Promoting musculoskeletal system soft tissue regeneration by biomaterial-mediated modulation of macrophage polarization

Jinchun Ye [a,b,c,d,e], Chang Xie [b,c,d,e], Canlong Wang [a,b,c,d,e], Jiayun Huang [a,b,c,d,e], Zi Yin [a,b,c,d,e,f], Boon Chin Heng [g], Xiao Chen [a,b,c,d,e,f,**], Weiliang Shen [a,b,c,d,e,f,*]

[a] Department of Orthopedic Surgery (Sports Medicine) of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China
[b] Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, Zhejiang University School of Medicine, Hangzhou, China
[c] Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Zhejiang University School of Medicine, Hangzhou, China
[d] Department of Sports Medicine, Zhejiang University School of Medicine, Hangzhou, China
[e] Institute of Sports Medicine, Zhejiang University, Hangzhou, China
[f] China Orthopedic Regenerative Medicine Group (CORMed), Hangzhou, China
[g] Central Laboratories, Peking University School of Stomatology, Beijing, China

ARTICLE INFO

ABSTRACT

Musculoskeletal disorders are common in clinical practice. Repairing critical-sized defects in musculoskeletal systems remains a challenge for researchers and surgeons, requiring the application of tissue engineering biomaterials. Successful application depends on the response of the host tissue to the biomaterial and specific healing process of each anatomical structure. The commonly-held view is that biomaterials should be biocompatible to minimize local host immune response. However, a growing number of studies have shown that active modulation of the immune cells, particularly macrophages, via biomaterials is an effective way to control immune response and promote tissue regeneration as well as biomaterial integration. Therefore, we critically review the role of macrophages in the repair of injured musculoskeletal system soft tissues, which have relatively poor regenerative capacities, as well as discuss further enhancement of target tissue regeneration via modulation of macrophage polarization by biomaterial-mediated immunomodulation (biomaterial properties and delivery systems). This active regulation approach rather than passive-evade strategy maximizes the potential of biomaterials to promote musculoskeletal system soft tissue regeneration and provides alternative therapeutic options for repairing critical-sized defects.

1. Introduction

As global life expectancy increases, the absolute number of years lived with disability (YLDs) due to non-communicable diseases is also growing rapidly [1]. In 2016, musculoskeletal conditions were rated as the second most debilitating disease in the world. Musculoskeletal system injuries are also very commonly encountered in the clinic. Small acute defects can be repaired and some degenerative injuries can be removed surgically. However, there are still a lack of effective treatment modalities for critical sized defects. Clinically, utilizing autografts and allografts also have many disadvantages including donor site complications, rejection and infection [2]. Therefore, how to effectively treat musculoskeletal system disease and repair critical sized injuries is crucial to improving people's quality of life and extending their productive life [3].

In the past few decades, biomaterial-based tissue engineering is gaining in popularity in facilitating healing of musculoskeletal injuries. The ultimate function of biomaterials depends on the response of the host tissue to biomaterials and the specific remodeling process of each anatomical structure [4]. A common problem with implanted biomaterials is that they trigger an adverse immune response that may lead to excessive inflammation, pain, tissue destruction, fibrotic

encapsulation and even graft failure [4]. These adverse reactions are the bottlenecks that hinder the successful application of biomaterials, so previous implants were designed to evade immune responses [5–7]. However, increasing numbers of studies have shown that active regulation of the immune system can better promote tissue regeneration [8–11]. The immune cells, particularly macrophages, play a vital role in the regeneration and repair of injured musculoskeletal tissues. Macrophages have dynamic and plastic phenotypes, which can take on different functions as the microenvironment changes, such as the transformation of pro-inflammatory M1 phenotype to pro-regenerative M2 phenotype [12]. The imbalance of macrophage phenotypes and dysregulation of the conversion between M1 and M2 are important causes of unresolved inflammation and poor regeneration. Furthermore, unresolved chronic inflammation can lead to the fusion of macrophages into giant cells, the recruitment of fibroblasts, the formation of fibrous capsule, and ultimately the failure of the implant [4,12]. Recent evidence demonstrated that the behavior of macrophages and the transformation of phenotypes could be fine-tuned by varying the biomaterial properties (such as physiochemical surface properties and topography), and that biomaterials could also be used as carriers to deliver bioactive molecules and drugs to directly modulate the immune response [10,11, 13,14]. In this way, modulating the macrophage polarization and immune response in the microenvironment via the immunomodulation of biomaterials to make it develop in the direction of enhancing regeneration may be a promising strategy for musculoskeletal system regenerative medicine.

In view of the fact that a large body of research has explored the role of immunomodulation of biomaterials in promoting bone regeneration, with few studies being conducted on cartilage, tendon/ligament, and muscle tissues, we attempted to discuss the important role of biomaterial-mediated modulation of macrophage polarization in the repair of these tissues that have relatively poor regenerative capacities. In this review, we will first briefly describe how the immune system is involved in the process of tissue repair after injury, and the important roles that specific phenotypes of macrophages play in the regeneration of cartilage, tendon/ligament, and muscle tissues. Then we will examine how the behavior of macrophages could be regulated by different properties of the biomaterial and through delivery of drugs. Finally, we will discuss immunomodulatory biomaterials and drug delivery system used to harness regenerative subpopulations of macrophages in cartilage, tendon/ligament and muscle regeneration.

2. The polarization of macrophages and their roles in musculoskeletal system soft tissue regeneration

After tissue injury, damage-associated molecular patterns (DAMPs) will be triggered and induce inflammation [15]. DAMPs are endogenous molecules released from necrotic cells or damaged extracellular matrix (ECM), including heat shock proteins (HSP), high-mobility group box protein 1 (HMGB1), nucleic acids including mitochondrial DNA, inflammatory cytokines such as interleukin 1 alpha (IL-1α), and IL-33 and fragments of ECM such as hyaluronic acid, collagen, etc. [16,17]. Toll-like receptors (TLR) and some other types of pattern recognition receptors present on antigen-presenting cells (such as dendritic cells and macrophages) would recognize these danger signals and initiate inflammation by activating transcription factors NF-κB or interferon-regulatory factors [18]. Post-injury inflammation progresses in three stages: The first stage is the early pro-inflammatory response, where necrotic cells, coagulation, and invading microorganisms activate the inflammatory response, leading to recruitment of innate immune cells like neutrophils and monocytes to the wound site to remove cell debris and infectious organisms, which help in coordinating the tissue repair. In the second stage, the inflammatory response gradually subsides, and the immune cells involved in inflammatory response (such as macrophages) switch to anti-inflammatory and pro-regenerative phenotypes, promoting the repair and remodeling of the tissue; In the final

stage, the inflammatory cells gradually die off or leave the injury site, and the injury is gradually repaired [19](Fig. 1A). The degree and duration of the inflammation can vary and affect the outcome of the eventual repair. Too little inflammation can cause harmful factors (especially bacteria) to linger and destroy tissues, whereas chronic and unresolved inflammation can eventually lead to a variety of diseases, including fibrosis and even cancer [20].

2.1. The polarization of macrophages

Although many immune cell types are involved in the inflammatory response after injury, macrophages play a vital important role in tissue repair, regeneration, and fibrosis [21]. Macrophages involved in injury repair can be derived from tissue-resident macrophages or from the recruitment and differentiation of circulating monocytes in the blood. Classically-activated macrophages (M1 macrophages) and alternatively-activated macrophages (M2 macrophages) are two of the most commonly studied phenotypes in tissue repair [22]. The traditional classification of M1 and M2 is based on *in vitro* characterization, in which M1 phenotype is stimulated by lipopolysaccharide (LPS), interferon-gamma (IFN-γ) and tumor necrosis factor-α (TNF-α), while M2 phenotype is stimulated by IL10, IL-4 or IL-13 [23,24] (Fig. 1B). Activation of M1 macrophages is related to signal transducers and activators of transcription 1(STAT1) and interferon regulatory factor (IRF) activation, while activation of M2 is associated with STAT6 [25]. These pathways could inhibit each other, for example, the activation of STAT6 could suppress STAT1-dependent transcription in mouse macrophages, and the activation of STAT1 could also suppress STAT6-dependent transcription [26,27]. However, within the complex *in vivo* environment, M1 and M2 are not representative of all macrophage states, but are considered to be two extremes of a continuum of functional states. Individual macrophages that co-express M1 and M2 markers can be seen *in vivo* [28]. When M1 macrophages are treated with IL-4 alone or in combination with IL-13, they can be repolarized and exhibit anti-inflammatory phenotypes [29–31]. Similarly, when M2 macrophages are treated with LPS or IFN-γ, they will express markers associated with an inflammatory phenotype. When stimulated by a combination of IL-4, IL-13, LPS, and IFN-γ, both M1 and M2 markers (CD86 and CD206) were observed to be expressed in individual cells [32]. The expression of CD86 declined after the first 24 h and returned to the basal levels after 96 h, while CD206 expression continued to increase after 48–72 h and remained sustained even after 96 h. These results thus indicated a high plasticity of macrophage phenotypes and a continuum model of activation. M1 macrophages are the predominant population during the first few days after injury, secreting a large number of pro-inflammatory cytokines such as TNF-α, inducible nitric oxide synthase (iNOS), and IL-12, which induce inflammatory response and early proliferation. Then, the macrophage phenotype changes to M2, which secretes anti-inflammatory cytokines IL-4 and IL-10, as well as transforming growth factor-beta (TGF-β) and arginase, which contribute to proliferation, differentiation, and tissue remodeling [24].

With continuous research on the function of macrophages, we find that the sequential activation of the M1 and M2 phenotypes of macrophages not only affects the outbreak and regression of inflammation, but also exerts a profound impact on the quality of tissue regeneration and the results of remodeling. For example, some recent studies have shown that M1 macrophages could induce osteogenesis and mineralization of mesenchymal stem cells (MSCs) through cyclooxygenase-2 (COX-2)-prostaglandin E2 (PGE2) pathway during the early stages of fracture healing [33]. Initial transient inflammation can more effectively promote the activation of MSCs and the secretion of higher levels of PGE2 and other anti-inflammatory signals, thus enhancing osteoblast differentiation and shifting macrophages from M1 to M2 phenotype. These results thus suggest that early initiation of the repair program by M1 macrophages, followed by the transition from M1 phenotype to M2 phenotype at the appropriate time point, is the key to enhancing bone
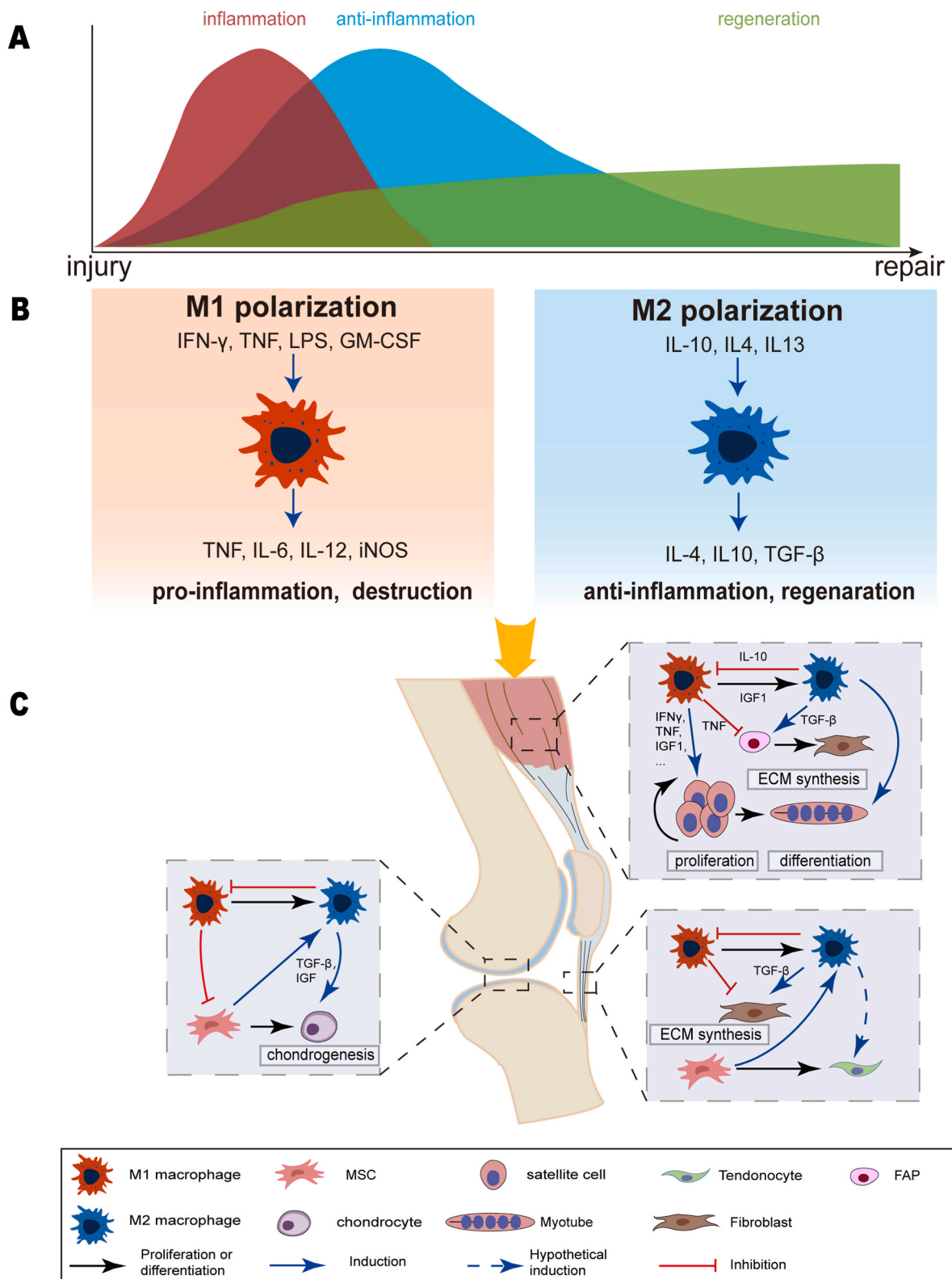
Fig. 1. The characterization of M1 and M2 macrophages and their roles in musculoskeletal system soft tissue regeneration. (A) Three phases following tissue injury: the first phase is the early pro-inflammatory response, then inflammation subsides and the immune cells involved in the inflammatory response (mainly macrophages) switch to pro-regenerative phenotypes to induce regeneration until the injury is finally repaired. (B) Schematic illustration of canonical M1 and M2 polarized macrophages. (C) Overview of the roles that M1/M2 macrophages play in the repair of injured musculoskeletal system soft tissues.

regeneration [33,34]. Moreover, sequential activation of macrophages from M1 to M2 could also facilitate the vascularization of scaffolds applied in bone regeneration [35].

However, the actual functions of macrophages may vary among diverse tissue repair process. In view of this, we attempted to summarize the role of different macrophage phenotypes in musculoskeletal system soft tissue regeneration process and explored whether they could be potential molecular targets for evoking this autogenous regenerative power.

2.2. The role of macrophages in cartilage regeneration

Macrophages play a complex and important role in the repair of cartilage, tendon/ligament and muscle after injury (Fig. 1C). Macrophages are present in the synovial lining of joints and are involved in the progression of osteoarthritis [36]. Blom et al. [37] selectively depleted synovial macrophages from the synovial lining with clodronate liposomes prior to experimental osteoarthritis (OA) induction in mice knee joints and found that osteophyte formation and fibrosis were significantly reduced after OA induction. However, more recent studies analyzed diverse subsets of macrophages in healthy and inflamed joints, and found that synovial macrophages exhibited functional diversification [38]. Utomo et al. [39] found that M1 macrophages induced by IFN + TNF enhanced cartilage inflammation and degeneration *in vitro*, while IL-4 or IL-10 induced M2 macrophages did not directly inhibit this effect. Although their study did not show a direct protective effect of M2 macrophages on cartilage, this phenotype should not be completely ignored. Through the activation and inhibition of rapamycin complex 1 (mTORC1), Zhang et al. [40] proved that M1 polarized synovial macrophages aggravated osteoarthritis while M2 polarization could alleviate it. Besides, they demonstrated that M1 macrophages exacerbated OA partially through R-spondin-2 (Rspo2), which represented a potential and novel therapeutic target. Interaction between different macrophage phenotypes and with other types of cells may contribute to the outcome of cartilage regeneration. M1 macrophages have been proven to be the major mediator of inhibition of MSC chondrogenesis, while polarized M2 macrophages up-regulate the expression of TGF-β and insulin-like growth factor (IGF), creating a microenvironment that promotes cartilage formation and the synthesis of type II collagen and glycosaminoglycan [41,42].

2.3. The role of macrophages in tendon/ligament regeneration

Macrophages are also involved in the process of tendon/ligament regeneration after injury and are closely associated with it. Tendons and ligaments are mainly composed of type I collagen, but they undergo repair with the formation of fibrotic scar following injury [43,44]. Such pathological scar formation leads to the weakening of mechanical strength, which is not the optimal regeneration that we want. Durantaye et al. [45] found that macrophages in injured tendons could promote cell proliferation and ECM synthesis, but their presence also led to tissue fibrosis with inferior mechanical properties. While another study demonstrated that although non-specific depletion of macrophages could limit granulated tissue formation, it hindered early matrix formation, which was detrimental to ligament tensile strength [46]. Because both studies involve non-specific depletion of macrophages, their results did not compare the different roles of M1 and M2 macrophages in the process of tendon regeneration. Nevertheless, they both agreed that regulating macrophage phenotypes rather than non-specific depletion could be a more effective way to reduce scarring and regenerate natural tendon tissues. Sugg et al. [47] investigated changes of macrophage phenotypes within 28 days after tenotomy and repair. Three days after surgery, M1 macrophages could be seen clustering in the area of the newly-formed tendon tissue, mainly in the area of ECM absorption, and remained throughout the study period. However, at the site of tendon ECM organization, M2 macrophages accumulated slowly and became the dominant macrophage phenotype by 28 days. During the early stages of tendon injury, macrophages are responsible for phagocytosis and the removal of dead cells and tissue debris. M1 macrophages are involved in early inflammatory responses and are associated with sharp increases in the expression of pro-inflammatory genes, like TNFα, IL-1β, and COX2; whereas M2 macrophages play a crucial role in the regeneration and remodeling of tendon tissue by stimulating cell proliferation and the synthesis of ECM components [47,48]. The use of anti-inflammatory drug Parecoxib during the first 5 days after transection of the Achilles tendon in rats impairs tendon repair, suggesting that early inflammation is necessary for functional remodeling of the tendon [49]. However, Lin et al. [50] showed that an abnormally increased ratio of M1 to M2 correlated with pathological fibrosis during tendon healing. However, recent studies have demonstrated that M2 macrophages are also associated with fibrosis, and excessive M2 macrophage activation can lead to increased scar formation and impair normal tendon healing [51,52]. This suggests that the excellent outcome of tendon/ligament regeneration——reduced damage by mitigating inflammation, enhanced tissue regeneration and limited pathological fibrosis, may be achieved by the sequential transformation of macrophage phenotype instead of a specific phenotype of macrophages. Connie et al. [53] demonstrated this by using extracellular vesicles isolated from MSCs to reduce the ratio of M1 to M2 phenotypes, thereby improving tendon healing outcomes. Even so, the exact role of the various phenotypes of macrophages in tendon/ligament healing is still not precisely defined. With the development of high throughput single cell RNA-sequencing technologies, it is reasonable to assume that we can more accurately identify and characterize the various macrophage subpopulations, and understand the interaction between these cells and fibroblasts, so as to provide a precise therapeutic target for the prevention of fibrotic tendon/ligament healing.

2.4. The role of macrophages in muscle regeneration

Muscle regeneration depends on myogenic precursor cells (MPCs), also known as satellite cells. After muscle injury, previously quiescent satellite cells are activated and enter the cell cycle. Some of the postmitotic daughter cells differentiate into multi-nucleated myotubes and then undergo rapid growth and terminal differentiation, while others return to quiescence and replenish the satellite cell stock [54]. Activation of satellite cells is a key factor in muscle repair, and the initial inflammatory response is closely related to it. M1 macrophages express IFN-γ, which induces more macrophages to undergo M1 polarization, and inhibits MPCs differentiation via a pathway mediated by the MHC class II transactivator, so as to keep them proliferating to rapidly increase their population and support tissue repair [55,56]. Another pro-inflammatory cytokine, TNF, promotes the early differentiation of MPCs [56–58]. These results indicate that M1 macrophages play an important role in regulating the proliferation and differentiation of MPCs during the early stage. In addition, M1 macrophages can also express insulin-like growth factor 1 (IGF1) as a strong mitogen of MPCs and mediate the transition from M1 to M2 [59]. The transition of the macrophage phenotype from M1 to M2 is also necessary for muscle regeneration. With the transformation of macrophage phenotype, the increase of IL-10 expression level also represents the transition of muscle regeneration from proliferation to differentiation and growth [60]. During the switch of M1 to M2 phenotype, the depletion of F4/80+ macrophages reduced muscle regeneration and muscle membrane repair, which demonstrated the important role of M2 macrophages in muscle repair and regeneration [61]. Muscle regeneration also requires the deposition of extracellular matrix, which is dependent on fibro/adipogenic progenitors (FAPs) activation [62]. After acute muscle damage, TNF produced by M1 macrophages induces FAPs apoptosis, while M2 macrophages can release TGF-β to inhibit this effect and induce FAPs to differentiate into a fibrogenic phenotype [63,64]. Therefore, restoration of the complete structure and function of injured muscles requires

both M1 macrophages to eliminate excessive FAPs and avoid excessive pathological fibrosis, and M2 macrophages to control proper matrix deposition necessary for muscle regeneration. This poses a challenge for us to regulate macrophage phenotypes, because the switch needs to be controlled at a very precise point in time [64].

Based on these results, we can conclude that macrophages play an extremely important role in the remodeling of the musculoskeletal system soft tissues, and that the regulation of the macrophage phenotype

may serve as a beneficial strategy to maximize the repair potential.

3. Biomaterial-mediated modulation of macrophage polarization

Currently, the development of tissue engineering technology opens a new avenue for tissue regeneration and repair. As foreign bodies, biomaterials are recognized by the immune system upon implantation,
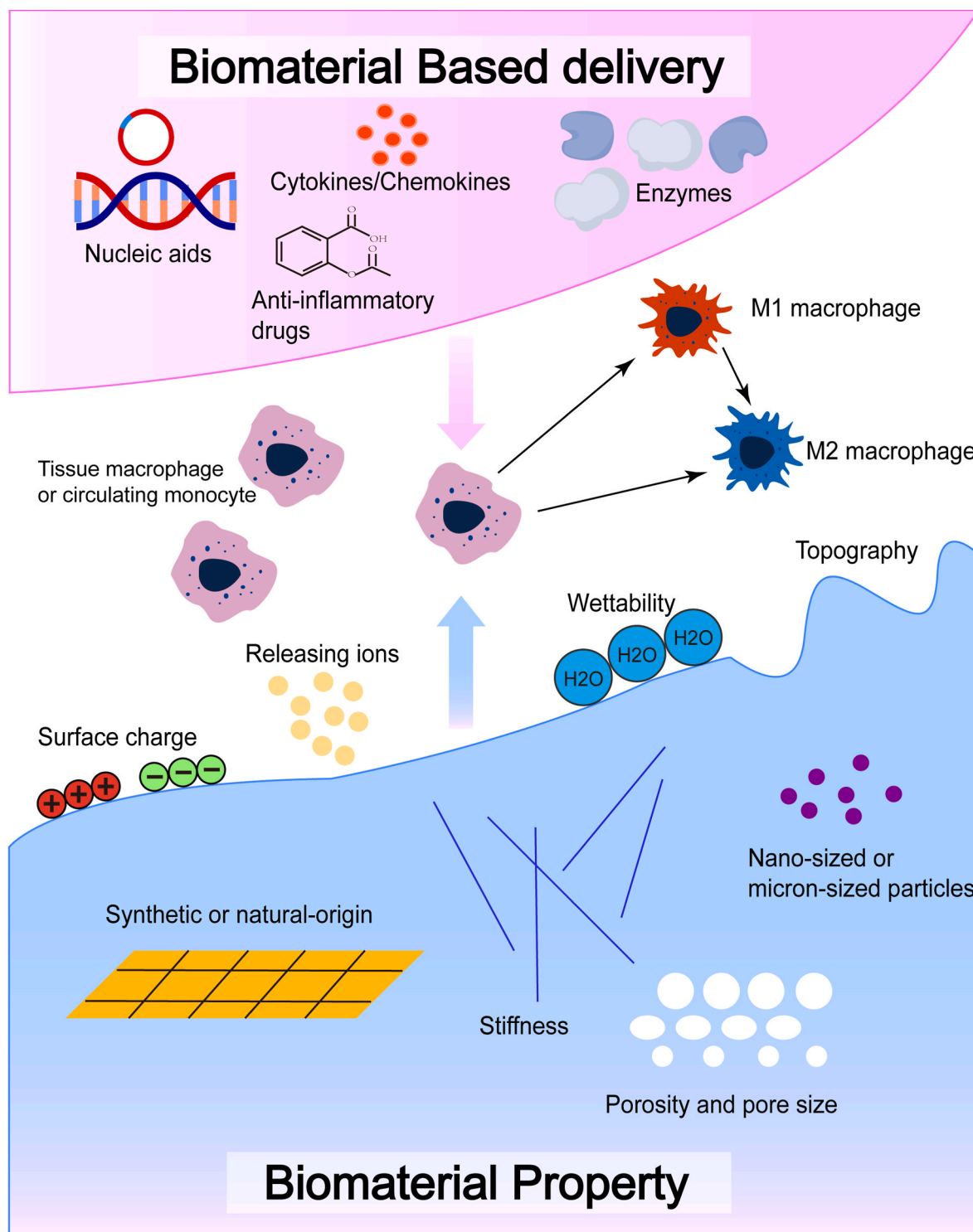

Fig. 2. Biomaterial and biomaterial-based delivery of bioactive signals to trigger macrophage polarization. The physical and biochemical properties of biomaterials as well as biomaterial-based delivery system induce macrophages to polarize into pro-reparative phenotype to improve repair outcomes.

which triggers an immune response. The immune response induced by biomaterials is a "double-edged sword". A beneficial immune response can form a microenvironment that promotes tissue healing and improves tissue repair and regeneration, while a poor immune response may lead to chronic inflammation and the formation of fibrous capsules around the implant, leading to loss of functions and even tissue destruction [4]. Therefore, it is imperative to delve into how biomaterials interact with the immune system and modify the properties of biomaterials to modulate the behavior of immune cells so that the immune response could develop in the direction we need it to.

3.1. Strategies for immunomodulating macrophage polarization through surface or biochemical parameters

A large number of studies have proven that the surface properties and biochemical properties of biomaterials play an important role in modulating macrophage polarization [13](Fig. 2). The regulatory effect of biomaterials on immune cells depends to a large extent on surface properties, possibly because they can affect protein adsorption [65–67]. As soon as the biomaterial is implanted into the body, proteins (such as fibrinogen, fibronectin, complement proteins and vitronectin) in the blood and interstitial fluids immediately adhere to the surface of the biomaterial. The properties of the biomaterial itself (such as surface chemistry and topography) play a crucial part in the type and manner of protein adsorption. When the protein is adsorbed onto the surface of the biomaterial, it may undergo conformational changes and expose some domains or epitopes that were previously hidden. When host cells (including immune cells) recognize and bind to these sites, specific cellular responses to the surface properties of the biomaterial are triggered. For example, fibrinogen denatures when attached to the surface of a biomaterial, exposing two epitopes (γ190-202, P1, and γ377-395, P2) [68]. These epitopes interact with the phagocyte integrin Mac-1 (CD11b/CD18), leading to aggregation of phagocytes and promoting an inflammatory response. The more epitopes are exposed, the more severe the inflammatory response will be.

The surface topography of the biomaterial is an important parameter that affects the adhesion and differentiation of macrophages [69–71]. McWhorter et al. [70] found that in vitro M1 macrophages have a rounded shape while M2 macrophages are elongated. Therefore, they used a micropatterning approach to directly elongate macrophages, and found an increase in M2-phenotypic markers and a decrease in the secretion of inflammatory cytokines. Porosity and pore size are also key factors in determining the effectiveness of biomaterials, as they can affect not only the diffusion of nutrients and oxygen supplies, but also the polarization of macrophages. Increased porosity and pore size of an electrospun scaffold increased expression of M2 marker Arginase 1 while decreasing expression of M1 marker iNOS, suggesting that they induced macrophages to polarize towards the pro-regenerative M2 phenotype instead of pro-inflammatory M1 phenotype [72]. In addition, the surface chemical properties of the material, such as surface hydrophilicity and surface charge, also have a significant impact on the adhesion of macrophages and their release of pro-inflammatory factors and chemokines [73–75]. The surface of a more hydrophilic nature did not induce activated monocyte adhesion, while enhanced monocyte adhesion was observed on the surface that was more hydrophobic, leading to a localized inflammatory response [73]. Hotchkiss et al. [74] also found that rough titanium surfaces with higher hydrophilicity were more conducive to the polarization of M2 macrophages. In the past, we mainly considered the effect of surface properties on stem cell differentiation. This view should be revised, and we should realize that the surface properties of biomaterial are equally important in regulating the phenotype and functional change of macrophages that can positively influence regeneration.

The biochemical properties of biomaterials (e.g., synthetic or naturally-derived) also play a significant role in determining macrophage functions. Decellularized ECM-derived scaffolds have been shown to promote wound healing and guide M2 macrophage polarization, which might be related to the mTOR/Rictor mediated T helper 2 pathway [11]. However, the typical foreign body response induced by synthetic biomaterials was found to be mediated by Th17-associated molecules secreted by IL-36+ macrophages through single-cell RNA sequencing analysis [76]. Beattie and colleagues showed that the decellularized scaffolds composed of porcine urinary bladder ECM caused more progenitor cells to migrate to the tendons than autologous tendon graft, which was likely because decellularized ECM scaffolds promoted the polarization of M2 macrophages [77]. Moreover, type II collagen, an indispensable collagenous component of articular cartilage, could also favor M2 polarization, which contributes to chondrogenesis and cartilage repair [42].

The immunomodulatory capacities of nanoparticles can be utilized to create an environment conducive for tissue regeneration, making it a successful strategy. Micron-sized hydroxyapatite (HA) particles could drive immature macrophages towards a pro-inflammatory M1 phenotype, whereas nano-sized HA particles preferentially promoted macrophages to polarize into the M2 phenotype and enhance MSC osteogenesis as well as tissue angiogenesis in an IL-10-dependent manner [78,79]. As foreign substances, it is macrophages that play a crucial role in the recognition, processing and removal of nanoparticles (NPs) when they enter the body. At the same time, nanoparticles can play different roles in the polarization and reprogramming of macrophages due to their different physical and chemical properties [80]. For example, compared with Ag nanoparticles, Au nanoparticles induced up-regulated expression of IL-1, IL-6 and TNF-α. This might be due to the different uptake patterns of these nanoparticles. A part of the negatively charged AuNPs were more likely to adsorb non-specific serum proteins on their surfaces to enter cells through receptor-mediated endocytosis, while Ag NPs entered macrophages through simple endocytosis [81].

Overall, the examples mentioned above demonstrated that the interaction between biomaterials and macrophages could provide a basis for regulating the phenotype transition of the macrophages. We need to validate the relationship between different parameters of biomaterials and functional changes of macrophages, and take them into account when designing biomaterials for tissue engineering applications.

3.2. Strategies for immunomodulating macrophage polarization through biomaterial-based delivery systems

Although the importance of physical and chemical cues of biomaterials in the regulation of macrophages has been fully confirmed, the fine regulation of macrophage phenotypes is still largely unknown, especially how to regulate the degree of polarization and the precise time point of regulation. By contrast, biomaterials that deliver anti-inflammatory drugs, cytokines, and genes allow for more fine-tuned control of the timing and extent of macrophage polarization (Fig. 2).

Recently, diverse biomaterial-based drug delivery strategies have been developed for sequentially modulating macrophage polarization, which is beneficial to improving our understanding of the temporal control of macrophage behavior and precise control of polarization degree. Spiller et al. [35] designed a decellularized bone scaffold on which IFNg was physically adsorbed for short release kinetics (24 h) and IL-4 was conjugated via biotin-streptavidin binding for sustained release kinetics (6 days). Although the early rapid release of IFNg promoted M1 polarization in macrophages and increased scaffold vascularization, the system they designed did not induce robust M1 and M2 activation in either the early or late stage, but instead yielded a mixed macrophage phenotype. Although a mixed M1/M2 phenotype may be favorable for angiogenesis, it is associated with fibrosis around the implant and is not conducive to implant integration [82,83]. Therefore, distinct activation periods are crucial to avoid overlapping phases when designing delivery system to modulate the sequential polarization of macrophages. Chen et al. [84] prepared a system of titania nanotubes (TNT) with double

hydrogel layers, loaded IL-4 in TNT, and then placed IFN-γ between the two hydrogel layers. After macrophages were seeded on the system, IFN-γ was rapidly released within 3 days and promoted the polarization of macrophages towards the M1 phenotype. However, IL-4 was continuously released over the first week and promoted the conversion of macrophages from the M1 to M2 phenotype after 4 days, thus realizing the regulation of macrophage phenotype conversion. Li et al. [85] found that silicon could promote M2 polarization of macrophages, so they designed a 5% (w/v) calcium silicate/β-tricalcium phosphate (CaSiO3-β-TCP) scaffold and loaded IFN-γ onto the scaffold. When the scaffold was implanted subcutaneously, IFN-γ was released and induced M1 polarization of macrophages in the first three days, and then the scaffold gradually degraded and released silicon ions to induce M2 polarization. This design enabled the sequential polarization of the macrophage phenotype and promoted the vascularization of tissue-engineered bone.

These results thus indicated the importance of characterizing the release profiles *in vivo* and tailoring to the application at hand. As mentioned above, macrophages play diverse roles in different tissue regeneration processes, and each phenotype of macrophages may appear and remain active at different time points. Therefore, it is crucial for engineers to design the delivery system and optimize the release curve according to the specificity of each tissue, and choose an optimal modulatory time to avoid a mixed phenotype. Moreover, excessive polarization of a specific phenotype of macrophages can lead to undesirable results [86]. However, due to the complexity of the environment *in vivo*, there are few studies focusing on the relationship between the required doses of drugs and the degree of macrophage polarization. It has been shown that macrophages from aged mice are hypo-responsive to IFN-γ, suggesting that higher doses are required to promote M1 polarization [87]. Therefore, more research is needed in the future to determine the exact dose required for polarizing macrophages *in vivo*, which is essential for the successful application of the delivery system.

Additionally, due to their special colloidal stability, some nanoparticles have been increasingly used as carriers to deliver drugs to targets and to achieve sustained drug release [88]. When modified zeolitic imidazolate framework-8 (ZIF-8) NPs were ingested by M1 macrophages, due to their pH-sensitive degradation ability, ZIF-8 NPs would degrade in the acidic condition of the endosomes and release the internal proteins, thereby reducing undesired drug release and increasing drug concentration at the target site [89]. Anti-CD16/32 antibody could also be jointed to the surface of nanoparticles, enabling them to be more precisely targeted at M1 macrophage to improve efficiency.

Shields et al. [90] designed a "backpack" made of three layers of biodegradable polymers, a polyvinyl alcohol (PVA) layer as the innermost middle layer to load IFN-γ, and two layers of poly (lactic-co-glycolic) acid (PLGA) on either side to provide structural support. The backpack could adhere to the surface of macrophages and continuously release IFN-γ to promote the M1 polarization of macrophages. The design of the backpack, which is currently used to treat cancer, is a novel delivery strategy and could be applied to tissue regeneration by changing the immunomodulatory payloads and the dosage.

Because of the remarkable results achieved in the regulation of macrophage phenotypes by biomaterials, and the close relationship between macrophages of different phenotypes and the regeneration process of musculoskeletal system soft tissue, we believe that the outcomes of tissue repair can be improved through the immunomodulation of biomaterials and this is a promising direction for the future design of musculoskeletal soft tissue regenerative biomaterials.

4. Application of immunomodulatory biomaterials in musculoskeletal soft tissue regeneration

Macrophages are considered to be closely involved in key bone

regeneration phases, including osteogenesis and osteoclastogenesis. Many studies have investigated the immunomodulation of biomaterials through surface chemistry, topography, and delivery of bioactive molecules, with encouraging results in bone regeneration [91]. In contrast, the application of immunomodulatory biomaterials in the regeneration of musculoskeletal soft tissue seems less as shown in Table 1, and the exploration of the cross-talk between biomaterial, macrophage polarization and regeneration is also limited. However, it is of vital importance to select appropriate engineering parameters and the bioactive molecules for delivery. Therefore, we attempted to summarize studies on biomaterials that promoted the regeneration of musculoskeletal soft tissue through immunomodulation of macrophage polarization, thereby providing cues for future material design.

4.1. Applications in cartilage regeneration

Osteoarthritis is a disease characterized by the degradation of articular cartilage and bone as well as the inflammation of the synovium and joint fat pad. Numerous studies have shown that pro-inflammatory cytokines and synovial macrophages play a crucial part in the progression of osteoarthritis [36,113]. Therefore, through immunomodulation of biomaterials, mitigating the inflammatory environment in the joints and regulating the behavior of macrophages have become a promising method to prevent joint destruction of osteoarthritis and promote cartilage regeneration [114].

4.1.1. Biochemical properties of biomaterials for cartilage regeneration

ECM derived biomaterials have been extensively studied to promote regeneration due to their immunomodulatory capabilities, including type 2 collagen, a specific ECM of cartilage. Dai et al. [42,92] found that squid type II collagen had an obvious anti-inflammatory effect, which could not only reduce the secretion of pro-inflammatory cytokines by promoting the dephosphorylation of p-STAT1 in M1 macrophages, but also induce the polarization of M2 macrophages. In the OA rat model, SCII also increased M2 macrophages and the expression levels of TGF-β1 and TGF-β3 within the synovial membrane, creating a pro-chondrogenic environment and inhibiting chondrocyte apoptosis and matrix metalloproteinase 13 (MMP13) production. This might be attributed to the fact that the released free amino acids (e.g. glycine, histidine, proline, hydroxyproline) from squid type II collagen could effectively inhibit the release of pro-inflammatory cytokines and promote the polarization of M2 macrophages. They then developed a double-network hydrogel composed of squid cartilage type II gelatin (SGII) and hyaluronic acid (HA), which was capable of immunomodulating macrophage polarization, and validated its role in segmental costal cartilage defect repair [115].

Arlov et al. [93] demonstrated that sulfated alginate 3D hydrogels had outstanding mechanical properties and could also produce significant anti-inflammatory effects on IL-1β induced chondrocytes. Solution forms of alginate sulfate also had anti-inflammatory properties, inhibiting the expression of pro-inflammatory markers IL-6, CXCL8 and PTGS2 in human chondrocytes stimulated by IL-1β and reducing the expression and synthesis of TNF-α in M1 macrophages induced from human THP-1 cells [94]. The solution could be injected directly into the joint cavity for viscosupplementation of the synovial fluid and inhibiting inflammation with minimal damage from invasive surgery. As studies constantly reveal the prominent role of macrophages in inflammatory joint disease, we believe that in the future, more and more biomaterials with immunomodulatory properties will be studied to treat inflammatory joint disease and promote cartilage regeneration.

In addition, some metals have been used to enhance the immunomodulatory properties of biomaterials due to their special ability to regulate the behaviors of macrophages. When these biomaterials degrade in the body, they release metal ions, which have important effects on the immune environment. The incorporation of Cu^{2+} into bioactive glass-ceramic scaffolds (Cu-BGC) significantly promoted

Table 1
Applied immunomodulatory biomaterials for musculoskeletal soft tissue engineering.

Engineering Parameters	Property	Application	Effect	Ref
Natural-origin biomaterials	Squid type II collagen (SCII)	Cartilage	Induces M2-biased polarization of macrophages; directly induces chondrogenesis or indirectly through M2 macrophage-mediated TGF-β/Smad pathway; inhibits chondrocyte apoptosis and hypertrophy	[42,92]
	Sulfated alginate	Cartilage	Alleviates the effects of pro-inflammatory cytokines on chondrocytes and inhibits the expression of pro-inflammatory cytokine TNF-α in M1 macrophages	[93,94]
	Decellularized ECM	Tendon/Muscle	Has excellent biocompatibility but relatively poor mechanical properties; shifts macrophage polarization to pro-regeneration phenotype	[95,96,97]
Releasing ions	Cu	Cartilage	Promotes chondrocyte differentiation and cartilage regeneration through hypoxia-inducible factor (HIF) pathway; induces M2 polarization within a lower concentration range of 0.5–16 ppm, while inducing inflammatory response due to its cytotoxic effects at a higher concentration (28.3 ppm)	[98,99]
	Mg, Sr	Cartilage	Reduces inflammation and induces chondrogenic differentiation of MSCs	[100,101]
Topography	Aligned substrates	Tendon	Induces M2-like polarization and extracellular synthesis of tendon fibroblasts.	[102]
	Aligned nanofibers + decellularized ECM	Muscle	Increases M2 macrophages and myofiber regeneration while functional muscle regeneration is limited	[103,104]
	Aligned nanofibers + mechanical load	Tendon	Suppresses inflammatory activation and increases M2 macrophages	[105]
	Aligned microfibers with different diameter sizes	Tendon	Smaller diameter (1.27 μm) induces higher M2-like markers and possesses better mechanical properties than large diameter (2.5 μm)	[106]
Delivery of bioactive factors	TGF -β	Cartilage	Induces M2 polarization and MSCs recruitment and chondrogenesis	[107]
	IL-4	Muscle	Shifts macrophage polarization from M1 to M2 and achieves prominently functional muscle regeneration.	[108]
	myostatin inhibitors	Muscle	Increases regulatory T cells and M2 polarization.	[109]
	IL-10 encoding plasmid DNA	Cartilage	Induces M2 polarization; reduces pro-inflammatory cytokines and joint damage	[110]
	Anti-TNF-α siRNA	Cartilage	Reduces cartilage destruction and inflammatory response	[111]
	Manganese ferrite and ceria	Cartilage	Induces macrophage polarization from M1 to M2 phenotype by scavenging reactive oxygen species (ROS) and improving hypoxia environment	[112]
	S-methylisothiourea hemisulfate salt and catalase	Cartilage	Promotes M2 polarization by restoring mitochondrial function	[89]

cartilage regeneration and increased the gene expression levels of ACAN, COL II and SOX-9 [98]. In one study, the effective concentration of Cu^{2+} ions released by the Cu-BGC scaffold was 0.5–16 ppm, and a decrease in iNOS(M1 marker)expression with concomitant increase in CD206(M2 marker) expression were observed, as well as inhibited expression of pro-inflammatory cytokines (TNF-α and IL-18) and enhanced anti-inflammatory cytokines (IL-10). However, in another study, when Cu^{2+} ions were at a high concentration (28.3 ppm), macrophages were induced into the M1 phenotype, suggesting that the use of Cu^{2+} ions to induce the anti-inflammatory phenotype of macrophages might be concentration-dependent [99]. Strontium releasing calcium silicate (Sr-CS) ceramic could also promote cartilage and subchondral bone regeneration [100]. On one hand, strontium ions promote the osteogenic and chondrogenic differentiation of MSCs; while on the other hand, strontium also inhibited the inflammatory response mediated by macrophages in the synovial membrane. Magnesium could regulate the polarization of macrophages induced by lipopolysaccharide (LPS) and interferon-γ (IFN-γ) [101]. Although the percentage of CD206-positive (M2)cells decreased in the presence of strontium, the decrease of CCR7-positive cells (M1) was more significant. In addition, Magnesium reduced macrophage-induced inflammation by impeding the nuclear translocation and phosphorylation of NF-κB in macrophages and inhibiting its activation, thereby enhancing chondrogenic differentiation of human bone marrow mesenchymal stem cells (hBMSCs). Parks et al. [116] mixed magnesium hydroxide (MH) nanoparticles into PLGA scaffolds and achieved good results in promoting cartilage regeneration.

MSCs have been widely studied as a therapeutic tool for OA due to their chondrogenic potential and immunomodulatory capacities [117]. MSCs can not only suppress the activation of M1 macrophages and reprogram them to polarize into M2 macrophages, but can also ameliorate synovial inflammation in a mouse OA model [117,118]. However, M1 macrophages have been shown to impede the chondrogenic differentiation of MSCs, indicating that although MSCs can improve the inflammatory environment and induce M2 polarization, exacerbated inflammation can in turn inhibit their functions [41].

Considering the potential of these immunomodulatory biomaterials in inhibiting inflammatory response and promoting chondrogenic differentiation of MSCs, the future application of these biomaterials together with MSCs may serve as a beneficial strategy to enhance the effect of cartilage repair. Since the effect of metal ions on macrophages may be concentration dependent, there is currently a lack of systematic studies to validate the relationship between different metal ion concentrations and macrophage phenotypes [98,99]. In addition, how to maintain its concentration within the effective range in the complex environment of the joint cavity is also an urgent problem that needs to be solved.

4.1.2. Delivery system for cartilage regeneration

Due to the sophisticated composition and architecture of articular cartilage, biomaterial-based scaffolds have been investigated for controlled release, targeting and local delivery of immunoregulatory factors, including proteins (e.g., cytokines and enzymes), and nucleic acids (e.g., silencing RNA and plasmid DNA). These immunoregulatory factors can mitigate the inflammatory environment in articular cartilage, eliminate adverse factors, and promote cartilage regeneration [119]. Ji et al. [107] introduced TGF-β1 into a thermosensitive and photocrosslinkable hydrogel (GM-HPCH) to shift macrophage phenotype from M1 to M2 and stimulate MSC recruitment. Besides, the hydrogel had good biocompatibility and biodegradability. When injected into the joints, the hydrogel would form a gel after thermal gelation and be photocrosslinked by UV light to improve its mechanical properties. As an effective anti-inflammatory cytokine, IL-4 inhibits macrophages from synthesizing IL-1,TNF-α,IL-6,IL-8 and IL-12, of which TNF-α is one of the main cytokines that mediate inflammation and IL-1 is a key mediator of cartilage and bone destruction. Systemic treatment of IL-4 has been shown to protect cartilage, and IL-10 has a similar effect. Both of them can reverse cartilage degradation in rheumatoid arthritis [120,121]. Jain et al. [110] modified non-condensing alginate nanoparticles with tuftsin peptide targeting macrophages to transduce IL-10 plasmid DNA. After intraperitoneal administration, these nanoparticles were successfully localized to the joint, significantly increasing the

proportion of M2 phenotype synovial macrophages and downregulating levels of pro-inflammatory cytokines (TNF-α,IL-6 and IL-1β), thereby reducing damage to the joint. Howard et al. [111] used chitosan nanoparticles loaded with small interfering RNA (siRNA) to knockdown TNF-α in macrophages throughout the body, which also had a significant protective effect on cartilage. Previous delivery system mainly focused on enhancing chondrogenic differentiation of MSCs, only a few researches paid attention to the delivery of bioactive factors and the changes of immune microenvironment. Therefore, more studies are in need to explore the mechanism of cross-talk among loaded bioactive molecules, host immune response and cartilage regeneration to achieve most desirable results.

Hypoxia also exerted a significant effect on the polarization of macrophages. M1 macrophages rely mainly on glycolysis for energy, while M2 macrophages rely more on oxidative phosphorylation, suggesting that hypoxia may drive M1-biased polarization [122]. In the synovium of rheumatoid arthritis, lack of oxygen causes immune cells to up-regulate hypoxia-inducible factor (HIF-1α), activating pro-inflammatory pathways and exacerbating synovitis [123]. Kim et al. [112] used mesoporous silica nanoparticles to deliver manganese ferrite and ceria nanoparticles to remove ROS and improve hypoxia, which eliminated pro-inflammatory M1 macrophages and induced M2 macrophages for rheumatoid arthritis (RA) treatment. Hypoxia can also impair mitochondrial functions, leading to metabolic disorders that further aggravate the inflammatory response. Therefore, some studies have proposed new strategies for impaired mitochondrial function. Zhou et al. [89] encapsulated S-methylisothiourea hemisulfate salt, which inhibited the production of NO, and catalase, which catalyzed the generation of oxygen, into modified zeolitic imidazolate framework-8 (ZIF-8) nanoparticles and delivered these to the knee joints of OA mice. Such treatment significantly improved mitochondrial function and facilitated the transformation of macrophages from M1 to M2, reducing cartilage damage. These successful studies on oxygen levels and metabolic reprogramming have opened a new field of research on the regulation of macrophage phenotype to promote cartilage regeneration.

Current evidence suggests that M1 macrophages are mainly involved in destructive and degenerative processes in chondrocytes, while M2 macrophages can aid in cartilage regeneration. Therefore, inhibiting M1 and inducing M2 macrophages are the mainstream strategy for the treatment of osteoarthritis and the promotion of cartilage regeneration. In view of this, precise targeting and controlled release to improve therapeutic effectiveness efficacy offers great potential to delivery systems for cartilage regeneration. Systemic delivery requires higher drug doses and may exert unknown effects on tissues other than the target, and nanocarriers offer a new solution to these problems. A targeting ligand can be grafted on the surface of nanoparticles to achieve specific delivery [124]. Chen et al. [125] synthesized a nanocomposite with quadrilateral ruthenium nanoparticles (QRuNPs) as the core and thermosensitive PLGA as vesicles to deliver resveratrol to induce M2 polarization. The nanocomposite is surface-modified with dextran sulfate (DS) that can bind to macrophage scavenger receptors, so that it can be targeted to macrophages. Besides, the nanocomposite has photothermal response characteristics, and can release resveratrol rapidly and continuously under 808 nm laser irradiation, so as to realize the controlled release of payloads. In addition, Zhou et al. [89] have achieved precise M1 macrophage targeting by modifying anti-CD16/32 antibody on the surface of nanoparticles and prolonged the existence of nanoparticles in the synovium. When used as a vector for gene delivery, nanoparticles can protect nucleic acids from enzymatic degradation, reduce immunogenicity and increase transmembrane efficiency [126]. Therefore, it is reasonable to assume that developing an efficient and safe nanocarrier with a restricted target is the future direction of designing delivery systems for cartilage regeneration.

4.2. Applications in tendon/ligament regeneration

Tendons and ligaments are the connective tissue that connects muscle to bone or between bone and bone. They have unique biomechanical properties and are responsible for the transfer of payload between muscle and bone and assist in bodily movement. These unique functions require that biomaterials used in tendon and ligament repair should not only promote better cell growth and tissue regeneration, but also provide the necessary mechanical support, which increases the requirement for mechanical properties of biomaterials. Therefore, autografts or allografts are often used to repair damaged tendons or ligaments, while the development of biomaterials is relatively immature. At present, the main biomaterials studied are naturally-derived ECM, silk-based scaffolds and some synthetic materials such as poly(l-lactic acid) (PLLA), PLGA, and polycaprolactone (PCL) [127,128].

4.2.1. Biochemical properties of biomaterials for tendon/ligament regeneration

As mentioned above, ECM-derived scaffolds could promote macrophages to favor the M2 phenotype. This is related to different tissue sources of ECM as well as processing methods, and may exert an important impact on tissue remodeling at the implantation site [129, 130]. Valentin et al. [130] used different ECM scaffolds and autologous tissues to repair defects in the musculotendinous tissue of the abdominal wall in rats, and explored the host reactions caused by them. They found that the ECM scaffold promoted anti-inflammatory macrophages, whereas the autograft promoted pro-inflammatory macrophages by 2 weeks after implantation. Beattie et al. [77] also found in their study that it was likely that urinary bladder matrix (UBM) derived decellularized ECM scaffolds induced anti-inflammatory macrophages, causing more progenitor cells to migrate to the tendons repaired by the ECM scaffold than autologous tendon graft. The decellularized UBM-ECM scaffold did not contain complete cellular components, while the apoptosis and necrosis of the cells in the autograft led to the release of pro-inflammatory mediators. In addition, porcine small intestinal submucosa ECM (SIS-ECM), as a clinical Food and Drug Administration (FDA)-approved biomaterial for rotator cuff repair, could attract bone marrow-derived cells after implantation. When it was used to repair the Achilles tendons of mice, it was observed that by 16 weeks, bone marrow-derived cells labeled with green fluorescent protein (GFP) were present throughout the tendon remodeling process without any inflammatory response [95].

4.2.2. Physical properties of biomaterials for tendon/ligament regeneration

Although naturally-derived ECMs have excellent biocompatibility, their elastic moduli are inferior to that of tendon, which means that they are not able to provide enough mechanical support like a normal tendon [96]. In contrast, synthetic polymers can be designed to improve mechanical properties, mimic the structure of natural tendons and regulate tendon fibroblast function and matrix deposition [131]. Various studies have explored the effects of material cues on stem cell tenogenic differentiation, with recent studies having started to explore material cues on macrophage polarization and tendon regeneration [132]. In Fotticchia's study [133], they found that tendon cells inoculated on polycaprolactone scaffolds processed by electrospinning technology with aligned fibres exhibited elongated morphology adapted to the scaffold topography after seven days of culture. However, in their study, there were no significant differences in the expression of key inflammatory cytokine genes in cultured THP-1 cells between the aligned and random fibers, which is contrary to conventional wisdom. The inflammatory response they induced was limited, and might be related to the short duration of the experiment. M1 macrophages could secrete pro-inflammatory cytokines through paracrine pathways, leading to a significant downregulation of signals related to extracellular matrix synthesis of tendon fibroblasts, accompanied by a significant increase in matrix degradation enzyme synthesis. However, the highly-aligned

electrospun PCL scaffold could not only promote the synthesis of tendon matrix by fibroblasts, but also significantly reduce the influence of the inflammatory environment on fibroblasts [102]. Angelina et al. [105] demonstrated in their experiments that aligned nanofiber substrates promoted M2-like polarization. They found that macrophages were more sensitive to mechanical loading than tendon fibroblasts and mechanical loading also had a similar "mechano-protective" effect which could mute inflammatory activation. Thus, topographical cues of the biomaterial scaffold and mechanical loading could be combined to have a profound effect on macrophages and fibroblasts to direct tendon tissue repair. In another study, Khatib et al. [106] investigated the effect of different diameters of highly aligned PLGA fibers on tendon regeneration and found that the smaller fiber diameter of 1.27 μm had better mechanical properties and promoted macrophage skewing towards the M2 phenotype compared with the 2.5 μm fiber diameter. Collectively, the results of these studies indicate that surface topography and fiber diameter of the biomaterial and the mechanical loading play an important role in modulating macrophage phenotypes and mitigating the effects of the inflammatory environment on fibroblasts. However, these results are mostly derived from *in vitro* models. Tendon repair after injury is a complex process of cellular events and cell-matrix interactions that cannot be perfectly simulated *in vitro*. Future systematic *in vivo* experiments are needed to provide sufficient evidence that regulating macrophage polarization can promote high-quality tendon regeneration.

The mechanical properties of the scaffold, including tensile load and mechanical strength, are important to tissue engineering biomaterials for tendon/ligament applications. When designing load-bearing biomaterials, the degradation rate of biodegradable tissue engineering biomaterials is required to match the rate of tissue growth, and excessive degradation will lead to the loss of mechanical properties of biomaterials and failure of grafts [127]. Monocyte-derived macrophages are the major cell types involved in biodegradation of implanted materials [134]. Wissing et al. [135] used electrospun poly-ε-caprolactone-bisurea scaffolds with different fiber diameters (2 or 6 μm) and orientations (isotropic or anisotropic) and inoculated macrophages onto the scaffolds to study the degradation of biomaterials with varying structure by macrophages. They found that the 6 μm Ø anisotropic group showed the most significant oxidative degradation, as revealed by the highest level of lipid peroxidation, gene expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and fiber erosion detected under scanning electron microscope (SEM) analysis. Although the macrophages involved in degradation exhibit specific phenotypes different from the traditional M1 or M2 classification, this study sheds light on the fact that the structure of the scaffold can influence macrophage-driven degradation. The role of the different macrophage subgroups in the degradation of biomaterials *in vivo* can be more clearly analyzed by single cell technology and regulated by the properties of biomaterials. This also provides a new solution for us to solve the problem of the mechanical strength of tendon/ligament tissue engineering biomaterials being lost too fast due to the degradation by macrophages after implantation.

4.3. Applications in muscle regeneration

Compared to cartilage and tendon/ligament, skeletal muscle has a better capacity for regeneration after injury. However, with its natural capacity for regeneration, it is still not able to deal with some severe trauma, requiring the assistance of tissue engineering/regenerative medicine strategy.

4.3.1. Biochemical and physical properties of biomaterials for muscle regeneration

There have been many attempts to use naturally-derived biomaterials such as ECM, synthetic biomaterials such as polylactic acid (PLA), poly(glycolic acid) (PGA), PCL, etc. or a combination of these to

promote skeletal muscle regeneration, with good results [136–139].

As mentioned earlier, ECM scaffold can promote M2 polarization of macrophages. When the M2 macrophages were inhibited with aspirin, the ECM mediated myogenesis was hindered [97]. Combining MSCs and ECM scaffold, Qiu et al. [140] found that they can collaboratively promote the polarization of M2 macrophages, and thus have a synergistic effect on promoting muscle regeneration. A large number of ECM scaffolds have been successfully used in clinical practice, including limb muscle repair, ventral hernia repair and esophageal reconstruction, thus proving their therapeutic efficacy [138,141]. However, there still exist some problems with ECM scaffolds in repairing volumetric muscle loss. Aurora et al. [142] found that porcine urinary bladder matrix ECM was useless for repairing the gastrocnemius musculotendinous junction injury in a rat model, and that it was only fibrotic tissue remodeling occurring, rather than muscle fiber regeneration when repairing the tibialis anterior volumetric muscle loss. They posited that the recovery of muscle function resulting from ECM implantation may be due to fibrotic deposition rather than muscle fiber regeneration. Furthermore, limited or even no muscle fiber regeneration was independent of the source of the ECM scaffold [143,144]. The cellular and molecular mechanisms of ECM scaffold mediated muscle remodeling are still not fully understood [145]. A recent study demonstrated that limited muscle fiber regeneration may be related to the low number of satellite cell migration at the transplant site and the low function of activated macrophages [146]. Thus, application of ECM scaffolds combined with myogenic cells or bioactive factors that can modulate macrophage functions may be a beneficial strategy to appreciably enhance *de novo* muscle fiber regeneration.

As for synthetic biomaterials, surface topography plays an important role in their functional success. Numerous studies have shown that aligned fibers promote the formation of myotubes, as opposed to randomly-aligned fibers [147–149]. Topographic cues of highly-aligned electrospun scaffolds have an effect on the adhesion and proliferation of myoblasts and facilitate the fusion of myotubes. Moreover, it has been well recognized that highly aligned nanofiber could promote M2 polarization of macrophages. Patel et al. [103] combined decellularized muscle ECM with electrospun aligned nanofibers and found that this composite promoted the growth and myogenesis of satellite cells *in vitro*. They then used the scaffold to treat volume muscle loss in a murine model, verifying the scaffold's enhancement of myofiber regeneration and promotion of anti-inflammatory M2 macrophages [104]. These hybrid scaffolds not only display bioactivity and biocompatibility, but can be modified to enhance mechanical strength as well. The ECM scaffold alone resulted in improved muscle function with limited muscle regeneration, whereas this combination of ECM with aligned nanofibers increased muscle fiber regeneration without functional improvement. The lack of functional improvement may be due to the short duration of study (28 days), and the regenerated muscle fibers are not yet innervated to produce force. Nevertheless, these results are exciting as they demonstrated the important role of modulating macrophage phenotypes through surface topography of the scaffolds in the repair of volume muscle loss, thus paving the way for future biomaterial design.

4.3.2. Delivery system for muscle regeneration

The complex interaction between the immune system and muscle is the key to muscle regeneration. Some cytokines are involved in the co-regulation of inflammatory response and myogenesis, making them ideal candidates for delivery to immunomodulate muscle regeneration. IL-10 regulates the transition of macrophages from M1 to M2, which is necessary for regeneration of injured muscle [60]. Another cytokine, IL-4, not only directs the growth of myoblast cells and fuses them into myoblast tubes, but also drive the accumulation of M2 through self-renewal rather than recruitment [150,151]. Raimondo et al. [108] injected IL-4-conjugated gold nanoparticles into murine skeletal muscle 3 days after ischemic injury to shift macrophage polarization from M1 to M2 and attained functional muscle regeneration. Because the cross-talk

between macrophages and muscle regeneration process is complex and many signaling pathways remain to be elucidated, more research into the regulation of muscle regeneration by using biomaterials in conjunction with these cytokines may be promising. In addition to cytokines, there are other proteins and nucleic acids that can be delivered to immunomodulate muscle repair. Estrellas et al. [109] used biological hydrogels composed of hyaluronic acid (HA) and processed extracellular matrix as a scaffold to deliver myostatin inhibitors to regulate the immune microenvironment to induce myogenesis. This therapy significantly increased FoxP3+ regulatory T cell and Foxp3 gene expression and promoted M2 polarization and expression of anti-inflammatory cytokines in CD206 + macrophages. There have been few (or no) studies to directly regulate macrophage polarization during muscle regeneration by genes loaded in scaffolds. Hepatocyte growth factor (HGF) treatment can up-regulate the expression level of M2 marker genes (IL-10 and Arg1) and inhibit the expression of M1 macrophage marker genes (IL-1β, iNOS, and TNFα) in Raw 264.7 cells through the CaMKKβ-AMPK signaling pathway [152]. Due to the short half-life of HGF, Choi et al. [153] delivered HGF-expressing plasmid vectors to mice with muscle injury and successfully enhanced muscle regeneration. After the knockout of microRNA-155 (miR-155) in mice, the prolonged immune response of M1 macrophages and delayed activation of M2 macrophages led to muscle regeneration defect. The significant role of miR-155 in regulating the balance of different macrophage phenotypes also provides a new intervention for the treatment of some degenerative muscle diseases.

As already mentioned, sequential activation of M1 and M2 is critical for the activation, proliferation, and differentiation of satellite cells and FAPs. Therefore, there is a reasonable prospect that biomaterial-based delivery to activate macrophages and induce the transformation of phenotypes at an appropriate time point is a potential therapeutic approach to promote muscle regeneration. Although such attempts have been limited so far, excellent results have been obtained, including muscle fiber regeneration and functional improvements [108]. However, researchers need more *in vitro* and *in vivo* studies to determine the most optimal payload, dosage and precise timing to ensure that the delivery system works as intended.

5. Future perspective

Current studies mainly focus on the immunomodulatory effects of biomaterials in bone repair, and there are few studies on cartilage, tendon/ligament and muscle. However, increasing evidence shows that macrophages of different phenotypes play important roles in cartilage destruction in osteoarthritis and cartilage repair and regeneration, making it a new therapeutic strategy to induce macrophages into the optimal functional phenotypes to enhance cartilage regeneration. Type II collagen is the indispensable component of articular cartilage, and some collagens and hydrogels with excellent immunomodulatory capacity are promising biomaterials for cartilage repair in the future. The delivery of bioactive molecules with immunomodulatory effects on macrophage function by biomaterials are also potential therapeutic options, which requires us to develop stable and biocompatible biomaterials for sustained drug release and more accurate delivery of the molecules to the target site. In the healing process after tendon and ligament injury, macrophages are involved in the process of inflammation and matrix deposition, while depletion of macrophages reduces scar formation. Therefore, we need to establish more accurate classification of macrophage subsets and functions via high throughput screening methods, so as to provide guidance for immune regulation to promote high-quality healing. Although ECM materials are widely recognized for their specific roles in modulating macrophage phenotypes, there still exists challenges in the treatment of large defects. There have been studies that have explored the surface topography of biomaterials such as the alignment of fibers that regulate macrophage phenotypes to promote tendon and muscle regeneration, with good results. It is known

that the physical and chemical properties of the biomaterial surface, such as stiffness, porosity, and hydrophilicity, have important implications on regulating the macrophage phenotype, but there are still limited attempts in this area. We believe that in the future, more researchers will focus on the modification of biomaterials through physical and chemical methods to more effectively regulate the function of macrophages to improve the effects of tissue regeneration.

At present, the failure of many in-situ tissue engineering biomaterials like silk scaffold for anterior cruciate ligament reconstruction is due to their rapid degradation *in vivo*, and the loss of structure and mechanical properties during the degradation process that cannot be quickly compensated by newly-formed tissue. We have shown that the properties of biomaterials can influence macrophage-mediated degradation, and more studies are needed in the future to figure out the relationship between the different properties of biomaterials and degradation-related macrophage sub-populations. If the problem of too rapid degradation *in vivo* can be resolved by modification of biomaterials, the application of in-situ tissue engineering biomaterials will surely be advanced to a new level.

This review mainly focused on the important role of biomaterial mediated modulation of macrophage phenotypes for soft tissue regeneration in the musculoskeletal system. However, with the development and application of new technology platforms such as single-cell sequencing and transcriptomic analysis, we have realized that macrophage polarization can span beyond the conventional binary states (i.e., M1 vs M2). Therefore, we may need to classify the functions of different subsets of macrophages more accurately according to the genes and markers they express and the cytokines they secrete, so as to better understand the cross-talk between biomaterials and macrophages *in vivo*. For example, we can find the specific subset of macrophages that lead to fibrosis after implantation of the synthetic biomaterial through single-cell RNA sequencing, which is a more accurate screening method. Through single-cell sequencing, we can have a more comprehensive and in-depth understanding of the macrophage phenotype and functional changes caused by the implantation of different biomaterials, so as to select the best biomaterial parameters to induce the most suitable macrophage phenotype for tissue regeneration.

Although macrophages play an important central role in tissue regeneration, other key cells in the immune system and the dynamic connections between them are equally important. The function of different subsets of T cells and other immune cells in regeneration is still not well understood and further research is needed to explore whether they can be used as effective therapeutic targets for musculoskeletal system soft tissue regeneration in the future.

Previous design of biomaterials focused on the mechanical properties and the induction of stem cell differentiation, while few studies on the immunomodulation capacities of biomaterials have been carried out. However, our review demonstrated the excellent results of modulating macrophage functions by biomaterials, and we firmly believe that biomaterial-mediated immunomodulation of macrophage polarization will be a promising direction for the future design of tissue engineering biomaterials.

6. Conclusion

From these studies, we can conclude that active control of the immune system rather than a passive approach, is an effective therapeutic strategy for enhancing musculoskeletal system soft tissue regeneration. Macrophages play a crucial role in the healing process, and their high plasticity is a means of modulating polarization via immunomodulatory properties of biomaterials. At present, strategies to regulate the polarization of macrophages mainly include the modification of the physicochemical characteristics of the surface of biomaterials, the biochemical characteristics of the biomaterials themselves, and the utilization of biomaterials as platforms for the delivery of bioactive molecules. These novel strategies provide effective interventions for musculoskeletal

system soft tissue repair and may serve as potential therapeutic modalities for critical-sized defects.

Declaration of competing interest

None.

References

[1] Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016, Lancet 390 (10100) (2017) 1211–1259.

[2] N. Shibuya, D.C. Jupiter, Bone graft substitute: allograft and xenograft, Clin. Podiatr. Med. Surg. 32 (1) (2015) 21–34.

[3] A.M. Briggs, A.D. Woolf, K. Dreinhöfer, et al., Reducing the global burden of musculoskeletal conditions, Bull. World Health Organ. 96 (5) (2018) 366–368.

[4] J.M. Anderson, A. Rodriguez, D.T. Chang, Foreign body reaction to biomaterials, Semin. Immunol. 20 (2) (2008) 86–100.

[5] S. Farah, J.C. Doloff, P. Müller, et al., Long-term implant fibrosis prevention in rodents and non-human primates using crystallized drug formulations, Nat. Mater. 18 (8) (2019) 892–904.

[6] A.J. Vegas, O. Veiseh, J.C. Doloff, et al., Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates, Nat. Biotechnol. 34 (3) (2016) 345–352.

[7] Q. Liu, A. Chiu, L.H. Wang, et al., Zwitterionically modified alginates mitigate cellular overgrowth for cell encapsulation, Nat. Commun. 10 (1) (2019) 5262.

[8] Z. Julier, A.J. Park, P.S. Briquez, M.M. Martino, Promoting tissue regeneration by modulating the immune system, Acta Biomater. 53 (2017) 13–28.

[9] L. Chung, D.R. Maestas Jr., F. Housseau, J.H. Elisseeff, Key players in the immune response to biomaterial scaffolds for regenerative medicine, Adv. Drug Deliv. Rev. 114 (2017) 184–192.

[10] E. Mariani, G. Lisignoli, R.M. Borzì, L. Pulsatelli, Biomaterials: foreign bodies or tuners for the immune response, Int. J. Mol. Sci. 20 (3) (2019).

[11] K. Sadtler, K. Estrellas, B.W. Allen, et al., Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells, Science 352 (6283) (2016) 366–370.

[12] B.N. Brown, B.D. Ratner, S.B. Goodman, S. Amar, S.F. Badylak, Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine, Biomaterials 33 (15) (2012) 3792–3802.

[13] T.D. Smith, R.R. Nagalla, E.Y. Chen, W.F. Liu, Harnessing macrophage plasticity for tissue regeneration, Adv. Drug Deliv. Rev. 114 (2017) 193–205.

[14] A. Vishwakarma, N.S. Bhise, M.B. Evangelista, et al., Engineering immunomodulatory biomaterials to tune the inflammatory response, Trends Biotechnol. 34 (6) (2016) 470–482.

[15] M.E. Bianchi, DAMPs, PAMPs and alarmins: all we need to know about danger, J. Leukoc. Biol. 81 (1) (2007) 1–5.

[16] H. Kono, A. Onda, T. Yanagida, Molecular determinants of sterile inflammation, Curr. Opin. Immunol. 26 (2014) 147–156.

[17] T.L. Adair-Kirk, R.M. Senior, Fragments of extracellular matrix as mediators of inflammation, Int. J. Biochem. Cell Biol. 40 (6–7) (2008) 1101–1110.

[18] A. Ozinsky, D.M. Underhill, J.D. Fontenot, et al., The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors, Proc. Natl. Acad. Sci. U. S. A. 97 (25) (2000) 13766–13771.

[19] S.A. Eming, T.A. Wynn, P. Martin, Inflammation and metabolism in tissue repair and regeneration, Science 356 (6342) (2017) 1026–1030.

[20] M. Karin, H. Clevers, Reparative inflammation takes charge of tissue regeneration, Nature 529 (7586) (2016) 307–315.

[21] T.A. Wynn, K.M. Vannella, Macrophages in tissue repair, regeneration, and fibrosis, Immunity 44 (3) (2016) 450–462.

[22] A. Mantovani, S.K. Biswas, M.R. Galdiero, A. Sica, M. Locati, Macrophage plasticity and polarization in tissue repair and remodelling, J. Pathol. 229 (2) (2013) 176–185.

[23] D.M. Mosser, J.P. Edwards, Exploring the full spectrum of macrophage activation, Nat. Rev. Immunol. 8 (12) (2008) 958–969.

[24] M.L. Novak, T.J. Koh, Macrophage phenotypes during tissue repair, J. Leukoc. Biol. 93 (6) (2013) 875–881.

[25] F.O. Martinez, S. Gordon, The M1 and M2 paradigm of macrophage activation: time for reassessment, F1000Prime Rep. 6 (2014) 13.

[26] Y. Ohmori, T.A. Hamilton, IL-4-induced STAT6 suppresses IFN-gamma-stimulated STAT1-dependent transcription in mouse macrophages, J. Immunol. 159 (11) (1997) 5474–5482.

[27] C. Venkataraman, S. Leung, A. Salvekar, H. Mano, U. Schindler, Repression of IL-4-induced gene expression by IFN-gamma requires Stat1 activation, J. Immunol. 162 (7) (1999) 4053–4061.

[28] J.L. Stöger, M.J. Gijbels, S. van der Velden, et al., Distribution of macrophage polarization markers in human atherosclerosis, Atherosclerosis 225 (2) (2012) 461–468.

[29] M.J. Davis, T.M. Tsang, Y. Qiu, et al., Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in Cryptococcus neoformans infection, mBio 4 (3) (2013) e00264-13.

[30] J. Khallou-Laschet, A. Varthaman, G. Fornasa, et al., Macrophage plasticity in experimental atherosclerosis, PLoS One 5 (1) (2010), e8852.

[31] F. Porcheray, S. Viaud, A.C. Rimaniol, et al., Macrophage activation switching: an asset for the resolution of inflammation, Clin. Exp. Immunol. 142 (3) (2005) 481–489.

[32] T.D. Smith, M.J. Tse, E.L. Read, W.F. Liu, Regulation of macrophage polarization and plasticity by complex activation signals, Integr. Biol. (Camb.) 8 (9) (2016) 946–955.

[33] L.Y. Lu, F. Loi, K. Nathan, et al., Pro-inflammatory M1 macrophages promote Osteogenesis by mesenchymal stem cells via the COX-2-prostaglandin E2 pathway, J. Orthop. Res. 35 (11) (2017) 2378–2385.

[34] F. Loi, L.A. Córdova, R. Zhang, et al., The effects of immunomodulation by macrophage subsets on osteogenesis in vitro, Stem Cell Res. Ther. 7 (2016) 15.

[35] K.L. Spiller, S. Nassiri, C.E. Witherel, et al., Sequential delivery of immunomodulatory cytokines to facilitate the M1-to-M2 transition of macrophages and enhance vascularization of bone scaffolds, Biomaterials 37 (2015) 194–207.

[36] J. Bondeson, A.B. Blom, S. Wainwright, C. Hughes, B. Caterson, W.B. van den Berg, The role of synovial macrophages and macrophage-produced mediators in driving inflammatory and destructive responses in osteoarthritis, Arthritis Rheum. 62 (3) (2010) 647–657.

[37] A.B. Blom, P.L. van Lent, A.E. Holthuysen, et al., Synovial lining macrophages mediate osteophyte formation during experimental osteoarthritis, Osteoarthritis Cartilage 12 (8) (2004) 627–635.

[38] S. Culemann, A. Grüneboom, J.Á. Nicolás-Ávila, et al., Locally renewing resident synovial macrophages provide a protective barrier for the joint, Nature 572 (7771) (2019) 670–675.

[39] L. Utomo, Y.M. Bastiaansen-Jenniskens, J.A. Verhaar, G.J. van Osch, Cartilage inflammation and degeneration is enhanced by pro-inflammatory (M1) macrophages in vitro, but not inhibited directly by anti-inflammatory (M2) macrophages, Osteoarthritis Cartilage 24 (12) (2016) 2162–2170.

[40] H. Zhang, C. Lin, C. Zeng, et al., Synovial macrophage M1 polarisation exacerbates experimental osteoarthritis partially through R-spondin-2, Ann. Rheum. Dis. 77 (10) (2018) 1524–1534.

[41] N. Fahy, M.L. de Vries-van Melle, J. Lehmann, et al., Human osteoarthritic synovium impacts chondrogenic differentiation of mesenchymal stem cells via macrophage polarisation state, Osteoarthritis Cartilage 22 (8) (2014) 1167–1175.

[42] M. Dai, B. Sui, Y. Xue, X. Liu, J. Sun, Cartilage repair in degenerative osteoarthritis mediated by squid type II collagen via immunomodulating activation of M2 macrophages, inhibiting apoptosis and hypertrophy of chondrocytes, Biomaterials 180 (2018) 91–103.

[43] P. Kannus, Structure of the tendon connective tissue, Scand. J. Med. Sci. Sports 10 (6) (2000) 312–320.

[44] A. Nichols, K.T. Best, A.E. Loiselle, The cellular basis of fibrotic tendon healing: challenges and opportunities, Transl. Res. 209 (2019) 156–168.

[45] M. de la Durantaye, A.B. Piette, N. van Rooijen, J. Frenette, Macrophage depletion reduces cell proliferation and extracellular matrix accumulation but increases the ultimate tensile strength of injured Achilles tendons, J. Orthop. Res. 32 (2) (2014) 279–285.

[46] C.S. Chamberlain, E.M. Leiferman, K.E. Frisch, et al., The influence of macrophage depletion on ligament healing, Connect. Tissue Res. 52 (3) (2011) 203–211.

[47] K.B. Sugg, J. Lubardic, J.P. Gumucio, C.L. Mendias, Changes in macrophage phenotype and induction of epithelial-to-mesenchymal transition genes following acute Achilles tenotomy and repair, J. Orthop. Res. 32 (7) (2014) 944–951.

[48] C.N. Manning, N. Havlioglu, E. Knutsen, et al., The early inflammatory response after flexor tendon healing: a gene expression and histological analysis, J. Orthop. Res. 32 (5) (2014) 645–652.

[49] O. Virchenko, B. Skoglund, P. Aspenberg, Parecoxib impairs early tendon repair but improves later remodeling, Am. J. Sports Med. 32 (7) (2004) 1743–1747.

[50] D. Lin, P. Alberton, M.D. Caceres, E. Volkmer, M. Schieker, D. Docheva, Tenomodulin is essential for prevention of adipocyte accumulation and fibrovascular scar formation during early tendon healing, Cell Death Dis. 8 (10) (2017), e3116.

[51] M.A. Gibbons, A.C. MacKinnon, P. Ramachandran, et al., Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis, Am. J. Respir. Crit. Care Med. 184 (5) (2011) 569–581.

[52] J.E. Ackerman, M.B. Geary, C.A. Orner, F. Bawany, A.E. Loiselle, Obesity/Type II diabetes alters macrophage polarization resulting in a fibrotic tendon healing response, PLoS One 12 (7) (2017), e0181127.

[53] C.S. Chamberlain, A. Clements, J.A. Kink, et al., Extracellular vesicle-educated macrophages promote early achilles tendon healing, Stem Cell. 37 (5) (2019) 652–662.

[54] S.B. Chargé, M.A. Rudnicki, Cellular and molecular regulation of muscle regeneration, Physiol. Rev. 84 (1) (2004) 209–238.

[55] P. Londhe, J.K. Davie, Interferon-γ resets muscle cell fate by stimulating the sequential recruitment of JARID2 and PRC2 to promoters to repress myogenesis, Sci. Signal. 6 (305) (2013) ra107.

[56] J.G. Tidball, Regulation of muscle growth and regeneration by the immune system, Nat. Rev. Immunol. 17 (3) (2017) 165–178.

[57] S.E. Chen, E. Gerken, Y. Zhang, et al., Role of TNF-{alpha} signaling in regeneration of cardiotoxin-injured muscle, Am. J. Physiol. Cell Physiol. 289 (5) (2005) C1179–C1187.

[58] D. Palacios, C. Mozzetta, S. Consalvi, et al., TNF/p38α/polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration, Cell Stem Cell 7 (4) (2010) 455–469.

[59] J. Tonkin, L. Temmerman, R.D. Sampson, et al., Monocyte/Macrophage-derived IGF-1 orchestrates murine skeletal muscle regeneration and modulates autocrine polarization, Mol. Ther. 23 (7) (2015) 1189–1200.

[60] B. Deng, M. Wehling-Henricks, S.A. Villalta, Y. Wang, J.G. Tidball, IL-10 triggers changes in macrophage phenotype that promote muscle growth and regeneration, J. Immunol. 189 (7) (2012) 3669–3680.

[61] J.G. Tidball, M. Wehling-Henricks, Macrophages promote muscle membrane repair and muscle fibre growth and regeneration during modified muscle loading in mice in vivo, J. Physiol. 578 (Pt 1) (2007) 327–336.

[62] A. Uezumi, T. Ito, D. Morikawa, et al., Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle, J. Cell Sci. 124 (Pt 21) (2011) 3654–3664.

[63] M.L. Novak, E.M. Weinheimer-Haus, T.J. Koh, Macrophage activation and skeletal muscle healing following traumatic injury, J. Pathol. 232 (3) (2014) 344–355.

[64] D.R. Lemos, F. Babaeijandaghi, M. Low, et al., Nilotinib reduces muscle fibrosis in chronic muscle injury by promoting TNF-mediated apoptosis of fibro/adipogenic progenitors, Nat. Med. 21 (7) (2015) 786–794.

[65] P. Roach, D. Farrar, C.C. Perry, Surface tailoring for controlled protein adsorption: effect of topography at the nanometer scale and chemistry, J. Am. Chem. Soc. 128 (12) (2006) 3939–3945.

[66] P. Roach, D. Farrar, C.C. Perry, Interpretation of protein adsorption: surface-induced conformational changes, J. Am. Chem. Soc. 127 (22) (2005) 8168–8173.

[67] C.J. Wilson, R.E. Clegg, D.I. Leavesley, M.J. Pearcy, Mediation of biomaterial-cell interactions by adsorbed proteins: a review, Tissue Eng. 11 (1–2) (2005) 1–18.

[68] W.J. Hu, J.W. Eaton, T.P. Ugarova, L. Tang, Molecular basis of biomaterial-mediated foreign body reactions, Blood 98 (4) (2001) 1231–1238.

[69] A.K. Refai, M. Textor, D.M. Brunette, J.D. Waterfield, Effect of titanium surface topography on macrophage activation and secretion of proinflammatory cytokines and chemokines, J. Biomed. Mater. Res. 70 (2) (2004) 194–205.

[70] F.Y. McWhorter, T. Wang, P. Nguyen, T. Chung, W.F. Liu, Modulation of macrophage phenotype by cell shape, Proc. Natl. Acad. Sci. U. S. A. 110 (43) (2013) 17253–17258.

[71] M.J. Vassey, G.P. Figueredo, D.J. Scurr, et al., Immune modulation by design: using topography to control human monocyte attachment and macrophage differentiation, Adv. Sci. 7 (11) (2020), 1903392.

[72] K. Garg, N.A. Pullen, C.A. Oskeritzian, J.J. Ryan, G.L. Bowlin, Macrophage functional polarization (M1/M2) in response to varying fiber and pore dimensions of electrospun scaffolds, Biomaterials 34 (18) (2013) 4439–4451.

[73] A. Hezi-Yamit, C. Sullivan, J. Wong, et al., Impact of polymer hydrophilicity on biocompatibility: implication for DES polymer design, J. Biomed. Mater. Res. 90 (1) (2009) 133–141.

[74] K.M. Hotchkiss, G.B. Reddy, S.L. Hyzy, Z. Schwartz, B.D. Boyan, R. Olivares-Navarrete, Titanium surface characteristics, including topography and wettability, alter macrophage activation, Acta Biomater. 31 (2016) 425–434.

[75] C.H. Lee, Y.J. Kim, J.H. Jang, J.W. Park, Modulating macrophage polarization with divalent cations in nanostructured titanium implant surfaces, Nanotechnology 27 (8) (2016), 085101.

[76] S.D. Sommerfeld, C. Cherry, R.M. Schwab, et al., Interleukin-36γ-producing macrophages drive IL-17-mediated fibrosis, Sci. Immunol. 4 (40) (2019).

[77] A.J. Beattie, T.W. Gilbert, J.P. Guyot, A.J. Yates, S.F. Badylak, Chemoattraction of progenitor cells by remodeling extracellular matrix scaffolds, Tissue Eng. 15 (5) (2009) 1119–1125.

[78] O.R. Mahon, S. O'Hanlon, C.C. Cunningham, et al., Orthopaedic implant materials drive M1 macrophage polarization in a spleen tyrosine kinase- and mitogen-activated protein kinase-dependent manner, Acta Biomater. 65 (2018) 426–435.

[79] O.R. Mahon, D.C. Browe, T. Gonzalez-Fernandez, et al., Nano-particle mediated M2 macrophage polarization enhances bone formation and MSC osteogenesis in an IL-10 dependent manner, Biomaterials 239 (2020), 119833.

[80] X. Miao, X. Leng, Q. Zhang, The current state of nanoparticle-induced macrophage polarization and reprogramming research, Int. J. Mol. Sci. 18 (2) (2017).

[81] H.J. Yen, S.H. Hsu, C.L. Tsai, Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes, Small 5 (13) (2009) 1553–1561.

[82] C. Troidl, G. Jung, K. Troidl, et al., The temporal and spatial distribution of macrophage subpopulations during arteriogenesis, Curr. Vasc. Pharmacol. 11 (1) (2013) 5–12.

[83] T. Yu, W. Wang, S. Nassiri, et al., Temporal and spatial distribution of macrophage phenotype markers in the foreign body response to glutaraldehyde-crosslinked gelatin hydrogels, J. Biomater. Sci. Polym. Ed. 27 (8) (2016) 721–742.

[84] J. Chen, M. Li, C. Yang, et al., Macrophage phenotype switch by sequential action of immunomodulatory cytokines from hydrogel layers on titania nanotubes, Colloids Surf. B Biointerfaces 163 (2018) 336–345.

[85] T. Li, M. Peng, Z. Yang, et al., 3D-printed IFN-γ-loading calcium silicate-β-tricalcium phosphate scaffold sequentially activates M1 and M2 polarization of macrophages to promote vascularization of tissue engineering bone, Acta Biomater. 71 (2018) 96–107.

[86] E.M. O' Brien, G.E. Risser, K.L. Spiller, Sequential drug delivery to modulate macrophage behavior and enhance implant integration, Adv. Drug Deliv. Rev. 149–150 (2019) 85–94.

[87] P. Yoon, K.T. Keylock, M.E. Hartman, G.G. Freund, J.A. Woods, Macrophage hypo-responsiveness to interferon-gamma in aged mice is associated with impaired signaling through Jak-STAT, Mech. Ageing Dev. 125 (2) (2004) 137–143.

[88] T.C. Yih, M. Al-Fandi, Engineered nanoparticles as precise drug delivery systems, J. Cell. Biochem. 97 (6) (2006) 1184–1190.

[89] F. Zhou, J. Mei, S. Yang, et al., Modified ZIF-8 nanoparticles attenuate osteoarthritis by reprogramming the metabolic pathway of synovial macrophages, ACS Appl. Mater. Interfaces 12 (2) (2020) 2009–2022.

[90] C.W. Shields 4th, M.A. Evans, L.L. Wang, et al., Cellular backpacks for macrophage immunotherapy, Sci. Adv. 6 (18) (2020), eaaz6579.

[91] J. Lee, H. Byun, S.K. Madhurakkat Perikamana, S. Lee, H. Shin, Current advances in immunomodulatory biomaterials for bone regeneration, Adv. Healthc Mater. 8 (4) (2019), e1801106.

[92] M. Dai, X. Liu, N. Wang, J. Sun, Squid type II collagen as a novel biomaterial: isolation, characterization, immunogenicity and relieving effect on degenerative osteoarthritis via inhibiting STAT1 signaling in pro-inflammatory macrophages, Mater. Sci. Eng. C Mater. Biol. Appl. 89 (2018) 283–294.

[93] Ø. Arlov, E. Öztürk, M. Steinwachs, G. Skjåk-Bræk, M. Zenobi-Wong, Biomimetic sulphated alginate hydrogels suppress IL-1β-induced inflammatory responses in human chondrocytes, Eur. Cell. Mater. 33 (2017) 76–89.

[94] A. Kerschenmeyer, Ø. Arlov, V. Malheiro, et al., Anti-oxidant and immune-modulatory properties of sulfated alginate derivatives on human chondrocytes and macrophages, Biomater. Sci. 5 (9) (2017) 1756–1765.

[95] T. Zantop, T.W. Gilbert, M.C. Yoder, S.F. Badylak, Extracellular matrix scaffolds are repopulated by bone marrow-derived cells in a mouse model of achilles tendon reconstruction, J. Orthop. Res. 24 (6) (2006) 1299–1309.

[96] K.A. Derwin, A.R. Baker, R.K. Spragg, D.R. Leigh, J.P. Iannotti, Commercial extracellular matrix scaffolds for rotator cuff tendon repair. Biomechanical, biochemical, and cellular properties, J. Bone Joint Surg. Am. 88 (12) (2006) 2665–2672.

[97] C.L. Dearth, P.F. Slivka, S.A. Stewart, et al., Inhibition of COX1/2 alters the host response and reduces ECM scaffold mediated constructive tissue remodeling in a rodent model of skeletal muscle injury, Acta Biomater. 31 (2016) 50–60.

[98] R. Lin, C. Deng, X. Li, et al., Copper-incorporated bioactive glass-ceramics inducing anti-inflammatory phenotype and regeneration of cartilage/bone interface, Theranostics 9 (21) (2019) 6300–6313.

[99] C. Wu, Y. Zhou, M. Xu, et al., Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity, Biomaterials 34 (2) (2013) 422–433.

[100] C. Wang, B. Chen, W. Wang, et al., Strontium released bi-lineage scaffolds with immunomodulatory properties induce a pro-regenerative environment for osteochondral regeneration, Mater. Sci. Eng. C Mater. Biol. Appl. 103 (2019), 109833.

[101] T. Hu, H. Xu, C. Wang, H. Qin, Z. An, Magnesium enhances the chondrogenic differentiation of mesenchymal stem cells by inhibiting activated macrophage-induced inflammation, Sci. Rep. 8 (1) (2018) 3406.

[102] A.D. Schoenenberger, J. Foolen, P. Moor, U. Silvan, J.G. Snedeker, Substrate fiber alignment mediates tendon cell response to inflammatory signaling, Acta Biomater. 71 (2018) 306–317.

[103] K.H. Patel, A.J. Dunn, M. Talovic, et al., Aligned nanofibers of decellularized muscle ECM support myogenic activity in primary satellite cells in vitro, Biomed. Mater. 14 (3) (2019), 035010.

[104] K.H. Patel, M. Talovic, A.J. Dunn, et al., Aligned nanofibers of decellularized muscle extracellular matrix for volumetric muscle loss, J. Biomed. Mater. Res. B Appl. Biomater. 108 (6) (2020) 2528–2537, https://doi.org/10.1002/jbm.b.34584.

[105] A.D. Schoenenberger, H. Tempfer, C. Lehner, et al., Macromechanics and polycaprolactone fiber organization drive macrophage polarization and regulate inflammatory activation of tendon in vitro and in vivo, Biomaterials 249 (2020), 120034.

[106] M. El Khatib, A. Mauro, M. Di Mattia, et al., Electrospun PLGA fiber diameter and alignment of tendon biomimetic fleece potentiate tenogenic differentiation and immunomodulatory function of amniotic epithelial stem cells, Cells 9 (5) (2020).

[107] X. Ji, Z. Lei, M. Yuan, et al., Cartilage repair mediated by thermosensitive photocrosslinkable TGFβ1-loaded GM-HPCH via immunomodulating macrophages, recruiting MSCs and promoting chondrogenesis, Theranostics 10 (6) (2020) 2872–2887.

[108] T.M. Raimondo, D.J. Mooney, Functional muscle recovery with nanoparticle-directed M2 macrophage polarization in mice, Proc. Natl. Acad. Sci. U. S. A. 115 (42) (2018) 10648–10653.

[109] K.M. Estrellas, L. Chung, L.A. Cheu, et al., Biological scaffold-mediated delivery of myostatin inhibitor promotes a regenerative immune response in an animal model of Duchenne muscular dystrophy, J. Biol. Chem. 293 (40) (2018) 15594–15605.

[110] S. Jain, T.H. Tran, M. Amiji, Macrophage repolarization with targeted alginate nanoparticles containing IL-10 plasmid DNA for the treatment of experimental arthritis, Biomaterials 61 (2015) 162–177.

[111] K.A. Howard, S.R. Paludan, M.A. Behlke, F. Besenbacher, B. Deleuran, J. Kjems, Chitosan/siRNA nanoparticle-mediated TNF-alpha knockdown in peritoneal macrophages for anti-inflammatory treatment in a murine arthritis model, Mol. Ther. 17 (1) (2009) 162–168.

[112] J. Kim, H.Y. Kim, S.Y. Song, et al., Synergistic oxygen generation and reactive oxygen species scavenging by manganese ferrite/ceria Co-decorated nanoparticles for rheumatoid arthritis treatment, ACS Nano 13 (3) (2019) 3206–3217.

[113] C. Manferdini, F. Paolella, E. Gabusi, et al., From osteoarthritic synovium to synovial-derived cells characterization: synovial macrophages are key effector cells, Arthritis Res. Ther. 18 (2016) 83.

[114] C.L. Wu, N.S. Harasymowicz, M.A. Klimak, K.H. Collins, F. Guilak, The role of macrophages in osteoarthritis and cartilage repair, Osteoarthritis Cartilage 28 (5) (2020) 544–554.

[115] M. Dai, B. Sui, Y. Hua, et al., A well defect-suitable and high-strength biomimetic squid type II gelatin hydrogel promoted in situ costal cartilage regeneration via dynamic immunomodulation and direct induction manners, Biomaterials 240 (2020), 119841.

[116] K.S. Park, B.J. Kim, E. Lih, et al., Versatile effects of magnesium hydroxide nanoparticles in PLGA scaffold-mediated chondrogenesis, Acta Biomater. 73 (2018) 204–216.

[117] C.R. Harrell, B.S. Markovic, C. Fellabaum, A. Arsenijevic, V. Volarevic, Mesenchymal stem cell-based therapy of osteoarthritis: current knowledge and future perspectives, Biomed. Pharmacother. 109 (2019) 2318–2326.

[118] A.M. Hamilton, W.Y. Cheung, A. Gómez-Aristizábal, et al., Iron nanoparticle-labeled murine mesenchymal stromal cells in an osteoarthritic model persists and suggests anti-inflammatory mechanism of action, PloS One 14 (12) (2019), e0214107.

[119] J.M. Patel, K.S. Saleh, J.A. Burdick, R.L. Mauck, Bioactive factors for cartilage repair and regeneration: improving delivery, retention, and activity, Acta Biomater. 93 (2019) 222–238.

[120] J.A. van Roon, J.L. van Roy, F.H. Gmelig-Meyling, F.P. Lafeber, J.W. Bijlsma, Prevention and reversal of cartilage degradation in rheumatoid arthritis by interleukin-10 and interleukin-4, Arthritis Rheum. 39 (5) (1996) 829–835.

[121] L.A. Joosten, E. Lubberts, M.M. Helsen, et al., Protection against cartilage and bone destruction by systemic interleukin-4 treatment in established murine type II collagen-induced arthritis, Arthritis Res. 1 (1) (1999) 81–91.

[122] J. Blagih, R.G. Jones, Polarizing macrophages through reprogramming of glucose metabolism, Cell Metabol. 15 (6) (2012) 793–795.

[123] U. Fearon, M. Canavan, M. Biniecka, D.J. Veale, Hypoxia, mitochondrial dysfunction and synovial invasiveness in rheumatoid arthritis, Nat. Rev. Rheumatol. 12 (7) (2016) 385–397.

[124] M.P. Patel, R.R. Patel, J.K. Patel, Chitosan mediated targeted drug delivery system: a review, J. Pharm. Pharmaceut. Sci. 13 (4) (2010) 536–557.

[125] X. Chen, X. Zhu, L. Ma, et al., A core-shell structure QRu-PLGA-RES-DS NP nanocomposite with photothermal response-induced M2 macrophage polarization for rheumatoid arthritis therapy, Nanoscale 11 (39) (2019) 18209–18223.

[126] H. Ragelle, G. Vandermeulen, V. Préat, Chitosan-based siRNA delivery systems, J. Contr. Release 172 (1) (2013) 207–218.

[127] D.A. Brennan, A.A. Conte, G. Kanski, et al., Mechanical considerations for electrospun nanofibers in tendon and ligament repair, Adv. Healthc Mater. 7 (12) (2018), e1701277.

[128] G. Narayanan, V.N. Vernekar, E.L. Kuyinu, C.T. Laurencin, Poly (lactic acid)-based biomaterials for orthopaedic regenerative engineering, Adv. Drug Deliv. Rev. 107 (2016) 247–276.

[129] J.L. Dziki, D.S. Wang, C. Pineda, B.M. Sicari, T. Rausch, S.F. Badylak, Solubilized extracellular matrix bioscaffolds derived from diverse source tissues differentially influence macrophage phenotype, J. Biomed. Mater. Res. 105 (1) (2017) 138–147.

[130] J.E. Valentin, J.S. Badylak, G.P. McCabe, S.F. Badylak, Extracellular matrix bioscaffolds for orthopaedic applications. A comparative histologic study, J. Bone Joint Surg. Am. 88 (12) (2006) 2673–2686.

[131] N.M. Lee, C. Erisken, T. Iskratsch, M. Sheetz, W.N. Levine, H.H. Lu, Polymer fiber-based models of connective tissue repair and healing, Biomaterials 112 (2017) 303–312.

[132] J. Lin, W. Zhou, S. Han, et al., Cell-material interactions in tendon tissue engineering, Acta Biomater. 70 (2018) 1–11.

[133] A. Fotticchia, D. Musson, C. Lenardi, E. Demirci, Y. Liu, Anisotropic cytocompatible electrospun scaffold for tendon tissue engineering elicits limited inflammatory response in vitro, J. Biomater. Appl. 33 (1) (2018) 127–139.

[134] R.S. Labow, E. Meek, J.P. Santerre, Model systems to assess the destructive potential of human neutrophils and monocyte-derived macrophages during the acute and chronic phases of inflammation, J. Biomed. Mater. Res. 54 (2) (2001) 189–197.

[135] T.B. Wissing, V. Bonito, E.E. van Haaften, et al., Macrophage-driven biomaterial degradation depends on scaffold microarchitecture, Front. Bioeng. Biotechnol. 7 (2019) 87.

[136] B.M. Sicari, V. Agrawal, B.F. Siu, et al., A murine model of volumetric muscle loss and a regenerative medicine approach for tissue replacement, Tissue Eng. 18 (19–20) (2012) 1941–1948.

[137] N.J. Turner, A.J. Yates Jr., D.J. Weber, et al., Xenogeneic extracellular matrix as an inductive scaffold for regeneration of a functioning musculotendinous junction, Tissue Eng. 16 (11) (2010) 3309–3317.

[138] M.T. Wolf, C.L. Dearth, S.B. Sonnenberg, E.G. Loboa, S.F. Badylak, Naturally derived and synthetic scaffolds for skeletal muscle reconstruction, Adv. Drug Deliv. Rev. 84 (2015) 208–221.

[139] Y. Hong, A. Huber, K. Takanari, et al., Mechanical properties and in vivo behavior of a biodegradable synthetic polymer microfiber-extracellular matrix hydrogel biohybrid scaffold, Biomaterials 32 (13) (2011) 3387–3394.

[140] X. Qiu, S. Liu, H. Zhang, et al., Mesenchymal stem cells and extracellular matrix scaffold promote muscle regeneration by synergistically regulating macrophage polarization toward the M2 phenotype, Stem Cell Res. Ther. 9 (1) (2018) 88.

[141] B.M. Sicari, J.P. Rubin, C.L. Dearth, et al., An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss, Sci. Transl. Med. 6 (234) (2014), 234ra58.

[142] A. Aurora, J.L. Roe, B.T. Corona, T.J. Walters, An acellular biologic scaffold does not regenerate appreciable de novo muscle tissue in rat models of volumetric muscle loss injury, Biomaterials 67 (2015) 393–407.

[143] K. Garg, C.L. Ward, C.R. Rathbone, B.T. Corona, Transplantation of devitalized muscle scaffolds is insufficient for appreciable de novo muscle fiber regeneration after volumetric muscle loss injury, Cell Tissue Res. 358 (3) (2014) 857–873.

[144] B.T. Corona, X. Wu, C.L. Ward, J.S. McDaniel, C.R. Rathbone, T.J. Walters, The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM, Biomaterials 34 (13) (2013) 3324–3335.

[145] S.F. Badylak, J.L. Dziki, B.M. Sicari, F. Ambrosio, M.L. Boninger, Mechanisms by which acellular biologic scaffolds promote functional skeletal muscle restoration, Biomaterials 103 (2016) 128–136.

[146] K. Garg, C.L. Ward, B.T. Corona, Asynchronous inflammation and myogenic cell migration limit muscle tissue regeneration mediated by a cellular scaffolds, Inflamm. Cell Signal 1 (4) (2014).

[147] J.S. Choi, S.J. Lee, G.J. Christ, A. Atala, J.J. Yoo, The influence of electrospun aligned poly(epsilon-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes, Biomaterials 29 (19) (2008) 2899–2906.

[148] K.J. Aviss, J.E. Gough, S. Downes, Aligned electrospun polymer fibres for skeletal muscle regeneration, Eur. Cell. Mater. 19 (2010) 193–204.

[149] T. Neumann, S.D. Hauschka, J.E. Sanders, Tissue engineering of skeletal muscle using polymer fiber arrays, Tissue Eng. 9 (5) (2003) 995–1003.

[150] V. Horsley, K.M. Jansen, S.T. Mills, G.K. Pavlath, IL-4 acts as a myoblast recruitment factor during mammalian muscle growth, Cell 113 (4) (2003) 483–494.

[151] S.J. Jenkins, D. Ruckerl, P.C. Cook, et al., Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation, Science 332 (6035) (2011) 1284–1288.

[152] W. Choi, J. Lee, J. Lee, S.H. Lee, S. Kim, Hepatocyte growth factor regulates macrophage transition to the M2 phenotype and promotes murine skeletal muscle regeneration, Front. Physiol. 10 (2019) 914.

[153] M. Nie, J. Liu, Q. Yang, et al., MicroRNA-155 facilitates skeletal muscle regeneration by balancing pro- and anti-inflammatory macrophages, Cell Death Dis. 7 (6) (2016), e2261.