# Growth Factors – A Brief Review

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## Abstract

The understanding and use of growth factors (GFs) in different pathologies of the musculoskeletal system are points of great interest to many scientists who believe in biology as a new way of solving diverse health problems. This article is a brief update explaining the physiological regenerative processes, where in regeneration the tissue properties are not distinguishable from the original and in repair the tissue has a different architecture and function and with physical and mechanical properties inferior to the original. The sources, functions, types and current clinical applications of GFs are explained. Although there are diverse techniques and processes to obtain plasma using platelet concentrate, the authors perform Anitua's Technique to obtain PRGF®, which is characterised by slow centrifugation of fresh blood that provides the plasma fraction used, a small amount of blood is required, bovine thrombin is not used, the clot is obtained adding calcium chloride and leukocytes are not included.

## Keywords

Growth factors, platelet-derived growth factor, platelets, PRGF®, platelet-rich plasma

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Treatment with the bioactive molecules of cells, i.e growth factors (GFs), cytokines and scaffolds to regenerate tissue such as skeletal muscle is becoming common practice. This triad of therapeutic substances represents 'tissue engineering', a term coined by Professor Fung of University of California, San Diego, at a scientific meeting hosted by the the US National Science Foundation, the objective of which was to discuss an upcoming concept that mixed biology and engineering.[1]

From the first group of publications about bioactive molecules it is important to highlight those of Professor Marshall R Urist. In 1965, he published on the extracellular matrix of bone tissue that has the capacity of inducing bone formation, known as bone morphogenetic protein (BMP).[2]

Samuel Balk showed that to initiate their division, chicken fibroblasts, rat thymus and bone marrow cells require appreciable extracellular concentrations of calcium ions or factors peculiar to serum but not to plasma. Nancy Kohler et al. presented evidence that platelets are an enriched source of growth-promoting activity for BTB mouse fibroblasts. Russell Ross et al. studied the role of factors derived from blood serum in promoting cell proliferation *in vitro*. Antoniades, Scher and Stiles described that human platelets contain a polypeptide growth factor that stimulates the proliferation of connective tissue cells. They identified two types: platelet-derived growth factor (PDGF) I and PDGF II. Both were constituted from two aminoacid chains of different molecular weight. And Heldin, Westermark and Wasteson studied the chemical properties of PDGF and the utilisation of these properties in a purification protocol for PDGF leading to an electrophoretically pure product.[3–7]

In 1995, the multidisciplinary group lead by oral surgeon Eduardo Anitua, who founded the Biotechnology Institute and the foundation named after him, increased the knowledge of platelet function and its therapeutic applications.[8,9]

## Physiological Processes – Regenerative Biology

Regenerative biology is based on identifying the cellular and molecular differences that exist between regeneration (newly restored tissue, its properties are not distinguishable from the original tissue) and repair (restoration of a tissue with a different architecture and a different function. Its physical and mechanical properties are inferior to originals. It is a transformation that happens spontaneously and results in a scar).

The success in stimulating the tissue-regenerative mechanisms is based on promotion by means of artificial biosubstances (scaffolds) or natural (GFs and cytokines) cell migration, proliferation and differentiation. Therefore, the bioactive molecules and the scaffolds encourage cells to proliferate rapidly and synthesise proteins vigorously. GFs and cytokines are soluble proteins – bioactive molecules – which regulate major events in tissue repair and remodelling. The use of GFs has been considered a way to manipulate the host-healing response at the site of injury, so one of the main goals in the biology of regeneration is to identify the signals that regulate cell proliferation and differentiation.

It is possible that regeneration is only a question of activating certain cells providing the adequate stimulating signals or neutralising certain signals suppressive to regeneration. However, a requirement

of regeneration is the potential of cell division that is classified as unstable, stable and permanent. It is known that if permanent cells disappear they cannot be substituted, they have a long life and live in protected environments an example of this is the nerve cells. The majority of distinguished cells are not permanent. They renew and originate in two ways: simple duplication of pre-existing cells or from stem cells undistinguished by a process of differentiation, which involves a change in the cell fenotype. There are great similarities between embryogenesis and repair. In both processes, BMPs, GFs and stem cells are important elements. The BMPs and GFs contribute to giving volume and conducting the embryological cell, tissue and organ development, supervising tissue biological function. They play a major role in the post-foetus physiology.

## Growth Factors – Source, Functions and Types

The sources of the GFs are the extracellular matrix, the platelets – megakariocytes – the plasma and others.

All human body tissue cells are in a constant rejuvenation process. The bone tissue cells are within the extracellular matrix, a net formed by macromollecules that actively participate in cell metabolism and regulate the behaviour of the cells that are in contact with it. Protein soluble factors that include BMPs and GFs can be found in the extracellular matrix.

Platelets, which are small blood cell fragments that arise from the megakariocytes without nucleus, have two functions: bleeding interruption when there has been a vascular injury (haemostatic function) and stimulating cell proliferation and tissue scarring (bio-therapeutic function) when they release GFs that have been synthesised by the megakariocytes and stored in the alpha granules.[8–12]

It has been said that GFs, also referred to as growth differentiation factors (GDFs) by some authors, are soluble and diffusible polypeptides, and regulate growth, differentiation and fenotype of numerous types of cells like those of the musculoskeletal system for example. The have molecular weights between 5 and 35kDa, and are produced by a great variety of cells. They join specific membrane receptors located on the surface of the cell on which they work. This happens when they are sent from one cell to another to transmit one concrete signal and start a biological action: migration, differentiation, activation, etc. The cell or cells that receive the signal could be near or far from the cell that has synthesised and released the factor. Some are synthesised by almost all cells, for example transforming growth factor-beta 1 (TGF-β1), which take part in almost all physiological processes. It is important to add that the GFs are multifunctional in that they stimulate the proliferation of certain cell types, inhibit the production of others and cause non-related effects with the proliferation in other types of cells. They take part in repair and regeneration and regulate major processes such as mitogenesis, chemotaxis, cell differentiation and metabolism. The names of the GFs reflect their originally described activity or isolation source. Those that are in bone tissue and in the tissues involved in regeneration are the following:

- PDGF. Produced by platelets, macrophages and endothelial cells. It is a protein stored in the alpha granules of the platelets, released when platelets pool together and start the coagulation cascade. The connective tissue cells of the mentioned area respond by starting a replication process.
- Vascular endothelial growth factor (VEGF). The aminoacid sequence has a 24% of the PDGF-β, but it joins different receptors inducing different biological effects. It is a potent mitogen specific to endothelial cells. It has an *in vivo* angiogenic action.
- TGF-β is a protein subgroup that includes the BMPs and others. It has three fundamental functions: to modulate cell proliferation (it is a suppressant); increase the extracellular matrix synthesis and inhibit its degradation; and has an immunosuppressant effect. However, the cell-specific action depends on the precise circumstances of its environment.
- Acidic and basic fibroblastic growth factors (aFGF and bFGF) are synthesised by many types of cells, such as fibroblasts and osteoblasts. Four types of receptors have been identified. They have an important role in tissue regeneration, stimulate the proliferation of the majority of involved cells in the endothelial capillary repair, endothelial vessels, fibroblast, keranocytes, condrocytes, mioblasts, etc.
- Insulin-like growth factor type I and II (IGF-I and II) are found in large quantities in bone. The osteoblasts produce type I, which stimulates bone formation thus inducing cell proliferation, increasing the number of osteoclastic multinucleated cells, differentiation and biosynthesis of collagen type I.
- Epidermal growth factor (EGF). Its structure is similar, not identical, to TGF-α in that it joins the same receptors and in terms of its biological action. The kidneys, submandible gland, lacrimal gland, Brunner's gland and the megakariocytes synthesise EGF. This GF is found in saliva, tears and urine, improves wound repair, stimulates migration, epithelial cells mitosis and increases protein synthesis such as fibronectin. It also attracts fibroblast chemotaxis and these synthesise collagen increasing the collagen total.

The GFs related to the number of platelets are PDGF, TGF-β1 and 2, VEGF, EGF and basic fibroblastic growth factor (bFGF). They are produced by the megakariocytes and stored in the alpha-granules of the platelets. And the GFs not related to the number of platelets are IGF-I and hepatic growth factor (HGF). These are plasma GFs. The TGF-β1, PDGF and IGF-I modulate cell proliferation and migration and synthesis of extracellular matrix. And the VEGF, HGF and bFGF are chemotactic and mitogenic for endothelial cells promoting angiogenesis and vascularisation a major step in healing.

## Growth Factor Therapy

The aim is to use platelets that mimick the physiological process when a tissue is damaged and ensure platelets deliver proteins to the injured area.[12]

It has been noticed that all types of connective tissue contain many of the signalling proteins which play a very important role in the remodelling and repair of the tissue.

Currently, there are diverse techniques and processes to obtain plasma using platelet concentrate. There are some that reach a platelet concentration up to four to six times higher than the physiological plasma concentration.

PRGF® is plasma rich in platelets with all proteins and plasmatic coagulation factors. The GFs that comprise PRGF® are PDGF platelet-derived growth factors, TGF-β1 and 2, IGF-I, VEGF A and C, FGF, EGF

and HGF. It is obtained following the technique described and patented by Anitua. The main characteristics are:

- the plasmatic fraction used is obtained through slow centrifugation of the fresh blood;
- that it is carried out with small amounts of blood;
- the clot is obtained by adding calcium (calcium chloride). Anitua does not use bovine thrombin to coagulate; and
- does not include leukocytes in the preparation because they make fibrin unstable by accelerating fibrinolysis, and they contain and express matrix metalloproteinase enzymes (MMPs) that contribute to extracellular matrix degradation.[13]

## Clinical Applications

PRGF can be applied to injuries of all types of connective tissue: cartilage, bone, ligament, tendon, muscle, skin and synovial membrane, etc.[14]

### Cartilage

The aim of treating chondral injuries with PRGF is to re-fill defects with new chondral tissue. There are numerous research studies that support the treatment[15–18] and some publications about clinical applications.[19–22]

### Bone

The use of PRGF is being studied as treatment of bone fractures that have impaired healing that cause pain and disability.

*In vitro* studies have demonstrated that PDGF stimulate the proliferation of human trabecular bone cells and human osteoblast-like cells. Initial studies *in vivo* were reported in the fields of oral-maxillofacial surgery and in dentistry. The studies were initially about the effects of isolated PRP.

Thomas A Einhorn published a prospective, randomised clinical trial involving 122 patients with 124 tibial non-union fractures, which was conducted with the use of BMP-7 (osteogenic protein-1 [OP-1]), and Mikel Sánchez et al. published the results obtained in non-union fractures treated with PRGF.[23,24] On the basis of these data, on the 17 October 2001 the US Food and Drug Administration (FDA) issued a humanitarian device exemption for the application of the OP-1 implant as 'an alternative to autograft in recalcifiant long bone non-union where use of autograft is unfeasible and alternative treatments have failed'.

### Ligament

Many trials have been published in recent years on the study and clinical use of the BMP and on GFs. The goal of each of these is to identify the optimum combinations of these proteins, with higher power, the most effective exposure time, the most effective therapeutic dosages and how to define the right ways to release them.[25–36]

In experimental studies carried out on animal models it has been found that the TGF-β1 and EGF as well as the use of GFs obtained from autologous platelet concentrate act in the anterior cruciate ligament (ACL) graft remodellation causing increased collagen synthesis, increased fibroblast synthesis, improved scarring speed, increased tension strength resistance and increased maturing speed.[37–40]

The use of PRGF in ACL surgery has two goals: to prevent anterior knee pain and to achieve a quicker fixation and maturity of the graft. Sánchez et al. reported the results obtained with the application of PRGF in ACL repair surgery in a group of 50 patients with PRGF and 50 patients without PRGF were minimised haematomas, reduced post-operative signs of inflammation, reduced pain, reduced the recovery process, accelerated integration of the graft and reduced probability of laxity post-surgery. Furthermore, Radice et al. reported that the results obtained with the application of GFs in ACL repair surgery in a group of 25 patients with GFs and 25 patients without, where a significant reduction was achieved in the biological maturing time of the graft, were least 49%.[41,42] Kuroda et al. found that the release of the GFs peaks between the third and sixth week and almost completely ceases at 12 weeks.[43]

Yoshikawa et al. showed upregulated expression of VEGF, a potent stimulator of angiogenesis, at two to three weeks post-reconstruction. Recent studies found that exogenous application of VEGF enhanced cell infiltration and fibroblast expression during the proliferation phase of healing, but this also induced significant deterioration to the mechanical properties of the graft. These findings support the reports of numerous other studies that all found the mechanical properties to be at a minimum around the proliferation phase of healing at six to eight weeks.[44]

In the early phase, Kawamura et al. and Kuroda et al. reported that graft necrosis leads to a release of source cytokines interleukin that trigger a cascade of GFs expression, which result in cell migration and proliferation and extracellular matrix synthesis and revascularisation.[45] Scheffler, Unterhauser and Weiler share the same results with the studies of Kuroda, Kawamura and Yoshikawa.[46]

### Tendon

The goal is to obtain a new healthy tendon tissue.[47] Sanchez et al. reported good results in Achilles tendon injuries.[48]

## Conclusion

PRGF is an autologous preparation that contains proteins that accelerate and improve tissue healing. Orthopaedic surgeons are well prepared in terms of biology and tissue engineering to repair injured anatomy and to recover disturbed function. However, further basic science studies and clinical trials need to be carried out. ■

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