Platelet-Rich Plasma (PRP) – Potential Orthopaedic Applications of Autologous Preparations Rich in Growth Factors (PRGF)

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Growth factors and cytokines have essential roles in regulating the mechanisms and pathways that govern wound healing and tissue formation[1,2]. The emerging fields of tissue engineering and regenerative medicine are based on the delivery of growth factors and bioactive proteins to localized sites to trigger healing and regenerative processes[3].

Growth factors are synthesized by megakaryocytes and stored mainly in the α granules of platelets. Blood platelets have a major role in hemostasis and also in the inflammatory and healing processes[4,5]. Platelets circulate through the bloodstream for about 8 to 10 days carrying growth factors, which they release together with clotting factors in areas where tissue has been damaged. Once activated, platelets secrete numerous proteins such as fibrinogen, fibronectin, and vitronectin, in addition to a multitude of growth factors including bone morphogenic proteins (BMPs), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) that drive tissue regeneration mechanisms [1, 3, 4].

Since the late 1990s innovations, mainly by oral surgeons, have led to the development of platelet enriched preparations that promise to increase repair capacities of tissues by increasing the quantity of growth factors and proteins secreted by the platelets [6,7, 8, 9, 10].

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TERMINOLOGY

There is wide range of biological preparations that are called platelet-rich plasma or have similar or confusing names that are used interchangeably. The term "platelet-rich plasma" is often used to identify these preparations even if they are prepared using different protocols and differ quantitatively and qualitatively[11]. Anitua at al. recommend using the term "preparation rich in growth factors" or "PRGF" to identify autologous blood in which the platelets are concentrated using one-step centrifugation with sodium citrate added as the anticoagulant and calcium chloride as the activator and in which leukocytes have been eliminated[7]. Ehrenfest et al. presented a classification of different platelet concentrates into four categories, depending on their leukocytes and fibrin content: pure platelet rich plasma (P-PRP), leukocyte-and platelet rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and platelet-rich fibrin (L-PRF)[12].

We will use the term PRP when speaking generically about plasma that contains a high concentration of platelets and PRGF when referring to the preparations described by Anitua et al. above[7].

PREPARING AND DELIVERING PRGF

Peripheral blood (20 cc) is drawn into a tube containing 3.8% sodium citrate. The tube is centrifuged for eight minutes at 1800 RPM (460 G) at room temperature. The 0.5 ml plasma fraction located just above the sedimented red cells, but not including the buffy coat, is collected. Glass tubes containing the PRP are incubated at 37°C in the presence of 22.8 mM CaCl to start clot retraction[13]. The solution is injected into or applied to the area in which the therapeutic effect is intended.

The leukocytes are eliminated because the proteases and acid hydrolases in the white blood cells cause an inflammatory reaction[14]. Calcium chloride is used as an activator; if thrombin is used to activate the preparation, there is a risk of developing antibodies.

A fibrin matrix can also be prepared to deliver the growth factors at the site of implantation and to retain them from an excessive initial burst of release, such as in tendons [3,15]. A 3-D fibrin matrix can be prepared by regulated polymerization of fibrinogen in plasma [3,16].

Increasing the platelet concentration in tissue increases the growth factor content and improves the therapeutic effects, but excessive platelets have an inhibitory effect[6]. Optimizing composition and use of PRP is crucial to enhancing the therapeutic potential of this technology[17,18].

POTENTIAL ORTHOPAEDIC APPLICATIONS OF PRGF

Bones and fractures

In-vitro studies have demonstrated that platelet-derived growth factors stimulate the proliferation of human trabecular bone cells[19] and osteoblast-like cells[20, 21]. Marx and co-workers have shown faster maturation and denser bone regeneration of mandibular grafts if PRP was added to bone grafts[22].

Anitua at al. studied the effects of PRGF on bone regeneration and on titanium implant osteointegration in goats and concluded that PRGF accelerated bone regeneration in artificial defects and improved integration of the dental implants[23].

In an animal model, Simman et al. showed accelerated and stronger bone fracture healing in rat femurs when PRP was added compared to bones left untreated[24].

Nonunion and spine fusion

Sánchez and co-workers showed the enhancement of healing of 16 nonhypertrophic nonunions with PRGF supplementing surgical treatment[25].

Several studies have shown that PRP decreased the rate of spine fusion[26,27,28]. The FDA now requires manufacturers of PRP devices for bone healing to warn that the safety and efficacy has not been established[29].

Joints

In an *in vitro* experiment Anitua and co-workers studied the effect of PRGF on synovial cells from osteoarthritic knees and found increased secretion of hyaluronic acid and increased production of hepatocyte growth factor they attributed to PRGF[30].

Sánchez at al. compared injections of PRGF and hyaluronan into osteoarthritic knees and showed better pain relief with PRGF[31].

In a case report, Sánchez at al. described the successful arthroscopic reattachment of a >2cm loose chondral body using five biodegradable pins plus PRGF[32].

Tendons and ligaments

Platelet-rich fibrin matrices have been shown to accelerate tendon cell proliferation, stimulate the synthesis of type I collagen, and promote neovascularization both *in vivo* and *in vitro*[16].

Anitua et al. showed that the presence of platelets within fibrin matrices significantly increased the proliferation of tendon cells[15]. They also demonstrated that PRGF promoted proliferation and induced VEGF and HGF production by tendon cells in culture[33].

Mishra et al. showed significant improvement in 15 patients who had chronic elbow lateral epicondylitis following a single injection of PRP[34].

In human studies, PRP has been shown to enhance healing of surgically repaired Achilles tendon[15]. The same authors also used PRGF to improve the healing of the Achilles tendons of two patients who had wound breakdown and infection after surgical repairs[35].

The effects of PRP and bone plugs upon the healing of hamstring tendons in a bone tunnel showed no effect on the osteoligamentous interface or tunnel widening evolution, though the use of PRP effectively prevented tunnel widening[36]. In a retrospective clinical trial of 100 patients undergoing anterior cruciate ligament reconstruction, Sanchez and co-workers reported fewer complications and improved healing if PRGF was injected[37].

In a randomized controlled pilot study on the treatment of chronic cutaneous ulcers, Anitua and co-workers accelerated the healing of chronic ulcers using PRGF[38].

SUMMARY

Platelets are the source of growth factors that regulate tissue healing and regeneration. Increasing the quantity of growth factors has the potential to improve healing and to allow regeneration. Further basic science studies and clinical double-blind studies are needed to advance our knowledge in this field and to prove the efficacy of this treatment.

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