

Bovine Pericardium Membrane as a Barrier Material for Periodontal Tissue Regeneration: A Retrospective Study

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Abstract:

Objective: This study aimed to evaluate the efficacy of bovine pericardium as a barrier membrane for guided periodontal tissue regeneration.

Material and Methods: The records of 20 periodontitis patients with a median age of 44.50 (IQR17) who underwent guided tissue regeneration (GTR) using bovine pericardium membranes (PericardLEMB, Tissue Bank, USM, Malaysia) were retrieved. The parameters, including plaque score (PS), gingivitis score (GS), probing pocket depth (PPD), clinical attachment loss (CAL), and alveolar bone loss from pre- to post-6-months of GTR, were compared using the Wilcoxon Paired Signed-Ranks test.

Results: We found statistically significant improvements in all of the parameters 6 months post-GTR (p -value<0.05). The median and interquartile range (IQR) for PS and GS decreased from 20.68% (IQR18.60) to 12.62% (IQR11.10) and 25.90% (IQR25.58) to 10% (IQR17.73), respectively. Similarly, after 6 months, median PPD and CAL had decreased to 3.25mm (IQR1.56) and 3.25mm (IQR1.92), respectively, from the baseline values of 7.00mm (IQR3.38) and 7.50mm (IQR3.61). Consistently, significant radiographic alveolar bone gain (20%) was observed 6 months after GTR.

Conclusion: Despite this study's limitations, such as small sample size, lack of control group, and short follow-up duration, it is conceivable to propose that utilizing BPM as bioabsorbable barrier membranes in the GTR procedure promotes periodontal tissue regeneration.

Keywords: barrier membrane, bovine pericardium, guided tissue regeneration, periodontal parameters

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Introduction

Periodontitis is an inflammatory disease affecting tissues supporting teeth characterized by the destruction of the periodontal ligament and alveolar bone, resulting in tooth loss if untreated. Guided tissue regeneration (GTR) is an established approach for restoring periodontal tissue loss due to disease^{1,2}. This treatment protocol utilised physical barriers (a membrane) to restrict epithelial and gingival connective tissue cell migration, allowing the required cells to repopulate the defect site for the regeneration of new alveolar bone, cementum, and periodontal ligament fibers. The goal of GTR is to restore periodontal tissue loss and function through regeneration, which is not healing through repair by long junctional epithelium³. In 1976, Melcher established the theoretical basis for GTR, emphasizing the importance of keeping unwanted cells out of the healing site in order to encourage the growth of the desired tissue. According to this concept, the apical migration of epithelial cells during periodontal healing is faster than the regeneration of the periodontal ligament. As a result, barrier membranes must be used to direct periodontal ligaments and bone formation in the damaged regions⁴.

Barrier membranes were first employed in the oral cavity in 1982 when directed tissue regeneration was used to replace periodontal tissues⁵. The membranes are meant to meet the requirements by acting as a barrier, diverting gingival tissue away from the root surface and creating space over the defect area⁶. Human periodontal ligament fibroblasts are the cells that play an important role in periodontal tissue regeneration and maintenance⁷. The ability to recruit and activate periodontal ligament fibroblast cells, as well as biocompatibility, biodegradability, and immunogenicity, are all important factors in selecting an acceptable tissue barrier in GTR⁸⁻¹⁰.

Barrier membranes are divided into 2 categories: resorbable and non-resorbable. The earliest non-resorbable membranes that were constructed and used in the GTR

process included expanded polytetrafluoroethylene (ePTFE) and cellulose^{8,11}. Resorbable membranes were eventually introduced to address the drawbacks of non-resorbable membranes, such as the need for a second surgery to remove them. Resorbable membranes include synthetic membranes consisting of polylactic and polyethylene glycol, as well as natural membranes composed of oxidised cellulose mesh, collagen, and acellular dermal matrix. Nearly all of the commercially available resorbable membranes show breakdowns 4–8 weeks after application^{9,12}. In recent years, a novel bovine pericardium membrane (BPM) was developed with a slow resorption rate. This membrane was obtained from bovine sources and is mainly composed of collagen type 1¹³. In fact, in addition to being produced using an innovative decellularization method that allows it to retain its collagen structure, it has been subjected to a cross-linking process, which distinguishes this barrier from other membranes (whose collagen is typically derived from other tissues or animal species) by having a reabsorption time of 3–6 months. This gives the barrier the improved performance of non-resorbable membranes, with the added benefit of being completely resorbable¹².

BPM offers several advantages over other natural resorbable membranes, including high biocompatibility, robust mechanical strength, and effective facilitation of periodontal tissue regeneration by providing a stable environment for cellular activity. However, BPM can be more expensive than some other natural resorbable membranes like porcine-derived or human-derived membranes, which might limit its use in some clinical settings. While a slower resorption rate can be advantageous, it can also potentially lead to prolonged inflammation if the membrane remains in place longer than necessary or if early resorption is desired^{14,15}.

Several organic and synthetic materials have been studied for their usefulness as GTR materials or scaffolds, with varying degrees of success. Because of its

biocompatibility with host tissues, BPM has been used as a graft material in several medical specialties, including cardiology¹⁶, general surgery¹⁷, pulmonology¹⁸, neurology¹⁹, and ophthalmology²⁰. BPM is also commonly utilised as a barrier membrane in periodontal regeneration procedures. However, the effectiveness of this membrane is scarcely reported; thus, this study was conducted in order to evaluate the efficacy of BPM as a barrier membrane for guided periodontal tissue regeneration by assessing the periodontal clinical parameters.

Material and Methods

This is a retrospective study utilizing a records review of periodontitis patients attending the dental clinic at the Hospital Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan, Malaysia. The study was conducted from June 1, 2023, to May 31, 2024. The selection criteria of patients were those that were treated with the GTR procedure using BPM (PericardLEMB, Tissue Bank, USM) with a minimum follow-up of 3 months. A convenience sampling method was used whereby the records of periodontitis patients who had undergone GTR were retrieved.

The sample size was estimated using a single mean formula with a standard deviation (S.D.) of 1.51²¹, and by anticipating a dropout rate of 10%, 39 periodontitis cases should have been retrieved. However, due to the unavailability of the complete data, only 20 cases were included. The study protocol was approved by the Human Research Ethics Committee USM (USM/JEPem/KK/23040320).

A convenience sampling method was used whereby the records of periodontitis patients who had undergone GTR were retrieved. The following details were obtained: the demographic data, type of periodontal defect, and other periodontal parameters, such as plaque score (PS), gingivitis score (GS), probing pocket depth (PPD), and clinical attachment loss (CAL), for pre- and post-surgery

follow-up at 3 and 6 months. PS was the record of the presence or absence of plaque along the gingival margins. This variable was evaluated at 4 sites per tooth: buccal, lingual, mesial, and distal surfaces. The number of sites with plaque was recorded and expressed as a percentage of the total sites examined²². GS, in contrast, measures gingival bleeding within 10 seconds of gentle probing along the gingival crevice, using the same sites as PS²³. PPD was determined by measuring the distance from the gingival margin to the base of the pocket, while CAL is the distance from the cemento-enamel junction to the base of the pocket. Both PPD and CAL were measured in millimeters on 6 sites per tooth: disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual and mesio-lingual. All the measurements were made using the graduated periodontal probe. Radiographic alveolar bone loss (ABL) was assessed at pre- and post-six months using Romexis software (Planmeca, Finland). Some missing data due to incomplete periodontal charts or poor radiographs were excluded.

Statistical analysis

Data analysis was completed using Statistical Package for the Social Sciences (SPSS) Version 27. Since the sample size was small and the data were not normally distributed, numerical data are presented in the median and interquartile range (IQR), whereas frequency and percentage (%) represent the categorical data. Non-parametric test, Friedman test with pairwise comparison, and Wilcoxon Paired Signed-Ranks test were used to compare the parameters from baseline to the 3- and 6-month reviews. To account for the increased risk of Type 1 error due to multiple comparisons (baseline vs. 3 months, baseline vs. 6 months), the Bonferroni correction was applied to the significance level. Statistical significance was set at $p\text{-value} < 0.05$ with 95% confidence interval (CI).

Results

Overall, 20 periodontitis cases with a median age of 44.50 (IQR17) that fulfilled the criteria and had the complete data at baseline until the 6-month review were selected. All the patients were non-smokers, with the majority being female (70%). Additionally, 35% of the patients had controlled systemic diseases, including 5 individuals (25%) with hypertension and 1 individual (5%) with diabetes mellitus and hypercholesterolemia. The baseline data were compared between the included and excluded cases in order to assess any potential biases from the missing data. The results showed no significant differences, except that more posterior teeth were treated in the excluded cases (p -value=0.023). Meanwhile, the 20 selected cases consisted of an equal number of posterior and anterior teeth. All GTR procedures were performed at sites with vertical periodontal bone defects, in combination with bone grafts

placed beneath the BPM during surgery. Detailed results are shown in Table 1.

Patients were reviewed for at least 6 months; healing after surgery occurred without any complications and uneventfully. No tooth loss was reported for teeth treated with GTR. The median and IQR of all the parameters were significantly reduced from baseline until the 6-month review (p -value<0.001), as shown in Table 2. The significance values were then adjusted by Bonferroni correction for multiple tests. The result found that statistical significance decreased (p -value<0.05) in all the parameters from baseline to the 6-month follow-up, except for the PS [baseline vs. 3-month (p -value=0.081) and 3-month vs. 6-month (p -value=0.246)], PPD (p -value=0.291) and CAL (p =0.537) for 3-month vs 6-month. The median percentage of radiographic alveolar bone loss also gained from 50% (IQR40) to 30% (IQR0) post-6-month GTR, as presented in Table 2.

Table 1 Demographic characteristics of the study subjects and comparison of baseline data between the included (n=20) and excluded (n=19) cases

Variables	Included cases n=20 median (IQR)	Excluded cases n=19 median (IQR)	Z statistic	p-value ^a
Age (years)	44.5 (17)	48.0 (24)	-0.267	0.789
Gender, n (%)				
Male	6 (30.0)	10 (52.6)	2.06 (1) ^b	0.151
Female	14 (70.0)	9 (47.4)		
Systemic disease, n (%)				
Yes	7 (35.0)	12 (63.2)	3.09 (1) ^b	0.079
No	13 (65.0)	7 (36.8)		
Number of teeth, n (%)				
Anterior	10 (50.0)	3 (15.8)	5.13 (1) ^b	0.023
Posterior	10 (50.0)	16 (84.2)		
Plaque score (%)	20.7 (18.6)	34.0 (33.0)	-1.475	0.140
Gingivitis score (%)	25.9 (25.6)	39.8 (34.0)	-0.618	0.536
Probing pocket depth (mm)	7.0 (3.38)	7.0 (2.0)	-0.014	0.989
Clinical attachment loss (mm)	7.5 (3.61)	7.0 (2.0)	-0.028	0.977
Alveolar bone loss (%)	50 (40.0)	50 (20.0)	-1.321	0.187

Statistically significant at the level p -value<0.05, ^aMann-Whitney U test, ^bChi-Square test (df), IQR=Interquartile range

Table 2 Comparison of periodontal clinical parameters of the periodontal defects treated with guided tissue regeneration using bovine pericardium membranes (PericardLEMB, USM) between baseline, 3-months and 6-months post-surgery (n=20)

Parameters	Baseline median (IQR)	3 months median (IQR)	6 months median (IQR)	Test statistic (df) ^a	p-value
Plaque score (%)	20.68 (18.60)	17.77 (10.00)	12.62 (11.10)	15.7 (2)	<0.001 ^b
Gingivitis score (%)	25.90 (25.58)	16.20 (25.79)	9.90 (17.73)	26.27 (2)	<0.001 ^{b,c}
Probing pocket depth (mm)	7.00 (3.38)	4.62 (1.75)	3.25 (1.56)	32.49 (2)	<0.001 ^c
Clinical attachment loss (mm)	7.50 (3.61)	4.62 (1.75)	3.25 (1.92)	31.51 (2)	<0.001 ^c
Alveolar bone loss (%)	50.00 (40.00)	-	30.00 (0.00)	-3.358	0.001 ^d

The significance level is p-value<0.05; ^aFriedman Test (p-value=0.000); ^bSignificance for baseline vs. 6-months after being adjusted by the Bonferroni correction (p-value=0.000); ^cSignificance for baseline vs. 3-months (p-value=0.000), 3-months vs. 6-months (p-value=0.034), after being adjusted by the Bonferroni correction; ^dWilcoxon Signed Rank Test

Discussion

This current study utilized BPM in combination with bone grafts to regenerate periodontal tissue loss due to periodontitis. The findings indicate a favourable outcome after the GTR procedures, which is denoted by the significant changes in the periodontal parameters from the baseline to the 6-month follow-up (p-value<0.05). Plaque scores and gingivitis scores were evaluated as these reflect the oral hygiene of each patient. Even though the PS changes were not statistically significant at the second phase of follow-up, the score can be considered minimal with a median of 12.62 (IQR11.1) at 6 months, which also reflects the significant reduction of the gingival inflammation. This may indicate the ability of patients to control their oral hygiene within the healing periods. Maintenance of oral hygiene can be accomplished through adequate home care. It is the clinician's responsibility to educate patients on the need to efficiently eliminate dental biofilm at home, particularly prior to beginning active periodontal treatment. The need for good home care should be emphasized repeatedly during the first and subsequent phases of periodontal therapy. This is crucial to ensuring that healing is completely achieved after any periodontal therapy or surgery, as this determines the success of the treatment²⁴.

The main aims of periodontal therapy are to achieve pocket depth reduction, new attachment, and bone gain. In this present study the reduction of PPD and CAL were statistically significant from the baseline until the 3- and 6-month follow-up. Although the changes were not significant from 3 to 6 months, the final median (IQR) of these parameters was found to be less than 4 mm, indicating a positive outcome. These findings are equivalent to those of some studies that were previously conducted in order to compare the changes in the periodontal clinical parameters, as well as the radiographic bone loss of the periodontal defects before and after being treated with GTR using BPM^{12,25,26}. The positive changes could imply the effectiveness of the procedures utilizing BPM to achieve periodontal tissue regeneration, observed at 6 months postsurgery. Stavropoulos and colleagues conducted a study to assess the clinical and histological healing of profound infra-bony defects treated with BPM in GTR and the granular bovine bone graft, which suggested that the use of bovine pericardium and adjunct bovine material implantation could lead to significant and long-lasting clinical improvements²⁵.

Management of periodontal tissue loss and bone defects affected by periodontitis is crucial to ensuring the

long-term survival of the tooth. GTR is one of the most common approaches being employed by clinicians. Various membranes available are being used as barriers in GTR procedures⁹.

The in-house BPM or PericardLEMB (Tissue Bank, USM) is a resorbable collagen membrane derived from the bovine pericardium, known as xenograft. This membrane is frequently used in our clinical setting, due to it being easily available locally. Tissue Bank USM processes and produces freeze-dried BPM that can be applied for tissue or bone regeneration to provide optimum healing conditions after implantation at wounds or defect areas²⁷. According to an in-vitro study by Athar et al. in 2014, PericardLEMB is suitable for periodontal fibroblast cell proliferation¹³.

During open-flap surgery for GTR, the membrane is inserted beneath the mucoperiosteal flap and adapted to cover the defect area. This separates the gingival tissue and epithelial from the root surface and alveolar bone. The flap is then approximated, completely covering the membrane and secured with sutures. Bone graft or bone substitute can be packed in the defect site in order to prevent the membrane from collapsing^{25,28}. Membranes should possess good mechanical properties to withstand the pressure and maintain the defect space for regeneration⁹.

Our results align with a pilot case series in which 8 patients with stage III periodontitis were treated with a bovine pericardium membrane, showing a mean PPD reduction of 4.8 mm, a CAL gain of 3.5 mm, and radiographic bone fill after one year. The authors suggested that treating the intrabony defects with a slow, resorbable BPM and a bovine bone graft that had been treated at a low temperature could be safe and lead to notable clinical improvements¹².

In tissue engineering, BPM can act as a scaffold because it mimics an extracellular matrix that provides mechanical support for cell attachment¹³. BPM is also the ideal choice as a barrier material in guided bone regeneration or socket preservation^{9,27}. This membrane

promotes tissue regeneration by inducing periodontal ligament fibroblasts, as well as osteoblast proliferation and regeneration, by creating a naturally suitable environment for host cell migration and proliferation. This could be attributed to the numerous benefits of bovine pericardium membranes, which include acellularity, superior consistency, and great mechanical properties with a minimal thickness of 0.5mm, allowing for reliable suture retention and optimal operating handling qualities¹⁵.

Based on this present study, GTR using BPM can be considered feasible as a barrier as the results for all clinical parameters were satisfactory and consistent with previous studies. It is important to acknowledge certain limitations of this study: particularly that the small sample size may reduce the statistical power and limit the generalizability of our findings. Although 39 cases of GTR were available, only 20 cases were selected due to incomplete or missing data, either at 3 months or 6 months. A comparison of the baseline data between the included and excluded cases revealed no significance for all the parameters, except tooth type. This could be a potential bias that could affect the study's impact, although the selected cases have an equal number of anterior and posterior teeth. The short duration of follow-up also restricts the results on the efficacy of BPM as GTR material. A larger sample size with a longer study duration should be considered in order to confirm the current observations. The retrospective study design may impose some bias as the evaluation of the parameters might have been performed by the same clinicians, who were not blinded for each interval. Furthermore, the absence of a control group and comparisons with other types of membranes were unable to be conducted due to the limited number used in our setting. Thus, the finding is rendered inconclusive and should be interpreted with caution.

On the other hand, advancements in periodontal tissue regeneration, particularly tissue engineering with the development of multiphasic membranes, pose a

challenge for BPM. Alternatively, these membranes can be incorporated with growth factors or regenerative cells to enhance wound stability and healing; thus, they can remain one of the preferred materials for clinical application⁸. The utilization of BPM in regenerative procedures could be improved with the addition of nanocarbonated hydroxyapatite to the porous side of a 3-layered membrane of nanocarbonated hydroxyapatite/collagen/poly (lactic-co-glycolic acid) that increases both the biocompatibility and osteoconductivity of the membrane, allowing for 3D cell ingrowth and differentiation¹⁵. Future studies should be conducted addressing these limitations as discussed above in order to demonstrate the clinical efficacy of this approach.

Conclusion

Within the study limitations, it can be suggested that the guided tissue regeneration procedure, employing bovine pericardium as a bioabsorbable barrier membrane, may effectively promote periodontal tissue regeneration. However, clinical trials with longer durations of post-surgical review for more conclusive findings are recommended in order to compare this membrane with other commercially available materials.

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Conflict of interest

All of the authors declare that there are no conflicts of interest in any form.

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