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The relevance of L-PRF use in dental revitalization

Bérangère Catto

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

Gandra, 13 de junho de 2022

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Trabalho realizado sob a Orientação do Prof. Doutor Paulo Miller

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Declaração de Integridade

Eu, Bérangère Jeanne Marie Catto, declaro ter atuado com absoluta integridade na elaboração deste trabalho, confirmo que em todo o trabalho conducente à sua elaboração não recorri a qualquer forma de falsificação de resultados ou à prática de plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria do trabalho intelectual pertencente a outrem, na sua totalidade ou em partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores foram referenciadas ou redigidas com novas palavras, tendo neste caso colocado a citação da fonte bibliográfica.

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Parecer do Orientador

Eu, Paulo Manuel Cruz Miller , com a categoria profissional de Professor Auxiliar do Instituto Universitário de Ciências da Saúde, tendo assumido o papel de Orientador da Dissertação intitulada *"The relevance of L-PRF use in dental revitalization"*, do estudante do Mestrado Integrado em Medicina Dentária, Bérangère Jeanne Marie Catto, declaro que o meu parecer é positivo relativamente à Dissertação e que concordo com a sua submissão na UC Dissertação no moodle como solicitação de Admissão a Provas Públicas conducentes à obtenção do Grau de Mestre, tal como está determinado regulamentarmente no Regulamento Específico do MIMD, IUCS, aprovado pelos órgãos competentes em vigor.

Gandra, 18 de Junho de 2022

_____
(O Orientador)

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RESUMO:

Introdução: Atualmente, os procedimentos endodônticos regenerativos são cada vez mais utilizados num esforço de manter tanto os dentes maduros como os imaturos na cavidade oral. Nas últimas décadas, melhores e mais eficientes *scaffolds* viram a luz do dia, entre eles o Platelet Rich Fibrin (PRF). Já foi utilizada noutros sectores da odontologia como a periodontologia, a PRF está agora a ser utilizada na odontologia regenerativa devido às suas propriedades físicas e químicas.

Objetivo: O objetivo desta revisão sistemática é determinar e avaliar a relevância dos usos da L-PRF como *scaffold* no tratamento endodôntico regenerativo, utilizando a literatura atualmente disponível.

Método: Uma pesquisa bibliográfica foi realizada nas bases de dados PubMed e EBSCO em janeiro 2022, utilizando várias combinações dos termos de pesquisa: "*Pulp revascularization*", "*pulp revitalization*" "*regenerative endodontics* ", "*Platelet rich fibrin*", "*L-PRF*" and "*Dental pulp Necrosis/therapy[Mesh]*".

Resultados: 44 estudos foram incluídos de acordo com os critérios de inclusão. A utilização de L-PRF leva ao desaparecimento de sintomas clínicos e radiográficos em todos os estudos selecionados. O PRF, induz a proliferação e diferenciação celular, assim como a libertação de numerosos fatores de crescimento. Contudo, não há diferença significativa entre a utilização de L-PRF, PRP ou BC em termos de desenvolvimento radicular. O protocolo de centrifugação e o equipamento tem um impacto na qualidade da L-PRF produzida.

Conclusão: O PRF é um scaffold válido para tratamento de revascularização em dentes imaturos, uma vez que permite o alongamento das raízes, espessura das paredes dentinárias, fechamento apical e, por vezes, vitalidade da polpa. No entanto, são necessários mais estudos para avaliar verdadeiramente o seu potencial regenerativo em dentes maduros. Além disso, a sua supremacia em PRP ou mesmo em BC também necessita de mais investigação. Deve ser feita uma uniformização do protocolo de centrifugação.

Palavras- chave: "platelet-rich fibrin" "PRF" "revascularization" "regenerative endodontic, "PRP"

ABSTRACT

Introduction: Nowadays, regenerative endodontic procedures are increasingly being used in an effort to maintain both mature and immature teeth in the oral cavity. In the last few decades, better and more efficient scaffolds have seen the light of day, among them the Platelet Rich Fibrin (PRF). It has already been employed in other sectors of dentistry like periodontology, PRF is now being used in regenerative dentistry thanks to its physical and chemical properties.

Aim: The aim of this systematic review is to determine and evaluate the relevance of L-PRF uses as a scaffold in regenerative endodontics treatment using the current available literature.

Methods: A literature search was conducted in PubMed and EBSCO databases in January 2022, using various combinations of search terms: *"Pulp revascularization", "pulp revitalization", "regenerative endodontics", "Platelet rich fibrin", "L-PRF" and "Dental pulp necrosis/therapy"*.

Results: 44 studies were included according to the inclusion criteria. L-PRF use leads to the disappearance of clinical and radiographic symptoms in all of the selected studies. It induces cell proliferation and differentiation as well as the release of numerous Growth Factors. However, there is no significant difference between the use of L-PRF, PRP or BC in terms of root development. Centrifugation protocol and equipment has an impact on the quality of the L-PRF produced.

Conclusions: PRF is a valid scaffold for revascularization treatment in immature teeth as it allows root lengthening, dentinal walls thickness, apical closure and sometimes, pulp vitality. However, further studies are needed to truly assess its regenerative potential on mature teeth. Additionally, its supremacy on PRP or even on BC also need more research.

Keywords: "L-PRF", "dental revascularization", "endodontic treatment", "pulp necrosis", "PRP"

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LIST OF ABBREVIATIONS:

A-PRF: Advanced PRF

BC: Blood clot

$Ca(OH)_2$: Calcium Hydroxide

CEJ: Cemento-enamel junction

CF: Ciprofloxacin

CGF: Concentrate Growth Factor

CHX: Chlorhexidine

DAP: Double antibiotic paste

DPSC: Dental Pulp Stem Cells

EDTA: Ethylenediamine tetra-acetic acid

EGF: Epidermal Growth Factor

GF: Growth Factor

hDPCs: Human dental Pulp cells

IGF: Insulin Growth Factor

IL-1: Interleukin 1

IL-4: Interleukin 4

IL-6: Interleukin 6

L-PRF: Leukocyte and Platelet rich Fibrin

MIN: Minocyclin

MT: Metronidazole

MTA: mineral trioxide aggregate

NaOCl: Sodium hypochlorite

PDGF: Platelet-Derived Growth Factor

PRF: Platelet Rich Fibrin

PRP: Platelet Rich Plasma

PPP: Plasma Poor Platelet

RBC: Red Blood Cells

RCF: Relevant Centrifugation Force

RET: Regenerative Endodontics Treatments.

TAP: Triple Antibiotic Paste

TGF-β1: Transforming Growth Factor Beta 1

TNF-α: Tumor necrosis Growth factor alpha

VEGF: Vascular Endothelial Growth Factor

SCAP: Stem cell of the apical papilla

1. INTRODUCTION:

Untreated carious cavities or trauma can cause pulpal necrosis. For children, whose teeth are not yet fully developed, pulp necrosis represents a concern for teeth conservation, as the formation of the dental organ is stopped (1,2). A necrotic immature tooth has a short root with thin fragile root walls, a broad pulp canal and an open apex (3). Even for fully developed teeth, necrosis is a barrier to tooth preservation in adult as well because it increases the risk of root fracture but also of bacterial infection through contact with the periodontium.

Root canal treatment with gutta percha is efficient but not recommended on immature teeth. In fact, instrumenting the canal and hence the dentinal walls would increase the likelihood of root fractures and perforations (2–4). Apexification was initially developed in order to promote apical closure using calcium hydroxide. However, many studies have shown that apexification weakens the root in the long term, as it denatures its components (4). Furthermore, none of these techniques address the issue of root maturation.

The concept of regenerative endodontic technique (RET), with revascularization as it most successful approach, emerges as an alternative to both apexification and obturation. It is a conservative approach that re-initiates root development and apexogenesis by using the regenerative potential of the periapical tissues with the adjuvant of tissue engineering (5,6).
Three key components are mandatory for its success: stem cells, growth Factors (GF) and a scaffold capable of supporting the cell development. Traditionally, bleeding is induced in the disinfected root canal, which will create a Blood Clot (BC) and after will be colonized by stem cells. However, the technique remains difficult to perform and the long-term results are not always obtained. Thus, the BC has been replaced with scaffolds, which serve as the foundation of the RET and are loaded with growth factors to promotes cells growth and differentiation. This is how the platelets were introduced in 1974 (5,7,8).

Platelet rich Plasma (PRP) was one of the first scaffold to be introduced but production difficulties have relegated it to the sidelines. Platelet Rich Fibrin (PRF) was introduced in 2001 (9), and is nowadays used in many medical fields (4,7). It is a tetra-molecular structure and contains cytokines, platelets, leukocytes, and stem cells. PRF works as a biodegradable scaffold that

promotes revascularization and cell migration, differentiation, and proliferation as it releases the GF it contains during a twenty-eight-day period (10). Its composition and properties stimulate the growth of the root and the thickness of its walls. Combined with Mineral Tri-Aggregate (MTA) it induces apical closure. As a result, it appears to be a suitable material for the RET but also for periodontal defects treatment.

2. AIM

The main goal of this work is to determine and evaluated the relevance of L-PRF uses as a scaffold in regenerative endodontics treatment (RET) effectuated in necrotic teeth, through the verification of features such as root lengthening, root thickening, apical closure, and disappearance of the clinical and radiographical symptoms.

The secondary goal of this study is to understand how PRF works and what factors may influence its success.

3. METHOD

3.1. Search Strategy

For this systematic review, a bibliographic research was conducted on the PubMed and EBSCOHost, using the following search terms: "*Pulp revascularization", "pulp revitalization", "regenerative endodontics ", "Platelet rich fibrin", "L-PRF"* and *"Dental pulp Necrosis/therapy"* indifferent combinations:

("Platelet-Rich Fibrin"[Mesh]) AND "Leukocytes"[Mesh]) AND ("Regenerative Endodontics"[Mesh] OR "pulp revascularization") OR "Dental Pulp Necrosis/therapy"[Mesh])	1120 articles
"Pulp revitalization" OR "pulp revascularization" AND "regenerative endodontics" AND "Platelet rich fibrin" OR "L-PRF" AND "scaffold"	246 articles
"Platelet-Rich Fibrin"[Mesh] AND "Leukocytes"[Mesh] OR "LPRF" AND "Regenerative Endodontics"[Mesh] OR "pulp revascularization"	124 articles
((pulp revascularization) OR (regenerative endodontics))AND ((Leukocytes and Platelet rich fibrin) OR (L-PRF))	82 articles

Table 1: Keyword combination used for searching on PubMed

Pulp revascularization AND platelet rich fibrin OR L-PRF and regenerative endodontics	9 articles
Pulp revascularization OR pulp revitalization OR regenerative endodontics AND platelet-rich fibrin OR L-PRF	526 articles
regenerative endodontics AND platelet-rich fibrin OR L-PRF AND pulp revascularization	16 articles

Table 2: Keyword combination used for searching on EBSCO

A manual search was also performed, using the references of the selected articles and the similar articles proposed by PubMed.

3.2. *Criteria for study selection and inclusion*

The inclusion criteria concerned the articles published between 2011 and 2022, written exclusively in English whose studies were conducted on Humans. The selected studies include randomized studies, clinical trials, case report, retrospective, and experimental studies

3.3. *Study selection*

The search strategy with the above detailed keyword combination identified 2123 articles in the different databases. After application of the inclusion criteria 562 articles were obtained. Following titles screening we have selected 149 hypothetically suitable ones for our study. After removing the duplicates, a total of 64 articles were selected for a review of the abstract. A thorough reading of the articles allowed us to select 44 of them.

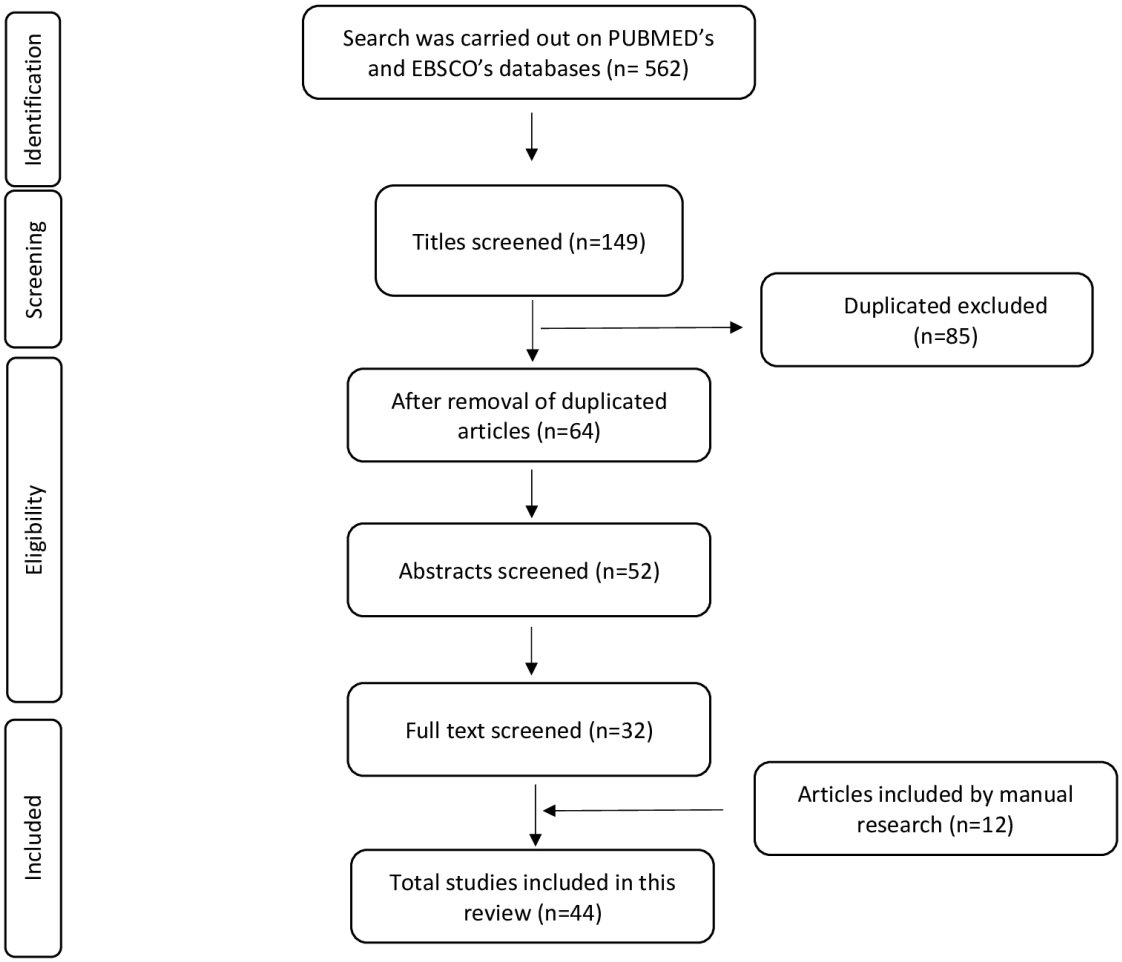

Figure 1: flow diagram of the search strategy

For a better comparison of the results the clinical randomized studies and the case reports were examined separately.

4. RESULTS

4.1. Randomized control trials

Study (authors and publication year)	Number of teeth, patient age	Parameters recorded	Scaffold protocol	Comparison/ control	Follow-up	Results	Findings/ conclusions
Jadhav GR. et al. 2012 (11)	20 anterior teeth, 15-28 years	Evaluate and compare apexogenesis induced by revascularization with or without PRP	**PRP**: 8mL blood sample centrifuged with acid citrate dextrose, 10% calcium chloride, 2400 rpm for 10 min, 3600 rpm for 15 min **BC:** induction of bleeding with a needle 2mm beyond the working length into the periapical tissues	PRP vs BC	6, 12 months	After 6 and 12 months all teeth were asymptomatic. All teeth were asymptomatic. Statistically difference in periapical healing, apical closure, and dentinal wall thickening with the use of PRP. Root lengthening is comparable with or without PRP.	Adding PRP to the revascularization technique might improve the outcome.
Narang I et al. 2015 (12)	20 teeth, below 20 years old	Evaluate and compare the regenerative potential of the blood clot, PRP and PRF in immature necrotic permanent teeth	Not discussed	Gutta percha, vs BC vs PRP vs PRF	6, 18 months	All patients of each group were asymptomatic **Periapical healing:** 98% of cases in the PRF group showed excellent periapical healing. **Apical closure:** BC group have the better result (66.67%) followed by PRF groups (40%). **Root lengthening:** 90% of the PRF group showed excellent results with statistical difference over other groups. **Dentinal wall thickening:** 60% of the PRF group showed excellent results.	All teeth were asymptomatic. PRF as a scaffold shows significant periapical healing, root lengthening and dentinal wall thickening in necrotic immature permanent teeth over the blood clot and PRP. BC and PRP show comparative results.

Kobayashi E. et al. 2016 (13)	18 blood samples from 6 donors, 30-60 years	To compare GF release over time from PRP, PRF and A-PRF	**PRP:** 1000 rpm 7 min then 3000 rpm for 10 min **PRF:** 2700rpm for 12 min **A-PRF:** 1500 rpm for 14 min	PRP vs PRF vs A-PRF	15, 60 min, 8h, 1 day, 3 days and 10 days	**Total protein released:** A-PRF released the highest GF: 11048.19 ng/mL, significantly more than PRF 9261.89 ng/mL and PRP 6176.15 ng/mL. **Time of release:** PRP release in more GF in the early time than the others. Then, A-PRF delivers significantly more GF after 3 days than the others for VEGF, TGFB1, PDGF. It is the opposite for EGF and IGF.	The various platelets have different release dynamics: PRP is good for immediate needs whereas the others are better for long term necessities. PRP releases protein in early time where PRF will release continual and constant growth factor. A-PRF releases more quantities of Growth factor than PRF and PRP.
Fujioka-Kobayashi M. et al. 2017 (14)	24 blood sample from 8 donors, 30-60 years	To insight how centrifuge speed and duration influence PRF scaffolds and their GF release and their effect on cellular biocompatibility.	**L-PRF:** 2700rpm for 12 min **A-PRF:** 1300 rpm for 14 min **A-PRF+:** 1300rpm for 8 min	L-PRF vs A-PRF vs A-PRF+	10 days	A-PRF+ demonstrates significantly higher total GF release compared with A-PRF and L-PRF. A-PRF and A-PRF+ scaffolds directly impact the capacity of fibroblasts to migrate, proliferate and release other GF.	Reducing the time and speed of centrifugation favour an increased release of GF from the scaffolds.
Wend S. et al. 2017 (15)	Blood sample from 3 volunteers, 20-60 years	To evaluate the influence of centrifugal force on cell type and GF release within I-PRF	**High speed:** 2800rpm for 3 min **Medium speed:** 1400 rpm for 3 min **Low speed:** 700 rpm for 3 min	high speed vs medium speed vs low speed	N/A	Significantly higher numbers of leukocytes, lymphocytes, neutrophil granulocytes, monocytes, and platelets were found in the low-speed groups compared to the medium or high speeds. **Histological observation:** the low speed PRF showed more platelets and leukocytes densely and evenly distributed in the fibrin network then in the other groups.	By decreasing the speed of centrifugation there is a significant increase of GF and cytokines release as well as a significantly higher number of inflammatory cells and platelets.

						GF and cytokine release: increased GF (EGF, TGFB1, PDGF) release with the lower speed PRF.	
Shivashankar VY. et al. 2017 (16)	60 anterior teeth, 6-28years	To compare the effect of PRF, BC and PRP in the revascularization of immature and necrotic teeth	Not discussed	PRF vs BC vs PRP	3,6,9,12 months	**Periapical healing:** Significantly better results with the PRP group at the end of the year. **Root lengthening and thickness:** no difference between groups after a year. **Response to vitality test:** 15% of PRF's group, 13.30% of BC's groups and 15.8% of PRP's group regain sensibility after 12 months	All teeth were asymptomatic. PRP gives better results in periapical wound healing. The three groups were comparable regarding the lateral wall thickening, root lengthening and response to vitality test.
Cabaro S. et al. 2018 (17)	Nine volunteers gave 4 tubes of blood each, 20-26 years	To insight the leukocytes subpopulation of L-PRF and A-PRF and investigate cytokines and GF release by each material.	**L-PRF**: 9mL blood sample centrifuged at 3000rpm (400g) for 12 min **A-PRF**: 9mL blood sample centrifuged at 100g for 10 min **PRGF**: 9ml blood sample with 3.8%sodium citrate centrifuged at 580g for 8min	L-PRF vs A-PRF	24h	A-PRF release more a higher number of GF (VEGF, PDGF, IFNγ) than L-PRF without significantly difference but they release the same amount of IL-4, IL-6, IL-8 TNFα)	The use of A-PRF is relevant in clinical fields that require tissue repair and new vessel formation because it releases more molecules and growth factors such as VEGF and PDGF than L-PRF. Changing the original protocol could modify the secretory properties of PRF.
Nageh M. et al. 2018 (18)	15 upper central incisors, 18-40 years	To evaluate the possibility of regaining pulp sensibility in mature necrotic teeth using PRF	**PRF**: 5mL blood sample centrifuged at 3000 rpm for 10min. The membrane was fragmented. No bleeding was induced before.	N/A	3, 6, 9, 12 months	The percentage of teeth that regained their sensitivity reached the maximum level after 12 months. Tooth sensibility was regained in 9 patients (60%),	Tooth sensibility is the indication of the formation of vital pulp-like tissue, but still needs investigation clinically and histologically.

Hong S. et al. 2018 (19)	Three 3rd mandibular molars, 14-20 years	To evaluate the effect of PRF and CGF on the proliferation, migration, and differentiation of SCAPs	**CGF**: 10mL blood sample of each patient centrifuged at 2700 rpm for 2 min, 2400 rpm for 4 min, 2700 rpm for 4 min and 3000 rpm for 3 min. **PRF**: 10mL blood sample of each patient centrifuged at 3000rpm for 10min	CGF vs PRF vs control	3 weeks	**Cell proliferation:** no dose dependent effect was revealed, CGF and PRF showed better than the control. **Cell migration:** the migration from the PRF's group was denser. both induced cell migration without significant difference. **Mineralization:** the expression of related genes was statistically higher in the PRF group.	CGF and PRF significantly promote the proliferation, migration, and differentiation of SCAPs, and they may be a promising biomaterial to be used in regenerative endodontics.
Ulusoy AT. et al. 2019 (20)	88 maxillary incisors, 8-11years	To compare the clinical and radiographic performance of PRF, PRP and PP without prior apical bleeding in RET	**PRP:** 20mL blood sample with 15mL of citrate solution centrifuged at 1250rpm for 15 min. **PRF:** 10mL blood sample centrifuged at 3000rpm for 10min **PP:** PRP preparation as described before and then centrifuged again at 4000rpm for 10min. The PP concentrate at the bottom was collected. **BC:** bleeding was induced with a K15 2mm beyond the apex	PRF vs PRP vs PP vs BC	3,6,9,12,18, 24, 36,48 months	1 case from the PRF group and 1 from the BC group showed signs of failure (pain) The others were asymptomatic without statistical difference. **Apical closure:** 73.9% of cases showed complete apical closure. The number was higher in the PP group (82.4%), than the BC (76.2%), PRF (70.6%), and PRP (66.7%) groups. **Response to vitality:** 86% of teeth showed a positive response. Time for the first positive response is the same among the groups. **Root width and length:** there are no significant differences between groups.	Periapical healing, radiographic root development and positive response to sensibility test was obtained in all teeth except from 2. PRP, PRF and PP can produce comparable results as BC without the prior apical bleeding.
Ragab RA. et al. 2019 (21)	22 upper anterior teeth, 7-12 years	To evaluate the effect of PRF during the revitalization of necrotic immature permanent anterior teeth	**BC:** bleeding was induced by irritating apical tissues with an H60. **PRF:** 12mL blood sample centrifuged at 3000rpm	BC vs PRF	6,12 months	**Root lengthening:** no significant differences were observed between groups. Calcific bridges were revealed either in apical or/and cervical in both groups.	Root development was achieved. PRF may not be essential for revitalization of necrotic immature permanent anterior

			for 12min. Bleeding was induced before.			**Periapical healing:** it was obtained in both groups with a decrease in the periapical radiolucency.	teeth but may be an adjuvant to BC.
Mittal N. et al 2019 (2)	16 upper incisors, no age was mentioned	To evaluate and compare the regenerative potential of natural and artificial scaffolds.	**PRF:** 5mL blood sample centrifuged at 2700rpm for 12min. The membrane was not fragmented. No bleeding was induced before. **Collagen:** bleeding was induced with a K30, and then sterile collagen granules were place into the canal. **Placentrex:** bleeding was induced and then placentrex mixed with collagen granules were placed inside the canal **Chitosan:** bleeding was induced, then chitosan granules were place inside the canal.	PRF vs collagen vs placentrex vs chitosan	3, 6, 12 months	All cases were asymptomatic after the follow up period. **Periapical healing:** collagen gave the best result followed by PRF, chitosan, placentrex. **Apical closure:** PRF gave the best result followed by placentrex, collagen and chitosan. No statistical difference was observed. **Root lengthening:** placentrex showed the best result followed by collagen, chitosan and PRF as the least effective. No statistical difference was observed. **Dentinal wall thickening:** PRF showed better results followed by collagen, chitosan and placentrex. PRF and collagen have statistical differences with placentrex. *	The authors concluded that PRF and collagen are better scaffolds for REP in terms of periapical healing, root lengthening, dentinal thickening. and apical closure.
Chai J. et al. 2019 (22)	Five 3rd molars, 18-22 years. For the scaffold's preparation patients were between 20-40 years	To compare cellular regenerative activity of human dental pulp cells (DPCS) when cultured with liquid PRF or traditional PRP in vitro	**PRP:** 10mL blood sample with anticoagulant was centrifuged at 900g for 5 min. Supernatant was removed and centrifuged again at 2000g for 15min **PRF:** 10mL blood sample centrifuged at 700g for 3 min	PRF vs PRP	12h, 24h, 7 days, 14 days	PRP and PRF increased the migration and proliferation of hDPCs, with PRF more effective. When PRF was cultured in an inflammatory environment, the regenerative potential was observed and hDCPs regeneration was facilitated.	Liquid PRF promoted greater migration, proliferation, and differentiation of DPCS than PRP

Rizk HM. et al. 2020 (1)	30 upper central incisors,8-14 years	To evaluate and compare the regenerative potential of PRP and PRF scaffold in immature necrotic permanent teeth.	**Protocol not discussed.** **PRP:** no bleeding was induced **PRF:** membrane was fragmented. No bleeding was induced.	PRP vs PRF	3, 6, 9, 12 months	No significant difference was observed between the groups in terms of apical closure, bone density, and root lengthening. PRF showed higher discoloration than PRP.	The two scaffolds showed good result in root length and width and apical closure. According to the authors, PRP should be the first choice even if the results obtained do not differ significantly from those obtained with PRF.
Miron RJ. et al. 2020 (23)	72 Blood samples from 6 healthy volunteers	To compare different method of PRF production in 3 different centrifuges at high and low g-force protocols	2 separate protocols on each centrifuge: -**High speed (L-PRF):** 700g for 12min max. -**low speed (A-PRF):** 200g for 8min	High speed vs low speed obtained PRF on 3 centrifuges: Process PRF, Salvin Dental and IntraLock	10 days	**Effect of high and low RCF on PRF matrices:** PRF weight is significantly greater when RCF is high, and the fibrin network is denser. More cells are encountered in the RBC when the speed is high. The cell distribution is more even with A-PRF, and it release a significantly greater number of GF (PDGF, lymphocytes, leukocytes, platelets **Role of centrifugation tubes on PRF matrix:** the tubes have a direct impact on the final size of PRF matrix, Process for PRF's tubes produced the biggest PRF matrix.	PRF clots obtained by low -speed centrifugation contain higher concentration of platelet and growth factors, although smaller in size.
Mittal N. et al. 2021 (24)	36 teeth, 16-34 years	To evaluate and compare the possibility of regaining pulp sensibility and outcomes of regeneration procedure in mature	**BC:** induced bleeding with a K15 2mm beyond the apex **PRF:** patient's blood centrifuged at 2700rpm for 14 min. **Collagen:** sterile granule of synthetic collagen	BC vs PRF vs Collagen vs Hydroxyapatite	3, 6, 9, 12 months	**At 3 months:** no significant difference inter-groups. **At 6 months:** PRF groups showed 22.3% positive response and collagen groups 11.1%n no responses were observed in the other groups. Not significant	The PRF's group showed better results than any of the other scaffolds for pulp sensibility, but without any statistical differences among the four groups.

		necrotic teeth using RET with various scaffolds.	from Eucare Pharmaceuticals were used **Hydroxyapatite:** crystals from Surgiwear company were used			**At 9 months:** PRF groups showed 44.4% of response and collagen group 33.3% hydroxyapatite 22.2% and none for the BC groups. Not significant. **12 months:** PRF 66.6%, collagen 44.4%, hydroxyapatite 33.3% and BC 11.1%. Not significant.	
Kritika S. et al. 2021 (25)	23 maxillary central incisors, 9-25 years	To evaluate the regenerative potential of PRF	**PRF**: 10mL blood sample centrifuged at 3300 rpm (400g) for 12min. The membrane was fragment. No bleeding was induced	N/A	3, 6, 9, 12, 15, 18, 24 months	All cases were asymptomatic. **6 months:** 5 cases complete resolution of symptoms and complete bone healing. 2 with partial resolution. **12 months:** significant increase in root length, and apical dentin thickness. Decrease of apical diameter. **18 -24 months**: distal and mesial dentin thickness significantly increase. Apical diameter decrease	The use of PRF is clinically and radiographically effective in terms of increased root length, apical closure, periapical healing, and root thickness. Only one tooth regains sensibility.
Youssef A. et al. 2022 (26)	20 maxillary anterior teeth, 18-40 years	To compare the clinical and radiographic outcomes of two REP using BC or PRF as scaffolds.	**BC:** bleeding was induced by irritating the periapical tissues with a K25, 2mm beyond the apex **PRF:** blood samples were centrifuged at 400g for 10min. Bleeding was induced before the collocation of PRF, and the membrane was fragmented.	BC vs PRF	6, 12 months	**Radiographic evaluation:** no statistical differences between groups. Increase in peri-radicular healing at 6 and 12 months. **Pulp sensibility:** no statistical differences between groups but between the preoperative, 6 month and 12 months follow-up of each group. 20% of patients treated with BC regained tooth sensibility and 50% of PRF's patients.	They both enable periapical healing with better results for the PRF group. PRF and BC can be valid scaffolds for RET in mature necrotic teeth.

Table 3: Data extracted from the included randomized Studies

4.1.1. Capacity of L-PRF in inducing root maturation:

The goal of all endodontic treatment symptom alleviation and periapical healing are the goal of all endodontic treatment. PRF promotes periapical healing both clinically and radiographically as well as the elimination of all symptoms (1,2,12,16,21,24–26).

One of the primary goals of RET is root maturation, which allows for root thickening, apical closure, and root lengthening. As a result, the tooth is less likely to fracture and will remain in the oral cavity. All the studies show that PRF is better than, or as effective as, PRP or BC to induce root development in non-vital permanent teeth without, however, any significant differences.

Ulusoy et al (20) and *Rizk et al* (1) studies showed that on average, the mean increase in root lengthening was of 7 to 8% with PRF and of 5 to 9% with PRP, without any statistical differences between the two groups. In twelve months, the dentinal wall thickened from 19.48% to 39.27% with PRP compared to 11.14% to 42.37% with PRF, also without any statistical difference. Apical closure is also obtained more quickly with PRF, as the apical diameter began to shrink in most teeth at about 6 months after the procedures. But in the following months all teeth treated with PRP, or BC caught up with better final results.

Regardless of these findings, all authors concur that more research on mature teeth and longer follow-up period are required.

4.1.2. Capacity of L-PRF inducing tooth sensibility

After root maturation, the main goal of RET is pulp revitalization. It is also the most difficult aim to attain, which explains why so few studies have reached it and in such little proportions (18,24–26). Despite these low outcomes the PRF outperforms the BC, with 50% up to 60% more pulp sensibility against minus 20% (16,18,24,26). Pulp sensibility is restored in the same length of time with PRF or with PRP: 4.6 month on average with PRF, compared to 4.8 months with PRP. These two scaffolds are more effective matrices indeed : with BC used as a scaffold, pulp sensibility, if it is regained, is restored after an average of 11 month (20) but there are no significant differences. As a matter of fact, the MTA layer on top of the PRF , functions as an insulator, blocking stimuli from reaching the neo-pulp; thus explaining the lack of positive responses to the electrical and cold tests which are used to determine the sensitivity of the pulp. (18,25).

The nature of the neo pulp must also be considered and should be investigated (12).

4.1.3. Effect of L-PRF on growth factor discharge:

PRF-based matrix delivers GF in a longer time period than PRP(13,22). Indeed, the latter releases more GF in the early time (the first few hours) that follows the RET, whereas the PRF-based matrix tends to release its GF after 3 days and onward until reaching a pick at 14 days. PRF also delivers more GF than PRP with a maximum of 11048.19ng/mL compared to 6176.15ng/mL (13).

PRF matrix has a positive effect on cell migration and cell proliferation with better results than PRP (22) on human dental pulp cells. It also stimulates the expression of mineralization genes more than when the BC is used as a scaffold (19).

4.1.4. The impact of centrifuge during L-PRF preparation

Due to the lack of uniformity on PRF manufacturing protocols, all methods differ and employ different centrifugation durations. Three studies (14,15,23) focused on the effect of its parameters on PRF quality. The results are clear: a smaller, lighter but enhanced PRF is produced by lowering, not just the speed, but also the duration of centrifugation. By reducing the speed below 200g, a matrix was produced, more concentrated in leukocytes, platelets as well as TGFβ1, VEGF, PDGF, and EGF (17). GF are more evenly distributed in the low speed matrix (15,23), and directly impact the capacity of fibroblasts to migrate, proliferate and release other GF (14).

Miron et al. (23) further emphasized the impact of the centrifuge tubes on the quality of the PRF. Indeed, the former would affect not just the PRF's capacity to form a clot but also its size and the quantity of GF it contains.

4.2. Case reports

Study (authors and publication year)	Sex and age	Chief complaint	Tooth number	Medication and time	PRP/PRF protocol	Restoration method	Follow-up
Torabinejad M. et al 2011 (27)	Male 9y	Sensitivity to mastication. discoloration	15	2% lidocaine with 1:100000 epinephrine 10mL of 5.25% NaOCl TAP (CF, MIN, MT) for 22days	**PRP:** 20mL blood sample. Centrifuged in the presence of an anticoagulant. No bleeding was induced in the root canal before.	MTA, Cavit and amalgam	5 ½ months
Shivashankar VY. et al. 2012 (28)	Male 9y	Broken upper tooth with discoloration	11	2%lidocaine with 1:100000 adrenaline 20mL of 5.25% NaOCl and 0.2%CHX TAP (CF 500mg, MT 400mg, MIN 50mg) for 21 days	**PRF**: 12mL blood sample 3000rpm for 10 min. No bleeding was induced before. PRF membrane was not cut in fragments	MTA, Cavit then GIC and composite	3, 6, 9, 12 months
Martin G. et al. 2013 (29)	Male 9y	Pain during mastication	46	2%lidocaine with 1:100000 epinephrine 5.25% NaOCl TAP (CF 200mg, MT 500mg, MIN 100mg) for 5months	**PRP**: 10mL of blood sample with 3.8% of sodium citrate centrifuged at 1500rpm for 30min. Bleeding was induced	MTA, IRM then composite	7, 14 and 25 months
Keswani D. et al. 2013 (30)	Male 7y	Pain, trauma	11	2% lidocaine with 1:100000 epinephrine 5.25% NaOCl TAP (CF, MIN, MT) for 3 weeks	**PRF:**5mL blood sample centrifuged at 3000rpm for 10 min. The membrane was then fragmented. No bleeding was induced before	MTA, Cavit, composite	7, 12, 15mo
Jadhav GR. et al. 2015 (31)	Male 16y	Pain in upper front teeth with mobility	21	TAP for 3 weeks	**PRF**: blood sample centrifuged at 3000rpm for 12min	MTA, glass ionomer cement	12 and 18 months
Nagaveni NB. et al. 2015 (4)	Male 10y	Broken maxillary anterior tooth with discoloration	21	2% Lidocaine with 1:100000 epinephrine 5.25% NaOCl	**PRF**: 5mL blood sample centrifuged at 3000rpm for 15min. No bleeding was induced	MTA, Cavit then glass ionomer cement	1, 3, 6, 9, 12 months

				TAP (CF 500mg, MT 400mg, MIN 40mg) for 7days,			
Faizuddin U. et al. 2015 (32)	Male 14y	Broken upper tooth with discoloration	11	2%lidocaine with 1:100000 adrenaline. 5.25%NaOCl, 0.2%CHX TAP (CF 500mg; MT 400mg, MIN 50mg) for 21 days	**PRF:** 10ml blood sample centrifuged at 3000rpm for 10 min. No bleeding was induced.	Gray MTA, Cavit then glass ionomer and composite restoration	3, 6, 9, 12, 14 months
Nagaveni NB. et al. 2016 (33)	Male 11y	Broken upper front tooth	11	2%lidocaine with 1:100000 epinephrine 5.25%NaOCl TAP (CF 500mg, MT 400mg, MIN 50mg) for 1 week	**PRF:** 5mL blood sample centrifuged 3000 rpm for 15 min. The membrane was cut into fragments. No bleeding was induced	MTA, glass ionomer cement.	1, 3,6,9,12 months
Ray HL Jr. et al. 2016 (34)	Male 11y	Trauma	21	0.5% NaOCl, 17%EDTA DAP (CF 200mg, MT 500mg) for 4 weeks 3% mepivacaine without vasoconstrictor	**PRF:** 7mL blood sample spun for 20 min at 402g (3000rpm). The membrane was cut into fragments. Bleeding was induced	MTA, glass ionomer base then composite resin	12, 24, 36 months
Subash D. et al. 2016 (35)	Male 13y	Pain in the left posterior tooth region	37	2% lidocaine with 1:100000 epinephrine 5.25% NaOCl TAP (CF, MT, MIN) for 21 days	**PRF**: 10mL blood sample centrifuged at 3000rpm for 10min. The membrane was fragmented. No bleeding was induced	Biodentine and composite resin	1, 3, 6, 9,12 months
Bakhtiar H. et al. 2017 (36)	Female 9y	Avulsion	21	2%lidocaine with 1:80000 epinephrine 1.5%NaOCl dentin-bonding agent TAP (CF 167mg, MT 167mg, Cefaclor 167mg) for 3 weeks. 3% mepivacaine 17%EDTA	**PRF:**9mL blood sample centrifuged at 2700rpm for 12 min. The membrane was rolled, no bleeding was induced.	Biodentine, glass ionomer cement	1, 3,6, 12, 18months
	Female 18y	Cosmetic complain	12				
	Female 9y	Recurrent swelling	11,21				
	Male 7y	Trauma and intrusion	11				
Meza G. et al. 2019 (37)	Male 50y	Sharp spontaneous dental pain	28	0.12% CHX 2% hydrochloride with 1:80000 epinephrine 1.5%NaOCl	**PRF**: two 10mL blood samples centrifuged at 500g for 12min. The	Collagen membrane, Biodentine, glass ionomer and composite resin	36 months.

				Calcium hydroxide for 4 weeks 3% mepivacaine 17%EDTA	membrane was not fragmented. Bleeding was induced		
Nagaveni NB. et al. 2020 (38)	Male 11y	Pain in the upper front teeth	12 (PRF) 22(BC)	2%lidocaine with 1:100000 epinephrine. 5.25%NaOCl TAP (CF 500µg, MT 400µg, MIN 50 µg) for 7 days	Not discussed	White MTA, glass ionomer cement	1, 3, 6, 12 months
Çalışkan M.K. et al. 2020 (39)	Male 14y	Periapical lesions	12	1.5%NaOCl, 17% EDTA TAP (CF, MT, MIN) for 3 weeks	**PRF:** 5mL blood sample centrifuged at 2400rpm for 12min. The membrane was then fragmented	MTA, composite resin	36 months
	Male 14y	trauma	11				
	Female 13y	Trauma	11				
Nawal RR. et al. 2020 (40)	2 female, 4males, 13-28 years	Fractured or discoloured teeth	Anterior teeth	1.5%NaOCl TAP 17%EDTA	**PRF:** 10mL blood sample centrifuged at 3000rpm for 10min. The membrane was fragmented. No bleeding was induced.	White MTA, glass ionomer cement, then composite	6, 12, 18, 24, 36, 48 and 60 months

Table 4: Data extracted from the included Case report using PRF or PRP

Study (author and publication year)	Tooth number	Asymptomatic	Periapical healing	Percussion/ palpation test	Sensibility (electric or cold)	Apical closure	Root lengthening	Root thickening	Discoloration	Complications/ obliteration
Torabinejad M. et al 2012 (27)	15 (PRP)	+	+	-	+	+	+	+	+	N/A
Shivashankar VY. et al. 2012 (28)	11	+	+	-	+	+	+	+	N/A	N/A
Martin G. et al. 2013 (29)	46 (PRP)	N/A	+	N/A	N/A	+	N/A	+	N/A	+
Keswani D. et al. 2013 (30)	11	+	N/A	-	+	+	+	+	N/A	N/A
Jadhav GR. et al. 2015 (31)	21	+	+	N/A	N/A	+	+	+	N/A	N/A
Nagaveni NB. et al. 2015 (4)	21	+	+	-	+	+	+	+	N/A	N/A
Faizuddin U. et al. 2015 (32)	11	+	+	-	N/A	+	N/A	+	N/A	N/A
Nagaveni NB. et al. 2016 (33)	11	+	+	-	+	+	+	+	N/A	+
Ray HL Jr. et al. 2016 (34)	21	+	+	N/A	-(cold) +(electric)	N/A	+	N/A	+	N/A
Subash D. et al. 2016 (35)	37	+	+	-	+	+	+	+	-	N/A
Bakhtiar H. et al. 2017 (36)	21	+	+	-	-	+	-	+	+	N/A
	12	+	+	-	-	+	+	+	+	N/A
	11,21	+	+/-	-	-	+	+	+	+	N/A
	11	+	+	-	+	+	+	+	+	N/A
Meza G. et al. 2019 (37)	28	+	+	-	-(cold) +(electric)	N/A	N/A	N/A	N/A	+
Nagaveni NB. et al. 2020 (38)	12 (PRF)	+	+	-	+	+	+	+	N/A	N/A
	22 (BC)	+	+	-	+	-	+	-	N/A	N/A
Çalışkan M.K. et al. 2020 (39)	12	+	+/-	N/A	-	N/A	-	-	N/A	N/A
	11	+	+	N/A	-	N/A	-	-	N/A	+
	11	+	+	N/A	-	N/A	-	-	N/A	+
Nawal RR. et al. 2020(40)	21/11	+	+	-	-	+	+	+	N/A	N/A

Table 5: Results extracted from the included case reports.

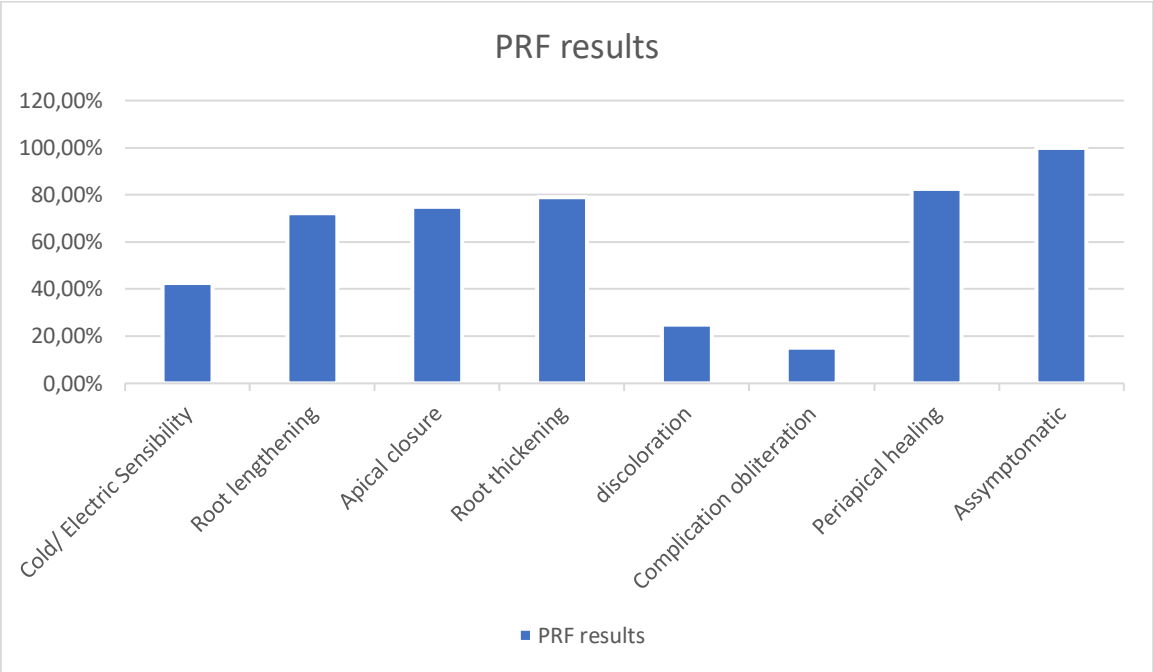

Figure 2: PRF result extracted from the case reports

As shown in figure 2, all cases selected were asymptomatic and most of them showed periapical healing, root lengthening, root thickening and apical closure. Less than a quarter of them showed discoloration, and obliteration of the root canal. 42.5% of all cases recovered sensibility to the cold or electrical test, which is considered a success in RET.

5. DISCUSSION

5.1. REGENERATIVE ENDODONTIC TREATMENT

5.1.1. Overall description:

Regenerative endodontic treatment (RET) is a method that encompasses a group of tissue engineering processes whose aim is to replace injured tooth structures (i.e. pulp-dentin complex, but also root elements) in case of pulp necrosis (3,6,10).

The overall principle behind this concept is the following one: some stem cells of the periodontal ligament (SCAP), of the Herwig's epithelial root sheath and of the epithelial remnants of Malassez survive necrosis and can, under favorable conditions, be reactivated and, ultimately, form "odontoblast-like" cells (4,12,19,28). These cells migrate into the radicular space, in which they differentiate and colonize in order to induce the development of a pulp-like tissue. The organization of this neo-pulp differs from the classical pulp, which is more akin to periodontal ligament.

The aim of this treatment is to allow pulp regeneration and thus to restart root development and apexogenesis in necrotic teeth, and consequently, strengthen them.

RET promotes the migration, proliferation, and differentiation of periodontal ligament stem cells.

This technique differs from other traditional filling procedures as it presupposes the use of biological tissue, which is organic, natural, autologous and bioactive rather than an inert, non-resorbable material (2).

The original protocol consists in the induction of a blood clot (BC) obtained by over-instrumentation of the canals, and thus irritation of the periodontal tissues. The generated BC will act as a matrix for SCAPs colonization and the development of new tissues.

This procedure requires the presence, at the same time of these elements:

1. stem cells capable of generating new tissues,
2. signaling molecules
3. a 3D matrix (2,19,31).

In addition, the matrix must be porous, biodegradable, and able to support and organize cell differentiation (10).

The BC generated by the original technique is an effective matrix. It does, however, have some limitations: on the one hand, its induction is difficult and painful for the patient, and on the other hand, its liquid texture prevents from homogeneously applying restorative materials.

5.1.2. Importance of disinfection management:

The success of RET, with or without Platelet Rich Fibrin (PRF), is above all determined by infection management in the root canal system. Therefore, Triple Antibiotic Paste (TAP) has been recommended, as, TAP contains in most cases ciprofloxacin, metronidazole, and minocycline. However, using TAP may result in a potential discoloration of the crown(27,35,36). It was therefore advised not to apply TAP beyond the cemento-enamel junction (CEJ) (4,28,30,33).) or to solely use ciprofloxacin and metronidazole in a Double Antibiotic Paste (DAP) (2,18,21,24).).The use of DAP should not have any negative effect on the canal's disinfection or on the outcome of the RET.

Disinfection also involves the use of solvents such as sodium hypochlorite (NaOCl). The concentration of NaOCl is not subject to limitations, on the other hand, there is no consensus on its use. The American Association of Endodontist (AAE) recommends a 1.5% concentration for the execution of RET (10), since higher concentrations denature dentin growth factor (GF) and impair SCAP survival and differentiation (21). NaOCl has a proteolytic action on the collagen matrix that lowers dentin's elastic modulus and flexural strength. Furthermore, a high concentration has no significantly greater antimicrobial effects than a 0.5% concentration (18). This recommendation was followed in some cases (1,24–27,39,40), but in others a 5.25% concentration was used (4,20,21,29,30,35,38), which did not appear to have a deleterious influence on the RET outcomes.

To avoid excessively long NaOCl contact with the canal walls and in order to remove potential residues of TAP or DAP, in all studies, the canal was flushed with a 17% ethylenediamine tetra acetic acid (EDTA), as recommended by the AAE (39). EDTA stimulates the release, by dentinal walls, of GF such as VEGF and TGFβ1, which helps not only the proliferation and differentiation of SCAPs, but also the angiogenesis (10). It is therefore contributing to the success of RET.

5.2. PLATELETS CHARACTERISTICS

In order to counteract the above mentioned shortcomings of BC, numerous forms of platelet concentrate have been developed over the last twenty years: they are innovative, endogenous and they increase the regeneration of soft or hard tissue (17). The overall concept is to use human blood proteins as a source of Growth Factors (GF), able to stimulate angiogenesis and tissue growth, based on the idea that blood supply is required for tissue regeneration (7).

Platelets do, in fact, contain 3 to 5 times) more platelets than other scaffolds. Moreover, they release GF, which subsequently stimulates the expression of type 1 collagen in periodontal ligament cells and modulates cell proliferation, attachment, and migration. Finally, platelet concentrates have the capacity to regulate angiogenesis.

5.2.1. Platelet Rich Plasma

Platelet Rich Plasma (PRP) which was one of the first to be used for RET (7,8), contains a platelet concentration of over 1 million/mL. It is five times the typical amount of platelets in a scaffold (16). However, it also includes platelet-derived growth factors (PDGF), transforming growth factors β1 (TGFβ1), and Insulin-like growth factors (IGF). All three of them play an important role in the differentiation and development of periodontal cells. In fact, these molecules can regulate biological activities, induces proliferation, adhesion, migration, and cell differentiation in bone and connective tissue (6).

The preparation of PRP requires a double centrifugation of the freshly collected blood and the addition of firstly anticoagulants, and then of thrombin or calcium chloride, in order to activate the platelet concentrate, and to produce a jelly-like structure through the formation of a fibrin network. This jelly-like fibrin has several benefits. It does, however, have a major disadvantage: it releases the GF it contains too quickly, that is to say in 7 to 14 hours (1,7,10,30) time after which, the amount of GF decreases drastically. Moreover, the addition of thrombin and other coagulation adjuvants might trigger an immunological response and impede the treatment since thrombin and Calcium chloride are known to inhibit wound healing (7).

5.2.2. Leukocyte and Platelet Rich Fibrin

Platelet Rich Fibrin (PRF) also known as Leukocyte and Platelet Rich Fibrin (L-PRF) due to its high leukocytes concentration, has been developed more recently by *Choukroun et al* (9). It differs from PRP in various ways, the most significant of which being its manufacturing protocol. PRF is produced by a single centrifugation and by a progressive polymerization of fibrinogen into fibrin without neither anticoagulants nor thrombin. This gradual and spontaneous polymerization offers the benefit of capturing cytokines, platelets and other functional GF in the fibrin matrix and delivering them more slowly -between 7 to 28 days- during the fibrin network's natural remodeling process (5,9,27,41). Maximal GF release coincides with peak cell proliferation on day 14 (30).

The PRF is composed of 97% of platelets and of more than 50% of leukocytes trapped in the fibrin network (7). The leukocytes play a main role in wound healing and immunological control, as they secrete cytokines such as Interleukin (IL) 1, IL-6, IL-4, and Tumor necrosis factor alpha (TNF-α).

Hence, the PRF encompasses IL-1, IL-6, and IL-4, which are mediators of the inflammation control and are also responsible, not only for the activation of lymphocytes T and B, but also for the recruitment of B cells and the synthesis of fibroblast by fibrinoid collagen. Leukocytes are also a source of Vascular endothelial Growth factor (VEGF), the molecule responsible for angiogenesis and thereby revascularization.

PRF also contains TGFβ1, which stimulates collagen and fibronectin synthesis for tissue repair and angiogenesis, and PDGF, which regulates mesenchymal cell migration, proliferation, and survival. Additionally PRF contains IGF1 and IGF2, which have a chemiotactic function on osteoblast and are apoptotic mediators (6,13,36,42,43). Finally, PRF contains Epidermal Growth Factor (EGF) that stimulates endothelial cells chemiotaxis and angiogenesis. PRF has been reported to have antimicrobial properties, the which have yet to be proven (6,7).

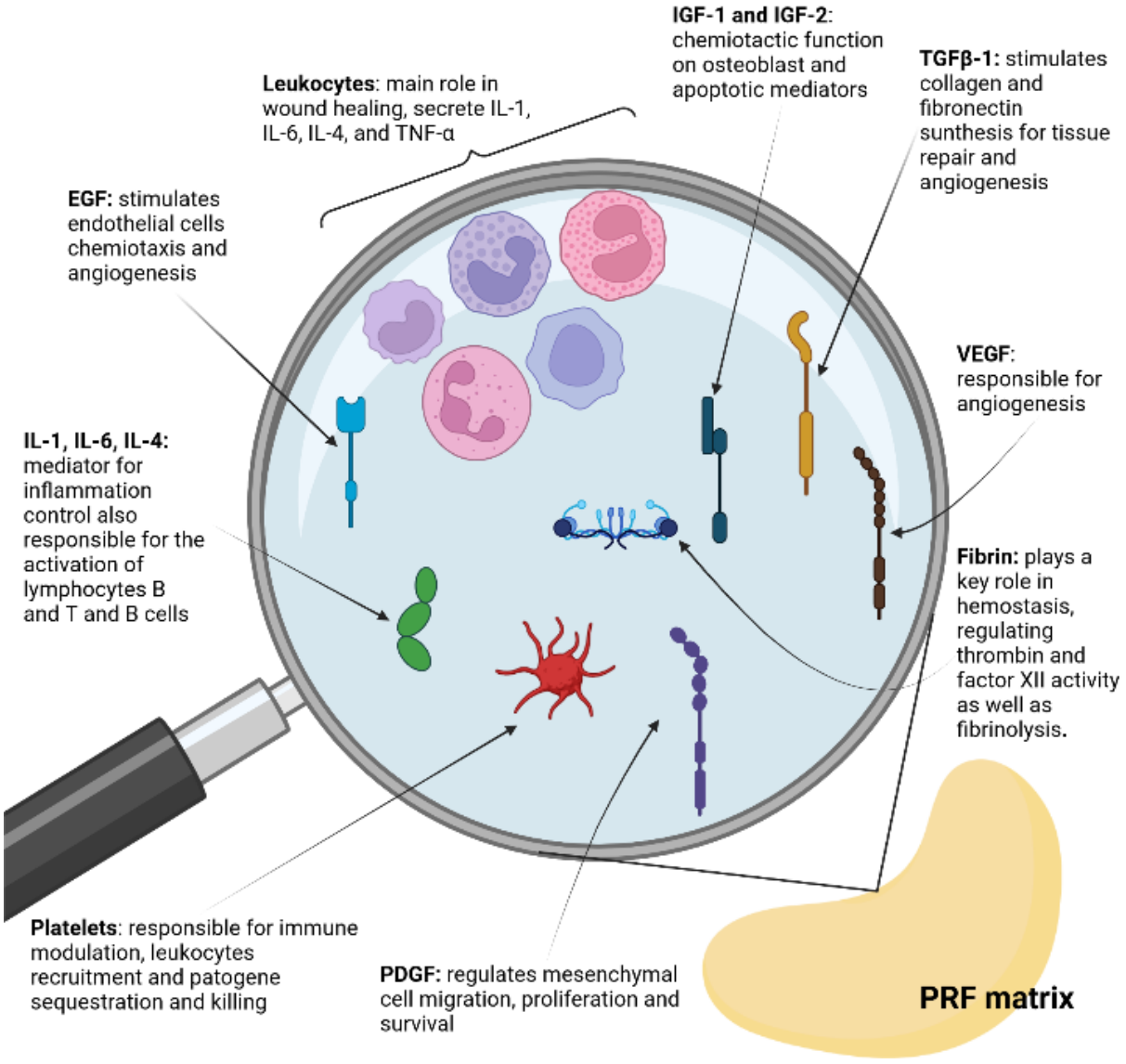

Figure 3: PRF main components (created with Biorender.com)

PRF is also an interposing material as it provides a selective apical barrier, preventing unwanted cells from invaginating and allowing the proliferation of suitable cells (42). A histological analysis of a PRF-treated tooth (29) revealed the existence of disorganized mineral tissue, which can go as far as to obstruct the whole root canal area. This disorganized tissue includes cementoid cells, which coat the dentinal walls and contribute to the closure of the apex, which PRP would not be able to do(29).

Another study published in 2019 by *Chai et al* (22) intended to emphasize the effect of PRP and PRF on pulp cells. The addition of PRF should allow the cells to migrate and differentiate significantly faster than with PRP.

5.2.3. Pro and cons of L-PRF over PRP

There are various advantages of using PRF over PRP. As previously stated, the use of a biochemical adjuvant for blood activation is not required in the production of PRF. This prevents inflammatory responses and protocol mistakes (4,5,8,30).

It is easier and less expensive to produce PRF, it is more effective at inducing and supporting migration, as well as cell proliferation. It also supports the immune system, thank to the presence of numerous leukocytes, and promotes spontaneous healing of supporting tissues as well as bone tissue, by allowing homeostasis, collagen synthesis and angiogenesis (5,8,22,27,41).

Nonetheless, PRF has drawbacks, which are mostly related to its manufacturing and use (5,7):

- Considering that only a very limited quantity of PRF is produced from the blood sample collected, it cannot be used on large wounds.
- Speed and duration of centrifugation have a significant impact on the quality of the PRF produced. It must be processed immediately after blood collection; otherwise, coagulation will occur and the PRF is not to be obtained.
- Its storage is impossible : once the membrane has been created, it must be promptly placed in the canal, otherwise it would get dehydrated .(41).
- The matrix cannot be used on anybody else but the blood donor, except if a compatible receiver is present when the procedure takes place.
- Blood collection can be traumatic for children, who could then not be cooperative during the further steps of the treatment.
- Finally this procedure requires additional training for blood drawing, as well as a specialized and expensive equipment (10).

5.3. L-PRF PROTOCOL

5.3.1. L-PRF obtention

Obtaining PRF is so far, not subject to any strict procedure. The only prerequisite is that PRF must be obtained by implementing high-speed centrifugation of the patient's blood. This centrifugation should last from 10 to 12 minutes, at a speed of 2700 rotations per minute (rpm) or 750 g (7,44). Following the centrifugation, three separate parts are to be observed in the centrifugation tube: platelet poor plasma (PPP), PRF in the form of a membrane and the red blood cells (RBC) (7). Once the PRF membrane has been collected, pressed between two sterile gauzes, (to remove the residues PPP and RBC), it is ready for use.

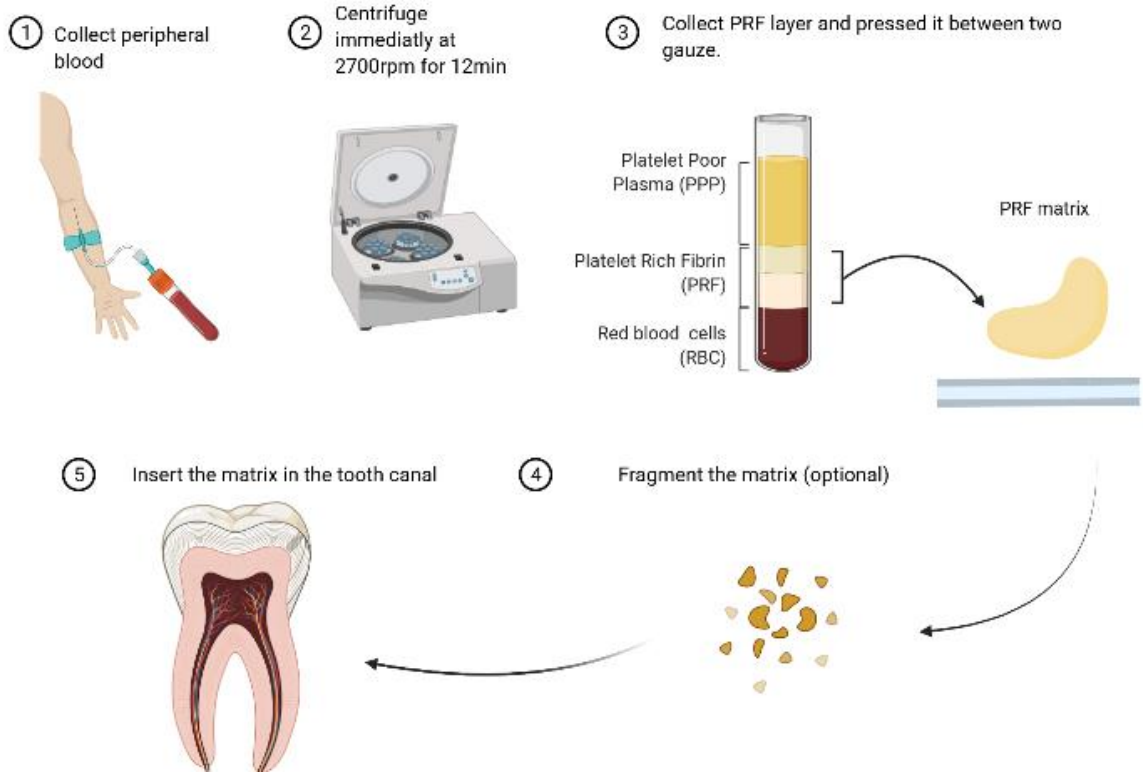

Figure 4: PRF Protocol (created by Biorender.com)

Regarding RET, several protocols are mentioned, some of which leave the membrane as a whole (2,4,17,19–21,28,31,32,37) prior to inserting it into the canal, while others (1,18,25,26,30,34,36,39,40) advise fragmenting it in order to make it easier to place and to reach the apex of the tooth. *Chai et al* (22) also propose using a liquid rather than a solid form of PRF, as it will penetrate more easily and will coagulate in the canal without problems

The induction of a bleeding prior to PRF membrane placement should also play a role in RET. According to *Çalışkan et al* (39) and *Metlerska et al* (10), the BC should remain the reference

matrix, as it contains all the necessary elements for the growth of new cells and as the PRF should only be used as an adjuvant which compensates the BC's limitations and allows better outcomes.

5.3.2. Role of centrifugation

Centrifugation must be conducted shortly after blood collection in order to keep the GFs active and present in the PRF. The centrifugation speed is also a critical factor, as it determines the amount of GFs present in the matrix. Indeed, the rotation speed should push the figured elements of the blood (containing leukocytes and GFs) to the bottom of the tube, where the RBC is located, rather than in the middle third, where the PRF matrix is located (7,14,15,23). Thus, the higher the velocity, the less GFs, and leukocytes the matrix contains. The speed must therefore be adapted to minimize blood layers separation and thus obtain a PRF matrix containing more GF (7,14,15,23). If the velocity of centrifugation is reduced to 208 g, an advanced PRF (A-PRF) could be produced. The A-PRF matrix is more porous: it facilitates cellular penetration into the scaffold and shows a better vascularization (7). The size of the matrix is also linked to the centrifugation speed: the higher the rpm, the bigger and heavier the PRF matrix and the denser the fibrin network formed (23).

Centrifugation duration is also subject to question. *Fujioka et al* (14) in their study, compared the influence of speed and duration on the quantity of GF released from the PRF matrix. They emphasized that these two parameters had a significant influence, not only on GF release, but also on their ability to migrate, differentiate and proliferate, and according to them only low centrifugation PRF based matrices should be used.

The type of both centrifuges and tubes involved in the process of PRF production is another important parameter to consider. *Miron et al* (23) discovered that, despite identical machine calibration, depending on the brand used, the PRF contained different quantities of platelets and/or leukocytes but also the release of GF fluctuated. The type of tube, in which the collected blood is placed, influences not only the size of the PRF, but also, its ability to form a coagulate.

5.4. L-PRF IN REGENERATIVE ENDODONTIC TREATMENT

The PRF appears to be the best candidate for RET, due to its physical characteristics and GF-enriched constitution. All studies demonstrated an improvement in patients' symptomatology in terms of pain and peri-apical lesions. The vast majority of studies showed apical closure, increased root size and thickening of the dentinal walls. Some PRF coating even restored sensitivity of the necrotic tooth to cold and/or electrical tests (4,19,40,30,33–39).

Several explanations have been proposed to explain the restored vitality (18,25,26):

- PRF is enriched in GF, such as TGβ1, PDGF, IGF-1 and VEGF, which are involved in neurogenesis and can stimulate the differentiation, growth and maturation of fibroblast, odontoblast, and cementoblast.
- Remaining apical nerve cells could have survived and subsequently multiplied and differentiated in dental pulp nerve cell.
- Periodontal ligaments stem cells could have the ability to differentiate in nerve cells and colonize the root canal.
- Depending on the circumstances, bone marrow stem cells could develop into astrocytes or neurons.
- The implantation of PRF beyond the apical foramen could have triggered the transplantation of SCAP into the canal lumen, where they remained resistant to infection and maintained their capacity to proliferate and differentiate into odontoblast.

Many studies (4,35)) have selected mineral tri-aggregate (MTA) as a cementum as it provides signaling molecules for the formation of SCAPs (32). Due to the fact that it can produce crown discoloration it should be placed directly over the PRF and under the CEJ (18,21,28,36). With the PRF, it leads to the formation of a coronal and apical barrier, resulting in apical closure. This MTA layer may be responsible for the lack of positive response to the vitality tests. Indeed, new hard tissue growth may inhibit stimuli from reaching the neo pulp (1,4,18,25,26,28). Despite this negative response, the observation of apex closure, root elongation, dentinal wall thickening, and regression of periodontal lesions indicate the presence of functioning pulp tissue. Thus, a negative response does not always imply that the tooth is non-vital, just as a positive response does not always indicate pulp regrowth(26).

6. LIMITS:

Our present study, however, has encountered several limitations. Firstly, the search was done using Mesh ((Medical Subject Heading) terms, which limits the number of articles available to those referenced as Mesh. In addition, our study included articles written solely in English during the last 10-year.

Secondly, the protocol for performing PRF, medication, restauration system or even follow-up (Table 3 and 4) is not identical throughout the referenced studies. Furthermore, the age of the patients included in the researches is subject to major fluctuations impacting the studies results. Younger patients, with immature teeth, are more likely to have better results: as teeth are still forming, they contain more stem cells. As these SCAPs are highly reactive, differentiation and cell migration are more likely to occur than in mature teeth containing fewer SC (24,26). As there are few studies on mature teeth, additional study on the effectiveness of RET involving adult patients is needed.

Moreover, contacts were lost with some of the patients during the follow-up period of several studies (16,20). This fact significantly impacts the results as the groups involved are small (around 20 patients per group).
All of these variables must be considered while evaluating the results, as the purpose of RET is affected. The articles' results differ; some (1,21,31) propose to maintain BC or PRP as the material of choice for RET, while others (2,19,24,25) advocate that PRF is the best currently available scaffold. However, most authors (12,13,16,20,26) agree that, regarding the sole application of them, there is no significant difference between PRP or PRF.

Furthermore, low-speed centrifugation is still in its very early use, there is only a limited number of clinical studies on its influence on wound healing.

There appears to be a need for further studies considering these many aspects in order to truly and properly compare the use of PRF, PRP and BC as scaffolds in RET.

7. CONCLUSION

Within the limitations that we observed, our study has shown that PRF has positive effects on RET, making it a serious alternative to classical obturation techniques, but also to other scaffolds, such as PRP and BC. The reviewed articles showed, indeed, that PRF is a valid scaffold for revascularization treatment on both mature and immature teeth, as it leads to the disappearance of all clinical and radiographic symptoms and induces cell proliferation and differentiation.

In immature teeth, it allows root lengthening, dentinal walls thickening, apical closure and sometimes, restoration of pulp vitality. Despite a lack of studies on mature teeth, we are able to assess that PRF has a positive impact on their revitalization, even if the results are more subtle. Indeed, once the root maturation and apex closure have been completed, the observation of changes on these two parameters is less than obvious.

The formation of the PRF matrix is sensitive to both centrifuge protocols and duration or speed changes can alter the whole matrix organization and hence the outcome of the treatment in progress.

It seems necessary to carry out new studies with longer follow-up period. Moreover, searches regarding the revascularization potential of L-PRF on mature teeth have only recently begun, as a results, further studies are needed to truly assess L-PRF regenerative potential on mature teeth. Additional searches are required to confirm PRF true effectiveness and superiority over PRP and BC.

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