FMUC FACULDADE DE MEDICINA
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Mestrado Integrado em Medicina Dentária

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Summary

Resumo

Introdução: O crescente conhecimento na área da Periodontologia levou ao desenvolvimento de várias técnicas regenerativas, mas também ao reavivar do uso de concentrados plaquetários autólogos (APCs) no tratamento de defeitos periodontais infraósseos. Objetivos: Este trabalho pretende verificar a aplicabilidade e eficácia dos concentrados plaquetários (particularmente da fibrina rica em plaquetas e leucócitos (L-PRF)) na regeneração dos defeitos periodontais infraósseos, através de uma revisão sistematizada. Adicionalmente, pretende-se demonstrar o método de aplicação do L-PRF na regeneração recorrendo a uma série de casos clínicos. Metodologia: Foi definida uma questão PICOT: "Em pacientes com defeitos periodontais profundos, qual a eficácia da aplicação de L-PRF, isolado ou em combinação com outros biomateriais, na regeneração de defeitos periodontais, comparativamente à cirurgia de acesso e a outros APCs, após um período de cicatrização mínimo de 6 meses?", seguida de uma pesquisa eletrónica nas bases de dados primárias (PubMed e Cochrane) com as palavras-chave: “platelet rich plasma”, “platelet rich fibrin”, “plasma rich growth factors”, “periodontal defect”, “infrabony”, “intrabony”, “bone regeneration” e “periodontal regeneration”; com os conectores boleanos “AND” e “OR”. Sempre que possível, foram usados termos MeSH. Os critérios de pesquisa incluíram Meta-Análises, Revisões Sistemáticas e Ensaios Clínicos Randomizados, com o resumo disponível, em humanos, publicados nos últimos 10 anos, em inglês ou português. Efectuou-se uma série de casos clínicos para demonstrar a aplicabilidade do L-PRF na regeneração minimamente invasiva de defeitos periodontais infraósseos profundos. Resultados: Sete revisões sistemáticas foram incluídas nesta revisão. Os parâmetros avaliados foram a redução da profundidade de sondagem, o ganho de inserção clínica e o preenchimento ósseo do defeito, bem como os parâmetros centrados no paciente. A série de casos clínicos permitiu verificar a ausência de complicações pós-operatórias. Discussão: Relativamente aos parâmetros clínicos (redução da profundidade de sondagem e ganho de inserção clínica), o PRF parece apresentar melhores resultados que o plasma rico em plaquetas (PRP). No que respeita aos parâmetros radiológicos (preenchimento ósseo), apesar da limitada informação disponível, o PRF parece fornecer melhores resultados que o PRP. Os parâmetros centrados no paciente são escassamente abordados na literatura revista, mas o PRF aparente proporcionar um melhor pós-operatório. Conclusão: Considerando os limites desta revisão, é possível concluir que a evidência científica disponível demonstra uma melhoria dos parâmetros clínicos com a aplicação de L-PRF comparativamente à cirurgia de acesso e outros APCs. Contudo, são necessários mais ensaios clínicos randomizados multicêntricos com qualidade e validade científica para provar a dimensão da eficácia desta técnica.

Palavras-chave: PRF, L-PRF, concentrados plaquetares, defeitos infraósseos, regeneração periodontal

Abstract

Introduction: The increasing knowledge in the field of Periodontology lead to the development of several new regenerative techniques, but also to the revival of the use of autologous platelet concentrates (APCs) in the regeneration of infrabony defects. Objective: This study aims to verify the applicability and effectiveness of the use of platelet concentrates (in particular L-PRF) in the regeneration of periodontal infrabony defects, through a systematic review. Additionally, it pretends to exemplify the usage of L-PRF in regenerative periodontal surgery, through a case series. Methodology: A PICOT question was established: "In patients with periodontal infrabony defects, what is the efficacy of L-PRF alone or in combination with other biomaterials in periodontal regeneration, comparatively to open flap debridement and other AOCs, after at least 6 months of healing?". An electronic literature search was performed in PubMed database and Cochrane Library with the keywords: "platelet rich plasma", "platelet rich fibrin", "plasma rich growth factors", "periodontal defect", "infrabony", "intrabony", "bone regeneration" and "periodontal regeneration"; and the boolean connectors "AND" and "OR". The MeSH Terms were applied when possible. Filter criteria were: the type of articles, including Meta-Analysis, Systematic Reviews and Randomized Controlled Trial, with abstract text availability, in humans, published in the last 10 years, in English or Portuguese language. A cases series is presented to demonstrate the applicability of L-PRF in the minimally invasive regeneration of deep infrabony defects. Results: Seven systematic reviews were included in this review. The analyzed variables were probing depth reduction, clinical attachment level gain, bone fill and patient centered outcomes. No post-operative complications were observed in the case series. Discussion: Regarding clinical parameters (pocket depth reduction and clinical attachment level gain), PRF seems to provide better outcomes than platelet-rich plasma (PRP). Concerning radiologic parameters (bone fill), although with limited information, PRF seems to give better outcomes than PRP. The patient center outcomes were scarcely assessed across the studies included, but apparently PRF provides a better post-operatory recovery. Conclusions: Within the limits of this study, it can be concluded that the literature demonstrated a statistically significant clinical improvement when L-PRF is applied, in comparison to open flap debridment and other APCs. Nonetheless, more multicentered randomized clinical trials with quality and scientific validity are needed to prove the dimension of the effectiveness of this technique.

Keywords: PRF, L-PRF, platelet concentrates, infrabony defects, periodontal regeneration

List of abbreviations and acronyms

BRG	Bone replacement grafts
CAL	Clinical attachment level
GTR	Guided tissue regeneration
IFB	Infrabony defect
L-PRF	Leukocyte and platelet-rich fibrin
Mm	Millimeters
NS	Non-statistically significant
OFD	Open flap debridement
%	Percentage
PRGF	Plasma rich in growth factors
PRP	Platelet-rich plasma
PPD	Probing pocket depth
SRP	Scaling and root planning
SS	Statistically significant

1. Introduction

1.1. Chronic periodontitis

The periodontium is a dynamic structure comprising four main tissues: gingiva, periodontal ligament, root cementum and alveolar bone.[1] The disturbance of the balance between the host tissues and the resident microbiota results in disease of the periodontal tissues.[1]

Among the different periodontal diseases, chronic periodontitis is the most common form in adults and it is clinically defined by the following signs and symptoms: (1) color, texture and volume alterations of the marginal gingiva; (2) bleeding on probing from the gingival pocket area; (3) diminished resistance to probing of the soft marginal tissues; (4) loss of probing attachment level; (5) recession of the gingival margin; (6) loss of alveolar bone; (7) root furcation exposure; (8) increased tooth mobility and (9) drifting and eventually exfoliation of teeth.[1] Thereby, periodontitis can be defined as an inflammatory disease that causes an irreversible loss of attachment to the connective tissue and supporting alveolar bone.[1–3]

Bone resorption in periodontitis can produce three types of defects: suprabony (or horizontal) defects, infrabony (or vertical) defects and interradicular (or furcation) defects.[1,4] When the base of the pocket is located coronal to the alveolar crest, it is considered a suprabony defect.[1,4] If the base of the pocket has an apical location regarding the alveolar crest, it is classified as an infrabony defect.[1,4] The interradicular defects develop when there is pathological bone resorption of the furcation area due to periodontitis.[5]

Regarding infrabony defects, one of its main classifications is made according to the number of residual alveolar bone walls (Figure 1), namely, (1) one-wall defects, with half of a septum remaining between teeth (hemiseptal defects) or with one buccal/lingual wall; (2) two-wall defects, with one proximal wall and one buccal/lingual wall or one buccal wall and one lingual wall (which is specifically nominated as “crater”); and (3) three-wall defects, which consist of three bony walls and the radicular surface and that may be additionally classified as intrabony defect or circumferential osseous defect.[1,5]

The increasing knowledge about the importance of the number of walls in the regenerative potential of bone defects, highlighted the need to define and standardize their classification. In fact, the classical classification by Goldman & Cohen (1958), considers that two types infrabony defects can be recognized: intrabony defects and craters.[4] Intrabony defects are bony defects whose infrabony component affects primarily one tooth, while in craters the defect affects two adjacent root surfaces to a similar extent.[4] But, according to Weinberg & Eskow (2000), the expression “infrabony” may be applied to all vertical defects, although the term “intrabony” should be used specifically when referring to a three-walled defect adjacent to a radicular surface, with high regenerative potential.[5,6]

Furthermore, a circumferential bone defect extends to buccal or lingual surface of the root, unlike intrabony defects.[6]

The morphology of infrabony defects can assume a more complex anatomy, when different components are seen coronally and apically, for example, when the defect has a two-wall component in its coronal portion and a three-wall component in its most apical portion.[1,5,6] In fact, these combined osseous defects represent the majority of infrabony defects, which reveals the enormous variety and anatomic complexity of those.[5]

Other factors, as the width of the defect (or radiographic angle) or the topographic extension around the tooth, can be also used to classify bone defects (figure 1).[1]

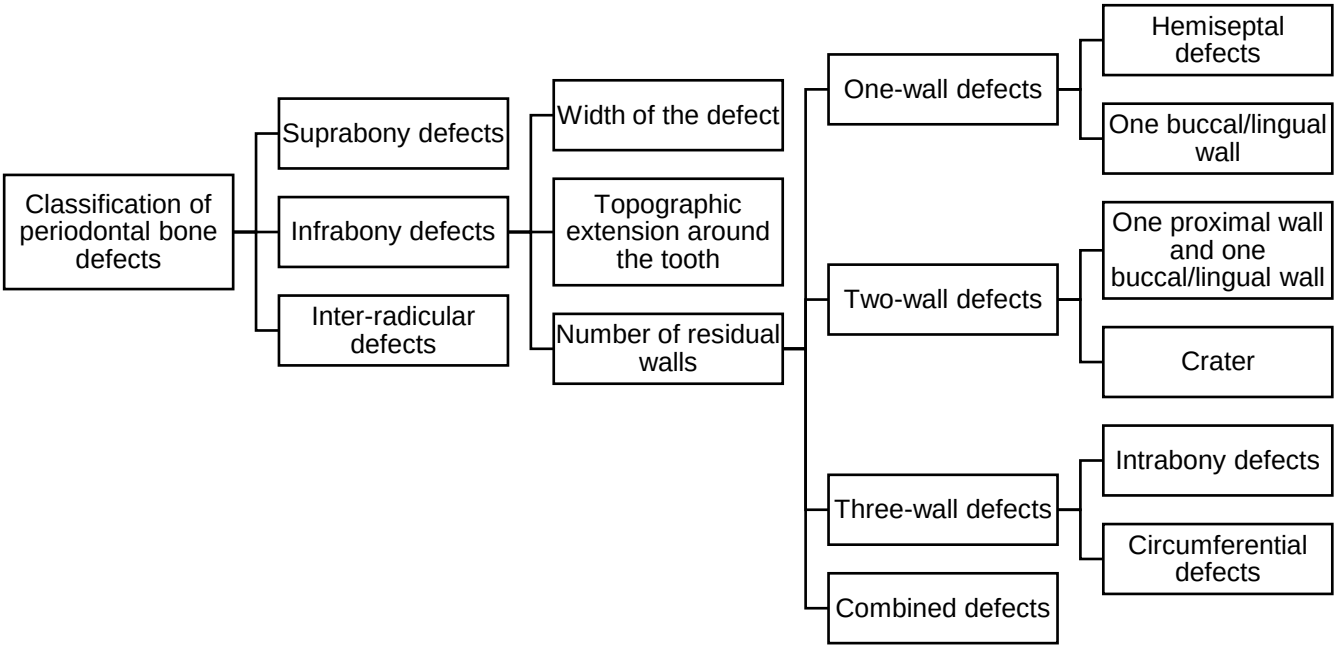


Figure 1. Classification of periodontal bone defects

1.2. Therapeutic approaches

The primary goal of periodontal treatment is to promote the maintenance of the natural dentition in optimum health and function.[7,8] It is, therefore, desirable to not only prevent periodontal disease progression, but also to regenerate all the tissues of the periodontium.[9,10]

Several non-surgical and surgical approaches have been studied and applied in Periodontology, specifically in infrabony defects with deep pockets (figure 2). Non-surgical treatments aim to disrupt the microbial biofilm and to suppress the inflammation, using pocket/root instrumentation combined with supragingival plaque control measures. Scaling and root planning (SRP), even when associated with proper oral hygiene program, does not predictably remove plaque and subgingival calculus and therefore, other approaches may be necessary, mainly in deep periodontal pockets.[1,5]

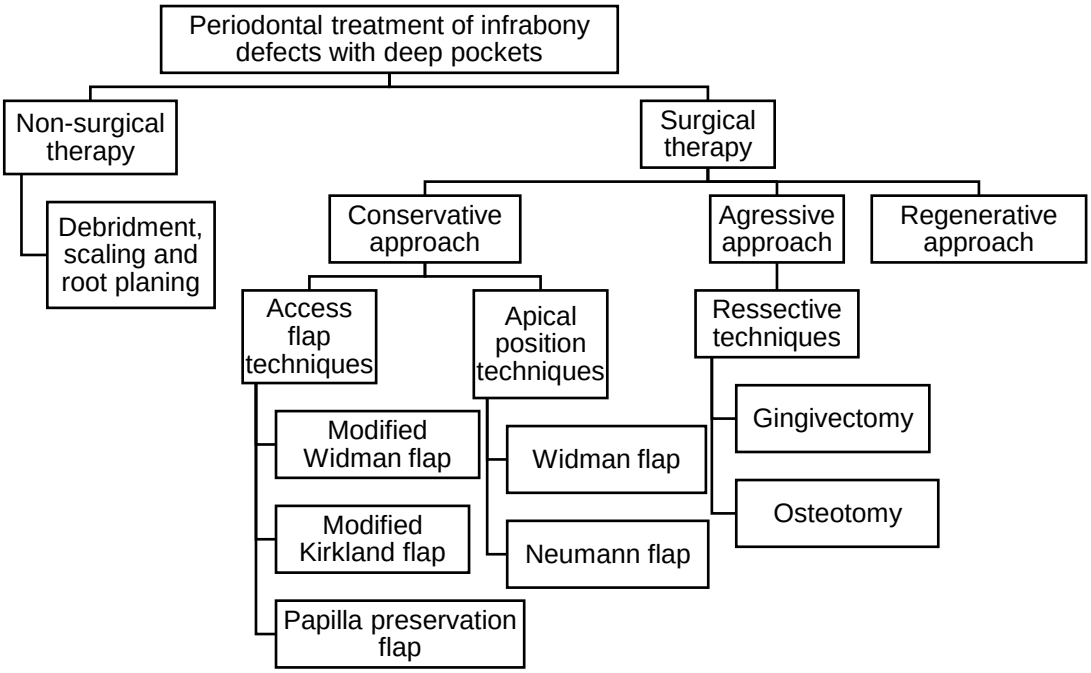


Figure 2. Periodontal treatment of infrabony defects with deep pockets

Despite their specific indications, surgical approaches must be seen as adjunctive therapies to non-surgical techniques.[1] The main goals of surgical approaches are to diminish the gingival inflammation and to benefit the patient's home care, both decisive factors for the long-term prognosis.[1] In order to achieve these goals, besides the access surgery therapy, a hard tissue intervention may be needed. The hard tissues' interventions, associated to an open flap techniques, may include eliminating totally or partially the osseous defect, by osteoplasty and/or ostectomy; instrumentation of the root surface; promoting healing through regenerative procedures; or, ultimately, extracting of the involved tooth.[1]

Focusing on the treatment of infrabony defects, which are usually associated with deep pockets (periodontal probing depth ≥ 6 mm), some approaches can be highlighted.[1,5]

Regarding a conservative approach, the main difference is the flap position at the end of the surgical intervention – if it is apically positioned at the level of the bone crest (original Widman flap, Neumann flap and apically repositioned flap) or maintained in a coronal position (Kirkland flap, modified Widman flap, and papilla preservation flap).[1] This conventional open flap debridement intervention is particularly advantageous at pockets that extend beyond the mucogingival border and/or where is necessary to treat bony or furcation defects, but fails to predictably regenerate the periodontal tissues.[1,7]

In a more invasive approach of soft tissues, gingivectomy is mainly an tissue-eliminating/resective technique, whose purpose is the complete elimination of the periodontal pocket.[1] Beside the aesthetic aspect, which can be problematic due to the gingival recessions, the potential residual pockets left can be inaccessible to proper patient-performed tooth cleaning during post-treatment maintenance.[1]

As for hard tissues, the goal is to convert an infrabony defect into a suprabony defect, using apical repositioning of the soft tissue flap(s) and osseous recontouring techniques.[1] These bone contouring procedures may lead to poor aesthetic outcomes, since they often result in recession of the gingival margin after healing.[11]

Although it is fair to say that conventional surgical approaches, that resect or eliminate tissue, can be beneficial in early or shallow infrabony defects (<3 mm), it is important to understand that the improvement in periodontal clinical parameters, when a non-regenerative therapy is applied, are mainly due to the formation of a long junctional epithelium.[1,12] The only approach that allows a predictable and long-term sustainable reconstitution of functional attachment apparatus is the regenerative therapy.[12,13]

1.2.1. Regenerative therapy

By definition, regeneration is the reproduction or reconstruction of a lost or injured part, in such a way that the architecture and function of the lost or injured tissues are completely restored, whereas repair refers to healing of a wound by tissue that does not fully restore the original architecture or function.[14]

Although it is established that the regeneration of the periodontium includes the formation of new cementum with inserting collagen fibers on the root surfaces and the regrowth of the alveolar bone, the understanding of the regeneration mechanism of an architecturally complex organ, such as periodontium, remained a challenge for many years.[1,15]

In 1976, Melcher developed a theory based on a “compartmentalization” concept, according to which the type of cell that repopulates the root surface after periodontal surgery determines the nature of the attachment that will be formed.[1,15] Several experimental studies have been conducted and it is known today, that the cells with the potential to produce a new connective tissue attachment reside in the periodontal ligament.[1,5]

Three different regenerative concepts have been employed – barrier membranes, grafts and biological modulators, plus other combinations between those.[16]

The barrier membranes behave as mechanical barriers that enable not only selective cell growth, but also provide space and stability to the blood clot.[16,17] The initial efforts on guided tissue regeneration (GTR) started around the 80’s with a cellulose acetate bacterial filter (Millipore® filter, type GS; Millipore SA,67 Molsheim, France), which was an occlusive membrane – functional, but not clinically ideal.[1,16] Later on, nonresorbable membranes of expanded-polytetrafluoroethylene (Gore-tex®, W. L. Gore & Ass. Inc., Flagstaff, Arizona, USA) were developed.[1,16,18] These membranes of e-PTFE are inert and biocompatible, but they persist after healing and it is necessary a second intervention for their removal.[1,16] In order to avoid this second

surgery, natural or synthetic bioresorbable barrier materials for GTR have been developed.[1,16] The natural bioresorbable barrier materials are frequently a cross-linked variety of porcine or bovine collagen, which is resorbed by the enzymatic activity of macrophages and polymorphonuclear leukocytes when implanted in the human body.[1,16] Despite their success, complications such as early degradation, epithelial down-growth along the material, premature loss of the material, infection from animal products and autoimmunization have to be taken in consideration.[1] Other type of barriers are those made of polylactic acid or co-polymers of polylactic acid and polyglycolic acid, which are biocompatible, but not totally inert.[1] These membranes are degraded by hydrolysis and eliminated from the organism through the Krebs cycle as carbon dioxide and water.[1]

Bone replacement grafts (BRG) are another regenerative concept, which includes materials of human (autologous or allogeneic), animal (xenogeneic) or synthetic origin (alloplastic) and are based on the biological principles of osteoconductivity and osteoinductivity, besides the grafts' capacity of space maintenance and blood-clot stabilization.[1,16]

It is worth noting one specific BRG, the decalcified freeze-dried bone allograft (DFDBA), that allegedly contains bone morphogenetic proteins (BMPs).[19] BMPs are members of the transforming growth factor beta (TGF-β) superfamily, with the exception of BMP-1, a pro-collagen C-protease.[20] These proteins yield several effects on bone as (1) their mitogenic activity on undifferentiated mesenchymal cells and osteoblast precursors; (2) their ability to induce the expression of the osteoblast phenotype; (3) their chemoattractive action for mesenchymal cells and monocytes; and (4) their capacity to link to extracellular matrix type IV collagen.[20] Summarily, this means that BMPs are osteoinductors, capable of stimulating local cell cycles to produce new bone.[21,22]

As an animal origin bone graft, bovine porous bone mineral (BPBM) is the result of the protein extraction from bovine bone, which produce a osteoconductive trabecular hydroxyapatite structure resembling human cancellous bone.[23]

Beta tricalcium phosphate (b-TCP) is a synthetic BRG, made of purified, multicrystalline porous form of calcium phosphate with a calcium-to-phosphate ratio analogous to natural bone - 39% calcium and 20% phosphorus, similar to human cancellous bone.[24,25] It has shown to be biocompatible, resorbable and osteoconductive.[24–26]

In what concerns wound-healing modifiers or bioactive agents, under the general designation of biological modulators, Trombelli *et al.* (2008) considered two major classes: growth factors (GF), which includes BMPs; and other agents, such as enamel matrix derivative (EMD) and a 15-amino-acid peptide (P-15).[20]

Regarding the first category, several GF can be found in the alpha granules of platelets, such as platelet derived growth factor (PDGF), transforming growth factor beta 1 (TGFβ1), endothelial growth factor, vascular endothelial growth factor (VEGF) and insulin growth factor 1 (IGF-1).[7] Among these, PDGFs are powerful biologic mediators, stimulating cell proliferation,

differentiation, angiogenesis and chemotaxis.[9,19] The action of PDGF alone, with or without a progression factor to induce mitosis, provoked the proliferation of both osteoblasts and isolated periodontal ligament (PDL) cells.[22] Another GF worth noting is TGF-β, capable of diverse functions such as increasing the differentiated function of osteoblasts, osteoblasts precursors and extracellular matrix formation/remodeling or stimulating the proliferation of gingival fibroblastic cells and formation of blood vessels.[22] Other GF related to periodontal renegeneration have been mentioned in the literature, such as fibroblast growth factor (FGF) and recombinant human platelet-derived growth factor-BB (rhPDGF-BB). Nevertheless, the majority of them still lack scientific evidence for efficacy and safety for clinical application.[16,20,27]

Enamel matrix derivative (EMD) is another bioactive agent. EMD is mainly an amelogin-compound (about 90%), plus porcine origin proteins – albumin, amelin and enamelin.[20] According to Hammarström *et al.* (1997), the initiating factor for cementum formation is the expressed amelogenin at the apical end of the forming root of human teeth.[28] The formation of cementum is associated with the development of the periodontal ligament and the alveolar bone.[28] The combination of EMD with other regenerative therapies is based on an hypothetic synergistic effect.[10] Although it is not proven, the current literature available is very promising regarding pocket-depth reduction, clinical attachment level gain and radiographic bone level.[16]

Another bioactive agent analyzed in some studies is the 15-amino-acid peptide (P-15), a synthetic cell-binding peptide that is equal to part of the sequence of the α1 chain of type I collagen.[20] The P-15 has been shown to increase the rate and the extent of attachment and migration of periodontal cells to root or biomaterial surfaces.[20,29] Similarly to EMD, P-15 have been combined with other products, such as anorganic bovine-derived matrix (ABM).[5,13,29]

1.2.1.1. Autologous platelet concentrates (APC)

Since 1970, autologous platelet concentrates have acquired more attention from medical community, but their introduction in oral and maxillofacial surgery occurred only in the 90s .[30]

The first generation of platelet concentrates (APC) comprises platelet-rich plasma (PRP) and plasma rich in growth factors (PRGF).[30]

PRP has in its constitution four to fivefold-increased platelet concentration above baseline, thus being enriched with several growth factors, such as PDGF, transforming growth factor-1 (TGF-1), transforming growth factor-2 (TGF-2), insulin growth factor-1 (IGF-1), insulin growth factor-2 (IGF-2), basic fibroblast growth (bFGF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF); and blood proteins related to osteoconduction (fibrin, fibronectin and vitronectin).[2,9,31,32] These growth factors reach their maximum release in the first day, yet it continues for seven days.[9]

The PRP preparation requires the following three components: (1) anticoagulants at the moment of blood collection, (2) bovine thrombin and (3) calcium chloride.[30,33] There are several

PRP preparation protocols, but all of them require two centifrugations (with time and number of rotations per minute variable according to the different authors).[25,31]

The different clinical applications of PRP, such as sinus floor elevation or treatment of periodontal defects, are based on the basic premise that its high concentrations of platelets will promote a local concentration of secreted growth factors, which will increase the initial bone repair mechanisms and its effects will remain even when PRP fade away.[32] Other properties attributed to PRP are not only related to an angiogenetic, proliferative and differentiative effect on osteoblasts, mainly due to PDGF and TGF-β; but also to its capacity of inducing clot formation when reacting with thrombin, thanks to its fibrinogen content, and its potential haemostatic activity, which provides blood-clot stability.[21,31,33]

It is worth noting that PRP is considered a safe autologous preparation, since it is prepared with the patient's own blood; is biologically acceptable; and it is economically viable. Nevertheless, the required use of bovine thrombin, which is not an autologous material, still remains a significant disadvantage, even if no disease transmission or immunogenic reactions have been reported to this date.[31] Additionally, Castro *et al.* (2017) emphasizes that the PRP fibrin network is thin and non-condensed and it has a low tensile strength, which makes it less helpful to use as space maintainer.[30]

The second generation of PC was introduced in 2001 by Choukroun and co-workers.[34] The platelet-rich fibrin (PRF) preparation consists exclusively in the blood centrifugation at high spin, without any additives, producing three layers: red blood corpuscles at the bottom of the tube, platelet-poor plasma (PPP) on the top and a "buffy coat" as intermediate layer, where most leucocytes and platelets are concentrated.[30] This intermediate layer can be carefully compressed and transformed into a membrane of, approximately, 1 mm in thickness.[30] The PRF membrane is a biocompatible, bioresorbable and three-dimensional polymerized fibrin matrix, capable of slowly releasing growth factors over a period of 7-14 days, but also delivering platelet, leukocytes, cytokines and matrix glycoproteins.[19,30] Several authors emphasize the strong fibrin network of PRF, due to the physiological concentration of thrombin during its preparation, which enhances its mechanical properties.[9,23,30,35]

Briefly, when comparing PRP with PRF, the second has some advantages, such as the less chair side time required (approximately 12 min. for preparation), no need of addition of bovine thrombin or anticoagulants, the longer-term effect of growth factors and the fact that it is easier to use as a membrane, similar to guided tissue regeneration (GTR) membrane.[8,36]

Conventionally, four main categories of APC can be distinguished: (1) pure PRP, with no leucocytes; (2) leucocytes rich PRPs (L-PRP); (3) pure PRF, without leucocytes; and (4) PRF with leucocytes (L-PRF).[35] However, other APC can be mentioned, such as advanced PRF (A-PRF), injectable PRF (i-PRF) or lyophilized PRF (Ly-PRF).

The standard protocol for PRF and L-PRF requires one step of centrifugation for 12 minutes at 2700 rpm.[37–39] The advanced PRF (A-PRF), also developed by Choukroun, has a slower centrifugation for a longer time (1500 rpm for 14 minutes).[37,38] This protocol modification

is thought to increase platelet concentrations and white blood cells, but also to change the distribution pattern for neutrophilic granulocytes, which modifies monocytes and macrophages behavior.[37,38,40]

On the other hand, i-PRF pretends to be an easier way to apply APC, once it has a liquid formulation.[41] Miron *et al.* (2017) centrifuged the blood samples at 700 rpm for 3 min. and collected the upper liquid as i-PRF.[41] The hypothetic advantages and/or disadvantages of this formulation are still to be studied.[41]

The Ly-PRF distinguishes itself from PRF, once in this case blood samples suffer a process of freeze-drying lyophilization, which it is thought to diminish the risks associated with ultra-low temperatures storage, but also allows to reduce costs and to improve the management of storage and transport of PRF.[42] Zhang *et al.* (2017) considered that Ly-PRF was less flexible and easily broken compared to fresh PRF, but also that the lyophilization process had no relevant influence on the clinical effects of PRF.[42]

This thesis aims to verify the applicability and effectiveness of the use of platelet concentrates (in particular L-PRF) in the regeneration of infrabony periodontal defects, through a review of the literature. Additionally, another objective of this work is to exemplify the ways of using L-PRF in regenerative periodontal surgery, using a case series.

2. Systematic Review

2.1. Materials and Methods

In order to establish an appropriated search protocol for this systematic review, a focused question was made. Taking into account PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines[43], the following PICOT based question was elaborated: "In patients with periodontal infrabony defects, what is the efficacy of L-PRF alone or in combination with other biomaterials in periodontal regeneration, comparatively to open flap debridment and other APCs, after at least 6 months of healing?"

(P) Population: patients with periodontal infrabony defects systemically healthy (ASA I) or with mild systemic disease (ASA II); (I) Intervention: application of L-PRF alone or in combination other biomaterials in periodontal regenerative surgery; C) Comparison: open flap debridement (OFD) and other APCs; (O) Outcomes: probing pocket depth reduction, clinical attachment gain, radiographic bone fill, patient centered outcomes; (T) Time: post-operative follow-up of at least 6 months.

The study selection respected the following inclusion criteria:

- Systematic reviews, meta-analysis or randomized clinical trials (RCTs) evaluating the effect of L-PRF in the regeneration of periodontal infrabony defects, alone or in combination with other biomaterials;
- Publications with human histological, radiograph or clinical outcome parameters assessing soft tissue and/or bone healing results after application of platelet concentrates;
- Human studies published in English and/or Portuguese.

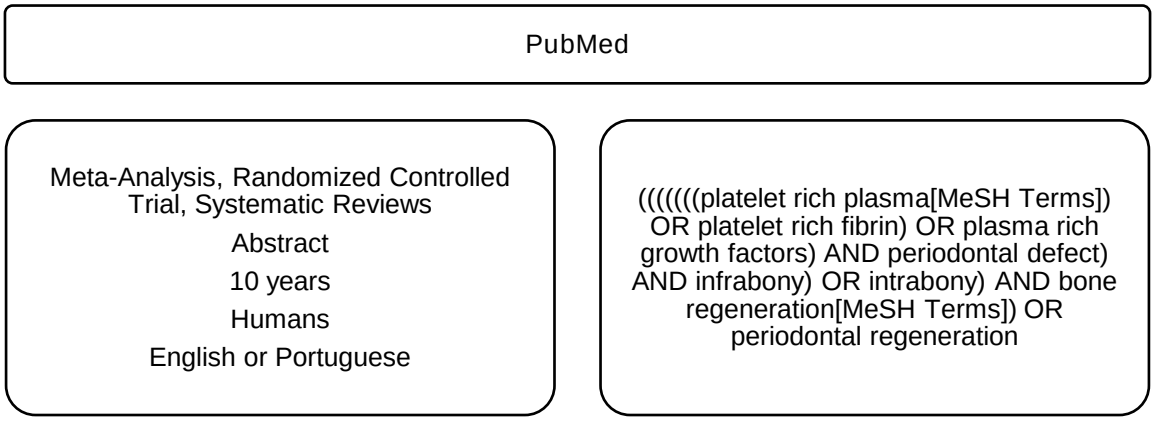
As exclusion criteria:

- Narrative reviews, case series or case reports;
- Animal or *in vitro* studies;
- Non-randomized controlled trials or with an inadequate control group;

For this systematic review, an electronic literature search was performed in PubMed database with the following keywords: "platelet rich plasma", "platelet rich fibrin", "plasma rich growth factors", "periodontal defect", "infrabony", "intrabony", "bone regeneration" and "periodontal regeneration"; and the Boolean connectors "AND" and "OR". MeSH Terms were applied when possible. Activated filter criteria were: the type of articles including Meta-Analysis,

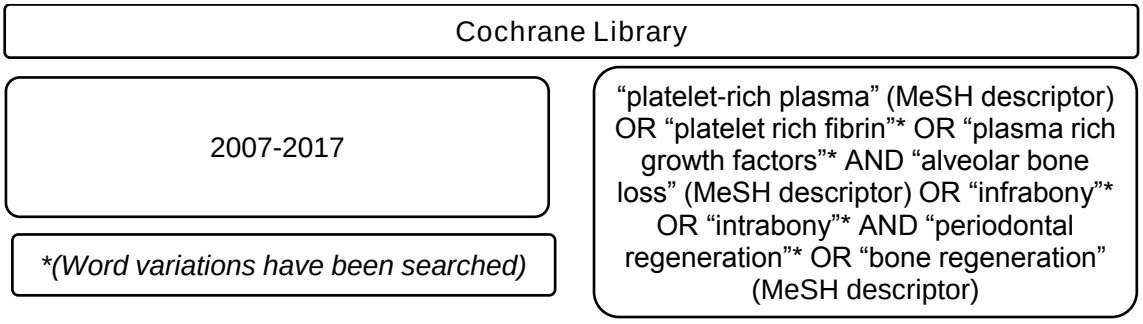
Systematic Reviews and Randomized Controlled Trial; with abstract text availability; humans; published in the last 10 years; in English or Portuguese language. The last search was performed at 15th May, 2017 (figure 3).

Figure 3. PubMed search



Another online search was made in Cochrane Library, using the terms: “platelet-rich plasma” (MeSH descriptor), “platelet rich fibrin”, “plasma rich growth factors”, “alveolar bone loss” (MeSH descriptor), “infrabony”, “intrabony”, “bone regeneration” (MeSH descriptor) and “periodontal regeneration”. The word variations for non-MeSH terms have been searched. The publications timeframe used was 2007 to 2017. The last update was made at 15th May, 2017 (figure 4).

Figure 4. Cochrane Library search



As a complementary search, cross-references and hand search were also taken into account in the Journal of Clinical Periodontology and the Journal of Periodontology.

2.2. Results

Electronic and manual search resulted in four hundred and eighty-three (483) publications. Five (5) duplicated publications were excluded. After reading titles and abstracts, four hundred forty-seven (447) were excluded for being unrelated to the PICOT question. After comprehensive reading, seven (7) papers were excluded (Appendix, Table IV) and twenty-four (24) publications were initially included for this review: 7 systematic reviews and 17 RCTs (figure 5). These 17 RCTs identified by our search were excluded *a posteriori* to avoid duplication of data, since all of them were already present in the 7 selected systematic reviews.

Figure 5. Flowchart for search strategy

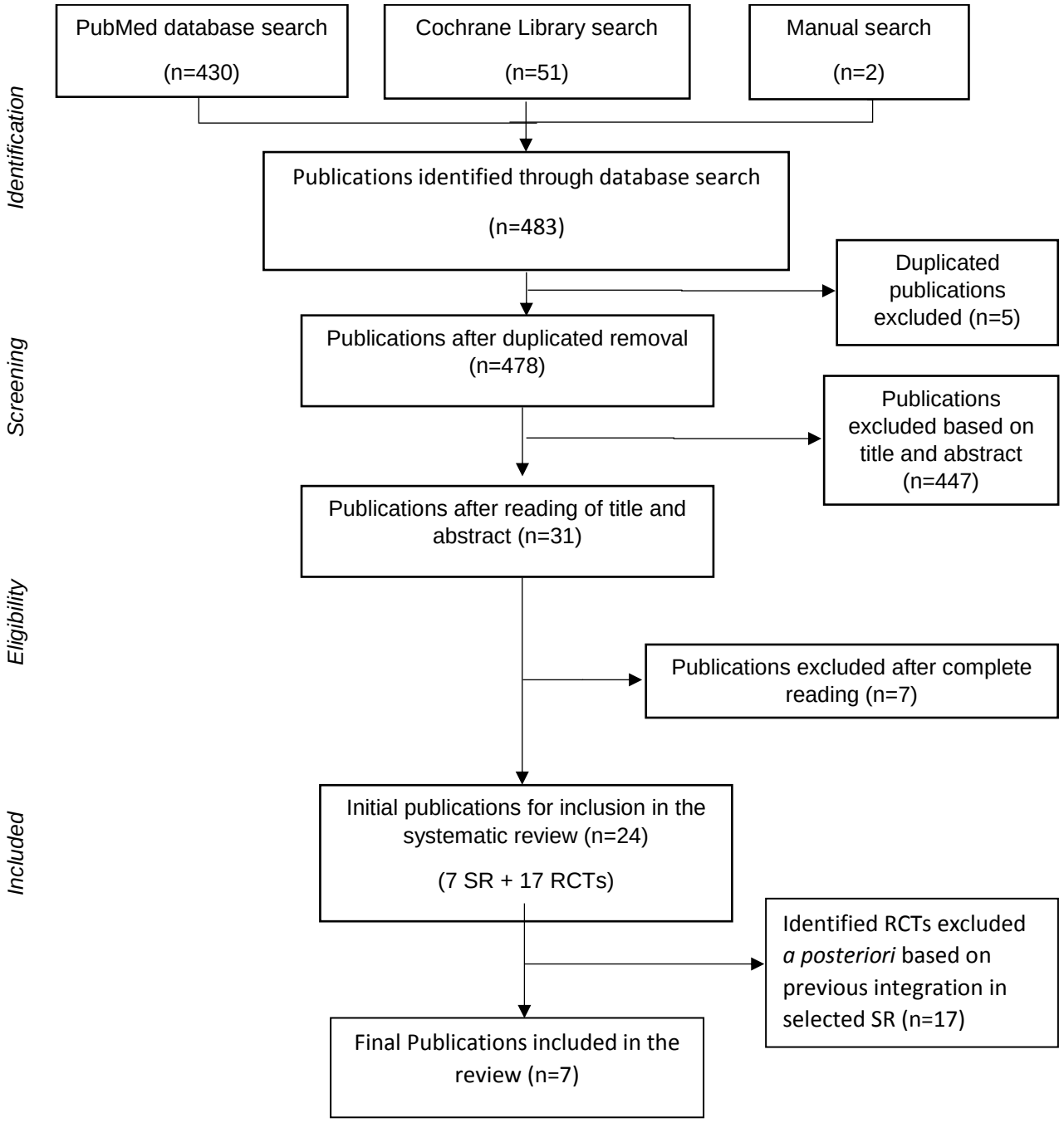


Table I. Systematic Reviews and Meta-analyses: general information and principal outcomes

Author, year	Meta-analysis	Number and type of studies included in the MA	Platelet concentrated	Treatment evaluated	Follow-up (months)	Outcomes (PPD reduction (mm), CAL gain (mm), Radiographic parameters – Bone fill (mm or %))	Statistical significance
Castro *et al.*, 2017	Yes	6 RCTs (in 13) Sharma & Pradeep 2011, Thorat *et a*l. 2011, Pradeep *m* 2012a*, 2015, Rosamma *et al*. 2012, Ajwani *et al.* 2015	L-PRF	L-PRF+OFD vs OFD PRF/PRP+OFD Vs OFD*	9-12 months	PPD red: SMD: 1.10mm; 95% CI: 0.6-1.6 in favor of L-PRF. Cal gain: SMD: 1.20mm; 95% CI: 0.5-1.9 in favor of L-PRF. Bone fill (mm): SMD: 1.70mm; 95% CI: 1.0-2.3 in favor of L-PRF. Bone fill (%): SMD: 46.0%, 95% CI: 33.2–58.7 in favor of L-PRF.	SS P<0.001
Del Fabbro *et al.*, 2011	Yes	10 RCTs (in 16) Hanna *et al*. 2004, Okuda *et al*. 2005, Ouyang & Qiao 2006 Christgau *et al.* 2006 Döri *et al.* 2007a Döri *et al.* 2007b Demir *et al.* 2007 Piermontese *et al.* 2008 Döri *et al.* 2008a Döri *et al*. 2008b	PRP	PRP + Bone graft Vs Bone graft PRP + Bone graft + GTR Vs Bone graft + GTR PRP + Bone graft + EMD Vs Bone graft + EMD	6-12 months	10 RCT: SMD: 0.50 mm (95% CI: 0.12 to 0.88 mm) in favor of PRP. 4 RCT with GTR: SMD: 0.04mm(95%CI: -0.33 to 0.41mm). 6 RCT without GTR: SMD: 0.84mm(95% CI: 0.27 to 1.42mm). A significant positive effect of the adjunct of PRP was found for intrabony defects. Such an effect was magnified in studies in which GTR was not used, whereas in studies using GTR, the use of PRP had no adjunctive effect.	SS for CAL gain NS for PRP+GTR
Hou *et al*., 2016	Yes	12 RCTs (in 15) Okuda K *et al*. 2005 Hanna R *et al*. 2004 Döri *et al*. 2009 Demir *et al.* 2007 Agarwal *et al*. 2014 Piemontese *et al.* 2008 Özdemir *et al.* 2012 Kaushick *et al.* 2007 Christgau *et al*. 2006 Döri, Huszar *et al.* 2007 Döri *et al.* 2007 Döri *et al*. 2008	PRP	PRP + Bone graft Vs Bone graft PRP + Bone graft + GTR Vs Bone graft + GTR	9-12 months	Clinically and significantly greater CAL gains and PPD reductions observed in subjects who received PRP as an adjunct to periodontal intrabony defect therapy: CAL: WMD 0.76 mm, 95 % CI = 0.34 to 1.18 mm, P = 0.0004; PPD: WMD 0.53 mm, 95 % CI = 0.21 to 0.85 mm, P = 0.001. Meta-analysis of patients who underwent GTR demonstrated that this approach did not significantly affect treatment outcomes (CAL: WMD 0.08 mm, 95 % CI = −0.30 to 0.46 mm, P = 0.67), as indicated by a comparison with patients who did not undergo GTR (CAL: WMD 1.22 mm, 95 % CI = 0.88 to 1.57 mm, P < 0.00001).	SS for CAL gain and PPD NS for PRP+GTR

Table I. Systematic Reviews and Meta-analyses: general information and principal outcomes (continuation)

Author, year	Meta-analysis	Number and type of studies included in the MA	Platelet concentrated	Evaluated treatment	Follow-up (months)	Outcomes (PPD reduction (mm), CAL gain (mm), Radiographic parameters – Bone fill (mm or %))	Statistical significance
Panda *et al.*, 2014	Yes	4 RCTs Thorat *et al*. 2011, Sharma & Pradeep 2011b***, Pradeep *et al*. 2012a*, Pradeep *et al*. 2012b**	PRF	PRF+OFD vs OFD PRF/PRP+OFD Vs OFD* PRF + HA Vs OFD**	9 months	PRF has a significant additive effect when used along with OFD. Statistically significant PPD reduction and CAL gain at the end of the follow-up in both test and control group in all four studies. Radiologically, significantly greater bone fill for PRF+OFD, as compared to OFD alone, in all four studies. Unreadable data from Forrest Plots.	Unreadable data
	Yes	7 RCTs Demir *et al.* 2007~ Döri *et al.* 2009 Hassan *et al*. 2012 Okuda *et al*. 2005 Parimala & Mehta 2010 Piemontese *et al.* 2008 Saini *et al.* 2011	PRP	PRP + Bone graft Vs Bone graft	9-12 months	Significant improvement in the CAL in the group using platelet concentrates in combination with graft materials over the group using graft materials alone. Consistent positive effect on radiological bone fill, when used along with bone substitutes. Unreadable data from Forrest Plots.	Unreadable data
	Yes	4 RCTs Döri *et al.* 2008 Christgau *et al.* 2006 Döri *et al.* 2007a Döri *et al.* 2007b	PRP	PRP + Bone graft + GTR Vs Bone graft + GTR	9-12 months	PRP showed no additive beneficial effect when combined with bone graft and GTR membrane for the treatment of intrabony defects. Meta-analysis of PPD reduction and clinical attachment gain in the experimental group over the control group was not significant. Unreadable data from Forrest Plots.	Unreadable data

Table I. Systematic Reviews and Meta-analyses: general information and principal outcomes (continuation)

Author, year	Meta-analysis	Number and type of studies included in the MA	Platelet concentrated	Evaluated treatment	Follow-up (months)	Outcomes (PPD reduction (mm), CAL gain (mm), Radiographic parameters – Bone fill (mm or %))	Statistical significance
Plachokova *et al.*, 2015	No	3 RCTs Hanna *et al*. 2004 Okuda *et al*. 2005 Sammartino *et al*. 2005	PRP	Graft+PRP vs. Graft	3-12 months	No meta-analysis due to heterogeneity. Differences in treatment effects for periodontal defects in terms of clinical attachment level (CAL) were significant, the mean differences ranging from 0.8 to 3.2mm.	-
Roselló-Camps *et al.*, 2015	Yes	14 RCTS (in 21) Lekovic *et al*. 2002 Camargo *et al.* 2002 Okuda K *et al*. 2005 Ouyang & Qiao 2006 Demir *et al.* 2007 Döri *et al.* 2007b Döri *et al.* 2007a Döri *et al.* 2008 Piemontese *et al.* 2008 Camargo *et al*. 2009 Özdemir *et al.* 2012 Pradeep *et al.* 2012 Baja *et al*. 2013	PRP	PRP + Bone graft Vs Bone graft PRP + Bone graft + GTR Vs Bone graft + GTR	9-12 months	14 RCTs for PPD reduction: WMD 0.55 mm, with a 95% CI= -0.09 mm to 1.20 mm (p= 0.09) in favor of PRP. 2 RCTs for bone level (BL) in mm: WMD was 0.76 mm (95% CI= 0.21 mm to 1.31 mm, p=0.007) in favor of PRP. 2 RCTs for bone level (BL) in %: WMD 47.41% (95% CI= 32.48% to 62.33%, p< 0.0001) in favor of PRP. 12 RCTs for CAL Gain: WMD 0.58 mm, with a 95% CI= 0.24 mm to 0.91 mm (p= 0.0008) in favor of PRP. High heterogeneity among studies. PRP might offer some beneficial effects on clinical and radiographic outcomes for regeneration of periodontal intrabony defects.	NS for PDD reduction SS for bone level (in mm and %) and CAL
Shah *et al.*, 2014	Yes	5 RCTs Thorat *et al*. 2011, Sharma & Pradeep 2011a, Pradeep *et al.* 2012a, Pradeep *et al.* 2012b**, Rosamma et al. 2012,	PRF	PRF+OFD vs OFD PRF/PRP+OFD Vs OFD* PRF + HA Vs OFD**	9-12 months	PPD reduction: SMD: 1.10mm; 95% CI: 0.56-1.64 in favor of PRF (no significant heterogeneity). CAL gain: SMD: 0.95mm; 95% CI: 0.20-1.71 in favor of PRF (no significant heterogeneity). Bony defect reduction (mm): SMD: 2.33mm; 95% CI: 1.43-3.23 (no significant heterogeneity).	SS for PDD reduction and IBD P<0.001 NS for CAL gain P=0.006

3. Case series

3.1. Materials and Methods

In order to demonstrate the L-PRF protocol for the treatment of periodontal infrabony defects, a case series is presented.

The clinical records of periodontal patients followed in a university clinical centre (Dentistry Department of the Faculty of Medicine – University of Coimbra) were analyzed and the patient selection occurred according to the following inclusion criteria:

- Systemically healthy humans (ASA I) or patients with mild systemic disease (ASA II);
- Infrabony defects with PPD ≥ 6 mm, confirmed on a standard periapical radiograph, and with a two or three walls morphology;
- Bleeding on probe (BOP) and a full-mouth plaque score ≤ 20%;
- Non-smokers.

As exclusion criteria:

- Patients with uncontrolled systemic diseases or adverse conditions for periodontal surgery;
- Infrabony defects with PPD ≤ 5 mm and/or with a one wall bony defect morphology;
- BOP and plaque score ≥ 20%;
- Smokers.

After the screening and eligibility process, four patients fulfilled the inclusion criteria, but one patient failed the pre-operatory appointments and was excluded. An informed consent was given to the remaining three patients.

During pre-surgical therapy, each patient was given careful instructions regarding proper oral hygiene measures. A full mouth supragingival and subgingival SRP procedure was performed (when indicated), under local anesthesia, using ultrasonic and hand instrumentation. Six to eight weeks after this preliminary treatment, a periodontal re-evaluation was performed to confirm the suitability of the sites for periodontal surgery.

The L-PRF clinical protocol used is in accordance with the guidelines of the 1st European Meeting on Enhanced Natural Healing in Dentistry (Leuven, Belgium; 2016)[39], using the IntraSpin™ centrifuge (Intra-Lock, Boca Raton, FL, USA), and includes the following steps:

1. Blood collection – Obtainment of 4 to 8 tubes of 9 mL of blood, according to the number of membranes needed (photography 4.2.i.6);

2. Centrifugation - This process should start within 60 seconds after blood collection and with the centrifuge always loaded with an even number of tubes. In some cases, such as to collect fibrinogen or when it is needed more than 60 seconds to collect all the blood tubes, two cycles of centrifugation may be required (photographies 4.2.i.7 and 4.2.i.9). The centrifugation to produce the L-PRF clots should be made at 2700 rpm (revolutions per minute) or 400g RCF (Relative Centrifugal Force), for at least 12 minutes (photography 4.2.i.9). If the patient is under anti-coagulant medication, the centrifugation time should be around 15 to 18 minutes (photography 4.2.ii.7)

3. L-PRF membranes preparation – After centrifugation, red blood cells are gently removed from the clots (photography 4.2.i.10) and these last are placed in a compression box (Xpression™ kit) for 5 minutes, to obtain the L-PRF membranes (photography 4.2.i.12);

4. L-PRF membranes lifetime - These membranes can be used within the next 2 hours, if drying out is prevented.

In our clinical cases, the surgical approaches performed were the minimally invasive surgical technique (MIST) or the modified minimally invasive surgical technique (M-MIST), according to their specific indications (Table II).[16] In both techniques, the initial access to the defect requires a modified papilla preservation technique (MPPT) or simplified papilla preservation flap (SPPF) approach.[1] Vertical incisions are avoided whenever possible.[1] MPPT is indicated in sites where the interdental space width is at least 2 mm at the most coronal portion of the papilla and SPPF can be applied in narrower interdental sites.[1] Both procedures aim to increase the space for regeneration and also to achieve and maintain primary closure of the interdental space.[1]

As postoperative immediate care, a systemic regimen was prescribed (doxycycline 100mg, two times per day, for 7 days; and 600 mg ibuprofen, two times per day, for 5 days) and a soft diet was recommended. Patients were requested to avoid normal brushing, flossing and chewing for two weeks at the intervention site. After that period, sutures were removed and a post-surgical soft toothbrush was recommended for the next two weeks. Patients were instructed to not use any kind of mouthwash during the initial healing period.

Table II. Case series methodology

Patient (female/male)	Age (years)	Defect morphology (teeth: number of walls)	Intervention	L-PRF preparation			Surgical access technique	Suture technique and thread
				Hardware	Blood collected (number of tubes, mL)	Centrifugation process (number of cycles, force / time)		
B.C. (F)	27	16: 2w	OFD + Xenograft + Fibrinogen + L-PRF fragments + L-PRF membrane	IntraSpin™ centrifuge (Intra-Lock, Boca Raton, FL, USA)	1 tube 8mL 4 tubes 9 mL	2 cycles 400 g / 3 min 400 g / 12 min	M-MIST	Modified internal mattress suture with a 5-0 monofilament thread
G.I. (F)	66	36: 2w	OFD + L-PRF fragments + L-PRF membrane		4 tubes 9 mL	1 cycle 400 g / 15 min	MIST	Modified internal mattress suture with a 6-0 monofilament thread
R.F. (F)	66	1) 44: 2w 2) 42: 2w 3) 32: 3w (apically) + 2p (coronally)	OFD + L-PRF fragments + L-PRF membrane		6 tubes 9 mL	1 cycle 400 g / 12 min	1) M-MIST 2) M-MIST 3) MIST	Modified internal mattress sutures with a 6-0 monofilament threads

Pre-operative data and follow-up of the case series are presented in Table III.

Table III. Pre-operative data

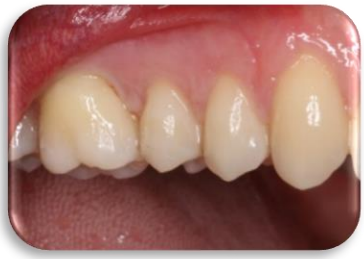
Patient (sex)	Age (years)	Defect(s) morphology (teeth: number of walls)	Intervention	Initial pocket depth (mm)	Initial PPD (mm)	Adverse effects reported	Follow-up (months)
B.C. (F)	27	16: 2w	OFD + Xenograft + L-PRF fragments + L-PRF membrane	7	8	No	6
G.I. (F)	66	36: 2w	OFD + L-PRF fragments + L-PRF membrane	5	6	No	3
R.F. (F)	66	1) 44: 2w 2) 42: 2w 3) 32: 3w (apically) + 2p (coronally)	OFD + L-PRF fragments + L-PRF membrane	1) 4 2) 4 3) 6	1) 5 2) 5 3) 7	No	1
F = Female; M = Male; W = Walls							

At follow-up appointments, the soft tissues exhibited an apparently healthy healing and no significant morbidity was mentioned for the patients.

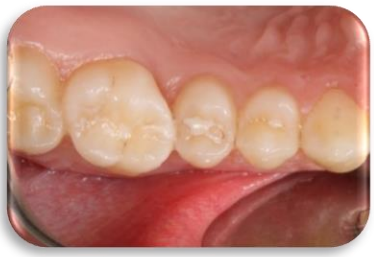
3.2. Results

3.2.1. Clinical case 1

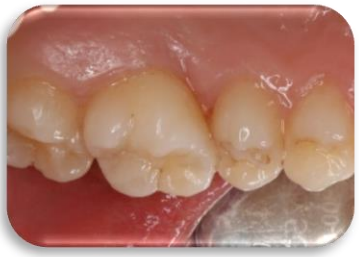
- B.C., female, 27 years



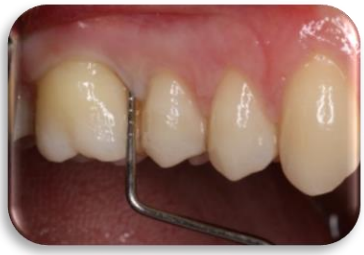
1. Pre-operative buccal view of 16



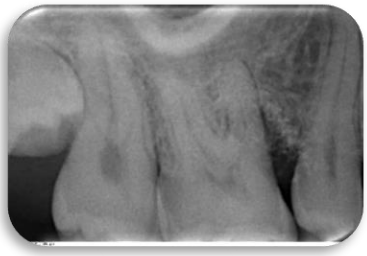
2. Pre-operative occlusal view of 16



3. Pre-operative palatal view of 16



4. Pre-operative probing depth (PD=7 mm)



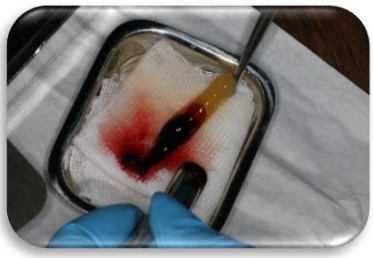
5. Pre-operative periapical radiography

6. Collection of 6 tubes of 9 mL of patient blood

7. Centrifugation at 2700 rpm for 3 min for fibrinogen separation

8. Fibrinogen collection

9. L-PRF clots collection, after new centrifugation for 12 min

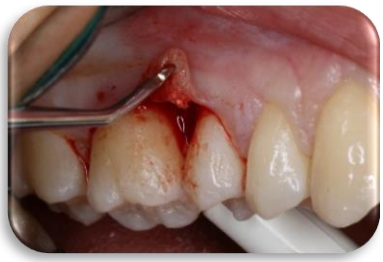


10. Careful separation of red blood cells

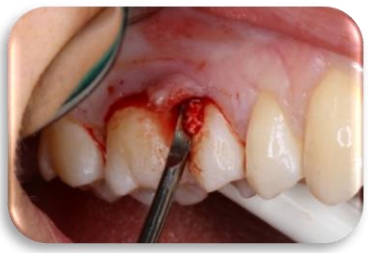
11. L-PRF clots without red blood cells

12. Clots compression for, at least, 5 min

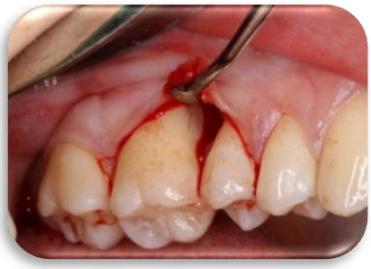
13. Obtained L-PRF membranes after clots compression



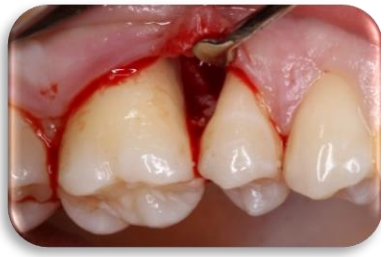
14. Surgical access (M-MIST)



15. Removal of capsulated regenerative material

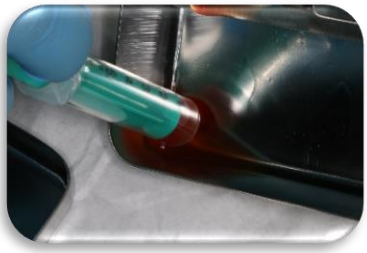


16. Presence of residual calculus on a radicular groove

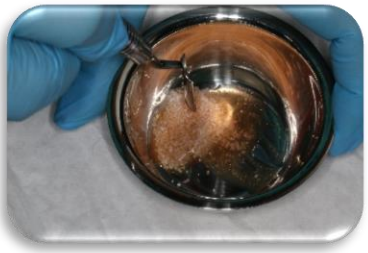


17. Cleaned infrabony defect (8mm deep)

18. Xenograft (Bio-Oss® - Geistlich Pharma AG, Switzerland)

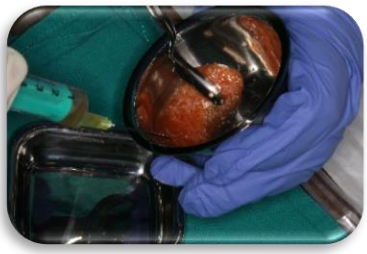


19. Collection of the membrane compression surplus fluid

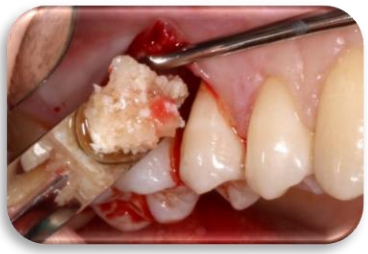


20. Hydration of the xenograft with the collected fluid

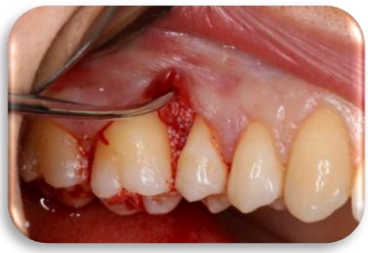
21. Mixture of hydrated xenograft with L-PRF membrane fragments



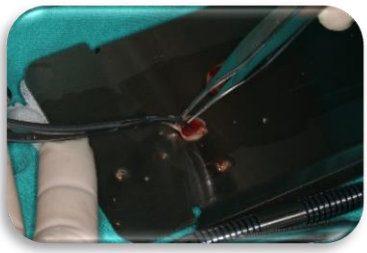
22. Addition of fibrinogen to obtain the bone block



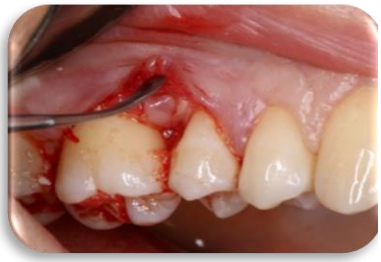
23. Insertion of the bone block into the defect



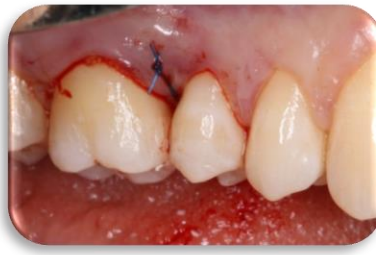
24. Bone block compaction



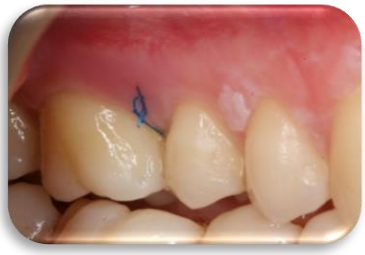
25. Conformation of an L-PRF membrane to cover the bone block



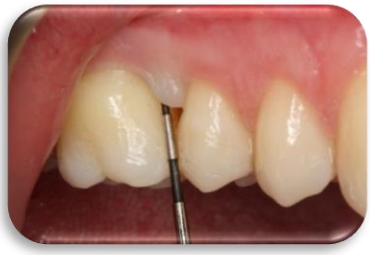
26. Placement of L-PRF membrane over the bone block



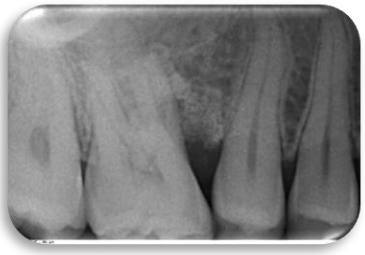
27. Modified internal mattress suture with a 5-0 monofilament thread



28. Post-operative view at 5 days



29. PD at 6 months (5mm)

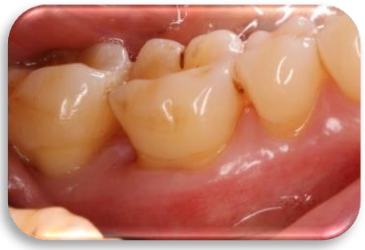


30. Post-operative periapical radiography at 6 months

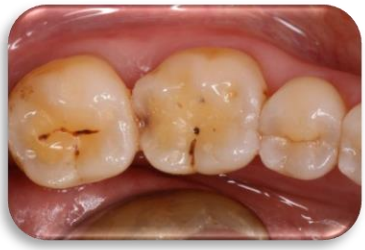
Main conclusions: The combined approach of xenograft + L-PRF + fibrinogen induced a favorable healing of soft tissues. No post-operative complication was reported. At 6 months, 2mm of CAL gain was obtained, along with a good radiographic filling of the defect.

3.2.2. Clinical case 2

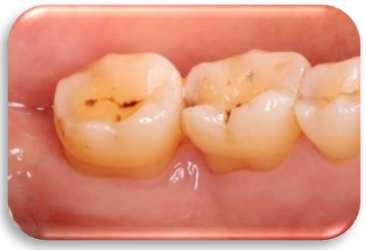
G.I., female, 66 years



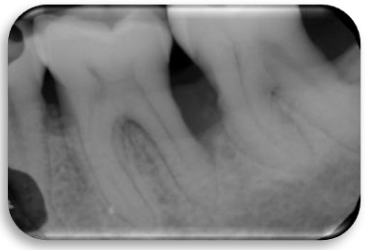
1. Pre-operative buccal view of 36



2. Pre-operative occlusal view of 36

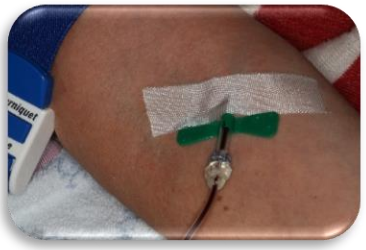


3. Pre-operative palatal view of 36



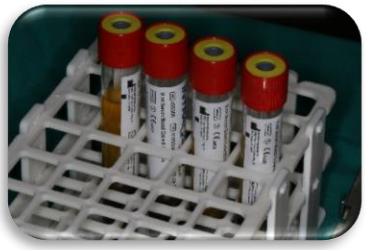
4.Pre-operative periapical radiography

5. Tissue Regeneration Kit (*Intra-lock®*, *Boca Raton*, FL, USA)

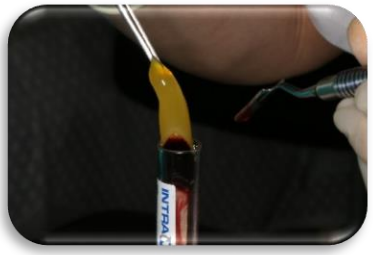


6. Collection of patient blood

7. Centrifugation at 2700 rpm for 15 min (according to anticoagulated patients' guidelines)



8. Collection of 4 tubes of 9 mL of patient blood



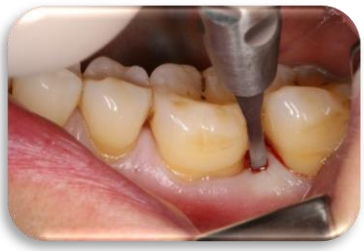
9. Collection of L-PRF clots

10. Careful separation of red blood cells

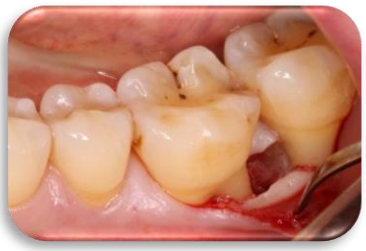
11. L-PRF clots without red blood cells

12. Clots compression

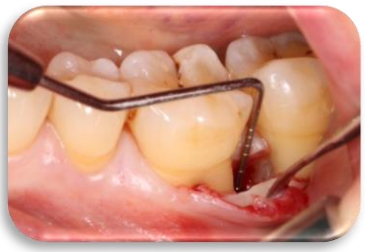
13. Microsurgical instruments (Hu-Friedy® Mfg. Co., LLC)



14. MIST approach of the infrabony defect



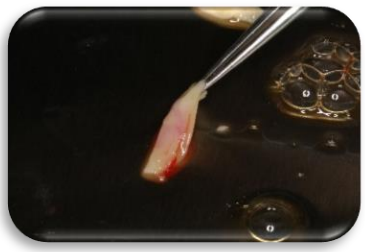
15. Cleaned infrabony defect



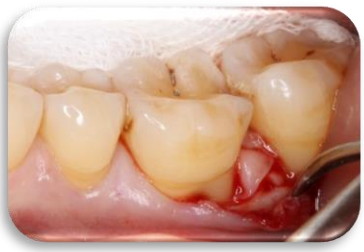
16. Probing depth of 6 mm

17. Obtained L-PRF membranes after clots compression

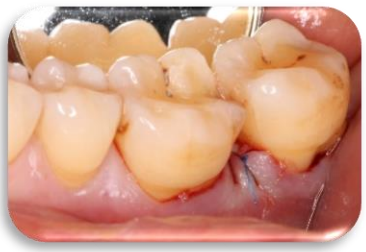
18. Resizing and shaping into smaller L-PRF membranes



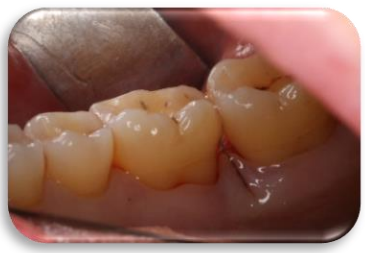
19. Selection of L-PRF fragment



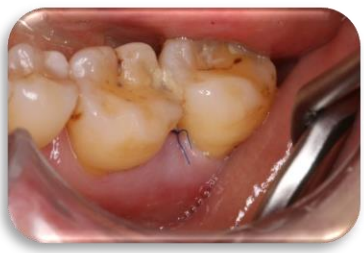
20. Placement of L-PRF membrane in and over the bone defect



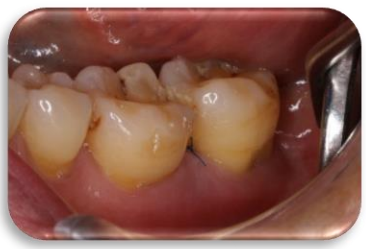
21. Modified internal mattress suture with a 6-0 monofilament thread



22. Immediate post-operative lingual l view of 36



23. Post-operative view at 7 days

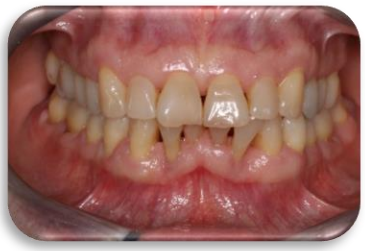


24. Post-operative view at 15 days

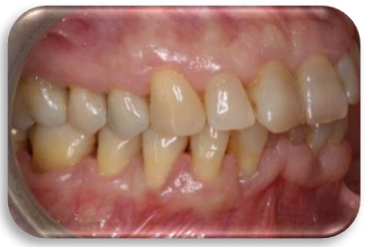
Main conclusions: Soft tissues exhibited an initial good healing, without inflammatory signs. No pain, discomfort or other complications was observed.

3.2.3. Clinical case 3

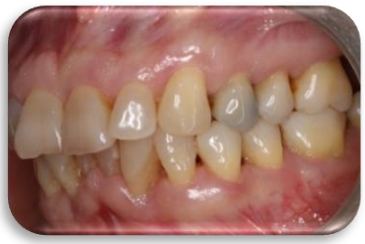
R.F., female, 66 years



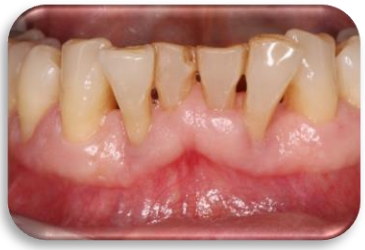
1. Pre-operative frontal view



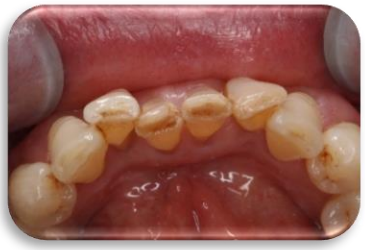
2. Pre-operative right side view



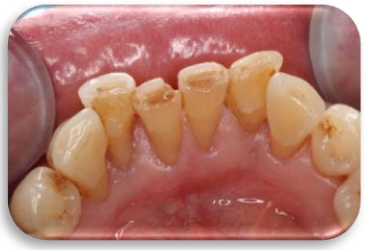
3. Pre-operative left side view



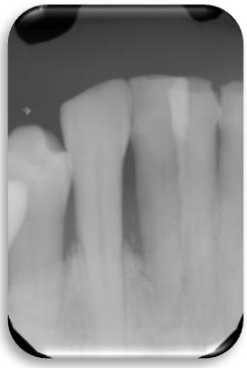
4. Pre-operative vestibular view of 44, 42 and 32



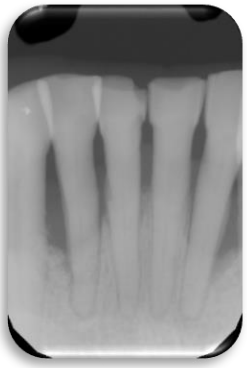
5. Pre-operative incisal/occlusal view of 44, 42 and 32



6. Pre-operative lingual view of 44, 42 and 32

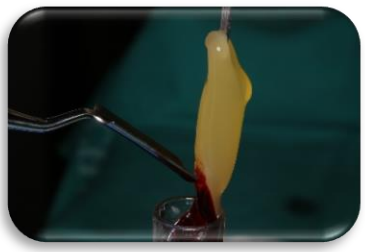


7. Pre-operative periapical radiography of 44 and 42

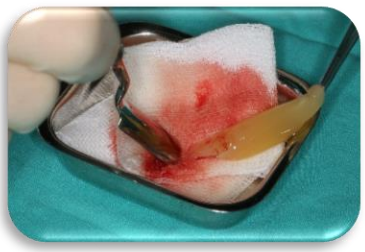


8. Pre-operative periapical radiography

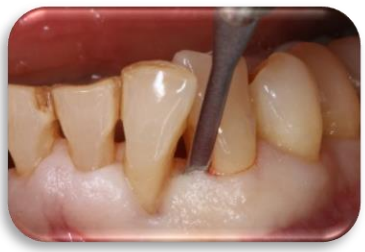
9. Collection of 6 tubes of 9 mL of patient blood and centrifugation at 2700 rpm for 12 min



10. Collection of L-PRF clot

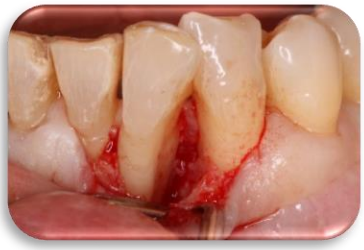


11. Careful separation of red blood cells



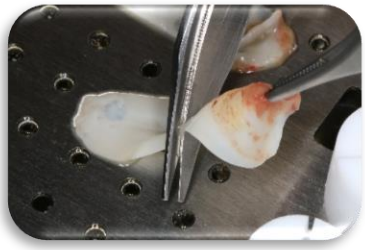
12. MIST approach of 32

13. Probing depth (32) of 7 mm

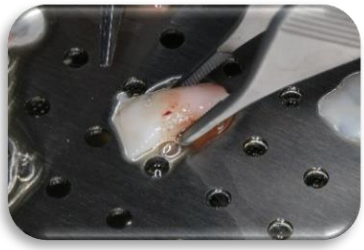


14. Cleaned infrabony defect

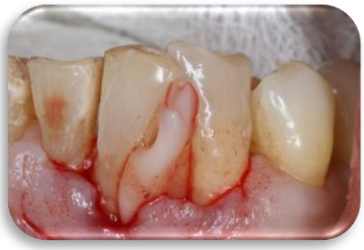
15. Obtained L-PRF membranes after clots compression



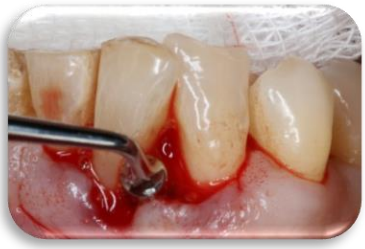
16. Conformation of L-PRF membrane



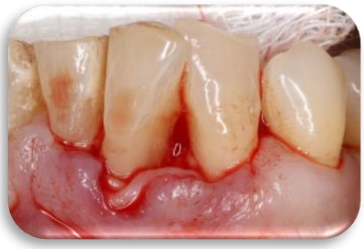
17. Selection of L-PRF membrane fragment



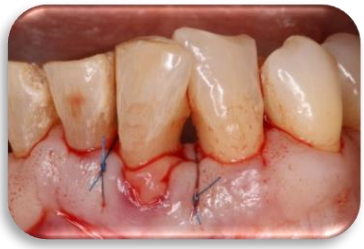
18. Initial placement of L-PRF membrane in the bone defect



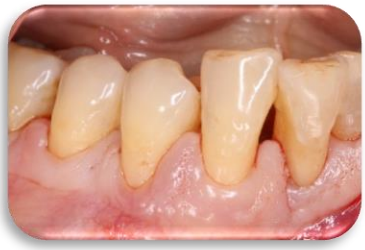
19. Conformation of L-PRF membrane in the defect



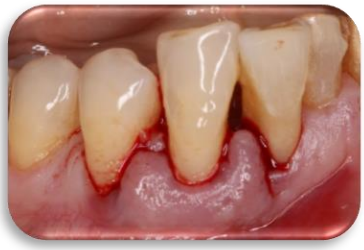
20. Portion of the membrane purposely left exposed for root coverage



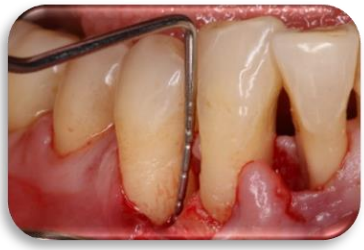
21. Modified internal mattress suture with a 6-0 monofilament thread



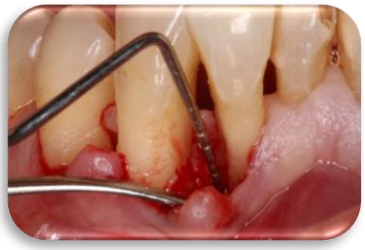
22. Close-up of pre-operative vestibular view of 42 and 44



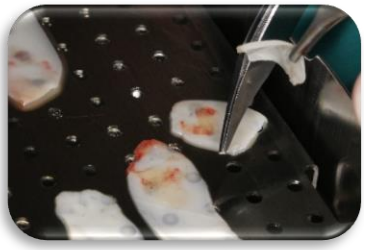
23. M-MIST approach of 42 and 44 with protection of the interproximal papillae



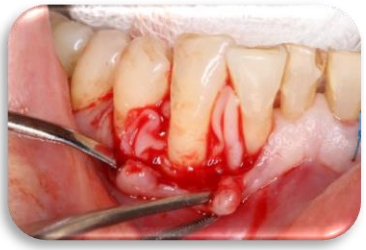
24. Pocket depth (44) of 5 mm



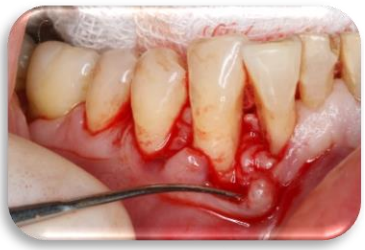
25. Pocket depth (tooth 42) of 5 mm



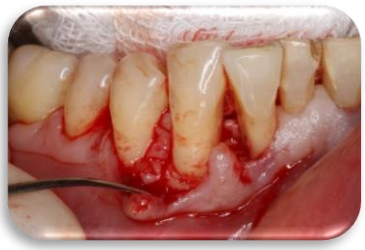
26.Transforming L-PRF membranes into smaller membranes



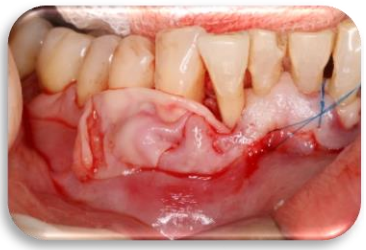
27. Insertion of L-PRF membrane fragments into the defect



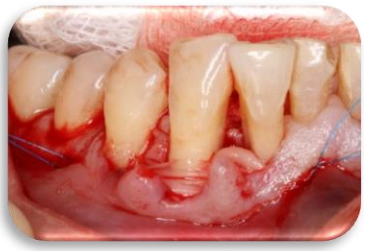
28. Conformation of L-PRF membrane in the infrabony defect of tooth 42



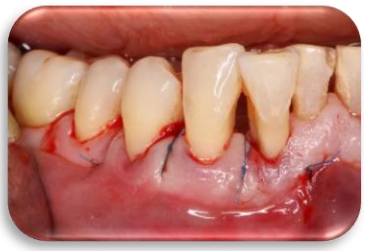
29. Conformation of L-PRF membrane in the infrabony defect of tooth 44



30. Initial stabilization of a L-PRF membrane over the infrabony defects



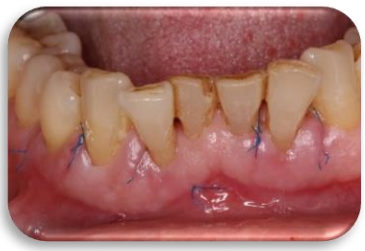
31. Conformation of the L-PRF membrane over the defects



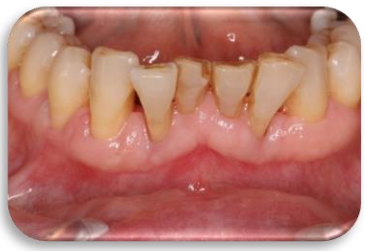
32. Modified internal mattress suture with a 6-0 monofilament thread

33. Collection of the remaining exudate from L-PRF membrane compression

34. Injection of exudate in the infrabony defects area



35. Post-operative view at 7 days



36. Post-operative view at 15 days

Main conclusions: Even with a more extensive intervention (three defects treated simultaneously), no post-operative discomfort was reported and soft tissues presented an initial good healing without signs of inflammation.

4. Discussion

Quality assessment of the systematic reviews

Most of the selected systematic reviews used the Cochrane Collaboration's tool for assessing risk of bias of the RCTs included. The vast majority of the evaluated studies have a moderate risk of bias, with a split-mouth design, which has to be taken in consideration to adjust the reported conclusions. Additionally, the systematic review and meta-analysis by Panda *et al.* (2014) revealed itself as a publication with several flaws, namely: absence of the values of the weighted mean differences for the three meta-analyses; poor graphical quality of the forest plots which hampered data collection from it and possible error in the bibliography, where the reference of the study by Sharma & Pradeep (2011) included in the meta-analysis is of a study in furcation defects and not in infrabony defects. Additionally, Castro *et al.* (2017) and Shah *et al.* (2014), considered the study by Rosamma *et al*. (2012) a RCT. In fact, this study is a controlled clinical trial with a split model design and with unclear information about the allocation process of the treatment delivered to each site, and according to our understanding, it shouldn´t be classified as a RCT. This fact may limit the conclusions reported by Castro *et al.* (2017) and Shah *et al.* (2014).

Probing pocket depth (PPD) reduction

Besides studies heterogeneity, four meta-analyses reported data about probing depth reduction. For PRF, Castro *et al.* (2017) and Shah *et al*. *(*2014) reported a statistically significant mean difference of 1,10mm (95% CI: 0.6-1.6mm) and 1.10mm (95% CI: 0.56-1.64) in favor of PRF, comparatively to OFD, respectively. For PRP, Hou *et al*. (2016) presented a statistically significant mean difference of 0,53mm (95 % CI = 0.21 - 0.85 mm), but Roselló-Camps *et al.* (2015) a non-significant mean difference of 0,55mm (95% CI= -0.09 -1.20 mm) in favor to PRP+bone grafts vs bone grafts in monotherapy. Regarding this clinical outcome for intrabony defects regenerative therapy, it can be seen that PRF almost duplicated the results obtained with PRP; but these results must be interpreted carefully due to the high heterogeneity among the studies and it is important to highlight that none of the meta-analyses identified any study comparing PRP with OFD.

Clinical attachment level (CAL) gain

CAL gain was one of the main outcome evaluated by all the selected systematic reviews. For PRF, Castro *et al.* (2017) obtained a statistically significant mean difference in CAL gain of 1.20mm (95% CI: 0.5-1.9mm) in favor of L-PRF. Panda *et al. (*2014) also refers a statistically significant CAL gain in favor of PRF, but without indicating its quantitative value and presenting unreadable Forrest Plots in their paper, thus preventing any data extraction. On the other hand,

Shah *et al*. *(*2014) reported a non-statistically significant mean difference of 0.95mm (95% CI: 0.20-1.71mm) in favor of PRF.

For PRP combined with bone grafts, there is a consensual statistically positive effect in favor of this combination among all the meta-analyses, with mean differences of 0.50 mm (95% CI: 0.12 to 0.88 mm) in Del Fabbro *et al.* (2001); 0.58 mm (95% CI= 0.24 to 0.91 mm) in Roselló-Camps *et al.* (2015); 0.76 mm (95 % CI = 0.34 to 1.18 mm) in Hou *et al*. (2016).

When combined with bone grafts and GTR membrane, PRP showed no additive beneficial effect for the treatment of intrabony defects, with several meta-analyses presenting non statistically significant mean differences in CAL gain: 0.04mm (95%CI: -0.33 to 0.41mm) in Del Fabbro *et al*. (2011); 0.08 mm (95 % CI = −0.30 to 0.46 mm) in Hou *et al*. (2016). Again, Panda *et al. (*2014) refers a similar conclusion, but without indicating its quantitative value and presenting unreadable Forrest Plots in their paper, thus preventing any data extraction.

Taking into account the limits of these conclusions due to the great heterogeneity in the RCTs evaluated, PRF showed better clinical results than PRP. But, as refered earlier there are no studies comparing PRP vs OFD, which difficults a direct comparison between PRF and PRP, because PPR was always used in association with bone grafts or membranes and never alone.

Bone fill and pocket depth

Limited information has been published regarding bone fill and pocket depth (or reduction of the infrabony component of the osseous defect), normally evaluated on calibrated periapical radiographs. For PRF, Castro *et al. (*2017) presented a statistically significant mean difference in bone fill of 1.70mm (95% CI: 1.0-2.3mm) or 46.0% (CI: 33.2–58.7 %) in favor of L-PRF (based on 6 RCTs). Shah *et al*. *(*2014) reported a statistically significant mean difference in bony defect reduction of 2.33mm (95% CI: 1.43-3.23mm), but with significant heterogeneity. Panda *et al.* (2014) reported a significantly greater bone fill for PRF+OFD, as compared to OFD alone, in all four studies submitted to meta-analysis, but again with unreadable data on the corresponding forrest plot.

Regarding PRP + bone grafts, Panda *et al.* (2014) refers, without any quantitative data, a consistent positive effect on radiological bone fill based on its meta-analysis of 7 RCTs. 2 RCTs for bone level (BL) in mm: WMD was 0.76 mm (95% CI= 0.21 mm to 1.31 mm, p=0.007) in favor of PRP.

Roselló-Camps *et al.* (2015), based on 2 RCTs (Pradeep *et al.* (2012) and Bajaj *et al.* (2013)), presented a significant mean difference for bone level (BL) in % of 47.41% (95% CI= 32.48% to 62.33%) in favor of PRP; and based in other 2 RCTs (Okuda K *et al*. (2005) and Piemontese *et al.* (2008)) a significant mean difference for bone level (BL) in mm of 0.76 mm (95% CI= 0.21 mm to 1.31 mm) also in favor of PRP.

Regarding radiographic bone fill, PRF seems to give better results than PRP.

Biological rational for clinical outcomes

L-PRF and PRP contain different cell concentrations, release different amount of growth factors, and have different mechanical properties although both come from a blood sample.[44]

Platelet rich fibrin has shown better outcomes when compared to different types of platelet concentrates, namely with PRP.[7] Part of this results may be due to Its strong fibrin architecture and its superior mechanical properties distinguish it from other kinds of APCs[45], along with its capability of a slow continuous release of growth factors over a period of 7–14 days (which contributes to stimulates the local environment for differentiation and proliferation of stem and progenitor cells).[46] PRP, for example, has a thin and non-condensed fibrin network with a low tensile strength so that it is less useful as a space maintainer.[47] The strong fibrin network in L-PRF is explained by the physiological concentrations of thrombin during its preparation. Rowe *et al.* (2007) concluded that a high thrombin concentration resulted in a high-interconnected fibre mesh with a fine fibre structure.[48] However, as thrombin concentration decreased, fibre size increased as well as the mechanical properties.[30]

One of the drawbacks reported by literature is the L-PRF short shelf-life.[19] Plus, although PRF is a denser and firmer agent than other biological preparations, it still has not the ideal ability to space-maintaining.[23] This leads to the importance of the configuration of the selected infrabony defects, which may have influenced the outcomes presented, because 3 and 2 wall defects have better regenerative potential. Another factor to take into consideration is the surgical access technique. Apparently, none of the RCTs evaluated used a minimally invasive surgical technique. Nowadays it is known that microsurgical access surgery, like MIST or M-MIST, can potentiate the results of the principal periodontal clinical outcomes[16], because these techniques have the capacity to maintain space for regeneration and may overcome the limitation of a less firmer regenerative material like PRF or other APCs.

Patient center outcomes

Patient-based variables such as esthetics and postoperative discomfort (i.e., pain, swelling, infection, and abscess) are not properly assessed across the studies included in the present review. According to Shah *et al* (2014), Rosamma *et al.* (2014) is the only study that used visual analog score to compare the patient's response to PRF and OFD treatment and the results showed that PRF resulted in slightly better results in experimental group for pain and healing. Roselló-Camps *et al.* (2015) suggest a more rapid healing and less post-operative pain in PRP-treated sites compared to controls and an uneventful post-operative healing when PRP was used in conjunction with grafting materials.

Case series considerations

The main goal of our case series was to demonstrate the L-PRF preparation protocol, but also the healing potential of L-PRF membranes. It is worth noting that the inherent specificity of the defined inclusion/exclusion criteria narrowed the number of suitable patients, plus the patient's database was restricted to a university clinical centre (Dentistry Department of the Faculty of Medicine – University of Coimbra).Nevertheless and contrary to the published evidence, we also selected ASA II patients because there is a need of information for patients similar to those treated in a everyday clinical practice; and all RCTs used only ASA I patients.

The immediate outcomes of our clinical cases suggest an improvement in a patient's quality of life, since no post-operative complications (such as pain and swelling) were reported. In one case, a 2mm CAL gain was obtained with a good radiographic bone of the treated defect.

Implications for future research

Although the existing scientific evidence regarding the applicability of L-PRF membranes in the regeneration of periodontal infrabony defects has a certain degree of reliability, a few aspects should be improved in the future.

Firstly, it is important to diminish the heterogeneity in L-PRF preparation protocols, for example, concerning the centrifuge used, the centrifugation time, the number of clots used and the amount of blood drawn.

It would be very interestingly to analyze each type of infrabony defect, two and three-wall, separately to understand the effect of L-PRF according to the defect morphology.

Another aspect to consider, it is the follow-up time. A longer follow-up time would allow to conclude about the stability of L-PRF effects on periodontium regeneration.

Further studies should consider histologic analysis, since it is the only way to clarify if the clinical attachment gain is a true histologic gain, and also incorporate more patient centered outcomes.

5. Conclusions

The present systematic review and the case series report allowed for the following conclusions:

- Platelet rich fibrin has shown better outcomes when compared with other kinds of APCs. Part of this may be due to its strong fibrin architecture, superior mechanical properties and a slow continuous release of growth factors. Further pre-clinical histological analysis should complement this data.

- Platelet rich fibrin improved significantly clinical periodontal parameters, such as probing depth reduction, clinical attachment level and radiographic parameters (bone fill) compared to OFD.

- Patient-centered outcomes such as esthetics and post-operative complications were not properly assessed across the studies included in the present review. The scarce information available suggests that PRF resulted in slightly better results for pain and healing compared to other regenerative treatments.

- The literature available had a moderate risk of bias, with detectable flaws, such as absence of weighted mean differences, heterogeneity between studies and insufficient data information.

- The immediate outcomes of the case series are according with the available literature regarding excellent immediate post-operative healing and potential application in periodontal regeneration.

- Despite the moderate level of evidence regarding the applicability of L-PRF in periodontal regeneration, further studies should improve methodological issues and consider specific infrabony defects morphological analysis. Longer follow-up studies and pragmatic clinical trials are needed to improve future conclusions regarding L-PRF and other autologous platelet concentrates.

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Appendix

Table IV. Publications excluded

Author, year	Type of study	Reason of exclusion
Aspriello *et al.,* 2010	RCT	Unrelated to PICOT question
Gamal *et al.,* 2016	RCT	Unrelated to PICOT question
Moder *et al.*, 2012	RCT	Undefined APC
Nevins *et al.*, 2013	RCT	Unrelated to PICOT question
Pradeep *et al.,* 2009	RCT	PRP used in all experimental groups
Rodrigues *et al.*, 2011	RCT	PRP used in all experimental groups
Trombelli *et al.*, 2008	Review	Not systematic review

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