Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev. Chim.1949

The Effect of Type 1 Atellocolagen in Association with Blood Self-derivatives in Alveolar Bone Augumentation

BOGDAN-CATALIN ALEXANDRU[1], MONICA POPA[1], COSMIN PESTEAN[2], ROBERT PURDOIU[3], LIVIU OANA[4], ANNE-MARIE CONSTANTIN[5], RAMONA AMINA POPOVICI[6*], ALINA-SIMONA SOVREA[5], CARMEN GEORGIU[7*], GABRIELA DOGARU[8]

[1]"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Medicine, Discipline of Medical Hygiene, 8 Victor Babes Str., 400012, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine, Department of Anesthesiology and Intensive Care, 3-5 Calea Manastur Str., 400372, Cluj-Napoca, Romania
[3]University of Agricultural Sciences and Veterinary Medicine, Department of Radiology, 3-5 Calea Manastur Str., 400372, Cluj-Napoca, Romania
[4]University of Agricultural Sciences and Veterinary Medicine, Department of Surgical Pathology, 3-5 Calea Manastur Str., 400372, Cluj-Napoca, Romania
[5]"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Medicine, Discipline of Histology, 8 Victor Babes Str., 400012, Cluj-Napoca, Romania
[6]"Victor Babes" University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Oral Health and Management, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania
[7]"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Medicine, Discipline of Pathology, 8 Victor Babes Str., 400012, Cluj-Napoca, Romania
[8]"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Medicine, Discipline of Medical Rehabilitation, 8 Victor Babes Str., 400012, Cluj-Napoca, Romania

Abstract: *The current experimental study was conducted in sheep, which present bone anisotropy and a predisposition to periodontal disease, with alveolar bone resorption, similar to that found in humans. In this study, alveolar bone augmentation was performed using a lyophilized bovine bone xenograft enriched with type 1 atelocollagen, which was combined with autologous platelet-rich plasma (PRP) and advanced platelet-rich fibrin (A-PRF) as a membrane. The results were radiologically and histologically evaluated at six weeks postoperatively. At that time, the transformation of the composite biomaterial was clearly visible, suggesting that the regeneration process started from the periphery of the augmentation mass, which was progressively transformed from a granular eosinophilic material into an undifferentiated hypercellular one, then into fibroblastic, cartilaginous tissue, and finally into new bone and desmodontal-like tissue. This process was most probably induced by all the compounds used, the growth factors found in autologous blood derivatives, including bone morphogenetic proteins (BMPs), as well as type 1 atelocollagen from the graft composition, especially in combination. The composite biomaterial at six weeks postoperatively provided excellent results regarding alveolar bone regeneration, and without any risk, as opposed to that found in overdose of recombinant growth factors.*

Keywords: *PRP, A-PRF, BMP, sheep, bone augmentation, alveolar bone*

1.Introduction

Alveolar bone destruction is one of the main causes of tooth loss, with many different etiologies, from acute or chronic trauma to periodontal disease, with its complex pathogenesis related to local factors or systemic diseases. Moreover, alveolar bone destruction always results in tooth loss, and tooth loss, regardless of the cause, is followed by complete and irreversible alveolar bone resorption.

**email: ramona.popovici@umft.ro; carmen.georgiu@hotmail.com*

Particular attention is now paid to preparations used for guided alveolar bone regeneration, different preparations having already been used over time. Xenografts are biomaterials with a bone augmentation role and are represented by lyophilized bone masses belonging to a different species. Among them, xenografts of bovine origin were generally considered as the equivalents of autografts [1]. They usually undergo physiological osteoclast and osteoblast remodeling [1], the grafts being minimally resorbed during the healing period [2]. In this study, a bovine xenograft with a macroporous structure enriched with type 1 atelocollagen was used. Macroporous structures are ideal in osteoconduction, favoring vascular proliferation within the augmentation mass. On the other hand, atelocollagen stimulates platelet aggregation and favors growth peptide release, also with an osteoconduction effect.

Growth factors are essential for tissue healing and nowadays there is strong evidence regarding the high levels of growth factors released by autologous blood derivatives, particularly by platelets and white cells. Autologous blood derivatives are usually in the form of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), obtained by centrifugation of autologous blood, and have, in addition to regenerative and anti-inflammatory effects, a demonstrated antibacterial effect. They act especially against periodontal germs such as Porphyromonas gingivalis and Aggregatibacter actino-mycetemcomitans, which are found in large amounts in the periodontal exudate of periodontopathic patients [3]. These blood derivatives are usually obtained immediately before the bone augmentation procedure, by different centrifugation protocols (according to the indications of the manufacturers), in test tubes containing different components.

PRP usually contains a pool of growth factors effective in osteogenic induction, proliferation and differentiation [7]. Studies demonstrate the role of PRP in bone regeneration [8-12], through the increased content of various growth factors and other cytokines (potentiating bone morphogenetic protein (BMP) synthesis and subsequently, cell proliferation) [7,13]. The therapeutic potential of PRP is dependent on dose [14] and local pH [15]. It is injectable due to the added anticoagulant that is most often represented by heparin, as well as sodium citrate. As for PRF, an advanced platelet-rich fibrin (A-PRF) form, it contains: slow-release cytokines, full natural fibrin, monocytes, granulocytes and plasma proteins, with long-term release of autologous BMP-2. A-PRF fraction also has an increased content of platelets, neutrophils, subfamilies of leukocytes, as well as red blood cells and exhibits an increased amount of stem cells [4], and more growth factors. Of autologous blood derivatives, PRP and A-PRF are considered to be among the easiest to obtain and most efficient preparations used in tissues [4-7,13]. Consequently, they were used in the current study, PRP in combination with a bovine xenograft enriched with type 1 atelocollagen and A-PRF as a membrane.

The animal species chosen for this experimental study was represented by sheep, which have already been used in many dental research fields such as periodontal wound healing [17], endodontic regeneration [18], stem cell therapy in bone regeneration [19], implant studies [20]. The experimental sheep model of chronic marginal periodontitis is preferred because of its similarities to humans. It is known that the main bacterial species involved in human chronic marginal periodontitis are Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Tannerella forsythia and Aggregatibacter actinomycetemcomitans [22,23]. A number of studies have reported similarities between ovine and human periodontal flora, containing: Treponema [24], Bacteroides melaninogenicus/Prevotella [25,26], Bacteroides gingivalis [27]. This bacterial colonization and the natural evolution of periodontal disease in sheep are the main arguments for using this experimental sheep model of chronic marginal periodontitis. However, similarly to the human species, individual variations in the extension and severity of periodontal disease may complicate the response to healing in sheep [21].

The aim of this study was to assess the efficiency in guided alveolar bone regeneration in sheep of a composite system, consisting of a bovine xenograft enriched with type 1 atelocollagen and combined with PRP and A-PRF used as a membrane, in the context of chronic marginal periodontitis.

2.Materials and methods

The study was conducted based on the approval of the Ethics Committee no. 23 of 23.01.2015, issued by "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania.

This experimental study included three 5-year-old sheep (Ovis aries, Romanian Turcana breed), non-pregnant multiparous females, with a weight ranging between 40-60 kg, without cardiac diseases or other organic disorders, which had chronic marginal periodontitis and received periodontal treatments, including bone augmentation. In order to avoid the suffering of the animals, general anesthesia was used before any examination or intervention on the dental bone and periodontal structures.

Periodontal status [28] and the probing depth were evaluated with an 11 mm periodontal probe with a tip diameter of 0.5 mm, and tooth mobility was assessed using a dental clamp and the same graded periodontal probe. At the same time, the calculus and bacterial plaque index was evaluated, as well as bleeding on probing and the degree of tooth mobility.

The post-evaluation treatment was multimodal. First, antibiotic therapy was applied, which consisted of subcutaneous injection of 1 mL Amoxicillin Retard 20%/10 kg live body weight, for five days, starting 12 h before initiation of local treatments. This was followed by piezoelectric ultrasonic supragingival scaling, performed using a flat loop with a spatula-shaped active portion [29], with moderate intensity, in order to avoid destruction of the enamel prisms and root cementum in the coronal third, given the animals' tooth morphology. Then, closed field periodontal curettage and root planning were performed. Periodontal curettage was performed in the presence of periodontal pockets diagnosed by periodontometry, inflammation, dental calculus and bleeding on probing. It was carried out manually, using Gracey curettes number 1/2, 3/4, 5/6 [29], with a 3 mm longer passive portion, in order to easily access pockets deeper than 5 mm [28]. Regarding the active portion, this has a sharp surface that acts on the root cementum, as well as a round surface positioned in the opposite direction, which protects the adjacent areas. With the same curettes, the granulation tissue of the inner epithelium of periodontal pockets was also curetted.

Periodontal bone augmentation was aimed at regenerating superficial and deep bone and ligament tissue; for this purpose, bone grafting was performed with lyophilized bovine bone (Hypro-Oss), incorporating type 1 atelocollagen, modified to non-immunogenic atelocollagen 30% and hydroxyl-apatite 70%, with organic matrix containing small molecules of proteoglycans and non-collagen proteins [30], and having a macroporous structure.

Platelet-rich plasma PRP containing thrombocytes and white blood cells in a proportion of 99.9% was obtained by centrifugation of the test tubes with autologous blood at a speed of 3500 rotations per minute for five minutes (XC-2000 centrifuge). The presence of thixotropic gel is mandatory for the adequate separation of white blood cells from erythrocytes; anticoagulant must also be present. The xenograft was combined with PRP [4,11], and subsequently applied to the surgical site, to a recipient dental bone bed (application to the bone as well as to the root cementum was performed).

The membrane was made of A-PRF, obtained by centrifugation of the test tubes at a speed of 1500 rotations/min, for 10 min in the XC-2000 centrifuge (low-speed centrifugation concept). The test tubes can be simple or with fibrin addition. Following centrifugation, the platelet clot was extracted, which was pressed and thus the membrane was obtained; this was cut and placed so as to cover the entire xenograft combined with PRP, with the margins exceeding the grafted bed, in order to penetrate under the periosteum adjacent to the surgical site.

Guided tissue regeneration was carried out with a coronally displaced flap and deepening of the vestibular sulcus. The intervention was strictly performed in the lower front region. Incision of the sulcus was made with a periodontal scalpel, and the mucoperiosteum was detached up to the vestibular cul-de-sac and up to 5 mm in the lateral areas.

After the periodontal bone defect was uncovered, vestibuloplasty was performed [29] with an incision of the lip and cheek mucosa, at 8 mm distance from the insertion of the fixed mucosa. The incision was horizontal, and did not exceed the vestibular chin area (adaptation of Kazanjian's

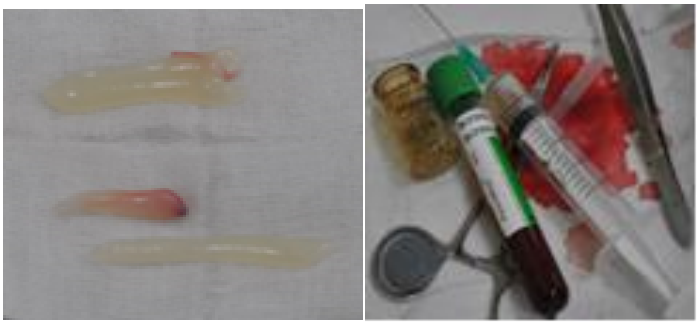
method). Subsequently, two symmetrical Z-shaped discharge incisions were made, which allowed displacing the flap in coronal direction, avoiding in this way the tensions exerted on the flap during achievement of lower lip functionality (a technique adapted to the anatomical structures of sheep).

After evidencing the root portions without bone support, following mucoperiosteal flap detachment, the root surfaces were washed with citric acid, sterile physiological serum, and with PRP. Then, the bovine xenograft enriched with type 1 atelocollagen, previously combined with PRP, was applied**,** and next, the A-PRF membrane was positioned**,** exceeding the augmentation area by about 5 mm. The membranes did not require suturing or fixation with pins. The mucoperiosteal flap was placed over the augmentation area, being pulled coronally and sutured in the desired position; subsequently, the incision of the cul-de-sac can be extended if needed, to ensure the absence of traction of the flap. Resorbable sutures were placed in order to avoid additional sedation.

The euthanasia protocol was introduced after six weeks. The specimens for histological examination were collected by sectioning the chin with the entire front teeth group. The dental-periodontal structures were reexamined during the sample taking session, on which occasion the absence of calculus and bacterial plaque deposits was found. All the histological slides were stained with hematoxylin-eosin.

3.Results and discussions

The post-centrifugal result for A-PRF and PRP respectively according to the techniques described above is that of (Figure1 a. A-PRF b.PRP).



a b

Figure 1. a. Image showing the color difference between the A-PRF clot in the middle and PRF; b.The test tube used for PRP preparation (Plasmolifting), the container in which HIPRO-OSS was stored, and the syringe in which autoplasma was collected

The results were histologically evaluated at six weeks postoperatively. The biomaterial was partially transformed, simultaneously presenting areas of hypercellularity, cartilage, new bone and desmodontal-like tissue.

The "transformation" into new bone started in the deepest portion of the augmentation area, at the contact point with the mesenchymal tissues of the host. From there, fibrocartilaginous neoosteogenesis progressively replaced the biomaterial, very similarly to what is usually observed in a callus formation. At this point, six weeks postoperatively, the process was not fully completed, but the new bone formation was sufficiently advanced to reveal details of the process of neoosteogenesis. Small amounts of granular biomaterial were still present (Figure 2 a), but also, next to them, small areas of hypercellular biomaterial, with numerous small undifferentiated mesenchymal cells or stem cells (Figure 2 b, c), and larger areas of more differentiated tissues such as collagen fiber-secreting fibroblasts (Figure 2 d, e), fibrocartilage (Figure 2 f), cartilage (Figure 2 g) and also small new bone structures (Figure 2 h) were found. The more differentiated ones were in direct contact with the host tissues, meaning the remnants of original alveolar bone, desmodontium and gingival fibromucosa.

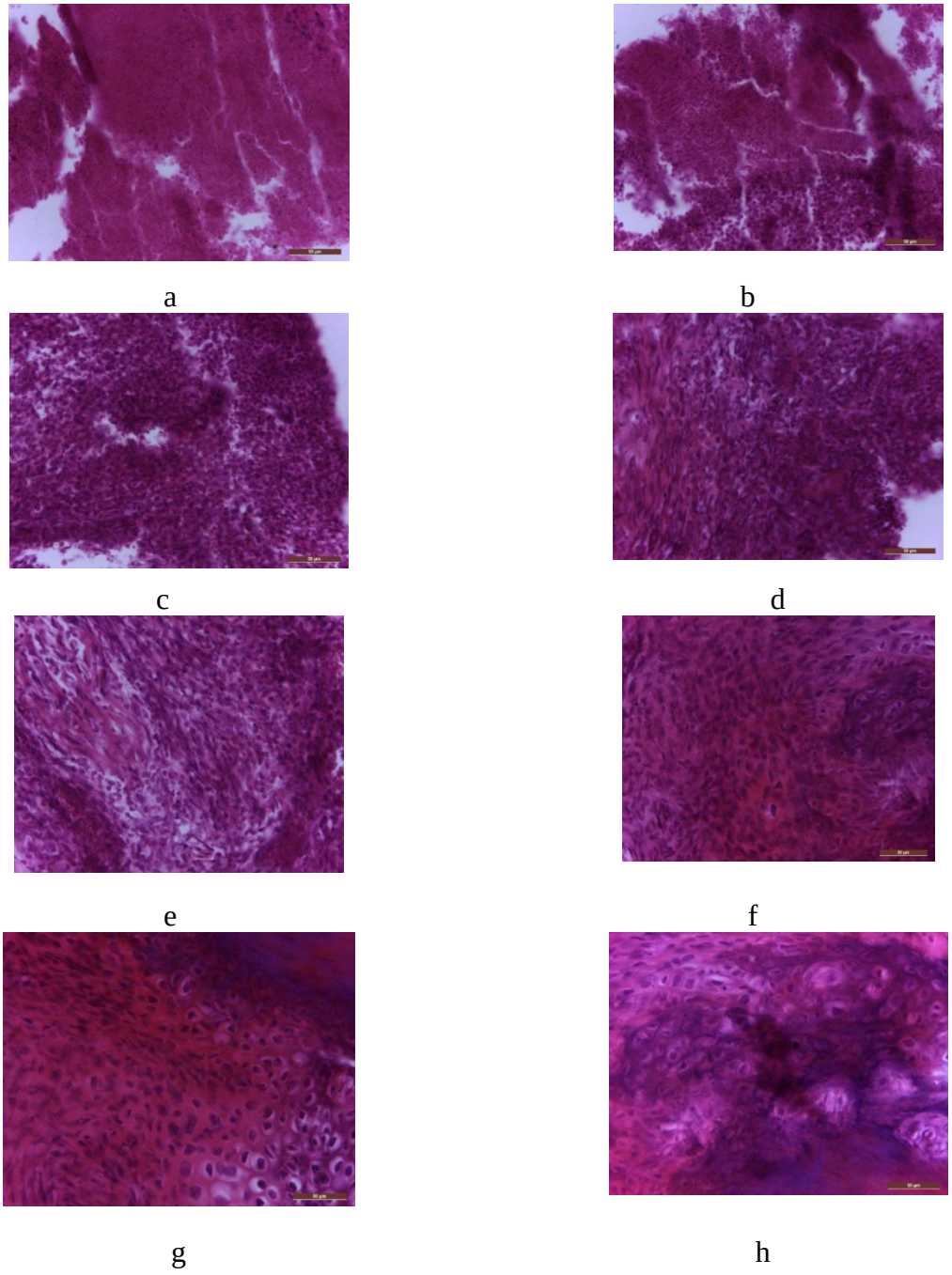


Figure 2. Detailed representation of the transformation of the biomaterial into bone structure, at six weeks postoperatively: a. Non-transformed, fine, granular, eosinophilic hypocellular biomaterial; b. Transformation of the biomaterial, with variable cellularity, representing the first stage of transformation; c The biomaterial with increased cellularity, consisting of numerous undifferentiated mesenchymal cells, with a small amount of cytoplasm and small basophilic nuclei; d. The first fibroblast differentiations of the biomaterial, with fusiform fibroblast cells with a fascicular arrangement, but also interspersed with undifferentiated cells; e. Organization in fascicles of collagen fibers (highly eosinophilic) secreting fibroblasts; f. Fibrocartilaginous transformation, the first chondroblasts being visible in the right upper part of the image; g. Cartilaginous island (right lower corner of the image) inside fibrocartilage; h. Extremely basophilic (calcified) organizing bone structure (in the center of the image), inside fibrocartilaginous tissue. Hematoxylin-eosin staining

The location of all these transformations was directly related to the location of the composite biomaterial used for bone augmentation present around the vestibular parts of the treated roots. Bone augmentation appeared as fibrocartilaginous ossification in the coronal part of the residual alveolar bones (Figure 3 a, b), between the bone and the gum, or also covering the desmodontium and the root, after more extensive alveolar bone resorption. However, all stages of ossification, fibrous, fibrocartilaginous (Figure 3 c, d) or fully differentiated bone trabeculae were present (Figure 3 e, f).

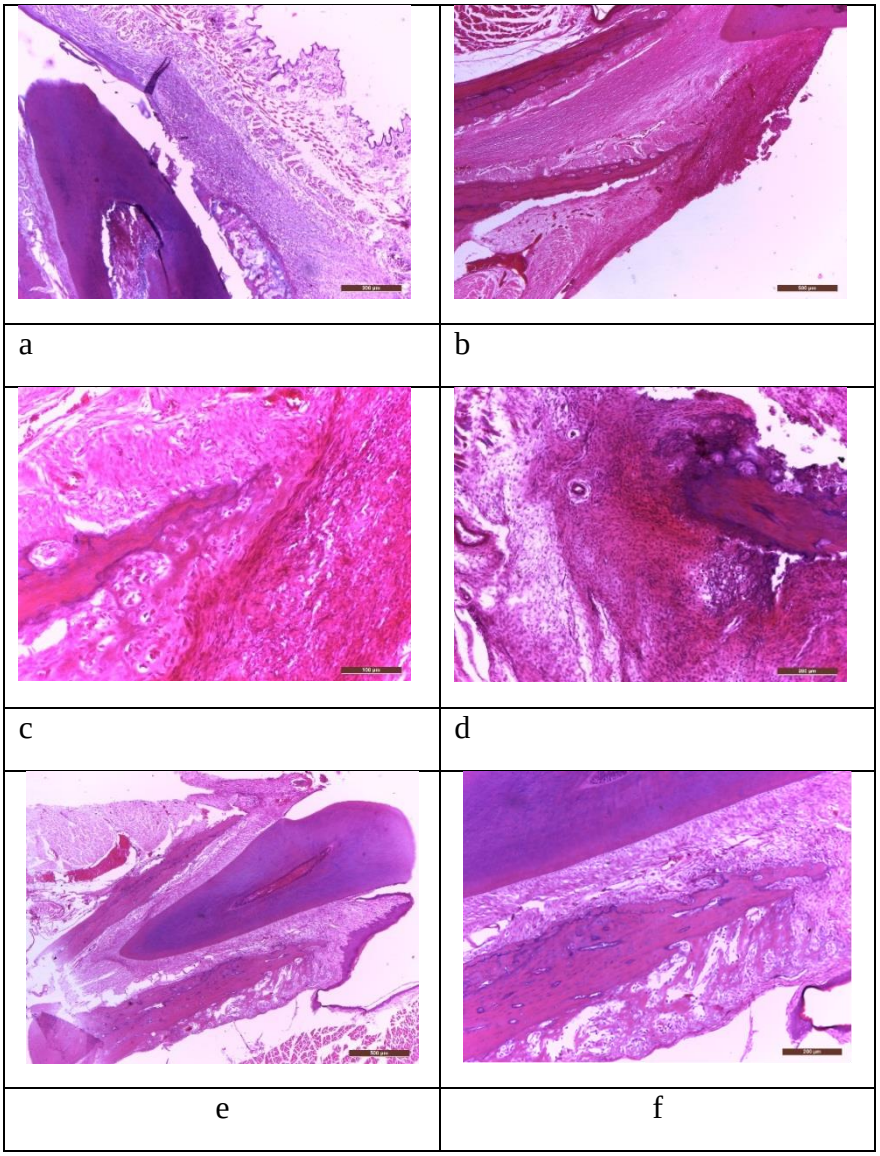


Figure 3. The location of the biomaterial and of its end results, according to the elements of the periodontal region: a. Tooth, alveolar bone and hypercellular biomaterial between the bone and the gum; b. Hypercellular biomaterial covering the rests of the resorbed alveolar bone, and of the desmodontium and the root; c. Desmodontium, initial alveolar bone, fibrocartilaginous growth on one side of the initial bone and fibroblastic area; d. Initial alveolar bone surrounded by extensive fibrous and fibrocartilaginous tissues; e. General appearance of a tooth in oblique-longitudinal section, along with the periodontium (gingiva, desmodontium, alveolar bone), which shows on the surface of the alveolar bone (lower part of the image) a new bone forming area, with fine bone trabeculae, perpendicular to the initial bone, representing the bone augmentation performed by the biomaterial. f At a larger magnification, the same tooth, desmodontium, initial alveolar bone, and new, bone trabeculae. Hematoxylin-eosin staining.

As this study confirms, the animals included in the study (sheep) can be successfully used in cellular experimental studies, and particularly in the study of periodontal therapy, representing a predictable model for human clinical studies [36,37]. Moreover, in the chosen sheep, at the age of five years, the presence of chronic marginal periodontitis as well as the guided bone regeneration were more conclusive, considering the bone isoform and the calcium concentration that decreases [38] with aging; calcium concentration increases until two years of age, after which it starts to decrease [38]. Physiologically, this species is known to have increased tooth mobility (equivalent to grade II tooth mobility in humans), with much more elastic ligaments and a much larger desmodontal space. This is why dento-dental immobilization was not chosen, which did not prove to be unfavorable regarding the evaluation of results and bone formation.

The success of the treatment presented here was mainly the result of the composite material used for bone augmentation, meaning all the compounds themselves, but especially in combination. Firstly, the macroporous material used as a xenograft potentiates the growth of blood vessels towards the inside of the structure. Secondly, atelocollagen stimulates and accelerates the formation of new bone tissue by triggering platelet aggregation, degradation and release of growth peptides responsible for osteoinduction. Furthermore, it was already emphasized that alkaline phosphatase is more active after 12 days in the presence of atelocollagen, which makes it more useful for oral and maxillofacial bone reconstruction [31]. Also, atelocollagen in contact with fibrin shows a much higher resistance, and inflammatory cell accumulation in the presence of atelocollagen is significantly lower [32].

Regarding PRP, it confers an angiogenic role to the composite material [33], and promotes collagen synthesis [6, 33,46] and bone formation. It suppresses chondrocyte apoptosis and improves transplanted cell integrity, regulates cellular differentiation, and modulates growth factors, including BMP2 [34], which contribute to osteogenic induction, proliferation and differentiation [16,34,35]. It also contributes to stem cell osteogenesis [7], establishes a balance between adipogenesis and osteogenesis, induces a significant increase in cell proliferation without affecting differentiated cells. The amount of BMPs in PRP varies with the pH (4), which is dependent on platelet lysis in buffer systems, which in turn influence the potentiators of BMPs, explaining the various release mechanisms of BMPs from PRP, such as: activation of the therapeutic potential of muscle-derived stem cells, which express BMP4 [6], as well as activation of the therapeutic potential of adipose tissue-derived stem cells, which express BMP2 [34], due to the high DNA content.

Moreover, the A-PRF used in this study is a blood cell concentrate, which contains a multitude of growth factors. It can be used as a mass for the augmentation of hard tissues both alone and combined with other bone augmentation materials [39]. The new A-PRF protocol, based on the low-speed centrifugation concept, is the most powerful concept as it combines the fibrin network, the growth factors from platelets and the white cell activity for only one goal: getting the more rapid and thorough vascularization of the local tissues [4,5,6]. The A-PRF protocol proposes the best composition for the healing cascade: slow-release cytokines, full natural fibrin, monocytes, granulocytes and plasma proteins, with long-term release of autologous BMP-2. A-PRF also presents a higher osteogenic potential [16,40] compared to PRF and PRP. This again is due to the concept of low-speed centrifugation, which produces a uniform distribution of cells in the clot, as well as to the presence of a higher amount of growth factors and a wide range of over 100 bioactive molecules, the most notable of which are PDGF, VEGF, IGF, EGF, TGF-ß and BMP2 [4,16]. It also contains more inflammatory cells compared to PRF [4,41]. In his studies, Pirraco showed BMP production by monocytes and macrophages, BMP receptors in monocytes thus being discovered. The results confirm that A-PRF releases all cytokines already present in PRF and a higher amount of monocytes and bone morphogenetic proteins, BMP-2 and BMP-7, respectively [6]. There are also studies that confirm the possibility of a role of TGF-ß1 in promoting bone regeneration in sheep [42].

Different histological analyses for A-PRF showed a significantly higher number of neutrophil granulocytes and leukocyte subfamilies compared to PRF. Different cells are seen in the middle and distal portions of the clot; here, the uniform presence of monocytes is highlighted, which subsequently

turn into macrophages and are found in PRF at the extremity of the clot. In this sense, A-PRF significantly facilitates cell penetration into the fibrin clot, promoting vascularization [4,6].

The rapid regeneration of bone and ligament structures as observed in this 6-week experimental study is most probably due to local stimulation of bone morphogenetic protein proliferation, owing to growth factors in the platelet autoplasma, which is potentiated by the qualities of the xenograft of bovine origin, enriched with type 1 atelocollagen, according to the described techniques. At the same time, the platelet autoplasma potentiates periodontal ligament regeneration by promoting collagen synthesis. The association of these elements induces a healing cascade that leads to cell chemotaxis, angiogenesis, cell proliferation and matrix synthesis without affecting the differentiated cell.

In the present study, glass test tubes were used to obtain A-PRF, although there are studies that preferentially indicate plastic test tubes or test tubes made of glass with the walls lined by plastic, because glass hardness more rapidly promotes coagulation cascades, unlike plastic elasticity that would prolong the coagulation time [43], but there are studies evidencing the detachment of silica microparticles during centrifugation of test tubes containing plastic masses. Although the silica particles of test tubes are considered to be stable, the possibility of inducing cytotoxicity in inflammatory cell responses was found [44], a behavior similar to that of direct dental adhesive systems [45].

As it is known, using rh-BMP-2 recombinant growth factors in sheep has yielded favorable results compared to evaluation under different conditions such as osteodistraction [46]. The current biomaterial used in this study does not involve the risks of overdosage or dose-dependent catabolic reactions, as it happens with recombinant growth factors including rh-BMPs. According to literature data, adverse effects generally occur in the case of the use of high doses, the most important of these effects being: cell apoptosis, inflammatory reactions, fibroblast infiltration [47].

Compared to autografts, additional surgery was here avoided; the doctor's physical effort and the trauma induced to the patient decrease, postoperative inflammation is extremely reduced given the predominance of platelets, and healing and osseointegration are very rapid [48].

Histological evaluation did not show osteolysis in the addition areas of the composite biomaterial after six weeks from completion of surgery, and clearly proved an evolution towards bone transformation, starting from the marginal portions of the hypocellular area of the biomaterial, also clearly revealing the bone transformation stages. The presence of new bone was histologically visible in all alveolar bone augmentation areas.

4.Conclusions

Alveolar bone augmentation, using a bovine xenograft enriched with type 1 atelocollagen and combined with PRP and A-PRF, respectively, as a membrane, provided better than expected results following evaluation at six weeks postoperatively. The biomaterial offers excellent results regarding alveolar bone regeneration. This study also revealed many details of the histological evolution from acellular granular biomaterial to well-structured new bone trabeculae and their well-guided apposition at periodontal level.

References

1.LUTZ, R., BERGER-FINK, S., STOCKMANN, P., NEUKAM, F.W., SCHLEGEL, K.A., Sinus floor augmentation with autogenous bone vs. a bovine-derived xenograft - a 5-year retrospective study, *Clin Oral Implants* Res. 2015 Jun;**26**(6):644-8.
2.MACHTEI, E.E., ROZITKY, D., ZIGDON-GILADI, H., HORWITZ, J., Dimensional Changes Following Lateral Wall Sinus Augmentation Without Concomitant Implant Placement Using a Composite Bone Graft. *Int J Oral Maxillofac Implants*. 2016 Jan-Feb;**31**(1):87-91.
3.KOUR, P., PUDAKALKATTI, P.S., VAS, A.M., DAS, S., PADMANABHAN, S. Comparative Evaluation of Antimicrobial Efficacy of Platelet-rich Plasma, Platelet-rich Fibrin, and Injectable

Platelet-rich Fibrin on the Standard Strains of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. *Contemp Clin Dent*. 2018 Sep;**9**(Suppl 2):S325-S330.
4.MIRON, J.R., CHOUKROUN, J., Platelet Rich Fibrin In Regenerative Dentistry, Wiley Blackwell, USA 2017, p. 1-54
5.BUSILACCHI, A., GIGANTE, A., MATTIOLI-BELMONTE, M., MANZOTTI, S., MUZZARELLI, R.A., Chitosan stabilizes platelet growth factors and modulates stem cell differentiation toward tissue regeneration. *Carbohydr Polym*. 2013 Oct 15;**98**(1):665-76.
6.MIFUNE, Y., MATSUMOTO, T., TAKAYAMA, K., OTA, S., LI, H., MESZAROS, L.B., USAS, A., NAGAMUNE, K., GHARAIBEH, B., FU, F.H., HUARD, J., The effect of platelet-rich plasma on the regenerative therapy of muscle derived stem cells for articular cartilage repair. *Osteoarthritis Cartilage*. 2013 Jan;**21**(1):175-85.
7.CHEN, L., LU, X., LI, S., SUN, Q., LI, W., SONG, D. Sustained delivery of BMP-2 and platelet-rich plasma-released growth factors contributes to osteogenesis of human adipose-derived stem cells. *Orthopedics*. 2012 Sep;**35**(9):e1402-9.
8.LIU, H.Y., WU, A.T., TSAI, C.Y., CHOU, K.R., ZENG, R., WANG, M.F., CHANG, W.C., HWANG, S.M., SU, C.H., DENG, W.P. The balance between adipogenesis and osteogenesis in bone regeneration by platelet-rich plasma for age-related osteoporosis. *Biomaterials* 2011, Oct;**32**(28):6773-80.
9.HOGGATT, J. Hot ortho-biologic topics at AAOS 2011†: platelet-rich plasma and related growth factors generate excitement. *BioDrugs*. 2011 Jun 1;**25**(3):197-202.
10.SCHUCKERT, K.H., JOPP, S., OSADNIK, M. The Use of Platelet Rich Plasma, Bone Morphogenetic Protein-2 and Different Scaffolds in Oral and Maxillofacial Surgery- Literature Review in Comparison with Own Clinical Experience. *J Oral Maxillofac Res*. 2011 Apr 1;**2**(1):e2. eCollection 2011.
11.HU, Z.M., PEEL, S.A., HO, S.K., SANDOR, G.K., CLOKIE, C.M. Comparison of platelet-rich plasma, bovine BMP, and rhBMP-4 on bone matrix protein expression in vitro. *Growth Factors*. 2009 Oct;**27**(5):280-8.
12.GIANAKOS, A., ZAMBRANA, L., SAVAGE-ELLIOTT, I., LANE, J.M., KENNEDY, J.G. Platelet-Rich Plasma in the Animal Long-Bone Model: An Analysis of Basic Science Evidence. *Orthopedics.* 2015 Dec;**38**(12):e1079-90.
13.TOMOYASU, A., HIGASHIO, K., KANOMATA, K., GOTO, M., KODAIRA, K., SERIZAWA, H., SUDA, T., NAKAMURA, A., NOJIMA, J., FUKUDA, T., KATAGIRI, T., Platelet-rich plasma stimulates osteoblastic differentiation in the presence of BMPs. *Biochem Biophys Res Commun.* 2007 Sep 14;**361**(1):62-7. Epub 2007 Jul 5.
14.MALHOTRA, A., PELLETIER, M., OLIVER, R., CHRISTOU, C., WALSH, W.R. Platelet-rich plasma and bone defect healing. *Tissue Eng Part* A. 2014 Oct;**20**(19-20):2614-33. Epub 2014 Apr 28.
15.KALÉN, A., WAHLSTROM, O., LINDER, C.H., MAGNUSSON, P. The content of bone morphogenetic proteins in platelets varies greatly between different platelet donors ,*Biochem Biophys Res Commun*. 2008 Oct 17;**375**(2):261-4.
16.WANG, W., SUN, L., ZHANG, P., SONG, J., LIU, W. An anti-inflammatory cell-free collagen/resveratrol scaffold for repairing osteochondral defects in rabbits. *Acta Biomater*. 2014 Dec;**10**(12):4983-4995.
17.DANESH MEYER, M.J., PACK, M.D. A comparison of 2 polyterafluoroethylene membranes in guided tissue regeneration in sheep. *J.Periodont Res* 1997;**32**:20-30 Munksgaard 1997 ISSN 0022-4384
18.ALTAII, M., CATHRO, P., BROBERG, M., RICHARDS, L. Endodontic regeneration and tooth revitalization in immature infected sheep teeth. School of Dentistry, University of Adelaide, Australia. *International Endodontic Journal*, **50**(5), 480–491.

19.SCHLIEPHAKE, H., KNEBEL, J.W., AUFDERHEIDE, M., TAUSCHER, M. Use of cultivated osteoprogenitor cells to increase bone formation in segmental mandibular defects: an experimental pilot study in sheep. *Int J Oral Maxillofac Surg*. 2001 Dec;**30**(6):531-7.
20.LANGHOFF, J.D., VOELTER, K., SCHARNWEBER, D., SCHNABELRAUCH, M., SCHLOTTIG, F., HEFTI, T., KALCHOFNER, K., NUSS, K., VON RECHENBERG, B. Comparison of chemically and pharmaceutically modified titanium and zirconia implant surfaces in dentistry: a study in sheep. *Int J Oral Maxillofac Surg*. 2008 Dec;**37**(12):1125-32.
21.DANESH-MEYER, M.J., PACK, A.R., MCMILLAN, M.D., A Comparison of 2 Polytetrafluoroethylene Membranes in Guided Tissue Regeneration in Sheep, Journal of Periodontal Resarch, **32**(1), 20-30, Jan 1997.
22.SANZ, M., CERIELLO, A., BUYSSCHAERT, M., CHAPPLE, I., DEMMER, R.T., GRAZIANI, F., HERRERA, D., JEPSEN, S., LIONE, L., MADIANOS, P., MATHUR, M., MONTANYA, E., SHAPIRA, L., TONETTI, M., VEGH, D. Scientific evidence on the links between periodontal diseases and diabetes: Consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International Diabetes Federation and the European Federation of Periodontology. *J Clin Periodontol.* 2018 Feb;**45**(2):138-149.
23.YONG, X., CHEN, Y., TAO, R., ZENG, Q., LIU, Z., JIANG, L., YE, L., LIN, X. Periodontopathogens and human β-defensin-2 expression in gingival crevicular fluid from patients with periodontal disease in Guangxi, China. *J Periodontal Res*. 2015 Jun;**50**(3):403-10.
24.BORSANELLI, A.C., RAMOS, T.N., GAETTI-JARDIM, E.JR., SCHWEITZER, C.M., DUTRA, I.S. *Treponema* species in the subgingival microflora of ovine periodontitis. *Vet Rec.* 2017 Feb 11;**180**(6):150.
25.FRISKEN, K.W., LAWS, A.J., TAGG, J.R., ORR, M.B. Environmental influences on the progression of clinical and microbiological parameters of sheep periodontal disease. *Res. Vet*. Sci. 1989;**46**:147-152.
26.ISMAIEL, M.O., GREENMAN, J., MORGAN, K., GLOVER, M.G., REES, A.S., SCULLY, C. Periodontitis in sheep: a model for human periodontal disease. *J Periodontol*. 1989 May;**60**(5):279-84.
27.MCCOURTIE, J., POXTON, I.R., BROWN, R., WHITTAKER, C.R., SPENCE, J.A., AITCHISON, G.U. A longitudinal study of the cultivable subgingival anaerobic bacteria isolated from sheep during the development of broken mouth periodontitis. *J Med Microbiol.* 1990 Apr;**31**(4):275-83.
28.LUNG, T., LAZAR, M. *Parodontologie clinică*, Editura Napoca-Star Cluj-Napoca 2007, ISBN 978-973-647-509-2, p.30-265.
29.DUMITRIU, H.T. *Parodontologie*, Editura Viața Medicală Românească, București 2009, ISBN 978-973-160-027-7, p.305-330
30.LAREU, R.R., ZEUGOLIS, D.I., ABU-RUB, M., PANDIT, A., RAGHUNATH, M. Essential modification of the Sircol Collagen Assay for the accurate quantification of collagen content in complex protein solutions. *Acta Biomater.* 2010 Aug;**6**(8):3146-51.
31.TAKECHI, M., NINOMIYA, Y., OHTA, K., TADA, M., SASAKI, K., RAHMAN, M.Z., OHTA, A., TSURU, K., ISHIKAWA, K. Effects of Apatite Cement Containing Atelocollagen on Attachment to and Proliferation and Differentiation of MC3T3-E1 Osteoblastic Cells. *Materials* (Basel). 2016 Apr 13;**9**(4). pii: E283.
32.GOTO, Y., OSHINO, S., SHIMIZU, T., SAITOH, Y. Application of atelocollagen sheet for sellar reconstruction. *J Clin Neurosci*. 2016 May;**27**:142-6.
33.HALPER, J. Advances in the use of growth factors for treatment of disorders of soft tissues. *Adv Exp Med Biol*. 2014;**802**:59-76.
34.SIMMAN, R., HOFFMANN, A., BOHINC, R.J., PETERSON, W.C., RUSS, A.J. Role of platelet-rich plasma in acceleration of bone fracture healing. *Ann Plast Surg*. 2008 Sep;**61**(3):337-44.
35.DELOS, D., RODEO, S.A., Enhancing meniscal repair through biology: platelet-rich plasma as an alternative strategy. *Instr Course Lect*. 2011;**60**:453-60.

36.GRONTHOS, S., MROZIK, K., SHI, S., BARTOLD, P.M. Division of Haematology, Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. *Calcif Tissue Int.* 2006 Nov;**79**(5):310-7.
37.JOHNSON, R.B., GILBERT, J.A., COOPER, R.C., DAI, X., NEWTON, B.I., TRACY, R.R., WEST, W.F., DEMOSS, T.L., MYERS, P.J., STRECKFUS, C.F. Department of Periodontics, University of Mississippi School of Dentistry, Jackson, USA. Alveolar bone loss one year following ovariectomy in sheep. *J Periodontol*. 1997 Sep;**68**(9):864-71.
38.SOUSA, C.P., AZEVEDO, J.T., SILVA, A.M., VIEGAS, C.A., REIS, R.L., GOMES, M.E., DIAS, I.R. Serum total and bone alkaline phosphatase levels and their correlation with serum minerals over the lifespan of sheep. *Acta Vet Hung.* 2014 Jun;**62**(2):205-14.
39.GIOGA, C., BECHIR, E.S, CURT-MOLA, F., DEFTA, C.L., BECHIR, A., BIRIS, C, BUNGET, A.M., The Benefits of Platelet-Rich Fibrin in Promoting Post-Extraction Healing, *Rev. Chim.* **68**, (12), 2017, 2974- 2977
40.ZHU, S.J., CHOI, B.H., JUNG, J.H., LEE, S.H., HUH, J.Y., YOU, T.M., LEE, H.J., LI, J. A comparative histologic analysis of tissue-engineered bone using platelet-rich plasma and platelet-enriched fibrin glue. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006 Aug;**102**(2):175-9.
41.EL BAGDADI, K., KUBESCH, A., YU, X., AL-MAAWI, S., ORLOWSKA, A., DIAS, A., BOOMS, P., DOHLE, E., SADER, R., KIRKPATRICK, C.J., CHOUKROUN, J., GHANAATI, S. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: a proof of concept of LSCC (low speed centrifugation concept). *Eur J Trauma Emerg Surg*. 2019 Jun;**45**(3):467-479.
42.MOHAMMED, S., PACK, A.R., KARDOS, T.B. The effect of transforming growth factor beta one (TGF-beta 1) on wound healing, with or without barrier membranes, in a Class II furcation defect in sheep. *J Periodontal Res.* 1998 Aug;**33**(6):335-44.
43.SIRBU, D., TOPALO, V., RUSNAC, C., STRISCA, S., SUHARSCHI, I., MIGHIC, A., GHETIU, A., CALDARARI, S., Tissue Engineering With Platelet-Rich Fibrin In Oral Region, *Medicina Stomatologică*, 2016, Nr.**1-2**(38-39), p. 16-23
44.TSUJINO, T., MASUKI, H., NAKAMURA, M., ISOBE, K., KAWABATA, H., AIZAWA, H., WATANABE, T., KITAMURA, Y., OKUDERA, H., OKUDA, K., NAKATA, K., KAWASE, T. Striking Differences in Platelet Distribution between Advanced-Platelet-Rich Fibrin and Concentrated Growth Factors: Effects of Silica-Containing Plastic Tubes, *J Funct Biomater.* 2019 Sep 17;**10**(3). pii: E43.
45.ALMASI, A., TODOR, L., RATIU, C.A., POPOVICI, R.A., TIGMEANU, C.V., TOFAN, S.A., PORUMB, A., In Vitro Study of the Structure and Adhesive Interface in Direct Restorations with Commercial Nanocomposite Materials, *Mater. Plast.*, **56**, (1), 2019, 277-281.
46.EPSTEIN, N.E. Commentary on research of bone morphogenetic protein discussed in review article: Genetic advances in the regeneration of the intervertebral disc. *Surg Neurol Int.* 2013 Mar 22;**4** (Suppl 2):S106-8. Print 2013.
47.RACHMIEL, A., AIZENBUD, D., PELED, M. Enhancement of bone formation by bone morphogenetic protein-2 during alveolar distraction: an experimental study in sheep. *J Periodontol*. 2004 Nov;**75**(11):1524-31.
48.SUSANU, S., POPESCU, M., CABA, B., PLAMADEALA, P., MORARU, A., COSTIN, D., GAVRILUT, G., AGOP, M., CIOCA, G. Accelerating the Bone Healing Process by the Intervention of the Platelet Growth Factors Impregnated in Collagen: An experimental and theoretical mathematical model, *Mater. Plast.*, **56**, (4), 2019, 973-979.

Manuscript received: 3.02.2020