Evaluation of Bone Regeneration on Polyhydroxyethyl-polymethyl Methacrylate Membrane in a Rabbit Calvarial Defect Model

SOMIN KIM1*, YAWON HWANG1*, MUHAMMAD KASHIF1, DOSUN JEONG2 and GONHYUNG KIM1

1Department of Veterinary Surgery, College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea;
2TE-Bios Company, LTD, C&V Center, Cheongju, Republic of Korea

Abstract. This study was conducted to evaluate the capacity of guiding bone regeneration of polyhydroxyethyl-polymethyl methacrylate (PHEMA-PMMA) membrane as a guided tissue regeneration membrane for bone defects. Two 8-mm diameter transosseous round defects were made at the parietal bone of 18 New Zealand White rabbits. Defects were covered with or without PHEMA-PMMA membrane. Radiological and histological evaluation revealed that the bone tissue over the defect was more regenerated with time in both groups. However, there was significantly more bone regeneration at 8 weeks in the experimental group than the control group (p<0.05). There was no sign of membrane degradation or tissue inflammation and no invasion of muscle and fibrous tissue into defects. PHEMA-PMMA is a potential material for guided tissue regeneration membrane as it induces no adverse tissue reaction and effectively supports selective bone regeneration.

Guided tissue regeneration (GTR) by maintaining bone forming space is applied in many clinical practices (1, 2). In the case of periodontal disease, bones become thinner, eventually losing function. Medical therapy and surgical therapy may cure diseased tissue, but there are usually epithelial cells at the root surface and defective formation of ligaments (3). These problems can be resolved using grafting materials or certain membranes that can be used as GTR by providing space and osteoconductive properties (4). Using periodontal membranes as GTR supports regeneration of diseased periodontal tissue such as alveolar bone and periodontal ligament. Membranes prevent invasion of gingival connective tissue and epithelial cells of mucosa (5, 6). Various materials and methods of GTR have been investigated, including use of polytetrafluoroethylene (PTFE) as non-resorbable membrane, polyglactin, collagen based materials as resorbable membrane (7-10). The advantage of resorbable GTRs is that additional surgery is not needed for their removal. However, most of these materials show an unpredictable degree of resorption, which can significantly alter the amount of bone formation (11, 12). Membranes for GTR possess many structural, mechanical and bio-functional limitations, and the ideal membrane for use in GTR application has yet to be developed (7). Although non-resorbable PTFE have been accepted as common materials for membranes used in dental and bone regeneration, there are currently many ongoing studies to develop new types of membrane. Polyhydroxyethyl methacrylate (PHEMA) provides better biocompatibility, higher permeability and high hydrophilicity, while polymethyl methacrylate (PMMA) is a biostable polymer to enhance the mechanical behavior of the hydrophilic structures (8). We hypothesized that PHEMA-PMMA is the ideal candidate for new GTR membrane for bone and periodontal defects based on its properties. In this study, PHEMA-PMMA membrane was evaluated for its efficacy as GTR in rabbit parietal bone.

Materials and Methods

Eighteen male New Zealand White rabbits were divided into three groups. The rabbits were treated in accordance with the guide for care and use of laboratory animals of Chungbuk National University. Two defects were made in each rabbit. A transosseous round defect of 8 mm was covered with new developed membrane in the experimental group, but no membrane was used for the control group. The membrane was made of porous PHEMA-PMMA with a size of 1 cm 8-10 mm.
× 1 cm and a thickness of 0.48-0.50 mm. HEMA (46% v/v, Aldrich, St. Louis, MO, USA) and MMA (10% v/v, Aldrich) were dissolved in dimethylformamide, while 0.02 M pentaerythiol tetraacrylate was added as a cross-linker agent. Solutions were then poured into a Teflon mold and placed at room temperature for 18 hours. The pores that formed, which were designed to deliver oxygen and promote tissue regeneration, were found to be 50 μm to 80 μm based on scanning electron microscopy (Figure 1). Membranes were applied within 3 weeks after production.

General anesthesia was induced by intramuscular injection of 5 mg/kg xylazine hydrochloride (Rompun, Bayer Korea, Seoul, Korea) and 10 mg/kg zolazepam and tiletamine (Zoetil 50, Virbac Laboratories, Carros, France). After stabilization of anesthesia, skin and periosteal membrane was separated from parietal bone. Two circular transosseous defects were made by 8 mm outer diameter trephine burr. One defect was covered by membrane and each apex of membrane was fixed to parietal bone using a titanium dental pin (Stabilok™ titanium small-diameter dentin pins, Fairfax Dental Inc., London, UK) (Figure 2). No membrane was used for the other defect. Subcutaneous tissue and skin were then sutured. Parietal bones were harvested at 2, 4 and 8 weeks after surgery. Briefly, rabbits were euthanized by an injection of potassium chloride after general anesthesia using xylazine and tiletamine/zolazepam. Muscles and connective tissues attached to the parietal bone and membrane were left attached to observe any inflammatory reaction and adhesion between the PMMA membrane and other tissues. Harvested parietal bones were radiographed (REX-525R; LISTEM, Wonju, South Korea) at the same time for histological evaluation. Samples were radiographed at 60 kV, 100 mA, and 2.0 mAS. Radiographic images were analyzed using the ImageJ program (v 1.50i, NIH, USA) (13,
The amount of bone regeneration was evaluated by measuring the newly formed tissue relative to the initial calvarial defect. All samples were fixed, decalcified, paraffin embedded and cut into 5 μm thick section in the direction perpendicular to the flat parietal bone. Three sections in the central part of the defects were obtained from each sample, and hematoxylin and eosin dye was used to estimate tissues microscopically. Bone formation in the defect area were measured using the ImageJ program and the percentage of repaired bone was calculated from the entire defect area (13, 14).

GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used to analyze the radiological and histologic results. The experimental group and control group at 2 weeks, 4 weeks and 8 weeks were compared by the Mann-Whitney test. Differences between 2 weeks, 4 weeks and 8 weeks were assessed by the Kruskal-Wallis test. A \( p < 0.05 \) was considered to indicate statistical significance.

**Results**

Anesthesia and surgery were noneventful for all 18 rabbits. One rabbit in the 2-week experimental group was dead the day after surgery and was therefore excluded from results. At the time of sampling, pins had migrated into undesired places in two samples. Surgical wounds healed without any inflammation and infection. Muscles and fibrous tissues were attached to parietal bone and the PHEMA-PMMA membrane. However membranes were easily separated from parietal bone and other soft tissues in most cases during histological processing. There was a remarkable amount of adhesion between parietal bone and the brain. Radiographic examination was conducted to evaluate the amount of regenerated bone. The radiopaque shadows over the defects filled in with time in both groups; however, it was more effectively filled in the experimental group. The Mean and SD of bone formation percentage in the control group was 10.9±10.6, 29.7±12.3 and 34.8±12.8 at 2, 4 and 8 weeks respectively. The mean and SD in the experimental group was 16.4±13.9, 38.3±26.4 and 50.3±13.7 at 2, 4 and 8 weeks (Table I). Bone formation at 8 weeks was significantly higher in the experimental group than the control group (\( p < 0.05 \)) (Figure 3).

Histological observation showed trabecular bone formations along the margin of calvarial defect, scattered bone islands and thin continuous bone regeneration in the defect area. The bone tissue over the defect showed more regeneration with time in both groups; however, more effective filling was observed in the experimental group. In the control group, invasion of muscle and fibrous tissue was shown in several defects, but no invasion was observed in the experimental group. There was no sign of membrane degradation or tissue inflammation.

**Table I. Radiographic and histological analysis of bone regeneration.**

| Period (weeks) | Control group | | | | | | Experimental group | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of defects | % | Number of defects | % | | Number of defects | % | | |
| R | H | R | H | R | H |
| 2 | 6 | 10.9±10.6 | 13.6±13.0 | 6 | 16.4±13.9 | 16.1±14.3 |
| 4 | 6 | 29.7±12.3 | 20.0±10.6 | 6 | 38.3±26.4 | 26.2±14.2 |
| 8 | 5 | 34.8±12.8* | 25.3±14.4** | 5 | 50.3±13.7* | 53.2±15.0** |

The percentage of regenerated bone (%) was determined by the ImageJ program and the results shown are the mean±SD. Defects were covered with PHEMA-PMMA membrane (experimental group) and without membrane (control group). *\( p<0.01 \), **\( p<0.05 \) differences between groups. R, Radiological evaluation; H, histological evaluation.
such as leukocyte or giant cells emigration. The mean ± SD of bone regeneration in the control group was 13.6±13.0, 20.0±10.6 and 25.3±14.4 at 2, 4 and 8 weeks, respectively, while the values were 16.1±14.3, 26.2±14.2 and 53.2±15.0 at 2, 4 and 8 weeks, respectively, in the experimental group (Table I). Histological evaluation showed significant bone regeneration in the experimental group at 8 weeks (p<0.05) (Figure 4). Bone regeneration was observed beneath the membrane in the experimental group.

Discussion

Autogenous tissue and cells are applied for various types of bone defects; however, the supply of donor tissue is often limited. Various scaffolds are essential to replace and assist the formation of new tissue in cases in which there is inadequate bone tissue. GTR membranes must exhibit: (i) biocompatibility to allow integration with the host tissues without inflammatory responses, (ii) a proper degradation profile to match those of new tissue formation, (iii) mechanical and physical properties adequate for its placement in vivo, and (iv) sufficient sustained strength to avoid membrane collapse and perform barrier function (7). Non-bioresorbable membranes of PTFE are more commonly used in the clinic because of cost effectiveness and their better bone regeneration compared to resorbable membranes (15). Unpredictable resorption time is a main disadvantage of resorbable membranes. Previous studies have shown that the ideal membrane for use in GTR application has yet to be developed. PHEMA-PMMA has not been evaluated as a GTR membrane for bone regeneration. The results of the present study indicate that PHEMA-PMMA membrane could provide a more effective environment for bone healing.

Rabbit parietal bone defects have been used to evaluate use of membrane as guided bone regeneration (5, 8, 16). The parietal bone provides similar conditions to mandibular bone as they are both flat bones with limited bone marrow and blood supply. In addition, parietal bones are not used for weight bearing and have few muscles attached (17). Young rabbits have thicker osteogenic cell layers and more active osteoblasts than elderly rabbits (18). Eight millimeter round defects are generally used as critical-size defects in studies of GTR (17). Therefore, young rabbits of 10 to 12 weeks old, which have just enough parietal bone to make an 8 mm round defect, were used in this study. At the time of sampling, membranes were easily separated from underlying parietal bone. Histologic exam showed no invasion of fibrous tissue or vessels to membrane, and there was no sign of membrane degradation or tissue inflammation. Hematoxylin and eosin staining of bone samples showed different results between the experimental group and the control group. In the experimental group, all newly formed bone was continuous from the defect margin without any invasion of soft tissue. Continuously regenerated bone adhered to the PHEMA-PMMA membrane, not the meningeal surface. However, in the control group, regeneration primarily formed with bony islands. Bony islands were both formed from periosteal membrane and meninges, and there was massive soft tissue invasion between islands.

Figure 4. Representative pictures of histological evaluation at 2, 4 and 8 weeks. Trabecular bone formations were observed along the margin of calvarial defect (arrow head), and scattered bone islands and thin continuous bone regeneration are visible in the defect area. The bone tissue over the defects showed greater regeneration with time in both groups, but more effective regeneration was observed in the experimental group. Bone regeneration was observed beneath the membrane (white arrow).
that made further bone regeneration difficult. Commonly, bone is regenerated under stable conditions that are free from pressure and tension. Therefore, more regeneration occurred around the defect margin and less in the central region. A stiffer membrane would provide a more protected environment and more bone regeneration (18). It should be noted that this study had several limitations. First, micro computed tomography evaluation was not conducted. A computed tomography scan of parietal bone would provide useful information regarding bone regeneration in terms of radiopacity (5). However, we believe our radiological and histological image analysis results obtained using ImageJ are sufficient to confirm that PHEMA-PMMA based membrane could be useful as GTR membrane. Second, an optimized method for removal of non-resorbable membrane was not clearly developed in this efficacy study. Remove of the membrane after bone healing is expected to be easy because separation was easy at the time of sampling. Finally, PHEMA-PMMA membrane is a non-resorbable material. Future research directions should include rational design of a membrane that evaluates control of degradation and mechanical properties while considering biological variables (7). However, PHEMA-PMMA is an ideal candidate for a new GTR membrane based on its properties. Moreover, the most important factor when selecting a GTR membrane should be its effects on bone healing, not its resorption properties.

In conclusion, PHEMA-PMMA is a good material for artificial membrane for bone and periodontal defects as it induces no adverse tissue reaction and effectively supports selective bone regeneration.

Conflicts of Interest
The Authors declare that they have no conflicts of interest regarding the publication of this article.

Acknowledgements
This research was supported by a Grant (2012-Bio-7) from Chungbuk Technopark, Korea. Scaffolds were developed by TE-Bios Company (Ochang, Korea).

References