Acknowledgments

First and foremost, I thank Allah for all the blessings with which he surrounded me and the strength he gave me to achieve success. I would like to acknowledge and extend my heartfelt gratitude to my supervisors Prof Ahmed Emad Eldin Rady and Dr Mohamed Ahmed Mashhour for their supervision and constant support. Their invaluable help of constructive comments and suggestions throughout this works have contributed to the success of this research.

Sincere thanks to my parents, my brother, sisters for their encouragement and support. I would like also to thank my wife for being there for me in my worst moments. I could not have done it without you.

Thank you very much.

List of contents

List of figures

List of abbreviations

ABIs : Autologous blood injections

ACS: Autologous conditioned serum

ACP: Autologous conditioned plasma

BMC: Bone marrow concentrate

BMP: bone morphogenic protein

b-FGF: basic fibroblast growth factor

BTE: bone tissue engineering

BRONJ: bisphosphonate-related osteonecrosis of the jaw

BMSC: Bone marrow mesenchymal stem cells

CAMs: Cell adhesion molecules

EGF: epidermal growth factor

GTR: guided tissue regeneration

HA: hyaluronic acid

HWHA: high molecular weight HA

IGF-1: Insulin-like growth factor

LWHA: low molecular weight HA

MSC: Mesenchymal stem cell

OA: osteoarthritis

PDGF: Platelet-derived growth factor

PPP: Platelet poor plasma

PG: platelet gel

PRFM: Platelet Rich Fibrin Matrix

PRP: Platelet rich plasma.

TGF: Transforming growth factor

TCP: tricalcium phosphate

TKA: total knee arthroplasty

TNFα: tumor necrosis factor alpha

VEGF: Vascular endothelial growth factor

Introduction

Platelet rich plasma (PRP) is an autologous concentrate of human platelets in a small volume of plasma, containing biologically active factors, responsible for haemostasis, synthesis of new connective tissue, and revascularization.[1] Autologous PRP has been shown to be safe and effective in promoting the natural processes of wound healing, soft tissue reconstruction, and bone reconstruction and augmentation.[2]

PRP technology represents an advanced regenerative therapy for acute and chronic injuries. It is commonly used for the repair, reconstruction, or supplementation of a recipient's tissues.[3] The supraphysiological concentration of platelets will provide a locally increased concentration of growth factors and cytokines that are contained within the platelets themselves.[4-6] A local injection of PRP is therefore believed to work by releasing growth factors and cytokines that recruit reparative cells and enhance the healing process at the injection site.[7–9]

The first descriptions of the development and use of PRP were in the early 1990s, when science was focused on developing new "biological glues." PRP preparations were potent glues because of their high proportions of fibrin, and they were primarily used in maxillofacial surgery. Surgeons and scientists observed that these preparations had bone-forming properties, as well as anti-inflammatory and antibacterial effects.[10, 11]

PRP has an extremely broad range of clinical healing applications in head and neck surgery, otolaryngology, cardiovascular surgery, burns and wound healing, oral and maxillofacial surgery, cosmetic surgery, and periodontics. In addition to its effectiveness for patients with chronic non-healing wounds, it has also been used as an antiangiogenic agent and as a carrier for growth factors.[2]

Because of its autogenous origin, easy preparation, and excellent safety profile, the advent of PRP has opened another therapeutic door for orthopaedic surgeons and sports medicine physicians.[12]

PRP has been used with increasing frequency in the musculoskeletal disorders. It has been used to enhance the healing of meniscus defects and muscle injuries, stimulate chondrocytes to engineer cartilaginous tissue, reduce pain and produce better and more balanced synovial fluid in arthritic knees, improve outcomes after total knee arthroplasty and subacromial decompression, accelerate bone formation, stimulate the healing of anterior cruciate ligament injury central defects, its primary repair or its reconstruction', improve the outcome of operated ruptured Achilles tendons, reduce pain in chronic tendinopathies, and prevent and reverse inter-vertebral disc degeneration.[13]

Since platelets are the main regulators of the inflammatory phase and play an essential role in the proliferation and differentiation phase, scientists have proposed the delivery of a concentrate of platelets at the site of the injury as a successful strategy for fostering the regeneration pathway during bone wound healing.[14]

PRP is prepared by withdrawal of patients' peripheral blood and centrifugation to obtain a highly concentrated sample of platelets, which undergo degranulation to release growth factors with healing properties. It also contains plasma, cytokines, thrombin, and other growth factors that are implicated in wound healing and have inherent biological and adhesive properties. The prepared concentrate is then injected back into the patient at the site of morbidity. This may be intralesional, intra-articular, or surrounding the involved tissue bed.[12]

The growing interest in the use of PRP to optimize the healing response of tissues has sparked the development and marketing of a plethora of commercial procedures that are designed to concentrate platelets and suspend them in plasma or a fibrin construct of varying densities.[12,18,19] Platelet count in PRP may vary according to the preparation technique, ranging from two to several folds above the physiological levels.[2] At least 16 commercial PRP preparation systems are currently available.[21]

For clinicians to fully understand the role of PRP, it is imperative that they have a general understanding of the body's healing process. Clinicians should also be aware of the various commercial systems available that may be utilized to produce PRP and the basis behind the performance of these systems. The presence of PRP provides additional treatment options when managing and treating different injuries. However, clinicians must realize that little clinical evidence exists supporting the efficacy of PRP and more well-designed, controlled, clinical trials are needed.[22]

I. Regenerative biomedicine

Regenerative biomedicine is progressively emerging at the forefront of medicine.[23] It refers to a strategy whereby the injured site is provided with the raw materials necessary for a ‘‘scarless repair’’, or regeneration, to occur in situ. The concept is one of augmentation and optimization of the natural healing response.[24]

Currently, regenerative Medicine represents a shift toward more affordable, approachable, and often bed-side strategies to tissue restoration.[24] Applications in physiatry, orthopedics, and sports medicine are currently being developed, and regenerative biomedicine is rapidly becoming an exciting and promising treatment option in musculoskeletal medicine.[25]

This innovative field includes interventions such as the use of mesenchymal stem cells, extracorporeal shock wave treatment, sclerosing agents, nitric oxide, and matrix metalloproteinase and platelet-rich plasma (PRP).[23] The focus of this review is on PRP, as bioactive regenerative therapy.

II. PRP as a regenerative therapy

PRP is a bioactive regenerative therapy that has garnered significant attention in last years.[1] Since platelets are the main regulators of the inflammatory phase and play an essential role in the proliferation and differentiation phase, scientists have proposed the delivery of a concentrate of platelets at the site of the injury as a successful strategy for fostering the regeneration pathway during healing process. The concentrate of platelets is prepared ex vivo and is defined as PRP.[26]

PRP is an autologous concentration of human platelets above baseline in a small volume of plasma produced from a patient's centrifuged blood.[18] Upon activation by an agonist, the platelets contained within the PRP release the contents of their granules consisting of inflammatory factors and growth factors.[26] PRP therefore has been advocated as a way to introduce increased concentrations of growth factors and other bioactive molecules to injured tissues in an attempt to optimize the local healing environment.[27]

Because of its autogenous origin, easy preparation, and excellent safety profile, the advent of PRP has opened another therapeutic door for orthopaedic surgeons and sports medicine physicians.[12]

III. Normal biologic healing response

Wound healing cascade involves 3 phases:
(1) Inflammatory phase
(2) Proliferative phase
(3) Maturation and/or Remodeling phase.

The initial phase, **the inflammatory phase**, occurs when injured, disrupted cells release chemical agents (i.e., so-called "growth factors") that cause a localized inflammatory reaction.[28] This phase occurs in the first week after injury and involves hemostasis and recruitment of inflammatory mediators. Tissue injury activates cyclooxygenase-2 and leads to vasodilation. Growth factors attract macrophages and fibroblasts.[29-31]

The proliferative and repair phases follow in the next days to 2 weeks, It occurs when new blood vessels form (i.e., angiogenesis) and fibroblasts migrate, proliferate, and begin depositing (regenerating) Type II collagen, resulting in the formation of so-called "granulation tissue".[28]

The remodeling phase follows up until about 1 year after injury, when collagen and scar tissue production takes place. Type I collagen replaces proteoglycan and fibronectin to form a more robust matrix with increased tensile strength.[29-31]

Soft-tissue or tendon healing generally involves angiogenesis, cell proliferation, and deposition of extracellular matrix, remodeling, and maturation. Various growth factors are stimulated in the process of repair and remain active during the healing stages. They have important roles in cell regulation, differentiation, proliferation, chemotaxis, and matrix synthesis.[29,32,33]

IV. Platelets and their role in healing process

Platelets are small, discoid, anucleate cells formed from the fragmentation of long proplatelet extensions of the megakaryocyte (figure 1). These extensions become interwoven through endothelial pores of the bone marrow sinusoids and are fragmented by shear forces, releasing a heterogeneous population of nascent platelets into the bloodstream. They have a circulating life span of 5–9 days and their predominant mechanism of clearance is via Kuppfer cells and Hepatocytes.[34,35,36]

Platelets serve in a variety of critical functions, including hemostasis (coagulation), inflammation, antimicrobial host defense, angiogenesis, and wound healing.[24] Although the initial focus on platelets centered on their role in coagulation, it was soon discovered that platelets harbor over 1,100 proteins, including growth factors, immune system messengers, enzymes, and other bioactive compounds that are involved in various aspects of tissue repair.[18,37,38]

After injury, platelets are on the front line and have a critical role in mediating healing by releasing growth factors from their granules.[18,39] The ability to use autolgous platelets to deliver increased concentrations of growth factors locally provided the rationale for the creation and use of PRP preparations for the augmentation of connective tissue healing[12,18]

The dense granules of platelets also have a role in tissue regeneration and can release serotonin, adenosine, dopamine, calcium, histamine, adenosine diphosphate, adenosine triphosphate, and catecholamines.[18,40] Together, the growth factors influence chemotaxis and cell migration via chemical mediators. These growth factors also can induce mitosis, extracellular matrix production, and angiogenesis. Moreover, they signal cells to proliferate and they influence maturation, differentiation, and ultimately tissue repair.[25,41-43]

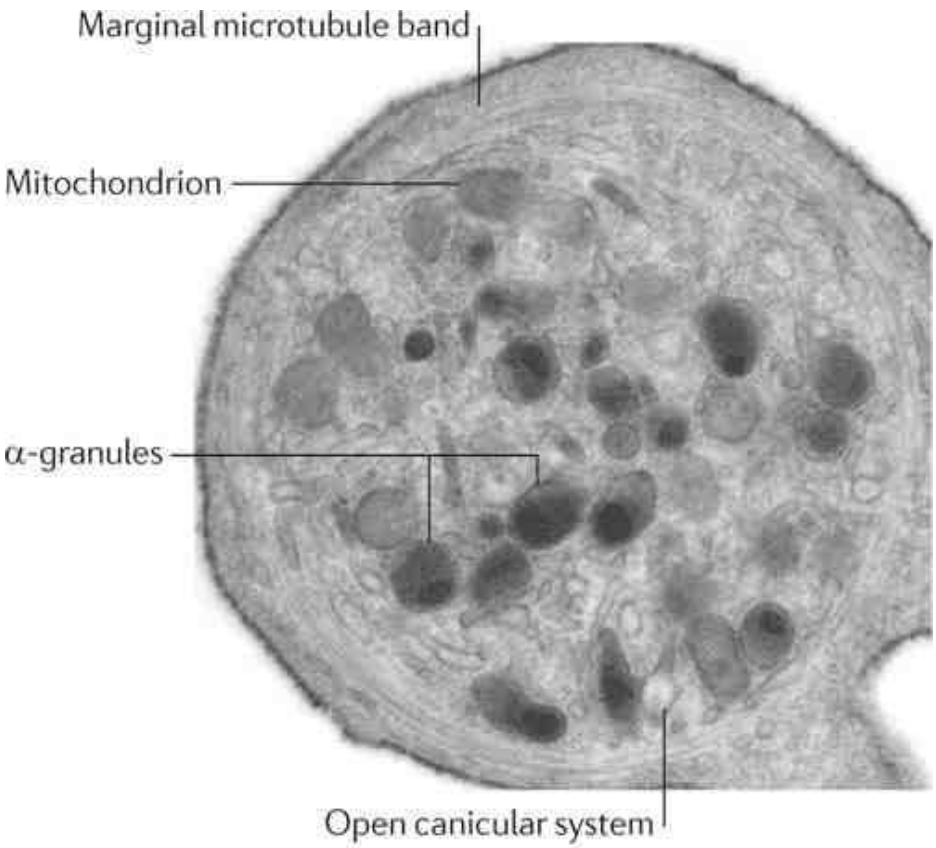

Figure 1: the subcellular organization of a resting platelet viewed by thin-section electron microscopy. (Sample JW, Itiliano JE, et al. Nat Rev Imm. 2011 11;264-274.)

V. Growth factors and their role in healing process

The growth factors are small peptides that bind to membrane receptors and promote downstream biologic pathways.[18,39] In other words, a growth factor is a cell-secreted peptide or protein that promotes or increases (i.e., "up-regulates") normal cellular functions, such as cell proliferation, differentiation, and tissue repair.[28]

There are at least 16 major families of growth factors. A platelet alpha granule, alone, contains over 250 different, evolutionarily-related growth factors.[29] These growth factors include insulin-like growth factor (IGF-1), transforming growth factor (TGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor, and basic fibroblast growth factor (b-FGF). Hepatocyte growth factor, epidermal growth factor, cytokines, chemokines, and metabolites also appear to be involved (Table 1). [18,39]

It was found that b-FGF and IGF-1 played a role in myogenesis and muscle regeneration in vivo in a mouse model. Therefore b-FGF and IGF-1 may have implications in recovery from muscle strains. The growth factors appeared to influence myoblast proliferation and differentiation.[23]

With regard to cartilage regeneration, growth factors appear to have chondro inductive effects. TGF contributes to chondrocyte phenotype expression and mesenchymal stem cell chondrogenic differentiation. IGF also has anabolic