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Current Trends and Perspectives in the Development of PRF Platelet Concentrates for Vital Pulp Therapy of Reversible Pulpitis

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Abstract

Platelet concentrates derived from autologous blood are used to stimulate tissue regeneration through the controlled release of growth factors and biologically active proteins. These autologous biomaterials provide a natural fibrin matrix containing platelets, leukocytes, and growth factors that are gradually released and support tissue repair processes. In recent years, they have been applied in the biological treatment of reversible pulpitis, where the main goal is to preserve the vitality of the dental pulp and to stimulate its regenerative potential. In this process, a key role is played by dental pulp stem cells (DPSCs), which possess the ability to proliferate, migrate, and differentiate into odontoblast-like cells under the influence of bioactive molecules released from PRF. The present review aims to outline the trends in the development of PRF-based autologous biomaterials and the experimental methods used for their investigation under laboratory conditions with cell cultures derived from dental pulp stem cells of permanent teeth (DPSCs). The establishment and identification of such stem cell cultures provide an opportunity to study their migration and behaviour in media containing autologous biomaterials, with the purpose of demonstrating their biostimulating activity and potential role in pulp tissue regeneration.

Keywords: Dental pulp; Stem cells, DPSCs, PRF, Growth factors, Regenerative endodontics, Platelet concentrates, Autologous biomaterials

Introduction

The dental pulp is a highly specialized connective tissue located within the tooth (in the pulp chamber and root canals). It is richly vascularized and innervated, containing various cell populations, including odontoblasts, fibroblasts, immunocompetent cells, and stem cells. Its main function is the formation and maintenance of dentin. Vital pulp ensures dentin metabolism, responds to irritation and injury, and actively participates in the tooth's immune defense. Owing to its rich innervation, it perceives pain and thermal stimuli, protecting the tooth from further injury. The pulp also serves as a reservoir of stem cells with proven potential for differentiation and tissue regeneration (1).

Preserving pulp vitality is essential for the long-term function and structural integrity of the tooth. Loss of vitality leads to impaired metabolism and increased susceptibility to fractures. Therefore,

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the treatment of pulp injuries aims not only to eliminate infection and pain but also to maintain its vitality through biological and regenerative approaches(2).

Diseases of the dental pulp and periapical tissues are among the most common oral pathologies, primarily caused by carious, traumatic, or iatrogenic factors. Conventional endodontic treatment (pulpectomy) preserves tooth morphology but not pulp vitality. In this context, modern regenerative endodontics, based on the principles of tissue engineering — including stem cells, biomaterials, and growth factors — seeks to preserve and restore pulp vitality (3).

Platelet concentrates derived from autologous blood are biological products obtained by centrifugation of the patient's own venous blood. They are used to stimulate tissue regeneration through the controlled release of growth factors and bioactive proteins. These concentrates are a natural source of biologically active molecules that promote the regeneration of bone, soft tissue, and dental pulp. In the literature, these products are referred to by various terms such as platelet concentrates from autologous blood or blood concentrates(4,5,6). In this paper, they are referred to collectively as autologous blood biomaterials (AB biomaterials).

Aim

This review aims to summarize the current understanding of PRF-based AB biomaterials and their laboratory evaluation using in vitro cultures of dental pulp stem cells (DPSCs) from permanent teeth.

Results

Classification of AB Biomaterials

The classifications of platelet concentrates from autologous blood have been proposed by various authors, including Magalon et al. (2016) and Lana (2017) (7,8). These early systematic classifications established the foundation for subsequent research, which by 2025 distinguished two main generations of platelet concentrates:

1. First generation – Platelet-Rich Plasma (PRP);
2. Second generation – Platelet-Rich Fibrin (PRF).

Platelet-Rich Fibrin (PRF) is a second-generation platelet concentrate, developed in 2001 by Joseph Choukroun and D. Dohan. Several methods for PRF preparation are currently known: Choukroun's method (A-PRF), the IntraSpin method (L-PRF), the Silfradent–Concentrated Growth Factor (CGF) method, and Miron's Solid PRF technique(4).

These AB biomaterials are obtained by centrifugation of venous blood collected from the patient. Two main types of centrifuges are used:

- Vertical centrifuges – with a fixed rotor angle (4);
- Horizontal centrifuges – where the tubes move to a horizontal position during operation (5,6).

For simplicity, these will be referred to as vertical and horizontal centrifuges throughout the text.

From vertical centrifuges, materials are produced following Choukroun's protocols, including A-PRF+, i-PRF, and their derivatives (4):

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- Dense A-PRF+ membrane;
- Gel-like A-PRF+ membrane;
- Sticky bone – a combination of gel A-PRF+ membrane and bone graft;
- Liquid i-PRF.

From horizontal centrifuges, AB biomaterials are prepared according to Miron's method, such as H-PRF (Solid PRF), Liquid PRF, e-PRF, and c-PRF (5,6).

Characteristics of AB Biomaterials

PRF-based AB biomaterials, produced by centrifugation, release growth factors from platelets while forming a three-dimensional fibrin matrix that continues to release them for an extended period of up to 14 days. These growth factors promote cellular proliferation and differentiation (9). In centrifuges with a fixed angle (vertical type), the duration of growth factor release is approximately 14 days, whereas horizontal centrifuges (e.g., for e-PRF preparation) extend this release period up to 4–6 months(4,5,6).

The highest concentration of platelets and leukocytes is found in the layer located directly above the erythrocytes, known as the buffy coat. This layer plays an important role in the immune defense of the dental pulp. Leukocytes release signaling molecules that attract stem cells both from the blood and from the pulp tissue(10).

Laboratory studies comparing the composition of various platelet concentrates have shown that Solid PRF, produced according to Miron's protocol in a horizontal centrifuge, contains approximately 3.5 times more platelets and leukocytes than L-PRF, obtained using fixed-angle centrifugation (11,12,13). Solid PRF also exhibits a higher concentration of immune cells within the buffy coat layer compared to A-PRF, L-PRF, and CGF biomaterials.

From a scientific standpoint, it is crucial to clarify how these differences — both in concentration and duration of growth factor release — affect regenerative processes and tissue healing. Further laboratory and clinical studies are needed to evaluate the potential of different types of AB biomaterials for the treatment of reversible pulpitis using direct pulp capping.

Growth Factors in AB Biomaterials

Growth factors stimulate angiogenesis and tissue regeneration during the healing process. They are present in all tissues, but blood is their primary reservoir (14). In addition, they play a role in analgesia of damaged tissues, which explains why patients treated with platelet concentrates often experience minimal postoperative pain(15).

The key growth factors involved in tissue biostimulation include:

1. Epidermal Growth Factor (EGF) – a mitogen for epithelial tissues, fibroblasts, and endothelial cells; stimulates granulation tissue formation (15).
2. Platelet-Derived Growth Factor (PDGF) – promotes connective tissue proliferation, acts chemotactically, and stimulates fibroblasts to produce type III collagen (15).
3. Vascular Endothelial Growth Factor (VEGF) – a potent mitogen for endothelial cells with strong angiogenic effects (15).
4. Fibroblast Growth Factor (FGF) – enhances proliferation and migration of keratinocytes and fibroblasts and supports angiogenesis (15).

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5. Transforming Growth Factor Beta (TGF-β) – supports epithelial cell growth, angiogenesis, and type I collagen synthesis by fibroblasts; proven to stimulate DPSC proliferation and osteogenesis (16,17,18).
6. Insulin-Like Growth Factor-1 (IGF-1) – directly stimulates osteoblasts, increases bone turnover in patients with low bone density, and, in combination with PDGF, accelerates wound healing (15,19).
7. Bone Morphogenetic Proteins (BMPs) – part of the TGF-β family, play key roles in osteogenesis, vasculogenesis, and embryonic development; regulate cellular differentiation via specific receptors (15,19).

Growth factors induce neovascularization, normalize hemodynamics and tissue oxygenation, stimulate extracellular matrix formation, and support tissue remodeling and maturation. The cytokines released during this process promote cell migration and proliferation within the fibrin matrix. Together with angiogenesis and lymphangiogenesis, these mechanisms are fundamental to wound healing (16,17,18).

Hong et al. (2018) studied the effects of PRF and CGF biomaterials on stem cells from the apical papilla (SCAPs) and found that both enhanced proliferation, migration, and mineralization, with PRF showing higher expression of odontogenic markers (ALP, BSP, DMP-1, DSPP), confirming its strong regenerative potential. Similar results have been observed with dental pulp stem cells (DPSCs), reinforcing the role of PRF as a universal biostimulator in dental tissue regeneration (20).

According to Tsutsui (2020), the main bioactive molecules involved in pulp regeneration include bFGF, TGF-β, PDGF, BMPs, and NGF (nerve growth factor). bFGF promotes cell proliferation and angiogenesis; TGF-β regulates odontoblast differentiation and reparative dentin formation; PDGF supports tissue remodeling; BMP-2 and BMP-4 induce the expression of dentin-specific genes (DSPP, DMP-1); and NGF contributes to neural differentiation and reinnervation(21).

Anaya-Sampayo et al. (2021) developed a composite biomaterial based on nano-hydroxyapatite, chitosan, gelatin, and alginate modified with PRF, demonstrating enhanced adhesion, viability, and osteogenic potential of DPSCs, stimulating the expression of ALP, RUNX2, and osteocalcin (22).

Chai et al. (2019) compared traditional Platelet-Rich Plasma (PRP) and Liquid PRF on human DPSCs cultured under normal and LPS-induced inflammatory conditions. Liquid PRF improved migration, mineralization, and odontogenic differentiation while suppressing inflammatory markers (TNF-α, IL-1β, NF-κB/p65), highlighting its dual regenerative and anti-inflammatory role (23).

Dental Pulp Stem Cells (DPSCs)

Stem cell populations can be isolated from various dental structures and at different developmental stages. To date, at least eight distinct mesenchymal stem cell populations have been identified:

- Dental pulp stem cells (DPSCs) (1)Gronthos et al., 2000);
- Stem cells from human exfoliated deciduous teeth (SHED) (24) Miura et al., 2003);
- Periodontal ligament stem cells (PDLSCs) (25) Seo et al., 2004);
- Dental follicle precursor cells (DFPCs) (26) Morsczeck et al., 2005);
- Alveolar bone marrow stem cells (ABMSCs) (27) Matsubara et al., 2005);
- Stem cells from the apical papilla (SCAPs) (28) Sonoyama et al., 2006);
- Tooth germ progenitor cells (TGPCs) (29) Ikeda et al., 2008);

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- Gingival mesenchymal stem cells (GMSCs) (30) Zhang et al., 2009).

Each of these populations demonstrates varying osteogenic, chondrogenic, and odontogenic potential, offering broad applications in dental regenerative medicine. Among them, DPSCs are the most accessible and suitable for in vitro studies.

The discovery of DPSCs marked a turning point in regenerative endodontics. Following their isolation by Gronthos et al. (1), further studies confirmed that these neural-crest-derived cells exhibit high proliferative and differentiation potential (31). They reside in perivascular and perineural niches within the pulp and contribute to its homeostasis and repair after injury.

DPSCs can differentiate into odontoblasts, osteoblasts, chondrocytes, neuron-like cells, and adipocytes, positioning them as a versatile source for tissue engineering in dentistry. DPSCs can be isolated from extracted healthy teeth and cultured in vitro to model pulp cell behavior. Such cell cultures serve as an effective platform for evaluating the biological potential of different AB biomaterials — including cell migration, proliferation, and comparative biostimulation studies(30,31).

Conclusion

The establishment and identification of dental pulp stem cell cultures provide a reliable model for investigating cell migration, proliferation, and differentiation in environments containing AB biomaterials. These studies help confirm the biostimulatory and regenerative potential of autologous blood biomaterials and their promising role in vital pulp therapy and tissue engineering.

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