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Basic Science and Clinical Application of Platelet-Rich Plasma in Fat Grafting: A Concise Review

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Abstract

Platelet-rich plasma (PRP) is an autologous preparation of concentrated platelets containing over 30 bioactive molecules including PDGF, TGF-β, VEGF, and EGF. The first use of PRP in augmenting regenerative medicine was described in 1998, when autologous PRP was used to aid cancellous bone graft maturation in patients with mandibular defects. Since that time, PRP has been implemented in a variety of procedures, ranging from joint replacements and hernia repair to pituitary tumor removal and radical neck dissections. Given the advancements in technology over the past 2 decades, this paper will summarize the cellular mechanisms underlying PRP, as well as discuss its clinical utility in autologous fat grafting and future roles in cosmetic plastic surgery.

Keywords: Platelet-rich plasma; Fat grafting

Introduction

Platelet rich plasma (PRP) is defined as an autologous concentration of platelets within a small plasma volume derived from whole blood via centrifugation with anticoagulation. The first use of PRP was described by Marx et al. who recognized that the high concentrations of platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-beta), and other growth factors in the product could be administered alongside cancellous bone grafts for patients with mandibular defects [1]. Over the next 20 years, PRP has been implemented in cosmetic procedures ranging from tissue expansion to skin grafting.

PRP is derived from centrifugation of whole blood, which separates into 3 layers: platelet poor plasma, platelet rich plasma, and red blood cells. Contained within these platelets are a number of hemodynamically active proteins that aid in the natural process of wound healing. Specifically, the platelet alpha-granules contain several of these molecules, including: PDGF, TGF-beta, vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), fibrinogen, fibronectin, and vitronectin [2-4]. In addition, platelet delta granules contain serotonin, histamine, dopamine, calcium, and adenosine, which act in tandem with the aforementioned growth factors to regulate wound healing [5].

Mechanism of Action

Similar to platelets, which are inert prior to hemodynamic changes that trigger degranulation, PRP also requires triggers to degranulate and set off signal cascades. Thrombin-mediated activation of platelets results in alpha-granule fusion to the platelet membrane, causing secretion of growth factors and bioactivation of proteins. Said technique predicates a rapid onset of the platelets within 10 minutes of clotting initiation, with 95% released within the first hour [6,7]. Given this abrupt release of the proteins, the time frame of therapeutic effect may in turn be limited. As a result, other methods of activation have been explored. Specifically, $CaCl_2$ can be used in lieu of thrombin to cause direct autologous thrombin formation from prothrombin in PRP [7].

Instead of a rapid release of factors, a diffuse fibrin matrix is formed instead, resulting in a more efficacious method to slowly release growth factors in a localized environment for as long as 7 days [5]. Once released, secreted growth factors preferentially bind cell membranes in nearby tissues via transmembrane receptors; PRP has a predilection for adult mesenchymal cells, epidermal cells, and endothelium in graft, flaps or wounds [1]. The downstream endogenous signal cascades serve to trigger proliferation, matrix and osteoid formation, and collagen synthesis. Given the timeframe of wound healing, regeneration, or other applications for which PRP is utilized, it is evident that decreasing the transient nature of the effect is required. However, equivalently important is the need to have a large enough dose for there to be a therapeutic effect at all. In this light, the standard accepted therapeutic dose is noted to be 1 million/ul, given that a 5 fold increase in platelet concentration is required to achieve cellular response [8].

Advantages, Disadvantages, and Future Directions

Given the minimal steps required in preparation and activation, there are several benefits to expanding the scope of PRP utilization in patient care. Primarily, the little to no technology required outside of centrifugation allows for rapid preparation of PRP in clinical settings, with providers being able to make the decision to use the technique hours prior to procedures. Furthermore, since true PRP is autologous, there is limited risk of triggering immunological attack, disease transmission, or cross-reactivity [9]. In addition, with PRP acting on cells via transmembrane signal cascades instead of cytoplasmic or intranuclear modulation of factors, there is a reduced risk of mutagenic effects. Finally, the produced concentrate is capable of remaining sterile and viable for up to 8 hours. However, this is not to discount obvious limitations with the technique. First, the rapid onset with thrombin mediated activation limits the effect timeframe; even with $CaCl_2$ mediated activation of PRP, the dispersion of growth factors is only

prolonged to 7 days, a significant shorter time frame than required for some of the procedures for which it is used.

Furthermore, given that there are numerous confounding variables involved with PRP use, there has been significant challenge in generating standardized protocols for patient use. The same can be same for research methodologies by which clinical testing can be conducted in patients; as a result, there are only a few randomized clinical trials available at this time. One prospective randomized multi-center clinical trial studied the use of autologous PRP in treatment of diabetic foot ulcers. 72 patients were randomized to either standard care with PPR gel or control saline gel dressings and examined for healing. Even after adjustment for wound size, the trial demonstrated that 81.3% of PRP treated wounds healed versus 42.1% in the control group after 12 weeks [10]. However, it should be noted that given the lack of tissue biopsy, the healing assessment was still subjective in nature.

Given these challenges, most specifically those transient nature of the PRP growth factor release, newer methods of prolonging delivery have been investigated. One promising technique is the use of platelet rich fibrin (PRF), which is obtained from whole blood by centrifuging whole blood without anticoagulation [11]. The coagulation cascade is subsequently activated and results in fibrin clot formation secondary to fibrinogen interaction with activated thrombin (from prothrombin). The resulting mixture allows for separation of a fibrin rich clot, which contains a high concentration of platelets that have yet to be activated. One study investigated the time change in growth factor release as a result of the fibrin clot polymer surrounding platelets, demonstrating that PRP had higher release of TGF-b and PDGF on the first day, but attenuated release thereafter. In contrast, PRF release of PDGF and TGF-beta was highest on days 7 and 14, respectively [12].

Fat Grafting

Autologous fat grafting has been a growing area of interest in the plastics and reconstructive realms since the late 1800's, when Gustav Neurben first described his attempts to use autologous fat in reducing deformities and scars associated with tuberculous osteitis [13]. Vast improvements have since been made in the use of fat grafting in soft tissue augmentation, and the technique is now considered a mainstay in the treatment of craniofacial abnormalities, trauma, malignancy, and aesthetic surgery; this is largely in part due to the standardization of the procedure by Coleman [14]. However, even though the procedure is now relatively low risk, a wide range of complications remain. This includes superficial injection of fat resulting in visible nodules, fat hypertrophy secondary to weight gain, and the most feared complication, downstream vascular occlusion due to embolic development [15]. A final complication, which is frequently dealt with by practitioner, is the rate of long-term fat resoprtion, which ranges widely from 50-90%, resulting in an average of 6 month lifetime for the graft [16]. Several techniques have been developed to attenuate this process, one of which is PRP augmentation of fat grafts.

One of the key limiting factors leading to the long-term failure and resorption of early autologous fat grafts was tissue ischemia; this primarily arose due to the use of bolus injections. Eto et al. first characterize the tissue ischemia in fat grafts into 3 zones: the central necrotic zone, the regenerating zone (characterized by mature adipocyte necrosis and subsequent replacement by adipose-deried stem cells (ASCs)), and the peripheral zone where mature adipose remained viable. Furthermore, their study ascertained that adipocytes within a 300 um of the native tissue survived due to their proximity to previously vascularized tissue and nutrient availability [17]. As a result, the inevitable conclusion is that larger fat grafts demonstrated higher rates of central necrosis and liquefaction than their smaller counterparts [5].

The high concentrations of PRP-derived growth factors previously noted that serve to augment cellular migration, proliferation, neovascularization, and extracellular matrix deposition make PRP therapy one of the most promising agents to increase autologous fat graft survival rates [18]. Given that the current hypothesis is that fat graft resorption is primarily based on insufficient neovascularization within the newly implanted tissue, PRP therapies have been investigated as a source to augment localized angiogenesis. In one by Lu et al. ASCs were labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethyllindocarbocynaine (Dil) and transduced with VEGF or left untransduced. Subsequently, the adipose cells were harvested and mixed with one of four preparations: 1) VEGF-transduced ASCs, 2) Dil-labeled untransduced ASCs, 3) insulin, or 4) control medium. The four preparations were subcutaneously injected at varying locations in 18 nude mice, with tissue harvest 6 months later for evaluation. This study demonstrated that the survival rate for the control transplants were significant lower (27.1%) than the VEGF-transduced and untransduced stem cells (74.1% and 60.1%, respectively). In addition, histological evaluation demonstrated that the capillary density for the VEGF-transduced ASC groups was higher than in other groups; they also contained CD31-double positive cells, which are found on endothelial cells and demonstrates neovascularization [19]. These results signify that although ASCs help increase the overall survival rates of fat grafts, it is actually VEGF that seems to primarily drive the neovascularization and overall tissue viability.

Chung et al. examined this hypothesis in another study, where VEGF-loaded microspheres in human lipoaspirate scaffold were compared to empty microspheres in lipoaspirate and lipoaspirate alone to determine if VEGF-alone would simulate angiogenesis in the tissue graft. The lipoaspirate preparations were injected into athymic mice, with the grafts collected at 3 and 6 weeks later for tissue analysis. Their study confirmed the aforementioned hypothesis, showing that VEGF-loaded microspheres induced statistically significantly more vascularization compared to the empty and lipoaspirate-only groups. This only serves to further support the applicability of PRP-secreted VEGF in fat graft survival [20]. Though these results offer a promising solution, there have been studies that demonstrate that excess vascularization has had deleterious effects in patients undergoing facial rejuvenation. In their clinical trial, 13 patients underwent facelift, where fat was sampled from the abdomen and subsequently subjected to 1 of 3 preparations: 1) SVF-enriched fat, 2) expanded ASCs, or 3) PRP-enriched fat. They ascertained that PRP-enriched fat yielded poorer outcomes due to the hyperangiogenesis and inflammatory infiltrates. While this phenomenon would be beneficial in combating pathological states in these grafts, it concomitantly demonstrates that that there could be a dose-dependent relationship for PRP [21]. However, it must not be overlooked that this specific study demonstrates that the use of PRP is beneficial outside of rat models, given that patients were studied. Nevertheless, the preparation of PRP was administered at the same concentration in each patient, which limits the study's ability to confirm a dose-dependent effect at this time. The latter point is vital, given that correct PRP dosing is required if it was to become more broadly accepted in the augmentation of soft tissue.

Based on these findings, a study by Li et al. investigated the long-term outcomes of using varied PRP volume fractions when combined with ASCs on fat grafts. The ASCs were isolated from human fat tissue and PRP was centrifuged from whole blood. The varying PRP concentration (ranging from 0 to 30% in 10% increments) was compared to ascertain the optimal ASC differentiation and histological viability of fat grafts at 30, 60, and 90 days in nude mice. They demonstrated that the combination of 20% or 30% volume fraction of PRP and ASC had the optimal outcomes with respect to adipogenic gene expression, remaining adipocytes, neovascularization. However, there was no significant difference between the 20% and 30% group in fat graft retention and histological improvement, indicating an attenuated increase with doses over 20% [22]. A recent study by Felthaus et al. in 2017 added onto this finding, demonstrating that PRP concentrations greater than 20% actually had an inhibitory effect on ASC differentiation [23].

Conclusion

Based on the current findings, it is evident that further research is required to understand the basic science underlying PRP mechanisms, as well as clinical research into how it should be utilized. As a result of lack of standardization in preparation and administration of PRP, mixed results for its efficacy have been demonstrated; however clinical trials are currently underway. Although many questions remain, current research has already highlighted many potential benefits of PRP in improvement of soft tissue augmentation and fat graft survival. The development of new PRP delivery systems and strategies to achieve a more sustained release of growth factors, such as PRF, are areas that merit further research moving forward.

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