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Guided Bone Regeneration (GBR) on Healing Bone Defects: A Histological Study in Rabbits

Muzaffer Aslan, DDS, PhD; Göksel Şimşek, DDS;
Ertunç Dayi, DDS, PhD

Abstract

In this study, the effects of guided bone regeneration (GBR) on the healing of bone defects were evaluated. Resorbable membranes were placed in experimentally formed cavities in the right posterior tibia of 30 rabbits. Decalcified histological sections were evaluated using optical microscopy at 10, 20, and 30 days after GBR. Osteocondrial bone union, active bone formation and spongiosal bone formation values of the GBR group are higher than the control group. It was found that GBR technique had a positive and accelerating influence in all phases of bone healing.

Keywords: Biomaterial, guided bone regeneration, GBR, bone healing

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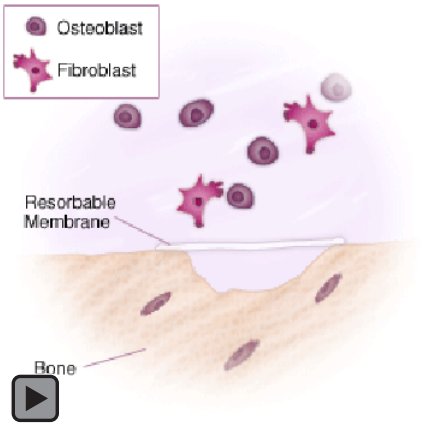
Introduction

Guided bone regeneration (GBR) is a current treatment for periodontal bone defects. In the GBR technique, a barrier membrane is placed over the periodontal defect to prevent the ingrowth of cells from the gingival connective tissue, epithelium, and the periodontal ligament.[1-9]

GBR was used in different studies in which the purpose was bone regeneration within intra-bony defects. This technique utilizes a mechanical barrier in an intra-bony defect with the aim of creating a secluded space to receive only cells with an osteogenic potential so osteogenesis may occur unimpeded within the space. In an intra-osseous wound, invasion of the clot by fibroblasts can result in non-union of bone.[2]



If the gap surrounding an implant is large, fibrous connective tissue cells may proliferate into the area and produce a fibrous capsule around the implant. The GBR technique may offer a method for avoiding these clinical complications. This method has been used in periodontal surgery to develop the attachment of periodontal connective tissue to the root surface of teeth, and to exclude epithelial cells from the wound. It has also been used to form improved osseous tissue around the implants in bone, to prevent fibrous encapsulation, and to produce additional bone in the area.[4]

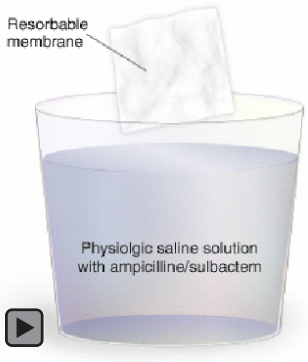
In maxillofacial surgery, fibrous non-union can be an undesirable outcome, especially in extensive reconstructive surgery. Non-union may occur when the fibroblasts organize the clot before the osseous cells migrate into the wound and initiate the bone-forming process. It has been suggested this occurs because fibroblasts have a faster rate of migration than osteoblasts. GBR offers a means of excluding fibroblasts from the clot; permitting slower bone-producing osteoblasts to affect clot organization and produce osseous healing.[4]

GBR membrane materials must maintain their barrier function long enough to allow osteoblasts to migrate into the wound. The distance to be spanned determines the time the membrane must function properly.[3] Resorbable and non-resorbable membranes have been used as a GBR barrier. However, non-resorbable membranes must be surgically removed after the healing period. A resorbable membrane that can transmit tissue fluid, but excludes undesired cells from the clot, would have the advantage of not requiring surgical removal. Recent studies have reported the successful use of resorbable membranes in GBR.[4]

Materials and Methods

Our experimental study was performed on 30 mature albino rabbits, each of which weighed 2 kg average, provided by Erzurum Veterinary Check-up and Research Institute, at the Physiology Laboratory of Faculty of Medicine of Atatürk University.

Rabbits were anesthetized with the injection of 1mg/kg dose of 50 mg/ml Ketalar (Parke-Davis, USA) in accordance with the principles of general surgery. Two cavities were created using physiologic cooling serum on the right posterior tibia of the rabbits. We covered one of the cavities with resorbable membrane (Tutoplast Pericardium Bovin, Biodynamics Inc., Germany), and left the other cavity empty to serve as a control. The diameter of each cavity was 3 mm. Prior to placement, the membrane was left for one hour in the physiologic saline solution with ampicilline/sulbactam. Derma and endoderm were sutured. During one week following the operation, the wound was treated and dressed, 0,25 cc 800.000 IU penicillin procaine were injected (I.V.).



After follow-up on the 10th, 20th and 30th days, the rabbits were grouped into equal numbers and sacrificed using high doses of parentally administered Ketalar. Bone segments where the experiment had been performed were removed. The block biopsies were harvested, fixed in buffered formalin, and decalcified in Morse/or EDTA solution. Once decalcified the blocks following routine histological processing and paraffin embedding were done and 5µm thick tissue blocks on the longitudinal/axial plane were obtained. The resulting specimens were dyed with haematoxylin eosin (HE) and examined under light microscope.

Findings

Post Operative 10th Day

In defects covered with resorbable membrane, spongiosal (trabecular) bone formation was seen in the floor and at the edge of the defect. There is no active bone marrow and cortex formation during this period (Figure 1).

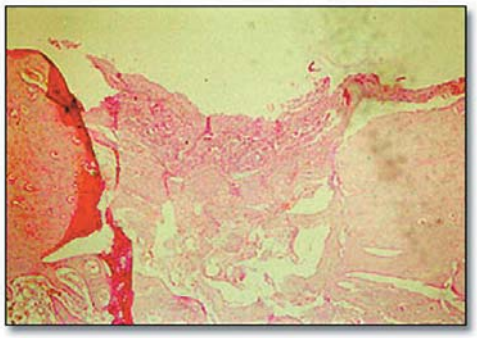


Figure 1. Active bone formation in the sites using resorbable membrane (10 days post-procedure).

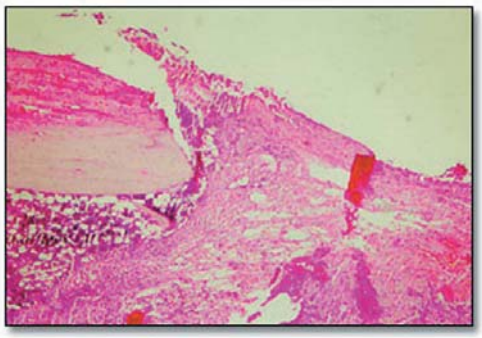


Figure 2. New bone formation in the control group (10 days post-procedure).

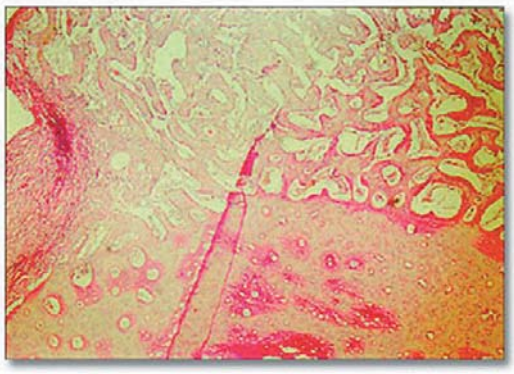


Figure 3. GTR healing after 20 days. Osteochondrial bone healing was seen.

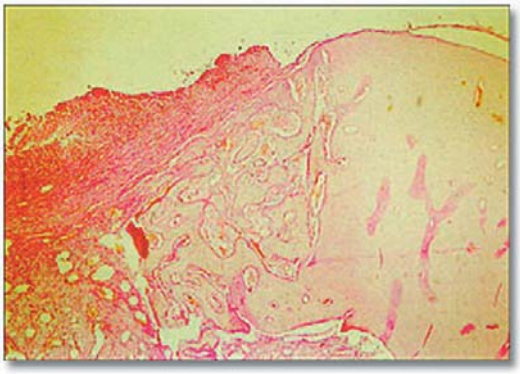


Figure 4. New bone formation in 20th day-control group.

New bone formation with a thin stratum on the sides and base of defects in the control group was observed. The thin bone was composed of connective tissue that contained spindle formed cells having nucleuses and vascular structures. It was observed the defect was full of fibrous connective tissue (Figure 2).

Post Operative 20th Day

In the GBR group osteochondrial union was seen in 6 cases, while the other 4 cases displayed evidence of a fibrous union. Spongious formation was observed in 6 cases. One of these cases had active bone formation. One in 10 cases showed evidence of bone marrow formation. There was no cortex formation in any of the cases (Figure 3).

In 6 cases in the control group there was osteochondrial healing, and in other cases fibrous bone union was in progress. In one case no new bone formation and cellular activity was observed (Figure 4).

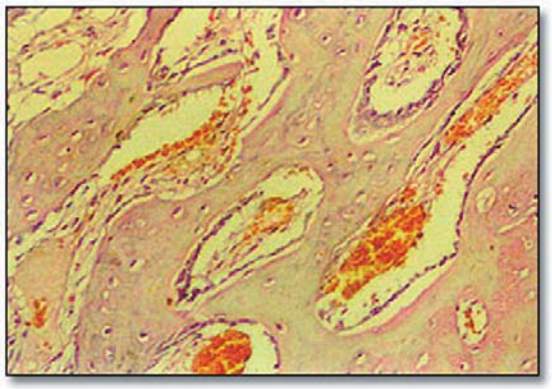


Figure 5. Osteochondrial bone union and early bone formation was seen in 30th day-GTR group.

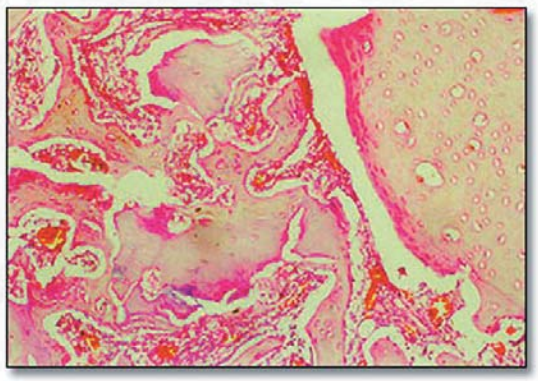


Figure 6. Osteochondrial bone healing in control group (30 days post-procedure).

Post Operative 30th Day

In the membrane group osteochondrial bone union was seen in all cases. Six of these showed active bone formation and 4 of the cases have early new bone formation (Figure 5).

In the control group osteochondrial formation was observed in 6 cases, and in 4 cases fibrous bone union was determined. In 6 cases spongious bone formation was observed, and in 1 case a new active bone formation was observed. Only 1 case had active bone marrow formation but no cortical bone formation occurred (Figure 6).

In all evaluation periods of the membrane group, there was no evidence of resorption and dehiscence of the membrane. Fibrous tissue was limited by the membrane and periosteum. Also, cartilage tissue was not observed in any of the specimens.

A statistically significant difference was observed using Mann-Whitney-U test after all evaluation periods (Tables 1, 2, and 3).

Discussion

In the osteotomy sites and in the bone defects the invasion of the mature fibrous tissue can result in undesired situations such as nun-union and encapsulation. The concept of GBR can prevent such problems.[2,3,4]

Table 1. Statistical analysis of the 10 day period.

	Union		Spongiosa		Cortex		Bone Marrow		Healing	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	1,000	,000	,000	,000	,000	,000	,000	,000	1,000	,000
GTR	2,000	,000	1,600	,516	,000	,000	,000	,000	3,600	,516

Table 2. Statistical analysis of the 20 day period.

	Union		Spongiosa		Cortex		Bone Marrow		Healing	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	1,600	,516	,100	,316	,000	,000	,000	,000	1,700	,674
GTR	1,900	,316	,900	,316	,000	,000	,000	,000	3,300	,948

Table 3. Statistical analysis of the 30 day period.

	Union		Spongiosa		Cortex		Bone Marrow		Healing	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	1,600	,516	,700	,674	,000	,000	,100	,316	2,400	1,075
GTR	2,000	,000	1,600	,516	,000	,000	,400	,516	4,300	0,948

The suture around the tooth and/or tissues to maintain potential space has usually attached barrier materials used in GBR studies. Resorbable membranes are biodegradable through the process of hydrolysis and do not require removal. Their composition is similar to the synthetic absorbable sutures with regard to their safety performance and rate of bioabsorption.

Sandberg et al.[2] formed standardized defects in both sides of a rat mandible and covered them with a resorbable membrane. In spite of fixation by the sutures, membrane dislocation was observed in one case.

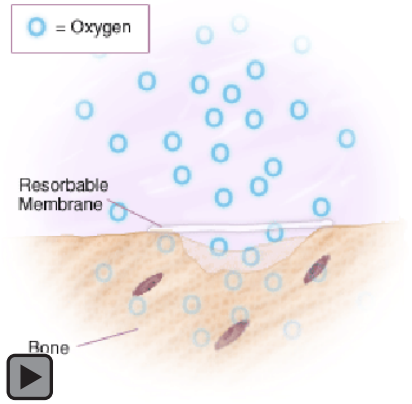
Polson et al.[1] used a GBR membrane in class II furcation defects in the mandibular and maxillary molar areas. They observed membrane collapse and dislocation. Granulation tissue was reported between the bone surface and the membrane in barrier-dislocated cases.

We applied the membrane extending 2 mm beyond the margins of the defects, and we did not use sutures for fixation. There was no dislocation in our membrane cases. We think this was related to anatomic and functional characteristics of the test sites.

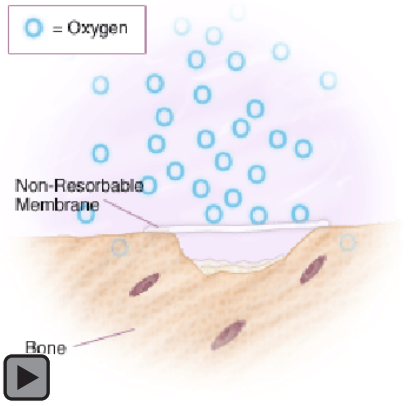
Another factor that may cause failure is infection. A wound subjected to bacterial contamination does not heal at an optimal rate.[1] Polson et al.[1] observed accelerated epithelial invagination into the wounds in bacterial infected cases. This situation reduced regeneration during healing period.

In our study the resorbable membrane was left for 1 hour in physiological serum solution with the ampicillin/sulbactam prior the implantation. As a result, no primary or secondary infection was observed.

Mundell et al.[4] used a collagen membrane in experimentally formed bone defects in the arcus of zygoma of rabbits. Reorganization and ossification was observed in membrane-performed cases after 4 weeks. In the same period of healing, fibrous tissue invasion was seen in the control group. In the GBR group there was new bone formation in the base of the defect within 2 weeks. At the end of 4 weeks, the defect was full of new uniform bone. In the osteotomy sites covered by the barrier membrane it was observed the periosteum was thickened and the overall extent of periosteal bone growth at the ends of the bone was greater compared with control sites. They did not provide a reason for this phenomenon.



Cartilage formation during bone regeneration has been considered to be due to low oxygen tension in the tissue.[2,10] Sandberg et al.[2] observed areas of cartilage in membrane performed experimental defects in a rat mandible. The presence of cartilage might be due to low oxygen tension caused by sealing off the periosteal vascular supply. Resorbable membranes are porous and, thus, allow a free interchange of tissue fluid and macromolecules while keeping unwanted cells out.



In our study we did not see areas of cartilage and tissue thickening in all periods of the GBR group. In almost all cases surrounding connective tissue had penetrated into the control cavity. Resorbable membrane appeared to improve healing in comparison with the control group.

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About the Authors

Muzaffer Aslan, DDS, PhD

Dr. Aslan is an Assistant Professor in the Department of Oral and Maxillofacial Surgery in the Faculty of Dentistry at Atatürk University in Erzurum, Turkey. His research interests include biomaterials and bone healing. Dr. Aslan is a member of the Turkish Oral and Maxillofacial Surgery Society.

e-mail: drmaslan@yahoo.com

Göksel Şimşek, DDS

Dr. S¸ims¸ek is a researcher in the Department of Oral and Maxillofacial Surgery in the Faculty of Dentistry at Atatürk University in Erzurum, Turkey. Her research interest focuses on the affect of biochemical materials on bone healing.

Ertunç Dayı, DDS, PhD

Dr. Dayı is a Professor and Chairman of the Department of Oral and Maxillofacial Surgery in the Faculty of Dentistry at Atatürk University in Erzurum, Turkey. He is a member of the Turkish Oral and Maxillofacial Surgery Society.