Journal of
Tissue Science & Engineering
ISSN: 2157-7552

Mahakalkar et al., J Tissue Sci Eng 2017, 8:3
DOI: 10.4172/2157-7552.1000208

Review Article Open Access

Bio-Engineering of Wounds by PRP Led Regeneration

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Abstract

Platelet rich plasma (PRP) therapy is one of the biological interventions in regenerative medicine with a sure success in clinical translations of wound healing. Platelet rich plasma is an autologous plasma fraction of peripheral blood and it's the simplest intervention of regenerative medicine. It is rapidly extending to multiple clinical fields because of its easy use and biosafety which facilitates translation in humans. The biggest advantage over other therapies is it's being autologous, no adverse events or reactions are encountered. The economic as well as social burden caused due to chronic and degenerative disease is well cared by this autologous therapy. The clinical applications have proven its efficacy and efficiency in healing all types of wounds. The microscopic and histopathological changes in the tissues after PRP therapy are described in this article. The changes in the tissues have evoked the transformation from fibrosis led healing to collagen led healing; a bioengineered mechanism of PRP led regeneration of wounds.

Keywords: Platelet rich plasma; Translation; Wounds

Introduction

The bio-technical intervention with the Regenerative Medicine is getting evolved. Recent advances in biotechnology and understanding the mechanisms of angiogenesis, inflammation and cellular activities such as proliferation, differentiation and metabolism have promoted researches and to generate new knowledge on how to manipulate these aspects at tissue and cellular level. This knowledge has been translated into the creation of regenerative medicine technologies which are imperative in order to address the current health care needs for various demographic changes. Recent increase in the economic and/or social burden of chronic and degenerative diseases has promoted the developments of novel therapies.

The biomaterials play an important role in biomedical devices and hence in tissue engineering, this helps regenerative potential to develop tissues and organs to restore normal body function. The basic understanding of the mechanisms of regenerative biomaterials at the molecular levels and their roles in the formation of newer tissues potentiates the newer fields in the domain of regenerative medicine and its further applications.

The use of PRP has been mentioned in many fields in the literature. Ferrari in 1987 used PRP for the first time after an open heart operation, as an autologous transfusion component to avoid homologous blood products transfusion. By now there are 18313 entries in NCBI for its uses in medical fields at various sites and tissues like orthopaedics, sports medicine, otolaryngology, neurosurgery, dentistry, urology, ophthalmology, wound healing, cosmetic surgery, maxillofacial surgery and cardiothoracic [1]. The advances in the field of medicine have led to many scientific researches and technology has built a strong platform for development of newer perspectives of PRP. The efficacy of PRP is mainly because of various growth factors and cytokines released by the platelets. These growth factors and cytokines affect and control variety of mechanisms like inflammation and infection, wound and muscle injury and soft tissue healing and also osteogenesis. The Platelets are also mediating the healing and repairing mechanism by attracting macrophages and osteoblasts and mesenchymal stem cells promoting degradation of necrosis and promoting a suitable environment for healing by regeneration of all the surrounding tissues [2].

The present literature suggest its use for tendinopathies [3], ligament sprains [4], muscle strains [5], osteoarthrosis [6], intervertebral discs entrapment neuropathies [7], fracture non-union [8].

The effective management of complex wounds remains a huge challenge for mankind. Throughout the world the wounds/ulcers are managed with lots of variations and uncertainties, by medical personnel .Many of these wounds are associated with loss of skin. Such loss of skin could be primary due to severe trauma or secondary due to necrosis or infections. Many of these injuries or wounds remain unhealed and become chronic non healing type of wounds because of various comorbidities like elderly age, active infections, other associated diseases like diabetes, neuropathies, bedsores and many more. Such chronic wound poses a huge financial and mental burden over the society in terms of hospital stay, cost of treatment, dressing materials and deformities resulting in compromised and dependent lifestyle. Such treatments comprised of local care of wounds initially by cleaning and debridement of wounds, subsequently by regular dressings or by assisted application of vacuum devices, to shrink and drain wounds. Along with this, variety of drugs likes antibiotics and analgesics. But the overall treatment of such wounds are quite challenging because of repeated debridements, unhealthy granulation tissues, spreading necrosis and exposed muscles, tendons and bones.

PRP Biotechnology

The various Biological intervention in the domain of Regenerative Medicine fall into four main categories - Gene Therapy, Tissue

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Received September 18, 2017; **Accepted** October 06, 2017; **Published** October 15, 2017

Citation: Mahakalkar C, Shrivastava S, Gupta A, Naik S, Kaple M, et al. (2017) Bio-Engineering of Wounds by PRP Led Regeneration. J Tissue Sci Eng 8: 208. doi: 10.4172/2157-7552.1000208

Engineering, Cell-based therapies, and Platelet rich plasma therapies. All these four interventions are demonstrating different outcomes in different clinical translations. Tissue engineering uses cells loaded within scaffolds, but still is in the development phases and has several limitations in terms of 3D tissue constructs. The unresolved issues of Tissue engineering are biocompatibility, improvements in mechanical properties and the size of the 3D constructs. In the similar way, cell therapies use cellular components like mesenchymal stem cells (MSCs), embryonic stem cells or induced pluripotent stem cells (iPSC). The use of mesenchymal stem cells derived from the bone marrow or from adipose tissue in the clinical trials seems to be promising, whereas induced pluripotent stem cells therapies are advancing at a slower pace because of serious concerns about safety due to genetic instability and its potential to form tumors [9].

PRP, an autologous plasma fraction of peripheral blood, is the simplest regenerative medicine intervention that is rapidly extending to multiple medical fields mainly due to the easy use and biosafety that facilitates translation in humans. In contrast to cell therapies where many preclinical experiments involved in demonstrating its safety and non-teratogenic efficacy, PRP therapies involve minimal manipulations being autologous in nature; hence GLP and regulatory compliances are easily met for its clinical uses and applications [10]. It is easy to prepare even at the site of primary care facility with minimal resources and with simpler technology. The resources required are the patient's blood, disposable syringes, test tubes and centrifuge equipment.

Mechanism of Action of Platelet Rich Plasma

PRP acts as a tissue sealant and a drug delivery system [11], by its action of releasing locally acting growth factors [12,13] via α-granules degranulation [13].

The variety of secretory proteins within α-granules of platelets are platelet-derived growth factor AA, BB and AB isomers (PDGF) [13], transforming growth factor- β (TGF-β) [13], platelet factor 4 (PF4) [13], interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF) [13], vascular endothelial growth factor (VEGF) [14], epidermal growth factor (EGF) [15], platelet-derived endothelial growth factor (PDEGF) [16], epithelial cell growth factor (ECGF), insulin like growth factor (IGF) [17], osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1) [18].

These growth factors help healing by attracting undifferentiated cells in the newly formed matrix and triggers cell division [19]. Platelet rich plasma may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration [19] and promotes new capillary growth, angiogenesis [20] and accelerates epithelialization [12] in chronic wounds.

PRP also play an important role in host defence mechanism at the site of wound by producing signalling proteins that attract macrophages [21]; PRP also may contain a small number of leukocytes [22] that synthesize interleukins as part of a non-specific immune response. Previous studies of PRP have demonstrated its effectivity as antimicrobial against *Escherichia coli*, *Staphylococcus aureus* [23,24], including methicillin-resistant *Staphylococcus aureus* [23], *Candida albicans* [24], and *Cryptococcus neoformans* [24]. This suggests its role as antimicrobial and has the potential to eradicate the infective pathogens from the site and boost the healing process. This justifies its use as pharmacological therapy in wound healing.

Platelet releases has been used in the treatment of wounds since 1985. Platelet rich plasma also serves as a growth factor agonist and has both important properties for healing mitogenic and chemotactic. Platelet rich plasma contains high levels of platelets and a full complement of clotting and growth factors [25]. All these regenerative events form different layers of biological control that PRP can influence for healing wounds.

Histopathological Changes Induced by PRP

We have studied the histopathological changes including gross and microscopic features of the granulation tissues developed after PRP infiltration as per STARS, i.e., Sandeep's Technique for assisted regeneration of skin [26]. This technique and the protocol utilizes exclusively PRP as mono therapy for tissue regeneration in wound healing along with sit normal saline coverage of wounds.

The clinical progression of the wound healing was observed in 100 consecutive cases treated by STARS protocol. In addition, from few randomly selected wounds the peripheral epithelial tissues were biopsied using punch biopsy forceps and were sent to histopathological examination on the same day, preserved in normal saline. The biopsies were taken at 0 day, 4 day, 7 day, 21 day and finally when wound showed clinical healing. A typical pattern of healing took place which is staged as following.

Stage of suppression of unhealthy tissue

This was the first effect of PRP on the wound behavior. The unhealthy tissues removed gradually by the microscopic environments. This change was seen within 3-7 days of the PRP therapy. It continued concurrently in the unhealthy tissues, as the healing of healthier tissue progressed further. The infection control was also achieved. In majority of wounds which were initially infected, the progressive culture reports revealed no growth. Clinically no need to perform debridements or surgical interventions to remove any such unhealthy/dead/necrotic tissue from the wounds, was felt (Figure 1).

Stage of defect filling with health granulation tissue

The wounds further regenerated with healthy red granulation tissue covering and filling the defects. In most of the wounds healthy granulation tissue appeared with 7-10 days. Gradually depending

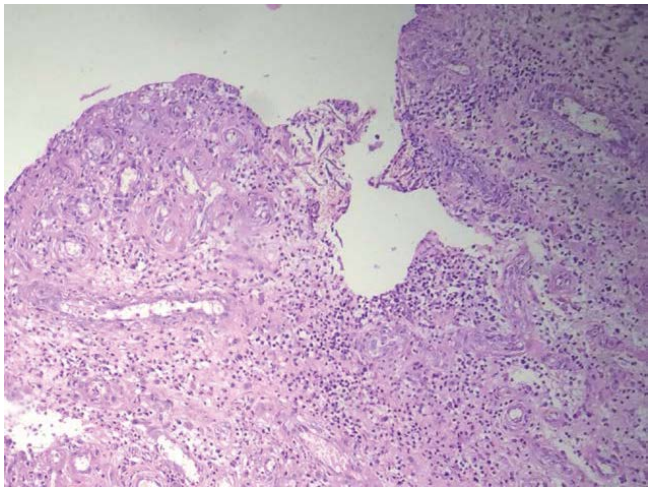

Figure 1: Histopathological changes after 48 h, showing increased phagocyte concentration.

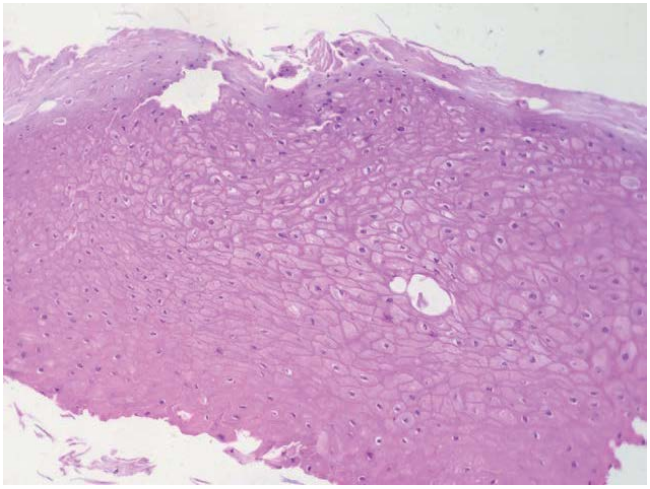

Figure 2: Histopathological changes after 10 day, showing defect filling with angiogenesis across the tissues.

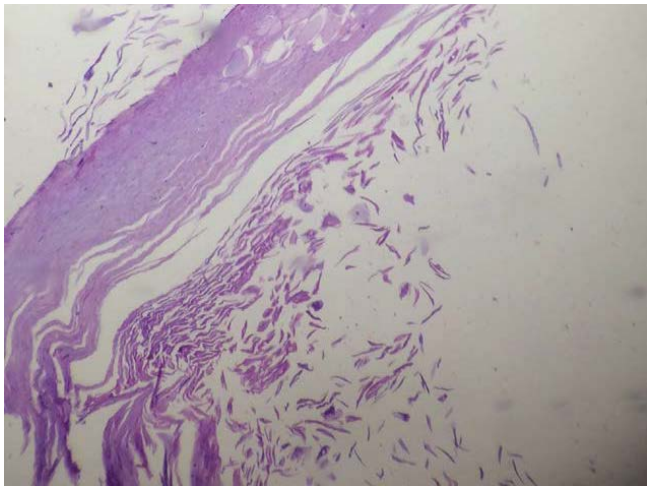

Figure 3: Histopathological changes after 21 day, showing collagen led healing.

on the size/depth of wound, it filled up with the defects and levelled. This granulation tissue eventually covered the underlying exposed bones and tendons. The rate of granulation coverage of tendons was slower than other soft issue and bone was the last tissue to be filled up/covered by it. Certain variations are noted during this stage and are still under investigations. The granulation is the clinical appearance of the neovascularized tissue, which is engineered by the different growth factors such as VGF present in the PRP through angiogenesis. The repeated dosage of PRP in the Protocol triggers and helps to maintain these factors inducing angiogenesis in all the tissues. It affects the whole tissue simultaneously and boosts the angiogenesis across all the tissues. Though it appears similar to natural healing but differs hugely in up scaling the potential of angiogenesis across the damaged tissue. This change in microscopic environment aids in the regenerative process tremendously. It further averts the threat of exposed tissues such as tendons and bones to necrosis. This change induced by PRP is a huge step forward towards regeneration and bioengineering of the tissues in the wounds (Figure 2).

Stage of epithelization progressing from the margins

This was an overlapping stage with the previous one. The healthy granulation filled up the floor and the defects. It leveled the wound margins and the epithelization progressed gradually from all the sides to the center of wound that has resulted in shrinking the effective size of the wound. This neo-regeneration happens almost at an average rate of 0.5 to 0.75 mm per day.

Stage of maturation of the epithelization

This is the last stage and finally led to complete healing of wound with coverage of assisted (by periodic PRP infiltration) regenerated skin. The neo-epithelized skin gradually matured to full thickness skin properties. The characteristics of matured epithelized tissue matched grossly with the local surrounding skin, including texture, elasticity and thickness. There was minimal of cicatrisation and residual contracture. The final tissue filled up defects showed the healing to be Collagen led and not fibrosis led. This is again a huge difference between PRP engineered tissue and natural healing. And a step ahead for painless healing as compared to the fibrotic tissue leading to painful stimuli unlike a collagen tissues (Figures 3 and 4).

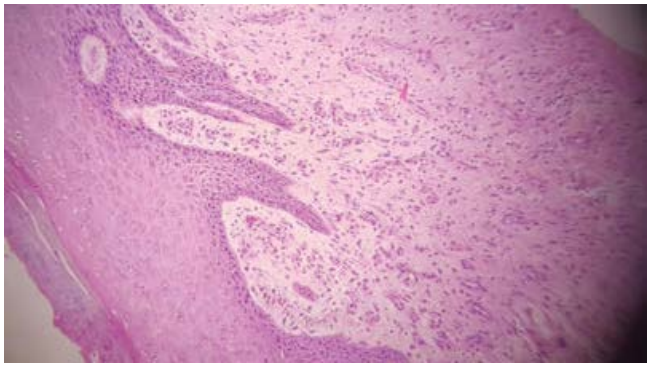

Figure 4: Histopathological changes when full thickness tissue is regenerated.

Perspectives of PRP Uses

In the last decade the PRP has been developed as “local Gel” and was used as an adjunct therapy for wound treatments and management including the treatment of chronic skin and soft tissue ulcerations [25]. PRP is being used to treat non-healing and chronic wounds for about more than 2 decades. One of the first clinical applications of platelets outside the blood stream, with healing purposes was in the treatment of chronic leg ulcers. Theoretically PRP stimulates healing in non-healing wounds, with the ultimate goal to reactivate healing. The rapid formation of granulation tissue heals the wounds fast and further prevents deeper tissue involvement and other associated co-morbidities.

Complex non-healing wounds present with multiple etiologies like pressure ulcers, diabetes, venous or arterial ulcers or due to surgical trauma. Impaired or delayed wound healing is one of the major complications resulting in the development of chronic wounds, leading to amputations of the extremities as that occurs in the diabetic foot. Such wounds can be treated with PRP, the studies are under process and the results are quiet promising. One of such case is reported using STARS therapy in a young female with Juvenile Diabetic presenting with non-healing infected ulcers on both the heels since last 3 years. The patient was administered PRP by STARS therapy. One of two heel ulcers healed completely and second ulcer healed almost 40%. This case has demonstrated enormous effect of PRP

infiltration resulting in a complete healing of non-healing diabetic ulcer without surgery, drugs and intense dressing, in an uncontrolled diabetic patient. STARS therapy led by Platelet regeneration, is an innovative protocol for such cases [27].

In addition many other publications also document its use in repair of other tissues such as maxillofacial surgery periodontal and oral surgery, cosmetic and plastic surgery, orthopedic and trauma surgery, spinal surgery, heart bypass surgery, and burns and also for tendinopathies, ligament sprains, muscle strains, osteoarthrosis, intervertebral discs entrapment neuropathies, fracture non-Union.

References

1. Ferrari M, Zia S, Valbonesi M, Henriquet F, Venere G, Spagnolo S, et al. (1987) A new technique for hemodilution, preparation of autologous platelet-rich plasma and intraoperative blood salvage in cardiac surgery. Int J Artif Organs 10: 47-50.
2. Mishra A, Pavelko T (2006) Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 34: 1774-1778.
3. Barrett S, Erredge S (2004) Growth factors for chronic plantar fasciitis. Podiatry Today 17: 37-42.
4. Murray MM, Spindler KP, Ballard P, Welch TP, Zurakowski D, et al. (2007) Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. J Orthop Res 25: 1007-1017.
5. Sánchez M, Anitua E, Andia I (2005) Application of autologous growth factors on skeletal muscle healing. Presented at 2nd World Congress on Regenerative Medicine, Liepzig, Germany.
6. Akeda K, An HS, Okuma M, Attawia M, Miyamoto K, et al. (2006) Platelet rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthritis Cartilage 14: 1272-1280.
7. Mulvaney S (2010) Treatment of peripheral nerve entrapments with real time ultrasound guided percutaneous hydro-neurolysis. Presented at AMSSM annual meeting.
8. Calori (2008) Injury. 39: 1391-1394.
9. Oerlemans AJM, van Hoeck MEC, van Leeuwen E, Dekkers WJM (2014) Hype and expectations in tissue engineering. Reg Med 9: 113-122.
10. Närhi MO, Nordström K (2014) Regulation of cell based therapeutic products intended for human applications in the EU. Reg Med 9: 327-351.
11. Marx RE (2004) Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 62: 489-496.
12. Pietramaggiori G, Kaipainen A, Czeczuga JM, Wagner CT, Orgill DP (2006) Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds.Wound Repair Regen 14: 573-580.
13. Gonshor A (2002) Technique for producing plateletrich plasma and platelet concentrate: Background and process. Int J Periodontics Restorative Dent 22: 547-557.
14. Weibrich G, Kleis WK, Kunz-Kostomanolakis M, Loos AH, Wagner W (2001) Correlation of platelet concentration in platelet-rich plasma to the extraction method, age, sex, and platelet count of the donor. Int J Oral Maxillofac Implants 16: 693-699.
15. McAleer JP, Sharma S, Kaplan EM, Persich G (2006) Use of autologous platelet concentrate in a nonhealing lower extremity wound. Adv Skin Wound Care 19: 354-363.
16. Steed DL, Goslen JB, Holloway GA, Malone JM, Bunt TJ, et al. (1992) Randomized prospective double-blind trial in healing chronic diabetic foot ulcers. CT-102 activated platelet supernatant, topical versus placebo. Diabetes Care 15: 1598-1604.
17. Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, KrolW, et al. (2007) Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: An *in vitro* study. J Bone Joint Surg Br 89: 417-420.
18. Tang YQ, Yeaman MR, Selsted ME (2002) Antimicrobial peptides from human platelets. Infect Immun 70: 6524-6533.
19. Mishra A, Woodall J Jr, Vieira A (2009) Treatment of tendon and muscle using platelet-rich plasma. Clin Sports Med 28: 113-125.
20. Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA (2001) Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. Diabetes Care 24: 483-488.
21. Lindeboom JA, Mathura KR, Aartman IH, Kroon FH, Milstein DM, et al. (2007) Influence of the application of platelet-enriched plasma in oral mucosal wound healing. Clin Oral Implants Res 18: 133-139.
22. Wrotniak M, Bielecki T, Gazdzik TS (2007) Current opinion about using the platelet-rich gel in orthopaedics and trauma surgery. Ortop Traumatol Rehabil 9: 227-238.
23. Crovetti G, Martinelli G, Issi M, Barone M, Guizzardi M, et al. (2004) Platelet gel for healing cutaneous chronic wounds. Transfus Apher Sci 30: 145-151.
24. O'Connell SM, Impeduglia T, Hessler K, Wang XJ, Carroll RJ, et al. (2008) Autologous platelet-rich fibrin matrix as cell therapy in the healing of chronic lower-extremity ulcers. Wound Repair Regen 16: 749-756.
25. Lacci KM, Dardik A (2010) Platelet-rich plasma: Support for its use in wound healing. Yale J Biol Med 83: 1-9.
26. Shrivastava S, Singh PK, Taywade S (2016) STARS therapy: Sandeep's technique for assisted regeneration of skin. J Orthop Allied Sci 4: 5-7.
27. Shrivastava S, Mahakalkar C, Singh P, Chandak A, Tayde S (2016) Platelet rich plasma as a mono therapy for diabetic ulcer. J Tissue Sci Eng 7: 186.