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Case Report / Приказ болесника

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Histological analysis of bone three months after the treatment of oroantral communication with autologous platelet-rich fibrin – a case series

Хистолошка анализа кости три месеца након реконструисања ороантралне комуникације аутологним фибрином богатим тромбоцитима – серија болесника

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Хистолошка анализа кости три месеца након реконструисања ороантралне комуникације аутологним фибрином богатим тромбоцитима – серија болесника

SUMMARY

Introduction Oroantral communication (OAC) closure may be accompanied with hard and soft tissue defects. Platelet-rich fibrin (PRF) is the second-generation platelet concentrate that can be an effective therapeutic option for the closure of defects up to 5mm. The aim of this investigation was to evaluate whether the PRF could be a viable therapeutic option for OAC closure and provide sufficient bone quality/quantity for the forthcoming implant placement.

Outlines of cases The case series included 8 patients treated with PRF due to the presence of OAC less than 3mm in diameter. Three months after the surgery, at the time of implant placement, bone samples were harvested and taken for histological analysis. The results demonstrated success in all eight cases, obtaining the hard and soft tissue healing. Histological analysis showed that newly formed bone was present on all histological samples, without visible signs of inflammation and necrosis.

Conclusion PRF could be a viable therapeutic option for OAC closure in specific clinical cases, but future randomized, controlled, clinical studies are required for more conclusive results.

Keywords: bone healing; autologous platelet-rich fibrin; oroantral communication

САЖЕТАК

Увод Хирушко затварање ороантралних комуникација (ОАК) може бити праћено стварањем коштаних или мекоткивних дефеката. Аутологни фибрин богат тромбоцитима (ФБТ) јесте тромбоцитни концентрат друге генерације ефикасан у реконструкцији ОАК дијаметра до 5 мм. Циљ истраживања је био да испита ефикасност ФБТ у реконструкцији ОАК и утврди да ли ће његова примена обезбедити адекватну коштану подлогу за будућу уградњу импланта.

Приказ болесника Истраживање је обухватило осам пацијената код којих је ОАК била мања од 3 мм и реконструисана применом ФБТ. Три месеца након хирушког захвата, приликом уградње импланата, са места ОАК узети су узорци кости ради хистолошке анализе. Код свих испитаника остварено је успешно мекоткивно зарастање. Резултат свих узорака показали су присуство новоформиране здраве кости, без знакова запаљења и некрозе.

Закључак ФБТ се може користити за реконструкцију ОАК у специфичним клиничким индикацијама. Ипак, неопходно је спровести рандомизоване, контролисане клиничке студије пре доношења јасних препорука.

Кључне речи: коштану зарастање; аутологни фибрин богат тромбоцитима; ороантрална комуникација

INTRODUCTION

Surgical closure of oroantral communication (OAC) may be accompanied with hard and soft tissue defects [1]. Although application of local soft-tissue flaps is still the most utilized technique for OAC closure, recent studies suggest that platelet-rich fibrin (PRF) can be an effective therapeutic option for the closure of defects up to 5mm in diameter. PRF is associated with minimal postoperative morbidity and allows preservation of adjacent teeth' soft tissue structures [2–5]. Additionally, the combination of PRF and bone grafting materials promotes hard tissue healing, obtaining better conditions for future implant placement [3, 4, 5].

PRF is the second-generation platelet concentrate consisting of a three-dimensional polymerized fibrin matrix in a molecular structure. It incorporates blood contents such as

leukocytes, erythrocytes, platelets, growth factors, and circulating stem cells [6]. PRF membrane induces tissue regeneration due to the stimulating effects on osteoblast cells, gingival fibroblasts, pulp cells, and periodontal ligament cells [1].

This case series aimed to evaluate whether the PRF could be a viable therapeutic option for the OAC closure and provide sufficient bone quality/quantity for the forthcoming implant placement.

REPORT OF CASES

This case series included eight patients treated at the Clinic for Oral Surgery, School of Dental Medicine in Belgrade (6 males and 2 females; aged between 21 and 43 years, mean age 34.6 ± 11.3). They were referred to the Clinic due to the presence of OAC and enrolled in the study according to the following inclusion criteria:

- Patients in good general health without a history of systemic disease or medication that could interfere with the treatment (ASA 1 and ASA2);
- Fresh OAC (not more than 24h from the tooth extraction);
- Without the clinical/radiological signs of maxillary sinusitis;
- Long and narrow alveolus of the extracted tooth;
- OAC less than 3mm in diameter;
- Length from the cortical margin of the extracted tooth to the OAC being at least 6mm;
- Clinically compliant patients consent to be enrolled in the study.

OAC was closed by autologous PRF plugs and membranes, following Choukroun's PRF centrifuge protocol (PRF DUO™, Nice, France) [7]. After the curettage and saline rinsing, wound edges were freshened, and the PRF plug was placed inside the alveolus. PRF membrane was shaped over the site in one layer, and the closure was obtained by interrupted sutures – Figure 1. The follow-up was scheduled, and sutures were removed on the 10th day.

Epithelization and soft tissue healing were uneventful in all eight cases. Three months after the OAC closure, there was a sufficient amount of keratinized gingiva and the CBCT evaluation revealed new bone formation in the area of the previously extracted tooth. Sub-antral height from 6 to 9mm (average 7.3mm) was obtained in all eight cases. The site was

reopened, bone samples for histological analysis were harvested (trephine burr; Ø 3.0 mm – Figure 2), and implants were placed. We were using bone level, tapered implants, following the maxilla protocol (avoiding the last sequence drill), and managed to obtain solid implant stability (from 20 Ncm to above).

Bone samples were stored in a 10% formalin solution for 12 to 24 hours and then decalcified in a microwave oven (8 cycles of 10 seconds; at 410 to 430°C for 20 min). The material was dehydrated with 70%, 95%, and 99% ethyl alcohol, respectively, and clarified with xylene. Gathered bone fragments were embedded in paraffin blocks, cut into slices (3 to 4µm), and stained with Goldner trichrome method. Analysis of samples was performed under Leica Microsystem® optical microscope (Leica Microsystems™ GmbH, Wetzlar, Germany).

The newly formed bone was present in all histological samples, without signs of inflammation and necrosis (Figure 3). Bone trabeculae were surrounded by the loose connective tissue in which no inflammatory infiltrate cells were seen, or their number was minimal. Additionally, intensive bone remodeling was noticed, i.e., the presence of mature (lamellar) bone and immature (fibrous) bone (Figure 4). Haversian canals with concentrically placed bone lamellae (characteristics for osteons) were seen as a sign of mature bone, and collagen-fiber networks as a sign of immature bone. The abundant presence of osteocytes lying in lacunae confirmed bone vitality, and the presence of osteoids covered with a dense layer of osteoblasts was a sign of active osteogenesis (Figure 5).

This paper was approved by the institutional ethics committee, and written consent was obtained from the patients for the publication of the report and any accompanying images.

DISCUSSION

Our findings indicate that the PRF could be a viable solution for the OAC closure in specific clinical cases. It provides proper hard and soft tissue healing, obtaining sufficient bone quality/quantity. At first, PRF acts as a boosting agent for soft tissue healing, supporting epithelization. Additionally, it promotes bone formation in the area of the extracted tooth, creating the vital bone, and shortens the healing period. The presence of mature and immature bone is a significant histological sign of intensive bone remodeling.

PRF plug acts as a core for bone healing and the PRF membrane acts as a biological membrane that promotes epithelization. Due to its properties, PRF has proven as the material of choice not only for this indication but as well as in many other clinical studies. Ondur et al.

[8] showed that the use of PRF for hard and soft tissue healing may have advantages due to its autogenous origin, being cheaper than the collagen membrane. The authors pointed to PRF's ability to release growth factors (TGF- β 1, PDGF- β , VEGF); particularly in the first 7 days, and later, up to 28 days. Similarly, Liu et al. promote PRF as a bone grafting material for oral and maxillofacial bone regeneration procedures as it improves proliferation, migration, differentiation, and mineralization of the cells during bone formation [9]. There is an indication that PRF could also diminish crestal bone resorption after tooth extraction [10], as demonstrated in periimplantitis therapy use [11]. Moreover, the application of PRF, either alone or in combination with another biomaterial, might be effective in reducing time for new bone formation and future implant placement [12, 13].

Although we were successful in all eight cases obtaining hard and soft tissue healing, it would be presumptive to state that PRF could be a universal tool for OAC closure. In this study we had a strict case selection, requiring fresh OAC with a diameter up to 3mm. Post-extraction socket had to be long and narrow, and the distance between the crest and OAC should be at least 6 mm. However, there is a question if the same healing would be obtained without the use of PRF since we didn't have a control group. Additionally, the sample size was too small. We didn't experience any complications and are considering if the procedure could be applied to larger/shallower defects, along with the use of bone substitute materials.

CONCLUSION

This case series indicates that PRF could be a viable therapeutic option for OAC closure providing optimal hard and soft tissue structures for the future implant placement. However, future randomized, controlled studies on larger sample sizes, with control groups, should contribute to more conclusive remarks.

Conflict of interest: None declared.

REFERENCES

1. Baek J.H, Kim B.O, Lee W.P. Implant placement after closure of oroantral communication by sinus bone graft using a collagen barrier membrane in the shape of a pouch: A Case report and review of the literature. *Medicina* 2021;57(6):626 (DOI: 10.3390/medicina57060626; PMID: 34208644)
2. Parvini P, Obreja K, Bejic A, Schwarz F, Becker J, Sader R, et al. Decision-making in closure of oroantral communication and fistula. *Int J Implant Dent.* 2019;5(1):13 (DOI: 10.1186/s40729-019-0165-7; PMID: 30931487)
3. Salgado-Peralvo A.O, Mateos-Moreno M.V, Uribarri A, Kewalramani N, Peña-Cardelles J.F, Velasco-Ortega E. Treatment of oroantral communication with Platelet-Rich Fibrin: A systematic review. *Journal of Stomatology, Oral and Maxillofacial Surgery* 2022; S2468-7855(22)00083-0. (DOI: 10.1016/j.jormas.2022.03.014; PMID: 35318134)
4. Khalifaoui M, Eya M, Selmi W, Lamia O, Nabiha D. Closure of oroantral communication with platelet-rich fibrin: A case report. *Saudi J Oral Dent Res* 2021; 6(12): 566-568. (DOI: 10.36348/sjodr.2021.v06i12.006)
5. Mainassara Chekaraou S, Benjelloun L, El Harti K. Management of oro-antral fistula: Two case reports and review. *Ann Med Surg (Lond).* 2021; 69:102817 (DOI: 10.1016/j.amsu.2021.102817; PMID: 34527240)
6. Niedzielska I, Ciapinski D, Bak M, Niedzielski D. The Assessment of the Usefulness of Platelet-Rich Fibrin in the Healing Process Bone Resorption. *Coatings* 2022; 12:247 (DOI: 10.3390/coatings12020247)
7. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AL, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Pathol Oral Radiol Endod.* 2006 Mar; 101(3):e37-44. PubMed PMID: 16504849.
8. Ondur E, Bolukbasi Balcioglu N, Soluk Tekkesin M, Guzel O, Ersanli S. Effects of Platelet-Rich Fibrin on Hard Tissue Healing: A Histomorphometric Crossover Trial in Sheep. *Materials (Basel).* 2020; 13(7):1695. (DOI: 10.3390/ma13071695; PMID: 32260464)
9. Liu Y, Sun X, Yu J, Wang J, Zhai P, Chen S, et al. Platelet-Rich Fibrin as a Bone Graft Material in Oral and Maxillofacial Bone Regeneration: Classification and Summary for Better Application. *BioMed research international* 2019; 3295756. (DOI: 10.1155/2019/3295756; PMID: 31886202)
10. Al-Badran A, Bierbaum S, Wolf-Brandstetter C. Does the choice of preparation protocol for platelet-rich fibrin have consequences for healing and alveolar ridge preservation after tooth extraction? A meta-analysis. *Journal of Oral and Maxillofacial Surgery* 2023; 31:S0278-2391(23)00071-X (doi: 10.1016/j.joms.2023.01.004; PMID: 36736375)
11. Guoxi S, Lei C, Haiyan L. Effects of platelet-rich fibrin combined with guided bone regeneration in the reconstruction of peri-implantitis bone defect. *Am J Transl Res.* 2021; 13(7): 8397–8402 (PMID: 34377334)
12. Pealez Otero A.I, Hasse Fernandes J.C, Borges T, Nassani L, De Moraes Castilho R, De Olivera Fernandes G.V. Sinus Lift Associated with Leucocyte-Platelet-Rich Fibrin (Second Generation) for Bone Gain: A Systematic Review. *J. Clin. Med.* 2022; 11(7): 1888 (DOI: 10.3390/jcm11071888; PMID: 35407494)
13. Guan S, Xiao T, Bai J, Ning C, Zhang X, Yang L, Li X. Clinical application of platelet-rich fibrin to enhance dental implant stability: A systematic review and meta-analysis. *Heliyon* 2023; 9(2):e13196 (DOI: 10.1016/j.heliyon.2023.e13196; PMID: 36785817)



Figure 1. Platelet-rich fibrin material placed in the alveolar socket

Paper accepted



Figure 2. Bone sample inside the trephine burr \varnothing 3.0 mm

Paper accepted

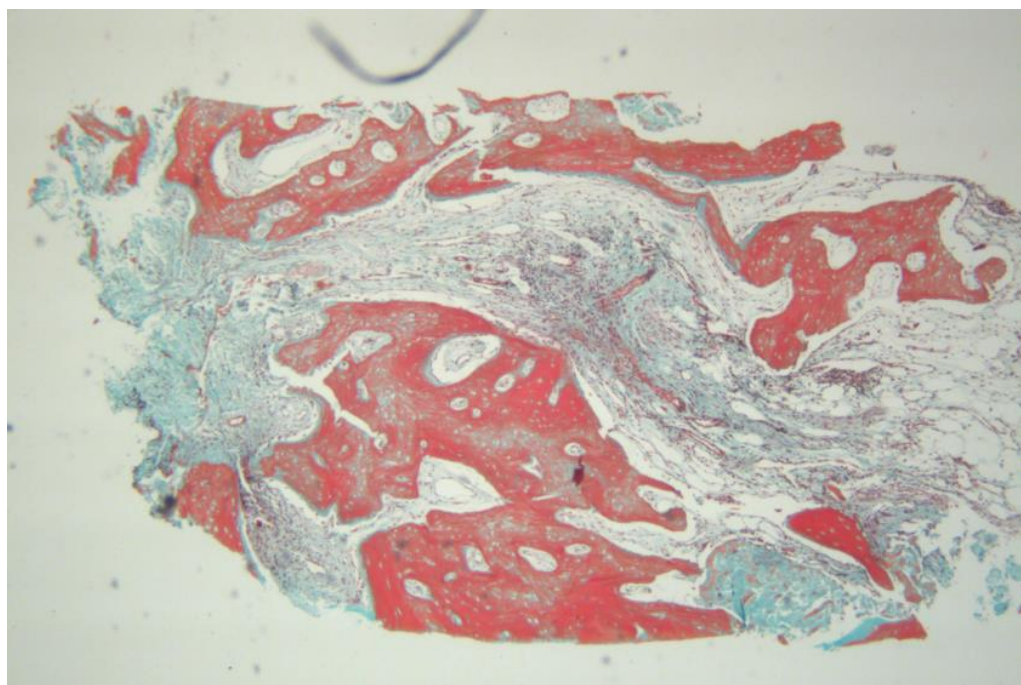


Figure 3. New formed bone tissue (Goldner–Trichrome method, 40 ×)

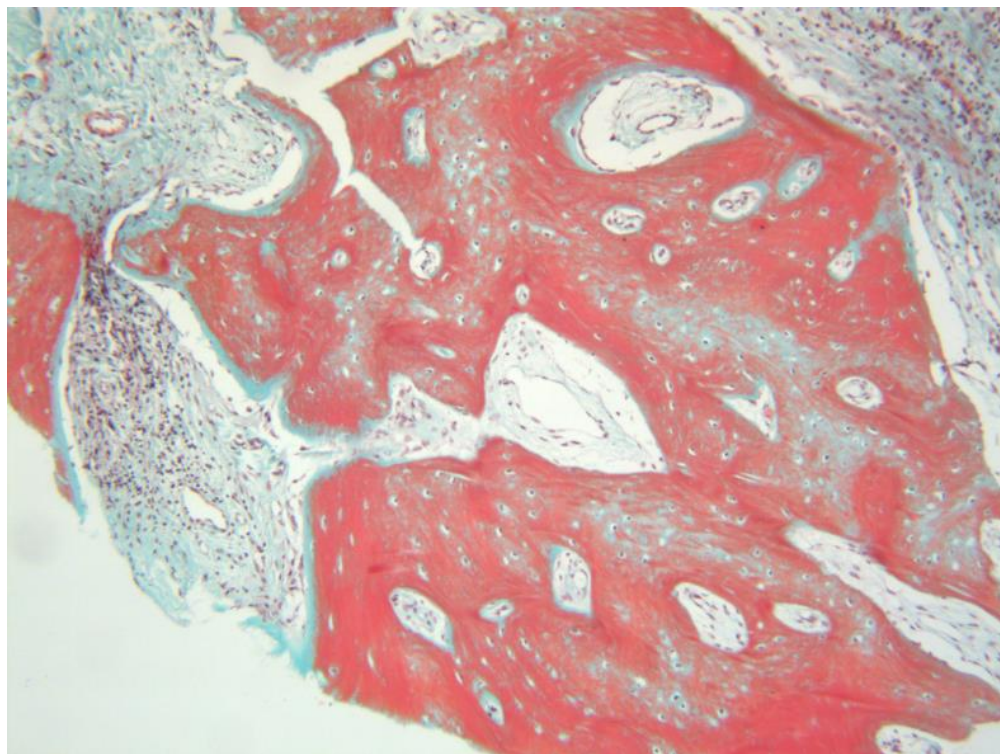


Figure 4. New formed bone tissue with elements of mature and immature bone (Goldner–Trichrome method, 100 ×)

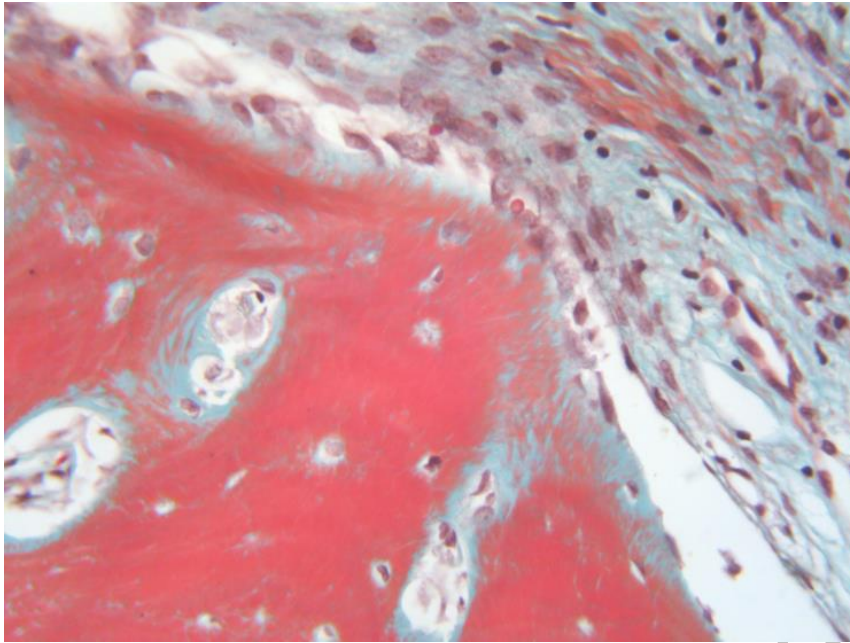


Figure 5. Osteocytes in the lacunae and osteoid and densely packed osteoblasts (Goldner–Trichrome method, 400 ×)

Paper accepted