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Microporous PTFE Membrane on Bone Regeneration in Rat Calvarial Defects

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Novel membrane is always being developed for guided bone regeneration(GBR). The purpose of this study was to evaluate the microporous polytetrafluoroethylene(PTFE) membrane on bone regeneration in rat calvarial defects. Thirty male Sprague-Dawley rats were used in 3 groups. One was the control group with no material used, another was the positive control group which used the resorbable membrane, collagen and the other was the experimental group which used the microporous PTFE membrane. The barrier membranes were applied in the surgically created standardized 8 mm cranial defect. The rats were sacrificed at 2 or 8 weeks. The amount of bone regeneration was assessed histologically and histomorphometrically. The bone formation increased under both barrier membranes compared with the control group. In terms of time, more bone was filled in 8 weeks than 2 weeks. Better bone formation was seen in defects protected by a microporous PTFE barrier when compared with the control and collagen group. It can be concluded that microporous PTFE membrane is effective in bone regeneration for its biocompatibility, bone formation enhancement, ease of use and space maintenance.

Key words: bone, microporous PTFE membrane, regeneration, rats

Introduction

Destroyed tissue is always a concerning factor in periodontal regeneration.[1)] Many methods for the periodontal regeneration have been mentioned such as root conditioning which enhances cell adhesive capacity, bone grafts in intrabony defects, and guided bone regeneration(GBR) using specific cell migration and bone morphogenetic proteins and other various growth factors. Among the various methods that have been introduced, GBR have been the most effective mode of therapy to facilitate bone healing.

GBR in the dental field refers to procedures that attempt to regenerate bone prior to placement of bridges or more commonly, implants. It carries basically two aims: 1)to improve the lack of bone/implant contact in the defect area and 2)to restore the contour of the alveolar ridge. This is accomplished by using bone grafts and biocompatible membranes that keep tissue out and allow the bone to expand.

GBR carries quite a wide range of surgical procedures and among those suggested procedures, membrane has been used for tissue and bone regeneration for decades. However, membranes, aside from their advantages, carry limitations such as difficulty in handling and the uncertainty of the outcome. In consequent, novel membrane for GBR is still being developed.[2)]

GBR commonly utilizes nonresorbable or resorbable membranes[3,4,5,6,7)] to provide an environment for bone to express its native osteogenic potency. Nonresorbable membranes such as Millipore filter, polytetrafluoroethylene[7)] and absorbable membranes such as collagen,[8,9)] polylactic acid[10,11,12)] polyglactin 910, glycolide[13)] and lactic copolymer are usually used in GBR.[14)]

The graft material needs to be stable and the soft connective tissue should be prevented from growing inside the defect area. Thus using a barrier membrane to cover and retain the graft material might satisfy both prerequisites.

There have been many reports on bone regeneration using different types of membranes[14,16)] such as the Gore-Tex membrane[17)] and porous membranes[18)] and comparisons of nonporous and porous membranes[19)] and absorbable and nonresorbable membranes.[20)] There are hardly any reports on the microporous PTFE membrane and its usage is not widely known.

The microporous polytetrafluoroethylene (PTFE) membrane

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is composed of high density PTFE and helps assure that the blood clot alone and/or graft material placed in the socket is protected during the initial healing phase after extraction. It is histocompatible, biocompatible and has capacity for space maintenance.[15] Its pore size is 0.2 μm large. Due to the high density of membrane and small porosity size, bacterial contamination is eliminated, protecting the underlying graft material and/or implant. In addition, primary soft tissue closure is not required. It also prevents fibrous connective tissue infiltration but allows permeation of oxygen and nutrients. The micro-sized pores also improve adhesion to bone.

Nonresorbable membranes may be difficult to use in the clinic for its usage. They need to be shaped and cut into proper size according to the defects and have a high risk of exposure, which causes infection. However, the microporous PTFE membrane is easy to use for it has a simple rectangular shape and can be cut into various sizes easily.

The purpose of this study was to evaluate the effects of micrcoporous PTFE membrane on bone regeneration in rat calvarial defects.

Materials & Methods

Animals

Thirty male Sprague Dawley rats, weighing 250~300 g, 8 weeks old were used. The rats were maintained in plastic cages in a room with a 12h-day/night cycle and an ambient temperature of 21°C, with ad libitum access to water and standard laboratory pellets. The routines of animal selection and management, surgical protocol, and preparation were approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

Experimental Design

The animals were divided into 3 groups, There were 10 animals in each group. They were allowed to heal for 2 weeks (5 rats from each group) or 8 weeks (5 rats from each group). Each animal received one of the 3 experimental treatments: a sham-surgery control in which no material was applied to the defect; collagen group as the carrier control; and the microporous PTFE membrane as the experimental group.

Surgical procedures

The rats were generally anaesthetized by an intramuscular injection (5 mg/kg body wt.) with Zoletil® (Virbac, Carros, France) and Rompun® (Bayer HealthCare, USA). During surgery, routine infiltration anesthesia (2% lidocaine, 1:100,000 epinephrine, Kwangmyung Pharmaceuticals, Seoul, Korea) was used at the surgical site. The surgical site was shaved and scrubbed with iodine. An incision was made in the sagittal plane across the cranium and a full thickness flap was reflected, exposing the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter was created on the cranium with the use of an 8 mm trephine drill (3i, FL, USA). Saline was irrigated to prevent the heat of the trephine drill. After removal of the calvarial disk, Collatape (Integra LifeSciences Corporation, NJ, USA) and the microporous PTFE membrane was cut in a square shape of 1 cm × 1 cm and was applied to the defects of the corresponding groups. The periosteum and skin were then closed and sutured with 4-0 Monosyn (Braun Aesculap AG&CO.KG, Tuttlingen).

Histologic and histomorphometric procedures

The animals were sacrificed by CO_2 asphyxiation at 2 and 8 weeks post-surgery. Block section including the surgical sites were removed and fixed in 10% neutral buffered formalin solution for 10 days. All samples were decalcified in EDTA-HCL for 7 days and embedded in paraffin. 5 μm thick coronal sections through the center of augmented area were obtained at 80 μm intervals, and stained with hematoxylin and eosin (H&E). The most central section from each block was selected for the histologic and histomorphometric evaluation. After conventional microscopic examination, computer-assisted histomorphometric measurements were done using an automated image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA) coupled with a video camera on a light microscope (Olympus BX50, Olympus Optical Co., Tokyo, Japan). The sections were examined at × 20 magnification. A digitizer was used to trace the defect outline along the new bone formation, and the percentage of bone fill was determined. The value of each measurement was automatically calculated by the image analysis system. The following histomorphometric parameters were measured for each section. 1) Defect closure(%): the distance(at each side of the defect) between the defect margin and the in-growing bone margin in millimeters × 100 2) New bone area(mm^2): all tissue within the boundaries of newly formed bone, i.e., mineralized bone and fatty marrow and fibrovascular tissue/marrow and residual biomaterial.

Statistical Analysis

Histomorphometric recordings from the samples were used to calculate means and standard deviations ($m \pm SD$). To analyze the effect of both time and condition and to detect the interaction effect between time and condition, the two-way analysis of variance was used ($P < 0.05$). ANOVA and Post-hock test were used to analyze the difference among the groups at each time point ($P < 0.05$). For the comparison between 2 and 8 weeks in a same group, statistical significance was determined by paired t-test ($P < 0.05$).

Results

Clinical findings

The wound healing was generally uneventful. There were

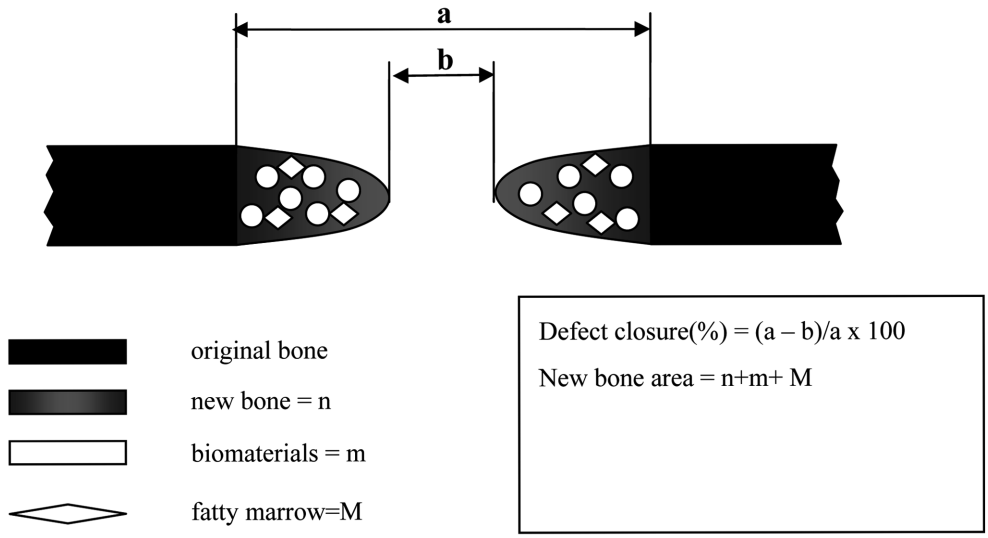

Figure 1. A schematic drawing of calvarial osteotomy defect showing histomorphometric analysis.

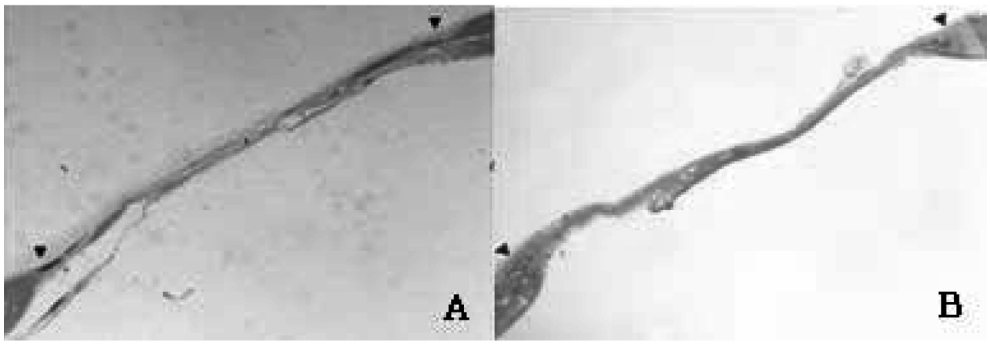

Figure 2. (A) Control 2 weeks: minimal bone formation is seen. (× 20), (B) Control 8 weeks: New bone formation is seen along the margin.

no signs of inflammation observed and appeared similar for all of the experimental groups.

Histological Analysis

Control group

Material exposure or other complications of the surgical sites were not observed. 2 weeks finding in the control group (Figure 2(A)) showed almost no regeneration. In the 8 weeks finding (Figure 2(B)), the defect was filled with loose connective tissue. All in all, the control group showed almost no regeneration.

Collagen Group

In 2 weeks finding of the collagen group (Figure 3(A)), the defects were filled with loose or dense, fibrous connective tissue and limited new bone formation was observed at the defect margin. The collagen membrane remained in the subepithelium and there was no resorption and the external form was maintained and was surrounded by connective tissue. In 8 weeks finding (Figure 3(B)), the resorption of collagen membrane progressed much. There was almost no infiltration of inflammatory cells. There was an increase in bone volume and there was no invagination of peripheral tissue.

Microporous PTFE membrane

The shape and form of the defect and microporous PTFE membrane were maintained in 2 weeks. (Figure 4(A)). Inflammatory cells were infiltrated in the margin of the defect and new bone was invaginated in the margin of the defect, an immature bone formed in a thimble-like morphology. In 8 weeks (Figure 4(B)), the periosteum and the dura mater were joined continuously and the microporous PTFE membrane was well maintained with fibrous connective tissue underneath. The microporous PTFE membrane was surrounded with loose connective tissue, mature bone and bony tissue in the margin of the defect. In the higher magnification of the microporous PTFE membrane, new bone invaginated along the marginal area in 2 weeks (Figure 5(A)) and in 8 weeks, the new bone was enhanced and bone forming cells were seen (Figure 5(B).

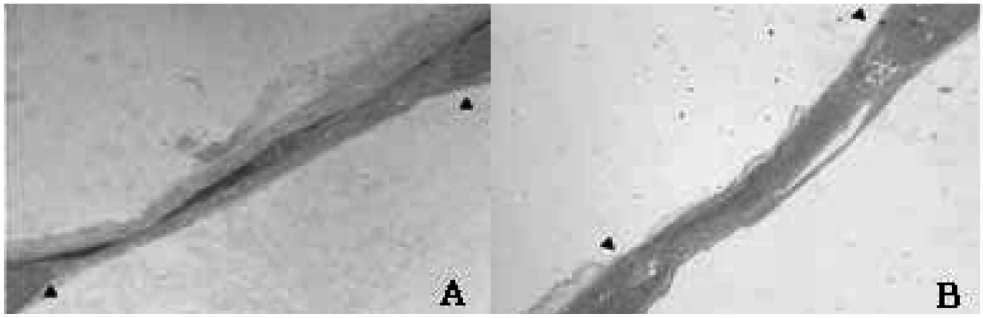

Figure 3. (A) Collagen 2 weeks: ingrowth of bone with loose/dense fibrous connective tissue, limited bone formation can be seen (× 20), (B) Collagen 8 weeks: bone formation increased in the margin and around collagen membrane (× 20)

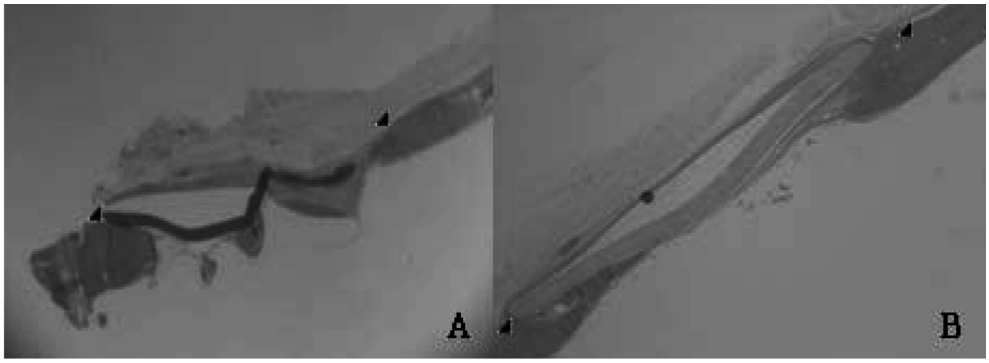

Figure 4. (A) Microporous PTFE membrane 2 weeks (× 20), (B) Microporous PTFE membrane 8 weeks: new bone ingrowth can be seen in marginal area (× 20).

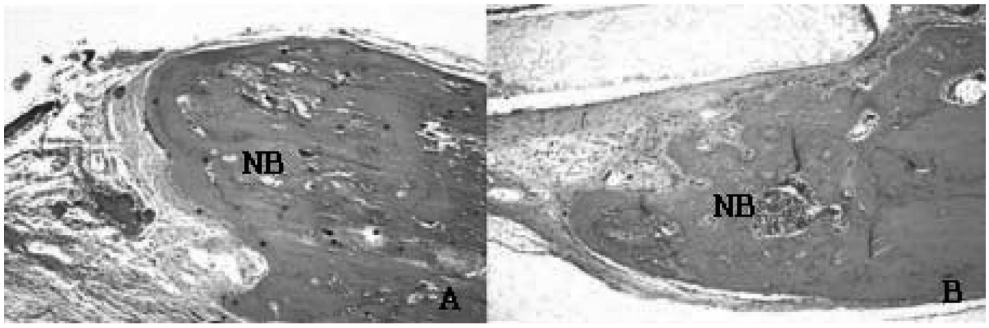

Figure 5. (A) Higher Magnification of Microporous PTFE membrane 2 weeks: new bone (NB) has invaginated along the marginal area (× 50), (B) Higher magnification of Microporous PTFE membrane 8 weeks: new bone (NB) enhanced along the marginal area and is surrounded by connective tissue. (× 50)

Histomorphometric Analysis(Tables 1, 2)

Discussion

Long term results of periodontal regeneration therapy with non-resorbable and bioabsorbable barriers have shown that both membranes were stable after 10 years in 12 of 16 defects.[21] Akimoto et al. reported that histomorphometric measurements showed a trend toward greater bone formation at membrane-treated sites compared with control sites.[22] In addition, Simion et al.[23] used membranes to cover surgically created saddle type ridge defects in the mandible of 6 dogs and a large amount of osseous healing occurred in all the membrane protected sites after a 6-month healing period. The present study evaluated the effects of bone regeneration of the microporous PTFE membrane in rat calvarial defects. The aspect that was focused in this study was to compare the non-resorbable microporous PTFE membrane with the resorbable collagen membrane, to see its bone regenerative potency. The microporous PTFE membrane has its advantage in terms of maintenance of form and shape. It is made of high-density PTFE with the porosity size of 0.2 μm. Owing to the high den-

Table 1. Defect Closure(%) (group means ± SD; n = 5)

	2 weeks	8 weeks
Control	13.9 ± 3.4	15.6 ± 7.2
Collagen membrane	22.6 ± 9.3*†	26.9 ± 10.4*†
Microporous PTFE membrane	34.5 ± 5.6*‡	40.3 ± 6.3*‡

*: Statistically significant difference compared to control group ($P < 0.05$)
‡: Statistically significant difference compared to collagen membrane group ($P < 0.05$)
†: Statistically significant difference compared to microporous PTFE membrane($P < 0.05$)

Table 2. New bone area(mm^2) (group means ± SD; n = 5)

	2 weeks	8 weeks
Control	0.2 ± 0.2	0.3 ± 0.2
Collagen membrane	2.9 ± 1.6*†	1.9 ± 3.3*†
Microporous PTFE membrane	3.1 ± 0.5*‡	3.3 ± 0.4*‡

*: Statistically significant difference compared to control group ($P < 0.05$)
‡: Statistically significant difference compared to collagen membrane group ($P < 0.05$)
†: Statistically significant difference compared to microporous PTFE membrane($P < 0.05$)

sity of PTFE membrane and small porosity size, bacterial contamination is eliminated, thus protecting the underlying graft material and/or implant. The primary soft tissue closure is not required. Thus, this membrane may be exposed to the oral cavity without the fear of possible complications that may occur from exposure of PTFE or resorbable membrane. It also allows nutrients and ions to move in and out. The microporous PTFE membrane also has its advantage compared to the resorbable membrane in that the resorbable membrane can be a problem if it is resorbed too early and if this occurs, the regenerated tissue becomes immature and the amount of bone formation and the level of attachment gain decreases. On the other hand, if the membrane is absorbed too late, healing of the tissue is delayed and complications such as abscess could be formed.

In addition, collagen membranes provide little stability and thereby carries negligible "space-making effect".[7] They also have a short degradation time to be suitable for GBR. It does not have the potency as a barrier for approximately 6 months for bone to form without disturbance underneath the membrane.[12,24] According to the histological and histomorphometric analysis, the microporous PTFE membrane showed an enhanced defect closure in the early healing phase when compared to the resorbable membrane, collagen. The closure of the defect was faster than the other groups. There was more bone formation in 8 weeks when compared to 2 weeks. Thus this could be implied that the microporous PTFE membrane avoids cell migration, consequently enhancing bone formation and thus increases the bone regenerative potency. This is in agreement with the purpose of using a nonresorbable membrane in periodontal lesion to prevent gingival connective tissue and epithelium from proliferating into the defect. The microporous PTFE membrane showed higher new bone area compared to the control group and the collagen group. In addition, there was a higher new bone area in 8 weeks than in 2 weeks. The microporous PTFE membrane can be concluded to be effective in bone regeneration for its biocompatibility, bone formation enhancement through avoiding cell migration, ease of use and space maintenance.

Acknowledgements

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