Use of Concentrated Growth Factor (CGF) in implantology

By Peter Mansour, BDS (Hons), FRACDS
Paul Kim, BDS (Hons), B.Pharm

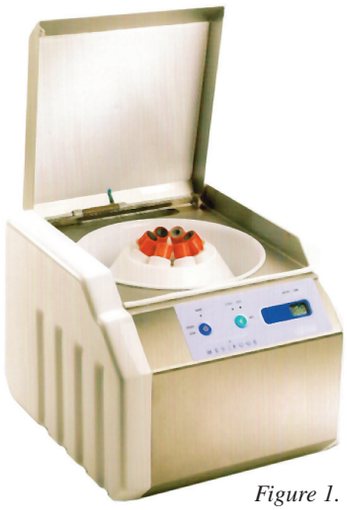

Figure 1.
Silfradent Medifuge c.g.f.

In the field of dental implantology, there has been increasing interest in products which promote wound healing. Various procedures and methods have been developed to amplify the elements of healing and enhance the body's own healing ability.

At beginning of every sequence of secondary intention (open) wound healing there is inflammation, platelet aggregation and the parallel formation of a fibrin matrix through the coagulation cascade. There are four basic essential elements to the process of wound healing:

1. Immune and matrix cells and their communication;
2. Changes in the vasculature, with angiogenesis;
3. Development of a framework and scaffold for cells; and
4. Establishment of a biological seal and space maintenance.

With regards to regeneration, the gold standard is autogenous bone and soft tissue grafting, which have their own limitations. Allogenic cadaver supplies and xenografts may be alternatives, but are also far from ideal and may not readily be available or even accepted by patients or clinicians. Even with the inception of the bone matrix proteins BMP-2 and BMP-7, we are still far from the ideal agent.

The concept of Plasma Rich Protein (PRP) was developed during a period where formulation and supplies of growth factors were still in development. The initially mixed results with PRP may have been due to wide variations in methods used in obtaining blood and in centrifuge procedures, as well as the lack of a proper study model.[1,2] The technique used to prepare PRP is very important as affects both the quantity and quality of the resulting product.

Platelet Rich Plasma

What is most consistent in PRP is the accelerated healing in the early phases, less pain and swelling and less complications associated with use of membranes.[3] This is attributed to the anti-inflammatory effect of PRP.[4-6] Although the end results may often be the same, this accelerated and boosted healing may lead to fewer complications associated with surgery, such as pain, infection, membrane exposure. These can be important considerations in patients such as diabetics and the elderly.

Platelets not only play a critical role in haemostasis, but are also essential in the healing process as they are rich source of growth factors.[5,7] Platelets essentially contain two types of granules - the alpha granules and dense bodies. The alpha granules contain fibrinogen, fibronectin, factor V and von Willebrand's factor, PDGF, TGB-beta, insulin like growth factor (IGF-II and IGF-I), TGF-f5 and VEGF. The dense bodies contain ADP, ATP, ionised calcium, histamine, serotonin and adrenaline.

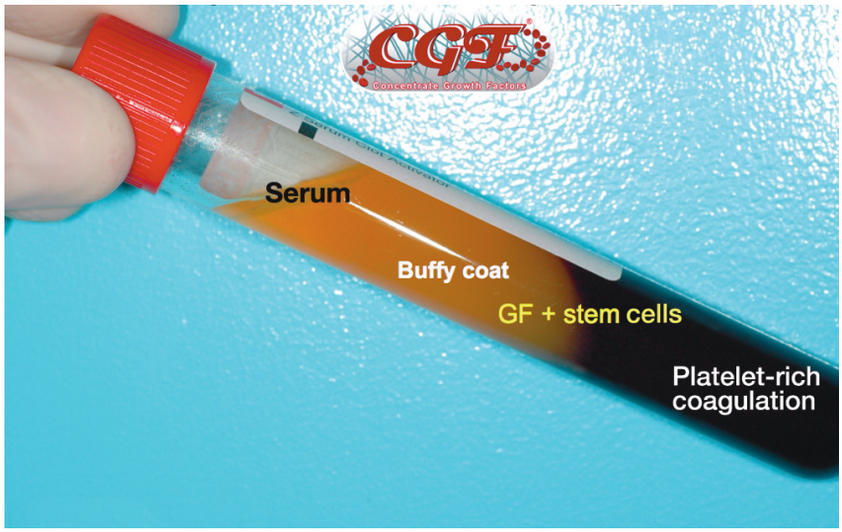

Figure 2. Layers formed from venous blood after CGF spin cycle is completed.

Figure 3. Silfradent Medifuge.

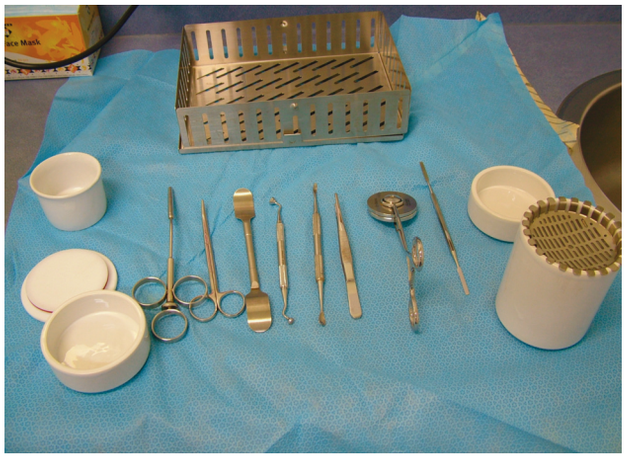

Figure 4. Instrument set up to process the CGF.

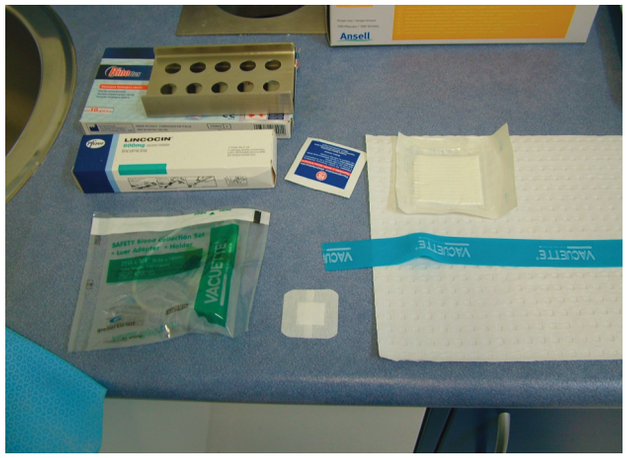

Figure 5. Basic set up for venapuncture.

The concept behind PRP is therefore to concentrate these growth factors, and many studies confirm that this is the case.[4] PRP uses differential centrifugation, in which the first spin segregates the PRP and red blood cells (RBC) from the platelet poor plasma. The second centrifuge step separates the RBC from the PRP, in which the latter is left at the bottom of the tube.[8] The following concentrate will therefore be dependent on the patients initial whole blood count. Marx and Carlson suggested that one million platelets per microliter was the concentration required to have clinically enhanced tissue healing. However, the platelet count usually varies from 595,000 to 1.1 million, with a mean of 785,000.[4]

Prior to surgery, thrombin and calcium chloride are added to the PRP to make a gel into which autogenous bone may also be added. The resultant product is tight, rigid and fragile fibrin. This mesh has the same concentrations of these cell adhesion molecules as normal clot blood (200-400 micrograms/mL), and therefore cannot act as a fibrin glue or as membrane.[9]

Activating platelets through the clot formation initiates the secretion of their growth factors. Within 10 minutes, they secrete 70% of their stored growth factors and close to 100% within the first hour.[10] However, complete release of growth factors takes about a week. PRP enhances osteogenesis by inducing and stimulating cells such as osteoblasts, whether derived from the local defect site or from autologous graft tissue. These cells are essential for PRP to enhance bony wound healing. PRP on its own is likely to only be osteoconductive at very best. This highlights the importance of mixing PRP with autogenous bone rather than bone substitutes.[10] Furthermore, PRP in its gel form also provides a scaffold for incoming cells and growth factors, allowing the local physiological mechanism to take over but in an accelerated fashion.

Marx and Garg stated that current procedures in concentrating the platelets in PRP are inadequate for producing significant enhancement in osteogenic potential.[11] Thus far, the literature on PRP indicates that only around 10% of the PRP volume is converted into bone. For these reasons, there have been alternative techniques developed to increase the platelet concentrate and the yield of platelets. High concentrate PRP has been described in the literature, and is basically an altered protocol to yield a higher percentage of platelets from regular PRP, with reports of from 10- to 23-fold increases.[11,12]

Platelet rich fibrin (PRF)

Platelet rich fibrin was first described by Choukroun in 2001. Unlike PRP, PRF does not require anticoagulants or any thrombin. Blood is collected in 10 mL tubes, which are immediately centrifuged without anticoagulant at 3000 rpm (approximately 400g according to our calculations), for 10 minutes. During centrifugation, the platelets are

Figure 6. Harvested CGF.

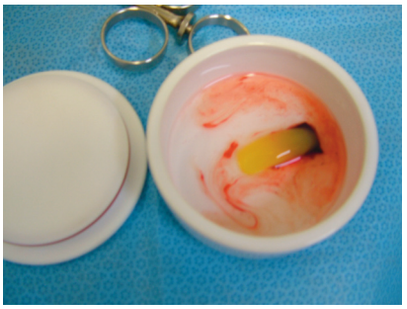

Figure 7. CGF in Lincomycin.

Figure 8. Trimming of the platelet-rich coagulation from the buffy coat.

Figure 9. CGF soaking in Lincomycin while the implant site is being prepared.

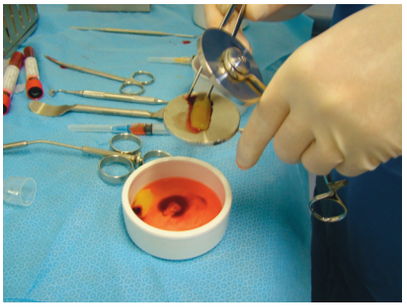

Figure 10. Specialised instrument used to form a membrane.

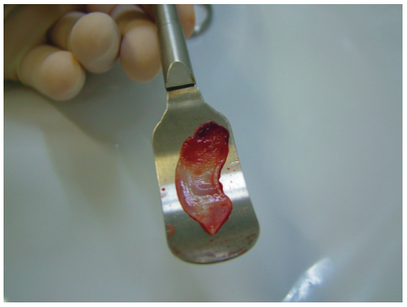

Figure 11. CGF membrane completed just prior to wound closure.

driven against the wall and thus the coagulation cascade becomes activated. The fibrin clot mixed with plasma and platelets collects in the middle of the sample. Some claim that this PRF not only acts as PRP does in osteogensis and wound healing, but may also function as a membrane.[13]

By driving out the fluids trapped in the fibrin matrix, autologous fibrin that is somewhat resistant to traction and shearing, and thus protects the graft from the oral environment.[14] Furthermore, this is attributed to the naturally slow polymerization process of physiologic levels of thrombin, allowing establishment of equilateral junctions with a fine and flexible fibrin network. This enables it to support cellular migration. However, Marx argues that to attempt PRP with a single spin would only produce a mixture of PRP and PPP, with disappointingly low platelet counts, due to the fact that red blood cells will interfere with the fine separation of the platelets.[9] This has been shown by an in vitro study using human cell cultures compared with PRP, where there seems to be less platelet yield and much less growth factors such as PDGF and TG.[7] However, the fibrin with entrapped cytokines,[15] would act as a protective function, physically and immunologically, as well as acting as scaffold for cells involved in the healing process and as a reservoir for growth factors. This entrapment may also increase the lifespan of activity of these growth factors and cytokines.[16] Choukroun compared a sinus lift using frozen dried bone allograft with FDBA and PRF, and showed similar quality of bone but with half the healing time.[17] This is a theme that is also carried through in other studies of PRP.

Platelet Rich in Growth Factors (PRGF E. Anitua)

A different technique for a "preparation rich in growth factors" (PRGF) has been described by Anitua. Using these methods, it is possible to obtain different autologous preparations rich in growth factors from the patient's blood, depending on the extent of coagulation and activation of the samples. These include scaffold-like PRGF composed of fibrillar and cellular components, which may be used to induce bone regeneration in postextraction sockets. Another is the liquid PRGF, which may be used to humidify and bioactivate dental implant surfaces to improve their osseointegration.[18] A 2008 study by Sherwin compared PRP preparation with a particular PRP system. The platelet yield was higher for PRP, but the activation of platelets were higher with the PRGF. The system for PRGF was an open system and is less desirable for surgeries, as it is harder to maintain sterility.

Concentrated Growth Factors

CGF, like PRF, does not require the addition of bovine thrombin or any anticoagulants. Furthermore, altered protocols in obtaining the blood sample and in the centrifuging procedure compare with PRF. Unlike PRF however, CGF uses variable rpm from 2400-2700 rpm to separate cells in the venous blood, resulting in fibrin rich blocks that are much larger, denser and richer in growth factors than common PRF. This shows better regenerative capacity and higher versatility when using the fibrin rich block. The resulting fibrin clot/block is of a higher quality due to the concentration of fibrinogen, factor XIII and thrombin that is obtained. Factor XIIIa, which is activated by thrombin, cross links the fibrin clot to increase stability, strength and protection against plasmin mediated degradation. Clinically, this translates to a clot with higher tensile strength (1.5 kg after 1 hour vs 500 gm), adhesive strength, and decrease in haemostatic time (105 secs vs 360 secs). Besides the tensile fibrin membrane, a red phase of concentrated red blood cells and platelets is also obtained. This is often mixed with either autogenous or other fillers for a more easy to handle and voluminous cavity filling method. Essentially the CGF is an upgraded version of PRF with a strengthened fibrin matrix and boosted growth factors and cytokines.

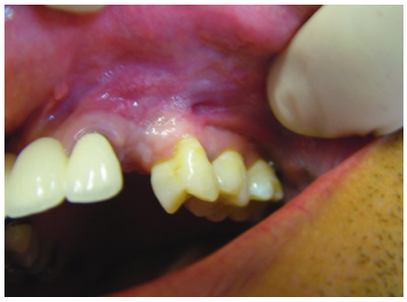

Figure 12. Buccal bone deficiency in the canine region.

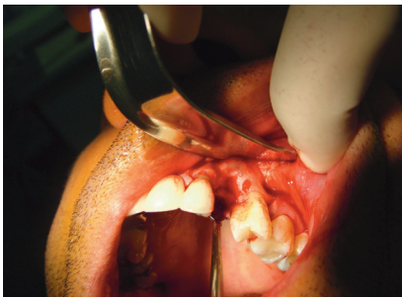

Figure 13. Proposed implant site exposed.

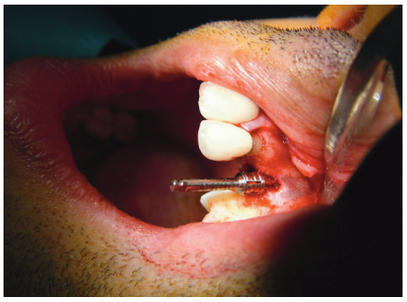

Figure 14. Angulation verified and horizontal defect noted.

Figure 15. A xenograft (Bio-oss) is mixed with the platelet-rich caogulation.

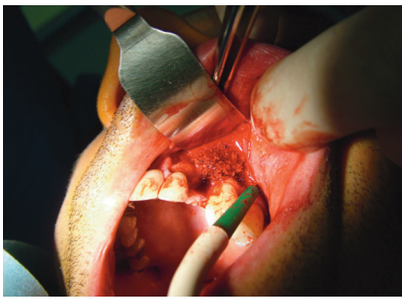

Figure 16. The defect is grafted.

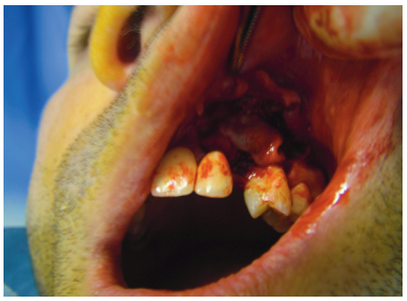

Figure 17. CGF placed over the graft to prevent resorption and enhance healing.

The CGF is characterised by 4 phases as shown in Figure 2:

1. A superior phase represented by the serum (blood plasma without fibrinogen and coagulation factors);
2. An interim phase represented by a very large and dense polymerised fibrin block;
3. A liquid phase containing the GFs, white line cells and stem cells waiting for stimulation and to differentiate into specialized cell types; and
4. A lower red portion comprising a viscous, dense, platelet-rich coagulation.

The phases and their components are:

1. Serum

Serum is the lightest and most liquid part of blood. It is fundamental for this technique as it is able to amalgamate all the grafts and it supplies many biochemical components and activators. It is fibrinogen-free and has only a few cells. It should be kept cool and mixed quickly in order to avoid denaturing the proteins. It is a clear and straw yellow in colour and consists of:

- 92% H2O
- 7% proteins, mineral salts, CO2:
  - Proteins: albumin, antibodies
  - Nutrients: glucides, amino acids, lipids
  - Enzymes
  - Hormones
  - Inorganic electrolytes

The serum is used to wash the cavity, to cover and protect all the regenerated portions.

2. Fibrin Buffy Coat

Thanks to the calibrated centrifugation carried out with the Medifuge phase separator (Silfradent, Italy) that polymerises the fibrinogen molecules (FG), the resultant fibrin block comprises three-dimensional polymer networks with interwoven fibres, all collected in a single phase in the form of a gel.

During polymerisation, the fibres' diameter grows until the end of the reaction. This concept explains why it is important to set up the equipment precisely so as to guarantee the maximum exploitation of the blood's potential by controlling the following:

- Speed;
- Temperature;
- Time;
- Acceleration and controlled speed; and
- Gravitational acceleration of approximately RCF200.

The development and growth of the fibrin gel block during the centrifugation and especially during the polymerisation phase, allows for a volume growth of the chains in all directions. In this way, many corpusculated components are combined, determining numerous therapeutic actions including:

- plasma and platelet cytokines: repair, anti-inflammatory and pain-killing effect during repair (TNF-a); and
- platelets: transmission of the signals and release of the GFs. The most important are the PDGF-BB, TGFI3-1 and IGF-1.

We therefore obtain significant volume fibrin gel blocks with excellent resistance for:

- cavity fillers;
- membrane supports;
- autologous membranes; and
- particles to be mixed with another filling material.

This translates into a simplified workflow, a high level of regenerative induction and greater versatility of applications for the fibrin block, ranging from the use of the whole block to the particles or membrane.

3. The Growth Factors and the unipotent Stem Cells are located just below the buffy coat and above the dense clot portion. This phase can be aspirated with a pipette and mixed with autologous bone in order to obtain an extremely high performance activated graft.

4. Coagulation

In the CGF technique, the red phase consists of concentrated red and white blood cells, platelets and clotting factors. It looks like a dark reddish dense gel, and can be used in its pure form or mixed with fibrin particles and/or autologous or heterologous bone when filling very large cavities.

CGF in regenerative surgery should therefore be considered as a multifactorial stimulation system. This versatility and multiplicity of applications makes it stand out from all the other techniques proposed so far.

Materials and methods

In order to obtain the CGF, we begin by taking a venous blood sample using a 21 x three quarter gauge butterfly vacuette needle and a vacuum-packed Vacuette 9 ml Z Serum Clot Activator (Greiner bio.one, Austria).

Once filled, the test tubes are quickly placed into the rotor of the Medifuge (Silfradent, Italy) centrifuge accelerator, without shaking them. This has exclusive characteristics with regards to:

- mechanical structures and characteristics, such as the monolithic sterilisable rotor;
- calibrated angled test tube;
- working temperature;
- disinfection of the rotation chamber;
- dynamic characteristics;
- settings: start, acceleration, speed and brake for the fluid to be centrifuged; and
- automatic, closed lid disinfection.

All this permits us to obtain more greatly differentiated components right from the test tube. After approximately 13 minutes of rotation, the serum is separated from the other phases of the CGF and stored in a specific sterile dappen dish. The fibrin phase is separated and stored in diluted antibiotic solution (Lincocin 600 mg). The initial portion of the coagulation containing the GFs and the stem cells are immediately stored in the dappen provided. The coagulum, which is rich in red blood cells and platelets, as well as iron, calcium and other fundamental components, is prepared to be used for the preparation of fillers, for mixtures of biomaterials or autologous bone taken for osteotomy.

The fibrin block, separated from the red phase, is prepared to be transformed according to the clinical need: direct cavity graft, shaped membrane with the use of the specific forceps provided, graft particle to be mixed with biomaterial or living autologous bone.

A specific process is necessary to obtain an autologous CGF graft for large cavities. In this case, the fibrin block is cut into particles of approximately 1-2 mm while the clot is fragmented and mixed with the fibrin particles, fresh blood and further graft material (ideally autologous bone). To increase the softness of the mixture, some serum can be added. This is all mixed and homogenised mechanically in the specific Round Up device (Silfradent, Italy) for approximately 6 seconds.

This dense and particularly adhesive paste is inserted into the cavities or bone defects, proving to be extremely mouldable. Everything is then covered by applying the CGF membrane obtained by squeezing the fibrin blocks with the forceps provided.

CGF membranes are used to cover wounds or reconstructed areas, which can stick together thanks to their adhesive power and, thanks to their elasticity, can be sutured.

At the end of the surgery, the wound can be "brushed" with serum.

Conclusion

During normal wound healing, the fibrin matrix is important in haemostasis, however more crucial is its role in acting as the initial scaffold for the new extracellular matrix. It allows binding of cells and healing proteins to the scaffold, such as platelets, WBCs, fibroblasts and osteoblasts, endothelial cells, and smooth muscle cells. Keratinocytes bind to fibrin. By expressing sites for cytokine binding, growth factors and adhesion molecules for cells, fibrin indirectly promotes wound healing

Fibrin has also been shown in animal models to be an important determinant of angiogenesis, as fibrin deposited in subcutaneous tissue induces angiogenesis. Furthermore, many studies have shown that wound healing is largely dictated by fibrin structure; in density, number of branch points, porosity and permeability. The fibrin physical structures are determined by many factors including clotting rate, Factor XIII concentration, thrombin, chloride ions, pH, etc. Optimizing these conditions is part of the aim of the CGF protocol.

Pathological alterations of these fibrin fibers occur in diseases such as diabetes and this clearly leads to disturbances in wound healing. Thus these are the patients that are most likely to benefit from CGF. In addition to the many studies that demonstrate an accelerated healing process with the application of PRP, and PRF, there have also been studies to show fibrin sealant on its own has been used as matrices in the promotion of wound healing.

Further to the fact that CGF not only uses an autogenous source of growth factors and membrane, there are no added animal derived products added as in PRP. With no anticoagulants added, the platelets begin to be activated naturally alongside the coagulation cascade. The resulting matrix/membrane rich in fibrin works synergistically with these growth factors.

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About the author

Dr Peter Mansour is a general dentist with an interest in implant dentistry. He has completed hands on implant placement and bone management courses in the USA and in 2009 completed a Masters Certificate in Advanced Implant Therapy with UCLA's Global Institute for Dental Education (GIDE). He is currently in private practice in Sydney and completing a distance learning masters degree in Fixed and Removable Prosthodontics with Kings' College London.

Dr Paul Kim is a general dentist with an interest in implant dentistry. He has completed hands on implant placement and bone management courses in the USA and in 2009 completed a Masters Certificate in Advanced Implant Therapy with at GIDE. He is currently completing a postgraduate Masters degree in Periodontology at Griffith University.