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Role of Platelets Rich Plasma in Management of Osteoporotic Fractures

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Authors' contributions

Authors AD and SA designed whole of the idea and wrote the first draft of the manuscript. Whereas authors AAM and RNS managed the literature searches. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Management of osteoporotic fracture is challenging. In most clinical settings, skeletal regenerations are biologically optimized, but still many patients continue to experience delayed or impaired healing. Methods to enhance these healing processes, are needed to decrease patient's agony, so that they can return to their work and regain their socioeconomic status in the community. Till this time, autologous bone grafting remain the standard procedure against which all new technologies are compared and analyzed.The success rate of union even after these grafts varies between 80-85% which further becomes decreased in case of repeated bone graft surgeries with donor site morbidities. Considering the concept that the healing of fracture started as soon as the formation of fracture clot, several investigators have suggested that degranulation of platelets at fracture clot elaborates the bioactive component, that aided the healing process. Because autologous platelet rich plasma products are safe and easy to prepare and administer, in this review, we reviewed the role of bioactive component released by activated platelet rich plasma in the fracture healing process and hypothesized that by combining the advantages of autologous bone grafts with autologous platelets concentrate, better and prompt results in orthopedic trauma managements can be obtained. We also observe that the use of these bioactive factors to enhance skeletal repair/healing represents the future of skeletal trauma management.

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Keywords: Osteoporotic fracture; fragility fractures; bone grafting; platelets bioactivity; Non-unions; platelet rich plasma.

ABBREVIATIONS

OS: Osteoporosis; PRP: Platelet-rich plasma; PG: Platelet gel; PF-4: Platelet factor 4; β-TG: β-thromboglobulin; CD40L: Cluster of Differentiation 40 ligand; ICAM: IntercellularAdhesion Molecule; VCAM: Vascular cell adhesion molecule; PECAM: Platelet endothelial cell adhesion molecule; TXA2: Thromboxane A2; ADP: Adenosine diphosphate; PMMA: Plymethylmethacrylate ; FGF:Fibroblast growth factor; BMP: Bone morphogenetic protein; BICR: Bone-implant contact rates;ACD-A: Acid citrate dextrose (ACD-A), PRP_{LP}*: PRP with a lower platelet and white blood-cell number;* PRP_{HP}*: PRP with a higher platelet and white blood-cell number;* PRP_{DS} *: PRP double-spin; BMSCs: Bone marrow stromal cells; ALP: Alkaline phosphatase; BMD: Bone mineral density; PTH: Parathyroid hormone;PDGF:Platelet-derived growth factor; TGF: Transforming growth factor; PDAF: Platelet-derived angiogenesis factor; PDEGF: Platelet-derived endothelial growth factor; PGF: Platelet growth factors; VEGF:Vascular endothelial growth factor; EGF: Epidermal growth factor; RUNX2: Runt-related transcription factor 2; OPN: Osteopontin; OCN: osteocalcin; PPAR-g2: Peroxisome proliferator activated receptor gamma 2; BMPR-IB: bone morphogenetic protein type IB receptor;BMSCs: Bone marrow stromal cells.*

1. INTRODUCTION

Osteoporosis (OS) is the bone diseases that lead to an increased risk of fracture [1]. According to the World Health Organization, osteoporosis is second to cardiovascular disease, affecting more than 200 million individuals with a lifetime risk for women to have a fragility fracture about 30–40% worldwide [2]. Many factors have been associated with this impaired fracture healing, including fracture anatomic configuration, factors exacerbated by treatment, drug usage, patients characteristic and their co-morbidities [3]. Osteoporosis stands out as one of the most important variable in fracture healing because it is not only associated with delayed/non-union, but also with increase risk of fracture [4]. The osteoporotic bone fracture passes through the normal stages of fracture healing, although this process is prolonged [5]. The healing of femur in osteoporotic rat model showed 40% reduction of new bone formation (callus) in the cross-sectional area and 23% reduction in bone mineral density [6]. Similarly, Meyer et al. [7] demonstrated that the time required for fracture healing was longer in older rats and both stiffness and strength of healing bone remained below the value of controls. The impaired healing capacity associated with osteoporotic fracture is reflected by striking increase in the rate of implant fixation failure [8]. The possible explanation for this consequence is that one might be having fewer mesenchymal stem cells in osteoporotic individual, which may lead to lower proliferative response [9]. D'Ippolito et al. [10] showed that age-related decrease in osteoblastogenesis was responsible for complications like non-union,implant failure and reoperation,in the operative management of osteoporotic fractures [11-13]. Because of their associated morbidity, disability and diminished quality of life, osteoporosis are now becoming a major public health problem [14]. Conventionally, autogenous bone graft has been the gold standard treatment for delayed union and non-union [15]. The success rate of bone grafting in the management of nonunionis about 85%-90% which decreases further to 66% in cases of revision bone grafting [16-18].

Platelet-rich plasma (PRP)is an autologous blood product with a greater concentration of platelets than physiological whole blood.Platelets are the rich source of platelet derived growth factors, including transforming growth factor and vascular endothelial growth factor. On mixing PRP with thrombin and calcium chloride solution, results in polymerization of fibrin from fibrinogen, creating a platelet gel (PG). The platelet gel can then be applied to wounds or may be used as an adjunct to surgery, to promote hemostasis and accelerate fracture healing rate.

In this background, research is being conducted to improve the fate of surgeries by using proactive components released by the activated autologous platelets rich plasma in the management of osteoporotic fractures.

2. OSTEOPOROTIC FRACTURE AND ITS MANAGEMENT

2.1 Osteoporotic Fracture

Osteoporosis is a skeletal disorder characterized by compromised bone strength, predisposing to an increased risk of fracture [19]. In the clinical setting, osteoporosis can be defined as a reduction in bone mass of > 2.5 SD below the mean for a young adult [20-22]. According to Eastell et al. [23] more than 40% of women and 14% of men over the age of 50 years will experience osteoporotic fractures. The common osteoporotic fracture site was seen in men arise in the ribs, spine, and wrist, whereas in women the most frequent osteoporotic fracture sites include the spine, ribs, wrist, humerus and femur [24]. The rising incidence of proximal fracture of the femur in the elderly, represents the most significant socioeconomic impact of osteoporosis [25]. Globally, in 2000, there were approximately 424 000 hip fractures in men and 1 098 000 in women. Based on these altering demographics, it is estimated that by 2025 there will be 800 000 hip fractures in men per year, and in women the numbers will rise to 1.8 million [25].

2.2 Management of Osteoporosis Fracture

The main technical problem in osteoporotic fracture fixation is difficulty in obtaining secure fixation of an implant. Because of loss of cortical and cancellous bone, the strength of implant fixation is significantly reduced. Bone mineral density (BMD) of bone directly correlates with the holding power of screws linearly [26-27]. The load transmitted at the bone-implant interface can often reduce strain tolerance. This may result in resorption of the bone, microfracture and loosening of the implant, with secondary failure of fixation [28-32].

Because of high rates of these complications, extensive advanced research into the development of implants is needed. This resulted in the use of relative stability techniques such as bone impaction, buttress fixation, intramedullary nails, fixed-angle devices, bone augmentation and joint replacement [26]. These techniques are the most effective in reducing strain at the bone-implant interface. The Buttress-plate fixation of metaphyseal avoids high strain at a single screw while the implant allows for a larger contact area at the bone-implant interface, again reducing strain on osteroporotic bone [33]. Further the Fixed-angle devices are very useful because the blade has a large surface area to resist angular deformation, torsion and the strain [34]. Moreover, the fixed-angle implants has led to the development of screws with threaded holes incorporated directly into the plate, the so-called locking compression plate's [35-37]. Plates with locking-head screw's also produce a fixed-

angle device that leads to increase the holding power of an implant having a locked screw at multiple fixed angles [38].

The most appreciable thing about the locking-plate device is actively to like, mechanical coupling between the screw head and the plates so in case of screw-bone interface failure, the screw-plate interface remains intact. However complete failure of implant fixation is still possible as in severe osteoporosis, but requires all screws failures simultaneously. Therefore, implants such as the locking compression plate have significant advantages in osteoporotic bone [33]. Further, the bone impaction significantly reduces the risk of implant. The controlled impaction can be accomplished by tensioning internal fixation devices such as the dynamic hip screw, which permits controlled impaction of the fracture while preventing the penetration of the joint by the screw [39].

2.2.1 Current trends in management of osteoporotic fractures

Advanced pharmacological alternatives for treatment of osteoporosis include antiresorptive agents (eg, bisphosphonates, calcitonin, PTH and raloxifene) which reduced osteoclastic activity and teriparatide (1-34 PTH—parathyroid hormone fragment), the first pure anabolic agent, which stimulates bone turnover in a positive manner to increase bone mass [40-41]. Teriparatide is a recombinant human protein made up of the initial 34 amino acids of human parathyroid hormone (PTH). Endogenous PTH has a vital role in calcium and phosphate metabolism and homeostasis [42]. PTH stimulates osteoclastic activity by increasing the renal tubular calcium reabsorption and renal phosphate wasting. However, continuous low dose of PTH secretion alters its actions and stimulates osteoblastic activity. The extracellular calcium induced by the effects of PTH on renal and intestinal calcium transport provides a supply of mineral for osteoblastogenesis [43]. Accordingly, as estrogen deficiency, temporal sequence effects on the bone remodelling results in a net increase in bone formation [44-45].

Effects on bone mass and bone quality are equally important. Therefore, therapies used to reduce fracture rates should have beneficial effects on bone mass as well as its quality. One time, daily exposure to teriparatide results in new bone formation on trabecular and cortical bone surfaces. However, the continuous PTH administration may stimulate bone resorption rather than bone formation, especially at cortical sites than the trabecular bone surfaces. Furthermore, effects of teriparatide treatment not only increase trabecular thickness but also increase trabecular connectivity [46-47]. Teriparatide effects are mediated via interaction with a specific G-protein coupled receptor with ligand binding induces a cascade that activates cyclic AMP/protein kinase A and protein kinase C pathways [48]. But still, the precise cellular mechanisms that exert an anabolic effect in response to an anti-resorptive effect is not known. Thus, more evidence will be needed before its role can be expanded to first line agent for the treatment of osteoporosis.

Biological processes which enhance the healing potential of osteoporotic fractures should also be considered as an adjunct to surgery, especially the influence of coating of the implant on the bone-implant interface. Bone augmentation can be accomplished by using bone autograft or allograft, bone cement or bone substitutes for further better management in osteoporotic fracture's [49-51]. Further the screw fixation using polymethylmethacrylate (PMMA) having the best results [52-59].

However, the resorbable polymers be also used to provide the additional stability needed in osteoporotic fractures. Mainil-Varlet et al. [60] demonstrated that an intramedullary augmentation device made of poly (l-lactide) have the same resistance against pullout as

PMMA. Joint replacement is another option for osteoporotic patients with articular fractures, and some metaphyseal fractures, where internal fixation is inappropriate or the patient has pre-existing arthritis [61].

In most of the comparative studies with an osteoporotic fracture patient who received hydroxyapatite (HA) -coated external fixator pins or screw gets better result as compared to a plain one [62-64]. Tengvall et al. (2004) have shown that bisphosphonates engraved on stainless steel screws showed a 28% higher pullout force after being implanted for two weeks in rat tibiae [65]. Edward et al. (2013) recently demonstrated that calcitonin is effective for reducing pain from acute vertebral compression fractures in patients with osteoporosis, similar to pamidronate [66].

However recently the implant surfaces can also be used to deliver growth factors, such as transforming growth factor (TGF-β), bone morphogenetic protein (BMP-2, BMP-7) or fibroblast growth factor (FGF) locally to influence bone formation and perhaps improve implant fixation [67]. However, their benefit in osteoporosis remains to be seen.

Another more advance biotechnological approach would be use bone tissue-engineering using a suitable scaffold material and adult mesenchymal stem cells [68-69]. Significant number of research will be needed to develop this area into routine clinical practice. Finally, gene transfer techniques [70] can deliver genuinely processed gene expression products to exact anatomical locations at therapeutic levels for sustained periods. However, the selection of the gene or gene combinations, and safety issues with some other factors remains under consideration.

Nevertheless, Bone augmentation with the titanium-mesh (Ti-mesh) technique is susceptible to a large rate of complications such as morbidity of the bone graft donor site, and mesh exposed to the oral cavity. Torres et al. (2010) suggest that the positive effect of PRP on the Ti-mesh technique is due to its capacity to improve soft tissue healing, thereby protecting the mesh and graft material secured beneath the gingival tissues [71]. However, a recent study by Philipp et al. (2013) found no significant differences in the bone-implant contact rates (BICR) for roughened implant surfaces compared with machined surfaces on dogs. In this animal model, the addition of PRP did not demonstrate evidence of faster bone formation or the resulting BICR [72]. Thus, this shows that the use of PRP is still controversial and require further research.

3. PRP AND ITS USAGE

3.1 PRP's Contents

Platelets are small anucleated discoid blood cells of size, approximately 1–3 μm. The average range of platelet count is from 1.5 to 3.0 × 10^{-5}/ml in peripheral blood, with a half-life time of about 7 days. These are heterogeneous in size. The larger platelets from healthy volunteers are more active, releasing more chemokines than smaller platelets [13]. Platelets originate from megakaryocytes in bone marrow and finally squeezed out into the circulation. Platelets, around their periphery, bear a ring of contractile microtubules containing actin and myosin. Many intracellular structures are present inside the platelets i.e. glycogen, lysosomes, and two types of granules. The one called "dense granule organelles" of 250 to 300 nm in size, which contain ATP, ADP, serotonin, and calcium, [73] and the other called Platelet "alpha (α) granules", having 300- to 500-nm in size with a proteome count of

approximately 284. These contain growth factors, clotting factors, and other proteins [74]. The platelets lysosomes in some recent literature also denoted as, “lambda granule” whose contents are released during platelet activation. These lambda granules, have mainly "clearing" responsibilities against the infectious agents and cellular debris [75]. Platelets have extensively invaginated membrane with a complex canalicular system, through which, on activation, subsequent release of granule content occurs. This process termed as exocytosis and degranulation result in an overall increase of platelet surface area. ADP is a main mediator in platelet activation, whereas serotonin is a weak platelet agonist with vasoconstrictive potential [76-81]. The α granules primarily contain the pro inflammatory and immune-modulatory molecules like P-selectin [79, 82], Platelet factor 4 (PF-4), β-thromboglobulin (β-TG), Cluster of Differentiation 40 ligand (CD40L), and adhesion molecules like, Intercellular Adhesion Molecule (ICAM), Vascular cell adhesion molecule (VCAM) and Platelet endothelial cell adhesion molecule (PECAM) [76, 83-84]. The lysosomes contain clearing factors such as cathepsins, collagenase, and glycohydrolases [85].

Platelet can be activated *via* both native and exogenous molecules, including collagen, platelet-activating factor, calcium, serotonin, magnesium, thromboxane A2 (TXA2), adenosine diphosphate (ADP), adrenergic activity, oxidative stress, shear stress, physical as well as mental stress or chemical used, such as nicotine [86-90]. The activated platelets express various surface markers like; glycoprotein receptor GPIIb/IIIa, p-selectin and CD40 ligand and secretes many pro-inflammatory and immune-modulators from their storage granules [87]. This process of paracrine secretion is termed “platelet bioactivity” and enables platelets to crosstalk with other platelets, endothelial cells as well as immune cell’s [91-92]. Platelets are most often function as a hemostatic and coagulating agent; however, proteomics studies have demonstrated that platelets contain over 800 proteins with various post-translational modifications, like as phosphorylation, leading to over 1,500 protein-based bioactive factors [93-94]. On activation platelet get aggregated followed by their remarkable change in shape that gives platelets the ability to bind fibrinogen *via* surface glycoprotein GPIIb/IIIa receptors [90]. This surface expressed activation markers, promotes the circulation of soluble CD40L and soluble P-selectin. Molecules like CD40 and CD40L, act as an important immune-modulator, enhance antigen presentation and adaptive immune responses [95].CD40 and CD40L could determine the T-cell-dependent isotype switching of B-cell-produced antibodies and to heighten the dendritic cell activation process [96]. Further, the in vitro study by Getgood et al. (2011) have shown that platelets are activated with an initial burst of growth factors followed by a sustained release [97]. Platelet activation results in an increase in anti-inflammatory cytokines because of the presence of hepatocyte growth factors [98] . Thus, because of localized delivery of great variety of biologically active growth factors to the site of injury, platelets may be used as a therapeutic option in immunology as well as regenerative medicine.

3.1.1 Platelets derived growth factors

Platelets have been demonstrated as the source of several growth factors and cytokines, which not only promote blood coagulation, tissue repair and the process of bone mineralization but also improve fracture healing. Thus they help in decreasing the amount of healing time significantly [99-101]. Recently it has been proposed that the platelet rich plasma (PRP), an autologous platelet concentrate, have a potential to increase regeneration and wound healing [102-105]. PRP application has been demonstrated to increase the local platelet concentration by 338% and accordingly increase the concentration of local growth factors [106]. Activated platelets lead to the secretory expression of the alpha granules

known to contain multiple growth factors including; platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet-derived angiogenesis factor (PDAF), platelet-derived endothelial growth factor (PDEGF) and many others that modulate the bone regenerative process [105, 107-109]. These growth factors possess paracrine related properties, which are stimulatory for mitogenic activity, cell differentiation, protein transcription and chemotaxis [12].

3.2 PRP's Advantage in Usage in Different Fields

Application of PRP in different type of treatments shown promising effect. Since the first application of PRP was in the treatment of skin ulcers in 1980, after that a considerable number of novel applications in different fields of medicine have emerged i.e. in Musculoskeletal pathology [110-112]; Osteoarthritis [113-115]; Gynecology [116-118]; Cardiovascular Surgery [119]; General Surgery and Plastic Surgery [119-122]; Burns [123-125]; Diabetic Ulcers [126-128]; Ophthalmology [129-131]; Otolaryngology [132]; Dermatology and Cosmetic Surgery [133-134]; Peripheral Nerves [135-136] etc. Contempt of the majority of experimental and clinical studies about the usefulness of PRP in different areas of regenerative medicine, few therapeutic indications also shows its effectiveness. This fact demanding to carrying out methodologically appropriate clinical trials in the coming future in order to improve the evidence level of treatment with PRP.

4. PRP's POTENTIAL USAGE IN OSTEOPOROTIC FRACTURE TREATMENT AND BONE HEALING

4.1 PRP's Effects on BMSCs

Osteoporotic bone derived BMSCs show an altered epigenetic expression (i.e., higher adipogenetic tendency and lower osteogenesis capacity) [137]. The enhanced adipogenesis and inhibited osteogenesis of BMSCs are the main cause of delay in the healing of osteoporotic fractures. Currently bone resorption inhibitors are used in the treatment of osteoporotic fractures, but these agents cannot promote bone callus formation. Thus, by simultaneously promoting osteoblastogenetic differentiation and suppressing BMSC adipogenesis can enhance bone formation of osteoporotic fractures.

Platelet-derived growth factors (PDGF) are the key factor that can promote the migration and proliferation of BMSCs [138]. The medium-concentration of PRP stimulates BMSC proliferation and osteogenic differentiation [139]. However, according to Kawasumi et al. [140] BMSC proliferation and bone formation were more prevalent in the highest concentration of PRP (4.3×10^9/mL). Arpornmaeklong et al. [141] further demonstrated that PRP (3.5×10^9/mL) had a dose-dependent stimulation of BMSC proliferation. Medium-concentration of PRP ($2.65 \pm 0.2 \times 10^9$/mL) increases the osteogenetic differentiation as well as inhibiting the adipogenic differentiation of age BMSCs. However, high-concentration ($8.21 \pm 0.4 \times 10^9$/mL) and low-concentration of PRP ($0.85 \pm 0.16 \times 10^9$/mL) of PRP shows no capability in the mitogenic and osteoinductive stimulation of BMSCs.

4.2 PRP's Effects on Bone Healing

The bone healing process is a delicate balance between bone deposition, resorption, and remodeling [3,142-143]. The progression of fracture healing can be divided into following as originally described by McKibbin, [144] namely: hematoma formation, Inflammation,

formation of soft callus; formation of hard callus and finally the bone remodelling process. During bone healing, platelets (as same as mature osteoblastic cells) act as an exogenous source of growth factors stimulating the activity of bone cell's [145-147]. At the site of bone fracture, platelets release numerous growth factors like PDGF, TGF- β, platelet growth factors (PGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) etc. providing the optimum level of secreted growth factors to the injury site [148-149]. As bone and cartilage, platelets are also the richest source of TGF-β, having both isoform TGF-β1 and TGF-β2 in it. TGF-β1 has the greatest potential for bone repair and TGF-β may contribute to bone healing at all stage's [150-151]. The PDGF and TGF-β1 have been shown to promote the proliferation and differentiation of osteoblasts while TGF-β1 also has ability to inhibit the differentiation of adipocyte [152-156]. Platelet-rich plasma (PRP) was considered as a novel osteoinductive therapeutic approach for the treatment of complications of the bone healing process [157-159]. Some experiments showed that platelets in PRP are activated by bone substitution materials [160] and biphasic osteochondral scaffolds [105].

Several studies have investigated the effects of platelet concentration on musculoskeletal tissue homeostasis [161-163]. Serotonin has been both positively and negatively to regulate bone mass [164], whereas plasminogen activators play a role in fracture repair [165-166]. Recently, Malhotra et al. in a comparative study, demonstrated the beneficial effect of PRP when used in combination with osteoconductive scaffolds [167]. Fisher et al. also show promising effect of PRP in preclinical trials and some clinical trials [168]. Similarly, many of the growth factors that are released by platelets play an important role during the entire healing process, are listed in Table 1 [169-187].

4.2.1 PRP preparation, storage and its concentration for bone healing

According to Augustus et al. in their recent study described three methods to obtain PRP from whole blood [188]. The peripheral blood has firstly has drawn from the patients by using A 60-mL syringe prefilled with 5 mL of acid citrate dextrose (ACD-A). Further, depending upon PRP separation methods, PRP obtained by a single-spin method, can be obtained by low spin (PRP_{LP}) and another is high spin (PRP_{HP}). The PRP_{LP} have lower platelet and White blood concentration whereas PRP_{HP} have high platelet and White blood concentration. The double-spin method (PRP_{DS}) is widely used to represent an overall survey of the techniques clinically available. With regard to the total number of platelets by using different separation method, Augustus et al. find a significantly increased platelet number compared with native whole blood ($142.7 \pm 44.40 \times 10^3/\mu L$). The PRP_{HP} ($873.8 \pm 207.82 \times 10^3/\mu L$) also showed a significantly higher number of platelets compared with PRP_{LP} ($378.3 \pm 58.64 \times 10^3/\mu L$) or PRP_{DS} ($447.7 + 183.7 \times 10^3/\mu L$). No significant difference in platelet number was seen when PRP_{LP} was compared with PRP_{DS} ($p = 0.52$)

Alteration of platelet's functionality has been shown during their preparation and storage [189-190]. As suggested by Tynngard et al., it was demonstrated that the measurement of P-selectin membrane levels and quantification of growth factor release are reliable tools for the definition of the maximal storage duration of PRP [189]. In addition, platelet reactivity toward different agonists significantly decreases during storage of platelet concentration [191]. A study has shown that PRP can be stored for 3-hour at room temperature with no significant effect on effectiveness. The growth factor release was unaffected over a period of 6 h post purification [191-192]. However, Autologous PRP may be prepared in the operation theater itself and can be used immediately.

Table 1. Brief summary of the function of different growth factor released by platelets on bone physiology, healing as well as angiogenesis

Growth Factors released by platelets	Functions	References
Platelet-Derived Growth Factor	Mitogenetic for mesenchymal and osteoblastic cells; Osteoinductive; regulates collagenase secretion and collagen synthesis.	169-172
Transforming Growth Factor beta	Stimulates undifferentiated mesenchymal cell proliferation; regulates fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; stimulates endothelial chemotaxis and angiogenesis.	169, 171-173
Fibroblast Growth Factor	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenetic effect on mesenchymal, chondrocytes and osteoblasts cells.	174-175
Insulin-Like Growth Factor 1	Play a role in bone remodelling and mineralization.	176-177
Insulin-Like Growth Factor 2	Stimulates proliferation of osteoblast-like cells; promote collagenous protein synthesis.	178-179
Vascular Endothelial Growth Factor	Increases angiogenesis and vessel permeability, stimulates mitogenesis of endothelial cells.	180-181
Epidermal Growth Factor	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis.	182-183
Interleukin 8	Promotes osteoclast formation as well as angiogenesis.	184-185
Connective Tissue Growth Factor	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion.	186-187

Till date, standard concentration of PRP for the optimal bone healing is not defined. According to Huang and Wang [193] conducted a study in ovariectomized mouse model with induced osteoporosis and reported that medium-concentration PRP ($2.65\pm0.2 \times 10^9$/mL) stimulates BMSC proliferation and osteogenic differentiation. Kawasumi et al. [140] reported that BMSC proliferation and bone formation were more prevalent in the highest concentration of PRP (4.3×10^9/mL). Arpornmaeklong et al. [141] reported that PRP (3.5×10^9/ml) had a dose-dependent stimulation of the BMSC proliferation while reducing ALP activity and calcium deposition. Chen et al. in a study have been demonstrated PRPs were capable of up-regulating the proliferation of aged BMSCs. Medium-concentration PRP (2.65±/mL) promotes osteogenetic differentiation moreover,inhibits the adipogenic differentiation of aged BMSCs. However, high-concentration PRP ($8.21\pm0.4 \times 10^9$ /mL) inhibited osteogenetic BMSC differentiation. Low-concentration PRP ($0.85\pm0.16 \times 10^9$ /mL) and PPP ($8\pm0.5 \times 10^6$ /mL) show no capability in the mitogenic and osteoinductive stimulation of BMSCs [194].

4.2.2 How to use PRP in vertebral collapse

PRP in vertebral collapse is not used widely but with the development of PG, PRP can be used in vertebral collapse at the time of vertebroplasty. Which can be done by adopting the

transpedicular root. The literature had not shown any study in which PRP has been used for this problem.

4.3 PRP-added Graft

A good bone graft material should retain all properties like osteogenic, osteoconductive, or osteoinductive properties. Bone grafts are time tested and are considered to achieve better bone regeneration and strength. There is no question related to the biological properties of autologous bone graft. But still, there are chances of delayed or non union even after bone grafting, which may be due to failure of achieving the desired environmental condition at the local fracture site by these grafts. This fact is a matter of great concern amongst all researchers.

Synthetic bone grafts have been considered as a potential alternative to the conventional use of bone grafts, due to their unlimited supply and as they carry no or limited risk of any disease transmission. The bone graft engineering practices have their own limitations or challenges. Though, bone tissue engineering aims to induce new functional bone regeneration via utilization of different synthetic biomaterial but still they are not clinically approved.

Currently a lot of studies are revealing the significance of platelet rich concentrates in clinical field especially in maxillofacial and dental surgery. The most appropriate reason behind its wide use is the optimum availability of numerous bioactive materials in this type of graft that accelerate musculoskeletal tissue regeneration and angiogenesis and thus bone healing as well. So the question arises that if these platelet's concentrates are being used in the field of dentistry for many years, why it's not being used for the orthopaedic trauma management? However, few studies regarding clinical application of PRP have been considered as a breakthrough in the stimulation and acceleration of bone and soft tissue healing. Many researchers have been observed that PRP may increase the success rate of bone grafting in the management of non-unions [99-105]. Further, the PRP enriched graft is a good source of various growth factors that secreted uniformly at the fracture site to provide a longer optimum environment that promote healing naturally [105-109,195]. It has also been documented that these grafts are devoid of any immunological or pathological consequence due to their autologous nature [196] and it is documented that bone fractures may seem to heal faster, stronger and better than bones treated with conventional bone grafting [99-105]. It has been found that these grafts are cost effective as they are easy to obtain and of lower cost than the recombinant grafts [197]. Therefore, according to several conducted studies, it may be now concluded that PRP is more than just platelets, and depending on the specific constituents of a PRP preparation, their clinical use can be theoretically matched to the pathology being treated.

As platelets concentrate may provide approximately all the bioactive agents, lack of which results in failure of the orthopaedic management (i.e. resulting in delayed or non-union). Though platelets can be used along with synthetic bioactive material, but, we hypothesize the combining of the advantages of autologous bone grafts with autologous platelets concentrate to obtain better and prompt results in orthopaedic trauma managements especially in fragility fractures.

Thus, mixing of PRP with bone graft materials might create a novel bone graft that is enriched with a high concentration of platelets, releasing various bioactive growth factors

and accelerating bone healing. The viscous nature of activated PRP, which is also called as plasma gel (PG), will stick the bone graft chips together, thus, preventing the bone graft particle migration. So in this way, it may be a promising technique that could support and encourage bone growth and accelerate fracture healing, particularly in patients' who are at risk of non-unions in fractures associated with osteoporosis.

4.4 PRP's Effects on Osteoporotic Fracture Healing

The osteoporotic fracture healing undergoes the same stages of healing as described by Lopez et al., (2003) except that, they are slow in progress and takes a longer period of healing [5]. There is few evidence published regarding the role of PRP in osteoporotic fractures in special relation with different growth factors (TGF, FGF, VEGF, EGF etc.) secreted by platelets [198-206]. The optimum concentration of these growth factors, secreted by platelets at fracture site not only enhances the rate of progression of healing but also improves quality of new bone formation.

Hen-Yu et al. (2011) showed the therapeutic role of PRP in osteoporosis and also provided the evidence that the PRP not only inhibit the maturation of pre-adipocytes (3T3-L1) into adipocyte but also promotes osteogenesis [207]. Muruganandan et al. observed that PRP-induced osteogenesis in osteoporotic fractures was achieved by simultaneously up-regulating osteogenesis-promoting genes RUNX2, OPN and OCN while downregulating adipogenesis regulators such as PPAR-g2 and leptin. They also concluded that PRP treatment enhanced BMP-2 and BMPR-IB and suppressed BMPR-IA pathways in pre-adipocytes [208]. In addition to these studies, more researchers observed the same and concluded that the transdifferentiation of adipocytes to osteoblasts were possible without genetic manipulation [207, 209-210].

5. ARENAS FOR FUTURE RESEARCH

The composition of PRP varies from patient to patient and may also vary with the methods of preparation. Also the method of storage and interaction with other biologics or materials may alter their functionality. Platelet rich plasma may enhance soft-tissue repair, especially for tendons, although it may inhibit bone formation [211]. Some physicians use PRP as a way to provide or promote growth factors and cytokines during tissue repair. PRP can lead to fibrous connective tissue and scar formation. Additionally, PRP is not osteoinductive. The American Academy of Orthopaedic Surgeons (2011) concluded that 'PRP is an option that yet remains unproven' [212]. Griffin et al.,reviewed Cochrane Central Register of Controlled Trials (The Cochrane Library, 2011 Issue 4), MEDLINE (1948 - 2011) and EMBASE (1980 - 2011) to assess the effects of PRP for healing long bone osteotomies, acute fractures, un-united fractures and defects in adults. They concluded that the potential benefit of platelet-rich therapies to augment long bone healing in adults cannot be justified and the currently available evidence from a single trial is insufficient to support the routine use of this intervention in clinical practice [213]. Therefore, use of PRP still blurred by controversial results from different studies, and a definite direction remains subtle.

6. SAFETY ISSUES

While numerous studies on the clinical applications of these grafts have been done, but knowledge about the fundamental effects of PRP at the cellular level remains uncertain. So the following safety issues must be kept in mind during the preparation of PRP and its uses,

i.e. PRP should be prepared by the method approved by the U.S. Food and Drug Administration [214]; Patients who are considered to be candidates for a PRP application must undergo a hematological evaluation[105]; All those patients who have history of immunologic disorders or blood/platelet dysfunction's, must be excluded [215]; Instead of bovine thrombin autologous thrombin should be used to activate PRP [216]; Optimum concentration of platelets should be used (>200,000 platelets//μL meets the Red Cross definition of PRP), to achieve the maximum positive effect of the PRP application [108, 217-220]; Efforts should also be focused on characterization of other components of PRP too, particularly leukocytes and fibrinogen [155]. This may bring more uniformity to the PRP, improves its specificity and bioactivity and maximize their positive clinical outcomes [220]; PRP studies should be properly documented, for the reasons like- to understand the role of platelets or other components in PRP, to further evaluate why it is or is not so efficacious, which type of patients will receive the most benefit, at what concentrations of PRP was used and similarly explore many other quarries.

7. CONCLUSIONS

At present, the molecular mechanisms of bone trauma repair studies had focused on three aspects i.e. inflammatory cytokines, growth factors and angiogenic factor. According to several studies, it has now been confirmed that the PRP works mainly via all these three aspects of bone repair.Platelets are unique blood elements, enriched with enormous valuable growth factors that initiates hemostasis and promote healing processes. PRP having high concentration of platelets, which can be activated to form a Platelet gel (PG), can be used for therapeutic use. Several data from different studies demonstrated the role of PRP in tissue regenerative processes. The authors do acknowledge that the mechanisms by which these combinations would work have not yet been established. We observe that their use, however, must be approached with caution and ultimately should be based on evidence-based medicine as level-I randomized controlled trials

8. FUTURE IMPLICATIONS

Autologous PRP aided bone grafts hold the key of future research in the field of regenerative medicine. The current authors have recently begun using a combination of autologous iliac bone grafts and PRP in the surgical treatment of benign osteolytic lesions. Combining two separate biologic is theoretically beneficial as the addition of growth factors through PRP could increase the differentiating potential of the pluripotent mesenchymal cells in bone grafts. We observe that the beneficial effects observed by us open a window for multicentric evidence based trials in the field.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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