



ABSTRACT BOOK

2nd European Meeting on Enhanced Natural Healing in Dentistry

7 - 9 September 2018

Leuven, Belgium

KU LEUVEN

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A WORD OF WELCOME

Dear ENHD 2018 participant,

We would like to thank you for your participation at the 2nd European Meeting on Enhanced Natural Healing in Dentistry, held in Leuven, from 7-9 September 2018.

More than 350 participants have registered from all over the world. It was a concentrated but also a very interesting meeting where we have seen a lot of friends and colleagues.

We hereby provide you the abstract book, with the abstracts of the oral and poster presentations.

We hope that you have enjoyed the conference and we are looking forward to seeing you again at another meeting!

Best regards,

Prof. Marc Quirynen, Prof. Nelson Pinto and Franck Renouard
Conference Chairs

CONFERENCE PROGRAMME

Scientific programme Friday September 7th *Workshop*

Venue workshop : Dentistry Skill Center, UZ St. Rafaël, entrance Cuythoek,
Kapucijnenvoer 7, 3000 Leuven

15.00 - 17.00 : Surgical Interventions: Video Presentations

- **Andy Temmerman & Marc Quirynen** (Nelson Pinto for Q&A)

17.30 - 18.30 : Hands-on: Preparation L-PRF & Ridge Preservation

- **Nelson Pinto** and Staff Periodontology

Scientific programme Saturday September 8th
Lectures on intra oral applications of L-PRF

Venue conference : Aula Pieter De Somer, Charles Debriotstraat 24, 3000 Leuven

08.00 - 09.00 : Welcome with coffee

09.00 - 09.05 : Opening by the chairman

- **Franck Renouard**

09.05 - 09.30 : L-PRF: why should it work: from extra-oral to intra-oral wounds

- **Nelson Pinto**

09.30 - 11.00 : Benefits of L-PRF for ridge preservation & bone block grafting

- **09.30-10.00 - Juan Blanco : Keynote**
- **10.00-10.20 - Ana Castro : Are the centrifuge and timing a key factor for the preparation of platelet concentrates?**
- **10.20-10.40 - Ana Castellano Viruleg : Changes in bone morphology after socket preservation procedure with leucocyte- and platelet-rich fibrin (L-PRF) vs. conventional treatment. Randomized clinical pilot study**
- **10.40-11:00 - Catherine Andrade : "Dentin block" in alveolar ridge preservation: a histological descriptive pilot study as proof of principle**

11.00 - 11.30 : Coffee break

11.30 - 13.00 : Benefits of L-PRF for the regeneration of periodontal defects (soft and hard tissues)

- **11.30-12.00 - Pierpaolo Cortellini : Keynote**
- **12.00-12.20 - Antonio Sanz : L-PRF in soft tissue regeneration**
- **12.20-12.40 - Mazen El-Abiad : Use of L-PRF in Periodontal Defects during Surgical and Non-Surgical Therapy**
- **12.40-13.00 - Sourav Panda : Adjunctive Effect of Autologous Platelet-Rich Fibrin to Barrier Membrane in the Treatment of Periodontal Intrabony Defects**

13.00 - 14.30 : Lunch (venue: "Jubileum" room of the University Hall, Naamsestraat 22)

Walking distance from conference venue, postgraduate students from the department of periodontology will guide you to the lunch area.

14.30 - 16.00 : L-PRF block for horizontal bone regeneration

- **14.30-15.00 - Marc Quirynen** : *Keynote*
- **15.00-15.20 - Pascal Valentini** : *Bone augmentation with L-PRF blocks in the posterior mandible: A clinical evaluation*
- **15.20-15.40 - Mic Demanet** : *Horizontal & vertical GBR combining L-PRF, autogenous bone, and liquid fibrinogen*
- **15.40-16.00 - Paul Stone** : *A Report of the Experience of L-PRF for Horizontal Ridge Augmentation in Private Practice*

16.00 - 16.30 : Coffee break

16.30 - 18.00 : Benefits of L-PRF for implant surgery (coating, sinus augmentation, ...)

- **16.30-17.00 - Andy Temmerman** : *Keynote*
- **17.00-17.20 - Joao Caramês** : *Guided Bone Regeneration with L-PRF in the atrophic maxilla – the GLAM technique*
- **17.20-17.40 - Simone Cortellini** : *Lateral and Trans-crestal sinus floor elevation in implant therapy with L-PRF: Randomized controlled clinical trial*
- **17.40-18.00 - Marcel Wainwright** : *Enhanced oral bone grafting in the sinus with L-PRF and the Intralift*

18.00 - 18.30 : Coffee break

18.30 - 20.00 : Basic research on how L-PRF can enhance the healing

- **18.30-19.00 - Ivo Lambrichts** : *Keynote*
- **19.00-19.15 - Pascal Gervois** : *Underlying processes of L-PRF mediated repair and its effect on dental pulp stem cells*
- **19.15-19.30 - Anna Ockerman** : *The influence of antithrombotic drugs on biomechanical characteristics of L-PRF*
- **19.30-19.45 - Luciano Pitzurra** : *Comparison of the effect of L-PRF and A-PRF on gingival fibroblast and periodontal ligament fibroblast*
- **19.45-20.00 - Júlio César Matias de Souza** : *Analyses on growth factors and collagen expression on i-PRF*

20.00 – 20.05 : Closure of scientific day

- **Nelson Pinto**

Scientific programme Sunday September 9th AM
Sharing clinician experience

Venue conference : Aula Pieter De Somer, Charles Debriotstraat 24, 3000 Leuven

08.00 - 08.30 : Welcome with coffee

Exchange of clinical experience

- **Marc Quirynen and Wim Teughels** : *Moderators*
- **FAQ via email:** *QforENHD2018@kuleuven.be*

08.30 - 10.00 : L-PRF: step by step approach, Part I

- **Ana Castro, Andy Temmerman, Nelson Pinto:** *Recent developments in preparation L-PRF*
- **Nelson Pinto, Juan Blanco, Andy Temmerman:** *Ridge preservation*
- **Antonio Sanz, Mazen El-Abiad, Andy Temmerman:** *Periodontal regeneration*

10.00 - 10.30 : Coffee break

10.30 - 12.00 : L-PRF: step by step approach, Part II

- **Andy Temmerman, Nelson Pinto:** *Sinus augmentation*
- **Andy Temmerman, Nelson Pinto, Juan Blanco:** *Implant surgery: coating/immediate placement*
- **Andy Temmerman, Nelson Pinto, Juan Blanco:** *L-PRF block*

12.00 - 12.05 : Closure

- **Marc Quirynen**

12.00 – 12.45 : Sandwich lunch

Scientific programme Sunday September 9th PM
Benefits of L-PRF in wound healing

Venue conference : Aula Pieter De Somer, Charles Debriotstraat 24, 3000 Leuven

13.30 – 14.00 : Coffee break

14.00 - 16.00 : Benefits of L-PRF in wound healing

- **14.00-14.30 - Nelson Pinto:** *The experience in Chili / Costa Rica*
- **14.30-15.00 - Yannick Spaey:** *The experience in Belgium*
- **15.00-15.30 - Sushil Koirala:** *The experience in Nepal*
- **15.30-16.00 - Enrico Rescigno:** *The experience in Italy*

LIST OF ORAL PRESENTATIONS

In alphabetic order by presenting author

“Dentin block” in alveolar ridge preservation: a histological descriptive pilot study as proof of principle

Catherine Andrade¹, Joaquin Camino¹, Mauricio Nally¹, Marc Quirynen², Benjamín Martínez³ & Nelson Pinto^{1,2}

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²*Section of Periodontology, Department of Oral Health Sciences, KU Leuven & Dentistry, University Hospitals, KU Leuven, Kapucijnenvoer 7, 3000 Leuven, Belgium.*

³*Department of Oral Diagnosis and Oral Pathology, School of Dentistry, Universidad Mayor, San Pio X 2422, Santiago, Chile.*

Aim: The aim of the present study was to describe the histological and clinical outcome of “Dentin block” (a mixture of: autogenous dentin + Leukocyte and Platelet Rich Fibrin (L-PRF) + liquid fibrinogen) in an alveolar ridge preservation test model.

Material and methods: 10 extraction sockets were grafted with a “Dentin block” (Figure 1,2) and clinically followed for 4, 5, or 6 months. At that moment biopsies were taken from the core of the grafted site for histologic and histo-morphometric analysis.

Two independent professionals analyzed the specimens at 10x magnification using ImageJ software. All specimens were digitized at the same magnification using a Leica DM500 microscope (Leica Microsystems, Wetzlar, Germany) and a digital camera (ICC 50 HD, Leica, Wetzlar, Germany) to measure the proportional areas of new bone, residual dentin graft particles, and connective tissue. The spatial scale of the active image was defined so measurements could be performed in calibrated units (μm^2). These measurements were expressed as a mean percentage of the total surface area of the section.

Results: All patients completed the study without any adverse event. All 10 sites showed an optimal amount of bone formation with good quality (Figure 3). The histological examination revealed a median relative percentage of bone, dentin, and connective tissue of 56.5, 3.6, and 39.9%, respectively (Table 1). A comparison of samples at different time points (4, 5, and 6 months) showed a progressive increase in the proportion of bone with a decrease in the proportion of dentin (Table 1). The bone was compact with normal osteocytes and moderate osteoblastic activity (Figure 4). In 4/10 samples no dentin was observed, in the other samples it represented 1-5%. The bone medullary spaces were filled with connective tissue showing a slight mononuclear infiltrate.

Discussion: In the present study, the use of a “Dentin block” in an alveolar ridge preservation model, showed optimal bone formation and an appropriate rate of dentin resorption. This new approach takes advantage of the biological properties of different autologous biomaterials: dentin, L-PRF, and liquid fibrinogen to regenerate bone.

The ability of dentin to promote bone formation in the alveolar ridge can be explained by its common embryological origin and similar composition.^[1,2] Dentin and bone also have non-collagenous proteins belonging to the SIBLING family (Small Integrin-Binding Ligand, N-linked Glycoprotein), including dentin sialophosphoprotein, dentin matrix protein 1, bone sialoprotein and osteopontin.^[3] Moreover, BMPs, TGF- β , IGF-I, and IGF-II have been detected in human dentin, and all are involved in the bone formation process.^[3]

On the other hand, the L-PRF a second generation of platelet concentrate, has shown to be able to enhance the healing process and to promote tissue regeneration due to the presence of platelets and leukocytes which release growth factors and cytokines involved in the healing process. In addition, the fibrin matrix with a specific tri-dimensional architecture also favors the different biological events during tissue regeneration.^[4,5,6,7,8] The third component, liquid fibrinogen, also releases growth factors and at the same time fixes all graft components together, allowing an easy handling, avoiding the loss of graft particle^[9].

Our preliminary study show promising results with an autologous graft "Dentin block" combining different regenerative properties from 3 biomaterials obtained from the patient himself with a low cost and a simple protocol, maximizing bone formation in a natural guided regeneration.

Conclusions: The present pilot study showed that in a "Dentin block" the dentin was replaced by new bone without host tissue reactions and at a favorable dentin resorption/bone formation rate. Although longer-term, multicenter, randomized, controlled clinical trials are required to confirm the preliminary results, our findings suggest a possible new substitute for bone regeneration.

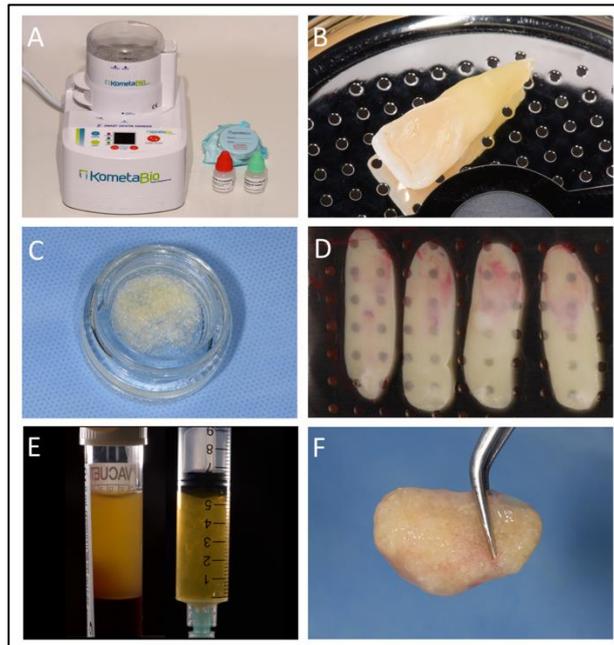


Figure 1: Preparation & application of Dentin Block: A. Smart Grinder Dentin TM (Kometa Bio, Holon, Israel). B. Extracted central incisor after cleaning. C. Particulate dentin as obtained at the end of process following a specific protocol (organic rest free) (particles 300 μ m-1200 μ m in size). D. Five L-PRF membranes after compression of the cloths in Xpression box (IntraSpin, Intra-Lock, Florida, USA). E. Liquide fibrinogen, aspirated from white cap tubes, after 3 min of centrifugation. F. Dentin block obtained after mixing 0.5 g dentin particles with 2 chopped L-PRF membranes and afterwards adding the liquid fibrinogen.

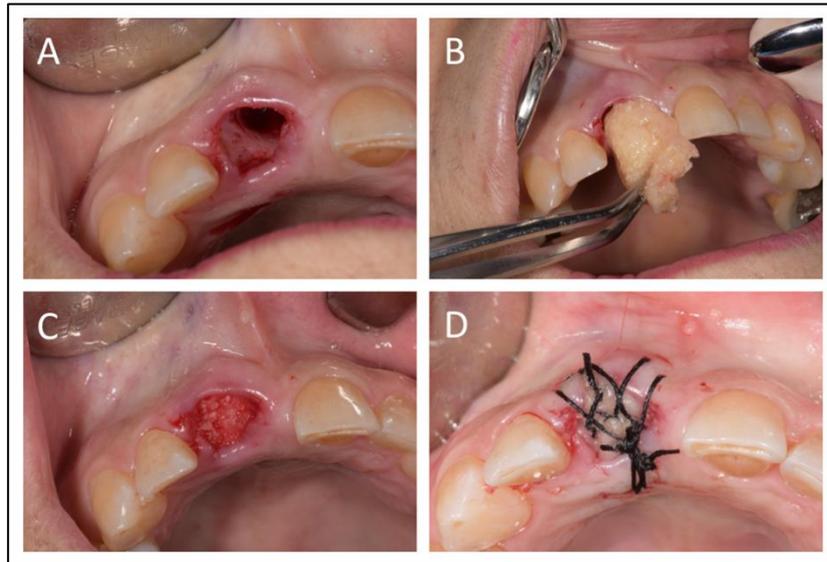


Figure 2: Ridge preservation via the use of a Dentin Block: A. Extraction socket (4-wall defect) of central upper incisor (tooth 1.1). B. Dentin block graft insertion. C. Relative position of Dentin block towards marginal bone. D. Dentin block graft is covered by two layer of L-PRF membranes secured with a silk suture. E. Bone healing after 5 months, at the moment of biopsy and implant placement.

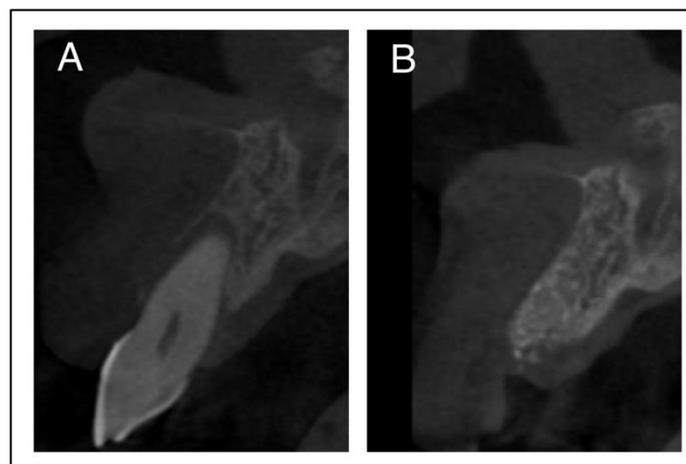


Figure 3: CBCT comparative analyses between baseline and after 4 months. A. Sagittal slice CBCT of pre-extraction socket of central upper incisor (tooth 1.1). B. Sagittal slice CBCT of alveolar ridge preservation with Dentin block (tooth 1.1) after 4 months. It is possible to observe a homogeneous density in the grafted site, comparable to normal trabecular bone.

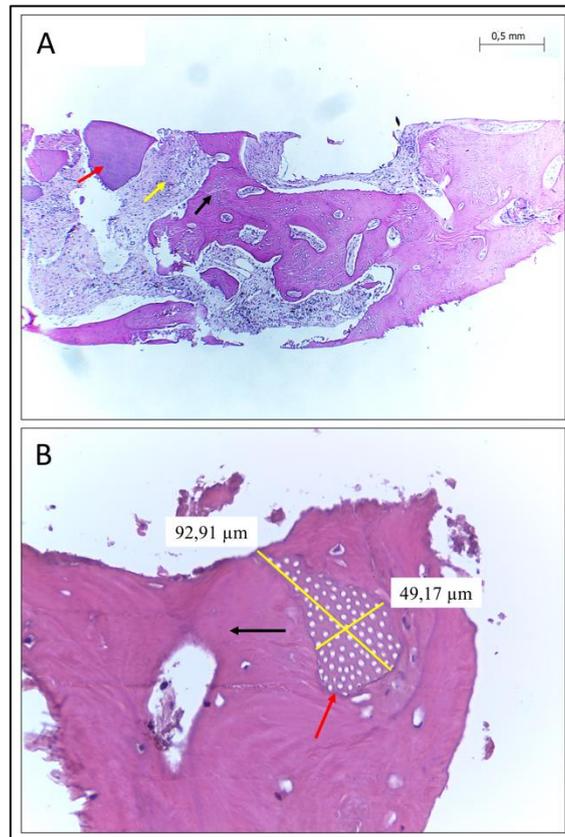


Figure 4: Histological samples (hematoxylin-eosin staining). A. Histologic section (10x magnification) from the core of grafted site showing compact bone (black arrow), connective tissue (yellow arrow) and a remaining dentin particle (red arrow). B. Histologic section (100x magnification) showing newly formed lamellar bone (black arrow) embedding a dentin graft particle, with clear dentinal tubules (red arrow). The yellow lines indicate the width and length of dentin particle.

Sample	Healing time (months)	Bone (%)	Dentin (%)	Connective tissue (%)
1	4	17.1	20.7	62.1
2	4	35.5	0.0	64.5
3	5	60.6	4.9	34.5
4	5	52.3	4.8	42.9
5	6	53.3	3.6	43.1
6	6	80.2	0.9	18.9
7	6	64.3	0.0	35.7
8	6	79.0	0.0	21.0
9	6	86.0	0.0	14.0
10	6	36.3	1.0	62.7
Overall mean		56.5	3.6	39.9
<i>S.D.</i>		22.2	6.4	18.7
Median		57.0	0.9	39.3
Mean/time point				
4 months		26.3	10.4	63.3
5 months		56.5	4.8	38.7
6 months		66.5	0.9	32.6

S.D. = Standard deviation

Table 1. Relative proportions in the 10 test sockets of bone, dentin and connective tissue estimated via histo-morphometric analysis. Biopsies were taken after 4, 5 or 6 months of submucosal healing.

References

- [1] Kim, Y.-K., Lee, J., Um, I.-W., Kim, K.-W., Murata, M., Akazawa, T., & Mitsugi, M. (2013). Tooth-derived bone graft material. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*, 39(3), 103.
- [2] Melek, L. N., & El Said, M. (2017). Evaluation of “Autogenous Bioengineered Injectable PRF – Tooth graft” combination (ABIT) in reconstruction of maxillary alveolar ridge defects: CBCT volumetric analysis. *The Saudi Journal for Dental Research*, 8(1-2), 86–96.
- [3] Murata M, Akazawa T, Mitsugi M, Kabir MA, UM IW, Minamida Y, et al. (2013). Autograft of dentin materials for bone regeneration. *Advances in Biomaterials Sciences and Biomedical Applications*, 1, 391–404.
- [4] Castro, A. B., Meschi, N., Temmerman, A., Pinto, N., Lambrechts, P., Teughels, W., & Quirynen, M. (2017b). Regenerative potential of leucocyte- and platelet-rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. *Journal of Clinical Periodontology*, 44(2), 225–234.
- [5] Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J. J., Mouhyi, J., & Gogly, B. (2006a). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 101(3), e51–e55.
- [6] Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J. J., Mouhyi, J., & Gogly, B. (2006b). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 101(3), e45–e50.
- [7] Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J. J., Mouhyi, J., & Gogly, B. (2006c). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 101(3), e37–e44.
- [8] Dohan Ehrenfest, D. M., Del Corso, M., Diss, A., Mouhyi, J., & Charrier, J.-B. (2010). Three-Dimensional Architecture and Cell Composition of a Choukroun’s Platelet-Rich Fibrin Clot and Membrane. *Journal of Periodontology*, 81(4), 546–555.
- [9] Andrade, C., Orrego, A., & Pinto, N. (2016). Hematologic profile of four autologous fibrinogen based binder obtained with different protocols. [https://doi.org/ 10.13140/RG.2.2.27106.12485](https://doi.org/10.13140/RG.2.2.27106.12485).

Benefits of L-PRF for ridge preservation & bone block grafting

Juan Blanco

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Tooth extraction inevitably leads to osseous deformities of the alveolar ridge producing marked decrease in width and some decrease in height. Prevention of ridge resorption has gained relevance in the last decades due to esthetic improvement in the front area, and the possible rehabilitation with dental implants anyplace in the mouth. To avoid or limit this ridge modification, several preservation techniques have been described, including the filling of the socket with auto-grafts, allografts, xenografts or alloplastic materials. In a recent systematic review, xenografts were found to perform the best, followed by allografts and alloplastic materials when compared to natural healing. Recently a “second generation” of platelets concentrate has been introduced and comprises Leukocyte and Platelet Rich Fibrin (L-PRF). Its characteristics (mechanical and biological) makes L-PRF a biologically suitable graft for alveolar ridge preservation and also osseous regeneration.

In this conference we will present an up to date review of socket preservation using L-PRF, preliminary data of our own clinical research on this topic, and clinical cases including socket preservation with L-PRF and its application in bone regeneration.

Guided Bone Regeneration With L-PRF in the Atrophic Maxilla – The GLAM Technique

Caramês, J.¹; Francisco, H.¹; Caramês, G.¹; Marques, D¹; Lopes, M.¹; Gouveia, R.¹

¹ *Implantology Institute, Avenida Columbano Bordalo Pinheiro nº50, Lisbon, Portugal*

Patients with maxillary atrophy and loss of lip support are often a challenge in terms of prosthodontic rehabilitation and surgical approach due to the aesthetic changes and bone availability for implant placement.

In edentulous patients, with severe maxillary atrophy and marked loss of lip support, the anterior maxilla commonly exhibits a thin buccal bone plate which requires horizontal bone augmentation, with several authors mentioning a minimum 2mm of facial bone to prevent vertical bone resorption.

The scientific literature presents several options for these cases (such as collagen or titanium membranes, non-resorbable pins, use of xenografts, allografts or autogenous bone) but still, none is considered as the gold standard.

The simultaneous approach, where implant placement is coincident with graft procedures, is preferred by both patients and clinicians, since it reduces treatment time and cost. However, it can't be applied in every case, due to the need of proper implant stability.

A significant clinical interest has grown regarding the use of L-PRF for regeneration, solely or in combination with xenografts, given its ease of protocol preparation, economic advantages, less invasive technique (no need for donor sites) and biological properties. Also, L-PRF has been used in immediately placed implants to restore the anatomy loss and to speed up soft tissue wound healing. However, the use of enough L-PRF membranes seems to be crucial to obtain an optimal effect.

For this reason, the use of a Guided bone regeneration with L-PRF in the Atrophic Maxilla (GLAM) technique is suggested as a surgical approach in patients with maxillary atrophy and evident loss of lip support, where Guided bone regeneration is performed with the use of L-PRF membranes and xenograft to restore the buccal bone volume of the Atrophic Maxilla, simultaneously to implant placement.

This presentation aims at describing the clinical technique and to report preliminary data on an ongoing prospective study that evaluates the dimensional changes in the aesthetic zone of resorbed maxillae (based on CBCT scans) occurred 12 months after full-arch implant placement and simultaneous regeneration with the GLAM technique.

Changes in bone morphology after socket preservation procedure with leucocyte- and platelet-rich-fibrin vs. conventional treatment

Ana Castellano, Paula Ruiz, Carlota Blanco, Lucía Maceiras, Lourdes Nóvoa, Leticia Caneiro, Juan Blanco

Department of Surgery and Medical-Surgical Specialties (Dentistry), Unit of Periodontology, Faculty of Medicine and Odontology, Santiago de Compostela.

Background: Socket preservation technique is a procedure that allows to maintain hard and soft tissue volume after tooth extraction. However, currently there is no available technique that can preserve the whole alveolar socket volume.

Aim: To evaluate and compare volume changes at 3 months after tooth extraction and socket preservation technique, with two treatment modalities (leukocyte and platelet rich fibrin -L-PRF- and deproteinized bovine bone mineral with 10% collagen covered with an autogenous soft-tissue graft -DBBM-C/PG-)

Material and methods: 40 patients with single hopeless teeth in the front area (15-25) will be selected in the Master of Periodontology of the University of Santiago de Compostela. In this work we will present preliminary results of 12 patients. Immediately after dental extractions, alveolar socket preservation technique will be done according to two randomly assigned treatment modalities (L-PRF / DBBM-C). The Horizontal bone changes (width) at 1 (primary outcome), 3 and 5 mm apical to the osseous crest and after 3 months of healing will be analysed by CBCT. The vertical changes in buccal, palatal and middle of the crestal bone will be also compared. Assessment of patient compliance with postoperative guidelines, intensity of pain after the intervention (VAS) and healing level (Landry index) will be also assessed.

Results: The radiographic analysis corresponding to 3 months in the test group shows an average of horizontal changes of -2.77 mm, -1.92 mm and -0.80 mm to -1 mm, -3 mm and -5 mm with respect to the crestal level, and in the group control -2.54 mm, -1.84 mm and -0.67 mm respectively. The mean of vertical changes in the test group was 1.84 mm in the vestibular crest and 1.06 mm in the palatal crest, for the control group it was 1.54 mm in the vestibular crest and 0.97 mm in the Palatal crest. The average of the Landry index was 3 for both groups.

Conclusions: Taking into account the limits of this study in terms of the number of patients studied, no differences can be seen in the radiographic changes between the 2 groups at 3 months, or in terms of postoperative morbidity and healing.

Are the centrifuge and timing a key factor for the preparation of platelet concentrates?

Castro A.B.¹, Cortellini S.¹, Coucke W.², Teughels W.¹, Pinto N.³, Quirynen M¹.

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² *Department of Clinical Biology, Scientific Institute of Public Health, Brussels, Belgium*

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Aim: To investigate if the use of a specific centrifuge, tubes or setting have any influence in the characteristics of the platelet concentrate obtained in terms of release of growth factors, cellular content and morphology.

Material and Methods: Three types of platelet concentrates were prepared with two different centrifuges adapting the *g* force for each protocol: leucocyte- and platelet rich fibrin (L-PRF) (408*g* for 12 minutes); advanced platelet rich fibrin (A-PRF) (276*g* for 14 min); advanced platelet rich fibrin+ (A-PRF+) (208*g* for 8 min). Two devices were used in which the *g* force could be adapted: the DUO centrifuge (Process for PRF, Nice, France) and the Intra-Spin centrifuge (Intra-Lock, Boca Raton, Florida, USA). Thus, we obtained three different preparations from each centrifuge: L-PRF-DUO, A-PRF-DUO, A-PRF+-DUO; and L-PRF-IL, A-PRF-IL, A-PRF+-IL. The levels of vascular endothelial growth factor (VEGF), transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor-AB (PDGF-AB) and bone morphogenetic protein-1 (BMP-1) released by all the membranes were analysed by means of ELISA for 5 different time intervals (0-4h, 4h-1day, 1-3d, 3-7d, 7-14d). The cellular counting of all the membranes was performed with a haematology analyser. Moreover, the morphology of the clots and membranes in terms of length, width and weight was evaluated. The impact of timing during the preparation of L-PRF was also assessed. Seven 9-ml glass-coated plastic tubes (BVBCTP-2, Intra-Spin, Intra-Lock, Florida, USA) were collected from three participants. One tube was immediately centrifuged (< 1 minute, at 408*g* for 12 min), whereas the rest were gently shaken for 1, 3, 5, 7, 10, and 15 minutes before centrifugation. The morphology of each L-PRF membrane was assessed after centrifugation. Another six 9-ml glass-coated plastic tubes were immediately centrifuge at 408*g* for 12 minutes. Standardized pictures were taken from the tubes immediately after centrifugation and after 10 min, 30 min, 1 h, 2 h, 3 h, and 4 h. The shrinkage of the clot inside the tube as well as the morphology of the L-PRF final membrane obtained were evaluated.

Results: Eight systemically healthy subjects participated in this study. TGF- β 1 was the most released growth factor after 14 days (cumulative concentration: L-PRF-IL: 119.409,6 pg/mb, L-PRF DUO: 124.223,5 pg/mb, A-PRF IL: 140.856,1 pg/mb, A-PRF DUO: 152.712,3 pg/mb, A-PRF+-IL: 128.691,2 pg/mb, and A-PRF+-DUO: 177.974,1 pg/mb). No statistically significant difference could be observed in the release of any growth factor between any membranes obtained with the different protocol and/or devices (Figure 1). The same applied for the cellular counting. The membranes prepared with the DUO Process centrifuge were often bigger in size than those from the Intra-Spin. However, no statistically significant difference could be observed. The timing in the preparation of the membranes was extremely important. As the time between blood collection and centrifugation became bigger, the membranes size decreased. After 5 min, no membrane could be obtained. The longer we wait after centrifugation to create the membranes, the smaller they became.

Conclusion: With the limitations of this study, we can conclude that the difference between L-PRF, A-PRF and A-PRF+ on one hand, or between the Intra-Spin and the DUO Process centrifuge on the other hand were insignificant. However, the timing at blood collection and after centrifugation needs to be considered as a very important. A randomized controlled clinical trial is started to investigate the benefits of L-PRF and A-PRF+ as a socket filling

material during ridge preservation when compared to natural healing. The preliminary data of the clinical study showed that the use of L-PRF or A-PRF+ as sole filling material can preserve the horizontal and vertical ridge dimension at three months after tooth extraction.

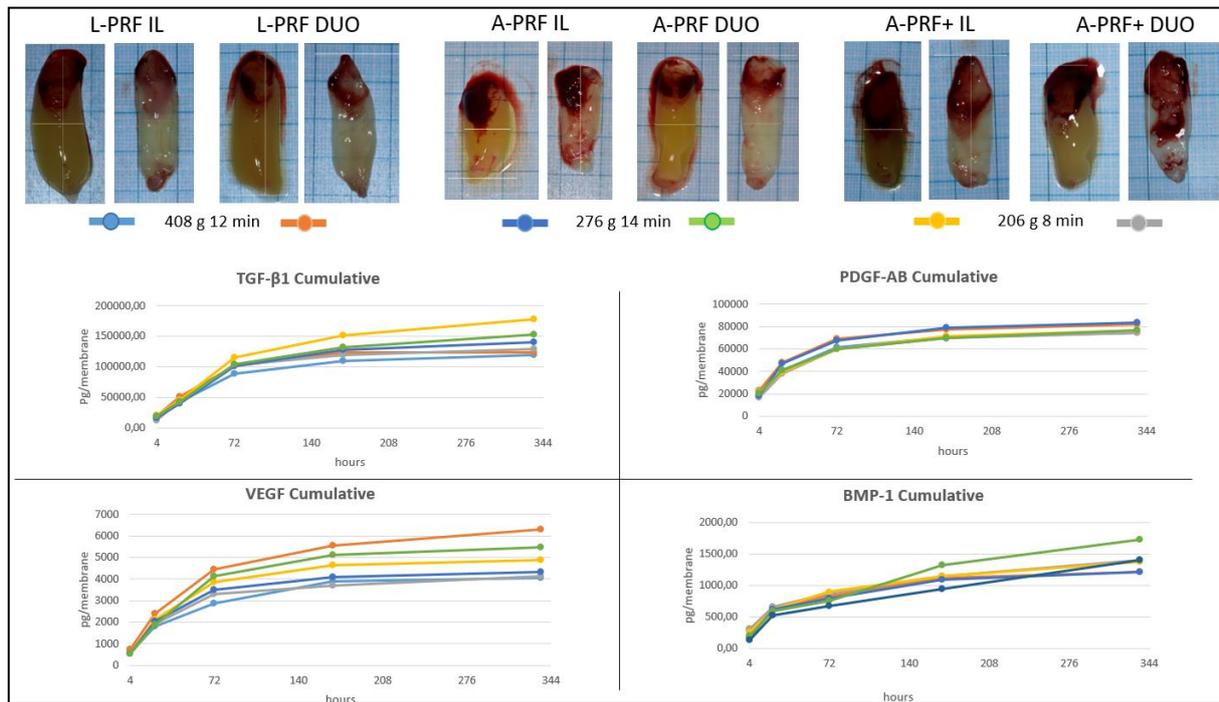


Figure 1. Release of growth factors (TGF-β1, PDGF-AB, VEGF, BMP-1) from all membranes up to 14 days (cumulative concentration). Representation of the morphology of all clots and membranes.

Benefits of L-PRF for the regeneration of periodontal defects (soft/hard tissues)

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Reconstruction of hard and soft tissues is one of the challenges in modern periodontal therapy. Clinicians are faced with the need to treat severe periodontal destructions associated with deep pockets and gingival recessions. Development of both surgical procedures and regenerative materials have so far increased the predictability of reconstructive medicine. Ample contribution comes from the understanding of the biologic principles that guide the wound healing process. Among many, key points are application of sophisticated surgery to provide stability of the blood clot and stability of the wound, careful manipulation of the soft tissues, perfect decontamination of the surgical field, and provision of regenerative materials able to speed / guide the reconstructive process. The application of blood-derived products, like the L-PRF, might contribute to facilitate or improve some of these aspects. A growing interest on the application of L-PRF has produced some studies that open a window on the potential and limits of this product.

Lateral and Trans-crestal sinus floor elevation in implant therapy with L-PRF: Randomized controlled clinical trial

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Aim: Sinus floor elevation is a technic to increase the alveolar bone height in the posterior maxilla. Different grafting materials for lateral and transcrestal sinus floor elevation have been described in literature. Xenografts, especially DBBM, are considered the “golden standard”. The application of L-PRF and L-PRF block has shown promising result in bone regeneration. However these two materials have never been compared to each other in RCT for sinus floor elevation. The primary objective of this study is to evaluate if the use of autologous leukocytes and platelet rich fibrin accelerate and promotes bone regeneration in the sinus in comparison with the standard GBR procedure.

Material and Methods: This study was designed as a randomized controlled clinical trial with parallel group design. Subjects in need of a lateral sinus floor elevation were treated with L-PRF block or DBBM and subjects in need of a transcrestal sinus floor elevation were treated with L-PRF or DBBM. Volumetric and linear augmentation was assessed using cone-beam computed tomography (pre-operatively (T0), post-operatively (T1) and post-healing (T2)). Outcomes were defined as the gain in bone height (mm) and the occurrence of any adverse event.

Results: The preliminary results show no difference between the two techniques.

Conclusion: The preliminary results of both studies will be presented.

Horizontal & vertical GBR combining L-PRF, autogenous bone, and liquid fibrinogen

Mic Demanet

Verwijsp praktijk Demanet & Leroy, Belgium

Aim: In order to optimise the outcome of horizontal and vertical GBR, a new protocol has been explored combining L-PRF, Autogenous bone, and liquid fibrinogen.

Material and Methods: Since more than 1 year, a new protocol for both horizontal as well as vertical bone augmentation, in both the upper and lower jaw was introduced in my private practise. An L-PRF block was prepared by mixing a particulated autogenous bone (often collected from the ramus mandibulae and the alveolar crest on the distal) with chopped L-PRF membranes at a 50:50 ratio and adding liquid fibrinogen to glue all together. All patients received antibiotics (2 x 1000 mg Amoxicilline for 5 days), Ibuprofen 3 x 600 mg/day for 3 days and an oral rinse with 0,12% Chlorhexidine as post-op protocol. Suture removal was performed after 3 weeks. The quality of the augmented bone was evaluated during implant placement, 4 – 6 months later.

Results: In the mean time more than 36 patients received their implants so that the augmented bone could be examined. In all patients a healthy, well vascularized, strong bone was found, with sufficient augmentation to allow a prosthetically driven implant insertion. All implants showed a good primary stability. Also during the healing phase after GBR no complications were recorded.

Conclusions: A L-PRF block with the use of autogenous bone as only bone substitute seems a suitable technique to augment deficient alveolar ridges.

Use of L-PRF in Periodontal Defects during Surgical and Non-Surgical Therapy

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Aim: My presentation aims to discuss the beneficial effect of L-PRF during non-surgical and surgical periodontal treatment in a private clinic.

Materials and methods: L-PRF membranes were used in 78 cases after scaling and root planning, and in 118 patients during the flap surgery in a 24 months' period. Teeth as well as implants were included in the treatment modalities. The surgical flaps or the scaling and root planning procedures were performed following the usual standards, but L-PRF membranes were added before the closure of the flaps, or at the end of the scaling and root planning procedure.

Results: An improvement in the objective as well as the subjective healing criteria were observed in the sites where the L-PRF membranes were used. A reduction or absence of inflammation was regularly observed in general and particularly in the proximal areas.

Conclusion: The additional use of the L-PRF membranes in routine periodontal procedures represents a pragmatic additional step that can have a positive influence on the clinical outcome with a minimal financial and biologic burdens.

References

[1] Ana B. Castro, Nastaran Meschi, Andy Temmerman, Nelson Pinto, Paul Lambrechts, Wim Teughels, Marc Quirynen (2017) Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. Journal of Clinical Periodontology 2017; 44; 67-82.

Leukocyte-Platelet Rich Fibrin (L-PRF) in the treatment of skin lesions

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Aim: The study intends to determine the effects of L-PRF in the treatment of skin lesions.

Methods: 18 patients with 23 skin ulcers of various types (10 post- traumatic, 6 diabetic, 2 venous stasis, 1 mixed (arteriovenous), 1 pressure, 1 peristomal, 1 rheumatoid), some already treated unsuccessfully with advanced dressings, were treated with L-PRF.

Results: 19 lesions (83 %) healed completely , 22 lesions (96%) presented highly vascularized and regenerated new tissue , in all 18 patients (100%) pain relief occurred at the first application. All traumatic wounds healed with fast epidermal growing. In one case of a diabetic foot with an exposed bone , healing was obtained avoiding amputation.

Conclusions: L-PRF, an autologous inexpensive and easy to prepare device in skin lesions, has shown in our limited experience excellent tolerability, antalgic efficacy , reduction of healing times in traumatic and inveterate non responders lesions , in diabetic foot could avoid amputation



Underlying processes of L-PRF mediated repair and its effect on dental pulp stem cells

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Aim: Leukocyte- and Platelet-Rich Fibrin (L-PRF) is an autologous platelet concentrate, consisting of a fibrin matrix enriched with platelets, leukocytes and a plethora of cytokines and growth factors. L-PRF is becoming a popular tool in regenerative medicine, and it has been proposed to stimulate tissue regeneration by promoting angiogenesis and nerve repair. Unfortunately, the underlying mechanisms of L-PRF induced tissue repair remain elusive. The L-PRF clot can be subdivided in two different fractions which contain soluble factors. During the compression process used to produce L-PRF membranes, the factors residing in the clot can be isolated and are termed the exudate (EX L-PRF). The factors present in the membrane can be isolated by keeping it in culture and the subsequent recovery of the culture medium (CM L-PRF). This project aimed to characterize L-PRF and explore its angiogenic and neuroregenerative potential. In addition, the effect of L-PRF on human dental pulp stem cells (hDPSCs) and hDPSC-mediated nerve regeneration was evaluated as it is known L-PRF contains cytokines that have been shown to enhance stem cell mediated repair, including TNF- α , IGF-1- and IL-1 β .

Material and methods: L-PRF growth factor release was determined by means of an antibody array and validated with ELISA. To evaluate L-PRF induced angiogenesis, endothelial proliferation, migration and tube formation assays were performed *in vitro* and in an *in ovo* chicken chorioallantoic membrane (CAM) assay. Whether L-PRF induced repair of the peripheral- or central nervous system *in vitro*, neurite outgrowth in dorsal root ganglia (DRG) and primary cortical neurons (pCNs) was measured respectively. The potential of the L-PRF subfractions on the survival, proliferation and migration of neural stem cells was assessed with a propidium iodide and transwell analysis. We also investigated the effect of L-PRF on peripheral nerve repair in an *in vivo* rat sciatic nerve injury model. Finally, the pro-regenerative effect of L-PRF on hDPSCs was evaluated with proliferation assays and the L-PRF-stimulated release of neurotrophins by hDPSCs was quantified with ELISA. The ability of L-PRF to enhance the neuroregenerative effect of hDPSCs was analysed on NSC proliferation and -migration and neurite outgrowth in pCNs.

Results and Discussion: The growth factor release profile by L-PRF revealed an abundance of C-X-C motif chemokine receptor 2 (CXCR-2) ligands and Epidermal Growth Factor (EGF) in addition to the presence of (NT-3), nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) in EX and CM L-PRF, with the highest concentrations being found in CM L-PRF. L-PRF induced blood vessel formation *in ovo* and stimulated endothelial proliferation, migration and tube formation *in vitro*. Inhibiting the EGF receptor lead to a reduction of the migratory response of endothelial cells. Neurite outgrowth was significantly enhanced in DRG neurons that were stimulated with CM L-PRF, but surprisingly this effect was not observed in pCNs. Strikingly, the addition of EX L-PRF to pCN and NSC cultures was neurotoxic. CM L-PRF did not induce neuronal cell death, but we found that it significantly stimulated NSC migration, but not proliferation. In addition, a pilot study showed that an L-PRF clot supports tissue regeneration *in vivo* of small gap nerve defects in rats. Finally, CM L-PRF and EX L-PRF significantly stimulated hDPSC proliferation and hDPSC-released BDNF release, but did not enhance the neuroregenerative effects of hDPSCs.

Conclusion: The results of this study demonstrated the angiogenic and neuroregenerative capacity of L-PRF both *in vitro* and *in vivo*. Moreover, these findings suggest caution when applying L-PRF when contact with the central nervous system cannot be avoided. Therefore, characterization of L-PRF and its molecular repair (or damaging) mechanisms remain important.

A clinical & social experience in the treatment of trophic ulcers in patients with Hansen's disease (Leprosy) using L-PRF wound care protocol in Nepal

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Punyaarjan Foundation – Nepal

Globally the trophic ulcers in patients with Hansen's disease (leprosy) are treated with conventional ulcer care approach which includes debridement of necrotic tissues, infection control, self-care education, nerve decompression and amputation if required. Conventional methods however are time consuming, costly and some ulcers even do not respond to these methods. Recently, Punyaarjan Foundation in joint collaboration with Anandaban Hospital has introduced L-PRF based wound care protocol as a pilot project to treat trophic ulcers in patients with Hansen's disease in Nepal. The presentation will share the clinical and social experiences of the team treating these ulcers using L-PRF based wound care protocol.

L-PRF: Characterization and its role in healing and tissue regeneration

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Leukocyte and Platelet Rich Fibrin (L-PRF) is an autologous platelet concentrate, consisting of a fibrin matrix enriched with platelets, leukocytes and a plethora of cytokines and growth factors. Since L-PRF can be produced bedside from whole blood without the use of an anti-coagulant, it is becoming a popular tool in regenerative medicine. More importantly, different types of platelet concentrates have been described to stimulate revascularization, which is a prerequisite for successful tissue regeneration. Therefore this lecture aims to characterize L-PRF and explore its angiogenic potential and its effects on stem cells. L-PRF induces endothelial proliferation, migration and tube formation *in vitro*. Furthermore, the individual components in the L-PRF construct will be presented and their role in the healing process will be discussed. Characterization of L-PRF remains important since patient variability still represents a clinical issue for the application of L-PRF.

The influence of antithrombotic drugs on biomechanical characteristics of Leukocyte Platelet Rich Fibrin membranes

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Aim - As antithrombotics modify blood coagulation, these drugs may influence the generation of Leukocyte Platelet Rich Fibrin (L-PRF), which is a living biomaterial derived from human blood, composed of immune and platelet concentrates on a fibrin membrane. Consequently, dependent on the concentration of antithrombotic drugs in the blood, among other patient-related factors, L-PRF membranes can vary in terms of mechanical characteristics such as shape, size and elasticity or stretch^[1]. This can have important clinical implications. The aim of this preliminary study was therefore to examine the elasticity of L-PRF membranes generated from blood samples whether or not supplemented with antithrombotic drugs.

Materials and methods - All blood samples were taken from one donor. L-PRF membranes were generated by withdrawn 9mL blood and immediately centrifuging it at 400g RCF (2700 rpm using the IntraSpin™ centrifuge, Intra-Lock, Boca Raton, FL, USA) for 12 minutes. Thereafter, L-PRF clots were removed from the tubes, separated from the red blood cells and placed in an Xpression™ kit for gentle compression during 5 minutes. Samples were prepared for tensile tests by cutting them in rectangular samples (width of 5mm and length of at least 16mm) and mounted on clamps of a testing bench of a planar biaxial tester (MessPhysik) without any tension on the membrane. Both clamps were moved to opposite sides until a preload of 0.1N was reached. The samples were then stretched by displacing both clamps an additional 12mm at a rate of 1mm/s and back. The displacement of 12mm was imposed 5 times, after which the stretching cycles were repeated for a displacement of 24mm and 36mm. Force-stretch curves were generated from the stretching data. During a first testing session, six blood samples were withdrawn and six L-PRF membranes were generated. A series of six tensile tests were performed with a time interval of half an hour in between to evaluate if L-PRF's elasticity declined over time. In a second session, four blood samples were taken: one control sample and three supplemented with 2.5IE, 5IE and 10IE of an antithrombotic drug (Clexane® 40 mg/0.4mL). Membranes were generated and uniaxial tensile tests were performed to examine the elasticity of these membranes.

Results and discussion - The series of six consecutive uniaxial tensile tests showed that within the tested period, d.i. four hours from the moment the L-PRF membranes were generated, the L-PRF's elasticity stayed within the same range. The tested L-PRF samples are numbered from one to six in Figure 1. For further testing, we can therefore assume that the timing of tests did and will not influence the results. This is in coherence with the finding that L-PRF membranes remain usable many hours after preparation (if conserved in physiological conditions)^[1]. The results of the samples supplemented with Clexane were rather surprising. The antithrombotic drug did not clearly influence the L-PRF membranes: L-PRF's elasticity seemed to be intact for the 2.5IE and 5IE samples, compared to the control sample (Figure 1). However, sample 10IE clearly behaved differently: the force-stretch curve shows no non-linearity (unlike the other samples) and this sample snapped easily. We believe that L-PRF membranes from blood of patients who are already taking antithrombotics for a long time will show these abnormalities as well.

Conclusion - From the present observations, it can be seen that there is a potential effect of antithrombotics on L-PRF's elasticity. Yet, further analysis on larger data samples and including more biomechanical factors should be carried out prior to drawing any clinical conclusions. Towards the future, analysis in an intention-to-treat population will be carried out to verify the influence of antithrombotic drugs on L-PRF membranes.

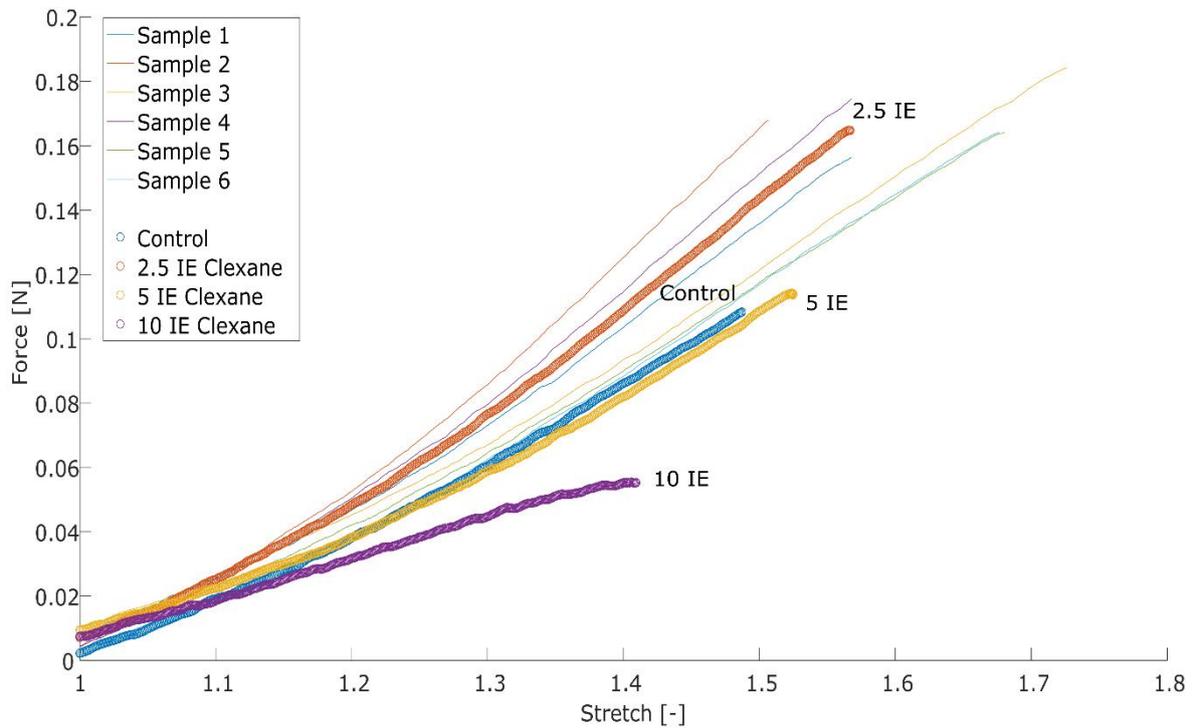


Figure 1: Force-stretch curves of six tested L-PRF membranes from testing session one (Sample 1-6) and of four L-PRF membranes of testing session two (Control, 2.5IE Clexane, 5IE Clexane and 10IE Clexane).

References

[1] Hartshorne J, Gluckman H. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part II: Preparation, optimization, handling and application, benefits and limitation of PRF. *Int Dent – African Ed.* 2016;6(5).

Adjunctive Effect of Autologous Platelet-Rich Fibrin to Barrier Membrane in the Treatment of Periodontal Intrabony Defects

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Background and Aim: Autologous platelet-rich fibrin (PRF) and barrier membranes in the treatment of intrabony defects in chronic periodontitis patients have shown significant clinical benefits. This study evaluates the additive effect of autologous PRF in combination with a barrier membrane versus the use of barrier membrane alone for the treatment of intrabony defects in chronic periodontitis patients.

Methods: A randomized split-mouth design was used. Sixteen patients with 32 paired intrabony defects were included. In each patient, one defect was treated using a resorbable collagen membrane along with PRF (test group) and the other defect by guided tissue regeneration alone (control group). The following clinical parameters were measured at baseline and after 9 months: plaque index, modified sulcus bleeding index, probing pocket depth, clinical attachment level, and gingival marginal level. The radiographic defect depth was also assessed at baseline and after 9 months.

Results: Test group showed a statistically significant improvement for probing depth ($P<0.002$), clinical attachment level ($P<0.001$), and radiographic defect depth ($P<0.001$) after 9 months as compared with the control sites. Radiographic defect depth reduction was $58.19\pm 13.24\%$ in the test group as compared with $24.86\pm 9.94\%$ reduction in the control group.

Conclusion: The adjunctive use of PRF in combination with barrier membrane is more effective in the treatment of intrabony defects in chronic periodontitis as compared with barrier membrane alone.

L-PRF: why should it work: from extra-oral to intra-oral wounds

Prof. Nelson R. Pinto

University of the ANDES - CHILE

Most clinicians are confronted with the treatment of wounds in their daily practice. According to their evolution these are classified as acute or chronic and the etiology could be attributed to surgical procedures, post-surgical complications, expression of systemic diseases, trauma, chemotherapy drugs or radiotherapy. The management of a wound until the complete healing challenges every clinician. Despite the advances of surgical techniques and biomaterials in this domain in order to enhance the healing process no consensus has been achieved so far.

Recently, the Natural Guided Regeneration Therapy based on the use of Leucocyte-Platelet Rich Fibrin (L-PRF) has been proposed as a common way of treatment to improve the healing process of any type of wound regardless their etiology neither their evolution. The L-PRF membrane considered a human living tissue graft has unique bio-mechanical characteristics. His fibrin structure and biological properties related with the cell content and slow release of growth factors make the L-PRF a perfect scaffold for hard and soft tissue regeneration. An added value is the antimicrobial and antinflammatory capacity which can benefit the patients in synergy with his potent analgesic effect. The possibility to treat difficult wounds extra-oral or intra-orally in a similar way has opened the possibility to regenerate tissues in a way that was not possible before. In this way we are transforming tissue repair into truly tissue regeneration. The greatest strength of any new therapy or technique lies in four fundamental pillars: availability, affordability, accessibility and reproducibility, the Natural Guided Regeneration therapy based on L-PRF has surpassed the test of these pillars.

L-PRF for Ridge preservation

Prof. Nelson R. Pinto

University of the ANDES - CHILE

Several biomaterials and techniques has been developed for ridge preservation. Most of them are very complex and expensive with no guarantee of successful results in all cases.

Every implant and biomaterial we use to treat our patients challenge human biology. Every step we move forward to reach the perfect combination that will match human biology in a better way improves the results and long term predictability of our clinical procedures . All research show us that the more we move to use autologous and nature based biomaterials we are getting more successful results .

The synergy of using autologous products has bring at the same time the possibility of doing minimally invasive approaches whit greatest benefits to our patients by shorten the number of procedures , the healing time and reducing the cost significantly.

In more than 15 years of study we are concluding that using the right combination of bio-activators and bio-materials we increase the success rate of tissue regeneration even in the most extreme challenging cases transforming tissue repair into truly tissue regeneration. The use of L-PRF for ridge preservation has been prove to be safe and effective as most equivalent techniques if is performed according to the protocol of the Natural Guided Regeneration which are reviewed step by step approach in this presentation.

Benefits of L-PRF in Wound Healing - the experience in Chili / Costa Rica

Prof. Nelson R. Pinto

University of the ANDES - CHILE

Leukocyte/platelet-rich fibrin (L-PRF), a second-generation platelet concentrate for topical use, is an autologous blood-derived product, which can be obtained, quickly and at low cost. It is classified as one of the four families of platelet concentrates for surgical use and is, therefore, a different class of products than traditional PRPs. L-PRF is produced from peripheral blood, which is immediately centrifuged without any anticoagulant. Coagulation starts during the centrifugation according to a specific protocol (FDA approved and CE marking): after centrifugation a red blood cell base at the bottom, acellular plasma as a supernatant (platelet-poor plasma), and the L-PRF clot in-between can be observed. The latter, rich in fibrin, platelets ($\pm 95\%$ of initial blood) and leukocytes ($\pm 50\%$ of initial blood), can be transformed into a membrane of 1 mm in thickness, by careful compression in a surgical box. (Expression Box, IntraSpin System, Intra-lock, Boca Raton, USA).

L-PRF membranes remain intact for more than 14 days in vitro (even more than 28 days in culture) and over 21 days in vivo. Due to a specific polymerization, architecture of the fibrin matrix, and cell content it possess antibacterial effects. L-PRF appeared therefore as a very interesting biomaterial to enhance wound healing. As it was proven in vitro, the IntraSpin/LPRF membranes, with a special fibrin network, progressively release a significant amount of growth factors (e.g., transforming growth factor $\beta 1$ (TGF β -1), platelet-derived growth factor AB (PDGF-AB), vascular endothelial growth factor (VEGF), BMPs, and insulin-like growth factors (IGF)), matrix glycoproteins (thrombospondin-1 (TSP-1)), fibronectin and vitronectin), and sequences of cytokines (e.g., IL-1 β , IL-6, TNF- α , and IL-4) for at least 7 days. The effects of L-PRF in vitro on cell cultures are very strong during at least 28 days, with a strong stimulation of proliferation of all tested cell lines (fibroblasts, pre-keratinocytes, pre-adipocytes, osteoblasts, and mesenchymal stem cells) and also a stimulation of differentiation of bone cells. L-PRF membranes behave in vitro like a "Human Living Tissue" interacting in co-cultures with cells. The release of leukocytes from the membrane enhance the environment to stimulate the M2 macrophage activity, this specific behavior reinforced the idea of using L-PRF membranes like a tissue graft in skin wounds. L-PRF can be considered as an "Autologous Blood Derivate Living Tissue Graft." In this sense, L-PRF is a very simple treatment without any risk for the patient that could be tried in all cases.

The possibility to use L-PRF as a biological scaffold as open the opportunity to regenerate soft and hard tissue in such a way that was not possible before. The clinical, immune histochemistry and histological findings (SEM, Confocal Laser, and Optical Microscopy) of our animals and humans studies over the last 14 years confirm the potential of L-PRF as a biological scaffold or as a "Living Tissue Graft " for hard and soft tissue regeneration in acute or chronic wounds. We have been able to probe L-PRF as a regenerative biomaterial in chronic wounds such as: Diabetic Foot, Venous Ulcers, Osteomyelitis, and Osteonecrosis by Bisphosphonate. In acute wounds: Traumatic Wounds and Burns. The possibility to use L-PRF in regenerative procedures for bone or skin grafts had led to new treatment concepts affecting a broad spectrum of clinical conditions. What we thought impossible yesterday could be routine tomorrow, through the "Natural Guided Regeneration therapy with IntraSpin/L-PRF."

Comparison of the effect of L-PRF and A-PRF on gingival fibroblast and periodontal ligament fibroblast

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Aim: To determine in an *in vitro* study whether L-PRF^[1, 2] and A-PRF+^[3, 4] differ in their capacity to induce proliferation and migration of two different periodontal fibroblast cell types. L-PRF and A-PRF+ are new autologous materials used in periodontal regenerative surgery^[5]. They are both derived from blood from patients, but have different intrinsic characteristics. The literature is controversial regarding the effects of the two PRF preparations on periodontal tissue fibroblasts^[6-10].

Material and methods: L-PRF and A-PRF+ membranes were prepared from venous blood of 8 patients with periodontitis, and subsequently incubated in culture medium. Gingival fibroblasts (G-F) and periodontal ligament fibroblast (PDL-F) cells were harvested from 5 different donors and pre-cultured until passage 3^[11]. The cells were then seeded on specific migration-assay plates, leaving a precise gap of $500 \pm 50 \mu\text{m}$ (Ibidi μ -slideTM). After 2 days, the pre cultured L-PRF and A-PRF+ supernatants were extracted from the plates, and added to the fibroblast cells, already seeded in assay plates. As controls, we added medium alone and medium with Fibroblast Growth Factor II (FGF II) on the same cells.

During 24 hours, the cell migration was observed with a dedicated time-frame microscope at 10x magnification, under 5% CO₂ and 37°C. Cell proliferation and cell viability were measured with a laser-induced fluorescence detection cell analyser. Images from living cell migration were analysed with an image software.

Main outcome measures were artificial wound closure percentage overtime, cell proliferation and cell viability. Statistical analyses were performed with Kruskal-Wallis test and Wilcoxon test, corrected with Friedman test for multiple comparisons.

Results: PRF conditioned medium from 8 patients were used on 7 cell lines (4 different Gingiva fibroblasts and 3 different periodontal ligament fibroblasts cultures). L-PRF and A-PRF+ induced higher cell proliferation than FGF and control: proliferation percentage versus control (NEG) were +69.2% for A-PRF+, +49.6% for L-PRF. Further, these differences were statistically significant, showing $p= 0.0367$ and $p= 0.0396$ respectively. Other comparisons were not significant.

A-PRF+ and L-PRF induced also significant faster cell migration (A-PRF+ vs FGF $p<0.001$; L-PRF vs FGF $p<0.01$; A-PRF+ vs NEG $p<0.001$; L-PRF vs NEG $p<0.05$), but were not differing from each other.

PRF conditioned medium did not affect cell viability, and were comparable to control cultures ($p=0.66$). In the late phase of migration (last 11 h), the induced migration was higher for the A-PRF+, compared to L-PRF ($p<0.001$).

Conclusions: L-PRF and A-PRF+ stimulate migration and proliferation of gingival fibroblasts and periodontal ligament fibroblasts, and cell migration by A-PRF+ was slightly increased above L-PRF.

References

1. Dohan Ehrenfest, D.M., L. Rasmusson, and T. Albrektsson, *Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF)*. *Trends Biotechnol*, 2009. 27(3): p. 158-67.
2. Dohan, D.M., et al., *Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2006. 101(3): p. e51-5.
3. Ghanaati, S., et al., *Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells*. *J Oral Implantol*, 2014. 40(6): p. 679-89.
4. Choukroun, J. and S. Ghanaati, *Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept*. *Eur J Trauma Emerg Surg*, 2017.
5. Miron, R.J., et al., *Use of platelet-rich fibrin in regenerative dentistry: a systematic review*. *Clin Oral Investig*, 2017. 21(6): p. 1913-1927.
6. El Bagdadi, K., et al., *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*, 2017.
7. Kawase, T., et al., *The heat-compression technique for the conversion of platelet-rich fibrin preparation to a barrier membrane with a reduced rate of biodegradation*. *J Biomed Mater Res B Appl Biomater*, 2015. 103(4): p. 825-31.
8. Fujioka-Kobayashi, M., et al., *Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response*. *J Periodontol*, 2017. 88(1): p. 112-121.
9. Kobayashi, E., et al., *Comparative release of growth factors from PRP, PRF, and advanced-PRF*. *Clin Oral Investig*, 2016.
10. Dohan Ehrenfest, D.M., et al., *The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane*. *Platelets*, 2017: p. 1-14.
11. Scheres, N., et al., *Gingival and periodontal ligament fibroblasts differ in their inflammatory response to viable Porphyromonas gingivalis*. *J Periodontal Res*, 2010. 45(2): p. 262-70.

L-PRF Block in posterior maxilla sites

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Leucocyte- and platelet-rich fibrin (L-PRF), a second generation platelet concentrate, is an autologous blood derived product, which can be obtained, quickly and at low cost. L-PRF is produced from peripheral blood, which is immediately centrifuged without any anticoagulant. L-PRF is rich in fibrin, platelets ($\pm 95\%$ of initial blood) and leucocytes ($\pm 50\%$ of initial blood), and can be transformed into strong membranes circa 1 mm in thickness. These membranes release large amounts of growth factors for a long period (≥ 7 days).

These membranes are also used to prepare a L-PRF block (a 1:1 mixture of particulated xenograft with chopped L-PRF membranes, adding Liquid Fibrinogen to trap the xenograft). Such L-PRF blocks can be used for horizontal (vertical) bone augmentation, when combined with a collagen membrane, fixed with titanium pins and protected by a superficial layer of L-PRF membranes.

29 patients presenting 36 sites with horizontal alveolar deficiencies were treated. One graft showed a complication during healing. The remaining grafts created an average linear horizontal bone gain of $\pm 4,5$ mm (measured on cross-sectional CBCT images). 50 implants were placed and survived with an average follow-up of 12 months. This presentation will show clear guidelines (step by step procedures) for the clinical use of L-PRF blocks.

L-PRF in soft tissue regeneration

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L-PRF, a second generation of platelets concentrates, enhances the healing process of hard and soft tissue in a natural way, because it increases the total amount of growth factors present in the recipient site. The gradual disintegration of L-PRF membranes in the site being regenerated, permits a slow release of growth factors, adhesion molecules and pro and anti inflammatory cytokines for at least 7 days, which modulate the reparatory inflammatory response, increase the speed and efficiency of tissue regeneration and diminish post-operative pain and edema. Some of these growth factors described are TGF- β 1 (Transforming Growth Factor β 1), PDGF (Platelet-Derived Growth Factor), VEGF (Vascular Endothelial Growth Factor), EGF (Epithelial Growth Factor), FGF (Fibroblast Growth Factor), IGF (Insulin like Growth Factor) and other adhesion molecules such as Vitronectin and Fibronectin are also released. These molecules have the role of enhancing proliferation, adhesion and function of fibroblasts, osteoblasts and mesenchymal cells as well as stimulating angiogenesis, vascular permeability and repair.

Hundred of publications today support the use of L-PRF in several different clinical conditions. In this presentation we will see the use of L-PRF in different clinical applications associated to periodontal plastic surgery.

The absence of an adequate keratinized mucosa has been associated with high plaque accumulation and gingival inflammation. If a decision is made to increase the zone of the keratinized gingiva, the standard of care for non-root-coverage techniques has been a free gingival graft (FGG). As a graft, the palatal tissue provides keratinized gingiva, but it retains its palatal phenotype and may be noticeably different in color and texture from the surrounding gingival tissue. It has been suggested that connective tissue of the palate in replacement of free gingival grafts, improves the color aspects of the grafted tissue, but nevertheless, a much greater reduction in size of the graft, post healing is observed. The use of L-PRF along with connective tissue to increase the width of the keratinized gingiva is one of the applications that will be shown, avoiding size reduction and speeding up the healing process

Gingival recessions are highly prevalent, affecting mainly people who have high oral hygiene standards, and generally the buccal surface is most involved, without compromising interproximal tissues.¹ Patients usually complain because of aesthetic and hypersensitivity reasons. The radicular area proves difficult to maintain an adequate oral hygiene, resulting in plaque accumulation and caries formation, all of which can significantly affect patients' life quality.^{2,3}

There are numerous surgical procedures described to treat gingival recessions, some of which include the laterally positioned or rotated flap, double papillae flap, semilunar flap, coronally advanced flap (CAF), tunnel technique and guided tissue regeneration. Some of these flaps can be combined with grafts such as sub-epithelial connective tissue, free gingival graft, acellular dermic matrices and today we can use L-PRF as a complement of the graft techniques or in replacement of them.

The ideal result is to achieve complete root coverage that is stable for a long time and in which the graft mimics neighbouring tissues.^{3,4,5} In this presentation we will show the use of L-PRF as the only graft material used along with a CAF technique to cover single gingival recessions

and also L-PRF with a tunnel technique described by Allen ⁶ to cover multiple gingival recessions.

References

1. Albandar, Kingman. Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, *J Periodontol* 1999;70(1):30-43.
2. Potârniche O, Solomon S, Pasarin L, Martu A, Nitescu D, Martu S. Statistical study on the prevalence of gingival recession in young adults. *International Journal of Medical Dentistry* 2013;3:219-224.
3. Saadoun, A. Current trends in gingival recession coverage-Part I: The tunnel connective tissue graft. *Pract Proced Aesthet Dent* 2006;18(7):433-438.
4. Cairo F, Pagliaro U, Nieri M. Treatment of gingival recession with coronally advanced flap procedures: a systematic review. *J Clin Periodontol* 2008;35(Suppl. 8):136-162.
5. Kontovazainitis G, Griffin TJ, Cheung WS. Treatment of gingival recession using platelet concentrate with a bioabsorbable membrane and coronally advanced flap: a report of two cases. *Int J Periodontics Restorative Dent* 2008;28(3):301-7.
7. Allen AL. Use of the supraperiosteal envelope in soft tissue grafting for root coverage. I. Rationale and technique. *Int J Periodontics Restorative Dent* 1994;14:216-27.

Analyses on growth factors and collagen expression on i-PRF

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Aim: The main aim of this study was to perform a cellular, morphological and protein characterization of an injectable platelet-rich fibrin (i-PRF).

Material and methods: Blood samples were collected from fifteen volunteers to prepare the i-PRF samples. Peripheral blood was used as a control group. Blood clot or i-PRF samples were incubated for 10 days. The supernatant of the samples was collected for ELISA immunoassay quantification of PDGF-AB and VEGF growth factors over periods of 1, 8, 24, 72 and 240 h. Blood clot or i-PRF samples were histologically characterized regarding the presence of IL-10, osteocalcin, and TGF- β . Reverse transcriptase polymerase chain reactions (RT-PCR) were used to evaluate the gene expression of type 1 collagen. Also, samples of i-PRF and blood clot were prepared to evaluate their morphologic aspects by scanning electron microscopy.

Results: A higher concentration of platelets and lymphocytes was recorded in i-PRF when compared to peripheral blood ($p < 0.05$). Release levels of VEGF were higher on blood clot samples (1933 ± 704 pg/ml) compared to i-PRF (852 ± 376 pg/ml; $p < 0.001$), and therefore there were no differences in PDGF-AB levels. Immunohistochemistry revealed a regulation of TGF- β , IL-10, and osteocalcin in the i-PRF group. RT-PCR showed increased type 1 collagen expression in i-PRF ($p < 0.05$). SEM images showed agglomeration of platelets and lymphocytes into the clusters of a dense 3-dimensional fibrin network formed in i-PRF.

Conclusion: The injectable PRF revealed clusters of a 3-dimensional fibrin network embedding a higher agglomeration of blood platelets, leukocytes, and growth factors when compared to blood clot. The *in vitro* characterization of the morphologic and biologic aspects of i-PRF leads to a better understanding of clinical effects and knowledge to develop effective guidelines for different clinical cases.

References

- [1] Castro, A.B., Meschi, N., Temmerman, A., Pinto, N., Lambrechts, P., Teughels, W. & Quirynen, M. (2017b) Regenerative potential of leucocyte- and platelet-rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. *Journal of Clinical Periodontology*. 44, 225–234.
- [2] Cortellini, S., Castro, A.B., Temmerman, A., Vandessel, J., Pinto, N., Jacobs, R., Quirynen, M. (2018) L-PRF block for bone augmentation procedure: a proof-of-concept study. *Journal of Clinical Periodontology* Feb 8. doi: 10.1111/jcpe.12877. [Epub ahead of print]
- [3] Varela, H., Noronha Oliveira, M., Pereira, J., Souza, J.C.M., Pinto, M., Quirynen, M. (2018) Platelet-rich fibrin to incorporate bioactive graft materials. In: *Nanostructured Biomaterials for Cranio-Maxillofacial and Oral Applications* 1st ed. Elsevier, 119-142.

Benefits of L-PRF in wound healing - the experience in Belgium

Yannick Spaey

Maria Ziekenhuis, Belgium

Based on recent literature an overview of the use of L-PRF in wound healing will be presented, with 2 main items: the use of L-PRF in MRONJ and secondly the treatment of all kind of skin "ulcers".

A Report of the Experience of L-PRF for Horizontal Ridge Augmentation in Private Practice

Paul Stone

Blackhills Specialist Dental Clinic and Edinburgh University Dental Institute, Scotland

Aim: The aim of this report is to review the impact of introducing L-PRF and biomaterials for horizontal ridge augmentation into a busy private specialist referral clinic

Material and methods: An informal review of the methods and techniques introduced to the clinical environment to allow for minimal interruption of day-to-day practice.

Results: A very well received new technique that was quickly and effectively integrated into clinical practice with excellent results for patients. Staff enjoyed the additional roles associated with the L-PRF process and all patients appreciated the technology and benefits.

Conclusion: After team training and a short learning curve, staff quickly became proficient at the preparation of the L-PRF and the results of the first 18 months of experience supported the introduction of the technique to this private clinic.

The benefits in L-PRF in implant surgery

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After teeth are extracted, a dynamic bone remodeling phenomenon takes place, often reducing the bone height and width leading to vertical and horizontal resorption of the alveolar ridge. This presents the clinician with significant challenges in the rehabilitation. For sure the posterior maxilla, due to the presence of the maxillary sinus, represents a particular region in the dental arch.

Continuous advances in the field of implant dentistry have provided clinicians with various treatment options to facilitate the placement of oral implants in patients with vertical bone deficits in the posterior maxilla. Today, one of the most popular and scientifically addressed ways to compensate for inadequate vertical bone height is to elevate the sinus floor. Sinus floor elevation procedures are of moderate to high complexity, entailing several significant risks and possible complications. These procedures are often employed in combination with bone grafts and biomaterials.

This lecture will provide an overview on the most important sinus floor elevation techniques. Techniques will be described in detail to avoid pitfalls.

Furthermore, the use of second generation platelet concentrates (L-PRF), during these procedures to minimize the need of biomaterials and for possible complication management will be addressed. The use of L-PRF as an implant coating material will be critically discussed.

Bone augmentation with LPRF blocks in the posterior mandible: A clinical evaluation

Pascal Valentini, Olivier Henry Savajol, Jean Paul Mangion, Jean Michel Ferrandi

University of Corsica

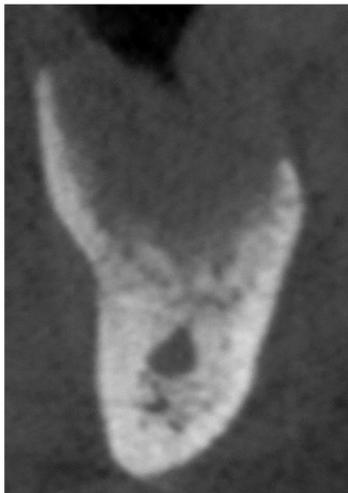
In some cases the implant placement in the posterior mandible requires a second surgical site to harvest the bone blocks as grafting material. This technique is often related with some morbidity. The use of LPRF blocks in conjunction with GBR could be the solution to make bone augmentation less invasive. The aim of this presentation is to present some cases of atrophic posterior mandible treated with this technique.

Material and Methods: 6 patients presenting bone deficiency in the posterior mandible due to peri-implantitis, extraction due to bulky endodontic lesions and long term denture wearing have been selected for bone augmentation with L-PRF blocks in combination with adsorbable collagen membrane prior implant placement 4 months after the graft procedure. The bone gain has been evaluated with CBCT.

Results: For all the patients, implants placement was possible after bone augmentation with this technique. For one patient who underwent vertical augmentation the bone quality was not good enough for implant placement an implant placement had to be postponed.

Discussion: Bone formation seems to be dependant of the bony environment especially the number of bony wall bordering the area to be augmented. This statement is particularly valid for vertical augmentation.

Conclusion: This grafting technique seems to be promising for bone augmentation in the posterior mandible but there is a need for more studies in order to select the indications.



pre op



post graft



4 months

Enhanced oral bone grafting in the sinus with L-PRF and the Intralift

Marcel Wainwright

Universidad de Sevilla, Department of Oral Surgery, Calle Avicena, Sevilla/Spain

Aim: The aim of the presentation is to present the benefits in using L-PRF in conjunction with sinus augmentation procedures, especially with the Intralift™. Since the author is an expert in sinus grafting procedures, especially in ultrasonic driven sinus graftings and a Co-inventor of the Intralift™, he adds blood concentrates since many years to his augmentation material.

Material and Method: The author describes in his presentation a crestal ultrasonic and hydrodynamic sinus grafting procedure (Intralift™) based on the cavitation effect. In his presentation he shows cases with β -TCP and Hydroxyapatite that was used in sinus grafting as a material where L-PRF was added. Different cases are presented and some of them with remaining bone heights less than 2 mm. Post surgical follow up and radiographic analysis, analgetic consumption post op and swelling behavior have been monitored to underline the enhanced outcome with L-PRF.

Results: In the presented cases the additional use of L-PRF in sinus grafting, here in combination with the Intralift™, the post op complains, the enhanced wound healing and the enhanced bone regeneration was obvious and is documented.

In his presentation and according to the long term experience of the author with blood concentrates he highlights the benefits of adding L-PRF to a standardized sinus grafting procedure. A less invasive concept with a better outcome in terms of enhanced wound healing, less post op complains and faster bone regeneration was observed and showed the capacity of this additional application to standardize the use of L-PRF in any sinus grafting protocols.

Conclusion: Adding L-PRF to sinus grafting procedures is a method where less post op complains, less swelling, faster wound regeneration and a faster bone regeneration can be obtained. In combination with β -TCP and Hydroxyapatite as a grafting material in the presented cases the author can underline the beneficial effects of L-PRF in sinus grafting procedures.

LIST OF POSTER PRESENTATIONS

In alphabetic order by presenting author

Coronally advanced flap with L-PRF versus connective tissue graft for treatment of single gingival recession

Catherine Andrade¹, Antonio Sanz¹, Nelson Pinto¹

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Aim: The aim of the present study was to compare the coronally advanced flap (CAF) with L-PRF versus connective tissue graft (CTG) for treatment of single Miller's class I and II gingival recession. (Preliminary report)

Material and methods: A randomized controlled clinical trial was performed with two parallel groups (20 gingival recessions per group): 1) CAF associated with L-PRF and 2) CAF associated with connective tissue graft.

A trained and calibrated examiner performed the following clinical measurements at baseline, 1, 3 and 6 months postoperative: recession depth (RD), recession width (RW), probing depth (PD), clinical attachment level (CAL), keratinized gingival width (KGW) and gingival/mucosal thickness (GTH). The recession area (RA) and the root coverage percentage was calculated using a standardized photograph with a magnification 1:1.

The statistical analysis was performed using the software Stata v14.0. Measures of central tendency and variability were calculated for each parameter by treatment at baseline and for delta to 1, 3 and 6-month post-treatment.

The method of Shapiro-Wilk test was used to confirm that the data were sampled from a normal distribution. The significance of the difference between groups was evaluated with the paired-samples t-test for data with normal distribution and Mann-Whitney U test for data without normal distribution. Differences were considered statistically significant at P value < 0.05.

Results: The preliminary data showed 11 gingival recessions with 6 months of follow-up (CAF+L-PRF= 6; CAF+CTG=5). The baseline clinical parameters showed a statistically significant difference for recession depth (p value= 0.0323), the median and iqr (interquartile range) for CAF+L-PRF and CAF+CTG were 3(1) and 2(0) respectively. The table 1 shows the measures of central tendency and variability for the parameters evaluated at baseline and for delta to 1, 3 and 6-month post-treatment and the differences observed between groups.

A statistically significant decrease was observed for CAF+L-PRF group for delta PD to 6 months (p value=0.0174). On the other hand, the CAF+CTG showed a significant decrease for delta CAL to 3 months, and for delta RD to 1 month. Additionally, this group showed a significant decrease for delta RW to 3 and 6 months and a significant increase for delta KGW to 1 month. The percentage of coverage achieved was major for CAF+CTG with a statistical significant difference at 1, 3 and 6 months. The figure 1 shows the clinical result obtained at 6 months with both treatment modalities.

Conclusions: This preliminary report shows that both group were able to achieve a root coverage. However, the CAF+CTG group showed a superior result for CAL, RD, RW, KGW and percentage of root coverage compare to CAF+L-PRF. The study is on course for that reason, we expect to have for patients at September.

Variables	CAF+L-PRF n= 6	CAF+CTG n=5	P Value
Probing depth (mm)			
Baseline	1.72 ± 0.25	1.8 ± 0.18	0.5790
1 Month	0.28 ± 0.44	1 ± 0.23	0.0513
3 Months	0.22 ± 0.46	0.2 ± 0.3	0.9277
6 Months	-0.11 ± 0.17	0.2 ± 0.18	0.0174*
Clinical attachment level (mm)			
Baseline	3.44 ± 0.54	2.93 ± 0.28	0.0911
1 Month	0 (2)	-1.67(0.33)	0.0648
3 Months	-0.11 ± 0.86	-1.2 ± 0.51	0.0349*
6 Months	-0.83 (2.33)	-1.33 (0.33)	0.0980
Recession depth (mm)			
Baseline	3 (1)	2 (0)	0.0323*
1 Month	-1.83 ± 0.84	-3.8 ± 0.84	0.0119*
3 Months	-1.33 ± 0.82	-2.4 ± 0.89	0.0686
6 Months	-2 (3)	-2 (0)	0.1028
Recession width (mm)			
Baseline	4.83 ± 2.23	3.2 ± 0.84	0.1581
1 Month	-1.83 ± 1.32	-3.2 ± 0.84	0.0784
3 Months	-1.5 ± 1.38	-3.2 ± 0.84	0.0398*
6 Months	-1.5 ± 1.38	-3.2 ± 0.84	0.0398*
Keratinized gingival width (mm)			
Baseline	4.5 ± 0.86	5.13 ± 1.76	0.4540
1 Month	-0.17 ± 0.94	1.93 ± 1.79	0.0332*
3 Months	-0.22 ± 1.36	1.07 ± 1.36	0.1524
6 Months	-0.06 ± 1.27	-0.26 ± 1.49	0.8058
Gingival/mucosal thickness (mm)			
Baseline	3 ± 0.76	2.6 ± 0.28	0.2967
1 Month	0.72 ± 0.90	0.67 ± 0.24	0.8975
3 Months	0.17 ± 1.17	0.53 ± 0.29	0.5151
6 Months	0.33 (1)	0.33 (0.33)	0.4092
Recession area (mm²)			
Baseline	10.8 (18.62)	5.63 (2.92)	0.2012
1 Month	-7.37 ± 3.23	-5.81 ± 2.59	0.4081
3 Months	-7.43 ± 3.32	-5.81 ± 2.59	0.3990
6 Months	-7.11 ± 2.49	-5.81 ± 2.59	0.4182
% Root Coverage			
1 Month	64.33 (50.61)	100 (0)	0.0128*
3 Months	67.33 (50.11)	100 (0)	0.0041*
6 Months	70.39 (60.11)	100 (0)	0.0041*

Table 1: Statistical description of baseline and delta to 1, 3 and 6 months of probing depth, clinical attachment level, keratinized gingiva, tissue thickness and recession area.

Mean ± SD, Median (iqr)
*Statistically significant difference (P <0.05)

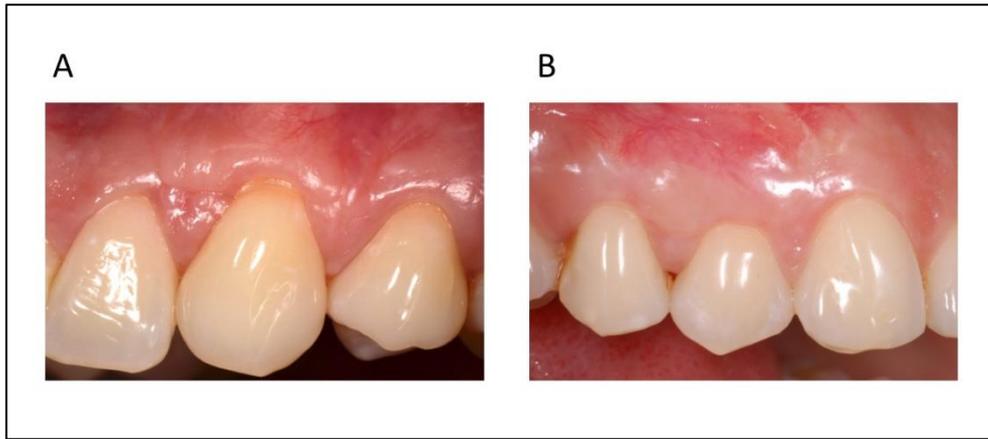


Figure 1: Clinical results obtained at 6 month post-treatment for CAF+L-PRF (A) and CAF+CTG (B).

Use of L-PRF and CTG in the treatment of Gingival Recessions: A Case Report

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Aim: The aim of this report is to present a case in which gingival recessions have been treated using the tunnel technique combined with sub-epithelial palatal connective tissue graft (CTG) and leukocyte- and platelet-rich fibrin (L-PRF). Few studies refer to the use of L-PRF in treating these defects and, to the author's knowledge, no case report has been published regarding the use of both L-PRF and CTG in treating gingival recessions in combination with the tunnel technique in the same surgical site nor in showing long-term outcomes.

Material and methods: In this case, a 30-year-old woman with multiple gingival recessions, thin gingival biotype and dental sensitivity has been treated and followed for four years using a novel protocol for radicular coverage.

Results: This clinical case achieved complete root coverage and excellent results in terms of contour and colour, this outcome being maintained and stable for a long time (four years).

Conclusion: Covering gingival recessions using the above-mentioned technique is a reliable treatment that allows gingival tissue regeneration and improves insertion levels in an efficient, aesthetic and highly stable way over time. The use of L-PRF could produce a positive difference in the site needing to be regenerated by adding a concentration of growth factors that induce regeneration of lost tissues.



Figure 1: Use of L-PRF in the case. A. L-PRF insertion. B. L-PRF exudate injection. C. L-PRF membranes as a biological surgical dressing.



Figure 2: A. Baseline B. 4 years after surgery

Clinical Measurement	Tooth 4.2	Tooth 4.1	Tooth 3.1	Tooth 3.2
Miller's Class	I	II	I	I
Pini Prato's Class	A-	A-	A-	A-
Recession depth	1mm	5mm	1,5mm	1,5mm
Recession width	1,5mm	2,5mm	2mm	1,5mm
Width of keratinized tissue	3mm	0mm	2mm	3mm
Dental sensitivity	VAS 2	VAS 5	VAS 2	VAS 2
Gingival biotype (De Rouck, 2009)	Thin (A1)			

Table 1: Initial Parameters

Clinical Measurement	Tooth 4.2	Tooth 4.1	Tooth 3.1	Tooth 3.2
Recession depth	0mm	0mm	0mm	0mm
Recession width	0mm	0mm	0mm	0mm
Width of keratinized tissue	4mm	4mm	3mm	3mm
Dental sensitivity	VAS 0	VAS 0	VAS 0	VAS 0
Gingival biotype (De Rouck, 2009)	Thick (A2)			

Table 2: 4 year follow-up parameters

References

- [1] American Academy of Periodontology. Glossary of Periodontal Terms. Chicago: The American Academy of Periodontology, 2001:44.
- [2] Potârniche O, Solomon S, Pasarin L, Martu A, Nitescu D, Martu S. Statistical study on the prevalence of gingival recession in young adults. *International Journal of Medical Dentistry* 2013;3:219-224.
- [3] Saadoun, A. Current trends in gingival recession coverage-Part I: The tunnel connective tissue graft. *Pract Proced Aesthet Dent* 2006;18(7):433-438.
- [4] Cairo F, Pagliaro U, Nieri M. Treatment of gingival recession with coronally advanced flap procedures: a systematic review. *J Clin Periodontol* 2008;35(Suppl. 8):136-162.
- [5] Allen AL. Use of the supraperiosteal envelope in soft tissue grafting for root coverage. I. Rationale and technique. *Int J Periodontics Restorative Dent* 1994;14:216-27.
- [6] Dohan D, Choukron J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:E56-60.
- [7] Saluja H, Dehane V, Mahindra U. Platelet-Rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. *Ann Maxillofac Surg* 2011;1:53-57.
- [8] Dohan D, Choukron J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:E45-50.
- [9] Dohan M, Bielecki T, Jimbo R, et al. Do the Fibrin Architecture and Leukocyte Content Influence the Growth Factor Release of Platelet Concentrates? An Evidence-based Answer Comparing a Pure Platelet-Rich Plasma (P-PRP) Gel and a Leukocyte- and Platelet-Rich Fibrin (L-PRF). *Current Pharmaceutical Biotechnology*, 2012;13:1145-1152.
- [10] Leitner C, Gruber R, Neumüller J, et al. Platelet content and growth factor release in platelet-rich plasma: a comparison of four different systems. *Vox Sanguinis* 2006;91:135–139.
- [11] Padma R, Shilpa A, Kumar PA, Nagasri M, Kumar C, Sreedhar A. A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's class-I and II recession defects. *J Indian Soc Periodontol* 2013;17(5):631-6.
- [12] Keceli HG, Kamak G, Erdemir EO, Evginer MS, Dolgun A. The Adjunctive Effect of Platelet-Rich Fibrin to Connective Tissue Graft in the Treatment of Buccal Recession Defects: Results of a Randomized, Parallel-Group Controlled Trial. *J Periodontol* 2015;86(11):1221-30.
- [13] Kuka S, Ipci SD, Cakar G, Yılmaz S. Clinical evaluation of coronally advanced flap with or without platelet-rich fibrin for the treatment of multiple gingival recessions. *Clin Oral Investig* 2017 Oct 20.
- [14] Öncü E. The Use of Platelet-Rich Fibrin Versus Subepithelial Connective Tissue Graft in Treatment of Multiple Gingival Recessions: A Randomized Clinical Trial. *Int J Periodontics Restorative Dent* 2017 Mar/Apr;37(2):265-271.
- [15] Pazmiño VFC, Rodas MAR, Cáceres CDB, et al. Clinical Comparison of the Subepithelial Connective Tissue versus Platelet-Rich Fibrin for the Multiple Gingival Recession Coverage on Anterior Teeth Using the Tunneling Technique. *Case Rep Dent* 2017;2017:4949710.
- [16] M. & Van Assche, N. (2011) Microbial changes after full-mouth tooth extraction, followed by 2-stage implant placement. *Journal of Clinical Periodontology* 38, 581-589.

Use of L-PRF as an adjunct to nonsurgical periodontal therapy: A pilot clinical study

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Aim: To evaluate the effect of the use of L-PRF as adjunct to scaling and root planning in the treatment of chronic periodontitis.

Materials and methods: A pilot double-blind split-mouth randomized clinical trial was conducted in 11 patients diagnosed with chronic periodontitis. The right and left quadrants of maxillary and jaw, were randomly assigned to scaling and root planning + L-PRF (test) or scaling and root planning + irrigation with physiological saline (control). Periodontal clinical parameters were evaluated at the beginning and at 6 weeks. In addition, the post-treatment dental sensitivity was evaluated using a visual analogue scale.

Results: An overview of the analysis showed that the test group presented better results in the periodontal parameters evaluated at 6 weeks, in comparison to the control group. However, the results were not statistically significant. The only variable that resulted in a statistically significant improvement was tooth sensitivity post-treatment at 1 and 6 weeks, with a p-value of 0.003 and 0.002, respectively.

Conclusions: The use of L-PRF as adjuvant therapy to scaling and root planning was superior in decreasing tooth sensitivity after treatment. Randomized clinical trials of sufficient size are required to confirm these observations.

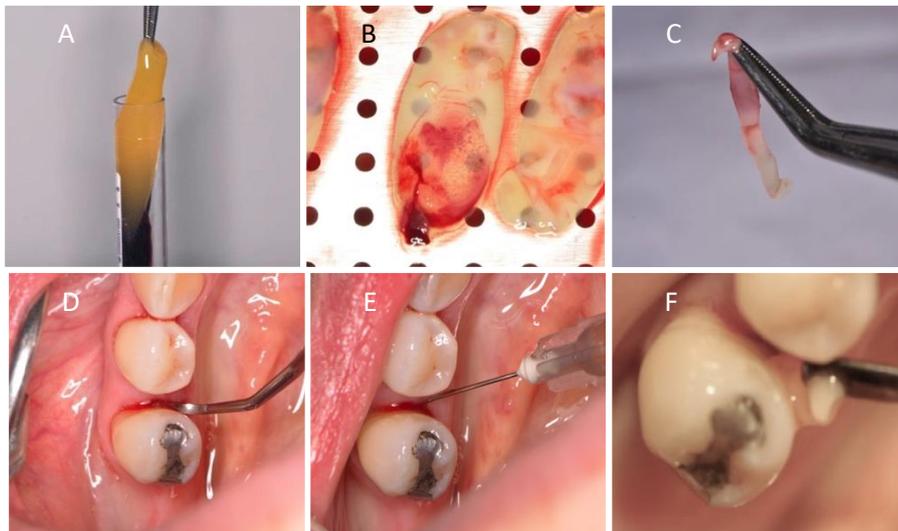


Figure 1: Preparation and application of L-PRF: A. Extraction of L-PRF clot. B. L-PRF membranes formation. C. L-PRF membrane cut into strips. D. Scaling and root planning. E. Irrigation of the pocket with L-PRF exudate. F. Insertion of L-PRF membranes into the pocket.

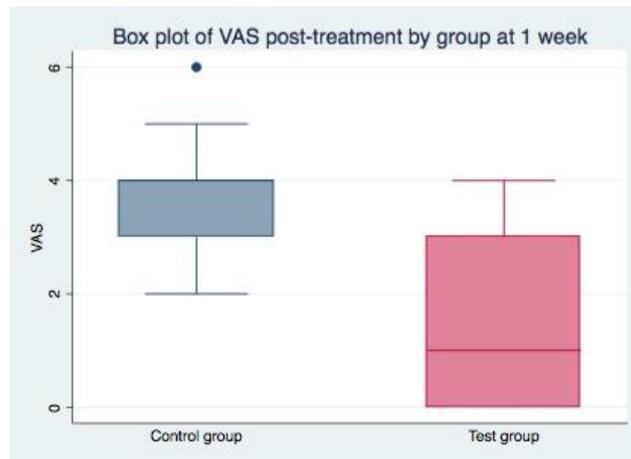


Figure 2: Box plot of VAS post-treatment by group at 1 week.

Variables	Control Group		Test Group		<i>p-value</i>
	Mean	SD	Mean	SD	
PD Delta (mm)	0.93	± 0.350	1.02	± 0.42	0.61
GM Delta (mm)	-0.63	± 0.56	-0.65	± 0.44	0.93
CAL Delta (mm)	0.34	± 0.51	0.40	± 0.37	0.75
BOP Delta (%)	27.16	± 25.99	35.11	± 22.84	0.45
GI Delta	0.62	± 0.37	0.77	± 0.53	0.45

Table 1: between test group variables

Difference control and for delta (at 0 and 6

week follow-up).

PD: Probing depth.

GM: Gingival margin.

CAL: Clinical attachment level.

Bop: Bleeding on probing.

GI: Gingival Index.

Variables	Control group		Test group		<i>p-value</i>
	Mean	SD	Mean	SD	
Sensitivity at 1 week	3.63	± 1.20	1.54	± 1.69	0.003 *
Sensitivity at 6 weeks	3.36	± 1.20	1.36	± 1.43	0.002 *

Table 2: Post-treatment sensitivity differences between groups.

* Statistically significant differences ($p\text{-value} \leq 0.005$)

References

- [1] Armitage GC. Periodontal diagnoses and classification of periodontal diseases. *Periodontol* 2000. 2004;34:9–21.
- [2] Heitz-mayfield LJA. Surgical and nonsurgical periodontal therapy Learned and unlearned concepts. *Periodontol* 2000. 2013;62:218–31.
- [3] Stabholz A, Soskolne WA SL. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontol* 2000. 2010;53(90):138–53.
- [4] Ultrasonic vs. hand instrumentation in periodontal therapy: clinical outcomes. 2016;71:113–27.
- [5] Tomasi C, Wennström JL. Full-mouth treatment vs. the conventional staged approach for periodontal infection control. *Periodontol* 2000. 2009;51(1):45–62.
- [6] Quirynen M, Teughels W, De Soete M, van Steenberghe D. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. *Periodontol* 2000. 2002;28:72–90.
- [7] Da Rocha HAJ, Silva CF, Santiago FL, Martins LG, Dias PC, De Magalhães D. Local Drug Delivery Systems in the Treatment of Periodontitis: A Literature Review. *J Int Acad Periodontol*. 2015;17(3):82–90.

- [8] Smiley CJ, Tracy SL, Abt E, Michalowicz BS, John MT, Gunsolley J, et al. Evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *J Am Dent Assoc*. 2015;146(7):525–35.
- [9] Slots J. Selection of antimicrobial agents in periodontal therapy. *J Periodontol Res*. 2002 Oct;37(5):389–98.
- [10] Fernando Augusto Perrella, Emanuel da Silva Rovai, Andréa Carvalho De Marco, Mauro Pedrine Santamaria, Magda Feres, Luciene Cristina de Figueredo WDK and JBOA. Clinical and Microbiological Evaluation of Povidone-Iodine 10% as an Adjunct to Nonsurgical Periodontal Therapy in Chronic Periodontitis: A Randomized Clinical Trial. *J Int Acad Periodontol*. 2016 Jun;18(4):109–19.
- [11] Keller DC, Buechel M. Oral Health Case Reports Periodontal Treatment with Direct Medication Delivery of Hydrogen Peroxide and Oxygen. 2017;3(1):3–8.
- [12] Nadig PS, Shah MA. Tetracycline as local drug delivery in treatment of chronic periodontitis: A systematic review and meta-analysis. *J Indian Soc Periodontol*. 2016;20(6):576–83.
- [13] Greenstein G. Local Drug Delivery in the Treatment of Periodontal Diseases: Assessing the Clinical Significance of the Results. *J Periodontol*. 2006;77(4):565–78.
- [14] Hanes PJ, Purvis JP. Local Anti-Infective Therapy: Pharmacological Agents. A Systematic Review. *Ann Periodontol*. 2003;8(1):79–98.
- [15] Herrera D, Matesanz P, Bascones-Martínez A, Sanz M. Local and systemic antimicrobial therapy in periodontics. *J Evid Based Dent Pract*. 2012;12(3 SUPPL.):50–60.
- [16] Ioannou I, Dimitriadis N, Papadimitriou K, Vouros I, Sakellari D, Konstantinidis A. The Effect of Locally Delivered Doxycycline in the Treatment of Chronic Periodontitis. A Clinical and Microbiological Cohort Study. *J Oral Maxillofac Res*. 2010;1(4):1–12.
- [17] Bonito AJ, Lux L, Lohr KN. Impact of Local Adjuncts to Scaling and Root Planing in Periodontal Disease Therapy: A Systematic Review. *J Periodontol*. 2005;76(8):1227–36.
- [18] Rosenberg DR, Andrade CX, Chaparro AP, Inostroza CM, Ramirez V, Violant D, et al. Short-Term Effects of 2% Atorvastatin Dentifrice as an Adjunct to Periodontal Therapy: A Randomized Double-Masked Clinical Trial. *J Periodontol*. 2015;86(5):623–30.
- [19] Bertl K, Parllaku A, Pandis N, Buhlin K, Klinge B, Stavropoulos A. The effect of local and systemic statin use as an adjunct to non-surgical and surgical periodontal therapy—A systematic review and meta-analysis. *J Dent*. 2017;67:18–28.
- [20] Muniz FWMG, Taminski K, Cavagni J, Celeste RK, Weidlich P, Rösing CK. The effect of statins on periodontal treatment—a systematic review with meta-analyses and meta-regression. *Clin Oral Investig*. 2018;22(2):671–87.
- [21] Mohiuddin K, Ravindra S, Ahmed MG ulza., Murthy S, Smitha BR aj. Single use of tetracycline with and without diclofenac sodium as local drug delivery in pocket therapy: a clinico-microbiological study. *J Investig Clin Dent*. 2011;2(4):280–6.
- [22] Funosas E, Feser G, Escovich L, Maestri L. Alteration of hemostasis in patients treated with subgingival NSAIDs during periodontal therapy. *Acta Odontol Latinoam*. 2012;25(1):103–8.
- [23] Heasman PA, Benn DK, Kelly PJ, Seymour RA, Aitken D. The use of topical flurbiprofen as an adjunct to non-surgical management of periodontal disease. *J Clin Periodontol*. 1993;20(6):457–64.
- [24] Flemmig TF, Epp B, Funkenhauser Z, Newman MG, Kornman KS, Haubitz I, et al. Adjunctive supragingival irrigation with acetylsalicylic acid in periodontal supportive therapy. *J Clin Periodontol*. 1995;22(6):427–33.
- [25] Laleman I, Yilmaz E, Ozcelik O, Haytac C, Pauwels M, Herrero ER, et al. The effect of a streptococci containing probiotic in periodontal therapy: A randomized controlled trial. *J Clin Periodontol*. 2015;42(11):1032–41.
- [26] Gizem İ, Gürsoy H, Dirikan Ş, Cakar G, Emekli-alturfan E. Clinical and Biochemical Evaluation of *Lactobacillus Reuteri* Containing Lozenges as an Adjunct to Non-Surgical Periodontal Therapy in Chronic Periodontitis Material & Method. 2015;1–13.
- [27] Morales A, Carvajal P, Silva N, Hernandez M, Godoy C, Rodriguez G, et al. Clinical Effects of *Lactobacillus rhamnosus* in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-Up. *J Periodontol*. 2016;87(8):944–52.
- [28] Martin-Cabezas R, Davideau J-L, Tenenbaum H, Huck O. Clinical efficacy of probiotics as an adjunctive therapy to non-surgical periodontal treatment of chronic periodontitis: a systematic review and meta-analysis. *J Clin Periodontol*. 2016;43(6):520–30.
- [29] Non-surgical DF. Histologic Evaluation of Human Intrabony Periodontal Therapy With and Without. 2003;12–4.
- [30] Gutierrez MA, Mellonig JT, Cochran DL. Evaluation of enamel matrix derivative as an adjunct to non-surgical periodontal therapy. *J Clin Periodontol*. 2003;30(8):739–45.

- [31] Mombelli A, Brochut P, Plagnat D, Casagni F, Giannopoulou C. Enamel matrix proteins and systemic antibiotics as adjuncts to non-surgical periodontal treatment: Clinical effects. *J Clin Periodontol.* 2005;32(3):225–30.
- [32] Mellonig JT, Valderrama P, Gregory HJ, Cochran DL. Clinical and Histologic Evaluation of Non-Surgical Periodontal Therapy With Enamel Matrix Derivative: A Report of Four Cases. *J Periodontol.* 2009;80(9):1534–40.
- [33] Isola G, Matarese G, Williams RC, Siciliano VI, Alibrandi A, Cordasco G, et al. The effects of a desiccant agent in the treatment of chronic periodontitis: A randomized, controlled clinical trial. *Clin Oral Investig.* 2018;22(2):791–800.
- [34] Gómez Hernández C, Domínguez Martín A, García Kass AI, García Núñez JA. Aplicación complementaria de terapia fotodinámica y de la radiación láser de Er:YAG al tratamiento no quirúrgico de la periodontitis crónica: estudio comparativo de sus efectos clínicos, antiinflamatorios y antimicrobianos. *Av Odontoestomatol.* 2011;27(3):147–60.
- [35] Giannelli M, Formigli L, Lorenzini L, Bani D. Combined photoablative and photodynamic diode laser therapy as an adjunct to non-surgical periodontal treatment. A randomized split-mouth clinical trial. *J Clin Periodontol.* 2012;39(10):962–70.
- [36] Euzebio Alves VT, de Andrade AKP, Toaliar JM, Conde MC, Zezell DM, Cai S, et al. Clinical and microbiological evaluation of high intensity diode laser adjunct to non-surgical periodontal treatment: A 6-month clinical trial. *Clin Oral Investig.* 2013;17(1):87–95.
- [37] Moreira AL, Novaes AB, Grisi MF, Taba M, Souza SL, Palioto DB, et al. Antimicrobial Photodynamic Therapy as an Adjunct to Non-Surgical Treatment of Aggressive Periodontitis: A Split-Mouth Randomized Controlled Trial. *J Periodontol.* 2015;86(3):376–86.
- [38] Segarra-Vidal M, Guerra-Ojeda S, Vallés LS, López-Roldán A, Mauricio MD, Aldasoro M, et al. Effects of photodynamic therapy in periodontal treatment: A randomized, controlled clinical trial. *J Clin Periodontol.* 2017;44(9):915–25.
- [39] Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol.* 2009;27(3):158–67.
- [40] Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.* 2006;101(3).
- [41] Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.* 2006;101(3).
- [42] Castro AB, Meschi N, Temmerman A, Pinto N, Lambrechts P, Teughels W, et al. Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. *J Clin Periodontol.* 2017;44(1):67–82.
- [43] Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.* 2006;101(3).
- [44] Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier J-B. Three-Dimensional Architecture and Cell Composition of a Choukroun's Platelet-Rich Fibrin Clot and Membrane. *J Periodontol.* 2010;81(4):546–55.
- [45] Anwandter A, Bohmann S, Nally M, Castro AB, Quirynen M, Pinto N. Dimensional changes of the post extraction alveolar ridge, preserved with Leukocyte- and Platelet Rich Fibrin: A clinical pilot study. *J Dent.* 2016;52:23–9.
- [46] Chang YC, Zhao JH. Effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects. *Aust Dent J.* 2011;56(4):365–71.
- [47] Thorat M, Pradeep AR, Pallavi B. Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: A controlled clinical trial. *J Clin Periodontol.* 2011;38(10):925–32.
- [48] Page RC, Eke PI. Case Definitions for use in population-based surveillance of Periodontitis. *J Periodontol.* 2007;78(7):1387-99.
- [49] Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol.* 1967;38(6):610–6.
- [50] Wang ZS, Feng ZH, Wu GF, Bai SZ, Dong Y, Chen FM, et al. The use of platelet-rich fibrin combined with periodontal ligament and jaw bone mesenchymal stem cell sheets for periodontal tissue engineering. *Sci Rep.* 2016;6(June):1–15.
- [51] Dohan Ehrenfest David M. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Muscle, Ligaments Tendons J.* 2014;4(1):3–9.

- [52] Muñoz F, Jiménez C, Espinoza D, Vervelle A, Beugnet J, Haidar Z. Use of leukocyte and platelet-rich fibrin (L-PRF) in periodontally accelerated osteogenic orthodontics (PAOO): Clinical effects on edema and pain. *J Clin Exp Dent*. 2016;8(2):e119–24.
- [53] Rosamma Joseph V, Sam G, Vijay Amol N. Clinical evaluation of autologous platelet rich fibrin in horizontal alveolar bony defects. *J Clin Diagnostic Res*. 2014;8(11):ZC43-ZC47.
- [54] Panda S, Doraiswamy J, Malaiappan S, Varghese SS, Del Fabbro M. Additive effect of autologous platelet concentrates in treatment of intrabony defects: a systematic review and meta-analysis. *J Investig Clin Dent*. 2016;7(1):13–26.
- [55] Chang YC, Wu KC, Zhao JH. Clinical application of platelet-rich fibrin as the sole grafting material in periodontal intrabony defects. *J Dent Sci* 2011;6(3):181–8.
- [56] Simonpieri A, Del Corso M, Vervelle A, Jimbo R, Inchingolo F, Sammartino G, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: Bone graft, implant and reconstructive surgery. *Curr Pharm Biotechnol*. 2012;13(7):1231–56.
- [57] Matesanz-Pérez P, García-Gargallo M, Figuera E, Bascones-Martínez A, Sanz M, Herrera D. A systematic review on the effects of local antimicrobials as adjuncts to subgingival debridement, compared with subgingival debridement alone, in the treatment of chronic periodontitis. *J Clin Periodontol*. 2013;40(3):227–41.
- [58] Cieslik-Bielecka A, Dohan Ehrenfest DM, Lubkowska A, Bielecki T. Microbicidal properties of Leukocyte- and Platelet-Rich Plasma/Fibrin (L-PRP/L-PRF): new perspectives. *J Biol Regul Homeost Agents*. 2014;26(2 Suppl 1):43S–52S.

Clinical application of PRF membrane seeded with autologous palatal fibroblasts in gingival recession treatment

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Background and aim: Today the gold standard for gingival recessions (GR) coverage is the connective tissue graft (CTG) placed during mucogingival surgery. Seeking for new approaches in GR coverage with less biological cost and comparable clinical outcomes some new approaches were introduced: the use of soft tissue substitutes instead of CTG, the use of regenerative therapies and tissue engineering approaches.

In this case report we introduce a novel approach for treatment of multiple gingival recessions using PRF membrane seeded with autologous palatal fibroblasts (APF) placed under coronally advanced flap (CAF).

Case report: In November 2016 a 38-year old non-smoker woman was referred to the private practise in St. Petersburg, Russia, with a chief complaint of teeth sensitivity. She presented with multiple gingival recessions 1-4 mm in depth Miller Class II and III in thin periodontal biotype.

Two month prior the intervention patient underwent a palatal tissue harvesting with a soft tissue punch for a further fibroblast extraction and cultivation of APF in laboratory.

On the day of the mucogingival surgery around 9 ml of whole venous blood was collected from the patient in a sterile vacutainer tube with anticoagulant for PRF clot preparation. The suspension of vital APF culture, delivered from laboratory the same day, was injected into the PRF clot before its placement.

PRF clot was secured over 3.5, 3.6, 3.7 with 6-0 Prolene sutures and flap was sutured over the clot with 6-0, 7-0 Prolene sutures. The postsurgical protocol involved administration of antibiotics (amoxicillin 500mg, every 12h for 14d) and 0.12% chlorhexidine gluconate mouthrinse (every 12 h for 5 d). Oral analgesics (ibuprofen 600 mg, every 8 h, as necessary) were also prescribed. Suture removal after 2 weeks. Clinical periodontal parameters were evaluated at the baseline, at 1, 6 and 12 months postoperatively.

Results: The tissue-healing was uneventful. During the first year after surgery BOP and PI were stable. After 1,5 month patient showed a significant reduction of REC and CAL gain, that remained stable after one year of follow-up along with a satisfying aesthetic result (90-100% of the root coverage).

Conclusion: PRF clot seeded with autologous human palatal fibroblasts might be considered as an alternative of CTG in the treatment of Miller class II and III gingival recession, representing a novel tissue-engineering concept and living cell-based therapy. However, the application of cell culture requires complex laboratory conditions and is associated with increased time and cost.

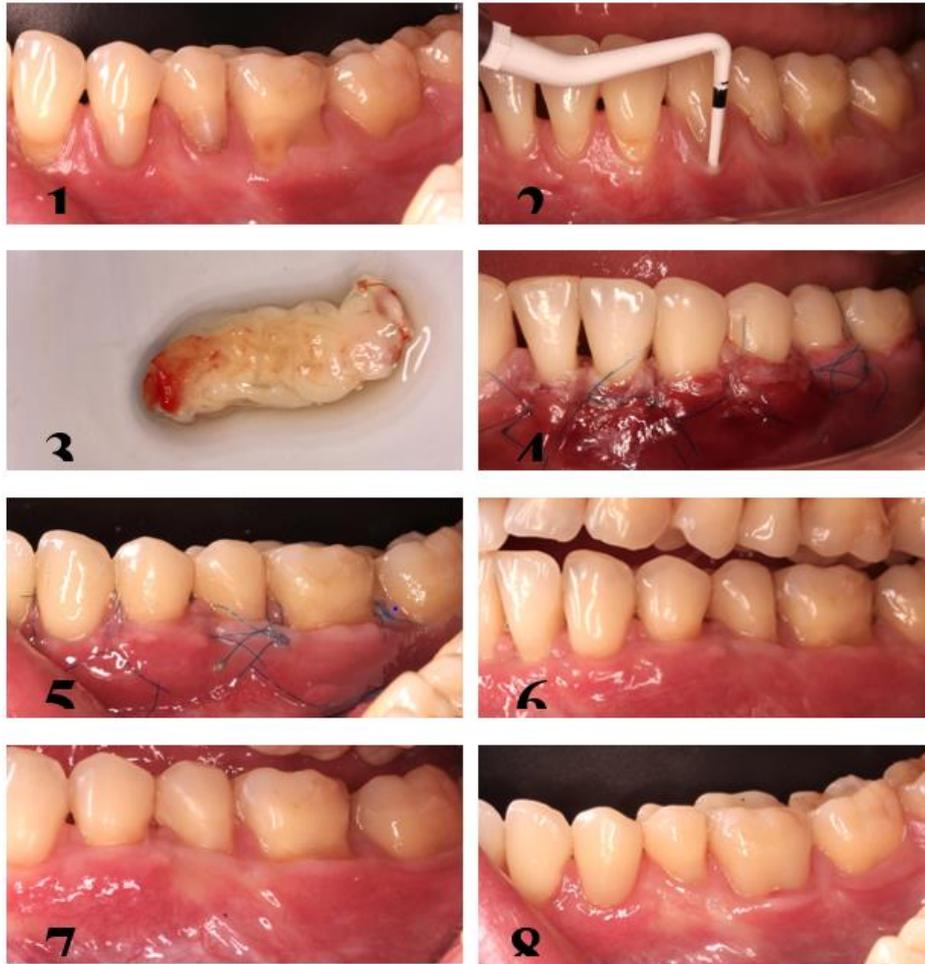


Figure 1: Preoperative view

Figure 2: Baseline periodontal biotype assessment

Figure 3: PRF clot seeded with the suspension of autologous palatal fibroblasts

Figure 4: PRF clot is placed under the CAF and separately sutured

Figure 5: Intraoral view 14 days after mucogingival surgery. Suture removal

Figure 6: 1 month after surgery

Figure 7: 6 months of follow-up

Figure 8: 12 months of follow-up, after composite restoration

References

- [1] Tatakis DN & Trombelli L. Gingival recession treatment: guided tissue regeneration with bioabsorbable membrane versus connective tissue graft. *J Periodontol.* 2000 Feb;71(2):299-307.
- [2] Rotundo R & Pini-Prato G. Use of a new collagen matrix (mucograft) for the treatment of multiple gingival recessions: case reports. *Int J Periodontics Restorative Dent.* 2012 Aug;32(4):413-9.
- [3] Milinkovic et al. Clinical application of autologous fibroblast cell culture in gingival recession treatment. *J Periodontal Res.* 2015 Jun;50(3):363-70.
- [4] Köseoğlu et al. Efficacy of collagen membrane seeded with autologous gingival fibroblasts in gingival recession treatment: a randomized, controlled pilot study. *J Periodontol.* 2013 Oct;84(10):1416-24.
- [5] Pini-Prato GP, Rotundo R, Magnani C, Soranzo C. Tissue engineering technology for gingival augmentation procedures: a case report. *Int J Periodontics Restorative Dent* 2000;20:552–559.
- [6] Pini-Prato G, Rotundo R, Magnani C, Soranzo C, Muzzi L, Cairo F. An autologous cell hyaluronic acid graft technique for gingival augmentation: a case series. *J Periodontol* 2003;74:262–267.
- [7] McGuire MK, Scheyer ET, Nunn ME, Lavin PT. A pilot study to evaluate a tissue-engineered bilayered cell therapy as an alternative to tissue from the palate. *J Periodontol* 2008;79:1847–1856.
- [8] Kobayashi E, Fujioka-Kobayashi M, Sculean A, Chappuis V, Buser D, Schaller B, Döri F, Miron RJ. Effects of platelet rich plasma (PRP) on human gingival fibroblast, osteoblast and periodontal ligament cell behaviour. *BMC Oral Health.* 2017 Jun 2;17(1):91.
- [9] Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. *J Periodontol.* 2009 Feb;80(2):244-52.

Secretome analysis of leukocyte-platelet rich fibrin membranes used in wound healing

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Aim: Leukocyte-platelet rich fibrin (L-PRF) membranes are extensively used in dentistry and in other clinical scenarios to accelerate wound healing and tissue regeneration. Active platelets secrete growth factors and other proteins that contribute to wound healing. The goal of the present study was to investigate the secretome of L-PRF membranes to better understand their tissue regeneration properties.

Material and methods: Fresh blood samples were collected from healthy volunteers into glass-coated plastic tubes and centrifuged at 400g during 12 minutes to obtain L-PRF membranes. Membranes were cultured in DMEM medium with 1% penicillin/streptomycin following an established procedure and the secretome collected and concentrated at days 3 and 7 (after membranes extraction). Following precipitation, protein samples were either concentrated in one gel band or separated in a 12% Bis-acrylamide gel. Bands were cut off from the gel and proteins in-gel trypsin digested and identified in a Sciex Triple TOF. A systems biology analysis (Ingenuity Pathways Analysis) was also carried out. Growth factors were identified by a commercial protein array (Human Growth Factor Array Q1, RayBiotech, Inc.).

Results: Over 400 proteins were identified following three days of membrane culture, being related to the following principal canonical pathways: acute phase response signaling, and clathrin-mediated endocytosis signaling. The majority of proteins identified are implicated in cellular movement, cell death and survival and cell-to-cell signaling and interaction, linking to inflammatory and immunological response. In a SDS-PAGE-based differential analysis, four main proteins bands varied between the secretome at days 3 and 7; 360 proteins were identified in those bands at day 3 and 292 at day 7. Between these two conditions, 259 proteins were common, 101 only present at day 3, and 33 proteins only present at day 7. Many of these proteins are related to the canonical pathways mentioned above, although proteins such as epidermal growth factor and complement proteins are only present at day 3. The results obtained from the array showed a higher concentration of many growth factors at day 3, for example PDGF-AA, EGF and TGF- β ; these three proteins were also identified at day 3 by proteomics. Only GDF-15 was detected at higher concentration at day 7 in all donors.

Conclusion: We present the first comprehensive analysis of the secretome of L-PRF membranes comparing different culture times. Our results highlight the majority of growth factors are secreted predominantly during the first three days of membrane culture with some of them remaining overexpressed after 7 days. The most relevant pathway related to differentially regulated proteins (especially those up-regulated at day 3) is clathrin-mediated endocytosis signaling, which is related to growth factors interchange and is involved in mechanisms of tissue regeneration.

A combined approach using endodontic surgery with L-PRF for improved clinical results- Case presentations

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Aim: Modern endodontic surgery using a dental operating microscope and ultrasonic tips for retrograde cavity preparation followed by sealing of the retrograde preparation demonstrates a high success rate. Some cases, however, may benefit from adjunctive therapy with L-PRF with the aim of inducing a positive effect on bone regeneration in the periapical defect. Such cases may include unusual defect anatomy and size, proximity to the maxillary sinus, complicating systemic conditions such as diabetes, and anti-resorptive medication.

Materials and Methods: We present cases in which this therapeutic modality was used, show the clinical and radiographic outcome and suggest indications for using this combined approach to endodontic therapy.

Tissue engineering: platelet rich fibrin as a sole graft material for sinus augmentation

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Aim: Membrane perforation, graft material escape into the sinus cavity and graft material contamination are some the possible complications of sinus augmentation. Management of above-mentioned complications can be clinically challenging. Use of platelet rich fibrin (PRF) has demonstrated excellent regenerative properties in augmented sites. PRF is an autologous material, when used as the sole graft material for sinus graft, that does not require removal in the event the graft becomes contaminated. This report is to investigate PRF as a sole graft material for sinus augmentation

Methods: Lateral window sinus augmentation was planned for tooth #2 and 3 area with simultaneous implant placement. 8 tubes venous blood was drawn and processed via Choukroun's method. The minor perforation of Schneider membrane was sealed with 2 layers PRF membrane. 4 layers PRF membrane were placed over sinus chamber as filler. Implant fixtures were soaked in iPRF (liquid form) and inserted with approximately 15Ncm of initial stability. Implant fixtures also served as tenting post for the sinus augmentation since PRF does not have space maintenance ability. 2 layers of PRF membranes were placed over the lateral window prior to suturing. Radiographs taken immediately post surgery demonstrated approximately 50% of the implant fixtures are in grafted material (PRF).

Results : After 7 months, the site was re-entered during second stage surgery. Upon re-entry, complete bone fill of the lateral window was evident. Radiographic examination revealed satisfactory bone fill in area previously augmented with PRF. Healing abutment was placed. Final restoration delivered 4 weeks post second stage surgery.

Conclusion: The use of PRF as the sole graft material when performing sinus augmentation in conjunction with implant placement offers the following advantages over traditional particular graft material.

1. Ease of material acquisition
2. Relative low cost
3. No chance of donor/recipient disease transmission
4. Does not require removal when augmented site becomes infected
5. Great regenerative ability in both hard and soft tissue

It is the authors' opinion that use of PRF as the sole graft material when performing sinus augmentation in conjunction with implant placement is a viable alternative to traditional particular graft material.



Figure 1: Platelet rich fibrin as a sole graft material for sinus with implant as space making

References

- [1] L David M. Dohan, Joseph Choukroun, Antoine Diss, Steve L. Dohand, Anthony J-J.Dohane, Jaafar Mouhyi, Bruno Gogly, Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution, *Oral and Maxillofacial Surgery* ,Volume 101, Issue 3, Pages 37-44 (March 2006).
- [2] David M. Dohan, Joseph Choukroun, Antoine Diss, Steve L. Dohand, Anthony J.J.Dohane, Jaafar Mouhyi, Bruno Gogly, Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features, *Oral and Maxillofacial Surgery*, January 11, 2006, pages e45-e50. (March 2006).
- [3] David M. Dohan, Joseph Choukroun, Antoine Diss, Steve L. Dohand, Anthony J.J.Dohane, Jaafar Mouhyi, Bruno Gogly, Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates?, *Oral and Maxillofacial Surgery* ,Volume 101, Issue 3, Pages e51-e55 (March 2006).
- [4] Joseph Choukroun, Antoine Diss, Alain Simonpieri, Marie-Odile Girard, Christian Schoeffler, Steve L. Dohand, Anthony J.J.Dohane, Jaafar Mouhyi, David M. Dohan, Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing, *Oral and Maxillofacial Surgery* ,Volume 101, Issue 3, Pages e56-e60 (March 2006).
- [5] Mazor, Z, Horowitz R. Dohan, D. et al. Floor Augmentation with simultaneous Implant placement using Choukroun's Platelet-Rich Fibrin as the Sole grafting Material: A radiologic and Histologic study at 6 month *JAAP* 2009;80,2056-2064
- [6] Tajima N, Ohba S, Sawase T, Asahina I, Evaluation of Sinus Floor Augmentation with Simultaneous Implant Placement Using Platelet-Rich Fibrin as Sole Grafting Material., *Int J of Oral & Max Implants*. Vol 28, Number 1, 2013, 77-83
- [7] Pinto, N, Quirynen M et al: Guideline for the use of L-PRF Catholic U of Leuven/U. Hospitals Leuven Perio. 2018 Flow Chart Four P 10-11

Plasma-rich fibrin in neurosurgery: a feasibility study

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Background: Cerebrospinal fluid (CSF) leakage represents an important and sometimes challenging complication in both cranial and spinal surgery. Current available options for dural closure pose inherent problems regarding safety, efficacy, immunogenicity, cost, and invasiveness. In this article, the use of leukocyte- and platelet-rich fibrin (L-PRF) derived from the patient's own blood is proposed to facilitate dural closure. We aim to describe the safety, feasibility, and applicability of L-PRF membranes and plugs in cranial and spinal neurosurgery.

Methods: A retrospective study reviewing clinical and surgical characteristics was conducted in 47 patients in whom the use of LPRF was attempted to reinforce dural closure at a single institution during 1 year. Procedures included skull base, posterior fossa, and spinal revision surgeries.

Results: L-PRF membranes and/or plugs were used in 44 surgeries. The preparation of L-PRF failed in three cases. L-PRF membranes were used as onlay grafts to augment sealing or sutured into a defect. No short-term complications related to the use of L-PRF were recorded. Postoperative CSF leakage was present in two endoscopic transsphenoidal pituitary surgeries and in one spinal CSF leak repair.

Conclusion: L-PRF is safe, inexpensive, and completely autologous and can be rapidly and non-invasively harvested to aid in dural closure. Theoretical advantages include a regenerative bioactive potential, which could lead to improved wound healing and reduced infection rates. These findings warrant larger prospective studies to determine the potential role of L-PRF in neurosurgery.

Effects of application injectable platelet-rich fibrin (i-PRF) as adjunct therapy in the initial treatment of periodontitis

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Aim: The aim of the study was to investigate whether there are differences between initial treatment of chronic periodontitis (SRP) and SRP in conjunction with injectable platelet-rich fibrin (i-PRF) application on the concentration of periodontal pathogens after 3 months. Simultaneously, we monitored the following clinical parameters:

Material and methods: Twenty patients with chronic periodontitis who had at least two sites with pocket depth (PD) \geq 4 mm on contralateral side participated in the study. Using a split-mouth design, the patients were treated with SRP + I-PRF-(test group) or SRP only-(control group). Gingival crevicular fluid (GCF) and subgingival plaque were collected with paper points (DentsplyMaillefer, Tulsa, OK, USA) at baseline and 3 months after the treatment. The presence and the concentrations of *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) and *Tannerella forsythia* (Tf) were analyzed by real-time polymerase chain reaction (qPCR). The periodontal parameters, including bleeding on probing (BOP), probing pocket depth (PPD) and the clinical attachment level (CAL), were recorded on both sides.

Results: In both groups, total amount of periodontal pathogens in the GCF samples obtained after 3 months was reduced. The test group demonstrated significantly fewer Aa ($P < 0.05$) and PI and the total number of all periopathogens as well. Moreover, the test group exhibited statistically significant increase in PPD, CAL and BOP values ($P < 0.01$, $P < 0.01$ and $P = 0.05$), respectively.

Conclusion: Considering the limited number of patients, periodontal therapy in conjunction with injectable platelet-rich fibrin (i-PRF) displayed decreased concentration of periodontal pathogens after 3 months and improvement in the above mentioned clinical parameters.

L-PRF derived from smokers and non-smokers stimulate proliferation and migration in periodontal ligament cells

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Cigarette smoking has been associated with delayed wound healing at the clinical level and *in vitro* and *in vivo* evidence has reported its adverse effects on wound healing, including changes on migration, proliferation and differentiation of periodontal cells. In non-smokers (NS) individuals, several clinical approaches aimed to improve wound healing have been made, including the use of Leucocyte platelet rich fibrin (L-PRF). However, differences of the biomolecules profile or biological activities between LPR-F isolated from smokers (S) compared to NS have not been determined.

Objective: To analyze and to compare the biological composition and biomolecules kinetic release from L-PRF clots derived from NS or S donors. Also, to evaluate the effects triggered by L-PRF in periodontal ligament cell (PDLC) proliferation and migration.

Methods: L-PRF clots were obtained from NS and S donors using venous blood samples centrifuged at 400 x G during 12 minutes. L-PRF clots were cultured in DMEM during 1, 3 or 7 Days (D) to obtain conditioned medium (CM). CMs were analyzed by proteomic arrays to evaluate 11 biomolecules involved in the wound healing process (PDGFAA, PDGFBB, PDGFAB, EGF, HGF, BMP7, VEGF-A, IGFB-6, IGFBP-2, IL-6, IL1Beta). Then, periodontal ligament cells were stimulated with CM to evaluate proliferation using Ki67 immunostaining, and cell migration through 8mm grid membranes. Results were analyzed statistically using anova and turkey's test for multiple comparisons.

Results: CM obtained from LPRF clots from both, NS or S donors released biomolecules throughout the seven days of culture after isolation. Of the growth factors analyzed, PDGFAA, PDGFBB, PDGFAB and EGF were released at higher levels from LPRF clots obtained from S and NS donors. The biomolecules levels did not show statistical differences between the CM obtained from the four S and NS LPRF analyzed. CMs taken at 1 or 3D, derived from both NS and S donors, were able to stimulate PDLC proliferation (80%, average). However, proliferation was significantly reduced with CM of both, NS and S taken at 7D after the initial isolation (40%, average). Our preliminary study (from four samples) show that CM obtained from LPRF clots from both NS or S donors had comparable levels of the biomolecules evaluated and were able to induce PDCL proliferation and migration at equivalent degrees.

Simultaneous sinus floor elevation and implant placement using L-PRF as a sole graft material

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Background: Sinus floor elevation (SFE) and simultaneous implant placement is predictable and reproducible. However, the graft material for the antral cavity remains a topic of debate. Considering the high osteogenic potential of the Schneiderian membrane, most graft materials are generally accepted.

Material and methods: This study was designed as a single cohort prospective study aimed to evaluate simultaneous SFE and implant placement using L-PRF as a sole graft material. Clinical and radiographic measurements (CBCT) were performed immediately after implant placement and at abutment connection (6 months later). The amount of newly formed bone was linear recorded on cross sectional images. Four measurements (mesial, distal, buccal, palatal) were registered with the axis of the implant as reference.

Results: Six lateral and 22 transalveolar SFE were performed in 26 patients with simultaneous implant placement. Six months after surgery, 27/29 implants were clinically integrated. The mean vertical bone gain was $3,4 \pm 1,2$ mm and $5,4 \pm 1,5$ mm, for transalveolar SFE and lateral SFE respectively. The level of the new sinus floor was in all cases in continuation with the apex of the implant, and the peri-implant crestal bone height was stable.

Conclusion: L-PRF as a sole graft material during simultaneous SFE and implant placement proved to be a practical, safe and economical subsinus graft material, resulting in natural bone formation.

Use of L-PRF in the treatment of severe dental mobility with bone regeneration and parodontal defects

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Guided tissue regeneration represents the best documented regenerative procedure for the attenuation of periodontal regeneration linked to intra-bone defects.

With the advent of the use of the emocomponents, the goals achieved are certainly more satisfying with long-term predictability and perfect bone-tissue integration.

The use of L-PRF and PRF-block allows: L-PRF is a biological protocol of autogenous fibrin, rich in platelets, leukocytes, growth factors and plasma proteins; PRF-block allows a single blood collection and a single centrifugation to obtain an L-PRF and a biomaterial graft that rapidly transforms into a solid block.

The case reveals how, in a patient with high dental mobility, with reduced dental splinting activity in the inferior arch, the grafting and the modeling of L-PRF block and of the PRF membrane demonstrate the total locking of the single teeth with restoration of dental architecture.

Patient woman, without health problems, non smoker.

At the initial CBCM, there was a notable bone reabsorption and gingival tissues, elevated dental mobility to be entracte from the teeth.

First biopsy sampling ascertains the presence of high levels of inflammatory infiltrate perivascular lymphocyte, floor epithelium composed of notable cellular acanthosis, presence of cytokines, interleukin 1- 2, macrophages.

After decontaminating with sodium hypochlorite (NaClO) plus physiological solution the periodontopathic sites, the gingival pockets, the patient performed a pre-surgical prophylaxis of one week with chlorhexidine gel for topical use 1% plus 3ml rovamycin with micro tablets.

After performing the prophylaxis, the patient underwent surgery for remodeling and bone augmentation with L-PRF block, after having induced bone osteogenesis with a casing cutter, several L-PRF blocks were placed on the bone from treating increasing height and size, approximately 20 PRF membranes are prepared which are placed over the bone graft and shake according to the functional gingival architecture. After 3 days from surgery, dental stability was guaranteed.

Follow-up at 3 - 4 - 8 - 12 months, optimal clinical situation, no dental movement, positive dental architecture. Histologically, the reduction of the interleukin 1 inflammatory infiltrate with ankylosis between the bone grafted with PRF BLOCK and the surface of the root cement is verified, manifesting the total blockage of the dental movement.

When digital meets biology

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Aim: When implants are placed in the molar region, patients often complain about food impaction in the buccal concavity, below the implant crown, due to loss of the buccal contour. This problem occurs because the roots of a molar are significantly wider than the implant. This trial explains a full digital workflow to preserve the emergence profile of the extracted molar.

Material and methods: Twelve patients with lost molars (upper or lower, n=13) were included in this trial. On the first appointment a CBCT (FOV 8x8) and IOS (3Shape) were taken. The STL and CBCT were matched in a planning software (Msoft® - Swissmeda AG) and implant position was digitally planned. In this planning, a CAD CAM individualized healing abutment was designed too and delivered together with the 3D guide (MIS-implants).

On the second appointment the tooth crown was removed and implant osteotomy was performed guided, through the roots of the tooth¹. The roots were then extracted, and an immediate implant placement was performed with a V3B+ implant (MIS-implants)². The remaining gap was filled with a mixture of xenograft (Bio-Oss®, Geistlich Pharma AG) and L-Prf³. Primary wound closure was achieved by the individualized healing abutment⁴.

After 3-6 months, osseointegration was confirmed by ISQ values and a new IOS (3Shape) was made. The dental lab fabricated a final crown with exactly the same emergence profile as the individualized healing abutment (using the STL of the designed healing abutment). The final crown was cleaned⁶ and inserted⁵ in the patient's mouth.

After 6 months of follow up no implant has failed, no patient complained about food impaction or a lack of volume of the gingiva around the implant.

Discussion: By drilling through the root, the implant will more likely follow the planned direction, even when guided. A second advantage of this procedure is that the remaining pieces of the tooth are easily extracted. Although, if the atraumatic extraction fails, the implant could have too little primary stability to perform an immediate implantation.

In this trial all implants had good primary stability (15 Ncm), even when placed in only 2-3 mm of native bone. The "jumping" distance, even up to 7 mm, was filled with L-Prf block.

The biggest problem in immediate implant placement in molars is primary wound closure. By using individualized healing abutments, this issue is easily managed.

The healing of all cases was uneventful and the initial contour was maintained. When removing the individualized healing abutment, graft particles were visible, as described in the concept of dual-chamber grafting technique^{7,8}.

Conclusion: L-Prf in combination with Bio-Oss®, induced a fast osseointegration, even when the implant had almost no bone to implant contact. The described procedure is a combination of grafting material (Bio-Oss®), wound healing enhancers (L-Prf) and prosthetic graft protector (individualized healing abutment).

References

- [1] Rebele SF, Zuhr O, Hürzeler MB. Pre-extractive interradicular implant bed preparation: case presentations of a novel approach to immediate implant placement at multirrooted molar sites. *Int J Periodontics Restorative Dent.* 2013 Jan-Feb;33(1):89-96
- [2] Passoni BB, Marques de Castro DS2, de Araújo MA2, de Araújo CD2, Piatelli A3, Benfatti CA4. Influence of immediate/delayed implant placement and implant platform on the peri-implant bone formation. *Clin Oral Implants Res.* 2016 Nov;27(11):1376-1383
- [3] Temmerman A. The use of leucocyte and platelet-rich fibrin in socket management and ridge preservation: a split-mouth, randomized, controlled clinical trial. *J Clin Periodontol.* 2016 Nov;43(11):990-999

- [4] Finelle G, Lee SJ. Guided Immediate Implant Placement with Wound Closure by Computer-Aided Design/Computer-Assisted Manufacture Sealing Socket Abutment: Case Report. *Int J Oral Maxillofac Implants*. 2017 Mar/Apr
- [5] Linkevicius T. The Novel Design of Zirconium Oxide-Based Screw-Retained Restorations, Maximizing Exposure of Zirconia to Soft Peri-implant Tissues: Clinical Report After 3 Years of Follow-up. *Int J Periodontics Restorative Dent*. 2017 Jan/Feb;37(1):41-47
- [6] Canullo L1, Micarelli C, Lembo-Fazio L, Iannello G, Clementini M. Microscopical and microbiologic characterization of customized titanium abutments after different cleaning procedures. *Clin Oral Implants Res*. 2014 Mar;25(3):328-36
- [7] Chu SJ, Salama MA, Salama H, et al. The dual-zone therapeutic concept of managing immediate implant placement and provisional restoration in anterior extraction sockets. *Compend Contin Educ Dent*. 2012;33(7):524-534.
- [8] Chu SJ, Salama MA, Garber DA, et al. Flapless postextraction socket implant placement, part 2: the effects of bone grafting and provisional restoration on peri-implant soft tissue height and thickness—a retrospective study. *Int J Periodontics Restorative Dent*. 2015; 35(6): 803-809.

The benefits of combining L-RPF with BOPT in the healing of hard and soft tissue

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Aim: To demonstrate the benefits of combining the two techniques of L-PRF and BOPT in enhancing the regeneration of bone and soft tissue. BOPT, developed by Dr. Loy, is a biological orientation preparation technique, which consists of drilling the tooth in vertical form. This allows the tissues to grow unimpeded to the determined limit of the crown and not to the limit determined by horizontal preparation. By using the BOPT technique in combination with L-PRF, the resulting tissue is more stable, thicker and healing is quicker than in older methods.

Material and methods: Three cases are presented to demonstrate the efficacy of this combined method.

Case 1 (Periimplantitis): A 55-year-old man presented with infection and bone loss in 3 of 4 implants placed 11 years previously.

Treatment: The most affected implant (22) was removed and the remaining 2 implants were treated in vertical form and with L-PRF block. Three months later the aesthetic problem was corrected with a fixed prosthesis using BOPT technique.



Figure 1: Presentation 2017



Figure 2: Surgery with L-PRF block, local antibiotic and BOPT technique (December 2017)



Figure 3: After (May 2018)

Case 2 (Periodontitis): An 85- year-old woman presented with periodontitis and fissures in the enamel of the superior incisors.

Treatment: The incisors were drilled in vertical form and L-PRF membranes were used to fill the spaces between the teeth to increase gum volume and accelerate healing. This occurred within eight days. Twenty days after surgery, an impression could be taken for the fixed prosthesis.



Figure 4: Presentation (June 2018)



Figure 5: Treatment with L-PRF membranes



Figure 6: Day of treatment with provisional fixed prosthesis



Figure 7: Cicatrization after twenty four hours.



Figure 8: Cicatrization after eight days



Figure 9: Cicatrization after one month. Impression was taken.



Figure 10: Before and after (July 2018)

Case 3 (Bone defects): A 58-year-old woman presented with bone and gum defects in teeth 11 and 22 as a consequence of previous cysts.

Treatment: The 2 teeth were extracted and the defect was treated with L-PRF block and covered with L-PRF membranes, sutured into place exteriorly. Healing was complete after 1 month to enable the impression for the fixed prosthesis to be taken. Normally, this cicatrization takes 3 months.



Figure 11: The defect in gum and bone

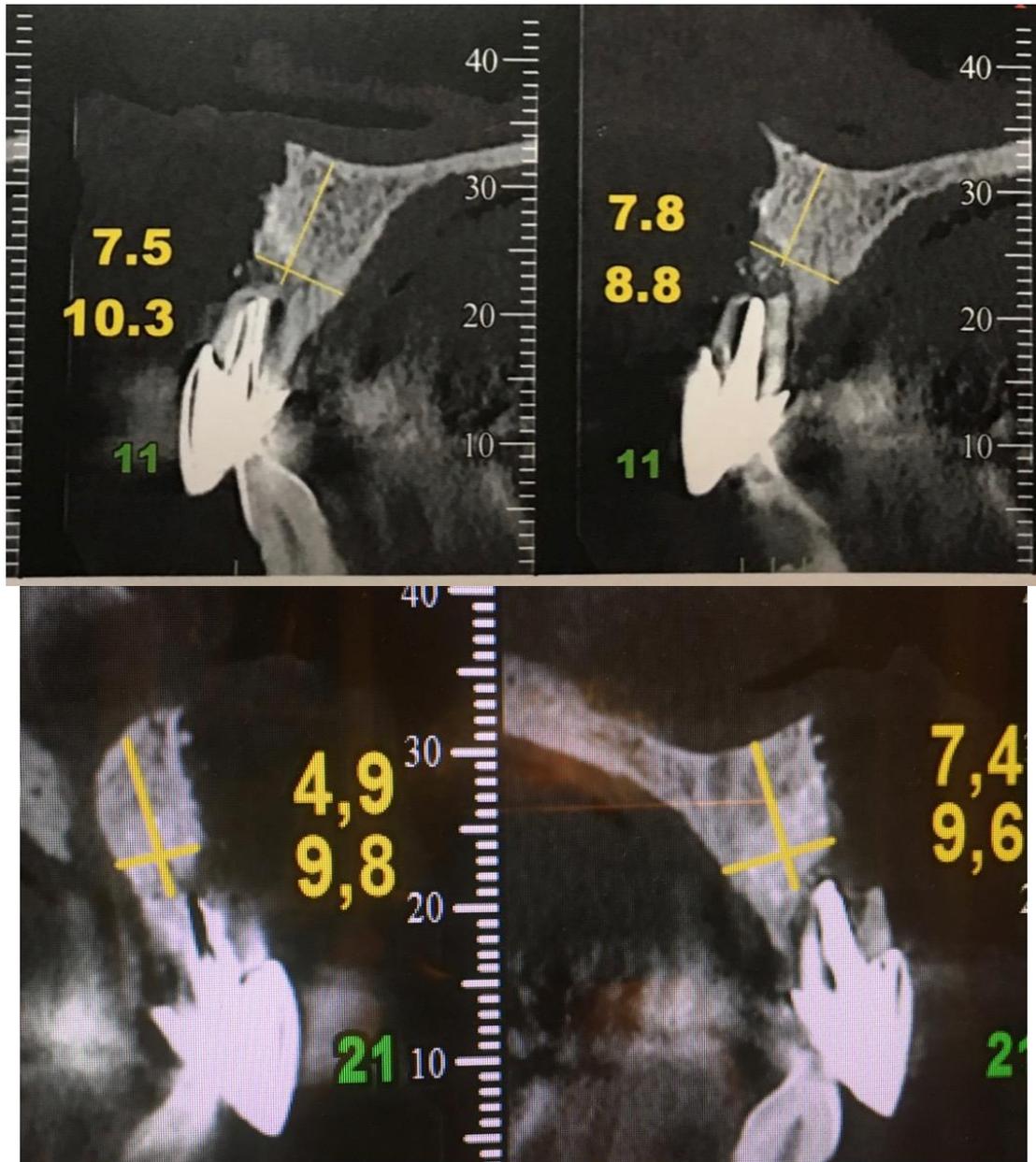


Figure 12: TAC before intervention



Figure 13: Surgery with L-PRF block and membranes, and early cicatrisation

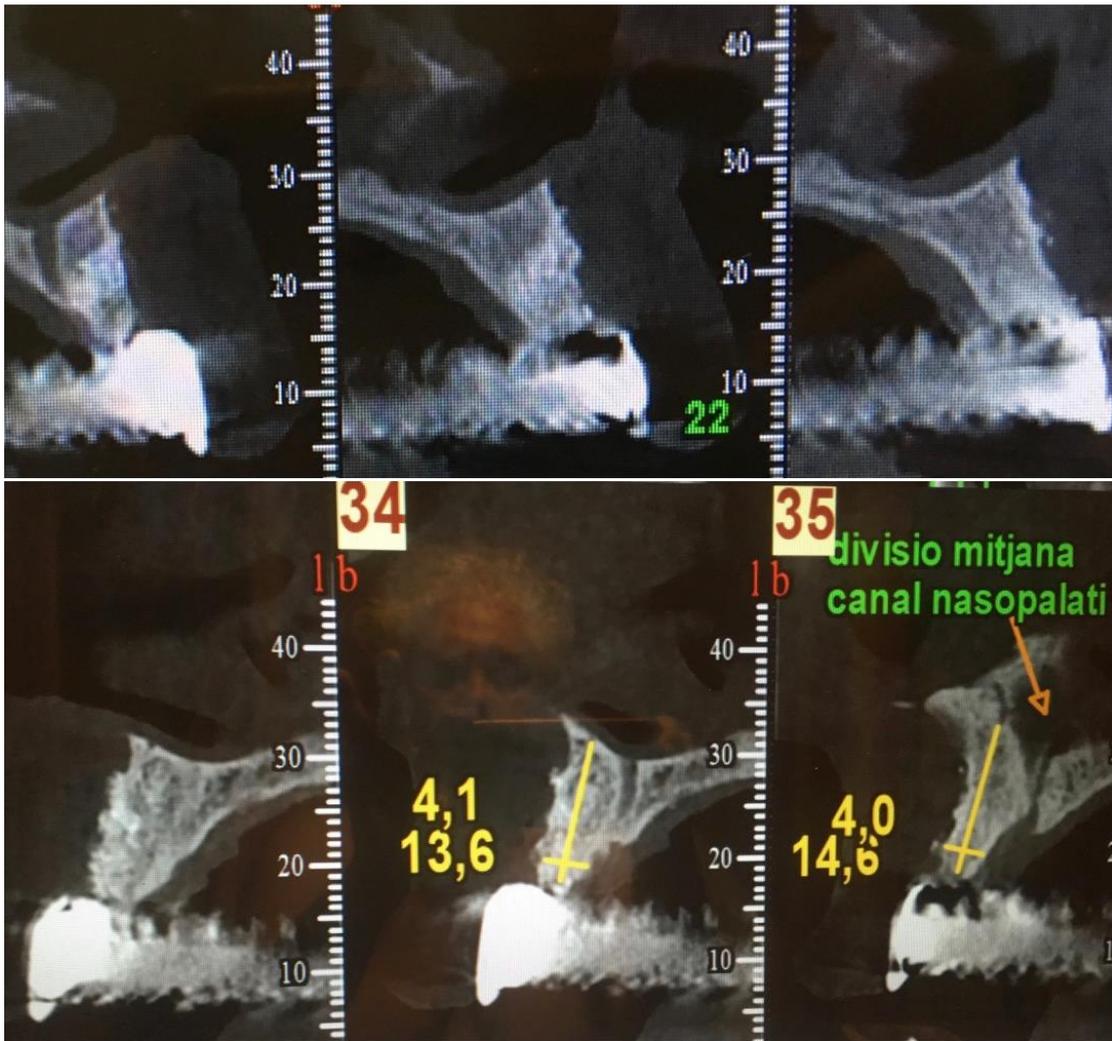


Figure 14: Tac demonstrating bone and gum regeneration (one year later)



Figure 15: The result

Results: The combination of the two techniques of L-PRF and BOPT results in improved gum and bone healing in shorter time than is normally the case.

Conclusion: L-PRF is an excellent technique for the healing of hard and soft tissue. In combination with BOPT, we have more control and are able to model the soft tissue to our fixed prosthesis.

Effects of platelet-rich plasma on the density of myelinated nerve fibers around dental implants: an experimental study on beagle dogs

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Background: Whilst increasing evidence on the effectiveness of local platelet-rich plasma (PRP) application for soft and bone tissue healing in implant surgery, characterization of the healing and regenerated myelinated nerve fibers with autologous PRP in physiological integration of oral implants remains poorly documented. Furthermore, the influence of such biological materials at different concentration on the assumed restoration of sensory function has hardly been explored.

Aim: To histologically evaluate the peri-implant regenerated myelinated nerve fibers after the local application of a different concentration of PRP.

Materials and methods: Ethical approval was obtained to carry out a split mouth study in 9 beagle dogs. Each dog randomly received 8 commercial threaded titanium implants (BLB system, Naton, China) (Grade V, 3.3mm Ø × 8mm long, non-submerged healing) at both sides of mandibular premolar regions from one out of 4 groups: delayed implant placement without any loading (control I); delayed implant placement with delayed loading (control II); low concentration of PRP + delayed implant placement with delayed loading (L-PRP); high concentration of PRP + delayed implant placement with delayed loading (H-PRP). Animals were euthanized at 1, 3 and 6 months after implant placement and loading, respectively (3 dogs/time point). The block biopsies were removed and trimmed into blocks with a 3- to 5- mm piece of peri-implant bone and fixed in 0.5 mol/L ethylenediaminetetraacetic acid(EDTA) phosphate-buffered saline(pH 7.4) at 4 °C for 10 months, until the implants were easily removed using surgical forceps. Tissue blocks were serially cut into 4µm~ 6µm thick sections using a microtome parallel to the implants. The myelinated nerve fibers were counted and measurement in ring shape with 0.5mm width around the middle level of implant. The number of the nerve fibers were counted with the software Image J.

Results: The nerve density of control group I was found lower than that of the other groups, yet significance could not be reached ($P < 0.05$). Also, the number of myelinated nerve fibers increased after 3 months of healing, with a significant increase for control group 1. However, no distinct difference were found between three time points for L-PRP and H-PRP groups, while for nerve diameters, none of the groups showed a distinct difference.

Conclusions: Within the limits of the study, H-PRP and L-PRP may promote the regeneration of the myelinated nerve fibers compared to that only obtained by delayed implant placement or delayed loading. The present findings do not seem to suggest an improved sensory function when using H-PRP or L-PRP. Towards the future, more studies with bigger samples might be needed to verify these findings.

Acknowledgement: The authors thank the laboratory technicians from the Research Base of West China Hospital, Sichuan University for their valuable help during this animal research. Fellowship support from Research Foundation Flanders (FWO) from the Belgian government and Sichuan Province Science and Technology Support Program (2016SZ0010). Scholarship support from China Scholarship council (D.Song).

Interaction between i-PRF and biomaterials: a microscopic evaluation

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Aim: The objective of the study was to inspect the interaction between injectable platelet-rich fibrin and implant surfaces or porous bone substitutes by microscopic analyses.

Materials and methods: Blood samples were collected from 5 volunteers to obtain the injectable platelet-fibrin (i-PRF). The i-PRF was mixed with titanium implant surfaces or porous hydroxyapatite/ β -TCP particulate. The i-PRF-biomaterial assemblies were inspected by confocal microscopy and field-emission guns electron microscopy (FEGSEM).

Results: The morphology of the mixture between i-PRF and HA/ β -BCP granules is shown in Figure 1. FEGSEM images revealed an agglomeration of particles surrounding by the i-PRF (Fig. 1). The dominant adhesion pathway consists in a mechanical interlocking of bioactive ceramic particles into the i-PRF network. However, some regions of the biomaterial/i-PRF assemblies revealed a dense fibrin network embedding red blood cells, blood platelets and leucocytes.

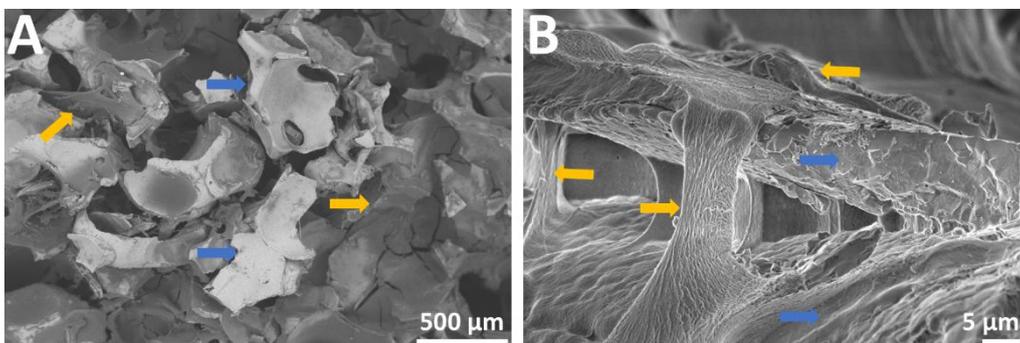


Figure 1: SEM images of the combination between a fibrin glue network and BCP granules. (A) The morphology of the mixture between i-PRF (yellow arrow) and bioactive ceramic granules (blue arrows). (B) Fibrin-based glue between the bioactive ceramic granules.^[1]

Conclusion: Bioactive ceramics composed of hydroxyapatite and β -TCP can be agglomerated by i-PRF polymerization on a mechanical interlocking pathway. The addition of bioactive ceramics to a biodegradable natural polymer such i-PRF may be used to favorably alter the degradation rate of the bone substitute composed of biomaterial/i-PRF.

References

^[1] Varela, H., Noronha Oliveira, M., Pereira, J., Souza, J.C.M., Pinto, M., Quiryne, M. (2018) Platelet-rich fibrin to incorporate bioactive graft materials. In: Nanostructured Biomaterials for Cranio-Maxillofacial and Oral Applications 1st ed. Elsevier, 119-142

Effect of berberine on osteogenic differentiation of bone marrow mesenchymal stem cells

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Aim: To make a preliminary research in the effect of *Porphyromonas gingivalis* (Pg) infection on osteogenesis of bone mesenchymal stem cells (BMSCs), then explored the possible interventional effect of berberine on this bacterium's functions, and its direct effect on osteogenesis of BMSCs.

Material and methods: The primary rat bone mesenchymal stem cells were isolated and cultured. Cell surface markers were detected by flow cytometry. Multiple differentiation potential was identified by osteoblastic and adipogenic induction. Effect of Pg on the proliferation of BMSCs was determined by CCK-8 assay. Effect of Pg on expression of osteogenic differentiation related gene was analyzed by quantitative PCR. Antibacterial capacities of berberine against *Porphyromonas gingivalis* were determined by broth microdilution method. Berberine's effect on gingipain (RgpA) activity was detected by enzymatic reaction. Alkaline phosphatase (ALP) activity assay, quantitative PCR and alizarin red staining was adopted to evaluate effect of berberine on BMSCs osteoblastic differentiation.

Results: The isolated cells have adherent property. Cell surface markers such as CD90, CD105 expression rates are 99.5%, 99.8% respectively; CD45, CD90 expression rates are 4.67%, 3.72% respectively. The isolated cells have the potential of differentiation into osteocyte and lipocyte in specific condition. Berberine has an inhibit role on Pg growth in a concentration dependent manner. MIC of berberine against Pg is 31.3 µg/ml. Berberine also has a certain inhibitory effect on gingipain activity. ALP and OCN mRNA expressions are downregulated when BMSCs are treated with Pg at MOI 100:1. Remarkably, berberine restores the changes induced by Pg, and further stimulates ALP activity, gene expression of *Osx*, *COL I*, *ALP*, *OCN* and *OPN*, and calcium nodules formation. The most effective concentration is 3 µM.

Conclusion: Pg infection results in downregulation of osteogenesis related genes expression in BMSCs without no cytotoxicity. Berberine possesses effectively antibacterial action against Pg. Berberine restores osteogenesis inhibition induced by Pg. Moreover, berberine promotes BMSCs' osteogenesis potential. All of these make berberine a potent candidate drug in periodontal tissue regeneration.

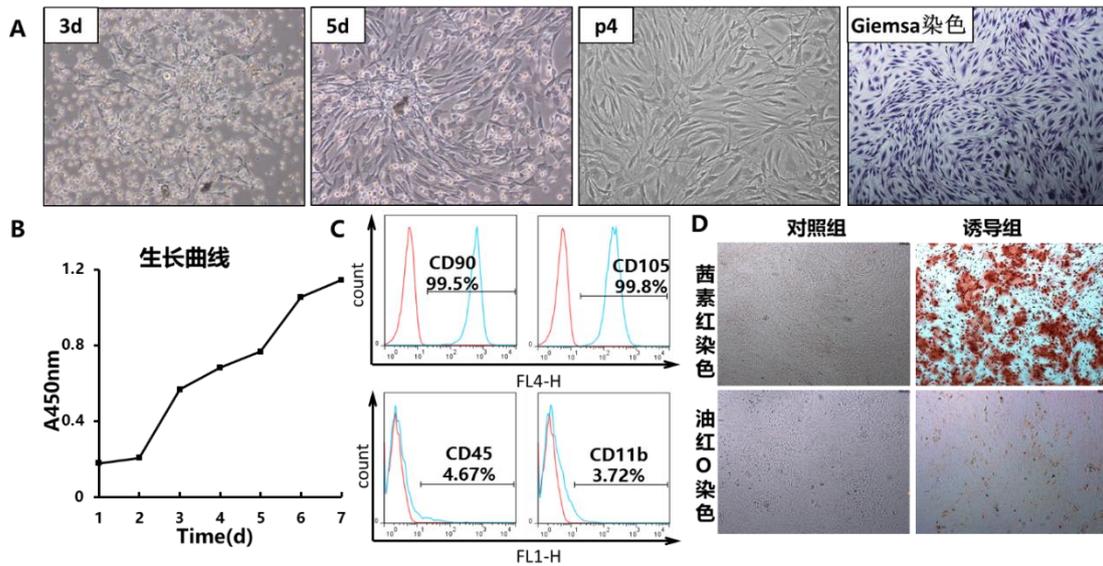


Figure 1: The primary culture of rat BMSCs

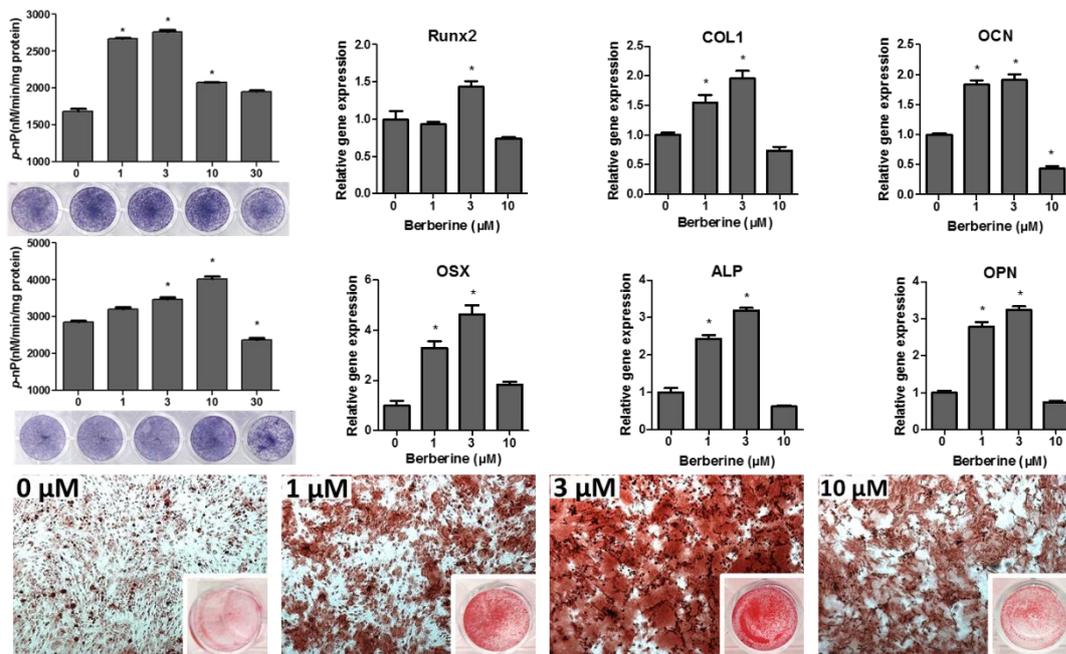


Figure 2: Berberine promote osteogenic differentiation of rat BMSCs

References

- [1] Torrin A., Li O., Brune J. C., et al. (2011) CD146 expression on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization[J]. *Blood* 117(19): 5067-5077.
- [2] Covas D. T., Panepucci R. A., Fontes A. M., et al. (2008) Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts[J]. *Exp Hematol* 36(5): 642-654.
- [3] Coyne T. M., Marcus A. J., Woodbury D., et al. (2006) Marrow stromal cells transplanted to the adult brain are rejected by an inflammatory response and transfer donor labels to host neurons and glia[J]. *Stem Cells* 24(11): 2483-2492.
- [4] Meirelles L. S., Caplan A. I. and Nardi N. B. (2008) In search of the in vivo identity of mesenchymal stem cells. *Stem Cells*, 2008, 26(9): 2287-2299.
- [5] Xu D., Yang W., Zhou C., et al. (2010) Preventive effects of berberine on glucocorticoid-induced osteoporosis in rats. *Planta Med*, 2010, 76(16): 1809-1813.
- [6] Lee H. W., Suh J. H., Kim H. N., et al. Berberine promotes osteoblast differentiation by Runx2 activation with p38 MAPK[J]. *J Bone Miner Res*, 2008, 23(8): 1227-1237.

Indirect bio-printing as a novel technique for guided bone regeneration: a proof of concept

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Aim: The standard of care in implant dentistry currently involves performing bone augmentation procedures freehand, based on visual inspection and operator experience. The aim of this poster is to present a novel technique for performing guided bone augmentation using a patient-specific 3D printed surgical guide.

Material and methods: A patient undergoing implant placement with simultaneous bone contour augmentation in the upper premolar region was chosen for this pilot study. Based on the pre-operative CBCT a virtual bone augmentation procedure was performed until the desired bone contour was obtained. A surgical guide was then designed and adapted to the desired bone shape. The augmentation volume was slightly overcompensated, and a 2 mm offset was added buccally to allow for two layers of L-PRF membranes. Additionally, holes for membrane tacks were made into the guide, taking into account the root anatomy of the neighboring teeth.

During the surgery, L-PRF membranes and a L-PRF block were prepared and placed onto the guide, which was then adapted to the bone surface. Membrane tacks were used to fix the membranes and the guide was removed. A periosteal releasing incision was performed and primary closure was achieved after suturing. Healing was uneventful.

Post-operative CBCT were taken immediately after the surgery and at 8 months, after the second stage surgery. Both the initial bone graft shape and the result at 8 months were analyzed for differences in shape from the initial plan at 0, 2mm, 4mm and 6mm from the implant shoulder.

Results: The average planned graft thickness (horizontal distance from the guide model to the bone at the measured sites was 8.13 mm (SD ± 0.69 mm). This includes space for the L-PRF membranes (2 mm). The average graft thickness measured on the post-operative CBCT was 6.39 mm (SD ± 0.9 mm), resulting in an average difference of 1.74 mm (SD ± 0.33 mm) between plan and result. At 8 months post-operatively an average resorption of 0.88 mm (SD ± 0.56 mm) was measured.

Discussion: Although the use of the guide resulted in a clinical success, differences were observed between the planned bone graft shape and the result, both immediately post-operatively and at 8 months. While surgical factors (maladaptation of the guide to the bone surface, forces applied during flap closure and suture) are most likely responsible for the former, resorption of the graft is most likely responsible for the latter. All these factors need to be considered and compensated for in future studies.

Conclusion: Within the limitations of this study, the custom surgical guide appears to provide a predictable way of shaping the bone graft volume. Additionally, it allows the surgeon to work in a sterile field while preparing the graft material, potentially reducing the risk of infection. Finally, the use of a L-PRF block covered using L-PRF membranes appears to be a valid technique for horizontal bone contour augmentation.

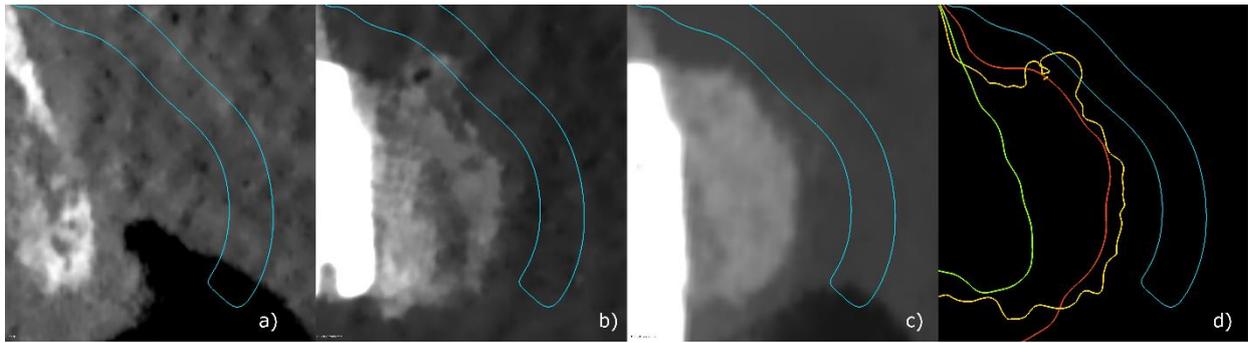


Figure 1: Sagittal views of pre-operative (a), immediately post-operative (b) and 8-month post-operative (c) CBCT slices with the surgical guide overlaid in blue. Their respective segmentations (green for pre-operative, yellow for immediately post-operative and red for 8 months post-operative) are also shown (d).

References

- [1] Elnayef B, Porta C, Suárez-López Del Amo F, Mordini L, Gargallo-Albiol J, Hernández-Alfaro F. (2018) The Fate of Lateral Ridge Augmentation: A Systematic Review and Meta-Analysis. *Int J Oral Maxillofac Implants*. 2018 May/Jun;33(3):622-635. doi: 10.11607/jomi.6290.

THANK YOU!

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