Advanced Drug Delivery Reviews 185 (2022) 114299

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

The tendon microenvironment: Engineered *in vitro* models to study cellular crosstalk

Manuel Gomez-Florit [1], Claudia J. Labrador-Rached, Rui M.A. Domingues *, 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, Parque de Ciência e Tecnologia, Barco, Guimarães, Portugal
ICVS/3B's–PT Government Associate Laboratory, Barco, Guimarães, Portugal

ARTICLE INFO

Article history:
Received 10 December 2021
Revised 11 April 2022
Accepted 12 April 2022
Available online 15 April 2022

Keywords:
Tendon
Tendinopathy
2D models
3D models
Regenerative medicine
Tissue engineering
Bioengineering

ABSTRACT

Tendinopathy is a multi-faceted pathology characterized by alterations in tendon microstructure, cellularity and collagen composition. Challenged by the possibility of regenerating pathological or ruptured tendons, the healing mechanisms of this tissue have been widely researched over the past decades. However, so far, most of the cellular players and processes influencing tendon repair remain unknown, which emphasizes the need for developing relevant *in vitro* models enabling to study the complex multicellular crosstalk occurring in tendon microenvironments. In this review, we critically discuss the insights on the interaction between tenocytes and the other tendon resident cells that have been devised through different types of existing *in vitro* models. Building on the generated knowledge, we stress the need for advanced models able to mimic the hierarchical architecture, cellularity and physiological signaling of tendon niche under dynamic culture conditions, along with the recreation of the integrated gradients of its tissue interfaces. In a forward-looking vision of the field, we discuss how the convergence of multiple bioengineering technologies can be leveraged as potential platforms to develop the next generation of relevant *in vitro* models that can contribute for a deeper fundamental knowledge to develop more effective treatments.

Contents

Abbreviations: AM, additive manufacturing; ASC, adipose tissue-derived stem cell; ACAN, aggrecan; ALP, alkaline phosphatase; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CXCL, C-X-C motif chemokine ligand; CX3CL1, C-X3-C ligand 1; DAMPs, damage-associated molecular patterns; dECM, decellularized ECM; ECM, extracellular matrix; ERK, extracellular signal-regulated kinases; FM, fascicular matrix; FAPs, fibro–adipogenic progenitors; CX3CR1, fractalkine receptor; iPSC, induced pluripotent stem cells; ICAM1, intercellular adhesion molecule 1; IFM, interfascicular matrix; IFN, interferon; IL, interleukin; MRC1, mannose receptor C-type 1; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; MKX, mohwak; MTJ, myotendinous junction; NK, natural killer; NF-kB, nuclear factor kB; OoC, organs-on-a-chip; PBMC, peripheral blood-derived mononuclear cell; PDGF, platelet-derived growth factor; PDGFRA, platelet-derived growth factor receptor alpha; PLLA, poly(l-lactide); PCL, polycaprolactone; PDMS, polydimethylsiloxane; PGE2, prostaglandin E2; PRG4, proteoglycan 4; SCX, scleraxis; SLRPs, small leucine-rich proteoglycans; TNC, tenascin-C; TSPCs, tendon stem/progenitor cells; TDC, tendon-derived cell; TNMD, tenomodulin; 3D, three-dimensional; TGF-β, transforming growth factor beta; TPPP3, tubulin polymerization-promoting protein 3; TNF, tumor necrosis factor; 2D, two-dimensional; VCAM1, vascular cell adhesion molecule 1.

* 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, Parque de Ciência e Tecnologia, Barco, Guimarães, Portugal.
E-mail addresses: manuel.gomezflorit@ssib.es (M. Gomez-Florit), rui.domingues@i3bs.uminho.pt (R.M.A. Domingues), megomes@i3bs.uminho.pt (M.E. Gomes).
[1] Present address: Health Research Institute of the Balearic Islands (IdISBa), 07010 Palma, Spain.

https://doi.org/10.1016/j.addr.2022.114299
0169-409X/

1. Introduction

Tendinopathy encompasses pathologies of tendon of different origins associated with pain and impaired function related to mechanical loading [1]. Tendinopathies and subsequent potential tendon ruptures remain an unmet clinical need that compromises the healthy lifestyle of people of all ages [2]. The real impact of this disease is difficult to measure but its magnitude reached 30–50% of musculoskeletal-related primary care visits worldwide on previous years [3], causing patient disability and reduced work productivity [4]. The prevalence of tendinopathies is high in both athletic and non-athletic adult population (around 25% in athletes and 6% in general population), and is expected to increase due to aging and sedentary-active lifestyle [5,6]. Moreover, the low intrinsic regeneration ability of tendon tissue poses an additional challenge to the limited therapeutic solutions available. Common treatments for tendinopathies include immobilization, physiotherapy, the administration of steroidal and non-steroidal anti-inflammatory drugs, injection with platelet-rich plasma, or surgery for severe cases [7-11]. Interestingly, a recent systematic review of clinical trials found that the use of surgery was as good as exercise-based therapies in mid- and long-term pain reduction and improved function and quality of life in patients with various tendinopathies [12]. Nevertheless, injured tendons never regain their original functionality, thus impairing the full recovery of patients [13]. It is clear that the mechanisms underlying tendon disease and healing are not completely understood, which is hampering the development of effective therapies that tackle its etiology and promote tendon regeneration.

Although animal models are viewed as gold standard and the majority of studies on mechanisms of tendon (patho)physiology largely derive from them [14], these models are limited in their ability to capture features of human tendon tissues (Table 1). For example, the mechanical properties of tendon widely diverge among species [15]. Moreover, small animal models of tendinopathies do not accurately reflect the human tendon repair mechanism since animal healing usually results in a fully repaired tendon [16]. Even though large animal models (horses, dogs and sheep) naturally develop tendon disease, thus better resembling the human condition, their costly and timely handling limits their use [17]. Moreover, given that human samples are rarely acquired before rupture [18], the animals unclear homology to human

Table 1
Comparison of animal models with 2D and 3D *in vitro* models.

Type of model	Advantages	Disadvantages
Animal models	Include tissues full complexity Account for interactions with organs/tissues in the body	Limited ability to capture features of human tissues and diseases Low representability Expensive Lengthy (time consuming) Require highly qualified personnel High ethical concerns
2D models	Incorporate human cells High throughput Cheap User-friendly Incorporate two cell types to study simple cellular crosstalk Real-time observation and adaptable to standard cellular and molecular biology techniques No ethical concerns Capture human individuality	Oversimplified systems Static condition (except when incorporating mechanical stimuli) Lack cell-to-cell contact (except direct co-cultures) Do not allow development of tissue-like constructs Lack of translational power Donor-to-donor variability
3D models	Incorporate human cells More complex system incorporating multi-cellular environment Incorporate the 3D architecture Increased representability of tissues Reduction in animal testing Capture human individuality	Medium/Low throughput Expensive Require trained personnel Loss of proportional heterogeneity from the isolated cell population during expansion Donor-to-donor variability

tendinopathies poses another challenge to study the early pathogenesis of these conditions. Apart from these limitations, the use of animal models also introduces numerous ethical considerations.

The urgent need to understand the mechanisms of human tendinopathy and tendon healing required to develop more effective treatments while addressing the low representability of animal models of human physiology emphasizes the need for human-relevant *in vitro* models of these tissues. Tendon models should be able to mimic the 3D architecture of the native tissue, the biophysical cues derived from its biomechanical function, and more importantly, the biological signaling stemming from the crosstalk between the different cellular and extracellular matrix (ECM) players involved in tendon healing. Actually, cell-to-cell crosstalk has been shown to be key to understand tissue homeostasis and repair after tendon injury [19,20].

This review starts by recapitulating the current knowledge on tendon structure and composition, the cellular players and their roles, and its known physiological and healing mechanisms to understand the needs required to design human-relevant *in vitro* models recapitulating specific microenvironments of tissue physiology or pathophysiology. Next, the main focus of the review is directed towards the critical analysis of existing 2D and bioengineered 3D models that have been used to study tendon cell crosstalk, tendinopathy and respective microenvironments. Moreover, their advantages and main limitations are highlighted as well as how current models could be upgraded to better mimic tendon health and disease microenvironments. Finally, we overview prospective emerging bioengineering technologies that could be used for the development of a next-generation of tendon microphysiological models that include all relevant biophysical and biological cues of tendon tissues microenvironments and allow to study tendon health and disease as well as to develop new therapeutics for tendon.

2. Tendon structure and composition

Tendons are dense connective tissues that connect muscles to bones to transmit forces that enable movement. Tendon tissues have different mechanical properties depending on the function they perform. They can be stiff to hold a joint together, which is the case of positional tendons, or more elastic to facilitate motion, in case of energy-storing tendons [21-23]. Both tendon types are almost exclusively composed by ECM produced by fibroblast-like stromal cells called tenocytes. Tendon tissues are endowed with a highly hierarchical architecture mainly composed by type I collagen (~60–85% dry weight) self-arranged in fibrous structures with dimensions from the nano to the microscale (Fig. 1) [24]. Starting at the nanoscale, type I collagen molecules with diameters of 1 nm form helicoidal tropocollagens that aggregate to form a microfibril [25,26]. Multiple microfibrils form fibrils ranging between 20 and 150 nm. Entering into the microscale, fibrils join to form fibers with diameters of 1–20 μm, which further bundle together into fascicles of 150–1000 μm. The collection of fascicles packed together are separated by the endotenon or interfascicular matrix (IFM), a fine connective tissue containing blood vessels, lymphatics, and nerves; and enclosed by the epitenon, a connective tissue sheath connecting the vascular, lymphatic, and nervous systems to the tendon [27]. Altogether, they represent the macroscopic tendon tissue, where resident tenocytes located between fascicles are the so-called functional unit of tendon (Fig. 1). This highly organized hierarchical architecture made of crosslinked type I collagen fibers is responsible for the tensile strength of tendon and the efficient energy transfer between muscle and bone during locomotion [28].

The ECM of tendon, although mainly composed by type I collagen in the fascicular matrix (FM), contains other collagens in lower proportions including the fibrillar collagens type III, V, and XI,

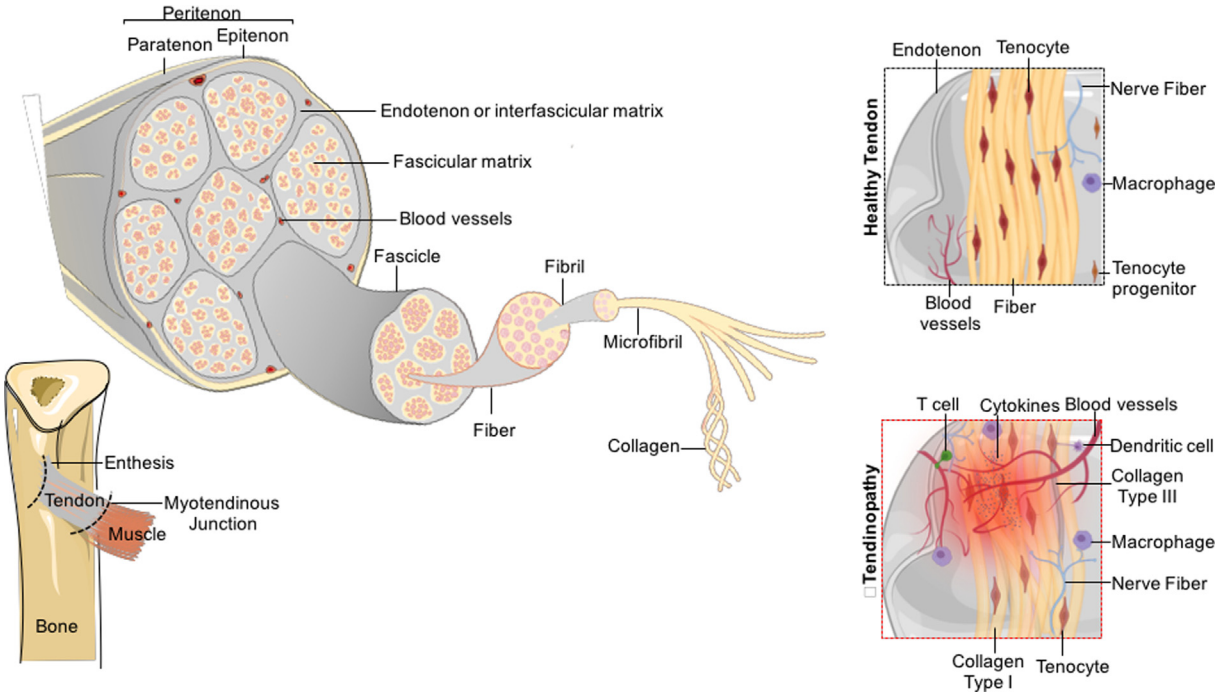

Fig. 1. Hierarchical anisotropic structure of tendon from the nano (collagen molecules) to the macroscale (tissue). Healthy tendons suffer cellular and molecular alterations during tendinopathy.

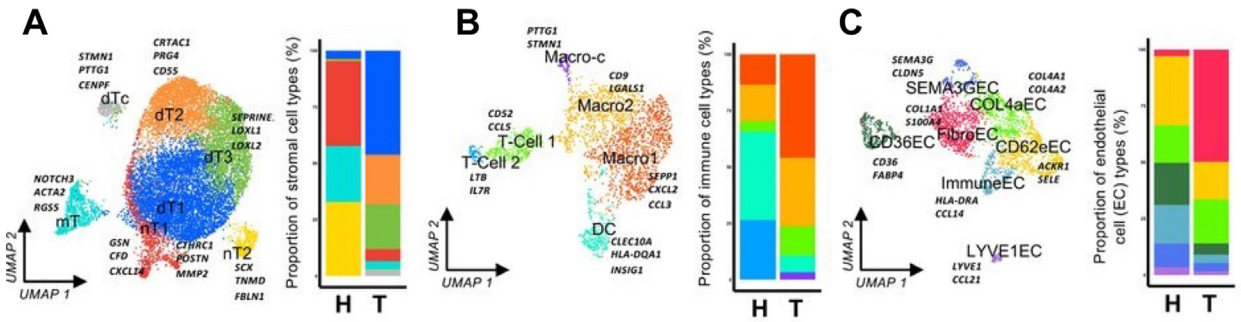

Fig. 2. Tendon cell clusters found through single cell RNA sequencing and spatial transcriptomics in healthy (H) and tendinopathy (T) conditions. Uniform manifold approximation and projection (UMAP) embedding of (A) stromal cells, (B) immune cells, and (C) endothelial cells of tendon. . Adapted from [42]

being these last two types regulators of collagen fibril formation [17]. The non-collagenous components of tendon create an hydrated space in the dense collagen matrix for nutrient flow and innervation [17]. They are composed by small leucine-rich proteoglycans (SLRPs), mostly by decorin (~80%), biglycan, fibromodulin, and lumican, but also contain larger proteoglycans such as versican and aggrecan (ACAN) [17,29]. Similarly, some elastin and glycoproteins including fibronectin, laminins, lubricin, tenascin-C (TNC) and proteins of the thrombospondin family are present to a lesser extent [17,29]. This complexity is responsible for specialized regions with distinct mechanical properties. For example, high levels of ACAN increase water uptake and thus the ability of tendons to withstand compression, in contrast to tensile-resistant regions that have rather scattered non-collagenous proteins [29]. Moreover, the efficiency of the energy storage and release during load cycles to allow movement has been associated to the presence of lubricin and elastin in the IFM, which facilitate the sliding behavior of the fascicles [30,31]. Elastin, as it is highly compliant with a resilient behavior, is responsible of the viscoelasticity, fatigue resistance, and recoverability of the IFM [32]. These characteristics appear critical for maintaining tendon integrity during locomotion. As a result, although intermolecular crosslinking between collagen fibrils are mainly responsible of the strength of tendon [28], the non-collagenous elastic components within its structure complement its mechanical properties and enhance its functionality [22,31]. Beyond its critical role on the biomechanical and structural functions of tendon, the ECM has many roles on biological signaling. For example, cells are able to sense the mechanical properties and the spatial organization of the fibrillar microenvironment (ECM topology) via mechanotransduction pathways [33]. Another example is the ability of negatively charged ECM components, including some proteoglycans present in tendon, to bind and retain soluble signaling molecules like growth factors, dynamically regulating their release, activation, and presentation to cell surface receptors [34].

2.1. Tendon cell populations and phenotypes

The maintenance of tendon functionality relies on the synthesis and remodeling of its ECM components, an action mainly attributed to resident tenocytes [35]. Tenocytes are fibroblast-like mature tendon cells residing between collagen fibers showing an elongated morphology, low nucleus to cytoplasm ratio, and low metabolic activity (Fig. 1) [36,37]. In contrast, the IFM hosts a highly active and abundant (10-fold greater) rounder-shaped cell population that is responsible for the release of proteins needed to repair damage with fast turnover (Fig. 1) [38]. It has been suggested that cells in the IFM are an heterogeneous population of fibroblasts, progenitor, and vascular cells [39] that also have significant roles in tissue repair [40].

The identity and diversity of the tendon cells population seem however to be richer than it was previously thought. Human tendon tissue has been shown to contain multiple specialized cell subtypes [41,42]. At least five different groups of tenocytes expressing the type I collagen genes (*COL1A1/2*) were identified through single-cell transcriptomics and proteomics (Fig. 2) [41]. These tenocyte subgroups included two scleraxis-expressing (*SCX*) clusters co-expressing microfibril related genes like fibrilin-1, versican, elastin microfibril interfacer 1, and decorin. Interestingly, one of these clusters also exhibits a marked pro-inflammatory profile, expressing C-X-C motif chemokine ligand (*CXCL*)-1, 6 and 8. The other populations include a group expressing genes associated with fibro–adipogenic progenitors (FAPs), a chondrogenic group expressing tubulin polymerization-promoting protein 3 and proteoglycan 4 (*TPPP3/PRG4*), and integrin alpha 7-expressing smooth muscle-mesenchymal cells. Moreover, the expression of these markers and the ratio between the different cell groups seems to be dynamic, varying in healthy and diseased human tendon [41,42] (Fig. 2), with diseased tenocytes co-expressing microfibril associated genes and genes for ECM proteins (e.g., collagens type I and III, fibronectin, byglycan) [41], which is considered the hallmark feature of tendinopathy. In addition to tenocytes, endothelial and immune cells were identified in healthy and diseased tendon tissue but with distinct quantitative and qualitative characteristics [41,42]. Compared to normal tendon, in tendinopathy, endothelial cells (CD31 +) showed an upregulation of C-C motif chemokine ligand (CCL)-21, which is known to trigger the activation of dendritic cells and subsequently of T cells [42]. Additionally, in diseased tendon, macrophages numbers are higher and show an upregulated expression of *CD74,* which activation (through cytokines released by tenocytes) can induce proinflammatory effects [42].

Tendon tissue is also characterized by the presence of small numbers of different subpopulations of tendon stem/progenitor cells (TSPCs) within specialized niches composed by biglycan and fibromodulin [43], but also within other niches, whose proposed role and function have been reviewed in detail [44,45]. Among these, a subpopulation expressing nestin (a type IV intermediate filament), which showed multilineage differentiation capacity and high expression of tendon-related genes and tenogenic potential, has been considered the teno-lineage stem cells [46]. Moreover, recent studies have revealed the presence of a tendon stem cells population expressing *TPPP3* and platelet-derived growth factor receptor alpha (*PDGFRA*), which are able to generate new tenocytes and self-renew throughout healing [47]. Interestingly, this population was found to reside within the tendon stem cell niche together with FAP cells that originate fibrotic cells [47]. The existence of stem cells differentiating into fibrotic cells in tendon provides a possible intrinsic mechanism for the persistent fibrotic scarring observed after tendon injuries [47]. Moreover, tendon

stem cells differentiation and alteration of ECM composition during tendinopathy has been shown to be dysregulated by CTRP3, a protein belonging to the tumor necrosis factor superfamily, that is secreted by chondrogenic lineage cells [48].

Although not currently consensually established, the identification of tendon cells phenotype has so far relied on the expression profile of common markers among resident populations. In addition to the most abundant ECM proteins in the IFM such as type I and III collagen or SLRPs like decorin, a series of genes related to tenogenic differentiation of stem cells have been categorized as markers of the tendon phenotype [49]. These include, for example, the glycoprotein tenomodulin (TNMD), a member of the type II transmembrane protein family, essential to modulate tendon development and maturation of collagen fibrils [25]. Its C-terminal cysteine-rich extracellular domain acts as a strong regulator of cell proliferation and differentiation [50,51]. TNMD-deficient mice showed a decreased tenocyte density in newborn tendons with an altered structure of collagen fibrils caused by a hindered accessibility of the type III and VI collagen epitopes [50]. Another commonly tendon-associated marker is the extracellular protein TNC, which contributes to the flexibility of the tissue and has helped to uncover early events in the formation of proximal tendons [52]. TNC is expressed by tenocytes in response to mechanical stress [25,53]. However, this marker is also expressed in other cell types, like glia and chondrocytes, among others [52]. The transcription factor member of the three amino acid loop extension homebox genes mohwak (MKX) has also been used as a tenogenic indicator because of its role in tendon maturation and collagen network development [54]. Indeed, MKX-knockout mice have been shown to develop smaller hypoplastic tendons with low type I collagen in their Achilles tendon [54]. Among all, the most used tendon marker is SCX, a basic helix-loop-helix transcription factor involved in the control of tendon formation and attachment of muscle to bone [36,52]. Its role in tendon morphogenesis has been associated to the activation of the transforming growth factor beta (TGF-β) pathway, a strong inducer of SCX expression [36]. Yet still viable, SCX-null mice present serious tendon defects and complete loss of force-transmitting and intermuscular tendons [55]. In addition, SCX has been proven crucial for adult tendon progenitor cell lineage differentiation into tenocytes during tendon repair [56]. Unlike TNMD, which is expressed by tenocytes to mature the tendon ECM, SCX has been reported to be continuously expressed by these cells throughout all stages of tendon development [52].

Even though SCX has been widely accepted as a tendon marker, this generalization is still arguable since SCX knockout mice showed abnormal musculoskeletal system tissues development, including muscle, bone, meniscus, and cartilage [57]. Moreover, SCX was critical for musculoskeletal stem cell self-renewal and proliferation potential [57]. The belief surrounding the role played by SCX in tendon healing, post-natal growth and homeostasis has been recently disputed after observing that depleting mice of the SCX + cell lineage was not detrimental for tendon healing but improved the biomechanical properties of the tissue [58]. Moreover, depletion of SCX + cell lineage did not affect collagen bridging during healing but rather it appeared favorable for matrix remodeling, a mechanism driven by tendon cells that differentiated into myofibroblasts expressing the intracellular contractile protein alpha smooth muscle actin [58]. The absence of SCX + cell lineage did not affect tendon growth or homeostasis three months post-repair [58], questioning the actual role of this gene after tendon formation. Some progresses have been made on establishing the link between SCX expression and its role on tendon cells mechanotransduction mechanisms, where it has been shown to facilitate mature tenocyte mechanosensing by regulating the expression of several mechanosensitive focal adhesion proteins [59]. However, the lack of tissue-specific markers with well understood mechanisms for tendon physiology is still a matter of debate. Further research on tendon cell populations and their phenotypes holds promise for the discovery of specific tenogenic markers, as well as for its related disorders.

3. Mechanisms of tissue repair and tendinopathy

Upon minor injuries, such as fascicle microdamages induced by overload, tendon might recover homeostasis, leading to a relatively functional tissue and without pain. Indeed, when a tensile load is applied to explanted tendon tissues, either by fiber stretching or by fiber-to-fiber sliding, the tendon can still follow an elastic deformation [60,61]. However, when strain passes the deflection point, the tissue elastic behavior is compromised [61]. Collagen fibers compensate the loss in load-transfer mechanisms by molecular sliding and extension, creating shear forces that gradually disrupt collagen and eventually lead to fascicle rupture [60]. Tissue damage is accumulated until reaching a "tipping point", when homeostatic mechanisms fail due to a lack of available nutrients for the damaged matrix remodeling [23]. In fact, repeated microstrain below the failure threshold is considered the origin of most overuse tendinopathies [15].

The traditional dogma undermining the role of inflammation in tendon healing has changed over the years as the research on the role of immune cells and inflammatory mediators in tendinopathies advances. Nowadays, it is accepted that molecular inflammation plays a critical role in tendon pathophysiology [62]. As inflammation is considered one hallmark of tendinopathies, understanding its mechanism and main players could lead to significant advances in the development of therapeutic alternatives. Tendon follows a wound healing process similarly to other tissues, in which the inflammatory activity begins at the molecular level when a disruptive external cue induces the immune system to trigger inflammation as a first-aid response to restore tissue homeostasis (Fig. 3) [63,64]. The process starts with an early inflammatory phase, in which resident macrophages recognize damage-associated molecular patterns (DAMPs) and release pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 [65,66]. The formation of a chemokine gradient leads to the recruitment and infiltration of neutrophils and macrophages into injury site [65]. These cells phagocyte necrotic material and ECM remnants [67]. The degradation of damaged ECM is further promoted through matrix metalloproteinases (MMPs) release. The process proceeds with the proliferative phase, where extrinsic cells from the peritendinous soft tissue as well as intrinsic cells in the epitenon and endotenon migrate to the injury site and become highly proliferative [67]. Within this phase, blood vessels and lymphocytes also invade the injury site, accompanied by a high expression of vascular endothelial growth factor (VEGF) and other growth factors [68]. Altogether, they form an immature granular tissue mainly composed of randomly oriented fibers rich in type III collagen (Figs. 1 and 3) [67]. TSPCs originally from the endotenon become the key players in tendon healing by simultaneously resorbing and producing this type III collagen [69]. In the final remodeling phase, physiologic loads are reinstituted contributing to the longitudinal orientation of fibers and the substitution of type III collagen by more mechanically compliant type I collagen [67]. The inflammatory process is highly regulated to maintain tissue integrity during tendon healing, which resolution is closely driven by resolution-promoting macrophages [70]. However, it has been hypothesized that following cumulative microtrauma, the fundamental process of debris removal and matrix repair initiated by tenocytes, leads to positive amplification of the immune response. Imbalanced and activated immune cells,

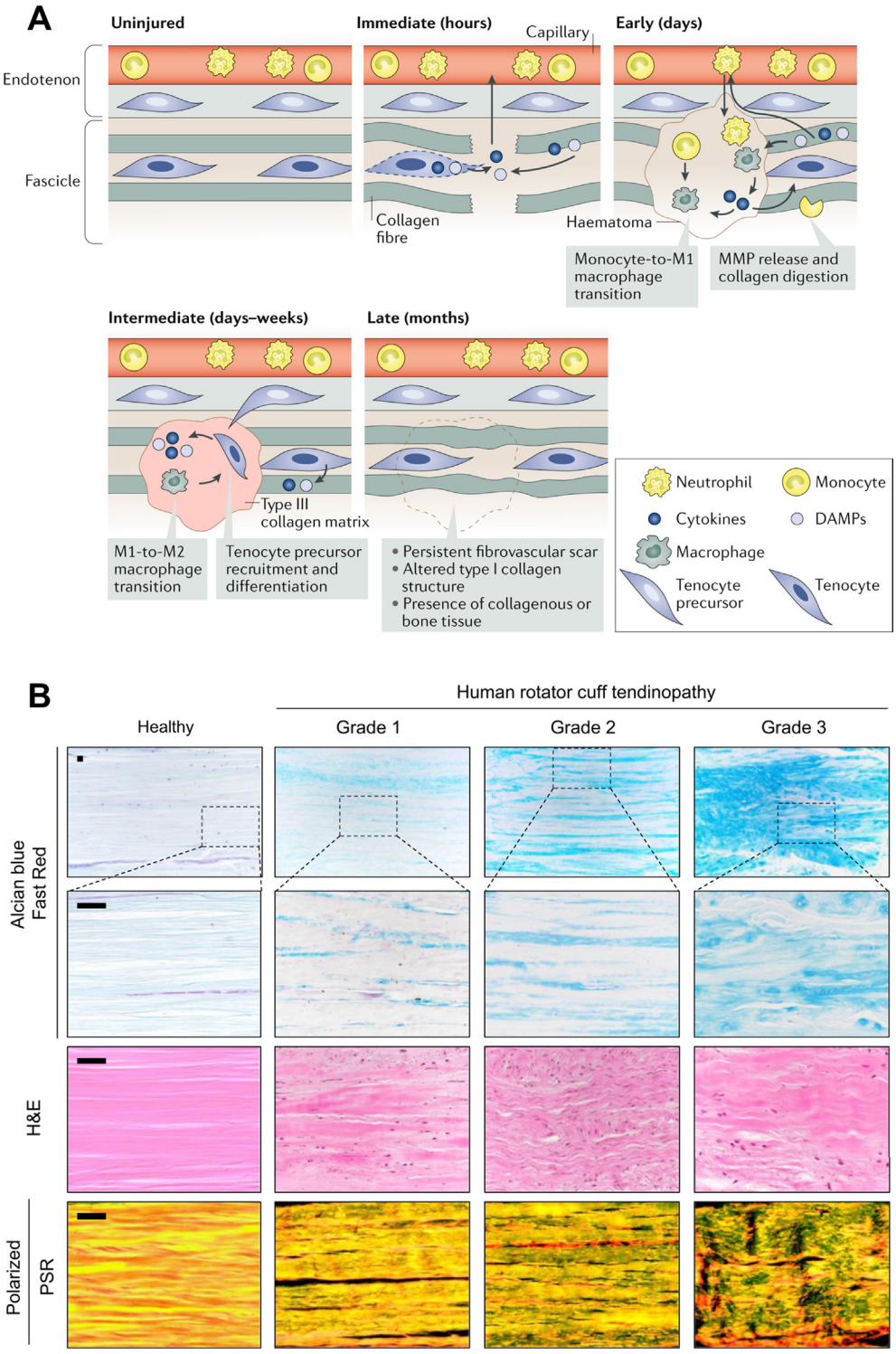

Fig. 3. (A) Cellular and molecular mechanisms of tendon tissue repair. After rupture or microdamage, tenocytes die, causing the release of cytokines and DAMPs, which activate adjacent stromal cells and tissue-resident immune cells, triggering the tissue repair mechanisms. The early or inflammatory phase is characterized by the activation of M1 macrophages and the phagocytosis of apoptotic cells and ECM debris (helped by MMPs-mediated collagen digestion). In the intermediary or proliferative phase, pro-resolving (M2) macrophages are increased while tenocyte precursors are recruited to the lesion to secrete a temporary type III collagen matrix. In the late or remodeling phase, this matrix is gradually substituted by type I collagen. The recovered tissues in adults never regain its re-injury architecture and show scar-like appearance, which can lead to tendinopathies. Reproduced from [85]. (B) Histological and immunohistochemical staining of human healthy and tendinopathic tendons. Alcian blue/Fast Red, hematoxylin and eosin (H&E), and Picrosirius red (PSR) staining (scale bars, 25 μm). Images for PSR staining were acquired using polarized light microscopy. Reproduced from [48].

primed by both endothelial cells and tenocytes, promote a cycle of inflammation and aberrant tissue repair rather than regeneration that drives disease chronicity [42].

Due to this failed regenerative process, the second hallmark of tendinopathies is fibrosis, which is characterized by the exacerbated accumulation of ECM in a highly disorganized matter that

shows high vascularization (Figs. 1 and 2) [71]. Although fibrosis is a regular step in wound healing, the deficient intrinsic regenerative capacity of tendon is responsible for the rather permanent scar tissue formation after injury [72,73]. Scar formation in between microruptured or tendon ends provides a physical bridge to allow its function [74]. However, excessive fibrotic tissue creating adhesions between tendon to its adjacent tissues and disrupting its innate architecture hinders its mobility and thus its ability to transmit forces from the muscle to the bone [74]. Scar formation occurs following the remodeling phase of tendon healing, where the remodeled tendon tissue remains hypercellular with a larger ratio of type III to type I collagen [67]. Type III collagen fibrils are thinner with lower crosslinking potential, which results in a remodeled tendon with lower biomechanical properties and increased risk of re-rupture [67]. Even after this period, healed tendons never restore its microstructure and composition, being reported that in normal situations only about 70% of its original mechanical properties are recovered [62,66]. Several studies have focused on the cellular and molecular mechanisms of tendon fibrosis [75]. At the cellular level, aberrant macrophages and myofibroblasts (αSMA + cells) activity is a main driver of fibrosis in a variety of tissues, including tendon [75,76]. Works by Loiselle and colleagues have demonstrated that the small calcium binding protein S100a4, which prompts the expression of αSMA in resident cells increasing the degree of fibrosis in lung [77], is expressed by some tendon cells within the bridging scar tissue during tendon healing [78,79]. At molecular level, TGF-β1 signaling is a major driver of fibrosis in many tissues [80]. In tendon, inhibition of TGF-β1 during the inflammatory phase has been shown to decrease the formation of peritendinous adhesions following flexor tendon injury and repair [81]; nonetheless, TGF-β1 is also critically involved in promoting cell proliferation and collagen deposition during the fibroblastic and/or proliferative and remodeling phases needed to achieve tissue homeostasis [75]. Additionally, activation of the platelet-derived growth factor (PDGF) signaling pathway upon injury seems to direct FAP cells coexisting in the tendon niche to further elicit fibrosis and tendon stem cell differentiation into new tenocytes [47]. Another study has pointed out the role of activin A in the overexpression of pro-fibrotic genes in fibroblasts, including periostin and asporin (proteins involved in collagen binding) [82].

Besides the inflammatory and fibrotic processes that occur during tendon repair, the abnormal differentiation of tendon stem cells, leading to chondrogenic degeneration or even heterotopic ossification, is commonly observed in the clinics [83,84]. A greater understanding on the molecular processes involved in tendon repair through representative models of tendon injury and fibrosis holds promise for the development of more effective therapeutic solutions and targets.

4. Cellular crosstalk in tendon microenvironments

4.1. Inflammation in tendon

During the healing process, tendon resident cells interact with a wide variety of cell populations. As inflammation is highly orchestrated by macrophage activity [86], the crosstalk between immune cells and tenocytes has become a major target to understand tendon pathophysiology. Tendon tissue, characterized by its hypocellularity, has few resident macrophages throughout the epitenon and inner fibers [62]. The increased infiltration of these cells after injury, along with neutrophils to kill infectious agents, makes them key players in tendon repair mechanisms [87]. Tissue-resident macrophages show different phenotypes with a spectrum of functionalities, including in tendon [62,88,89]. Typically, macrophages have been classified in M1 or classically-activated pro-inflammatory phenotype responsible for ECM breakdown, inflammation, and apoptosis, or in M2 or alternatively-activated anti-inflammatory phenotype in charge of promoting ECM deposition and tissue repair, although these two are extremes of overlapping functional states [66]. The identification of macrophages after tendon injury has been performed through widely accepted surface markers, such as the monocyte marker CD68, or the polarization-associated markers CD80/CD86 and CD163/CD206 for the M1 and M2-like phenotypes, respectively [66]. Moreover, the signaling pathways associated to the activation of the M1 or M2 phenotypes include the pro-inflammatory pathways of interferon (IFN) and nuclear factor kB (NF-kB), pro-fibrotic pathways with the signal transducer and activator of transcription 6, and inflammation resolving pathways involving glucocorticoid receptor activation [90]. The transition from the M1 to the M2 phenotype throughout the healing process prevents chronic inflammation and the severe consequences related to uncontrolled recruitment of immune cells. The collaborative character of macrophages within the resolution of inflammation has promoted the study of their crosstalk with other cell types in the tendon niche.

The mechanisms driving the tendon healing are yet to be fully elucidated, particularly with respect of the key players driving and resolving inflammation. It has been suggested that resident tenocytes have the capacity to trigger an inflammatory response, potentially through the recognition of DAMPs [91]. Recently, a novel immune cell population able to express the fractalkine receptor (CX3CR1) and its cognate C-X3-C ligand 1 (CX3CL1) has been identified within the tendon core [92]. Both chemokines are responsible for monocytes recruitment and are mainly expressed by myeloid and lymphoid lineage cells, including mast cells and natural killer (NK) cells [93]. These CX3CL1 and CX3CR1-expressing cells referred to as "tenophages" were identified after the inhibition of the fractalkine receptor in healthy human tendon, demonstrating the presence of immune-like tendon cells within its rich collagen-matrix [92].

Another potential player in the modulation of tendon healing are mast cells. Unlike macrophages, mature mast cells do not normally circulate but rather stay distributed throughout exposed tissues such as the skin or airways [94]. Their strategic location allows them to be one of the first responders to pathogens and external stimuli [94]. Nevertheless, they have been found within and surrounding the tendon tissue, which suggests their potential involvement in modulating tendon healing [95]. Furthermore, an increased number of mast cells accompanied with myofibroblasts and neuropeptide-containing nerve fibers throughout healing stages was found in an animal model of tendon injury [96]. Mast cells are specialized to amplify or suppress immune responses since they can store, produce, and release inflammatory regulators [94]. The phenotype shift of mast cells is influenced by microenvironmental cues and their proteinase content [94,97]. Given that mast cells release their granules upon activation, the study of their crosstalk with tendon-derived cell lines could help to shed light on their role within the molecular mechanisms of inflammation. Indeed, the concept of immune cells coordinating inflammation throughout the healing process has gained acceptance over the last years as their molecular and biological mechanisms are further researched [87]. The presence of T and B lymphocytes and NK cells has been demonstrated in human Achilles tendinopathies [96] and T cells have also been identified to reside preferentially at the interface with bone [98]. Recent data has also shown that T-cells are present in greater proportion in diseased than in normal tendon, reflecting its potential role in a sustained chronic inflammatory response [42]. Yet, there are many unknowns regarding the involvement of other immune cell types on the modulation of tendon repair. Further knowledge in this field would certainly con-

tribute for the development of new immunoregenerative tendon therapies.

4.2. Neovascularization in tendon disease

A hypervascular tendon ECM is characteristic of diseased tendon microenvironments, in comparison to the poorly vascularized healthy tendons [99]. This neovascularization and the subsequent increase in oxygen and nutrients supply have been recently shown as a trigger of degenerative ECM remodeling within the tissue stroma [100]. Nevertheless, newly formed blood vessels harbor higher numbers of perivascular cells, which hold a population of TSPCs able to mediate tendon injury healing [101]. Therefore, although neovascularization is an indispensable process during the regeneration of the majority of tissues, including tendon, it potentially has a negative impact on functional tendon repair [68,99].

After a tendon injury, the negligible levels of VEGF found in healthy tendon show a significant increase [102]. VEGF signaling is critical for blood and lymph vessel formation, although it can also elicit responses in other cells [102]. Among these, tendon cells express VEGF receptors in response to inflammatory stimulation and injury, which binding with its ligand promotes tendon degeneration-associated events [103]. Moreover, the activation of macrophages after injury induces an up-regulation of VEGF production, which positively feedbacks this mechanism [104]. In vitro, cyclic mechanical load also induced the expression of VEGF and hypoxia inducible factor 1 in a frequency dependent fashion in tenocytes, pointing to a mechanism involved in cellular response to overload [105]. Interestingly, the use of Bevacizumab, a humanized antibody attenuating VEGF-A signaling, reduced vessel neoformation after injury and positively promoted tendon healing *in vivo* [106].

In healthy conditions, TNMD expressed by tendon-resident cells exhibits antiangiogenic properties when expressed in a secreted form, indicating a crucial role in maintaining an antiangiogenic state in tendon tissue [107]. Further, TNMD has recently been demonstrated to limit the formation of a fibrovascular scar during early events in tendon healing [108].

4.3. The complex tendon interfaces

The tendon-to-bone interface, also referred as the enthesis, is the physical continuous transition between the viscoelastic tendon to the rigid osseous tissue. It presents a complex composition gradient with distinct tissue architectures populated by heterogenous cell types [109]. On one side, the enthesis bridges the highly aligned type I collagen structure of tendon to the mineralized and more isotropic bone tissue. Bone is essential for locomotion, organ support and protection, and contains the bone marrow for blood cells production [109]. Bone undergoes a dynamic remodeling process that resorbs and forms bone in a cyclic manner. The process is the result of the coordinated activity of bone cells (mainly osteoblasts and osteoclasts) that carry out distinctive yet complementary activities. Through this cycle, a highly mineralized and vascularized matrix is formed with embedded osteocytes, the most abundant cells in bone (~95%), whose functions are highly regulated by mechanical stimuli [109]. The zonal gradient in tendon-to-bone interface is characterized by a fibrocartilaginous region that gradually changes its composition and structure between both tissues. Starting at the tendon end, the structure is similarly formed by aligned type I collagen fibers and DCN with elongated tendon resident cells between fibers [109]. This region transitions into a non-mineralized fibrocartilage with a larger amount of type II and III collagen and less type I, IX, and X collagen, decorin, and ACAN [109]. Tendon cells are substituted by fibrochondrocytes that become hypertrophic within the next region. The mineralized fibrocartilage carries hydroxyapatite in the matrix composed by type I, II, and X collagen and ACAN [109-111]. At the bone end, the bone-resident cells populate a randomly organized matrix rich in type I collagen with high concentrations of carbonated apatite minerals [109-111]. Although type I collagen is found along all enthesis regions, its organization differs from one to another thus changing the tissue mechanical properties. The enthesis requires these specialized gradient transitions for effective energy transfer during locomotion.

The muscle-to-tendon interface is an area of overlap between muscle and tendon known as the myotendinous junction (MTJ). The skeletal muscle is a highly organized tissue with muscles fibers being surrounded by connective tissue and grouped together to form fascicles [112]. Each fascicle is further covered by connective tissue and bundled together until forming the entire muscle [112]. The cylindrical myofibrils located within each fiber are divided longitudinally into repeating segments called sarcomeres, which constitute the basic unit of muscle contraction [112]. Unlike tendon, the high contractile activity and cellularity of muscle requires extensive vascularization to meet its metabolic demands [112]. Muscle fibers at the MTJ form finger-like projections that contain an aligned network of actin microfilaments [112]. Focal adhesion complexes bind the actin matrix to transmembrane proteins anchoring the muscle cytoskeletal proteins to the dense tendon ECM [112]. These complexes are critical for MTJ function since they secure muscle fibers to tendon fibrils [112].

Loads applied to the muscle–tendon–bone unit during active contraction create uniform tension across tissues that hold different stiffness and cross-sectional areas [112]. Muscle is the most compliant but the thinnest at the tendon interface, where it experiences the greatest strain and likelihood of failure [112]. Conservative therapies following injuries tend to fail leaving surgical treatments as the remaining solution [109]. Suture repairs allow for the reattachment of the tissues but significant fibrosis compromises their mechanical properties and leads to structural and mechanical failure with rates between 20 and 94% at the surgical site [109,112]. Alternative treatments are needed to address the poor regeneration capability of tendon after injury. At the enthesis, it has been shown that the crosstalk between tendon fibroblasts and bone-derived cells influence fibrocartilage formation after graft transplantation by initiating transdifferentiation [113]. Yet, the unique transitional gradient of these interfaces is never recreated during the natural healing process [109].

In order to better understand the nature of these multiple cellular interactions and their mechanisms during healing, various *in vitro* models have been developed, ranging from simple two-dimensional (2D) co-cultures to more sophisticated three-dimensional (3D) multicellular systems, which are discussed in subsequent sections.

5. Tendon models of cellular crosstalk

In vitro models are key experimental tools not only to study the cellular crosstalk and molecular pathways of health and disease but also in the pipelines of drug discovery. In this section, we review different *in vitro* models that have been used to model tendon microenvironments for the study of cellular crosstalk. We start by identifying the typical cell sources used for tendon modeling and review how common 2D models are applied to combine different cell types for studying their biological communication. More than exhaustively reviewing all published literature on these models, our aim was to identify the main advantages and limitations of the different variations of these type of assays in the context of tendon modeling. Next, we review how different biophysical and bio-

logical cues can be added to 2D models in order to improve their potential to mimic the tendon microenvironment. Finally, we focus on how the latest bioengineering tools have been or can be further leveraged to fabricate advanced multicellular models with improved predictive power for their *in vitro–in vivo* extrapolations. Although tendon explant-based systems have gained relevance as alternative physiologically-relevant models for the study of mechanisms associated with tendon homeostasis and pathophysiology [100,114], its main advantages and limitations have been recently reviewed with great detail by other excellent reviews [17], and therefore they are not discuss here.

5.1. Cell sources for health and disease modeling

A relevant aspect of building tendon *in vitro* models is the selection of available cell sources that can represent intrinsic and extrinsic tendon cell populations (Table 2). Tenocytes constitute the most obvious representative cell type to build models of tendon core. However, obtaining unmixed populations of these cells is challenging since the typical tissue digestion (collagenase-based) or explant outgrowth protocols used for their isolation render heterogeneous populations of spindle-shaped adherent cells from the FM and IFM [38]. Actually, different authors have defined these cells with different names, including tendon-derived cells (TDCs) [115,116], tenocytes [117,118], tendon fibroblasts [119] or even TSPCs (after verification of surface markers) [43,120,121]. Moreover, sorting and expansion of TDCs (or other proposed names) is rather difficult since, as explained before, unique tendon cells markers have not been stablished yet, they usually have a limited life span, low expansion rate and tend to suffer fast phenotypic drifts in typical 2D culture after just a few passages [122]. Considering the heterogeneity of cell populations identified in tendon [42], it would thus be important to better define the naming for the cells that are obtained by quite similar isolation methods. It should also not be ignored that the selected isolation method itself has an impact on the biological behavior and phenotype of obtained cells [123]. These factors, together with the low availability of human tendon samples (normally obtained from individuals with advanced pathology) and the low number of cells resident in the tissue, make human TDCs a less than optimal cell source to build *in vitro* models. An alternative is the use of mesenchymal stem cells (MSCs) of diverse origins (e.g. bone marrow, adipose tissue), which can be stimulated to differentiate into the tenogenic lineage using multiple biophysical and/or biochemical strategies [124,125]. However, widely accepted protocols for the *in vitro* tenogenic differentiation of MSCs have not been completely established so far. Interestingly, tenocytes have been recently derived from human induced pluripotent stem cells (iPSC) by recapitulating the embryonal development [126,127]. Because iPSC incorporate patient-specific genetic and epigenetic signatures [128], they might become a quite valuable tool in the future of tendon modeling, particularly if considering the possibility of developing personalized therapies.

Cells from the immune (resident and circulating) and vascular systems play an important role in pathophysiological response to tendon injuries (as expanded in section 4). Consequently, these type of cells should be incorporated in *in vitro* models, especially when attempting to model tendon disease. Again, the most representative option would be to use cells isolated from whole blood. Using this approach, primary monocytes and lymphocytes can be obtained and activated into macrophages [129,130] and T-cells [131], respectively. In alternative to these time-consuming protocols, many laboratories use cell lines due to their availability and easier handling. Among these, human monocyte THP-1 cell line or Jurkat cell line might be used to study multiple events related to the immune response, including macrophages polarization

Table 2
Main cell populations in tendon and common cell types used to model tendon.

Cell Population	Characteristics	Location	Function	Model cells	Ref.
Tenocytes	Spindle-shape, elongated, fibroblast-like with at least five subpopulations expressing COL1A1/2	Anchored around collagen fibers within the FM	Tendon ECM remodeling, maintenance of tissue homeostasis, and energy production	Cells derived from tissue digestion or explant outgrowth (human or animal), mesenchymal stem cells of different origins	[25,36,37,41,64]
Tendon Stem/ Progenitor Cells	Highly proliferative. Express the widely accepted tendon markers (e.g. SCX). Include different subpopulations (e.g. expressing TPPP3 and PDGFRA)	Specialized stem cells niches throughout tendon. Proposed locations in endotenon, epitenon, and perivascular region	Generate new tendon cells through self-renewal. Secrete paracrine factors for immunomodulatory effects		[41,42,49,101]
IFM Cells	Round-shape, higher metabolic activity than tenocytes, including a population CD146+	Within the IFM non-collagenous elastic region	Attributed to fast matrix turnover during tendon healing		[38,40,140]
Immune Cells	Resident dendritic cells, macrophages and lymphocytes	Throughout tendon	Activation of the inflammatory response upon recognition of DAMPs	Primary monocytes and lymphocytes, immune cell lines (e.g. THP-1 and Jurkat)	[41,65,66]
Endothelial Cells	CD31 + and CD45-	Mainly within IFM	Specialized blood or lymphatic vascular functions	Endothelial cells (e.g. HUVECs), adipose-tissue stem cells (endothelially differentiated)	[42,141]

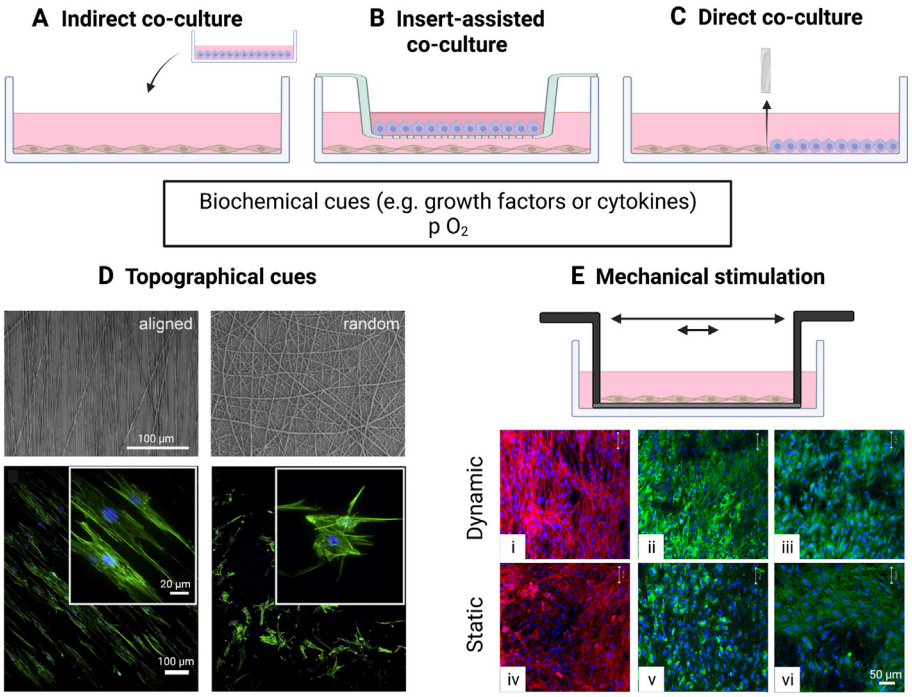

Fig. 4. Tendon 2D models to study cellular crosstalk. (A) Indirect and (B) insert-assisted co-culture are mainly used to study cellular crosstalk through soluble factors (paracrine signaling) [142,148]. While in indirect co-culture the effect of conditioned medium from one cell culture in a second separate cell culture is studied, using insert-assisted cultures a permeable membrane in the form of a well-plate insert separates the cell cultures, achieving a more realistic cellular interaction since released factors from both cultures get diffused. These models possess the advantages of being simple and able to recreate cell dialogue but lack cell-to-cell contact. On the other hand, using (C) direct co-culture models (with or without gradients created using a removable barrier) both cell types are cultured together, which allows cell-to-cell interactions in addition to paracrine signaling. However, multiple culturing parameters (e.g. cell densities, media) need to be optimized. Moreover, the incorporation of (D) topographical cues (SEM images, scale bar 100 µm; fluorescence images, scale bar 100 µm with 20 µm inserts, adapted from [149]) or (E) mechanical stimulation (arrow in fluorescence image indicates the loading direction, adapted from [150]) in 2D models has been shown to influence cells behavior. Moreover, the supplementation of the culture media with different factors (e.g. inflammatory mediators) or modulating the oxygen partial pressure (p O2) can be applied to mimic tendinopathy.

[119] and T cell activation [132], respectively. However, these cell lines derive from leukemia patients, decreasing their representability in the onset of tendon diseases. On the other hand, the vascular system might be represented by the incorporation of endothelial cells, such as human umbilical vein endothelial cells [107,133,134]. In alternative, endothelially differentiated adipose tissue-derived stem cells (ASCs) have shown the capacity to form endothelial structures [135-137].

Due to the low availability of human tendon samples, cells obtained from animal tissues constitute a common and more available alternative to study organ, cellular and molecular changes over the entire course of tendon diseases as well as to study possible tendon repair strategies. Cells obtained from different animal tissues typically used for *in vivo* tendinopathy research, ranging from small rodents (mice, rats) to large animals (rabbits, horses) [14,138], have also been generally used for *in vitro* modeling. However, as occurs with the extrapolation of results from studies with animal models, caution must also be taken on the possible conclusions derived from *in vitro* studies using animal cells. For example, a recent work comparing animal and human tenocytes side-by-side has shown significant differences in cellular and molecular features and response to inflammation among them [139].

Therefore, the availability and representability of cells used to build tendon *in vitro* models represents a dichotomy that needs to be rationally considered when selecting a model cell to fit the purpose for each specific research question.

5.2. 2D models

The use of cells cultured on flat plastic surfaces has been vastly explored in research due to its practicality, simplicity, and accessibility. These models allow for the optimization of culture conditions and the evaluation of the effects of one specific parameter at a time. Typical studies of cellular crosstalk in 2D have mostly involved co-cultures systems using standard clear polystyrene culture plates in different configurations. Co-culture systems can recapitulate the relationships among different cell types in the native tissue, which is of particular interest when aiming to understand the mechanisms of cellular communication [142]. Moreover, the supplementation of the culture media with different factors mimicking a particular condition (e.g. inflammatory mediators to mimic tendinopathy) or modulating the oxygen partial pressure can be easily applied using these models to study the effects of the stimulation of one cell type in another cell type at a time. In this section, we discuss how the use of different typical 2D models (Fig. 4) influences cellular response, with the aim to highlight its main scientific findings but also its modelling limitations.

In order to exemplify the potential of 2D models, we focus our discussion in the study of tendon inflammatory microenvironment *in vitro* [129,131]. For example, using an indirect co-culture setup (Fig. 4A), primary human tenocytes were cultured with media derived from human peripheral blood-derived mononuclear cells (PBMC) pre-activated with anti-CD3/CD28 (which released high levels of pro-inflammatory cytokines) [129]. Tenocytes showed a significant increase of IL-6 release and an enhanced expression of adhesion molecules like intercellular and vascular adhesion molecules (*ICAM1* and *VCAM1*), compared with tenocytes cultured with media from unstimulated PBMC. A similar setup was used to evaluate the effect of conditioned media obtained from exogenously damaged healthy tendon explants in healthy primary tenocytes [131]. The conditioned media upregulated healthy tenocytes gene

expression of inflammatory cytokines (*IL1B*, *IL6*), chemokines (*IL8*, *CCL2* and *CCL5*) and adhesion molecules (*ICAM1*) involved in T cell recruitment, which was also observed after stimulation of tenocytes with IL-1β (mimicking tendinopathy), in comparison with unstimulated media. Furthermore, the supernatants of these IL-1β-activated tenocytes promoted the migration of CD4 + and CD8 + T cells compared with fresh culture media [131]. The use of indirect co-cultures in these studies allowed to show that the inflammatory response in tendon is likely mediated through soluble factors released from immune cells and confirmed the potential role of tenocytes during the activation and amplification of the inflammatory response, through tenocyte-mediated recruitment of T cells into tendon after tissue damage. Interestingly, direct co-cultures (Fig. 4C) of T cells with tenocytes lead to a significant increase in CD69 expression and IFN-γ release, indicating a more effective activation of T cells [131]. This effect primarily required direct contact between T cells and tenocytes, since it was substantially reduced when cultures were performed with cells separated by membranes in insert-assisted systems (Fig. 4B). Moreover, these "recruited" T cells feedbacked tenocytes, upregulating the expression of inflammatory mediators and chemokines and increasing the collagens type III/type I ratio, compared with insert-assisted cultures. Also, the direct culture of primary human tenocytes (stimulated with media from activated PBMC) with monocyte-derived macrophages induced a partial macrophage M1 polarization [129]. These findings suggested that tenocytes might be further triggered by the T cells-derived mediators to release soluble factors, which activate the neighboring macrophages. To determine whether these effects were contact dependent, tenocytes and macrophages were cultured using an insert-assisted system, which also induced greater production of inflammatory markers and M1-like macrophages polarization, indicating that the communication between these cells occurs in a paracrine manner [129]. In contrast, only the direct co-culture of these cells increased collagen type I production compared with monocultures and insert-assisted cultures [129], highlighting the role of the signaling triggered by the direct contact between tenocytes and macrophages in collagen production, which has been related to tissue fibrosis typically occurring in tendon diseases [143]. In summary, the use of different 2D culture models with indirect or direct contact has allowed to shed light on the importance of both the cell-to-cell contact and paracrine signaling mechanisms, which depend on the specific cellular and molecular response to damage in the tendon inflammatory microenvironment. Additionally, these works also highlight the importance of selecting the adequate 2D model to study different aspects of cellular crosstalk.

Direct co-culture systems have also been used to study the crosstalk between human TDCs and pre-osteoblasts under osteogenic medium conditions [144]. Co-cultures increased the expression of bone-related markers –e.g. osteopontin and the runt-related transcription factor 2–, the activity of alkaline phosphatase (ALP) and matrix mineralization, when compared to individual cultures [144]. This work highlighted the importance of direct cell–cell interactions when recreating cellular crosstalk at the enthesis, although other important biochemical and biophysical cues should be introduced to increase the biological significance of the *in vitro* microenvironment context.

In general, the use of *in vitro* 2D co-culture models provides the practical advantages of simple assay handling, sample replication and reproducibility, although they possess some intrinsic limitations (Table 1). On one hand, while indirect or insert-assisted co-cultures allow to study the effect of soluble factors (paracrine signaling) of one cell type in another cell type, they lack the essential cell-to-cell contact that occurs in living tissues. Alternatively, even though the use of direct co-cultures mimics more realistically cellular interactions, the analysis of the results at cellular type level is more complex since it requires an additional step of cell sorting. Additionally, for both insert-assisted and direct co-cultures, the analysis of the specific origin of paracrine effectors is challenging since both cell types release soluble factors in the same media. On the other hand, the complexity of tendon microenvironments where different cell types and soluble factors interact in a complex three-dimensional ECM with defined anisotropic hierarchical architecture is not reproduced by these 2D models. Because the correct combination of tissue specific biophysical, biochemical and biological cues is required for its function and homeostasis, the recreation of tissue hallmarks *in vitro* is also fundamental to increase the significance of results [145]. In particular for tendon, in the absence of tendon ECM mimetic topographies, tenocyte culture on typical flat culture plastic surfaces lead to fast phenotype drift [116]. In fact, unlike healthy tendon tissue, alteration in cell morphology occurring in tendinopathy due to the loss of structural organization increases tenocyte proliferation rate and poor quality of produced matrix [116,146]. Moreover, this type of 2D models lack the mechanical stimuli that are responsible for the activation of various mechano-sensitive signaling pathways vital in tendon homeostasis [147].

5.3. 2D models with additional functional complexity

The tendon microenvironment is complex, not only considering cellular diversity and spatial distribution but also its structure and function. As tendon tissue is mainly composed of longitudinally aligned type I collagen, substantial fiber misalignment works as a physical indicator for the highly sensitive tenocytes that homeostasis is disrupted. Moreover, prolonged or excessive elongation of collagen fibrils leads to the breakdown of connected molecules and collagen disfunction [151]. Therefore, some 2D models have been adapted to include an extra level of complexity to account for the cell gradients at tendon interfaces, its physical cues from the ECM, including collagen fiber orientation and topography, or even mechanical stimulation during locomotion (Fig. 4).

5.3.1. Cellular gradients

Tendon interfaces are characterized by a gradual transition between tissues with different composition, cell populations, and mechanical properties, as previously discussed. While the crosstalk in direct co-cultures of osteoblasts and tenocytes can provide useful insights on their phenotype modulation, more realistic platforms would include a fibrocartilage interface as a transition region mimicking the enthesis. A simple method to recreate this cellular gradient in culture systems is using temporary barriers for cell compartmentalization that can be removed to allow cell migration and direct contact (Fig. 4C) [111,152-154]. For instance, an *in vitro* model was developed to encompass three distinct yet continuous regions using a detachable agarose hydrogel divider between primary osteoblasts and fibroblasts obtained from explants of the anterior cruciate ligament [111]. Once the divider was removed, migration of both cell types allowed for their direct contact, creating a transition zone, while remaining localized in their respective regions of origin. Co-culture resulted in decreased cell proliferation rates, ALP activity and osteoblast-mediated mineralization whereas it increased fibroblast-mediated mineralization [111]. The expression of interface-relevant markers such as type II collagen, ACAN, and cartilage oligomeric matrix protein suggested that cellular crosstalk may induce cell transdifferentiation and eventual fibrocartilage formation that bridges both tissues [111]. In summary, recreating the cellular distribution might provide new insights on how cellular crosstalk affects MTJ or enthesis regeneration after injury.

5.3.2. Topographical cues

As explained, tenocytes reside within the highly aligned collagen fascicles and exhibit an elongated shape [36,37]. Tendon ECM architecture has been extensively studied over the past years leading to a better understanding on its role on cellular behavior, tissue properties, and overall functionality. Previous studies have demonstrated that aligned topography of nanofibers positively affects attachment, shape, gene expression, and matrix deposition of tendon cells since it recapitulates the native ECM organization of tendon [115,155,156]. Furthermore, aligned topographies have been shown to induce tenogenic differentiation of stem cells and prevent their phenotypic drift [147,157]. Thus, cell culture settings incorporating aligned and misaligned (random) fibrous meshes on 2D surfaces have been developed to mimic the architecture of healthy or diseased tendon (Fig. 4D). For example, polymer meshes with varying fiber diameter and alignment were used to evaluate the response of human tenocytes as a model for tendon repair, where unaligned fibers represent the first stages of repair and aligned fibers that of complete healing [158]. It was found that unaligned nanoscale fibers (390 ± 140 nm and 740 ± 160 nm) promoted cell growth and collagen synthesis, while in the mature repair model consisting of unaligned micron-sized fibers (1420 ± 370 nm) these behaviors were significantly reduced [158]. Moreover, recent studies have shown that the macrophages inflammatory response in tendons could be mediated by substrate topography [149]. In a similar topography driven approach on electrospun fiber meshes, human tenocytes were either stimulated with pro-inflammatory cytokines or co-cultured with macrophages using inserts [149]. Substrate topography influenced cell behavior, changing tenocytes elongated morphology acquired on aligned meshes towards a round-like shape and reducing the expression of transcriptional regulators of the tenogenic phenotype on random meshes. Furthermore, inflammatory conditions in insert-assisted co-culture with M1 macrophages not only downregulated pathways responsible for ECM synthesis but also increased the production of matrix-degrading proteins [149]. These findings were enhanced in the random mesh topography, suggesting that tendon cells were predisposed to degenerative ECM remodeling pathways due to its recapitulation of fibrotic tissue. Indeed, subtract topography differently modulated macrophage crosstalk with tenocytes showing an enhanced downregulation of tendon markers on randomly oriented fibers [149]. Interestingly, it has been further shown that disorganized surface topography alone drives pro-inflammatory signature in macrophages [119]. Hence, although these are still flat cell culture surfaces, the topographical cues from their fibrous nature have granted 2D models with more realistic culture environments, which in return promises more biologically relevant results. Beyond the tendon inspired topographies based on the replication of its healthy or diseased ECM state and produced by a variety of different fabrication methods [116,149,159,160], the testing of multiple non-nature related micro-topographies created with machine learning technologies, such as on the TopoChip [161], might also lead to improved high-throughput screening platforms to study tendon cells crosstalk.

5.3.3. Mechanical stimulation

Mechanical stimulation is necessary for the maintenance of tendon homeostasis [162,163]. In general, the use of uniaxial dynamic loading regimens is known to contribute for the maintenance of physiologic tenocytes phenotype and to promote the expression of tenogenic markers in stem cells [164-167]. Moreover, efficient recovery after tendon injury in animals has been correlated to the application of mechanical stimulation, i.e. exercise training, mostly due to increased collagen and ECM synthesis by tenocytes [151]. Actually, cells respond to mechanical stimuli through complex mechanotransduction processes that can modulate the anabolic (ECM synthesis) and catabolic (MMPs expression and ECM degradation) pathways [33]. Nevertheless, to study the effects of mechanical stimulation, cells need to be seeded or encapsulated in stretchable materials (Fig. 4E). For example, human tendon stem cells seeded on fetal bovine serum-coated flexible silicone dishes were cyclical stretched at a frequency of 1 Hz and 8% strain, which resulted in a strong upregulation of tendon-related genes mediated through extracellular signal-regulated kinases (ERK1/2) and p38 pathways [168]. Using a similar setup but at 10% strain, tenocytes aligned perpendicularly to the applied load and maintained their phenotype [164]. Recently, polycaprolactone (PCL) nanofiber membranes with aligned and random orientation seeded with macrophages and/or tenocytes were clamped within a custom-made bioreactor able to provide static and dynamic loading [119]. In this work, dynamic loading (7% cyclic strain at 1 Hz for 8 h followed by 16 h of rest) significantly upregulated the expression of the M1-marker C-C motif chemokine receptor 7 (CCR7) while not affecting the M2-marker mannose receptor C-type 1 (MRC1), compared with static loading (1% constant strain) or static culture. Nevertheless, the direct co-culture of macrophages and tenocytes on aligned substrates under dynamic mechanical loads reduced the pro-inflammatory macrophage population while increasing the proportions of M2-like (MRC1 +) cell phenotypes, a behavior that was also observed *in vivo* [119]. Herein, mechanical stretching seemed to provide a mechano-protective effect to inflammatory triggers, not only by the increase in M2-like cell subpopulations but also by the reduced translocation of NF-kB p65 subunit to the nucleus of tenocytes [119]. Interestingly, the activation of the NF-kB pathway has been associated to impaired tendon healing [169]. In a different work using PCL nanofiber random membranes, mechanical stimulation at 5% strain at 1 Hz induced cell and ECM alignment and tenomodulin deposition in bone-marrow MSCs, in contrast to static conditions (Fig. 4E) [150].

In order to simulate the tendon-to-bone healing environment after tendon reconstruction with postoperative loadings, murine pre-osteoblasts and tendon-derived fibroblasts were cultured onto collagen-coated six-well plates [110]. Cyclical loading applied for 1 h twice a day for 3 days with a frequency of 1 Hz and 3% strain decreased the expression of type I collagen, *ALP*, and osteocalcin in monocultures of both cell types but increased their expression and protein deposition in co-culture [110]. Moreover, the bone-related gene bone morphogenetic protein-2 was increased under mechanical loading independently of culture conditions [110]. The addition of mechanical stimulation into 2D models has allowed to better understand the tendon development, cellular crosstalk during inflammation or the beneficial effects of postoperative loading (rehabilitation) for patients. Actually, many different systems have been devised to apply mechanical stimulation to cultured cells and scaffolds, which has been extensively reviewed recently [170]. However, a main drawback associated with mechanical loading is that stimulation protocols widely differ between studies (strain from 0 to 10% and frequency between 0.0167 and 1 Hz) [170], which make comparisons among studies difficult to establish and might in some cases also lead to deleterious effects. For example, prolonged tenocytes mechanical loading resulted in increased cell detachment and spheroid formation [110]. Therefore, research efforts should be made in order to clearly stablish the optimal mechanical stimulation conditions to induce specific effects in tendon cells, while also considering that different tendons have different mechanical behaviors and requirements (i.e. energy-storing and positional tendons [21-23]).

Overall, the use of adequate topography or mechanical stimulation in 2D models allows a better representation of the tendon microenvironment (Table 1). Indeed, the use of culture systems on meshes with aligned and misaligned topographies reproduces

the observed cellular trends in healthy tissues and in tendinopathies. The inclusion of mechanical loadings sets a step towards what tendon tissue experiences during joint movement. Moreover, the combination of adequate topography and mechanical stimulation better recreated the tendon microenvironment [133,171]. Yet, the entire compilation of biological, chemical, and physical cues of tendon tissue 3D microenvironments [124,172] cannot be closely recapitulated in 2D, which has motivated the search of new tools to build more accurate and biologically representative models.

5.4. Bioengineered 3D models: Leveraging on tissue engineered systems

The addition of complexity by designing 3D structures to host cells has several advantages when recreating tissue environments (Table 1). Cellular components in any tissue are exposed to physical factors given by the architecture and composition of its ECM, the presence of multiple cell populations, and a large variety of released soluble factors that create spatial concentration gradients. Different 3D culture systems have gained momentum in recent years, allowing to get more representative mechanistic insights of innate tissue cellular interactions [145,173]. These strategies rely primarily on the use of biomaterials as structural support for cells to more accurately replicate native 3D microenvironments. The relevance of 3D microenvironments has recently been proved using 3D-cultured hTSPCs, which exhibited better tenogenic differentiation and tendon regeneration ability than the cells cultured on 2D surfaces [174]. Moreover, the recent advances in tissue engineering and biofabrication tools are being leveraged for the fast patterning of different types of cells and ECM into a given living 3D structure [175]. Because understanding tissue development and architecture under physiologically relevant microenvironments is key for building functional *in vitro* models, some of these 3D bioengineered constructions have been used as improved tools to further comprehend the interplay between tenocytes and other cells types in tendon tissue remodeling and repair. However, despite the remarkable advances seen on the development of bioengineered tendon systems, which could be easily adopted to fabricate 3D *in vitro* models with increased physiological relevance, beyond the collagen-based systems (further discussed below), very few studies have explored this existing knowledge to date. In order to highlight the potential of available biofabrication tools to produce 3D tissue engineered systems recreating multiple aspects of tendon tissues and foster their assimilation in the establishment of advanced *in vitro* tendon models, in the following sections we discuss the potential of these systems and their fabrication strategies, not only to study cellular crosstalk but also to improve the current drug discovery pipelines for tendon therapies.

5.4.1. Cell sheets

A potential technology to produce tissue-like constructs is cell sheet tissue engineering, which takes advantage of ECM deposition by the cells themselves, preserving cell-to-cell contact to produce self-assembled microtissues [176]. Although it might be arguable if cell sheets can be considered true 3D systems, we have included it in this section given that they incorporate the 3D context of newly-deposited ECM. The construction of cell sheets typically relies on coating 2D culture dishes with temperature-responsive polymers which can transition from hydrophobic to hydrophilic [176] (usually poly(N-isopropylacrylamide), although a variety of polymers have been proposed, reviewed here [177]). High cell densities are seeded on these surfaces, where cells can grow and deposit ECM that self-assembles into a tissue-like structure. After thermal-induced increase in hydrophilicity, these tissue-like constructs can be detached from the surfaces conserving the cell-to-cell network and self-assembled ECM, including adhesive proteins, which allows to stack multiple sheets to build thick constructs [176]. More recently, loading magnetic nanoparticles in cells has allowed to recover the cell sheets with the aid magnetic forces [178,179]. This technology has been used to build different tendon-mimetic ECM-rich constructs with different cell sources for *in vitro* and *in vivo* applications [180-183]. An obvious key factor for cell sheet engineering is the selection of the adequate cell source in terms of differentiation and availability, which is particularly relevant in tendon field due to the scarcity of tendon-derived cells. For example, although human TSPCs expressed higher levels of tendon-related markers than SCX-transduced human MSCs on 2D cultures, both cell types showed similar ability to produce compact tendinous sheets with spindle-shaped cells and collagen type I fibrils [182]. Moreover, human TSPCs from aged/degenerative tendons were less competent than cells from young/healthy tendons to form 3D cell sheets, which was suggested as an interesting model to investigate the molecular and cellular mechanisms of tendon aging and degeneration [120]. More recently, these type of cell sheets were rolled up to form 3D rod-like tissue structures, which could be applied to develop tendon organoids [184]. Despite various works have produced tendinous 3D cell sheets showing the characteristic proteoglycan-, glycosaminoglycan- and type I collagen-rich ECM of tendon [178-183], their production is lengthy and, more importantly, they fail to closely recapitulate the anisotropic architecture, cell alignment and compartmentalization observed in this tissue. The use of the topographical cues reviewed in the previous section together with macromolecular crowding, a strategy to enhance and accelerate native ECM synthesis and deposition adding macromolecules in the culture media (readers are addressed to recent works and reviews [185-187]), may constitute possible strategies to overcome some of these limitations and build more biomimetic cell-based tendon constructs. Additionally, stacking of cell sheets produced with different cell types [188,189] might constitute a possible strategy to mimic the cellular crosstalk between extrinsic and intrinsic tendon compartments.

5.4.2. Collagen structures

Since tendons are mainly composed of type I collagen, this tissue derived polymer has been widely used to create 3D constructs suitable for cell seeding or encapsulation. Collagen-based hydrogels became popular among tendon research community because it allows the easy production of anisotropic cellular constructs (in contrast to the typical isotropic hydrogels) [190-194]. These anisotropic systems typically consist of low-density collagen gels with encapsulated cells, which are restrained during culture by the use of different mechanical retention systems to harness cellular contraction and guide their alignment along the axis of tension (Fig. 5A-D). However, it is worth mentioning that fibrin, which shows similar contractile behavior, has also been used as alternative to collagen for developing similar tendon constructs [195,196]. Some studies have shown that compared to collagen, fibrin leads to improved tenogenic expression and ECM organization by tendon progenitor cells [196], and might therefore be considered as a better alternative for *in vitro* tendon modeling.

In one example of collagen based systems for *in vitro* modeling, tenocytes extracted from healthy human hamstring tendon were first cultured in 3D collagen lattices and then incubated with soluble factors released by the human mast cell line HMC-1 [95]. Tenocytes showed higher proliferation (a characteristic of tendinopathies) and expression of cyclooxygenase-2, a key enzyme in the release of the pro-inflammatory molecule prostaglandin E2 (PGE2). These effects were attributed to the presence of TGF-β in the mast cells secretome, providing evidence of the contribution of mast cells in the development of tendinopathies. Moreover, when production of PGE2 both in mast cells and tenocytes was blocked, PGE2-induced downregulation of type I collagen was averted and

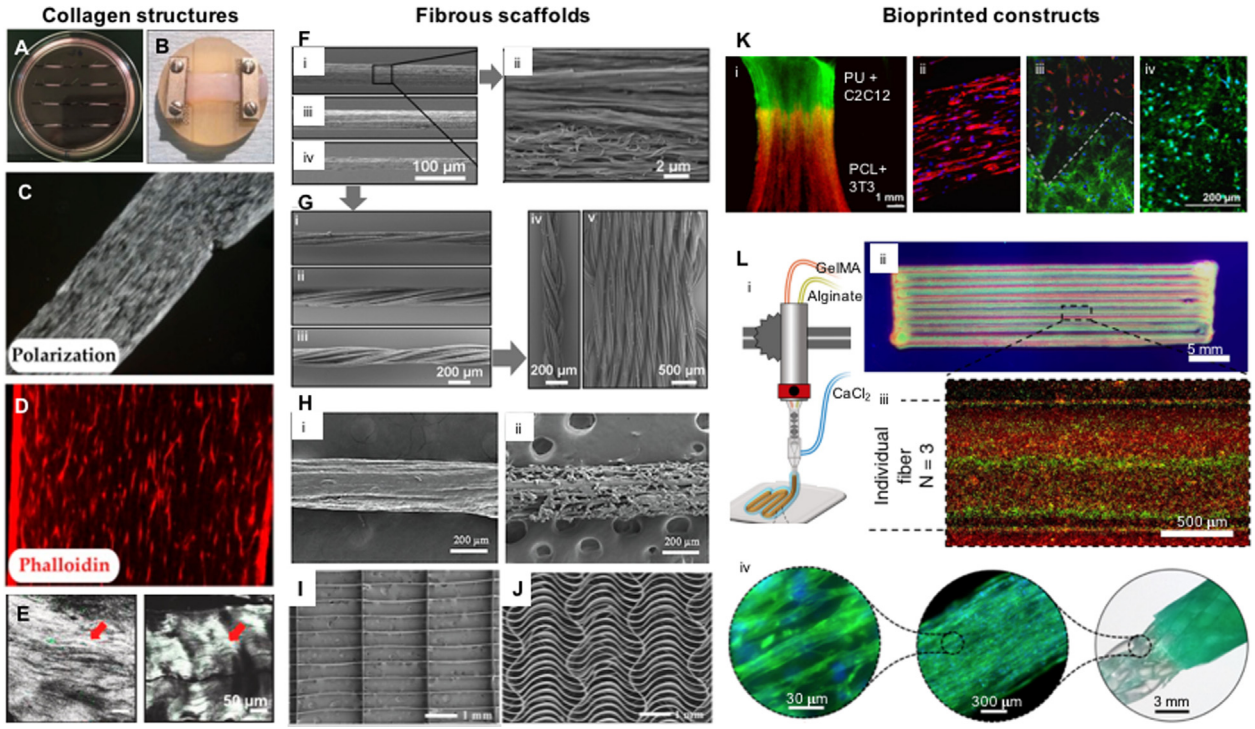

Fig. 5. Examples of 3D tissue engineered systems with potential for tendon *in vitro* modelling. Collagen hydrogels mechanically retained using (A) sutures and needles (adapted from [194]) or (B) custom campling device (adapted from [190]) induce (C) collagen (polarization microscopic image) and (D) cell alignment (phalloidin staining), adapted from [194]. Moreover, (E) using confocal reflectance, it was possible to observe of collagen fiber development on cell-seeded collagen hydrogels clamped for 6 weeks (E-left), which was comparable to native tendon tissue (E-right) (adapted from [190]). (F) Electrospinning was used to produce continuous and aligned nanofiber threads (PCL-based) as elementary unit of the 3D assembly, mimicking the collagen fibers in native tendon. (G) Yarns consisting of (Gi) 6, (Gii) 9 and (Giii) 12 fibers, which were later utilized to produce (Giv) braided and (Gv) woven 3D scaffolds mimicking tendon hierarchy (adapted from [203]). (H) Wet spinning was used to produce scaffolds for the tendon-to-bone interface. (Hi) While wet spinning of PCL/gelatin produced aligned fibers to mimic tendon, (Hii) the addition of hydroxyapatite nanoparticles in the blend rendered more random fibers to mimic bone side (adapted from [210]). (I-J) SEM images of PCL melt electrowriting scaffolds with (I) straight fibers or (J) serpentine architecture, which could be used to mimic tendon health and disease, respectively (adapted from [214]). (K) Bioprinting of an MTJ construct, in which different polymers and cells encapsulated in hydrogels were used to address the different mechanical and cellular characteristics of muscle (polyurethane-PU + C2C12 cells) and tendon (PCL + 3 T3 cells), adapted from [224]. (Ki) Fluorescently labeled constructs after 7 days in culture (green: C2C12 cells, red: 3 T3 cells, yellow: interface region). (Kii) At the muscle side, C2C12 cells formed highly-aligned, multinucleated myotube structures (red: desmin, blue: DAPI). (Kiii) At the interface region, depicted by the dotted line, differential expression between the two cell types is observed (red: desmin, green: type I collagen, blue: DAPI). (Kiv) At the tendon side, 3 T3 cells secreted type I collagen (green: collagen, blue: DAPI). (L) Biofabrication of multicompartmental hydrogel fibers (Li) using a static mixer integrated with a coaxial microfluidic device extruding mixed streams of alginate (yellow) and gelatin methacryloyl (GelMA, red) through a sheath flow of $CaCl_2$ solution to crosslink alginate and form the matrix of the fiber before UV light exposure to crosslink GelMA striations, adapted from [238]. This setup was used to fabricate (Lii) unidirectional structures consisting of (Liii) individual fibers with compartmentalized microfilaments (using a 3 elements static mixer). (Liv) The organized internal microstructure of the fibers directed cellular alignment, while the robustness of the fibers enabled their bioassembly, toward formation of biomimetic hierarchical structures (F-actin/DAPI staining was used to assess the morphology of C2C12 cells cultured in multicompartmental fibers).

the contractile activity of tenocytes was enhanced. Remarkably, this molecular pathway might possibly influence the tenocytes-mediated control of cellular contraction, collagen accumulation and alignment, which plays an important role in the balance between tissue repair and fibrosis [197]. Moreover, in another study performed on collagen tendon-like constructs, TDCs from rat Achilles tendons were used to determine the presence of an immune-like tendon cell subpopulation [92]. These collagen constructs could be easily formed by placing a collagen-cell solution between silk sutures pinned on silicone-coated petri dishes and allowing its temperature-dependent gelation [198]. This setting was used to demonstrate that the inhibition of CX3CL1 significantly reduced cell migration, a key process for wound healing [92]. Further stimulation with pro-inflammatory cytokines IL-1ß and TNF-α upregulated the expression of genes encoding for inflammatory and ECM-degrading proteins, as well as CD68 and CXCL1 markers, which confirmed the immune-like phenotype of the recently discovered "tenophages" [92]. Although these models emulate the collagen-rich environment of tendon tissues, typical soft collagen gels fail to recreate the inherent biophysical cues of native fibrous tissues that are known to critically modulate cell interactions in tendon and the compartmental tissue–tissue interfaces that govern essential spatiotemporal biochemical gradients. Recent works have proposed possible strategies to mitigate some of these limitations. For example, the Stevens group has shown that high-density collagen gels under static tension are able to undergo a cellular-mediated (tenocytes, ligament fibroblasts, and meniscal fibrochondrocytes) hierarchical collagen fiber formation process that remarkably reconstructs the 3D architecture of their native tissue microenvironment (Fig. 5E) [190]. Nevertheless, it has been shown that some of these collagen-based anisotropic systems may tend to have non-uniform collagen fiber alignment within the constructs [192], suggesting that better bioengineering alternatives are required to recreate the anisotropic biophysical cues of tendon cell niches. Also, reinforcement of collagen with other biomaterials like silk has allowed to increase its mechanical properties [199]. On different approach, structural tissue compartmentalization was recreated in a "semi-bioengineered" tendon model using tendon explants (mimicking the intrinsic tendon core) surrounded by a collagen hydrogel with encapsulated cells (CD146 + TSPCs or macrophages, mimicking an extrinsic compartment) [130]. This "tenostruct" allowed cell communication and

migration between compartments and the analysis of crosstalk and cellular behavior in the specific compartments.

5.4.3. Fibrous scaffolds

Multiple bioengineered tendon analogs have been developed based on both natural and synthetic fibrous material platforms, including e.g. collagen, chitosan, silk fibroin and PCL [200-202]. Over the last years, different fiber formation techniques have been developed for the fabrication of fibrous scaffolds including electrospinning, wet spinning or melt electrowriting (reviewed here [201]). Among these, electrospinning technology has several advantages for tendon modeling because it enables the relatively easy production of different hierarchical fibrous materials in the form of random and aligned meshes where the elementary fiber diameters can be tuned form tens of nanometers up to a few micrometers, representative of the topographical features sensed by resident tenocytes (in the range of collagen fibrils). Moreover, in combination with textile techniques, electrospinning has been widely explored to fabricate biomimetic hierarchical structures as a mechanical support and 3D template for tendon cells and for inducing the tenogenic commitment of progenitor/stem cells (Fig. 5F-G) [115,151,203-207].

The modulation of fibers dimensions and composition in scaffolds has great potential to develop models of tendon interfaces, where gradual transitions of architecture and physical properties are observed from one end to the other. For example, an ideal model for the MTJ would consist in a 3D system with distinct mechanical profiles mimicking the compliant muscle and the stiff tendon. To address this need, a continuous PCL/collagen and poly (l-lactide)(PLLA)/collagen co-electrospun triphasic scaffold was developed [208]. This scaffold exhibited regional variations in mechanical properties with moduli in the range of 4.490–27.62 MPa and similar strain profiles to native tissue, although actual strain values at the muscle-to-bone interface are different, with failure occurring at higher strain levels than in this polymeric scaffold [208]. The scaffold was seeded with 3 T3 fibroblasts and the embryonic precursors of muscle cells, C2C12 myoblasts, which formed myotubes upon seeding. Even if each cell type was seeded separately along the scaffold, the platform remains a viable option to study the crosstalk between both cell types. Nevertheless, this model of tendon-to-muscle has the limitation of having a random topography unrepresentative of the organized longitudinal structures of both muscle and tendon.

Fibrous scaffolds made of different polymers have also been used to create constructs mimicking the tendon-to-bone by spatially controlling the tenogenic differentiation of ASCs using different biochemical cues (e.g. growth factors or hydroxyapatite) (Fig. 5H) [209-212]. In one study, PDGF, which is upregulated during tendon repair, was immobilized on aligned and misaligned PLLA nanofibers to analyze how topographical and biological cues affected the tenogenic differentiation of ASCs. The immobilization of PDGF enhanced the proliferation and tenogenic differentiation of ASCs regardless of fiber topography. Yet, ASCs on PDGF-immobilized aligned nanofibers exhibited a tenocyte phenotype with more elongated and spindle-shaped morphology and a greater aspect ratio compared with unaligned fibers. Also, the gradient-wise immobilization of PDGF spatially controlled the phenotypic differentiation of ASCs into tenocytes, as confirmed by their expression of tenogenic markers [209]. This setup is interesting for the advancement of tendon models given that it recreates the spatially-controlled matrix-bound signaling mechanisms of growth factors presentation rather than following the typical ineffective culture media supplementation with soluble growth factors. Moreover, besides the widely demonstrated role of scaffolds topography and mechanical stimuli on the maintenance of tenocytes phenotype and induction of tenogenic differentiation of mesenchymal/stromal cells, co-culture of ASCs, tenocytes and endothelial cells was also shown to further potentiate these effects [133]. These data suggest that the heterocellular signaling crosstalk occurring under relevant tendon architecture is important for the tenogenic potential of engineered tendon constructs and should therefore be considered when designing 3D organotypic *in vitro* models of this tissue.

Despite the proven potential of electrospinning to fabricate scaffolds closely replicating the hierarchical fibrous architecture of tendon tissues, it presents two main limitations. On one hand, the control over 3D fiber geometry and deposition is still limited. A potential alternative technology to address this issue is melt electrowriting that can accurately and continuously "write" micro-scaled fibers with different geometries in 3D (linear and crimped geometries represented in Fig. 5I-J) although this technology is typically limited to constructs of 1 mm thickness and to fiber dimeters between 5 and 50 μm (in the range of collagen fibers) [213,214]. On the other hand, cells are usually manually seeded on the biomaterial's surface prior to the *in vitro* maturation steps. Thus, before a significant *de novo* matrix is secreted and deposited (a process that requires long culture periods), cells reside in a far from physiological microenvironment lacking some important biochemical and biophysical cues of the pericellular space provided by the dense tendon ECM niches, which can lead to cell phenotype drifts or loss of function [215,216]. The development of new engineering strategies to mitigate the disruption of these important signals will improve the biological significance of cell responses in eletrospun-based models. The use of composite living fiber systems [155,217], consisting of defined cell-laden hydrogels and tendon mimetic fiber cores, or combination of electrospun scaffolds with macromolecular crowding culture concepts [185-187], might represent possible approaches to be explored in order to improve the physiological relevance of these models.

5.4.4. Bioprinted constructs

A wide range of bioprinting strategies and technologies have been devised in recent years with the goal of improving our capacity to fabricate biologically and physiologically relevant spatial architectures with multiple cells and/or biomaterials, which might eventually lead to 3D living constructs emulating the functions of human tissues [173]. The most widespread 3D bioprinting technologies are extrusion-based systems that basically consist of deposing cell-laden bioinks (cell-laden biomaterials) in a layer-by-layer fashion to obtain a 3D tissue-like structure [218]. The possibilities that 3D bioprinting techniques have brought to the field of tissue engineering, enabling the controlled deposition of materials, cells and bioactive cues in the 3D space, might be leveraged for the fabrication of tendon-like constructs and of complex structures of its interfaces. Of particular interest for *in vitro* modeling applications is the ability of these technologies to enable the fast and automated production of sample replicates, including the possibility for direct *in situ* fabrication of miniaturized 3D constructs in multiwell plate formats [219,220], representing therefore obvious advantages in terms of assay throughput and reproducibility. A general limitation of bioprinted tendon constructs aimed at transplantation for tissue regeneration is the inherent characteristics of typical bioink materials, which mainly consist of soft polymer hydrogels processed under biocompatible conditions. In order to overcome these limitations, multimaterial fabrication strategies that combine the use of acellular mechanical support components (e.g. biodegradable aliphatic polyesters such as PCL or PLLA) with cellular components (bioinks) have been proposed [173]. Although miniaturized constructs used for *in vitro* assays are not aimed to restore load-bearing functions of damaged tissues, these multimaterial fabrication strategies might be leveraged not only to better recreate the 3D microstructure, gradients and biophysical cues of

tendon tissue and its interfaces, but also offer the possibility to directly fabricate living constructs within its own perfusable bioreactor [221-223], allowing to perform screening assays under more physiologic-like conditions.

The field of tendon tissue engineering has made use of these techniques for the development of tendon constructs with the potential to be used as relevant 3D *in vitro* models. For example, using the integrated organ printing technology, different polymers were used for each tissue of the tendon-to-muscle interface (Fig. 5K) [224]. Elastomeric polyurethane was used for the compliant muscle while stiffer PCL was used for tendon, leaving a 10% overlap printing region to create an interface. Moreover, each section was co-printed with C2C12 myoblasts and 3 T3 fibroblast-laden bioinks, respectively. In addition to presenting the gradual mechanical pattern from elastic to more plastic properties, after 7 days of co-culture in the construct, the expression of the MTJ-associated genes, focal adhesion markers as well as the expression of relevant markers in each tissue side was increased [224]. These hybrid constructs have the advantage of being biologically and mechanically customizable, having control over shape, size, and architecture. Although designed as potential implantable replacements for injured tissues [224], they might be also well-adapted for studying cellular crosstalk in MTJ. However, typical extrusion-based bioprinting multi-material systems still have several limitation on their potential to recreate the intrinsic heterogeneity and the length-scales of complex hierarchical microstructures, like those of the tendon tissue and its interfaces [173].

One option to better recapitulate the inherent tissue microenvironmental niche in 3D cell-printed constructs is by using tissue- and organ-specific decellularized ECM (dECM) as bioink hydrogels [225]. This type of biomaterials provides a unique spatial distribution of structural and functional components that characterize the tissue of origin, allowing to capture the key biomolecular hallmarks of their healthy and pathological conditions [226]. The environmental cues of dECMs are not limited to the tissue-specific structural proteins and polysaccharides, but also include soluble factors absorbed within the ECM protein components that participate in important biological spatiotemporal signaling cascades [227]. This biological complexity of dECMs, difficult to recreate with natural and synthetic single-component biomaterials, has significantly increased their popularity for applications in tissue regeneration and also in *in vitro* modeling [228,229]. A few recent studies have used bovine [230] and porcine [231,232] tendon dECM bioinks, showing that the remnant biological signature from their tissue of origin could induce the tenogenic differentiation of human bone marrow-derived MSCs. Similar strategies were applied for printing spatially-graded tendon-to-bone constructs, consisting of three distinct layers of bioink compositions: tendon and bone dECM intercalated by a middle layer composed by a mixture of both dECMs [232]. Interestingly, this graded microenvironment, mimicking the transition of native fibrocartilage region, could elicit a zonal differentiation of encapsulated hBMSCs, inducing their differentiation into tenogenic and osteogenic lineages at tendon and bone dECM sides, respectively, which were interfaced by a region where cells showed upregulation of chondrogenic markers (e.g. collagen type II and ACAN), suggesting a fibrochondrogenic commitment. Although the level of control over construct microstructure is still far from reaching the architectural length scales and anisotropic organization of native tissue, this biofabrication approach might be advantageously explored to build 3D heterotypic models of tendon-to-bone interface starting from a single cell source. This would contribute to improve the physiological relevance of cellular crosstalk studies, being an alternative to e.g., previously proposed *in vitro* tri-culture models where agarose hydrogels were used to encapsulate relevant interface cells found in this tissue post-injury (represented by bone marrow-derived MSCs) and to separate from other cells of interest (fibroblast and osteoblasts) cultured in 2D tissue culture plastic [233]. However, batch-to-batch variability, problems of printability and poor mechanical properties are common limitations associated with dECM ink biomaterials [225], which might hinder their wide application in *in vitro* modeling and point to the need of developing new tendon specific bioinks.

Despite achieving a better representation of the native tendon tissue and its interfaces, the discussed 3D constructs lack some tissue-specific functional cues and cellular patterns difficult to recreate with conventional bioprinting techniques. For instance, while integrated 3D bioprinting can tackle the issue of heterogeneous cellular distribution, it is generally unable to reach the required printing resolution at the micro- and nanoscale when recreating hierarchical architectures [175]. The convergence of extrusion bioprinting systems with other additive manufacturing (AM) fiber-based technologies can be explored to refine the structural complexity and resolution of 3D printed constructs in order to better replicate the intricate microarchitectures and cellular diversity of native tissues. One option is the use of microfluidics printing heads [234], which can be leveraged to recreate the multicellular composition and compartmentalization [235-237] of the extrinsic and intrinsic compartments of tendon fascicles, as well as the biochemical and cellular gradients of tendon-to-bone interfaces. Moreover, some recent microfluidic systems allow to bioprint hydrogel fiber-based constructs with internal microtopographies that offer a remarkable control over 3D cellular orientation (Fig. 5L) [238], which might be advantageously explored for the automated bioprinting of healthy (ordered) and diseased (disordered) 3D tendon models. Similar biomimetic morphological and mechanical cues might be obtained by combining advanced multimaterial bioprinting methods, such as integrating melt electrowriting with hydrogel extrusion bioprinting [239]. This would allow the fabrication of hierarchical constructs composed of stiff polymeric microfibers with well-defined patterns that replicate the collagenous component of tendon ECM while approaching its topographical length scales (fibers with diameter below 5 μm have been produced [240]), intercalated by cell-laden hydrogel inks recreating the soft and highly hydrated nature of the IFM, which is rich in glycosaminoglycans and proteoglycans [38].

However, in order to have a higher level of fabrication control allowing to reach the nanoscale dimensions of the elementary structures of tendon ECM (the dimensions of collagen fibrils), hybridization of both AM and non-AM fabrication technologies (e.g. two-photon photopolymerization or tomographic volumetric bioprinting [241]) will most likely be required. Furthermore, even highly realistic 3D models may lack other vital features like nutrient and oxygen gradients, and the mechanically active microenvironment, which are central to the function of all tissues [242], including tendon. The complex nature of cellular interactions would require the combination of intrinsic and extrinsic factors within a single tendon model.

6. Perspectives on future tendon microphysiological systems

The development of a new generation of human tendon microphysiological systems will allow not only to obtain a deeper knowledge of tendon health, disease and healing but also will enable the development of more adequate therapies that tackle its etiology and promote tendon regeneration.

The intersection between microfluidic-based technologies and bioengineering concepts has been leveraged for the development of multiple tissues and organs-on-a-chip (OoC) [243-245]. These dynamic miniaturized systems are aimed to recreate the functional unit of a tissue, mimicking its basic physiological mechanisms in

highly controlled conditions [246]. Indeed, microfluidic-based *in vitro* model systems count with a young but rich history of progress since their early developments in the late 1990 s [247]. One of the first landmarks of OoC technology is the lung-on-a-chip that recreated the epithelial/endothelial interface on stretchable polydimethylsiloxane (PDMS) membranes mounted within microfluidic devices also made of this transparent material [243]. Since then, there has been a surge of different OoC devices, promising to revolutionize the way how tissue/organ physiology and pathophysiology studies will be performed in the near future [248]. Most traditionally, their fabrication has been performed through soft lithography/replica molding [249] or photolithographic methods [242]. Commonly, microfluidic devices are created on PDMS platforms due to its multiple advantages such as controlled polymerization kinetics, optical transparency, and biocompatibility [247,250]. However, microfluidic *in vitro* models are not restricted to the use of PDMS-based platforms. A large variety of both natural and synthetic hydrogels are being explored to develop these systems, widening the available design space for these devices [251]. As microfluidics use small amounts of fluids (between 10^{-9} to 10^{-18} L) within the perfusable microchannels, these systems benefit from lower reagent consumptions, facilitated integration of sensors, electrical components, or mechanical loading, as well as portability. Moreover, microfluidic-based co-culture models, unlike other macroscopic and static systems, allow culture conditions under dynamic laminar fluid flow, which better represents *in vivo* biological processes [246,252]. Altogether, these microphysiological systems have allowed the generation of precise settings to tune dynamic fluid flows and spatiotemporal gradients to deliver nutrients and other chemical cues to multicellular constructions in a controlled manner, leading to numerous tissue models that better mimic of their innate behaviors [246].

Remarkably, although microfluidic-based systems have a unique potential to provide tight control over many of the dynamic biophysical and biological cues that recapitulate native tissues physiology, these approaches remain largely underexplored for tendon *in vitro* modeling. Beyond the obvious advantages of sample miniaturization and their inherent cellular compartmentalization capabilities, these systems can incorporate different physical forces, including physiologically relevant levels of fluid shear stress, cyclic strain and mechanical compression, and permit analysis of organ-specific responses, including recruitment of circulating immune cells, in reaction to environmental perturbations [246]. One of the very few examples where microfluidic systems have been explored to study tendon physiology has been recently proposed by Snedeker group [117]. In this interesting study, microfluidics flow chambers were used to apply tissue levels shear stress to tenocytes (estimated to be in the range of 2–6 Pa), contributing to clearly demonstrate that shear forces are a key mechanical stimulus for these cells and are sensed through the signaling activation of mechanosensitive PIEZO1 channels. However, there are multiple OoC platforms and bioengineering solutions previously developed that could be easily adapted for tendon modeling. For example, OoC devices consisting of arrays of functional human 3D tissues and produced using different hydrogel systems have been applied to model several anisotropic tissues (e.g. cardiac [253] and skeletal muscle [254]), as well as to study how their specific biomimetic environmental cues differently impact immune cell motility [255]. Therefore, it is plausible that similar approaches could be implemented to bioengineer 3D tendon models.

The development of these tendon-on-a-chip models holds promise to improve not only the research of cell–cell interactions but also of tendon tissue interfaces crosstalk. Furthermore, the idea of linking different musculoskeletal systems is a quite attractive quest, which has been perspectively reviewed elsewhere [256]. Although engineered solutions exist for some of these systems separately [254,257-259], their integration in a miniaturized joint-on-a-chip could provide a focused unit to enable the study of their synergistic interactions. At a smaller level of complexity, but perhaps more feasible to implement, bone- and muscle-on-a-chip could be assembled into a tendon-on-chip model to recreate both tendon interfaces. Within these systems, multiple parameters could be tuned to recreate different tendon microenvironments, including that of inflammation or the ECM hallmarks of tissue fibrosis. Although promising, there are challenges associated to multiple tissue-on-a-chip units integration, starting with suitable protocols for cell culture conditions in small volumes [260], the complexity of cell seeding protocols and the establishment of different test conditions [261]. Moreover, adequate flow rates and tissue specific vascularization should be optimized given, for example, the dissimilarities among the hypocellular tendon and the highly metabolically active muscle.

The development of microphysiological systems is not limited to the use of plastic-based chip platforms with predefined microfluidic circuits produced by microfabrication approaches. Multiple biofabrication technologies can be explored or combined to produce multi-cellular 3D tissue models with features at different length scales difficult to reproduce with other conventional alternatives [175,262,263]. For example, the concept of integrating 3D bioprinting platforms (in this case, a digital micromirror device-based bioprinter) with microfluidic printing heads enabled the design of multi-material models with bioinspired architectures [264]. By allowing the controlled switching and mixing of tissue-specific bioinks directly at the printhead, this strategy demonstrated potential to recreate the gradients and patterns of tendon tissue interfaces, such as the enthesis and MTJ [264]. It is argued by the author that this fabrication technology has as major advantage the high printing fidelity and speed compared to conventional vat-photopolymerization 3D bioprinting strategies, such as digital light processing-based techniques. On this matter, emerging biofabrication technologies such as 3D volumetric bioprinting systems [265], might also contribute to overcome some challenges related to the fast biofabrication of complex heterogeneous and perfusable microfluidic-based models with tissue relevant 3D geometries and patterns [262]. Nevertheless, the most widespread bioprinting technology nowadays available in most bioengineering labs is the extrusion-based printing system [173]. These biofabrication platforms can be explored for tissue/organ modeling following numerous directions. We and others have leveraged on the principles of matrix assisted 3D free-form bioprinting followed by “locking” of embedded constructs within support fluid materials for the direct manufacture of cell-laden devices housing microphysiological systems with tissue relevant geometries and heterogeneous cellular patterns, produced without requiring any specialized microfabrication skills [266-268]. Besides enabling automated sample replication at high printing fidelity, these versatile approaches have shown multiple advantages for *in vitro* modeling, allowing to design models with more biomimetic 3D geometries and architectures, promoting improved cell–cell crosstalk and tissue morphogenesis, and showing increased potential for process and size scale up [266,267,269].

Beyond the fabrication aspects and their support platforms, the next generation of *in vitro* tendon models will certainly also integrate precise genome engineering tools such as CRISPR/Cas9 (reviewed here [270]). With these tools, it is now possible to quickly induce loss-of-function and gain-of-function mutations or to introduce chromosomal translocations in cells of interest that mimic the alterations and signaling described, for example, in tendinopathic cells.

Overall, the design space resulting from the integration of different biofabrication and microfluidic technologies together with

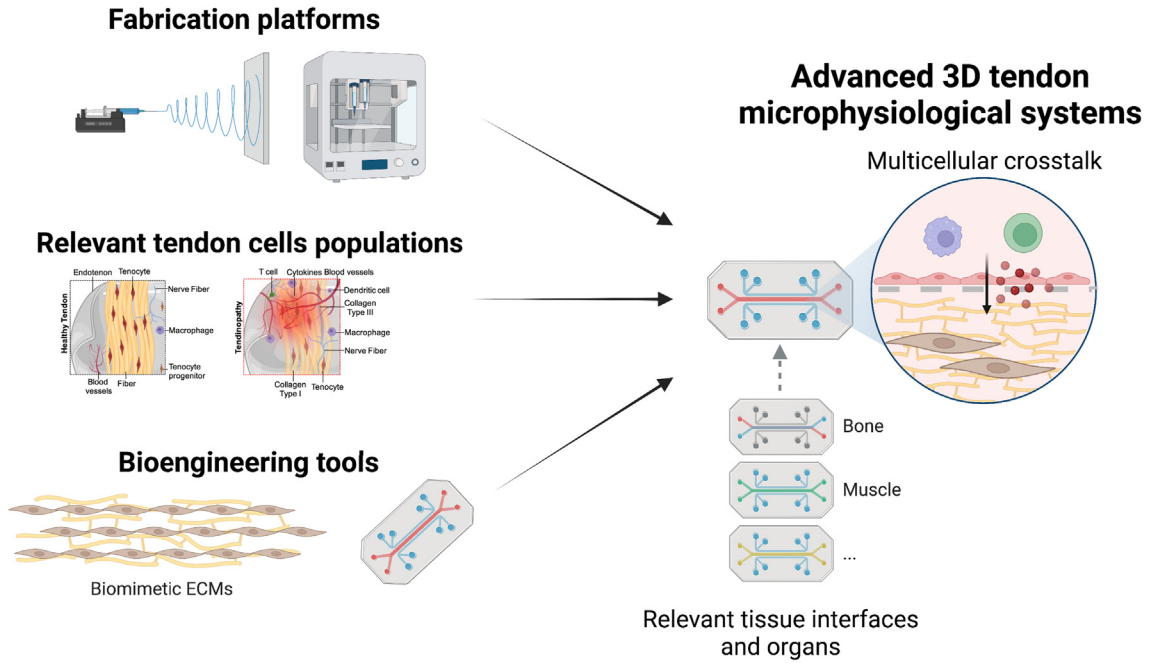

Fig. 6. Vision for future advanced *in vitro* tendon models. The combination of state-of-the-art fabrication platforms (fibrous techniques, 3D bioprinting), bioengineering tools (microfluidic technology, biomimetic ECMs) and relevant cell populations might enable to replicate the hierarchical and fibrous architecture of tendon, its compartmentalization and cellularity to transition from the typical 2D and 3D models into biologically relevant microphysiological models of tendon health and disease. The integration of these systems with other relevant tissues-on-a-chip might further increase the complexity and the research landscape to be explored.

relevant tendon cell populations might contribute for the development of a new generation of 3D tendon models with improved predictive power for their *in vitro–in vivo* extrapolations (Fig. 6). By recreating microphysiological systems where cell–cell and cell-matrix interactions occur in more relevant physical and biological context, these strategies will provide valuable tools for the study of tendon physiology and pathology, and thus to identify the main players in tendon healing mechanisms. Therefore, they stand as promising models to understand molecular phenomena and develop alternative tendon therapies that promote tendinopathy resolution and tendon regeneration. Ultimately, this will not only allow the obvious testing of potential drug candidates, but also assessing biomechanical stimulation protocols to implement better physiotherapy rehabilitation regimes.

7. Conclusions

In this review we overview the existing *in vitro* models used for studying the interactions between the different actors of tendon microenvironments and briefly highlight their main contributions to the current knowledge on tendon physiology and pathophysiology. Engineered *in vitro* models to study the crosstalk between tendon and immune cells have allowed to elucidate some of the molecular mechanisms involved in their healing process, contributing to consolidate the concept that immune cell are major players on the coordination of tendinopathy. Moreover, it is now known that the crosstalk between diverse cell types at tendon interfaces with bone and muscle are key drivers of the formation of their characteristic structural and cellular gradients. However, deeper understanding of these phenomena will require the development of sophisticated human *in vitro* models able to properly recapitulate the unique hallmarks of tendon tissue microenvironment. Possible directions to be explored for the transition from traditional 2D models toward complex biomimetic 3D systems for studying cellular crosstalk were addressed, emphasizing the potential of available biofabrication and microfluidic technologies for developing relevant organotypic models. The integration of these bioengineering technologies in tendon modeling will allow to better recreate the hierarchical architecture, compartmentalization and cellular patterns of the tissue and its interfaces, as well as to model the dynamic biological and biophysical cue that govern tendon homeostasis while increasing representability of human tissues and reducing the need for animal experimentation. Consistent advances in this field will ultimately contribute for the development of improved therapeutic solutions for the challenging tendon injuries and pathologies.

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

The authors acknowledge the European Union's Horizon 2020 research and innovation program under European Research Council grant agreement 772817 and Twinning grant agreement no. 810850 – Achilles. Fundação para a Ciência e a Tecnologia (FCT) for project PTDC/NAN-MAT/30595/2017 and for CEECIND/01375/2017 (MGF) and 2020.03410.CEECIND (RMAD). Schematics in some figures were created with BioRender.com. The authors also want to acknowledge Mahwish Bakht (3B's Research Group, University of Minho) for designing Fig. 1.

References

[1] A. Scott, K. Squier, H. Alfredson, R. Bahr, J.L. Cook, B. Coombes, R.-J. de Vos, S.N. Fu, A. Grimaldi, J.S. Lewis, N. Maffulli, S.P. Magnusson, P. Malliaras, S. Mc Auliffe, E.H.G. Oei, C.R. Purdam, J.D. Rees, E.K. Rio, K. Gravare Silbernagel, C. Speed, A. Weir, J.M. Wolf, I.V.D. Akker-Scheek, B.T. Vicenzino, J. Zwerver, International Scientific Tendinopathy Symposium Consensus: Clinical Terminology, Br. J. Sports Med. 54 (5) (2020) 260–262.

[2] C. Hopkins, S.-C. Fu, E. Chua, X. Hu, C. Rolf, V.M. Mattila, L. Qin, P.-H. Yung, K.-M. Chan, Critical review on the socio-economic impact of tendinopathy, Asia-Pacific, J. Sport. Med. Arthrosc. Rehabil. Technol. 4 (2016) 9–20.

[3] T. Vos, A.A. Abajobir, K.H. Abate, C. Abbafati, K.M. Abbas, F. Abd-Allah, R.S. Abdulkader, A.M. Abdulle, T.A. Abebo, S.F. Abera, V. Aboyans, L.J. Abu-Raddad, I.N. Ackerman, A.A. Adamu, O. Adetokunboh, M. Afarideh, A. Afshin, S.K. Agarwal, R. Aggarwal, A. Agrawal, S. Agrawal, H. Ahmadieh, M.B. Ahmed, M.T. E. Aichour, A.N. Aichour, I. Aichour, S. Aiyar, R.O. Akinyemi, N. Akseer, F.H. Al Lami, F. Alahdab, Z. Al-Aly, K. Alam, N. Alam, T. Alam, D. Alasfoor, K.A. Alene, R. Ali, R. Alizadeh-Navaei, A. Alkerwi, F. Alla, P. Allebeck, C. Allen, F. Al-Maskari, R. Al-Raddadi, U. Alsharif, S. Alsowaidi, K.A. Altirkawi, A.T. Amare, E. Amini, W. Ammar, Y.A. Amoako, H.H. Andersen, C.A.T. Antonio, P. Anwari, J. Ärnlöv, A.I. Artaman, K.K. Aryal, H. Asayesh, S.W. Asgedom, R. Assadi, T.M. Atey, N.T. Atnafu, S.R. Atre, L. Avila-Burgos, E.F.G.A. Avokphako, A. Awasthi, U. Bacha, A. Badawi, K. Balakrishnan, A. Banerjee, M.S. Bannick, A. Barac, R.M. Barber, S.L. Barker-Collo, T. Bärnighausen, S. Barquera, L. Barregard, L.H. Barrero, S. Basu, B. Battista, K.E. Battle, B.T. Baune, S. Bazargan-Hejazi, J. Beardsley, N. Bedi, E. Beghi, Y. Béjot, B.B. Bekele, M.L. Bell, D.A. Bennett, I.M. Bensenor, J. Benson, A. Berhane, D.F. Berhe, E. Bernabé, B.D. Betsu, M. Beuran, A.S. Beyene, N. Bhala, A. Bhansali, S. Bhatt, Z.A. Bhutta, S. Biadgilign, B.K. Bicer, K. Bienhoff, B. Bikbov, C. Birungi, S. Biryukov, D. Bisanzio, H.M. Bizuayehu, D.J. Boneya, S. Boufous, R.R. A. Bourne, A. Brazinova, T.S. Brugha, R. Buchbinder, L.N.B. Bulto, B.R. Bumgarner, Z.A. Butt, L. Cahuana-Hurtado, E. Cameron, M. Car, H. Carabin, J. R. Carapetis, R. Cárdenas, D.O. Carpenter, J.J. Carrero, A. Carter, F. Carvalho, D. C. Casey, V. Caso, C.A. Castañeda-Orjuela, C.D. Castle, F. Catalá-López, H.-Y. Chang, J.-C. Chang, F.J. Charlson, H. Chen, M. Chibalabala, C.E. Chibueze, V.H. Chisumpa, A.A. Chitheer, D.J. Christopher, L.G. Ciobanu, M. Cirillo, D. Colombara, C. Cooper, P.A. Cortesi, M.H. Criqui, J.A. Crump, A.F. Dadi, K. Dalal, L. Dandona, R. Dandona, J. das Neves, D.V. Davitoiu, B. de Courten, D.D. De Leo, B.K. Defo, L. Degenhardt, S. Deiparine, R.P. Dellavalle, K. Deribe, D.C. Des Jarlais, S. Dey, S.D. Dharmaratne, P.K. Dhillon, D. Dicker, E.L. Ding, S. Djalalinia, H.P. Do, E.R. Dorsey, K.P.B. dos Santos, D. Douwes-Schultz, K.E. Doyle, T.R. Driscoll, M. Dubey, B.B. Duncan, Z.Z. El-Khatib, J. Ellerstrand, A. Enayati, A.Y. Endries, S.P. Ermakov, H.E. Erskine, B. Eshrati, S. Eskandarieh, A. Esteghamati, K. Estep, F.B.B. Fanuel, C.S.E.S. Farinha, A. Faro, F. Farzadfar, M.S. Fazeli, V.L. Feigin, S.-M. Fereshtehnejad, J.C. Fernandes, A.J. Ferrari, T.R. Feyissa, I. Filip, F. Fischer, C. Fitzmaurice, A.D. Flaxman, L.S. Flor, N. Foigt, K.J. Foreman, R.C. Franklin, N. Fullman, T. Fürst, J.M. Furtado, N.D. Futran, E. Gakidou, M. Ganji, A.L. Garcia-Basteiro, T. Gebre, T.T. Gebrehiwot, A. Geleto, B. L. Gemechu, H.A. Gesesew, P.W. Gething, A. Ghajar, K.B. Gibney, P.S. Gill, R.F. Gillum, I.A.M. Ginawi, A.Z. Giref, M.D. Gishu, G. Giussani, W.W. Godwin, A.L. Gold, E.M. Goldberg, P.N. Gona, A. Goodridge, S.V. Gopalani, A. Goto, A.C. Goulart, M. Griswold, H.C. Gugnani, R. Gupta, R. Gupta, T. Gupta, V. Gupta, N. Hafezi-Nejad, G.B. Hailu, A.D. Hailu, R.R. Hamadeh, S. Hamidi, A.J. Handal, G.J. Hankey, S.W. Hanson, Y. Hao, H.L. Harb, H.A. Hareri, J.M. Haro, J. Harvey, M.S. Hassanvand, R. Havmoeller, C. Hawley, S.I. Hay, R.J. Hay, N.J. Henry, I.B. Heredia-Pi, J.M. Hernandez, P. Heydarpour, H.W. Hoek, H.J. Hoffman, N. Horita, H.D. Hosgood, S. Hostiuc, P.J. Hotez, D.G. Hoy, A.S. Htet, G. Hu, H. Huang, C. Huynh, K.M. Iburg, E.U. Igumbor, C. Ikeda, C.M.S. Irvine, K.H. Jacobsen, N. Jahanmehr, M.B. Jakovljevic, S.K. Jassal, M. Javanbakht, S.P. Jayaraman, P. Jeemon, P.N. Jensen, V. Jha, G. Jiang, D. John, S.C. Johnson, C.O. Johnson, J.B. Jonas, M. Jürisson, Z. Kabir, R. Kadel, A. Kahsay, R. Kamal, H. Kan, N.E. Karam, A. Karch, C.K. Karema, A. Kasaeian, G.M. Kassa, N.A. Kassaw, N.J. Kassebaum, A. Kastor, S.V. Katikireddi, A. Kaul, N. Kawakami, P.N. Keiyoro, A.P. Kengne, A. Keren, Y.S. Khader, I.A. Khalil, E.A. Khan, Y.-H. Khang, A. Khosravi, J. Khubchandani, A.A. Kiadaliri, C. Kieling, Y.J. Kim, D. Kim, P. Kim, R.W. Kimokoti, Y. Kinfu, A. Kisa, K.A. Kissimova-Skarbek, M. Kivimaki, A.K. Knudsen, Y. Kokubo, D. Kolte, J.A. Kopec, S. Kosen, P.A. Koul, A.i. Koyanagi, M. Kravchenko, S. Krishnaswami, K.J. Krohn, G.A. Kumar, P. Kumar, S. Kumar, H.H. Kyu, D.K. Lal, R. Lalloo, N. Lambert, Q. Lan, A. Larsson, P.M. Lavados, J.L. Leasher, P.H. Lee, J.-T. Lee, J. Leigh, C.T. Leshargie, J. Leung, R. Leung, M. Levi, Y. Li, Y. Li, D. Li Kappe, X. Liang, M.L. Liben, S.S. Lim, S. Linn, P.Y. Liu, A. Liu, S. Liu, Y. Liu, R. Lodha, G. Logroscino, S.J. London, K.J. Looker, A.D. Lopez, S. Lorkowski, P.A. Lotufo, N. Low, R. Lozano, T.C.D. Lucas, E.R.K. Macarayan, H. Magdy Abd El Razek, M. Magdy Abd El Razek, M. Mahdavi, M. Majdan, R. Majdzadeh, A. Majeed, R. Malekzadeh, R. Malhotra, D.C. Malta, A.A. Mamun, H. Manguerra, T. Manhertz, A. Mantilla, L.G. Mantovani, C.C. Mapoma, L.B. Marczak, J. Martinez-Raga, F.R. Martins-Melo, I. Martopullo, W. März, M.R. Mathur, M. Mazidi, C. McAlinden, M. McGaughey, J.J. McGrath, M. McKee, C. McNellan, S. Mehata, M.M. Mehndiratta, T.C. Mekonnen, P. Memiah, Z.A. Memish, W. Mendoza, M.A. Mengistie, D.T. Mengistu, G.A. Mensah, T.J. Meretoja, A. Meretoja, H.B. Mezgebe, R. Micha, A. Millear, T.R. Miller, E.J. Mills, M. Mirarefin, E.M. Mirrakhimov, A. Misganaw, S.R. Mishra, P.B. Mitchell, K.A. Mohammad, A. Mohammadi, K.E. Mohammed, S. Mohammed, S.K. Mohanty, A.H. Mokdad, S.K. Mollenkopf, L. Monasta, M. Montico, M. Moradi-Lakeh, P. Moraga, R. Mori, C. Morozoff, S.D. Morrison, M. Moses, C. Mountjoy-Venning, K.B. Mruts, U.O. Mueller, K. Muller, M.E. Murdoch, G.V.S. Murthy, K.I. Musa, J. B. Nachega, G. Nagel, M. Naghavi, A. Naheed, K.S. Naidoo, L. Naldi, V. Nangia, G. Natarajan, D.E. Negasa, R.I. Negoi, I. Negoi, C.R. Newton, J.W. Ngunjiri, T.H. Nguyen, Q.L. Nguyen, C.T. Nguyen, G. Nguyen, M. Nguyen, E. Nichols, D.N.A. Ningrum, S. Nolte, V.M. Nong, B.o. Norrving, J.J.N. Noubiap, M.J. O'Donnell, F.A. Ogbo, I.-H. Oh, A. Okoro, O. Oladimeji, T.O. Olagunju, A.T. Olagunju, H.E. Olsen, B.O. Olusanya, J.O. Olusanya, K. Ong, J.N. Opio, E. Oren, A. Ortiz, A. Osgood-Zimmerman, M. Osman, M.O. Owolabi, M. Pa, R.E. Pacella, A. Pana, B.K. Panda, C. Papachristou, E.-K. Park, C.D. Parry, M. Parsaeian, S.B. Patten, G.C. Patton, K. Paulson, N. Pearce, D.M. Pereira, N. Perico, K. Pesudovs, C.B. Peterson, M. Petzold, M.R. Phillips, D.M. Pigott, J.D. Pillay, C. Pinho, D. Plass, M.A. Pletcher, S. Popova, R.G. Poulton, F. Pourmalek, D. Prabhakaran, N.M. Prasad, N. Prasad, C. Purcell, M. Qorbani, R. Quansah, B.P.A. Quintanilla, R.H.S. Rabiee, A. Radfar, A. Rafay, K. Rahimi, A. Rahimi-Movaghar, V. Rahimi-Movaghar, M.H.U. Rahman, M. Rahman, R.K. Rai, S. Rajsic, U. Ram, C.L. Ranabhat, Z. Rankin, P. C. Rao, P.V. Rao, S. Rawaf, S.E. Ray, R.C. Reiner, N. Reinig, M.B. Reitsma, G. Remuzzi, A.M.N. Renzaho, S. Resnikoff, S. Rezaei, A.L. Ribeiro, L. Ronfani, G. Roshandel, G.A. Roth, A. Roy, E. Rubagotti, G.M. Ruhago, S. Saadat, N. Sadat, M. Safdarian, S. Safi, S. Safiri, R. Sagar, R. Sahathevan, J. Salama, H.O.B. Saleem, J.A. Salomon, S.S. Salvi, A.M. Samy, J.R. Sanabria, D. Santomauro, I.S. Santos, J.V. Santos, M.M. Santric Milicevic, B. Sartorius, M. Satpathy, M. Sawhney, S. Saxena, M.I. Schmidt, I.J.C. Schneider, B. Schöttker, D.C. Schwebel, F. Schwendicke, S. Seedat, S.G. Sepanlou, E.E. Servan-Mori, T. Setegn, K.A. Shackelford, A. Shaheen, M.A. Shaikh, M. Shamsipour, S.M. Shariful Islam, J. Sharma, R. Sharma, J. She, P. Shi, C. Shields, G.T. Shifa, M. Shigematsu, Y. Shinohara, R. Shiri, R. Shirkoohi, S. Shirude, K. Shishani, M.G. Shrime, A.M. Sibai, I.D. Sigfusdottir, D.A.S. Silva, J.P. Silva, D.G.A. Silveira, J.A. Singh, N.P. Singh, D.N. Sinha, E. Skiadaresi, V. Skirbekk, E.L. Slepak, A. Sligar, D.L. Smith, M. Smith, B.H.A. Sobaih, E. Sobngwi, R.J.D. Sorensen, T.C.M. Sousa, L.A. Sposato, C.T. Sreeramareddy, V. Srinivasan, J.D. Stanaway, V. Stathopoulou, N. Steel, M.B. Stein, D.J. Stein, T.J. Steiner, C. Steiner, S. Steinke, M.A. Stokes, L.J. Stovner, B. Strub, M. Subart, M.B. Sufiyan, B.F. Sunguya, P.J. Sur, S. Swaminathan, B.L. Sykes, D.O. Sylte, R. Tabarés-Seisdedos, G.R. Taffere, J.S. Takala, N. Tandon, M. Tavakkoli, N. Taveira, H.R. Taylor, A. Tehrani-Banihashemi, T. Tekelab, A.S. Terkawi, D.J. Tesfaye, B. Tesssema, O. Thamsuwan, K.E. Thomas, A.G. Thrift, T.Y. Tiruye, R. Tobe-Gai, M.C. Tollanes, M. Tonelli, R. Topor-Madry, M. Tortajada, M. Touvier, B.X. Tran, S. Tripathi, C. Troeger, T. Truelsen, D. Tsoi, K.B. Tuem, E.M. Tuzcu, S. Tyrovolas, K.N. Ukwaja, E.A. Undurraga, C.J. Uneke, R. Updike, O.A. Uthman, B.S.C. Uzochukwu, J.F.M. van Boven, S. Varughese, T. Vasankari, S. Venkatesh, N. Venketasubramanian, R. Vidavalur, F.S. Violante, S.K. Vladimirov, V.V. Vlassov, S.E. Vollset, F. Wadilo, T. Wakayo, Y.-P. Wang, M. Weaver, S. Weichenthal, E. Weiderpass, R.G. Weintraub, A. Werdecker, R. Westerman, H.A. Whiteford, T. Wijeratne, C.S. Wiysonge, C.D.A. Wolfe, R. Woodbrook, A.D. Woolf, A. Workicho, D. Xavier, G. Xu, S. Yadgir, M. Yaghoubi, B. Yakob, L.L. Yan, Y. Yano, P. Ye, H.H. Yimam, P. Yip, N. Yonemoto, S.-J. Yoon, M. Yotebieng, M.Z. Younis, Z. Zaidi, M.E.S. Zaki, E. A. Zegeye, Z.M. Zenebe, X. Zhang, M. Zhou, B. Zipkin, S. Zodpey, L.J. Zuhlke, C.J. L. Murray, Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016, Lancet. 390 (10100) (2017) 1211–1259.

[4] T.S.O. Sleeswijk Visser, A.C. van der Vlist, R.F. van Oosterom, P. van Veldhoven, J.A.N. Verhaar, R.-J. de Vos, Impact of chronic Achilles tendinopathy on health-related quality of life, work performance, healthcare utilisation and costs, BMJ Open Sport Exerc. Med. 7 (1) (2021) e001023, https://doi.org/10.1136/bmjsem-2020-001023.

[5] H. Riel, C.F. Lindstrøm, M.S. Rathleff, M.B. Jensen, J.L. Olesen, Prevalence and incidence rate of lower-extremity tendinopathies in a Danish general practice: a registry-based study, BMC Musculoskelet. Disord. 20 (2019) 239, https://doi.org/10.1186/s12891-019-2629-6.

[6] U.M. Kujala, S. Sarna, J. Kaprio, Cumulative Incidence of Achilles Tendon Rupture and Tendinopathy in Male Former Elite Athletes, Clin. J. Sport Med. 15 (2005) 133–135, https://doi.org/10.1097/01.jsm.0000165347.55638.23.

[7] W.B. Leadbetter, Anti-inflammatory therapy in tendinopathy: The role of nonsteroidal drugs and corticosteroid injections, Tendon Inj. Basic Sci, Clin. Med. (2005) 211–232, https://doi.org/10.1007/1-84628-050-8_22.

[8] R. Aicale, R.D. Bisaccia, A. Oliviero, F. Oliva, N. Maffulli, Current pharmacological approaches to the treatment of tendinopathy, Expert Opin. Pharmacother. 21 (2020) 1467–1477, https://doi.org/10.1080/14656566.2020.1763306.

[9] A. Mishra, J. Woodall, A. Vieira, Treatment of Tendon and Muscle Using Platelet-Rich Plasma, Clin. Sports Med. 28 (1) (2009) 113–125.

[10] Y. Zhou, J.-C. Wang, PRP Treatment Efficacy for Tendinopathy: A Review of Basic Science Studies, Biomed Res. Int. 2016 (2016) 1–8.

[11] A.J. Lomas, C.N.M. Ryan, A. Sorushanova, N. Shologu, A.I. Sideri, V. Tsioli, G.C. Fthenakis, A. Tzora, I. Skoufos, L.R. Quinlan, G. O'Laighin, A.M. Mullen, J.L. Kelly, S. Kearns, M. Biggs, A. Pandit, D.I. Zeugolis, The past, present and future in scaffold-based tendon treatments, Adv. Drug Deliv. Rev. 84 (2015) 257–277.

[12] D. Challoumas, C. Clifford, P. Kirwan, N.L. Millar, How does surgery compare to sham surgery or physiotherapy as a treatment for tendinopathy? A systematic review of randomised trials, BMJ Open Sport Exerc. Med. 5 (1) (2019) e000528, https://doi.org/10.1136/bmjsem-2019-000528.

[13] A. Hoffmann, G. Gross, Tendon and ligament engineering: from cell biology to in vivo application, Regen. Med. 1 (2006) 563–574, https://doi.org/10.2217/17460751.1.4.563.

[14] M.W. Hast, A. Zuskov, L.J. Soslowsky, The role of animal models in tendon research, Bone Joint Res. 3 (2014) 193–202, https://doi.org/10.1302/2046-3758.36.2000281.

[15] J.H. Shepherd, K. Legerlotz, T. Demirci, C. Klemt, G.P. Riley, H.RC. Screen, Functionally distinct tendon fascicles exhibit different creep and stress relaxation behaviour, Proc. Inst. Mech. Eng. Part H J. Eng. Med. 228 (1) (2014) 49–59.

[16] M.A. Brehm, L.D. Shultz, J. Luban, D.L. Greiner, Overcoming Current Limitations in Humanized Mouse Research, J. Infect. Dis. 208 (2013) S125–S130, https://doi.org/10.1093/infdis/jit319.

[17] S.L. Wunderli, U. Blache, J.G. Snedeker, Tendon explant models for physiologically relevant in vitro study of tissue biology–a perspective, Connect. Tissue Res. 61 (3-4) (2020) 262–277.
[18] G. Riley, The pathogenesis of tendinopathy, A molecular perspective, Rheumatology. 43 (2004) 131–142, https://doi.org/10.1093/rheumatology/keg448.
[19] I.N.E. Wang, H.H. Lu, Role of cell-cell interactions on the regeneration of soft tissue-to-bone interface, Annu. Int. Conf. IEEE Eng. Med. Biol. - Proc. (2006) 783–786, https://doi.org/10.1109/IEMBS.2006.259456.
[20] M.E. Wall, N.A. Dyment, J. Bodle, J. Volmer, E. Loboa, A. Cederlund, et al., Cell Signaling in Tenocytes: Response to Load and Ligands in Health and Disease, in: Adv. Exp. Med. Biol., 2016: pp. 79–95. doi:10.1007/978-3-319-33943-6_7.
[21] C.T. Thorpe, C.P. Udeze, H.L. Birch, P.D. Clegg, H.R.C. Screen, Specialization of tendon mechanical properties results from interfascicular differences, J. R. Soc. Interface. 9 (2012) 3108–3117, https://doi.org/10.1098/rsif.2012.0362.
[22] 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, https://doi.org/10.1016/j.actbio.2016.06.012.
[23] J.G. Snedeker, J. Foolen, Tendon injury and repair – A perspective on the basic mechanisms of tendon disease and future clinical therapy, Acta Biomater. 63 (2017) 18–36, https://doi.org/10.1016/j.actbio.2017.08.032.
[24] H.R.C. Screen, D.E. Berk, K.E. Kadler, F. Ramirez, M.F. Young, Tendon Functional Extracellular Matrix, J. Orthop. Res. 33 (2015) 793–799, https://doi.org/10.1002/jor.22818.
[25] M. Benjamin, E. Kaiser, S. Milz, Structure-function relationships in tendons: A review, J. Anat. 212 (2008) 211–228, https://doi.org/10.1111/j.1469-7580.2008.00864.x.
[26] J.K. Mouw, G. Ou, V.M. Weaver, Extracellular matrix assembly: a multiscale deconstruction, Nat. Rev. Mol. Cell Biol. 15 (2014) 771–785, https://doi.org/10.1038/nrm3902.
[27] J.H.C. Wang, Mechanobiology of tendon, J. Biomech. 39 (2006) 1563–1582, https://doi.org/10.1016/j.jbiomech.2005.05.011.
[28] B. Depalle, Z. Qin, S.J. Shefelbine, M.J. Buehler, Influence of cross-link structure, density and mechanical properties in the mesoscale deformation mechanisms of collagen fibrils, J. Mech. Behav. Biomed. Mater. 52 (2015) 1–13, https://doi.org/10.1016/j.jmbbm.2014.07.008.
[29] C.T. Thorpe, H.L. Birch, P.D. Clegg, H.R.C. Screen, The role of the non-collagenous matrix in tendon function, Int. J. Exp. Pathol. 94 (2013) 248–259, https://doi.org/10.1111/iep.12027.
[30] Y.-L. Sun, Z. Wei, C. Zhao, G.D. Jay, T.M. Schmid, P.C. Amadio, et al., Lubricin in human achilles tendon: The evidence of intratendinous sliding motion and shear force in achilles tendon, J. Orthop. Res. 33 (2015) 932–937, https://doi.org/10.1002/jor.22897.
[31] T.M. Grant, M.S. Thompson, J. Urban, J. Yu, Elastic fibres are broadly distributed in tendon and highly localized around tenocytes, J. Anat. 222 (2013) 573–579, https://doi.org/10.1111/joa.12048.
[32] M.S. Godinho, C.T. Thorpe, S.E. Greenwald, C. Screen, Elastase treatment of tendon specifically impacts the mechanical 1 properties of the interfascicular matrix 2 3, (n.d.). doi:10.1101/2020.09.18.303081.
[33] H. Wolfenson, B. Yang, M.P. Sheetz, Steps in Mechanotransduction Pathways that Control Cell Morphology, Annu. Rev. Physiol. 81 (2019) 585–605, https://doi.org/10.1146/annurev-physiol-021317-121245.
[34] M.J. Dalby, A.J. García, M. Salmeron-Sanchez, Receptor control in mesenchymal stem cell engineering, Nat. Rev. Mater. 3 (2018) 17091, https://doi.org/10.1038/natrevmats.2017.91.
[35] M. Kaya, N. Karahan, B. Yılmaz, Tendons, IntechOpen, 2019.
[36] M. Grinstein, J.L. Galloway, Developmental Biology in Tendon Tissue Engineering, Elsevier Inc., 2018. doi:10.1016/b978-0-12-811467-4.00008-5.
[37] F.S. Chuen, C.Y. Chuk, W.Y. Ping, W.W. Nar, H.L. Kim, C.K. Ming, Immunohistochemical characterization of cells in adult human patellar tendons, J. Histochem. Cytochem. 52 (2004) 1151–1157, https://doi.org/10.1369/jhc.3A6232.2004.
[38] C.T. Thorpe, M.J. Peffers, D. Simpson, E. Halliwell, H.R.C. Screen, P.D. Clegg, Anatomical heterogeneity of tendon: Fascicular and interfascicular tendon compartments have distinct proteomic composition, Sci. Rep. 6 (2016) 20455, https://doi.org/10.1038/srep20455.
[39] C.T. Thorpe, H.R.C. Screen, Tendon Structure and Composition, in: Adv. Exp. Med. Biol., 2016: pp. 3–10. doi:10.1007/978-3-319-33943-6_1.
[40] E.M. Spiesz, C.T. Thorpe, S. Chaudhry, G.P. Riley, H.L. Birch, P.D. Clegg, et al., Tendon extracellular matrix damage, degradation and inflammation in response to in vitro overload exercise, J. Orthop. Res. 33 (2015) 889–897, https://doi.org/10.1002/jor.22879.
[41] 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), https://doi.org/10.1038/s41598-020-70786-5.
[42] 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) 1494–1497.
[43] Y. Bi, D. Ehirchiou, T.M. Kilts, C.A. Inkson, M.C. Embree, W. Sonoyama, L.i. Li, A. I. Leet, B.-M. Seo, L.i. 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.
[44] Z. Huang, Z.i. 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), https://doi.org/10.3389/fcell.2021.631272.
[45] B. Walia, A.H. Huang, Tendon stem progenitor cells: Understanding the biology to inform therapeutic strategies for tendon repair, J. Orthop. Res. 37 (2019) 1270–1280, https://doi.org/10.1002/jor.24156.
[46] Z.i. 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.-W. Ouyang, Single-cell analysis reveals a nestin + tendon stem/progenitor cell population with strong tenogenic potentiality, Sci. Adv. 2 (11) (2016), https://doi.org/10.1126/sciadv.1600874.
[47] 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 (2019) 1490–1503, https://doi.org/10.1038/s41556-019-0417-z.
[48] Y. Cho, H.-S. Kim, D. Kang, H. Kim, N. Lee, J. Yun, Y.-J. Kim, K.M. Lee, J.-H. Kim, H.-R. Kim, Y.-i. Hwang, C.H. Jo, J.-H. Kim, CTRP3 exacerbates tendinopathy by dysregulating tendon stem cell differentiation and altering extracellular matrix composition, Sci. Adv. 7 (47) (2021), https://doi.org/10.1126/sciadv.abg6069.
[49] 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 (2013) 199–210, https://doi.org/10.1089/ten.tea.2012.0182.
[50] D. Docheva, E.B. Hunziker, R. Fässler, O. Brandau, Tenomodulin Is Necessary for Tenocyte Proliferation and Tendon Maturation, Mol. Cell. Biol. 25 (2005) 699–705, https://doi.org/10.1128/MCB.25.2.699-705.2005.
[51] S. Dex, D. Lin, C. Shukunami, D. Docheva, Tenogenic modulating insider factor: Systematic assessment on the functions of tenomodulin gene, Gene. 587 (2016) 1–17, https://doi.org/10.1016/j.gene.2016.04.051.
[52] R. Schweitzer, J.H. Chyung, L.C. Murtaugh, A.E. Brent, V. Rosen, E.N. Olson, A. Lassar, C.J. Tabin, Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments, Development. 128 (19) (2001) 3855–3866.
[53] R. Chiquet-Ehrismann, R.P. Tucker, Connective tissues: Signalling by tenascins, Int. J. Biochem. Cell Biol. 36 (2004) 1085–1089, https://doi.org/10.1016/j.biocel.2004.01.007.
[54] Y. Ito, N. Toriuchi, T. Yoshitaka, H. Ueno-Kudoh, T. Sato, S. Yokoyama, K. Nishida, T. Akimoto, M. Takahashi, S. Miyaki, H. Asahara, The Mohawk homeobox gene is a critical regulator of tendon differentiation, Proc. Natl. Acad. Sci. U. S. A. 107 (23) (2010) 10538–10542.
[55] N.D. Murchison, B.A. Price, D.A. Conner, D.R. Keene, E.N. Olson, C.J. Tabin, et al., Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons, Development. 134 (2007) 2697–2708, https://doi.org/10.1242/dev.001933.
[56] T. Sakabe, K. Sakai, T. Maeda, A. Sunaga, N. Furuta, R. Schweitzer, T. Sasaki, T. Sakai, Transcription factor scleraxis vitally contributes to progenitor lineage direction in wound healing of adult tendon in mice, J. Biol. Chem. 293 (16) (2018) 5766–5780.
[57] Z.i. Yin, J. Lin, R. Yan, R. Liu, M. Liu, B.o. Zhou, W. Zhou, C. An, Y. Chen, Y. Hu, C. Fan, K. Zhao, B. Wu, X. Zou, J. Zhang, A.H. El-Hashash, X. Chen, H. Ouyang, Atlas of Musculoskeletal Stem Cells with the Soft and Hard Tissue Differentiation Architecture, Adv. Sci. 7 (23) (2020) 2000938, https://doi.org/10.1002/advs.202000938.
[58] K.T. Best, K.E. Mora, E. Knapp, M.R. Buckley, A.E. Loiselle, Scleraxis-Lineage Cell Depletion Improves Tendon Healing, BioRxiv. (2020) 1–9, https://doi.org/10.1101/2020.02.01.93025.
[59] A.E.C. Nichols, R.E. Settlage, S.R. Werre, L.A. Dahlgren, Novel roles for scleraxis in regulating adult tenocyte function, BMC Cell Biol. 19 (2018) 14, https://doi.org/10.1186/s12860-018-0166-z.
[60] T. Stauber, U. Blache, J.G. Snedeker, Tendon tissue microdamage and the limits of intrinsic repair, Matrix Biol. 85–86 (2020) 68–79, https://doi.org/10.1016/j.matbio.2019.07.008.
[61] H.R.C. Screen, Hierarchical approaches to understanding tendon mechanics, J. Biomech. Sci. Eng. 4 (2009) 481–499, https://doi.org/10.1299/jbse.4.481.
[62] 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.
[63] S.A. Eming, T.A. Wynn, P. Martin, Inflammation and metabolism in tissue repair and regeneration, Science 356 (6342) (2017) 1026–1030.
[64] M. Schneider, P. Angele, T.A.H. Järvinen, D. Docheva, Rescue plan for Achilles: Therapeutics steering the fate and functions of stem cells in tendon wound healing, Adv. Drug Deliv. Rev. 129 (2018) 352–375, https://doi.org/10.1016/j.addr.2017.12.016.
[65] S. Gordon, L. Martinez-Pomares, Physiological roles of macrophages, Pflugers Arch. Eur, J. Physiol. 469 (2017) 365–374, https://doi.org/10.1007/s00424-017-1945-7.
[66] H.-T. Xu, C.-W. Lee, M.-Y. Li, Y.-F. Wang, P.-S.-H. Yung, O.-K.-S. Lee, The shift in macrophages polarisation after tendon injury: A systematic review, J. Orthop. Transl. 21 (2020) 24–34, https://doi.org/10.1016/j.jot.2019.11.009.
[67] S.A. Müller, A. Todorov, P.E. Heisterbach, I. Martin, M. Majewski, Tendon healing: an overview of physiology, biology, and pathology of tendon healing and systematic review of state of the art in tendon bioengineering, Knee Surgery, Sport. Traumatol. Arthrosc. 23 (2015) 2097–2105, https://doi.org/10.1007/s00167-013-2680-z.

[68] S. Korntner, C. Lehner, R. Gehwolf, A. Wagner, M. Grütz, N. Kunkel, H. Tempfer, A. Traweger, Limiting angiogenesis to modulate scar formation, Adv. Drug Deliv. Rev. 146 (2019) 170–189.

[69] N.A. Dyment, C.-F. Liu, N. Kazemi, L.E. Aschbacher-Smith, K. Kenter, A.P. Breidenbach, J.T. Shearn, C. Wylie, D.W. Rowe, D.L. Butler, E. Mezey, The Paratenon Contributes to Scleraxis-Expressing Cells during Patellar Tendon Healing, PLoS One. 8 (3) (2013) e59944, https://doi.org/10.1371/journal.pone.0059944.

[70] G. Jomaa, C.-K. Kwan, S.-C. Fu, S.-K.-K. Ling, K.-M. Chan, P.-S.-H. Yung, et al., A systematic review of inflammatory cells and markers in human tendinopathy, BMC Musculoskelet. Disord. 21 (2020) 78, https://doi.org/10.1186/s12891-020-3094-y.

[71] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: Therapeutic translation for fibrotic disease, Nat. Med. 18 (2012) 1028–1040, https://doi.org/10.1038/nm.2807.

[72] M.D. Davidson, J.A. Burdick, R.G. Wells, Engineered Biomaterial Platforms to Study Fibrosis, Adv. Healthc. Mater. 1901682 (2020) 1901682, https://doi.org/10.1002/adhm.201901682.

[73] M. Benjamin, J.R. Ralphs, Tendons and ligaments - An overview, Histol. Histopathol. 12 (1997) 1135–1144.

[74] J.G. Graham, M.L. Wang, M. Rivlin, P.K. Beredjiklian, Biologic and mechanical aspects of tendon fibrosis after injury and repair, Connect. Tissue Res. 60 (2019) 10–20, https://doi.org/10.1080/03008207.2018.1512979.

[75] A.E.C. Nichols, K.T. Best, A.E. Loiselle, The cellular basis of fibrotic tendon healing: challenges and opportunities, Transl. Res. 209 (2019) 156–168, https://doi.org/10.1016/j.trsl.2019.02.002.

[76] T.A. Wynn, K.M. Vannella, Macrophages in Tissue Repair, Regeneration, and Fibrosis, Immunity. 44 (2016) 450–462, https://doi.org/10.1016/j.immuni.2016.02.015.

[77] L. Chen, J. Li, J. Zhang, C. Dai, X. Liu, J. Wang, Z. Gao, H. Guo, R. Wang, S. Lu, F. Wang, H. Zhang, H. Chen, X. Fan, S. Wang, Z. Qin, S100A4 promotes liver fibrosis via activation of hepatic stellate cells, J. Hepatol. 62 (1) (2015) 156–164.

[78] J.E. Ackerman, K.T. Best, R.J. O'Keefe, A.E. Loiselle, Deletion of EP4 in S100a4-lineage cells reduces scar tissue formation during early but not later stages of tendon healing, Sci. Rep. 7 (2017) 8658, https://doi.org/10.1038/s41598-017-09407-7.

[79] J.E. Ackerman, A.E. Nichols, V. Studentsova, K.T. Best, E. Knapp, A.E. Loiselle, Cell non-autonomous functions of S100a4 drive fibrotic tendon healing, Elife. 8 (2019), https://doi.org/10.7554/eLife.45342.

[80] X. Meng, D.J. Nikolic-Paterson, H.Y. Lan, TGF-β: the master regulator of fibrosis, Nat. Rev. Nephrol. 12 (2016) 325–338, https://doi.org/10.1038/nrneph.2016.48.

[81] H. Jorgensen, S. McLellan, J. Crossan, A. Curtis, Neutralisation of TGFβ or binding of VLA-4 to fibronectin prevents rat tendon adhesion following transection, Cytokine. 30 (2005) 195–202, https://doi.org/10.1016/j.cyto.2004.12.017.

[82] M.S. Wietecha, M. Pensalfini, M. Cangkrama, B. Müller, J. Jin, J. Brinckmann, E. Mazza, S. Werner, Activin-mediated alterations of the fibroblast transcriptome and matrisome control the biomechanical properties of skin wounds, Nat. Commun. 11 (1) (2020), https://doi.org/10.1038/s41467-020-16409-z.

[83] Q. Zhang, D. Zhou, H. Wang, J. Tan, Heterotopic ossification of tendon and ligament, J. Cell. Mol. Med. 24 (2020) 5428–5437, https://doi.org/10.1111/jcmm.15240.

[84] S. Asai, S. Otsuru, M.E. Candela, L. Cantley, K. Uchibe, T.J. Hofmann, K. Zhang, K.L. Wapner, L.J. Soslowsky, E.M. Horwitz, M. Enomoto-Iwamoto, Tendon Progenitor Cells in Injured Tendons Have Strong Chondrogenic Potential: The CD105-Negative Subpopulation Induces Chondrogenic Degeneration, Stem Cells. 32 (12) (2014) 3266–3277.

[85] E. Gracey, A. Burssens, I. Cambré, G. Schett, R. Lories, I.B. McInnes, H. Asahara, D. Elewaut, Tendon and ligament mechanical loading in the pathogenesis of inflammatory arthritis, Nat. Rev. Rheumatol. 16 (4) (2020) 193–207.

[86] K.M. Vannella, T.A. Wynn, Mechanisms of Organ Injury and Repair by Macrophages*, Annu. Rev. Physiol. 79 (2017) 593–617, https://doi.org/10.1146/annurev-physiol-022516-034356.

[87] P. Martin, S.J. Leibovich, Inflammatory cells during wound repair: The good, the bad and the ugly, Trends Cell Biol. 15 (2005) 599–607, https://doi.org/10.1016/j.tcb.2005.09.002.

[88] D. Marsolais, C.H. Coté, J. Frenette, Neutrophils and macrophages accumulate sequentially following Achilles tendon injury, J. Orthop. Res. 19 (2001) 1203–1209, https://doi.org/10.1016/S0736-0266(01)00031-6.

[89] L.C. Davies, P.R. Taylor, Tissue-resident macrophages: Then and now, Immunology. 144 (2015) 541–548, https://doi.org/10.1111/imm.12451.

[90] S.G. Dakin, F.O. Martinez, C. Yapp, G. Wells, U. Oppermann, B.J.F. Dean, R.D.J. Smith, K. Wheway, B. Watkins, L. Roche, A.J. Carr, Inflammation activation and resolution in human tendon disease, Sci. Transl. Med. 7 (311) (2015), https://doi.org/10.1126/scitranslmed.aac4269.

[91] N.L. Millar, G.A.C. Murrell, I.B. McInnes, Inflammatory mechanisms in tendinopathy – towards translation, Nat. Rev. Rheumatol. 13 (2017) 110–122, https://doi.org/10.1038/nrrheum.2016.213.

[92] C. Lehner, G. Spitzer, R. Gehwolf, A. Wagner, N. Weissenbacher, C. Deininger, et al., Tenophages: a novel macrophage-like tendon cell population expressing CX3CL1 and CX3CR1, Dis. Model. Mech. (2019). doi:10.1242/dmm.041384.

[93] E. Mass, I. Ballesteros, M. Farlik, F. Halbritter, P. Günther, L. Crozet, C.E. Jacome-Galarza, K. Händler, 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).

[94] S.J. Galli, N. Borregaard, T.A. Wynn, Phenotypic and functional plasticity of cells of innate immunity: Macrophages, mast cells and neutrophils, Nat. Immunol. 12 (2011) 1035–1044, https://doi.org/10.1038/ni.2109.

[95] H. Behzad, A. Sharma, R. Mousavizadeh, A. Lu, A. Scott, Mast cells exert pro-inflammatory effects of relevance to the pathophyisology of tendinopathy, Arthritis Res. Ther. 15 (2013) R184, https://doi.org/10.1186/ar4374.

[96] C. Tang, Y. Chen, J. Huang, K. Zhao, X. Chen, Z.i. Yin, B.C. Heng, W. Chen, W. Shen, The roles of inflammatory mediators and immunocytes in tendinopathy, Elsevier (Singapore), Pte Ltd 14 (2018) 23–33.

[97] I.T. Harvima, G. Nilsson, Mast cells as regulators of skin inflammation and immunity, Acta Derm. Venereol. 91 (2011) 644–650, https://doi.org/10.2340/00015555-1197.

[98] R.J. Lories, I.B. McInnes, Primed for inflammation: Enthesis-resident T cells, Nat. Med. 18 (2012) 1018–1019, https://doi.org/10.1038/nm.2854.

[99] H. Tempfer, A. Traweger, Tendon Vasculature in Health and Disease, Front. Physiol. 6 (2015), https://doi.org/10.3389/fphys.2015.00330.

[100] S.L. Wunderli, U. Blache, A. Beretta Piccoli, B. Niederöst, C.N. Holenstein, F.S. Passini, U. Silván, L. Bundgaard, U. auf dem Keller, J.G. Snedeker, Tendon response to matrix unloading is determined by the patho-physiological niche, Matrix Biol. 89 (2020) 11–26.

[101] H. Tempfer, A. Wagner, R. Gehwolf, C. Lehner, M. Tauber, H. Resch, H.C. Bauer, Perivascular cells of the supraspinatus tendon express both tendon- and stem cell-related markers, Histochem. Cell Biol. 131 (6) (2009) 733–741.

[102] X. Liu, B. Zhu, Y. Li, X. Liu, S. Guo, C. Wang, S. Li, D. Wang, The Role of Vascular Endothelial Growth Factor in Tendon Healing, Front. Physiol. 12 (2021), https://doi.org/10.3389/fphys.2021.766080.

[103] H. Tempfer, G. Spitzer, C. Lehner, A. Wagner, R. Gehwolf, J. Fierlbeck, N. Weissenbacher, M. Jessen, L.M. Heindl, A. Traweger, VEGF-D-mediated signaling in tendon cells is involved in degenerative processes, FASEB J. 36 (2) (2022), https://doi.org/10.1096/fj.202100773RRR.

[104] M.N. van Sterkenburg, C.N. van Dijk, Mid-portion Achilles tendinopathy: why painful? An evidence-based philosophy, Knee Surgery, Sport. Traumatol. Arthrosc. 19 (2011) 1367–1375, https://doi.org/10.1007/s00167-011-1535-8.

[105] W. Petersen, D. Varoga, T. Zantop, J. Hassenpflug, R. Mentlein, T. Pufe, Cyclic strain influences the expression of the vascular endothelial growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1α) in tendon fibroblasts, J. Orthop. Res. 22 (2004) 847–853, https://doi.org/10.1016/j.orthres.2003.11.009.

[106] H. Tempfer, A. Kaser-Eichberger, C. Lehner, R. Gehwolf, S. Korntner, N. Kunkel, A. Wagner, M. Gruetz, L. Heindl, F. Schroedl, A. Traweger, Bevacizumab Improves Achilles Tendon Repair in a Rat Model, Cell. Physiol. Biochem. 46 (3) (2018) 1148–1158.

[107] Y. Oshima, K. Sato, F. Tashiro, J.-I. Miyazaki, K. Nishida, Y. Hiraki, Y. Tano, C. Shukunami, Anti-angiogenic action of the C-terminal domain of tenomodulin that shares homology with chondromodulin-I, J. Cell Sci. 117 (13) (2004) 2731–2744.

[108] D. Lin, P. Alberton, M.D. Caceres, E. Volkmer, M. Schieker, D. Docheva, Tenomodulin is essential for prevention of adipocyte accumulation and fibrovascular scar formation during early tendon healing, Cell Death Dis. 8 (2017) e3116, https://doi.org/10.1038/cddis.2017.510.

[109] I. Calejo, R. Costa-Almeida, M.E. Gomes, Cellular Complexity at the Interface: Challenges in Enthesis Tissue Engineering, Adv. Exp. Med. Biol. 1144 (2019) 71–90, https://doi.org/10.1007/5584_2018_307.

[110] J. Struewer, P.P. Roessler, K.F. Schuettler, V. Ruppert, T. Stein, N. Timmesfeld, J. R.J. Paletta, T. Efe, Influence of cyclical mechanical loading on osteogenic markers in an osteoblast-fibroblast co-culture in vitro: Tendon-to-bone interface in anterior cruciate ligament reconstruction, Int. Orthop. 38 (5) (2014) 1083–1089.

[111] I.-N. Wang, J. Shan, R. Choi, S. Oh, C.K. Kepler, F.H. Chen, H.H. Lu, Role of osteoblast-fibroblast interactions in the formation of the ligament-to-bone interface, J. Orthop. Res. 25 (12) (2007) 1609–1620.

[112] K.W. VanDusen, L.M. Larkin, Muscle-tendon interface, Elsevier Ltd (2015), https://doi.org/10.1016/B978-1-78242-301-0.00017-3.

[113] M. Kobayashi, N. Watanabe, Y. Oshima, Y. Kajikawa, M. Kawata, T. Kubo, The fate of host and graft cells in early healing of bone tunnel after tendon graft, Am. J. Sports Med. 33 (2005) 1892–1897, https://doi.org/10.1177/0363546505277140.

[114] E. Soreide, J.M. Denbeigh, E.A. Lewallen, R. Thaler, R.M. Samsonraj, D.L. Jones, W. Xu, D. Larson, L. Nordsletten, S. Kakar, A.J. van Wijnen, A multi-chamber tissue culture device for load-dependent parallel evaluation of tendon explants, BMC Musculoskelet. Disord. 20 (1) (2019), https://doi.org/10.1186/s12891-019-2896-2.

[115] R.M.A. Domingues, S. Chiera, P. Gershovich, A. Motta, R.L. Reis, M.E. Gomes, Enhancing the Biomechanical Performance of Anisotropic Nanofibrous Scaffolds in Tendon Tissue Engineering: Reinforcement with Cellulose Nanocrystals, Adv. Healthc. Mater. 5 (2016) 1364–1375, https://doi.org/10.1002/adhm.201501048.

[116] A. Dede Eren, A. Vasilevich, E.D. Eren, P. Sudarsanam, U. Tuvshindorj, J. de Boer, J. Foolen, Tendon-Derived Biomimetic Surface Topographies Induce Phenotypic Maintenance of Tenocytes In Vitro, Tissue Eng. Part A. 27 (15-16) (2021) 1023–1036.

[117] 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. Niederöst, A.N. Horvath, T. Götschi, S. Ma, B. Passini-Tall, S.F. Fucentese, U. Blache, U. Silván, 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.
[118] D. Patel, S. Sharma, S.J. Bryant, H.R.C. Screen, Recapitulating the Micromechanical Behavior of Tension and Shear in a Biomimetic Hydrogel for Controlling Tenocyte Response, Adv. Healthc. Mater. 6 (2017) 1601095, https://doi.org/10.1002/adhm.201601095.
[119] 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) 120034, https://doi.org/10.1016/j.biomaterials.2020.120034.
[120] Z. Yan, H. Yin, C. Brochhausen, C.G. Pfeifer, V. Alt, D. Docheva, Aged Tendon Stem/Progenitor Cells Are Less Competent to Form 3D Tendon Organoids Due to Cell Autonomous and Matrix Production Deficits, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.00406.
[121] J. Kohler, C. Popov, B. Klotz, P. Alberton, W.C. Prall, F. Haasters, S. Müller-Deubert, R. Ebert, L. Klein-Hitpass, F. Jakob, M. Schieker, D. Docheva, Uncovering the cellular and molecular changes in tendon stem/progenitor cells attributed to tendon aging and degeneration, Aging Cell. 12 (6) (2013) 988–999.
[122] S.K. Denduluri, B. Scott, J.D. Lamplot, L. Yin, Z. Yan, Z. Wang, J. Ye, J. Wang, Q. Wei, M.K. Mohammed, R.C. Haydon, R.W. Kang, T.-C. He, A. Athiviraham, S.H. Ho, L.L. Shi, Immortalized Mouse Achilles Tenocytes Demonstrate Long-Term Proliferative Capacity While Retaining Tenogenic Properties, Tissue Eng. Part C, Methods. 22 (3) (2016) 280–289.
[123] A.E.C. Nichols, S.N. Muscat, S.E. Miller, L.J. Green, M.S. Richards, A.E. Loiselle, Impact of isolation method on cellular activation and presence of specific tendon cell subpopulations during in vitro culture, FASEB J. 35 (2021), https://doi.org/10.1096/fj.202100405R.
[124] K. Spanoudes, D. Gaspar, A. Pandit, D.I. Zeugolis, The biophysical, biochemical, and biological toolbox for tenogenic phenotype maintenance in vitro, Trends Biotechnol. 32 (2014) 474–482, https://doi.org/10.1016/j.tibtech.2014.06.009.
[125] D. Docheva, S.A. Müller, M. Majewski, C.H. Evans, Biologics for tendon repair, Adv. Drug Deliv. Rev. 84 (2015) 222–239, https://doi.org/10.1016/j.addr.2014.11.015.
[126] T. Nakajima, M. Ikeya, Development of pluripotent stem cell-based human tenocytes, Dev. Growth Differ. 63 (2021) 38–46, https://doi.org/10.1111/dgd.12702.
[127] T. Nakajima, A. Nakahata, N. Yamada, K. Yoshizawa, T.M. Kato, M. Iwasaki, C. Zhao, H. Kuroki, M. Ikeya, Grafting of iPS cell-derived tenocytes promotes motor function recovery after Achilles tendon rupture, Nat. Commun. 12 (1) (2021), https://doi.org/10.1038/s41467-021-25328-6.
[128] C. Liu, A. Oikonomopoulos, N. Sayed, J.C. Wu, Modeling human diseases with induced pluripotent stem cells: from 2D to 3D and beyond, Development. 145 (2018), https://doi.org/10.1242/dev.156166.
[129] M. Stolk, F. Klatte-Schulz, A. Schmock, S. Minkwitz, B. Wildemann, M. Seifert, New insights into tenocyte-immune cell interplay in an in vitro model of inflammation, Sci. Rep. 7 (2017) 9801, https://doi.org/10.1038/s41598-017-09875-x.
[130] T. Stauber, M. Wolleb, A. Duss, P.K. Jaeger, I. Heggli, A.A. Hussien, U. Blache, J.G. Snedeker, Extrinsic Macrophages Protect While Tendon Progenitors Degrade: Insights from a Tissue Engineered Model of Tendon Compartmental Crosstalk, Adv. Healthc. Mater. 10 (20) (2021) 2100741.
[131] 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.
[132] K.L. Hui, L. Balagopalan, L.E. Samelson, A. Upadhyaya, Y.-L. Wang, Cytoskeletal forces during signaling activation in Jurkat T-cells, Mol. Biol. Cell. 26 (4) (2015) 685–695.
[133] S. Wu, Y. Wang, P.N. Streubel, B. Duan, Living nanofiber yarn-based woven biotextiles for tendon tissue engineering using cell tri-culture and mechanical stimulation, Acta Biomater. 62 (2017) 102–115, https://doi.org/10.1016/j.actbio.2017.08.043.
[134] H. Yin, Z. Yan, R.J. Bauer, J. Peng, M. Schieker, M. Nerlich, D. Docheva, Functionalized thermosensitive hydrogel combined with tendon stem/progenitor cells as injectable cell delivery carrier for tendon tissue engineering, Biomed. Mater. 13 (3) (2018) 034107, https://doi.org/10.1088/1748-605X/aaadd1.
[135] S. Muller, I. Ader, J. Creff, H. Leménager, P. Achard, L. Casteilla, L. Sensebé, A. Carrière, F. Deschaseaux, Human adipose stromal-vascular fraction self-organizes to form vascularized adipose tissue in 3D cultures, Sci. Rep. 9 (1) (2019), https://doi.org/10.1038/s41598-019-43624-6.
[136] D.-H. Kang, F. Louis, H. Liu, H. Shimoda, Y. Nishiyama, H. Nozawa, M. Kakitani, D. Takagi, D. Kasa, E. Nagamori, S. Irie, S. Kitano, M. Matsusaki, Engineered whole cut meat-like tissue by the assembly of cell fibers using tendon-gel integrated bioprinting, Nat. Commun. 12 (1) (2021), https://doi.org/10.1038/s41467-021-25236-9.
[137] S.M. Mihaila, A.M. Frias, R.P. Pirraco, T. Rada, R.L. Reis, M.E. Gomes, A.P. Marques, Human Adipose Tissue-Derived SSEA-4 Subpopulation Multi-Differentiation Potential Towards the Endothelial and Osteogenic Lineages, Tissue Eng. Part A 19 (1-2) (2013) 235–246.
[138] S.J. Warden, Animal models for the study of tendinopathy, Br. J. Sports Med. 41 (2007) 232–240, https://doi.org/10.1136/bjsm.2006.032342.
[139] G.L. Oreff, M. Fenu, C. Vogl, I. Ribitsch, F. Jenner, Species variations in tenocytes' response to inflammation require careful selection of animal models for tendon research, Sci. Rep. 11 (2021) 12451, https://doi.org/10.1038/s41598-021-91914-9.
[140] N. Marr, R. Meeson, E.F. Kelly, Y. Fang, M.J. Peffers, A.A. Pitsillides, J. Dudhia, C. T. Thorpe, CD146 Delineates an Interfascicular Cell Sub-Population in Tendon That Is Recruited during Injury through Its Ligand Laminin-α4, Int. J. Mol. Sci. 22 (18) (2021) 9729.
[141] 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. Physiol. 319 (5) (2020) C885–C894.
[142] N.K. Paschos, W.E. Brown, R. Eswaramoorthy, J.C. Hu, K.A. Athanasiou, Advances in tissue engineering through stem cell-based co-culture, J. Tissue Eng. Regen. Med. 9 (2015) 488–503, https://doi.org/10.1002/term.1870.
[143] N.L. Millar, K.G. Silbernagel, K. Thorborg, P.D. Kirwan, L.M. Galatz, G.D. Abrams, G.A.C. Murrell, I.B. McInnes, S.A. Rodeo, Tendinopathy, Nat. Rev. Dis. Prim. 7 (1) (2021), https://doi.org/10.1038/s41572-020-00234-1.
[144] I. Calejo, R. Costa-Almeida, A.I. Gonçalves, D. Berdecka, R.L. Reis, M.E. Gomes, Bi-directional modulation of cellular interactions in an in vitro co-culture model of tendon-to-bone interface, Cell Prolif. 51 (2018) 1–16, https://doi.org/10.1111/cpr.12493.
[145] L. Yang, S. Pijuan-Galito, H.S. Rho, A.S. Vasilevich, A.D. Eren, L.u. Ge, P. Habibović, M.R. Alexander, J. de Boer, A. Carlier, P. van Rijn, Q. Zhou, High-Throughput Methods in the Discovery and Study of Biomaterials and Materiobiology, Chem. Rev. 121 (8) (2021) 4561–4677.
[146] Y. Xu, G.A.C. Murrell, The Basic Science of Tendinopathy, Clin. Orthop. Relat. Res. 466 (2008) 1528–1538, https://doi.org/10.1007/s11999-008-0286-4.
[147] A.R. Tomás, A.I. Gonçalves, E. Paz, P. Freitas, R.M.A. Domingues, M.E. Gomes, Magneto-mechanical actuation of magnetic responsive fibrous scaffolds boosts tenogenesis of human adipose stem cells, Nanoscale. 11 (39) (2019) 18255–18271.
[148] M.R. Citeroni, M.C. Ciardulli, V. Russo, G. Della Porta, A. Mauro, M. El Khatib, M. Di Mattia, D. Galesso, C. Barbera, N.R. Forsyth, N. Maffulli, B. Barboni, Review in vitro innovation of tendon tissue engineering strategies, Int. J. Mol. Sci. 21 (18) (2020) 6726.
[149] 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, https://doi.org/10.1016/j.actbio.2018.03.004.
[150] A. Garcia Garcia, J.-B. Perot, M. Beldjilali-Labro, Q. Dermigny, M. Naudot, S. Le Ricousse, C. Legallais, F. Bedoui, Monitoring mechanical stimulation for optimal tendon tissue engineering: A mechanical and biological multiscale study, J. Biomed. Mater. Res. Part A. 109 (10) (2021) 1881–1892.
[151] W.L. Lim, L.L. Liau, M.H. Ng, S.R. Chowdhury, J.X. Law, Current Progress in Tendon and Ligament Tissue Engineering, Tissue Eng, Regen. Med. 16 (2019) 549–571, https://doi.org/10.1007/s13770-019-00196-w.
[152] T. Schwarting, M. Benölken, S. Ruchholtz, M. Frink, P. Lechler, Bone morphogenetic protein-7 enhances bone-tendon integration in a murine in vitro co-culture model, Int. Orthop. 39 (2015) 799–805, https://doi.org/10.1007/s00264-015-2688-8.
[153] T. Schwarting, D. Schenk, M. Frink, M. Benölken, F. Steindor, M. Oswald, S. Ruchholtz, P. Lechler, Stimulation with bone morphogenetic protein-2 (BMP-2) enhances bone–tendon integration in vitro, Connect. Tissue Res. 57 (2) (2016) 99–112.
[154] T. Schwarting, S. Pretzsch, F. Debus, S. Ruchholtz, P. Lechler, The Effect of Cyclooxygenase Inhibition on Tendon-Bone Healing in an In Vitro Coculture Model, Mediators Inflamm. 2015 (2015) 1–10, https://doi.org/10.1155/2015/926369.
[155] R. Costa-Almeida, R.M.A. Domingues, A. Fallahi, H. Avci, I.K. Yazdi, M. Akbari, R.L. Reis, A. Tamayol, M.E. Gomes, A. Khademhosseini, Cell-laden composite suture threads for repairing damaged tendons, J. Tissue Eng. Regen. Med. 12 (4) (2018) 1039–1048.
[156] K.L. Moffat, A.S. Kwei, J.P. Spalazzi, D. Ph, S.B. Doty, D. Ph, et al., Novel Nanofiber-Based Scaffold for Rotator Cuff Repair and Augmentation, Tissue Eng. - Part A. 15 (2009) 115–126, https://doi.org/10.1089/ten.tea.2008.0014.
[157] Z.i. Yin, X. Chen, J.L. Chen, W.L. Shen, T.M. Hieu Nguyen, L. Gao, H.W. Ouyang, Biomaterials The regulation of tendon stem cell differentiation by the alignment of nanofibers, Biomaterials. 31 (8) (2010) 2163–2175.
[158] N.M. Lee, C. Erisken, T. Iskratsch, M. Sheetz, W.N. Levine, H.H. Lu, Polymer fiber-based models of connective tissue repair and healing, Biomaterials. 112 (2017) 303–312, https://doi.org/10.1016/j.biomaterials.2016.10.013.
[159] A. Dede Eren, E.D. Eren, T.J.S. Wilting, J. de Boer, H. Gelderblom, J. Foolen, Self-agglomerated collagen patterns govern cell behaviour, Sci. Rep. 11 (2021) 1516, https://doi.org/10.1038/s41598-021-81054-5.
[160] M. Iannone, M. Ventre, L. Formisano, L. Casalino, E.J. Patriarca, P.A. Netti, Nanoengineered Surfaces for Focal Adhesion Guidance Trigger Mesenchymal Stem Cell Self-Organization and Tenogenesis, Nano Lett. 15 (2015) 1517–1525, https://doi.org/10.1021/nl503737k.
[161] S. Vermeulen, A. Vasilevich, D. Tsiapalis, N. Roumans, P. Vroemen, N.R.M. Beijer, A. Dede Eren, D. Zeugolis, J. de Boer, Identification of topographical

architectures supporting the phenotype of rat tenocytes, Acta Biomater. 83 (2019) 277–290.
[162] J.J. Paredes, N. Andarawis-Puri, Therapeutics for tendon regeneration: a multidisciplinary review of tendon research for improved healing, Ann. N. Y. Acad. Sci. 1383 (2016) 125–138, https://doi.org/10.1111/nyas.13228.
[163] H.R.C. Screen, J.C. Shelton, D.L. Bader, D.A. Lee, Cyclic tensile strain upregulates collagen synthesis in isolated tendon fascicles, Biochem. Biophys. Res. Commun. 336 (2005) 424–429, https://doi.org/10.1016/j.bbrc.2005.08.102.
[164] D. Gaspar, C.N.M. Ryan, D.I. Zeugolis, Multifactorial bottom-up bioengineering approaches for the development of living tissue substitutes, FASEB J. 33 (2019) 5741–5754, https://doi.org/10.1096/fj.201802451R.
[165] Y. Xu, S. Dong, Q. Zhou, X. Mo, L. Song, T. Hou, J. Wu, S. Li, Y. Li, P. Li, Y. Gan, J. Xu, The effect of mechanical stimulation on the maturation of TDSCs-poly(L-lactide-co-e-caprolactone)/collagen scaffold constructs for tendon tissue engineering, Biomaterials. 35 (9) (2014) 2760–2772.
[166] K. Kataoka, R. Kurimoto, H. Tsutsumi, T. Chiba, T. Kato, K. Shishido, M. Kato, Y. Ito, Y. Cho, O. Hoshi, A. Mimata, Y. Sakamaki, R. Nakamichi, M.K. Lotz, K. Naruse, H. Asahara, In vitro Neo-Genesis of Tendon/Ligament-Like Tissue by Combination of Mohawk and a Three-Dimensional Cyclic Mechanical Stretch Culture System, Front. Cell Dev. Biol. 8 (2020), https://doi.org/10.3389/fcell.2020.00307.
[167] S. Dex, P. Alberton, L. Willkomm, T. Söllradl, S. Bago, S. Milz, M. Shakibaei, A. Ignatius, W. Bloch, H. Clausen-Schaumann, C. Shukunami, M. Schieker, D. Docheva, Tenomodulin is Required for Tendon Endurance Running and Collagen I Fibril Adaptation to Mechanical Load, EBioMedicine. 20 (2017) 240–254.
[168] 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, https://doi.org/10.1186/s12867-015-0036-6.
[169] S. Franz, S. Rammelt, D. Scharnweber, J.C. Simon, Immune responses to implants – A review of the implications for the design of immunomodulatory biomaterials, Biomaterials. 32 (2011) 6692–6709, https://doi.org/10.1016/j.biomaterials.2011.05.078.
[170] M. Meeremans, G.R. Van de Walle, S. Van Vlierberghe, C. De Schauwer, The Lack of a Representative Tendinopathy Model Hampers Fundamental Mesenchymal Stem Cell Research, Front. Cell Dev. Biol. 9 (2021), https://doi.org/10.3389/fcell.2021.651164.
[171] S. Wu, J. Liu, Y.e. Qi, J. Cai, J. Zhao, B. Duan, S. Chen, Tendon-bioinspired wavy nanofibrous scaffolds provide tunable anisotropy and promote tenogenesis for tendon tissue engineering, Mater. Sci. Eng. C. 126 (2021) 112181, https://doi.org/10.1016/j.msec.2021.112181.
[172] I. Calejo, R. Costa-Almeida, R.L. Reis, M.E. Gomes, A Physiology-Inspired Multifactorial Toolbox in Soft-to-Hard Musculoskeletal Interface Tissue Engineering, Trends Biotechnol. 38 (2020) 83–98, https://doi.org/10.1016/j.tibtech.2019.06.003.
[173] C. Mota, S. Camarero-Espinosa, M.B. Baker, P. Wieringa, L. Moroni, Bioprinting: From Tissue and Organ Development to in Vitro Models, Chem. Rev. 120 (19) (2020) 10547–10607.
[174] H. Zhang, Y. Chen, C. Fan, R. Liu, J. Huang, Y. Zhang, C. Tang, B.o. Zhou, X. Chen, W. Ju, Y. Zhao, J. Han, P. Wu, S. Zhang, W. Shen, Z.i. Yin, X. Chen, H. Ouyang, Cell-subpopulation alteration and FGF7 activation regulate the function of tendon stem/progenitor cells in 3D microenvironment revealed by single-cell analysis, Biomaterials. 280 (2022) 121238, https://doi.org/10.1016/j.biomaterials.2021.121238.
[175] C. Arrigoni, D. Petta, S. Bersini, V. Mironov, C. Candrian, M. Moretti, Engineering complex muscle-tissue interfaces through microfabrication, Biofabrication. 11 (3) (2019) 032004, https://doi.org/10.1088/1758-5090/ab1e7c.
[176] Y. Haraguchi, T. Shimizu, T. Sasagawa, H. Sekine, K. Sakaguchi, T. Kikuchi, W. Sekine, S. Sekiya, M. Yamato, M. Umezu, T. Okano, Fabrication of functional three-dimensional tissues by stacking cell sheets in vitro, Nat. Protoc. 7 (5) (2012) 850–858.
[177] A. De Pieri, Y. Rochev, D.I. Zeugolis, Scaffold-free cell-based tissue engineering therapies: advances, shortfalls and forecast, Npj Regen. Med. 6 (2021) 18, https://doi.org/10.1038/s41536-021-00133-3.
[178] A. Vinhas, A.I. Gonçalves, 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, https://doi.org/10.1016/j.actbio.2021.06.036.
[179] A.I. Gonçalves, M.T. Rodrigues, M.E. Gomes, Tissue-engineered magnetic cell sheet patches for advanced strategies in tendon regeneration, Acta Biomater. 63 (2017) 110–122, https://doi.org/10.1016/j.actbio.2017.09.014.
[180] I. Komatsu, J.-H.-C. Wang, K. Iwasaki, T. Shimizu, T. Okano, The effect of tendon stem/progenitor cell (TSC) sheet on the early tendon healing in a rat Achilles tendon injury model, Acta Biomater. 42 (2016) 136–146, https://doi.org/10.1016/j.actbio.2016.06.026.
[181] S. Chen, J. Wang, Y. Chen, X. Mo, C. Fan, Tenogenic adipose-derived stem cell sheets with nanoyarn scaffolds for tendon regeneration, Mater. Sci. Eng. C. 119 (2021), https://doi.org/10.1016/j.msec.2020.111506.
[182] C.-F. Hsieh, Z. Yan, R. Schumann, S. Milz, C. Pfeifer, M. Schieker, D. Docheva, In Vitro Comparison of 2D-Cell Culture and 3D-Cell Sheets of Scleraxis-Programmed Bone Marrow Derived Mesenchymal Stem Cells to Primary Tendon Stem/Progenitor Cells for Tendon Repair, Int. J. Mol. Sci. 19 (8) (2018) 2272.
[183] C. Zhang, E. Zhang, L. Yang, W. Tu, J. Lin, C. Yuan, V. Bunpetch, X. Chen, H. Ouyang, Histone deacetylase inhibitor treated cell sheet from mouse tendon stem/progenitor cells promotes tendon repair, Biomaterials. 172 (2018) 66–82.
[184] J. Chu, O. Pieles, C. Pfeifer, V. Alt, C. Morsczeck, D. Docheva, Dental follicle cell differentiation towards periodontal ligament-like tissue in a self-assembly three-dimensional organoid model, Eur. Cells Mater. 42 (2021) 20–33, https://doi.org/10.22203/eCM.v042a02.
[185] D. Tsiapalis, A. De Pieri, K. Spanoudes, I. Sallent, S. Kearns, J.L. Kelly, M. Raghunath, D.I. Zeugolis, The synergistic effect of low oxygen tension and macromolecular crowding in the development of extracellular matrix-rich tendon equivalents, Biofabrication. 12 (2) (2020) 025018, https://doi.org/10.1088/1758-5090/ab6412.
[186] D. Tsiapalis, D.I. Zeugolis, It is time to crowd your cell culture media – Physicochemical considerations with biological consequences, Biomaterials. 275 (2021) 120943, https://doi.org/10.1016/j.biomaterials.2021.120943.
[187] D. Tsiapalis, S. Rana, M. Doulgkeroglou, S. Kearns, J. Kelly, Y. Bayon, et al., The effect of aligned electrospun fibers and macromolecular crowding in tenocyte culture, in (2020) 225–247, https://doi.org/10.1016/bs.mcb.2019.11.003.
[188] Y. Sakai, K. Yamanouchi, K. Ohashi, M. Koike, R. Utoh, H. Hasegawa, I. Muraoka, T. Suematsu, A. Soyama, M. Hidaka, M. Takatsuki, T. Kuroki, S. Eguchi, Vascularized subcutaneous human liver tissue from engineered hepatocyte/fibroblast sheets in mice, Biomaterials. 65 (2015) 66–75.
[189] N. Asakawa, T. Shimizu, Y. Tsuda, S. Sekiya, T. Sasagawa, M. Yamato, F. Fukai, T. Okano, Pre-vascularization of in vitro three-dimensional tissues created by cell sheet engineering, Biomaterials. 31 (14) (2010) 3903–3909.
[190] J.L. Puetzer, T. Ma, I. Sallent, A. Gelmi, M.M. Stevens, Driving Hierarchical Collagen Fiber Formation for Functional Tendon, Ligament, and Meniscus Replacement, Biomaterials. 269 (2021), https://doi.org/10.1016/j.biomaterials.2020.120527 120527.
[191] M.L. Bayer, C.-Y. Yeung, K.E. Kadler, K. Qvortrup, K. Baar, R.B. Svensson, S. Peter Magnusson, M. Krogsgaard, M. Koch, M. Kjaer, The initiation of embryonic-like collagen fibrillogenesis by adult human tendon fibroblasts when cultured under tension, Biomaterials. 31 (18) (2010) 4889–4897.
[192] V.S. Nirmalanandhan, M. Rao, M.S. Sacks, B. Haridas, D.L. Butler, Effect of length of the engineered tendon construct on its structure–function relationships in culture, J. Biomech. 40 (2007) 2523–2529, https://doi.org/10.1016/j.jbiomech.2006.11.016.
[193] H.A. Awad, D.L. Butler, M.T. Harris, R.E. Ibrahim, Y. Wu, R.G. Young, et al., In vitro characterization of mesenchymal stem cell-seeded collagen scaffolds for tendon repair: Effects of initial seeding density on contraction kinetics, J. Biomed. Mater. Res. 51 (2000) 233–240, https://doi.org/10.1002/(SICI)1097-4636(200008)51:2<233::AID-JBM12>3.0.CO;2-B.
[194] R. Gehwolf, B. Schwemberger, M. Jessen, S. Korntner, A. Wagner, C. Lehner, N. Weissenbacher, H. Tempfer, A. Traweger, Global Responses of Il-1β-Primed 3D Tendon Constructs to Treatment with Pulsed Electromagnetic Fields, Cells. 8 (5) (2019) 399.
[195] A. Herchenhan, M.L. Bayer, R.B. Svensson, S.P. Magnusson, M. Kjaer, In vitro tendon tissue development from human fibroblasts demonstrates collagen fibril diameter growth associated with a rise in mechanical strength, Dev. Dyn. 242 (2013) 2–8, https://doi.org/10.1002/dvdy.23896.
[196] A.P. Breidenbach, N.A. Dyment, Y. Lu, M. Rao, J.T. Shearn, D.W. Rowe, K.E. Kadler, D.L. Butler, Fibrin Gels Exhibit Improved Biological, Structural, and Mechanical Properties Compared with Collagen Gels in Cell-Based Tendon Tissue-Engineered Constructs, Tissue Eng. Part A 21 (3-4) (2015) 438–450.
[197] D. Shakiba, F. Alisafaei, A. Savadipour, R.A. Rowe, Z. Liu, K.M. Pryse, V.B. Shenoy, E.L. Elson, G.M. Genin, The Balance between Actomyosin Contractility and Microtubule Polymerization Regulates Hierarchical Protrusions That Govern Efficient Fibroblast-Collagen Interactions, ACS Nano. 14 (7) (2020) 7868–7879.
[198] R. Gehwolf, G. Spitzer, A. Wagner, C. Lehner, N. Weissenbacher, H. Tempfer, et al., 3D-Embedded Cell Cultures to Study Tendon Biology, in: Methods Mol. Biol., 2019: pp. 155–165. doi:10.1007/7651_2019_208.
[199] Z. Zheng, J. Ran, W. Chen, Y. Hu, T. Zhu, X. Chen, Z.i. Yin, B.C. Heng, G. Feng, H. Le, C. Tang, J. Huang, Y. Chen, Y. Zhou, P. Dominique, W. Shen, H.-W. Ouyang, Alignment of collagen fiber in knitted silk scaffold for functional massive rotator cuff repair, Acta Biomater. 51 (2017) 317–329.
[200] P.J. Yang, J.S. Temenoff, Engineering Orthopedic Tissue Interfaces, Tissue Eng. Part B Rev. 15 (2009) 127–141, https://doi.org/10.1089/ten.teb.2008.0371.
[201] Y.J. No, M. Castilho, Y. Ramaswamy, H. Zreiqat, Role of Biomaterials and Controlled Architecture on Tendon/Ligament Repair and Regeneration, Adv. Mater. 32 (2020) 1904511, https://doi.org/10.1002/adma.201904511.
[202] C. Rinoldi, E. Kijeńska-Gawrońska, A. Khademhosseini, A. Tamayol, W. Swieszkowski, Fibrous Systems as Potential Solutions for Tendon and Ligament Repair, Healing, and Regeneration, Adv. Healthc. Mater. 10 (2021) 2001305, https://doi.org/10.1002/adhm.202001305.
[203] M. Laranjeira, R.M.A.M.A. Domingues, R. Costa-Almeida, R.L.L. Reis, M.E.E. Gomes, 3D Mimicry of Native-Tissue-Fiber Architecture Guides Tendon-Derived Cells and Adipose Stem Cells into Artificial Tendon Constructs, Small. 13 (2017) 1700689, https://doi.org/10.1002/smll.201700689.
[204] H. Almeida, R.M.A. Domingues, S.M. Mithieux, R.A. Pires, A.I. Gonçalves, M. Gómez-Florit, R.L. Reis, A.S. Weiss, M.E. Gomes, Tropoelastin-Coated Tendon Biomimetic Scaffolds Promote Stem Cell Tenogenic Commitment and Deposition of Elastin-Rich Matrix, ACS Appl, Mater. Interfaces. 11 (22) (2019) 19830–19840.

[205] A.R.R. Tomás, A.I.I. Gonçalves, E. Paz, P. Freitas, R.M.A.M.A. Domingues, M.E.E. Gomes, Magneto-mechanical actuation of magnetic responsive fibrous scaffolds boosts tenogenesis of human adipose stem cells, Nanoscale. 11 (2019) 18255–18271, https://doi.org/10.1039/C9NR04355A.
[206] J.G. Barber, A.M. Handorf, T.J. Allee, W.-J. Li, Braided Nanofibrous Scaffold for Tendon and Ligament Tissue Engineering, Tissue Eng. Part A 19 (2013) 1265–1274, https://doi.org/10.1089/ten.tea.2010.0538.
[207] A. Sensini, C. Gualandi, M.L. Focarete, J. Belcari, A. Zucchelli, L. Boyle, G.C. Reilly, A.P. Kao, G. Tozzi, L. Cristofolini, Multiscale hierarchical bioresorbable scaffolds for the regeneration of tendons and ligaments, Biofabrication. 11 (3) (2019) 035026, https://doi.org/10.1088/1758-5090/ab20ad.
[208] M.R. Ladd, S.J. Lee, J.D. Stitzel, A. Atala, J.J. Yoo, Co-electrospun dual scaffolding system with potential for muscle-tendon junction tissue engineering, Biomaterials. 32 (2011) 1549–1559, https://doi.org/10.1016/j.biomaterials.2010.10.038.
[209] S.K. Madhurakkat Perikamana, J. Lee, T. Ahmad, E.M. Kim, H. Byun, S. Lee, et al., Harnessing biochemical and structural cues for tenogenic differentiation of adipose derived stem cells (ADSCs) and development of an in vitro tissue interface mimicking tendon-bone insertion graft, Biomaterials. 165 (2018) 79–93, https://doi.org/10.1016/j.biomaterials.2018.02.046.
[210] I. Calejo, R. Costa-Almeida, R.L. Reis, M.E. Gomes, A Textile Platform Using Continuous Aligned and Textured Composite Microfibers to Engineer Tendon-to-Bone Interface Gradient Scaffolds, Adv. Healthc. Mater. 8 (2019) 1900200, https://doi.org/10.1002/adhm.201900200.
[211] S. Font Tellado, S. Chiera, W. Bonani, P.S.P. Poh, C. Migliaresi, A. Motta, E.R. Balmayor, M. van Griensven, Heparin functionalization increases retention of TGF-β2 and GDF5 on biphasic silk fibroin scaffolds for tendon/ligament-to-bone tissue engineering, Acta Biomater. 72 (2018) 150–166.
[212] P. Chen, L. Li, L. Dong, S. Wang, Z. Huang, Y. Qian, C. Wang, W. Liu, L.i. Yang, Gradient Biomineralized Silk Fibroin Nanofibrous Scaffold with Osteochondral Inductivity for Integration of Tendon to Bone, ACS Biomater. Sci. Eng. 7 (3) (2021) 841–851.
[213] O. Bas, D. D'Angella, J.G. Baldwin, N.J. Castro, F.M. Wunner, N.T. Saidy, S. Kollmannsberger, A. Reali, E. Rank, E.M. De-Juan-Pardo, D.W. Hutmacher, An Integrated Design, Material, and Fabrication Platform for Engineering Biomechanically and Biologically Functional Soft Tissues, ACS Appl. Mater. Interfaces. 9 (35) (2017) 29430–29437.
[214] N.T. Saidy, F. Wolf, O. Bas, H. Keijdener, D.W. Hutmacher, P. Mela, E.M. De-Juan-Pardo, Biologically Inspired Scaffolds for Heart Valve Tissue Engineering via Melt Electrowriting, Small. 15 (24) (2019) 1900873.
[215] M. Raghunath, D.I. Zeugolis, Transforming eukaryotic cell culture with macromolecular crowding, Trends Biochem. Sci. 46 (2021) 805–811, https://doi.org/10.1016/j.tibs.2021.04.006.
[216] D. Gaspar, K.P. Fuller, D.I. Zeugolis, Polydispersity and negative charge are key modulators of extracellular matrix deposition under macromolecular crowding conditions, Acta Biomater. 88 (2019) 197–210, https://doi.org/10.1016/j.actbio.2019.02.050.
[217] R. Costa-Almeida, I. Calejo, R. Altieri, R.M.A. Domingues, E. Giordano, R.L. Reis, M.E. Gomes, Exploring platelet lysate hydrogel-coated suture threads as biofunctional composite living fibers for cell delivery in tissue repair, Biomed. Mater. 14 (3) (2019) 034104, https://doi.org/10.1088/1748-605X/ab0de6.
[218] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (2014) 773–785, https://doi.org/10.1038/nbt.2958.
[219] H.H. Hwang, S. You, X. Ma, L. Kwe, G. Victorine, N. Lawrence, X. Wan, H. Shen, W. Zhu, S. Chen, High throughput direct 3D bioprinting in multiwell plates, Biofabrication. 13 (2) (2021) 025007, https://doi.org/10.1088/1758-5090/ab89ca.
[220] K.T. Lawlor, J.M. Vanslambrouck, J.W. Higgins, A. Chambon, K. Bishard, D. Arndt, P.X. Er, S.B. Wilson, S.E. Howden, K.S. Tan, F. Li, L.J. Hale, B. Shepherd, S. Pentoney, S.C. Presnell, A.E. Chen, M.H. Little, Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation, Nat. Mater. 20 (2) (2021) 260–271.
[221] D.B. Kolesky, K.A. Homan, M.A. Skylar-Scott, J.A. Lewis, Three-dimensional bioprinting of thick vascularized tissues, Proc. Natl. Acad. Sci. 113 (2016) 3179–3184, https://doi.org/10.1073/pnas.1521342113.
[222] D.B. Kolesky, R.L. Truby, A.S. Gladman, T.A. Busbee, K.A. Homan, J.A. Lewis, 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs, Adv. Mater. 26 (2014) 3124–3130, https://doi.org/10.1002/adma.201305506.
[223] F. Meng, C.M. Meyer, D. Joung, D.A. Vallera, M.C. McAlpine, A. Panoskaltsis-Mortari, 3D Bioprinted In Vitro Metastatic Models via Reconstruction of Tumor Microenvironments, Adv. Mater. 31 (2019) 1806899, https://doi.org/10.1002/adma.201806899.
[224] T.K. Merceron, M. Burt, Y.-J. Seol, H.-W. Kang, S.J. Lee, J.J. Yoo, A. Atala, A 3D bioprinted complex structure for engineering the muscle-tendon unit, Biofabrication. 7 (3) (2015) 035003, https://doi.org/10.1088/1758-5090/7/3/035003.
[225] B.S. Kim, S. Das, J. Jang, D.-W. Cho, Decellularized Extracellular Matrix-based Bioinks for Engineering Tissue- and Organ-specific Microenvironments, Chem. Rev. 120 (2020) 10608–10661, https://doi.org/10.1021/acs.chemrev.9b00808.
[226] L.P. Ferreira, V.M. Gaspar, J.F. Mano, Decellularized Extracellular Matrix for Bioengineering Physiomimetic 3D in Vitro Tumor Models, Trends Biotechnol. 38 (2020) 1397–1414, https://doi.org/10.1016/j.tibtech.2020.04.006.
[227] G.G. Giobbe, C. Crowley, C. Luni, S. Campinoti, M. Khedr, K. Kretzschmar, M.M. De Santis, E. Zambaiti, F. Michielin, L. Meran, Q. Hu, G. van Son, L. Urbani, A. Manfredi, M. Giomo, S. Eaton, D. Cacchiarelli, V.S.W. Li, H. Clevers, P. Bonfanti, N. Elvassore, P. De Coppi, Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture, Nat. Commun. 10 (1) (2019), https://doi.org/10.1038/s41467-019-13605-4.
[228] J. Li, C. Parra-Cantu, Z. Wang, Y.S. Zhang, Improving Bioprinted Volumetric Tumor Microenvironments In Vitro, Trends in Cancer. 6 (2020) 745–756, https://doi.org/10.1016/j.trecan.2020.06.002.
[229] H.-G. Yi, Y.H. Jeong, Y. Kim, Y.-J. Choi, H.E. Moon, S.H. Park, K.S. Kang, M. Bae, J. Jang, H. Youn, S.H. Paek, D.-W. Cho, A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy, Nat. Biomed. Eng. 3 (7) (2019) 509–519.
[230] B. Toprakhisar, A. Nadernezhad, E. Bakirci, N. Khani, G.A. Skvortsov, B. Koc, Development of Bioink from Decellularized Tendon Extracellular Matrix for 3D Bioprinting, Macromol. Biosci. 18 (2018) 1800024, https://doi.org/10.1002/mabi.201800024.
[231] F. Zhao, J. Cheng, J. Zhang, H. Yu, W. Dai, W. Yan, M. Sun, G. Ding, Q.i. Li, Q. Meng, Q. Liu, X. Duan, X. Hu, Y. Ao, Comparison of three different acidic solutions in tendon decellularized extracellular matrix bio-ink fabrication for 3D cell printing, Acta Biomater. 131 (2021) 262–275.
[232] S. Chae, Y. Sun, Y.-J. Choi, D.-H. Ha, I. Jeon, D.-W. Cho, 3D cell-printing of tendon-bone interface using tissue-derived extracellular matrix bioinks for chronic rotator cuff repair, Biofabrication. 13 (3) (2021) 035005, https://doi.org/10.1088/1758-5090/abd159.
[233] I.-N.-E. Wang, D.R. Bogdanowicz, S. Mitroo, J. Shan, S. Kala, H.H. Lu, Cellular interactions regulate stem cell differentiation in tri-culture, Connect. Tissue Res. 57 (2016) 476–487, https://doi.org/10.1080/03008207.2016.1230106.
[234] E. Davoodi, E. Sarikhani, H. Montazerian, S. Ahadian, M. Costantini, W. Swieszkowski, S.M. Willerth, K. Walus, M. Mofidfar, E. Toyserkani, A. Khademhosseini, N. Ashammakhi, Extrusion and Microfluidic-Based Bioprinting to Fabricate Biomimetic Tissues and Organs, Adv. Mater. Technol. 5 (8) (2020) 1901044.
[235] M. Costantini, S. Testa, P. Mozetic, A. Barbetta, C. Fuoco, E. Fornetti, et al., Microfluidic-enhanced 3D bioprinting of aligned myoblast-laden hydrogels leads to functionally organized myofibers in vitro and in vivo, Biomaterials. 131 (2017) 98–110, https://doi.org/10.1016/j.biomaterials.2017.03.026.
[236] K. Yao, W. Li, K. Li, Q. Wu, Y. Gu, L. Zhao, Y. Zhang, X. Gao, Simple Fabrication of Multicomponent Heterogeneous Fibers for Cell Co-Culture via Microfluidic Spinning, Macromol. Biosci. 20 (3) (2020) 1900395, https://doi.org/10.1002/mabi.201900395.
[237] C.F. Guimarães, L. Gasperini, A.P. Marques, R.L. Reis, 3D flow-focusing microfluidic biofabrication: One-chip-fits-all hydrogel fiber architectures, Appl. Mater. Today. 23 (2021), https://doi.org/10.1016/j.apmt.2021.101013.
[238] M. Samandari, F. Alipanah, K. Majidzadeh-A, M.M. Alvarez, G. Trujillo-de Santiago, A. Tamayol, Controlling cellular organization in bioprinting through designed 3D microcompartmentalization, Appl. Phys. Rev. 8 (2) (2021) 021404, https://doi.org/10.1063/5.0040732.
[239] M. de Ruijter, A. Ribeiro, I. Dokter, M. Castilho, J. Malda, Simultaneous Micropatterning of Fibrous Meshes and Bioinks for the Fabrication of Living Tissue Constructs, Adv. Healthc. Mater. 8 (2019) 1800418, https://doi.org/10.1002/adhm.201800418.
[240] A. Hrynevich, B.Ş. Elçi, J.N. Haigh, R. McMaster, A. Youssef, C. Blum, T. Blunk, G. Hochleitner, J. Groll, P.D. Dalton, Dimension-Based Design of Melt Electrowritten Scaffolds, Small. 14 (22) (2018) 1800232, https://doi.org/10.1002/smll.201800232.
[241] P.D. Dalton, T.B.F. Woodfield, V. Mironov, J. Groll, Advances in Hybrid Fabrication toward Hierarchical Tissue Constructs, Adv. Sci. 7 (2020) 1902953, https://doi.org/10.1002/advs.201902953.
[242] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, Trends Cell Biol. 21 (2011) 745–754, https://doi.org/10.1016/j.tcb.2011.09.005.
[243] D. Huh, B.D. Matthews, A. Mammoto, M. Montoya-Zavala, H.Y. Hsin, D.E. Ingber, Reconstituting Organ-Level Lung Functions on a Chip, Science 328 (5986) (2010) 1662–1668.
[244] K.J. Jang, K.Y. Suh, A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells, Lab Chip. 10 (2010) 36–42, https://doi.org/10.1039/b907515a.
[245] Q. Wu, J. Liu, X. Wang, L. Feng, J. Wu, X. Zhu, W. Wen, X. Gong, Organ-on-a-chip: Recent breakthroughs and future prospects, Biomed. Eng. Online. 19 (1) (2020), https://doi.org/10.1186/s12938-020-0752-0.
[246] S.N. Bhatia, D.E. Ingber, Microfluidic organs-on-chips, Nat. Biotechnol. 32 (2014) 760–772, https://doi.org/10.1038/nbt.2989.
[247] B. Zhang, A. Korolj, B.F.L. Lai, M. Radisic, Advances in organ-on-a-chip engineering, Nat. Rev. Mater. 3 (2018) 257–278, https://doi.org/10.1038/s41578-018-0034-7.
[248] G. Vunjak-Novakovic, K. Ronaldson-Bouchard, M. Radisic, Organs-on-a-chip models for biological research, Cell. 184 (2021) 4597–4611, https://doi.org/10.1016/j.cell.2021.08.005.
[249] D. Qin, Y. Xia, G.M. Whitesides, Soft lithography for micro- and nanoscale patterning, Nat. Protoc. 5 (2010) 491–502, https://doi.org/10.1038/nprot.2009.234.
[250] G.M. Whitesides, The origins and the future of microfluidics, Nature. 442 (2006) 368–373, https://doi.org/10.1038/nature05058.
[251] S.B. Campbell, Q. Wu, J. Yazbeck, C. Liu, S. Okhovatian, M. Radisic, Beyond Polydimethylsiloxane: Alternative Materials for Fabrication of Organ-on-a-Chip Devices and Microphysiological Systems, ACS Biomater. Sci. Eng. 7 (2021) 2880–2899, https://doi.org/10.1021/acsbiomaterials.0c00640.

[252] E.M. Purcell, Life at low Reynolds number, Am. J. Phys. 45 (1977) 3–11, https://doi.org/10.1119/1.10903.
[253] Y. Zhao, N. Rafatian, E.Y. Wang, N.T. Feric, B.F.L. Lai, E.J. Knee-Walden, P.H. Backx, M. Radisic, Engineering microenvironment for human cardiac tissue assembly in heart-on-a-chip platform, Matrix Biol. 85-86 (2020) 189–204.
[254] M.A. Ortega, X. Fernández-Garibay, A.G. Castaño, F. De Chiara, A. Hernández-Albors, J. Balaguer-Trias, J. Ramón-Azcón, Muscle-on-a-chip with an on-site multiplexed biosensing system for in situ monitoring of secreted IL-6 and TNF-α, Lab Chip. 19 (15) (2019) 2568–2580.
[255] H.C. Pruitt, D. Lewis, M. Ciccaglione, S. Connor, Q. Smith, J.W. Hickey, J.P. Schneck, S. Gerecht, Collagen fiber structure guides 3D motility of cytotoxic T lymphocytes, Matrix Biol. 85-86 (2020) 147–159.
[256] S. Piluso, Y. Li, F. Abinzano, R. Levato, L. Moreira Teixeira, M. Karperien, J. Leijten, R. van Weeren, J. Malda, Mimicking the Articular Joint with In Vitro Models, Trends Biotechnol. 37 (10) (2019) 1063–1077.
[257] P. Occhetta, A. Mainardi, E. Votta, Q. Vallmajo-Martin, M. Ehrbar, I. Martin, A. Barbero, M. Rasponi, Hyperphysiological compression of articular cartilage induces an osteoarthritic phenotype in a cartilage-on-a-chip model, Nat. Biomed. Eng. 3 (7) (2019) 545–557.
[258] M. Rothbauer, G. Höll, C. Eilenberger, S.R.A. Kratz, B. Farooq, P. Schuller, I. Olmos Calvo, R.A. Byrne, B. Meyer, B. Niederreiter, S. Küpcü, F. Sevelda, J. Holinka, O. Hayden, S.F. Tedde, H.P. Kiener, P. Ertl, Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing, Lab Chip. 20 (8) (2020) 1461–1471.
[259] D.B. Chou, V. Frismantas, Y. Milton, R. David, P. Pop-Damkov, D. Ferguson, A. MacDonald, Ö. Vargel Bölükbaşı, C.E. Joyce, L.S. Moreira Teixeira, A. Rech, A. Jiang, E. Calamari, S. Jalili-Firoozinezhad, B.A. Furlong, L.R. O'Sullivan, C.F. Ng, Y. Choe, S. Marquez, K.C. Myers, O.K. Weinberg, R.P. Hasserjian, R. Novak, O. Levy, R. Prantil-Baun, C.D. Novina, A. Shimamura, L. Ewart, D.E. Ingber, On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology, Nat. Biomed. Eng. 4 (4) (2020) 394–406.
[260] S. Halldorsson, E. Lucumi, R. Gómez-Sjöberg, R.M.T. Fleming, Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices, Biosens. Bioelectron. 63 (2015) 218–231, https://doi.org/10.1016/j.bios.2014.07.029.
[261] I.K. Zervantonakis, C.R. Kothapalli, S. Chung, R. Sudo, R.D. Kamm, Microfluidic devices for studying heterotypic cell-cell interactions and tissue specimen cultures under controlled microenvironments, Biomicrofluidics. 5 (1) (2011) 013406, https://doi.org/10.1063/1.3553237.
[262] H. Ravanbakhsh, V. Karamzadeh, G. Bao, L. Mongeau, D. Juncker, Y.S. Zhang, Emerging Technologies in Multi-Material Bioprinting, Adv. Mater. 33 (49) (2021) 2104730.
[263] A.K. Miri, E. Mostafavi, D. Khorsandi, S.-K. Hu, M. Malpica, A. Khademhosseini, Bioprinters for organs-on-chips, Biofabrication. 11 (2019), https://doi.org/10.1088/1758-5090/ab2798 042002.
[264] A.K. Miri, D. Nieto, L. Iglesias, H. Goodarzi Hosseinabadi, S. Maharjan, G.U. Ruiz-Esparza, et al., Microfluidics-Enabled Multimaterial Maskless Stereolithographic Bioprinting, Adv. Mater. 30 (2018) 1800242. doi:10.1002/adma.201800242.
[265] P.N. Bernal, P. Delrot, D. Loterie, Y. Li, J. Malda, C. Moser, R. Levato, Volumetric Bioprinting of Complex Living-Tissue Constructs within Seconds, Adv. Mater. 31 (42) (2019) 1904209, https://doi.org/10.1002/adma.201904209.
[266] S.M. Bakht, M. Gomez-Florit, T. Lamers, R.L. Reis, R.M.A. Domingues, M.E. Gomes, 3D Bioprinting of Miniaturized Tissues Embedded in Self-Assembled Nanoparticle-Based Fibrillar Platforms, Adv. Funct. Mater. 2104245 (2021) 1–16, https://doi.org/10.1002/adfm.202104245.
[267] J.A. Brassard, M. Nikolaev, T. Hübscher, M. Hofer, M.P. Lutolf, Recapitulating macro-scale tissue self-organization through organoid bioprinting, Nat. Mater. 20 (2021) 22–29, https://doi.org/10.1038/s41563-020-00803-5.
[268] C.D. Morley, S.T. Ellison, T. Bhattacharjee, C.S. O'Bryan, Y. Zhang, K.F. Smith, C. P. Kabb, M. Sebastian, G.L. Moore, K.D. Schulze, S. Niemi, W.G. Sawyer, D.D. Tran, D.A. Mitchell, B.S. Sumerlin, C.T. Flores, T.E. Angelini, Quantitative characterization of 3D bioprinted structural elements under cell generated forces, Nat. Commun. 10 (1) (2019), https://doi.org/10.1038/s41467-019-10919-1.
[269] B. Ayan, D.N. Heo, Z. Zhang, M. Dey, A. Povilianskas, C. Drapaca, I.T. Ozbolat, Aspiration-assisted bioprinting for precise positioning of biologics, Sci. Adv. 6 (10) (2020), https://doi.org/10.1126/sciadv.aaw5111.
[270] F.V. Jacinto, W. Link, B.I. Ferreira, CRISPR/Cas9-mediated genome editing: From basic research to translational medicine, J. Cell. Mol. Med. 24 (2020) 3766–3778, https://doi.org/10.1111/jcmm.14916.