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Use of Collagen, PTFE and PRF Membranes in Bone Reconstruction an Experimental and Histomorphometric Study

Ioana Irina Neculae[1], Vlad Marian Anghelescu[1], Sabina Andrada Zurac[2], Octavian Marius Dinca[1], Cristian George Vladan[1], Alexandru Bucur[1]

Corresponding author:
Ioana Irina Necolae, DMD DDS
Medical Department of Oro-Maxillo-Facial Surgery
"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
E-mail: ioana_neculae84@yahoo.com

[1]Medical Department of Oro-Maxillo-Facial Surgery,
Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[2]Medical Department of Pathology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Bone regeneration techniques cannot be done without barrier membranes, even if horizontal or vertical ridge augmentation and socket ridge preservation is taken into consideration. This study presents a comparison between outcomes of bone regeneration, after producing standardized bone defects followed by covering them with membranes, on an animal experimental model. The study was conducted on 18 New Zeeland rabbits, by creating 2 defects in the left tibial bone of each rabbit: one standardized defect with a diameter of 4 mm, and the second by creating 5 monocortical holes with a small round bur. The defects were augmented with bovine bone, beta-tricalcium phosphate and perioglass and they were covered with 3 types of membrane: collagen (12 defects - group A), PTFE membrane (12 defects - group B) and PRF membrane, made from the blood of the same rabbit (12 defects – group C). The animals were sacrificed after 6 months and analysed histomorphometrically. The new bone around graft particles has a thickness of 98.26 µm for collagen membrane, 49.19 µm for PTFE membrane and 63.98 µm for PRF membrane. The density of osteoblasts and osteocytes has an average of 0.0012 for collagen membrane, 0.0009 for PTFE membrane and 0.0010 for PRF membrane. Regarding the collagen membrane, it is observed that when used the bone regeneration appears to have a higher density of osteoforming cells and a higher quantity of new bone

Key words: barrier membrane, bone regeneration, prf membrane, ptfe membrane, collagen membrane

INTRODUCTION

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Reconstruction of bone defects of the upper and lower jaws is one the most challenging interests for oral and maxillofacial surgeons. (1) Detailed knowledge of the process which leads to such bone defects and awareness of several bone augmentation and bone regeneration techniques will improve the final results. Whether it involves horizontal or vertical ridge augmentation or socket ridge

preservation, guided bone regeneration cannot be done without the use of barrier membranes. (2,3) The barrier membranes allow a better integration of bone grafts on the receptor site, because of their role of maintaining a certain distance between the graft and the overlying soft tissues. (4,5)

This study presents o comparison between outcomes of bone regeneration, after producing standardized bone defects followed by covering them with membranes, on an animal experimental model. Advantages and disadvantages of resorbable and non-resorbable membranes have been discussed in detail for a very long time among surgeons. PRF (platelet rich fibrin), used by Choukroun in 2004, seems to bring a great benefit in GBR (guided bone regeneration) and GTR (guided tisular regeneration) techniques. (6) Yet, the short time of resorption of PRF seems to limit the use of these membranes, as the only membranes in bone regeneration. (7)

MATERIALS AND METHODS

This study involved 18 New Zeeland white rabbits (9 male and 9 female) with an average of 6 months of age and an average weight of 2.5 kg, from "Cantacuzino" National Institute of Immunologic and Microbiologic Research, Bucharest. The animal study protocol was approved by the Ethical Committee of Animals from Bucharest. The animals were housed in special rooms (temperature 18ºC – 24ºC, humidity 50% - 70%, and a 12 hours light/dark cycle) and fed with a standard diet. The animals were randomly divided in three groups (Group A, Group B and Group C) depending on the used barrier membrane. The animals were sacrificed after 6 months using 200 mg/ml IV Phenobarbital. The bone defects were produced in the left tibial bone. The membranes used were: collagen membrane (Collprotect membrane – Botiss®), d-PTFE membrane (Cytoplast TxT 200®) and PRF membrane obtained from the blood of the experience animal. The animals were anesthetised with 10 mg/kg xylazine and 50 mg/kg ketamine.

Platelet rich fibrin preparation method

For our experiment 10 ml of venous blood were obtained from the central vein of the ear and was centrifuged for 13 minutes at 3000 rpm. After the centrifugation, the blood was separated in three layers, the inferior layer (erythrocytic mass) and the top layer consisting of acellular platelet poor plasma were removed. The PRF clot in the middle was kept and compressed to obtain a membrane. (*fig. 1*) This membrane was used to cover 12 bone defects.

Figure 1 - PRF Membrane

Surgical procedure

After the general anaesthesia, the left tibial area was shaved and the skin was sterilized with povidone iodine solution. Using a 5 cm incision of skin, the subcutaneous and muscular tissues were dissected and the periosteum was removed from the bone. Two type of defects were created: one defect was a monocortical standardized defect made with a 4 mm diameter threphine burr under irrigation with saline solution, and the other defect, within a distance of 3 mm, was made by drilling 5 monocortical holes with a small round burr. (*fig. 2*) The defects were augmented with three different materials: bovine bone (Biooss® – Geistlich Biomaterials, Switzerland, 0.25 – 1 mm), beta-tri-calcium phosphate (40%) with hydroxyapatite (60%) (4 Bone BCH® – Mis, 0.5 – 1 mm) and Perioglass® (Novabone). (*fig. 3*) 12 defects were covered with collagen membranes (4 defects for each grafting material), 12 bone defects were covered with PTFE membrane and 12 defects were covered with PRF membranes from the same rabbit. After placement of the membranes suture was performed in three layers (periosteum, muscular tissue and skin). Postoperatively we administered intramuscular ketoprofen for 3 days.

We monitored the surgical wound for two weeks.

The animals were sacrificed after 6 months using Phenobarbital IV.

The left tibia was harvested and fixed with formaldehyde 10%.

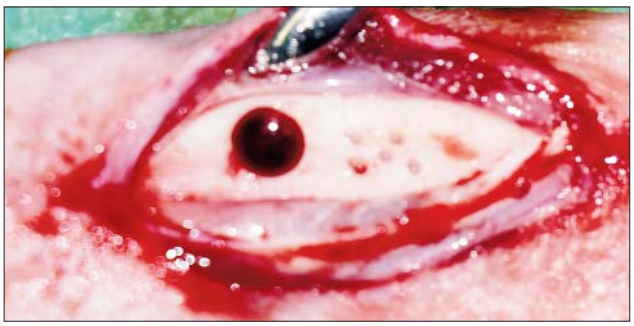

Figure 2 - The two types of bone defects

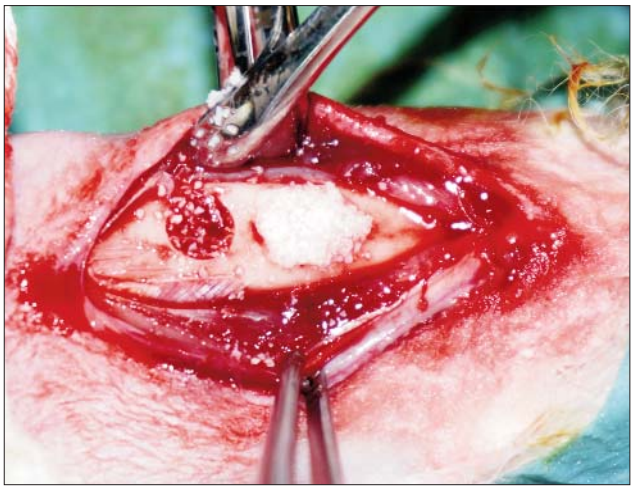

Figure 3 - The defects augmented with Biooss

Histomorphometric evaluation

After fixation of each specimen in formaldehyde 10%, they were decalcified by using formic acid for 20 days, the solution being changed constantly. After decalcification, we harvested the areas of interest and continued with the histopatological evaluation. The specimens were introduced in paraffin blocks, then using a microtome several serial cross sections were made. The sections were fixed in haematoxylin-eosin and evaluated under the microscope (Olympus xc30 Optical Microscope). We evaluated new bone formation alongside the bone grafts, which was then measured and analysed. We also counted the number of osteoblasts and osteocytes from the areas of new bone (by use of Olympus CellSens Dimension). The results were compared depending on the barrier membranes. For statistical analysis we used SPSS program, version 24, from IMB.

RESULTS AND DISCUSSION

The rabbits were divided into three groups: group A, with collagen membranes, group B, with d-PTFE membranes and group C with PRF membranes. The thickness of the new bone formed around the particles of the augmentation materials had an average of 98.26 µm for collagen membranes, 49.19 µm for d-PTFE membranes and 63.98 µm for PRF membranes. (*table 1*) The difference observed in non-resorbable membranes was a secondary phenomenon, by a small dehiscence which appeared in two rabbits of group B. This dehiscence permitted the infiltration of fibrous tissue, the membrane presenting with a degree of porosity. (*fig. 4*)

These differences are the same in regards to the cellular density (of osteoblasts and osteocytes) with an average of 0.0012 for collagen membranes, 0.0009 for d-PTFE membranes and an average of 0.0010 for PRF membranes. (*table 2*) The greater density of osteoblasts around the grafts particles showed a higher new bone apposition in that area. (*fig. 5*)

The differences between the two types of barrier membrane remained at a constant measure regardless of the type of grafting material that was used. A greater thickness of the newly formed bone was observed around the particles of bone graft and an increased cell

Table 1 - Statistical analysis of new bone thickness around particles of graft augmentation materials

	N	Minimum	Maximum	Mean	Std. Deviation
The thickness of new bone for collagen membrane	44	10.20	363.80	98.2664	85.53107
The thickness of new bone for d-PTFE membrane	40	11.70	128.90	49.1930	29.07121
The thickness of new bone for PRF membrane	60	9.10	391.60	63.9883	55.55623
Valid N (listwise)	40				

Table 2 - Statistical analysis of density of osteoblasts and osteocytes form the new bone areas around particles of graft augmentation materials

	N	Minimum	Maximum	Mean	Std. Deviation
The density of cells for collagen membrane	11	.000814	.002411	.00126682	.000485137
The density of cells for d-PTFE membrane	13	.000280	.001787	.00091092	.000368236
The density of cells for PRF membrane	15	.000737	.001432	.00107593	.000201207
Valid N (listwise)	11				

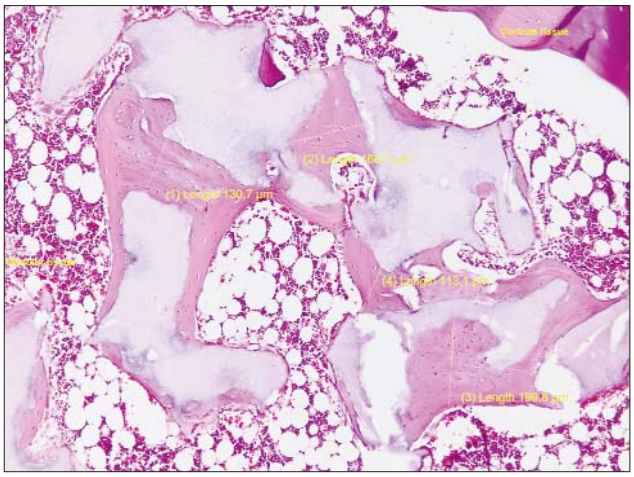

Figure 4 - Transversal section of rabbit tibia: area of augmentation with beta-tricalcium phosphate and collagen membrane. In the medullar bone tissue new bone around graft particles is shown

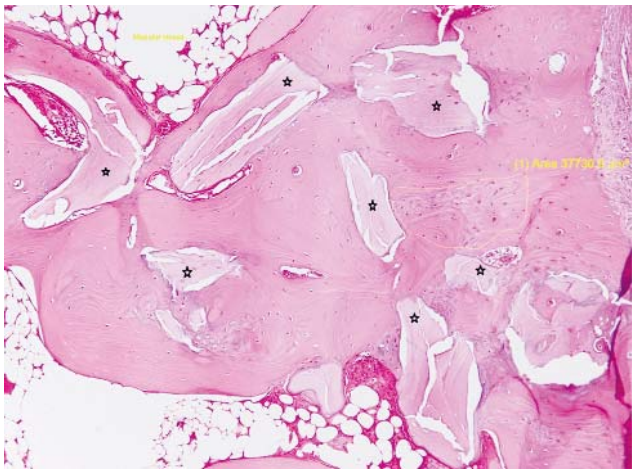

Figure 5 - Transversal section of rabbit tibia: area of Biooss augmentation and PRF membrane. In the cortical bone, it can be noted that the particles of Biooss (black stars) have integrated in an area of new bone. This area presents a high density of osteoblasts and osteocytes

density of osteoblasts for collagen membrane as compared to other types of membranes. Although three different types of bone augmentation materials were used, they did not influence the results.

A great variety of animal species are included in studies which concern bone regeneration in oral and maxillofacial surgery. (8) However, rats and rabbits are frequently used due to their easy handling, compared with other species, being also less expensive. (9) We used rabbits in our study because this species allows enough blood harvest to prepare a PRF membrane. The calvaria and tibia are the most used areas for bone regeneration studies. (10,11)

Barrier membranes are tested in a lot of experimental studies of bone regeneration and they are widely used in clinical therapy. (12-22) The placement of a barrier membrane promotes bone formation because it does not allow non-osteogenic soft tissue to infiltrate in the bone defect.

The d-PTFE membranes – polytetrafluoroethylene is a non-resorbable type of membrane, 100% dense with a 0.3 µm pore size. A second surgery is needed in order to remove it. (*fig. 6*) (23-25)

Resorbable membranes are easier to use, but we cannot manage the time of their resorption and the effect that degradation has on the bone formation. (26, 27) The most important collagen membrane is Bio-Guide. It is a porcine collagen type I membrane, with a bilaminar structure, one dense layer and one porous layer. The dense layer has a polished surface which prevents the infiltration of epithelial cells in the bone defects, meanwhile, the porous surface enables the

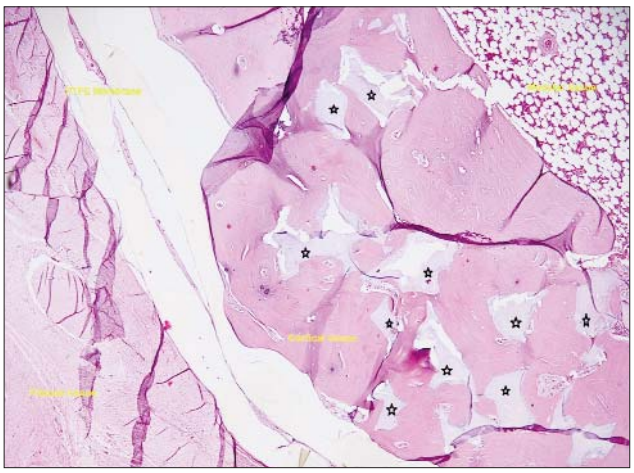

Figure 6 - Transversal section of rabbit tibia: area of beta-tricalcium phosphate and d-PTFE membrane. In the cortical bone there are particles of beta-tricalcium phosphate (black stars) and around them there is a high density of osteocytes

integration of the membrane in the tissues. (28)

PRF was developed by Choukroun in 2000, especially for usage in oral and maxillofacial surgery. Compared with PRP (platelet rich plasma), which is blood plasma that has been enriched with platelets, obtaining PRF is easier and it doesn't resorb as quickly. Growth factors from PRF are slowly released because of the fibrin matrix. (29) In a recent study regarding PRF, three different principal growth factors were found: TGFß-1, PDGF and VEGF, who remained in the PRF membranes for 7 days, because of the dense structure of fibrin and slow release into the tissues. (30) The structure of fibrin offers a bio-skeleton for cellular migration. (31,32)

In the present study it is important to mention that the membranes were used over bone defects created ad hoc. Bone regeneration is activated by the release of growth factors. The bone matrix is considered to be one of the richest source of growth factors, produced by osteoblasts and other cells. (33-35) The bone matrix is directly exposed to bone fractures, osteotomies, dental implant placement and other bone defects. Histologically, cellular activation is the expression of neo-angiogenic stimulation, recruitment of osteoblasts and new bone formation. We can influence this process by using a barrier membrane, so we can concentrate the growth factors in the bone defect, and the bone defect is more quickly repaired. (36) Besides osteo-precursor cells, bone formation needs two things: blood supply and solid base for bone neo-apposition. (37) The pathway of bone regeneration in bone defects covered with membrane always starts from the bone walls towards the centre of the defect. (38)

CONCLUSION

In the present study it can be noted that bone regeneration differs depending on the type of barrier membranes used. There is an advantage of collagen membranes compared to non-resorbable d-PTFE membranes. The use of PRF membrane in bone augmentation techniques seems to bring a certain benefit. Coverage of bone defects with barrier membranes create a favourable environment for bone regeneration, because they create a space where osteogenic cells can migrate, and they prevent the non-osteogenic cells from the soft tissues to penetrate the bone defect. The membranes also represent a support for the soft tissues, preventing them from collapsing within the bone defect.

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