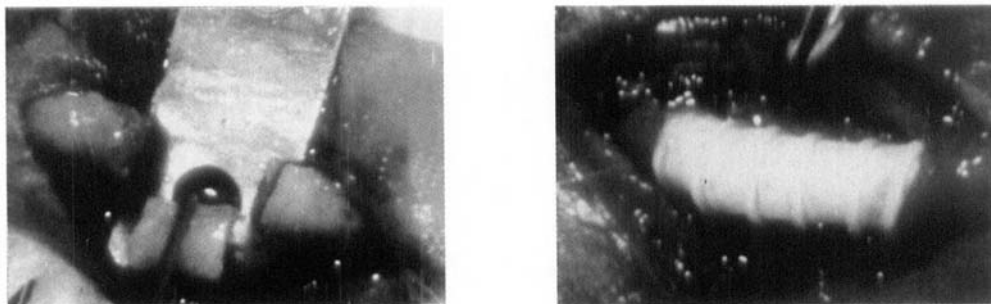


Figure 1. A defect was created in the rabbit radius with removal of the intermediate bone cylinder (left). The defect on the test side was bridged with an e-PTFE tube, reinforced with PTFE rings (right). The defect on the contralateral side served as the control.

5 rabbits the periosteum was kept intact. No fixation was used, since the ulna served as a splint.

The defect on the right or left side in each rabbit was covered with a tube of expanded polytetrafluoroethylene (e-PTFE) tube (Gore-Tex™, W. L. Gore & Ass., Inc., Flagstaff, AZ, U.S.A.), reinforced with compact PTFE rings (Figure 1). The tubes measured 5 mm in diameter and overlapped 5 mm of the bone ends. The defect on the contralateral side was left uncovered and served as the control.

### Radiography

Radiographs of the bone defects were obtained 0, 2, 4, 6, 9, and 13 weeks after operation in 3 rabbits (with 7 mm defects), which were killed after 13 weeks. In the other 5 rabbits, which were killed after 27 weeks, radiographs were obtained 10, 18, and 27 weeks postoperatively.

### Specimen preparation and analysis

The defect sites with adjacent bone were dissected, fixed in 4% buffered formalin, dehydrated in a graded series of ethanol and embedded in plastic resin. One undecalcified ground section of each site was produced by means of a sawing and grinding

technique (Exakt Apparatebau, Norderstedt, Germany). The ground sections were ground to a thickness of about 10  $\mu\text{m}$  and stained with 1% toluidine blue. The sections were viewed and photographed in a Leitz Orthoplan microscope.

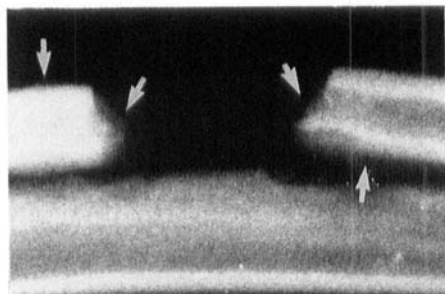
### Results

The animals used their forelegs in a normal way a few hours after operation. The healing was uneventful and there were no signs of infection.

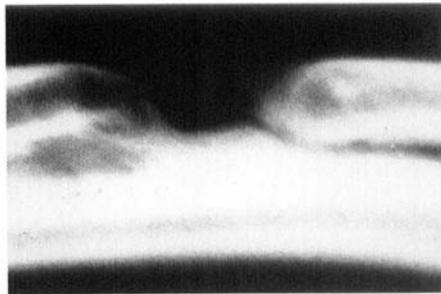
### Radiographic examination

*Control side* (Figure 2). The healing process was similar in all animals. 2 weeks postoperatively, a thin callus formation was seen covering the cut surfaces and extending up to 1.5 cm along the outer surface of the bone ends. At 6 weeks, the bone ends were rounded off and sealed with cortical bone. Bone-bridging of the defects did not occur, but frequently synostosis between radius and ulna was observed. After 6 weeks, no major changes occurred, except a slightly increased maturity in the newly formed bone.

Figure 2. Radiographs of a control defect demonstrating the healing of a 7 mm radial defect.

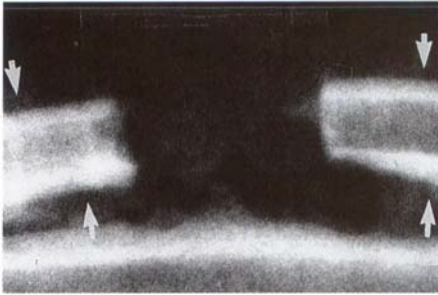


At 2 weeks, a callus formation can be seen covering the cut bone surfaces and part of the outer surfaces of the bone ends (arrows).

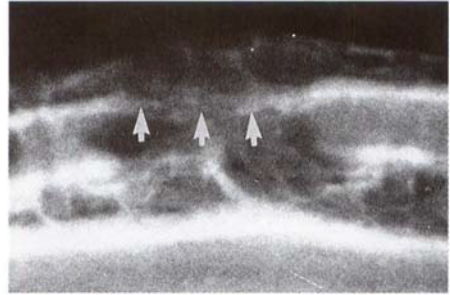


At 9 weeks, the bone ends are sealed with cortical bone. A synostosis between radius and ulna can be seen at the proximal site of the defect.

Figure 3. Radiographs of a test defect demonstrating the healing process of a 7 mm radial defect.



At 2 weeks, callus formation is seen on the outer surface of the bone ends (arrows).



At 9 weeks, a thin cortical bone bridges the defect (arrows).



Figure 4. Radiographs of a 10 mm test defect 27 weeks post-operatively. Observe the thin cortical bone formed along the inner surface of the membrane (arrows). The bone marrow space seems to be expanded.



Figure 5. Radiograph of a 10 mm test defect 27 weeks post-operatively. The defect is healed with apparently normal cortical bone and bone marrow in the center.

*Test side* (Figure 3). At 2 weeks, a thin callus formation along the outer surface of the bone ends was observed, and after 4 weeks, although not quantified, it seemed to be somewhat more extensive than on the control side. A discrete, dense material was seen on the cut bone surfaces. The bone marrow space at the bone ends remained open and in 4 cases was somewhat expanded. At 6 weeks, the bone formation had increased and at 9 weeks, a thin cortical bone bridged the defect along the inner side of the membrane. The bone marrow space at the bone ends remained open and in most cases was somewhat expanded. Only minor changes were observed in the radiographs, taken at later time intervals (13, 18, and 27 weeks postoperatively; Figure 4). In 1 animal (10 mm defect), the defect healed with normal cortical bone and an apparently normal bone marrow space in the center (Figure 5).

#### Histologic examination

*Control side.* All defects showed nonunion, with muscle and fibrous tissue occupying the defect. A considerable amount of bone was formed at the bone ends, which were rounded off with a closed marrow space at both ends (Figure 6).

*Test side.* There was a consistent healing pattern, as the inner surface of the membrane was aligned with an interrupted thin layer of cortical bone (Figure 7). No bone formation was observed on the outer side of the membrane facing the surrounding soft tissue. Islands of trabecular bone could be seen in the center of the defects which, in some specimens, formed a transverse zone including cartilagenous tis-

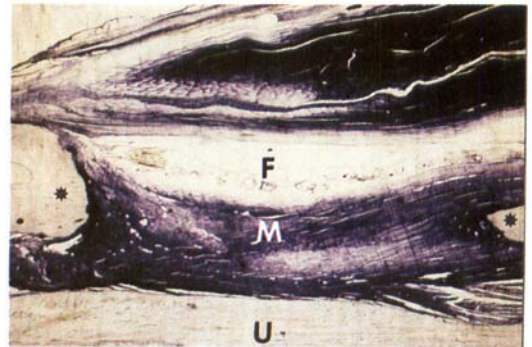


Figure 6. Histological section of a 10 mm control defect. The defect area is occupied by fibrous (F) and muscle (M) tissue. The bone ends are completely closed by a layer of newly formed bone (proximal end is seen). \* bone ends. U ulna. Toluidine blue,  $\times 10$ .

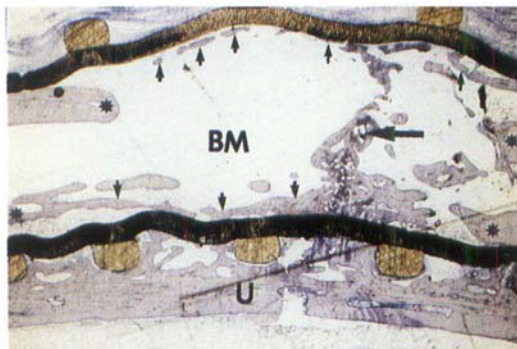


Figure 7. Histological section of a 10 mm test defect. A thin layer of cortical bone (small arrows) has formed along the inner surface of the membrane. Observe the large volume of bone marrow (BM) within the healed defect area. In this particular specimen, a transverse zone of trabecular bone is seen (large arrow). \* bone ends. U ulna. Toluidine blue,  $\times 10$ .



Figure 8. Histological section of a 10 mm test defect demonstrating mineralized bone bridging the defect without interruption. Toluidine blue,  $\times 10$ .

sue. However, bone marrow occupied the largest volume of the defect. In 1 rabbit with a 10 mm defect, the cortical bone on the inner side of the membrane bridged the defect without interruptions (Figure 8).

The 2 groups of rabbits, operated with 7 and 10 mm defects, respectively, demonstrated a similar healing result. No conclusions could be drawn from the attempt to remove or leave the periosteum on the bone ends.

## Discussion

Our findings demonstrated the possibility of achieving bone-union of long-bone defects by preventing ingrowth of muscular and fibrous tissues with the aid of a physical membrane barrier. However, the regenerated cortical bone in the defect on the membrane side was thin and fragile, while bone marrow tissue occupied the major part of the previous defect. The mineralized bone formed along the inner surface of the membrane, indicating that the presence of a solid surface facilitates bone regeneration. One way to reduce regeneration of bone marrow and to offer solid surfaces to enhance mineralized bone formation is to use some kind of graft. Nielsen (1992) used demineralized bone matrix (DBM) to fill bone defects, similar to those in our study, which were covered or not covered with a resorbable polyurethane membrane. He found that the combination of DBM and membrane (Guided Bone Induction) resulted in proper regeneration of the defects, while DBM alone resulted in a mixture of bone and fibrous tissue after 12 weeks of healing. The DBM has bone-inductive properties (Reddi et al.

1987) and may also have a mechanical preventive effect on the regeneration of bone marrow into the defects.

Another explanation of the incomplete healing observed in our study, is the absence of trabecular bone in the diaphysis of the rabbit radius where the defects were created. It has been found that cortical bone in rabbits is less reactive to surgical trauma than trabecular bone (Sennerby et al. 1992, Lundgren et al. 1993, 1994). This may be due to the fact that trabecular bone has a large surface in relation to its volume, and therefore a large number of osteogenic cells can rapidly respond to trauma. Cortical bone has, in comparison, a smaller surface in relation to its volume and thereby a smaller number of osteogenic cells available.

In contrast to the results of our study, Dahlin et al. (1988, 1990) showed virtually complete bone healing when using the membrane technique for healing of transosseous defects in the rat mandible and in the monkey mandible and maxilla. Besides the possibilities discussed above, this difference may be due to differences in the healing pattern of enchondral and intramembranous bone. It is known that enchondral and intramembranous bone perform differently when used as bone grafts, since the enchondral bone graft seems to be resorbed with time, while the intramembranous bone graft seems to maintain or even expand its volume (Smith and Abramson 1974).

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