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Yunia Dwi Rakhmatia
Prosthodontic Department, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia, rakhmatia@unpad.ac.id

Yasunori Ayukawa
Section of Implant and Rehabilitative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, ayukawa@dent.kyushu-u.ac.jp

Akihiro Furuhashi
Section of Implant and Rehabilitative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, furuhasi@dent.kyushu-u.ac.jp

Kiyoshi Koyano
Division of Advanced Dental Devices and Therapeutics, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, koyano@dent.kyushu-u.ac.jp

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ORIGINAL ARTICLE

Selection of Commercially Available Membrane between Resorbable and Non-resorbable Materials for Guided Bone Regeneration

Yunia Dwi Rakhmatia[*1,2], Yasunori Ayukawa[2], Akihiro Furuhashi[2], Kiyoshi Koyano[3]

[1]Prosthodontic Department, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia
[2]Section of Implant and Rehabilitative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan
[3]Division of Advanced Dental Devices and Therapeutics, Faculty of Dental Science, Kyushu University, Fukuoka, Japan
**Correspondence e-mail to: rakhmatia@unpad.ac.id*

ABSTRACT

Objective: This study aims to evaluate the commercially available membranes used for treatment in Guided Bone Regeneration (GBR). **Methods:** Four membranes resorbable and non-resorbable were used and a critical size defect in six-week-old Wistar rats was created for membrane application. Meanwhile, the defect without membrane treatment was used as the control (C). **Results:** After 4 and 8 weeks, all rats were euthanized and block biopsies of calvaria including membrane were excised and analysed using microcomputed tomography (micro-CT). The sections were dehydrated with graded ethanol, embedded in resin, and cut for histologic evaluation. After 4 weeks, all membrane groups and the control showed different degrees of bone volume (BV) and mineral density (BMD). Titanium mesh (TM) was observed with higher bone volume but lower BMD compared to the control, Cytoplast (CP), Biomend (BM), and GC membranes. The results showed that newly formed bone adjacent to the original filled the defect area. **Conclusion:** TM was the stiffest among the commercially available membranes used and increased the abundance of bone formation at 4 weeks. The selection of membranes used in GBR needs to consider the treatment requirement and the patient's point of view.

Key words: guided bone regeneration, membrane, non-resorbable, resorbable, titanium

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INTRODUCTION

The loss of teeth leads to bone resorption and affects the quantity and quality of jaw bone prior to implant treatment. Meanwhile, Guided Bone Regeneration (GBR) is a procedure carried out using a membrane that allows desired cells and prevents the undesired from ingrowth in a secluded space intended for bone regeneration. The membrane needs to fulfil some criteria to optimize its function as a barrier, which includes biocompatibility, space maintenance ability, cell occlusivity or selective permeability, tissue integration, and clinical manageability.[1]

Various membranes have been developed, which can be grouped as resorbable and non-resorbable. The biomaterial and physical properties of membranes influence their function and the selection of materials is based on biological properties as well as the treatment requirements.[2] Commonly used resorbable materials are made from natural or synthetic polymers, such as collagen, polyglycolide, and polylactide. Resorbable membranes have the advantage of being resorbed by the body, thereby eliminating the second surgery for membrane removal. However, their disadvantage includes the unpredictable degree of resorption, which can alter the result in bone regeneration.[3] The use of non-resorbable membranes also has a drawback because of the necessity for its removal with a second-stage surgical procedure. These membranes, including polytetrafluoroethylene (PTFE) and titanium mesh, offer advantages to provide effective barrier function and to maintain the space for a sufficient period.[4]

The commercially available membranes used in this study are resorbable namely BioMend (BM) and GC as well as non-resorbable such as Titanium Micro-Mesh (TM) and Cytoplast (CP). BM is a type I resorbable collagen membrane derived from bovine tendon and degrades after 8 weeks. The cells occlusivity serves as a barrier to prevent epithelial cell migration and allows passage of essential nutrients.[5] Previous study demonstrated that BM has an affinity for the bacteria, *Porphyromonas gingivalis*, hence, the membrane degraded to 86.4%.[6] Bacterial infection on this membrane might lead to the failure of GBR processes. Meanwhile, GC is a bioresorbable synthetic polymer that is composed of Polylactide-co-glycolide acid (PLGA). A clinical study reported that GC membrane induced sufficient bone augmentation leading to successful implant treatment.[7] This membrane has already been used clinically and provided favorable outcomes with no severe complications including infection.[8,9] TM has also been used in numerous surgical applications to facilitate the augmentation of alveolar ridge defects due to its excellent mechanical properties. Its rigidity provides extensive space maintenance and prevents contour collapse even in cases with a large bone cavity, the elasticity prevents mucosal compression, the stability inhibits graft displacement, and its plasticity permits bending, contouring, and adaptation to any unique bony defect.[10,11] However, TM has macroporous with pore diameters in the millimeter range. This macroporosity creates sharp spots when the material is cut or bent, and might provide an easy pathway for microbial contamination into the healing site.[12] CP has also been reported with success in bone and tissue regeneration.[13,14] This membrane is made from a high-density PTFE (d-PTFE), hence, bacterial infiltration into the bone defect site is eliminated. However, CP can be removed easily by pulling on the membrane without lifting the mucosal flap because its attachment to tissues is weak.[15] This study aims to compare and evaluate the commercially available membranes between resorbable and non-resorbable types with their consideration for GBR treatment.

METHODS

The four commercially available membranes used in this study were Titanium Micro Mesh™ (TM) (ACE Surgical Supply Co, Brockton, MA, USA), Cytoplast™ (CP) GBR-200 (Osteogenics Biomedical, Inc., Lubbock, TX, USA), BioMend® (BM) (Zimmer Dental Inc., Carlsbad, CA, USA), and GC membrane® (GC) (GC Corporation, Bumkyou-ku, Tokyo, Japan) (Figure 1). TM has a 100-µm thickness and 1700-µm pore diameter, while CP is a non-resorbable d-PTFE membrane with 200-µm thickness and pore diameter <0.2 µm. Furthermore, BM membrane has a 170-µm thickness and 0.004-µm pore diameter, while GC has characteristics of opaque, smooth, and dense resorbable membrane with a 210-µm thickness. The control (C) group consisted of animals with uncovered defect sites, while the entire membranes had a diameter of 12 mm and were bent to adapt with the surrounding bone and tissue at experimental sites.

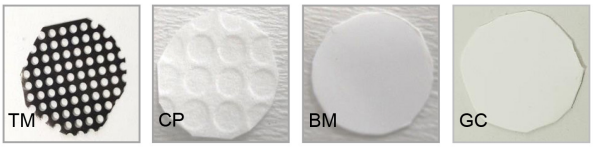

Figure 1. Experimental membranes TM, CP, BM, and GC

A total of 50 six-week-old male Wistar rats were used and treated in accordance with Kyushu University (Fukuoka, Japan) guidelines for animal care. The rats were housed under identical conditions and fed a commercially available standard rodent food containing 1.25% calcium, 1.06% phosphate, and 2.0 IU g-1 vitamin D3 (CE-2, CLEA Japan, Tokyo, Japan), also, water was given ad libitum. The animals were divided into 5 groups namely TM, CP, BM, GC and C groups with 2 periods of healing time at 4 and 8 weeks. Each group consisted of five animals.

Surgical procedures

The animals were anesthetized in an aseptic condition, the forehead was shaved along the sagittal suture, then an incision was made to reflect the parietal bone. Afterward, a circular 7 mm-diameter bone defect was created with a surgical trephine bur, then it was covered with a membrane. At the borders, Histoacryl® glue (Braun, Melsungen, Germany) was bonded to prevent membrane movement. The defect without any membrane was used as a control. The skin flaps were sutured with non-resorbable suture material, then after 4 and 8 weeks, all animals were euthanized and perfused with fixative solution. Calvaria bone including membranes and surrounding soft tissue were taken for microcomputed tomography (micro-CT) analysis.

Micro-CT analysis

Calvaria bone biopsies were imaged and analysed using micro-ct SkyScan 1076 (SkyScan, Aartselaar, Belgium) at 60 kV/167 µA and a Ti-0.5 filter. The specimens were placed in a cylindrical plane and scanned parallel to the coronal aspect of the calvaria bone, then, high-resolution scanning in a slice thickness of 18 µm was performed. From each set of scans, a three-dimensional reconstruction was made and analysed using micro-CT software (Version 1.10, Bruker/Skyscan µCT, Kartuizersweg, Kontich, Belgium). Region of interest analysis was performed to assess primary parameters, namely bone volume (BV) and total tissue volume (TV), both measured in mm^3. TV is the volume of the whole examined sample. BV was calculated as the volume of the region characterized as bone (defined as the number of voxels with grey values in the range

30–90) and normalized ratio metrically against the total volume of the region of interest (BV/TV) to derive the percentage bone volume (% BV). Bone with different degrees of mineralization displays different densities and linear attenuation coefficients, resulting in grey-value variations in the CT scans, the distribution of which is a measure for the degree of mineralization, i.e., bone mineral density (BMD) (g/cm^3). The degree of mineralization, expressed in milligrams of hydroxyapatite per cubic centimeter (mgHA/cm^3), was found to be 0.25 to 0.75 mgHA/cm^3.

Histological evaluation

All specimens were dehydrated with a graded series of ethanol and embedded into methacrylate resin. Undecalcified sagittal sections with thickness ~60 µm were cut, polished and stained using Masson's trichrome method. The center of the test membrane from the histological section of each specimen was selected to represent the group for evaluation. The histological evaluation of bone and the cellular tissue responses were examined under a light microscope (BZ-9000, Keyence, Osaka, Japan).

Statistical analysis

The mean and standard deviation values for BV and BMD were calculated for each group at different healing times. Statistical evaluation of these values was performed using a one-way analysis of variance with post-hoc Tukey test. Furthermore, statistical significance was considered at $p < 0.05$ among the groups.

RESULTS

The membranes examined in this study were divided into resorbable namely BM and GC, as well as non-resorbable including TM and CP. Among the different membrane materials tested, TM was the stiffest and was difficult to adapt to the bone surface contour compared to CP, BM, and GC. After bending, the margins of the CP, BM, and GC membranes tend to have inadequate stiffness. Some of these membranes had visibly collapsed into the defect site.

Micro-CT evaluation

The quantitative results of bone regeneration derived from micro-CT analysis, including BV and BMD are shown in Figure 2. Values of BV in TM at 4 and 8 weeks were higher compared to other membrane groups, while the BMD values for C were derived by comparing x-ray attenuation in the scanned bone samples with that in hydroxyapatite standards. Furthermore, C and CP had higher mineralization levels compared to other experimental groups, the BMD values tended to increase as the BV decreases. Micro-CT reconstruction in Figure 3 shows that expansive bone formation was found in TM group at 4 and 8 weeks compared to other

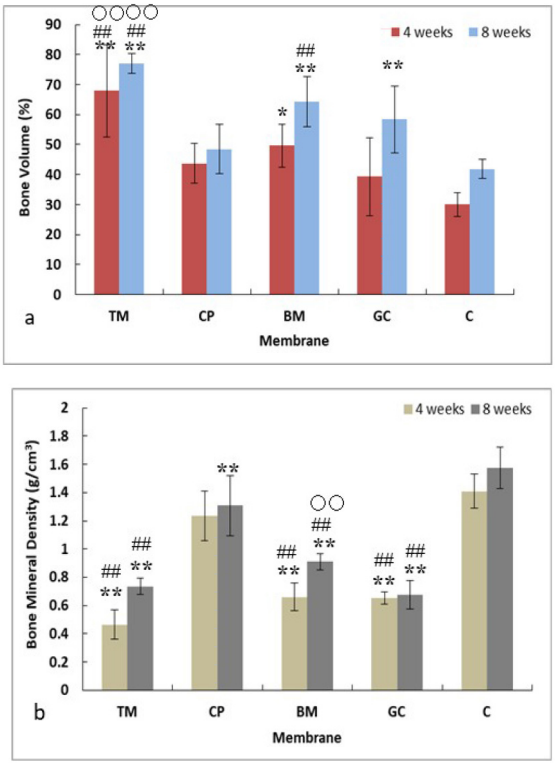

Figure 2. (a) Bone volume and (b) bone mineral density. Statistical significance: *, **: compared to C, ##: compared to CP, ○○: compared to GC. *: $p < 0.05$; **, ##, ○○: $p < 0.01$

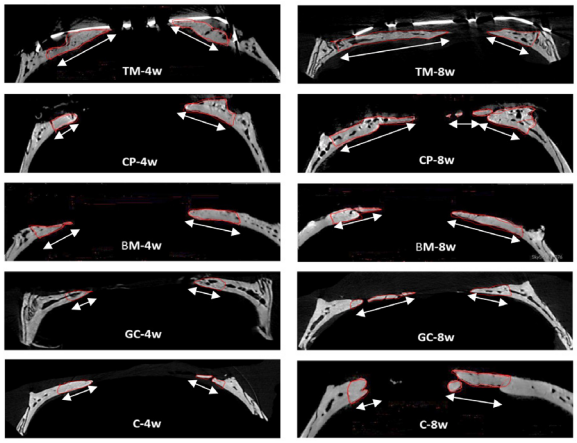

Figure 3. Micro-CT reconstruction from sample beneath the experimental membranes and control groups after 4 and 8 weeks. Red lines (white arrows) show new bone formation.

membranes and C groups. Bone was larger at 8 weeks compared to 4 weeks of healing time.

Histologic evaluation

The histologic analysis of all groups complemented the micro-CT results presented in Figure 4. At 4 weeks, bone formation with more intense red staining was observed in all groups, specifically beneath the TM membrane. In the C group, only minimal bone was formed adjacent to the original bone and the defect sites were filled mainly with fibrous connective tissues. After 8 weeks of healing, all groups exhibited

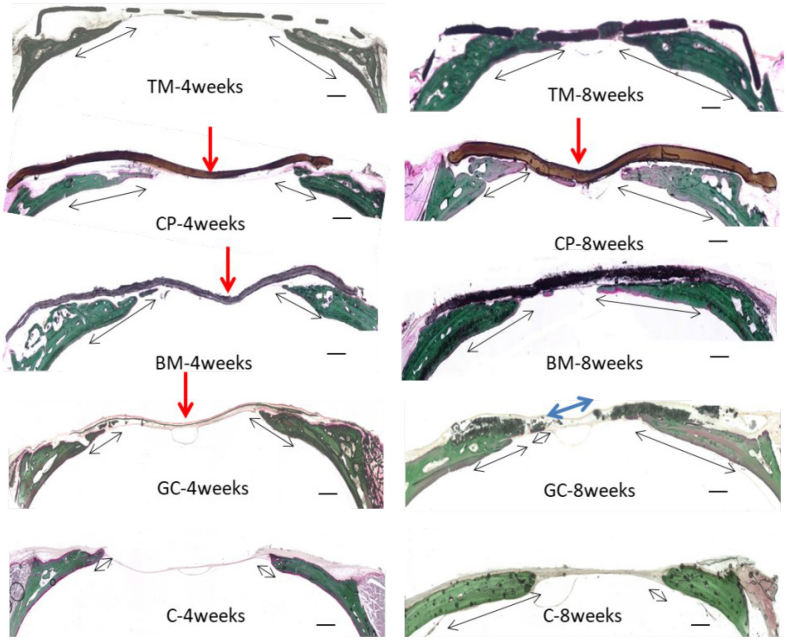

Figure 4. Histological image of samples with experimental membranes and control groups after 4 and 8 weeks of healing period. Black arrow shows new bone formation and red arrows (upper membrane) show membrane collapse to the defect area. Membrane degradation was shown in GC group at 8 weeks (blue arrow). Magnification x4, Bar = 500 µm.

a greater new bone formation with a higher degree of mineralization compared to the groups at 4 weeks. The defect margins were also found indistinguishable from the newly formed bone. TM, CP, and BM were observed with expansive bone formation from adjacent original bone to the defect site, with some specimens exhibiting complete resolution of the defect. In addition, some samples of GC membrane were found to resorb at 8 weeks. C group was observed with bone formation only from the adjacent original bone with higher mineralization.

DISCUSSION

The results showed that TM as the stiffest membrane exhibited abundant bone formation compared to the other materials. This suggests that a membrane must be stiff adequately to maintain the space intended for bone regeneration. When a membrane collapses into the defect, it might hamper the formation of new bone.[16,17] CP, BM, and GC which had fairly soft consistency, tend to collapse into the bone defect, leading to less bone formation, specifically after 4 weeks of healing time. A membrane needs to have adequate resistance against the soft tissue pressure laying from above to prevent its collapse. The results suggest that bone substitutes or other materials that provide additional support must be used beneath CP, BM, and GC membranes. The ideal range of membrane thickness for the reconstruction of large bone tissue defects is reportedly between 100 and 200 µm.[18] All of the commercially available membranes used in this study were in the range of the suggested thickness. A membrane thickness that is required for stability must be balanced with the ability to adapt to the contours of the adjacent bone.[19] Furthermore, histoacryl was used to fix the membrane and to prevent membrane dislodgement. Sufficient fixation of the membrane is vital for stabilizing the blood clot, preventing the membrane micro-movement and proper wound healing. A previous study reported that membrane movement during the healing process is detrimental to bone formation and might lead to the development of fibrous tissue instead of bone.[20]

Based on the results, the selective permeability of a membrane plays a critical role in bone formation, specifically at the initial healing time. The optimal pore size must be advantageous regarding the diffusion of fluids, nutritional materials, angiogenesis, and peripheral sealing to prevent ingrowth of soft tissue-forming cells. CP as a d-PTFE material had lower BV compared to TM. It is assumed that the high density of CP blocks the integral vascularization process, thereby inhibiting bone formation in the defect area. In contrast, a study reported complete healing after 10 weeks when a rat was treated for mandibular defect using a dense-PTFE.[21] An occlusive membrane might hamper the penetration of nutrients and growth-regulatory factors to the defect site, thereby inhibiting bone formation.[22] Previous studies reported that d-PTFE completely blocks the penetration of food and bacteria, hence, even with the exposure to the oral cavity, it still acts as an appropriate membrane barrier.[23,24] TM

has millimeter-level pore sizes, which are presumably important in maintaining blood supply and believed to enhance regeneration by improving wound stability through tissue integration and allowing diffusion of extracellular nutrients across the membrane.[25,26] However, the macroporosity of TM leads to soft tissue ingrowth through the pores, thereby making the removal of the membrane difficult during the second surgery. It is also believed that the smooth surface of TM makes it less susceptible to bacterial contamination than resorbable materials.[10] The higher BVs and the lower BMDs of TM group were due to faster ingrowth of bone forming cells into the membrane than the mineral apposition. Mineral apposition might be incomplete when using materials with a large pore size because the new bone takes time to grow into the defect areas. In addition, it is intuitive that a material with small pores (CP) will have a greater number of 'growth centers', thus producing better quality (i.e. higher density) bone.

BM is a bovine sourced from a bioresorbable membrane and known to modulate various cell behaviors such as adhesion, spreading, and the chemotactic ability in attracting cells due to the collagen structure.[3] A previous study reported that fibroblasts when cultured in the presence of collagen, facilitate cells attachment on BM.[27] It was also reported that the degradation of the cross-linked BM membrane was caused by the enzymatic activity of macrophages and polymorphonuclear leucocytes.[28] After 8 weeks, the degradation was associated with decreased tissue integration and vascularization leading to poor membrane resistance towards collapse, thereby limiting bone formation. Furthermore, a previous study reported a significantly increased cellular attachment to the BM membrane compared to Gore-Tex®,[29] which has the same chemical origin as the CP membrane. GC membrane composes of a synthetic copolymer of polylactic acid (PLA) and polyglycolic acid (PGA) and has been developed in various therapeutic devices including membrane GBR, bone grafts, and the drug delivery system.[30-33] Its degradation process is influenced by polymer end groups, degradation pH, temperature, etc which varies from approximately 1-2 months.[34] Based on the result, GC had the lowest bone volume among other commercially available membranes. In addition, from histological images, the degradation occurred at 8 weeks after the membrane application. Previous studies reported that BM and GC were degraded after 6-8 weeks and 13-30 weeks, respectively.[19,35] Resorbable materials have a disadvantage of unpredictable degrees of resorption. When they are resorbed rapidly, the membrane can not maintain the intended space from preventing soft tissue ingrowth and this might alter bone regeneration.[36,37] Additional support is also required when resorbable materials are used to protect larger defect sites.[10,36] When the membranes are exposed and/or associated with inflammatory reactions, they rapidly degrade, thereby affecting barrier function to regenerate bone and the implant becomes unstable.[38]

CONCLUSION

The concept of GBR has been developed to optimize treatment strategies for the reconstruction of the alveolar ridge and bone defect. The commercially available membranes used in this study augmented new bone in this critical-sized rat calvaria defect model after 4 and 8 weeks of healing. Among the membranes tested, TM is recommended for the reconstruction of bone tissue defects because of its ability to support new bone growth into the defect area. However, the results obtained have limitations since the relatively small sample size of five rats in each group decreases the statistical power. Therefore, the membrane selection must be based on the benefits and limitations inherent to the materials in relation to the functional requirements in the specific clinical application.

CONFLICT OF INTEREST

K.K. belongs to the Division of Advanced Dental Devices and Therapeutics, Faculty of Dental Science, Kyushu University. This division is endowed by GC Corporation, Tokyo, Japan. However, GC Corporation had no specific roles in this study. All other authors state that they have no conflicts of interest.

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