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Estimation of Release of VEGF From Concentrated Growth Factor compared with that of Platelet Rich Fibrin at Different Time Intervals

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Abstract: Introduction
Autologous platelet concentrate (APC) emerged as innovative autologous blood products that enhance tissue healing and regeneration in regenerative therapy.There are two generations of APC. 1) First Generation: Platelet Rich plasma (PRP) and Plasma rich growth factor (PRGF). 2) Second Generation: Platelet Rich Fibrin (PRF) and Concentrated Growth Factor (CGF)
Aim
To compare the release of VEGF from CGF with that of PRF at different time intervals of 1, 4 and 7 days
Materials and methods
CGF and PRF have been prepared from human blood. The blood samples were used to quantify the release of VEGF at different time intervals using ELISA kit specific to VEGF. The results were viewed using a spectrophotometer
Results
CGF showed better release of VEGF compared to PRF at 1, 4 and 7 days
Conclusion
CGF can be used as scaffold in Regenerative endodontic therapy.

Keywords: Platelet, autologous, regeneration, growth factor.

1. Introduction

Regenerative dentistry is an emerging field of medicine involving stem cell technology, tissue engineering and dental science. It exploits biological mechanisms to regenerate damaged oral tissues and restore their functions.(1). Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex(Murray et al, 2007)

According to Langer and Vacanti, 1993, Tissue Engineering is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function(2).Triad of tissue engineering includes stem cells, growth factors and scaffolds.

Growth factors are polypeptides or proteins that bind to specific receptors on the surface of target cells that affect a broad range of cellular activities including migration, proliferation, differentiation and apoptosis of dental pulp cells, including stem/progenitor cells(3). Different growth factors released in the Pulpo dentinal complex are VEGF, PDGF, BMP, FGF, EGF, IGF etc. Vascular Endothelial Growth Factor plays an important role in cell proliferation and angiogenesis(4,5).

According to the American Society of Testing Material, Scaffold can be defined as the support, delivery vehicle or matrix for facilitating the migration, bind or transport of cells or bioactive cues used for repair or regeneration of tissues(6). Based on the nature of the material, scaffolds can be classified as natural or synthetic. Natural

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scaffolds include autologous platelet concentrates(APC)(7). Blood concentrates have 95% RBC, 4% platelets and 1% WBC whereas APCs have 95% platelets, 4% RBC and 1% WBC. This makes APC more concentrated with platelets and thereby more release of growth factors. APC is of two generations. First generation includes PRP and PRGF, the second generation includes PRF and CGF(8). In the first generation, anticoagulants are added to the blood to prepare blood concentrate whereas in the second generation, anticoagulants are not added.The efficacy of platelet concentrates in promoting wound healing and tissue regeneration has been at the center of scientific interest over the past few decades. Platelets include growth factors (GFs) such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), transforming growth factor β-1 (TGF-β1) and platelet-derived growth factor-BB (PDGF-BB). There are significant differences in the amounts of GFs produced using the three different APC techniques (CGF, PRF and PRP). PRF and CGF produce significantly more GFs during the procedure as compared to PRP. The levels of bFGF in CGF and PRF are significantly higher than in PRP(9)PRF and CGF differs from its predecessor (PRP/PRGF) by several parameters like simplicity of its preparation and its implementation. The time of preparation and cost of preparation are both significantly lower as they do not necessitate the direct activation with additional factors such as bovine thrombin or extrinsic anticoagulants. Because of its fibrous structure, PRF and CGF retain a larger number of cytokines and growth factors in a supportive three dimensional fibrin scaffold for cell migration(10).As a good combination product of biomaterial and cytokines, CGF appears to have superior clinical applications to promote pulp regeneration(11). However there are a very limited number of studies that have actually compared CGF with that of PRF.

The aim of the study is to compare the release of growth factor VEGF of Concentrated Growth Factor(CGF) with that of Platelet Rich Fibrin(PRF) at time intervals of 1,4 and 7 days.

2. Materials And Methods

Before the study was initiated, Ethical clearance was obtained from the Ethical Committee of Saveetha Dental College, Chennai.

Preparation of PRF/ CGF:

Ten healthy human volunteers consented for the study. Blood samples of the individuals were collected for preparation of PRF and CGF. Blood samples from each individual were separately used for PRF and CGF preparation so that VEGF release can be quantified and compared between CGF and PRF of the same individual.

Figure 1: Blood sample collection

Figure 2: Collected blood

Figure 3: Centrifuge

The collected blood was placed in a medifuge for preparation of PRF/ CGF. The rotations per minute(RPM) and duration of each cycle varies between CGF and PRF. For CGF, it is 2700 rpm for 2 mins,2400 rpm for 4 mins, 2700 rpm for 4 mins, 3000rpm for 3 minutes. For PRF, it is 3000 rpm for 10 mins. Or 2700 rpm for 15 mins. After the cycles of rotation in medifuge, CGF/PRF is obtained.

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Figure 4: Layers after centrifugation

Figure 5: CGF layer

The CGF is obtained in three layers, the superficial layer has serum, the middle layer is the layer of interest which is thick fibrinous. The third layer is the residual red blood cells. The middle layer CGF is cut into equal parts and placed in centrifuge tubes for further quantification. All the procedures were carried under sterile and aseptic conditions to avoid contamination.

Quantification of release of VEGF:

The prepared CGF and PRF from blood samples were cut into equal parts and stored in separate centrifuges with Dulbecco's Modified Eagle Medium(DMEM). DMEM is a widely used basal medium for cell culture. It is rich in amino acids, vitamins, glucose and sodium pyruvate. Also it makes use of sodium bicarbonate to buffer the pH. The samples were then placed in an incubator at 37 degree celsius for 1,4 and 7 days duration. The release of VEGF was quantified with an ELISA kit specific to VEGF at time intervals of 1,4 and 7 days.

Figure 6: DMEM

Figure 7: DMEM in centrifuge tube

Figure 8: Samples stored in DMEM

The change in color was estimated with a spectrophotometer at 450 nm optical density.

3. Results

The VEGF level released by CGF on the first day was 52 ±22.8pg/ml, whereas that by PRF on the first day was 48 ±20.14pg/ml. The VEGF release by CGF and PRF on the fourth day was 220 ± 188.3pg/ml and 160 ± 110 pg/ml respectively. On the seventh day, the release of VEGF by CGF and PRF was 260 ± 190 pg/ml and 200 ± 130.5 pg/ml.

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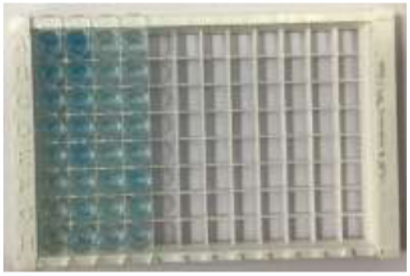

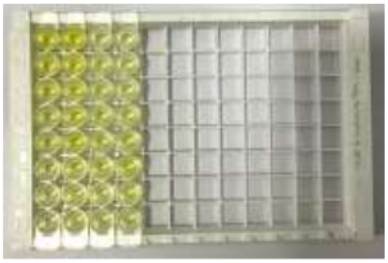

Figure 9: Positive wells indicating presence of VEGF
Figure 10: wells ready for spectrophotometric analysis

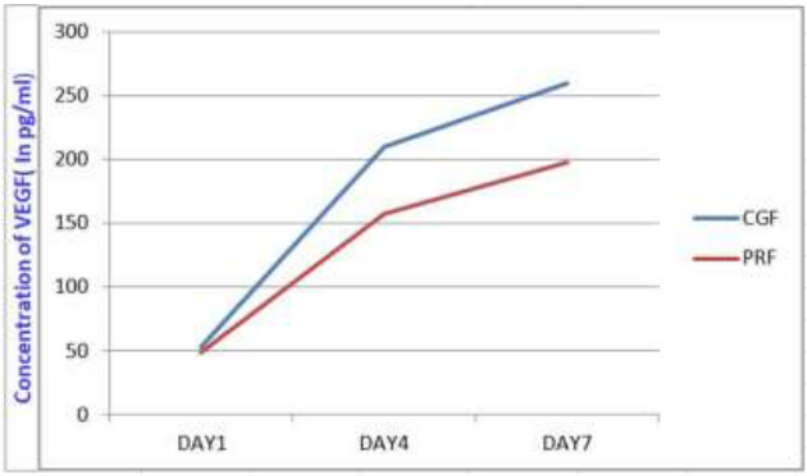

Figure 11: Graph showing the concentration of VEGF release at different time intervals

4. Discussion

Regenerative endodontics is an evolving branch in the field of endodontics. It is a branch which deals with regeneration of pulp dentin complexes. Tissue engineering is defined as understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use according to MacArthur & Oreffo, 2005. The triad of tissue engineering includes growth factor, stem cells and scaffolds. Various growth factors known to be released in pulp dentin complex which cause cell proliferation and differentiation include Fibroblast growth factor(FGF), Bone morphogenic protein(BMP), TGF beta, Vascular endothelial growth factor(VEGF), platelet derived growth factor(PDGF), insulin derived growth factor(IGF), neuronal growth factor(NGF) etc. VEGF is an important growth factor in regenerative endodontics as it plays an important role in cell proliferation and angiogenesis, thereby promoting regeneration of tissues.

Scaffolds are three dimensional structures which act as vehicle, support or matrix to facilitate migration of cells for cell growth, adhesion and differentiation thereby promoting regeneration of tissues. Scaffolds may be classified based on their form, degradability and origin. Based on origin they may be classified as natural or synthetic scaffolds. Natural scaffolds include different types of autologous platelet concentrates. Platelet Rich Plasma, PRPF are included in the first generation of APC where anticoagulants are added in blood for preparation of scaffold. Second generation includes PRF and CGF wherein no anticoagulants are added in preparation.

PRF has been studied in literature to some extent. Regenerative endodontic procedure has been tried using PRF in mature permanent necrotic teeth, which showed better results than blood clot(12). A clinical trial by Karan et al was done to compare the effects of mineral trioxide aggregate (MTA) and platelet-rich fibrin (PRF) use on periapical healing in surgically treated periapical lesions using cone-beam computed tomographic (CBCT) imaging.High success rates were achieved using MTA in periapical lesions in endodontic microsurgery. The application of PRF to the surgical cavity may not necessarily improve outcomes(13). PRP, PRF and MTA were

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evaluated as direct pulp capping agent by Shobana et al. However there was no significant difference reported between the pulp capping agents used(14).

CGF is the latest addition to it which is said to have fibrin network which is more dense and rich in platelet concentrates thereby promoting increased release of growth factors., The release of chemokines responsible for cell recruitment is another advantage of this new generation of platelets concentrates. Concentrated growth factor (CGF), which is known as bioscaffolds and a reservoir of cytokines. As the latest generation of platelet concentrate products, the modified production process of CGF is simpler and requires repeatedly switching the centrifugation speed(15). As a result, the relatively stiffer structure of CGF is more similar to a natural fibrin and contains abundant growth factors and proteins from autologous platelets and leukocytes(16). CGF contains large amounts of growth factors including platelet-derived growth factor-BB (PDGF-BB), transforming growth factor β-1 (TGF-β1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). All these factors were intimately involved in the regulation of cell differentiation, proliferation, and angiogenesis which were vital for tissue regeneration. Concentrated growth factor (CGF) is a natural biomaterial, is known to contain platelets, cytokines, and growth factors to facilitate the healing process, but there has been little information acquired in regenerative endodontics. A study by Xu et al., investigated the effects of CGF on proliferation, migration, and differentiation in human dental stem pulp cells. CGF showed positive results for cell proliferation,migration and differentiation of stem cells(17). A retrospective study by Yang et al. aimed to investigate the success rate of concentrated growth factor (CGF) and blood clot as scaffolds in regenerative endodontic procedures. CGF may be a suitable alternative scaffold in regenerative endodontic procedures when adequate bleeding cannot be achieved(18). The effects of PRF and CGF on the proliferation, migration, and differentiation of human stem cells of the apical papilla (SCAPs) has been studied by Hong et al. Both CGF and PRF can promote the proliferation, migration, and differentiation of SCAPs. CGF may be a promising alternative in regenerative endodontics(19)

In the present study, PRF and CGF were compared in terms of release of VEGF at different time individuals. The manufacturer stated the minimum concentration of release of VEGF for quantification is 18pg/ml. The results of the study show that release of VEGF from both CGF and PRF started on the first day. The concentration of VEGF released from CGF was 52 pg/ml and that of VEGF released from PRF was 50pg/ml on the first day. There was a drastic increase in the release of VEGF from CGF and PRF on the fourth day. CGF released around 210 pg/ml ,and PRF released 160 pg/ml. VEGF release increased on the seventh day, CGF released 260 pg/ml whereas PRF released 190 pg/ml of VEGF. The difference between CGF and PRF was statistically significant. Nagarajan et al. aimed to quantify and compare VEGF release by PRF, PRFM, and dental pulp at different time intervals. According to the author, among the platelet concentrates, differential expression of VEGF-A was superior in PRF. The use of PRF in partial pulpitis should be explored in order to restore pulp vascularity and hasten pulpal healing. However, CGF was not evaluated by the author(20). Similarly PRP and PRF were compared by Kobayachi et al in which the author estimated the release of PDGF-BB, TGFB1, VEGF, and PDGF-AB at different time intervals. According to the author, PRP can be recommended for fast delivery of growth factors whereas A-PRF is better-suited for long-term release (21). Similarly Wen et al compared the release of growth factor between PRP and blood. Release of growth factor was more in PRP(22).

Within the limitations of the study it can be said that both PRF and CGF could release Vascular Endothelial Growth factor(VEGF) at different time intervals. Both were quantified and compared. The release was higher from CGF compared to PRF at 1,4 and 7 days time intervals. The peak release of CGF was seen on the 14th day. However in our study we did not quantify the release of VEGF after 14 days. So, CGF was more effective than PRF in terms of release of VEGF.

5. Conclusion

CGF showed better release of VEGF compared to PRF at time intervals of 1, 4 and 7 days.

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