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Concentrated Growth Factor: A Review

Review Article

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Abstract

CGF-"A miracle in regenerative dentistry". CGF- Concentrated Growth Factor is a new regeneration platelet aggregate which is used widely in periodontal and oral surgeries. It is adopted without the use of chemicals which grades it more eco-friendly. It contains various growth factors which enhances its action and promotes wound healing. CGF is currently used along with autologous bone particles to induce bone regeneration and connective tissue attachment that shows excellent results .In future research can prove, that CGF can be used as a sole regenerative material. It is an excellent biomaterial which showcases back to normal periodontium with external finishing. This article focuses on the preparation, applications and advantages of using CGF in the field of regeneration.

Keywords: Concentrated Growth Factor; Periodontal Regenerative Procedures; Platelets Growth Factors.

Introduction

Periodontitis is a disease of the periodontium characterized by the irreversible loss of connective tissue attachment and supporting alveolar bone [1]. Periodontitis begins with the development of pocket formation induced by bacterial plaque, and progresses to the initiation of alveolar bone destruction, resulting in various bone destructive patterns and the alteration of available alveolar bone [2]. Intrabony defects associated with periodontal pockets represent the anatomical sequelae of the apical spread of the dental plaque in the course of periodontitis [3]. Such defects, if left untreated, are risk factors for periodontitis progression and further loss of attachment [4]. Several surgical techniques have been developed to regenerate periodontal tissues, such as guided tissue regeneration (GTR) [5] and the use of enamel matrix derivative (EMD) [6]. In recent years, the use of autologous platelet concentrates (APCs), which are rich in growth factors, combined with these surgical techniques has emerged as a possible tool to enhance the predictability of the treatment of periodontal defects. Addition to the existing platelet concentrates was the Concentrated Growth Factors (CGFs) which was first developed by Corigliano in 2010, originally developed by Sacco in 2006 [7].

CGFs are produced by centrifuging blood samples at alternating and controlled speeds using a special centrifuge (Medifuge, Silfradentsrl, Italy). Different centrifugation speeds permit the isolation of a much larger and denser fibrin matrix richer in growth factors than typically found in PRP or PRF. In theory, CGFs appear to exhibit superior potential for tissue regeneration in clinical and biotechnological applications, as evident in a report of sinus and alveolar ridge augmentation [9]. Unlike PRP, CGFs do not dissolve rapidly following application. Instead, the strong fibrin gel in the matrix addition is slowly remodelled in a similar manner to a natural blood clot. Thus, CGFS prolong the duration of growth factor activity, which is conducive for growth factor synergy, and enhances cell proliferation and osteogenic differentiation [10]. Another likely favourable component in CGFs are stem cells. The CGFs not only improve the wound stability, which is essential for the establishment of a new connective tissue attachment to a root surface, but also provide a scaffold supporting cytokine attachment and cellular migration. Through the polymerization of the fibrinogen molecules, the fibrin block comprises a 3D polymer network of interwoven fibres. Upon scanning electron microscopic analysis of the fibrin block, Rodella et al., observed a fibrin network constituted by thin and thick fibrillar elements,

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including multiple trapped platelets. A 3D environment is crucial for cell-cell and protein-protein interactions to create tissue symmetry [8].

Recently, with the advent of platelet concentrates there has been considerable interest in the use of growth factors as therapeutic agents in the treatment of oral and maxillofacial pathologies. This platelet concentrates are autologous blood preparations containing supra-physiological concentration of platelets, which by definition are neither toxic nor immunogenic and are capable of accelerating the normal processes of bone regeneration. Generally, for bone and tooth regeneration it requires 3 components like 1. Scaffolds 2. Stem cells, 3. Growth factors. This platelet concentrates contain all these 3 components hence considered as ideal material for bone and tooth regeneration. According to Badran (2017) effective platelet concentrates for bone regeneration should be 2-6-fold increase in normal platelet concentration and ideally it should be 5-fold increase. Platelets begin to actively secrete these proteins within 10 mins after clotting, with more than 95% of the pre-synthesized growth factor secreted within 1 hour. For the balance of their life (5-10 days), the platelets synthesize and secrete additional proteins. As the direct platelet influence begins to subside, macrophages, which arrive by means of vascular ingrowth stimulated by the platelets, assume responsibility for wound healing regulation by secreting their own factors. Thus, the platelets at the repair site ultimately set the pace for wound repair.

Evolution of Platelet Concentrates

1954

Kingsley [11] first used the term PRP to earmark thrombocyte concentrate during experiments related to blood coagulation.

1970

"Fibrin glue" was introduced by Matras [12] which improved healing of skin wounds in rt models. Fibrin glue was made by polymerizing fibrinogen with thrombin and calcium. However, due to low concentration of fibrinogen in donor plasma, the quality and stability of fibrin glue was suboptimal.

1975-1978

Numerous research works suggested an enhanced concept for using blood extracts and designated them as "platelet-fibrinogen-thrombin mixtures" [13].

1979

Another author called it "gelatin platelet-gel foam". This new proposition asserted the performance of platelets and demonstrated exquisite preliminary results in general surgery, neurosurgery and ophthalmology. However, till then all these products were used primarily for their "gluey effect", without considering the effects of growth factors or their healing properties.

1986

Knighton et al., [14] first demonstrated that platelet concentrates successfully promote healing and termed it as "platelet-derived wound healing factors (PDWHF)", which was successfully tested for the management of skin ulcers.

1988,1990

Kingsley et al., [11] Knighton et al., [14] used a slightly different term "platelet-derived wound healing formula (PDWHF)".

1997

Whitman et al., [15] named their product PRP during preparation but when the end product had a consistency of a fibrin gel and therefore labelled it as "platelet gel".

1998

The development of these techniques continued slowly until the article of Marx et al., [16], which started the craze for these techniques. However, all these products were designated as PRP without deliberation of their content or architecture, and this paucity of terminology continued for many years.

1999

One of the popular methods advertised on large scale to prepare pure platelet rich plasma was commercialized as plasma rich in growth factors (PRGF) or also called as preparation rich in growth factors (Endoret, Victoria, Biotechnology Institute BTI, Spain). However, because of lack of specific pipetting steps and also lack of ergonomics, there were significant issues with this technique [17]. Another widely promoted technique for P-PRP was commercialized by the name Vivostat PRF (Alleroed, Denmark). However, as the name implies it is not a PRF but produces a PRP product.

2000

Simultaneously, Choukroun et al., [18] developed another form of platelet concentrate in France which was labelled as PRF, based on strong fibrin gel polymerization found in this preparation. It was stamped as a "second-generation" platelet concentrate because it was obviously different from other PRPs. This proved an important milestone in the evolution of terminology.

2006

Sacco [19] introduced a new concept of CGF (concentrated growth factors). For making CGF from venous blood, rpm in range of 2400-2700 was used to separate cells. The fibrin rich blocks that were obtained were much larger, richer and denser.

2010

Concept of sticky bone (autologous fibrin glue mixed with bone graft) was introduced by Sohn [20] in 2010.

Role of Platelets in Wound Healing

Platelets play a key role in wound healing and hence wound healing after periodontal treatment can be accelerated by the use of platelet concentrates. The wound healing process initiated by the

formation of blood clot and after tissue injury in periodontal surgery causes adherence and aggregation of platelets surgery causes adherence and aggregation of platelets favouring the formation of thrombin and fibrin. In addition, there is release of certain substances from platelets that promote tissue repair, angiogenesis, inflammation and immune response. Platelets also contain biologically active proteins and the binding of these secreted proteins within a developing fibrin mesh or to the extracellular mesh can create chemotactic gradients favouring the recruitment of the stem cells, stimulating cell migration, differentiation and promoting repair. Thus, the use of autologous platelet concentrates is a promising application in the field of periodontal regeneration and can be used in clinical situations requiring rapid healing. According to Ymer H Mekaj in 2016 [21], the mechanism of action of activated platelets in wound healing is as follows:

1. Participates in haemostasis (all phases of haemostasis)
2. Participates in inflammation (neutrophil, lymphocyte, monocyte infiltration as well as differentiation of macrophages)
3. Participates in proliferation (re-epithelialization, angiogenesis and extra-cellular matrix formation)
4. Participates in re-modelling (collagen remodelling followed by vascular maturation).

Role of Various Growth Factors in CGF in Periodontal Regeneration

Platelet is one of the major resources of autogenous growth factors [22]. Platelet-rich plasma (PRP) was the first generation of platelet gels for periodontal regeneration therapy [23]. While the potential benefits of this procedure have been criticized, many of the discrepancies are likely more related to the lack of more suitable standardization methods and definition of different PRP preparation than to any functional deficiencies, as the protocols and biological and surgical techniques differ widely between different research groups [24, 25]. Platelet-rich fibrin (PRF), the second generation of platelet concentrates, has the same properties as PRP with the advantages of osteogenicity [26, 27]. The preparation process of PRF is simple and without the addition of bovine thrombin and anticoagulant drugs, because PRF is derived from autologous blood [28, 29].

Concentrated growth factor (CGF) is a novel generation of platelet concentrate product [30]. CGF is made by centrifuging blood samples at alternating and controlled speeds using a special centrifuge (Medifuge, Silfradentsrl, Italy) [31]. Different centrifugation speeds permit the isolation of a much larger and denser fibrin matrix with abundant growth factors. Rodella et al. observed the presence of transforming growth factor β-1 (TGF-β1) and vascular endothelial growth factor (VEGF) in CGF and red blood cell layers [32]. In theory, CGF appears to have more abundant growth factors because of its special centrifugation process. However, there are few studies supporting this. According to C. Durante et al., in 201 [33], transforming growth factor beta (TGF-b), platelet derived growth factor (PDGF) isoforms AA, AB and BB, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) as well as fibroblast growth factor (FGF) and insulin-like growth factor 1(IGF-1)were considered as the most active signalling molecules for clinical applications, and they are known to be contained in a-granules in platelet cytoplasm [34]. The time-course analysis of growth factor releases in supernatant rich in growth factor by $CaCl_2$ addition and incubation at 40°C suggested that PDGF isoforms, VEGF and TGF-b are strongly released at early time-points and that FGF and EGF are significantly enhanced only after prolonged incubation.

Basic Clinical Procedure to Obtain Concentrated Growth Factor

CGF is prepared in accordance with the protocol developed by Sacco (2006). From the patient, blood is collected in the Vacuette tube (Greiner Bio-One, GmbH, Kremsmunster, Austria) which contain silicon coating for clot activation. Tubes containing blood is placed in a special centrifugation machine (Medifuge MF200, Silfradentsrl, Forlì, Italy). This machine is preprogramed with the following characteristics:

1) Acceleration for 30 seconds
2) 2 minutes centrifugation at 2,700 rpm (692 gm)
3) 4 minutes at 2,400 rpm (547 gm)
4) 4 minutes at 2,700 rpm (592 gm)
5) 3 minutes at 3,000 rpm (855 gm)
6) 36 seconds deceleration and stopped.

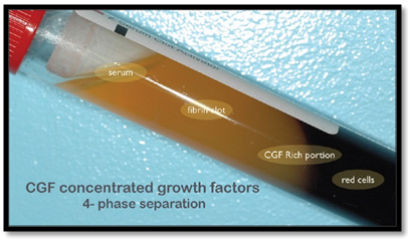
At the end of the process, four blood fractions are identified:

Table 1. Summarizes the alternative names and sources of various growth factors.

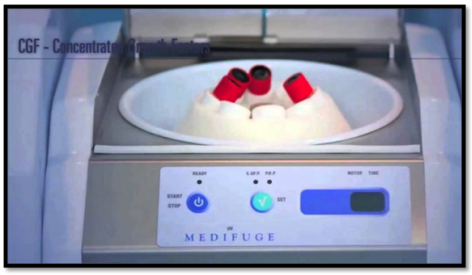
GROWTH FACTOR	ALTERNATIVE NAME	SOURCES
PLATELET DERIVED GROWTH FACTOR	Fibroblast-derived growth factor; Glioma-derived growth factor	Degranulating platelets; Endothelial cells; Smooth muscles; Macrophages; Fibroblasts
INSULIN-LIKE GROWTH FACTOR	Erythropoietic factor; Growth-promoting activity for vascular endothelial cells	Macrophages; Osteoblasts; Plasma stored in bone
TRANSFORMING GROWTH FACTOR-α	Milk-derived growth factor; Transformed cell growth factor	Macrophages; Osteoblasts
TRANSFORMING GROWTH FACTOR-β	Epithelial cell-specific growth inhibitor; Tumour-inducing factor-1	Platelet α granules
FIBROBLST GROWTH FACTOR FAMILY-aFGF and bFGF	Heparin-binding growth factor-α; Adipocyte growth factor; Bone-derived growth factor	Macrophages and osteoblasts stored in bone

(1) Superior phase, representing the liquid phase of plasma named platelet poor plasma (PPP),
(2) Interim phase or fibrin buffy coat phase,
(3) Liquid phase and
(4) Lower red phase

Figure 1. CGF was obtained after centrifugation.



Clinical Applications



Applications in periodontics

CGF is an excellent bioactive protein which enhances bone healing due to its stimulatory effect on epithelialization and angiogenesis. CGF is mixed with autologous bone particles or biomaterials to fill the bone defects to induce bone regeneration. The advantages of CGF over platelet-rich plasma are lack of biochemical modification, easy method of preparation, application with minimal expense. It also serves as a resorbable interpositional membrane. Avoids the early invigilation of the gingival epithelium is inhibited by the CGF layer results as a barrier to epithelium migration.

Applications in gingival recession

CGF act as a membrane support in recession coverage as it constantly releases growth factors to produce tissue regeneration. According to the study it proved that CGF and CAF placed together enhance the healing of soft tissues. CGF is used as a barrier membrane to facilitate tissue healing and results in obtaining the attached gingival width in root coverage procedures like sliding flap technique.

CGF and Osseointegration

Osseointegration of dental implants is vital for stability, success and for a longer shelf life. (CGF) increases implant stability, accelerates osseointegration by increasing the differentiation of osteoblasts and healing of the bone around the implant. CGF contains fibrinogen, growth factors, leukocytes, coagulation factors, endothelial growth factor, platelets for angiogenesis and tissue remodelling. It provides a matrix for cell migration. Platelets contain a high concentration of bioactive proteins required for healing, growth, and cell morphogenesis. CGF increases FGF-β or VEGF release, which are essential for angiogenesis and enhancing neutrophil migration by performing integrin release.

Summary

Regeneration of the periodontal tissues is a dynamic process involving cell-cell and extra-cellular matrix interactions.Growth factors elegantly co-ordinate these interactions resulting in wound healing and regeneration of tissues. A review of current existing literature shows that a combination of growth factors in an optimal concentration is best suited for periodontal regeneration. CGF is a novel ingress of tissue engineering to clinicians and researchers in the field of dentistry. Its inherent ability to harbour growth factors facilitates stimulation and acceleration of both hard and soft tissue regeneration. Being a completely natural, physiologic and economical source of autologous product, it possesses beneficial effects of eliminating concerns about immunogenic reactions and disease transmission. However, since knowledge on this topic is still in its preliminary stage, the effectiveness of CGF in regenerative procedures should be evaluated in studies comprising of large samples and their clinical applications in randomized control trials has to be encouraged.

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