Title: Platelet Rich Plasma: "A Healing Aid"

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PLATELET RICH PLASMA: "A HEALING AID"

Introduction

For understanding the principles behind the use of Platelet-rich plasma (PRP), one must first understand the biology of its most important component-the platelet. Platelets are one of the most important components of blood. They are necessary for a variety of functions, the most important of them being the clotting of blood. There are about 140,000 to 400,000 platelets/mm^3 of circulating blood, and they normally have a half-life of about 4 days. After vascular injury, platelets encounter extracellular matrix constituents that are normally sequestered in beneath an intact endothelium, such as collagen, proteoglycans, fibronectin, and other adhesive glycoproteins. On contact with these, platelets undergo three general reactions: (1) adhesion and shape change, (2) secretion (release reaction), and (3) aggregation. This sets up an autocatalytic reaction leading to build up of an enlarging platelet aggregate, the primary haemostatic plug. Platelet contraction then results in an irreversible, fused mass called the secondary haemostatic plug.

Constitution of platelet-rich plasma

Platelet-rich plasma is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline.[1] As such, PRP contains not only a high level of platelets but also the full complement of clotting factors, the latter of which typically remain at their normal, physiologic levels. Other terms used to describe platelet preparations include platelet concentrate, platelet gel, and platelet releasate.[1, 2]

Besides being a concentration of platelets, PRP is also a concentration of the some of the fundamental protein growth factors (GFs) proven to be the active secretions of the platelets. These are as following:

GROWTH FACTOR	BIOLOGIC ACTION
Platelet derived growth factor	Stimulates DNA and protein synthesis in osseous tissues; mitogenic effects on mesenchymal cells; angiogenic effects on endothelial cells.
Transforming growth factor β	Stimulates angiogenesis; enhanced woven bone formation; stimulate matrix synthesis in most culture systems; chemotactic effects on osteoblastic cells; stimulates endothelial chemotaxis; stimulates bone formation by inhibitory effects on osteoclasts.
Platelet- derived angiogenesis factor	Mitogenic effects on endothelial cells; increased angiogenesis and vessel permeability.

Insulin- like growth factor 1	Stimulates proliferation of osteoblasts and matrix synthesis; increases expression of bone matrix proteins, such as osteocalcin, in combination with PDGF it enhances the rate and quality of wound healing.
Platelet factor 4	Chemoattractant for neutrophils and fibroblasts.
Vascular endothelial growth factor	Enhances angiogenesis.
Fibroblast growth factor	Enhances angiogenesis
Epithelial cell growth factor	Stimulates endothelial chemotaxis and promotes angiogenesis.
Hepatocyte growth factor	Enhances angiogenesis and inhibits fibrosis.

The release of these growth factors is triggered by the activation of platelets. In addition to growth factors, platelets release numerous other substances (e.g., fibronectin, vitronectin, sphingosine 1-phosphate, thromboxane A2, calcium etc…) that are important in wound healing. A blood clot is the centre focus of initiating any soft tissue healing and bone regeneration. PRP is a simple strategy to concentrate platelets or enrich natural blood clot which forms in normal surgical wounds, to initiate a more rapid and complete healing process. A natural blood clot contains 95% red blood cells (RBCs) 5% platelets, less than one percent white blood cells (WBCs) and numerous amounts of fibrin strands. A PRP blood clot contains 4% RBCs, 95% platelets and 1% WBCs.

This is where one must first identify what exactly is platelet-rich plasma. True PRP is always autologous and is not homologous. Homologous platelets are not viable and thus cannot secrete bioactive growth factors. Abundance of the cell membranes in homologous platelets also makes them antigenic, causing various secondary immune reactions.

Mechanism of action of platelet rich plasma

Growth factors are involved in key stages of wound healing and regenerative processes including chemotaxis, proliferation, differentiation, and angiogenesis.[3] Importantly, all these may occur simultaneously, and in different tissues where the effects may be different, depending upon the conditions.

PRP works via the degranulation of the α-granules in platelets, which contain the synthesized and pre-packaged growth factors. The active secretion of these growth factors is initiated by the clotting process of blood and begins within 10 minutes after clotting.

PRP is shown to remain sterile and the concentrated platelets viable for upto 8 hours once developed in the anticoagulated state and placed on a sterile surgical table. Like most growth factors, those within the platelets are incomplete because they must be soluble.

After the initial burst of PRP-related growth factors, the platelets synthesize and secrete additional factors for the remaining 7 days of their life span. Once the platelet is exhausted and dies off, the macrophages that have arrived in the area via the vascular in-growth stimulated by the platelets, assumes the function of wound healing regulation by secretion of some of the same factors as well as others.[11]Therefore, the number of platelets in the blood clot within the graft, wound, or adherent to a flap sets the rate of wound healing. PRP merely increases this number.

Processing Technique

Platelets can be prepared by two techniques – General – purpose cell separators which require large quantity of blood (450ml) and is suitable to be used in hospital set up and second, Platelet –concentrating cell separators which are more widely used since the equipment can be accommodated in a dental clinic setup. Venous blood is drawn into a tube containing an anticoagulant. After first centrifugation the disposable carrier yields three layers of blood. The topmost layer is platelet poor plasma (PPP) which comprises 45%, the middle layer is PRP which makes 5% of the total volume called as a buffy coat and bottom layer, red blood cells (RBC) which make up around 40%. After second centrifugation the PRP settles at the bottom. The PPP and RBCs are either discarded or injected back to patient's body. This PRP is then mixed with bovine thrombin and calcium chloride at the time of application. Calcium chloride nullifies the effect of citrate anticoagulant used, and thrombin helps in activating the fibrinogen, which is converted to fibrin and cross-linked.[8, 9, 10]

Handling and application of PRP

The platelet-rich plasma is drawn into a 10-ml syringe and the activating solution is drawn into a 1-ml syringe. Both syringe plungers are connected to move together with both output ports connected to a dual-spray applicator tip that allows both solutions to be mixed as they are applied to the wound.[12,13] Because the [alpha]-granules quickly release their contents on activation, Marx[1] states that the clotted PRP should be used within 10 minutes of clot initiation. This is not an issue with the dual-syringe spray delivery, as the PRP is delivered to the wound site immediately after activation. In the case of other mixing techniques, it is important to transfer the clot to the surgical site before clot retraction; otherwise, the transferred clot may be deficient in the secretary proteins that were expressed.

Role of platelet rich plasma in regeneration

It should be understood that from an evolutionary level, it is inefficient for the body to have numerous stem mesenchymal cells that are just dedicated to healing. In fact stem cell populations decrease with age, leading to the fact younger people don't scar as much as older people. Platelets, which are only in the graft site for less than five days, allow the body to efficiently start a cascade reaction utilizing growth factors.[14] It can be considered that PRP "jump starts" the cascade of regenerative events leading to form a mature graft site.[15]

Three essential components required to engineer pulp dentin tissue include an appropriate stem cell population, a physical scaffold and signaling molecules / growth factors supported by adequate nutrition.[4]

Scaffold is a three dimensional biodegradable porous polymer framework that serves as a potential biologic carrier to facilitate delivery of stem cells and/or growth factors at a local receptor site. A scaffold serves to function as a temporary structural framework until replaced by new matrix formed by inhabitant cells capable of supporting the load.[4]

Platelet rich plasma is a material of interest which could serve as potentially viable scaffold material as it is rich in pre existing growth factors like platelet derived growth factor (PDGF), transforming growth factor-beta (TGF-β), is biodegradable and easy to prepare in a dental setting.[5] Based upon the finding that dentin chips can stimulate dentin formation in an exposed pulp, these may be considered as a scaffold matrix.[6] PRP provides a suitable matrix for pulp stem cell adherence and are a reservoir of sequestered growth factors which promote differentiation of dental pulp stem cells (DPSCs) into odontoblasts.[7]

Conclusion

The PRP preparation can be easily obtained in a dental office environment and can be used for various procedures being done in a surgical dental practice. The growth factor enhancement is especially applicable for patients that are healing impaired such as elderly. PRP appears to enhance both hard tissue and soft tissue healing. It has been shown that application of PRP to the wound healing site increases the concentration of platelets by up to 338%.

Further study is needed to evaluate the biologic effect of PRP on the proliferation and the differentiation of human dental stem cells, and find the key cytokines inducing these effects to estimate the clinical feasibility of PRP for dental tissue engineering.

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