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Comparison Of the Effectiveness of The Three Types of Absorbable Collagen Membrane In The Reconstruction Of Rabbit Skull Bony Lesions In The Histologic And Histomorphometric Method

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Abstract

Introduction: The use of the barrier membranes is a standard method for the regeneration of bone lesions related periodontal diseases as well as pre - and post - implant treatments. Despite the research carried out in this regard, a complete study in Iran has yet to study the comparative study of commercial types of this membrane (absorbable), and further studies are needed. Therefore, this research is done on the histological and histomorphologic examination of this membrane and its comparison with the foreign brand on the rabbit skull.

Materials and methods: In this experimental animal study, 24 New Zealand male rabbits were used with weight of 2 kg and 20 weeks old. The specimens were divided into four groups (Tehran, Biomend, Senomembrane (Kish) and control). In all specimens, two defects with a diameter of 11 mm were created in the skull and randomly treated with three types of membrane. After sacrificing rabbits at 3 weeks and 10 weeks, the amount of bone formation and membrane remnant was assessed histomorphometrically and inflammation rate by histologic method. Data were analyzed using Kruskal-Wallis and Mann-Whitney tests with 95% confidence interval was used to compare the data ($P<0.05$).

Results: The results of this study showed that in all groups, the average percentage of bone formation was incremental. However, this difference was significant only in Senomembrane group (PV = 0.016). Compared to two periods of time, the average percentage of membrane remnant in all three groups of Tehran (PV= 0.002), Senomembrane (PV = 0.002) and Biomend (PV = 0.003) decreased significantly in week 10 compared to week 3. Degree of inflammation was significantly decreased in all three groups of Tehran (PV = 0.005), Senomembrane (PV = 0.002) and Biomend (PV = 0.002) over time.

Conclusion: In this study, the effectiveness of Biomend membrane was shown in the short term in comparison with the membrane of Tehran and Senomembrane in relation to bone formation. However, after 10 weeks, the results of regeneration of Iranian membranes were close to the foreign brand.

Keywords: Membrane, Histomorphometry, guided bone regeneration, guided tissue regeneration

Introduction

After extraction or losing a tooth, in the absence of intervention, the loss of the volume of the alveolar ridge is definite and irreversible. (1) Also alveolar ridge deficiency due to congenital defects, trauma, pathological conditions, infection, or periodontal problems may be an important problem for dental implants. (2)

Periodontal studies over the past decades have resulted in the treatment of GTR (guided tissue regeneration) and GBR (guided bone regeneration) (3). In repairing bone defects, bone repair can occur with problems and complications due to soft tissue admission around the lesion within it. (4) According to the Melcher theory, the removal of epithelium and connective tissue (which is a rapid growth) of bone defect causes slow-growing tissues (PDL and bone cells) to occupy the defect space is by placing a barrier membrane between the bone defect and the gum. (3) The use of these barrier membranes has greatly transformed the implant dentistry over the past 20 years to rebuild bone loss. (5) This barrier membrane prevents other cells from bone loss and, more importantly, preserves space for the slow process of bone formation (1). In addition, the presence of the membrane stabilizes blood clots containing bone precursor cells. On the other hand, the physical presence of blood clots maintains space and prevents the apical migration of glandular epithelium cells (1). This technique can be used in conjunction with bone graft and or bone replacement, which increases the space and also the osteoconductive / osteoinductive bone activity (1). The lack of height or width of the alveolar ridge is a major impediment to the placement of the implant. There are various techniques for inadequate ridge reconstruction, many of which are related to morbidity, even in the area of secondary surgery. With the help of the GBR, the inadequate ridge location can be reduced to morbidity and rebuilt without secondary surgery (6). Various studies have shown that the lack difference implantation of implants in the patient's original bone and the implants associated with bone regeneration were consistent with the implantation durability, marginal bone height, and soft tissue parameters around the implant (7, 8). Also, the durability ratio of implanted placements in remanufactured or developed areas using barrier membranes is reported to be between 97% and 100%, and most survival studies show more than 90% after at least one year of operation. The membranes used in regeneration treatments are divided into two groups: absorbable and non-absorbable. In primary surgical techniques, the widely used synthetic and non-absorbent polyethylene terephthalate (e-PTFE) membrane was used. Although e-PTFE is accepted as a gold standard, there are some disadvantages, including the early exposure of the membrane and subsequent progression of bacterial colonization and infection, which the remedial treatment of this case causes discomfort and increased patient costs and reduced bone regeneration, also, the need for second-line surgery to remove membranes, which results in the loss of alveolar bone and the loss of newly formed bone, led to the development of absorbable membranes (9). In general, absorbent membranes exhibit better clinical performance than non-absorbent membranes and, if applicable, are the preferred treatment techniques (10). The advantage of absorbable membranes, mainly made from collagen bovine and pigs (11), is the lack need of secondary surgery to remove membranes. Considering the advantages and disadvantages of these membranes, the use of a membrane barrier has become a standard treatment in GTR and GBR for the treatment of periodontal bone defects and implant surrounding defects, as well as for bone reinforcement before implant placement. For these reasons, clinicians and researchers agreed with the use of absorbable membranes in regeneration surgeries (12). Despite the research carried out in this regard, a complete study in Iran has yet to study the comparative study of commercial types of

this membrane (absorbable), and further studies are needed. Therefore, this research is done on the histological and histomorphologic examination of this membrane and its comparison with the foreign brand on the rabbit skull.

Materials and methods

In this study, used twenty four New Zealand male rabbits weighing 2 kg and aged 20 to 16 weeks were taken from the Medizist Company. Checking and confirming the health of rabbits was done by the veterinarian. In order to observe the standard diet and familiarity with the environment for two weeks before the surgery, the rabbits were brought to the environment and kept in separate cages under standard conditions. Animal selection, maintenance, and surgical protocol based on the ethics protocol of Isfahan University of Medical Sciences and approved by the Ethics Committee of the University with the code number 396093.

Used items:

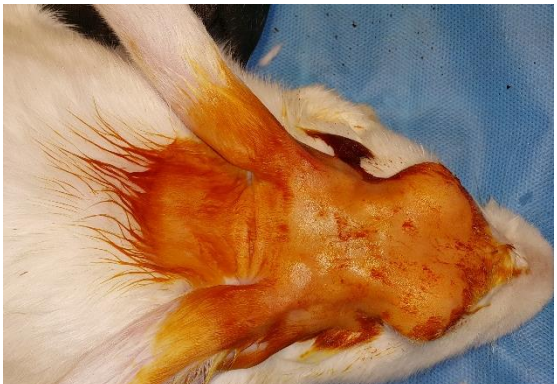
1-Regen-Membrane (Iranian Tissue Bank Research & Preparation Center, Tehran, Iran)

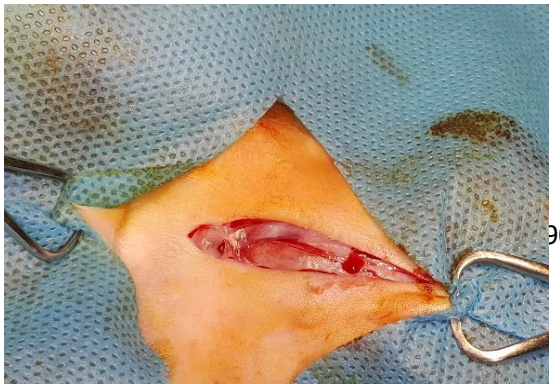
2-BioMend (membrane is manufactured by Integra Life Sciences Corporation for Zimmer Dental Inc.)

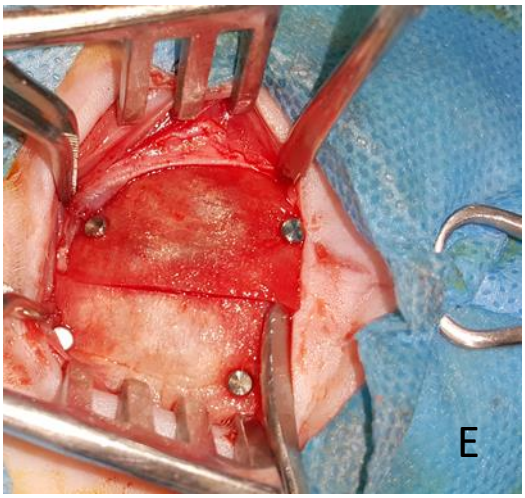
3-cenoMembrane (Tissue Regeneration Corporation, Kish, Iran)

Surgery protocol:

Rabbits were under anesthesia with intramuscular injection of 35 mg / kg ketamine, Alfasan, Woerden, Holld) and 75% mg / kg aspromazine (Neurotranq, Alfasan, Woerden, Holland). The surgical area was disinfected by a shaved razor, with a povidine iodine solution. Then, for local anesthesia, lidocaine hydrochloride 2% with epinephrine 1: 80000 was used at the surgical site. With razor No. 15, oxygen is fed into the midline of the cranium from the frontal bone to the occipital bone with an approximate length of 6 cm for the colostrum exposure. The flap was lifted in full thickness. Using a Trephine (Trephine Bur 11, TPB-8, MCTBIO, Korea) under a lot of washing with normal saline, two standard Defecate circles with a diameter of 11 mm on both sides of the Squared Mead Sagittal were created. Bone windows were carefully removed to prevent damage to the Meninge. The lesions were then randomly coated with three membranes and not in the membrane control group. The membranes were cutoff and cover in 12 × 12 mm sizes for the full cover of the entire environment Defecates test group. It was proved by the tag (bone Tac 2 mm, MCTBIO, Rep of KOREA) and Bone Tac Holder, BT-HD MCTBIO, Korea. The flaps were returned to the first position and sutured with absorbable Sutures of vikril (0-4) 19 in the form of a layer (separate skin and periostium). Immediately, chloramphenicol spray was used to prevent local infection in the potion. Rabbits received intramuscularly 20 mg / kg cefazolin. The sutures were drawn after 10-14 days. In each session, three rabbits were surgically treated. A total of 48 defects were created, divided into four groups of 12, and were sacrificed at 12 times each time (3 and 10 weeks) of the rabbits with an overdose of anesthetic drugs. Each calvarium of each animal was prepared to prepare histologic and histomorphometric samples. Therefore, at each stage, 6 defects are assigned to each group (Figure1).



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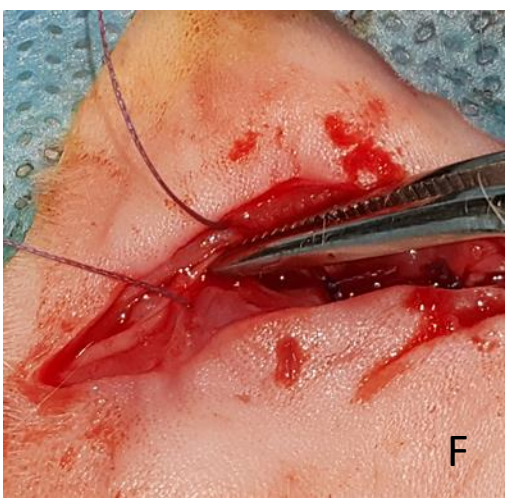
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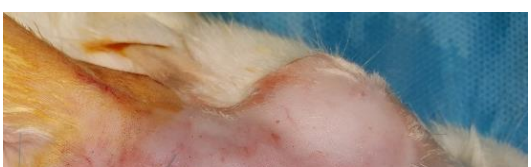

Figure1. Surgical procedure: A. surgical site preparation, B. incision made with 4 to 6 cm length, C. Creating 11mm defects on both sides of the middle Suture, D. Bone window removal, E. Defect coverage by membranes, F. Membranes fixation by Tacs, G. layered suturing of periosteum and skin, F. Skull bone block removal and tissue passage from the center of the defect

Histology

After sacrificing the animal at any time, it was separated by the technician of the entire nursery of the Calvary, which contained the surrounding defect and healthy bone area. Surgical blocks were fixed for 5 days in formalin 10%. Fixed samples in 20% formic acid were decalcified for 10 days, during which time acid was exchanged daily and decalcification was investigated. After calcification, the samples were processed for 8-12 hours by the Tissue processor (Histocinet SHAAKDON model). The tissue was then removed from the device, molded with paraffin and placed in a refrigerator. In the next step, the blocks were prepared by a microscope machine of the Lica model from the center of the defect, and cut-offs of 5 microns thickness were performed by standard staining (hematoxylin-eosin and tri-chromosomes). The stained slides were used for pathological studies. For histological examination of inflammation, residual membrane, new bone formation, optical microscope and magnification of 10 and 40 were used. It was also used to determine the new bone percentage and the percentage of the remaining membrane of the histomorphometric, then that photographs with a magnification of 10 was developed by stereomicroscope Nikon model SMZ745 and the camera True Chorome Metric model HDM was developed and tested by TCapture software. Lumens were detected with an x400 magnification to determine inflammation. And the average number of inflammatory cells in 10 fields was evaluated. (Figure 2 and 3)

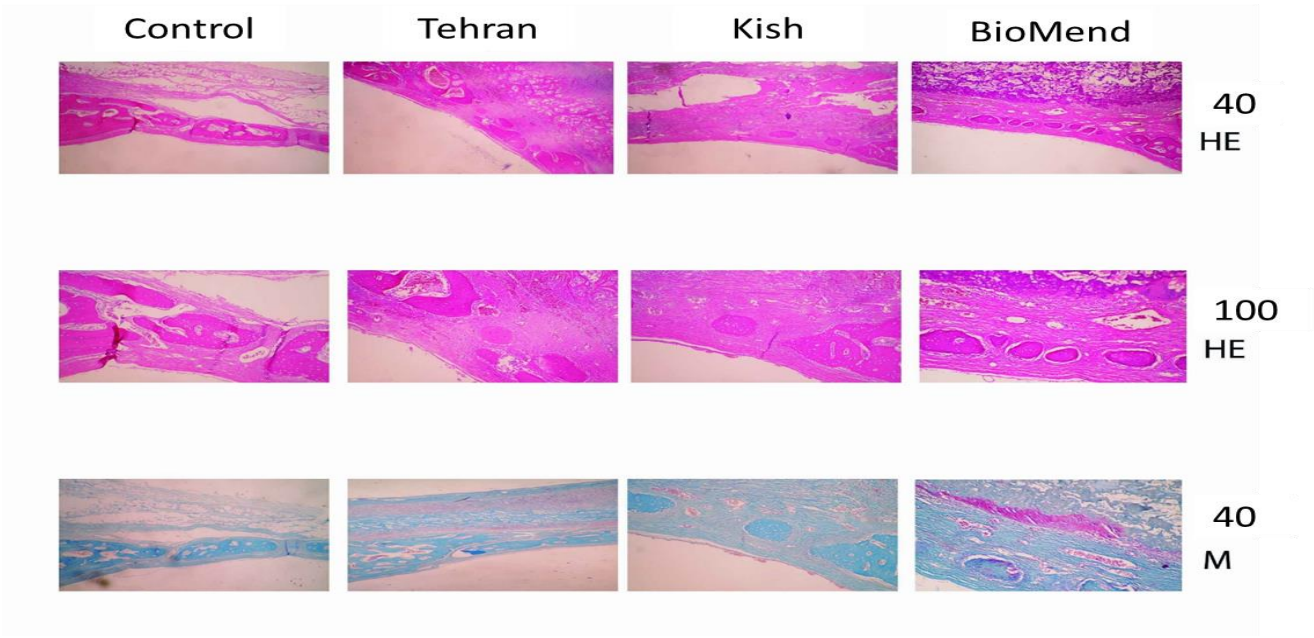

Figure2. Histological sections at week 3 (Hematoxylin-Eosin (HE), Masson staining (M))

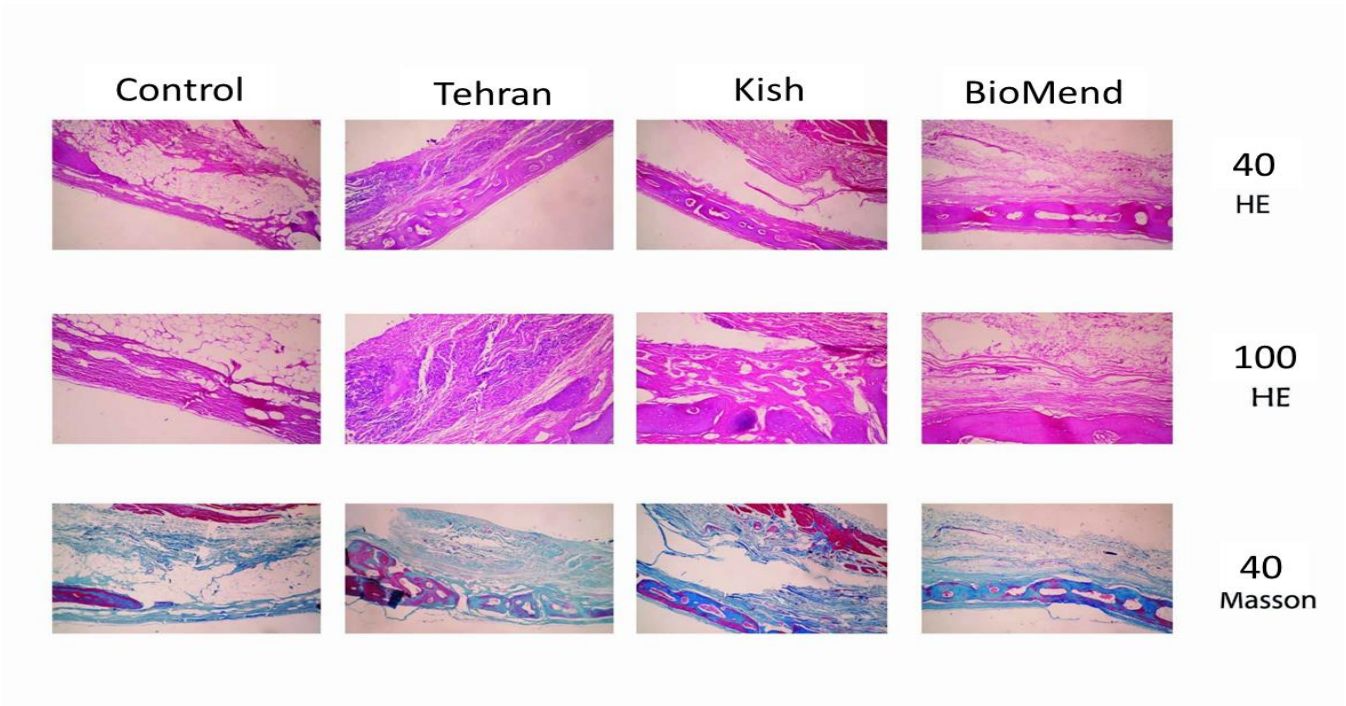

Figure3. Histological sections at week 10 (Hematoxylin-Eosin (HE), Masson staining (M))

Inflammation rate based on inflammatory cells of lymphocyte include:
No inflammation: no inflammatory cells.
Mild inflammation: inflammatory cells ≥ 30.
Medium inflammation: 60 <inflammatory cells <30
Severe inflammation: inflammatory cells <60
2% bone composed

3% remaining membrane
Data were analyzed by SPSS 22 software. The comparison between groups was done by Kruskal Wallis and Mann-Whitney tests. The values of P <0.05 were considered as statistically significant.

Result

In this interventional animal study, the amount of bone rebuilt the amount of membrane remaining and the level of inflammation in standard defects CSDs rabbit calvaria treated with three types of membranes. After 3 weeks in 10 weeks, they were checked and compared. In the control group, after 3 weeks, the formation of a fetal bone from the defect margins was observed. (Figure 3) These defects were occupied mainly by soft tissue that had collapsed into the defect. The periostium contains many blood vessels. The formation of bone islands in the center of defects was seen. At week 10, bone islands connection was seen at the center of defecation. (Figure 1), which indicates the osteogenic activity of periostium around and the Dura matter. At some histological levels, a bone bridge was created between the two edges of the defect. But the height of the bone bridge was much less than the native bone density around the defect. The rest of the defect was filled with soft tissue and fibrous tissue. In the control group, inflammation was negligible at both times.

In the membranes group, collagen membranes were invariably structurally intact at week 3 and had a good marginal fit with the native bone around the defect. The formation of blood vessels around the collagen membrane and its proximity was evident. In the central regions, collagen membranes were somewhat collapsed, in addition to the underlying Dura matter invasion, it was also seen in the defect. Both of these factors have led to a significant reduction in the area of the central defect. The formation of a wedge shape woven bone from the defect to the center of the defect was evident. But these bone islands did not reach each other. The integration of these bony islands in Biomend membranes more than membranes in Tehran and in Tehran city was more than membranes Kish. Inside and around defect and membranes, there was a significant presence of inflammatory cells, mainly lymphocytes, which meant the beginning of membrane decomposition. In the third week, the rate of inflammation in the membranes of Biomend was lower than that of Tehran and Kish membranes, and there was no significant difference between Tehran and Kish (Figure 2).

At week 10, collagen membranes did not retain their original form and appearance like in the third week. At the tenth week, the bone was more mature than the third week. At some histological levels, the membrane thickness was replaced by the presence of Osteoid and the formation of blood vessels. The number of lymphocytes was significantly reduced compared with the third week, indicating the progression of recovery (Figure 3).

Histologic findings

The average percentage of bone is formed and the percentage of membrane remnant is shown in Table 1.

Table1. Average amount of Bone formation, Average amount of Membrane remnant in 4 Groups at Week 3 and Week 10

	group	Week 3		Week 10	
		Mean	Standard deviation	Mean	Standard deviation

Amount of Bone formation (%)	control	53.79	28	63.79	29.18
	Tehran	57.12	32.85	79.97	21.98
	Kish	20.99	10.39	71.35	35.41
	Biomend	81.49	13.73	85.35	21.71
Membrane remnant (%)	control	-	-	-	-
	Tehran	91.66	20.41	10.10	10.00
	Kish	100.00	0.00	12.50	13.69
	Biomend	78.33	34.88	5.88	3.76

Kruskal Wallis analysis showed that there was a significant difference in the rate of new bone formation among groups of 3 weeks (PV = 0.1111). In completing the results, the Mann-Whitney test showed that this significant difference was observed between control and kish groups (PV = 0.225), control and Biomend (PV = 0.445), and Kish and Biomend groups (0.004) PV) and Tehran and Kish groups (PV = 0.078), this difference was close to meaningful. A comparison of new bone percentages in 4 groups at times 3 weeks and 10 weeks is shown in diagram 1.

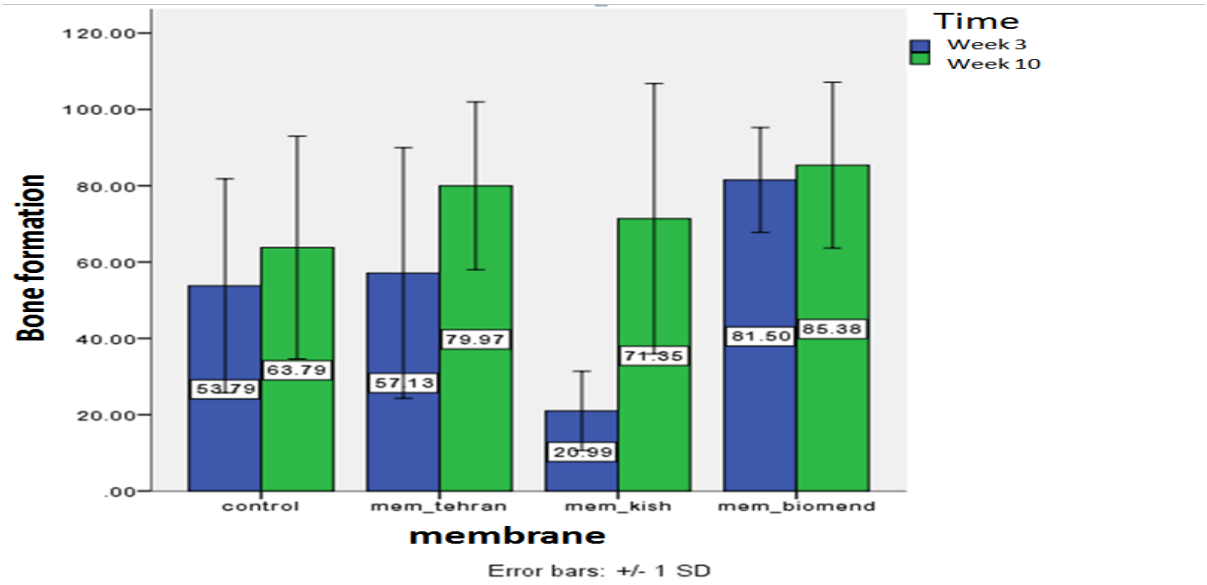

Diagram1. Comparison the amount of bone formation in 4 groups at week 3 and week 10

Also, Kruskal Wallis analysis showed no significant difference in the residual membrane in the test groups (PV = 304.0). A comparison of the remaining membrane percentages in 4 groups at times 3 weeks and 10 weeks is shown in diagram 2.

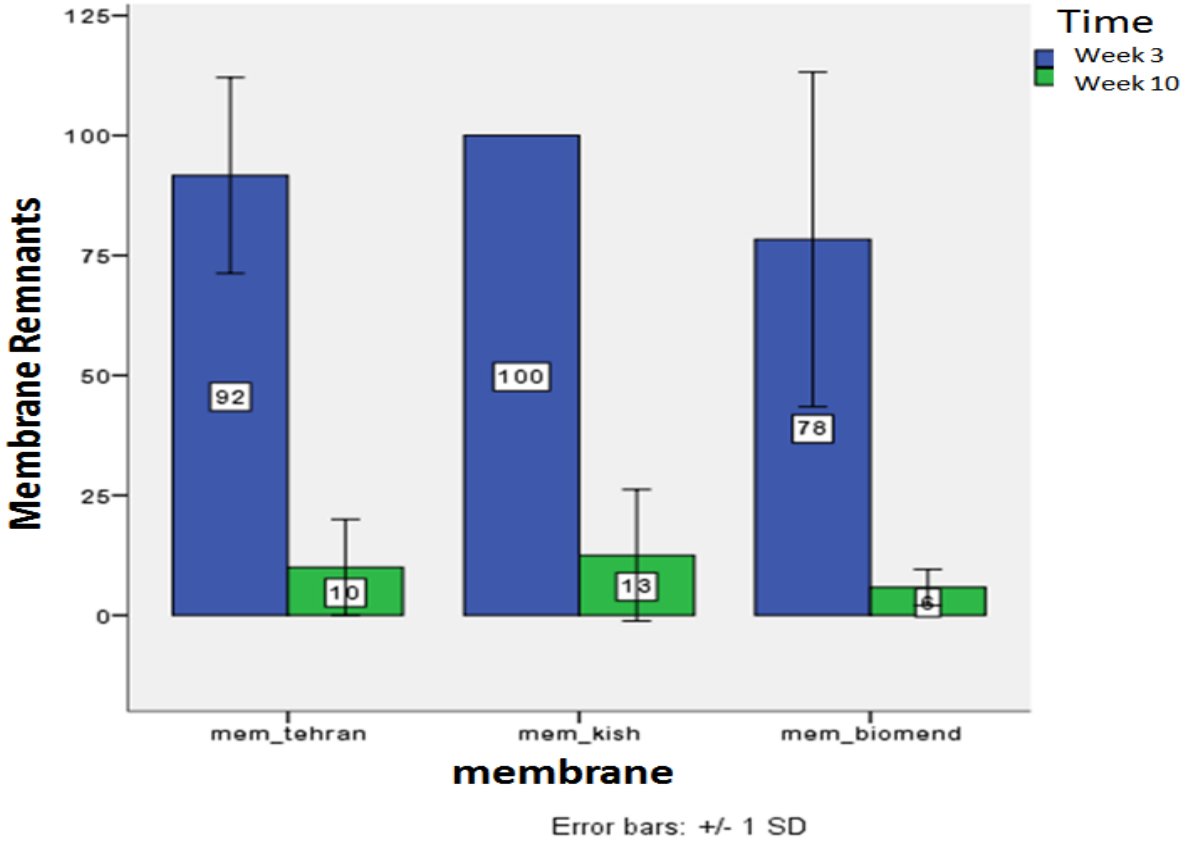

Diagram2. Comparison of percentage of membrane remnant in three groups at week 3 and week 10

Distribution of the abundance of inflammation in 4 groups at times 3 weeks and 10 weeks is shown in Table 2.

Table2. Frequency Distribution of Inflammation Degree in Groups

Time	Degree of Inflammation	Groups			
		Control	Tehran	Kish	Biomend
Week 3 (N=6)	0	6	0	0	0
	1	-	1	1	2
	2	-	2	2	3
	3	-	3	3	1
Week 10 (N=6)	0	5	4	6	6
	1	0	2	0	0
	2	1	0	0	0
	3	-	0	0	0

In amount of inflammation, Kruskal-Wallis test showed a significant difference (PV = 0.002), which is a significant difference between the control group and Tehran, Kish and biomand test groups. (002/0 = PV). But there was no significant difference between membranes.

In the 10th week results of the Kruskal Wallis test, there were no significant differences in the percentage of residual membrane (PV = 0.53), new bone formation percentage (PV = 30.9) and inflammation values (PV = 0.286) among Control group and test groups did not show. Finally, the

results of Van Whitney test showed that bone formation in the Kish group was significantly different in two periods of 3 weeks and 10 weeks (PV = 0.016). However, in the other groups, there was no difference in the intervals of 3 weeks and 10 weeks with each other. There was a significant difference in inflammation amount time intervals between 3 weeks and 10 weeks in the Tehran group (PV = 0.005), Kish group (PV = 0.002) and Biomand (PV = 0.002). But in the control group 3 weeks and 10 weeks, this difference was not significant (PV = 0.37). There was a significant difference in the amount of remaining membrane in the period of 3 weeks and 10 weeks in the Tehran group (PV = 0.002), Kish (PV = 0.002, Biomand) PV = 0.003.

Discussion

In this interventional animal study, the amount of bone remodeling, the amount of membrane remaining and the level of inflammation in standard defects (CSD) calvary of rabbits treated with three types of membranes. After 3 weeks in 10 weeks, they were compared and checked. The results of this study showed that bone formation increased in all groups in the 10th week compared to the third week, but when the study groups were compared together, the results showed that in the Kish group, the bone formation was more affected by time than in other groups, so that in the tenth week, significant bone leakage was observed in the third week. Perhaps this result is due to the effect of the membrane structure on the amount of angiogenesis of the defect. However, in the Wehrhan study, the effect of thickness and membrane structure and its collagen density on angiogenesis has been emphasized (13). In any case, the examination of this issue requires further evaluations.

The results of this study showed that in each of the four groups, the course of inflammation had a decreasing trend in the 10th week compared to the third week. These results are consistent with the results of Branel and Ahn studies (14, 15). It is clear that with the progress of the restoration process on the one hand and the reduction of the amount of collagen membranes remaining after its decomposition, the amount of inflammation decreases over time. Comparison of test groups at two time points showed that the percentage of membrane remained at a significant level during the period of 10 weeks compared to three weeks. This is due to the decomposition of membranes over time. In all specimens, at the time of 3 weeks, the formation of a new bone from the defect margin began to develop, extending in the form of a bow to the center. However, the bone formation rate was not the same at different times in different groups, so that in the third week, the highest bone formation was first in the Biomend group, then in the Tehran group, and then in the control group, and finally in the Kish group, and at the tenth week this trend was almost the same as the progression, but the only difference was the outgrowth of bone formation in the Kish group compared to the control group.

On the other hand, the comparison of inflammation rates among different groups showed that in the third week, reported the inflammation rate in the control group was close to zero, which seems to be due to the lack of membranes in this group and the high metabolism of rabbits. Among the other experimental groups, although there was no significant difference, inflammation rates was lowest in the Biomend group and then in the groups of Tehran and Kish it was approximately equal to inflammation. At the tenth week, in all groups, inflammation was minimal. Comparing these results, it seems that the Biomend membrane in the short term is more effective in bone formation compared to the membrane of Tehran and Kish, although after 10 weeks, the results of Tehran and Kish regeneration are also close to the Biomend. This difference is probably due to differences in the physical structure and thickness of the membranes, which affects the amount of angiogenesis and blood vessel infiltration through the membrane into the defect and anastomosis of the vessels with blood vessels of the defect on the one hand, and maintains the consistency of membranes and

prevents collapse of the membranes into the defect over time, on the other hand. On the other hand, the presence of at least inflammation around the mummies of Biomend, which indicates more biocompatibility, can be another reason for the formation of more bone in the regenerated defects with these membranes. In cases where absorbent membranes are used, there is a critical maintenance to prevent the membrane from absorbing and does not interfere with regurgitation. Hurzeler et al. Considered this time to rebuild alveolar tissue for approximately 2 to 8 weeks. (16) The results of this study showed that the remaining membrane was not significantly different in the third week and the 10th week. Given that the rate of rabbit metabolism is much higher than that of humans. Thus, the membrane that produces better results in the rabbit in the short term may have better results in humans over the medium term.

Conclusion

It can be concluded that although all the membranes examined in this study were cross-linked collagen membranes, but the results of their regeneration were different. Tehran and Kish membranes are Native collagen membranes. These membranes are extracted from the pericardium of the heart and lack cross-linking chemicals. But the Biomend membrane is made from Achilles cow, and during the process, a cross-linked chemical is applied to the membrane, which appears to be different from the normal pericardial cross-link. The difference in bone formation, the course of inflammation during regeneration and membrane survival can be mainly due to the same differences that affect the chemical composition, strength, physical structure, permeability and biocompatibility of collagen membranes.

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