Mechanosignalling in cartilage: an emerging target for the treatment of osteoarthritis

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Targeting mechanosignalling in cartilage repair: an emerging paradigm in the treatment of osteoarthritis

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Abstract

Mechanical stimuli play a fundamental role in articular cartilage health and disease. Chondrocytes respond to the physical properties of the extracellular matrix (ECM) and the mechanical forces exerted during joint loading. In osteoarthritis (OA), catabolic processes degrade the functional ECM, while the composition and viscoelastic properties of the matrix produced by chondrocytes are altered. The abnormal loading environment created propagates cell dysfunction and inflammation. Chondrocytes sense their physical environment via an array of mechanosensitive receptors and channels, which in turn activate a complex network of downstream signalling pathways and regulate a plethora of cell processes central to OA pathology. This review focuses on recent advances in the understanding of the complex role of specific mechanosignalling mechanisms in cartilage health and OA, highlighting key molecular processes that can be therapeutically targeted to interrupt pathological feedback loops. The potential for combining these mechanosignalling targets with the rapidly expanding field of smart mechanoresponsive biomaterials and delivery systems will be discussed as an emerging paradigm in OA treatment. The continued advancements in this field have the potential to enable restoration of healthy mechanical microenvironments and signalling through the development of precision therapeutics, mechano-regulated biomaterials and drug systems in the near future.

Key Points

- Mechanical forces are a critical environmental factor in maintaining joint homeostasis, determining cell phenotype, inflammatory responses and a tightly regulated anabolic-catabolic signaling axis essential to cartilage homeostasis.

- Chondrocytes sense their mechanical environment through numerous direct and indirect mechanisms that regulate cell function in health and degenerative diseases, such as osteoarthritis.

- Targeted inhibition of mechano-inflammatory signalling pathways or restoration of functional chondroprotective extracellular matrix environments in OA may prevent ECM degradation and promote reparative anabolic processes.

- Development of self-regulating and mechanically responsive biomaterials and drug delivery systems offer advanced ‘on-demand’ therapeutic approaches for the treatment of OA.

Introduction

Mechanical signalling is a critical mediator of numerous physiological and pathophysiological processes in the cells and tissues of the joint. In cartilage, chondrocytes synthesize, and are surrounded by, a highly specialized extracellular matrix (ECM) that allows low friction movement and protects the tissue during mechanical loading. In healthy cartilage, the mechanical forces generated by movement are an essential component of maintaining the homeostatic balance of chondrocyte-mediated ECM deposition and re-modelling – a dynamic, continuous process of adaptation to the local mechanical environment, involving both sensation and transduction of forces. However, though some movement is required for healthy cartilage, excessive mechanical loading is also a key risk factor in the pathogenesis and progression of osteoarthritis (OA). Decades of research have demonstrated that the role of mechanical loading in the pathobiology of OA goes beyond tissue 'wear-and-tear' and is in fact a dynamic driving force in the disease, through the activation of mechanoresponsive cell signalling (mechanosignalling) and resultant production of inflammatory mediators and catabolic enzymes.[1-4] This pathological signal transduction, which can be viewed as a corruption of chondrocyte mechano-adaptive processes, occurs when chondrocytes experience excessive physical forces, or when the chondroprotective ECM is compromised. In the latter, inadequate distribution of loads means that even within normal physiological ranges excessive stress can be exerted on chondrocytes. In addition, loss of key ECM molecules and structures also significantly impacts mechanically controlled chondro-protective mechanisms.[5,6]

The pathological changes in the cartilage ECM in OA, which include degeneration of the functional matrix (most notably type II collagen and proteoglycans), loss of tissue hydration and production of incorrect fibrous ECM, occur concomitantly with aberrant chondrocyte proliferation, senescence, inflammation, and hypertrophy. [7] Dysregulation of mechanosignalling appears to play a central role in these related processes and the phenotypic drift of chondrocytes seen in OA. Although the mechanisms by which degenerative or reparative signalling programs are initiated within the cell are not fully elucidated, key molecular pathways and signalling mechanisms have been identified. In OA, targeting these pathways is an emerging strategy to reduce the release of the proinflammatory cytokines and catabolic enzymes that drive the progression of the disease. [8,9] Further to this, recent work demonstrates the direct interaction of mechanosignalling and inflammatory mediators, highlighting the potential for mechanobiology approaches to simultaneously boost repair and reduce mechano-inflammation. [10] It is likely that correction of pathological mechanosignalling, either through adjustment of the cellular mechanical environment or through control over mechanosignalling,

will enhance the clinical impact of locally delivered anabolic factors that have shown promise, for example FGF18.[11]

Regenerative biomaterials offer the opportunity to restore repair-stimulating mechanical environments while potentially, through additional functionalisation, delivering disease-modifying OA drugs (DMOADs) or nucleic acids. Systems under development can provide sophisticated control over this delivery, including stimuli-responsive release systems, highlighting the potential for closed-loop therapeutic delivery.[12] Similarly, synthetic gene circuits that respond to OA-associated stimuli, e.g., inflammatory signalling, by upregulating the production of DMOADs represent an exciting development to enable in situ self-regulating therapeutic cell reprogramming. [9]

In this review, we focus on recent advances in the understanding of the complex role of specific mechanosignalling mechanisms in chondrocytes in healthy and OA cartilage. We highlight several key molecular processes with therapeutic potential and discuss strategies to target these to interrupt pathological feedback loops, including the potential for combining these mechanosignalling targets with the rapidly expanding field of smart mechanoresponsive biomaterials and delivery systems.

Cartilage mechano-adaptation in health and disease

Articular cartilage has a structure that is highly specialised for low friction movement and weight bearing. Cartilage itself can be subdivided into three overlapping zones moving from the subchondral bone towards the articulating surface- the deep calcified zone, the intermediate zone and the superficial zone. [13] Within these zones, the ECM composition and architecture reflect the forces they experience during movement. In the deep zone, to resist compressive loads type II collagen fibres are thick and arranged perpendicular to the joint surface while proteoglycan concentrations are high to promote water retention. The intermediate zone experiences compressive and shear forces, and type II collagen fibres are arranged randomly to resist forces from a number of directions. In the superficial zone, chondrocytes and type II collagen fibres are orientated transversely to disperse shear forces during articulation and secrete proteoglycan 4 (PRG4) for lubrication (Figure 1).[13] On the tissue scale, the physical properties of cartilage are determined by the composition of the ECM - a predominantly type II collagen network trapping high concentrations of proteoglycans (e.g. aggrecan, lubricin, perlecan) and glycosaminoglycans (GAGs) (e.g. hyaluronan).[14-16] These proteoglycans and GAGs impart a fixed negative charge on the ECM, promoting water retention and conferring remarkable shock-absorbing and low-friction properties. Tissue compression forces this interstitial fluid from the tissue, which upon unloading returns through charge interactions. Concurrently, hydrostatic pressure generated by ECM obstruction of interstitial fluid movement protects the tissue from compressive forces.[15] Chondrocytes

therefore experience a range of loading modes, often simultaneously (compression, stretch, shear, pressure) through the transducer of this ECM. In combination with magnitude and frequency of force, the integrity of the ECM plays a significant role in determining if an experienced load initiates catabolic signalling cascades in tissue resident chondrocytes. Evidence indicates that ECM degradation not only affects the transmission of forces across the tissue but can alter the type of loading experienced by a chondrocyte in a particular zone, significantly impacting cell responses.[2]

The matrix in which chondrocytes reside does not have a homogenous structure and composition. Surrounding chondrocytes, a distinct region termed the pericellular matrix (PCM) has perhaps the most significant influence on cell mechanotransduction. Together with the cell itself, this region, which is around 2-4 μm thick, forms the structural, functional and metabolic unit commonly referred to as the 'chondron'.[17] The PCM can be around an order of magnitude softer than the bulk tissue ECM (0.04-0.1 MPa and 0.1-2MPa respectively) and is characterized by the presence of type VI collagen but also contains other important components including perlecan, aggrecan, hyaluronan, biglycan, type IX collagen, laminin, and fibronectin.[17,18] Moving outwards from the cell the PCM integrates with the territorial matrix (TM), a region characterised by a network of tightly packed fine, fibrillar collagen, proteoglycan and fibronectin.[19-21] In turn the TM integrates with the interterritorial matrix (ITM) or the bulk tissue ECM (Figure 1). Though the integrity of all of these ECM regions is important for tissue function, the PCM directly modulates the forces experienced by the cell. Therefore, chondrocytes effectively respond to mechanical stresses either 'directly' through sensing PCM deformation via cell-matrix adhesions and/or cell sensory structures, or 'indirectly' following the mechanically induced release of sequestered growth factors and their interaction with cell receptors (Figure 2).

Considering this function, it is perhaps no surprise that the PCM, and in particular the destruction of the PCM, has been implicated in playing a pivotal role in disease.[22] Indeed, PCM degeneration is one of the earliest events in OA, altering both the transmittance and mode of mechanical forces experienced by chondrocytes.[23,24] It is interesting to note that experimental and *in silico* models show that the forces experienced by chondrocytes across various species are comparable and independent of animal mass due to variation in PCM/ ECM properties between species.[25,26] When a mechanical stimulus is outside these thresholds, ECM remodelling is initiated. Targeting chondrocyte mechanosensing offers the opportunity to re-tune cell thresholds in disease to re-establish the dynamic homeostatic balance.

Alterations in the composition and architecture of the PCM and TM lead to altered bioavailability of sequestered growth factors.[27-30] Following deformation or destruction, ECM sequestered factors are released

to interact with cell membrane receptors, activating downstream intracellular signalling. A well-studied example of this is PCM/ TM involvement in FGF signalling (recently reviewed in[31]).[27,32-35] All FGFs depend on heparin sulphate to act as an obligate co-receptor to bind, dimerise and activate receptors (FGFRs).[31] In the PCM and TM, FGFs bind to perlecan, a heparin sulphate proteoglycan and form an FGF-reservoir that is released to activate FGFRs on mechanical stimulation. FGF signalling can activate multiple intracellular signalling pathways including PKC (protein kinase C), MAPK (Ras-Mitogen-activated protein kinase) and PI3K (phosphoinositide 3-kinase)/ AKT (protein kinase B).[31] A healthy PCM/ TM composition is likely to have a significant impact on both the availability of sequestered FGFs and the type of FGF present, as family members exhibit differing affinities for heparin sulphate binding. With FGFs, the balance between the deleterious and beneficial effects of signalling is dependent on the specific family members and receptors activated.[36] Notably FGF18 (sprifermin), which activates FGFR3 is the only FGF therapy undergoing clinical trials and has shown encouraging results by increasing cartilage thickness and reducing loss through intra-articular injection in OA (Figure 2A).[37-39]

The activity of several other growth factor families have mechanical elements to their activity and regulation including members of the TGFβ superfamily, in which mechanical stress activates TGFβ signalling in an integrin-dependent manner (discussed further below).[40] Further understanding of how the initiation of anabolic or catabolic/ inflammatory signalling is regulated, the signalling proteins released under injurious/non-injurious conditions and their impact on the anabolic-catabolic axis of cartilage tissue will provide valuable information to guide the development of pharmacological treatments for OA. Below, we discuss several key chondrocyte mechanosignalling mechanisms including, integrins,[41-43] mechanosensitive ion channels,[44-49] cytoskeletal and nucleoskeletal constituents,[50] and the primary cilium.[51-54]

Integrin-mediated mechanosignalling

Integrins, the most well-studied of the cell adhesion molecules, are key components in determining cell responses to their environment. Their activity is tightly controlled through both biochemical and mechanical regulatory pathways (reviewed in[55-59]). Briefly, upon ligand binding, integrins undergo conformational changes, exposing regions in their cytoplasmic tails that promote binding to the actin cytoskeleton and integrin adhesion complex formation.[60,61] The maturation of these nascent adhesion complexes into focal complexes, focal adhesions, and fibrillar adhesions is tightly regulated.[59] Integrin-mediated force generation and mechanotransduction occurs through the 'molecular clutch' mechanism[62] in a substrate stiffness and integrin type-dependent manner (reviewed in [59]) (Figure 2B).[63,64] In articular cartilage, several integrin

heterodimers are present including α1β1, α5β1, α10β1, α11β1 and αVβ1, with a weaker expression of α3β1 and αVβ3.[65-67] The integrin profile of OA chondrocytes is altered and difficult to interpret. For example, the expression of α1β1, α3β1, α2β1, α4β1 and β2 is increased but the full implications of this are currently unclear.[68-70] The expression of some integrin subunits is responsive to mechanical stimulation (e.g., α5, which increases)[71] and several have been linked with both healthy and pathological chondrocyte mechanosignalling. Recently, a critical role for αV integrin in the activation of TGFβ signalling in regions of mechanical stress in OA cartilage was demonstrated,[40] revealing one mechanism by which pathological ECM destruction is localised in the tissue. In regions of high mechanical stress in the cartilage, generated by altered subchondral bone architecture in OA, talin-mediated increases in cytoskeleton contractile forces and chondrocyte stiffness trigger αV-mediated activation of TGFβ. Through this mechanism, regions of TGFβ activation are correlated with regions of high mechanical stress, where TGFβ activation dysregulates chondrocyte metabolism and homeostasis, driving ECM degradation. Knockout of αV integrin in mice significantly attenuated this TGFβ activation and downstream ECM degradation. Other interesting integrin expression profiles include the α10 and α11 subunits and β1-containing integrins.[69,72-76] Integrin α10 is the dominant collagen-binding integrin in healthy chondrocytes,[77,78] while α11, which is also collagen-binding, is expressed at low levels.[65,67,79] In contrast, α11 is increased in OA cartilage and also correlates with poorer chondrogenic differentiation of mesenchymal stem cells. α10 expression is also upregulated by chondroprotective factors like FGF2 and BMP2, while α11 is increased by TGFβ.[65,80] Mechanistic details of how these integrin subunits exert their effect on cell phenotypes is yet to be determined, though a link to cell stiffness and cytoskeletal contractility seems likely. Clearly, the significant influence of these integrins and others on chondrocyte behaviour make them attractive therapeutic targets in the treatment of OA. For this, the "repurposing" of integrin receptor antagonists that are currently available or undergoing clinical examination may be an attractive approach. For example, Cilengitide, a selective inhibitor of integrin αVβ3 and αVβ5, currently in clinical trials for the treatment of glioblastoma is capable of supressing inflammatory (IL-1β, TNF-α) and catabolic mediators (MMP3, MMP13) in chondrocytes.[81]

Cytoskeletal and nucleoskeletal elements

The cytoskeleton has a singificant impact on mechanotransduction and plays a fundamental role in physiological and pathological chondrocyte phenotypes.[50] The actin cytoskeleton undergoes reorganisation with deformative (e.g., compression) and non-deformative loading (osmotic, hydrostatic pressure), and

disruption of F-actin is associated with altered cell viscoelastic properties and nuclear deformation following compression.[82-85] Treatment with thymosin B4, an inhibitor of F-actin polymerisation, results in elevated expression of catabolic mediators, leading to increased cartilage catabolism with mechanical stress.[86] Cytoskeletal elements are abnormally distributed and assembled (or even absent altogether) in OA chondrocytes, compromising cell metabolic activities and biomechanical integrity.[87,88] Moreover, in culture the application of an anabolic cyclical loading regime alone is unable to reverse these cytoskeletal changes, suggesting a combined intervention approach is required.[88]

Direct mechanotransduction in part relies on a physical link between the ECM, cytoskeleton and the nucleus. The cytoskeleton transmits external forces to the nucleus via linker of nucleoskeleton and cytoskeleton (LINC) complexes associated with nuclear lamins. These deformations influence chromatin dynamics, epigenetics, and gene transcription (Figure 2B).[89,90] Dysregulation of nuclear mechanosensing has been observed recently in OA subchondral bone, where it has a role in HDAC epigenetic regulation of MSC osteogenic potential.[91] Mechanically, cellular tension developed by the cytoskeleton also directly regulates YAP nuclear translocation, where it can promote the transcription of YAP-target genes. This direct mechanical activation has been demonstrated in 2D, where cytoskeletal tension stretches the cell nucleus and nuclear pores facilitating YAP movement into the nucleus.[92] This direct mechanism of nuclear entry may have signficant implications for controlling YAP activity in the altered mechanical environment of the OA.

The chondrocyte channelome

Chondrocytes express a diverse array of ion channels and porins collectively referred to as the 'chondrocyte channelome' (Reviewed in [45,49]). The activity of channelome proteins is associated with fluctuations in ion signalling, one of the earliest cellular events in chondrocyte responses to various modes of mechanical stimulation. Ion channels can be classified based on their gating mechanisms such as voltage, ligand, or mechanically-gated. While each type of channel can be found in the chondrocyte channelome, mechanically-gated ion channels are of particular interest as they are capable of inducing rapid mechanosensory transduction. Calcium signaling (Ca^{2+}), a ubiquitous second messenger, has a key role in activating multiple intracellular signaling pathways in mechanotransduction (Figure 2B, 2C). Any initial ion influx can also be amplified further by the subsequent release of ions from intracellular stores. In cartilage, recent work has paid particular focus to members of the vallinoid subfamily of transient receptor potential (TRPV) channels, which are regulators of intracellular Ca^{2+} concentration in non-excitable cells. While chondrocytes express various TRP channels, the TRPV4 non-specific cation channel has gained interest due to its role in controlling the

mechano-osmotic transduction cascade (Figure 2B, 2C).[46,47,93] TRPV4-mediated intracellular Ca^{2+} signaling is a key mechanosignalling pathway in chondrocytes and is involved in regulation of ECM biosynthesis.[47] Deletion of TRPV4 in mice results in severe OA-like presentations,[46] while strikingly, loss of TRPV4 in chondrocytes prevents age-related OA development but not mechanical load-dependent OA.[94] These anabolic roles for TRPV4 suggest it is a promising therapeutic target. In other mechanosensitive cells, the TRP proteins, TRPA1 and TRPP2, also have well-established mechanosensory properties and appear likely to be involved in chondrocytes.[95]

While physiological loading can induce an anabolic response in chondrocytes through the activity of TRPV4 ion channels, another family of mechanosensitive ion channels are implicated in the mechanotransduction of injurious mechanical stimuli. Piezo channels, specifically Piezo 1 and 2, are cation permeable ion channels also involved in chondrocyte mechanotransduction (Reviewed further in [96]). Piezo 1 and 2 are distinct from other ion channels in that they consist of 4 transmembrane helical bundles termed Piezo repeats, which together form flexible propeller blade-like structures. Both Piezo 1 and 2 are present in chondrocytes, providing high strain mechanosensitivity and the ability to respond to 'hyperphysiological' levels of mechanical stress.[97] Briefly, high strain (compressive loading >45% strain) increases Ca^{2+} influx via Piezo channels. Knockdown of either Piezo1 or 2 prevents mechanically induced Ca^{2+} transients, suggesting a synergistic action. Inhibition of Piezo acitivity with GsMTx4 (a Peizo 1/2 blocking peptide) protects chondrocytes from mechanically induced cell death, highlighting their therapeutic potential.[96,97]

Primary cilia

The primary cilium is a non-motile cellular organelle, which protrudes from the cell surface and contains a high concentration of mechanosensory machinery and receptors. In chondrocytes, the primary cilia have an important role in mechanotransduction (Figure 2C).[51,98] Primary cilia exhibit cartilage zone dependent variation (in both occurrence and length, which both increase with distance from the articulating surface), which is thought to be as a result of the different stress, strain, and fluid flow experienced throughout each zone.[51] Interestingly, cilia length and the percentage of ciliated chondrocytes in cartilage have been shown to increase with OA severity. Additionally, the orientation of OA chondrocyte primary cilia is observed to be towards the articular surface rathar than away as is observed in healthy chondrocytes.[51] In primary cillia knockouts (Col2a1Cre/ift88$^{fl/fl}$ transgenic mice) cartilage stiffness is decreased while, tissue thickness and the expression of several OA markers (*COLX, RUNX2, MMP13, ADAMTS5*) are increased.[53,99] Further, transgenic mice with mutant Polaris, an essential cilia protein, fail to initiate mechanosignalling and

compression of IFT88(orpk) mutant chondrocytes (that lack primary cilia) does not initate intracellular Ca^{2+} signaling, ECM synthesis or ATP release.[95,100,101] The localisation of mechanosensors to the primary cilia facilitates the mechanical activation of purinergic signalling in the form of ATP and intracellular Ca^{2+} release.[102] This ATP release is mediated by the mechanically stimulated opening of hemichannels (connexons), present in high densities in the primary cillia, and is linked with several anabolic activities including an increase in proteoglycan production and cell proliferation.[102-105]

Mechanotherapeutics for the treatment of osteoarthritis

Mechanotherapeutics originally refered to the use of physcial therapy to treat a disease but now encompasses interventions that target molecular, cellular and tissue level mechanosignalling to repair tissue damage or treat disease.[106,107] In short, mechanotransduction can be targeted in a therapeutic manner to either promote anti-catabolic, anabolic pathways and repair processes, or to block the pathways central to OA. However, underlying the complex nature of OA pathogenesis is an equally complex signalling network. For example, a study aimed at characterising the response of articular cartilage explants to mechanical injury revealed the significant regulation (>2 fold change) in 690 genes.[108] These pathways are implicated in regulating inflammation, production of catabolic and degradative enzymes (*MMPs, ADAMTSs*), cellular apoptosis and bone dysfunction within the joint. In the following sections, we highlight some of the prominent mechanosignaling pathways that have been implemented in the pathogenesis and progression of OA and how these pathways might be targeted therapeutically (Figure 3).

The Mitogen-Activated Protein Kinase (MAPK) Pathway

The mitogen-activated protein kinases (MAPKs) play a key role in the regulation of various cell signaling pathways that are implicated in cellular proliferation, matrix synthesis, survival and mediation of pain.[109] In chondrocytes, MAPKs are involved in regulating gene expression in response to mechanical stimulation. Activation of ERK1/2, P38 MAPK and the SAPK/ERK kinase 1 (SEK1) occurs following compression. [110] Furthermore, integrin activation through matrix fragments activates MAP kinases and results in the upregulation of catabolic signalling. As previously discussed, $\alpha 5\beta 1$ mediates matrix degradation induced by fibronectin fragments.[111] The signalling proteins downstream of α5β1 have since been identified. In short, PKCδ activation of proline-rich tyrosine kinase 2 (PYK2) and downstream activation of the MAP kinases ERK1/2, JNK1/2, and P38α leads to heightened activity of NF-κB and activator protein-1 (AP-1) transcription factors.[111-114] This signalling also requires the production of reactive oxygen species and the presence of active Rac1.[115] [116] It has also been noted that MAP kinases are required for stimulation of nitric oxide (NO),

MMP3, and MMP13 production, which further potentiate the progression to OA[117-119] Compression or shear stress activate ERK1/2 and P38 MAPK contributing to an increase in the expression of proinflammatory cytokines and molecules whilst also regulating the expression of mechanoresponsive anabolic genes (Figure 3).[120] While therapeutic inhibition of MAPKs holds great potential in slowing the progression of OA, there remain safety concerns over the use of general MAPKs inhibitors in humans.[121] Considering the involvement of MAPKs within a complex signaling network, it is likely that a system offering long-term feedback for the controlled delivery of therapeutics will be required. Furthermore, targeting the deleterious processes downstream of MAPK activation, such as the mechano-inflammation mediators discussed below, may be a more attractive therapeutic option. Aside from this, the use of targeted, biomaterial-based approaches, utilized typically in tissue engineering applications may enable increased specificity and efficacy of selective modulation of MAPKs in a therapeutic manner. For example, the application of highly versatile functionalized scaffolds, which have previously demonstrated success in modulating c-Jun N-terminal kinase 3 (JNK3) for enhancing the osteogenic potential of mesenchymal stem cells, might provide the basis as a platform for targeted regulation of MAPK signaling pathways in other tissues such as cartilage.[122]

Mechano-inflammation and the NF-κB signalling pathway

Aside from the well-known cytokine-mediated activation of the NF-κB signaling, NF-κB activation can also be regulated by physical forces in chondrocytes. For example, low magnitude mechanical strain blocks nuclear translocation of NF-κB, preventing upregulation of proinflammatory genes[123]. Conversely, high magnitude mechanical strain, representative of that experienced in injury, induces transactivation of NF-κB signaling complex and expression of proinflammatory genes, cartilage destruction and inhibition of ECM synthesis[124]. These findings indicate a central role for mechano-inflammation in the progression of OA and perhaps more importantly open the door to a number of promising therapeutic targets.

A well described mechano-inflammatory pathway involves activation of the TGFβ-activated kinase 1 (TAK1), upstream of the P38 MAPK, c-Jun N-terminal kinase (JNK) and NF-κB signaling (Figure 3).[125] Of particular note, is that although TAK1 can be activated by inflammatory cytokines and TLR ligation, the pattern of ubiquitination of TAK1 observed is distinct from that seen following cytokine stimulation.[126] Recently, GREMLIN-1, a secreted BMP agonist, has also been identified as a mediator of NF-κB signaling following excessive mechanical loading in chondrocytes. Inhibition of VEGFR2 or NF-κB attenuated GREMLIN-1 induced pro-inflammatory and catabolic activity. Moreover, GREMLIN-1 antibody or chondrocyte-specific knockdown suppresses OA development *in vivo*, while the delivery of recombinant

GREMLIN-1 accelerates this process. Further investigation revealed that mechanically loaded GREMLIN-1 production occurs through the Rac1-ROS-NF-κB pathway (Figure 3).[127]

Inflammation predisposes chondrocytes to become hypersensitive to injurious levels of mechanical loading as a result of IL-1α induced upregulation of Piezo1 and subsequent increased basal Ca^{2+} levels.[128] Interestingly, the presence of IL-1α also increased chondrocyte deformation in response to the same loading magnitude due to F-actin rarefication. Further to these findings, a signaling mechanism from the IL-1 receptor type I (IL-1RI) complex on the cell membrane via MKK3/6 to P38 MAPK has been delineated through phosphorylation of P38 to the nuclear signaling of the transcription factor CREBP1, which together with ATF2 and HNF4α up-regulates PIEZO1 (Figure 3). This 'feed forward' mechanism, as described by the authors, indicates mechano-inflammation contributes to the maladaptive reprogramming of chondrocytes and provides a rational set of mechanotransduction informed targets for therapeutic approaches to OA.[128]

In the same study, TRPV4 expression levels and function were not altered by the presence of IL1α, suggesting that inflammation has a selective effect on the expression of cell mechanosensory machinery. However, TRPV4 has also been identified as a key mechanoreceptor involved in mechano-inflammation. Recent studies investigating how ECM viscoelasticity modulates the function of healthy and OA chondrocytes has shown that under static conditions, chondrocytes interact with the ECM of fast- and slow-relaxing alginate hydrogels, regulating their volume and phenotype.[8] In fast-relaxing gels, chondrocyte cell volume expands and ECM expression is upregulated, while the opposite holds true for slow-relaxing gels. While inflammation predisposes cells to mechanical stress, the reverse has also been observed where cells cultured in slow-relaxing gels undergo a proinflammatory phenotypic shift, making the cells more sensitive to extrinsic inflammatory cues. [8,128] Mechanistically, one mechanism through which chondrocytes sense and respond to changes in matrix viscoelasticity is through the TRPV4-GSK3β molecular axis. Treatment of cells in slow-relaxing gels with a TRPV4 selective inhibitor reduced inflammation and shifted the cells towards the phenotype observed in fast-relaxing gels. Similarly, treatment of cells cultured in fast-relaxing gels with a TRPV4 activator, resulted in an increase in intracellular Ca^{2+}, GSK3β phosphorylation and a subsequent increase in inflammation (Figure 3).[8] It is interesting to note that a TRPV4-phosphatidylinositol 3-kinase (PI3K)/ Akt-p27Kip1 signalling axis has been demonstrated to control tumour cell (MDA-MB-231) proliferation, which is promoted in fast relaxing hydrogels but arrested in slow relaxing gels.[129] In chondrocytes, cell confinement in fast relaxing viscoelastic hydrogels has been reported to enhance proliferation, ECM production, and anabolic gene expression. Furthermore, in this study restricted cell expansion was also shown to induce IL-1β signalling, highlighting the interplay between cell expansion and

OA progression.[130] Unlike healthy chondrocytes, which can sense and transduce changes in ECM viscoelasticity, OA chondrocytes are unable to do so. Interestingly, treatment of OA chondrocytes with a TRPV4 inhibitor failed to change Ca^{2+} levels suggesting a dysregulation of the TRPV4-GSK3β molecular axis in OA chondrocytes.[8] While TRPV4 plays a central role in responding to mechanical signals in healthy chondrocytes, its impairment in OA chondrocytes may limit its use as a therapeutic target (at least in the later stages of OA). Such findings highlight the importance of not only understanding the mechanism of drift in chondrocyte phenotype but also the timing of the molecular events governing this shift and will be invaluable in determining treatment strategies.

The WNT signalling pathway

WNT signalling has established roles in embryonic development, tissue homeostasis, growth and is implicated in the onset and progression of various pathologies (specifics reviewed in [131]). In cartilage, WNT activity is essential for cartilage homeostasis, however controlled activity is required with a moderate amount of WNT activity shown to be essential for chondrocyte proliferation while excessive activity contributes to hypertrophy and increased expression of MMPs. In OA, the expression of WNT pathway members is dysregulated, increasing the expression of catabolic enzymes.[132] Mechanical injury leads to the upregulation of Frizzled-related protein (*FRZB*), a WNT-binding protein.[133] Meanwhile, knockout of FRZB leads to an increase in MMP expression and the accumulation of β-catenin in IL-1β stimulated chondrocytes. A microarray and systematic analysis revealed distinct differences between healthy and injured cartilage including the up-regulation of WNT-16, downregulation of FRZB, upregulation of WNT target genes, and nuclear localization of β-catenin. Furthermore, WNT-16 and β-catenin were up-regulated in areas of the same joint that had moderate to severe OA compared to preserved cartilage areas.[134] In a mouse model of OA, both protein and mRNA levels were also up-regulated, however, WNT16 deficient mice presented with more severe OA suggesting a homeostatic role. Interestingly, WNT16 deficient mice failed to up-regulate lubricin (PRG4), a low friction proteoglycan and chondroprotective agent.[135] The WNT signaling pathway thus presents potential therapeutic targets for the treatment of OA.[132,136,137] However, the involvement of WNT signaling in so many important biological processes makes targeting these pathways a daunting task. Any such intervention should aim to fine tune WNT signaling activity (Reviewed further by [138]). Recent strong performance of Lorecivivint (SM04690), a WNT pathway modulator, in Phase 1 (NCT02095548) and Phase 2 (NCT02536833, NCT03122860) clinical trials is encouraging and demonstrated safety and efficacy in knee OA patients, with significant improvements observed compared to placebo.[139,140] Lorecivivint, inhibits the

activity of CDC-like kinase enzymes (CLKs), in particular CLK2 and dual-specificity tyrosine phosphorylation-regulated kinases enzymes (DYRK), specifically DYRK1A, enhancing chondrogenesis, chondrocyte function, and anti-inflammation (Figure 3).[141] Another promising therapeutic is DOT1-Like Histone Lysine Methyltransferase (DOT1L), which has been shown limit WNT activation. DOT1L appears to have a chondroprotective role in cartilage, with intra-articular delivery of a DOT1L inhibitor found to trigger development of OA (Figure 3).[142-144]

YAP/TAZ signalling pathway

The transcriptional cofactors Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) form a key mechanosignalling complex and are involved in regulating chondrogenesis, chondrocyte maturation, and hypertrophy.[145] YAP/TAZ activity is tightly regulated by biochemical and mechanical regulation. Biochemically, they are members of the Hippo pathway, which can be activated by cadherin-cadherin interactions and leads to YAP phosphorylation and retention in the cytoplasm, where it cannot act to influence transcription. This regulation demonstrates that YAP/TAZ activity can be controlled by cell structure and polarity, cell-ECM interactions, and physical cues from the microenvironment and directly converted into gene expression profiles, adapting cell phenotypes rapidly. In complex or in vivo 3D environments, however, YAP/TAZ regulation is not fully understood and responses divergent from well-established 2D responses have been reported.[146,147] During chondrogenesis of MSCs in vitro, YAP is downregulated.[148] YAP also inhibits terminal chondrocyte maturation and hypertrophy by suppressing type X collagen expression (COL10A1) through interacting with runt-related transcription factor 2 (RUNX2), an important regulator of chondrocyte hypertrophy and osteogenesis.[145] In OA, deletion of YAP promotes cartilage disruption, while proinflammatory cytokines typically upregulated in the OA joint (IL-1β, TNF-α) drive the destruction of YAP through TAK1-mediated phosphorylation. YAP also interacts with TAK1 and attenuates NF-κB signalling by inhibiting substrate accessibility, which establishes a reciprocal antagonism between YAP/TAZ and NF-κB, important in regulation of proinflammatory responses in OA (Figure 3).[10]

Central energy metabolism and activity metabolites

Metabolism has a key role in OA progression and recent studies have begun to reveal links between chondrocyte mechanotransduction and metabolic pathways.[149] Central energy metabolism pathways (glycolysis, the pentose-phosphate pathway (PPP), and the Krebs/TCA cycle etc.) are how cells harvest energy (e.g., ATP) generating the precursors to non-essential amino acids.[150] There is now strong evidence

that mechanosignalling can regulate cell metabolism and that metabolic processes in healthy and OA chondrocytes respond differently to mechanical stimuli. For example, in OA chondrocytes cyclic loading reduces phosphorylation of AKT, a regulator of Forkhead box O (FoxO) signalling in energy homeostasis.[151] Conversely, mechanical stimulation induces AKT phosphorylation in healthy cells.[152] These different metabolic responses have implications for downstream anabolic responses to mechanical loading observed in healthy cells, fuelled by a glycolytic energy flux that is reduced in OA.[153] Investigating the effect of mechanically induced changes on the cellular metabolome can enable the identification of metabolites involved in mediating cell mechanotransduction and phenotypes, opening the possibility of harnessing these 'activity metabolites' to control cell behaviour or developing chemical analogs with enhanced therapeutic action.[154-156] Metabolomics data obtained to date has highlighted the role of various signalling pathways associated with mechanotransduction. Considering the multifactorial nature of OA and the distinct shift in chondrocyte metabolism in disease, it is likely that further elucidation of the role of central energy metabolism in mechanotransduction will yield promising therapeutic targets by comparing mechanically-induced metabolite expression in both healthy and OA chondrocytes.[154,155]

Future Directions

Physical therapy is commonly used in the non-pharmacological treatment of OA, albeit without a comprehensive understanding of the underlying cellular mechanosensory and signaling mechanisms at play.[107] Understanding mechanosignalling holds the key for the development of therapeutics that control cellular responses, halt disease progression, and enable regeneration of the cartilage tissue. While promising DMOADs have emerged in recent years (e.g., COX-2 inhibitors, corticosteroids, IL-1β inhibitors, TNF-α inhibitors etc.), these have not proved to be as effective as hoped and a rethinking of how we develop therapeutics for OA is required. A good starting point may be the repurposing of selective small molecule inhibitors (SMIs) that have shown success in blocking mechanosignalling pathways in other mechanosensate cells/ tissues. For example, WRG-28 specifically inhibits the receptor-ligand interactions of discoidin domain receptor 2 (DDR2) in tumours, but aberrant DDR2 activation has also been implicated in OA.[157] The continuing development of advanced cartilage-on-a-chip models, capable of recapitulating in vivo-like physiological and pathological mechanical and biochemical cues in higher-throughput systems, will provide valuable methods for rapid and accurate screening of prospective DMOADs prior to *in vivo* testing.[158]

In addition to the physical impediments to developing and delivering successful DMOADs (e.g., tissue location, dense ECM, rapid drug clearance), there is an intricate link between mechanosignalling and critical catabolic-anabolic signalling axes driving the progression of OA. While research to date largely focuses on controlling one specific parameter (e.g., matrix stiffness, growth factor presentation, inflammation, mechanical stimulation, biomaterial degradation etc.), this approach often overlooks this complex interplay between stimuli. However, researchers should not despair at the overwhelming complexity of these pathways as collectively we continue to make advancements in our therapeutic toolbox. For example, recent development of biomaterials capable of regulating a combination of these factors simultaneously has enabled acquisition of valuable insights into this signalling interplay. For example, investigations into the role of several mechanosignalling pathways (integrin α5β1, YAP, SMAD/TGFβ, MAPKs and WNT) and their involvement in regulating the differentiation of hMSCS cultured on articular or hypertrophic promoting hydrogels with cyclic compression. This approach revealed a role for down-regulation of WNT signaling to promote articular-like chondrocyte phenotypes in soft matrices and confirmed YAP-regulation of hypertophy in stiff matrices.[145,159] The application of -omics analysis with such platforms to map cellular and molecular responses to tightly controlled biomechanical stimuli (mechanomics) will provide even greater insight into responses to specific stimuli.[160]

Another important consideration in mechanotherapeutics is timing of intervention. As the articular joint tissue heals, the needs of the tissue evolve, adding a further degree of complexity. Further to this, therapies for OA, a disease that is intricately linked to the aging process, may require sustained drug administration over a long time-period, whilst maintaining the delicate anabolic-catabolic balance. It is also likely that OA patients will show an increase in co-morbidities as they age, which may further complicate treatment. To overcome these challenges, significant advancements have been made in developing cartilage-specific drug delivery strategies. For example, developments in advanced therapeutic approaches such as gene therapy for the delivery of plasmid (p)DNA or RNA-based therapeutics (siRNA, miRNA) may help overcome issues with target specificity, safety and bioavailability. In recent years, advances in vector technologies and a greater understanding of the mechanisms of action have led to a surge in the development of gene therapy approaches. The planned FDA Phase 3 clinical trials (TGC12301, TGC15302) of INVOSSA™ (Kolon TissueGene), for the delivery of TGFβ1, will be observed closely by researchers, with a successful outcome potentially opening a new era of gene therapy for OA. The potential of siRNAs has been demonstrated through knockdown of modulators of mechano-inflammation including WNT/ β-catenin, NF-κB and p38 MAPK[161,162] (reviewed recently in [163]). MicroRNAs (miRNAs - small single-stranded non-coding

RNAs) are highly potent post-transcriptional regulators, as one miRNA can exert an effect on multiple mRNAs. Several miRNAs have been shown to be mechanically-regulated in chondrocytes, with alterations in miR expression associated with OA and with different cartilage zones subjected to different degrees of mechancial loading.[164-168] miRNA profiling of OA cartilage has revealed unique miRNA signatures and several miR's have been identified as potential therapeutic targets.[169] One promising candidate, for example, is miR-365, which is upregulated with cyclical loading of chondrocytes and in OA, regulates cell hypertophy through a mechanism involving NF-κB and HDAC4.[170] Itself a potent inhibitor of chondrocyte hypertophy and MMP expression, HDAC4 activity is inhibited by miR-365 and MMP13 and type X collagen expression increased. Inhibition of miR-365 was able to attenuate this upregulation.[166,170] Conversely, miR-222 is down-regulated in OA chondrocytes and is implicated in regulating cartilage destruction and MMP-13 expression. Intra-articular delivery of miR-222 to mouse destabilised medial meniscus models recovers cell number and significantly reduces cartilage destruction.[171,172] More recently, miRNAs regulated by different loading conditions has led to the discovery of several miRs (miR-199b-5p, miR-1229-5p, miR-1275, miR-4459, miR-6891-5p, and miR-7150) which were only affected under catabolic loading conditions.[173] These systematic approaches investigating diverse loading conditions in both healthy and OA cells is likely to lead to further development of therapuetic avenues in this area.[174]

Mechanoresponsive smart biomaterials that leverage the mechanical environment of the joint during regeneration can facilitate drug delivery in sync with the needs of the tissue, ultimately enhancing the therapeutic process (Figure 4). For cartilage applications, a suite of mechanically-activated microcapsules (MAMCs) with varying thresholds of mechanoactivation capable of releasing TGFβ3 have been developed.[175] Using this MAMC system to deliver anti-inflammatory agents was demonstrated to successfully reduce matrix degradation in engineered cartilage constructs treated with IL1β (Figure 4).[12] However, issues remain surrounding prolonging delivery *in vivo* in these systems over timescales suitable for cartilage regeneration and fine-tuning release profiles to mechanical inputs. In order to overcome these limitations, researchers have begun to develop autonomous mechanotherapeutics, which can respond in real-time to the changing microenvironment, thus overcoming issues with long-term integration, target specificity, timing, homeostatic maintenance and repeated administration. Recently, for example, through deconstructing the signaling networks downstream of TRPV4 activation, a synthetic TRPV4-responsive gene circuit was developed, which was shown to be effective as an autonomously regulated drug delivery system capable of producing interleukin-1 receptor antagonist in response to mechanical loading (Figure 4).[176] While only used for the mechanical activation of TRPV4 and the delivery of a specific anti-inflammatory, the framework of this system

opens the door to other mechanoreceptors and signalling pathways, allowing for a new generation of mechanotherapeutics. The development of such mechanically responsive biomaterials and closed-loop drug delivery systems may be the key in maintaining treatment efficacy in the changing environment in the joint during regeneration.

Conclusion

Mechanical stimuli are a critical environmental factor in maintaining joint homeostasis. How chondrocytes sense and respond to these mechanical forces determines cellular phenotype, regulates inflammation and tightly controls the anabolic-catabolic axis required for cartilage health. Chondrocytes are equipped to respond to these forces through a variety of mechanosensory mechanisms and signalling. Altered mechanical forces, as a result of trauma or a reduced capacity to withstand normal loading conditions, regulates cell processes central to OA pathology, thus providing a number of effectors which can be targeted to uncouple pathological feedback loops. Coupling these targets with advanced drug delivery systems ranging from gene therapy to mechanosensitive biomaterials offers great promise in the development of safe and effective treatments for OA.

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Competing Interests

The authors declare no competing interests.

Figure 1 Articular cartilage composition and structure. Schematic representation of articular cartilage composition and structure in healthy and osteoarthritic tissue states. The structure of articular cartilage can be divided into four distinct zones (superficial, middle, deep and calcified). Each zone exhibits characteristic composition and organization. The ECM composition and architecture represent the forces experienced within these zones. Chondrocytes and type II collagen fibres are orientated transversely in the superficial zone, enabling the dispersion of shear forces during articulation. The presence of lubricin/proteoglycan 4 (PRG4) within this zone further facilitates lubrication of the joint. Within the middle zone, type II collagen fibres resist compressive and shear forces from a number of directions, exhibited by the random arrangement of the fibres. Thick collagen fibres arranged perpendicular to the articulating joint surface in the deep zone resist compressive loads. High concentrations of proteoglycan in this zone enable water retention. The matrix in which chondrocytes reside also differs in composition and structure and can be divided into three regions moving from the chondrocyte outwards – the pericellular matrix (PCM), territorial matrix (TM) and the interterritorial matrix (ITM). The PCM, characterized by the presence of type VI collagen, surrounds the chondrocyte and has a significant influence on chondrocyte mechanotransduction. The PCM also contains other important components (i.e. hyaluronan, biglycan, fibronectin). The PCM integrates with the TM, a region characterized by tightly packed fibrillary collagen and proteoglycan. The outermost region, termed the ITM, is used to refer to the bulk tissue ECM. While the integrity of the ECM as a whole plays a significant role in the transmission of forces across the tissue, each specific zone determines the type of loading experienced by a chondrocyte, influencing the cell response.

Figure 2 Chondrocyte mechanosignalling. An illustrative overview of key mechanosensing mechanisms in chondrocytes.(A) Indirect mechanosensing. On deformation, extracellular matrix sequestered growth factors including fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and transforming growth factor (TGF) activate cell surface receptors. FGF binds to FGF receptors (FGFRs) and phospholipase C$\boldsymbol{\gamma}$ (PLC$\boldsymbol{\gamma}$) is activated by binding to the kinase domains of FGFRs and in turn stimulates protein kinase C (PKC) activity and activates downstream MAP kinases. FGF receptor ligand binding also activates growth factor receptor-bound protein 2 (GRB2), activating RAS, resulting in activation of extracellular signal regulated kinase (ERK1/2). ERK1/2 translocates to the nucleus and impacts the activity of numerous transcription factors. BMPs and TGFs bind to heterodimer BMP and TGF cell surface receptors (BMPR, TGFR). In the SMAD signalling pathway either SMAD 1/5/8 (BMPRs) or SMAD 2/3 (TGFRs) are phosphorylated and activated on ligand-receptor binding, recruiting SMAD4 and translocating to the nucleus and impacting

transcription. Non-SMAD signalling pathways are activated through TGFβ activated kinase (TAK1), which can activate JUN N-Terminal Kinase (JNK), P38 Mitogen Activated Protein Kinase (P38) and Nuclear Factor κB (NF-κB). (B) Direct mechanosensing. Integrin activation can trigger biochemical signal transduction and direct mechanical deformation of the cell through cytoskeletal contraction. On integrin binding, SRC kinase and focal adhesion kinase (FAK) recruitment result in GRB2 binding and downstream activation of ERK1/2 and JNK signalling. Integrin binding also triggers PI3K/ AKT pathway activation through Integrin Linked Protein Kinase (ILK). Mechanically regulated ion channels such as Transient Receptor Potential Cation Channel 4 (TRPV4) and Piezo Type Mechanosensitive Ion Channel Component 1/ 2 (PIEZO1/2) drive Ca^{2+} influxes into cells, activating numerous downstream signalling pathways. (C) Primary cilium-mediated mechanotransduction. Primary cilia house high levels of multiple mechanosensory receptors and channels involved in direct and indirect mechanosensing. On mechanical stimulation, for example through deflection when experiencing fluid flow, these are activated, resulting in downstream activation of multiple signalling pathways as elsewhere in the cell.

Figure 3 Mechano-inflammation signaling in chondrocytes in osteoarthritis. Mechano-inflammation in OA involves several key signaling pathways which can be activated by both inflammatory cytokines and/or mechanical stimuli. Mechanical regulation of pathway activity controls both downstream anabolic and catabolic processes offering a number of promising therapeutic targets which can halt the progression of OA while concomitantly stimulating anabolic repair. Activation of mechano-inflammatory signaling contributes to increased inflammatory gene expression, alterations in cytoskeletal phenotype, energy homeostasis and an increase in catabolic mediators. Key mediators within mechano-inflammation include TGFβ-activated kinase 1 (TAK1), YAP/TAZ, Nuclear Factor κB (NF-κB), JUN N-Terminal Kinase (JNK), P38 Mitogen Activated Protein Kinase (P38) and downstream mediators of the WNT signaling pathways. TAK1 activation occurs upstream of the P38 MAPK, c-Jun N-terminal kinase (JNK) and NF-κB. Inflammatory cytokines drive the destruction of YAP through TAK1-mediated phosphorylation, while YAP can also interact with TAK1 attenuating NF-κB. GREMLIN-1 exerts a pro-inflammatory effect through the GREMLIN-1-VEGFR axis. Induction of GREMLIN-1 as a result of excessive mechanical loading occurs via the Rac1-ROS-RelA/p65 axis and NF-κB signaling. Mechanoinflammation predisposes chondrocytes to a state of hypersensitization, rendering them susceptible toinflammatory and mechanical stimuli present within normal physiological ranges. IL-1αinduces the upregulation of Piezo Type Mechanosensitive Ion Channel Component (PIEZO1), increased calcium (Ca^{2+}) levels and contributes to F-actin rarefication via the transcription factors CREBP1,

ATF2 and HNF4α. Chondrocytes sense and respond to changes in matrix viscoelasticity through the Transient Receptor Potential Cation Channel 4 (TRPV4)-GSK3β axis, where TRPV4 activation, either pharmacologically or through culturing of cells on slow-relaxing gels, results in increased Ca^{2+}, GSK3β phosphorylation and increased inflammation. Therapeutic targeting of mechanoinflammation and its signaling pathways holds promising potential. Lorecivivint, a WNT pathway modulator, inhibits CDC-like kinase enzyme (CLK2) and dual-specificity tyrosine phosphorylation-regulated kinases enzymes (DYRK1A) activity enhancing chondrogenesis and anti-inflammation. Additionally, DOT1-Like Histone Lysine Methyltransferase (DOT1L) interacts with SIRT1 preventing Wnt pathway hyper-activation, maintaining cartilage homeostasis.

Figure 4 Mechano-responsive therapeutics for the treatment of osteoarthritis. Mechanotherapeutic approaches aim to take advantage of the dynamic physical stimuli present in the joint in order to deliver biomolecules. Approaches including drug loaded cross-linkable block copolymer hydrogels and tethered drug-filled depots enable delivery of biomolecules in a spatio-temporally controlled manner. Mechanically activated microcapsules (MAMCs) can be tailored to rupture in response to specific loading conditions enabling delivery of therapeutics in a controllable manner based on the specific needs of the tissue at any given time. Development of MAMCs encapsulating chondrogenic therapeutics (TGFβ) or anti-inflammatory agents (interleukin-1 receptor antagonist) have shown promising potential in cartilage regeneration. Further advancements within the fields of synthetic biology and tissue engineering have enabled the reprogramming of mechanotransduction and signaling pathways for the development of autonomously regulated drug delivery systems which can respond in real-time to the ever-changing mechanical microenvironment of the joint enabling enhanced tissue repair. A comprehensive understanding of the underlying signaling networks downstream of mechanoreceptor activation can be used for the development of synthetic gene circuits responsive to mechanical loading. The development of a synthetic TRPV4-responsive gene circuit enables autonomous production of the anti-inflammatory agent interleukin-1 receptor antagonist in response to mechanical loading.

BOX 1 | MECHANO-INFLAMMATION IN OSTEOARTHRITIS

Mechano-inflammation can be described as the inflammatory response to mechanical injury. Beyond wear and tear, excessive mechanical loading of the cartilage activates receptors and signalling pathways that result in the production of the catabolic enzymes that drive degradation of the functional cartilage ECM. Evidence from OA and other forms of arthritis also indicates that mechanical strain is important in determining the localisation of inflammation and tissue degradation.[40,177] Deletion or inhibition of mechanically induced proteases, or the inflammatory signalling pathways that control them, prevents OA progression in animal

models, even when joints are destabilised.[1,40] Similarly, progression of OA is prevented when joints are immobilised following induction of OA or when superficial zone chondrocytes are specifically destroyed prior to joint destabilisation.[2,4] Mechano-inflammation involves several key signalling pathways including TGFβ, P38, JNK, YAP/TAZ and NF-κB. TGFβ-activated kinase 1 (TAK1), which can be activated by both inflammatory cytokines and mechanical stimuli, appears to be involved in a number of these responses.[178] TAK1 mediates mechanical activation of MAPK cascades and is central to YAP/TAZ anabolic activity and reciprocal inhibitory interaction with NFκB.[10] It follows then that control of mechano-inflammation in the OA joint has the potential to both control catabolic processes and promote anabolic repair processes. Interestingly, along with magnitude, duration and frequency of force applied to the joint, the type of loading experienced by chondrocytes situated in the different zones of the cartilage appears to be significant in determining whether mechano-inflammation is activated, or anabolic processes are stimulated.[2] Targeting mechano-inflammation, either through restoration of a functional mechanical environment (e.g., by biomaterials) or by controlling key signalling mechanisms and receptors, might be one of the most promising strategies for improving the efficacy of OA therapies.

BOX 2 | USE OF MECHANO-RESPONSIVE 'SMART' BIOMATERIALS IN CARTILAGE

Mechano-responsive smart biomaterials can be designed to adapt and respond to the changing mechanical microenvironment of the joint during tissue restoration. By harnessing the constant dynamic stimuli present in the joint, mechano-responsive biomaterials can be used to deliver biomolecules in a spatio-temporally controlled manner (Figure 4).[179] Physical-based stimuli responsive drug delivery systems can deliver drugs in a controlled manner in response to compressive, tensile and shear forces.[180] For example, the use of mechano-responsive hydrogels, incorporating mechano-responsive drug depots enables the delivery of the anti-inflammatory drug dexamethasone in response to compressive forces applied to the gels.[181] Furthermore, the development of "smart" mechano-responsive biomaterials tailored to use specific mechanical cues present at various stages of the tissue regeneration process can deliver therapeutics sequentially based on the specific needs of the cells and tissue at any given time. The development of mechanically-activated microcapsules tuned to exhibit different rupture profiles enables specific drug release in a sequential manner using the different mechanical forces present throughout the healing process.[12,175] As our understanding of the receptors and signalling pathways involved in mechanotransduction increases, so too does the "degree of smartness" of mechanoresponsive approaches. In addition to responding to a particular physical force and delivering a specific drug, the use of advanced tissue engineering and synthetic biology approaches targeting mechanotransduction pathways involved in health and disease can enable "mechanobiological reprogramming" of cells for the creation of autonomous mechano-responsive constructs for enhanced tissue repair.[176]

References

1 Glasson, S. S. *et al.* Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* **434**, 644-648 (2005).

2 Burleigh, A. *et al.* Joint immobilization prevents murine osteoarthritis and reveals the highly mechanosensitive nature of protease expression in vivo. *Arthritis & Rheumatism* **64**, 2278-2288 (2012).

3 Ismail, H. M. *et al.* Interleukin-1 acts via the JNK-2 signaling pathway to induce aggrecan degradation by human chondrocytes. *Arthritis & rheumatology* **67**, 1826-1836 (2015).

4 Zhang, M. *et al.* Induced superficial chondrocyte death reduces catabolic cartilage damage in murine posttraumatic osteoarthritis. *The Journal of clinical investigation* **126**, 2893-2902 (2016).

5 Guilak, F., Nims, R. J., Dicks, A., Wu, C.-L. & Meulenbelt, I. Osteoarthritis as a disease of the cartilage pericellular matrix. *Matrix Biology* **71**, 40-50 (2018).

6 Vincent, T. L. Targeting mechanotransduction pathways in osteoarthritis: a focus on the pericellular matrix. *Current opinion in pharmacology* **13**, 449-454 (2013).

7 Pap, T. & Korb-Pap, A. Cartilage damage in osteoarthritis and rheumatoid arthritis—two unequal siblings. *Nature Reviews Rheumatology* **11**, 606-615 (2015).

8 Agarwal, P. *et al.* A dysfunctional TRPV4–GSK3β pathway prevents osteoarthritic chondrocytes from sensing changes in extracellular matrix viscoelasticity. *Nature Biomedical Engineering*, 1-13 (2021).

9 Nims, R. J. *et al.* A synthetic mechanogenetic gene circuit for autonomous drug delivery in engineered tissues. *Science Advances* **7**, eabd9858 (2021).

10 Deng, Y. *et al.* Reciprocal inhibition of YAP/TAZ and NF-κB regulates osteoarthritic cartilage degradation. *Nature communications* **9**, 1-14 (2018).

11 Eckstein, F. *et al.* Intra-articular sprifermin reduces cartilage loss in addition to increasing cartilage gain independent of location in the femorotibial joint: post-hoc analysis of a randomised, placebo-controlled phase II clinical trial. *Annals of the rheumatic diseases* **79**, 525-528 (2020).

12 Peredo, A. P. *et al.* Mechano-activated biomolecule release in regenerating load-bearing tissue microenvironments. *Biomaterials* **265**, 120255, doi:https://doi.org/10.1016/j.biomaterials.2020.120255 (2021).

13 Poole, A. R. *et al.* Composition and structure of articular cartilage: a template for tissue repair. *Clinical Orthopaedics and Related Research®* **391**, S26-S33 (2001).

14 Mow, V. C., Ratcliffe, A. & Poole, A. R. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. *Biomaterials* **13**, 67-97 (1992).

15 Schätti, O. R., Marková, M., Torzilli, P. A. & Gallo, L. M. Mechanical loading of cartilage explants with compression and sliding motion modulates gene expression of lubricin and catabolic enzymes. *Cartilage* **6**, 185-193 (2015).

16 Melrose, J., Hayes, A. J., Whitelock, J. M. & Little, C. B. Perlecan, the "jack of all trades" proteoglycan of cartilaginous weight-bearing connective tissues. *Bioessays* **30**, 457-469 (2008).

17 Poole, C. A. Articular cartilage chondrons: form, function and failure. *The Journal of Anatomy* **191**, 1-13 (1997).

18 Schinagl, R. M., Gurskis, D., Chen, A. C. & Sah, R. L. Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *Journal of Orthopaedic Research* **15**, 499-506 (1997).

19 Xia, Y., Moody, J. B., Alhadlaq, H. & Hu, J. Imaging the physical and morphological properties of a multi-zone young articular cartilage at microscopic resolution. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine* **17**, 365-374 (2003).

20 Ratcliffe, A., Fryer, P. R. & Hardingham, T. E. The distribution of aggregating proteoglycans in articular cartilage: comparison of quantitative immunoelectron microscopy with radioimmunoassay and biochemical analysis. *Journal of Histochemistry & Cytochemistry* **32**, 193-201 (1984).

21 Maroudas, A., Muir, H. & Wingham, J. The correlation of fixed negative charge with glycosaminoglycan content of human articular cartilage. *Biochimica et Biophysica Acta (BBA)-General Subjects* **177**, 492-500 (1969).
22 Guilak, F., Nims, R. J., Dicks, A., Wu, C.-L. & Meulenbelt, I. Osteoarthritis as a disease of the cartilage pericellular matrix. *Matrix Biol* **71-72**, 40-50, doi:10.1016/j.matbio.2018.05.008 (2018).
23 Wilusz, R. E., Zauscher, S. & Guilak, F. Micromechanical mapping of early osteoarthritic changes in the pericellular matrix of human articular cartilage. *Osteoarthritis and cartilage* **21**, 1895-1903, doi:10.1016/j.joca.2013.08.026 (2013).
24 Chery, D. R. *et al.* Early changes in cartilage pericellular matrix micromechanobiology portend the onset of post-traumatic osteoarthritis. *Acta Biomaterialia*, doi:https://doi.org/10.1016/j.actbio.2020.05.005 (2020).
25 Simon, W. H. Scale effects in animal joints. I. Articular cartilage thickness and compressive stress. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* **13**, 244-255 (1970).
26 Vincent, T. L. & Wann, A. K. Mechanoadaptation: articular cartilage through thick and thin. *The Journal of physiology* **597**, 1271-1281 (2019).
27 Vincent, T. L. Targeting mechanotransduction pathways in osteoarthritis: a focus on the pericellular matrix. *Current opinion in pharmacology* **13**, 449-454, doi:10.1016/j.coph.2013.01.010 (2013).
28 Guilak, F. *et al.* The pericellular matrix as a transducer of biomechanical and biochemical signals in articular cartilage. *Annals of the New York Academy of Sciences* **1068**, 498-512, doi:10.1196/annals.1346.011 (2006).
29 Youn, I., Choi, J., Cao, L., Setton, L. & Guilak, F. Zonal variations in the three-dimensional morphology of the chondron measured in situ using confocal microscopy. *Osteoarthritis and cartilage* **14**, 889-897 (2006).
30 Martin, J., Miller, B., Scherb, M., Lembke, L. & Buckwalter, J. Co-localization of insulin-like growth factor binding protein 3 and fibronectin in human articular cartilage. *Osteoarthritis and cartilage* **10**, 556-563 (2002).
31 Xie, Y., Zinkle, A., Chen, L. & Mohammadi, M. Fibroblast growth factor signalling in osteoarthritis and cartilage repair. *Nature Reviews Rheumatology* **16**, 547-564 (2020).
32 Vincent, T., Hermansson, M., Bolton, M., Wait, R. & Saklatvala, J. Basic FGF mediates an immediate response of articular cartilage to mechanical injury. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 8259-8264, doi:10.1073/pnas.122033199 (2002).
33 Vincent, T. L., Hermansson, M. A., Hansen, U. N., Amis, A. A. & Saklatvala, J. Basic fibroblast growth factor mediates transduction of mechanical signals when articular cartilage is loaded. *Arthritis Rheum* **50**, 526-533, doi:10.1002/art.20047 (2004).
34 Vincent, T. L., McLean, C. J., Full, L. E., Peston, D. & Saklatvala, J. FGF-2 is bound to perlecan in the pericellular matrix of articular cartilage, where it acts as a chondrocyte mechanotransducer. *Osteoarthritis and cartilage* **15**, 752-763, doi:10.1016/j.joca.2007.01.021 (2007).
35 Vincent, T. L. Fibroblast growth factor 2: good or bad guy in the joint? *Arthritis Research & Therapy* **13**, 127, doi:10.1186/ar3447 (2011).
36 Makarenkova, H. P. *et al.* Differential interactions of FGFs with heparan sulfate control gradient formation and branching morphogenesis. *Science signaling* **2**, ra55-ra55 (2009).
37 Eckstein, F., Wirth, W., Guermazi, A., Maschek, S. & Aydemir, A. Brief report: intraarticular sprifermin not only increases cartilage thickness, but also reduces cartilage loss: location-independent post hoc analysis using magnetic resonance imaging. *Arthritis & Rheumatology* **67**, 2916-2922 (2015).

38 Lohmander, L. S. *et al.* Intraarticular sprifermin (recombinant human fibroblast growth factor 18) in knee osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Arthritis & Rheumatology* **66**, 1820-1831 (2014).
39 Hochberg, M. C. *et al.* Effect of intra-articular sprifermin vs placebo on femorotibial joint cartilage thickness in patients with osteoarthritis: the FORWARD randomized clinical trial. *Jama* **322**, 1360-1370 (2019).
40 Zhen, G. *et al.* Mechanical stress determines the configuration of TGFβ activation in articular cartilage. *Nature communications* **12**, 1-16 (2021).
41 Loeser, R. F. Integrins and cell signaling in chondrocytes. *Biorheology* **39**, 119-124 (2002).
42 Millward-Sadler, S. J. & Salter, D. M. Integrin-Dependent Signal Cascades in Chondrocyte Mechanotransduction. *Annals of Biomedical Engineering* **32**, 435-446, doi:10.1023/B:ABME.0000017538.72511.48 (2004).
43 Ross, T. D. *et al.* Integrins in mechanotransduction. *Current Opinion in Cell Biology* **25**, 613-618, doi:https://doi.org/10.1016/j.ceb.2013.05.006 (2013).
44 Wright, M., Jobanputra, P., Bavington, C., Salter, D. M. & Nuki, G. Effects of intermittent pressure-induced strain on the electrophysiology of cultured human chondrocytes: evidence for the presence of stretch-activated membrane ion channels. *Clinical science (London, England : 1979)* **90**, 61-71, doi:10.1042/cs0900061 (1996).
45 Barrett-Jolley, R., Lewis, R., Fallman, R. & Mobasheri, A. The Emerging Chondrocyte Channelome. **1**, doi:10.3389/fphys.2010.00135 (2010).
46 Clark, A. L., Votta, B. J., Kumar, S., Liedtke, W. & Guilak, F. Chondroprotective role of the osmotically sensitive ion channel transient receptor potential vanilloid 4: age- and sex-dependent progression of osteoarthritis in Trpv4-deficient mice. *Arthritis Rheum* **62**, 2973-2983, doi:10.1002/art.27624 (2010).
47 O'Conor, C. J., Leddy, H. A., Benefield, H. C., Liedtke, W. B. & Guilak, F. TRPV4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. **111**, 1316-1321, doi:10.1073/pnas.1319569111 %J Proceedings of the National Academy of Sciences (2014).
48 Matta, C., Zákány, R. & Mobasheri, A. Voltage-dependent calcium channels in chondrocytes: roles in health and disease. *Curr Rheumatol Rep* **17**, 43, doi:10.1007/s11926-015-0521-4 (2015).
49 Mobasheri, A. *et al.* The chondrocyte channelome: A narrative review. *Joint Bone Spine* **86**, 29-35, doi:https://doi.org/10.1016/j.jbspin.2018.01.012 (2019).
50 Blain, E. J. Involvement of the cytoskeletal elements in articular cartilage homeostasis and pathology. *Int J Exp Pathol* **90**, 1-15, doi:10.1111/j.1365-2613.2008.00625.x (2009).
51 McGlashan, S. R., Cluett, E. C., Jensen, C. G. & Poole, C. A. Primary cilia in osteoarthritic chondrocytes: from chondrons to clusters. *Developmental dynamics : an official publication of the American Association of Anatomists* **237**, 2013-2020, doi:10.1002/dvdy.21501 (2008).
52 Ruhlen, R. & Marberry, K. The chondrocyte primary cilium. *Osteoarthritis and cartilage* **22**, 1071-1076, doi:10.1016/j.joca.2014.05.011 (2014).
53 Irianto, J., Ramaswamy, G., Serra, R. & Knight, M. M. Depletion of chondrocyte primary cilia reduces the compressive modulus of articular cartilage. *Journal of biomechanics* **47**, 579-582, doi:10.1016/j.jbiomech.2013.11.040 (2014).
54 Tao, F., Jiang, T., Tao, H., Cao, H. & Xiang, W. Primary cilia: Versatile regulator in cartilage development. **53**, e12765, doi:10.1111/cpr.12765 (2020).
55 Kechagia, J. Z., Ivaska, J. & Roca-Cusachs, P. Integrins as biomechanical sensors of the microenvironment. *Nature Reviews Molecular Cell Biology*, 1 (2019).
56 Humphries, J. D., Chastney, M. R., Askari, J. A. & Humphries, M. J. Signal transduction via integrin adhesion complexes. *Current opinion in cell biology* **56**, 14-21 (2019).

57 Seetharaman, S. & Etienne-Manneville, S. Integrin diversity brings specificity in mechanotransduction. *Biology of the Cell* **110**, 49-64 (2018).
58 Chen, Y., Ju, L., Rushdi, M., Ge, C. & Zhu, C. Receptor-mediated cell mechanosensing. *Molecular biology of the cell* **28**, 3134-3155 (2017).
59 Cantini, M., Donnelly, H., Dalby, M. J. & Salmeron-Sanchez, M. The Plot Thickens: The Emerging Role of Matrix Viscosity in Cell Mechanotransduction. *Advanced Healthcare Materials* **9**, 1901259 (2020).
60 Parsons, J. T., Horwitz, A. R. & Schwartz, M. A. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nature reviews Molecular cell biology* **11**, 633-643 (2010).
61 Puklin-Faucher, E. & Sheetz, M. P. The mechanical integrin cycle. *Journal of cell science* **122**, 179-186 (2009).
62 Elosegui-Artola, A. *et al.* Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. *Nature cell biology* **18**, 540-548 (2016).
63 Elosegui-Artola, A. *et al.* Rigidity sensing and adaptation through regulation of integrin types. *Nature materials* **13**, 631-637 (2014).
64 Franz, F., Daday, C. & Gräter, F. Advances in molecular simulations of protein mechanical properties and function. *Current Opinion in Structural Biology* **61**, 132-138 (2020).
65 Gouttenoire, J. *et al.* BMP-2 and TGF-β1 differentially control expression of type II procollagen and α10 and α11 integrins in mouse chondrocytes. *European journal of cell biology* **89**, 307-314 (2010).
66 Salter, D., Hughes, D., Simpson, R. & Gardner, D. Integrin expression by human articular chondrocytes. *Rheumatology* **31**, 231-234 (1992).
67 Zhang, W.-M. *et al.* Analysis of the human integrin α11 gene (ITGA11) and its promoter. *Matrix biology* **21**, 513-523 (2002).
68 Loeser, R. F. Integrins and chondrocyte–matrix interactions in articular cartilage. *Matrix Biology* **39**, 11-16 (2014).
69 Orazizadeh, M. *et al.* CD47 associates with alpha 5 integrin and regulates responses of human articular chondrocytes to mechanical stimulation in an in vitro model. *Arthritis research & therapy* **10**, R4 (2008).
70 Ostergaard, K. *et al.* Expression of α and β subunits of the integrin superfamily in articular cartilage from macroscopically normal and osteoarthritic human femoral heads. *Annals of the rheumatic diseases* **57**, 303-308 (1998).
71 Lucchinetti, E., Bhargava, M. M. & Torzilli, P. A. The effect of mechanical load on integrin subunits α5 and β1 in chondrocytes from mature and immature cartilage explants. *Cell and tissue research* **315**, 385-391 (2004).
72 Millward-Sadler, S. *et al.* Integrin-regulated secretion of interleukin 4: a novel pathway of mechanotransduction in human articular chondrocytes. *The Journal of cell biology* **145**, 183-189 (1999).
73 Millward-Sadler, S., Wright, M., Davies, L., Nuki, G. & Salter, D. Mechanotransduction via integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* **43**, 2091-2099 (2000).
74 Steward, A. *et al.* Cell–matrix interactions regulate mesenchymal stem cell response to hydrostatic pressure. *Acta Biomaterialia* **8**, 2153-2159 (2012).
75 Jablonski, C. L., Ferguson, S., Pozzi, A. & Clark, A. L. Integrin α1β1 participates in chondrocyte transduction of osmotic stress. *Biochemical and biophysical research communications* **445**, 184-190 (2014).
76 Wright, M. *et al.* Hyperpolarisation of cultured human chondrocytes following cyclical pressure-induced strain: Evidence of a role for α5β1 integrin as a chondrocyte mechanoreceptor. *Journal of orthopaedic research* **15**, 742-747 (1997).

77 Camper, L., Hellman, U. & Lundgren-Åkerlund, E. Isolation, cloning, and sequence analysis of the integrin subunit α10, a β1-associated collagen binding integrin expressed on chondrocytes. *Journal of Biological Chemistry* **273**, 20383-20389 (1998).
78 Bengtsson, T., Camper, L., Schneller, M. & Lundgren-Åkerlund, E. Characterization of the mouse integrin subunit α10 gene and comparison with its human homologue: Genomic structure, chromosomal localization and identification of splice variants. *Matrix Biology* **20**, 565-576 (2001).
79 Lehnert, K. *et al.* Cloning, sequence analysis, and chromosomal localization of the novel human integrin α11 subunit (ITGA11). *Genomics* **60**, 179-187 (1999).
80 Varas, L. *et al.* α 10 Integrin Expression Is Up-Regulated on Fibroblast Growth Factor-2-Treated Mesenchymal Stem Cells with Improved Chondrogenic Differentiation Potential. *Stem cells and development* **16**, 965-978 (2007).
81 Hirose, N. *et al.* Protective effects of cilengitide on inflammation in chondrocytes under excessive mechanical stress. *Cell biology international* **44**, 966-974, doi:10.1002/cbin.11293 (2020).
82 Chao, P. H., West, A. C. & Hung, C. T. Chondrocyte intracellular calcium, cytoskeletal organization, and gene expression responses to dynamic osmotic loading. *American journal of physiology. Cell physiology* **291**, C718-725, doi:10.1152/ajpcell.00127.2005 (2006).
83 Erickson, G. R., Northrup, D. L. & Guilak, F. Hypo-osmotic stress induces calcium-dependent actin reorganization in articular chondrocytes. *Osteoarthritis and cartilage* **11**, 187-197, doi:10.1053/s1063-4584(02)00347-3 (2003).
84 Grodzinsky, A. J., Levenston, M. E., Jin, M. & Frank, E. H. Cartilage Tissue Remodeling in Response to Mechanical Forces. **2**, 691-713, doi:10.1146/annurev.bioeng.2.1.691 (2000).
85 Guilak, F. Compression-induced changes in the shape and volume of the chondrocyte nucleus. *Journal of Biomechanics* **28**, 1529-1541, doi:https://doi.org/10.1016/0021-9290(95)00100-X (1995).
86 Blain, E. J., Mason, D. J. & Duance, V. C. The effect of thymosin beta4 on articular cartilage chondrocyte matrix metalloproteinase expression. *Biochemical Society transactions* **30**, 879-882, doi:10.1042/bst0300879 (2002).
87 Fioravanti, A., Nerucci, F., Annefeld, M., Collodel, G. & Marcolongo, R. Morphological and cytoskeletal aspects of cultivated normal and osteoarthritic human articular chondrocytes after cyclical pressure: a pilot study. *Clinical and experimental rheumatology* **21**, 739-746 (2003).
88 Fioravanti, A., Benetti, D., Coppola, G. & Collodel, G. Effect of continuous high hydrostatic pressure on the morphology and cytoskeleton of normal and osteoarthritic human chondrocytes cultivated in alginate gels. *Clinical and experimental rheumatology* **23**, 847-853 (2005).
89 Isermann, P. & Lammerding, J. Nuclear Mechanics and Mechanotransduction in Health and Disease. *Current Biology* **23**, R1113-R1121, doi:https://doi.org/10.1016/j.cub.2013.11.009 (2013).
90 Khilan, A. A., Al-Maslamani, N. A. & Horn, H. F. Cell stretchers and the LINC complex in mechanotransduction. *Archives of biochemistry and biophysics* **702**, 108829, doi:https://doi.org/10.1016/j.abb.2021.108829 (2021).
91 Killaars, A. R., Walker, C. J. & Anseth, K. S. Nuclear mechanosensing controls MSC osteogenic potential through HDAC epigenetic remodeling. *Proceedings of the National Academy of Sciences* **117**, 21258, doi:10.1073/pnas.2006765117 (2020).
92 Elosegui-Artola, A. *et al.* Force triggers YAP nuclear entry by regulating transport across nuclear pores. *Cell* **171**, 1397-1410. e1314 (2017).
93 Phan, M. N. *et al.* Functional characterization of TRPV4 as an osmotically sensitive ion channel in porcine articular chondrocytes. *Arthritis Rheum* **60**, 3028-3037, doi:10.1002/art.24799 (2009).
94 O'Conor, C. J. *et al.* Cartilage-Specific Knockout of the Mechanosensory Ion Channel TRPV4 Decreases Age-Related Osteoarthritis. *Scientific Reports* **6**, 29053, doi:10.1038/srep29053 (2016).
95 Drexler, S. K., Wann, A. K. T. & Vincent, T. L.

96 Lee, W., Guilak, F. & Liedtke, W. Role of Piezo Channels in Joint Health and Injury. *Curr Top Membr* **79**, 263-273, doi:10.1016/bs.ctm.2016.10.003 (2017).
97 Lee, W. *et al.* Synergy between Piezo1 and Piezo2 channels confers high-strain mechanosensitivity to articular cartilage. **111**, E5114-E5122, doi:10.1073/pnas.1414298111 %J Proceedings of the National Academy of Sciences (2014).
98 McGlashan, S. R., Jensen, C. G. & Poole, C. A. Localization of extracellular matrix receptors on the chondrocyte primary cilium. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **54**, 1005-1014, doi:10.1369/jhc.5A6866.2006 (2006).
99 Chang, C. F., Ramaswamy, G. & Serra, R. Depletion of primary cilia in articular chondrocytes results in reduced Gli3 repressor to activator ratio, increased Hedgehog signaling, and symptoms of early osteoarthritis. *Osteoarthritis and cartilage* **20**, 152-161, doi:10.1016/j.joca.2011.11.009 (2012).
100 Wann, A. K. T. *et al.* Primary cilia mediate mechanotransduction through control of ATP-induced Ca2+ signaling in compressed chondrocytes. **26**, 1663-1671, doi:10.1096/fj.11-193649 (2012).
101 Shao, Y. Y., Wang, L., Welter, J. F. & Ballock, R. T. Primary cilia modulate Ihh signal transduction in response to hydrostatic loading of growth plate chondrocytes. *Bone* **50**, 79-84, doi:10.1016/j.bone.2011.08.033 (2012).
102 Pingguan-Murphy, B., El-Azzeh, M., Bader, D. & Knight, M. Cyclic compression of chondrocytes modulates a purinergic calcium signalling pathway in a strain rate-and frequency-dependent manner. *Journal of cellular physiology* **209**, 389-397 (2006).
103 Zhang, J. *et al.* Connexin43 hemichannels mediate small molecule exchange between chondrocytes and matrix in biomechanically-stimulated temporomandibular joint cartilage. *Osteoarthritis and cartilage* **22**, 822-830 (2014).
104 Garcia, M. & Knight, M. M. Cyclic loading opens hemichannels to release ATP as part of a chondrocyte mechanotransduction pathway. *Journal of Orthopaedic Research* **28**, 510-515 (2010).
105 Chowdhury, T. & Knight, M. Purinergic pathway suppresses the release of. NO and stimulates proteoglycan synthesis in chondrocyte/agarose constructs subjected to dynamic compression. *Journal of cellular physiology* **209**, 845-853 (2006).
106 Huang, C., Holfeld, J., Schaden, W., Orgill, D. & Ogawa, R. Mechanotherapy: revisiting physical therapy and recruiting mechanobiology for a new era in medicine. *Trends in Molecular Medicine* **19**, 555-564, doi:10.1016/j.molmed.2013.05.005 (2013).
107 Thompson, W. R., Scott, A., Loghmani, M. T., Ward, S. R. & Warden, S. J. Understanding Mechanobiology: Physical Therapists as a Force in Mechanotherapy and Musculoskeletal Regenerative Rehabilitation. *Physical Therapy* **96**, 560-569, doi:10.2522/ptj.20150224 (2016).
108 Dell'accio, F., De Bari, C., Eltawil, N. M., Vanhummelen, P. & Pitzalis, C. Identification of the molecular response of articular cartilage to injury, by microarray screening: Wnt-16 expression and signaling after injury and in osteoarthritis. *Arthritis Rheum* **58**, 1410-1421, doi:10.1002/art.23444 (2008).
109 Loeser, R. F., Erickson, E. A. & Long, D. L. Mitogen-activated protein kinases as therapeutic targets in osteoarthritis. *Curr Opin Rheumatol* **20**, 581-586, doi:10.1097/BOR.0b013e3283090463 (2008).
110 Fanning, P. J. *et al.* Mechanical Regulation of Mitogen-activated Protein Kinase Signaling in Articular Cartilage *. *Journal of Biological Chemistry* **278**, 50940-50948, doi:10.1074/jbc.M305107200 (2003).
111 Forsyth, C. B., Pulai, J. & Loeser, R. F. Fibronectin fragments and blocking antibodies to α2β1 and α5β1 integrins stimulate mitogen-activated protein kinase signaling and increase collagenase 3 (matrix metalloproteinase 13) production by human articular chondrocytes. *Arthritis & Rheumatism* **46**, 2368-2376 (2002).

112 Im, H.-J. *et al.* Inhibitory effects of insulin-like growth factor-1 and osteogenic protein-1 on fibronectin fragment-and interleukin-1β-stimulated matrix metalloproteinase-13 expression in human chondrocytes. *Journal of Biological Chemistry* **278**, 25386-25394 (2003).
113 Loeser, R. F., Forsyth, C. B., Samarel, A. M. & Im, H.-J. Fibronectin fragment activation of proline-rich tyrosine kinase PYK2 mediates integrin signals regulating collagenase-3 expression by human chondrocytes through a protein kinase C-dependent pathway. *Journal of Biological Chemistry* **278**, 24577-24585 (2003).
114 Pulai, J. I. *et al.* NF-κB mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments. *the Journal of Immunology* **174**, 5781-5788 (2005).
115 Del Carlo, M., Schwartz, D., Erickson, E. A. & Loeser, R. F. Endogenous production of reactive oxygen species is required for stimulation of human articular chondrocyte matrix metalloproteinase production by fibronectin fragments. *Free Radical Biology and Medicine* **42**, 1350-1358 (2007).
116 Long, D. L., Willey, J. S. & Loeser, R. F. Rac1 is required for matrix metalloproteinase 13 production by chondrocytes in response to fibronectin fragments. *Arthritis & Rheumatism* **65**, 1561-1568 (2013).
117 Gemba, T., Valbracht, J., Alsalameh, S. & Lotz, M. Focal adhesion kinase and mitogen-activated protein kinases are involved in chondrocyte activation by the 29-kDa amino-terminal fibronectin fragment. *Journal of Biological Chemistry* **277**, 907-911 (2002).
118 Ding, L., Guo, D. & Homandberg, G. The cartilage chondrolytic mechanism of fibronectin fragments involves MAP kinases: comparison of three fragments and native fibronectin. *Osteoarthritis and cartilage* **16**, 1253-1262 (2008).
119 Ding, L., Guo, D. & Homandberg, G. Fibronectin fragments mediate matrix metalloproteinase upregulation and cartilage damage through proline rich tyrosine kinase 2, c-src, NF-κB and protein kinase Cδ. *Osteoarthritis and cartilage* **17**, 1385-1392 (2009).
120 Fitzgerald, J. B. *et al.* Shear- and Compression-induced Chondrocyte Transcription Requires MAPK Activation in Cartilage Explants*. *Journal of Biological Chemistry* **283**, 6735-6743, doi:https://doi.org/10.1074/jbc.M708670200 (2008).
121 Zhang, J., Shen, B. & Lin, A. Novel strategies for inhibition of the p38 MAPK pathway. *Trends in pharmacological sciences* **28**, 286-295 (2007).
122 González-Vázquez, A. *et al.* Accelerating bone healing in vivo by harnessing the age-altered activation of c-Jun N-terminal kinase 3. *Biomaterials* **268**, 120540, doi:https://doi.org/10.1016/j.biomaterials.2020.120540 (2021).
123 Agarwal, S. *et al.* A central role for the nuclear factor-kappaB pathway in anti-inflammatory and proinflammatory actions of mechanical strain. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **17**, 899-901, doi:10.1096/fj.02-0901fje (2003).
124 Yang, Y. *et al.* Mechanical stress protects against osteoarthritis via regulation of the AMPK/NF-κB signaling pathway. *Journal of cellular physiology* **234**, 9156-9167, doi:10.1002/jcp.27592 (2019).
125 Vincent, T. L. Mechanoflammation in osteoarthritis pathogenesis. *Semin Arthritis Rheum* **49**, S36-s38, doi:10.1016/j.semarthrit.2019.09.018 (2019).
126 Ismail, H. M., Didangelos, A., Vincent, T. L. & Saklatvala, J. Rapid Activation of Transforming Growth Factor β-Activated Kinase 1 in Chondrocytes by Phosphorylation and K(63) -Linked Polyubiquitination Upon Injury to Animal Articular Cartilage. *Arthritis & rheumatology (Hoboken, N.J.)* **69**, 565-575, doi:10.1002/art.39965 (2017).
127 Chang, S. H. *et al.* Excessive mechanical loading promotes osteoarthritis through the gremlin-1–NF-κB pathway. *Nature Communications* **10**, 1442, doi:10.1038/s41467-019-09491-5 (2019).

128 Lee, W. *et al.* Inflammatory signaling sensitizes Piezo1 mechanotransduction in articular chondrocytes as a pathogenic feed-forward mechanism in osteoarthritis. *Proceedings of the National Academy of Sciences* **118**, e2001611118, doi:10.1073/pnas.2001611118 (2021).
129 Nam, S. *et al.* Cell cycle progression in confining microenvironments is regulated by a growth-responsive TRPV4-PI3K/Akt-p27(Kip1) signaling axis. *Sci Adv* **5**, eaaw6171, doi:10.1126/sciadv.aaw6171 (2019).
130 Lee, H. P., Gu, L., Mooney, D. J., Levenston, M. E. & Chaudhuri, O. Mechanical confinement regulates cartilage matrix formation by chondrocytes. *Nat Mater* **16**, 1243-1251, doi:10.1038/nmat4993 (2017).
131 Miller, J. R. The Wnts. *Genome biology* **3**, Reviews3001, doi:10.1186/gb-2001-3-1-reviews3001 (2002).
132 De Santis, M. *et al.* The Role of Wnt Pathway in the Pathogenesis of OA and Its Potential Therapeutic Implications in the Field of Regenerative Medicine. *BioMed research international* **2018**, 7402947, doi:10.1155/2018/7402947 (2018).
133 Dell'Accio, F. *et al.* Activation of WNT and BMP signaling in adult human articular cartilage following mechanical injury. *Arthritis Res Ther* **8**, R139, doi:10.1186/ar2029 (2006).
134 Bougault, C. *et al.* Protective role of frizzled-related protein B on matrix metalloproteinase induction in mouse chondrocytes. *Arthritis Res Ther* **16**, R137, doi:10.1186/ar4599 (2014).
135 Nalesso, G. *et al.* WNT16 antagonises excessive canonical WNT activation and protects cartilage in osteoarthritis. *Annals of the rheumatic diseases* **76**, 218-226, doi:10.1136/annrheumdis-2015-208577 (2017).
136 Wang, Y., Fan, X., Xing, L. & Tian, F. Wnt signaling: a promising target for osteoarthritis therapy. *Cell Communication and Signaling* **17**, 97, doi:10.1186/s12964-019-0411-x (2019).
137 Lories, R. J. & Monteagudo, S. Review Article: Is Wnt Signaling an Attractive Target for the Treatment of Osteoarthritis? *Rheumatology and Therapy* **7**, 259-270, doi:10.1007/s40744-020-00205-8 (2020).
138 Monteagudo, S. & Lories, R. J. Cushioning the cartilage: a canonical Wnt restricting matter. *Nature reviews. Rheumatology* **13**, 670-681, doi:10.1038/nrrheum.2017.171 (2017).
139 Deshmukh, V. *et al.* A small-molecule inhibitor of the Wnt pathway (SM04690) as a potential disease modifying agent for the treatment of osteoarthritis of the knee. *Osteoarthritis and cartilage* **26**, 18-27, doi:10.1016/j.joca.2017.08.015 (2018).
140 Yazici, Y. *et al.* A Phase 2b randomized trial of lorecivivint, a novel intra-articular CLK2/DYRK1A inhibitor and Wnt pathway modulator for knee osteoarthritis. *Osteoarthritis and cartilage* **29**, 654-666, doi:10.1016/j.joca.2021.02.004 (2021).
141 Deshmukh, V. *et al.* Modulation of the Wnt pathway through inhibition of CLK2 and DYRK1A by lorecivivint as a novel, potentially disease-modifying approach for knee osteoarthritis treatment. *Osteoarthritis and cartilage* **27**, 1347-1360, doi:10.1016/j.joca.2019.05.006 (2019).
142 Monteagudo, S. *et al.* DOT1L safeguards cartilage homeostasis and protects against osteoarthritis. *Nat Commun* **8**, 15889, doi:10.1038/ncomms15889 (2017).
143 Cornelis, F. M. F. *et al.* Increased susceptibility to develop spontaneous and post-traumatic osteoarthritis in Dot1l-deficient mice. *Osteoarthritis and cartilage* **27**, 513-525, doi:10.1016/j.joca.2018.11.008 (2019).
144 Castaño Betancourt, M. C. *et al.* Genome-wide association and functional studies identify the DOT1L gene to be involved in cartilage thickness and hip osteoarthritis. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 8218-8223, doi:10.1073/pnas.1119899109 (2012).
145 Deng, Y. *et al.* Yap1 regulates multiple steps of chondrocyte differentiation during skeletal development and bone repair. *Cell reports* **14**, 2224-2237 (2016).

146 Baker, B. M. & Chen, C. S. Deconstructing the third dimension–how 3D culture microenvironments alter cellular cues. *Journal of cell science* **125**, 3015-3024 (2012).
147 Caliari, S. R., Vega, S. L., Kwon, M., Soulas, E. M. & Burdick, J. A. Dimensionality and spreading influence MSC YAP/TAZ signaling in hydrogel environments. *Biomaterials* **103**, 314-323 (2016).
148 Karystinou, A. *et al.* Yes-associated protein (YAP) is a negative regulator of chondrogenesis in mesenchymal stem cells. *Arthritis research & therapy* **17**, 147 (2015).
149 Mobasheri, A. *et al.* The role of metabolism in the pathogenesis of osteoarthritis. *Nature reviews. Rheumatology* **13**, 302-311, doi:10.1038/nrrheum.2017.50 (2017).
150 Salinas, D., Mumey, B. M. & June, R. K. Physiological dynamic compression regulates central energy metabolism in primary human chondrocytes. *Biomechanics and Modeling in Mechanobiology* **18**, 69-77, doi:10.1007/s10237-018-1068-x (2019).
151 Lehtinen, M. K. *et al.* A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell* **125**, 987-1001, doi:10.1016/j.cell.2006.03.046 (2006).
152 Niehoff, A. *et al.* Dynamic and static mechanical compression affects Akt phosphorylation in porcine patellofemoral joint cartilage. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **26**, 616-623, doi:10.1002/jor.20542 (2008).
153 Holledge, M. M., Millward-Sadler, S. J., Nuki, G. & Salter, D. M. Mechanical regulation of proteoglycan synthesis in normal and osteoarthritic human articular chondrocytes--roles for alpha5 and alphaVbeta5 integrins. *Biorheology* **45**, 275-288 (2008).
154 Jutila, A. A. *et al.* Candidate mediators of chondrocyte mechanotransduction via targeted and untargeted metabolomic measurements. *Archives of biochemistry and biophysics* **545**, doi:10.1016/j.abb.2014.01.011 (2014).
155 Zignego, D. L., Jutila, A. A., Gelbke, M. K., Gannon, D. M. & June, R. K. The mechanical microenvironment of high concentration agarose for applying deformation to primary chondrocytes. *J Biomech* **47**, doi:10.1016/j.jbiomech.2013.10.051 (2014).
156 Hodgkinson, T. *et al.* The use of nanovibration to discover specific and potent bioactive metabolites that stimulate osteogenic differentiation in mesenchymal stem cells. *Science Advances* **7**, eabb7921 (2021).
157 Grither, W. R. & Longmore, G. D. Inhibition of tumor-microenvironment interaction and tumor invasion by small-molecule allosteric inhibitor of DDR2 extracellular domain. *Proceedings of the National Academy of Sciences of the United States of America* **115**, E7786-e7794, doi:10.1073/pnas.1805020115 (2018).
158 Occhetta, P. *et al.* Hyperphysiological compression of articular cartilage induces an osteoarthritic phenotype in a cartilage-on-a-chip model. *Nature Biomedical Engineering* **3**, 545-557, doi:10.1038/s41551-019-0406-3 (2019).
159 Lee, J. *et al.* Combinatorial screening of biochemical and physical signals for phenotypic regulation of stem cell–based cartilage tissue engineering. *Science Advances* **6**, eaaz5913, doi:10.1126/sciadv.aaz5913 (2020).
160 Wang, J., Lü, D., Mao, D. & Long, M. Mechanomics: an emerging field between biology and biomechanics. *Protein Cell* **5**, 518-531, doi:10.1007/s13238-014-0057-9 (2014).
161 Shi, S., Man, Z., Li, W., Sun, S. & Zhang, W. Silencing of Wnt5a prevents interleukin-1β-induced collagen type II degradation in rat chondrocytes. *Exp Ther Med* **12**, 3161-3166, doi:10.3892/etm.2016.3788 (2016).
162 Yan, H. *et al.* Suppression of NF-κB activity via nanoparticle-based siRNA delivery alters early cartilage responses to injury. *Proceedings of the National Academy of Sciences* **113**, E6199-E6208 (2016).
163 Rai, M. F. *et al.* Applications of RNA interference in the treatment of arthritis. *Translational Research* **214**, 1-16 (2019).

164 Cheleschi, S. *et al.* Hydrostatic Pressure Regulates MicroRNA Expression Levels in Osteoarthritic Chondrocyte Cultures via the Wnt/β-Catenin Pathway. **18**, 133 (2017).
165 De Palma, A. *et al.* Hydrostatic pressure as epigenetic modulator in chondrocyte cultures: A study on miRNA-155, miRNA-181a and miRNA-223 expression levels. *J Biomech* **66**, 165-169, doi:10.1016/j.jbiomech.2017.10.044 (2018).
166 Yang, X. *et al.* Mechanical and IL-1β Responsive miR-365 Contributes to Osteoarthritis Development by Targeting Histone Deacetylase 4. *Int J Mol Sci* **17**, 436, doi:10.3390/ijms17040436 (2016).
167 Stadnik, P. S. *et al.* Regulation of microRNA-221, -222, -21 and -27 in articular cartilage subjected to abnormal compressive forces. **599**, 143-155, doi:https://doi.org/10.1113/JP279810 (2021).
168 Dunn, W., DuRaine, G. & Reddi, A. H. Profiling microRNA expression in bovine articular cartilage and implications for mechanotransduction. *Arthritis Rheum* **60**, 2333-2339, doi:10.1002/art.24678 (2009).
169 Iliopoulos, D., Malizos, K. N., Oikonomou, P. & Tsezou, A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One* **3**, e3740, doi:10.1371/journal.pone.0003740 (2008).
170 Yang, X. *et al.* Mechanical and IL-1β responsive miR-365 contributes to osteoarthritis development by targeting histone deacetylase 4. *International journal of molecular sciences* **17**, 436 (2016).
171 Song, J. *et al.* MicroRNA-222 regulates MMP-13 via targeting HDAC-4 during osteoarthritis pathogenesis. *BBA Clin* **3**, 79-89, doi:10.1016/j.bbacli.2014.11.009 (2014).
172 Lolli, A. *et al.* Pro-Chondrogenic Effect of miR-221 and Slug Depletion in Human MSCs. *Stem Cell Reviews and Reports* **10**, 841-855, doi:10.1007/s12015-014-9532-1 (2014).
173 Hecht, N., Johnstone, B., Angele, P., Walker, T. & Richter, W. Mechanosensitive MiRs regulated by anabolic and catabolic loading of human cartilage. *Osteoarthritis and cartilage* **27**, 1208-1218, doi:10.1016/j.joca.2019.04.010 (2019).
174 Lolli, A., Colella, F., De Bari, C. & van Osch, G. J. V. M. Targeting anti-chondrogenic factors for the stimulation of chondrogenesis: A new paradigm in cartilage repair. **37**, 12-22, doi:10.1002/jor.24136 (2019).
175 Mohanraj, B. *et al.* Mechanically Activated Microcapsules for "On-Demand" Drug Delivery in Dynamically Loaded Musculoskeletal Tissues. **29**, 1807909, doi:https://doi.org/10.1002/adfm.201807909 (2019).
176 Nims, R. J. *et al.* A synthetic mechanogenetic gene circuit for autonomous drug delivery in engineered tissues. **7**, eabd9858, doi:10.1126/sciadv.abd9858 %J Science Advances (2021).
177 Cambré, I. *et al.* Mechanical strain determines the site-specific localization of inflammation and tissue damage in arthritis. *Nature communications* **9**, 1-14 (2018).
178 Ismail, H. M., Didangelos, A., Vincent, T. L. & Saklatvala, J. Rapid Activation of Transforming Growth Factor β–Activated Kinase 1 in Chondrocytes by Phosphorylation and K63-Linked Polyubiquitination Upon Injury to Animal Articular Cartilage. *Arthritis & Rheumatology* **69**, 565-575 (2017).
179 Lin, X., Bai, Y., Zhou, H. & Yang, L. Mechano-active biomaterials for tissue repair and regeneration. *Journal of Materials Science & Technology* **59**, 227-233, doi:https://doi.org/10.1016/j.jmst.2020.03.074 (2020).
180 Zhang, Y., Yu, J., Bomba, H. N., Zhu, Y. & Gu, Z. Mechanical Force-Triggered Drug Delivery. *Chemical Reviews* **116**, 12536-12563, doi:10.1021/acs.chemrev.6b00369 (2016).
181 Xiao, L. *et al.* Hyaluronic Acid-Based Hydrogels Containing Covalently Integrated Drug Depots: Implication for Controlling Inflammation in Mechanically Stressed Tissues. *Biomacromolecules* **14**, 3808-3819, doi:10.1021/bm4011276 (2013).

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