Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (December Suppl.) : 2018 : S6-S9

ISSN-0972-3005

BONE FORMATION IN RAT'S CALVARIAL DEFECT AFTER APPLICATION OF DEMINERALIZED FREEZE DRIED BOVINE CORTICAL BONE MEMBRANE

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(Received 25 September, 2018; accepted 15 November, 2018)

Key word: *Bovine cortical bone membrane, Guided bone regeneration, Bone formation*

Abstract– Bovine pericardium collagen membrane (BPCM) had been widely used in guided bone regeneration (GBR) for alveolar bone augmentation. However, it is associated with prolonged biodegradation. A newly developed Demineralized Freeze-Dried Bovine Cortical Bone Membrane (DFDBCBM) assumed to be rich in osteogenic growth factors was found to be biocompatible. Its osteogenic capacity yet to be proven. This study evaluated bone defects healing in rats' calvarial critical sized defectafter the application of DFDBCBM and BPCM. Critical sized defect was made on calvaria bone of 30 Wistar rats. The samples were divided into 3 groups, each contains of 10 rats. The defects were then covered by DFDBCBM and BPCM in the first two groups, the control group was left to heal without membrane application. Samples were collected at 4 and 8 weeks from each group. Degree of bone healing was examined using histology examination with HE staining using bone healing score. Data was analyzed statistically with the Kruskal-Wallis test and Mann Whitney accordingly. The degree of bone healing was significantly higher in DFDBCBM groups compared to BPCM groups both in 4 and 8 weeks observation. DFDBCBM has higher capacity for bone defect healing compared to BPCM in rat's calvaria defect.

INTRODUCTION

Alveolar augmentation is a surgical procedure to improve the shape and size of the alveolar bone in preparation for receiving and maintaining a dental prosthesis and implant placement (Pellegrini, *et al*., 2013). Whereas Guide Bone Regeneration (GBR) is a technique where bone growth is obtained by maintaining space and preventing soft tissue growth into the area which will be developed using a resorbable or nonresorbable barrier membrane and giving bone graft inside the defect (Donos *et al*., 2002). The initial types of graft material are autogenous, allograft, xenograft, and alloplastic (Newman, 2002). Autogenous is obtained from the patient itself and has the ability to form new bone in osteogenesis, osteinduction and osteoconduction (Tedyasihto, 2010).

Demineralized freeze dried bovine cortical bone membrane (DFDBCBM) has several advantages including containing collagen that is intact so that it is expected to produce more growth factors. In addition, DFDBCBM also has no difficulty in biodegradation, thus allowing a better membrane degradation process which will support the osseointegration process of new bone with surrounding tissue and will induce better bone healing than Bovine Pericardium Collagen Membrane (BPCM).

To determine the effectiveness of using DFDBCBM in the GBR method, it is necessary to know the degree of bone healing.

MATERIALS AND METHODS

Preparation of surgical procedure

As an animal, 30 Wistar rats aged 10 - 12 weeks were used with a weight of 120 - 140 g, which were adjusted for a week. Rats were kept each in a cage

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measuring 30 x 30 x 10 cm for each treatment group consisting of 5 rats. All animals are given a normal diet and drinking water during the study period.

The procedure begins with intramuscular injection of ketamine HCl (20 mg / kg body weight). After the anesthetic has worked, the area of the dorsum region is shaved and povidone iodine disinfection is 10%. Skin incisions were performed on the rat's head in the medial section, and then mucoperiosteal flap retraction was performed until calvaria bone was obtained. Medial suture was identified in calvaria rats. The defect was made on the right lateral side of the medial suture using a bur of 5 mm in diameter as thick as 0.8 mm. The membrane was applied over the defect until the defect is completely covered by the membrane. Then suturing the incision wound on the skin with silk thread 3.0.

In the 4th and 8th weeks after the membrane application, each 5 rats from the treatment and control groups were sacrificed for the research tissue specimen retrieval procedure. The mouse is decapitated without removing the skin tissue above the defect. The specimens processed to obtain histology slides under routine procedure.

Histology Examination

In this study, an examination of the degree of bone healing was carried out by using indicators score of connective tissue formation, woven bone, bone trabeculae and formation of adult bones. The four indicators were observed at week 4 and week 8.

Table 1. Bone Healing Score

Score	Callus Proportion			
	Fibrous Tissue	Woven Bone	Trabeculae Bone	Adult Bones
1	++++	+	+	-
2	++	++	++	-
3	+	+++	+++	-
4	+/-	++++/++	++++/+	+/+++

Score 1: healing zone is dominated by fibrous tissue with / without a little woven bone and with / without bone trabeculae, no adult bone is found
Score 2: healing zone consists of a small amount of fibrous tissue, and has begun to form woven bones and trabecular bones, no adult bones
Score 3: healing zone consists of a little fibrous tissue, lots of woven bones and trabecular bone, adult bones have not been found
Score 4: fibrous tissue begins to disappear, the proportion of woven bones and trabecular bone is higher, adult bones have been found

Thedata from histological examination result is analyzed statistically with the Kruskal-Wallis test and followed by the Mann Whitney test accordingly. Significant difference is determined when p value is < 0.05.

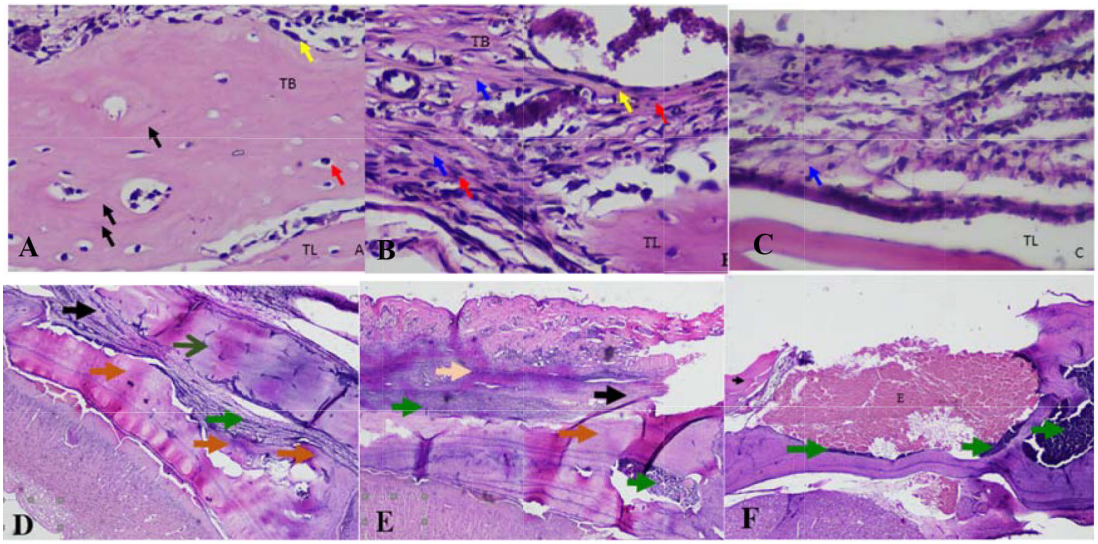

Fig. 1. Microscopy of defect healing in the fourth week and eight weekafter implantation of membrane. (A) Implantation of DFDBCBM, (B) BPCM and (C) without membrane. It appears that there are already woven bone tissue with little lamellae (1-2 lamellae) (→), bone vitality is confirmed by the presence of osteocytes (→). Note: osteoblasts (→), new bone (TB), old bone (TL), connective tissue (→), New Bone (→), inflammatory cell (→), fibrous tissue (→), bone membrane (→), pericard membrane (→), erythrocyte (E).

RESULTS

Based on the results of observations and measurement of the value of the variable degree of bone healing by histology examination divided into 3 research groups: (1) the treatment group (DFDBCBM); (2) treatment group (BPCM); (3) negative control group (without membrane), with each study sample that has been observed in the 4th and 8th weeks, obtained results. In this study, the total healing zone area, fibrous tissue area and reinforcement area within the healing zone were calculated. On histomorphometric calculation at week 4th and 8th. The total area of fibrous tissue of DFDBCBM treatment group was narrower.

The data described in figure 2 showed that the degree of bone healing in DFDBCBM groups is higher than BPCM and control groups both in fourth and eighth week. Statistical analysis with Kruskal-Wallis test revealed significant difference ($p < 0.05$) among groups in both observation times. Mann-Whitney U-test confirmed that there are significant difference ($p < 0.05$) in bone healing degree between DFDBCBM group and BPCM as well as control group in fourth week. Significant difference in bone healing degree in eight weeks is only found between DFDBCBM and control group ($p<0.05$).

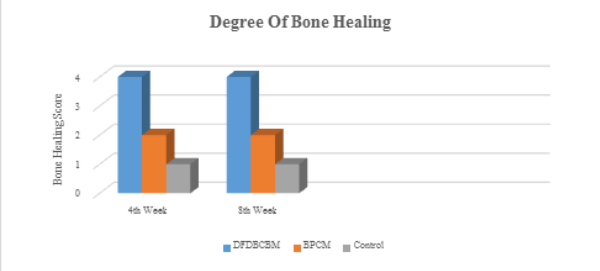

Fig. 2. The average of The Degree of Bone Healingin DFDBCBM, BPCM and control groups in fourth and eighth week post implantation

DISCUSSION

The observation in this study consisted of 2 periods, 4 weeks and 8 weeks. This is done to assess the dynamic processes that occur so that they can see the changes that occur during the healing process. The 4th week observation time is based on the bone formation phase, where there is a mineralization process, woven bone formation, osteoclasts convert callus to lamellar bone and woven bone. Lamellar and woven bone in this study were counted as reinforcement areas (Bouxsein, 2010).

The degree of bone healing was carried out by using indicators scale of connective tissue formation, woven bone, bone trabeculae and formation of adult bones (McAllister *et al.*, 2007). The four indicators were observed at week 4 and week 8. The higher score showed the better degree of bone healing in the calvaria rat defect. If the composition in the calvaria bone defect is dominated by connective tissue, it indicates that the healing of bone defects has not yet formed bone that covers the defect. Whereas if the calvaria bone defect has begun to form bone and some adult bones begin to form, this indicates that the bone defect has undergone a healing process and the defect has begun to be covered by bone (Kalfas, 2001).

In addition to the irregular collagen fibers, there are other characteristics for primary bone tissue, namely the lack of mineral content so that it is easily penetrated by X-rays and more osteocytes when compared to secondary bone tissue. Primary bone tissue will eventually undergo remodeling into a secondary (lamellar bone) that is physically stronger and resilient. Therefore in healthy adult bones there is only lamella (Nieminen, *et al.*, 2006).

The result of the study determines that DFDBCBM has the potential to be applied as a membrane in the GBR procedure. This is because DFDBCBM indicates that the number of adult osteoblasts in the bone defect area is obtained and osteogenesis process occurs better in the defect applied by DFDBCBM membrane. This shows that DFDBCBM membranes have good osteoinduction properties that increase the process of osteogenesis after subcutaneous implantation in animals testing the Wistar Rattus norvegicus strain.

CONCLUSION

From this study, it was found that Demineralized Freeze-Dried Bovine Cortical Bone Membrane has high erosteogenic capacity compared to bovine pericardial membrane. Therefore, it is potential to be applied as a membrane in the Guide Bone Regeneration procedure.

REFERENCES

Donos, N., Kostopoulos, L. and Karring, T. 2002. Augmentation of the rat jaw with autogeneic cortico-cancellous bone *graft*s and guided tissue regeneration. *Clin Oral Implants Res.* 13 : 192-202.

Kalfas, I.H. 2001. The principles of bone healing. *Neurosurg focus* 10 : 1-10.

McAllister, B.S. and Haghighat, K., 2007. Bone augmentation techniques. *J. Periodontal*. 78:377-96.

Nieminen, T., Kallela, Keranan, J., Hiidenheimo, I., Kainulainen, N., Wuolijok, E. and Rantala, I. 2006. *In vivo* and *in vitro* degradation of a novel bioactive guided tissue regeneration *membrane*', *Int. J. Oral Maxillofac. Surg*. 35 : 727–732.

Newman, M.G., Takei, H.H. and Carranza, F.A. 2002. *Clinical Periodontology*, 9th ed., W. B. Saunders Company, Philadelphia.

Pellegrini, G., Pagni, G. and Rasperini, G. 2013. Surgical Approaches Based on Biological Objectives: GTR versus GBR Techniques. *International Journal of Dentistry,* University of Milan, Italy.

Tedyasihto, B. 2010. Buku Ajar Implantologi Mulut: Teori & Praktek, EGC, Jakarta.