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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Different angiogenic response and bone regeneration following the use of various types of collagen membranes – *in vivo* histomorphometric study in rabbit calvarial critical-size defects

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SUMMARY

Introduction/Objective Success of guided bone regeneration depends on the size and morphology of defect, characteristics of barrier membranes and adequate angiogenesis.
The aim of the study was to reveal impact of three different collagen membranes on angiogenesis and bone production in critical-size defects.

Methods Defects were created in rabbit calvarias, filled with bovine bone graft and randomly covered with one of three investigated collagen membranes (Biogide – BG, Heart – PC, Mucograft – MG) or left without a membrane for the control group (C). After two and four weeks of healing, a total of 10 animals were sacrificed for histological and histomorphometric analysis of angiogenesis, bone regeneration, and inflammatory response.

Results In the early healing phase, the highest values of trabecular thickness and trabecular area were recorded with PC and BG membranes, respectively. After four weeks, significantly improved bone healing was noted in the MG group, as well as significantly pronounced inflammation. Initially, vessel density was significantly higher in the C group compared to all three membranes. After four weeks, significantly better results were observed in the MG compared to the other groups, BG compared to the rest of groups, and between PC and C groups.

Conclusion The use of collagen membranes significantly affects angiogenesis, reducing it in the early and enhancing it at the later healing phase. All three tested membranes in combination with bone graft significantly improved the amount of regenerated bone. Among the investigated groups, MG favored more pronounced angiogenic, osteogenic, and inflammatory response in the observation period of four weeks.

Keywords: collagen membrane; angiogenesis; guided bone regeneration; collagen matrix; pericardium

INTRODUCTION

Opposite to other connective tissues bone has a remarkable ability to completely restore its structure and function, recapitulating the embryonic processes of intramembranous and endochondral ossification. On the other hand, besides its substantial self-regenerative capacity, healing of intraoral bone defects largely depends on the size and morphology of the defect, number of bony walls, mechanical wound stability, healing environment and treatment protocols [1, 2].

Concept of guided bone regeneration (GBR) is based on the use of barrier membranes that selectively exclude migration of fast-growing soft tissue cells, thus allowing enough time for osteoprogenitors to populate and regenerate the entire defect. Among numerous available bioresorbable and non-resorbable barriers today, collagen is recognized as more frequently used due to its biocompatibility, hemostatic effect, osteoblast attraction, growth factor adsorption, and the active role in bone formation [3, 4]. In contrast, unpredictable resorption and poor mechanical stability are their main limiting factors [5]. These are partially compensated by combining a membrane with particulate bone graft and using membranes of improved structural characteristics and prolonged barrier longevity, such as cross-linked membranes, the two-membrane technique, membranes of more resistant source of collagen, or incorporation of antibacterial agents, growth factors, and ceramics within their structure [3, 6, 7].

For successful bone regeneration, angiogenesis is considered the prerequisite factor for bone formation, repair and remodeling [8]. New blood vessels, besides providing nutrition and gaseous exchange, bring important growth factors and stem cells into the healing zone [8, 9]. They temporally precede bone formation, starting exclusively from the surrounding bony walls and the periosteum [10]. While more

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blood vessels could mean more nutrition, growth factors, stem cells, and intensified regeneration, there is an assumption that one of the mechanisms of membrane efficiency might be the exclusion of blood vessels from the overlaying soft tissue that do not have the bone-forming potential [9].

Therefore, the aim of this study was to reveal how different collagen membranes effect angiogenesis in the critical-size defect model and its further reflection on bone healing. The inflammatory response was also evaluated with respect to its impact on bone regeneration.

METHODS

Study design and surgical procedures

Ten skeletally mature New Zealand White rabbits, weighing 3.5–4.5 kg, were included in this study. The study protocol was approved by the Institutional Ethics Committee, School of Dental Medicine, University of Belgrade (Approval number 36/10) and conducted in accordance with the European Union Directive 2010/63/EU for animal experiments.

The surgical procedure was performed under general anesthesia, achieved with intramuscular administration of 5 mg/kg of xylazine, 35 mg/kg of ketamine, and 0.75 mg/kg of acepromazine. In brief, after disinfection, incision, and flap elevation, four 8-mm circular bicortical defects were created in rabbit calvaria, two in the frontal and two in the parietal bones, using trephine drills. The defects were filed with bone substitute (Bio-Oss, Geistlich Söhne AG, Schlieren, Switzerland) and randomly assigned to one of following investigated groups: 1) BG (Biogide, Geistlich), 2) PC (heart pericardium membrane, Bioteck, Arcugnano, Italy), 3) MG (Mucograft, Geistlich), and 4) C (control group without membrane). All membranes were trimmed into the 10 × 10 mm quadrant shape, adapted over defects, and stabilized by suturing the periosteum with horizontal mattress sutures (Coated Vicryl 5-0, Ethicon Inc., Raritan, NJ, USA). The skin was closed with a continuous suture (Coated Vicryl 4-0, Ethicon Inc.). Postoperatively, the animals received antibiotics (15 mg/kg of oxytetracycline, intramuscularly) and analgesics (0.01 mg/kg of butorphanol, subcutaneously) for three days.

After two and four weeks of healing, five randomly assigned rabbits were sacrificed under general anesthesia, with an overdose of phenobarbital (100 mg/kg). Their cranial vaults were removed with a saw, rinsed with water, and immersed in 10% buffered formalin solution. Thereafter, formalin-fixed calvarial bones were cut with a low-speed diamond saw disc in the regions of previously created experimental bone defects.

Histologic processing and histomorphometric evaluation

Bone samples were further decalcified with 10% formic acid, dehydrated in ethanol, molded in paraffin blocks, and longitudinally sectioned through the center of the defects. Three central tissue sections of 5 μm thicknesses were cut from each block for hematoxylin-eosin staining. Histomorphometric analysis and histologic observation were done by an experienced pathologist blinded to the experimental groups. Slides were observed by optical microscopy (Olympus 5 microscope, Olympus Corporation, Tokyo, Japan) using Olympus Cell-B morphometric software.

Histomorphometric parameters were analyzed quantitatively, counted in the areas of the highest density (hot spots) at high power magnification (HPM) of 200 ×. The following parameters were measured: vessel density (VD, number of blood vessels within one microscopic field under HPM), blood vessel diameter (BVD, the largest vessel diameter, in μm), blood vessel area (BVA, in μm^2), trabecular thickness (Tb.Th, the widest dimension of bone trabeculae, in μm), trabecular area, (Tb.A, in μm^2), and multinucleated giant cells (MNGC, number of cells within one microscopic field under HPM).

Statistical analysis

Statistical analyses were made using IBM SPSS Statistics, Version 24.0 (IBM Corp., Armonk, NY, USA). All data were presented as mean and standard deviation (SD). Statistical methods for intergroup analyses included the Mann–Whitney U-test, or one-way ANOVA, due to the normality test. Intragroup comparisons within time were assess using the two-sample t-test or Mann–Whitney U-test. The level of significance was set at ≤ 0.05.

RESULTS

The healing was uneventful in all the animals. During the study there were no signs of infection, allergic reaction, wound dehiscence, or membrane exposure.

Histological findings

After two weeks, all membranes showed an angiogenic potential. BG and PC membranes had blood vessels in direct contact with membrane fibers that partially grew into the membrane (Figure 1A, B). In contrast, MG membrane was forming a blood vessels demarcation between the membrane and graft (Figure 1C). This relationship was maintained after four weeks (Figure 1D–F). Analyzing bone production, we noticed that all trabeculae were similar in cellularity, but there was a difference in their thickness. At the second week of healing, trabeculae in the MG group were thinner, more graceful and narrower (Figure 2C), than the other two membranes (Figure 2A, B). Contrary, in the fourth week newly formed bone was more voluminous and wider in MG group (Figure 2D-F). Considering inflammatory response, a limited infiltrate of nonspecific inflammatory cells was observed in all the groups at the second week of healing. On the other hand, after four weeks, in the MG group there were MNGC present, with diffuse membrane infiltration (Figure 1F), while

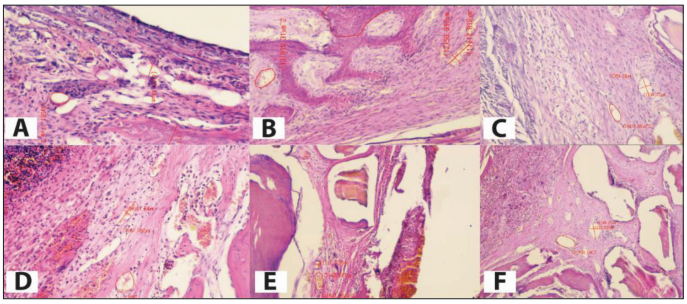

Figure 1. Histological images showing angiogenesis and inflammatory response concerning investigated membranes BG (A, D), PC (B, E), MG (C, F), and healing time: two weeks (A, B, C), four weeks (D, E, F); H&E, 200 ×

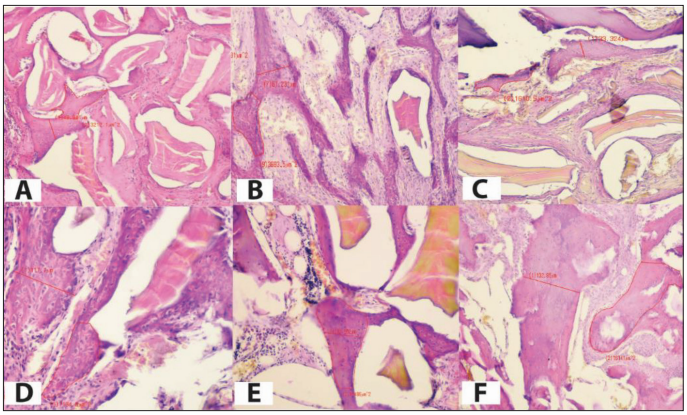

Figure 2. Histological images showing bone production according to different membranes; BG (A, D), PC (B, E), MG (C, F), and follow-up time: two weeks (A, B, C), four weeks (D, E, F); H&E, 200 ×

the other two membranes did not induce such an intense tissue response (Figure 1D, E).

Histomorphometric analysis

Angiogenesis (Table 1)

In two weeks, all the investigated parameters were significantly increased in group C compared to the other groups, except for BVD, which lacked significance between the C and MG groups. Inside the membranes, groups' statistical analyses showed significantly increased VD in group BG in comparison to PC and MG groups, which was also seen between PC group and MG group. On the other hand, BVD and BVA were significantly increased in MG compared to BG and PC. However, while BVD was significantly increased in BG compared to PC, BVA was significantly higher in PC than in BG. After four weeks of healing, MG group showed significantly increased values of all vascular parameters in comparison to the other groups. Considering VD, significantly higher results were also observed with BG compared to PC and C, as well as between PC and C. Contrary, BVD and BVA were significantly increased in C compared to BG and PC, although without significance for BVD between C and BG. Comparison between BG and PC regarding BVD showed significantly higher results for BG, while PC showed significantly increased values for BVA. Within the time frame, T4 *vs.* T2, all investigated parameters were significantly improved in the MG group, while all the other groups expressed decreased values.

Bone regeneration (Table 2)

Use of collagen membranes after two weeks of healing resulted in significantly more bone comparing to group C in both parameters. Analysis among investigated membranes after two weeks of healing demonstrated significantly increased Tb.Th in group PC, compared with BG and MG, as well as between PC and MG. In contrast, Tb.A was increased in BG compared to PC, and significantly higher in BG and PC compared to MG. Similarly, after four weeks of healing, collagen membranes significantly improved bone regeneration compared to the control. Among the investigated membranes, both parameters were significantly higher for MG in comparison to BG and PC. However, while Tb.Th was significantly improved in BG in comparison to PC, Tb.A was significantly higher for PC. Considering bone production with time, all groups showed significant increase of Tb.Th, which corresponds to significant increment of Tb.A.

Inflammatory response (Table 2)

Two weeks after the surgery, no MNGC was detected within any investigated group. After four weeks of healing, results of an inflammatory response were significantly higher for the MG group compared to all the other groups, as well as between the BG and the PC groups.

DISCUSSION

In recent years, a growing body of evidence has been suggesting that blood vessels in bone promote osteogenesis [10, 11]. However, there are scarce data regarding the influence of barrier membranes on angiogenesis, pointing out the need for its research [12].

Our study investigated the impact of three structurally different collagen membranes on angiogenesis and bone production in rabbit calvarial critical-size defects. Since previous research showed that membranes possess the greatest impact in the upper and central defect regions, we focused our analysis on that top half part of bone defect [4].

According to our results, it seems that in the early healing phase more pronounced angiogenesis can be expected at those sites where barrier membrane is not used. This result is in line with previous research of De Marco et al. [13], who pointed out that more intensive and extensive revascularization of autologous block bone graft were found in the group without occlusive membrane use, where new blood vessels proliferate not only from the bony walls of the recipient bed, but from the overlaying soft tissue as well.

Table 1. Histomorphometrical analysis of vascular parameters

Parameters	BG	PC	MG	C	Comparison between groups
T2					
VD (N/mm^2)	9.87 ± 1.73	8 ± 0.76	6 ± 0.66	14.40 ± 1.24	BG *vs.* PC p = 0.002; BG *vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
BVD* (μm)	23.26 ± 2.63	19.7 ± 2.77	35.16 ± 3.45	37.07 ± 2.01	BG *vs.* PC p = 0.005; BG *vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C p = 0.000; MG *vs.* C NS
BVA (μm^2)	552.86 ± 10.49	605.33 ± 18.77	808.35 ± 23.87	2960.40 ± 944.49	BG *vs.* PC, BG*vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
T4					
VD (N/mm^2)	8.2 ± 1.01	6.8 ± 0.94	11.33 ± 1.5	5.93 ± 0.80	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, MG *vs.* C p = 0.000; PC *vs.* C p = 0.019
BVD* (μm)	24 ± 2.15	12.85 ± 2.22	38.98 ± 1.9	25.88 ± 2.45	BG *vs.* C NS; BG *vs.* PC, BG *vs.* MG, PC *vs.* MG, PC vs C, MG *vs.* C p = 0.000
BVA (μm^2)	461.18 ± 23.02	527.93 ± 29.61	1479.11 ± 174.29	726.49 ± 21.07	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
T4 *vs.* T2§					
VD	0.003	0.001	0.000	0.000	
BVD	NS	0.000	0.001	0.000	
BVA	0.000	0.000	0.000	0.000	

Values are given as mean ± SD;
BG – BioGide; PC – pericardial membrane (heart); MG – Mucograft; C – control; T2 – two weeks; T4 – four weeks; VD – vessel density; BVD – blood vessel diameter; BVA – blood vessel area; NS – not significant;
Kruskal–Wallis test, post hoc Mann–Whitney test;
*one-way ANOVA, Bonferroni post hoc test (due to normality);
§two-sample t-test / Mann–Whitney test

Table 2. Histomorphometrical analysis of bone production and inflammatory response

Parameters	BG	PC	MG	C	Comparison between groups
T2					
Tb.Th* (μm)	49.05 ± 1.33	75.2 ± 5.37	32.77 ± 4.88	19.79 ± 4.49	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
Tb.A (μm^2)	3738.17 ± 332.45	3594.83 ± 192.62	1598.29 ± 68.11	1168.23 ± 117.12	BG *vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000; BG *vs.* PC NS (p = 0.059)
MNGC (N/mm^2)	-	-	-	-	
T4					
Tb.Th (μm)	118.06 ± 3.08	94.62 ± 2.92	130.38 ± 9.36	23.90 ± 2.33	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
Tb.A (μm^2)	7243.18 ± 425.70	15316.57 ± 1563.44	16490.59 ± 886.98	2239.16 ± 164.32	BG *vs.* MG, BG *vs.* PC, BG *vs.* C, PC *vs.* C, MG *vs.* C p = 0.000; PC *vs.* MG p = 0.011
MNGC (N/mm^2)	2.67 ± 0.97	0.93 ± 0.7	11.6 ± 1.96	0	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
T4 *vs.* T2§					
Tb.Th	0.000	0.000	0.000	0.005	
Tb.A	0.000	0.000	0.000	0.000	

Values are given as mean ± SD;
BG – BioGide; PC – pericardial membrane (heart); MG – Mucograft; C – control; T2 – two weeks; T4 – four weeks;
Tb.Th. – trabecular thickness; Tb.A – trabecular area; MNGC – multinucleated giant cell; NS – not significant;
Kruskal–Wallis test, post hoc Mann–Whitney test;
*one-way ANOVA, Bonferroni post hoc test (due to normality);
§two-sample t-test / Mann–Whitney test

When we compare results among investigated membranes, the difference in their angiogenic response may be the result of their distinctive structure. The findings of this study revealed that in the early healing phase, the BG membrane produced the highest VD in the underlining bone defect, followed by the PC membrane, while the thick MG membrane expressed the lowest early angiogenesis. These results are in line with previous animal studies investigating angiogenesis inside the collagen membranes themselves, which showed early angiogenesis of the BG [14, 15], a somewhat slower angiogenesis of the bovine PC [14], and a delay in angiogenesis of the MG [16]. Even though angiogenesis and bone regeneration mainly arose from the surrounding bony walls, Schwarz et al. [17] found some localized areas of newly formed bone below the barrier membranes, which allow transmembranous angiogenesis, in contrast to the occlusive ones.

However, despite better result of angiogenesis with the use of BG in comparison to PC, we found significantly thicker bone trabeculae and improved bone Tb.A after two and four weeks, respectively, with the use of the PC membrane. Although angiogenesis plays a significant role in bone healing, modification of material surface properties, mechanical characteristics, thickness, porosity, and composition are recognized to be important issues in GBR [7]. In line with that, a recent study by You et al. [6]

revealed better bone regeneration using a porcine pericardial membrane compared to the BG membrane, where the smooth surface of the pericardial membrane promoted proliferation and differentiation of attracted human bone mesenchymal stem cells at a higher level, implying its potentially osteoconductive and osteinductive characteristics. Although we used the pericardial membrane from different animal species, similar multilayer composition of pericardium could probably have allowed more bone-forming cells to attach and proliferate on its lower surface, like in previous research. Other possible factors that may affect bone production are excellent mechanical properties of pericardium [18]. Regardless of its negligible impact on space maintenance ability due to bone graft use, mechanically stable environment and stiffer surfaces showed enhanced osteoblast differentiation [19]. Furthermore, heart pericardial sac consists of collagen and elastin, whose elastic fibers, in addition to improving tensile strength, might have a potential pro-osteogenic role [20].

Regarding MG, our results showed that in the early healing phase it provoked the lowest VD of the underlining defect, as well as significantly lower bone regeneration compared to the other two membranes. Similar results were demonstrated by Basudan et al. [21] in the same animal model after two, four, six, and eight weeks of healing, showing lower bone regeneration comparing MG and the cross-linked collagen membrane. They considered that a possible reason for a decreased bone regenerative potential may be slower vascularization of the dense, compact layer of MG [16]. Our results may also indicate that the compact layer of this thick matrix may be the reason for lower initial angiogenesis and bone regeneration outcome.

When we compared the result in the later healing phase, we found that angiogenesis was significantly higher with the use of collagen membranes compared to the control. That result is in line with data from Koerdt et al. [22], who found that in the augmentation model of sheep bone, the use of a bovine bone substitute and collagen barrier membrane improved vascularization of an autologous iliac bone graft in the later healing period. This finding could be explained by a lower metabolic demand of tissue in the control group, in which a barrier membrane was not used, so that the competing fibrous cells had access to the defect area. Moreover, we observed that the lowest bone formation after four weeks of healing was in the control group, which is in agreement with previous research of impaired bone production without membrane use [4].

Precisely with respect to results after four weeks of healing, the MG group showed significantly improved angiogenic and osteogenic response compared to other investigated groups. There are several potential reasons for this outcome. First of all, this collagen matrix has an open-pore structure on its lower surface, suitable for stem cell ingrowth [16]. Even though collagen matrices were primary constructed for soft tissue augmentation, according to the latest findings they are highly appropriate for bone forming cell adhesion, migration, proliferation and osteoblast differentiation [23]. Moreover, MG allowed for the highest percentage of cell penetration from the liquid platelet-rich fibrin, compared to BG and the bovine PC membrane [24]. Secondly, due to the highest thickness, it possesses the largest area of collagen strands available for absorption of various growth factors released from the deeper layers, after the initial burst release, over the next two weeks [25]. Although bone morphogenetic protein 2 is discharged at a low percentage in the early phase for the MG membrane, it has been shown that in the collagen–hyaluronic acid membrane more intensified discharge was noted between three and seven weeks, probably as a result of membrane degradation [25, 26].

Finally, after four weeks of healing, we found the presence of material-induced MNGC, which could be related to the material degradation process [27]. MNGC symbolize the syncytium of macrophages that could be pro-inflammatory (M1) or pro-regenerative (M2) [28]. Considering that a higher number of MNGC in the MG group is followed by the presence of intensified angiogenesis and bone production, it could be speculated that at least one part of these cells may have pro-regenerative potential. Moreover, a recent study showed a rather similar distribution of M1 and M2 macrophages after four weeks of soft tissue healing with MG, although without MNGC formation [29]. In addition, both types of macrophages can contribute to angiogenesis – M1 via vascular endothelial growth factor production in the initiation process, and M2 by releasing thrombocyte growth factor and matrix metalloproteinase responsible for vascular branching and maturation [30]. In our study, only the MG group showed significant angiogenesis enhancements with time, both in VD and size.

CONCLUSION

The use of collagen membranes significantly affects angiogenesis, reducing it in the early healing phase and enhancing it at a later one. All three tested membranes in combination with bone graft significantly improved the amount of regenerated bone. Among the investigated groups, MG favored more pronounced angiogenic, osteogenic, and inflammatory response in observation period of four weeks. Further studies with longer follow-up are needed to investigate whether this trend continues with time.

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REFERENCES

1. Sculean A, Stavropoulos A, Bosshardt DD. Self-regenerative capacity of intra-oral bone defects. J Clin Periodontol. 2019;46 Suppl 21:70–81. [DOI: 10.1111/jcpe.13075] [PMID: 30697789]
2. Arnal HM, Angioni CD, Gaultier F, Urbinelli R, Urban IA. Horizontal guided bone regeneration on knife-edge ridges: A retrospective case-control pilot study comparing two surgical techniques. Clin Implant Dent Relat Res. 2022;24(2):211–21. [DOI: 10.1111/cid.13073] [PMID: 35167184]
3. Sbricoli L, Guazzo R, Annunziata M, Gobbato L, Bressan E, Nastri L. Selection of Collagen Membranes for Bone Regeneration: A Literature Review. Materials (Basel). 2020;13(3):786. [DOI: 10.3390/ma13030786] [PMID: 32050433]
4. Turri A, Elgali I, Vazirisani F, Johansson A, Emanuelsson L, Dahlin C, et al. Guided bone regeneration is promoted by the molecular events in the membrane compartment. Biomaterials. 2016;84:167–83. [DOI: 10.1016/j.biomaterials.2016.01.034] [PMID: 26828682]
5. Solomon SM, Sufaru IG, Teslaru S, Ghiciuc CM, Stafie CS. Finding the Perfect Membrane: Current Knowledge on Barrier Membranes in Regenerative Procedures: A Descriptive Review. Appl Sci. 2022;12(3):1042. [DOI: 10.3390/app12031042]
6. You P, Liu Y, Wang X, Li B, Wu W, Tang L. Acellular pericardium: A naturally hierarchical, osteoconductive, and osteoinductive biomaterial for guided bone regeneration. J Biomed Mater Res A. 2021;109(2):132–45. [DOI: 10.1002/jbm.a.37011] [PMID: 32441432]
7. Omar O, Elgali I, Dahlin C, Thomsen P. Barrier membranes: More than the barrier effect? J Clin Periodontol. 2019;46 Suppl 21(Suppl Suppl 21):103–23. [DOI: 10.1111/jcpe.13068] [PMID: 30667525]
8. Filipowska J, Tomaszewski KA, Niedźwiedzki Ł, Walocha JA, Niedźwiedzki T. The role of vasculature in bone development, regeneration and proper systemic functioning. Angiogenesis. 2017;20(3):291–302. [DOI: 10.1007/s10456-017-9541-1] [PMID: 28194536]
9. Gruber R, Stadlinger B, Terheyden H. Cell-to-cell communication in guided bone regeneration: molecular and cellular mechanisms. Clin Oral Implants Res. 2017;28(9):1139–46. [DOI: 10.1111/clr.12929] [PMID: 27550738]
10. Peng Y, Wu S, Li Y, Crane JL. Type H blood vessels in bone modeling and remodeling. Theranostics. 2020;10(1):426–36. [DOI: 10.7150/thno.34126] [PMID: 31903130]
11. Yan ZQ, Wang XK, Zhou Y, Wang ZG, Wang ZX, Jin L, et al. H-type blood vessels participate in alveolar bone remodeling during murine tooth extraction healing. Oral Dis. 2020;26(5):998–1009. [DOI: 10.1111/odi.13321] [PMID: 32144839]
12. Saghiri MA, Asatourian A, Garcia-Godoy F, Sheibani N. The role of angiogenesis in implant dentistry part II: The effect of bone-grafting and barrier membrane materials on angiogenesis. Med Oral Patol Oral Cir Bucal. 2016;21(4):e526–37. [DOI: 10.4317/medoral.21200] [PMID: 27031074]
13. De Marco AC, Jardini MA, Lima LP. Revascularization of autogenous block grafts with or without an e-PTFE membrane. Int J Oral Maxillofac Implants. 2005;20(6):867–74. [PMID: 16392343]
14. Schwarz F, Rothamel D, Herten M, Sager M, Becker J. Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. Clin Oral Implants Res. 2006;17(4):403–9. [DOI: 10.1111/j.1600-0501.2005.01225.x] [PMID: 16907771]
15. Calciolari E, Ravanetti F, Strange A, Mardas N, Bozec L, Cacchioli A, et al. Degradation pattern of a porcine collagen membrane in an in vivo model of guided bone regeneration. J Periodontal Res. 2018;53(3):430–9. [DOI: 10.1111/jre.12530] [PMID: 29446096]
16. Ghanaati S, Schlee M, Webber MJ, Willershausen I, Barbeck M, Balic E, et al. Evaluation of the tissue reaction to a new bilayered collagen matrix in vivo and its translation to the clinic. Biomed Mater. 2011;6(1):015010. [DOI: 10.1088/1748-6041/6/1/015010] [PMID: 21239849]
17. Schwarz F, Rothamel D, Herten M, Wüstefeld M, Sager M, Ferrari D, et al. Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. Clin Oral Implants Res. 2008;19(4):402–15. [DOI: 10.1111/j.1600-0501.2007.01486.x] [PMID: 18324961]
18. Hwang JW, Kim S, Kim SW, Lee JH. Effect of Extracellular Matrix Membrane on Bone Formation in a Rabbit Tibial Defect Model. Biomed Res Int. 2016;2016:6715295. [DOI: 10.1155/2016/6715295] [PMID: 27047963]
19. Wang Y, Hua Y, Zhang Q, Yang J, Li H, Li Y, et al. Using biomimetically mineralized collagen membranes with different surface stiffness to guide regeneration of bone defects. J Tissue Eng Regen Med. 2018;12(7):1545–55. [DOI: 10.1002/term.2670] [PMID: 29691999]
20. Omar O, Dahlin A, Gasser A, Dahlin C. Tissue dynamics and regenerative outcome in two resorbable non-cross-linked collagen membranes for guided bone regeneration: A preclinical molecular and histological study in vivo. Clin Oral Implants Res. 2018;29(1):7–19. [DOI: 10.1111/clr.13032] [PMID: 28703398]
21. Basudan A, Babay N, Ramalingam S, Nooh N, Al-Kindi M, Al-Rasheed A, et al. Efficacy of Mucograft vs Conventional Resorbable Collagen Membranes in Guided Bone Regeneration Around Standardized Calvarial Defects in Rats: An In Vivo Microcomputed Tomographic Analysis. Int J Periodontics Restorative Dent. 2016;36 Suppl:s109–21. [DOI: 10.11607/prd.2261] [PMID: 27031625]
22. Koerdt S, Siebers J, Bloch W, Ristow O, Kuebler AC, Reuther T. Immunohistochemial study on the expression of von Willebrand factor (vWF) after onlay autogenous iliac grafts for lateral alveolar ridge augmentation. Head Face Med. 2013;9:40. [DOI: 10.1186/1746-160X-9-40] [PMID: 24330606]
23. Lin Z, Nica C, Sculean A, Asparuhova MB. Positive Effects of Three-Dimensional Collagen-Based Matrices on the Behavior of Osteoprogenitors. Front Bioeng Biotechnol. 2021;9:708830. [DOI: 10.3389/fbioe.2021.708830] [PMID: 34368101]
24. Al-Maawi S, Herrera-Vizcaíno C, Orlowska A, Willershausen I, Sader R, Miron RJ, et al. Biologization of Collagen-Based Biomaterials Using Liquid-Platelet-Rich Fibrin: New Insights into Clinically Applicable Tissue Engineering. Materials (Basel). 2019;12(23):3993. [DOI: 10.3390/ma12233993] [PMID: 31810182]
25. Nica C, Lin Z, Sculean A, Asparuhova MB. Adsorption and Release of Growth Factors from Four Different Porcine-Derived Collagen Matrices. Materials (Basel). 2020;13(11):2635. [DOI: 10.3390/ma13112635] [PMID: 32526991]
26. Chung EJ, Chien KB, Aguado BA, Shah RN. Osteogenic potential of BMP-2-releasing self-assembled membranes. Tissue Eng Part A. 2013;19(23–24):2664–73. [DOI: 10.1089/ten.TEA.2012.0667] [PMID: 23790163]
27. Tanneberger AM, Al-Maawi S, Herrera-Vizcaíno C, Orlowska A, Kubesch A, Sader R, et al. Multinucleated giant cells within the in vivo implantation bed of a collagen-based biomaterial determine its degradation pattern. Clin Oral Investig. 2021;25(3):859–73. [DOI: 10.1007/s00784-020-03373-7] [PMID: 32514904]
28. Miron RJ, Bosshardt DD. Multinucleated Giant Cells: Good Guys or Bad Guys? Tissue Eng Part B Rev. 2018;24(1):53–65. [DOI: 10.1089/ten.TEB.2017.0242] [PMID: 28825357]
29. Al-Maawi S, Rother S, Halfter N, Fiebig KM, Moritz J, Moeller S, et al. Covalent linkage of sulfated hyaluronan to the collagen scaffold Mucograft® enhances scaffold stability and reduces proinflammatory macrophage activation in vivo. Bioact Mater. 2021;8:420–34. [DOI: 10.1016/j.bioactmat.2021.06.008] [PMID: 34541411]
30. Spiller KL, Anfang RR, Spiller KJ, Ng J, Nakazawa KR, Daulton JW, et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds. Biomaterials. 2014;35(15):4477–88. [DOI: 10.1016/j.biomaterials.2014.02.012] [PMID: 24589361]

Различит ангиогени одговор и коштана регенерација после примене различитих врста колагених мембрана – *in vivo* хистоморфометријска студија на критичним дефектима калварије кунића

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САЖЕТАК

Увод/Циљ Успех вођене коштане регенерације зависи од величине и морфологије дефекта, карактеристика баријерне мембране и адекватне ангиогенезе.

Циљ ове студије је био да се открије утицај три структурално различите колагене мембране на ангиогенезу и коштану продукцију у дефектима критичне величине.

Методе Дефекти су направљени на калваријама кунића, попуњени говеђим коштаним графтом и насумично покривени једном од три испитиване колагене мембране (*Biogide – BG, Heart – PC, Mukograft – MG*) или остављени без мембране за контролу (К). После две и четири недеље зарастања укупно 10 животиња је жртвовано ради хистолошке и хистоморфометријске анализе ангиогенезе, коштане регенерације и инфламаторног одговора.

Резултати У раној фази зарастања највеће вредности дебљине и површине трабекула су забележене редом код *PC* и *BG* мембрана. После четири недеље значајно боље коштано зарастање је уочено у групи *MG*, као и значајно израженија инфламација. Густина крвних судова је иницијално била значајно већа у групи К у поређењу са све три мембране. После четири недеље значајно бољи резулати су примећени у групи *MG* у поређењу са осталим групама, у групи *BG* у поређењу са преосталим групама и између група *PC* и К.

Закључак Примена колагених мембрана значајно утиче на ангиогенезу, смањујући је у раној а подстичући је у каснијој фази зарастања. Све три испитиване мембране у комбинацији са коштаним графтом су значајно повећале количину регенерисане кости. Међу испитиваним групама *MG* је фаворизовао израженији ангиогени, остеогени и инфламаторни одговор у периоду посматрања од четири недеље.

Кључне речи: колагене мембране; ангиогенеза; вођена коштана регенерација; колагени матрикс; перикард