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Review Article

Advances and Updates of Regeneration with Blood Biomaterials-A Review

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Abstract

Background: Blood-derived products to seal wounds and stimulate healing were described 40 years ago. Though the use of fibrin adhesives in many field-related protocols is well documented from the past 30 years, there is a deficiency in a complete and definite protocol for the preparation of platelet concentrates precisely due to its concealed enigmatic characteristics and interactions which has yet to be discovered.

Aim: The aim of this review article is to discuss on platelet concentrates and their advancing effects on regeneration.

Search Strategy: This review which was mainly web-based was done from publications in the last 25 years. One hundred articles were electronically searched and the main databases were (PubMed, Google Scholar, Web of Science, and Science direct). The search was filtered using reviews, research articles, *in-vitro* and *in-vivo* researches and the search terms included growth factors, platelet concentrates, titanium and regeneration. Our research extended to relevant articles from a few national and state Journals as well as manual search. Pathology literature was also referred.

Results: A combination of platelet concentrates and various biomaterials have resulted in an increase in the efficiency of the net performance than their individual effects.

Conclusion: Platelets are crucial for wound healing and neovascularisation. Titanium is hemocompatible. A combination of the two can open a novel gateway to regeneration. Further researches are necessary to advance this healing process.

Keywords: *Growth Factor; Platelet Concentrates; Platelets; Titanium; TPRP; Regeneration*

Abbreviations

TPRP: Titanium Prepared Platelet-Rich Plasma; ADP: Adenosine Diphosphate; PRP: Platelet-Rich Plasma; PPP: Platelet-Poor Plasma; PRF: Platelet-Rich Fibrin; TGF-beta-1: Transforming growth factor-beta-1; GIC: Glass Ionomer Cement; IRM: Intermediate Restorative Material; T-PRF: Titanium Prepared Platelet-Rich Fibrin; VEGF-A: Vascular Endothelial Growth Factor; FGF-2: Basic Fibroblast Growth Factor; EGF: Epidermal Growth Factor; Ang-1: Angiopoietin-1; ASD: Atrial Septal Defects

Introduction

Wound healing is a natural *recuperative* process during which the resident and circulating cells release mediators and signals generated from extracellular matrix [1]. The cell continues to interact with its own extracellular matrix products and with extracellular matrix produced by other cells [2]. Like hormones and growth factors, the extra cellular matrix plays an important role in the regulation of cell

growth, differentiation. In the absence of any extracellular matrix interactions, human endothelial cells rapidly undergo programmed cell death [3]. After a vascular injury, platelets encounter extracellular matrix constituents (collagen, proteoglycans, fibronectin and other adhesive glycoproteins) which are normally sequestrated beneath an intact endothelium. Then, platelets undergo activation involving adhesion and shape change, secretion (release reaction) and aggregation [2].

Platelets

Formed in bone marrow from megakaryocytes, platelets are discoidal and anuclear structures, having a lifespan of 8 - 10 days. Granules present in the cytoplasm, secrete their contents at the time of activation. Alpha granules contain many proteins, platelet specific (such as beta thromboglobulin) or non platelet specific (e.g. fibronectin, thrombospondin, fibrinogen and other factors of coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulin) [4].

Platelet extracellular matrix adhesion is mediated through Von willebrand factor. Platelet granule secretion occurs shortly after adhesion. ADP is a potent mediator of platelet aggregation and calcium is important for the coagulation cascade. The release reaction also results in surface expression of phospholipid complex providing a locus for calcium and coagulation factor interactions in the clotting cascade [2].

Effects of erythrocytes on Platelets: Juana Valles., *et al*. proved that erythrocytes promoted platelet reactivity in a plasma medium, as demonstrated in an *in vitro* system that independently evaluated the biochemistry of platelet activation and recruitment. Amplification of P-thromboglobulin release was induced by erythrocytes. Erythrocytes stimulate PRP resulting in a highly significant enhancement in P-thromboglobulin release at each collagen concentration tested. In the presence of erythrocytes, when platelets were stimulated by collagen, the ability of the derived cell-free supernatant, was significantly improved [5]. This suggests that erythrocytes and collagen activates platelets.

History of protocols

One of the first platelet concentrates protocols PRGF (plasma rich in growth factor/preparation rich in growth factor) was described in 1999 by Anitua. In their study, centrifugation of venous blood was done to obtain the three distinct layers: acellular plasma, buffy coat and RBCs. To collect the entire PRGF fraction, several pipetting trials, incorporated with handling and pipetting errors are necessary after which polymerization of the fibrin is instigated by 10% $CaCl_2$ solution. An unstable PRGF gel is formed after 15 to 20 minutes that has to be used instantly. This basic protocol underwent several modifications with regard to types of anticoagulants and centrifugation and was commercialized or used in various researches [6]. A "two-step gradient centrifugation method" was initially used for the preparation of PRP. In order to separate the, platelets, clotting factors and leukocytes from the erythrocytes, a strong first spin was inceptively performed. After separating the RBCs, the plasma was subjected to a second centrifugation to harvest the PRP fraction from the leukocytes and platelets [7]. In combination with tissue engineering scaffolds, PPP fraction can be a substitute to the use of autologous growth factors and proteins.[1] PRF is the most recent developments of these protocols and it is promoted as a second generation platelet concentrate since it is produced without any anticoagulants or gelifying agents [6]. A fibrin clot is acquired, just between the acellular plasma at the top and the red corpuscles at the bottom [8]. Scientific statistics reports that this biomaterial would be a suitable matrix for the development of consistent healing without inflammatory residue. PRF is not only a platelet concentrate but also an immune node able to stimulate defence mechanism [9]. PRF presents a complex tridimensional architecture which makes it a real platelet and leukocyte-rich fibrin biomaterial [10].

Discussion

Revascularization

In the early phases of thrombosis, hemostasis, and atherosclerosis, platelet activation and platelet recruitment play crucial roles [3]. Among the many identified growth factors that serve to initiate and control angiogenesis are; vascular endothelial growth factor-A (VEGF-A), basic fibroblast growth factor (FGF-2), epidermal growth factor (EGF) and angiopoietin-1 (Ang-1) [11]. Ang-1 increases proliferation,

migration, and differentiation of human microvascular endothelial cells [1]. During angiogenesis, endothelial cells migrate towards angiogenic signals from the parent vessel and proliferate along the chemo-attractants gradient. This results in the formation of new basement membrane with nascent capillary sprouts and tubes [12]. Endothelial cells migrates "tip" cells to guide the direction of new blood vessel formation and trailing "stalk" cells to establish the lumen of the new vessel, which are crucial for stabilization and branching of new vessels [1]. Silencing the α2 integrin subunit increased VEGF-A levels and decreased FGF-2 levels. Both VEGF-A and FGF-2 are two of the growth factors necessary for initiating angiogenesis and both are chemotactic for endothelial cells [11].

Effects on pulp and interactions of platelet concentrates

The appropriate concentration of the PRP treatment enhanced proliferation, mineralization, differentiation of human dental stem cells (periodontal ligament stem cells and dental pulp stem cells) [13]. Ostby., *et al.* in 1961 conceptualized regeneration of pulp which was the key to regenerative endodontic procedures. Subsequent researchers', Rule and winter (1966), Nygaard-Ostby and Hjortdal (1971), Ham., *et al*. (1972) further worked in this regard. The term revascularization was described by Iwaya., *et al*. in 2001 as a procedure that resulted in thickening of the root canal walls and continued root development [14]. Bezgin T., *et al*. (2014) revascularized immature teeth with necrotic pulps using concentrated platelet rich plasma (cPRP) following disinfection of the canal space with triple antibiotic paste [15]. PRF was used by D. Keswani and RK Pandey., *et al*. to revascularize an immature tooth with a necrotic pulp [16]. In 2012, pulpotomy with PRF was performed by H Hiremath., *et al*. in a human mature permanent molar tooth, to study its clinical and radiographic success [17]. PRF is a natural fibrin-based biomaterial and is able to guide epithelial cell migration to its surface favoring the development of microvascularization. In the case of infected wounds, its utilization seems to be of high interest [18].

TGF-beta-1 is known to be involved as a key factor in tissue healing because of its various functions. It stimulates osteoblastic proliferation and collagen type-1 and fibronectin synthesis, enhances woven bone formation, enhances chemotaxis of osteoblasts cells and stimulates angiogenesis. On layering with dental materials PRF might release other growth factors along with TGF-beta-1 such as vascular endothelial growth factor, epithelial growth factor, platelet-derived growth factor and insulin-like growth factor [19]. Harish Mullaguri., *et al*. (2016) proved that biodentin induces larger amount of TGF-beta-1 release and also maintains the integrity of fibrin structure when compared with GIC and IRM when layered over PRF. The other factor that influences growth factors release is the pH of the membranes. Biodentin was chosen for inducing alkaline pH. In his study, Biodentin released 1.6 times more TGF-beta-1 than GIC and IRM at both 1 hour and 5 hour time interval. The increase in TGF-beta-1 from PRF when layered with Biodentin, observed in his study might improve the healing during pulp repair and regeneration of pulp [19]. After extraction of a tooth, the filling of a tooth socket by PRF. quickly, neovascularises through the PRF clot and the epithelial covering is developed. Prompt healing of the wound is observed without purulent complication, pain or dryness [18]. Notwithstanding the tooth being necrotic, the mechanism behind regenerative endodontics is that, some pulp tissue can survive apically which under favorable environment proliferate to proceed in the restorative process of regeneration [20].

The use of bioactive filling materials is logical [22] as the root canal system is inherently wet [21]. PRP was mixed with the sybograft. This graft was used only as a scaffold for the PRP and as filler for prolonging the action of the growth factors present in the platelet rich plasma. The graft begins to set when PRP is mixed with the graft [23]. Though there is no confirmed systematic treatment protocol for endodontic regeneration, many of the cases have shown complimentary results, with absence of clinical symptoms and continued radiographic evidence of development of the dentin-pulp complex [20].

Diabetic cells fail to produce adequate levels of growth factors. Therefore, supplementing wound sites with growth factors provided by other means seems to be a plausible alternative to normalize the healing process. In this context PRP has also facilitated tissue healing [24]. PRF does not appear to enhance cellular proliferation in long term but may play an important role in the revascularization of the graft by supporting angiogenesis. Sinus floor augmentation with freeze dried bone and PRF leads to a reduction of healing time prior to implant placement [25].

The bioactive surface of titanium labels it as a promising biocompatible material for biomedical devices. It is considered the most biocompatible of all metals due to the protective oxide film that forms naturally in the presence of oxygen [26]. Titanium has one of the

highest strength to weight ratios and corrosion resistance among metals [27]. Due to the corrosive environment in the body, corrosion of the metal in the implanted material takes place. A protective oxide layer is formed over time, on the surface of the metals and alloys used as implants. This layer inhibits corrosion and limits the release of metal ions [28]. It was observed by some scientist that titanium induced platelet aggregation. They aimed to define the structural characteristics of T-PRF and compare it with leukocyte and platelet rich fibrin method [26]. When compared to PRF samples, the titanium prepared platelet rich fibrin samples appeared to have a highly organized network with continuous integrity. Their results showed a mature fibrin network in TPRF clots and 15 days after placement of the membrane, they found newly-formed connective tissue and islets of bony tissue in the TPRF membrane [29]. Gomez TW., *et al*. studied angiogenesis using TPRP and concluded that TPRP has better angiogenic potential than its counterpart PRP [30]. This may be due to a significantly increased availability of growth factors in the presence of hemocompatible titanium. These studies establish that platelet-derived fractions may exert an effective pro-angiogenic response in different organs which accounts for further research [1].

Future Prospective

Drugs inhibitory to platelet: Aspirin is the "gold standard" antiplatelet agent for prevention of arterial thromboses [31]. Resveratrol is known to inhibit platelet aggregation and the growth of a variety of cancer cells and have a potent anti-inflammatory and antioxidant effects [32]. Aspirin and resveratrol inhibits platelet aggregation and reduces the viscosity of blood. The increased velocity of blood may prevent platelet aggregation. As resveratrol can inhibit growth of a variety of cancer cells, this may conclude that these drugs have an inhibitory effect on platelet aggregation therefore inhibiting degranulation and thus minimization of growth factors. Therefore hypothetically, inhibition of platelet degranulation may be able to arrest cancer.

Titanium alloy used for ASD closure: Transcatheter closure of ASD is currently preferred over open heart surgery. A nickel-titanium alloy (nitinol) device 'the amplatzer septal occluder' (AGA Medical Corp, Golden Valley, MN; USA) is the commonly implanted device for the transcatheter closure of ASD. Though it produces good long-term results in children and adults, systemic side effects associated with nickel allergy are pericarditis, and increased migraine headaches. Therefore, that titanium is more resistant than nickel to corrosion and the possible localized stress and friction caused by the motion of the heart [28].

The low speed centrifugation concept was introduced after extensive research aiming to understand the effects of centrifugation on the components and bioactivity of PRF. In comparison with untreated endothelial cells, the formation of an increased number of vessel-like structures resulted from *in vitro* endothelial cells cultures when treated with liquid PRF. Therefore, trigger of cell mediated vascularization and regeneration is supported by liquid PRF which is an autologous source of growth factors [33]. Solid PRF can be obtained by refrigerating it for a few minutes prior to use. PRF can be stored at 22°C to maintain its fibrin matrix polymerized in a tetramolecular structure. Since PRP is serous in nature it can be used as a vehicle for the setting of graft materials.

Conclusion

The use of regenerative modalities in dentistry has become a standard of care for many clinicians working in the field of implant dentistry. Currently, a variety of biomaterials are routinely being utilized including barrier membranes, bone grafting materials, and bioactive growth factors to facilitate new tissue regeneration [34]. In spite of the fact that these procedures are successful in arresting the infectious process and resolving apical periodontitis, they are unsuccessful in attaining normal physiological pulpal responses and complete root development. The challenges associated with healing these cases far surpasses the technical challenges of cleaning and shaping and obturating a large root canal space with deficient apical constriction and thin dentinal walls [35]. Owing to these facts it is necessary to research on biocompatible obturating materials that promote revascularization and thus regeneration.

Hypothesis

Thus, interaction of a Blood derived Bioactive Biomaterial with a hemocompatible biomaterial like titanium can be one of the most superlative amalgam in wound healing and regeneration. This amalgam may be useful as a hemocompatible root canal filling material!

Further studies need to be done on titanium prepared platelet rich products and different methods of activation of platelets. Thus, its clinical applications can greatly contribute to the service of man.

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Conflict of Interest

There are no conflicts of interest.

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