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The Use of L-PRF in Endodontic Microsurgery

Luciana Maria dos Santos Ribeiro

Dissertação conducente ao Grau de Mestre em Medicina Dentária
(Ciclo Integrado)

Gandra, 24 de setembro de 2020

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Trabalho realizado sob a Orientação do Prof. Doutor Paulo Miller e Coorientação do Prof. Doutor António Ferraz

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RESUMO

Introdução: Lesões periapicais maiores constituem um procedimento cirúrgico desafiador devido à maior quantidade de osso reabsorvido. L-PRF, um novo conceito de regeneração de tecidos guiada naturalmente, contém uma densa rede de fibrina que libera uma quantidade significativa de fatores de crescimento autólogos, citoquinas e proteínas. O L-PRF melhora as fases iniciais da cicatrização, reduzindo o processo inflamatório e o risco de infeção. Este material é particularmente útil em situações complexas, quando algumas paredes ósseas são destruídas e a regeneração é difícil.

Objetivo: o objetivo desta revisão sistemática foi interpretar a literatura disponível atualmente e analisar os efeitos do uso de L-PRF na microcirurgia endodôntica.

Método: A pesquisa foi realizada nos bancos de dados PUBMED e EBSCO e combinações de vários termos de pesquisa foram utilizados para encontrar estudos apropriados.

Resultados: L-PRF demonstrou induzir a proliferação de fibroblastos e células estaminais, proporcionar hemostasia acelerada e ter liberação enriquecida e lenta de vários fatores de crescimento, citoquinas e proteínas que, juntos, intervêm no processo de regeneração, potencializando e acelerando a resposta biológica natural.

As características, protocolo e materiais de centrifugação influenciam a viabilidade do L-PRF produzido, o que requer a implementação de um protocolo L-PRF padronizado.

Conclusões: o uso de L-PRF como auxiliar na microcirurgia endodôntica provou ser bem-sucedido, alcançando a cicatrização completa dos tecidos moles e duros na área periapical em todos os estudos. Além disso, mostrou potencial na redução da dor pós-cirúrgica, inflamação e administração de medicamentos.

Palavras-chave: "*L-PRF*"; "*periapical lesions*"; "*healing process*"; "*bone recovery*"; "*tissue regeneration*".

ABSTRACT

Introduction: Larger periapical lesions constitute a challenging surgical procedure due to the greater amount of resorbed bone. L-PRF, a new concept of naturally guided tissue regeneration, contains a dense fibrin network that releases a significant amount of autologous growth factors, cytokines and proteins. L-PRF improves the early stages of healing, reducing the inflammatory process and the infection risk. This material is particularly useful in complex situations when some bone walls are destroyed, and regeneration is difficult.

Aim: The aim of this systematic review was to interpret the current available literature and analyze the effects of the use of L-PRF in Endodontic Microsurgery.

Method: Search was carried out on PUBMED's and EBSCO's databases and combinations of several search terms were applied to find appropriate studies.

Results: L-PRF demonstrated to induce fibroblast and staminal cells proliferation, provide faster hemostasis and has enriched and slow release of various growth factors, cytokines and proteins which altogether are featured in the regeneration process, enhancing and expediting the biological natural response.

The centrifuge's characteristics, protocol and materials deeply influence the viability of the L-PRF produced, which calls for the implementation of a standardized L-PRF protocol.

Conclusions: L-PRF's use as an aid in Endodontic Microsurgery has proven to be successful, achieving complete soft and hard tissue healing in the periapical and surrounding areas in all studies assessing the matter. Additionally, it has shown potential in decreasing post-surgical pain, inflammation and drug administration.

Keywords: "*L-PRF*"; "*periapical lesions*"; "*healing process*"; "*bone recovery*"; "*tissue regeneration*".

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LIST OF ABBREVIATIONS:

L-PRF: Leukocyte and Platelet-Rich Fibrin

PRF: Platelet-Rich Fibrin

L-PRP: Leukocyte Platelet-Rich Plasma

TGF-β1: Transforming Growth Factor Beta 1

MSC: Mesenchymal Stem Cells

HUVECS: Human Umbilical Vein Endothelial Cells

VEGF: Vascular Endothelial Growth Factor

IGF-1: Insulin Growth Factor 1

IL-1ß: Interleukin 1 Beta

IL-4: Interleukin 4

IL-6: Interleukin 6

PDGF-AB: Platelet-Derived Growth Factor AB

IL-1b: Interleukin-1b

PDGF: Platelet-Derived Growth factor

A-PRF: Advanced Platelet-Rich Fibrin

RCF: Relative Centrifugal Force

PPP: Plasma Poor Platelets

RBC: Red Blood Cells

1. INTRODUCTION

The long-term success of root therapy is directly associated with maximum elimination and inactivation of microorganisms and infected tissues (1). Occasionally, extensive and large periapical lesions may constitute a challenge to completely heal as a high amount of bone tissue is lost. When primary root treatment fails, other regenerative endodontic procedures have to be considered in order to preserve the tooth (2,3).

Endodontic surgery encompasses the removal of the necrotic and infected tissue followed by apical resection and ultimately, filling with a retrograde material. The success rate ranges from 31% to 91% (4) (5).

Leukocyte platelet-rich fibrin (L-PRF) was pioneered by *Dr. Joseph Choukroun et al.* back in 2001, is now considered a second-generation platelet concentrate and of enormous potential. Its application has been already proven successful in wound healing and bone regeneration in a wide range of areas in dentistry (6–8).

L-PRF stimulates cell conduct: increases fibroblast proliferation (9), has a slow and rich discharge of several growth factors (10) as well as an abundant proteome content (11). Furthermore, L-PRF embodies a natural three-dimensional fibrin architecture merged with half the leukocytes and almost total of the platelets from the original blood gathering (12). Its accomplishments are presumably due to these unique features.

In addition, L-PRF presents a great economic choice as a result of being collected directly from the patient's blood and, for that same reason, lacks the complications that come along with allogenic materials as it mimics the biological natural response (10). Therefore, L-PRF's application is quite simple, quick and cost-effective.

Although L-PRF gained popularity among different dental fields, the potential as a regenerative material in the periapical and surrounding areas seems to not be clear yet. Consequently, the aim of this review was to interpret the current available literature and analyze the effects of L-PRF in the periapical region. To achieve success in the periapical area, a size decreasement in the radiolucent area needs to be accomplished as well as complete soft and hard tissue regeneration. Since L-PRF has already shown evident great results in wound and bone healing outside the periapical sites (6), the hypothesis of performing well in the periapical region is going to be further analyzed in this review.

1.1) *<u>The role of growth factors and cytokines in L-PRF:</u>*

Figure 1. The Role of some of the cytokines and growth factors present in leukocyte platelet-rich fibrin.

1.2) *<u>Advantages of L-PRF:</u>*

- ✓ The technique is rapidly and easily executed;
- ✓ Cost-effective;
- ✓ Autologous nature;
- ✓ Extended fibroblast proliferation – up to 48 hours(9);
- ✓ Slow discharge of growth factors – up to 7 days(10);
- ✓ Lack of the risks associated with allogenic materials;
- ✓ Autologous nature;
- ✓ Remains with half the leukocytes and most platelets from the original blood harvest(12);

- ✓ Ample disposure of proteins(11,13);
- ✓ Immune regulative effect(14,15);
- ✓ Has anti-inflammatory and anti-bacterial effects(11)

1.3) Ideal Preparation of L-PRF:

1. Quickly collect (17 seconds per tube mean value) 9ml of blood without any anti-coagulant agent from the antecubital vein into sterile glass-coated tubes;
2. Rapidly (less than 1 minute) place the samples in the centrifuge;
3. Centrifuge at 2700 rpm (400g) for at least 12 minutes;
4. Remove the leukocyte-rich fibrin clot formed at the middle part of the glass tube;
5. Scrape off the remains of red blood cells with the aid of a gauze;
6. Place into an adequate sterile surgical box;
7. Lastly, compress into membranes.

2. METHOD

2.1) Search Strategy

A literature search was carried out on PUBMED (via National Library of Medicine) as well as on EBSCO until the 16th of January of 2020, using the following combination of search terms: "*L-PRF*" AND "*microsurgery*" AND "*periapical lesions*" AND "*bone recovery*" AND "*tissue regeneration*" AND "*healing process*". Also, a manual search was performed considering the references within the selected articles.

2.2) Criteria for study selection and inclusion

The inclusion criteria considered only articles published in English, between the period of 2008 up to 2020, describing in vitro and clinical studies evaluating the effect of PRF on soft and hard tissue healing among various dental fields. Human studies were not limited to randomized clinical trials.

2.3) Study Selection

The article selection process is depicted in Fig. 2. A total of 532 potentially eligible articles were identified through the screening of the titles. Following the exclusion of duplicates, 117 articles were assessed through the abstract. After a review of the remaining 76 full-text articles, a total of 41 publications were eligible to be included in this review.

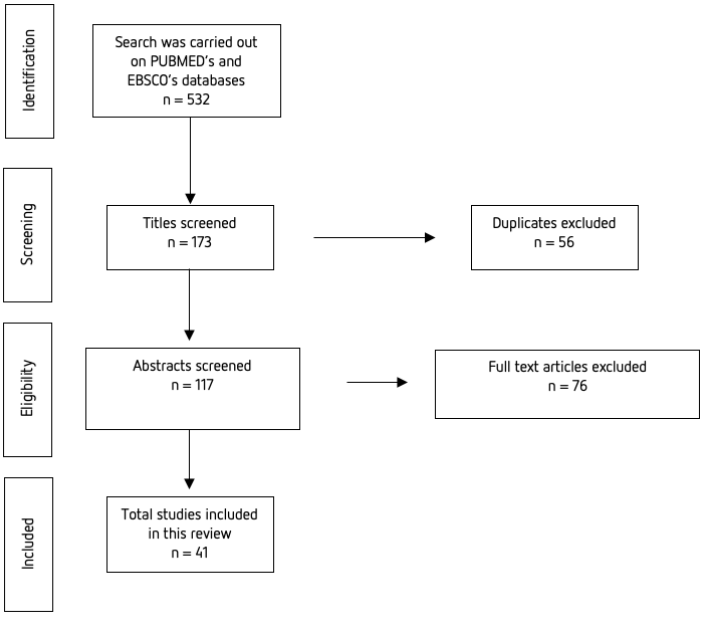

Figure 2: Flow diagram of the Study Selection

2.4) Screening Method

Three of the authors (P.M.; A.F.; L.R.) independently analyzed the titles and abstracts of potentially relevant articles. The screening was based on the question: ''Is platelet rich fibrin (PRF) effective on the periapical region?'' Full text articles were obtained if the response to the screening question was ''yes'', ''uncertain'' or "no". Selected articles were individually read and evaluated concerning the purpose of this study. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager.

The following factors were taken into consideration for this review: author names; year; characteristics of the population; purpose and findings of the respective studies;

3. RESULTS

3.1) IN VITRO

Author Publication Year	Population	Parameters recorded	L-PRF Preparation Protocol	Comparison/Control	Findings/Conclusions
Bucur M. et al. 2019	10 healthy volunteers	Capacity of alveolar blood clots (ABCs), platelet-rich fibrin (PRF) and plasma rich in growth factors (PRGF) to induce in vitro fibroblasts proliferation and migration as a measure of alveolar regeneration.	200 x g for 14 min	alveolar blood clots (ABCs) versus platelet-rich fibrin (PRF) versus plasma rich in growth factors (PRGF)	After 48 h of cultivation we registered activated proliferation, but slightly decreased compared to the 24 h profile. Our data confirm that the presence of the blood clot is involved in the regenerative processes. The migratory capacity of fibroblasts was statistically activated by the PL compounds while not affected by the tested PRFs. The chemical mediators present within the blood clot, either produced by inflammatory cells captive within, or by endothelial or mesenchymal cells induced fibroblastic proliferation and subsequent collagen deposition.
Schar O. et al. 2015	11 donors	Analyze the concentration and kinetics of growth factors released from L-PRF, L-PRP, natural blood clot, investigate the migration of mesenchymal stem cells (MSCs), the human umbilical vein endothelial cells (HUVECs).	Not disclosed	L-PRF versus L-PRP versus natural blood clot.	In comparison to L-PRP, L-PRF had higher amounts of released TGF-b1, a long-term release of growth factors, and stronger induction of cell migration. Future preclinical studies should confirm these data in a defined injury model.
Jung H. et al. 2013	27-year-old male volunteer	Investigate the in vitro effects of PRF on osteoblasts, in terms of the key cellular functions, and especially the effects on two growth factors, the homodimer of platelet-derived growth factor subunit B (BPDGF-BB) and transforming growth factor (TGF)-β1, which are associated with wound healing and regeneration	PRF was incubated with 10 ml of fresh α–MEM at 37oC under 5% CO2 in air	N/A	The release of autologous growth factors from PRF was maintained for a reasonable period of time, and exerted positive effects on the proliferation and differentiation of osteoblasts. The use of PRF thus appears to be a promising method for enhancing bone healing and remodeling

Kim J. et al. 2017	iliac bones of normal people	Analyze platelet-derived growth factors and transforming growth factors-b in normal human serum (NHS) and PRF	1080 rpm for 10 minutes, and cultured the pieces and the supernatant	N/A	PRF contains lots of PDGFs and TGF-b-growth factors engaged in osteoblast activity and tissue regeneration—and these factors serve to stimulate osteoblastic responses with stable effects; therefore, application of PRF to bone regeneration is expected to be effective in improving bone formation.
Wu C. et al. 2012	10 healthy volunteers	determine the effects of PRF on cell attachment, proliferation, phosphorylated Akt, heat shock protein 47 (HSP47) and lysyl oxidase (LOX) expression on human osteoblasts	3000 rpm for 12 minutes	N/A	PRF is capable of increasing osteoblast attachment, proliferation and simultaneously upregulating collagen-related protein production. These actions in combination would effectively promote bone regeneration.
Kargarpour Z. et al. 2020	6 healthy volunteers	investigate the impact of soluble extracts of PRF membranes on in vitro osteoclastogenesis	1570 rpm for 12 minutes	N/A	Osteoclastogenesis is greatly suppressed by soluble extracts of PRF membranes as wells as decreased expression levels of the osteoclasts markers, however, cannot reverse the process once osteoclastogenesis has evolved.
Yaprak E. et al. 2018	8 healthy volunteers	Analyze the abundant proteome content of PRF and summarize previously reported effects of identified proteins on wound healing via a literature review.	400g for 10 min	N/A	Totally, thirty-five blood proteins were commonly identified among all studied samples. These proteins included serine protease inhibitors, such as alpha-1-antitrypsin, alpha- 1-antichymotrypsin, alpha-1-acid glycoprotein, inter-alpha-trypsin-inhibitor, protease C1 inhibitor, and complement proteins. In addition, abundant presence of immunoglobulin G was observed. The abundance of albumin, haptoglobin, ceruloplasmin vitronectin, fetuin-A, ficolin-3 and transthyretin was also detected.
Kang Y. et al. 2011	human mandible of young adults at	identify the variety of protein profiles of PRFe and cPRP	Not disclosed	N/A	The protein array kits that consisted of 36 cytokines and 55 angiogenesis related proteins were used for human PRFe.

Ehrenfest D. et al. 2017	8 healthy volunteers	Analyzing the intrinsic differences between four L-PRF centrifuges available on the market and its consequences on the quality of the platelet concentrates	400 g for 12 minutes	(Intra-Spin, Intra-Lock), A-PRF 12 (Advanced PRF, Process), Salvin 1310 (Salvin Dental) and LW - UPD8 (LW Scientific)	At the classical speed of production of L-PRF, the level of undesirable vibrations on the original centrifuge was between 4.5 and 6 times lower than with other centrifuges. The slow release of the three tested growth factors from original L-PRF membranes was significantly stronger at all experimental times than the release from A-PRF membranes. The A-PRF membranes dissolved in vitro after less than 3 days, while the L-PRF membrane remained in good shape during at least 7 days.
Chandra R. et al. 2017	88 participants	evaluate OD, fibrinogen content and effectiveness in bone-added osteotome sinus floor elevation (BAOSFE) of leukocyte-rich and platelet-rich fibrin (L-PRF) generated by the standard protocol (2700 RPM for 12 min) versus a RCF-adjusted protocol to generate precisely 400 g of force across two centrifuges with swing-out rotors	2700 rpm for 12 min	2700 rpm for 12 min vs. relative centrifugal force (RCF)-adjusted protocol across two widely used laboratory centrifuges with swing-out rotors	A reduction in RCF resulted in a less dense clot and had a positive influence on the regenerative potential of L-PRF.
Choukroun J. et al. 2018	6 volunteers	analyze systematically the influence of the relative centrifugation force (RCF) on leukocytes, platelets and growth factor release within fluid platelet-rich fibrin matrices (PRF)	2400 rpm; 8 min 1200 rpm; 8 min; 600 rpm; 8 min	710 g; 2400 rpm; 8 min vs. 177 g 1200 rpm; 8 min vs. 44 g;	We postulate that the so-called low speed centrifugation concept (LSCC) selectively enriches leukocytes, platelets and growth factors within fluid PRF-based matrices.
Miron R. et al. 2019	6 volunteers	compare different commercially available centrifuges and their respective protocols utilizing a novel method to quantify cells	solid-PRF protocol of 700g for 8 min and a liquid-PRF protocol of 200g for 8 min	solid-PRF 700g for 8 min and a liquid-PRF 200g for 8 min	Furthermore, PRF produced via horizontal centrifugation accumulated a higher number and concentration of platelets/leukocytes when compared to either fixed-angle centrifugation.
Tsujino T. et al. 2019	6 volunteers	quantified silica microparticles incorporated into the PRF matrix	200× g for 14 min	N/A	demonstrated silica ability to incorporate into the matrix of PRF
Masuki H. et al. 2016	2 patients	further assess the biosafety of the silica microparticles, we presently examined their effects on human normal periosteal cells derived from alveolar bone	3200 rpm for 4 min	N/A	Silica microparticles contained in plastic tubes for the purpose of blood coagulation are hazardous for various cell types around sites where silica- contaminated PRF matrices are implanted

3.1.1) The capacity of PRF in inducing in vitro fibroblasts proliferation

Fibroblasts are an essential wellspring of most extracellular grid components, are plenteous in the skin, taking part in the turnover of extracellular matrix as one of the main events in wound healing. The job of fibroblasts is complex; they mediate in fibroplasias and angiogenesis yet additionally in inflammation, granulation tissue formation and scar remodeling. Their contribution in regeneration was revisited by numerous ongoing investigations.
According to the only study evaluating fibroblasts proliferation, it was demonstrated most cellular proliferation induced by the compounds after 24h of seeding and activated proliferation at the 48h mark but lightly inferior when compared to the 24h numbers. However, when assessing fibroblasts migration, L-PRF displayed no action(9).

3.1.2) Effect of PRF on growth factor discharge

Platelet-rich concentrates have an enriched content of growth factors. When in contrast with L-PRP and blood clot, L-PRF has shown to have the higher levels of TGF-b1 (transforming growth factor beta 1) release and also registered the most powerful migration of MSC (mesenchymal stem cells) and HUVECS (human umbilical vein endothelial cells).

Platelet-rich fibrin has an ability to slowly release growth factors such as transforming growth factor b1 (TGF-b1), vascular endothelial growth factor (VEGF), insulin growth factor (IGF-1), platelet-derived growth factor AB (PDGF-AB), and interleukin-1b (IL-1b). Moreover, PRF can powerfully induce migration of MSC (mesenchymal stem cells) and HUVECS (human umbilical vein endothelial cells). It is relevant to notice L-PRF achieved best values in the long term (peaking in cell migration at day 3 and releasing growth factors at day 7) (10).

Figure 3: Release of growth factors post application of L-PRF

3.1.3) L-PRF's impact in osteoblasts proliferation and osteoclasteogenesis

Some growth factors and cells are not only engaged in the soft tissue healing process but also constitute potent agents in hard tissue regeneration, namely PDGF (Platelet-derived growth factor), TGF-b1 and fibroblasts, which are indeed, important in inducing the differentiation of osteoblasts and producing collagen which is the main component of the hard tissue, therefore contributing in bone remodeling and mineralization.

According to different studies, PRF is able to induce osteoblasts proliferation and differentiation therefore enhancing and accelerating bone regeneration(16–18).

Furthermore, one recent study demonstrated PRF also suppresses osteoclastogenesis, consequently leading to lessened osteoclastic bone resorption. However, post development and maturation of osteoclasts, PRF is unable to reverse the process(19).

3.1.4) Proteome content of PRF

Besides encompassing a rich source of growth factors and cytokines(10), the blood also incorporates various sorts of proteins. Therefore, since L-PRF is obtained from blood, some of those proteins are expected to be present in L-PRF's composition.
Out of two studies evaluating the proteome content of PRF, in total 55 different blood proteins were found in all PRF samples(11,13), from which 16 have been previously reported to play a role in the healing process(11).
Among these proteins, were serine protease inhibitors (alpha-1-antitrypsin, alpha-1-antichymotrypsin, alpha-1-acid glycoprotein, inter-alpha-trypsin-inhibitor, and protease C1 inhibitor), complement proteins (complement C3), immunoglobulins (immunoglobulin G) and some other blood proteins (albumin, haptoglobin, vitronectin, fetuin-A, apolipoproteins, ceruloplasmin, transthyretin, and ficolin-3).

The wound healing is a complex order of mechanisms and can be divided in different stages: hemostasis, inflammation, proliferation and ultimately, tissue remodeling(11,20). The cytokines, growth factors and proteins work cooperatively to intensify the natural biological response in the healing process. Initially, occurs the formation of a blood clot in order to cease the hemorrhage and initiate cell migration. The release of growth factors by L-PRF, acts as a magnet of inflammatory cells and fibroblasts to the fibrin clot. Consequently to the complement activation, proteins such as complement C3 perform as chemoattractants, increase the infiltration of inflammatory cells and fibroblasts and boost the fibronectin and collagen I levels. Afterwards, the regulation of angiogenesis, cellular migration, tissue maturation and synthesis of extracellular matrix are carried out by some growth factors and cytokines aided by some other blood proteins, such as serine protease inhibitors, which help decrease the damaging effects generated by serine proteases secreted by neutrophils, increase collagen synthesis and fibroblast proliferation (alpha1-antitrypsin), reduce tissue damage and edema and intensify re-epithelialization (proteinase C1 inhibitor). Other blood proteins further contribute to the healing process, increasing infection control, anti-bacterial defense (immunoglobulin G) angiogenesis, fibroblast migration (haptoglobin), collagen synthesis (albumin) and tissue remodeling (fetuin-A).

Therefore, growth factors and cytokines are undoubtedly, directly associated to proteins in the healing process as many interactions between them have been previously reported(11,21).

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3.1.5) Analyzing the impact of protocol, centrifuge and materials used during L-PRF preparation.

The first L-PRF was developed as an open-access method, numerous variations of the primary technique were eventually implemented.

One of the studies analyzed the intrinsic differences between four popular L-PRF centrifuges available on the market and the consequences on the quality of the platelet concentrates produced, it was demonstrated that at the traditional speed of creation of L-PRF (2700rpm for 12mins), the degree of unwanted vibration on the first L-PRF centrifuge (Intra-Spin) is between 4.5 and 6 times lower comparatively with different centrifuges. In addition, when assessing the slow release of 4 distinct growth factors, it was much higher in L-PRF membranes when compared to A-PRF membranes, possibly explained by the stronger biological structure of L-PRF. Also, L-PRF membranes were the only ones that prevailed for at least 7 days.
In addition, in order for the fibrin clot to fully polymerize, it needs to be centrifuged for an adequate period of time. For L-PRF, 12 minutes seem to be sufficient.
Lastly, it is important to mention, using L-PRF in certain vibrations or machines, can damage and even destroy the cells present in the clot (12)

Moreover, when assessing if the relative centrifugal force (RCF) influences the quantity, quality, and the regenerative capacity of the PRF matrix, it was concluded a lower RCF provided a less dense clot but a higher gain of bone height. Therefore reducing RCF has positive impacts in the regeneration process of the bone.(22) These results corroborate what *Chroukroun et al.* (23) reported in 2018, where reduced RCF presented a evident higher count of platelets and leukocytes as well as an increase of the concentration of TGF-β1 and VEGF(23).

Another study evaluating the differences that occur when using horizontal versus 33° fixed-angle centrifugation, it was demonstrated that, usually when using fixed-angle centrifugation (per-example Intra-Spin™), many layers are formed, the Plasma Poor Platelets (PPP) is placed higher in the tube, the majority of the leukocytes and platelets were found within the buffy coat which is the layer placed in the middle of the tube and almost none were found in the first 4ml of the preparation, which is the PPP. The last layer encompasses the Red Blood Cells (RBC) which is

placed in the lower part of the tube. Whereas, horizontal centrifugation provided the ability for the cells to properly spread and increased the number of platelets and leukocytes of the final clot(24).

Additionally, the type of tubes used for blood collection seem to influence the viability of the clot. In order to correctly prepare PRF, plain glass tubes are required. Currently in the market, there is a scarcity of glass tubes commonly used in the laboratory. As a result, PRF users are opting for silica-coated tubes. However, recent studies have shown silica can interfere with cell viability(25,26). Evidence provided demonstrated silica ability to incorporate into the matrix of PRF(25) and induce cell apoptosis and consequent decrease of cell viability and proliferation(26). Overall, it was evidently proved that the centrifugation protocols, type of centrifuge and materials used highly influences the viability, quality and quantity of the final L-PRF clot.

3.2) CASE REPORTS

Author Publication Year	Population	Aim	L-PRF Preparation Protocol	Healing Period/Follow-up	Comparison/Control	Findings/Conclusions
Mourão C. et al. 2018	10 patients	PRF as a hemostatic material in oral soft tissues.	400g for 12min	Hemostasis mean time: 10.3 ± 2.5 s.	N/A	PRF membranes were active as hemostatic agents being regarded as a possible alternative to use in soft tissue excisional biopsies.
Uchiyama Y. et al. 2018	60 patients	evaluate differences in soft tissue healing and bony regeneration of impacted mandibular third molar extraction sites, with and without the incorporation of PRF	12 min at 2700 rpm	8 weeks	PRF vs non-PRF	Incorporation of PRF proved to be beneficial for patients, yielding a quicker postoperative recovery with fewer complications such as postoperative swelling and edema, pain, and trismus; better overall postoperative results in terms of faster soft tissue healing as well as an earlier bony regeneration.
Singh A. et al. 2012	20 patients	evaluate the efficacy of PRF in soft tissue healing and bone regeneration in mandibular third molar extraction sockets.	3,000 rpm for 10 mins	3 months	N/A	Pain was less in study side comparing to control site soft tissue was healing was better in study site. Evaluation of trabecular bone formation started earlier in PRF site compare to control site.
Karunakar P. et al. 2014	2 cases	Not disclosed	Not disclosed	9 months	N/A	Absence of an intra-radicular lesion, pain, and swelling, along with tooth stability and adequate radiographic bone fill at 9 months of follow-up indicated a successful outcome.

Srinivias B. et al. 2018	30 patients	Evaluate and compare wound healing and bone regeneration in extraction sockets with and without PRF.	10 min at 3000 rpm.	24 h and 3 months.	Extraction sockets with and without PRF.	Patients in PRF group had better healing index when compared to without PRF group. Use of PRF showed a comparable increase in bone density as well.
Shubhashini N. et al. 2013	1 patient, 35-year-old patient	Evaluate the bone regeneration achieved using PRF in endodontically induced periapical lesions over a period of 9 months	3,000 rpm for 10 mins	9 months	N/A	The use of PRF as monotherapy for achieving periapical regeneration has shown promising results in the above case.
Karan N. et al 2019	37 patients with 44 periapical lesions were recruited into the study.	Compare the effects MTA and PRF use on periapical healing in surgically treated periapical lesions.	3000 rpm for 10 mins	12 months	MTA vs PRF vs MTA+PRF	High success rates were achieved using MTA and PRF in periapical lesions in endodontic microsurgery. Further studies are needed with long-term follow-up.
Kulkarni M. 2019	3 cases	Use of PRF as the sole grafting material in periapical bone defects	Non disclosed	1 year	N/A	Excellent bone fill was observed in the periapical defects.
Liu Z. 2019	N/A	Investigate growth factors release kinetics for the combination of F-PRF and L-PRF with different ratios to promote bone tissue regeneration.	3000 rpm for 10 min	12 weeks	F-PRF vs L-PRF	The results showed that the new bone formation in the fresh/lyophilized PRF (1:1) was much more than that of other groups in defects at both 6 and 12 weeks.
Meschi N. 2018	50 patients	Investigate the impact of the adjunct of leukocyte and platelet-rich fibrin (LPRF) to root- end surgery (RES) on the patients' quality of life during the first week post RES.	702 RCF in an Intra-Lock® centrifuge.	7 days	L-PRF vs control	There was no statistical significant evidence for improvement of patients' quality of life with and without L-PRF.
Singh S. et al. 2013	15 cases	Introduce a second-generation platelet concentrate, platelet-rich fibrin (PRF)	3,000 rpm for 10 minutes	6 months	N/A	At the end of six months, all patients showed complete bone regeneration. It requires around 1 year for complete healing to occur after the periapical surgery while with the use of PRF, healing is fastened and requires approx. 6 months for complete regeneration of bone.

Pinto N. et al. 2017	1 patient	Describe innovative regenerative endodontic therapy using L-PRF in the root canal and an extensive apical lesion in an immature tooth with dens invaginatus and asymptomatic apical periodontitis.	2700 rpm for 12 minutes	1 year	N/A	The clinical evaluations performed at 6 months and 1 year revealed an absence of symptoms. The radiographic evaluations showed that the apical lesion was resolved. The cone-beam images indicated that the root length increased and the walls had thickened. The success of the results in this case report indicate that L-PRF can be used as a complement in apical surgery and REPs and could provide an innovative alternative treatment strategy for complex clinical cases like these.
Popowicz W. et al. 2019	2 cases	Describe root-end surgery with the use of the implant DDS-Pro planning software.	2700rpm for 10 minutes	12 months	N/A	The presented case reports show potential for targeted endodontic microsurgery not just in execution but also a positive outcome in a short follow-up period. Preserving the cortical plate to be used as an autologous graft was an added advantage in this technique.
Sharma S. et al. 2018	3 cases	Explore the conservative management of teeth with coexistent open apices and large periapical lesions. A collective approach strategy 1 step apexification with PRF and Biodentine supplemented with lesion decompression under a stringent disinfection protocol expedited healing.	3000rpm for 10 minutes	6 months	N/A	Host-modulating responses of PRF contribute in expediting the healing process. Reasonable osseous healing in the periapex could be appreciated as early as 3 months in all patients.
Yacob N. et al. 2019	24-year-old man	Nondisclosed	3000 rpm for 10 minutes	1 year	N/A	Combination of bone graft and PRF demonstrated successful result with regeneration of periodontal tissue.
Ahmed G. et al. 2018	12 patients	Assess the healing of periapical lesions using volumetric analysis of CBCT images, after either filling with PRF gel or filling with combined PRF gel and bioactive glass.	3000 rpm for 10 minutes.	1 year	PRF vs PRF + bioactive glass	The bioactive glass bone graft material did not give a significant difference when combined with PRF, so the use of PRF alone was sufficient to achieve periapical bone healing.

Velozo C. et al. 2019	52-year-old female	Describe a regenerative endodontic therapy using PRF in three teeth bearing chronic apical periodontitis	12 minutes at 2,700 rpm	12 months	N/A	Images of the computed tomography of the periapical lesions and reestablishment of the buccal cortical bone. The successful results in this clinical case indicate a therapy which can be used as a complement, providing an alternative treatment strategy for complex clinical cases.
Gupta S. et al. 2016	22 year old male	Illustrate the use of PRF for the treatment of periapical lesion.	3,000 rpm for 10 minutes	6 months	N/A	PRF presented regenerative benefits along with good functional recovery.
Lv H. et al. 2018	5 patients	Compare the performance of platelet-rich fibrin (PRF) with BC in inducing root development and periapical lesion healing after tooth revascularization.	400 g for 10 min	12 months	PRF vs Blood Clot	Root elongation, dentinal wall thickening and apex closure were found in most cases (80% in both groups). There was no significant difference between the groups in terms of clinical sign resolution, root development and periapical healing.
Rangaranath A. et al. 2017	1 patient	Add knowledge to the existing literature about the use of platelet-rich fibrin (PRF) in the treatment of large periapical lesion.	10 min at 3000 rpm	12 months	N/A	PRF produced by high-speed centrifugation accelerated the wound healing and induced the rapid rate of bone formation
Al.Hamed F. et al. 2017	47 patients	Assess the effect of platelet-rich fibrin (PRF) on postoperative pain, analgesic consumption, soft tissue healing and socket complications following the extraction of mandibular third molars.	Not disclosed	7 days	PRF vs non-PRF	PRF could reduce alveolar osteitis, pain, and analgesic consumption following removal of impacted mandibular third molars.
Marenzi G. et al. 2015	26 patients	Evaluate the effects of L-PRF on the pain and soft tissue healing after tooth extractions	2700 rpm for 12 mins	3,7,14 and 21 days	PRF vs non-PRF	The use of L-PRF in post-extraction sockets filling can be proposed as a useful procedure in order to manage the postoperative pain and to promote the soft tissue healing process, reducing the early adverse effects of the inflammation.

Uyanik L. et al. 2015	20 patients	Compare of postoperative outcomes in impacted mandibular third molars that were treated using either platelet-rich fibrin (PRF), a combination of PRF and piezosurgery, or conventional rotatory osteotomy	3000 rpm for 10 mins	1, 2, 3, and 7 days	PRF vs non-PRF vs Piezosurgery + PRF vs Traditional surgery	The use of PRF with traditional surgery and PRF combined with piezosurgery significantly reduced pain during the postoperative period.
Ozgul O. et al. 2015	56 patients	Assess the effectiveness of PRF in the healing process by evaluating the changes in pain and swelling after third molar surgery.	3000 rpm for 10 mins	1, 3 and 7 days	PRF vs non-PRF	PRF seems to be effectiveness on postoperative horizontal swelling after third molar surgery. PRF could be used on a routine basis after third molar extraction surgery
Kumar Y. et al. 2016	34 patients	Evaluate (by fractal analysis) osseous regeneration in extraction sockets with and without platelet-rich fibrin in a study with a substantial sample and a reliable technique to calibrate its effects on bone cells.	Not disclosed	1, 3 days and 1, 4 weeks	PRF vs non-PRF	PRF improves healing of both soft and hard tissues. The pain score was significantly better in the experimental group.

3.2.1) Evaluating the power of PRF as a hemostatic material in oral soft tissues

The ceasing of postoperative bleeding in the oral cavity constitutes a huge challenge after surgical procedures due to its high vascularity nature. The use of platelet-rich fibrin is considered as a natural alternative to acquire hemostasis. It was found out of 10 patients submitted to the treatment in this case series, 10 reported none post-operative bleeding, no infection after 7 days and total healing of the lesion after 4 months. The mean time for the ceasing of blood at the surgical place was 10.3 ± 2.5 s, which is indicative to be closer to minimum values of prothrombin's reference time (12-15s) for healthy people. In summary, PRF membranes may, therefore, be considered as a hemostatic option in surgical sites(27).

3.2.2) Evaluate and compare PRF's effect in wound healing and bone regeneration outside periapical sites

Platelet rich fibrin provides a consistent and continuous source of growth factors and proteins, mimicking the physiological wound requirements and reparative tissue cycles.
In a total of 3 studies, all 3 of them (100%) observed a positive effect of PRF in wound healing and bone regeneration outside the periapical region(6–8). These results appear to show that PRF promotes wound closure and soft and hard tissue regeneration. Therefore, PRF acquires its validation as a viable alternative technique. The amount of growth factors and proteins embedded in PRF may play a role.

3.2.3) The effect of the use of PRF on periapical healing in surgically treated periapical lesions.

Periapical surgeries include elimination of infected and necrotic tissues, resection of the apical piece of the tooth (apicoectomy), and preparation of the apical cavity for the filling with a retrograde material. Moreover, legitimate condensation of the retrograde filling material is crucial for the long haul accomplishment of apical resections(28).

From a total of 11 studies examining the effect of PRF on periapical healing in surgically treated periapical lesions, 11 (100%) demonstrated complete wound healing and bone regeneration in the periapical and surrounding areas(1–4,28–34). Despite this, one of the studies compared L-PRF's impact to other materials such as Bioactive glass bone graft, an advantage over this material was reported(4).

Additionally, it is important to point out, 2 studies assessed L-PRF's role when combined with other materials, such as Biodentine Apical Barrier(29) and Bovine Graft(1), which could falsely influence the results since it is not evaluating PRF's effect on its own. Also, 10 out of the 11 mentioned studies did not have a control group, which might also be indicative of influence over the results.

Moreover, different protocols of preparation of L-PRF were noticed. Out of 11 studies, 8 of them centrifuged the blood harvest at 3000rpm for 10 minutes(2–4,28–30,33,34), another 2, used 2700 rpm for 10 minutes(1,32) and only one out of all the studies centrifuged at 2700rpm for 12 minutes(31) which is the one proven most effective(12). Out of the total 11 studies, only one disclosed the type of tubes used in the preparation of the L-PRF's clot (32). Previously, it has been demonstrated that the protocol, centrifuge, type of centrifugation and materials used to collect the blood are deeply associated with the viability of the final clot, therefore contributing immensely to the final outcomes of the use of L-PRF.

Although PRF has been widely used in distinct areas of dentistry, more investigation is needed to further investigate L-PRF's effectiveness. As for the moment, there are still numerous factors influencing the clear interpretation of the literature. Despite this, analyzing the results from the literature now available, it can be concluded that PRF conferred regenerative advantages along with great functional recovery. Not only is it effective but also a better economical choice than other available materials.

3.2.4) Influence of L-PRF on the patient's pain and need of analgesics usage post-surgical procedures

Many of the studies conducted assessing PRF's contribution to soft and hard tissue healing also evaluated PRF's capacity of decreasing pain and necessity of medication administration post-surgical procedures.

As a result, from a total of 6 studies, (83,3%) 5 of them reported a decline in pain and analgesics consumption post-surgery(35–39) and only one demonstrated the lack of statistical significant difference between the experimental and control groups(7).

Additionally, two of the total studies also revealed lessened inflammatory response subsequent to the use of PRF (36,38).

Nevertheless, this parameter is based on a subjective response to a pain scale and may differ due to the patient's interpretation of pain and may differ from patient to patient.

4. DISCUSSION

4.1) Endodontic Microsurgery

Endodontic Microsurgery consists in the removal of pathological and necrotic tissues, resection of a small part of the apex followed by the preparation of the apical cavity in order to ultimately, be filled with retrograde material. Its success is inevitably dependable on the complete healing of the periapical region. *Abramovitz et al.* has previously reported 24.5% of the cases cannot achieve total periapical healing in the absence of periapical surgery. However, it should be only considered an alternative when primary root treatment fails and symptoms persist or, in case of impossibility of execution(2,3).

Due to its proprieties, L-PRF should be considered a viable material in the aid of soft and hard tissue healing in periapical microsurgery.

4.2) L-PRF's role in soft and hard tissue regeneration

In order to enhance both soft and hard tissue healing, L-PRF fibrin matrix slowly discharges numerous regenerative cytokines (interleukins IL-1ß, IL-4, IL-6), growth factors (transforming growth factor b1 (TGF-b1), vascular endothelial growth factor (VEGF), insulin growth factor (IGF-1), platelet-derived growth factor AB (PDGF-AB)) and proteins (serine protease inhibitors (alpha-1-antitrypsin, alpha-1-antichymotrypsin, alpha-1-acid glycoprotein, inter-alpha-trypsin-inhibitor, and protease C1 inhibitor), complement proteins (complement C3), immunoglobulins (immunoglobulin G) and some other blood proteins (albumin, haptoglobin, vitronectin, fetuin-A, apolipoproteins, ceruloplasmin, transthyretin, and ficolin-3) which are mainly behind L-PRF's powerful impact in enhancing soft tissue healing and bone regeneration.

Wound healing is a complex order of different mechanisms and can be divided into different stages: the inflammatory, proliferative and remodeling phase respectively.

Growth factors are endogenous signaling molecules involved in all stages of wound healing, acting as different cell attractants consequently, triggering cell migration, proliferation, and

differentiation. Besides, they participate in bone regeneration, intensifying the expression of osteoblasts and suppressing osteoclastogenesis. In addition, proteins also ally with growth factors, stimulating and boosting these features.
Platelets also play an important role in wound healing, participating in the initial phase of cell response (inflammatory stage) to wound healing, namely the adhesion and aggregation of the blood clot, creating hemostasis. Leukocytes are present in this stage as well, neutrophils migrate to the wound in order to avoid infection followed by monocytes, which later turn into macrophages, releasing more growth factors and cytokines and triggering angiogenesis. Additionally, leukocytes suppress inflammatory response as well, by discharging serine proteases(40).
Platelets store other bioactive proteins as well, such as the serine protease inhibitors, coagulation factors, chemotactic factors, adhesion molecules, vasoactive substances, as well as some bactericidal and fungicidal proteins that also play an active role in preventing infection.
Furthermore, L-PRF is able to induce fibroblasts proliferation and cell migration (MSCs and HUVECs). Fibroblasts start synthesizing collagen meanwhile angiogenesis occurs and the wound starts progressively gaining stability. Lastly, through the remodeling phase, the previous collagen is replaced by organized/structured collagen.

4.3) L-PRF's proprieties

L-PRF (leukocyte and platelet-rich fibrin) was pioneered by *Choukroun et al.* in the early 2000s, is now considered as a second generation platelet concentrate(7) and has been widely and successfully used among various dental fields(6–8). Its three-dimensional biological signature of fibrin, known to stimulate natural immunity(14), merged with leukocytes which have an immune regulative effect(14) assemble an unique and revolutionary material due to the superior advantages offered over other type of materials as it has an autologous nature, it can be easily, quickly and non-expensively collected from the patient's blood, consequently centrifuged and still maintain 97% of the platelets and 50% of the leukocytes from the original blood gathering(12,41). Also, its application comes

without the risks associated with allogenic materials(10) as it mimics the biological natural response.

In vitro, L-PRF membranes appear to have impactful effects on the inflation of the proliferation processes of most cell types(10,12) as it was proven to increase fibroblasts proliferation(9) which is fundamental in the process of tissue regeneration since it takes part in the turnover of the extracellular matrix.

Additionally, PRF also has a rich and slow discharge of growth factors, such as platelet derived growth factors (VEGF,PDGF-AB,TGF-β1), plasmatic growth factor (IGF-1) and pro-inflammatory interleukin-1b (10). Growth factors and cytokines contribute immensely to the different stages of the process of soft and hard tissue regeneration(10,14). Besides, PRF has an affluent proteome content as well, which further contributes to intensify its effectiveness in the healing process as they are directly related via different mechanisms(11). Additionally, it is fundamental to mention that L-PRF seems to have a long-term effect(9,10), this is, when analyzing the studies which tested fibroblasts proliferation, it reaches its peak not immediately after application but plenty of hours later, usually in the following 24h and prolonged through 48h(9). Again, this happens in the study investigating PRF's role in the release of various growth factors, where PRF registers the best values in cell migration at day 3 and growth factors discharge at day 7(10). This particular feature is interesting and relevant as it proves PRF application remains active and effective long after it has been applied.

According to the current knowledge, it is evident the use of L-PRF has indeed, many advantages. It was clearly pointed out through various studies, benefits as expedited wound healing, bone regeneration(7,29,33) and might even be effective when used as a hemostatic material(27).

4.4) *L-PRF's Protocol*

Since the initial PRF was introduced as an open-access protocol(12), multiple variations of usage were eventually implemented.

L-PRF preparation technique is quickly and easily executed, consists on collecting the blood

from the patient (before the surgery, otherwise the surgical procedure might activate the platelets), through the antecubital vein into sterile plain glass-coated tubes (instead of silica-coated tubes which have proven to be cytotoxic), absent from any additives or anti-coagulant agents, centrifuge ideally at 2700 rpm (400g) as lower relative centrifugal force provides higher cell count and viability therefore, augmented regenerative capacity, it was also demonstrated that higher levels of vibration can damage or eradicate the cells; for at least 12 minutes lo let the fibrin fully polymerize and using horizontal centrifugation in order to spread cells evenly throughout the clot, otherwise they are mainly concentrated within the buffy coat(12).
Post-centrifugation, it is possible to observe different layers in the clot: red blood cells (RBC) placed at the lower part of the tube followed by the L-PRF clot in the middle part and (platelet-poor plasma) PPP is placed on top (40). Afterwards, it is possible to proceed to the remove of the L-PRF clot, which can then be used as a clot or lightly compressed into membranes in a specific surgical box(12,40). However, through the evidence previously provided can be concluded that the characteristics of centrifugation, protocol and type of materials used are deeply associated with the viability, quality and quantity of the final clot. This introduces a new question and highlights an important flaw in the study designs used to assess PRF up until this moment. This calls for a standardized implemented protocol in order to precisely evaluate the literature regarding L-PRF's usage.

4.5) *The use of L-PRF in the periapical region*

Given its natural three-dimensional fibrin architecture(3), abundant disposal of leukocytes, anti-inflammatory, immune-regulator effects, capacity of inducing cell proliferation and migration, slow release of cytokines, growth factors and proteins, which come together to enhance and accelerate soft and hard tissue healing, L-PRF seems to be the ideal biomaterial to perform successfully in the periapical region.
In reality, when analyzing its effect in the periapical area, the results were evidently great, as 100% reported complete wound healing and bone regeneration in the periapical and surrounding areas(1–4,29–34) as well as a faster recovery in some of the cases(7,29,33).

Additionally, when in comparison with other materials, an advantage over Bioactive Glass Bone Graft was reported(4).

Moreover, different protocols of preparation of L-PRF were noticed. Out of 11 studies, 8 of them centrifuged the blood harvest at 3000rpm for 10 minutes, another 2 used 2700 rpm for 10 minutes and only one out of all the studies centrifuged at 2700rpm for 12 minutes which is the one proven most effective(12). Also, 10 out of the 11 mentioned studies did not have a control group. Both these aspects might be indicative of influence over the results, as it was previously mentioned, centrifuging above 2700 rpm can damage or eradicate the cells(12), consequently leading to a different response. Lower RCF is recommended as it demonstrated previously a higher cell count and as a result, better outcomes. The type of tubes used were also previously reported to affect the viability of the clot, as silica coated (plastic) tubes were proven to be cytotoxic and reduce cell count. Out of the total of 11 studies assessing PRF, only one disclosed the type of tubes used in the preparation of the L-PRF clot(32), which means the other 10 studies kept the material of the tubes undisclosed. Therefore, it is another variable that should be taken into account.

Besides, the absence of a control group might be incorrectly influencing the interpretation of the overall result, as the lack of a control group makes it impossible to rule out other factors which may influence the outcome of the study.

Besides, 2 out of 11 studies assessing L-PRF's impact in the periapical site, used L-PRF combined with other materials. This factor is also relevant because the outcome is evidently being affected by association and not by L-PRF solely.

Overall, the success rate of these studies is evident (100%). Nonetheless, there are flaws in the current literature, particularly in the study designs preventing the reader from interpreting it accurately and precisely. Moreover, there are a number of variables present that might influence the outcomes as well, for instance the patient's age, being that younger patients are expected to have better results and also the diameter of the lesion which is expected to take part in the duration of the healing process and respective final outcome. Therefore, currently these results are only indicative that L-PRF might be a good viable option. However, further investigation which takes into consideration all the variables that may take part in final outcome, is needed in order to indeed, prove L-PRF's effectiveness.

4.6) *The role of L-PRF in pain and inflammation*

L-PRF also demonstrated to decrease and pain after surgical procedures, consequently a reduction in the administration of medication (35–39).

Additionally, two of the studies assessing the subject reported lessened inflammatory response subsequent to the use of L-PRF (36,38).

These results might be explained by the autogenous nature and biological impact of L-PRF, since it is collected by the patients' blood, the risk of infection is highly minimized as well as inflammation, since PRF offers anti-inflammatory proprieties provided by the leukocytes collected from the blood.

Nevertheless, this parameter is based on a subjective response to a pain scale and may differ due to the patient's interpretation of pain, therefore, may differ from patient to patient.

5. CONCLUSION

L-PRF is an autologous material offering superior advantages over allo and xenografts as well as alloplastic materials, due to its nature the risk of infection is highly minimized.

L-PRF has a powerful biological impact, demonstrating to induce fibroblast and staminal cells proliferation, provide fastened hemostasis acquirement and has a enriched and slow release of various growth factors, cytokines and proteins which altogether are featured in the healing and regeneration process, enhancing and expediting the biological natural response.

Moreover, it is fundamental to mention that the centrifuge's characteristics, protocol and materials deeply influence the viability of the L-PRF clot produced, which calls for the implementation of a standardized L-PRF's preparation protocol.

The choice of using L-PRF as an aid in Endodontic Microsurgery has proven to be, indeed, a positive one, achieving complete soft and hard tissue healing in the periapical and surrounding areas in 100% of the studies assessing the matter and might even decrease post-surgical pain, inflammation and drug administration.

Despite the predominant great results, many weaknesses in the study designs were found. Therefore, currently the literature points towards L-PRF effectiveness in the periapical region. However, further investigation is needed in order to undoubtedly prove L-PRF's effectiveness.

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