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| RESEARCH ARTICLE

Comparative Evaluation of Blood Clot, Platelet-Rich Plasma, and Platelet-Rich Fibrin Scaffolds in the Success of Regenerative Endodontic Treatments: A Literature Review

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| ABSTRACT
The use of natural scaffolds in regenerative endodontic treatments can significantly impact treatment outcomes. Natural scaffolds such as Platelet-Rich Plasma (PRP), Platelet-Rich Fibrin (PRF), and blood clot are effective in improving clinical symptoms, resolving periapical lesions, regenerating dentin structure, closing the root apex, and increasing root length in regenerative endodontic treatments. This study aims to provide a comparative evaluation of blood clot, platelet-rich plasma (PRP), and platelet-rich fibrin (PRF) as natural scaffolds in the success of regenerative endodontic treatments.

| KEYWORDS

Regenerative endodontics, blood clot, platelet-rich plasma, platelet-rich fibrin

| ARTICLE INFORMATION

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1. Introduction

The primary goal of root canal therapy is to prevent or treat apical periodontitis. Achieving this requires preserving the health of periodontal tissues in cases of pulpitis and creating conditions for the regeneration of healthy periapical tissue in cases of necrosis. Most current treatments focus on replacing lost tissues with inert materials, such as filling the root canal system with gutta-percha or using implants, which have shown satisfactory success even in the presence of infected periapical tissues (Jurič et al., 2024).

In recent years, tissue engineering has gained attention as a therapeutic approach for treating tissue and organ defects, and even for the complete replacement of an organ. This has led to the development of a new field where engineering and biology principles are applied to repair damaged living tissues, promoting regeneration, repair, and restoration of tissue function (Zhu et al., 2023). Scaffolds, cells, and growth factors constitute the three main components of tissue engineering, with scaffolds being the most extensively studied among them. An ideal scaffold for tissue engineering should possess specific characteristics, including porosity for nutrient diffusion and waste removal, and mechanical stability to withstand and transmit forces. Additionally, the surface chemistry of scaffold materials should enhance cell adhesion and intracellular signaling to induce targeted differentiation into the desired natural phenotype (Ferraz, 2024).

Extracellular matrix (ECM) molecules, such as fibronectin and laminin, interact with glycoprotein receptors on the surface of tissue cells to promote cell migration and differentiation. Beyond providing a structural and adhesive framework for cells, the ECM also acts as a reservoir for growth factors essential for cell function. Given these properties, scaffolds derived from biological sources through decellularization of living tissues have garnered significant interest (Yang et al., 2005). Decellularization is a process that removes all cellular components from a tissue, leaving only the extracellular matrix and intercellular scaffolds intact. These biologically derived scaffolds have been successfully used in tissue engineering and regenerative medicine (Yang et al., 2005).

Cellular scaffolds are categorized into three types: natural, synthetic, and composite. Natural scaffolds are derived from nature, the human body, or animals. These scaffolds may be ceramic, sharing hydroxyapatite structures with natural bone, or protein-based, resembling organic bone matrix proteins like collagen, osteopontin, and osteonectin. Examples of natural organic scaffolds include PRP, PRF, collagen, gelatin, fibrin, and blood clots, which often serve as natural substrates for cell adhesion, differentiation, and proliferation (Adel et al., 2022).

Today, there is growing interest in methods aimed at regenerating the healthy pulp-dentin complex. The objective of regenerative dentistry is to biologically replace dental tissues and surrounding structures. Progress in regenerative dentistry is largely dependent on biological treatments. Many regenerative dentistry concepts have emerged due to advancements in tissue engineering, which emphasizes the integration of stem cells, growth factors, morphogens, and scaffolds to form dental tissues (Wei et al., 2022).

Treatment of pulp necrosis in an immature tooth with an open apex is considered a significant challenge for dentists. Nowadays, in cases of necrotic teeth with immature apices, attention has shifted toward the technique of regenerative endodontics (revascularization). In this technique, if the root canal space is properly disinfected and a blood clot forms as a matrix, the trapped cells within it can lead to the formation of new tissue (Singh et al., 2023).

This technique offers advantages such as continued root development, increased root length, thickening of the canal wall, and apex closure. This approach is technically simple and can be performed using available tools without incurring the high costs associated with advanced biotechnological methods. Also, revascularization of the tissue within the root canal system is achieved using the patient's own blood, thereby eliminating the risk of tissue rejection (Murray, 2023). Blood clots serve as natural scaffolds for regenerating dental pulp tissue. The source of blood in pulp revascularization treatments is typically the periapical tissues, which enter the root canal space. Blood clots form naturally after tissue injury through the activation of thrombin and fibrinogen, creating a cross-linked fibrin scaffold. Blood clots have been widely used in biomedicine as scaffolds for stem cells. Some studies have shown that platelet-rich plasma accelerates regenerative treatments by enhancing wound healing, fibroblast proliferation, and neovascularization, leading to improved outcomes (Dohle et al., 2018). Additionally, the role of platelet-rich fibrin in wound healing and angiogenesis has also been highlighted in research (Saputro et al., 2022).

Given the importance of effective root canal treatments, particularly in teeth with immature apices, the use of natural scaffolds in regenerative endodontic therapies appears promising. However, to date, no comprehensive review has been published comparing the success rates of these three natural scaffolds. Therefore, this review study aims to evaluate and compare blood clots, platelet-rich plasma, and platelet-rich fibrin as natural scaffolds in the clinical and radiographic success of regenerative endodontic treatments.

2. Natural Scaffolds Used in Pulp Revascularization Treatments

platelet-rich plasma (PRP)

Platelet-rich plasma (PRP) is defined as a concentration of platelets in a small volume of plasma, enriched with autologous growth factors. These growth factors are natural biological mediators that play a key role in regulating cellular events involved in tissue repair and regeneration. After binding to membrane receptors, these growth factors induce intracellular signaling pathways in specific target cells, typically activating genes that ultimately alter cellular activity and phenotype (Saputro et al., 2022; Martínez-Zapata et al., 2009). However, the effects of each growth factor are regulated through a complex feedback system involving other growth factors, enzymes, and binding proteins. Recent advances in cellular and molecular biology have enhanced our understanding of these factors' functions. Studies have shown that growth factors can increase tissue regeneration capacity by regulating cell chemotaxis, differentiation, and proliferation (Dohle et al., 2018).

PRP represents a novel approach for tissue regeneration and has become a valuable tool for enhancing healing in various dental surgical procedures, particularly in older patients. PRP is obtained by centrifuging the patient's own blood and contains growth factors that promote wound healing, playing a significant role in tissue repair mechanisms. Its use in surgery offers benefits such as reduced bleeding, improved soft tissue healing, and enhanced bone regeneration. Human studies have reported notable outcomes regarding PRP application in oral and dental surgeries (e.g., tooth extraction, periodontal surgery, implant surgery) (Fernandes & Yang, 2016).

The normal platelet concentration ranges from 150,000 to 450,000 per microliter of blood, while PRP increases platelet concentration three to five times (approximately 1,000,000 platelets per microliter). Growth factors present in platelets, such as PDGF, TGF, VEGF, and EGF, are essential for wound healing and hemostasis (Foster et al., 2009).

The effectiveness of PRP depends on platelet concentration ratios, preparation methods, injection volume, the nature and severity of the injury, and individual patient characteristics. Variability in these factors has led to inconsistent medical outcomes for PRP. Platelets play a crucial role in host defense mechanisms at the wound site by providing signaling peptides that recruit macrophages.

PRP is prepared autologously from patient plasma and used as a source of growth factors for regenerating periodontal defects (Xu et al., 2021).

Platelet-Rich Fibrin (PRF)

The use of concentrated platelets was prominent until the discovery of PRF, with blood-derived growth factors being utilized for over two decades in medicine. Despite PRP's early success, certain limitations were reported. The technique was time-consuming and required anticoagulants like bovine thrombin and calcium chloride to prevent clotting, both of which could inhibit wound healing. Despite these drawbacks, PRP was used in maxillofacial bone surgeries. However, its liquid form necessitated combination with other biomaterials, such as allografts or xenografts, or synthetic products. Furthermore, laboratory studies revealed the rapid release of growth factors from PRP (Arshad et al., 2021).

These limitations prompted researchers to explore alternative models for successful tissue regeneration. Platelet concentrates of the second generation, prepared without anticoagulants and with shorter preparation times, were termed platelet-rich fibrin (PRF). This method entraps many cells, including platelets and leukocytes, within a fibrin matrix. PRF, later referred to as leukocyte-rich PRF (L-PRF), contains various cells essential for tissue regeneration, including macrophages, granulocytes, and neutrophils. These cells are trapped in a three-dimensional fibrin matrix during centrifugation. PRF significantly improves wound healing and angiogenesis when combined with bone biomaterials (Lv et al, 2018; Liao et al., 2014).

A critical difference between PRF and PRP is the inclusion of leukocytes in PRF, which have demonstrated resistance to pathogens and importance in immune regulation, as well as their key role in binding biomaterials to host tissues. PRF does not require anticoagulants due to the fibrin matrix and naturally clots in their absence. PRF includes natural blood growth factors like TGF-beta, which accelerates oral cell proliferation, and PDGF, a key regulator of mesenchymal cell migration, proliferation, and survival. VEGF, another crucial growth factor in PRF, promotes angiogenesis and blood supply to damaged tissues. PRF also contains epidermal growth factor (EGF) and insulin-like growth factors (IGF), which regulate cell proliferation and differentiation (Li et al., 2023; Rizk et al., 2020).

Blood Clots

Fibrinogen and fibrin are vital for clot formation, fibrinolysis, cell-matrix interactions, inflammation, wound repair, angiogenesis, and neoplasia. Their role in these processes depends on fibrin's properties and interactions with binding sites, proenzymes, coagulation factors, enzyme inhibitors, and cellular receptors. Wound healing outcomes are heavily influenced by fibrin network characteristics, such as fiber thickness, branching points, porosity, and permeability (Laurens et al., 2006).

Fibrinogen is a primary component of the coagulation cascade and quickly forms an insoluble fibrin scaffold upon tissue injury. Fibrin, a natural biopolymer, is created during blood clotting through fibrinogen polymerization. This fibrin network is critical for hemostasis after tissue injury, serving as a temporary scaffold for wound repair. Due to its structural and functional properties, fibrin is utilized in regenerative medicine. Fibrin can deliver ECM proteins like fibronectin and growth factors. Main fibrin scaffolds, such as PRF and PRP, are widely used as autologous biomaterials in regenerative medicine, wound healing, orthopedics, and dental treatments. Fibrin degradation products also stimulate cell migration and tissue regeneration, playing an essential role in wound healing and contributing to new product development for over a century (Jang et al., 2020).

3. Discussion

The goal of root canal treatment is to preserve and restore the health of the periapical tissues. The objectives of pulp revascularization go beyond those of conventional root canal therapy. Specifically, its primary aim is to complete root development in cases of immature teeth (de Souza Araújo et al., 2017). The success of pulp revascularization is assessed by the presence of radiographic and clinical evidence of healthy periapical tissues and viable pulp within the canal. Radiographic indicators of pulp tissue function (or pulp-like tissue) include continued root development, both in length and wall thickness. Other criteria include the presence of living tissue within the canal, confirmation of blood supply via laser Doppler flowmetry, pulp vitality tests (e.g., thermal, cold, EPT), and the absence of clinical symptoms (Antunes et al., 2015).

The key distinction between pulp revascularization and tissue engineering (which focuses on the transplantation of cells, growth factors, and scaffolds) lies in the reliance of pulp revascularization on inducing bleeding within the root canal space. Studies evaluating the role of natural scaffolds in regenerative endodontics have generally agreed that scaffolds significantly improve treatment outcomes, regardless of their type. An ideal scaffold should selectively bind cells, concentrate them, contain growth factors, and degrade over time. Thus, a scaffold is more complex than a simple cell-placement matrix (Saputro et al., 2022). From a clinical perspective, platelet-rich plasma (PRP) appears to meet many of these requirements. Autologous PRP is relatively easy to prepare, rich in growth factors, biodegradable, and forms a three-dimensional fibrin matrix. Combining scaffolds with specific growth factors is crucial for the optimal regeneration of odontoblast-like cells, which is essential for advancing regenerative endodontics as a predictable clinical process (Adel et al., 2022).

Several studies have investigated the effect of PRP on the success of regenerative endodontic treatments. PRP has been shown to reduce inflammatory cells around the tooth and promote tissue healing and repair (Hargreaves et al., 2013). Torabinejad & Faras (2012) reported that PRP in regenerative endodontics led to the formation of connective pulp-like tissue with minimal inflammatory cells observed at the sample margins. Consequently, PRP can be utilized as a scaffold in regenerative endodontics to create pulp-like tissue in human teeth. However, some studies suggest that platelet-rich fibrin (PRF) may be a more effective natural scaffold due to differences in leukocyte content between PRP and PRF. Studies comparing PRP and PRF in regenerative endodontics for immature anterior teeth have indicated that PRF performs better in certain parameters, such as increasing root dentin thickness (Orhan et al., 2012; ElSheshtawy et al., 2020; Nosrat et al., 2011; Kim et al., 2010).

For instance, Jayadevan et al. (2021) noted that while both PRP and PRF could be used as natural scaffolds in regenerative endodontics for immature anterior teeth, PRF could be recommended as a more effective option because it enhances root dentin thickness, which is vital for strengthening immature teeth. However, Rizk et al. (Rizk et al., 2020) observed no statistically significant differences between PRP and PRF in clinical symptom improvement, root length and thickness increases, or periapical lesion healing. They did report, however, that PRF caused significantly more tooth discoloration than PRP. Consequently, they suggested that PRP is a suitable alternative to PRF for revascularization in necrotic immature teeth, demonstrating excellent 12-month prognoses. Similarly, Xu et al. (Xu et al., 2020) highlighted the positive effects of PRP in regenerative endodontics and emphasized the need for further double-blind and clinical trials to establish definitive guidelines for using PRP as a scaffold. Some studies have also suggested comparable effects of PRP and PRF in regenerative endodontics. For example, Marí-Beffa et al. (2017) concluded that stem cells present in both PRP and PRF could serve as viable options for regenerative endodontic treatments. Hargreaves et al. (2013) indicated in their study that regenerative endodontic treatments (RETs) utilizing stem cells, particularly PRP and PRF, demonstrated higher healing rates compared to other invasive methods. However, additional clinical studies are still required in this field. Based on the reviewed studies, it can be concluded that PRP and PRF scaffolds are effective in RETs, with platelet-rich fibrin showing superior outcomes in increasing dentin thickness in necrotic immature teeth (Lv et al., 2018; Pinto et al., 2017; Del Fabbro et al., 2016).

Regarding the use of blood clots in RETs, varying results have been observed in studies. For instance, LV et al. (2018) found that PRF and blood clots were equally effective in alleviating symptoms, improving periapical lesions, and promoting root development in RETs. Similarly, Bezgin et al. (2015) observed that although PRP provided better outcomes in RETs, the differences between PRP and blood clots were not statistically significant. Zhang et al. (2014) suggested that PRP could be a viable option in clinical cases where apical tissue stimulation results in minimal or no bleeding during RETs. Alexander et al. (2020) noted significantly greater formation of hard tissue within the root canal, resulting in canal obstruction, in immature teeth treated with blood clots compared to PRP and PRF groups.

In general, RETs have been shown to improve apical periodontitis, eliminate clinical symptoms, promote continued root growth on radiographs, enhance fracture resistance, and improve the survival of necrotic immature teeth (Akcay et al., 2014). However, they may also result in undesirable tooth discoloration (Galler, 2016). It has been noted that minocycline, a primary component of intracanal disinfectants, is a key factor in tooth discoloration after RETs (Kahler et al., 2014). Mineral trioxide aggregate (MTA), the most commonly used material for coronal sealing in RETs, has also been shown to cause tooth discoloration, especially when in contact with blood (Marciano et al., 2015). Additionally, natural scaffolds like PRP, PRF, and blood clots, used in RETs, may contribute to tooth discoloration. For example, Shokouhinegad et al. (2019) found that coronal discoloration was significantly higher after one month in teeth treated with blood clots compared to PRF, however, no significant differences were observed after six months. Other studies have attributed the absence of discoloration in RETs using natural scaffolds to PRF (Felman et al, 2013; Guimarães et al., 2015). However, Rizk et al. (Rizk, 2020) reported that PRF caused more tooth discoloration than PRP.

4. Conclusion

In summary, natural scaffolds such as PRP, PRF, and blood clots are effective in improving clinical symptoms, healing periapical lesions, regenerating dentin structure, achieving root apex closure, and increasing root length. Although blood clots are the most commonly used natural scaffold, PRF and PRP appear to be more effective in cases where minimal bleeding occurs in the canal. However, PRP and PRF preparation processes are more costly and time-consuming compared to blood clots. Further studies are needed to reach more definitive conclusions.

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