Trends in Biotechnology

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Opinion

Human-Based Biological and Biomimetic Autologous Therapies for Musculoskeletal Tissue Regeneration

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Repairing and regenerating damaged musculoskeletal tissues is one of the greatest challenges in regenerative medicine. Blood contains the essential ingredients to biologically engineer drug delivery devices that provide spatiotemporal control over the availability of a wide range of autologous agents, including small molecules, cytokines, and growth factors. This opinion article summarizes our current knowledge of blood-derived biological drug delivery therapies. The potential mechanisms that control protein release are discussed, along with the biological rationale and effects of their use in different relevant musculoskeletal tissues, including articular cartilage, bone, tendon, muscle, and nerve tissue injuries. Finally, we finally describe the current challenges facing the field and recent advances that should drive novel solutions for the musculoskeletal system.

Personalized Medicine in Musculoskeletal Regeneration

An enormous clinical need exists for designing and developing technologies to promote the regeneration of injured or diseased tissues and organs. This demand is particularly great in the case of chronic and acute musculoskeletal conditions, including osteoarthritis, degenerative disc disease, bone, cartilage, tendon, ligament trauma, and other injuries such as cancer or infection. The treatment of these conditions involves 34 million surgical procedures per year in the USA alone [1]. To address these challenges, the rapidly growing field of personalized regenerative medicine seeks to replace, engineer, or regenerate human cells, tissues, or organs using autologous biological materials to restore or establish normal function. As an example of this expanding field, the biotech industry has already used regenerative medicine products in more than 300 000 patients [2].

Interestingly, the field of regenerative medicine seems likely to move away from more traditional and simple small-molecule **drugs** (see Glossary) toward increasingly complex and multimolecular **biological drug delivery therapies (BDDTs)**. The last two decades have seen an explosion in our understanding and use of blood-derived BDDTs in the treatment of musculoskeletal conditions. In fact, blood 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** [3]. This opinion article highlights our current knowledge of BDDTs. The potential mechanisms that control protein release are introduced. We then describe the biological rationale and effects of their

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Plasma and especially platelets contain an enormous amount of growth factors, cytokines, and chemokines that regulate fundamental mechanisms involved in the tissue repair and regeneration processes. These include cell proliferation, migration, synthesis of extracellular matrix components, apoptosis/cell survival, angiogenesis, and regulation of inflammation.

A fibrin scaffold obtained from a donor's blood acts as a biologically engineered matrix to exert spatiotemporal control over the release of the bioactive ingredients at the site of administration.

Blood-derived biological drug delivery therapies provide dosage form versatility over drug availability. They can be administered topically, as an eye-drop, or by subcutaneous, intradermal, and intramuscular injections. Moreover, they can be shaped as a pure liquid, an *in situ* gelling liquid, or a three-dimensional fibrin scaffold, thus increasing their therapeutic potential.

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use in different relevant musculoskeletal tissue injuries including tendon, ligament, cartilage, and muscle. Finally, we discuss current challenges facing the field as well as recent advances that should drive novel solutions for the musculoskeletal system.

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Blood-Derived BDDTs

Blood contains a wide range of biological elements that influence the development of functional substitutes for damaged tissues. It contains the basic concepts of the **tissue engineering** triad: cells, growth factors, and **scaffold**-forming elements [4]. In fact, 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, as blood-derived fibrin scaffolds are enriched in patient's own growth factors and cytokines providing cues to direct endogenous cells, including stem cells, to sites of repair [5].

Platelets constitute the essential biological elements within blood-derived BDDTs. 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 [6]. The multitude of released cues exerts complex biological effects that drive tissue regeneration. For example, platelet-derived factors modulate the activation of fibroblasts; induce proliferation and migration of cells critically involved in tissue repair, such as smooth muscle cells and mesenchymal stem cells (MSCs) [7]; regulate angiogenesis, a pivotal process for recovery of tissue function [8]; and may regulate apoptosis and survival of cells by means of released platelet microparticles [9]. Thus, blood-derived BDDTs, which are enriched in the platelet secretome, may successfully be used as feasible and autologous therapeutic tools, promoting the healing and repair of injured tissues.

The fibrin scaffold from blood-derived BDDT consists primarily of enriched fibrinogen, thrombin, and calcium and coagulation factors. Interestingly, it fulfills the critical abilities that a scaffold must have, including form, fixation, and formation [10]. For a scaffold to have form, it should be able to fill the space it is designed to fill. Blood-derived BDDTs are used therapeutically to fill gaps including ulcers, bone defects, or dental alveolus [11,12]. Another key property is fixation: the ability of a scaffold to integrate and attach to the surrounding microenvironment. Fibrin scaffolds obtained from blood 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 classification of the proteins into families and networks according to gene ontology, more than 40 proteins specifically involved in tissue regeneration and wound healing were identified [13]. Finally, the fibrin scaffold is able to drive the formation of the intended tissue.

Interestingly, blood-derived BDDT 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 [3] (Figure 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 feature.

Box 1. Biopharmaceutical Considerations about BDDT

The therapeutic success of a medicine relies not only on the type and number of drugs (biologically active mediators) but also on when and how they are delivered 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 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 a wide range of plasma and platelet-derived mediators [61]. 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 [62]. 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.

Box 2. The Biological Defense System: Hemostasis and Clotting, the Immune System, and Fibrogenesis

In response to physiological or repetitive mechanical stress and trauma that disrupt tissue homeostasis and damage blood vessels, the overlapping activation of hemostasis and clotting, the innate immune system, and fibrogenesis results in a healing process [63]. Key in these defense modules is the interplay between multiple lineages of cells, including blood-derived platelets, neutrophils, macrophages, and tissue stromal and parenchymal fibroblasts; stromal mesenchymal stem cells; and postmitotic cells such as chondrocytes, tenocytes, myocytes, endothelial cells, and **Schwann cells**, among others [64]. These cells sense damage by expressing trans-membrane **Toll-like receptors (TLRs)** that recognize **damage-associated molecular patterns (DAMPs)** as ligands, including blood extravasation elements, extracellular matrix (ECM) degradation products, and cell dying proteins [65]. This DAMP–TLR interaction activates the **intranuclear factor kappa B pathway**, which induces the gene expression of growth factors, cytokines, and prostaglandins [65]. The acute storm of **vascular endothelial growth factor**, **platelet-derived growth factor**, transforming growth factor-β, **connective tissue growth factor**, hepatocyte growth factor, **insulin-like growth factor 1**, interleukin 1β (IL-1β), IL-6, IL-10, nitric oxide, **prostaglandin E_2**, and tumor necrosis factor-α, among other inflammatory mediators, acts as key factors [65]. These factors govern the initial inflammation, angiogenesis, macrophage activation and polarization, cell fates and progenitor stem cell differentiation, and fibrogenesis, as well as the active resolution of inflammation, angiogenesis, and fibrogenesis [64]. This sterile inflammatory cell–cell, and cell–ECM, interaction landscape is the common ground of cartilage destruction and osteoarthritis, tendinopathies, muscle and ligament strains, bone fracture, or nerve compression or transection (see Figure 1B,C in main text).

Opponents to blood-derived BDDT hold that these therapies may be useful but that the mechanisms of tissue repair remain poorly understood. However, our progressing knowledge of biology and molecular biology is alleviating some of these concerns [14]. For example, platelets within the BDDT release agents such as **hepatocyte growth factor** and **stromal cell-derived growth factor 1**, which are known to control proliferation, recruitment, and activation of cell types critically involved in wound healing and tissue regeneration. In particular, hepatocyte growth factor exerts antiapoptotic [15], proangiogenic [16], and immunosuppressive activity [17] and promotes recruitment of MSCs to human arterial endothelial cells [7]. Stromal cell-derived growth factor 1 stimulates progenitor cell recruitment to arterial thrombi and differentiation of the cells to endothelial progenitors *in vivo* [18,19] (Figure 1C).

Implications of Blood-Derived BDDT in Musculoskeletal Conditions

Thanks to a deeper understanding of molecular and cellular processes going on in musculoskeletal pathologies, orthopedic surgery is going through a serious paradigm shift: instead of simply removing and replacing damaged tissue with artificial devices and materials, blood-derived BDDT is aimed at triggering and enhancing the natural *in vivo* tissue morphogenesis and regenerative capacity of damaged tissue [20] (Figure 1B–D and Box 2).

With this idea 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 derived from platelets or plasmatic proteins are candidates for mammalian tissue rejuvenation and healing [21–25].

Glossary

Biological drug delivery therapies: multimolecular autologous therapies derived from a patient's blood.
Connective tissue growth factor: a protein of the transforming growth factor-β superfamily released under mechanical stimuli that regulates fibrogenesis and angiogenesis.
Cues: stimuli that guide the growth of new tissues.
Damage-associated molecular patterns: host molecules released as a result of tissue and cell damage.
Drug: plasma and especially platelets contain an enormous amount of growth factors, cytokines, and chemokines that regulate fundamental mechanisms involved in tissue repair and regeneration processes, including cell proliferation, migration, synthesis of extracellular matrix components, apoptosis/cell survival rate, angiogenesis, and regulation of inflammation.
Drug delivery: a fibrin scaffold obtained from a donor's blood acts as a biologically engineered matrix to exert spatiotemporal control over the release of the bioactive ingredients at the site of administration.
Extracellular matrix: a complex three-dimensional cell-secreted structure that provides support, along with biochemical and biological signals, to cells and tissues.
Growth factors: molecules that regulate a variety of cellular functions, such as cell growth, proliferation, migration, and differentiation.
Hepatocyte growth factor: a powerful mitogen, motogen, and morphogen cytokine with pleiotropic effects on a variety of cell phenotype fates.
Insulin-like growth factor 1: a protein that regulates several key cellular processes, including growth and apoptosis.
Intranuclear factor kappa B pathway: a highly conserved intracellular signaling pathway whose activation induces tissue inflammation.
Intraosseous infiltration: a minimally invasive surgical procedure for delivering drugs into the bone marrow.
Lipoxin A4: a fatty acid present within platelets and an active mediator in the resolution of inflammation.
Mesenchymal progenitor cells: multipotent stromal cells that can

Box 3. Harnessing Endogenous Repair Process of Joint Tissues

Articular cartilage 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 proinflammatory 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 stromal cell-derived factor 1 (SDF-1), transforming growth factor-β (TGF-β), and fibronectin. This is the case in microfracture techniques and in the combined strategy using intra-articular and intraosseous infiltrations of blood-derived BDDT [23,25]. In doing so, this novel local BDDT tackles the four synovial joint tissues – articular cartilage, synovial fluid, 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, TGF-β, and fibronectin [24,25,27,67]. In addition, this BDDT dampens inflammatory stress at the level of joint tissues, by both inhibiting the nuclear factor-kB on chondrocytes and macrophages [68] and upregulating the antioxidant response element **NF-E2-related factor 2 (NrF2-ARE) pathway** in osteoblasts [69]. Improvements of clinical outcomes of patients with knee and hip osteoarthritis were reported applying this strategy [25,33], which might primarily be mediated by hepatocyte growth factor, connective tissue growth factor, insulin-like growth factor 1, and platelet-derived growth factor, among others [68–71], thereby paving the way to cartilage regeneration, however elusive it remains.

Articular Cartilage

The treatment of synovial joint pathologies remains daunting. These pathologies include traumatic osteochondral defects, osteochondritis dissecans, osteonecrosis, bone marrow edema-like lesions, and osteoarthritis; their ultimate victim appears to be the articular cartilage, with the subchondral bone as the culprit [26]. Current therapeutic strategies are oriented toward harnessing the endogenous repair response by stimulating bone marrow cells through drilling and microfracturing approaches, the transplantation of osteochondral autografts and osteochondral allografts, joint distraction, and the implantation of autologous chondrocytes. In this complex therapeutic landscape, blood-derived BDDTs emerge as a promising adjuvant autologous biomaterial with trophic-anabolic, anti-inflammatory, immunomodulatory, antioxidative, and analgesic effects on joint tissues (Box 3) [25]. Their versatility makes these products optimal biomaterials to be applied at the dysfunctional and deregulated injured site as a niche therapy [21,25] (Figure 2B). They may be applied either alone, harnessing the proliferative, migratory, and chondrogenic effect on endogenous **mesenchymal progenitor cells** [27], or as a carrier of stem cells and/or extracellular components, such as collagens and hyaluronic acid [28]. Animal model studies suggest that autologous blood-derived BDDTs, whose fibrin is embedded with platelet-derived and plasmatic growth factors, either alone or seeded with MSCs, have potential as a gel scaffold for focal cartilage and osteochondral defects repair [29–32]. These BDDT products are minimally manipulated, conceived and prepared *in situ*, and ready to be applied in the medical office or in the surgical theater. Intra-articular injections of blood-derived BDDTs have been proven to reduce pain and improve joint functionality in patients with knee or hip osteoarthritis [33,34] (Figure 2B and Box 3). Moreover, an increasing body of evidence indicates that blood-derived BDDT stimulates bone marrow, or combined with hydrogel, collagen, and/or hyaluronic acid membrane acts as a carrier of bone marrow-derived MSCs, resulting in structural and functional improvement in human focal cartilage defects [24,27,31]. A new strategy to safely deliver blood-derived BDDTs to the damaged synovial joint – a strategy that circumvents systemic toxicity, offers an excellent bioavailability, and does not present molecular size limitation – is the combination of intra-articular and **intraosseous infiltrations** of blood-derived BDDTs as an *in situ* biological 'joint centric' approach to treat traumatic osteochondral defects, osteochondritis dissecans, osteonecrosis, bone marrow edemalike lesions, and osteoarthritis. Such clinical application opens new therapeutic avenues in treatment of joints pathologies [25,35] (Figure 2B and Box 3).

Tendon Tissue

Musculoskeletal injuries are a growing medical problem associated with overuse or/and aged-related tissue alteration, with an estimated US$30 billion spent annually on their management in

differentiate into osteoblasts, chondrocytes, and adipocytes.
Metalloproteinases: proteases that degrade extracellular matrix components such as collagen.
Nerve growth factor: a protein that plays an important role in maintaining motoneuron size and in nerve target tissue trophism.
NF-E2-related factor 2 (NrF2-ARE) pathway: an intracellular pathway involved in the antioxidant cell response.
Platelet-derived growth factor: a relevant growth factor present in platelets that stimulates cell proliferation.
Prostaglandin E_2: an inflammatory mediator with local and systemic effects.
Satellite cells: muscle stem cell precursors of myofibers that lie in a specific muscular niche.
Scaffolds: templates generally used for tissue formation that are seeded with cells and growth factors.
Schwann cells: quiescent cells of adult peripheral nerves that synthesize myelin and possess a transdifferentiation potential.
Stromal-cell derived factor 1: a chemokine that plays an essential role in the homing and chemoattraction of pluripotent progenitor cells.
Syndesmosis: anteroinferior tibiofibular ligament of the ankle.
Target: blood-derived biological drug delivery therapy provides dosage form versatility over drug availability. These drugs can be administered topically, as an eye drop, or by subcutaneous, intradermal, and intramuscular injections. Moreover, they can be shaped as a pure liquid, an *in situ* gelling liquid, or a three-dimensional fibrin scaffold, thus increasing their therapeutic potential.
Tendon multipotent stem/progenitor cells: the tendon has a population of cells with multidifferentiation, proliferative, and self-renewing potential; these are termed tendon stem cells.
Tissue engineering: the field of medicine that aims to regenerate damaged tissues by combining cells from the body and growth factors with highly porous scaffold biomaterials, which act as templates for restoring, maintaining, or improving tissue function.
Toll-like receptors: trans-membrane cell receptors that detect

the USA alone. Tendons and ligaments account for 45% of these injuries, and unfortunately none of the various therapeutics, including exercise-based physical therapy, corticosteroid injections, nonsteroidal anti-inflammatory drugs, extracorporeal shock wave therapy, or surgical interventions, has provided a successful long-term solution [36]. The tendon is a hypocellular dynamic mechanosensitive composite structure that harbors immunocompetent cells and **tendon multipotent stem/progenitor cells [tendon-derived stem cells (TDSCs)]** [37], the key **targets** of blood-derived BDDTs. Moreover, the anabolic/catabolic balance of the tendon shifts according to its mechanical loading history (Figure 2A and Box 4). Acute and chronic tendon injuries result in pain, focal tenderness, and a decrease in strength and movement stemming from an inflamed and/or ruptured tendon. Blood-derived autologous bioscaffolds 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. The application of these scaffolds has been a product of *in vitro* and *in vivo* research that has provided a better understanding of the response of tenocytes and TDSCs to blood-derived BDDT [38–40] (Figure 2A and Box 4).

Blood-derived BDDT has been applied as ultrasound-guided intratendinous and intraligament infiltrations on chronic patellar tendinopathy and acute ankle sprain, respectively [41,42] and as intratendinous infiltrations intraoperatively combined with fibrin scaffolds on surgically repaired Achilles tendon tears [40] (Figure 2A). Both cases showed alleviated symptoms and improved function, a return to normal architecture of tendon and **syndesmosis** assessed by magnetic resonance imaging, and a shorter time in the recovery of motion and return to sporting activities [40]. Moreover, the application of blood-derived bioscaffolds 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 remodeling and integration of the graft, in addition to satisfactorily filling the gap and reconstructing the patella tendon and tibial and patella bone gap [43–45].

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 5). In acute muscle injuries, a blood-derived BDDT in its liquid formulation is infiltrated into the injury site, adjacent areas, and peripheral healthy muscle under **ultrasound guidance**. In the ensuing 1–3 min, this liquid-to-gel transition scaffold fills the muscle gaps and defects and serves as a highway for mechanical energy to transit from the environment to the cell, thereby bridging the cell-to-cell tissue transition, promoting multicellular assembly, and targeting muscle stem and immunocompetent cells. This process provides chemical signals, mechanical support, and plastic–elastic stiffness, which not only has a drastic impact on the fates of muscle stem cells, but also endows tissues with a suitable mechanical and chemical microenvironment for biological restoration [46] (Figure 2C). This dynamic spongelike fibrin-matrix BDDT is autologous, bioresorbable, and biocompatible [14]. 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 [47]. Notable here is another recent study that reported that this therapeutic approach had no benefit [48]. However, it is possible that the source of inconsistent clinical outcomes in muscle injuries treated with blood-derived BDDTs could result from the delayed administration, inconsistent dosage, and heterogeneity of the blood-derived BDDT biological composition itself, for which there is no standard protocol [49]. In order to characterize, standardize, and optimize the composition of blood-derived BDDTs to the specific cellular target and tissue repair processes, several *in vitro* and *in vivo* efforts are ongoing. Recent emphasis has been on applying only plasmatic factors or modifying the therapy's composition [50] by blocking **transforming growth factor-β** as a fibrotic factor or depleting myostatin with the goal of enhancing myoblast differentiation [51,52].[i]

signals coming from cell or tissue damage.

Transforming growth factor-β: a protein superfamily that regulates many cellular functions, including secretion of extracellular matrix constituents.

Ultrasound guidance: a technical procedure used to visualize and guide the allocation of therapeutic products in the tissue.

Vascular endothelial growth factor: promotes new blood vessel growth and exerts an antiapoptotic effect.

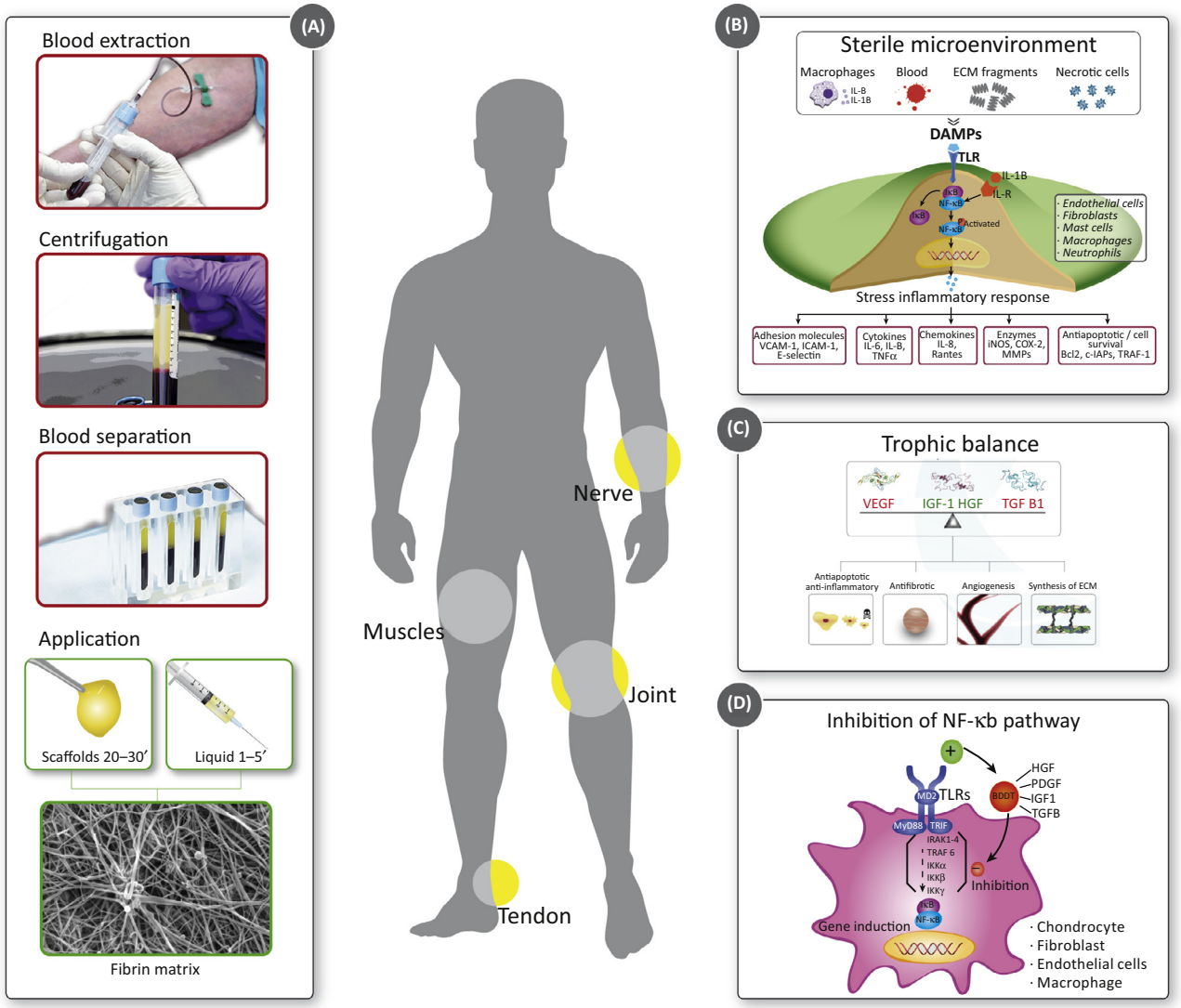

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Figure 1. (A) One of several procedures available to obtain a specific blood-derived biological drug delivery therapy (BDDT). (B) Tissue injury disrupts the chemical and physical composition of cell microenvironments, which prompts several cell lineages to respond with a proinflammatory gene expression through activation of the nuclear factor-kappa B (NFkB) signaling pathway without any involvement of pathogens. (C) A hypothetical mechanism by which the concurrent presence and a balanced ratio between platelet-secreted transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF), and plasma growth factors such as insulin-like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF) all conveyed by blood-derived BDDT might exert some biological effects. (D) Blood-derived BDDT; GFs within it such as HGF, platelet-derived growth factor, IGF-1, TGF-β1, and platelet microparticles have been proven to exert an immunomodulatory effect and promote an anti-inflammatory environment. Abbreviations: Bcl2, B-cell lymphoma 2; COX-2, cyclooxygenase 2; DAMP, damage-associated molecular pattern; ECM, extracellular matrix; IACM-1, intercellular adhesion molecule 1; IAP, inhibitor of apoptosis; IL, interleukin; iNOS, inducible nitric oxide synthase; MMP, metalloproteinase; RANTES, regulated on activation, normal t expressed and secreted; TLR, Toll-like receptor; TNF-∝, tumor necrosis factor ∝; TRAF-1, TNF receptor-associated factor 1.

To manage peripheral nerve injury, and fueled by the drawbacks posed by autologous nerve autografts, many biomedical engineering strategies have been applied, including nerve guidance conduits and scaffolds, incorporating and delivering neurotrophic factors [53], incorporating support cells into nerve guidance conduits or fibrin gels, and stimulating target organs through intramuscular injections of growth factors [53,54]. 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 [53,55] (Box 5). In humans,

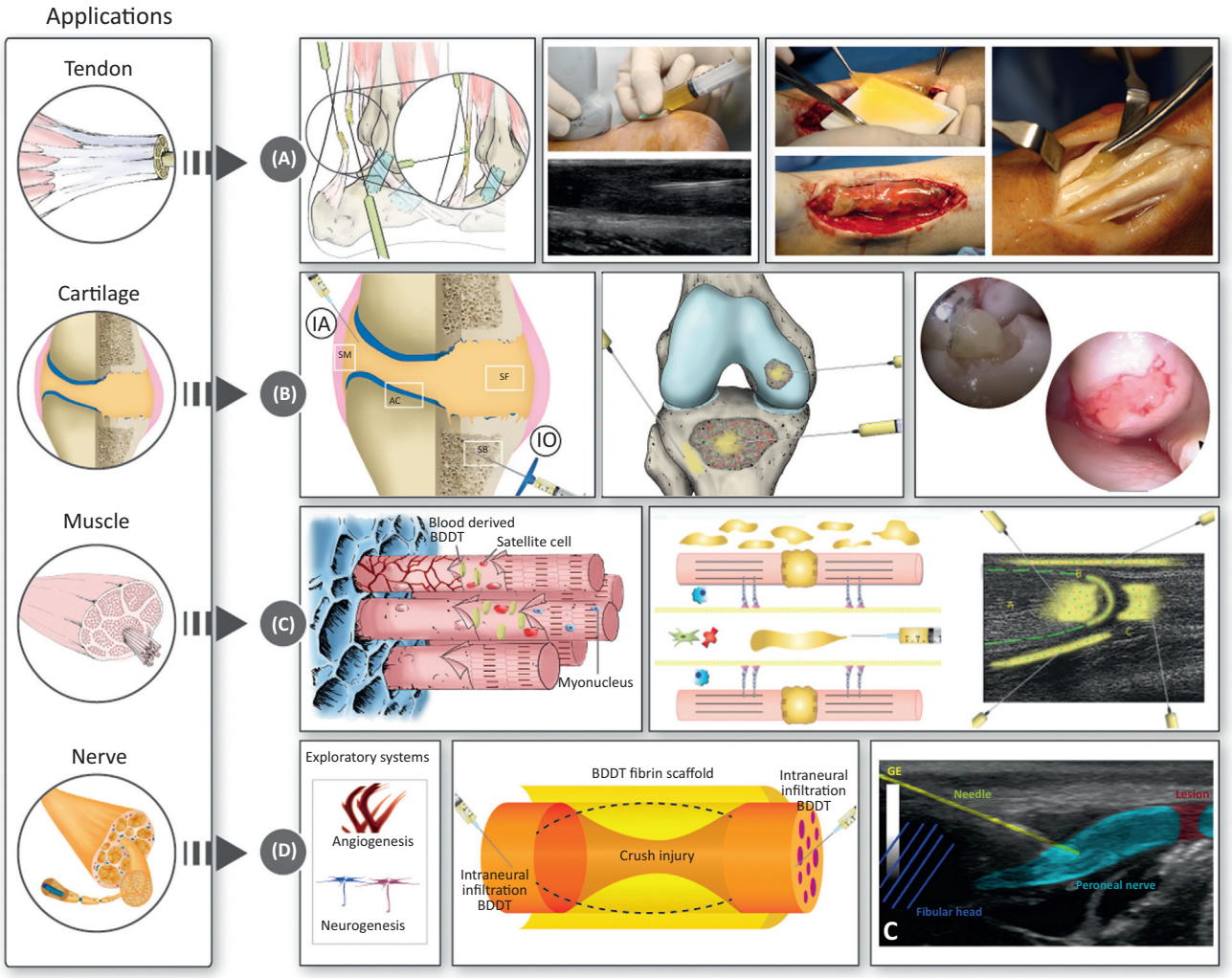

Figure 2. Advances in Regenerative Orthopedics Based on the Application of Blood-Derived Biological Drug Delivery Therapies (BDDTs). (A) Intratendinous infiltrations and application of scaffolds obtained from blood-derived BDDT to assist surgical reconstruction of tendon ruptures. In chronic tendinopathies, intratendinous injections are applied under ultrasound guidance. (B) A novel approach to treating severe knee osteoarthritis and osteochondral injuries by targeting the synovial membrane, superficial articular cartilage, synovial fluid, and subchondral bone by combining intra-articular 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 growth factors 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.

molecular intervention with blood-derived BDDT is partially bridging the gap between basic and clinical applications. In a double-blind, randomized clinical trial, the application of ultrasound-guided blood-derived BDDT injections in tibial and ulnar nerves has shown sensory improvement in leprosy peripheral neuropathy [56]. In addition, several case studies applying blood-derived BDDTs either as a filler of nerve conduits across nerve gaps post-trauma [57] or by infiltrating intraneurally in a peroneal nerve palsy [58] have reported neurological recovery (Figure 2D). Therefore, blood-derived BDDT applied in a combinatorial strategy as a filler, suturable membrane, and scaffold stands out as a promising candidate for an adjuvant nerve repair approach that can be harnessed by surgeons in the operating room and in the clinical setting [58,59].

Box 4. Adaptation, Inflammation, and Homeostatic Process in Tendons

There is increasing evidence showing that the tendon and ligament adaptation, injury, and repair processes share several intracellular pathways. Although it is difficult to draw a line between the cellular and molecular responses that lead to either tissue adaptation or tissue damage, the inflammatory process appears to be at the interface of tendon adaptation and damage [36,68]. Repetitive mechanical loading, as is the case in early stages of tendinopathy, and tendon overuse induce the activation of nuclear factor-kB and thereby the synthesis of matrix **metalloproteinases** (MMPs), two isoforms of cyclooxygenase (COX), namely, COX-1 and COX-2, and prostaglandin E_2 (PGE_2) by inflammatory tenocytes, mast cells, and other immunocompetent cells [36,72–74]. PGE_2 is a major systemic and local inflammatory mediator that decreases the production of collagen and causes aberrant differentiation of tendon-derived stem cells (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 [36,37,72,74].

An excellent series of *in vitro* and *in vivo* studies demonstrated that blood-derived BDDTs induced tenocyte proliferation, stimulated both the synthesis of type I collagen and neovascularization [39], promoted differentiation of TDSCs into active tenocytes, but significantly, the addition of leukocytes into the releasate increased the synthesis of PGE_2 and the gene expression of MMP-1, MMP-13, and interleukin-1B, and decreased the expression of alpha-smooth muscle actin as a marker of active tenocytes [73–75]. Among the myriad mediators conveyed by blood-derived BDDTs, hepatocyte growth factor and **lipoxin A4** have been shown to exert an anti-inflammatory and pro-resolution-of-inflammation effect on injured tendons (see Figures 1D and 2A in main text) [73,76].

Box 5. 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 vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), and hepatocyte growth factor (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 modulating the 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.

In the management of peripheral nerve injury, 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 [55] (see Figure 2D in main text), tissue fibrinolysis breaks the fibrin down, thereby releasing cell signaling molecules such as neurotrophic (**nerve growth factor**, brain-derived neurotrophic factor, IGF-1, PDGF, VEGF, and HGF) and neurotropic (fibrin, fibronectin, and vitronectin) factors [13]. 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 [53,77].

Concluding Remarks and Future Directions

The daunting complexity of many musculoskeletal tissues targeted by blood-derived BDDT, coupled with regulatory, clinical, and commercial challenges, adds up to multiple barriers to further product development and progress. In the last few years, many aspects of 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 [60], minimizing manipulation, and preparing devices that enhance safety and versatility of blood-based BDDTs. However, further work is necessary to better address some of the challenges that have arisen over time. For example, efforts in academia and the biotechnology industry to rapidly translate basic to clinical applications have limited our basic-science understanding of the biological roles of this therapy. There remains a troublesome inconsistency of terminology in this field, exacerbated by the incomplete characterization of a plethora of products under development (see Outstanding Questions). In addition, more robust randomized clinical trials and meta-analyses are needed to fully determine the potential of this technology in the treatment of both chronic and acute musculoskeletal conditions.

Outstanding Questions

Is there a common ground among the mechanical musculoskeletal pathologies as a target for biological drug delivery therapy (BDDT)? When mechanical stress is too intense or too repetitive, beyond the issues associated with aging, this stimulus can deregulate the transient inflammatory response, leading to a sterile non-resolving chronic inflammation. This phenomenon appears to be the tissue landscape of several chronic conditions involving immunocompetent cells, such as lymphocytes and macrophages.

Could BDDT harness the regenerative potential of endogenous stem cells? Blood-derived BDDTs convey several host stem cell fate modifiers that could be applied as 'new on-site solutions' to harness resident stem cells in tendons, the synovial membrane, subchondral bone, and satellite cells, in addition to modifying the microenvironment of the damaged tissue.

From whom will we obtain blood-derived BDDT in the future? So far, the common source of blood-derived BDDT has been autologous, which might be the root of some inconsistent clinical outcomes of this therapy. A homologous source could provide significant clinical benefits, a result which is yet to be proven.

Are surgical techniques and blood-derived BDDT mutually exclusive? Blood-derived biological drug delivery therapy is a new tool in the therapeutic arsenal of daily orthopedic surgical activity aimed at the injured tissue battlefield, synergizing with but not replacing surgical techniques, and intending to transform the battlefield into a new suitable tissue microenvironment, thereby contributing to healing process.

Do blood-derived BDDTs have a key feature that makes their application so extensive? Their versatility of liquid and scaffold formulation, safety, and pleiotropy of gradual and sustained delivery of cytokines and growth factors render this application a valuable systems biology therapy.

Fortunately, there are reasons for optimism. Novel formulations and fabrication methods are likely to help broaden the catalog 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. Exploring its potential for the *ex vivo* expansion of MSCs, 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, including 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.

Disclaimer Statement

The authors declare the following competing financial interests: E.A. is the Scientific Director and G.O and S.P. are scientists at BTI-Biotechnology Institute, a dental implant company that performs research in the fields of oral implantology and PRGF-Endoret technology.

Resources

[i] https://www.sportsmed.org/AOSSMIMIS/Members/Downloads/MeetingResources/AM2016Posters/Poster_60.pdf

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