Advanced Drug Delivery Reviews 196 (2023) 114815

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/adr

Prospects of magnetically based approaches addressing inflammation in tendon tissues

Adriana Vinhas, Ana F. Almeida, Márcia T. Rodrigues *, Manuela E. Gomes *

3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark – Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal
ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

ARTICLE INFO

Article history:
Received 24 October 2022
Revised 24 March 2023
Accepted 25 March 2023
Available online 30 March 2023

Keywords:
Tendon
Tendinopathies
Inflammatory mediators
Regenerative medicine
Magnetic field
Therapies

ABSTRACT

Tendon afflictions constitute a significant share of musculoskeletal diseases and represent a primary cause of incapacity worldwide. Unresolved/chronic inflammatory states have been associated with the onset and progression of tendon disorders, contributing to undesirable immune stimulation and detrimental tissue effects. Thus, targeting persistent inflammatory events could assist important developments to solve pathophysiological processes and innovative therapeutics to address impaired healing and accomplish complete tendon regeneration.

This review overviews the impact of inflammation and inflammatory mediators in tendon niches, unveiling the importance of tendon cell populations and their signature features, and the influence of microenvironmental factors on inflamed and injured tendons. The demand for non-invasive instructive strategies to manage persistent inflammatory mediators, guide inflammatory pathways, and modulate cellular responses will also be approached by exploring the role of pulsed electromagnetic field (PEMF). PEMF alone or combined with more sophisticated systems triggered by magnetic fields will be considered in the design of successful therapies to control inflammation in tendinopathic conditions.

Abbreviations: ActRIIA, anti-activin receptor type IIA; ADAMTS5, A disintegrin and metalloproteinase with thrombospondin motif; ADSCs, Adipose derived stem cells; APP, amyloid beta precursor protein; ASA, aspirin; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic proteins; BGN, biglycan; BMSC, Bone marrow-derived mesenchymal stem cells; CCL, chemokine (C-C motif) ligand; CCR2, chemokine receptor 2; CCL, C-C motif chemokine ligand; Col 1, type I collagen; Col III, type III collagen; COX-2, Cyclooxygenase-2; CTGF, connective tissue growth factor; Cx, Connexins; CXCL, C-X-X motif chemokine ligand; DAMPs, Damage-associated molecular patterns; DCs, denditric cells; DEGs, differentially expressed genes; ECM, Extracellular matrix; ERK1/2, Extracellular signal-regulated kinase 1/2; FAK, Focal adhesion kinase; FDA, Food and Drug Administration; FN, Fibronectin; GAGs, glycosaminoglycans; GelMA, gelatin methacryloyl; HIPK, homeodomain-interacting protein kinase; HMGB1, intracellular high mobility group box 1; Hz, hertz; ICAM, intercellular adhesion molecule; IFM, interfascicular matrix; IFN, interferon; IGF, insulin-like growth factor; IκB, inhibitors-of-kappa B; IL, interleukin; IL-R, interleukin receptor; iNOS, inducible nitric oxide; JAK/STAT, janus kinase/signal transducer and activator of transcription; JNK, Jun N-terminal kinases; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; Erk1/2, extracellular signal-regulated kinase 1/2; Exos, exosomes; $Exos^{TSC}$, exosomes isolated from tendon stem cells; $Exos^{ADSCs}$, exosomes derived from adipose-derived mesenchymal stem cells; $Exos^{MSC}$, extracellular vesicles derived from mesenchymal stem cell; mT, Intensity; magCSs, magnetic cell sheets; magSPCL, magnetically actuated membrane made of a polymer blend of starch and polycaprolactone (SPCL) impregnated with MNPs; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; MF, magnetic field; Min, minute; MMPs, matrix metalloproteinases; MNPs, magnetic nanoparticles; MSC, mesenchymal stem cell; MHCII, II major histocompatibility complex; MRC-1, C-type mannose receptor 1; MKX, Mohawk; Mφ, Macrophages; Ncf, neutrophil cytosolic factor; NF-κB, nuclear factor kappa-B; NLRP3, leucine-rich repeat containing protein-3; NOTCH3, notch receptor 3; NK, natural killer; PAMPs, pathogen-associated molecular patterns; PCL, polycaprolactone; PDGF, platelet-derived growth factor; PDMS, polydimethylsiloxane; PDPN, podoplanin; PEMF, pulsed electromagnetic field; PGE_2, prostaglandin E2; PGs, proteoglycans; PIEZO1, piezo type mechanosensitive ion channel component 1; PI3K, phosphatidylinositol 3-kinase; RELMα, resistin-like molecule alpha; RUNX2, runt-related transcription factor; s, seconds; SCX, scleraxis; Singlec-1, sialic acid-binding Ig-like lectin; SOX-9, SRRM, serine/arginine repetitive matrix; SPCL, polymer blend of starch and polycaprolactone; SRY-box transcription factor 9, TDSCs, tendon-derived stem/progenitor cells; TGF-β, transforming growth factor-beta; Th, T helper; Thr, threonine; TIMP, tissue inhibitors of metalloproteinases; TNC, Tenascin C; TNFα, tumor necrosis factor alfa; TNMD, tenomodulin; TPPP3, tubulin polymerization-promoting protein family member 3; TRPM2, cation channel transient receptor potential melastatin 2/Transient receptor potential cation channel subfamily M member 2; TSC, tendon stem cells; Tyr, tyrosine; VCAM-1, vascular cell adhesion molecule 1; UMAP, uniform manifold approximation and projection.

* Corresponding authors at: 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark – Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal.

E-mail addresses: mrodrigues@i3bs.uminho.pt (M.T. Rodrigues), megomes@i3bs.uminho.pt (M.E. Gomes).

https://doi.org/10.1016/j.addr.2023.114815
0169-409X/

Contents

1. The problematic behind tendon healing . . . 2
   1.1. Tendon architecture . . . 2
   1.2. Cell populations in tendon tissue . . . 3
2. The role of inflammation in tendon repair . . . 5
   2.1. Cellular contributions to inflammation . . . 5
      2.1.1. Macrophages . . . 5
      2.1.2. Dendritic and T cells . . . 5
      2.1.3. Mast cells . . . 5
      2.1.4. Other cells . . . 5
   2.2. Investigating inflammatory pathways for designing successful therapies . . . 6
      2.2.1. NF-κB pathway . . . 6
      2.2.2. MAPK pathway . . . 6
   2.3. Cell to cell communication in inflammatory mediators . . . 6
      2.3.1. Cell-cell junctions . . . 6
      2.3.2. Extracellular vesicle-mediated-communication . . . 7
3. The dynamic cell-ECM interplay . . . 7
   3.1. Tendon matrix cues . . . 7
   3.2. Mechanotransduction . . . 8
   3.3. Mechano-immunology in tendinopathy . . . 9
4. Magnetic force-based strategies for inflammation regulation and tendon healing . . . 10
   4.1. Magnetotherapy as non-conventional treatment . . . 10
   4.2. Exploring magnetic fields in the modulation of pro-inflammatory microenvironments . . . 10
   4.3. Immunomodulatory effects of PEMF stimulation . . . 10
   4.4. Prospects of magnetic based systems to tackle inflammation . . . 11
   4.5. Bottlenecks hindering the expansion of PEMF-based therapies . . . 12
5. Conclusions . . . 12
   Declaration of Competing Interest . . . 12
   Acknowledgements . . . 12
   References . . . 12

1. The problematic behind tendon healing

Tendon tissues have limited intrinsic healing potential, and injuries to these tissues can lead to debilitating conditions with poor long-term clinical outcomes. Together with age, gender, lifestyle, and exercise levels, changes in cellularity and extracellular matrix (ECM) activity may contribute to advancing tendon injuries and disorders [1]. Clinical treatments are often lengthy, expensive, and insufficient to provide functional and permanent solutions for an increasingly active aging population. The clinical, social, and economic impact of tendon lesions and impaired regeneration inspires an understanding of tendon biology and advanced approaches to stimulate the formation of new functional tendons, enabling the restoration of tissue biomechanics to a pre-injury state.

Tendinopathies are an umbrella term that characterizes a multifactorial spectrum of tendon disorders usually associated with swelling, prolonged pain, overuse activities and impaired performance. Tendinopathies are common, accounting for up to 30% of general practise musculoskeletal consultations [2], and constitute a complex medical problem. These pathologies have been associated with a failed healing response affecting the ability of the tendon to adapt to loading, which weakens the tissue and compromises its integrity, ultimately leading to partial or full ruptures [3]. Despite the multifactorial aetiology related to tendinopathy, the complexity and symptoms behind chronic tendon disorders suggest the involvement of unresolved inflammation. A microenvironment with persistent inflammatory events is likely to contribute through deviant cellular and mechanical signals to chronic/degenerative changes in tendon structure, impairing tendon functionality and increasing the tissue vulnerability to re-injury. Since early diagnosis is difficult, chronic tendinopathy is underestimated with treatments often oriented towards the management of pain that would greatly benefit from a deeper knowledge of the inflammatory events mediating the underlying pathophysiology aiming to prevent and manage the progression of tendon disorders.

1.1. Tendon architecture

Tendons hold a unique structure that guarantees a specific biomechanical performance necessary for their role in movement and locomotion as well as for the stability of the joints by transmitting forces from the muscle to the bone and absorbing external forces to prevent injury to the muscle. Such features are enabled by a dense extracellular matrix (ECM) composed of tightly bundled collagen fibers, predominantly type I collagen (Col I). Collagen molecules are hierarchically arranged into fibrils, fibers, fiber bundles, and fascicles that confer biomechanical strength to the tendon [4]. Also present in the matrix, type III collagen (Col III) is essential for normal collagen fibrilogenesis and responsible for regulating the dimensions of Col I fibrils. Type I and type III collagens provide mechanical strength to the newly synthesized matrix [5] whereas type V collagen provides a template for fibrillogenesis. In addition to collagens, proteoglycans (PGs) and glycosaminoglycans (GAGs), such as fibromodulin and chondroitin sulfate, respectively, are present in tendon matrix and provide viscoelastic properties [1] adjusting the tissue to the mechanical forces tendons are subjected to.

The tendon unit and tendon subunits are surrounded by loose connective sheaths, the epitenon, and the endotenon or interfascicular matrix (IFM), respectively. The IFM is a looser, less organized matrix than collagen-rich fascicles with a greater number of cells, and high quantities of Col III, PGs, and elastin. The greater rate of matrix turnover, which helps to maintain a healthy tendon structure, and facilitated sliding between fascicles to allow tendons to

stretch, highlights the critical action of IFM for tendon function [6,7].

Tendons that bend sharply around joints (e.g. Achilles) have a sheath called the paratenon that reduces friction, allowing free tendon movement against surrounding tissues [3], and sustaining the vascular network that penetrates the endotenon and epitenon. Taken together, the sheaths ensure vascular, lymphatic, and nerve supplies.

When homeostasis is disrupted by trauma, mechanical stress, and/or aging, tendons lose matrix integrity due to changes in collagen fiber thickness and organization, as well as increased GAG and PG content [8]. The decrease in collagen content results in tendon stiffening whereas variations in the ratio of Col III to Col I contribute to the irregular alignment of the fibers and inferior biomechanical strength [9]. The exposure in time and the severity of these events jeopardize a functional and healthy tendon contributing to the establishment of tendinopathic conditions. The pain and swelling that often accompany tendinopathy have been theorized to be associated with an inflammatory response promoted by the changes in tendon milieu, which in turn benefits a vascular and neural supply and a hypercellular state with more prominent nuclei rounding tenocytes [10]. Along with an increased number of cells, necrotic zones and the formation of non-tendon tissues, typically cartilaginous, bone, or adipose deposits are also observed in tendinopathic lesions [1,11].

1.2. Cell populations in tendon tissue

The tendon ECM is synthesized and maintained by a sparse population of resident cells, whose interactions with their biological context define cell fate and function, being critically important for ensuring tendon functional competence and structural integrity.

Historically, tendons were classified as relatively hypocellular tissues composed of a mixed population of tenocytes and tendon-derived stem/progenitor cells [12], with endowment from other cell types such as immune, vascular, synovial, and non-resident mesenchymal stem cells [13].

Tenoblasts and tenocytes are organized in longitudinal rows between collagen fibers being responsible for the formation and turnover of the matrix. Despite their influence in establishing a complex network of cytoplasmic processes that link adjacent cells via gap-junctions, they have limited mitotic activity in adults and aged individuals, which may compromise the healing process after injury, especially in later stages of life.

Distinct biological responses have been related to different tenocyte populations according to their distribution within tendons (Fig. 1**A**). Tenocytes resident within the fascicles are more active [3,12], and the populations of IFM proliferate more quickly and have a higher ECM turnover than tenocytes in fascicles [14]. Tenocytes commonly express tendon markers such as scleraxis (SCX), a basic helix-loop-helix transcription factor, tenomodulin (TNMD), a type II transmembrane protein, and Col I. It has been demonstrated that SCX is expressed in mature tendons of the limb and trunk and in the progenitor cell population [15]. SCX is required for the proper embryonic development of tendons and the earliest detectable marker for differentiated tendon cells [16].

A population of tendon-derived stem/progenitor cells (TDSCs) also resides in the tendon microenvironment holding an important function in tissue renewal and repair [18]. TDSCs are scarce cells representing less than 5% of the total tendon cell population that exhibit a classical adult mesenchymal stem cell (MSCs) profile with self-renewal capability, clonogenicity, and differentiation into osteoblasts, chondrocytes, tenocytes, myocytes, or adipocytes [3,19,20]. Interestingly, no TDSC-specific marker that enables distinction between mature tenocytes and stem/progenitor cells has been identified to date. TDSC populations share with tenocytes high amounts of SCX [21] as well as Mohawk (MKX) and Tenascin C (TNC) during the tendon maturation phase [22]. TDSCs also express TNMD [12], which is positively regulated by SCX and reported to be a specific marker of mature tenocytes and ligamentocytes [23,24].

Published works have reported that stem cell populations from tendon proper hold increased potential for the regeneration of tendon structures nevertheless, stem cells from the paratenon show higher expression of progenitor cell markers comprising CD45, CD90, CD105, and Oct-4, compared to cells from the tendon core [25] and reinforce the expression of SCX, TNMD, and Col I in tendon proper stem cells and tenocytes [26].

Nestin, a type IV intermediate filament protein crucial to fate decisions and phenotype maintenance in TDSCs, was found to be highly expressed in TDSCs isolated from human Achilles in comparison to mature tenocytes [27]. Nestin^{+} cells evidence strong self-renewal, tenogenesis, and colony formation abilities [27]. Studies also showed TDSCs to be positive for pluripotency-inducing factors including Oct-4, Nanog, nucleostemin, SSEA-4, c-myc, and Sox-2 [28,29].

In vitro studies with rotator cuff-derived tendon cells reported a human TDSCs-subpopulation expressing high levels of CD44, CD90, and CD146. Additionally, this subset express the transmembranar proteins CD49d (integrin α-4) and CD49f (integrin α-6), the latter of which is described as being present in multiple stem-cell populations. CD146^{+} populations were shown to influence recruitment and survival outcomes whereas CD49f seems to be involved in self-renewal regulation of TDSCs [30].

In human patellar tendon Harvey *et al.* revealed a tubulin polymerization-promoting protein family member 3 (TPPP3^{+}) and platelet-derived growth factor receptor alpha (PDGFRA^{+}) TDSC population able to generate new tenocytes during tissue healing. Interestingly, in the same tendon cell niche a population of TPPP3^{-} fibro-adipogenic progenitors originated fibrotic cells, which could ultimately influence the formation of fibrotic scars during tendon repair [31].

The expression of CD109 and CD200 ligands were likewise described in TDSCs with potential for engaging inflammation modulation. The inhibition of CD109 suppressed inflammation reducing inflammatory factors, chemo-attractiveness and cell migration. Although CD200R is a receptor typically identified on immune-competent cells, the interaction of CD200/CD200R suggests an active role for TSPCs in regulating inflammatory processes during tendon injury/repair [30].

More recently, the identification of human cell populations, their heterogeneity, and interactions have been boosted by single-cell analysis technologies based on cell-type gene regulation signatures. Millar *et al*., described mix populations of endothelial, immune, stromal tenocytes and stromal mural cells in the human supraspinatus tendon. The mural cells included pericytes that may be the source of epitenon TDSCs, as they are phenotypically similar to notch receptor 3 (NOTCH3) cells, which in turn can differentiate into fibroblasts following interaction with endothelial cells [17]. In the same study, stromal cell populations were shown to modulate immune responses by promoting the recruitment and activation of immune cells together with cytokine secretion in diseased tendon. Moreover, the density of cell populations [32] and their molecular expression [17] were described to be distinctive from healthy tendons, whose amounts may provide insights on mechanisms driving tendon disease (Fig. 1**B, 1C**).

In diseased tendon, the expression of amyloid beta precursor protein (APP) which can act as an inducer of a resolution-promoting phenotype is decreased in stromal tenocytes [17], whereas the APP receptor (CD74) involved in intracellular sorting

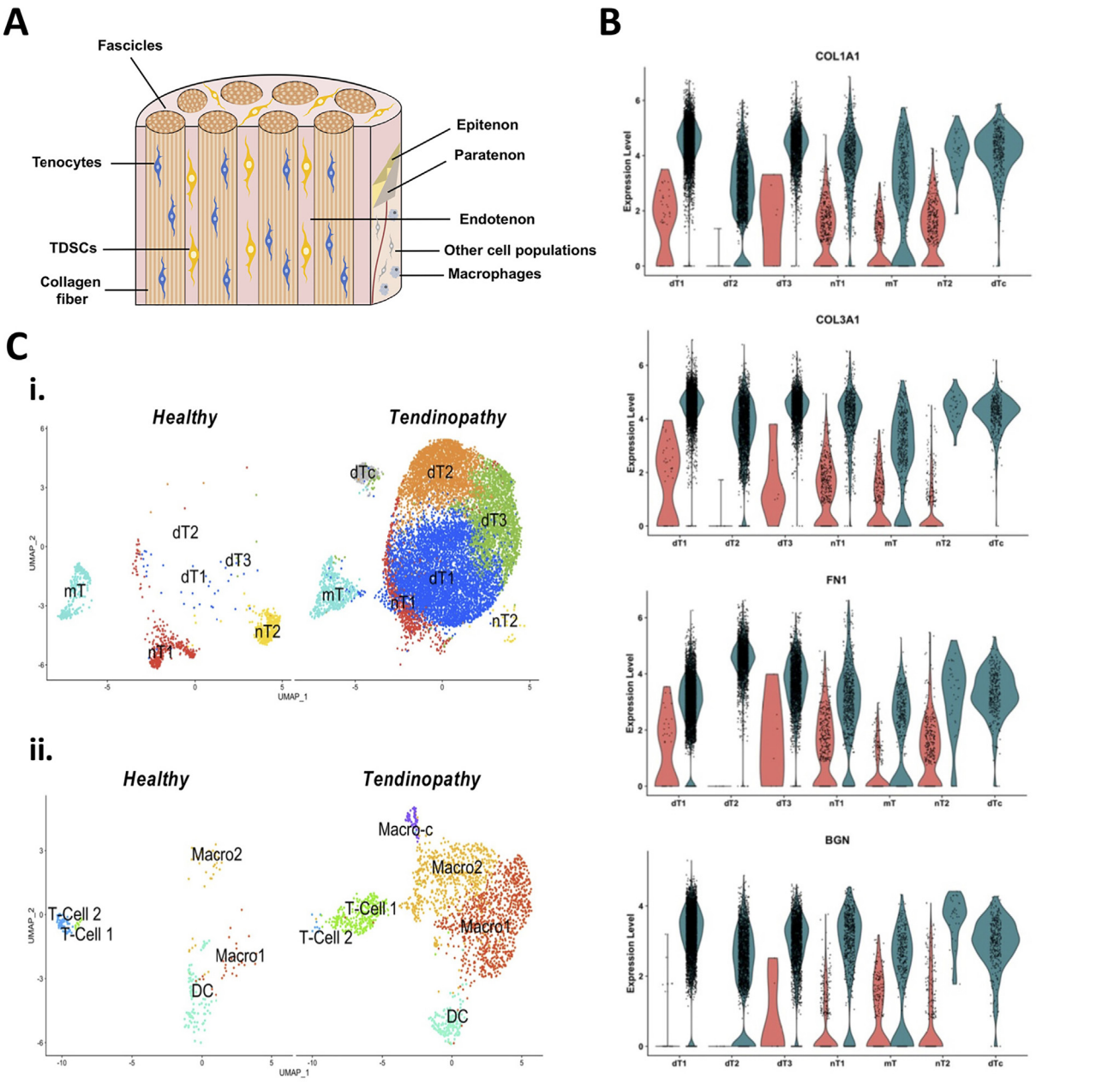

Fig. 1. Schematic representation of tendon organization and resident cell populations influencing tendon healing. A. Tenocytes align between collagen fibers, which are organized into structural units of increased complexity (fascicles). Cells within endotenon are responsible for intrinsic healing while paratenon and epitenon also house stem cells and other cell populations commonly associated to the extrinsic healing of tendons. **B.** Violin plots of extracellular markers, namely type I collagen (COL1A1), type III collagen (COL3A1), fibronectin 1 (FN1), and biglycan (BGN) from healthy (pink) and diseased (blue) human tendons. **C.** Uniform manifold approximation and projection (UMAP) embedding of single cells delineating **Ci.** stromal cells of the tendon, and **Cii.** immune cells of tendon. B and C adapted from [17]. Abbreviations: dT1: diseased tenocyte 1, dT2: diseased tenocyte2, dT3: diseased tenocyte3 nT1: normal tenocyte1, mT: mural tenocyte, nT2: normal tenocyte2, dTc: diseased cycling tenocytes, macro1: macrophage1, macro2: macrophage2, T-Cell1: T-Cells1, T-Cells2: T-Cell2, and DC: dendritic cell.

of major histocompatibility complex II (MHC II) molecules was upregulated in Mφ [33].

Also, in tendinopathic tendon a population of lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1^{+}) endothelial cells showed an upregulation of C–C motif chemokine ligand (CCL21). CCL21 regulates dendritic cells (DC) and T cell populations and may be an important player in the development of an inflammatory milieu. Matrix genes including Col I, Col III, FN1, and BGN evidenced an increased expression in diseased stromal cell populations [17].

Using healthy and diseased tendon samples, Kendal *et al.* applied scRNA-seq and CITE-sequencing to screen cell subtypes with specialized functions to assist signaling pathways in different stages of disease [32]. From the *ex vivo* human tendons and considering the top differentially expressed 4 genes per cluster and literature selected markers, tenocyte clusters expressing collagen matrix genes, monocytes, T lymphocytes and endothelial cells were identified. The expression of inflammatory genes C-X-C motif chemokine ligand 1 (CXCL1), CXCL6, and CXCL8 as well as the alarmin gene CD248, vascular cell adhesion molecule 1 (VCAM-1), and podoplanin (PDPN) were increased in the tenocyte populations from diseased tendons. In the same tendons, monocytes and macrophages (Mφ) expressed increased CCL2, CCL3, and CCL4 while diseased endothelium revealed increased expression of

intercellular adhesion molecule 1 (ICAM-1), VCAM-1, and the chemoattractant genes CXCL12, CCL2 and Selectine E together with serine/arginine repetitive matrix 2 (SRRM2), interleukin 33 (IL-33), and Nestin [32].

Despite the multiple (stem) cell subsets identified in tendon tissues and their plasticity, the involvement of these populations in the mechanisms of tendon (neo)formation, homeostasis, adaptation to loading, and healing is in its infancy, posing new challenges and opportunities to the development of novel therapies and preventing the advancement of tendon disease.

2. The role of inflammation in tendon repair

Inflammatory pathways have a pivotal molecular basis in the pathogenesis of many chronic diseases, including tendinopathy. As in other tissues, the self-recovery of tendons involves a complex and coordinated series of events starting with an initial insult the surface pattern receptors recognize [34]. Upon mechanical stress caused by injury, tenocytes are activated and release endogenous damage-associated molecular patterns (DAMPs) [35]. Tenocytes also participate in the inflammatory response by synthesizing inflammatory signals that stimulate cell recruitment and the migration of monocytes and activated T cells to the site of damage [35].

A subset of pro-inflammatory macrophages (M1φ) are more abundant in the early inflammatory process (1–5 days after injury) [36]. M1φ significantly contribute to the release of interleukin 1 beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor alpha (TNFα), interleukin 12 (IL-12), cyclooxygenase-2 (COX-2) and reactive nitrogen and oxygen species [13,37] to assist T helper (Th1) cell responses [38], enhance phagocytosis of apoptotic cells [39] and help to clean the debris. M1φ together with M2φ, the latter supporting T helper 2 (Th2)-associated effector functions [39] appear in later stages (4–10 days). M2φ secrete anti-inflammatory factors ((e.g. IL-4, IL-10, IL-13, and transforming growth factor-beta 3 (TGF-β3)) and cell attracting chemokines (e.g. CCL7, CCL22, and CCL24) enabling the attenuation of inflammation and the beginning of the cell proliferation and tissue deposition phases [37]. Both populations also contribute to recruiting and stimulating fibroblasts to synthesize ECM components [40]. Afterwards, a gradual reorganization of the newly deposited collagen occurs. The tenocytes and collagen fibers become more organized and aligned in the direction of stress, corresponding to a decrease in Col III, vascularity, cellularity, and water content in the forming scar. The Col III is replaced with Col I, with stronger crosslinking and tensile strength. The remodeling phase is a critical and necessary process during healing for the restoration of tendon biochemical properties.

Normal tendon healing results from both intrinsic and extrinsic processes. Intrinsic healing occurs when tenocytes from the epitenon and endotenon proliferate and migrate to the injury site. In turn, extrinsic healing involves the migration of cells comprehending endothelial-, perivascular-, smooth muscle-, synovial, neuronal, fat, and immune cells [1,32,41] into the damage from the surrounding sheath and synovium. Extrinsic healing has been linked to scarring and peritendinous adhesions due to the infiltrating fibroblastic activity and fibrin deposition between the lesion and the sheath, which severely compromise tendon functionalities, and the motion of the affected joint.

2.1. Cellular contributions to inflammation

Immune resident cells positive for CD45, CD206, and neutrophil cytosolic factor 1 ($Ncf1^+$) were identified in postnatal mouse Achilles, indicating a physiological role of immune cells in tendon maintenance and homeostasis preservation [42].

Owing to the fact that immune cell populations have been detected in tendinopathies, the adaptive immune response may play a crucial task in the chronicity of the inflammatory response in the context of tendon damage [2]. Moreover, inflamed tissues express inflammatory triggers and molecules that can stimulate the migration, retention, and survival rates of leukocytes, favouring cyclic disease events and the tendinopathic process.

2.1.1. Macrophages

As mentioned before, Mφ populations are pivotal players in tendon injury and healing. The functional switch into M1φ or M2φ phenotypes is a versatile tool for Mφ-targeting strategies to study biomarkers influencing disease progression and to create therapeutics with multivalence downstream signaling for tendinopathy.

Abrogation of interleukin 4 receptor alpha (IL-4Rα) signaling in Mφ was found to impair wound repair in an *in vitro* model [43]. On the other hand, interleukin 13 (IL-13) can drive pathological fibrosis through excessive M2φ activation. In models of helminth-induced fibrosis, IL-13 driven inflammation and fibrosis were alleviated by depletion of Th2-associated Mφ [44].

IL-4 stimulated-chemokine receptor 2 (CCR2) plays a crucial function in Mφ recruitment and regulated inflammation with impact in patient healing outcomes. Although CCR2 is expressed in several tendon-intrinsic cell populations, loss of CCR2 resulted in a low number of Mφ and myofibroblasts compromising the total recovery of tendon functionality. Thus, CCR2 has been shown to be very important for Mφ downstream functionality in tendon maintenance by reacquiring mechanical properties after tendon injury [45].

Biopsy specimens of chronic tendinopathic Achilles tendons evidenced increased numbers of Mφ ($CD68\text{-}KP1^+$) and endothelial cells ($CD34^+$) compared with healthy tendons [46]. Additionally, cycling Mφ were found to be unique to diseased tendons [42].

2.1.2. Dendritic and T cells

Diseased tendons exhibit higher levels of DC activation and decreased C1Q genes which were described as regulatory DC markers [42]. Endothelial cells seem to stimulate DCs presenting increased levels of CCL21, which regulates the migration of DC and subsequently T cells [42]. Activated T cells lead to the upregulation of IL-6, IL-8, COX-2, CCL2, CCL5 and CXCL10 in tenocytes amplifying the inflammatory response. This interaction also influences the collagen III/I ratio in tenocytes, changing the matrix properties of the tendon [35].

2.1.3. Mast cells

Mast cells act as sentinel cells in the tendon proper and are closely associated with neovessels to respond rapidly to external insults [47]. It is not clear whether mast cells proliferate locally or migrate into tendon as differentiated cells or by an influx of mast cell progenitors from the circulation. Nevertheless, mast cell density is considerable in human tendinopathic tissue. Scott *et al.* demonstrated a three-fold increase of mast cells in diseased patella [48], and a two-fold increase in diseased rotator cuff compared to healthy tendons.

2.1.4. Other cells

Lehner *et al.* reported a fractalkine receptor (CX3CR1) and its cognate C-X3-C ligand 1 (CX3CL1) in resident cells of the tendon core in healthy human Achilles [49]. The CX3CR1 and CX3CL1 have functionality related to the recruitment of monocytes and are typically expressed by myeloid and lymphoid cells, including mast cells and natural killer (NK) cells [50]. These regulatory cells are engaged in reciprocal interactions with other immune cells, which

might participate in tendon healing by modulating cell proliferation, collagen deposition, the release of vascular endothelial growth factors, and the fibrosis response.

The phenotypic signatures of immune-competent clusters and selectively targeting the adaptive/stromal interface may provide novel translational strategies for assisting the management of human tendon disorders.

2.2. Investigating inflammatory pathways for designing successful therapies

2.2.1. NF-κB pathway

Dysregulation of NF-κB and MAPK activity has been associated with inflammatory, autoimmune, and metabolic diseases. In inflammatory niches, NF-κB typically responds to cytokines such as IL-1β and TNFα. IL-1β is released in response to pathogen-associated molecular patterns (PAMPs) and DAMPs inducing tenocytes to produce COX-2, prostaglandin E2 (PGE_2) and matrix metalloproteinase-1 (MMP-1), which in turn accelerates the degradation of the ECM [51]. Similarly, TNFα stimulates *in vitro* tenocytes to amplify their own TNFα and produce IL-1β and IL-6 leading to the inhibition of ECM formation [52]. As major producers of IL-1β, Mφ may be a key player in the stimulation of fibroblast proliferation with consequent influence on collagen and α-smooth muscle actin synthesis [53].

During homeostasis, NF-κB is bound to inhibitors-of-kappa B (IκB), which maintains the NF-κB pool mainly in the cytoplasm by inhibiting its nuclear localization and association with DNA. In the case of activation (non-homeostasis), the binding between NF-κB and IκB is phosphorylated, and consequently, NF-κB dimers translocate to the nucleus, bind to the DNA, and regulate the transcription of numerous inflammatory genes into cytokines and chemokines [54,55]. Increased NF-κB expression has been associated with early rotator cuff tendinopathy [56]. In accordance, tendon stromal cells from patients with supraspinatus tendon disease revealed increased expression of interferon gamma (IFNy) and NF-κB genes compared to tendon cells obtained from healthy controls [57]. In recent work performed by us, NF-κB activation by exogenous IL-1β supplementation in human tendon-derived cells translated into an increase in IL-6, TNFα, IL-8, and COX-2 [58]. Also, suppression of IL-1β-induced activation of the NF-κB pathway in cultured human tenocytes, resulted in an increase in collagen, TNMD, and SCX production, while the genetic expression of *COX-2, MMPs, Bax, and caspase-3* was inhibited [59]. Preclinical studies in a mouse rotator cuff model supports that NF-κB activation by IL-1β causes tendon degeneration, which is accompanied by a rise in IL-6 and CCL2 [56].

In Mφ cultures, the activation of NF-κB induces the expression of TNFα, IL-1β, IL-6, and c-Rel complexes. c-Rel is important for scaling the inflammatory response based on transient/persistent TLR-4 stimulation, which can prevent hyperinflammatory responses [60]. Courtine *et al.* showed that mice lacking both c-Rel and p50 NF-κB proteins have impaired innate immune responses to sepsis, with deficient Mφ responses for phagocytosis and bacterial killing [61].

2.2.2. MAPK pathway

Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine kinases implicated in several cellular processes, including proliferation, differentiation, cell motility, metabolism, and inflammation. There are three well-known MAPK pathways: extracellular signal-regulated kinase 1/2 (Erk1/2), which is activated in response to growth factors, hormones, and inflammatory stimuli, the Jun N-terminal kinases (JNK) and p38 mitogen-activated protein kinases (p38 MAPK) pathways, both activated by inflammatory stimuli and stress signals [62].

Activation of the Erk1/2 signaling pathway has been implicated in a rat supraspinatus tendinopathy model and in tendon-derived cells from rat Achilles treated with IL-1β [63]. Erk1/2 pathway operates on the degradation of tendon fascicles driving tendon matrix deterioration and moderates the expression of important fibrotic mediators, including TGF-β, connective tissue growth factor (CTGF), and bone morphogenic proteins (BMP) in torn tendons [63].

The use of pharmacological inhibitors of Erk1/2 has been described to abolish the induction of catabolic gene expression (e.g. MMPs) and prevent the loss of tendon mechanical properties in tendon explants from mice [64]. FR180204 (Erk inhibitor), in particular, also inhibits the production of IL-6, IL-8, and TNFα in human tendon cells [65]. The JNK pathway has been reported to have an important involvement in regulating inflammation during tendon healing. Conditioning co-cultures of human Mφ and synovial fibroblasts with TNFα modulated the expression of janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway necessary for M1φ polarization [66]. In a patellar tendon injury model of Sprague-Dawley rats, Tarafder *et al.* showed that treatment with IQ-1s, a JNK inhibitor, significantly downregulated IL-6, and iNOS, while the expression of IL-10 and TIMP-3 was significantly upregulated [67]. A similar study investigated the effects of aspirin (ASA) and IL-1β on TDSCs from rat Achilles, both *in vitro and in vivo*, with similar outcomes. The TDSCs treated with IL-1β and ASA significantly reduced *IL-6* and *MMP-3,* and upregulated *IL-10* and *TIMP-3*. When a JNK inhibitor (SP600125) was applied, the expression of IL-10 and TIMP-3 was induced by IL-1β treatment, and the effect of ASA was significantly reduced by SP600125 while IL-6 was enhanced [68]. This work suggests that ASA inhibits inflammation via JNK and that SP600125 diminishes the anti-inflammatory effects of ASA.

Similar to Erk1/2 and JNK, inhibition of p38 MAPK results in a significant reduction of IL-6 in the rat Achilles and a modest decrease in the expression of Col I and Col III [69]. In a rat supraspinatus tear, the inhibition of p38 MAPK by SB203580 caused a reduction in *IL-1β, IL-6*, and *COX-2*, and an increase in *IL-10* [70]. Likely, Millar *et al.* reported the activation of p38, and Erk1/2 MAPK in cultures of human tendon-derived cells derived from hamstring tendons subjected to hypoxic environments. When SB203580 and FR180204 were administered, the expression of IL-6 and IL-8 diminished [71]. Thus, therapies to assist in the resolution of inflammatory events targeting key molecules associated with the activation of signaling pathways could lead to balanced inflammatory signals that may modulate the progression of tendon diseases and improve treatment outcomes.

2.3. Cell to cell communication in inflammatory mediators

2.3.1. Cell-cell junctions

Cell-cell junctions are functional units for the correct and efficient flow of information in multiple cell processes, including adhesion, communication, and differentiation of cells. These complexes can generate surface polarity, which permits cells to sense and respond to their local environments. In tendon tissues, cells are coupled via the connexins Cx32 and Cx43. Connexins integrate gap-junctions, which are protein monomers specialized in intercellular membrane channels that function as conduits between the cytoplasm of contiguous cells. They enable the direct commute of ions, nucleotides, small molecules, and second messengers between neighboring cells [72], working as active social networks. The involvement of gap-junctions in the communication of inflammatory signals and tendon regeneration has not been fully elucidated. *In vitro* studies with rabbit Achilles tendon cells showed that Cx43-mediated gap junctional intercellular communication may be important in the broadcasting of inflammatory markers

following intense exercise and an increment in body temperature [73]. In this study, Cx43 was not affected by heat treatment, but inhibition of gap-junctions with B-glycyrrhetinic acid led to an increase in the expression of MMP-1, IL-1β, and IL-6. Moreover, when Cx43 was overexpressed under heat stress, lower levels of Col I, IL-1β, MMP-1 and IL-6 were observed.

The permeability of gap junctions between tenocytes is modified in response to mechanical loading. Maeda *et al.* showed that tenocytes isolated from Achilles tendons and mechanically stimulated with 4% strain for 1 h showed increased expression of Cx43 and its localization at the cell membrane. Interestingly, when an 8% strain was applied to the cells, Cx43 expression diminished but Cx43 localization was not affected [74].

The duration of mechanical loading also influences tenocyte communication. A 10 min loading did not interfere with gap-junction communication, whereas loading for 1 h significantly reduced intercellular gap-junction permeability, thus inhibiting cell networks.

The regulation of collagen production in the tendon matrix may also rely on gap-junction mechanisms. In chick tenocytes, the inhibition of Cx43 resulted in a significant production of Col I mRNA even though the use of non-specific inhibitors of gap-junctions (e.g., octanol) suppressed collagen synthesis [75]. However, under mechanical loading, Cx32 stimulates collagen synthesis in tenocyte monolayers [76], suggesting a strong interaction between collagen, loading, and gap junctions in tendons.

2.3.2. Extracellular vesicle-mediated-communication

The extracellular vesicles (EVs) are 50–150 nm membrane-bound vesicles of endosomal origin secreted by multiple types of cells. EVs are selectively packaged with molecules like proteins, peptides, oligonucleotides (e.g. mi/si/mRNA), and other factors that precisely contribute to immunomodulation responses and to the regulation of phenotype and function during tissue repair [77]. Since EVs are designed for efficient cell–cell communication and intracellular delivery, these physiological broadcasters have the therapeutic capability of targeting sensitive cells. Moreover, EVs can be engineered using chemical or biological modification strategies to develop sophisticated payload systems for tendon disease intervention [78].

Exosomes isolated from tendon derived stem cells ($Exos^{TSC}$) promoted the proliferation and migration of tenocytes *in vitro*, which relied on the activation of the phosphatidylinositol 3-kinase (PI3K)/protein B (AKT) and (MAPK/Erk1/2) signaling pathways. $Exos^{TSC}$ was mixed with gelatin methacryloyl (GelMA) and placed in an Achilles defect. Two and eight weeks after *in situ* administration, $Exos^{TSC}$ treated tendons demonstrated a continuous and regular arrangement of ECM in comparison with control groups, suggesting that $Exos^{TSC}$ could assist the healing of injured tendons [79].

Exosomes derived from mesenchymal stem cells ($Exos^{MSCs}$) also improved patellar tendon injury. $Exos^{MSCs}$ promoted the proliferation, migration, and tenogenic differentiation of TSCs *in vitro*, likely mediated by the activation of SMAD2/3 and SMAD1/5/9 pathways. $Exos^{MSCs}$ were further loaded into GelMA hydrogels, and cross-linked in patellar lesions. Animals treated with $Exos^{MSCs}$ evidenced higher numbers of M2φ ($CD163^{+}$), higher levels of M2φ-stimulating IL-10, and tendons with more regular collagen fibers [80].

The injection of exosomes from adipose derived stem cells into a rat supraspinatus tendon model also promoted the alignment of collagen fibers and muscle bundles unlike the exosomes-free group, which showed a defective arrangement of collagen fibers [81]. All together, these results highlight the potential of exosomes to biomechanically stimulate healing during the remodeling phase.

Chamberlain *et al.* demonstrated that fibrin with MSC-derived EVs reduced the M1/M2 ratio and increased the number of endothelial cells 14 days after Achilles injury following unilateral surgical transection in mice. Furthermore, tendons treated with fibrin containing MSC-derived EVs demonstrated alignment and compact collagen fibers and higher expression of the Col I, SCX and TNMD.

EVs were also shown to demonstrate potential for immunoregulation in tendon, counting the successfully education of Mφ into an M2-like phenotype, and thus accelerating tendon healing [82]. Intraperitoneal injection of MSC-derived EVs decreased the number of $CCR7^{+}$ cells as well as the levels of IFNy, IL-1β and IL-6, leading to an increase in the number of $CD163^{+}$ Mφ and higher expression of the M2φ genes, IL-4 and IL-10 [83].

The contribution of EVs communication has also been explored in inflammatory diseases. Mice with ulcerative colitis showed an increase in IL-10 and TGF-β levels and a reduction in IFNy, IL-12, TNFα, chemokine (C–C motif) ligand 24 (CCL24), and chemokine (C–C motif) ligand 17 (CCL17) levels after EVs treatment [84].

In summary, intercellular signaling is highly relevant for elucidating the mechanisms driving immunomodulatory responses and anticipating effective regenerative responses (Fig. 2). Furthermore, the fine-tuned biomodulation of cells and biochemical cues to regulate microenvironmental stimuli offers attractive possibilities for scar-free healing and therapeutic strategies in mature tendons.

3. The dynamic cell-ECM interplay

3.1. Tendon matrix cues

Healthy tendons rely on inflammatory mediators like TGF-β, basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) to protect tissue integrity after sudden exposure to mechanical stresses [85] or overloads. When a mechanical threshold is overcome, injury may occur leading to matrix degradation and a rearrangement of the discontinuous, crimped, and thinned collagen fibers deposited at an early phase of healing. Inadequate remodeling events or persistent inflammatory triggers support abnormal cell responses, for instance, autophagic and apoptotic cell death [87] guiding unbalanced matrix organization and changes in the matrix properties that weaken the tissue favouring the course of tendon disease.

Matrix stiffness is an important part of the regulation of the proliferation and differentiation processes in stem cell niches [86,87]. The proliferation of tendon cells was shown to increase with increasing matrix stiffness [88], likely a native response to advance repair after trauma. Stretching TDSCs (1 Hz and 8% strain) cultured on flexible silicone dishes demonstrated a positive effect on gene expression of FN, lumican, and versican with an important role in the assembly of collagen into fibrils, and in collagen-binding receptors α1, α2 and α11 [89]. On the other hand, matrix softness seems to maximize paracrine factors implicated in monocyte production and chemotaxis upon TNFα stimulation [90]. A decrease in stiffness implies a more extensible tendon that may facilitate its capacity to mechanically buffer the force transmitted through the tendon body [91]. Reduced tendon stiffness correlated with better clinical outcomes in athletes suffering from patellar tendinopathy.

ECM degradation products formed from collagen, FN, hyaluronic acid, and biglycans are potential DAMPs that upregulate pro-inflammatory triggers [92]. However, depending on the pathway involved, DAMPs can also modulate the production or activity of mediators such as TGF-β and IL-1β [93], and consequently the regulation of immune cells that naturally respond to these soluble factors.

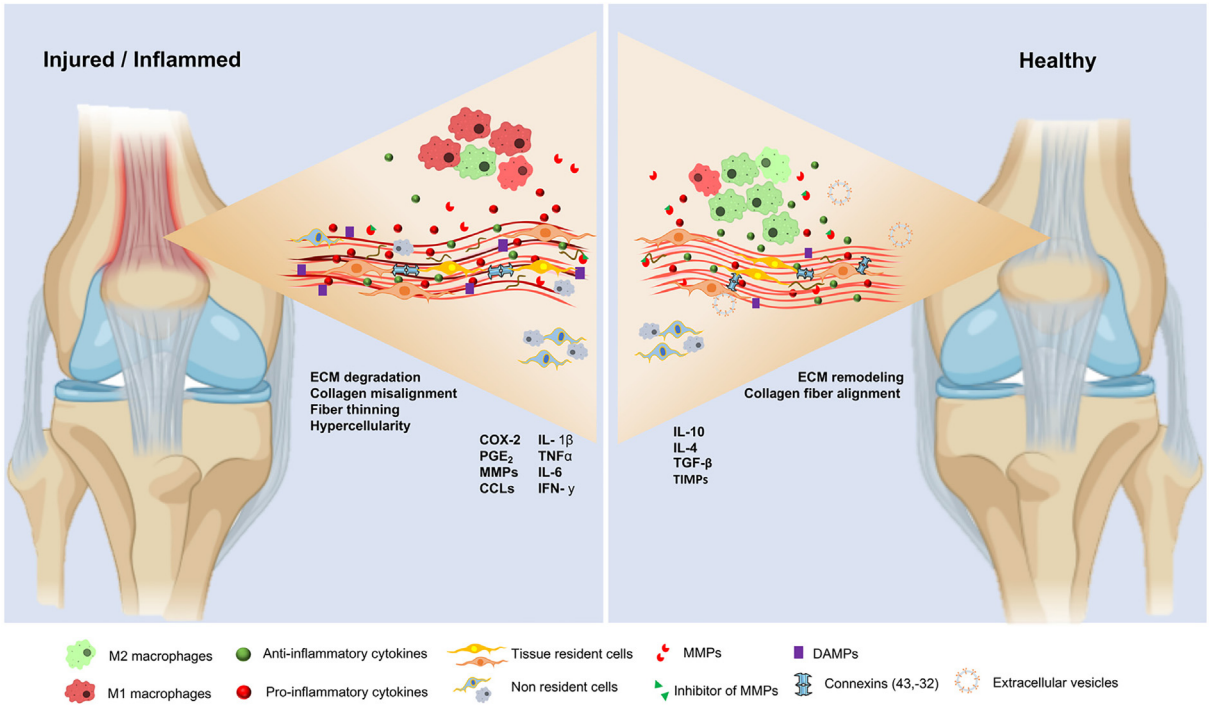

Fig. 2. Molecular and cellular interplay in injured/inflamed versus healthy tendons. The scheme represents the regulation of cell populations and microenvironmental cues through juxtacrine and paracrine communication in the tendon niche. The overall balance dictates a favorable or detrimental influence on tendon repair mechanisms.

Another important aspect of ECM degradation is abnormal cell communication due to misalignment of the fibers in the scar tissue, or complete interruption of cell networks in the most severe injuries. A study performed by Schoenenberger *et al.* reported that disorganized fiber surfaces can prime tendon cells towards pro-inflammatory signaling [94]. This hypothesis is supported by the fact that cytokine-mediated activation of MMPs is more significant in tendon fibroblasts in contact with biomaterials with misaligned fiber topography. Such topographies mimic the architecture of tendinopathic ECM, and it is plausible that matrix organization contributes to the perpetuation of tissue inflammation [94]. Accordingly, Thankam *et al.* associated matrix disorganization and inflammation with intracellular high mobility group box 1 (HMGB1) upregulation and leucine-rich repeat containing protein-3 (NLRP3) inflammasome activation in rotator cuff tendon injuries [92].

3.2. Mechanotransduction

Tendon matrix undergo tension, compression, shear, and fluid motion in response to the mechanical loads [95] driven by exercise, loading or injury and regulated by mechano-assisted mechanisms [96].

In healthy tendons, surrounding mechanical forces induce conformational changes in mechano-sensitive proteins and receptors guiding the subsequent transcription of mechanosensitive genes [97] (Fig. 3**A**).

Integrins are involved in the regulation of tendon cell processes important for exercise-induced adaptation and mechanotherapy as an upstream complex of the mechanosensory cellular apparatus. Mousavizadeh *et al.* demonstrated that integrin-linked kinase is necessary for the phosphorylation of AKT following mechanical stimulation [98]. A 10-day repetitive stretching of human hamstring cells via uniaxial cyclic strain (0.1 Hz, 10% strain, 10 s rest insertion for 1000 cycles/day) activated the AKT and mammalian target of rapamycin (mTOR) pathways increasing collagen expression. Similarly, a lack of mechanical load after a rotator cuff tear led to decreased activity of the AKT/mTOR pathway and a loss of mechanotransduction signaling.

In response to loading, the pathways that connect to the deformation sensors change the cytoplasmic filament organisation and content of human tenocytes, entailing brief changes in the intracellular calcium concentration, the release of ATP and -alter the expression of matrix genes (e.g. MMP-1) and of inflammatory signals (e.g. PGE_2) [99]. In particular, the sensitivity of the calcium ion channel piezo type mechanosensitive ion channel component 1 (PIEZO1) has been investigated in tendon models, including rat fascicles. PIEZO1 detects shear stresses that are further translated into an increase of collagen crosslinking enzymes (Lox and Plod2) associated with tendon stiffness [100].

Since stiffness varies in healthy and diseased tendon, Hussien *et al.* developed a Col I coated-polydimethylsiloxane (PDMS) for studying human tendon-derived stromal cell phenotypes driven by soft (2 kPa), intermediate (35 kPa), and rigid (180 kPa) stiffness (Fig. 3**B**). Using a pairwise differential expression analysis, the number of differentially expressed genes and *Enrichr* showed that cells on the range (35 vs. 180 kPa) were functionally enriched in mechanically regulated pathways, such as Hippo-Merlin and PI3K-Akt, and kinases (JAK-1, −2,-3 and YK2) (Fig. 3**Bi**). Conversely, the cells in the (2 vs. 180 kPa) comparison showed higher transcript levels in pathways related to the ECM, cell adhesion, and cytoskeleton, as well as in cyclin-dependent-, casein-, and homeodomain-interacting protein kinase (HIPK) (Fig. 3**Bii**). These results suggest that stiffness-signaling pathways may be altered by matrix stiffness in tendon stromal cells [101].

Tendon cilia also constitute physical stimulus-sensitive structures. Cilia are highly oriented with the ECM, and deflect in response to cyclic loading. A 4% stretching caused a greater elongation of the primary cilia in the tendon matrix, which is associated with a greater loss of biomechanical integrity. Six hours after an IL-1β treatment, no significant change in cilia elongation was observed, suggesting a physical rather than a IL-1β influence on

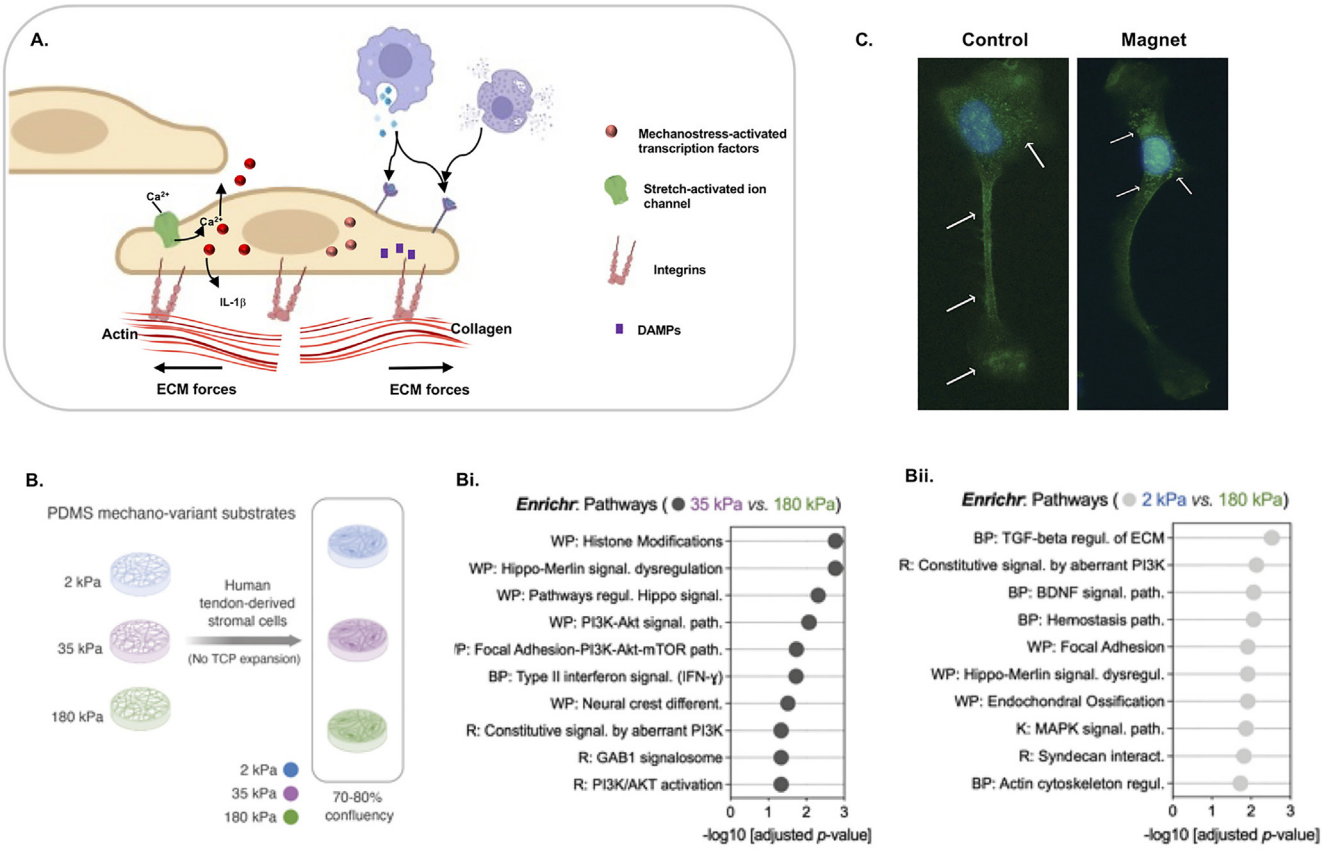

Fig. 3. Mechanotransduction in tendon. A. Resident cell populations sense and adapt to external mechanical changes through the activation of mechano-regulated structures such as cell receptors, integrins or ion channels. In the case of matrix damage downstream signaling pathways lead to the production of cytokines and other danger-associated molecules (e.g. DAMPs) that encourage the recruitment of inflammatory cells. **B.** Schematic overview of the range of substrate stiffnesses that were used to interrogate stiffness-sensing on healthy human tendon-derived cells directly seeded on PDMS substrates. **Bi** and **Bii** 10 enriched pathways assessed for paired stiffness comparison; namely **Bi.** 35 kPa vs. 180 kPa, and **Bii.** 2 kPa vs. 180 kPa. Enrichment analysis was performed in *Enrichr* using both up-regulated and down-regulated genes. Adapted from [101]. **C.** Immune cells are also influenced by mechano-magnetic forces. The fluorescent microscopic images show the distribution of the cation channel transient receptor potential melastatin 2/Transient Receptor Potential Cation Channel Subfamily M Member 2 (TRPM2) (green) in Mφ. In the control condition, the Mφ displays a cytoplasmic distribution of TRPM2 (depicted by arrows). After exposure to a magnetic-field gradient (magnet), TRPM2 is grouped around the nucleus (blue). Reprinted from Biophysical Journal, 114, Magnetic field changes macrophage phenotype, Jarek Wosik, Wei Chen, Kuang Qin, Rafik M. Ghobrial, Jacek Z. Kubiak, Malgorzata Kloc, Pages 2001–2013, Copyright (2023), with permission from Elsevier.

changes in cilia length [102]. Overuse and loads may initially cause cilia disassembly and shortening, affecting the mechanotransduction mechanisms. The changes in tendon cilia in response to loading, stress deprivation, and matrix environment may contribute to altering the mechano set point during injury.

3.3. Mechano-immunology in tendinopathy

In diseased tendons, the contact-dependent nature of immune cells makes them extremely sensitive to biomechanical variations, which may act as important immunomodulatory stimuli during tissue healing and regeneration [97].

Schoenenberger *et al.* studied THP-1 derived macrophages cultured in aligned and randomly oriented polycaprolactone (PCL) nanofiber substrates under static and dynamic loading. The dynamic loading was provided by a bioreactor inducing a 7% cyclic strain at 1 Hz for 8 h, which was combined with a 16 h resting period. Such load caused an upregulation of the M1φ marker C–C chemokine receptor type 7 (CCR7) but did not affect C-type mannose receptor 1 (MRC-1), a M2φ marker, independently of the subtract topography. Afterwards, authors studied the immune response with aligned subtracts under dynamic loading in the rat Achilles. The mechanical stretching increased the M2φ population demonstrating a mechano-protective role in the Achilles tendon [103].

Approaching a rotator cuff repair model, Liu *et al.* studied the effect of hydrogel containing an IL-4 neutralizing antibody under mechanical stimulation. A treadmill protocol established at 10 m/min on a 0° decline lane for 20 min per day, 5 days a week was set for the C57BL/6 mice run. The mechanical stresses induced by the exercise stimulated the synthesis of IL-4 and M2φ-polarization by IL-4/JAK/STAT [104].

On peritoneal Mφ exposed to a magnetic field (1.24 T, coercivity HcB 950 kA/m, and coercivity HcJ 1750 kA/m), the cation channel transient receptor potential melastatin 2/Transient Receptor Potential Cation Channel Subfamily M Member 2 (TRPM2), a Ca^{2+} permeable cation channel, was observed in the vicinity of the nucleus. Such action impacts on actin polymerization, which is ion current dependent causing Mφ elongation [105] (Fig. 3**C**).

The interplay between ECM dynamics, immune cells, and mechanical loading during tendon healing is just beginning to be explored. These pioneering works highlight the relevance of the mechanosensing machinery of immune cells, in particular Mφ, in the design of immunomodulatory approaches in injured or diseased microenvironments. The importance of restructuring tissue architecture is unquestionable to unveil the mechanisms behind tendon healing and to boost the development of improved regenerative tools for tendinopathy therapies.

4. Magnetic force-based strategies for inflammation regulation and tendon healing

4.1. Magnetotherapy as non-conventional treatment

Magneto-therapy defines an alternative therapy using magnets or a magnetic field (MF) to modulate cell response directly at the injury site, thus providing the medical community with a safe and non-invasive therapy. This therapeutic modality employs external magnetic stimulation, which can be provided by static-, alternating- or pulsed electro- magnetic fields. While static magnetic fields are constant fields over time, the pulsed electromagnetic field (PEMF) is generated by an alternating electric current traveling through a solenoid and applied in pulses. The magnetic field more frequently used for medical treatments is PEMF. PEMF devices have been approved by the U.S. Food and Drug Administration (FDA) to treat non-union fractures and post-operative pain and edema [106]. Magneto-therapy has been also applied to rehabilitation treatments of osteoarthritis [107]. In these treatments, the magnetic stimulation follows pre-established therapeutic programs, in which exposure time and intensity parameters are defined for a particular pathology or tissue. The magneto-therapy devices provide a non-invasive extracorporeal stimulus within human physiological ranges yet with a high precision control. This is partially due to the excellent organic tissue penetration of MF despite its remote action and the minimized potential to cause harmful effects upon cell contact [108]. Unlike other technologies, MF are not affected by changes in pH, temperature or surface charges.

Magnetic stimulation from a cellular and molecular perspective would enable new contact-free and cost-effective approaches in comparison to current pharmacological or rehabilitation regimens. Short-term exposure to low frequency electromagnetic fields has been shown to influence cell dynamics [109-112] in the transport of proteins, in the flow of Ca^{2+}, Na^{2+} or K^{+} ions [113], cell proliferation rate, and protein synthesis [114], which could further be explored for improving ECM deposition and collagen fiber alignment during tendon healing [115,116].

4.2. Exploring magnetic fields in the modulation of pro-inflammatory microenvironments

MF are defined by several parameters, including frequency and intensity. The main challenge for magnetic guidance of cell processes is to establish the most optimal parameters for biological responsiveness; overstimulation may be detrimental, resulting in apoptosis and cellular death, while under stimulation may cause no effect at all. Low-intensity (<1 Gauss) and low frequency (<3,000 Hz) PEMF is sufficient to interact with cells and tissues and exert a beneficial effect on inflammation control. Girolamo *et al.* compared different dosages of PEMF (75 Hz), varying the field intensity (1.5 mT or 3 mT) and exposure time (4 h, 8 h, and 12 h) applied to healthy human tendon cells. These studies demonstrated that exposure to a PEMF with 75 Hz, 1.5 mT, and 0.1% duty cycle decreased the release of IL-1β, TNFα, and IL-6 after 8 h and 12 h, and increased IL-10 levels, suggesting a modulatory behaviour of PEMF over cytokine profiles [111,112]. Concordant outcomes were observed in human fibroblast-like cells exposed to PEMF (50 Hz, 2.25 mT) for 15 min, indicating a decrease in IL-1β and TNFα after 14 and 21 days, and increased IL-10 values on day 21 [117].

Low-intensity and low frequency PEMF have evidenced regulatory outcomes in cytokine conditioned works. IL-1β-primed 3D tendon-like constructs using rat Achilles-derived cells exposed to a 1 h-PEMF (2 treatment cycles at 82 mT and 2 Hz) followed by 1.5 h resting time revealed matrix remodelling with the production of Col I, and MMPs, and attenuating of apoptosis [118].

Our group has investigated different combinations of PEMF parameters in IL-1β conditioned tendon cells obtained from human tendons. Some combinations of PEMF parameters were more favourable for biological processes, namely 5 Hz, 4 mT and 50% duty cycle while others, 5 Hz or 17 Hz combined with 5 mT and 50% duty cycle induced lower metabolic activity and reduced cell viability rates [119]. Supporting these studies on human tendon-derived cells, we also observed that a 1 h-exposure to PEMF with 5 Hz, 4 mT, and 50% duty cycle decreased the levels of IL-6, TNFα, IL-8, and COX-2, and upregulated IL-10 and IL-4. Moreover, under magnetic stimulation, the gene expression of MMP-1, −2, and −3 was reduced while TIMP-1 levels increased [119]. Although the impact of magnetic stimuli on cell responses from different tissues is not clear, studies with human annulus fibrosus cells conditioned to IL-1β evidenced an analogous modulatory effect of PEMF (3.85 Hz, 15 Hz burst and maximum 10 T/s rate, 25% duty cycle) on the expression of the inflammatory mediators IL-1α, IL-1β, IL-16, and IL-17 [120].

4.3. Immunomodulatory effects of PEMF stimulation

MF have been shown to regulate cytokine production by tendon cells populations. However, the predominant cytokine producers are immune-competent cells that exhibit plasticity and coordinated action in the articulation of multiple cell responses. Thus, insights into the magnetic responsiveness of cells, with a noteworthy emphasis on immune cells may assist in the establishment of more effective cell communication networks to prevent persistent detrimental signals and accelerate a pro-healing response.

In vitro studies have reported a decrease IL-6 and TNFα secreted by Mφ after PEMF stimulation, even when different PEMF conditions (15 Hz, 2 mT for 15 min or 5 Hz, 4 mT, and 50% duty cycle) were applied [121,122]. Furthermore, specific PEMF conditions (5 Hz, 4 mT, and 50% duty cycle) could increase IL-4 and IL-10 and predispose M2φ polarization by upregulating arginase-1 (Arg-1), MRC-1, and Sialic acid-binding lg-like lectin 1 (Singlec-1) [122].

The control over cytokine production in Mφ populations may be related to a reduction in NF-κB activation induced by continuous exposure of RAW264.7 macrophage-like cells to PEMF (75 Hz, 1.5 mT and 10% duty cycle). This study showed that the phosphorylation of p38 was PEMF-inhibited, and consequently the TNFα production by Mφ was also inhibited [123]. A study by Wosik *et al.*, proposed that the morphological elongation of Mφ *in vitro* can be induced by a MF [105], switching the upregulation of M2-specific genes, such as ARG-1, and decreasing iNOS expression (M1φ) to undetectable levels. In a more complex approach, Kang *et al.* developed a magnetic remotely controlled nanocage (MNC) conjugated to a bioactive ligand (Arg-Gly-Asp (RGD) peptide) to regulate *in vivo* the adhesion and resultant polarization of RAW 264.7 cells to an implantable surface [124]. The RGD uncaging improved the adhesion and M2 polarization inhibiting M1φ functions through Rho-associated protein kinase (ROCK) signaling, involved in the regulation of cytoskeletal arrangement and contractility. This elegant system holds promise to manipulate inflammatory or tissue-regenerative immune responses to implants [124] (Fig. 4**A**).

Although there are still questions to be addressed, especially considering the interactive and dynamic role of Mφ and other immune cells in tendon inflammation and healing, these works reinforce the potential effect of magnetic forces to promote the resolution of inflammation using precise cell-oriented tools.

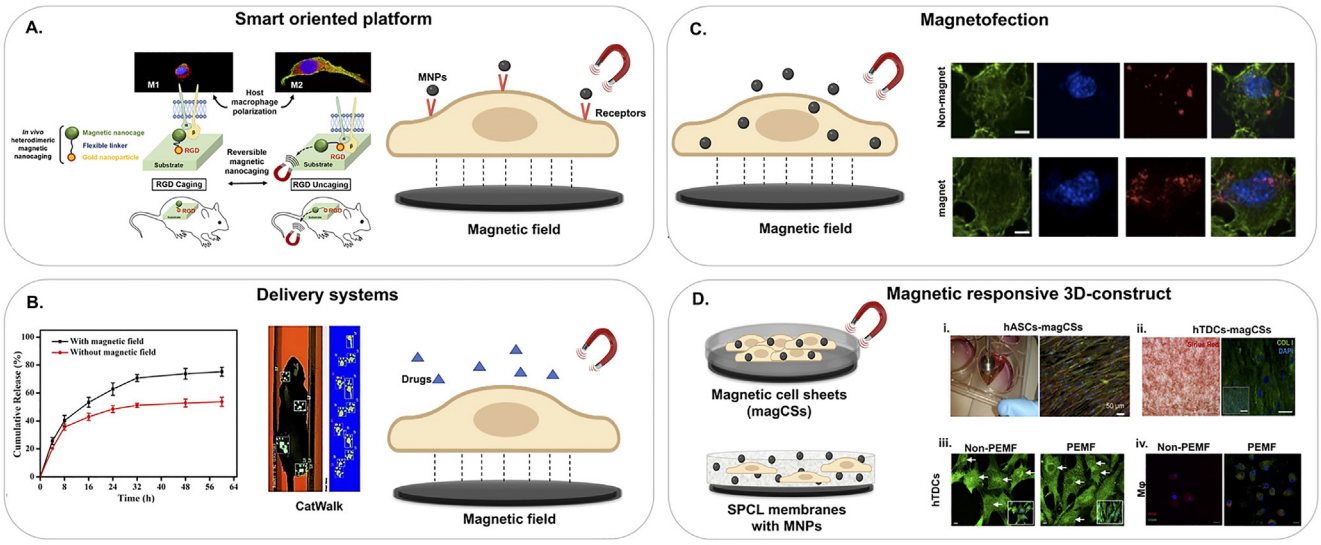

Fig. 4. Magnetically-assisted technologies with applicability to control inflammatory events in tendons. A. Smart-oriented platform to control cellular responses manipulating inflammatory and immune functions. A. Magnetic nanocage (MNC) was conjugated to RGD-bearing gold nanoparticle (GNP) via a flexible linker. The MNC was magnetically manipulated to reversibly uncage and cage RGD. The uncaging of RGD promoted the adhesion and M2φ polarization, and inhibited the M1φ polarization *in vitro* and *in vivo*. Reprinted (adapted) with permission from, Heemin Kang, Hee Joon Jung, Sung Kyu Kim, Dexter Siu Hong Wong, Sien Lin, Gang Li, Vinayak P. Dravid, Liming Bian, vol. 12, pages 5978–5994, Copyright (2023) American Chemical Society. **B. Magnetically-driven drug delivery to modulate the cell response.** A system with celecoxib drug actuated by a pulsed electromagnetic field (PEMF) was developed for the treatment of tendon injury. The celecoxib-loaded magnetism–responsive hydrogel showed higher drug release in the presence of PEMF, which led to a reduced inflammatory response. In accordance, the CatWalk gait analysis verified a synergistic effect of the drug and PEMF demonstrating promise for the rehabilitation of Achilles tendon rupture. Adapted from [127].**C. Magnetofection to efficiently deliver genetic material into cells.** Magnetofection involves the conjugation of nucleic acids to a magnetic nanoparticle for intracellular uptake by the application of an external magnetic field. A peptide binding to plasmid DNA (pDNA) (red) combined with MNPs was internalized in fibroblast cells (green) by the actuation of magnetic fields aiming for gene delivery to assist disease therapies. Adapted from [125]. **D. Sophisticated magnetically responsive systems to enhance tendon regeneration approaching scaffold-based and scaffold-free strategies. i)** Magnetic cell sheet technology: construction of magnetic cell sheet using human adipose derived stem cells (hASCs) magnetized with MNPs evidencing vinculin (green), phalloidin (red) and nuclei (blue). Reprinted from Publication Acta Biomaterialia, Vol 63, Ana I. Gonçalves, Márcia T. Rodrigues, Manuela E. Gomes, Tissue-engineered magnetic cell sheet patches for advanced strategies in tendon regeneration, Pages No.110-122, Copyright (2023), with permission from Elsevier. **ii)** Magnetic cell sheets constructed with human tendon derived stem cells (hTDCs) and MNPs, in which matrix deposition is detected by Sirius red/Fast green collagen staining and immunolocalization of Col I (green). Reprinted from Publication Acta Biomaterialia, Vol 131, Adriana Vinhas, Ana I. Gonçalves, Márcia T. Rodrigues, Manuela E. Gomes, Human tendon-derived cell sheets created by magnetic force-based tissue engineering hold tenogenic and immunomodulatory potential, Pages No.236–247, Copyright (2023), with permission from Elsevier. **iii.** Human tendon cells (hTDCs) stimulated with IL-1β on magnetic responsive membranes made of SPCL and MNPs (magSPCL) led to the activation of NFkB pathway. Representative confocal microscopy images of NFkB (in green) and nuclei (in blue). Arrows point the intracellular localization of NFkB protein. **iv.** Microscopy images of human Mφ (CD206 (green), and CD169 (red)) on magSPCL membranes. Insets are representative images in lower amplification (x20, scale bar 100 μm). Reprinted from Publication Acta Biomaterialia, Vol 117, A. Vinhas, M.T. Rodrigues, A.I. Gonçalves, R.L. Reis, M.E. Gomes, Magnetic responsive materials modulate the inflammatory profile of IL-1β conditioned tendon cells, Pages No. 235–245, Copyright (2023), with permission from Elsevier.

4.4. Prospects of magnetic based systems to tackle inflammation

Encouraging desirable interactions with living entities and prospecting functionalities to recreate the intricacy of biological processes, the actuation of MF can be combined with magnetically responsive nanomaterials such as magnetic nanoparticles (MNPs).

MNPs are extremely versatile with applicability as contrast imaging agents, in magnetically driven-cell transfection (magnetofection) [125], drug and gene delivery, or labelling of cells jointly with the possibility of transporting relevant molecules improving biorecognition and cellular regulation [126]. An example of inflammation imaging is the resource to *in vivo* MRI to detect tissue infiltration of inflammatory cells labelling circulating Mφ with MNPs. As Mφ naturally migrate into inflamed tissues, the magnetic properties of MNPs allow a non-invasive visualization and monitoring of inflammation, thereby assisting an earlier intervention in inflammation-associated diseases and improving outcomes.

The synergistic effect of PEMF and MNPs for remotely controlled delivery of an anti-inflammatory drug, celecoxib was investigated by Wang *et al.* anticipating improved tendon healing. The drug was loaded into a magnetically responsive hydrogel made of gelatin and magnetite nanoparticles. When PEMF was applied (pulsed magnetic therapy apparatus, for 30 min) there was an increment in the drug release rate with the time. The celecoxib-loaded system implanted in the rat Achilles improved the animals walking function pointing to an efficient repair [127] (Fig. 4**B**).

Another strategy employing magnetically actuated systems combined a peptide, a plasmid DNA (pDNA), and dextran MNPs. The pDNA internalization was homogeneously distributed throughout the cytosol by magnetofection using neodymium magnets, allowing magnetic field-guided local transfection and potentially facilitating targeted therapies [125] (Fig. 4**C**).

In recent years, cell and intracellular targeting, and mechano-magnetic transduction studies have widen the expectations for magnetically assisted technologies for human-driven cell-based and cell-free therapies [128]. The MNPs can be externally MF manipulated and locally create transient physical forces that cells are able to sense and respond to. Functionalized MNPs have already been designed for targeting mechano-responsive ion channels (e.g., TREK1, Wnt Frizzled, or PDGFRα and β receptors) in the promotion of osteogenic and smooth muscle cell phenotypes in human MSCs. In previous work by our group addressing tendon-oriented therapies, MNPs were functionalized with anti-activin receptor type IIA (ActRIIA) to stimulate tenogenic outcomes in human adipose stem cells (hASCs) actuated by an oscillating magnetic bioreactor [129]. ActRIIA targeting activated SMAD pathways regulating tenogenic features, which resulted in the expression/increased production of TNMD, TGF-β as well as of Col I and III.

PEMF has gained visibility in tissue engineering and regenerative medicine strategies due to the increasing literature supporting PEMF role in tissue healing and regeneration, along with the wide range of possibilities for designing sophisticated approaches combining PEMF with magnetic components. Such approaches propose advanced magnetically responsive systems designed to operate as smart delivery systems of bioactive molecules, including drugs, oligonucleotides, and/or genes, to educate cells [124,130], act as oriented triggers to precisely bind and activate cell receptors [131,132], intracellular molecules [133], or even to support the maturation of tissue-engineered constructs in scaffolding strategies [134]. Despite the fundamental and therapeutic potential of the systems with magnetic responsiveness to study the inflammation in the onset and progression of tendon pathology and their contribution to the repair of damaged tissues, there is a limited number of reports describing the magnetically-assisted regulation and control of inflammatory events. These numbers are even more depicted concerning inflammation in tendon disease.

Our group has been working with MNP-based strategies for the modulation of cell phenotypes pre-conditioned to cytokines. These include the exploitation of magnetic cell sheets (magCSs) [135] and MNPs incorporated into polymeric matrices [58] envisioning inflammation resolution foreseeing tendon regeneration.

The fusion of magnetically assisted- and cell sheet- technologies enables a more complex and more physiologically representative model for studying tendon responses, in which the dynamic cell–cell and cell-matrix interactions are preserved. Additionally, magCSs can be directly applied in a tendon lesion as a living patch enabling a closer interaction between implant and host cell populations [133] (Fig. 4**Di)**. The IL-1β priming of magCSs made of TDSCs and MNPs results in an increment of inflammatory associated cytokines, as occurs with 2D monolayers of TDSCs, that is reversed with a 1 h exposure to PEMF (5 Hz, 4 mT, 50% duty cycle) [135]. PEMF was shown to modulate the inflammatory profile of IL-1β-treated magCSs reducing the expression of *IL-1β, IL-6, IL-8*, and *TNFα,* and reinforcing IL-10 and IL-4. Additionally, PEMF assists in the production of COL I and COL III and a decrease in *MMP-1,-2,-3* expression via the MAPK (ERK1/2) pathway, known to be present in both acute and chronic inflammation (Fig. 4**Dii**).

To better understand the role of magnetically actuated biomaterials in modulating tendon inflammatory responses, a magnetically actuated membrane was fabricated using a polymer blend of starch and polycaprolactone (SPCL) impregnated with MNPs (magSPCL) [58]. IL-1β-treated tendon cells cultured on magSPCL evidenced a decrease of *TNFα, IL-6, IL-8, COX-2* as well as *MMP-1,-2,-3* under PEMF (5 Hz, 4 mT, 50% duty cycle). Conversely, the expression of *TIMP-1*, *IL-10*, and *IL-4* was enhanced, likely mediated by the NF-κB pathway (Fig. 4**Diii**). Interestingly, when Mφ were cultured on magSPCL, PEMF actuated constructs demonstrated an immunomodulatory effect favouring M2φ markers (e.g. $CD206^{+}$) (Fig. 4**Div**). Furthermore, these membranes demonstrated the *in vivo* modulation potential of inflammation in an ectopic rodent model [136].

Overall, the promising PEMF actuation in the different phases of healing, together with magnetically responsive biomaterials, can provide new prospects to influence the immune system and favour immunomodulatory responses to assist enhanced functional recovery of tissues.

4.5. Bottlenecks hindering the expansion of PEMF-based therapies

A major challenge of external magnetic stimulation relies on the standardization of magnetic parameters and the selection of the MF applied for comparable outcomes. Most of the studies are performed in custom-designed or house-made systems that pose barriers to the reproducibility of the work and, consequently, to advances in the understanding of magnetic effects in cellular structures and functionality. Notwithstanding these limitations, preclinical models support the modulatory effects of PEMF on inflammatory cues. In a work by Lee *et al.*, daily application of PEMF (17 Hz, 15 min/day, five sessions per week) led to a reduction in inflammation in Achilles tendonitis [137]. The effect of PEMF (3.846 kHz and 25% duty cycle) applied for 4 hr/day has also been reported in a rat model of intervertebral disc injury. The inhibition of TNFα gene expression and of IL-6 and IL-1β occurred 7 days after PEMF stimulation [138]. These results were also verified in the knee joint of an osteoarthritis model in Dunkin Hartley guinea-pigs, where a 3-month PEMF therapy (75 Hz, 1.6 mT, 6 h/day) reduced the progression of osteoarthritis lesions [139].

Independently of the cell population and tissues investigated, it seems that PEMF stimulation holds a desirable effect on inflammatory events in pathological environments. Intriguingly, PEMF (75 Hz, 1.3 ms pulsed for 24 h) not only modulated the cytokine production, but the PEMF stimulation over adenosine receptors previously treated with IL-1β could be associated with the inhibition of the NF-κB pathway in chondrocytes and hFOB 1.19 osteoblasts cells [140].

5. Conclusions

Understanding the intricate inflammation signaling in injured and diseased tendons will provide new opportunities to timely resolve inflammation and overcome persistent inflammatory cues that influence the composition and properties of tendon ECM and induce phenotypic and functional changes in resident cell populations. Magnetically-assisted technologies constitute a sophisticated and versatile platform of precision nanotools at cellular and molecular scales to guide and stimulate cellular behaviour and regulate signaling events. Magnetically-assisted technologies brings a promising future to assist the restoration of the tissue balance with great contributions to the fields of regenerative medicine and inflammatory-associated pathologies that go beyond tendon disease.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the ERC CoG MagTendon (No. 772817). Doctoral grant SFRH/BD/144816/2019 (A. F. Almeida) were provided by FCT.

References

[1] S. Steinmann, C.G. Pfeifer, C. Brochhausen, D. Docheva, Spectrum of Tendon Pathologies: Triggers, Trails and End-State, Int. J. Mol. Sci. 21 (3) (2020) 1–20.
[2] B.J.F. Dean, S.G. Dakin, N.L. Millar, A.J. Carr, Review: Emerging concepts in the pathogenesis of tendinopathy, Surgeon 15 (6) (2017) 349–354.
[3] D. Docheva, S.A. Muller, M. Majewski, C.H. Evans, Biologics for tendon repair, Adv. Drug Deliv. Rev. 84 (2015) 222–239.
[4] M. Franchi, A. Trire, M. Quaranta, E. Orsini, V. Ottani, Collagen structure of tendon relates to function, Sci. World J. 7 (2007) 404–420.
[5] K.E. Kadler, Y. Hojima, D.J. Prockop, Collagen fibrils invitro grow from pointed tips in the C- to N-terminal direction, Biochem. J. 268 (1990) 339–343.

[6] M.S.C. Godinho, C.T. Thorpe, S.E. Greenwald, H.R.C. Screen, Elastin is Localised to the Interfascicular Matrix of Energy Storing Tendons and Becomes Increasingly Disorganised With Ageing, Sci Rep 7 (1) (2017) 9713.
[7] C.T. Thorpe, G.P. Riley, H.L. Birch, P.D. Clegg, H.R.C. Screen, Fascicles and the interfascicular matrix show adaptation for fatigue resistance in energy storing tendons, Acta Biomater 42 (2016) 308–315.
[8] P.P. Lui, L.S. Chan, Y.W. Lee, S.C. Fu, K.M. Chan, Sustained expression of proteoglycans and collagen type III/type I ratio in a calcified tendinopathy model, Rheumatology (Oxford) 49 (2) (2010) 231–239.
[9] E. Finnamore, C. Waugh, L. Solomons, M. Ryan, C. West, A. Scott, Transverse tendon stiffness is reduced in people with Achilles tendinopathy: A cross-sectional study, PLoS One 14 (2) (2019) e0211863.
[10] S.H. Han, W. Choi, J. Song, J. Kim, S. Lee, Y. Choi, S.E. Byun, T. Ahn, H. Ahn, C. Ding, L. Baik, S. Ward, K. Ting, S. Lee, The Implication of Substance P in the Development of Tendinopathy: A Case Control Study, Int. J. Mol. Sci. 18 (6) (2017) 1–13.
[11] G. Andersson, P. Danielson, H. Alfredson, S. Forsgren, Nerve-related characteristics of ventral paratendinous tissue in chronic Achilles tendinosis, Knee Surg Sports Traumatol Arthrosc 15 (10) (2007) 1272–1279.
[12] Y. Bi, D. Ehirchiou, T.M. Kilts, C.A. Inkson, M.C. Embree, W. Sonoyama, L. Li, A.I. Leet, B.-M. Seo, L. Zhang, S. Shi, M.F. Young, Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, Nat. Med. 13 (10) (2007) 1219–1227.
[13] A.C. Noah, T.M. Li, L.M. Martinez, S. Wada, J.B. Swanson, N.P. Disser, K.B. Sugg, S.A. Rodeo, T.T. Lu, C.L. Mendias, Adaptive and Innate Immune Cell Responses in Tendons and Lymph Nodes After Tendon Injury and Repair, J. Appl. Physiol. 128 (3) (2019) 473–482.
[14] C.T. Thorpe, S. Chaudhry, A. Lei II, G.P. Varone, H.L. Riley, P.D. Birch, H.R.S. Clegg, Tendon overload results in alterations in cell shape and increased markers of inflammation and matrix degradation, Scandinavian J Med. Sci. Sports 25 (4) (2015) e381–e391.
[15] A.E. Brent, R. Schweitzer, C.J. Tabin, A Somitic Compartment of Tendon Progenitors, Cell 113 (2) (2003) 235–248.
[16] J.P. Gumucio, M.M. Schonk, Y.A. Kharaz, E. Comerford, C.L. Mendias, Scleraxis is required for the growth of adult tendons in response to mechanical loading, JCI Insight 5 (13) (2020) 1–15.
[17] M. Akbar, L. MacDonald, L.A.N. Crowe, K. Carlberg, M. Kurowska-Stolarska, P. L. Ståhl, S.J.B. Snelling, I.B. McInnes, N.L. Millar, Single cell and spatial transcriptomics in human tendon disease indicate dysregulated immune homeostasis, Ann. Rheum. Dis. 80 (11) (2021) 1493–1494.
[18] C. Liu, J.W. Luo, K.K. Zhang, L.X. Lin, T. Liang, Z.P. Luo, Y.Q. Zhuang, Y.L. Sun, Tendon-Derived Stem Cell Differentiation in the Degenerative Tendon Microenvironment, Stem Cells Int. 2018 (2018) 1–12.
[19] M. Kim, Y.K. Bae, S. Um, J.H. Kwon, G.H. Kim, S.J. Choi, W. Oh, H.J. Jin, A Small-Sized Population of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Shows High Stemness Properties and Therapeutic Benefit, Stem Cells Int. 2020 (2020) 1–17.
[20] Z. Huang, Z. Yin, J. Xu, Y. Fei, B.C. Heng, X. Jiang, W. Chen, W. Shen, Tendon Stem/Progenitor Cell Subpopulations and Their Implications in Tendon Biology, Front. Cell, Dev. Biol. 9 (2021) 1–14.
[21] C. Shukunami, A. Takimoto, Y. Nishizaki, Y. Yoshimoto, S. Tanaka, S. Miura, H. Watanabe, T. Sakuma, T. Yamamoto, G. Kondoh, Y. Hiraki, Scleraxis is a transcriptional activator that regulates the expression of Tenomodulin, a marker of mature tenocytes and ligamentocytes, Sci. Rep. 8 (3155) (2018) 1–17.
[22] J. Chen, E. Zhang, W. Zhang, Z. Liu, P. Lu, T. Zhu, Z. Yin, L.J. Backman, H. Liu, X. Chen, H. Ouyang, Fos Promotes Early Stage Teno-Lineage Differentiation of Tendon Stem/Progenitor Cells in Tendon, Stem Cells Transl. Med. 6 (11) (2017) 2009–2019.
[23] H. Yin, M.D. Caceres, Z. Yan, M. Schieker, M. Nerlich, D. Docheva, Tenomodulin regulates matrix remodeling of mouse tendon stem/progenitor cells in an ex vivo collagen I gel model, Biochem. Biophys. Res. Commun. 512 (4) (2019) 691–697.
[24] A.I. Goncalves, A. Vinhas, M.T. Rodrigues, M.E. Gomes, The impact of cryopreservation in signature markers and immunomodulatory profile of tendon and ligament derived cells, J. Cell. Physiol. 237 (1) (2022) 675–686.
[25] J.A. Cadby, E. Buehler, C. Godbout, P.R. van Weeren, J.G. Snedeker, Differences between the cell populations from the peritenon and the tendon core with regard to their potential implication in tendon repair, PLoS One 9 (3) (2014) 1–11.
[26] M.J. Mienaltowski, S.M. Adams, D.E. Birk, Tendon proper- and peritenon-derived progenitor cells have unique tenogenic properties, Stem Cell Res. Ther. 5 (86) (2014) 1–15.
[27] Z. Yin, J.-J. Hu, L. Yang, Z.-F. Zheng, C.-R. An, B.-B. Wu, C. Zhang, W.-L. Shen, H.-H. Liu, J.-L. Chen, B.C. Heng, G.-J. Guo, X. Chen, H. Ouyang, Single-cell analysis reveals a nestin+ tendon stem:progenitor cell population with strong tenogenic potentiality, Sci. Adv. 2 (2016) 1–14.
[28] Q. Tan, P.P. Lui, Y.W. Lee, In vivo identity of tendon stem cells and the roles of stem cells in tendon healing, Stem Cells Dev. 22 (23) (2013) 3128–3140.
[29] D. Jiang, B. Xu, M. Yang, Z. Zhao, Y. Zhang, Z. Li, Efficacy of tendon stem cells in fibroblast-derived matrix for tendon tissue engineering, Cytotherapy 16 (5) (2014) 662–673.
[30] R. Giancola, F. Oliva, M. Gallorini, N. Michetti, C. Gissi, F. Moussa, C.A.L. Passeri, A. Colosimo, A.C. Berardi, CD200 as a Potential New Player in Inflammation during Rotator Cuff Tendon Injury/Repair: An In Vitro Model, Int. J. Mol. Sci. 23 (2022) 1–10.
[31] T. Harvey, S. Flamenco, C.M. Fan, A Tppp3(+)Pdgfra(+) tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis, Nat Cell Biol 21 (12) (2019) 1490–1503.
[32] A.R. Kendal, T. Layton, H. Al-Mossawi, L. Appleton, S. Dakin, R. Brown, C. Loizou, M. Rogers, R. Sharp, A. Carr, Multi-omic single cell analysis resolves novel stromal cell populations in healthy and diseased human tendon, Sci. Rep. 10 (1) (2020) 1–14.
[33] D. Frölich, D. Blaβfeld, K. Reiter, C. Giesecke, C. Daridon, H.E. Mei, G.R. Burmester, D.M. Goldenberg, A. Salama, T. Dörner, The anti-CD74 humanized monoclonal antibody, milatuzumab, which targets the invariant chain of MHC II complexes, alters B-cell proliferation, migration, and adhesion molecule expression, Arthritis Research & Therapy 14 (2012) 1–12.
[34] O. Al-Sadi, G. Schulze-Tanzil, B. Kohl, A. Lohan, M. Lemke, W. Ertel, T. John, Tenocytes, pro-inflammatory cytokines and leukocytes- a relationship?, Muscles, Ligaments Tendons J 1 (3) (2011) 68–76
[35] E. Garcia-Melchor, G. Cafaro, L. MacDonald, L.A.N. Crowe, S. Sood, M. McLean, U.G. Fazzi, I.B. McInnes, M. Akbar, N.L. Millar, Novel self-amplificatory loop between T cells and tenocytes as a driver of chronicity in tendon disease, Ann Rheum Dis 80 (8) (2021) 1075–1085.
[36] K.L. Spiller, S. Nassiri, C.E. Witherel, R.R. Anfang, J. Ng, K.R. Nakazawa, T. Yu, G. Vunjak-Novakovic, 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.
[37] J.Y. Sunwoo, C.D. Eliasberg, C.B. Carballo, S.A. Rodeo, The role of the macrophage in tendinopathy and tendon healing, J. Orthop. Res. 38 (8) (2020) 1666–1675.
[38] K.L. Spiller, T.J. Koh, Macrophage-based therapeutic strategies in regenerative medicine, Adv. Drug Deliv. Rev. 122 (2017) 74–83.
[39] A. Mauro, V. Russo, L. Di Marcantonio, P. Berardinelli, A. Martelli, A. Muttini, M. Mattioli, B. Barboni, M1 and M2 macrophage recruitment during tendon regeneration induced by amniotic epithelial cell allotransplantation in ovine, Res. Vet. Sci. 105 (2016) 92–102.
[40] L.A. Murray, Q. Chen, M.S. Kramer, D.P. Hesson, R.L. Argentieri, X. Peng, M. Gulati, R.J. Homer, T. Russell, N. van Rooijen, J.A. Elias, C.M. Hogaboam, E.L. Herzog, TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P, Int. J. Biochem. Cell Biol. 43 (1) (2011) 154–162.
[41] M.J. Mienaltowski, S.M. Adams, D.E. Birk, Regional differences in stem cell/progenitor cell populations from the mouse achilles tendon, Tissue Eng. Part A 19 (1–2) (2013) 199–210.
[42] A.J. De Micheli, J.B. Swanson, N.P. Disser, L.M. Martinez, N.R. Walker, D.J. Oliver, B.D. Cosgrove, C.L. Mendias, Single-cell transcriptomic analysis identifies extensive heterogeneity in the cellular composition of mouse Achilles tendons, Am J Physiol Cell Physiol 319 (5) (2020) C885–C894.
[43] C.M. Minutti, L.H. Jackson-Jones, B. Garcia-Fojeda, J.A. Knipper, T.E. Sutherland, N. Logan, E. Ringqvist, R. Guillamat-Prats, D.A. Ferenbach, A. Artigas, C. Stamme, Z.C. Chroneos, D.M. Zaiss, C. Casals, J.E. Allen, Local amplifiers of IL-4Ralpha-mediated macrophage activation promote repair in lung and liver, Science 356 (6342) (2017) 1076–1080.
[44] L.A. Borthwick, The IL-1 cytokine family and its role in inflammation and fibrosis in the lung, Semin. Immunopathol. 38 (4) (2016) 517–534.
[45] S. Muscat, A.E.C. Nichols, E. Gira, A.E. Loiselle, CCR2 identifies tendon resident macrophage and T cell populations while CCR2 deficiency impedes late tendon healing, FASEB J 36 (11) (2022) 1–31.
[46] M.S. Kragsnaes, U. Fredberg, K. Stribolt, S.G. Kjaer, K. Bendix, T. Ellingsen, Stereological quantification of immune-competent cells in baseline biopsy specimens from achilles tendons: results from patients with chronic tendinopathy followed for more than 4 years, Am J Sports Med 42 (10) (2014) 2435–2445.
[47] M.A. Alim, M. Peterson, G. Pejler, Do Mast Cells Have a Role in Tendon Healing and Inflammation?, Cells 9 (5) (2020)
[48] A. Scott, O. Lian, R. Bahr, D.A. Hart, V. Duronio, K.M. Khan, Increased mast cell numbers in human patellar tendinosis: correlation with symptom duration and vascular hyperplasia, Br J Sports Med 42 (9) (2008) 753–757.
[49] C. Lehner, G. Spitzer, R. Gehwolf, A. Wagner, N. Weissenbacher, C. Deininger, K. Emmanuel, F. Wichlas, H. Tempfer, A. Traweger, Tenophages: a novel macrophage-like tendon cell population expressing CX3CL1 and CX3CR1, Dis Model Mech 12 (12) (2019).
[50] E. Mass, I. Ballesteros, M. Farlik, F. Halbritter, P. Gunther, L. Crozet, C.E. Jacome-Galarza, K. Handler, J. Klughammer, Y. Kobayashi, E. Gomez-Perdiguero, J.L. Schultze, M. Beyer, C. Bock, F. Geissmann, Specification of tissue-resident macrophages during organogenesis, Science 353 (6304) (2016).
[51] G. Yang, H.J. Im, J.H. Wang, Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts, Gene 363 (2005) 166–172.
[52] T. John, D. Lodka, B. Kohl, W. Ertel, J. Jammrath, C. Conrad, C. Stoll, C. Busch, G. Schulze-Tanzil, Effect of pro-inflammatory and immunoregulatory cytokines on human tenocytes, J. Orthop. Res. 28 (8) (2010) 1071–1077.
[53] Z. Zhu, J. Ding, Z. Ma, T. Iwashina, E.E. Tredget, Alternatively activated macrophages derived from THP-1 cells promote the fibrogenic activities of human dermal fibroblasts, Wound Repair Regen. 25 (3) (2017) 377–388.
[54] T. Liu, L. Zhang, D. Joo, S.C. Sun, NF-kappaB signaling in inflammation, Signal Transduct. Target. Ther. 2 (2017) 1–9.
[55] D.U. Ferreiro, E.A. Komives, Molecular mechanisms of system control of NF-kappaB signaling by IkappaBalpha, Biochem. 49 (8) (2010) 1560–1567.

[56] A.C. Abraham, S.A. Shah, M. Golman, L. Song, X. Li, I. Kurtaliaj, M. Akbar, N.L. Millar, Y. Abu-Amer, L.M. Galatz, S. Thomopoulos, Targeting the NF-kappaB signaling pathway in chronic tendon disease, Sci. Transl. Med. 11 (481) (2019) 1–23.
[57] S.G. Dakin, F.O. Martinez, C. Yapp, G. Wells, U. Oppermann, B.J. Dean, R.D. Smith, K. Wheway, B. Watkins, L. Roche, A.J. Carr, Inflammation activation and resolution in human tendon disease, Sci. Transl. Med. 7 (311) (2015) 1–31.
[58] A. Vinhas, M.T. Rodrigues, A.I. Goncalves, R.L. Reis, M.E. Gomes, Magnetic responsive materials modulate the inflammatory profile of IL-1beta conditioned tendon cells, Acta Biomater. 117 (2020) 235–245.
[59] C. Buhrmann, A. Mobasheri, F. Busch, C. Aldinger, R. Stahlmann, A. Montaseri, M. Shakibaei, Curcumin modulates nuclear factor kappaB (NF-kappaB)-mediated inflammation in human tenocytes in vitro: role of the phosphatidylinositol 3-kinase/Akt pathway, J. Biol. Chem. 286 (32) (2011) 28556–28566.
[60] M.G. Dorrington, I.D.C. Fraser, NF-kappaB Signaling in Macrophages: Dynamics, Crosstalk, and Signal Integration, Front. Immunol. 10 (705) (2019) 1–12.
[61] E. Courtine, N. Cagnard, J. Mazzolini, M. Antona, F. Pene, C. Fitting, S. Jacques, C. Rousseau, F. Niedergang, S. Gerondakis, J.D. Chiche, F. Ouaaz, J.P. Mira, Combined loss of cRel/p50 subunits of NF-kappaB leads to impaired innate host response in sepsis, Innate Immun. 18 (5) (2012) 753–763.
[62] E.K. Kim, E.J. Choi, Pathological roles of MAPK signaling pathways in human diseases, Biochim. Biophys. Acta 1802 (4) (2010) 396–405.
[63] W. Morita, S.J.B. Snelling, K. Wheway, B. Watkins, L. Appleton, A.J. Carr, S.G. Dakin, ERK1/2 drives IL-1β-induced expression of TGF-β1 and BMP-2 in torn tendons, Sci. Rep. 9 (19005) (2019) 1–10.
[64] U. Blache, S.L. Wunderli, A.A. Hussien, T. Stauber, G. Flückiger, M. Bollhalder, B. Niederöst, S.F. Fucentese, J.G. Snedeker, Inhibition of ERK1/2 kinases prevents tendon matrix breakdown, Sci. Rep. 11 (6838) (2021) 1–9.
[65] N.L. Millar, M. Akbar, A.L. Campbell, J.H. Reilly, S.C. Kerr, M. McLean, M. Frleta-Gilchrist, U.G. Fazzi, W.J. Leach, B.P. Rooney, L.A.N. Crowe, G.A.C. Murrell, I.B. McInnes, IL-17A mediates inflammatory and tissue remodelling events in early human tendinopathy, Sci. Rep. 6 (27149) (2016) 1–11.
[66] L.T. Donlin, A. Jayatilleke, E.G. Giannopoulou, G.D. Kalliolias, L.B. Ivashkiv, Modulation of TNF-induced macrophage polarization by synovial fibroblasts, J. Immunol. 193 (5) (2014) 2373–2383.
[67] S. Tarafder, E. Chen, Y. Jun, K. Kao, K.H. Sim, J. Back, F.Y. Lee, C.H. Lee, Tendon stem/progenitor cells regulate inflammation in tendon healing via JNK and STAT3 signaling, FASEB J 31 (9) (2017) 3991–3998.
[68] Y. Wang, G. He, H. Tang, Y. Shi, X. Kang, J. Lyu, M. Zhu, M. Zhou, M. Yang, M. Mu, W. Chen, B. Zhou, J. Zhang, K. Tang, Aspirin inhibits inflammation and scar formation in the injury tendon healing through regulating JNK/STAT-3 signalling pathway, Cell Prolif 52 (4) (2019) e12650.
[69] A.J. Schwartz, D.C. Sarver, K.B. Sugg, J.T. Dzierzawski, J.P. Gumucio, C.L. Mendias, p38 MAPK signaling in postnatal tendon growth and remodeling, PLoS One 10 (3) (2015) 1–21.
[70] J.M. Wilde, J.P. Gumucio, J.A. Grekin, D.C. Sarver, A.C. Noah, D.G. Ruehlmann, M.E. Davis, A. Bedi, C.L. Mendias, Inhibition of p38 mitogen-activated protein kinase signaling reduces fibrosis and lipid accumulation after rotator cuff repair, J. Shoulder Elbow Surg. 25 (9) (2016) 1501–1508.
[71] N.L. Millar, J.H. Reilly, S.C. Kerr, A.L. Campbell, K.J. Little, W.J. Leach, B.P. Rooney, G.A. Murrell, I.B. McInnes, Hypoxia: a critical regulator of early human tendinopathy, Ann. Rheum. Dis. 71 (2) (2012) 302–310.
[72] A.M. Buo, J.P. Stains, Gap junctional regulation of signal transduction in bone cells, FEBS Lett. 588 (8) (2014) 1315–1321.
[73] E. Maeda, S. Kimura, M. Yamada, M. Tashiro, T. Ohashi, Enhanced gap junction intercellular communication inhibits catabolic and pro-inflammatory responses in tenocytes against heat stress, J. Cell Commun. Signal. 11 (4) (2017) 369–380.
[74] E. Maeda, H. Pian, T. Ohashi, Temporal regulation of gap junctional communication between tenocytes subjected to static tensile strain with physiological and non-physiological amplitudes, Biochem. Biophys. Res. Commun. 482 (4) (2017) 1170–1175.
[75] A.D. Waggett, M. Benjamin, J.R. Ralphs, Connexin 32 and 43 gap junctions differentially modulate tenocyte response to cyclic mechanical load, Eur. J. Cell Biol. 85 (11) (2006) 1145–1154.
[76] E. Maeda, T. Ohashi, Mechano-regulation of gap junction communications between tendon cells is dependent on the magnitude of tensile strain, Biochem. Biophys. Res. Commun. 465 (2) (2015) 281–286.
[77] K.S. Park, E. Bandeira, G.V. Shelke, C. Lasser, J. Lotvall, Enhancement of therapeutic potential of mesenchymal stem cell-derived extracellular vesicles, Stem Cell Res Ther 10 (1) (2019) 288.
[78] J. Santos, P.V. Dalla, B.K. Milthorpe, A molecular analysis of cytokine content across extracellular vesicles, secretions, and intracellular space from different site-specific adipose-derived stem cells, Int. J. Mol. Sci. 23 (397) (2022) 1–17.
[79] M. Zhang, H. Liu, Q. Cui, P. Han, S. Yang, M. Shi, T. Zhang, Z. Zhang, Z. Li, Tendon stem cell-derived exosomes regulate inflammation and promote the high-quality healing of injured tendon, Stem Cell Res. Ther. 11 (402) (2020) 1–15.
[80] H. Liu, M. Zhang, M. Shi, T. Zhang, W. Lu, S. Yang, Q. Cui, Z. Li, Adipose-derived mesenchymal stromal cell-derived exosomes promote tendon healing by activating both SMAD1/5/9 and SMAD2/3, Stem Cell Res. Ther. 12 (338) (2021) 1–13.
[81] F. Guojian, L. Liangyu, P. Zhangyi, F. Aoyuan, Y. Feng, Adipose-derived stem cell exosomes facilitate rotator cuff repair by mediating tendon-derived stem cells, Regen. Med. 16 (4) (2021) 359–372.
[82] C.S. Chamberlain, A.E.B. Clements, J.A. Kink, U. Choi, G.S. Baer, M.A. Halanski, P. Hematti, R. Vanderby, Extracellular Vesicle-Educated Macrophages Promote Early Achilles Tendon Healing, Stem Cells 37 (5) (2019) 652–662.
[83] Z. Shi, Q. Wang, D. Jiang, Extracellular vesicles from bone marrow-derived multipotent mesenchymal stromal cells regulate inflammation and enhance tendon healing, J. Transl. Med. 17 (211) (2019) 1–12.
[84] L. Cao, H. Xu, G. Wang, M. Liu, D. Tian, Z. Yuan, Extracellular vesicles derived from bone marrow mesenchymal stem cells attenuate dextran sodium sulfate-induced ulcerative colitis by promoting M2 macrophage polarization, Int. Immunopharmacol. 72 (2019) 264–274.
[85] M. Skutek, M. van Griensven, J. Zeichen, N. Brauer, U. Bosch, Cyclic mechanical stretching modulates secretion pattern of growth factors in human tendon fibroblasts, Eur. J. Appl. Physiol. 86 (2001) 48–52.
[86] A. Islam, T. Mbimba, M. Younesi, O. Akkus, Effects of substrate stiffness on the tenoinduction of human mesenchymal stem cells, Acta Biomater. 58 (2017) 244–253.
[87] T. Wang, J.H. Lai, L.H. Han, X. Tong, F. Yang, Chondrogenic differentiation of adipose-derived stromal cells in combinatorial hydrogels containing cartilage matrix proteins with decoupled mechanical stiffness, Tissue Eng. Part A 20 (15–16) (2014) 2131–2139.
[88] C. Liu, J.W. Luo, T. Liang, L.X. Lin, Z.P. Luo, Y.Q. Zhuang, Y.L. Sun, Matrix stiffness regulates the differentiation of tendon-derived stem cells through FAK-ERK1/2 activation, Exp. Cell Res. 373 (1–2) (2018) 62–70.
[89] C. Popov, M. Burggraf, L. Kreja, A. Ignatius, M. Schieker, D. Docheva, Mechanical stimulation of human tendon stem/progenitor cells results in upregulation of matrix proteins, integrins and MMPs, and activation of p38 and ERK1/2 kinases, BMC Mol Biol 16 (2015) 6.
[90] S.W. Wong, S. Lenzini, M.H. Cooper, D.J. Mooney, J.-W. Shin, Soft extracellular matrix enhances inflammatory activation of mesenchymal stromal cells to induce monocyte production and trafficking, Sci. Adv. 6 (2020) 1–12.
[91] W.C. Lee, G.Y. Ng, Z.J. Zhang, P. Malliaras, L. Masci, S.N. Fu, Changes on Tendon Stiffness and Clinical Outcomes in Athletes Are Associated With Patellar Tendinopathy After Eccentric Exercise, Clin. J. Sport. Med. 30 (1) (2020) 25–32.
[92] F.G. Thankam, Z.K. Roesch, M.F. Dilisio, M.M. Radwan, A. Kovilam, R.M. Gross, D.K. Agrawal, Association of Inflammatory Responses and ECM Disorganization with HMGB1 Upregulation and NLRP3 Inflammasome Activation in the Injured Rotator Cuff Tendon, Sci. Rep. 8 (1) (2018) 1–14.
[93] V. Russo, M. El Khatib, G. Prencipe, M.R. Citeroni, M. Faydaver, A. Mauro, P. Berardinelli, A. Cerveró-Varona, A.A. Haidar-Montes, M. Turriani, O. Di Giacinto, M. Raspa, F. Scavizzi, F. Bonaventura, J. Stöckl, B. Barboni, Tendon Immune Regeneration: Insights on the Synergetic Role of Stem and Immune Cells during Tendon Regeneration, Cells 11 (3) (2022) 1–30.
[94] 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.
[95] M. Chatterjee, P.M. Muljadi, N. Andarawis-Puri, The role of the tendon ECM in mechanotransduction: disruption and repair following overuse, Connect Tissue Res 63 (1) (2022) 28–42.
[96] L.G. Benage, J.D. Sweeney, M.B. Giers, R. Balasubramanian, Dynamic Load Model Systems of Tendon Inflammation and Mechanobiology, Front Bioeng Biotechnol 10 (2022).
[97] H. Du, J.M. Bartleson, S. Butenko, V. Alonso, W.F. Liu, D.A. Winer, M.J. Butte, Tuning immunity through tissue mechanotransduction, Nat Rev Immunol (2022) 1–15.
[98] R. Mousavizadeh, P. Hojabrpour, F. Eltit, P.C. McDonald, S. Dedhar, R.G. McCormack, V. Duronio, S.M. Jafarnejad, A. Scott, beta1 integrin, ILK and mTOR regulate collagen synthesis in mechanically loaded tendon cells, Sci Rep 10 (1) (2020) 12644.
[99] M.E. Wall, A.J. Banes, Early responses to mechanical load in tendon: Role for calcium signaling, gap junctions and intercellular communication, J. Musculoskelet. Neuronal. Interact. 5 (1) (2005) 70–84.
[100] F.S. Passini, P.K. Jaeger, A.S. Saab, S. Hanlon, N.A. Chittim, M.J. Arlt, K.D. Ferrari, D. Haenni, S. Caprara, M. Bollhalder, B. Niederost, A.N. Horvath, T. Gotschi, S. Ma, B. Passini-Tall, S.F. Fucentese, U. Blache, U. Silvan, B. Weber, K. G. Silbernagel, J.G. Snedeker, Shear-stress sensing by PIEZO1 regulates tendon stiffness in rodents and influences jumping performance in humans, Nat Biomed Eng 5 (12) (2021) 1457–1471.
[101] A.A. Hussien, B. Niederoest, M. Bollhalder, N. Goedecke, J.G. Snedeker, The Stiffness-sensitive Transcriptome of Human Tendon Stromal Cells, Adv Healthc Mater 12 (7) (2023) e2101216.
[102] D. Rowson, M.M. Knight, H.R.C. Screen, Zonal variation in primary cilia elongation correlates with localized biomechanical degradation in stress deprived tendon, Journal of Orthopaedic Research 34 (12) (2016) 2146–2153.
[103] A.D. Schoenenberger, H. Tempfer, C. Lehner, J. Egloff, M. Mauracher, A. Bird, J. Widmer, K. Maniura-Weber, S.F. Fucentese, A. Traweger, U. Silvan, J.G. Snedeker, Macromechanics and polycaprolactone fiber organization drive macrophage polarization and regulate inflammatory activation of tendon in vitro and in vivo, Biomaterials 249 (2020).
[104] Y. Liu, L. Wang, S. Li, T. Zhang, C. Chen, J. Hu, D. Sun, H. Lu, Mechanical stimulation improves rotator cuff tendon-bone healing via activating IL-4/JAK/STAT signaling pathway mediated macrophage M2 polarization, J Orthop Translat 37 (2022) 78–88.

[105] J. Wosik, W. Chen, K. Qin, R.M. Ghobrial, J.Z. Kubiak, M. Kloc, Magnetic Field Changes Macrophage Phenotype, Biophys J 114 (8) (2018) 2001–2013.
[106] E.I. Waldorff, N. Zhang, J.T. Ryaby, Pulsed electromagnetic field applications: A corporate perspective, J. Orthop. Translat. 9 (2017) 60–68.
[107] G.L. Bagnato, G. Miceli, N. Marino, D. Sciortino, G.F. Bagnato, Pulsed electromagnetic fields in knee osteoarthritis: a double blind, placebo-controlled, randomized clinical trial, Rheumatology (Oxford) 55 (4) (2016) 755–762.
[108] S. Yaman, M. Anil-Inevi, E. Ozcivici, H.C. Tekin, Magnetic Force-Based Microfluidic Techniques for Cellular and Tissue Bioengineering, Front. Bioeng. Biotechnol. 6 (2018).
[109] M. Jiao, H. Yin, J. Hu, W. Xu, X. Zhang, P. Zhang, Effects of Low-Frequency Pulsed Electromagnetic Fields on High-Altitude Stress Ulcer Healing in Rats, Biomed Res. Int. 2019 (2019) 1–8.
[110] C. Seeliger, K. Falldorf, J. Sachtleben, M. van Griensven, Low-frequency pulsed electromagnetic fields significantly improve time of closure and proliferation of human tendon fibroblasts, Eur. J. Med. Res. 19 (37) (2014) 1–7.
[111] L. de Girolamo, M. Vigano, E. Galliera, D. Stanco, S. Setti, M.G. Marazzi, G. Thiebat, M.M. Corsi Romanelli, V. Sansone, In vitro functional response of human tendon cells to different dosages of low-frequency pulsed electromagnetic field, Knee Surg. Sports Traumatol. Arthrosc. 23 (11) (2015) 3443–3453.
[112] L. de Girolamo, D. Stanco, E. Galliera, M. Vigano, A. Colombini, S. Setti, E. Vianello, M.M. Corsi Romanelli, V. Sansone, Low frequency pulsed electromagnetic field affects proliferation, tissue-specific gene expression, and cytokines release of human tendon cells, Cell Biochem. Biophys. 66 (3) (2013) 697–708.
[113] L. Petecchia, F. Sbrana, R. Utzeri, M. Vercellino, C. Usai, L. Visai, M. Vassalli, P. Gavazzo, Electro-magnetic field promotes osteogenic differentiation of BM-hMSCs through a selective action on Ca(2+)-related mechanisms, Sci. Rep. 5 (13856) (2015) 1–13.
[114] J.F. Escobar, J.J. Vaca-González, J.M. Guevara, D.A. Garzón-Alvarado, Effect of magnetic and electric fields on plasma membrane of single cells: A computational approach, Eng. Rep. 2 (2) (2020) 1–14.
[115] K.I. Lee, J.S. Lee, K.T. Kang, Y.B. Shim, Y.S. Kim, J.W. Jang, S.H. Moon, D.D. D'Lima, In Vitro and In Vivo Performance of Tissue-Engineered Tendons for Anterior Cruciate Ligament Reconstruction, Am. J. Sports Med. 46 (7) (2018) 1641–1649.
[116] M.L. Bayer, P. Schjerling, A. Herchenhan, C. Zeltz, K.M. Heinemeier, L. Christensen, M. Krogsgaard, D. Gullberg, M. Kjaer, Release of tensile strain on engineered human tendon tissue disturbs cell adhesions, changes matrix architecture, and induces an inflammatory phenotype, PLoS One 9 (1) (2014) 1–16.
[117] I. Gomez-Ochoa, P. Gomez-Ochoa, F. Gomez-Casal, E. Cativiela, L. Larrad-Mur, Pulsed electromagnetic fields decrease proinflammatory cytokine secretion (IL-1beta and TNF-alpha) on human fibroblast-like cell culture, Rheumatol. Int. 31 (10) (2011) 1283–1289.
[118] R. Gehwolf, B. Schwemberger, M. Jessen, S. Korntner, A. Wagner, C. Lehner, N. Weissenbacher, H. Tempfer, A. Traweger, Global Responses of Il-1beta-Primed 3D Tendon Constructs to Treatment with Pulsed Electromagnetic Fields, Cells 8 (5) (2019) 1–20.
[119] A. Vinhas, M.T. Rodrigues, A.I. Goncalves, R.L. Reis, M.E. Gomes, Pulsed Electromagnetic Field Modulates Tendon Cells Response in IL-1beta-Conditioned Environment, J. Orthop. Res. 38 (1) (2019) 160–172.
[120] S.L. Miller, D.G. Coughlin, E.I. Waldorff, J.T. Ryaby, J.C. Lotz, Pulsed electromagnetic field (PEMF) treatment reduces expression of genes associated with disc degeneration in human intervertebral disc cells, Spine J. 16 (6) (2016) 770–776.
[121] C.L. Ross, Y. Zhou, C.E. McCall, S. Soker, T.L. Criswell, The Use of Pulsed Electromagnetic Field to Modulate Inflammation and Improve Tissue Regeneration: A Review, Bioelectricity 1 (4) (2019) 247–259.
[122] A. Vinhas, A.F. Almeida, A.I. Goncalves, M.T. Rodrigues, M.E. Gomes, Magnetic Stimulation Drives Macrophage Polarization in Cell to-Cell Communication with IL-1beta Primed Tendon Cells, Int. J. Mol. Sci. 21 (5441) (2020) 1–15.
[123] J. Ouyang, B. Zhang, L. Kuang, P. Yang, X. Du, H. Qi, N. Su, M. Jin, J. Yang, Y. Xie, Q. Tan, H. Chen, S. Chen, W. Jiang, M. Liu, X. Luo, M. He, Z. Ni, L. Chen, Pulsed Electromagnetic Field Inhibits Synovitis via Enhancing the Efferocytosis of Macrophages, Biomed. Res. Int. 2020 (2020) 1–13.
[124] H. Kang, H.J. Jung, S.K. Kim, D.S.H. Wong, S. Lin, G. Li, V.P. Dravid, L. Bian, Magnetic Manipulation of Reversible Nanocaging Controls In Vivo Adhesion and Polarization of Macrophages, ACS Nano 12 (6) (2018) 5978–5994.
[125] L.A. Blokpoel Ferreras, S.Y. Chan, S. Vazquez Reina, J.E. Dixon, Rapidly Transducing and Spatially Localized Magnetofection Using Peptide-Mediated Non-Viral Gene Delivery Based on Iron Oxide Nanoparticles, ACS Appl Nano Mater 4 (1) (2021) 167–181.
[126] A.F. Almeida, A. Vinhas, A.I. Goncalves, M.S. Miranda, M.T. Rodrigues, M.E. Gomes, Magnetic triggers in biomedical applications - prospects for contact free cell sensing and guidance, J Mater Chem B 9 (5) (2021) 1259–1271.
[127] J. Wang, L. Wang, Y. Gao, Z. Zhang, X. Huang, T. Han, B. Liu, Y. Zhang, Y. Li, L. Zhang, Synergistic Therapy of Celecoxib-Loaded Magnetism-Responsive Hydrogel for Tendon Tissue Injuries, Front Bioeng Biotechnol 8 (2020).
[128] A.M. Matos, A.I. Goncalves, A.J. El Haj, M.E. Gomes, Magnetic biomaterials and nano-instructive tools as mediators of tendon mechanotransduction, Nanoscale Adv 2 (1) (2020) 140–148.
[129] A.I. Goncalves, M. Rotherham, H. Markides, M.T. Rodrigues, R.L. Reis, M.E. Gomes, A.J. El Haj, Triggering the activation of Activin A type II receptor in human adipose stem cells towards tenogenic commitment using mechanomagnetic stimulation, Nanomedicine 14 (4) (2018) 1149–1159.
[130] M.S. Miranda, M.T. Rodrigues, R.M.A. Domingues, R.R. Costa, E. Paz, C. Rodriguez-Abreu, P. Freitas, B.G. Almeida, M.A. Carvalho, C. Goncalves, C.M. Ferreira, E. Torrado, R.L. Reis, J. Pedrosa, M.E. Gomes, Development of Inhalable Superparamagnetic Iron Oxide Nanoparticles (SPIONs) in Microparticulate System for Antituberculosis Drug Delivery, Adv. Healthc. Mater. 7 (15) (2018) 1–12.
[131] J. Dobson, S.H. Cartmell, A. Keramane, A.J. El Haj, Principles and design of a novel magnetic force mechanical conditioning bioreactor for tissue engineering, stem cell conditioning, and dynamic in vitro screening, IEEE Trans. Nanobiosci. 5 (3) (2006) 173–177.
[132] S. Hughes, S. McBain, J. Dobson, A.J. El Haj, Selective activation of mechanosensitive ion channels using magnetic particles, J. R. Soc. Interface 5 (25) (2008) 855–863.
[133] A.I. Goncalves, M.T. Rodrigues, M.E. Gomes, Tissue-engineered magnetic cell sheet patches for advanced strategies in tendon regeneration, Acta Biomater. 63 (2017) 110–122.
[134] S. Hao, J. Meng, Y. Zhang, J. Liu, X. Nie, F. Wu, Y. Yang, C. Wang, N. Gu, H. Xu, Macrophage phenotypic mechanomodulation of enhancing bone regeneration by superparamagnetic scaffold upon magnetization, Biomaterials 140 (2017) 16–25.
[135] A. Vinhas, A.I. Goncalves, M.T. Rodrigues, M.E. Gomes, Human tendon-derived cell sheets created by magnetic force-based tissue engineering hold tenogenic and immunomodulatory potential, Acta Biomater 131 (2021) 236–247.
[136] L. Santos, M. Silva, A.I. Gonçalves, T. Pesqueira, M.T. Rodrigues, M.E. Gomes, In vitro and in vivo assessment of magnetically actuated biomaterials and prospects in tendon healing 11 (9) (2016) 1107–1122.
[137] E.W.C. Lee, N. Maffulli, C.K. Li, K.M. Chan, Pulsed Magnetic and Electromagnetic Fields in Experimental Achilles Tendonitis in the Rat- A Prospective Randomized Study, Arch. Phys. Med. Rehabil. 78 (1997) 399–404.
[138] A.K. Chan, X. Tang, N.V. Mummaneni, D. Coughlin, E. Liebenberg, A. Ouyang, S. Dudli, M. Lauricella, N. Zhang, E.I. Waldorff, J.T. Ryaby, J.C. Lotz, Pulsed electromagnetic fields reduce acute inflammation in the injured rat-tail intervertebral disc, JOR Spine. 2 (4) (2019) 1–11.
[139] M. Fini, G. Giavaresi, P. Torricelli, F. Cavani, S. Setti, V. Cane, R. Giardino, Pulsed electromagnetic fields reduce knee osteoarthritic lesion progression in the aged Dunkin Hartley guinea pig, J. Orthop. Res. 23 (4) (2005) 899–908.
[140] F. Vincenzi, M. Targa, C. Corciulo, S. Gessi, S. Merighi, S. Setti, R. Cadossi, M.B. Goldring, P.A. Borea, K. Varani, Pulsed Electromagnetic Fields Increased the Anti-Inflammatory Effect of A2A and A3 Adenosine Receptors in Human T/C-28a2 Chondrocytes and hFOB 1.19 Osteoblasts, PLoS ONE 8 (5) (2013).