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Regenerative endodontic therapy- plasmotherapy. Review - Part I.

Janet Kirilova[1], Elitza Deliverska[2], Dimitar Kirov[3], Dimitar Yovchev[4], Snezhanka Topalova-Pirinska[1]

1. Department of Conservative Dentistry, FDM, Medical University of Sofia, Bulgaria

2. Department of Maxillofacial Surgery, FDM, Medical University of Sofia, Bulgaria

3. Department of Prosthetic Dental Medicine, FDM, Medical University of Sofia, Bulgaria

4. Department of Imaging and Oral Diagnostic,FDM, Medical University of Sofia, Bulgaria

Abstract

The aim of this review is to summarize biological products - plasmotherapy for regenerative endodontic therapy. Plasmotherapy (part of tissue engineering) is a relatively new method in medicine that stimulates the healing of hard and soft tissues by significantly relieving pain. The mechanism of action of bioproducts for plasmotherapy are considered, as well as the types of plasmotherapy products and protocols for their application.

Keywords: regenerative dentistry, biomaterials, growth factors, tissue engineering, endodontics

Introduction

During application of the conservative approach in endodontic treatment, the pulp chamber and root canals are cleaned and filled with biologically inert substances, which does not preserve viable tissue. Endodontic treatment is currently developing with the introduction of regenerative endodontic therapy. This aims to replace damaged tissues, including dentin, root structures and cells in the pulpo-dentin complex via biologically activated procedures (1,2). Regenerative endodontic therapy replaces affected tissues with vital and viable ones, both in the periapical periodontium and in the dental pulp, by stimulating the healing process or by placing biologically active substances (3,4,5).

Regenerative endodontic therapy is in fact tissue engineering. Tissue engineering is based on three main pillars: 1) cells/stem cells responsible for synthesizing new tissue matrices; 2) growth factors initiating the

functions; 3) a biomaterial scaffolds needed as an extracellular matrix for cell differentiation and biosynthesis(6).
Regenerative endodontic therapy is achieved through the introduction of biological products. These are the blood plasma along with growth factors contained in the platelets, as well as some graft materials(7,8,9). Protocols for the production of various biological products are continuously being perfected (10,11,12). As a result, the possibilities of endodontic treatment are expanded, resulting in significantly reducing the time for regeneration of affected tissue (7).

Aim

The aim of this review is to summarize biological products (plasmotherapy, bone replacement materials) and techniques for regenerative endodontic therapy.

Plasmotherapy

Plasmotherapy (part of tissue engineering) is a relatively new method in medicine that stimulates the healing of hard and soft tissues by significantly relieving pain (13,14). It is introduced to improve the healing of various surgical wounds by stimulating the ability of one's own tissues to heal (15). Plasmotherapy products are obtained after centrifugation of venous blood taken from a patient by venepuncture (8). Centrifugation aims to separate the blood components, the erythrocytes from the blood plasma. Plasma is rich in leukocytes (granulocytes and neutrophils), macrophages, plasmocytes and platelets, from which local growth factors and cytokines are released by degranulation(16). After centrifugation, these cells remain in a three-dimensional fibrin matrix. Placed in a surgical wound, the complex significantly improves tissue angiogenesis and accelerates the healing process. It has been shown that the time for tissue recovery is reduced by almost half, and that postoperative pain, swelling and discomfort are minimized (17). Plasmotherapy is an essential part of modern regenerative therapy (6).

Mechanism of action of bioproducts for plasmotherapy

Following a surgical intervention, platelets and leukocytes are essential for tissue regeneration within the process of wound healing. This process goes through three phases: inflammation, proliferation and new tissue formation. Platelets form a coagulum for initial hemostasis immediately after tissue damage or after the creation of a surgical wound. This coagulum is subsequently replaced by a fibrin clot. Once activated, platelets secrete natural molecules - various growth factors capable of stimulating cell growth, proliferation, differentiation and tissue repair (14,18). Platelets show significant metabolic activity. During their life cycle, they contain inactive growth factors that are activated in response to clotting. Increasing the concentration of platelets in blood products stimulates and accelerates the healing process by releasing biologically active growth factors and cytokines (18).
Growth factors that stimulate angiogenesis and tissue regeneration during the healing process exist in all tissues, but blood is their main reservoir (6,18).
Some important growth factors include: vascular endothelial growth factor (VEGF); platelet-derived growth factor (PDGFs); transforming growth factor beta (TGF-β); epidermal growth factor (EGF); insulin-like growth factor (IGF-1) and others. They cause new vascularization, normalize hemodynamics, tissue respiration, metabolism, stimulate the formation of extracellular matrix, support tissue maturation and remodelling. The

release of cytokines stimulates cell migration and proliferation within the fibrin matrix. Along with angiogenesis and lymphogenesis, this process is essential for wound healing(19,20,21). Under the influence of growth factors, great results can be achieved in soft tissue and bone augmentation management.

Types of plasmotherapy products and protocols for their application

Various platelet concentration protocols are available (7,11).

The products obtained after venepuncture and venous blood centrifugation differ depending on the chosen centrifugation regime and the type of collection tube used. Initially, a protocol for platelet-rich plasma (PRP) was developed - the first generation of protocols for the separation of blood plasma. In 2001, Choukroun and his collaborators developed a second generation of protocols, resulting in an autogenous concentrate rich in platelet mass that contains platelets, leukocytes, and growth factors in a healthy fibrin matrix (10,18). PRF (Platelet-rich fibrin) has the following variants: A-PRF and A-PRF+ (Advanced platelet rich fibrin and Advanced platelet rich fibrin plus) modern platelet rich fibrin matrix; i-PRF (injectable platelet rich fibrin) and i-PRF+(injectable platelet rich fibrin plus); S-PRF - a product that allows to make a "sticky bone" consistency of the used graft material (19). How do the proposed protocols differ from the original ones? The classical centrifugation protocol requires a relatively high centrifugation force of 1000 g - 708 g (10,11,20). In this way, a fibrin network with a dense structure and minimal interfibrous spaces is obtained (7). The fibrin matrix includes platelets, leukocytes, lymphocytes, macrophages, and stem cells, but the cell distribution is uneven. They are clustered near the erythrocytes, while at the free end of the tube, their density decreases dramatically (precisely this part of the plasma is used for further procedures). In addition, the obtained plasma contains only platelets, and a very small quantity of those. Moreover, anticoagulants, some of which of animal origin, are added to the tubes to slow down the clotting process, which could trigger an antigen-antibody allergic reaction. In the Choukroun protocol, the centrifugation force is low - it decreases to 208 g and 60 g(Low-Speed centrifugation consept-8,9). Another difference is that tubes without anticoagulant are used, eliminating the possibility of antigen-antibody reaction. The resulting fibrin matrix has a more porous structure and more extensive interfibrous spaces (facilitating cell migration), the distribution of platelets is uniform, significantly increasing their number, and the plasma contains leukocyte cells. It has been shown that the presence of leukocyte cells is a factor which attracts stem cells to the wound surface (this is one of the fundamental factors in tissue engineering). Clinical studies confirm the importance of cell distribution for the processes of vascularization and tissue regeneration (11). A significant increase in growth factors TGF-β1, VEGF, PDGF-AA, PDGF-AB PDGF-BB in the centrifuged plasma has been demonstrated. With prolonged centrifugation time (A-PRF + Advanced platelet rich fibrin plus regimen) growth factors TGF-β1, VEGF, EGF, IGF-1 are significantly increased (8,9). Both Choukroun protocols (A-PRF with 1300 rpm for 8 minutes and A-PRF + with 1300 rpm for 14 minutes) showed excellent biocompatibility and cellular activity in an in vitro study(11). Up to 300% increase in type 1 collagen synthesis is also found. And the same is a key factor in wound healing and remodeling. The A-PRF + centrifugation regimen facilitates the separation of plasma from red blood cells.

The A-PRF and A-PRF + protocols produce platelet-enriched fibrin. The end result combines the fibrin network, platelet growth factors and leukocyte activity, the three main factors for successful tissue engineering with one goal - to achieve rapid and complete vascularization. That is, two of the factors for successful tissue engineering - the matrix (three-dimensional fibrin network), and the growth factors – are present. Regarding the third factor - stem cells, in a study by Ponte et al. found that leukocytes secrete signalling factors that stimulate tissue regeneration and recruitment of mesenchymal stem cells. Strategies that use stem cells for tissue regeneration can be optimized by using bioactive skeletons or by adding various growth factors (23), i.e. the presence of leukocytes in the plasma after centrifugation according to the Choukroun protocol is particularly important.

A-PRF is a 'blood concentrate', not a 'platelet concentrate'. The A-PRF protocol aims to achieve a better composition for the treatment cascade: slow-release cytokines, natural fibrin, monocytes, granulocytes and plasma proteins with long-term release of autologous bone morphogenetic protein [24].

Another protocol is i-PRF (also based on the concept of low spin power), but here the reduction of spin power is 60g. This protocol uses plastic tubes without anticoagulants or other additives, unlike the A-PRF protocols where the tubes are glass, also without anticoagulants. The end product is the same fibrin matrix, rich in platelets and leukocytes, but in liquid form (injectable form of PRF). The resulting liquid plasma fraction is retained in liquid form for 10-15 minutes and coagulates after injection. It is convenient to combine it with different graft materials. The goal is to improve the soft tissue biotype without surgery, just by injection (19). This is achieved by reducing the spin time and speed, namely 700 rpm and 3 minutes for women or 700 rpm and 4 minutes for men(19). The i-PRF + protocol focuses on aesthetics. Special tubes are used, and the spin mode is 700 rpm for 5 minutes.

The application of bone replacement materials and techniques for regenerative endodontic therapy in regenerative endodontic therapy will be discussed in the second part of this review.

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Corresponding author:

Assoc.Prof. Dr.Janet Kirilova, PhD
Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University,
1, Sv. Georgi Sofyiski St., 1431 Sofia, Bulgaria.
Tel.: +359 888343397
E-mail: janetkirilova@gmail.com