CHAPTER 4

Effects of Plasma Rich in Growth Factors on Cells and Tissues of Musculoskeletal System: from Articular Cartilage to Muscles and Nerves

AUTHORS

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SUMMARY

An innovative approach to the treatment of acute and chronic sports injuries is the use of engineering biology assisted by the application of blood-derived biological drug delivery therapies (BDDTs) in their different formulations. The common ground of cartilage destruction and osteoarthritis (OA), tendinopathies, muscle and ligament strains, bone fracture or nerve compression or transection, among other tissue damage, is the sterile inflammation. Key processes in repairing the damaged tissues are inflammation, angiogenesis, macrophage activation and polarization, cell fates and progenitor stem cell differentiation, fibrogenesis as well as the active resolution of inflammation, angiogenesis, and fibrogenesis, which are essentially governed by cytokines, growth factors and other biological mediators The present chapter summarizes our current knowledge of effects of blood-derived biological drug delivery therapies (BDDTs) on tissues of the musculoskeletal system.

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1. INTRODUCTION

Through the design and construction of synthetic biological materials, tissue engineering provides us with the best technically available scaffolds and replacement devices. However, the weakest point of the current orthopaedic surgical treatments lies not with these highly sophisticated biomaterials but with the quality of patient tissues at the site where the synthetic biomaterials will be attached or anchored. Therefore the field of regenerative medicine is moving away from more traditional synthetic biology toward increasingly complex and multimolecular biological drug delivery therapies (BDDTs). In fact, blood itself contains the basic ingredients to biologically engineer drug delivery devices that provide spatiotemporal control over the presentation of a wide range of bioactive agents including small molecules, cytokines and growth factors[1].

Blood contains a wide range of biological elements that influence the development of functional substitutes for damaged tissues. It contains the basic ingredients of the tissue engineering triad, that is, cells, growth factors and scaffold-forming elements[2]. Significantly, blood provides fibrin as a provisional scaffold for tissue growth. It also contains cell-signaling elements both in plasma and in platelets in the form of biochemical or environmental cues that affect the biological fate and phenotype of cells. Importantly, BDDTs need not be seeded with cells before administration, since blood-derived fibrin scaffolds are enriched in a patient´s own growth factors and cytokines providing cues to direct endogenous cells, including stem cells, to sites of repair[3]. The present chapter highlights our current knowledge of BDDTs in the therapeutic potential of their use in different relevant musculoskeletal tissue injuries including tendon, ligament, cartilage, muscle, and nerve.

2. BLOOD-DERIVED BIOLOGICAL DRUG DELIVERY THERAPIES

Platelets constitute one of the essential biological elements within the blood-BDDT. They are the first cells that accumulate at sites of injury and, after activation, release dozens of biologically active mediators into the microenvironment, including well-known chemokines, cytokines, and growth factors[4]. The multitude of released cues exerts complex biological effects that drive tissue repair and regeneration. For example, platelet-derived factors modulate activation of fibroblasts, induce proliferation and migration of cells critically involved in tissue repair, such as smooth muscle cells and mesenchymal stem cells (MSCs)[5], regulate angiogenesis, a pivotal process for recovery of tissue function[6], and may regulate apoptosis and survival of cells by means of released platelet microparticles[7]. Thus, blood-derived BDDTs, which are enriched in platelet secretome, may successfully be used as productive and autologous therapeutic tools, promoting the healing and repair of injured tissues.

The fibrin scaffold, which is generated from the blood-derived BDDT, consists primarily of enriched fibrinogen, thrombin, and calcium and coagulation factors (figure 1). Interestingly, it fulfills the critical capacities that a scaffold must have, including form, fixation, and formation[8]. For a scaffold to have form, it should be able to fill the space it is designed to fill. Blood-derived BDDT is used therapeutically to fill gaps in ulcers, bone defects or dental alveolus among others[9, 10]. Another key property is fixation: the ability of a scaffold to integrate and attach to the surrounding microenvironment. Autologous fibrin scaffolds are biocompatible and biodegradable, and they serve as delivery vehicles and as scaffolding matrices. Furthermore, they contain dozens of adhesive proteins, including fibronectin, vitronectin, and serpins, among others (all of them pivotal elements from the extracellular matrix). Following a high-throughput proteomic characterization and classifying the proteins into families and networks according to gene ontology, more than 40 pro-

teins specifically involved in tissue regeneration and wound healing have been identified[11]. Finally, the fibrin scaffold is able to drive the formation of the intended tissue.

Interestingly, blood-derived biological drug delivery therapy is modernizing the ancient "art of healing" by providing dosage-form versatility over drug availability (Box 1). Current novel blood-based therapies can be administered topically, as an eye-drop or by subcutaneous, intradermal, and intramuscular injections. In addition, they can exist as a pure liquid, an in-situ gelling liquid, or a three-dimensional fibrin scaffold, thus enabling novel therapeutic strategies[1]. In general, all of these therapeutic formulations are administered locally. Once situated, the fibrin scaffold acts as a depot of bioactive mediators at any injury site, temporally controlling their presentation. The short half-lives of the autologous biological mediators, including growth factors, cytokines, and chemokines, emphasize the importance of this biology-mimicking delivery system (figure 1) .

Opponents to blood-derived BDDT hold that these therapies may show relevant but still poorly understood mechanisms of tissue repair. However, our accumulating knowledge of biology and molecular biology is alleviating some of these concerns[12]. For example, platelets within the BDDT release agents such as hepatocyte growth factor (HGF) and stromal-derived growth factor 1 (SFD-1), which are known to control proliferation, recruitment, and activation of cell types critically involved in wound healing and tissue regeneration (figure 1). In particular, HGF exerts antiapoptotic[13], proangiogenic[14], and immunosuppressive activity[15] and promotes recruitment of MSCs to human arterial endothelial cells[5]. SDF-1 stimulates progenitor cell recruitment to arterial thrombi and differentiation of the cells to endothelial progenitors in vivo[16, 17] .

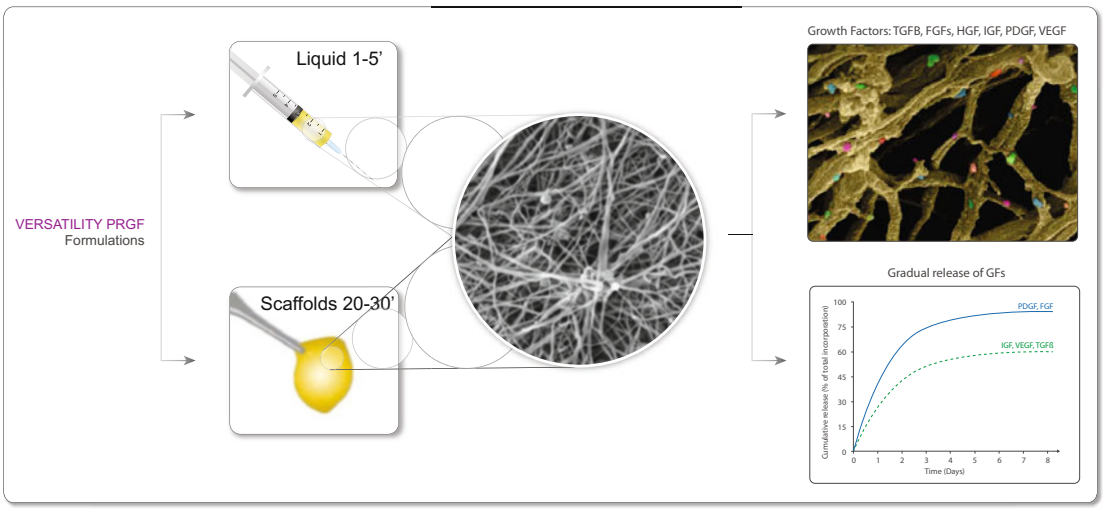

FIG. 1
Blood-derived biological drug delivery therapies (BDDTs) are based on a combination of naturally derived biomaterials such as fibrin and a pool of growth factors. For example, the three-dimensional fibrin scaffold obtained from fractionated human plasma represents a physiologically inspired solution to control the release of the wide range of plasma and platelet-derived mediators[64].

3. MECHANICS RULES CELL BIOLOGY

Virtually all the cells of the musculoskeletal tissues are mechano-sensitive and experience mechanical stress through the distortion of the extracellular matrix (ECM) complex. They respond to these stresses by changing their cellular biochemistry and physiology (mechanobiology and plastic adaptation) (figure 2)[18]. Moreover, mechanical forces are required to maintain the physical integrity of anatomical structures and homeostasis of the tissues by regulating cell functions, including gene induction, protein synthesis, and cell proliferation, differentiation, growth, survival and apoptosis[19].

However, the exposure of musculoskeletal cells to nonphysiological stimuli, either mechanical or biochemical, leads to swings of the tissue pendulum towards profound alterations of components of the extracellular matrix both cellular and acellular as well as the physical and chemical properties of the ECM. Such stimuli lead to cell microenvironment damage, degeneration and disease[20].

The exposure of cells (tenocytes, chondrocytes, fibroblasts, myofibres) to a novel cytoplasmic and extracellular microenvironment can modify their gene on-off state (gene switches) which might induce them to turn on previously silent genes, thereby altering their gene expression and gene products such as metalloproteinases (MMPs) and other molecules of the ECM (figure 2)[20, 21].

The common ground of cartilage destruction and osteoarthritis (OA), tendinopathies, muscle and ligament strains, bone fracture or nerve compression or transection, among other tissue damage, is the sterile inflammation. Key processes in healing the damaged tissues are inflammation, angiogenesis, macrophage activation and polarization, cell fates and progenitor stem cell differentiation, fibrogenesis as well as the active resolution of inflammation, angiogenesis, and fibrogenesis, which are essentially governed by cytokines, growth factors and other biological mediators (figure 3)[22].

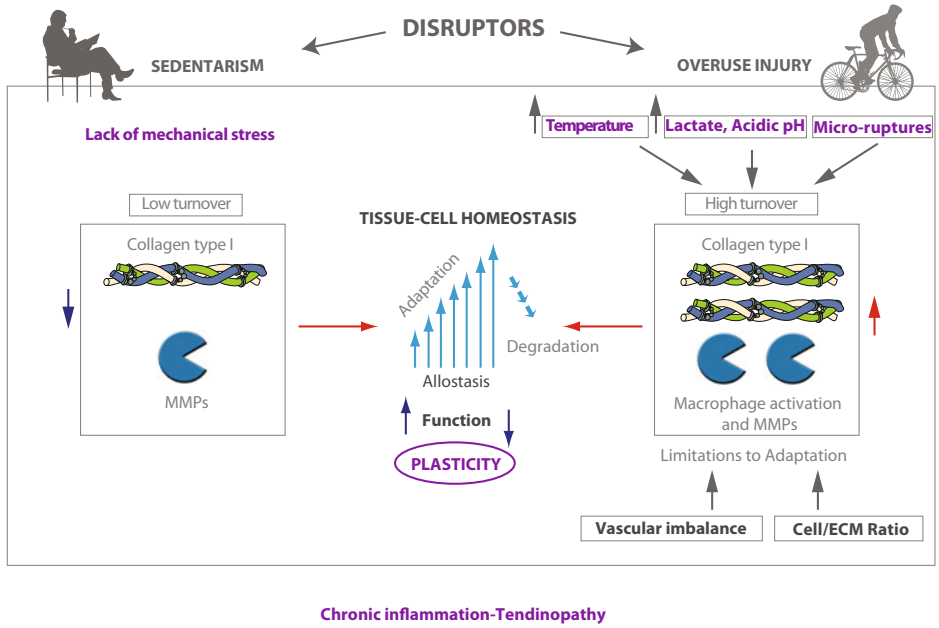

FIG. 2
The exposure of musculoskeletal cells to non-physiological stimuli, either mechanical or biochemical, leads to swings of the tissue pendulum towards profound alterations of cells and components of the extracellular matrix as well as the physical and chemical properties of the ECM. Such stimuli lead to cell microenvironment damage, low chronic inflammation and disfunction[20, 39].

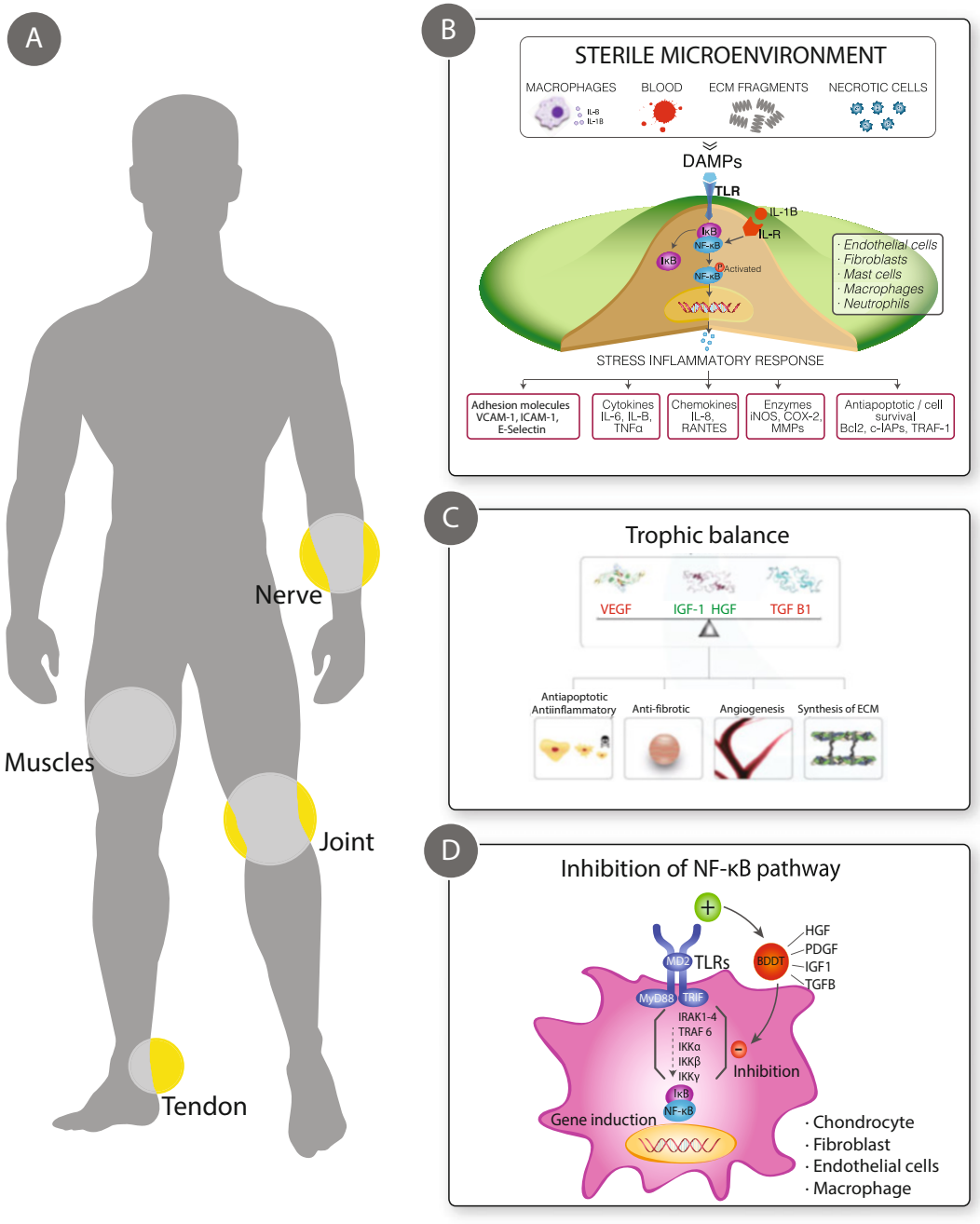

FIG. 3
The common ground of cartilage destruction and osteoarthritis (OA), tendinopathies, muscle and ligament strains, bone fracture or nerve compression or transection, among other tissue damage, is the sterile inflammation. B Tissue injury disrupts the chemical and physical composition of cell microenvironments, which prompts several cell lineages to respond with a pro-inflammatory gene expression through activation of the NFkB signaling pathway without any involvement of pathogens. C A hypothetical mechanism by which the concurrent presence and a balanced ratio between platelet-secreted TGFB1 and VEGF, and plasma growth factors such as IGF-1 and HGF all conveyed by blood-derived BDDT might exert some biological effects. D Blood-derived BDDT, GFs within it such us HGF, PDGF, IGF-1,TGFB1, and platelet microparticles have proven to exert an immunomodulatory effect and promote an antiinflammatory environment[22].
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4. EFFECTS OF BLOOD-DERIVED BDDT ON MUSCULOSKELETAL CONDITIONS

Thanks to a deeper understanding of molecular and cellular processes going on in musculoskeletal pathologies, orthopaedic surgery is going through a serious paradigm shift: instead of simply removing and replacing damaged tissue with artificial devices and materials, blood-derived biological drug delivery therapy application is aimed at triggering and enhancing the natural in vivo tissue morphogenesis and regenerative capacity of damaged tissue[23] (see chapter 3).

With this in mind, blood has always been present in the equation of healing therapies. Several lines of evidence derived either from systemic or local stem cell niche therapies, and represented by parabiosis or microfractures and tendon scarifications respectively, support the concept that factors stemmed from platelets or plasmatic proteins are candidates for mammalian tissue rejuvenation and healing[24-28].

Articular cartilage

The treatment of synovial joint pathologies such as traumatic osteochondral defects, osteochondritis disecans, osteonecrosis, bone marrow edema-like lesions, and osteoarthritis, whose ultimate victim appears to be the articular cartilage, with the subchondral bone as the culprit, remains daunting[29]. Current therapeutic strategies are oriented towards harnessing the endogenous repair response by stimulating bone marrow cells through drilling and microfracturing approaches, the transplantation of osteochondral autograft and osteochondral allograft, joint distraction, and the implantation of autologous chondrocytes. In this complex therapeutic landscape, blood-derived biological drug delivery therapies emerge as a promising adjuvant autologous biomaterial with trophic-anabolic, antiinflamatory, immunomodulatory, antioxidative, and analgesic effects on joint tissues (Box 2)[28]. Versatility makes these products optimal biomaterial, to be applied at the dysfunctional and deregulated injured site as a niche therapy[24, 28] (figure 4 B). They may be applied either alone, harnessing the proliferative, migratory, and chondrogenic effect on endogenous mesenchymal progenitor cells[30], or as a carrier of stem cells and/or extracellular components such as collagens and hyaluronic acid[31]. Animal model studies suggest that autologous blood-derived biological drug delivery therapies, whose fibrin is embedded with platelet-derived and plasmatic GFs, either alone or seeded with MSCs, have potential as a gel-scaffold for focal cartilage and osteochondral defects repair[32-35]. These BDDT products are minimally manipulated, conceived and prepared in situ and ready to be applied in the medical office or in the surgical theatre. Intraarticular injections of blood-derived BDDT have been proven to reduce pain and improve joint functionality in patients with knee or hip osteoarthritis[36, 37] (Figure 4 B, Box 2). Moreover, an increasing body of evidence indicates that blood-derived BDDT serves as a bone marrow stimulating approach, or combined with hydrogel, collagen and/or hyaluronic acid membrane, as a carrier of bone marrow-derived mesenchymal stem cells, resulting in structural and functional improvement in human focal cartilage defects[27, 30, 34]. A new strategy to safely deliver blood-derived BDDT to the damaged synovial joint–a strategy which circumvents systemic toxicity, offers an excellent bioavailability and does not present molecular size limitation–is the combination of intraarticular and intraosseous infiltrations of blood-derived BDDT as an in situ biological "joint centric" approach to treat traumatic osteochondral defects, osteochondritis disecans, osteonecrosis, bone marrow edema-like lesions, and osteoarthritis. Such clinical application opens new therapeutic avenues in treatment of joints pathologies[28, 38] (Box 2, Figure 4 B) (see chapter 9 and 11).

Tendon tissue

Musculoskeletal injuries are a growing medical problem associated with overuse and/or aged-related tissue alteration with an estimated annual $30 billion spent on its management in the USA alone. Tendon and ligament represent 45% of these injuries and unfortunately none of the various therapeutics– including exercise-based physi-

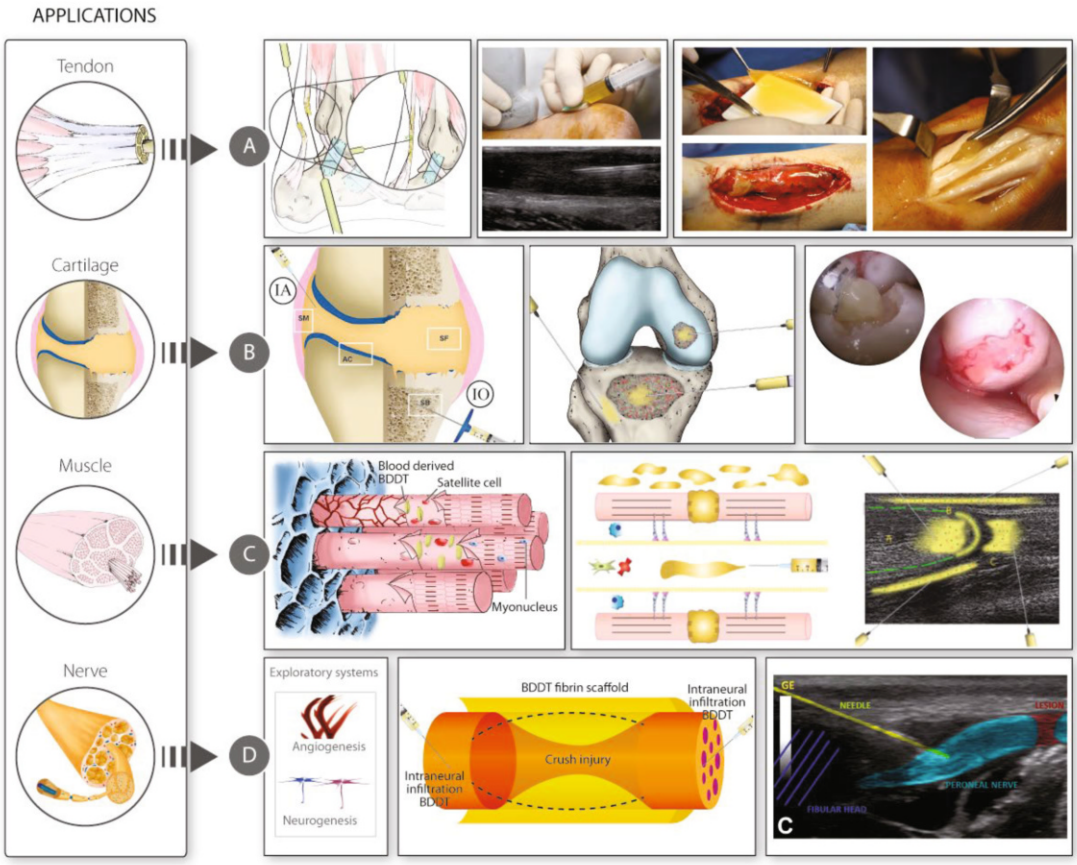

FIG. 4
Advances in regenerative orthopaedics based on the application of blood-derived BDDT. **A)** Intratendinous infiltrations and application of scaffolds obtained from blood-derived BDDT to assist surgical reconstruction of tendon ruptures. In chronic tendinopaties, intratendinous injections are applied under ultrasound guidance. **B)** A novel approach to treating severe knee osteoarthritis and osteochondral injuries by targeting synovial membrane, superficial articular cartilage, synovial fluid, and subchondral bone by combining intraarticular injections and intraosseous infiltrations of blood-derived BDDT. **C)** Blood-derived BDDT has been proposed as a bridge from spontaneity to molecular intervention in muscle tear repair. Early intramuscular injection performed with ultrasound guidance allocates the product into interfascicular and interfibrillar space, and into the injured site. Local fibrinolysis acts on fibrin scaffold gradually releasing GFs and cytokines. **D)** Peripheral nerve regeneration relies on angiogenesis and Schwann cell transdifferentiation, whose exploratory behavior drives spontaneous nerve regeneration. Ultrasound-guided intraneural infiltrations of blood-derived BDDT combined with the application of a fibrin scaffold wrap the injured area and enhance nerve regeneration[22].
(Reprinted with permission from Padilla et al.[22])

cal therapy, corticosteroid injections, non-steroidal anti-inflammatory drugs, extracorporeal shock wave therapy and surgical interventions–has provided a successful long-term solution[39].Tendon is a hypo-cellular dynamic mechanosensitive composite structure that harbors immunocompetent and tendon multipotent stem/progenitor cells (TDSCs)[40], the key targets of blood-derived BDDTs (Figure 4 A, Box 3). The anabolic/catabolic balance of stromal and parenchymal cells shift according to its mechanical loading history (Figure 2). Together with other highly specialized biological structures, tendons are extremely effective at performing the roles for which they have evolved while yet remaining particularly delicate. The fragile balance that their cells maintain with the extracellular matrix can be disrupted in different ways. This is the case, for instance, for high level sports activities with high performance demands and damage from overuse, or, in the opposite sense, the case of a sedentary life style and the subsequent damage from disuse. Acute and chronic tendon

injuries result in pain, focal tenderness, and a decrease in strength and movement stemmed from an inflammed and/or ruptured tendon. Blood-derived autologous bio-scaffolds have been used for a number of years as a raw material in tissue-engineered constructions, either alone in liquid or matrix formulations, or enriched with mechanical or chemical signals, and its application has been a product of in vitro and in vivo research which has provided a better understanding of tenocytes and TDSC response to blood-derived BDDT[41-43] (Box 3, Figure 4 A).

Blood-derived BDDT has been applied either as US-guided intratendinous or intraligament infiltrations on chronic patellar tendinopathy and acute ankle sprain[44, 45] respectively or as intratendinous infiltrations combined with fibrin scaffolds intraoperatively on surgically repaired Achilles tendons tears[43] (Figure 4 A). In both situations, results showed an improvement of symptoms and function, a return to normal architecture of tendon and syndesmosis assessed by MRI, and a shorter time in the recovery of motion and return to sporting activities[43]. Moreover, the application of blood-derived bio-scaffold on the tendon graft or the donor-site level after anterior cruciate ligament reconstruction was found to significantly reduce clinical symptoms and accelerate the process of remodelling and integration of the graft, in addition to satisfactorily filling the gap and reconstructing patella tendon and tibial and patella bone gap[46-48].

Muscle and Nerve tissues

Two other highly specialized tissues that form a functional unit in the musculoskeletal system are muscles and nerves, and several parallels can be drawn between their regeneration processes (Box 4). In acute muscle injuries, blood-derived BDDT liquid is infiltrated into the injury site, adjacent areas and peripheral healthy muscle under ultrasound guidance. In the ensuing 1-3 minutes, this liquid-to-gel transition 3D injectable scaffold allows a successful filling of the muscle gaps and defects and serves as a highway for mechanical energy to transit from the environment to the cell, thereby bridging cell-to-cell tissue transition, promoting multi-cellular assembly and targeting muscle stem and immunocompetent cells, providing chemical signals, mechanical support and plastic-elastic stiffness which not only has a drastic impact on fates of muscle stem cells, but also endows tissues with a suitable mechanical and chemical microenvironment for biological restoration[49] (Figure 1, and 4 C). This dynamic sponge-like fibrin-matrix biological drug delivery therapy is autologous, bio-reabsorbable, and biocompatible[12]. The application of blood-derived BDDT has been shown to shorten the recovery time and even to reduce pain in the case of human application[50]. Notable here is another recent study which reported no benefit of this therapeutic approach[51]. However, it is quite possible that the source of inconsistent clinical outcomes in muscle injuries treated with blood-derived BDDT could well be derived from the delayed administration, low dosage, and the heterogeneity of the blood-derived BDDT biological composition itself, for which there is yet no standard protocol[52]. In order to characterize, standardize, and tailor the composition of blood-derived BDDT to the specific cellular target and tissue repair processes, several in vitro and in vivo efforts are ongoing with an emphasis on applying only plasmatic factors or modifying its composition[53] by blocking TGFB as a fibrotic factor or depleting the myostatin (MSTN) with the goal of enhancing myoblast differentiation[54, 55].

In the management of peripheral nerve injury (PNI) and fuelled by the drawbacks posed by autologous nerve autografts, a great deal of biomedical engineering strategies have been applied, including nerve guidance conduits and scaffolds, incorporation and delivery of neurotrophic factors[56], incorporation of support cells into nerve guidance conduits (NGCs) or fibrin gels, and stimulation of target organs through intramuscular injections of GFs[56, 57]. In animals, platforms using fibrin scaffolds bathed in a cocktail of growth factors and injected or placed into the damaged area enhance the axonal growth necessary to achieve optimal functional recovery[56, 58].(Box 4). In humans, the molecular intervention with blood-derived BDDT is partially bridging the gap between the basic and

clinical application, and in a double-blind, randomized, clinical trial, the application of US-guided blood-derived BDDT injections in tibial and ulnar nerves has shown sensory improvement in leprosy peripheral neuropathy[59]. In addition, several case studies applying blood-derived biological drug delivery therapies either as a filler of nerve conduits across nerve gaps post trauma[60] or by infiltrating intraneurally in a peroneal nerve palsy[61], have reported neurological recovery (Figure 4 D). Therefore, blood-derived BDDT applied in a combinatorial strategy as a filler, suturable membrane, and scaffold, stand out as a promising candidate for an adjuvant nerve repair approach which can be harnessed by surgeons in the operating room and in the clinical setting.[61, 62] (see chapter 16).

5. FUTURE DIRECTIONS

The daunting complexity of many musculoskeletal tissues targeted by blood-derived BDDT, coupled with misleading factors associated with the regulatory, clinical and commercial contexts, adds up to multiple barriers to further product development and progress. In the last few years, many aspects related to the technological, biological and pharmaceutical fields have been addressed, including strategies for in vitro characterization of drug release, regulatory processes for in-situ drug preparation[63], minimizing manipulation, and preparing devices that enhance safety and versatility of blood-based biological drug delivery therapies. Moreover, efforts in academia and the biotechnology industry to rapidly translate basic to clinical applications tend to overtake our basic-science understanding of the biological roles of this therapy.

Fortunately, there are reasons for optimism. Novel formulations and fabrication methods are likely to help broaden the catalogue of blood-based BDDT applications. Designing operator-free technologies for BDDT fabrication together with the use of new technologies of additive manufacturing, or 3D bioprinting, may help to control the final properties of the autologous preparations. Efforts must continue to expand the science behind the current generation of blood-derived BDDT. The exploration of its potential for the ex vivo expansion of mesenchymal stem cells, together with the value of fibrin scaffolds for stem cell handling and transplantation, may also reduce some of the challenges faced in the field. Finally, homologous blood-derived BDDT may become an alternative to patients whose blood components such as plasma, platelets, or fibrinogen lack several regenerative key inductors. As a result of these and other advances, the safe clinical implementation of blood-derived BDDT is expected to accelerate and expand.

BOX 1

Biopharmaceutical considerations about biological drug delivery therapies

The therapeutic success of a medicine does not only rely on the type and number of drugs (biologically active mediators) but also on the manner and timing of their delivery to the tissue. When drugs are released without control over their location or rate of delivery, large doses are needed to achieve the desired biological effects, leading to increased toxicity or undesirable side effects. Blood-derived biological drug delivery therapies (BDDT) are based on a combination of naturally derived biomaterials such as fibrin and a pool of growth factors. For example, the three-dimensional fibrin scaffold obtained from fractionated human plasma represents a physiologically inspired solution to control the release of the wide range of plasma and platelet-derived mediators[64]. In practice, the release profile is a combination of diffusion and degradation of the matrix. Diffusion controls the release of the biologically active agents when it is slow compared with the rate of drug dissociation from the material, yet it happens much faster than material degradation[65]. Matrix degradation is preferentially regulated by hydrolytic cleavage of the carrier body and by enzymatic degradation. In this way, growth factors are progressively released and the fibrin scaffold acts as a temporary matrix for the new growing tissue (figure 1).

BOX 2

Harnessing endogenous repair process of joint tissues

Articular cartilage (AC) is a tissue that is remarkably resilient to compressive and shearing forces. Yet it is highly fragile to alterations of the synovial membrane (SM) and subchondral bone (SCB), two well-vascularized tissues from where systemic and local inflammation insults arise[66]. These aggressions are mediated by pro-inflammatory cytokines and inflammatory macrophages and synoviocytes, which damage articular cartilage as in the case of rheumatoid arthritis or osteoarthritis[66]. However, SM and SCB are also the egress point and source of nutrients and mesenchymal progenitor cells for mounting a chondrogenic reparative response, which is driven by the recruitment and chemotactic homing of synovium and bone marrow-derived stem cells mediated by SDF-1, TGFB, and fibronectin. This is the case in microfracture techniques and in the combined strategy using intraarticular (IA) and intraosseous (IO) infiltrations of blood-derived BDDT[26, 28]. In doing so, this novel local BDDT tackles the four synovial joint tissues—AC, SF, SM, and SCB—and acts as a dynamic autologous liquid scaffold that, in a sustained and gradual manner, conveys chemotactic endogenous MSC homing and chondrogenic factors such as SDF-1, TGFB, and fibronectin[27, 28, 30, 67]. In addition, this BDDT dampens inflammatory stress at the level of joint tissues, by both inhibiting the NFkB on chondrocytes and macrophages[68] and up-regulating the antioxidant response element NF-E2-related factor 2 (NrF2-ARE) pathway in osteoblasts[69]. Improvements of clinical outcomes in patients with knee and hip OA were reported applying this strategy[28, 36], which might primarily be mediated by HGF, CTGF, IGF-1, PDGF, among others[68-71], thereby paving the way to cartilage regeneration, however elusive it may remain (figure 3).

BOX 3

Adaptation, inflammation, and homeostatic process in tendon.

There is increasing evidence showing that tendon and ligament adaptation, injury, and repair processes share several intracellular pathways, and although it is difficult to draw the line between the cellular and molecular responses that lead to either tissue adaptation or tissue damage, inflammatory process appear to be at the interface of tendon adaptation and damage (figure 2)[39, 68]. Repetitive mechanical loading, as is the case in early stages of tendinopathy, and tendon overuse induce the activation of NFkB and thereby the synthesis of matrix metalloproteinases (MMPs), two isoforms of cyclooxygenase (COX) COX-1 and COX-2, and PGE2 by inflammatory tenocytes, mast cells and other immunocompetent cells[39, 72-74]. PGE2 is a major systemic and local inflammatory mediator that decreases the production of collagen and causes aberrant differentiation of TDSCs into adipogenic and osteogenic lineages [74], which might partially account for the presence of fibrocartilage, calcifications and adipose tissue in injured and chronic degenerative tendons[39, 40, 72, 74].

An excellent series of in vitro and in vivo studies demonstrated that blood-derived BDDT induced tenocyte proliferation, stimulated the synthesis of type I collagen and the neovascularization [42], promoted differentiation of TDSCs into active tenocytes, but significantly, the addition of leukocytes into the releasate increased the synthesis of PGE2 and the gene expression of metalloproteinase-1 (MMP-1), MMP-13, interleukin-1B (IL-1B), and decreased the expression of alfa-SMA as a marker of active tenocytes[73-75]. Among the myriad mediators conveyed by blood-derived BDDT, hepatocyte growth factor (HGF), and lipoxin A4 (LX4) have been shown to exert an anti-inflammatory and pro-resolution of inflammation effect on injured tendons (Figure 1 D and Figure 2 A)[73, 76].

BOX 4

Blood-derived BDDT application on muscle and nerve pathologies

Early inflammation, muscle satellite and stem cell-like myelinating Schwann cell activation, angiogenesis, and macrophage polarization, are key drivers of full function recovery, where growth factors (GFs) and the fibrin scaffold are instrumental instructive and permissive factors[77-80]. In the full reconstruction of muscle tissue, endothelial and muscle satellite cells, together with macrophages and other myogenic progenitor cells, signal reciprocally primarily by VEGF, PDGF, IGF-1, and HGF, making angiogenesis, myogenesis and neurogenesis proceed concomitantly[77, 80]. In extensive in vitro and in vivo preclinical studies, the combination of the aforementioned GFs or the use of blood-derived BDDT promoted an earlier regeneration of damaged muscles mainly by the modulation of inflammatory response, a reliable angiogenic stimulus, a significant expansion of the myogenic pool, and a macrophage polarization from an inflammatory to a trophic phenotype[79-81]. These biological effects prevented the formation of aberrant repair and fibrosis, which would otherwise result in clinical muscle relapses (figure 4 C).

In the management of peripheral nerve injury (PNI) blood-derived BDDT has emerged as a novel and versatile adjuvant approach. Once infiltrated intraneurally as a liquid-to-gel injectable scaffold, or wrapped around the injured nerve gap as a matrix-like viscous and malleable structure, or both, [58] (Figure 4 D) tissue fibrinolysis breaks the fibrin down, thereby releasing cell signalling molecules such as neurotrophic (NGF, BDGF, IGF-1, PDGF, VEGF, HGF) and neurotropic factors (fibrin, fibronectin, and vitronectin)[82]. These biomolecules govern early inflammation, stem cell-like myelinating Schwann cell activation, angiogenesis, macrophage polarization, as well as the active resolution of inflammation, angiogenesis, and fibrogenesis, thereby acting as key drivers of full nerve function recovery[56, 77].

1. Anitua, E. et al. (2007) The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials. 28, 4551-4560

2. Langer, R. and Vacanti, J.P. (1993) Tissue engineering. Science. 260, 920-926

3. Murphy, C.M. et al. (2013) Cell-scaffold interactions in the bone tissue engineering triad. Eur Cell Mater. 26, 120-132

4. Anitua, E. et al. (2004) Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 91, 4-15

5. Langer, H.F. et al. (2009) Platelet derived bFGF mediates vascular integrative mechanisms of mesenchymal stem cells in vitro. J Mol Cell Cardiol. 47, 315-325

6. Stellos, K. et al. (2010) Platelets in regeneration. Semin Thromb Hemost. 36, 175-184

7. Gawaz, M. and Vogel, S. (2013) Platelets in tissue repair: control of apoptosis and interactions with regenerative cells. Blood. 122, 2550-2554

8. Hollister, S.J. and Murphy, W.L. (2011) Scaffold translation: barriers between concept and clinic. Tissue Eng Part B Rev. 17, 459-474

9. Anitua, E. et al. (2015) Clinical, radiographical, and histological outcomes of plasma rich in growth factors in extraction socket: a randomized controlled clinical trial. Clin Oral Investig. 19, 589-600

10. Anitua, E. et al. (2008) Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. J Biomed Mater Res B Appl Biomater. 84, 415-421

11. Anitua, E. et al. (2015) High-throughput proteomic characterization of plasma rich in growth factors (PRGF-Endoret)-derived fibrin clot interactome. J Tissue Eng Regen Med. 9, E1-12

12. Anitua, E. and Orive, G. (2012) Endogenous regenerative technology using plasma- and platelet-derived growth factors. J Control Release. 157, 317-320

13. Xiao, G.H. et al. (2001) Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. Proc Natl Acad Sci U S A. 98, 247-252

14. Aoki, M. et al. (2000) Angiogenesis induced by hepatocyte growth factor in non-infarcted myocardium and infarcted myocardium: up-regulation of essential transcription factor for angiogenesis, ets. Gene Ther. 7, 417-427

15. Okunishi, K. et al. (2005) A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. J Immunol. 175, 4745-4753

16. Massberg, S. et al. (2006) Platelets secrete stromal cell-derived factor 1alpha and recruit bone marrow-derived progenitor cells to arterial thrombi in vivo. J Exp Med. 203, 1221-1233

17. Stellos, K. et al. (2008) Platelet-derived stromal cell-derived factor-1 regulates adhesion and promotes differentiation of human CD34+ cells to endothelial progenitor cells. Circulation. 117, 206-215

18. Wang, J.H. and Li, B. (2010) Mechanics rules cell biology. Sports Med Arthrosc Rehabil Ther Technol. 2, 16

19. Ingber, D.E. (2006) Cellular mechanotransduction: putting all the pieces together again. Faseb j. 20, 811-827

20. *Kjaer, M. (2004) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev. 84, 649-698*

21. *Riley, G.P. (2005) Gene expression and matrix turnover in overused and damaged tendons. Scand J Med Sci Sports. 15, 241-251*

22. *Padilla S, S.M., Orive G, Anitua E. Human-based biological and biomimetic autologous therapies for muskuloskeletal tissue regeneration. Trends Biotechnol. In press. http://dx.doi.org/10.1016/j.tibtech.2016.09.008*

23. *Ivkovic, A. et al. (2011) Regenerative medicine and tissue engineering in orthopaedic surgery. Front Biosci (Elite Ed). 3, 923-944*

24. *Wagers, A.J. (2012) The stem cell niche in regenerative medicine. Cell Stem Cell. 10, 362-369*

25. *Cosgrove, B.D. et al. (2014) Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. Nat Med. 20, 255-264*

26. *Steadman, J.R. et al. (1997) Microfracture technique forfull-thickness chondral defects: Technique and clinical results. Operative techniques in orthopaedics. 7, 300-304*

27. *Liu, H.Y. et al. (2014) Delayed animal aging through the recovery of stem cell senescence by platelet rich plasma. Biomaterials. 35, 9767-9776*

28. *Sanchez, M. et al. (2016) A new strategy to tackle severe knee osteoarthritis: Combination of intra-articular and intraosseous injections of Platelet Rich Plasma. Expert Opin Biol Ther. 16, 627-643*

29. *Campbell, T.M. et al. (2016) Mesenchymal Stem Cell Alterations in Bone Marrow Lesions in Patients With Hip Osteoarthritis. Arthritis Rheumatol. 68, 1648-1659*

30. *Kreuz, P.C. et al. (2015) Platelet-Rich Plasma Preparation Types Show Impact on Chondrogenic Differentiation, Migration, and Proliferation of Human Subchondral Mesenchymal Progenitor Cells. Arthroscopy. 31, 1951-1961*

31. *Zhu, Y. et al. (2013) Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review. Osteoarthritis Cartilage. 21, 1627-1637*

32. *Milano, G. et al. (2010) The effect of platelet rich plasma combined with microfractures on the treatment of chondral defects: an experimental study in a sheep model. Osteoarthritis Cartilage. 18, 971-980*

33. *Lee, H.R. et al. (2012) Platelet-rich plasma loaded hydrogel scaffold enhances chondrogenic differentiation and maturation with up-regulation of CB1 and CB2. J Control Release. 159, 332-337*

34. *Xie, X. et al. (2014) Biology of platelet-rich plasma and its clinical application in cartilage repair. Arthritis Res Ther. 16, 204*

35. *Chiang, C.-W. et al. (2014) Application of Synovial Fluid Mesenchymal Stem Cells: Platelet-rich Plasma Hydrogel for Focal Cartilage Defect. Journal of Experimental & Clinical Medicine. 6, 118-124*

36. *Sanchez, M. et al. (2012) Ultrasound-guided platelet-rich plasma injections for the treatment of osteoarthritis of the hip. Rheumatology (Oxford). 51, 144-150*

37. *Sanchez, M. et al. (2012) A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. Arthroscopy. 28, 1070-1078*

38. *Sánchez, M. et al. (2016) Combination of Intra-Articular and Intraosseous Injections of Platelet Rich Plasma for Severe Knee Osteoarthritis: A Pilot Study. BioMed Research International. 2016*

39. *Dakin, S.G. et al. (2015) Inflammation activation and resolution in human tendon disease. Sci Transl Med. 7, 311ra173*

40. *Bi, Y. et al. (2007) Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med. 13, 1219-1227*

41. Crowe, C.S. et al. (2015) Tendon regeneration with a novel tendon hydrogel: in vitro effects of platelet-rich plasma on rat adipose-derived stem cells. Plastic and reconstructive surgery. 135, 981e-989e

42. Anitua, E. et al. (2006) Autologous fibrin matrices: a potential source of biological mediators that modulate tendon cell activities. J Biomed Mater Res A. 77, 285-293

43. Sanchez, M. et al. (2007) Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med. 35, 245-251

44. Charousset, C. et al. (2014) Are multiple platelet-rich plasma injections useful for treatment of chronic patellar tendinopathy in athletes? a prospective study. Am J Sports Med. 42, 906-911

45. Laver, L. et al. (2015) Plasma rich in growth factors (PRGF) as a treatment for high ankle sprain in elite athletes: a randomized control trial. Knee Surg Sports Traumatol Arthrosc. 23, 3383-3392

46. Sanchez, M. et al. (2010) Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. Arthroscopy. 26, 470-480

47. de Almeida, A.M. et al. (2012) Patellar Tendon Healing With Platelet-Rich Plasma A Prospective Randomized Controlled Trial. The American journal of sports medicine. 40, 1282-1288

48. Cervellin, M. et al. (2012) Autologous platelet-rich plasma gel to reduce donor-site morbidity after patellar tendon graft harvesting for anterior cruciate ligament reconstruction: a randomized, controlled clinical study. Knee Surg Sports Traumatol Arthrosc. 20, 114-120

49. Sanchez, M. et al. (2014) Muscle repair: platelet-rich plasma derivates as a bridge from spontaneity to intervention. Injury. 45 Suppl 4, S7-14

50. Hamid, M.S.A. et al. (2014) Platelet-rich plasma injections for the treatment of hamstring injuries a randomized controlled trial. The American journal of sports medicine. 42, 2410-2418

51. Reurink, G. et al. (2014) Platelet-rich plasma injections in acute muscle injury. N Engl J Med. 370, 2546-2547

52. Anitua, E. et al. (2014) More on platelet-rich plasma injections in acute muscle injury. N Engl J Med. 371, 1264

53. Anitua, E. et al. (2009) Fibroblastic response to treatment with different preparations rich in growth factors. Cell Prolif. 42, 162-170

54. Li, H. et al. (2016) Customized platelet-rich plasma with transforming growth factor beta1 neutralization antibody to reduce fibrosis in skeletal muscle. Biomaterials. 87, 147-156

55. Miroshmychenko, O. et al. (2016) The use of platelet-rich and platelet-poor plasma to enhance differentiation of skeletal myoblasts: Implications for the use of autologous blood products for muscle regeneration. AAOS. Stanford Sports Medicine. July 7-10. The Broadmoor, Colorado.

56. Lu, P. et al. (2012) Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell. 150, 1264-1273

57. Pfister, B.J. et al. (2011) Biomedical engineering strategies for peripheral nerve repair: surgical applications, state of the art, and future challenges. Crit Rev Biomed Eng. 39, 81-124

58. Sanchez, M. et al. (2015) Ultrasound-guided plasma rich in growth factors injections and scaffolds hasten motor nerve functional recovery in an ovine model of nerve crush injury. J Tissue Eng Regen Med. DOI: 10.1002/term.2079

59. Anjayani, S. et al. (2014) Sensory improvement of leprosy peripheral neuropathy in patients treated with perineural injection of platelet-rich plasma. Int J Dermatol. 53, 109-113

60. Kuffler, D.P. (2014) Promoting peripheral axon regeneration across nerve gaps post trauma. JSM Neurosurg Spine. 2, 1044

61. Sanchez, M. et al. (2013) Ultrasound-guided platelet-rich plasma injections for the treatment of common peroneal nerve palsy associated with multiple ligament injuries of the knee. Knee Surg Sports Traumatol Arthrosc.

62. Kuffler, D.P. (2014) An assessment of current techniques for inducing axon regeneration and neurological recovery following peripheral nerve trauma. Prog Neurobiol. 116, 1-12

63. Anitua, E. et al. (2015) Closing regulatory gaps: new ground rules for platelet-rich plasma. Trends Biotechnol. 33, 492-495

64. Ahmed, T.A. et al. (2008) Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng Part B Rev. 14, 199-215

65. Kearney, C.J. and Mooney, D.J. (2013) Macroscale delivery systems for molecular and cellular payloads. Nat Mater. 12, 1004-1017

66. Scanzello, C.R. and Goldring, S.R. (2012) The role of synovitis in osteoarthritis pathogenesis. Bone. 51, 249-257

67. Liu, H.Y. et al. (2011) The balance between adipogenesis and osteogenesis in bone regeneration by platelet-rich plasma for age-related osteoporosis. Biomaterials. 32, 6773-6780

68. Bendinelli, P. et al. (2010) Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-kappaB inhibition via HGF. J Cell Physiol. 225, 757-766

69. Tohidnezhad, M. et al. (2014) Role of platelet-released growth factors in detoxification of reactive oxygen species in osteoblasts. Bone. 65, 9-17

70. Coudriet, G.M. et al. (2010) Hepatocyte growth factor modulates interleukin-6 production in bone marrow derived macrophages: implications for inflammatory mediated diseases. PLoS One. 5, e15384

71. Montaseri, A. et al. (2011) IGF-1 and PDGF-bb suppress IL-1beta-induced cartilage degradation through down-regulation of NF-kappaB signaling: involvement of Src/PI-3K/AKT pathway. PLoS One. 6, e28663

72. Yang, G. et al. (2005) Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. Gene. 363, 166-172

73. Zhang, J. et al. (2013) HGF mediates the anti-inflammatory effects of PRP on injured tendons. PLoS One. 8, e67303

74. Zhang, J. and Wang, J.H. (2010) Production of PGE(2) increases in tendons subjected to repetitive mechanical loading and induces differentiation of tendon stem cells into non-tenocytes. J Orthop Res. 28, 198-203

75. Zhou, Y. et al. (2015) The differential effects of leukocyte-containing and pure platelet-rich plasma (PRP) on tendon stem/progenitor cells - implications of PRP application for the clinical treatment of tendon injuries. Stem Cell Res Ther. 6, 173

76. Dakin, S.G. et al. (2012) Inflamm-aging and arachadonic acid metabolite differences with stage of tendon disease. PLoS One. 7, e48978

77. Cattin, A.L. et al. (2015) Macrophage-Induced Blood Vessels Guide Schwann Cell-Mediated Regeneration of Peripheral Nerves. Cell. 162, 1127-1139

78. Tidball, J.G. and Villalta, S.A. (2010) Regulatory interactions between muscle and the immune system during muscle regeneration. Am J Physiol Regul Integr Comp Physiol. 298, R1173-1187

79. Dimauro, I. et al. (2014) Platelet-rich plasma and skeletal muscle healing: a molecular analysis of the early phases of the regeneration process in an experimental animal model. PLoS One. 9, e102993

80. Anitua, E. et al. (2015) Infiltration of plasma rich in growth factors enhances in vivo angiogenesis and improves reperfusion and tissue remodeling after severe hind limb ischemia. J Control Release. 202, 31-39

81. Borselli, C. et al. (2010) Functional muscle regeneration with combined delivery of angiogenesis and myogenesis factors. Proc Natl Acad Sci U S A. 107, 3287-3292

82. Anitua, E. et al. (2015) High-throughput proteomic characterization of plasma rich in growth factors (PRGF-Endoret)-derived fibrin clot interactome. J Tissue Eng Regen Med. 9, E1-E12