CHAPTER 3

Repair and Regeneration: Connecting the Dots Among Coagulation, Immune System, the Sensory Nervous System and Fibrogenesis

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SUMMARY

Two conditions have challenged the survival of living beings with a closed circulatory system, namely trauma and infection. These conditions account for the primary life-threatening emergencies, namely, bleeding and microbial invasion. Through natural selection and conservation, evolution has shaped the biological defence system of vertebrates to cope with bleeding and microbial invasion by systems consisting of several interlinked modules such as coagulation, the innate immune system, and fibrogenesis. In humans, pivotal players in the biological defence system modules are platelets, leukocytes and fibrinogen, located within a connective tissue, namely, the blood, which acts as an incessantly dynamic and renewing kind of container in mammals. Drawing on the regenerative potential of platelets, plasma biomolecules and fibrin matrix, several systems of producing autologous platelets-and plasma derived products (APPDPs) have been developed and aimed at enhancing the natural in vivo tissue regenerative capacity of damaged tissues. However, in spite of skill and care in the elaboration and application of these blood products by medical staff, consistency in the surgical application of platelet rich plasma remains illusive due to a nonuniform approach in both the composition of these products and the modalities of their application. As a consequence, there is both light and shadow in the outcomes of this treatment.

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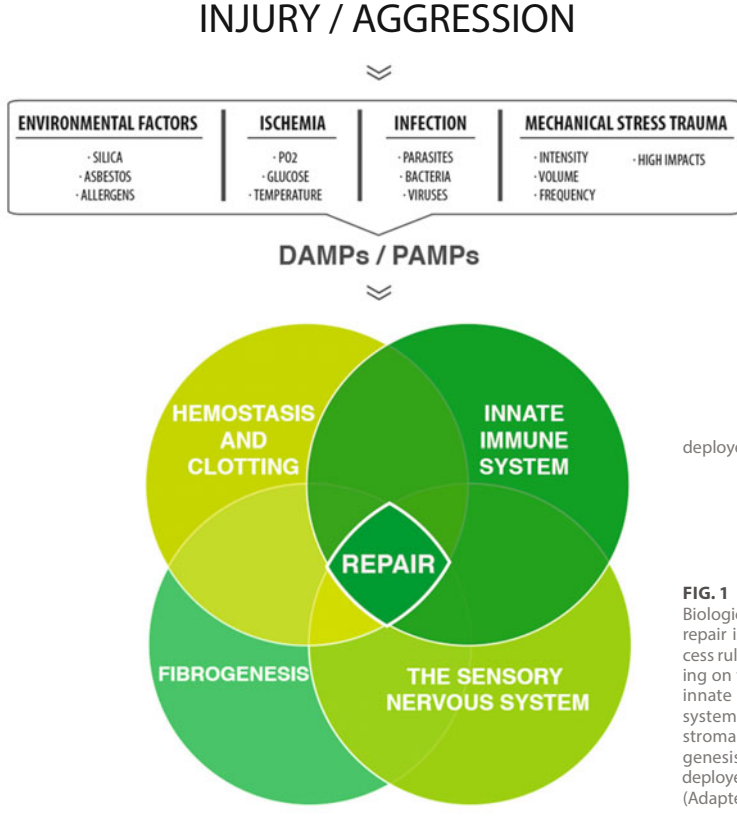
1. INTRODUCTION

Through natural selection and conservation, evolution has shaped the biological defence system of vertebrates (figure 1) to cope with bleeding and microbial invasion, two conditions, which have challenged the survival of animals with a closed circulatory system[1]. The biological defence system of vertebrates consists of 3 interlinked modules namely, coagulation, the innate immune system, and fibrogenesis[1, 2]. Once tissue damage and/or pathogen invasion is detected, organisms mount both a systemic and a local host defence response[2, 3]. In humans, the systemic response stems mainly from the influence of locally produced prostaglandin E2 (PGE2) and other inflammatory mediators[4] on the central nervous system, producing the response known as sickness behaviour (chiefly fever, anorexia, fatigue, and sleepiness) which appear to have only one purpose: survival[3, 5, 6]. Despite the pivotal role of systemic response in the survival function, this response is not in the scope of this chapter. The local response encompasses procoagulant and proinflammatory mechanisms which, together with the activation of platelets, endothelial cells, tissue-resident macrophages, recruitment of circulant monocytes and neutrophils, release inflammatory mediators in a quick autocrine and paracrine reaction (seconds to a few hours)[6-8]. While platelets manage to halt the bleeding process through platelet aggregation, thrombin generation, and fibrin clot formation[9], neutrophils and monocytes prevent the assault of microbial germ lines by killing them, thereby sterilizing the damaged area[8, 10]. Both processes of hemostasis and inflammation are triggered by the aforementioned disruptor, either trauma and/or infection.



deployed in different ar-

FIG. 1
Biological defense system of mammals. Tissue repair is an open and condition-sensitive process ruled by microenvironment cues. Depending on the balance of hemostasis and clotting, innate immune system, the sensory nervous system and fibrogenesis, parenchymal and stromal processes such as myogenesis, osteogenesis, angiogenesis, and neurogenesis will deployed in different arrays.[1, 2, 15, 53]
(Adapted with permission from Padilla et al.[110])

Immediately after this cascade module, where the pivotal players are platelets, leukocytes and fibrinogen, a dramatic switch from killing to healing occurs in the damaged area known as the resolution of inflammation[6, 11, 12]. As a continuum process, the biological defence program will later address reconstruction of the damaged area, which will require not only resolution of the inflammatory stage, but also the coordinated incorporation and action of other cell effectors such as macrophages, lymphocytes, fibroblasts, EPCs and structural biomolecules aimed at promoting angiogenesis, parenchymal and stromal cell proliferation and differentiation, and extracellular matrix (ECM) synthesis[7, 8, 10], where the new formed fibrin clot acts as a transient scaffold. (Figure 2)[13, 14].

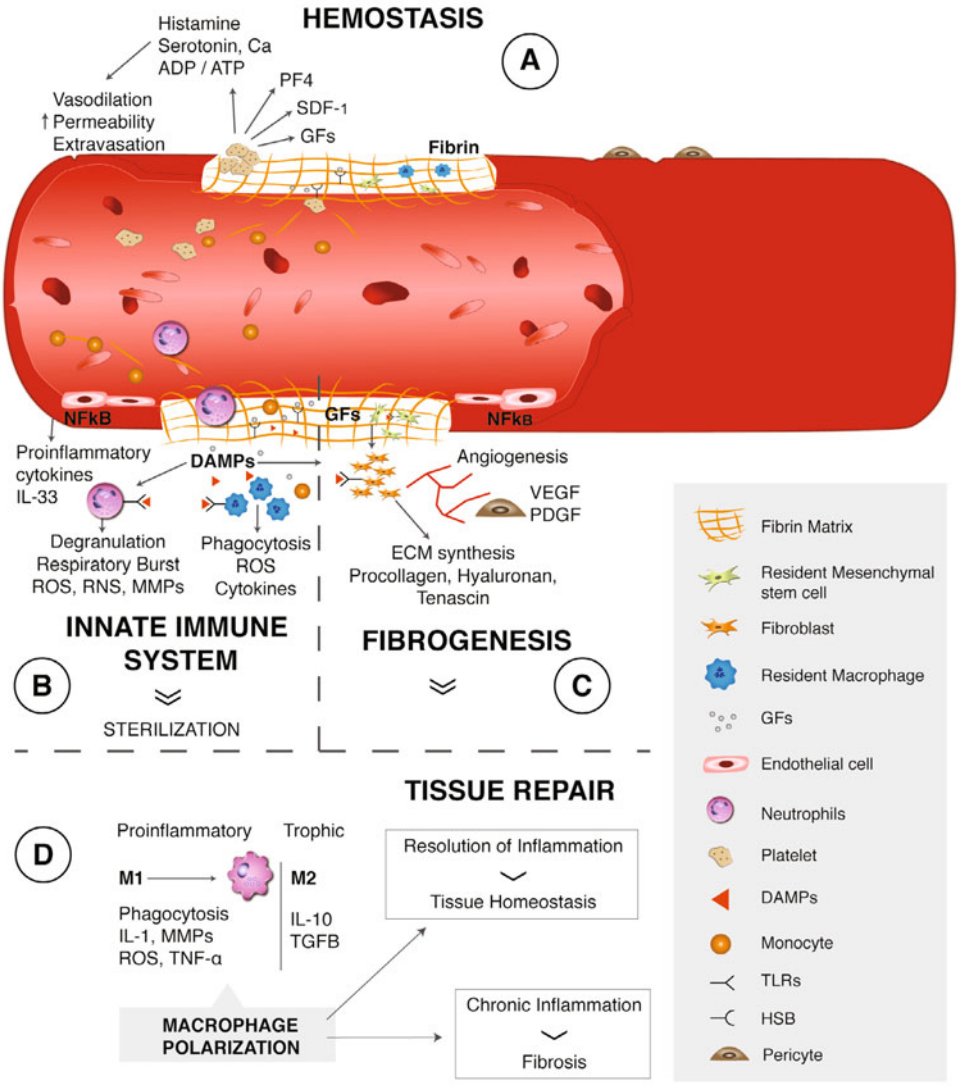


FIG. 2
Connecting the dots among the different modules of the biological defense system. Tissue reconstruction might be considered a byproduct of the mechanisms underlying the biological defense system.[1,2,15,30,37]
(Adapted with permission from Padilla et al.[110])

Drawing on the regenerative potential of platelets, plasma biomolecules and fibrin matrix[13, 15-17], several systems that produce autologous platelets- and plasma-derived products (APPDPs) have been developed, aimed at triggering and enhancing both the natural in vivo tissue morphogenesis and the regenerative capacity of damaged tissues. Platelet-rich plasma (PRPs) are platelet concentrates within a plasma suspension whose composition is determined by the method used to obtain it. PRGF includes plasma and twofold-or-more increases in platelet concentrations above baseline levels, and different collection methods mean the concentration of leukocytes and erythrocytes varies widely, from a complete absence to a high concentration of them[18]. The therapeutic potential of PRPs[19-21] and plasma rich in growth factors (PRGF) for in situ regenerative medicine has yielded extremely promising clinical and surgical outcomes in musculoskeletal system pathologies such as osteoarthritis and cartilage repair[17, 21-27], in oral and maxillofacial surgery[17, 19], as well as in the treatment of diabetic ulcers[19].

In an effort to connect the dots, this chapter is aimed at both clarifying the interactions among coagulation, immune system, and fibrogenesis that may play important roles in tissue healing, and shedding light on some pitfalls in the application of PRPs products as enhancers of tissue repair.

2. BIOLOGICAL DEFENCE SYSTEM: CONNECTING THE DOTS AMONG COAGULATION, IMMUNE SYSTEM, AND FIBROGENESIS

In mammals, the first step in tissue repair is the detection of tissue damage signals such as damage-and pathogen-associated molecular patterns (DAMPs or PAMPs) released as a result of necrotic and apoptotic cell death and damaged microbial and (ECM) host products. These products act as inducers of a complex cascade consisting of hemostasis and clotting, the innate immune system, and fibrogenesis, which are triggered in a sequential and intertwined spatiotemporal manner (figure 2)[10, 28, 29]. DAMPs and PAMPs are damage signals which are recognized by transmembrane toll-like receptors (TLRs) expressed by several tissue cells that act as sensor elements including platelets, mast cells, macrophages, epithelial and endothelial cells, and neutrophils (figure 3)[30-33]. The interaction of DAMPs and PAMPs with TLRs brings about, in these sentinel cells, the activation of a highly conserved intracellular signalling pathway known as NFkB. Its activation will end up inducing the gene expression of growth factors and cytokines, a phenomenon known as inflammatory response (figure 3)[6, 34]. The acute storm of pro-inflammatory cytokines including but not limited to interleukin 1beta (IL-1B), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-a), interferon gama (IFN-j), transforming growth factor beta (TGFB), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF), mainly coming from activated platelets, endothelial cells, pro-inflammatory macrophages, fibroblasts and mast cells, will attract, recruit, and activate circulating cells such as platelets, neutrophils and monocytes into the injury site[6]. From the beginning, platelets adhere to exposed collagen and tissue factor from damaged ECM, aggregate and facilitate blood clot and thrombin formation and polymerization of fibrinogen to fibrin, thereby halting the bleeding process[9, 13]. In addition to activating platelets and inducing pro-inflammatory cytokines from immune and endothelial cells[1, 35], thrombin performs other important roles as a potent endothelial permeability enhancer, chemoattractant of monocytes and neutrophils, inducer of expression of adhesive molecules and promoter of the degranulation of platelets, as well as influencing the cell cycle of several phenotypes[1, 15, 36]. Simultaneously neutrophils generate a cytotoxic microenvironment[8] as a result of both degranulation of anti-microbial molecules, proteases and metalloproteinases (MMPs), highly reactive oxygen and nitrogen species (ROS and RNS), and by respiratory burst, induce microbial death and sterilize the damaged area (figure 2)[8, 10]. Neutrophils

also attract circulating monocytes to the injured site, and activate dendritic cells and macrophages. These resident and migratory mononucleated cells take on a pro-inflammatory phenotype (M1) releasing nitric oxide (NO) and citrulline, ROS, MMPs and a tissue inhibitor of metalloproteinases (TIMPs). Moreover, macrophages express IL-1, IL-6, IL-12, and TNFa, induce Th1 cell infiltration and activation, and phagocytose apoptotic/necrotic cells and other ECM breakdown products, contributing together with the lipoxins (eicosanoids and platelet-activating factors)[7] to tissue repair[6, 28, 37, 38]. This first cell-and bacterial-killing, and matrix destroying microenvironment stems from both the initial noxious agents and the collateral damage inflicted by the inflammatory effectors of the innate immune system which can wreak havoc if the inflammatory process is too intense or too persistent[6, 12]. In addition, platelets release Stromal cell-derived factor (SDF-1), CTGF, TGFB, Platelet factor 4 (PF4) and VEGF, which together with fibrin adhesion cell receptors may well contribute to the resolution of inflammation by recruiting endothelial progenitor cells (EPCs)[39], preventing monocyte apoptosis, promoting trophic macrophages (M2)[40] and activating resident mesenchymal fibroblasts precursor cells[41, 42]. In doing so, these growth factors favour fibrogenesis[30] and generate both a trophic microenvironment, and tissue angiogenesis in a context dependent manner[43]. The formed transient fibrin matrix traps several growth factors and cytokines, promotes adhesion of immune cells, and is chemotactic for phagocytic leukocytes. This natural fibrin scaffold mediates cellular attachment through P-selectin and integrins binding on platelets, monocytes, macrophages, neutrophils, adult stem and hematopoietic progenitor cell antigen (CD34) progenitor cells [1, 13, 14, 43-45], and provides mechanical support and plastic-elastic stiffness all of which have a drastic impact on the fates of diverse cell types such as muscle stem cells[46, 47]. The resolution of inflammation is mainly driven by, but not limited to, the plasticity and polarization of macrophages from phagocytic and pro-inflammatory phenotype (M1) to anti-fibrotic and anti-inflammatory ones (M2)[2, 6, 11, 12], and by the production of lipoxins[7]. In an uninterrupted process, the biological defence system will intensify and address the already ongoing reconstruction of the damaged area, which will require not only resolution of the inflammatory stage, but also the coordinated action of other cell effectors such as trophic macrophages (M2), lymphocytes, activated fibroblasts, EPCs and structural biomolecules aimed at promoting angiogenesis, parenchymal and stromal cell proliferation and differentiation, and ECM synthesis, (figure 2)[10, 28, 48].

As a result of local activation of the tissue plasminogen activator/plasminogen system, fibrinolysis will release the growth factors and cytokines previously trapped in the fibrin network through the cell surface heparan sulphate-binding domains[45, 49] such as SDF-1, PDGF, VEGF, hepatocyte growth factor (HGF), Brain-derived neurotrophic factor (BDNF), Fibroblast growth factor (FGF) among other previously released growth factors by platelets, macrophages, endothelial cells, and newly activated fibroblasts, which will be freed up in the ECM[13, 14, 43-45]. The gradual and sustained release of growth factors[45] from fibrin controls morphogen gradients at the repair scenario[50] and facilitates vascular, epithelial and mesenchymal reconstruction (figure 5)[13, 14, 45]. After the phagocytosis of apoptotic neutrophils, lymphocytes or inflamed parenchymal and stromal cells, inflammatory macrophages (M1) will turn into an anti-inflammatory phenotype and express pro-inflammatory cytokines, including TGF B1, IL-4, IL-10, and PGE2[12]. These cytokines are involved in immunoregulatory functions as well as in the resolution or progression of fibrogenesis[28, 37] which, along with PDGF, and TGFB1, are released from fibrin matrix[14, 45], and lead the switch in the injured area from tissue breakdown to tissue reconstruction[12, 30, 38]. These cytokines allow circulating fibrocytes, perivascular pericytes, and resident mesenchymal cells to differentiate first into fibroblasts and later into alfa-smooth muscle actin myofibroblasts (αSMA) (figure 2)[31, 37, 41, 45]. Fibroblasts and myofibroblasts are highly synthetic and secretory cells that will partially address the loss of tissue in the injured area by synthesizing fibrillar ECM components such as collagen, elastin, fibronectin, tenascin-C, and hyaluronan. They will as well release mitogenic and motogenic cytokines which, together with IL-33

released from the dying cells[51] modulate cells involved in mesenchymal and parenchymal healing response, and which are known as part of the Th2 response[30, 51, 52]. In fibrogenesis (fig. 2), besides the chemical signalling pathways coming chiefly from profibrotic macrophages, the biomechanichal signalling will strongly influence the myofibroblast activity and fate, mainly through the presence of CTGF[10, 41].

These robust and flexible modules will be deployed in many different arrays, and depending on their balance the structural outcome of the repair process will not resolve with a unitary outcome[53]. Therefore, the repair process might be considered as a byproduct, or epiphenomenon of the mechanisms underlying the biological defence system[15, 54] and simply be the way inflammation and fibrogenesis are resolved[28, 55]. Therefore as a secondary outcome of the biological defence system[55], the newly formed tissue often presents several structural and patterning differences from the original one, the fibrotic scarring being the most unsuccessful and nonfunctional secondary outcome (figure 1 and 2)[10, 31, 56]. The resolution of the trophic or reparative period will be followed by the remodelling stage provided that the apoptotic clearance of myofibroblasts by regulatory macrophages is carried out, thereby eliminating the stimuli inducing TGF- ß1 and other profibrotic factors which otherwise would lead to a persistent fibrotic microenvironment[10, 57].

3. HARNESSING THE MORPHOGENETIC POTENTIAL OF AUTOLOGOUS PLATELETS – AND PLASMA DERIVED PRODUCTS (APPDPS)

In mammals, pivotal players in the biological defence system modules are platelets, leukocytes and fibrinogen, located within a connective tissue, namely, the blood, which acts as an incessantly dynamic and renewing kind of container. In addition to the biological defence function, the blood plays a central role in other physiological processes such as the transport of gases by erythrocytes, caloric energy transport and body temperature regulation by water, communication of body systems by hormones, and transport of waste products, among other functions[58]. Therefore, we no longer think of blood as an indivisible tissue-body whose functions reside in the blood as a whole, but rather as a tissue with cellular and acellular elements that carry out a myriad of interactions and specific functions[58, 59].

Platelets are circulating monitors, trackers and surveyors of the integrity of the vascular system and of the internal milieu as well as carriers of cytokines, chemokines and growth factors, taking part in the coordination of coagulation and inflammation as the core of the biological defence system[36, 60] (fig. 2)(see chapter 1). Platelets appear to be crucial in post-embryonic morphogenesis, and their activation in a biological context is carried out by factors such as thrombin, serotonin, or other tissue constituents such as DAMPs[1] (fig. 3). These molecules activate the platelets thereby releasing, by degranulation, growth factors and cytokines which, along with the formation of thrombin, trigger cell migration and proliferation, regulating angiogenesis, chemoattracting circulating progenitor cells, and guiding tissue remodelling and restoration of function[9, 15, 36, 61].

In addition to many bioactive mediators, (α-granules: TGF-®, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), hepatocyte growth factor (HGF), bone morphogenic proteins (BMPs), brain-derived neurotrophic factor (BDNF) and the dense granules: Histamine, Serotonin, Calcium (Ca) and ATP/ADP), there are other constituents in the plasma of APPDPs, namely IGF-1, HGF, prothrombin, fibrinogen, fibronectin and other proteins which, together with adhesive proteins expressed by activated platelets, play a central role in the cell signalling pathways which are involved in both tissue injury recognition and in repair of damaged tissues[9, 61].

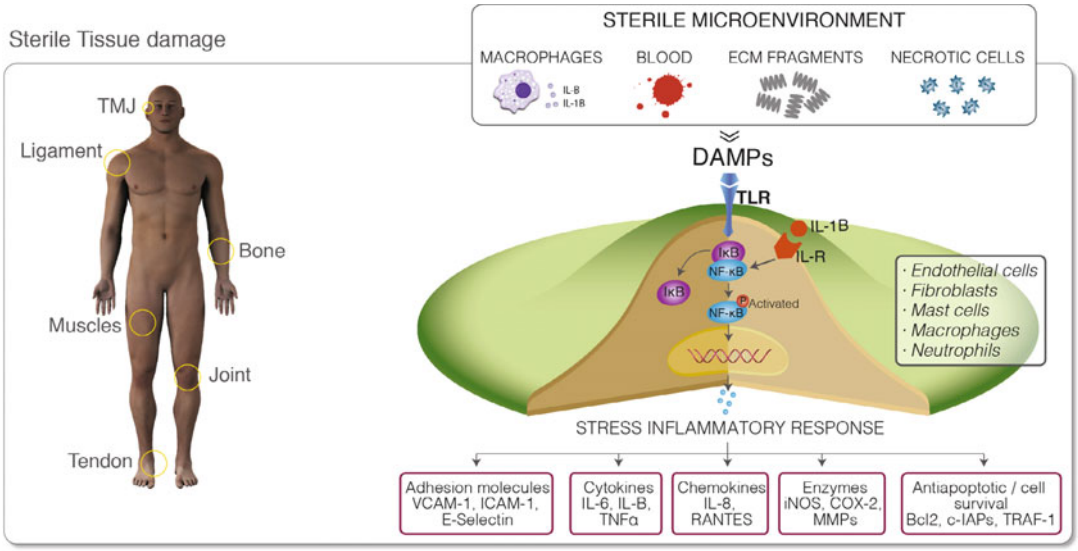


FIG. 3
Tissue repair in sterile conditions. Different types of signals reporting injury such as damage-associated molecular patterns (DAMPs) as well as toxins, minerals, crystals, chemical and antigens can trigger sterile inflammation. [29,33,34,80]
(Adapted with permission from Padilla et al.[110])

For instance, several studies have reported an important HGF-mediated anti-inflammatory effect of platelet-rich plasma on tenocytes, macrophages and chondrocytes[62-64] by attenuating the trans-activating activity of NF-kB[62] a highly conserved intracellular signalling pathway whose activation induces tissue inflammation[34]. HGF, a plasmatic key growth factor within APPDPs, has been shown to have a remarkable anti-inflammatory and anti-fibrotic effect on different tissues[65-67].

PRGF (Plasma rich in growth factors) which is included in APPDPs, conveys growth factors, cytokines, and morphogens contained in the platelets, as well as fibrinogen and other plasmatic proteins in a biologically balanced aggregate, managed and delivered in a pharmacological manner[18, 21] which might account for two special features: the resolution of inflammation and avoidance of fibrosis[68]. In addition, the three-dimensional fibrin network, formed either in vitro as a clot or in situ as an extracellular matrix, contains binding sites for cell adhesion as well as proteins such as thrombospondin-1 (TSP-1), α-1-antitrypsin fibronectin, acute phase proteins or proteins related to lipid metabolism[14, 16, 69]. This fibrin-scaffold formed as a provisional EC serves as a highway for mechanical energy to transit from the environment to the cell, bridges cell-to-cell tissue transition, promotes multi-cellular assembly, provides mechanical support and plastic-elastic stiffness which has a drastic impact on the fates of diverse cell types such as muscle stem cells[46, 47, 70], and endows tissues with a suitable microenvironment for biological restoration[9]. In addition, fibrin matrix, by heparin-binding domains, may sequester growth factors such as PDGF, FGF, HGF, BBNF, and VEGF[14, 45, 71] to gradually release them later.

4. SOME CLARITY IN THE APPLICATION OF PLATELET RICH PLASMA

It is important to shed some light on inconsistent therapeutic application, and bring into focus some common pitfalls regarding the composition of autologous plasma-and platelet derived products (APPDPs) since the biological effects rely quite essentially on their composition and directly depend on particular blood-spinning protocols, as well as on the modalities of their application. They can all deeply influence the success of tissue repair— a process which is already unpredictable — in the abscene of a unitary mechanism (fig. 4)[53].

A. Neutrophils as Engines of Destruction Although Life- Saving cells

A first concern regarding the APPDPs, easily understandable though controversial, is leukocyte concentration. In a repair scenario, leukocytes may aggravate tissue damage and promote a pro-inflammatory microenvironment by releasing TNF-α, IL-6, IFN-γ cytokines which induce the overexpression of MMPs, elastase and cathepsin G among others, thereby breaking down the ECM[8, 72, 73]. The release of reactive oxygen species (ROS) by neutrophils facilitates the removal of necrotic tissue in the repair stage but may exacerbate the initial lesion[74, 75]. In vitro, neutrophils injure cultured myotubes, and, in addition, can cause further muscle injury and disrupt some of the processes involved in skeletal muscle healing[76]. For instance, due primarily to a continual presence of offending elements in the damaged area, leukocyte infiltration may give rise to a nonresolving inflammation[28]. These secretory cells may persist over time, leading to an excessive accumulation of ECM, ultimately producing a pathological and nonfunctional scar tissue (fig. 2)[31, 77]. Anitua et al[78] reported that the inclusion of leukocytes in a fibrin scaffold obtained from APPDPs, both peaked the amount of two molecules involved in inflammation, IL-1 and IL-16, and produced a de-structured and heterogeneous aspect of the fibrin mesh[78]. In the same work and as a way of mimicking pathological conditions in a fibroblast culture exposed to pro-inflammatory cytokines, the addition of leukocytes to an autologous platelet- and plasma derived supernatant triggered over-expression of TGF-β1 and down-regulation of VEGF, an imbalance which may favour the formation of fibrosis. This phenomenon is not observed with an autologous platelet- and plasma-derived product supernatant which is leukocyte-free[78]. The presence of leukocytes in autologous platelet- and plasma-derived product formulations tips the balance of matrix synthesis towards catabolism[79] which may very well lead to a nonresolving inflammation[28]. Filardo and colleagues[22] compared the efficacy and safety of intra-articular injections of a leukocyte-free APPDP with a leukocyte-APPDP in the management of osteoarthritis. Patients treated with the former had fewer side effects than those treated with Leukocyte-APPDP, who presented more pain and more swelling. Tissue injury and microbial infection appear to represent distinct stresses to the host and much of the collateral damage inflicted by neutrophils and macrophages during the hemostatic-inflammatory period might be unnecessary to repair sterile injuries[80] such as musculoskeletal injuries (fig. 3).

Although a less common element in PRPs, erythrocytes too might be detrimental to the repair process, since their phagocytosis by macrophages may induce inflammation, oxidative stress, and promote the persistence of myofibroblasts, thereby leading to fibrosis[57].

B. Fibrogenesis: a double-edged sword

The concern about generating fibrosis has been present since the beginning of the therapeutic application of PRPs, mainly because of the TGF-β1 content in PRP. The TGF-β1 family has been implicated in the development of fibrosis in various tissues[10, 81, 82]. Several studies were conducted both in vitro[83-86] and in vivo[85, 87, 88] to try to tease out the influence of leukocyte-free APPDP on fibrosis. These studies confirmed that, although the TGF-β1family drives fibrogenesis, collagen synthesis and deposition, and it potentially might stimulate the formation of scar tissue[30, 48, 52], the concurrent presence of TGF-β1, VEGF, and HGF in the same lo-

cal environment makes leukocyte-free APPDP an antifibrotic autologous system. In the molecular network of leukocyte-free APPDP, the fibrotic effect of TGF-β1 would be either modulated, counterbalanced, or even hindered by the presence and local production of HGF, a remarkable antifibrotic regulator[89] and the VEGF, as shown by our work on cells cultured on fibrin matrices[83-86] thus suggesting the pleiotropic behaviour of TGF-β[6]. For instance, in sheep[86] four intratendinous injections of a pre-clotted preparation of leukocyte-free PRGF into the Achilles tendon fascicles triggered a healing response which stemmed from an increase of cellularity, cell organization and angiogenesis. No signs of fibrosis were observed in the histological examination of the sheep Achilles tendons infiltrated with leukocyte-free APPDP[86, 88]. Likewise, the application of leukocyte-free PRGF fibrin matrices on the surgically repaired Achilles tendon tears on 6 athletes showed no wound complication and significantly shortened by 35% the functional recovery time, compared with the group that underwent the same surgical procedure without leukocyte-free PRGF application[90]. In addition, the cross-sectional area of the repaired Achilles tendon, assessed a few years later by ultrasonography, was significantly greater, while minor complications including 1 superficial infection and 2 keloids occurred in the non-treated group[90]. In another study[87], a comparison was made of the overall arthroscopic appearance and the gross morphology and histology of tendon grafts and of the joints of patients treated with leukocyte-free PRGF infiltration with those not treated during anterior cruciate ligament (ACL) surgery[87]. During the remodelling period (6-24 months) the treated group showed more signs of remodelling, maturation, and a synthesis of new connective tissue which wrapped the infiltrated tendon graft with more and better-oriented cells, more akin to the native ACL than in the non-treated one[87].

Recently two other studies assessed the biological effect of leukocyte-free PRGF on other cell lineages such as keratocytes, conjunctival fibroblasts[91] and gingival fibroblasts[92] and synoviocytes[93]. Leukocyte-free APPDP formulations promote the fibroblast phenotype and revert the myofibroblast phenotype to its original fate by protecting and inhibiting TGF-β1-induced myofibroblast differentiation. In conjunctival fibroblasts, leukocyte-free APPDP inhibits and reverses TGF-β1-induced α-SMA expression of fibroblasts as an expression of myofibroblast differentiation[94], thereby preventing the generation of scar tissue. The outcomes of these two studies suggest that leukocyte-free APPDP modulates the fate of myofibroblasts in a way that might be determinant in resolving both inflammation and fibrogenesis, and driving the repair events towards mimicking original tissue, rather than to a tissue-fibrotic outcome[10, 81].

C. APPDPs are not magic products

A third issue related to APPDPs efficacy in healing is the way they are applied in different fields. It has become commonplace to infiltrate APPDPs in the treatment of musculoskeletal injuries as a kind of scatter shot instead of adopting a well thought out and executed biological approach. For instance the application of APPDP to rotator cuff tears is intended to provide the damaged structure with growth factors and cytokines as signalling molecules, one of the three elements involved in the repair process (the two others are cells and 3-dimensional scaffolds). However, as in the case of some studies, it is not enough to add a storm of growth factors to a tendon which for years has been undergoing a degenerative process, and, as a consequence, may have exhausted its healing capacity[95]. There should also be a systematic infiltration of the healthy peripheral tissue surrounding the injury, with the aim of recruiting, activating and mobilizing mesenchymal resident cells to contribute to tissue reparation processes and cell signalling pathways as well as activating endothelial cells and macrophages. A procedure has been developed for the arthroscopic repair of rotator cuff tears assisted by leukocyte-free APPDP which involves 5 infiltration sites, and ultrasound evaluations performed at the third and sixth week after the intervention, during which a subsequent ultrasound-guided infiltration of leukocyte-free PRGF is administered into the repaired tendon[96].

Moreover, we illustrate two examples of the treatment of chronic Achilles tendinopathy[97] in which the authors applied either a single injection of APPDP, following a local anesthetic injection, or two unguided peritendinous injections of autologous whole blood, which showed a negligible clinical effect[98]. In the wake of these two poor clinical results and making inferences about other autologous platelet rich plasma products, some researchers have suggested that all these blood derived products are ineffective in the treatment of mid-portion Achilles tendinopathy. However, a recently published study[99] by Charousset et al. showed clinical and radiological improvement of athletes with chronic Patellar tendinopathy who had been treated using three ultrasound-guided leukocyte-free APPDP infiltrations without any sedation or local injected anesthesia[99].

5. PLEIOTROPY AND ROBUSTNESS OF MECHANISMS UNDERLYING PRP THERAPIES

Wound healing or tissue reconstruction might be considered a byproduct of the mechanisms underlying the biological defence program that entails a set of overlapping complex phenomena encompassing both the recruitment of competent cells to undergo spatiotemporal phenotype commitment and the patterning of cell gene-products to generate ECM and thereby, new tissues (fig. 1)[15, 54]. There is a high degree of self-organization in the regeneration-repair process which may be seen as an open conditon-sensitve process where the environmental cues, both biomechanical and physico-chemical, play a crucial role in influencing and modulating cell phenotypes, in the gene expression, and in patterning the new tissue to mimic the one to be replaced[16, 37, 100, 101].

As is the case in TGF- β1, VEGF and HGF, most of the growth factors and cytokines in PRPs act on a variety of tissues just as they do in any biological system. These proteins exert their regulatory and pleiotropic-biological functions as members of a molecular network linking different modules or systems. Indeed, the results shown in this analysis suggest that applications concerning APPDPs are aptly framed within Nesse and Dawkins' proposal that it is a mistake to seek single, uni-directional causal agents in biological processes; single specific biological factors do not exist for each function[5]. There are simply biological constituents which, in a particular tissue-cell environment, may act together as inhibitions/activations in so-called "genetic switch activities," and induce the expression of cell phenotypes with various behaviours to promote tissue regeneration[5]. This concept, which has been discussed by Huang[102], may account for biological findings that would otherwise appear contradictory, such as the fact that the same molecule, for instance TGF-β1 or a given cell type, such as macrophages, may exert diametrically opposed biological functions [6, 103] or that the same adult stem cell may express different cell phenotypes in different microenvironments or tissue niches[104].

Much scientific evidence, together with the foundations of engineering biology, namely, standardization, decoupling and abstraction[105], has paved the way for several groups to use the blood as a raw material from which to obtain APPDPs as endogenous regenerative technology[19, 106, 107] instead of using the whole blood which conveys a multiplicity of cells and biomolecules whose role in the repair process is negligible or even detrimental (see chapter 4)[57, 75, 76]. At the same time, there are several crucial differences between autologous whole blood and autologous platelet- and plasma derived products (APPDPs), and therefore it is quite inaccurate to infer clinical results from one product as applicable to the other, or to lump all these blood-derived products together[18, 98, 108, 109].

6. CONCLUSIONS

A biological approach to the application of APP-DPs is crucial to obtaining optimum functional repair outcomes in addition to avoiding poor clinical results and drawing misleading inferences. Attempting to optimize the degree of functionality by producing a repaired tissue that is intended to be structurally identical to the damaged tissue might be considered an "artificial" goal imposed by human purposes. In other words, we are creating new goals and placing new demands on cell-based biological programs (to regenerate rather than repair in quiescent and non-dividing tissues) which were selected and conserved over millions of years in different species with a single goal: survival.[1, 36]

Several unanswered questions remain, some regarding molecular mechanisms that give rise to the clinical benefits, and others encompassing dosage aspects such as how many injections would be ideal in a first approach, the interval between them, and whether combining PRPs with stem cells might enhance the healing power of PRPs.

The time has come when we should no longer compare the biological and therapeutic efficacy of very distinct products in musculoskeletal orthopaedic surgery by lumping all autologous platelet-and plasma-derived products together.

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