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Concentrated Growth Factor - Review

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ABSTRACT:
Regenerative medicine is one of the most dominant objectives of today's rehabilitation therapies. Growth factors are bioactive proteins which control the process of wound healing. Platelet concentrates are rich in growth factors enhancing wound healing process and promotes regeneration of bone. One such platelets concentrates is Platelet Rich Fibrin (PRF) a second generation platelet derivative used widely for bone healing. And in 2006 Sacco introduced advanced second generation platelet derivative Concentrated Growth Factor (CGF), which showed enhanced bone healing capacity, higher tensile strength; more growth factors, higher viscosity and higher adhesive strength than PRF . Recently more importance has been given to CGF for its excellent biological activity and predictable method of preparation, their by findings its way in the field of regeneration.

Key words: Concentrated Growth Factors, Platelet Rich Fibrin, regeneration, growth factors.

INTRODUCTION:

New era of regenerative dentistry focuses on biocompatible and tissue like material for regeneration. This search is mainly for a material which reconstitutes lost tissues in certain scenario like large bone defect after enucleation. After injury/surgical exposure, wound healing get initiated. Wound healing is a multistage process which begins immediately after injury and repair/regenerate lost body tissue. Wound healing occur in following four stages haemostasis stage where blood clots, inflammatory stage where microbes are eliminated, proliferative or granulation stage where body repairs injured structure, remodeling phase where final restructuring occurs. Various cells participate in wound healing which is mediated by growth factors. Platelets are considered to be natural healer of injury and upon activation allow access to autologous growth factors. According to Intini.G (2009) Platelets contain many growth factors like TGF, FGF, and IGF which are responsible for osteoblast proliferation and bone deposition(1). These growth factors are protein molecules which signals cell for growth, proliferation, differentiation and act as a key mediator of inflammation. The results of experimental studies have established that growth factors play an important role in bone formation, fracture healing, tooth regeneration and the repair of other oral and maxillofacial tissues. 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 three components like 1. Scaffolds, 2.stem cells, 3.growth factors. This platelet concentrates contain all this three 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 (2).

Platelet concentrates are prepared by various technique and where used widely in dentistry. The usage of platelet concentrates begins with the introduction of fibrin sealants or "Fibrin glue" by Matras in 1970 (3). Fibrin sealants are fibrin clots containing meshwork of fibrin with entrapped platelets. Commonly used as topical haemostatic agent, tissue sealer, and mixed with bone graft for filling bony defect. The risk of viral transmission and inability to resist physical stress are the main drawbacks of fibrin glue (4). In order to overcome these limitations platelet concentrates are developed.

The use of autologous products with high platelet concentrations like Platelet concentrates (PC) are developed to combine the fibrin sealant properties with growth factor effects of platelets providing an ideal growth factor delivery system at the site of injury. In 1998 Marx et al introduced Platelet Rich Plasma (PRP), which is considered to be first generation platelet concentrates produced by two stage centrifugation process and use of bovine thrombin (5). This was followed by Platelet Rich Fibrin (PRF) developed in France by Choukroun et al. in 2001. This second generation platelet concentrate eliminates the risk associated with the use of bovine thrombin (6).

Newer additive to the second generation platelet concentrates is Concentrated Growth Factors (7). Sacco introduced CGF (Concentrated Growth Factors) in 2006. A special centrifugation machine called Medifuge (Italy) (Fig.1), is used to prepare CGF, similar to PRF, but with a different centrifugation speed ranging from 2400 to 2700 rpm which allows the separation of a fibrin matrix which is much denser, larger and richer in growth factors. This newer platelet concentrates considered to be better than PRF and contains autologous osteoinductive platelet growth factors and an osteoconductive fibrin matrix their by found its implication in various clinical situations.

Fig (1) Medifuge MF200, Silfradent srl, Forlì, Italy

Method of preparation:

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, Silfradent srl, Forlì, Italy). This machine is preprogrammed with the following characteristics: acceleration for 30 seconds , followed by 2 minutes centrifugation at 2,700 rpm, 4 minutes at 2,400 rpm, 4 minutes at

2,700 rpm, 3 minutes at 3,000 rpm at a force of 692 gm, 547 gm, 592 gm and 855 gm respectively and finally 36 seconds deceleration and stopped (8). At the end of the process, four blood fractions are identified: (1) the superior phase, representing the liquid phase of plasma named platelet poor plasma (PPP), (2) the interim phase or fibrin buffy coat phase, (3) the liquid phase and (4) lower red phase (Fig.2).

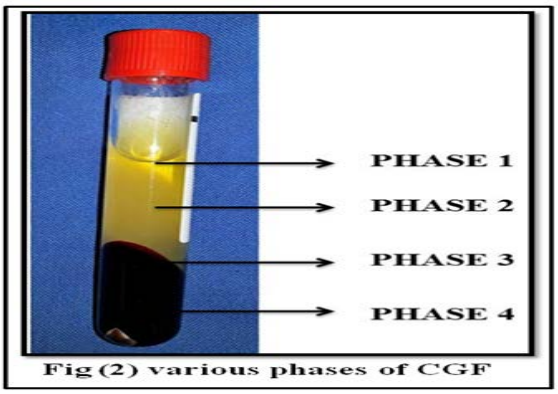

Fig (2) various phases of CGF

This layer of separation is mainly due to centrifugation force and specific gravity of blood components (9). Though smaller in size when compared with WBC, RBC's settle down at the bottom of the tube because of its iron content, high specific gravity and easy deformability nature. Although smaller than WBC's, if platelets clump together it settles down faster than WBC's. Once the fibrin clot is formed it entraps platelets and WBC's and forms a buffy coat at the middle by buoyant force (Fig.3).


Fig (3) CGF after centrifugation

Various phases of Concentrated Growth Factors:

Phase 1:

Superior phase is otherwise called as platelet poor plasma phase. This phase contain a clear straw coloured fluid which is lightest and most liquid part of blood representating serum. It contains 92% of water and 7% of other concentrates which includes proteins, lipids, enzymes, hormones and inorganic electrolytes. It is used to seal the bleeding capillaries, wash the surgical site, coat and protect the regenerated portions.

Phase 2:

Interim phase is otherwise called as fibrin phase containing 3 dimensional polymer networks of fibrinogen molecules with interwoven fibres united to form a single phase in the form of gel. When viewed under electron microscope this layer is constituted by thick and thin fibrillar elements. At the end of polymerization reaction three dimensional fibrin networks is formed by growth of fibrin at all directions.

The fibrin blocks of CGF contain increased level of fibrinogen, factor XIII and thrombin. From plasmin degradation, thrombin activated Factor XIII stabilizes fibrin clot. This stabilized fibrin clot have increased tensile strength and stability. Due to prolonged release of growth factors, proliferation and differentiation of osteogenic cells occurs at faster rate. This CGF is used for making membrane in guided tissue regeneration cases and used either individually or mixed with bone particles for closing bony defects. According to K Isobe (2017), tensile strength of CGF is equal to PRF and greater than PPTF (platelet poor plasma derived fibrin) and the order of degradability was PPTF > CGF ≈ A-PRF (10).

Phase 3:

Liquid phase contains growth factors, white blood cells and stem cells. These stem cells are able to differentiate into their specialized cell types. This liquid phase is mixed with autologous bone graft to get high performance

activated graft. CD 34 positive cells are present in CGF (11) and 3-4 mm of RBC compartment present adjacent to it. For bone grafting this CGF along with a part of RBC is used for regeneration procedures. According to Majka et al., (2001) CD34 + cells help in vascular maintenance, neovascularisation, and angiogenesis their by accelerating bone regeneration (12).

Phase 4

This lower phase is dark reddish dense gel. It consists of high concentration of red blood cells and also few white cells, platelets and clotting factors. It is used in pure form or mixed with bone grafts to fill large cavities.

Sticky bone

Blood collected in non coated vacutainer tube and centrifuged in Medifuge centrifugation machine for a period of 2 -12 minutes at 2400-2700 rpm. After centrifugation of vacutainer tube, two layers of blood fraction are obtained. The upper layer is autologous fibrin glue (AFG) and lower layer is RBC fraction which is discarded. Using syringe, AFG is removed from the tube and mixed with bone graft materials and allowed to polymerize for 5-10 min. Sohn *et al* noted that the exudates obtained after compression using armamentarium provided in the kit while making CGF membrane can be used for sticky bone preparation (14) (Fig.4).

Fig (4) armamentarium for CGF preparation

These exudates containing growth factors and autologous thrombin in RBC layer will accelerate polymerization of AFG, when it is mixed with bone powder and AFG to get sticky bone. The resultant sticky bone is moldable, prevents micro and macro movement of grafted bone, entraps platelets and leukocytes in its fibrin network, is natural and prevents ingrowths of soft tissues in graft.

Mechanism of action of Concentrated Growth Factors:

CGF exerts its beneficial effects via degranulation of the alpha granules in platelets that contain growth factors. When the biphasic platelets in CGF activated by thrombin, they release growth factors and other substances that serve to accelerate the wound-healing process by stimulating proliferation and differentiation of cells, collagen synthesis, osteoid formation followed by calcification. The first step, the active secretion of these growth factors, begins within minutes of the start of the coagulation sequence, and more than 90% are secreted during the first hour for 3 days. After this initial burst, in a second step, the different platelets secrete additional growth factors for the remaining 7 days of their viability. Platelets stimulate inflammatory cells like macrophages through growth factors; will tend to up regulate wound healing by release of its own growth

Kinetics of growth factor release:

According to Borsani et al (2015) Concentrated Growth Factors are loaded with abundant growth factors and have temporal release from the time of its preparation. VEGF level reaches its maximum at the end of 8^{th} day, whereas TNF-α reaches its maximum level at the 1st day and then it decreases until the 8th day, PDGF and TGF-ß1 release level remains constant, BDNF maximum level seen at the 1st day and after this it remains stable, maximum level of BMP-2 seen at 8th day, at the end of 6^{th} day IGF-1 level reaches maximum and then it

decreases. From this study he concluded that PDGF-AB, TGF-β1 and IGF-1 had a constant kinetic release; VEGF and BMP-2 had a slow kinetic release; TNF-α and BDNF had a fast kinetic release (13).

Role of Concentrated Growth Factor in bone regeneration:

CGF contains autologous osteoinductive platelet growth factors and an osteoconductive fibrin matrix. These growth factors while functioning on their own, are also synergistic and create close contact tissue repair regulatory systems. CGF contain strong fibrin meshwork with high tensile strength which is highly resistant to degradation and get remodeled slowly, whereas PRP fibrin meshwork is fragile and get disintegrated rapidly. Thus, CGFs prolonged the duration of growth factor action, which is conducive for the growth factor synergy, and enhances cell proliferation and osteogenic differentiation. According to Sohn et al (2009) CGF seems to be more handful and with more regenerative capability than the other previous presidia and concluded that healing of sinus lift intervention took just 4 months for bony healing (15). Smita Singh et al. (2013) used PRF for surgical management of Periapical lesions and found that healing is accelerated and bone regeneration occurs as early as 6 months while CGF takes only 4 months (16). According to Rodella et al (2011) CGF applied to maxillary sinus lift and ridge augmentation can substantially increase new bone deposition by 26.54 to 59.50% (average, 38.7%) (21).According to Park et al (2016) CGF showed a better new bone formation rate in bone defects than PRF (17). Under SEM examination CGF showed fibrinogen structures that were thicker per unit area and had a more regular pattern when compared with the PRF and concluded that CGF contained approximately 1.5 times more VEGF than PRF. Vascular endothelial growth factor (VEGF) is a key regulator of physiological angiogenesis, which represents a critical step in remodeling and induces vascularization of the CGF membrane. Similar to VEGF, EGF also promotes angiogenesis in CGF membrane; in addition it causes growth of epidermal layer and keratinization thereby bringing changes in gingiva.

Moreover, CD34+ cells have therapeutic potential in terms of both vasculogenesis and osteogenesis (18). CD34+ cells are capable of inducing neovascularization of the CGF membrane. CGF membrane promotes soft tissue defect sealing, thereby playing a key role in guided bone regeneration. Yu et al (2014) conducted in vitro study in beagle periodontal ligament stem cells (PDLSC's) and found that CGF increases alkaline phosphatase level (ALP), bone specific proteins like bone sialoproteins and osteocalcin level in time and dose dependant manner, their by accelerate the osteogenesis transformation process of PDLSCs (19). Jing Qiao and Na An (2016) conducted an in vitro study in human PDLSC's and found that 3CGFs seemed to be the optimal concentration of CGFs on hPDLCs proliferation and CGF plays a critical role in early phase of Wnt/β-catenin signaling participating in the proliferation and osteogenic differentiation of hPDLCs (20). According to wang et al (2017) CGF could markedly increase tissue repair mechanisms, such as cell proliferation and differentiation, angiogenesis, intracellular matrix deposition, immune modulation, antimicrobial activity, and remodeling their by minimizing post operative complication (22).

Role of Concentrated Growth Factors in Regenerative Endodontics:

Regenerative Endodontic procedures are defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex and it is based on the concept of tissue engineering. The three key ingredients for regeneration are morphogens, progenitor/stem cells, and the

extracellular matrix (ECM) scaffold (23). CGF contain all this three key ingredients hence plays a critical role in Regenerative Endodontics.

Stem cells:

Stem cells are undifferentiated embryonic or adult cells that continuously divide. Stem cells of the apical papilla (SCAPs), which are derived from an embryonic like soft tissue located at the apex of the incompletely developed root, exhibits a more pronounced population doubling capacity, an enhanced proliferation rate, and mineralization potential, indicating a more potent stem cell population. In a study conducted by Hong et al (2018) on SCAP cells found that both CGF and PRF promote differentiation, migration and proliferation of SCAP cells. The expression levels of osteogenic/odontoblast-related genes were reduced on day 7, but they were dramatically enhanced on day 14, and the related gene expression levels in the PRF were higher than those in the CGF. Finally concluded that the preparation of PRF lacks the consistent conditions, and the differences between the contents of leucocytes and cytokines may cause different results. Therefore, CGF may be more convenient to quantify its contents and acquire a predictable outcome in Regenerative Endodontics (24).

Growth factors:

Growth factors regulate either transplanted cells or endogenous cells in dental pulp-dentin regeneration. These growth factors recruiting the proper cells are critical in pulp regeneration (transforming growth factors [TGFs] β1, β3 for odontoblast differentiation and stimulation of dentin matrix). According to Qin et al. (2016) CGF could release TGF-β1 over a sustained period of time (at least 13 days) promoting odontoblastic differentiation. According to park et al (2016), CGF contained approximately 1.5 times more VEGF than PRF, their by enhancing revascularization of necrotic pulp.

Scaffolds:

Scaffolds serve as a platform where stem cells can proliferate and differentiate. Ideally it should be biocompatible, biodegradable, non toxic, should posses' adequate strength and should promote cell adhesion and act as reservoir of cells. . Rodella et al. (2011) conducted an immuno-histochemical study and reported that variable speed of centrifugation yielded CGF with bigger and high density fibrin meshwork (25). According to park et al (2016), SEM examination, fibrinogen structures were thicker per unit area and regularly arranged when compared with the PRF.

The fibrin clots have high cohesion because of the agglutination of fibrinogen, factor XIII, and thrombin. This provides protection from plasmin degradation, resulting in higher fibrin tensile strength and stability (26).

Thus, Sacco's membrane histologically contain dense fibrin network which is embedded with numerous platelets, leukocytes and growth factors. It is considered to be suitable for patients in promoting healing and minimizing postoperative complication and for surgeons because of its easy preparation and manipulation.

Contraindications:

Concentrated Growth Factors are autologous blood preparation and generally considered to be safe. But still it is contraindicated in patients with following hematological disorders like, a) severe hypovolemia, b) anemia, c) coagulopathies or platelet disorders like thrombocytopenia, d) anticoagulant/ fibrinolytic therapy, e) unstable angina, f) septic shock. Before treating patients with CGF hematological evaluation is done to rule out the above disorders.

Conclusion:

Concentrated Growth Factor is an upgraded version of Platelet Rich Fibrin with potential implication in various fields of dentistry. CGF used in following procedures like sinus and alveolar ridge augmentation, pre-implant augmentation procedures, healing of critical-size bone defects(after Periapical cyst enucleation), promotion of in vitro periodontal ligament stem cells proliferation, Regenerative Endodontics and in management of chronic venous ulcers. Due to lack of predictable preparation method for PRF it has been replaced by CGF in recent years. Furthermore, CGF have antibacterial, analgesic, anti-inflammatory which could indirectly contribute to the final clinical outcomes of surgical procedures and thus the use of CGF is inevitable in future.

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