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Research Article

PLATELET RICH FIBRIN: FOCUS ON GROWTH FATORS AND CYTOKINES

Amita Mali., Shraddha Gokhale., Vishakha Patil., Yogesh Khadtare and Smruti Lulla

Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune

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ABSTRACT

Wound healing is a complex and growing area that deals with many cell types and growth factors. Development of bioactive surgical additives which can regulate inflammation and accelerate healing is one of the great challenges in the field of clinical research. Platelet rich fibrin(PRF) is a second generation platelet concentrate which contains growth factors. The following review attempts to summarize the relevant literature regarding the growth factors and cytokines present in PRF focusing on its effect on healing.

INTRODUCTION

Platelet rich fibrin (PRF) is a biomaterial which was first developed in France by Choukroun *et al* and has a specific composition and three dimensional architecture.[1] It is an autologous cicatricial matrix, which is neither fibrin glue nor like a classical platelet concentrate.[2] It is prepared from patient's own blood without biochemical modification as no anticoagulant or bovine thrombin is required.[3] During centrifugation the PRF clot is obtained by a natural polymerization process.[4] The required quantity of whole blood is drawn into the tubes without anticoagulant and is immediately centrifuged at 2500 rpm for 10 minutes [Choukroun *et al* 2006]. The absence of anticoagulant allows activation of the majority of platelets in the sample to start the coagulation cascade within a few minutes. Initially, fibrinogen is concentrated in the upper part of the tube, until the circulating thrombin transforms it into a fibrin network. The outcome is a fibrin clot containing platelets in the middle of the tube, between the red blood cell layer at the bottom and acellular plasma at the top. This clot is removed from the tube and the attached red blood cells are scraped off and discarded to obtain a PRF clot. The natural fibrin architecture of the PRF clot seems responsible for a slow release of growth factors and matrix glycoproteins.[4] PRF dwells among a new generation of platelet concentrate that jump-starts the healing process to maximize predictability. Though platelets and leukocyte cytokines play an important part in the biology of this biomaterial, the fibrin matrix supporting them certainly constitutes the determining element responsible for the real therapeutic potential of PRF. The synergy between cytokines and their supporting fibrin matrix is important than any other parameter.[5] The slow polymerization mode of PRF and cicatricial capacity creates a physiologic architecture favourable for wound healing.[1] PRF can be considered as a healing biomaterial from clinical standpoint and it has emerged as one of the promising regenerative materials in the field of periodontics.[1,6]

Growth Factors and Cytokines in PRF

Growth factors have an essential role in the healing process and tissue formation as all stages of the repair process are controlled by a wide variety of cytokines and growth factors acting locally as regulators of the most basic cell functions. Growth factors influence many of the processes common to both tissue repair and disease, including angiogenesis, chemotaxis and cell proliferation. Thus growth factors and cytokines play an important role as therapeutic molecules for the repair or regeneration of a wide range of tissues.

Corresponding author:* **Amita Mali
Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune

Growth Factors in PRF

TGF β: Fibrosis agent

Transforming growth factor β (TGF β) belongs to a vast superfamily [7] of related proteins that also includes BMPs, growth and differentiation factors, activins, inhibitins and anti-Mullerian hormone. There are five isoforms of transforming growth factor β [β1-β5]. Most cells synthesize and respond to TGF β, but high levels are found in bone, platelets and cartilage.TGF β-1 is the most abundant isoform at protein level. It is not only in the platelet α-granules but also in general during intercellular dialog.[8] Its effects are extremely varied according to the amount applied, the matrix environment and cell type. It has been shown that it could stimulate the proliferation of osteoblasts just as easily as it could cause their inhibition. Although its effects in terms of proliferation are highly variable, for the great majority of cell types, it constitutes the most powerful fibrosis agent among all cytokines; in other words, it will induce a massive synthesis of matrix molecules such as collagen I and fibronectin, whether by osteoblasts or fibroblasts. Thus, although its regulation mechanisms are particularly complex, TGF β can be considered as an inflammation regulator through its capacity to induce fibrous cicatrisation.[7]

It inhibits collagen degradation by decreasing proteases and increasing protease inhibitors, all of which favour fibrogenesis. Further, it brings about chemotaxis and mitogenesis of osteoblast precursors while also stimulating osteoblast deposition. In addition, it inhibits osteoclast formation and bone resorption, thus favouring over resorption.[3] TGF β1 is ubiquitous, multifunctional growth factor. Several alternative names have been proposed, such as cartilage-inducing factor, differentiation inhibiting factor and tissue-derived growth inhibitor.[9]

Effects of TGF β

1. During the early stages of bone formation, the action of transforming growth factor- β is to recruit and stimulate osteoprogenitor cells to proliferate, providing a pool of early osteoblasts.[8]
2. In contrast, during later phases of osteoblast differentiation, transforming growth factor- β blocks differentiation and mineralization. These effects appear to be highly dependent on bone cell source, dose applied and the local environment, which may be a result of the inhibition of DNA synthesis at high transitioning growth factor-b concentrations.[8]
3. Bone formation by TGF- β 1 is promoted through chemotactic attraction of osteoblasts, enhancement of osteoblast proliferation and the early stages of differentiation with production of ECM proteins, stimulation of type II collagen expression and proteoglycan synthesis by chondrocyte precursor cells and suppression of hematopoietic precursor cell proliferation.[9]
4. A pivotal role in the bone-remodeling process has been assigned to TGF-β1 because it was proven to affect both bone resorption and formation. TGF-β l is secreted in a latent form by bone cells and is stored in the ECM. Active, resorbing osteoclasts are capable of activating TGP-β which in turn attenuates further bone resorption by impairing osteoclastogenesis and promotes bone formation through chemotactic attraction and stimulation of proliferation and diffentiation of osteoblast precursors. Thus TGF β 1 plays an important role in keeping the balance between two tightly regulated processes of bone resorption and subsequent bone formation.[9]
5. Additionally, transforming growth factor- β inhibits the expression of the Runx2 and osteocalcin genes, whose expression is controlled by Cbfal/Runx2 in osteoblast-like cell lines, and this was found to be mediated by Smad3.[8]
6. TGF-β1 regulates a broad range of biological processes, including cell proliferation, cell survival, cell differentiation, cell migration and production of extracellular matrix (ECM).
7. The combined actions of these cellular responses mediate the global effects of TGF-βl on immune responses angiogenesis, wound healing, development and bone formation. Bone formation is promoted through chemotactic attraction of osteoblasts, enhancement of osteoblast proliferation and the early stages of differentiation with production of ECM proteins, stimulation of type II collagen expression and proteoglycan synthesis by chondrocyte precursor cells, and suppression of hematopoietic precursor cell proliferation.[9]
8. Regarding the diversity of processes in which TGF-β l is involved, it is not surprising that this cytokine is of major importance both during embryogenesis and in maintaining tissue homeostasis during life.[9]
9. TGF- β stimulates PDL cell extracellular matrix synthesis, mitogenesis and proliferation. TGF- β receptors are up-regulated in regenerated PDL tissues suggesting that TGF- β may also be capable of mediating periodontal regeneration.[10]
10. TGFβ's are multifunctional cytokines that regulate growth, proliferation, adhesion and apoptosis of various cell types. In vitro, TGF β enhances collagen gel contraction, which indicates potential induction of fibrosis in vivo. This effect is increased by platelet derived growth factor (PDGF) and insulin like growth factor (IGF) but decreased by EGF.
11. Cell surface proteoglycans are differentially affected by TGF β. TGF β also forms substrates with glycosaminoglycans of different compositions and is an important signal in regulation of integrins, thus affecting cellular behaviour in adhesion, aggregation and migration.
12. In vivo. TGF β acts as potent inhibitor of growth for many types of cells, in particular epithelial cells, endothelial cells, haematopoctic cells and lymphocytes.
13. TGF β thereby mainly acts as a morphogen in that matrix synthesis of mesenchymal cells and autogenesis is induced, additionally has an immunosuppressive effect, which may be relevant in tumor growth.
14. TGF β also plays a role in bone graft incorporation, while TGF β2 and TGF β3 are supposed to be of minor importance in this matter. In soft tissue healing, TGF β acts as a fibrogenic factor that causes wound contraction.[11]

Disadvantage

TGF β1 not only modulates bone formation, but can also stimulate osteoclast formation and function under certain circumstances. Consequently the treatment may induce both bone formation and resorption.

It has a short half life implying the need for a matrix to allow slow release of the growth factor.

It is implicated in diverse functions outside the bone environment, suggesting that its systemic application might cause unwanted side effects.[9]

PDGFs: Stimulant of mesenchymatous lineages

Platelet derived growth factor (PDGF) seems to be the first growth factor present in a wound and it initiates connective tissue healing, including bone regeneration and repair. There are about 1200 molecules of PDGF in every individual platelet. Therefore a greater concentration of platelets as seen in PRF can be expected to have a profound effect on wound healing enhancement and bone regeneration.[3] The PDGF family includes four isoforms: PDGF-A, PDGF-B and recently discovered PDGF-C and PDGF-D.[10]

PDGFs are essential regulators for the migration, proliferation and survival of mesenchymatous cell lineages.[7] In 1974, it was discovered that the platelet was the principal source of mitogenic activity present in the whole blood serum and missing in plasma or plasma derived serum. Consequently this mitogen was termed "platelet-derived growth factor". [12] It is a powerful mitogen and has a strong chemotactic effect on osteoblasts and other connective tissue cells and may act to recruit mesenchymal cells during bone development and remodelling.[8]

According to the distribution of their specific receptors, they are able to induce stimulation as easily as inhibition of the development of these cells. This position of regulation node plays a fundamental role during the embryonic development and all tissue remodelling mechanism. For this reason, PDGFs play a critical role in the mechanism of physiological cicatrisation, the pathogenesis of atherosclerosis and many other fibro proliferative diseases (e.g neoplastic and pulmonary and renal fibrosis).[7]

Effect of PDGF

1. PDGF is an extraordinarily potent mitogen for most mesenchymally derived connective tissue forming cells.[12]
2. Not only is PDGF a potent mitogen but it is also chemotactic for the same cells for which it is mitogenic. When PDGF is exposed to susceptible cells, it induces them to migrate in a concentration gradient of PDGF
3. A number of intracellular events occur after exposure to PDGF. Some are virtually immediate (or at least as rapidly as they can be measured), while others require hours to days to be discerned. Two potentially important early, rapid responses to PDGF include auto phosphorylation of the PDGF receptor and increased turnover of membrane phospholipids.
   - Tyrosine phosphorylation has been associated with increased mitogenesis in some case and auto phosphorylation of the PDGF receptor may be an important component in the signaling mechanism that induces further changes within the cell that ultimately lead to DNA synthesis.[12]
   - Increased turnover of phosphatidylinositol can lead to formation of intracellular diglyceride with its subsequent breakdown to form free arachidonic acid, monoglyceride and phosphatidic acid. Diglyceride formation may play a role in stimulating activation of C kinase, which can lead to calcium mobilization, also considered to be potentially important in inducing DNA synthesis and cell proliferation.
4. When PDGF binds to cells, it also induces increased expression of several genes, including c-fos, c-myc, and the gene for beta interferon. The role of each of these genes in cell proliferation, participating both as agonists (c-myc. c-fos) and as inhibitors (beta interferon), is not yet well understood.
5. PDGF also induces changes in cell shape and reorganization of actin cables. This cell shape change may also be associated mitogenesis.[12]
6. The healing of soft tissues involves reepithelizationtion, angiogenesis and extracellular matrix deposition which is regulated by different types of growth factors. Platelet-derived growth factor acts on several cell types involved in wound healing. It stimulates mitogenicity, chemotaxis of fibroblasts, smooth muscle cells, chemotaxis of neutrophils and macrophages. It also stimulates macrophages to produce and secrete other growth factors of importance for various phases in the healing process. Moreover, PDGF has been shown to stimulate production of several matrix molecules, like fibronectin, collagen, proteoglycans and hyaluronic acid.[13]
7. Platelet-derived growth factor may also be of importance at later stages of wound healing, since it stimulates contraction of collagen matrices in vitro, implicating a role in wound contraction in vivo. Moreover, PDGF stimulates the production and secretion of collagenase by fibroblasts suggesting a role in the remodeling phase of wound healing. PDGF to affect wound healing has to be present at the site of the wound. [13]
8. PDGF also induces increased synthesis of RNA and protein, which may be important in connective tissue-forming cells since, upon exposure to PDGF, they increase their synthesis, release of collagen, proteoglycans and elastic fiber proteins.[12]
9. PDGF may also have direct and indirect effects on bone resorption by upregulation of collagenase transcription and an increase in IL-6 expression in osteoblasts.[8]
10. The PDGF receptors are expressed on capillary endothelial cells and PDGF has been shown to have an angiogenic effect .The effect is however, weaker than that of fibroblast growth factors or VEGF and PDGF does not appear to be of importance for the initial formation of blood vessels. However, in specific organs, the effect of PDGF on angiogenesis may be significant.[13]
11. Recently it has shown that PDGF is an extremely potent vasoconstrictive agent. It is more potent than angiotensin-II in inducing smooth muscle contraction

and could play an important role in constriction of blood vessels.[12]

12. Moreover. PDGF B-chain produced by capillaries may have a generally important role to recruit pericytes that is likely to be required to promote the structural integrity of the vessels. Platelet-derived growth factor has also been implicated in the regulation of the tonus of blood vessels.
13. On one hand, PDGF induces constriction of different types of blood vessels. On the other hand, PDGF-BB stimulation of endothelial cells induces a nitric oxide mediated relaxation.[13]
14. Another effect of PDGF that is of importance in the vascular system is its feedback control effect on platelet aggregation. Platelet-derived growth factor stimulation leads to decreased platelet aggregation. Human platelets which are a rich source of PDGF, have PDGF α-receptors but not β-receptors and PDGF receptors have also been demonstrated on megakaryocytes, the precursors of platelets. After thrombin-induced platelet aggregation, the content of the α-granules including PDGF is released. The fact that thrombin-induced platelet aggregation is accompanied by activation of platelet PDGF a-receptors and that this effect can be inhibited by PDGF antibodies, indicates that the PDGF released from platelets serves an autocrine feedback role in control of platelet aggregation.[13]

The IGF axis -Cell-protective agent

Insulin-like growth factors (IGFs) I and II are positive regulators of proliferation and differentiation for most cell types, which unfortunately include tumor cells which use the IGF system to increase their survival potential. Although these cytokines are cell multiplication mediators, in the main they constitute the major axis of programmed cell death (apoptosis) regulation, by inducing survival signals protecting cells from many matricial apoptotic stimuli. Moreover, even though IGFs are released during platelet degranulation, they are initially massively present in blood circulation.[7]

Insulin like growth factors increase proliferation and play a major role in stimulating mature osteoblast function.

Leucocytes and Cytokines in PRF

Inflammation is defined by all reaction phenomena initiated in response to a specific aggression. The inflammatory process proceeds in 3 successive phases: vascular phase, cellular phase, and cicatrization phase. The vascular phase is characterized by the development of hemostasis (i.e the constitution of a fibrin-based cicatricial matrix) and the installation of a leucocytic node (i.e the arrival on the injured site of inflammatory cells able to coordinate all concerned cellular forces leading them to the antiinfectious cover of initial healing steps). Finally, all the hemostatic processes lead to coagulation along the vascular wound and to the formation of a fibrin clot.[14]

The initial vasoexudative phenomena allow leucocyte migration to the inflammatory site. The first in place are polymorphonuclear leucocytes; they are replaced by monocytes/macrophages with their high phagocytosis ability. Lymphocytes and plasmocytes take part in the specific antigenic reaction. All these cells are activated on inflammatory sites and secrete many cytokines and growth promoters. The inflammation mediators take part in the fibroblast recruitment, induce proliferation, and stimulate biosynthetic activity, leading to the secretion of proteases (metalloproteases, plasmin) as well as the neosynthesis of the matricial macromolecules.

As inflammatory processes are inherent to the surgical act itself, the PRF addition might decrease many harmful effects, mainly by correcting certain destructive and noxious excesses during the healing process of wounded tissues.

Three principal inflammatory cytokines are IL-1β, IL-6 and TNF-α.[14]

Inflammatory Cytokines

Interleukin-lβ (IL-lβ)

IL-lb remains the prevalent isoform and is a key mediator of inflammation control. Its main activity is the stimulation of T helper lymphocytes. In combination with TNF-α, IL-1 would be implied in osteolysis, where it activates osteoclasts and inhibits bone formation.[3]

Interleukin 6 (IL-6)

IL-6 is a multifunctional cytokine that was originally identified as a B cell differentiation factor which induced the final maturation of B cells into antibody producing cells.[3] IL-6 significantly stimulates the secretion of antibodies by 120-400 times and this rate can increase.[14] In addition, IL-6 is an essential accessory factor for T cell activation and proliferation. IL-6 induced not only proliferation but also differentiation of cytotoxic T cells (CTL) in the presence of IL-2 from murine as well as human thymocytes and splenic T cells. The positive effect of IL-6 on hematopoiesis was first described by Ikebuchi *et al* in 1987.[3] IL-6 functions as a hepatocyte-stimulating factor and induces expression of various acute phase genes. Therefore constitutes a major amplification pathway for signals transmitted to immune cells. Thus, it supports the reaction chains leading to inflammation, destruction and remodelling. IL-6 and IL-3 act in a synergistic way to promote hematopoietic stem cell proliferation in vitro.[14]

Tumor necrosis factor-α (TNF-α)

TNFα is one of the cytokines first released during the inflammatory response to bacterial endotoxin aggression.

TNF derives its name from the ability to stimulate tumor necrosis and regression. TNF-α activates monocytes and stimulates the remodeling capacities of fibroblasts. In addition, it increases phagocytosis, neutrophil cytotoxicity and modulates the expression of key mediators such as IL-1 and IL-6.[14]

Healing Cytokines

A cicatricial property can be defined in relation to 2 aspects:

- Either it inhibits the inflammatory signal pathways and neutralizes their amplification. This is the case of IL-4.
- Or it supports and coordinates the development of initial cicatricial structures such as vascular tubes. This is the case of VEGF

Interleukin 4 (IL-4)

IL-4 induces differentiation of naive helper T cells into TH2 cells. This cytokine also supports proliferation and differentiation of the activated B cells. During inflammatory phenomena, it supports healing by moderating inflammation for e.g it increases fibrillary collagen synthesis by fibroblast and inhibits stimulation of MMP-1 and MMP-3 by IL-β. IL.-4 is a potent inducer of Interleukin-1 receptor antagonist (IL-Ra), which contributes to its anti-inflammatory actions by neutralizing the biological effects of IL-4.[3]

Interleukin-l receptor antagonist (IL-Ra)

Contributes to its anti-inflammatory actions by neutralizing the biological effects of IL-1.[3]

Vascular endothelial growth factor (VEGF)

VEGF is the most powerful and ubiquitous of known vascular growth promoters.[14] It is considered as a master regulatory molecule for angiogenesis-related processes.[3] It plays a direct role in the control of endothelial cell behaviours such as proliferation, migration, specialization or just survival. The simple presence of this cytokine will be enough to start angiogenesis and the combination of its different isoforms will make it possible to direct and refine the development plan of the network growth. Factors like IGF-1 and IL-lβ regulate angiogenesis by up regulating the expression of VEGF. [14]

CONCLUSION

Periodontal regeneration is unpredictable with any regenerative therapy currently used. A promising biologic therapy, offering various applications in dentistry is the use of platelet concentrates. PRF is a fibrin technology which belongs to a new generation of platelet concentrates. The growth factors present in PRF are described as promoters of tissue repair mechanisms and remodeling. Thus PRF features all the necessary parameters permitting optimal healing and can prove to be a simple and inexpensive technique for the successful regeneration of periodontal tissues.

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