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Clinical evidence in regenerative endodontics: platelet-rich fibrin in immature non-vital permanent teeth – "narrative review"

Universidade Fernando Pessoa

Faculdade de Ciências da Saúde

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Trabalho apresentado à Universidade Fernando Pessoa como parte dos requisitos para obtenção do grau de Mestre em Medicina Dentária

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Abstract

Objectives: This integrative review aims to understand the influence of PRF membrane on immature non-vital permanent teeth and the pros and cons of employing PRF in current and future regenerative endodontics.

Methods: PubMed, B-on and Science Direct search with eligibility criteria (written in English, Italian, Spanish, or Portuguese; publication date between 2013-2023, full open access to articles on the topic and clinical and *in vitro* studies.

Results: From 1653 articles in the databases, 4 were selected for analysis of the respective objectives, materials and methods and results obtained.

Conclusions: Although further scientific evidence is needed, PRF promotes dentinal wall increase, root elongation and apical diameter decrease in necrotic immature permanent teeth. The future of conservative endodontics is promising through the use of autologous materials.

Keywords: "endodontics", "platelet-rich fibrin", "dental pulp stem cells", "revascularisation", "odontoblastic differentiation", "reparative dentin"

Resumo

Objetivos: Esta revisão integrativa tem como objetivos entender a influência da membrana PRF em dentes permanentes imaturos não vitais e os aspetos a favor e contra o seu emprego na endodontia regenerativa atualmente e futuramente.

Métodos: Pesquisa na PubMed, B-on e Science Direct com critérios de elegibilidade (escrita na língua inglesa, italiana, espanhola ou portuguesa; data de publicação entre 2013-2023, acesso total livre aos artigos do tema e estudos clínicos e *in vitro*).

Resultados: Partindo de 1653 artigos nas bases de dados, 4 foram selecionados para análise dos respetivos objetivos, materiais e métodos e resultados obtidos.

Conclusões: Embora uma maior evidência científica seja necessária, PRF promove o aumento das paredes dentinárias, alongamento radicular e diminuição do diâmetro apical em dentes permanentes imaturos necrosados. O futuro da endodontia conservadora é promissor através do uso de materiais autólogos.

Palavras-chave: “endodontics”, “platelet-rich fibrin”, “dental pulp stem cells”, “revascularisation”, “odontoblastic differentiation”, “reparative dentin”

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List of abbreviations

% – percent

Mm – millimetres

Ml – millilitres

Min – minutes

G – gram

Rpm – rounds per minute

MESH – medical subject healings

MTA – mineral trioxide aggregate

NaOCl – sodium hypochlorite

EDTA – ethylenediaminetetraacetic acid

TAP – triple antibiotic paste

RET – regenerative endodontic therapy

BC – blood clot

PRP – platelet-rich plasma

PRF – platelet-rich fibrin

A-PRF – advanced platelet-rich fibrin

I-PRF – injectable platelet-rich fibrin

L-PRF – leucocyte platelet-rich fibrin

CEJ – cemental junction

PAI – periapical index

RRA – radiographic root area

RL – root length

ASA – American society of anaesthesiologist

AAE – American association of endodontics

CBCT – cone beam computer tomography

IL-1 – interleukin 1

IL-4 – interleukin 4

IL-6 – interleukin 6

IGF-1 – insulin-like growth factor 1

IGF-2 – insulin-like growth factor 2

EGF – epidermal growth factor

FGF – fibroblast growth factor

TGF-β – transforming growth factor β

TGF-β1 – transforming growth factor β1

TNF-α – tumour necrosis factor α

VEGF – vascular endothelial growth factor

PDGF – platelet-derived growth factor

pH - hydrogenionic potential

I. Introduction

An immature tooth is a dental piece whose root has not completed its proper development, thus lacking the apical cemento-dental junction. Conditions such as extensive dental caries, dental trauma and developmental abnormalities can lead to necrosis of immature permanent teeth (Morankar *et al.*, 2023). Pulp necrosis, in this condition, entails several repercussions, including short root, thin walls and inadequate crown-root relationship. Thus, in the face of the adversities, the non-vital immature tooth presents a challenge to clinical endodontic practice due to the difficulty in achieve an effective apical seal though conventional endodontic treatment.

Several methods have been used in the management of these teeth, namely, apexification with calcium hydroxide, apexification by apical sealing with mineral trioxide aggregate (MTA) and microsurgical endodontics. However, these traditional therapeutic options do not promote dentin wall thickening and increased root length, which eventually increases susceptibility to tooth fracture (Jayadevan *et al.*, 2021).

According to Arshad *et al.* (2021), as an alternative to these traditional methods, regenerative endodontics encompasses a set of techniques that provide relief from clinical symptoms, promotion of increased root length and thickness of canal walls, simultaneously to stimulating healing and repair of the pulp-dentin complex.

Among the promising regenerative procedures, platelet-rich fibrin (PRF), first described by Choukroun *et al.* in 2001, consists of an autologous complex that includes platelets, grown factors and cytokines that, when inserted into the necrotic immature tooth, can promote cell proliferation and differentiation, continuous release of grown factors and formation of tertiary dentin by stimulating odontoblasts. Furthermore, according to Arshad *et al.* (2021), due to its constituents, it assists in angiogenesis which is shown to be fundamental in the revascularization process.

Over the years, several authors have studied the applicability of second-generation platelet concentrates in regenerative endodontics with the aim of understanding their efficiency in revascularization of necrotic immature permanent teeth.

Contradictory results have been reported, so the focus of this integrative literature review is to answer the following questions:

a. "What is the influence of PRF on necrotic immature permanent teeth?"

b. “What are the pros and cons of employing PRF in current and future regenerative endodontics?”

1. Materials and methods

1.1. Methodology

To prepare this integrative review, a literature search was conducted in three databases – PubMed, B-on (online knowledge library) and Science Direct – in order to identify all *in vitro* and clinical studies on platelet-rich fibrin in immature non-vital permanent teeth.

Initially, the key words and Medical Subject Healings (MESH) terms, listed below, were strategically selected, and combined using the Boolean operators “AND” and “OR”. The combinations used in each electronic sources are listed in Table 1.

- “Platelet-rich fibrin” [Mesh]
- “Regenerative endodontics” [Mesh]
- “Tooth, non-vital” [Mesh]
- “PRF”
- “Immature permanent teeth”
- “Endodontics”

Table 2. Search strategy used in each database.

Database	Search strategy
PubMed	- “Platelet-rich fibrin” [Mesh] AND “Regenerative endodontics” [Mesh] - “Tooth, non-vital” [Mesh] AND “Platelet-rich fibrin” [Mesh] OR “Regenerative endodontics” [Mesh] - “PRF” AND “Immature permanent teeth”
B-on	- “PRF” AND “Immature permanent teeth” AND “Endodontics”
Science Direct	-“Platelet-rich fibrin” [Mesh] AND “Regenerative endodontics” [Mesh]

	- "PRF" AND "Immature permanent teeth" - "Immature permanent teeth" AND ""Regenerative endodontics" [Mesh] - "PRF" AND "Endodontics" OR "Regenerative endodontics" [Mesh]

Based on the aforementioned search strategy, a total of 1653 articles were found in the selected databases.

1.2. Eligibility criteria

Secondly, in order to specify the type of article for further analysis, eligibility criteria – inclusion and exclusion criteria - were defined (table 2).

Table 3. Eligibility criteria.

Inclusion criteria	Exclusion criteria
- Language: English, Italian, Spanish, and Portuguese - Time schedule: from 2013 to 2023 - Type of study: clinical studies and *in vitro* - Access: articles in their entirety that portray the theme concerning the work	- Language: any language other than English, Italian, Spanish, and Portuguese - Time schedule: below 2013 - Type of study: any type of study other than clinical studies and *in vitro* - Access: articles for which access is not completely free

1.3. Data collection

After defining the inclusion and exclusion criteria, the eligibility criteria were applied to the 1653 articles identified in the first step. In a chained manner, the duplicates were removed so that, subsequently, the remaining articles were selected based on the language, time schedule and access. After this selection, the titles of the identified articles were read to understand which ones would meet the inclusion criteria and which ones would meet the exclusion criteria. More specifically, in a penultimate step, the remaining papers were screened by reading their abstract. The remaining papers were analysed in their entirety to understand which ones would be included and which ones would be excluded. After the full text of the previously selected

articles were analysed, from the literature search database used in the present review, a total of 4 articles fit the eligibility criteria as illustrated in figure 1.

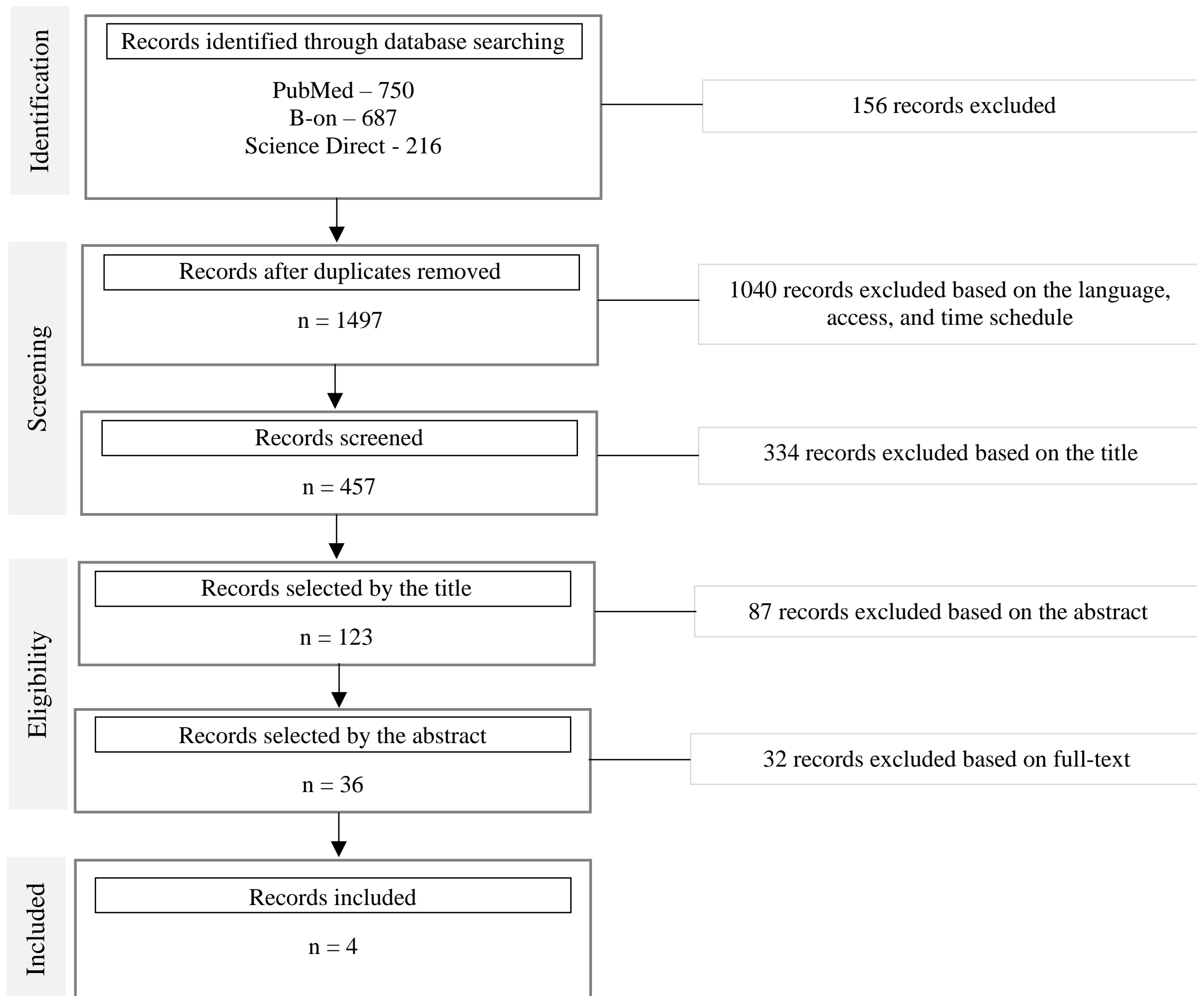

Figure 3. PRISMA flowchart for identification, selection, and inclusion of articles

II. Development

1. Results

Given the integrative literature search as completed, 4 final articles were selected for careful analysis. In order to explore the scientific evidence on second-generation platelet concentrates known as PRF, the objectives of each study are described below, as well as the materials and methods employed and their results, for further discussion.

1.1. Wang *et al.* (2016)

Wang *et al*. in 2016 performed an *in vitro* study whose objective was to evaluate the use of PRF in immature permanent teeth as a regenerative therapy. Bearing this aim in mind, they selected

a total of 10 immature bi-root premolars in order to establish the model of pulpless immature permanent teeth. Pulpectomy was performed on all teeth using the following steps – exposure of the pulp of every single tooth followed by manually shaping and disinfection through a set of irrigators (20 ml (millilitre) 5.25% (percent) NaOCl (sodium hypochlorite), 5 ml 17% EDTA (ethylenediamine tetraacetic acid) and 5 ml of physiological saline for a final rinse) and drying of these same canals. Given pulpectomy as complete, they then proceeded to the PRF clot's attainment. Briefly, 9 ml of blood sample was drawn and placed into test tubes so that it could be centrifuged. After this, the 10 teeth were divided into 2 groups:

- Group A: 2 teeth on which only pulpectomy was performed (positive control)
- Group B: 8 teeth on which was applied PRF as a scaffold
  - Sub-group 1: 1 canal of each 8 bi-root teeth was left empty (negative control)
  - Sub-group 2: 1 canal of each 8 bi-root teeth was filled with PRF clot

Finalizing the procedure, PRF clot was applied into sub-group 2 of group B and all the bi-root teeth were sealed with MTA plus glass ionomer cement.

Every 2 weeks after, 3 clinical assessments were evaluated such as tooth mobility, gingival condition, and restoration retentions. Moreover, radiographic records were taken of the preoperative, immediate postoperative and 12 weeks postoperative. Reaching 12 weeks post procedure, the 10 teeth underwent a set of procedures in order to be prepared to be analysed under microscope using haematoxylin-eosin as a stainer. To determinate if there were any significant differences between the groups, a level of significance was set at 5 % ($p < 0.05$).

The results obtained were separated into four different categories:

A. Clinical and radiographic outcomes
- Group A: a normal root development, thickness and elongation was observed;
- Group B sub-group 1: 2 out a total of 8 roots showed an increase of lateral dentin wall
- Group B sub-group 2: a diffuse increase in canal wall density in the apical third was noted and 7 out a total of 8 roots showed an increase of lateral dentin wall

B. Histological outcomes: all teeth's canal space of group B sub-group 2 have demonstrated 2 types of newly formed mineralised and vital tissues – one adhering to the dentin wall and another forming bone-like mineral tissue in the connective tissue, which filled the root canal with blood vessels and fibroblast-like cells;

C. Dentin-associated mineral tissue: although the thickness of canal walls has increased, the dentin associated-mineral tissue formed was different form the tertiary dentin having a

disorganised structure and embedded cells. In addition to this, the new dentinal tubules formed in group B sub-group 2 appeared to be irregular structures with rough inner walls and disconnected from the pre-existing tubules;

D. Connective tissue and bone-like mineral tissue: in terms of connective tissue, the cells that settled along the dentin-associated mineral tissue were different from odontoblasts found in normal roots in terms of polarisation, notably, poorly polarised, and non-palisade pattern-like arrangements. Relative to bone-like mineral tissue analysed under microscope, around the apical foramen and the outside of the bone tissue was incorporated with typical bone mineral tissue. However, in almost all the roots a bone island mineral with entrapped cells was found.

1.2. Jayadevan *et al.* (2021)

Throughout a prospective clinical study, Jayadevan et al. in 2021 compared A-PRF (advanced platelet-rich fibrin) and PRF as scaffolds in traumatized immature non-vital teeth with the aim of, simultaneously, evaluate its effect on periapical healing and root development. Based on a careful selection listed below, 30 traumatized immature non-vital maxillary incisors in 28 patients aged between 8 and 27 years have been put on treatment.

- Inclusion criteria: immature single-rooted non-vital teeth, apical width greater than 1 mm, presence or absence of periapical lesion and trauma as an etiology of pulp necrosis.
- Exclusion criteria: teeth with dental and periodontal conditions such as mature apex, resorption (internal or external), mobility, ankylosis, root fracture, periodontal pocket and unrestorable structure.

A total of 30 teeth were randomly allotted into two groups:

- Group A: 15 teeth treated with A-PRF as a scaffold
- Group B: 15 teeth treated with PRF as a scaffold

In both groups the teeth were submitted to a regenerative procedure consisting of 2 appointments and performed by the same endodontist. The protocol was in accordance with the AAE (American Association of Endodontists) guidelines. Thus, the first visit started with local anaesthesia with 2% lidocaine administration and absolute isolation in order to access pulp canal. Since disinfection is fundamental in these teeth, a gentle irrigation protocol was deployed using 20 ml of 1.5% NaOCl, saline solution and 20 ml of 17% EDTA, in this order respectively.

The canal was then dried, and TAP (triple antibiotic paste) was introduced into canal space as intracanal medication. Temporarily, cavit restoration was used to seal the access cavity over 4 weeks. Once the indicated time had elapsed, a second visit was scheduled to perform the RET (regenerative endodontic treatment). It should be highlighted that only asymptomatic teeth were included for this second round of treatment. Therefore, local anaesthesia was administered, in this case without vasoconstrictor. Once again, just like the first appointment, gentle irrigation was performed via saline and 20 ml 17% EDTA and then paper points were used to dry the canals. Bleeding was then induced in the root canal system to collect blood from the apical area up to the level of the CEJ (cementoenamel junction). Once the tooth has been prepared to be loaded with the scaffold, A-PRF and PRF were obtained following these instructions:

- A-PRF (group A): 10 ml blood sample into test tube without anticoagulant, centrifugation at 1500 rpm for 14 minutes and remotion of A-PRF from the tube followed by squeeze to obtain the membrane
- PRF (group B): 10 ml blood sample into test tube without anticoagulant, centrifugation at 2700 rpm for 12 minutes and remotion of PRF from the tube followed by squeeze to obtain the membrane

Up to the moment of its application inside the canal, A-PRF or PRF was placed into it depending on the group. Over the scaffold, a cap of biodentine and glass ionomer cement were gently placed.

Regarding to follow-up evaluations, patients were reviewed after 6 and 12 months to check up. Four parameters were taken in consideration during the follow-up – periapical status, root length, dentin thickness and apical response. To rate the periapical status, 3 categories were used based on PAI (periapical index) scores: (a) Healed which means clinical and radiographic were normal (PAI 1 or 2), (b) Healing which means normal clinical but not total healing radiographically although diminished in size (PAI score 3 or 4) and (c) Diseased which means clinical symptoms/signs or normal clinical but there was persistence or enlargement of the periapical radiolucency radiographically (PAI score increased or unchanged). To measure the root length, an average of 2 straight lines from the CEJ to the midpoint of the apex of the root – one from the mesial point and another from the distal point – were performed. Through the subtraction of pulp space width from the total root width, root/dentin thickness was measured. Concerning to apical response, it was evaluated according to Chen *et al.* (2012) classification – type 1 (increased thickening of the canal walls and continued root maturation,), type 2 (no

significant continuation of root development with the root apex becoming blunt and closed), type 3 (continued root development with the apical foramen remaining open), type 4 (severe obliteration of the canal space) and type 5 (hard tissue barrier formed in the canal space between the coronal biodentin plug and the root apex). In order to know if there was significant difference in these parameters cited above between group A and B, statistical significance was set at 5 % ($p < 0.05$).

According to Jayadevan *et al.* (2021)**,** the results are as follow: both scaffolds considerably improved periapical healing; A-PRF improved greater root thickness while PRF improved greater root length; no significant difference were found about the type of apical response among the groups ($p > 0.05$).

1.3. Kritika *et al.* (2021)

Aiming to evaluate the regenerative potential of PRF in necrotic immature permanent maxillary central incisors, Kritika *et al.* (2021) present to the literature a prospective clinical study. For this purpose, they initially selected a total of 25 patients and established eligibility criteria, which wera as follows:

- Inclusion criteria: age from 9 to 25 years old; apical diameter greater than 1.1mm; patients rated as 1 or 2 according to American Society of Anaesthesiologist (ASA) classification; teeth with signs and/or symptoms such as pain, swelling and sinus opening and patients whose teeth have or do not have periapical pathosis (scale 2 according to the periapical index).
- Exclusion criteria: age greater than 25 years; patients ASA III/IV/V/VI; teeth with periapical index greater than 2, root resorption or fracture, closed apex or carious exposure, apical diameter lesser than 1.1mm, non-restorable or periodontally compromised teeth and teeth other than maxillary central incisors.

Bearing in mind the criteria cited above, out of the 25 initial patients, only 19 patients (23 teeth) were included in the study. The goals were set based on the AAE guidelines – primary objectives (elimination of the symptoms simultaneously with bone healing and resolution of apical radiolucency after 6-12 months from treatment), secondary (increase in root length, root dentin thickness and decrease in apical diameter after 12-24 months from treatment) and tertiary (positive pulp vitality response).

Regarding the treatment employed, in a first appointment after initial clinical and radiographic report, all teeth underwent a process of anaesthesia without vasoconstrictor undergoing minimal instrumentation under absolute isolation while the canal was irrigated using 10ml of 1% NaOCl. Afterwards, the canals were then dried and a triple antibiotic paste – ciprofloxacin, metronidazole, amoxicillin - was administered. The pulp chamber was temporarily restored, and a radiograph was taken. Three weeks later, in a second appointment, the PRF was prepared by the following protocol:

a. 10 ml of whole blood was drawn from the median cubital vein and collected in a sterile test tube
b. Centrifugation at 3300 rpm for 12 min at 400 g force
c. PRF membrane was placed between 2 sterile gauze pieces and squeezed

After attaining the PRF membrane, teeth were anaesthetised with anaesthesia without vasoconstrictor. The temporary restoration was then removed as well as the TAP. In a row, canal irrigation was done using 10 ml of 1% NaOCl followed by 5 ml of 17% EDTA. The canal was dried and the PRF was placed inside the canal – one piece placed 1 mm beyond the working length and the other one coronally just below the CEJ. MTA plug was packed coronally over the PRF scaffold, the pulp chamber was sealed with glass ionomer cement and the tooth was restored with composite resin.

Thenceforth a routine follow-up was carried out, being it at 3, 6, 9, 12, 15, 18-month intervals and for a period of 24 months until it reaches a full 2-year follow-up. For all patients, in each follow up, 6 parameters were evaluated – apical diameter, root length, thickness of mesial dentinal wall (apical third), thickness of distal dentinal wall (apical third), thickness of mesial dentinal wall (middle third) and thickness of distal dentinal wall (middle third).

All the parameters cited above were set at zero for standardisation to understand whether the values have increased or decreased and subsequent comparison. To detect the statistically significant difference a couple of tests were employed – the Friedman test followed by the Dunn post hoc test with Bonferroni correction. The p-value was set at 5% ($p < 0.05$).

The results are as follow ones:

1- Primary goals were achieved in all the patients;

2- Out of a total of 7 cases with apical lesion, in 5 cases were observed a complete bone healing and resolution of apical radiolucency and in 2 cases substantial evidence of it. That means 90% of the cases were success;
3- Regarding the secondary objectives, the increase in the apical third was achieved from 12 months. However, the middle third enlargement was only achieved from 18 and 24 months onwards. Apical diameter decreased from 12 months;
4- In relation to tertiary goals, sensibility tests were found to the negative during the total follow up period.

1.4. Yoshpe *et al.* (2022)

Through a retrospective clinical study, Yoshpe *et al.* (2022) set out to understand the impact of regenerative endodontic procedures, namely the application of PRF on immature non-vital permanent anterior and posterior teeth.

To accomplish this goal, fifty immature teeth from 45 patients aged between 7.6 and 16 years (23 males and 23 females) were selected based on some inclusion criteria:

- Dental trauma as an etiology of pulp necrosis in anterior teeth or deep caries as etiology of pulp necrosis in posterior teeth with or without periapical lesion in both
- Roots partially developed – Cvek's class from 3 to 5, which means, at least two thirds formed root

It is important to highlight that all procedures were performed by the same endodontist and the protocol used was based on the AAE's recommendations. In this context, at the first appointment, teeth were anaesthetised using 2% lidocaine followed by absolute isolation. In a row, irrigation with 4% NaOCl combined with minimal instrumentation was carried out. To conclude the first consultation, TAP (metronidazole, cefuroxime axetil and ciprofloxacin) was dressed into the teeth for 14 to 28 days. Post TAP's application time, a second appointment was held in which the PRF was applied. To do so, initially, a non-vasoconstrictor anaesthetic was applied to all teeth under treatment and the final irrigant was injected into canals – 20 ml of 17% EDTA. After getting the teeth in readiness to receive the PRF, its preparation was carried out by the following order:

a. 49 ml of whole blood was withdrawn from the median cubital vein and collected in a sterile test tube
b. Centrifugation at 1300 rpm for 8 min

c. PRF membrane was sectioned into small pieces

After attaining the PRF membrane, bleeding was induced into the root canal and PRF was condensed into the full root length. Over it, collagen plug was condensed in all the teeth. Distinctively, posterior teeth were restored with MTA, glass ionomer and composite resin while anterior teeth were restored with biodentine and glass ionomer. A follow-up period was settled out between 12 and 38 months. Hence, 5 evaluations/relations have been performed:

- Treatment's effect on relative increase in radiographic root area (RRA) and root length (RL);
- Relative increases in RL and dentin area;
- Mean increases in RL or dentin area in the anterior versus posterior roots;
- Root closure status and anterior/posterior root location;
- Relative increases in the dentin area or RL with root closure status (closed, incomplete, unchanged)

In terms of results, the researchers of this study founf that 95% roots increase in RRA and 86% in length whereby anterior roots' dimensions were larger than the posterior ones. Beyond this, relation between RRA and RL was observed. Concerning to mean increases in RL and RRA in posterior and anterior roots, it was observed in 11%, 39%, 6%, 26%, respectively. Regarding to apical closure status and type of tooth, a significant association was observed as follows – 89% posterior teeth and 30% anterior teeth (24% of anterior's apical diameter didn't change).

2. Regenerative endodontics

2.1. Historical overview and regenerative endodontics definition

Over the past decades, several methods have been developed for the therapeutic purpose of necrotic teeth with open apexes – calcium hydroxide apexification, MTA apexification and surgical endodontics. Relating to the first technique, although it has a potential to induce osteodentin barrier formation, calcium hydroxide apexification requires a long period (6 to 24 months) treatment and observation, since several sessions are necessary to achieve the goal of this therapy – closed apex. Moreover, besides the risk of root canal system's recontamination, achieving an apical limit is difficult and increases susceptibility to tooth fracture due to this risk of proteolysis and dehydration it entails (Boufdil *et al.*, 2020 and Morankar *et al.*, 2023).

Concerning to MTA apexification, introduced in the 1990s, according to Altan and Tosun (2016), long setting time (3 to 4 hours) is considered as the most important disadvantage. Although it is a single-session treatment, it does not stimulate root elongation and thickness increase (Morankar *et al*., 2023) leading to the root fragility (Boufdil *et al*., 2020). Regarding surgical endodontics, this treatment solution provides, according to Morankar *et al.*, 2023, an inadequate crown-root relationship since, as in a similar manner as the two traditional techniques mentioned above, root development is not provided.

According to Machut *et al.* in 2021, as a prevention of tooth fracture and future loss of it, tooth revascularisation consists of the replacement of damaged dental tissues, including dentin, pulp-dentin complex and root structures, with similar cells, thus generating the design of a new healthy tissue.

Therefore, based on the concept of revascularisation, regenerative endodontics presents itself to contemporary dentistry as a set of procedures whose aim is to stimulate and repair the pulp-dentin complex, preventing the instrumentation associated with conventional endodontic treatment (Arshad *et al.*, 2021). As a promising method, pulp regeneration promotes healing through neovascularisation and the mediation of growth and development of the constituent tissues of the tooth.

Being firstly described by Nygaard-Ostby *et al.* in 1960s and applied by Iwaya *et al.* in 2001 into immature permanent teeth, regenerative endodontic therapy is aimed to regenerate the pulp-dentin complex damaged by trauma, infection, or developmental anomaly of non-vital immature permanent teeth (Wei *et al.*, 2022).

2.2. Biological basis

According to Saber, 2009, tissue engineering is understood as the science whose aim is to design and manufacture new tissues in order to replace impaired or damaged ones. Once regenerative endodontic focuses on the stimulation and repair of the dentin-pulp complex as mentioned above, this therapy is based on the tissue engineering. From this point forward, as a mean to achieve the effectiveness of RET, three main components (tissue engineering's principles) are required including an appropriate source of stem cells, growth factors and an extracellular matrix scaffold (figure 2).

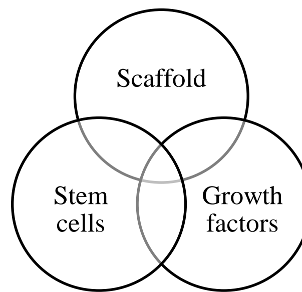

Figure 4. Principles of tissue engineering

By the way of stem cells, they are considered the most valuable cells for regenerative endodontics owing to their ability to differentiating into the desired tissue component. In the oral environment assorted sources of stem cells are found such as tooth germ progenitor cells, dental follicle stem cells, salivary gland stem cells, stem cells of the apical papilla, dental pulp stem cells, inflamed periapical progenitor cells, stem cells from exfoliated deciduous teeth, periodontal ligament stem cells, bone marrow stem cells, oral epithelial stem cells, gingival-derived mesenchymal stem cells and, last but not least, periosteal stem cells. As a potential onset of stem cells in reference to RET, dental pulp, apical papilla and inflamed periapical tissue are demonstrated to be essential sources of it (Saber, 2009).

In proportion to growth factors, these act as a stimulus to take place the differentiation of stem cells into a number of cell phenotypes. Thus, according to Hargreaves *et al.*, 2013, growth factors is a critical component in regulating tissue differentiation.

Finally, yet importantly, a scaffold is a three-dimensional structure that aids cellular arrangement and favours vascularisation. Besides performing that, scaffolds play as a key concerning to the regulation of stem cell differentiation. This occurs through the local release of growth factors or by the signalling cascade triggered when those cells bind to the scaffold and to each other. (Saber, 2009 and Hargreaves *et al.*, 2013).

2.3. Therapeutic options

In the field of RET, dissimilar procedures have been reported in the literature, for case in a point, utilisation of blood clot (BC), pulpal implantation, stem cell utilisation, scaffold implantation, injectable scaffold and so on.

Regarding BC, according to Bakhtiar *et al.* (2017), this technique is based on inducing bleeding from the periapical area with the purpose of promoting the migration of periapical cells, including mesenchymal stem cells, into the sterile root canal system. Although it is an affordable and effective technique, according to the authors cited above, excessive force to obtain bleeding may eventually cause damage to the Hertwig's epithelial sheath which is a crucial element for the root development intended with RET.

With the advances made in this area, 2 mainly platelet concentrates were developed entitled platelet-rich plasma (PRP) and platelet-rich fibrin (PRF).

Also, kwon as first generation of platelet concentrates, PRP, as the name implies, is a platelet-rich plasma concentrate derived from autologous blood after centrifugation performed to remove red blood cells (Alves and Grimalt, 2018). In spite of its clinical success, according to Narang *et al.* (2015) and Rizk *et al.* (2019), PRP preparation requires an anticoagulant contained in test tubes leading this platelet concentrate not 100% autologous.

As a means to overcoming the PRP's disadvantage mentioned above, a second generation of platelet concentrate 100% autologous was introduced to regenerative endodontic, labelled platelet-rich fibrin.

3. Platelet-rich fibrin

3.1. Definition

In the early 21st century, due to the existing demand to generate an autologous platelet concentrate suitable of minimising the inflammatory reaction induced, Choukroun *et al.* in 2001 elaborated the second generation of platelet concentrates, PRF (Choukroun *et al.*, 2001)

A few definitions of PRF are described in the literature. According to Kumar, Surendranath and Eswaramoorthy (2023), PRF consists of an organised fibrin gel that acts as a biodegradable scaffold releasing growth factors in a sustained manner. Correspondingly, Arshad *et al.* (2021) describes PRF as a compacted biomechanical fibrin complex consisting of leukocytes, cytokines, and glycoproteins. In an analogous manner, a reservoir for biochemicals composed of leukocytes, platelets, and a range of healing proteins within a dense fibrin matrix is the definition that Ragab, Lattif and Dokky (2019) rely on to describe this second platelet concentrate.

3.2. Classification of PRF

In terms of classification, there are four types of PRFs – pure platelet-rich fibrin, leucocyte platelet-rich fibrin, advanced platelet-rich fibrin, and injectable platelet-rich fibrin – illustrated in the diagram below and characterised further below. (Figure 3)

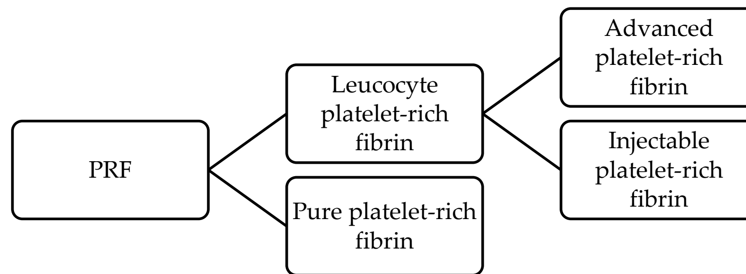

Figure 5. Classification of PRF

3.2.1. Pure platelet-rich fibrin

Therefore, pure platelet-rich fibrin is a concentrate obtained from the patient's own centrifuged blood that incorporates leukocytes, platelets, and a wide range of proteins into a dense fibrin matrix. Thereupon, pure platelet-rich fibrin is the same denomination of platelet-rich fibrin.

3.2.2. Leucocyte platelet-rich fibrin (L-PRF)

Leucocyte platelet-rich fibrin is a type of platelet-rich fibrin whose composition contains white blood cells which cells are important during the healing process. L-PRF comprises two groups of platelet-fibrin concentrates, namely advanced platelet-rich fibrin and injectable-platelet-rich fibrin which are described below (Crisci, Rescigno and Crisci, 2019).

3.2.3. Advanced platelet-rich fibrin (A-PRF)

According to Jayadevan *et al.* (2021), A-PRF, a subtype of L-PRF, consists of a fibrin membrane, which is similar to pure platelet-rich fibrin except its composition – plus neutrophils. This addition to its composition is advantageous as long as neutrophils can

stimulate monocytes to differentiate into macrophages and release more growth factors to promote tissue regeneration.

3.2.4. Injectable platelet-rich fibrin (I-PRF)

Towards injectable platelet-rich fibrin, this platelet concentrate may be obtaining through lowering the centrifugal force and duration. Hence, I-PRF, a subtype of L-PRF, consists of an injectable formulation of pure platelet-rich fibrin whose composition is higher in regenerative cells with higher concentrations of grown factors (Miron *et al.*, 2017).

3.3. Clinical applications

In contemporary times, according to Arshad *et al.* (2021), regenerative endodontics has been successfully implemented in endodontic practice and it is able to provide advantageous results in a variety of clinical situations.

As a regenerative method, platelet-rich fibrin can be applied in dentistry reality, particularly in the field of conservative dentistry, namely in cases of non-vital immature permanent teeth by promoting their revascularisation. (Arshad *et al.*, 2021).

Focusing on the regenerative endodontic treatment, this second-generation platelet concentrate can also be used as a apical plug in apexification, post enucleation of large periapical lesions, and for pulpotomy in young permanent teeth (Hotwani and Sharma, 2014).

3.4. Requirements

According to Saber (2019), in order to promote pulp-dentin complex regeneration in immature non-vital permanent teeth, PRF may exhibit the following ideal requirements:

- Porosity – allows placement of cells and growth factors
- Transport of nutrients, oxygen, and waste
- Biodegradable and biocompatible – absence of toxic byproducts release
- Replaceable – capacity to be replaced by regenerative tissue
- Strength – attendance of adequate physical and mechanical strength

3.5. PRF composition

Intending to fulfil the regenerative endodontics's objective, PRF composition includes fibrin matrix, platelets, leukocytes, glycoproteins, cytokines, and generated growth factors (Demirci, Guneri and Çaliskan, 2020, Arshad *et al.*, 2021 and Yoshpe *et al.*, 2022).

Attending to fibrin matrix, according to Sakthivel *et al.* (2020), it acts as a scaffold for the adhesion and agglomeration of cells at the desired location, which proves to be fundamental in the RET. In addition, fibrin contributes to pulp-dentin revascularisation, notably by being the source of several growth factors and assisting in fibroblast migration.

Concerning to platelets, they express and release growth factors, particularly transforming growth factor beta 1 and platelet-derived growth factor. In a chained manner, upon release, these factors not only interact with tumour necrosis factor-α and interleukin-1, but also attract neutrophils to the local and promote tissue repair (Nagaraja *et al.*, 2019). The occurrence of these events triggered by platelets is essential for angiogenesis, which is paramount in the revascularisation process, according to Arshad *et al.* (2021).

Apropos of cytokines, it incorporates a set of components by way of illustration transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF) and, finally yet importantly, epidermal growth factor (EGF).

Into the bargain of these components, generated growth factors – interleukin 1 (IL-1), interleukin 4 (IL-4), interleukin 6 (IL-6), tumour necrosis factor (TNF-α), VEGF, transforming growth factor β1 (TGF-β1), PDGF, IGF-1 and IGF-2 – have crucial specific roles in regeneration. IL-1, IL-4 and IL-6 play a key role in inflammation control once their stimulate immune cells as T-lymphocytes and B-lymphocytes. TNF-α apart from modulate the expression of the interleukins cited above, it also increases phagocytosis and the remodelling capacities of fibroblasts. VEGF have the ability to start angiogenesis. TGF- β1 leads the synthesis of collagen and fibronectin. PDGF are able to regulates migration, proliferation, and survival of one of the thre key components of regenerative endodontic – stem cells. IGF-1 and IGF-2 have effect on osteoblasts (cells responsible for bone tissue formation) and cell apoptosis (Hotwani and Sharma, 2014).

3.6. Mechanism of action

Since the fibrin membrane incorporated with platelets and leukocytes is obtained, once in contact with the tooth, platelet degranulation occurs and with it the release of cytokines which promote the proliferation and migration of cells to the platelet-rich fibrin membrane. In addition, TGF-β accelerates reactive dentinogenesis by stimulating odontoblastic activity. Alongside, VEGF assists angiogenesis, which is key in revascularisation process.

In the matter of how PRF leads pulp-dentin complex revascularisation in immature non-vital permanent teeth, there are four theories that explain the revascularisation mechanism, according to Albuquerque *et al.* (2014). Three theories are related to stem cells although they are differentiated from each other by the location of these cells.

The first possibility is related to stem cells who exist in the periapical region of immature teeth. This hypothesis argues that these stem cells have a great potential for differentiating into new fibroblasts and cementoblasts, being responsible for increasing dentinal walls and apical closure.

As a second theory, in a connect manner to the first one mentioned, stem cells from pulp tissue, who may be abundant in immature teeth, have the capacity of adhering to dentin walls to generate odontoblast-like cells for toot development.

The third theory, associated to stem cells from apical papilla defends that these cells are able to proliferate inside root canals thanks to their ability to proliferate. Concomitantly, growth factors who make up the fibrin membrane play an important role for the stem cells proliferation.

The final hypothesis, despite also being related to the differentiation and proliferation capacity of the stem cells referred to in the theories reported above, this one centres on the importance of the tooth having an open apex, since this morphological characteristic allows better communication with the periapical tissue and any stem cells which are lodged in it.

3.7. Immature non-vital permanent teeth indicative for PRF

Once the composition and the mode of action of this second-generation platelet concentrate are understood, it can be rationalised that not all immature non-vital permanent teeth are suitable for PRF application as a regenerative endodontic method for the pulp-dentin complex revascularisation.

For the purpose of a better comprehension and explanation of what immature teeth are candidates for PRF treatment, Cvek's classification (1992) based on the root development will be used:

- Stage 1: less than ½ of root formation with open apex (immature teeth)
- Stage 2: ½ root formation with open apex (immature teeth)
- Stage 3: 2/3 of root development with open apex (immature teeth)
- Stage 4: nearly completed root formation with open apex (immature teeth)
- Stage 5: completed root formation with closed apex (mature teeth)

As reported by Kim *et al.* (2018) and Wei *et al.* (2022), young permanent non-vital teeth at stage 1, 2 and 3 of Cvek's classification are suitable for PRF regenerative treatment. Concerning to stage 4 teeth, PRF as a RET and apexification with MTA plug are two therapeutic options in term of approach to teeth with this root development.

Beyond root development, restauration-related conditions such as requirement post for adequate coronal restoration are not suitable for platelet-rich fibrin regeneration (Kim *et al.*, 2018).

According to Wei *et al.* (2022), apical diameter is also an important parameter to be taken in consideration – PRF may be applicable in the teeth with apical diameters as small as 0.24 mm or above it.

3.8. Method of acquisition

This second-generation platelet concentrate is obtained based on a stepwise polymerisation protocol that embeds a high concentration of cytokines into the fibrin.

As a 100% self-labelling platelet concentrate, through the use of disposable needles and syringes, a whole blood sample is initially collected from the patient's cubital or antecubital vein, deviating between the studies analysed in this integrative literature review. Once the sample is collected, it is inserted into sterile glass test tubes so that it can be centrifuged. In a chained manner, the tubes are placed in a centrifuge at a certain rotation speed, force, and time interval.

According to Choukroun *et al.* (2001), authors of the premier platelet-rich fibrin preparation, the centrifugation of the collection should be performed at around 2700 to 3000 rpm for a time interval of 12 minutes or for approximately 400g of force.

After centrifugation of the harvested sample, three different layers are observed in test tubes, specifically, a bottom coat composed of red blood cells, an intermediate layer comprising the platelet-rich fibrin clot – the concentrate of interest – and an acellular plasma in a top layer.

In order to obtain the PRF layer, according to Choukroun *et al.* (2001), the middle coat is carefully separated from the remaining two using sterile clamps.

Subsequently, to obtain a strong fibrin network, the membrane resulting from centrifugation is placed between two pieces of sterile gauze and squeezed. Thereby, the second-generation platelet concentrate is achieved for subsequent application to the immature non-vital permanent teeth.

3.9. Application method

Taking a look into the American Association of Endodontics clinical considerations for a regenerative procedure revised in 2018, PRF can be applied into the canal space in two different ways – as an apical plug and as a scaffold.

Regarding to using PRF as an apical plug, it may be the application method chosen on the teeth listed below:

- Restorable teeth
- Maxillary or mandibular single-rooted immature permanent tooth with closed apex

Concerning to using PRF as a scaffold for regenerative endodontics, it may be the application method chosen on the teeth listed below:

- Restorable teeth
- Maxillary or mandibular single-rooted immature permanent tooth with open apex
- At least 5mm of root development

Therefore, after analysing the indications for each mode of application of PRF, the method of allocation of second-generation platelet concentrate in immature non-vital permanent teeth is the second one mentioned above. In more detail, the mode of application of PRF as a scaffold

in this type of teeth consists of two distinct appointments, which are described hereafter, based on the AAE protocol.

3.9.1. First appointment

The first appointment's goal is to achieve the absence of signs and/or symptoms, such as pain and swelling, for example. To this end, the AAE clinical protocol indicates a thorough disinfection of the root canal system followed by calcium hydroxide or TAP application.

In a more detailed approach, the root canal system ought to be irrigated firstly with 20 ml per canal of 1.5% NaOCl for 5 minutes then followed by 20 ml per canal of physiological saline also for 5 minutes and ultimately with 5% sodium thiosulfate. The calcium hydroxide or TAP shall be slowly injected into the canal space till the working length.

The intracanal treatment may be let into the canal for 1 to 4 weeks. Once that time ends up, a clinical exam should be performed to ensure that all the signs and/or symptoms which originally existed have gone off. If signs and/or symptoms persist, according to the AAE guidelines, the procedure reported above should be repeated until they are absent.

3.9.2. Second appointment

Upon reaching the absence of signs and/or symptoms, according to Sabeti, Lee and Torabinejad (2020), at the second appointment, the application of the PRF itself is carried out by the following steps:

a. Anaesthesia with local anaesthetic without vasoconstrictor and absolute isolation
b. Irrigation with 20ml per canal of saline for 5min in order to remove the intracanal medication
c. Disinfection with 20ml per canal of 17% EDTA for 5 minutes and final flush with physiological saline
d. Drying the root system canal
e. Preparation of the PRF
f. Bleeding induction to fill one-fourth to one-half of the apical portion of the root canal
g. Insertion of the PRF
h. MTA or a bioceramic plug over the PRF membrane

Concerning to the pulp canal place where the PRF membrane is allocated, it depends on the studies analysed in the review. Therefore, platelet-rich fibrin may be inserted into the apex of the tooth as Yoshpe *et al.* (2022) did or just below the CEJ such as Kritika *et al.* (2021) executed.

3.9.3. Follow-up

In line with the AAE guidelines revised in 2018, it is crucial to follow-up the PRF clinical cases within a relatively short time interval, specifically, six, twelve and twenty-four months for up to 2 years starting from the second appointment. At the time of the first check out, CBCT (Cone Beam Computer Tomography) is strongly recommended in order to analyse the induvial response to the platelet concentrate. In each follow-up, some clinical and radiographic parameters should be reviewed, being the requirements to be met in order to be granted successful regenerative endodontic treatment as will be described in more detail below. Once the 2-year follow-up period has been reached after execution of the platelet-rich fibrin as a regenerative technique, an annual check-up is recommended to make sure that everything is within the normal expected parameters.

3.10. Clinical and radiographic outcomes

Within a period of time after the application of PRF inside the root canal systems as a regenerative method, a set of clinical and radiographic outcomes may ideally be achieved in order to fulfil the purpose of regenerative endodontic therapy.

Regarding to clinical outcomes, according to the AAE guidelines, three distinct parameters must be achieved in order to be considered a clinical regenerative success. First of all, as an essential outcome, the absence of symptoms and/or signs may be accomplished. In accordance with the same guidelines mentioned above, this is the primary goal once without its reach, platelet-rich fibrin ought not to be applied inside the root canals of immature non-vital permanent teeth. Accordingly, this first outcome is the only one that must be reached previous to the implantation of the second-generation platelet concentrate. Unorthodoxly, the second and third goals are objectives that should be achieved upon application of the regenerative method. In spite of the second goal is related to the tooth structure, the third one has to do with the pulp itself. In a greater detail, an increase of root wall thickness and/or root length (secondary goal)

and a positive response to vitality testing (tertiary goal) may be accomplished to conclude the regeneration as a whole.

Concerning to radiographic outcomes, as stated by the AAE guidelines, rectification of apical radiolucency when present is one of the first parameters visualised radiographically and it is apparent from the 6-12 months after treatment and so on. In a similar manner, an increase of width of root canals is another parameter firstly observed. In relation to root length, it is generally observed from 12-24 months after treatment and so on, which means that thickening of the dentinal walls appears to occur earlier than root elongation.

3.11. Adverse effects

In spite of the clinical success that PRF presents as a regenerative method of the dentin-pulp complex, some conditions may occur, being known as adverse effects of this kind of technique.

As previously stated, PRF has the ability to stimulate not only pulp tissue formation, but also the formation of cementum-like tissue, bone, and periodontal ligament from the differentiation of mesenchymal stem cells. On the basis of this expertise, according to Jayadevan *et al.* (2021) and Kumar, Surendranath and Eswaramoorthy (2023), it can lead to calcification of the root canal, ankylosis between the intracanal hard tissue and the apical bone and even root resorption. A further adverse effect that can occurs is the persistence of infection. This last one cited, although not directly related to the second-generation platelet concentrate, it ends up underestimating its clinical success, indirectly. As attested by Kim *et al.* (2018), this complication is due to an inadequate disinfection of the root canal system, which promotes the resistance and a lack of removal of the biofilm responsible for the infection.

4. Discussion

After tooth eruption, the permanent tooth takes between three to five years to complete full apical closure, being until then called immature tooth or tooth with open apex. As previously mentioned, according to Morankar et al. (2023), under certain conditions such as dental caries, trauma and developmental abnormalities, pulp necrosis may occur, bringing sequelae to these teeth. According to Wilkstrom et al. (2021) research, traumatic injuries in the permanent immature teeth stand out as a global health problem. If we take a look carefully at the studies

reported in the present integrative review, out of a total of 3 clinical studies, 2 of them have trauma as the etiological basis of the immature permanent tooth.

Thereby, regenerative endodontics, first described in 2001 by Iwaya *et al.*, is a therapeutic choice in terms of healing of immature non-vital permanent teeth.

According to Kumar, Surendranath and Eswaramoorthy (2023), PRF consists of an organised fibrin gel that acts as a biodegradable scaffold releasing growth factors in a sustained manner. This gradual release of growth factors allows stable healing to take place as the fibrin matrix is resorbed. Thus, PRF, from undifferentiated cells promotes the differentiation, growth and maturation of fibroblasts, odontoblasts and cementoblasts. Furthermore, this second-generation scaffold favours the development of microvasculature concomitantly with the migration of epithelial cells which, according to Arshad *et al.* 2021, is shown to be essential in the revascularisation process.

In order to realize this regeneration process, PRF needs 3 fundamental requirements to achieve its clinical success – stem cells, scaffold and growth factors (Sakthivel *et al.*, 2020). Therefore, PRF's composition, listed below, also demonstrates to be essential for its success – fibrin matrix, platelets, leucocytes, glycoproteins such as thrombospondin, cytokines and growth factors – as already mentioned above.

However, other factors may benefit or compromise its clinical outcome, including patient-related, teeth-related, and technique-related factors.

Focusing on the patient, age may be an important one since the regenerative capacity and functionality of the stem cells required for RET decrease with advancing age (Demirci, Guneri and Çaliskan, 2020; Kritika *et al.*, 2021). Alongside this statement, we can see the same by comparing the age of the patients in the studies analysed and the clinical success obtained with the application of the platelet-rich fibrin – Jayadevan *et al.* (2021) (10-27 age, 60% success rate), Kritika *et al.* (2021) (9-25 age, 90% success rate) and Yoshpe *et al.* (2022) (7.6-16 age, 50.8% complete success rate). Therefore, according to Ragab, Lattif and Dokky (2019), the recommend age range for regenerative endodontic therapy is from 8 to 16 years old. Another patient-related factor that can be include in the discussion from the studies analysed is the gender. In this integrative review, 2 out of a total of 4 studies analysed have the same numer of female and male patients included in their eligibility criteria, so it can be used as a basis for comparison when analysing this factor. In the Jayadevan *et al.* (2021) clinical study where there were included 21 male and 21 female, the rate success was 60% and 40% respectively.

On the same hand, in the Yoshpe *et al.* (2022) study, 23 males and 23 females were included into the clinical study, but the rate success was not considered. So, based on this we can hypothesise that there is no significant gender difference in terms of clinical success. However, the data collected is not enough to draw conclusions on this topic, so further studies focused on this topic are needed.

Regarding to the teeth, the etiology of the immature apex and the type of teeth seems to be two important aspects to be taken into consideration. Focusing on the immature apex's etiology, as mentioned before, several conditions may be at its genesis. However, according to Rahul *et al.* 2023, if trauma is the cause of the necrotic open apex, damage to the residual cells of the root Hertwig epithelial sheath may occur, jeopardising pulp tissue regeneration, decreasing the clinical success of the second-generation platelet concentrate application. Considering the analysed clinical study developed by Yoshpe *et al.* (2022) where only immature permanent anterior teeth diagnosed with pulp necrosis after dental trauma and immature permanent posterior teeth with deep caries were included into the study, it can be concluded exactly what was mentioned above since the clinical success of PRF was higher in the posterior teeth (caries) than in the anterior teeth (trauma). In the same stream of thought and based on the same study, regarding the type of tooth on which the second-generation platelet concentrated is applied, results obtained show that the rate success in posterior teeth is higher than the anterior teeth. Nevertheless, there are few studies in the literature that perform the procedure on posterior teeth, since the teeth most frequently affected with trauma are the anterior teeth and most of the studies found in the literature include traumatised teeth, so there is not enough scientific support to state that success in posterior teeth.

In addition to the factors related to the patient and the tooth itself, another very important aspect that varies from study to study is the method of obtaining and applying the second-generation platelet concentrate membrane, in other words, the technique used, whether in the acquisition process or in the application process as a whole. Regarding the method of obtaining, the speed with which the fibrin membrane is formed influences the number of platelets and white blood cells that integrate it (Nagajara *et al.*, 2020) since the regulation of the inflammatory reaction is directly proportional to the quantity of platelets and leucocytes implanted in the PRF. Still on the method of obtaining, particularly the pH (hydrogenionic potential) of platelet-rich fibrin, this factor influences crucial phenomena in the development of the regeneration of the pulp-dentin complex, such as angiogenesis and macrophage activity. In order for these biological processes to take place correctly, a platelet concentrate with an acidic pH is essential for their

development, according to Nagajara *et al.* (2020). In relation to the clinical application protocol, disinfection is a crucial point in the process as uncontrolled infection does not allow regeneration to take place (Myers and Fountain, 1974). On the other hand, despite the importance of disinfecting the root canal system, the type of solution and the concentration at which it is employed into the canals are fundamental for the preservation of stem cells, one of the three crucial requirements in regenerative endodontic treatments. Therefore, according to Wei *et al.* (2022), irrigante solutions must comply with the following points – antibacterial effect, lower cytotoxicity and stimulation capacity of growth factors release. Taking a look into the 4 studies being analysed and discussed in this integrative review of the actual literature, all the studies employ a couple of solutions – NaOCl and EDTA. Being the most widely used endodontic irrigante, sodium hypochlorite has properties like its unique ability to dissolve pulp tissue which makes its use advantageous in cases of necrotic immature teeth due to its antimicrobial capacity, being able to eliminate all types of existing microorganisms. However, the ideal concentration at which it should be applied is 5% since at lower concentrations it becomes ineffective against microorganisms, decreasing the disinfection level, and higher concentrations it is cytotoxic to stem cells present in apical tissues, decreasing their ability to differentiate and survive (Ragab, Lattif and Dokky, 2019). In the 4 studies analysed in this integrative review (Wang *et al*., 2016, Jayadevan *et al.*, 2021, Kritika *et al.*, 2021 and Yoshpe *et al.*, 2022), the authors used different concentrations of the irrigante (5.25%, 1%, 1.5% and 4%, respectively) which may have interfered and explained the different outcomes. With regard to EDTA, according to Kim *et al.* (2018), it is a chelating agent able to remove smear layer and to reverse the deleterious effects of NaOCl employed prior to this solution. In addition to these properties, in terms of pulp-dentin complex regeneration, EDTA is a crucial solution for its development. Since it is a chelating agent, EDTA promotes the exposure of the dentin matrix simultaneously with the expression of dentin sialophosphoprotein. Thereby, it promotes the release of growth factors which are able to be signalling stem cells of apical papilla to differentiate into odontoblastic-like cells (Kim *et al.*, 2018). On the other hand, but in an interconnected way to achieve the goal of PRF application inside the root canal system, EDTA irrigation leads to the exposure of binding sites for attachments of newly formed tissue to the canal walls as well as the adhesion, migration, and differentiation of dental pulp stem cells towards or onto dentin (Kim *et al.*, 2018, Demirci, Guneri and Çaliskan, 2020 and Jayadevan *et al.*, 2021). In view of these properties of EDTA, in a concentration of 17% used in the total of 4 studies, authors of the studies analysed in this integrative review recommend its use as a final irrigante just before the PRF application.

Likewise, another factor to take into account is the intracanal medication that is placed inside the canal between the two appointments which constitute this regenerative procedure. Regarding this topic, two different medications may be applied – calcium hydroxide and triple paste antibiotic. Calcium hydroxide as an intracanal medicament acts as an antibacterial medicament by modifying bacterial cell wall lipopolysaccharides, due to being alkaline, what is advantageous in immature non-vital permanent teeth (Ahmad *et al.*, 2022). Regarding to TAP, one of the most widely used intracanal medicament, was described as a mixture of metronidazole, ciprofloxacin, and minocycline belonging to an effective endodontic antimicrobial capacity. Taking a look into the studies in discussion in this review, 3 from a total of 4 studies used TAP as an intracanal medication – Jayadevan *et al.* (2021), Kritika *et al.* (2021) and Yoshpe *et al.* (2022) – which can somehow prove that it is one of the most used medications in this ambit. Nevertheless its effectiveness in canal disinfection of immature non-vital permanent teeth, according to Kim *et al.* (2018), TAP is more often associated with crown discolouration than calcium hydroxide. This fact can be explained by the presence of minocycline in the composition of the triple antibiotic paste (Kahler e Rossi-Fedele, 2016). The same described above was reported in the results of the studies of Jayadevan *et al.* (2021) and Yoshpe *et al.* (2022). However, although this is a disadvantage of this intracanal medication, it can be circumvented by changing its composition. Analysing the study of Kritika *et al.* (2021), the authors used TAP whose composition was metronidazole, ciprofloxacin, and amoxicillin in place of minocycline to prevent possible future crown discolouration.

As regards the radiographic results, the following parameters were found in the 4 studies – decrease in apical lesion and apical diameter. In terms of the increase in dentin wall thickness with seeming root length it was found in 2 out a total of 4 studies. This difference in root length increase may have occurred because there may have been apposition of tissue other than root dentin. That is, instead of stimulating regeneration of the pulp-dentin complex, the application of the PRF inside the canal may have stimulated apposition of hard tissue such as cementum and bone in the canal space and thus there was no radiographic perception of root lengthening. In addition, the increase in root length is promoted by the cells of the epithelial sheath of Hertwig, which in many cases is damaged. Therefore not all radiographic results after regular patient control and follow-up can be reliable.

As for the clinical outcomes, as mentioned before, according to AAE guidelines, three distinct parameters must be achieved in order to be considered a clinical regenerative success through the use of platelet-rich fibrin – the absence of symptoms and/or signs, increase of root wall

thickness and/or root length and positive response to vitality testing, in this order respectively. With regard to the first goal of RET in immature non-vital permanent teeth, it was reported in the 4 articles present in this integrative review. Therefore, based on these data it can be stated that the rate of achievement of this goal is fairly high. This can also be reported with regard to the second objective of the application of the second-generation platelet concentrate on this set of teeth. Notwithstanding the achievement of the first two objectives of regenerative therapy, in these studies reported, after a follow-up period, the response to pulpar sensitivity tests (tertiary goal) was negative. As an explanation of the subsequent failure to achieve the third parameter, there are different hypotheses described in the literature. As a first hypothesis, according to Kumar, Surendranath and Eswaramoorthy (2023), the failure of pulp regeneration may have occurred due to the blood supply in the apical area and reduced resistance to infection that the dental trauma to which necrotic immature permanent teeth are subjected. Taking a look into the studies discussed, it can explain the negative response in all surveys where trauma is the etiology of this condition. Furthermore, in the clinical studies of Wang, Yang and Liu (2016), Kritika *et al.* (2021) and Yoshpe *et al.* (2022), MTA was used as the material to be placed over the PRF membrane implemented in the teeth. This statement is in line with a second hypothesis related to MTA since the thickness at which it is applied may compromise the responsiveness of any regenerated vital tissues. Still relatively to this topic, contrariwise, hypothesising that there were pulp responses in the studies analysed, it would not necessarily indicate regeneration of the pulp tissue itself. This can be justified on the basis of the hypothesis described in the study of Lei *et al.* (2015) in which they argue that after a regenerative endodontic procedure there is an induction of the formation of cementum-like, bone-like and nerve fibres in the canal. These vital tissues are normally vascularised and innervated, which means they are capable of responding to the pulp sensitivity tests used to assess pulp tissue regeneration when applying PRF as a regenerative method.

Nevertheless, all studies showed favourable responses with the application of PRF so it can be stated that PRF is clinically successful in necrotic immature permanent teeth.

In addition to clinical success, PRF has the following advantages and disadvantages for current and future regenerative endodontics (Eid *et al.*, 2022 and Kumar, Surendranath and Eswaramoorthy, 2023) (table 3).

Table 4. Advantages and disadvantages of PRF

Advantages	Disadvantages
- 100% autologous - Anticoagulant and thrombin free - Good cost-benefit ratio - Prolonged factor release - Ease of preparation, management, and application	- Requirement of specific devices - Requirement of clinical practice in blood draw - Common needles fear in target young patients

Owing to this, PRF has numerous advantages over the disadvantages for regenerative endodontics.

III. Conclusion

Regenerative procedures are crucial techniques in the approach of non-vital immature permanent teeth enabling, if needed in the future, the realisation of non-surgical endodontic treatment.

Among the therapeutic options for RET, PRF acts as a reservoir of growth factors that stimulate the differentiation, growth and maturation of fibroblasts, odontoblasts and cementoblasts from their undifferentiated precursors. Despite the disadvantages, the studies analysed have shown that PRF is an ideal therapeutic option for immature non-vital permanent teeth, increasing their root length and dentin wall thickness and decreasing apical diameter and apical radiolucency.

Being a technique with a high success rate, although more studies in this area are needed to obtain more scientific evidence, the future of conservative dentistry is promising, with the possibility of regeneration of the pulp-dentin complex.

In the future, the endodontic approach will increasingly become conservative, with a greater use of means from the patient's own (e.g. autologous blood) as a therapeutic option, thus reducing the use of synthetic materials.

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