Bone Defect Healing by Nonabsorbable Membrane for Guided Tissue Regeneration

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Abstract: This study was investigated whether bone regeneration is improved in the healing of experimental osseous defects with subperiosteal membranes. Muscle/periosteal flaps were raised, and 4.5-mm osseous defects were created bilaterally in the tibiae of ten rabbits. On the test side, a silicone membrane was placed over the defect, and the flaps were sutured outside the membrane. The defect on the contralateral side served as a control (no membrane). After healing periods of 1, 2, 4 and 8 weeks the animals were killed, and 15 µm hematoxylin-eosin-stained, undecalcified sections were prepared and examined histologically. The results suggest that the subperiosteal membrane keeps the osteogenic tissue structurally stable so that osteogenesis remains functionally stable during the early stages of healing.

Key words: guided tissue regeneration, subperiosteal membrane, silicone.

Bone regeneration in osseous defects is improved by the use a subperiosteal membrane technique of guided tissue regeneration\(^1,2\). Guided tissue regeneration is based on the hypothesis that different cellular components migrate at different rates into a wound area during healing. Barrier membranes are used to prevent undesirable tissue cells from growing into the defect, and thereby give an advantage in repopulating the defect to cells that can regenerate the desired tissue\(^3\).

A recent study of subperiosteal membrane implant\(^4\) suggests that healing of osseous defects is improved owing to a stabilizational function as well as to the barrier function. In fact, stabilization may be the chief mechanism for improving bone regeneration. Since absorbable materials were used in previous studies of the barrier technique, nonabsorbable materials should also be used so that the possible effects of degradation and absorption on improved defect healing can be excluded. The present study was performed to investigate whether bone regeneration in healing tibial osseous defects is improved by a nonabsorbable subperiosteal membrane in rabbits.

Materials and Methods

Ten adult male Japanese rabbits were used in this study. The animals were sedated with both a general anesthetic, Pentobarbital (Abbott Laboratories, North Chicago, IL; 50 mg/ml, 30 mg/kg body weight) and a local anesthetic, lidocaine (Fujisawa, Tokyo; 20 mg/1.8 ml with epinephrine, 0.0125 mg, 1.0 ml-1.8 ml infiltration). The medial border of the proximal tibia was chosen as the site of osseous defect formation. The hind legs of each
animal were shaved, washed and covered with a perforated drape.

The bone surface was exposed via a skin incision and careful subperiosteal dissection. Muscle/periosteal flaps were separated from the tibial cortex and raised. A round osseous defect was formed using a stainless steel bur 4.5 mm in diameter at a speed of 2,000 rpm under vigorous cooling with physiologic saline. The wound region was gently irrigated with the saline. In each animal, a piece of Silicone membrane (Koken, Tokyo) 15 mm × 15 mm in size was placed over one of the defects (test side). The muscle/periosteal flaps were gently stretched to cover the membrane and sutured in layers. The defect on the contralateral tibia in the same animal served as a control, with no membrane placed.

The animals were killed to obtain contralateral pairs of test and control specimens by intravenous injection of 0.5 ml (25 mg) Pentobarbital and 5 ml air on 1, 2, 4 and 8 weeks after surgery. The block specimens were immediately placed in 10% buffered phosphatic formalin solution for fixation. The specimens were dehydrated in a graded series of ethanol and preinfiltrated with Styrene monomer (Wako, Osaka), then infiltrated with Rigorac polyester resin (Nissin EM, Tokyo) and embedded in fresh Rigorac polyester resin. After polymerization, the samples of 200 μm were cut in a plane perpendicular to the tibial long axis and ground to a thickness of about 15 μm with an Exakt cutting-grinding system (Exakt, Hamburg). Multiple serial sections were stained with hematoxylin-eosin and examined in a Nikon biophot microscope (Nikon, Tokyo).

**Results**

**Control Site**

From 1 to 8 weeks postoperatively, overlying soft tissue initially prolapsed into the intraosseous wound area. The arrangement of the immature

![Figure 1](image-url)
fibrous tissue formed from the blood clot in the defect was irregular (Figure 1 A). A fibrous tissue core then formed in the center of the intraosseous wound area; the direction of its collagen fibers parallel to the surface of the original defect margins. New bone formed in the intraosseous wound area was discontinuous between the original defect margins (Figure 1 B). Later, although a continuous periosteum covered the defect with no prolapse of soft tissue, the intraosseous wound area still had no continuous new-formed bone between the original defect margins (Figure 1 C and 1 D).

**Test Site**

At 1 week postoperatively, the arrangement of the immature fibrous tissue formed from blood clots was regular. Osteogenesis occurred with osseous spicules (young trabeculae) (Figure 2 A, B). At 2 weeks postoperatively, Osteogenesis occurred significantly on each side of the membrane. The woven structure of new trabecular bone was formed (Figure 3 A, B).

At 4 weeks postoperatively, new-formed bone was continuous on each side of the membrane. The new-formed bone under the membrane was less than that above the membrane. Remodeling was clearly evident in both new-formed and original bones (Figure 4 A, B).

At 8 weeks postoperatively, continuous new-formed bone between the original defect margins was observed. Remodeling was evident in both new-formed and original bones (Figure 5 A, B).

All new-formed bone in both test and control sites was consistent with normal bone. There was no evidence of adverse tissue reaction with the membrane.
Discussion

In earlier years, researchers showed the potential of bone regenerative improvement by barrier technique in their experiments. This technique was developed into subperiosteal membrane implantation technique for the study of alveolar bone regeneration. It was thought that the function of the barrier was the protection of the blood clot beneath the barrier and the prevention of non-osteogenic extraskeletal connective tissue migration into the osseous healing area. Recently, subperiosteal membrane implantation has been employed in guided tissue regeneration for improving osseous healing, based on the similar hypothesis that different cellular components in the tissue have different rates of migration into a wound area during healing. The concept of guided tissue regeneration by barrier membranes is the prevention of ingrowth of non-desirable tissue cells into the defect, thereby giving preference for repopulation of the defect to those particular cells which have the capacity to regenerate the desired tissue. In the present study, it is difficult to explain with this hypothesis why significant osteogenesis occurred on the outside of the membrane, and why less osteogenesis occurred in the intraosseous wound area under the continuous periosteum despite the absence of non-osteogenic extraskeletal connective tissue (Figure 3).

The histological findings of this study showed that the membrane supported and protected the blood clots against the mechanical stress of muscles and other dislodging forces so that the clots were stably converted into orderly immature fibrous
tissue. This tissue was then supported and protected so as to be stably converted into osseous spicules, then stably into trabecular woven bone. The osteogenic tissues of blood clots as well as immature fibrous tissue and osseous spicules were not only protected under the membrane but also supported above the membrane. The membrane also indirectly supported the periosteum and prevented the soft tissues above the incised periosteum from prolapse (not ingrowth) into the osteogenic tissue area through the incision. The membrane diverted pressures from these osteogenic tissues to keep their structures stable until the firm trabecular woven structure of new bone could form.

The incision of the periosteum created an opportunity for ingrowth and/or proliferation of overlying soft tissue into the subperiosteal area above the membrane. No such ingrowth was observed, but there was significant osteogenesis. On the other hand, still less osteogenesis occurred even under the continuous periosteum, with no prolapse or ingrowth of overlying soft tissues. This might be the result of two conditions; either the continuous periosteum formed after overlying soft tissue prolapse into the defect area, or the periosteum covered the defect without overlying soft tissue prolapse throughout the postsurgery period. The periosteum may be too supple to protect the osteogenic tissues against dislodging forces. Without sufficient stabilization, especially in the center of the osseous defect area, the blood clot and immature fibrous tissue would have been hard convert into osseous spicules and into continuous bone. Instead, they were converted into mature fibrous tissue or resorbed by surrounding tissue. This means that the structural stabilization of those osteogenic tissues may be more important than the elimination of ingrowth of non-osteogenic tissue. This is in agreement with a study using an absorbable biomaterial\(^9\), and confirms that the subperiosteal membrane is capable of keeping the osteogenic tissue structurally stable so as to maintain functional stability of osteogenesis during the early stages of healing.

The principles of the subperiosteal membrane were evaluated through tibial defect healing in rabbits. It was concluded that the basic effect of the membrane is to keep the osteogenic tissue structurally stable so as to keep osteogenesis functionally stable during the early stages of healing.

References