The effect of intra-articular pressure and mechanical load on articular chondrocyte cellular signalling

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ABSTRACT

The various forms of joint loading have been found to differentially influence anatomical and molecular responses, which together are aimed to maintain the joint homeostasis. To elucidate these mechanisms of mechanotransduction, we review the roles of the distinct joint components, as well as numerous *in vitro* studies that have been performed to unravel chondrocyte responses in changing environments. The main signalling pathways in transduction of load signals are initiated by integrins sensing matrix deformation and by altered interstitial pH, influencing ion fluxes through membrane channels. Downstream signalling after static compression occurs mainly via the MAPK pathways of ERK1/2, SAPK (Jnk) and p38, which directly influence transcription factors of genes involved in cartilage breakdown. In contrast, cyclic compression and mild shear forces lead to membrane hyperpolarisation and subsequently stimulates cartilage matrix synthesis. These findings add further comprehension with regard to the satisfactory clinical outcomes of joint distraction in osteoarthritic (OA) joints. It has been shown that these joints contained repaired cartilage after treatment in animal models. Using improved *in vivo* models in further research would allow a more thorough understanding of the underlying molecular processes of this reparative capacity of damaged cartilage, which could lead to improved arthropathy treatment options.

Introduction

Cartilage degeneration is a common feature of joint diseases and is accompanied by loss of function and persistent pain. Since many years, abnormal and excessive joint loading has been associated with the onset and progression of cartilage degeneration, eventually leading to osteoarthritis (OA), a prevalent degenerative joint disease in which the subchondral bone is exposed to a higher than normal pressure, causing painful subchondral sclerosis and formation of subchondral cysts.[1] More recently, it has been found that unloading OA joints, for instance by distraction of the affected joint with an external fixator, possibly promotes the regeneration of cartilage and reduces subchondral bone density, leading to a significantly improved clinical status.[2,3] An explanation for eventual cartilage regeneration has not been described before, since this idea is relatively new and until recently it has been assumed that cartilage damage is irreversible. However, the importance of joint loading for maintenance of articular cartilage homeostasis has been known for many years.[4,5] Also, numerous researchers explored the cellular responses to mechanical load and intermittent or static pressure in chondrocytes, providing clues for molecular mechanisms involved in joint homeostasis and possible cartilage repair. In this review, we first describe the main components of diarthrodial joints, and the general mechanisms of homeostasis maintenance. Then, the most important findings of *in vitro* studies are reviewed, and we try to explain the beneficial effect of joint distraction on damaged cartilage with a preliminary model. Understanding the molecular mechanisms of cartilage damage and eventual regeneration will provide new insights in the promising treatment with joint distraction, and it may give us further cues for improved medication in subjects with degenerative cartilage diseases.

1. Composition of diarthrodial joint components

To fully comprehend the effect of chondrocyte metabolism on joint integrity and its role in synthesis of multiple proteins involved in joint homeostasis, we first discuss the main components of normal diarthrodial joints: articular cartilage, synovial fluid, subchondral bone and the synovial membrane.

1.1 Articular cartilage composition

Articular cartilage, or hyaline cartilage, is designed to bear and distribute loads on the bone surfaces inside joints, and is composed of a solid phase, including the extracellular matrix (ECM) and chondrocytes, and a fluid phase, the interstitial fluid, containing water and small electrolytes, which are primarily Na^+ and Cl^-.[6] The ECM consists of a highly hydrated collagen network and of proteoglycan aggregates. The interstitial fluid contains water and ions. Less than 5% of the tissue volume of cartilage consists of chondrocytes, that are responsible for maintaining the homeostasis with regard to the cartilage components.[7] Chondrocytes represent the only cell type inside articular cartilage, as cartilage tissue is not vascularised or innervated.

The collagen network consists primarily of collagen type II fibrils, and also contains minor amounts of type I, V, IX and XI fibrils.[8] Collagen is an important contributor to the tensile properties of the cartilage, where proteoglycans attract electrolytes in the interstitial fluid to generate a swelling pressure and to resist compressive loads.[9] Thus, the solid components of cartilage have a low permeability for water, while there is continuous interaction with water through covalent, ionic and hydrogen bonding, resulting in a high interstitial

fluid pressurisation, which is essential for adequate distribution of the loads inside the joint.[8]

Proteoglycans consist of a core protein to which one or more glycosaminoglycan (GAG) chains are attached. The most abundant proteoglycan in articular cartilage is the large aggregating aggrecan, having numerous GAGs attached to its long core protein, mainly chondroitin sulphate (CS) and keratan sulphate (KS).[10] Another GAG in articular cartilage is hyaluronan (HA), which has its major function in synovial fluid viscosity.[11] Proteoglycan molecules form aggregates by binding to hyaluronic acid molecules, and together with collagen they form the dense ECM network.[9] Smaller, non-aggregating proteoglycans in articular cartilage are for instance fibromodulin, decorin and biglycan.[12,13]

The articular cartilage surface, also called the superficial or tangential zone, encompass 10-20% of the articular cartilage thickness and has the highest collagen and interstitial fluid content.[6,7] In this zone, the collagen fibrils are arranged parallel to the articular surface, creating a low compressive modulus, meaning that this layer is easily deformed.[9] Chondrocytes in the surface zone produce relatively little proteoglycans, and synthesise more collagen type II and smaller proteins with lubricating and protective functions, such as the superficial zone protein (SZP).[14,15]

The middle zone of the articular cartilage accounts for 40-60% of the cartilage thickness.[7] Collagen fibrils in this zone are thicker and packed more loosely than in the superficial zone, and are obliquely oriented to the cartilage surface. The compressive modulus of the tissue is higher in this layer.[9]

The radial or deep zone fills 30% of the cartilage thickness and contains collagen fibrils with a large diameter that are oriented perpendicular to the surface.[7] This layer has the highest compressive modulus and also contains the most proteoglycans, and less water, compared to the other zones.[6,9] Chondrocytes in this zone are 10-fold more synthetically active than in the superficial zone.[16]

Below the deep zone is a layer of calcified cartilage, which contains rather collagen type X than collagen type II, and here is also the tide mark, which lays directly on the subchondral bone.[17]

As already mentioned, chondrocytes synthesise the cartilage compounds with varying ratios in the distinct zones, and these cell populations are therefore very heterogeneous, also with regard to size and shape, according to their position in the cartilage. Besides, the behaviour of chondrocytes is influenced by the age, pathology or mechanical stress of the surrounding cartilage,[18] which will be further discussed in the section about the effect of joint loading on chondrocyte metabolism (see *Section 3*).

1.2 Subchondral bone composition

Directly below the calcified cartilage layer and the tide mark is the interface with the subchondral bone plate, which separates the articular cartilage from the bone marrow.[17] Below this dense bone plate is a subarticular spongiosa, with its trabecular or plate-like bone structures enclosing spaces between them. Near the subchondral bone-cartilage interface, these spaces are very narrow, and deeper in the bone they are considerably enlarged.

Articular cartilage is supported by the underlying bone in both a biomechanical and biochemical way.[19] Biomechanically, the rigid bone, mainly composed of collagen type I, gives strength support to the soft and compression-sensitive articular cartilage, and attenuates the loads to a much greater extent than cartilage.[17] The quality of subchondral bone directly influences the response of cartilage to load, which is illustrated by the effect of an increased bone density, which often leads to OA, because bone with a higher density is very stiff and lays more load on the articular cartilage, resulting in cartilage damage.[20]

Subchondral bone is highly vascularised, especially in regions that experience considerable mechanical load. Cartilage has no blood supply, but exchange of nutrient solutions is possible between subchondral bone and articular cartilage by crossing of blood vessels into the calcified cartilage layer through openings in the bone at the subchondral interface.[17,19] If a region in the calcified cartilage is devoid of blood vessel entry from the subchondral bone plate, the chondrocytes in this region are dependent on diffusion of nutrients from the synovial fluid through the cartilage matrix, which is also the case for the superficial, middle and deep cartilage zones.

1.3 Synovial fluid composition

The joint cavity of diarthrodial joints is filled with synovial fluid, which is a dialysate of blood plasma containing additional proteins that are synthesised in synoviocytes and chondrocytes. Synovial fluid has various functions, including cartilage lubrication, and facilitation of transport of nutrients, waste products, enzymes, cytokines, growth factors and morphogens to maintain joint homeostasis and to allow communication between distinct cell populations within the joint.

Since synovial fluid is a dialysate of blood plasma, the major protein components are identical, except for the larger plasma proteins, because the synovial membrane hinders these from entering the synovial fluid compartment.[21] Albumin, as well as β_1, γ, α_1 and α_2 globulins and transferrins are the major protein components of synovial fluid.[22] There are also pro- and anti-inflammatory cytokines and growth factors present, which have important roles in regulation of the local cell populations.[21] Additionally, synovial fluid contains several lubricant molecules that are synthesised and secreted by synoviocytes or chondrocytes, including HA and proteoglycan-4 (PRG-4).[21] HA is a GAG that contributes to the viscosity of the synovial fluid, and thereby prevents fluid outflow to maintain the synovial volume, and the two variants of PRG-4, SZP and lubricin, are glycoproteins that mediate boundary lubrication of the articular cartilage.[11,15] SZP is uniquely expressed in chondrocytes in the superficial zone of cartilage, and lubricin and HA are synthesised by fibroblast-like synoviocytes at the luminal surface of the synovial membrane.[15,23,24] There are also few leukocytes, lymphocytes, macrophages and macrophagic synoviocytes in the synovial fluid.[21,25] Macrophagic synoviocytes are of bone marrow origin and can phagocytose cell debris and other wastes, and have an antigen-presenting function.[24,25] Synovial fluid further contains matrix metalloproteinases (MMPs), amounts of a distintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and tissue inhibitors of metalloproteinases (TIMPs), that are produced by chondrocytes and synoviocytes, and together determine the extent of ECM maintenance and breakdown in the articular cartilage.[26]

The synovial fluid is in direct contact with the articular surface and with the synovial membrane, and, in some joints, also with the meniscus and with ligaments. Therefore, in various arthropathies, major changes occur to the synovial fluid composition, which is exacerbated by or contributes to the pathology. In most arthropathies, including OA, rheumatoid arthritis (RA) and post-traumatic arthritis, the protein concentration in the synovial fluid is increased, and larger proteins are present inside the fluid as well.[22] This points to a changed permeability of the synovial membrane during disease.[21] Thus, the synovial membrane plays important roles in maintaining the joint homeostasis as well.

1.4 Synovial membrane composition

The synovial membrane is composed of two layers, including an outer vascularised and innervated fibrous capsule which contains fibroblasts, macrophages, adipocytes and mast cells, and an inner layer, the synovial intima, that covers the outer layer.[24,25] The intima contains the earlier mentioned fibroblast-like synoviocytes, which are specialised to synthesise HA, and the macrophagic synoviocytes, within an ECM composed of collagen, HA and proteoglycans.[21,25]

As described above, the permeability of the synovial membrane is the main determinant of plasma protein and water entry into the synovial fluid, but this barrier also retains the larger synovial fluid contents that are synthesised inside the joint, including lubricin, SZF and HA.[21] Thus, the synovial membrane physically and functionally lines the joint edge and provides a homeostatic environment to the cartilage, the subchondral bone and the synovial fluid.

In RA patients, the synovial membrane dramatically increases in mass and metabolic activity due to hyperplasia of the intima cells, leading to a change in synovial membrane permeability. The entry of larger plasma proteins into the synovial fluid has been associated with synovial inflammation, which is characteristic for RA.[27] Indeed, in OA and in other aetiologies of arthritis, there are changes in synovial membrane permeability as well, but to a much lesser extent than in RA.[28]

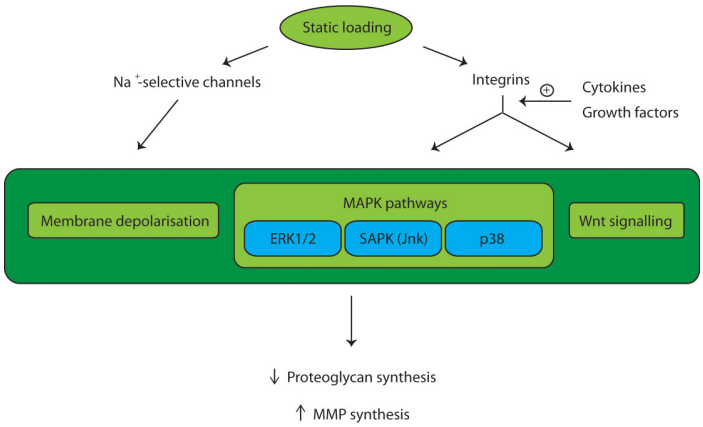

Figure 1 – **Several signalling mechanisms after (e.g.) static loading lead to catabolic cartilage processes**

2. Joint loading and intra-articular pressure

Even when a person is resting, the diarthrodial joints are loaded in several ways, because the anatomical conditions, including body mass, basal muscle tension and synovial fluid pressure, and gravity always apply forces to the joint.

When a subject is standing, the hip, knee and ankle joint capsules are compressed, leading to synovial fluid expression and a hydrostatic pressure in the interstitial fluid of the cartilage, as well as slight mechanical cartilage tissue deformation.

During movement, the forces change on one or more diarthrodial joints, and subsequently the joint compartments experience various forms of pressure and load changes, partly as a result of fluid flow and tensile strain. For instance, when someone is walking, the hip, knee and ankle joints are intermittently loaded, resulting in intermittent fluid movements and hydrostatic pressure changes in the interstitium of the cartilage, and cyclic tensile strain and deformation of the cartilage. It is important to note that, under the same load, the extent of cartilage deformation as well as the changes in fluid flow and the change in hydrostatic pressure, are very different between varying areas within one joint, and among various joints within one individual. Also, the age of the subjects and the pathologic state of the joints are responsible for much heterogeneity in load distribution on cartilage and chondrocytes.[18]

With excessive load, which occurs for instance in subjects with a high body mass index (BMI) or with extreme physical activity or heavy load bearing, the interstitial hydrostatic pressure exceeds normal values (30-50 MPa) and the mechanical load on cartilage induces a further compression. Cartilage tissue usually has a sufficient compressive modulus to maintain under higher loads, but on the longer term the ECM becomes thinner and other OA-related problems could develop in the joint, possibly due to chondrocyte apoptosis.[29-31] Also, repetitive impact loading leads to remodelling of the subchondral bone, which, in turn, induces cartilage degeneration as is found in OA.[31]

Also, shear forces inside the joint, including mechanical and fluid induced shear, have their effect on joint homeostasis. During aberrant joint conditions, this could result in shear stress, eventually leading to cartilage degeneration.

Conversely, cartilage thinning has also been observed during absence of joint loading, for instance in subjects with a spinal cord injury resulting in a complete cessation of physical exercise[32] and in immobilised rabbit knees.[33] Therefore, it has generally been assumed that the synovial fluid flow inside joints is beneficial for joint homeostasis, because with fluid movement, the nutrients, signalling molecules, lubrication compounds and waste products are transported within the joint, which is essential for chondrocyte survival. And, indeed, subjects with OA, RA and ankylosing spondylitis (AS) have been encouraged by clinicians to continue with physical exercise, since this has been found to result in a slightly higher satisfaction of therapy outcomes.[34,35] Additionally, distraction of a joint with external fixation for several weeks, which allows subtle joint motion but establishes a decreased intra-articular hydrostatic pressure and decreases mechanical cartilage stress, has been shown to allow cartilage regeneration in subjects with OA.[3]

Another interesting finding is that articular cartilage areas which mainly experience shear stress have a different cartilage composition than areas that are preferentially subjected to weight bearing,[36] suggesting that mechanical forces define the biological composition of the ECM. Finally, changes in joint loading have been shown to result in subchondral bone remodelling as well, which, again, has its influence on articular cartilage integrity.[2,33]

Together, these findings suggest that joint loading and mechanisms involved in ECM degeneration and cartilage maintenance are coupled by several regulatory pathways. This is also consistent with the early finding that loaded regions of cartilage acquire a different composition than unloaded regions.[37] Therefore, in the following section, we review the underlying molecular mechanisms of chondrocyte metabolism under different loading conditions, to elucidate the events in cartilage degeneration and regeneration in healthy subjects and in subjects with degenerative joint diseases.

3. Effect of joint loading on chondrocyte metabolism

As mentioned above, loading of cartilage could influence its future composition, by changing the metabolism of the present chondrocytes. For instance, regular cyclic loading has been found to positively stimulate chondrocytes to synthesise ECM components, whereas the absence of loading leads to cartilage weakening, and static or excessive loading, as well as impact loading, induces cartilage breakdown by a stimulation of MMP production and an immediate decrease of ECM synthesis.[31,38-43] Also, shear stress has been shown to decrease ECM synthesis and to induce chondrocyte changes related to apoptosis.[44]

These influences of load and shear on chondrocytes are mediated by several mechanotransduction pathways, including the mitogen-activated protein kinase (MAPK) signalling pathways, integrin activation and influence of growth factors and cytokines. Also, the pH in the interstitial fluid, as well as intracellular pH, play important roles in these processes. It should be noted that these processes are all interconnected and continuously influence each other under the changing loading conditions in the articular joint.

3.1 Roles of the MAPK pathways

During static cartilage compression, several components of the MAPK signalling pathways have been found to be dose-dependently activated by phosphorylation, including the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathway, the p38 MAPK pathway, and the c-Jun N-terminal kinase (JNK) pathway, which is also known as the stress-activated protein kinase (SAPK) pathway (see *Figure 1*).[45,46] ERK1/2 activation was shown to be independent of serum components, indicating that mechanical compression alone is able to initiate signalling.[45] This was also the case with supra-physiological fluid shear induced mechanical stress.[47] With static compression, ERK2 phosphorylation was more pronounced than that of ERK1, but both remained phosphorylated to some extent during at least 24 hours under a 50% compression.[45] Under the same conditions, p38 MAPK phosphorylation has been shown to sustain for 4 hours and returns then to baseline levels, and JNK pathway activation was found to be further elevated after 1 hour as compared to its activation after 10 minutes.[45] Indeed, SAPK family members have been associated with cellular responses to altered environmental conditions, and it is therefore not unreasonable that their response to stress increases with time.[46]

Static fluid shear for less than 2 hours has been shown to transiently induce ERK1/2 signalling, and temporarily inhibits aggrecan promoter activity.[47] This is possibly stimulated by tyrosine kinase signalling, including c-Src, after integrin stimulation (see *Section 3.2*).[47] Specific inhibition of ERK1/2 signalling completely reversed this effect, showing that reduction of aggrecan promoter activity is primarily regulated through the ERK1/2 signalling pathway.[47] On the other hand, prolonged fluid shear (48 hours) has been found to result in an upregulation of proteoglycan synthesis, confirming that on the longer term the ERK1/2 pathway is no longer activated and that other processes have become more dominant.[48]

JNK signalling drives c-Jun phosphorylation, which, in turn, allows formation of the activator protein-1 (AP-1) transcription factor complex.[46] Interestingly, the promoters involved in synthesis of

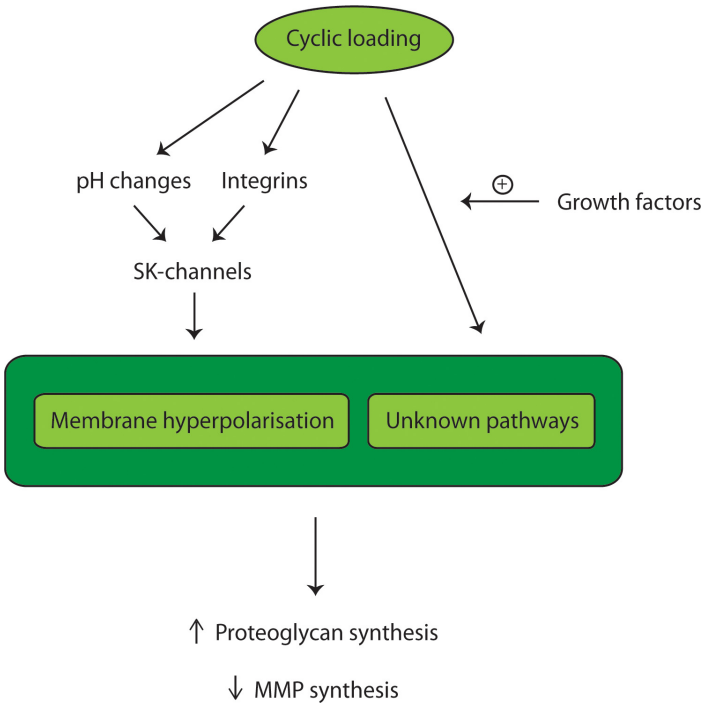

Figure 2 – **Several signalling mechanisms after (e.g.) cyclic loading lead to anabolic cartilage processes**

MMP-3 and MMP-13 have been found to contain functional AP-1-DNA binding sites, mainly via c-Fos and c-Jun proteins.[42] Via this mechanism, static as well as cyclic cartilage compression induces cartilage remodelling by initiating ECM breakdown.[42,49] A parallel dose-dependent inhibition of chondrocyte proteoglycan synthesis has been found during static cartilage compression and static hydrostatic pressure as well, but this is mediated by a distinct mechanism, including a stimulation of nitric oxide (NO) release, and subsequent IL-1 mediated suppression of proteoglycan production.[50,51] In contrast, cyclic cartilage compression stimulates proteoglycan and collagen synthesis, and is regulated by other factors, possibly influenced by interstitial fluid pH (see *Section 3.5*).[49]

3.2 Role of integrins

Integrins are heterodimeric receptors that transmit mechanical signals from the ECM to intracellular chondrocyte signalling pathways. Human chondrocytes prominently express the α5β1 integrin, and synthesise also the α1β1, α3β1, αvβ3 and αvβ5 integrins.[52] Among these, α1β1 integrin could bind to collagen type II and to several other typical cartilage matrix proteins, and α5β1, αvβ3 and αvβ5 integrins are ECM receptors for fibronectin, vitronectin and osteopontin, respectively.[52-54]

Cellular adhesion to the ECM as well as shear stress have been shown to stimulate integrin activation, followed by the earlier mentioned ERK1/2 signalling (*Figure 1*).[47,52,55,56] This ERK1/2 pathway is linked to integrin signalling through activation of tyrosine kinases, including c-Src and protein kinase C (PKC).[56,57] PKC may play a role in signal transduction from focal adhesion complexes as well.[47]

Also, the other MAPK pathways, JNK and p38, are activated after α5β1 integrin stimulation (*Figure 1*).[52]

With cyclic pressurisation, α5β1 integrin mediated signalling leads to membrane hyperpolarisation, by first influencing the actin cytoskeleton, stretch-activated ion channels and tyrosine kinase activation.[58,59] Tyrosine kinase signalling then promotes IL-4 release from chondrocytes, and IL-4 subsequently activates phospholipase C (PLC) and PKC in these cells, leading to small-conductance apamin-sensitive Ca^{2+}-activated K^+ (SK) channel activation, which, finally, induce membrane hyperpolarisation (see also *Section 3.5* and *Figure 2*).[58-60] This signalling pathway has been found to result in an upregulation of aggrecan expression, and a downregulation of MMP-3 expression.[61]

Also, activated integrins stimulate phosphatidyl inositol-3 kinase (PI3K)/Akt signalling pathways, which may promote Wnt signalling through inhibition of GSK3β and subsequent nuclear translocation of β-catenin (*Figure 1*).[62,63] Wnt signalling is linked to TCF4 mediated transcription, leading to increased expression of MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5, and decreased expression of collagen type II and aggrecan, when the chondrocytes are simultaneously exposed to a tensile strain for 24 hours.[64] Cytoskeletal reorganisation due to mechanical cell deformation may also influence Wnt signalling, as β-catenin interacts with the F-actin cytoskeleton at the cell surface via Rac-1.[65]

3.3 Role of growth factors

Several growth factors, including FGF and IGF-I, have been found to synergise with integrins in growth factor receptor activation and MAPK signalling (*Figure 1*), a cooperation which is mediated by Shc, a docking protein for Grb2 and EKR1/2.[66-68] Integrin stimulation alone does not considerably elevate the level of Shc binding to Grb2 and ERK1/2, but during additional IGF-I stimulation, these proteins interact and promote cytoskeletal reorganisation, chondrocyte survival and, in some cases, differentiation.[68] Also, IGF-I stimulation alone is not sufficient for chondrocyte survival, but matrix induced integrin mediated co-signalling is required.[69]

IGF-I also stimulates the production of collagens, proteoglycans and hyaluronan, which is synergistically promoted by dynamic cartilage compression (*Figure 2*).[49,70] Also, IGF-I promotes biosynthesis of SZP to lubricate the cartilage surface.[14] However, during static compression, stimulation with IGF-I has been found to lead to a primary inhibition of proteoglycan synthesis for 4 hours, which is the effect of static compression, and to a subsequent gradual increase towards 24 hours, which is the effect of IGF-I signalling.[49] Between 24 and 48 hours, no further increase in synthetic activity was observed in this experiment, which may be the result of ERK1/2 signalling, either sustained by the static compression (*Section 3.1*) or by IGF-I downstream signalling.[45,49]

Together, these findings suggest that growth factors and mechanical stimulation may be functionally related in chondrocytes, to allow a mild reparative response to mechanical compression during a parallel stimulation with growth factors, and to prevent growth factor maintained survival when there is no matrix signal.[69] However, *in vivo*, the cartilage ECM may hinder entry of the relatively large growth factors from the synovial fluid by size exclusion, and, for IGF-I, the IGF binding proteins (IGFBP) may prevent its actual interaction with chondrocytes.[71]

3.4 Role of cytokines

Cytokines synergistically influence integrin signalling as well, since IL-1 has been found to further enhance MMP production during fibronectin induced integrin activation, and IL-1 antagonists inhibited MMP synthesis under the same conditions.[72] This is consistent with the finding that IL-1 activation of the ERK1/2 pathway requires focal adhesion and flux of Ca^{2+}.[73] Conversely, ERK1/2 pathway activation by cyclic strain, shear stress and shear fluid, which is mediated by integrins (*Section 3.2*), has been shown to be Ca^{2+} independent.[47,57,74]

Also, chondrocytes produce IL-6 and GM-CSF during adhesion to fibronectin, which is therefore another coupling of integrin and cytokine signalling in chondrocytes.[75]

IL-1 has been shown to inhibit the biosynthesis of proteoglycans and of the SZP (*Section 1.1*), which could play a role in synovial inflammation and in the pathogenesis of several arthropathies.[14,51]

3.5 Role of interstitial and intracellular pH

During high-amplitude static cartilage compression, the pH in the interstitial fluid falls due to fluid expression from the cartilage matrix, which has been shown to result in an altered biosynthetic activity of the chondrocytes.[76,77]

Indeed, interstitial and intracellular pH are influencing each other, because membrane permeability changes when the extracellular pH falls, and leads to intracellular concentration changes of Na^+, K^+, Ca^{2+} and H^+.[18] These simultaneous fluctuations of extra- and intracellular ion concentrations are caused by the interdependence of membrane transporter proteins, as they have shared substrates.[18]

Changing pH values due to cyclic pressurisation may lead to changes in cell osmolarity, causing cell swelling, which would activate membrane stretch-sensitive Ca^{2+} channels. A Ca^{2+} influx activates the SK-channels, that are sensitive to Ca^{2+} and allow a K^+ efflux, leading to a subsequent membrane hyperpolarisation (*Figure*

2).[78] This hyperpolarisation induces aggrecan synthesis, and a downregulation of MMP-3 (*Section 3.2*, *Figure 2*).[61] On the other hand, continuous pressurisation has been found to activate a Na^+-selective channel, leading to a Na^+ influx, and subsequent membrane depolarisation, which has the opposite effect on the biosynthetic activity of chondrocytes (*Figure 1*).[18] Thus, cyclic and continuous pressurisation have differential effects on the cellular responses with regard to pH changes and ion fluxes, finally leading to distinct biosynthetic activity and subsequent cartilage degeneration or regeneration.

Conclusions

The regulation of joint homeostasis and chondrocyte responses to environmental alterations are very complex processes, and are still largely unknown, although a considerable amount of knowledge has been conceived by the various research group investigations. For instance, the various forms of joint loading have been found to differentially influence anatomical and molecular responses, which together are aimed to maintain the joint homeostasis.

In degenerative joint arthropathies, including OA, the reparative capacity of cartilage is still present, and could be activated by unloading the cartilage, for instance by joint distraction, which reduces intra-articular hydrostatic pressure and mechanical compressive forces. In the clinic, joint distraction has been applied to OA ankles, knees and hips, with very satisfactory outcomes, including for instance an increased joint space width, subchondral bone remodelling, significant decrease of pain and improved mobility and function.[2,3,79] This points to actual articular cartilage repair, which has been confirmed by a number of animal studies. These preclinical studies also showed that gradual weight bearing considerably improved histologically visible cartilage repair as compared with fixed distraction and distraction without weight bearing.[80-82]

Taking into account the presently reviewed molecular responses to cartilage loading, the beneficial effect of joint loading after joint distraction could be further comprehended. Altered mechano-transduction during joint distraction while there is still joint motion is a possible mechanism leading to proteoglycan synthesis and MMP downregulation (*Figure 2*), as well as to subchondral bone remodelling which further unloads the articular cartilage.

Since nowadays most findings with regard to chondrocyte mechanotransduction, and its further signalling to regulate biosynthesis, are based on *in vitro* models, and since many of these studies are performed under supra-physiological conditions, the development of an *in vivo* model, which allows more direct measurements of chondrocyte metabolism, is recommended. This would allow a further comprehension of these molecular processes, which is essential in understanding arthropathy development and other treatment options.

References

1. Buckwalter, J.A. and Martin, J.A. Osteoarthritis. *Advanced Drug Delivery Reviews* **58**, 150-167 (2006).
2. Intema, F.*, et al.* Subchondral bone remodeling is related to clinical improvement after joint distraction in the treatment of ankle osteoarthritis. *Osteoarthritis and Cartilage* **19**, 668-675 (2011).
3. Intema, F.*, et al.* Tissue structure modification in knee osteoarthritis by use of joint distraction: an open 1-year pilot study. *Annals of the Rheumatic Diseases* **70**, 1441-1446 (2011).
4. Eichelberger, L., *et al.* Biochemical studies of articular cartilage. III. Values following the immobilization of an extremity. *The Journal of Bone and Joint Surgery. American Volume* **41**, 1127-1142 (1959).
5. Hall, M.C. Articular changes in the knee of the adult rat after prolonged immobilization in extension. *Clinical Orthopaedics and Related Research* **34**, 184-195 (1964).
6. Ateshian, G.A. The role of interstitial fluid pressurization in articular cartilage lubrication. *Journal of Biomechanics* **42**, 1163-1176 (2009).
7. Heath, C.A. and Magari, S.R. Mini-review: Mechanical factors affecting cartilage regeneration in vitro. *Biotechnology and Bioengineering* **50**, 430-437 (1996).
8. Lane Smith, R.*, et al.* Effects of shear stress on articular chondrocyte metabolism. *Biorheology* **37**, 95-107 (2000).
9. Pearle, A.D., *et al.* Basic science of articular cartilage and osteoarthritis. *Clinics in Sports Medicine* **24**, 1-12 (2005).
10. Watanabe, H., *et al.* Roles of aggrecan, a large chondroitin sulfate proteoglycan, in cartilage structure and function. *Journal of Biochemistry* **124**, 687-693 (1998).
11. McDonald, J.N. and Levick, J.R. Effect of intra-articular hyaluronan on pressure-flow relation across synovium in anaesthetized rabbits. *The Journal of Physiology* **485 (Part 1)**, 179-193 (1995).
12. Poole, A.R.*, et al.* Contents and distributions of the proteoglycans decorin and biglycan in normal and osteoarthritic human articular cartilage. *Journal of Orthopaedic Research* **14**, 681-689 (1996).
13. Oldberg, A., *et al.* A collagen-binding 59-kd protein (fibromodulin) is structurally related to the small interstitial proteoglycans PG-S1 and PG-S2 (decorin). *The EMBO Journal* **8**, 2601-2604 (1989).
14. Flannery, C.R.*, et al.* Articular cartilage superficial zone protein (SZP) is homologous to megakaryocyte stimulating factor precursor and Is a multifunctional proteoglycan with potential growth-promoting, cytoprotective, and lubricating properties in cartilage metabolism. *Biochemical and Biophysical Research Communications* **254**, 535-541 (1999).
15. Jay, G.D., *et al.* Homology of lubricin and superficial zone protein (SZP): products of megakaryocyte stimulating factor (MSF) gene expression by human synovial fibroblasts and articular chondrocytes localized to chromosome 1q25. *Journal of Orthopaedic Research* **19**, 677-687 (2001).
16. Wong, M., *et al.* Zone-specific cell biosynthetic activity in mature bovine articular cartilage: a new method using confocal microscopic stereology and quantitative autoradiography. *Journal of Orthopaedic Research* **14**, 424-432 (1996).
17. Madry, H., *et al.* The basic science of the subchondral bone. *Knee Surgery, Sports Traumatology, Arthroscopy* **18**, 419-433 (2010).
18. Wilkins, R.J., *et al.* Chondrocyte regulation by mechanical load. *Biorheology* **37**, 67-74 (2000).
19. Pan, J.*, et al.* In situ measurement of transport between subchondral bone and articular cartilage. *Journal of Orthopaedic Research* **27**, 1347-1352 (2009).
20. Dequeker, J., *et al.* Bone density and osteoarthritis. *The Journal of Rheumatology* **43**, 98-100 (1995).
21. Hui, A.Y., *et al.* A systems biology approach to synovial joint lubrication in health, injury, and disease. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* **4**, 15-37 (2012).
22. Levick, J.R. Permeability of rheumatoid and normal human synovium to specific plasma proteins. *Arthritis and Rheumatism* **24**, 1550-1560 (1981).
23. Schumacher, B.L., *et al*. A novel proteoglycan synthesized and secreted by chondrocytes of the superficial zone of articular cartilage. *Archives of Biochemistry and Biophysics* **311**, 144-152 (1994).
24. FitzGerald, O. and Bresnihan, B. Synovial membrane cellularity and vascularity. *Annals of the Rheumatic Diseases* **54**, 511-515 (1995).
25. Iwanaga, T., *et al.* Morphology and functional roles of synoviocytes in the joint. *Archives of histology and cytology* **63**, 17-31 (2000).

26. Davidson, R.K., *et al.* Expression profiling of metalloproteinases and their inhibitors in synovium and cartilage. *Arthritis Research & Therapy* **8**, R124 (2006).
27. Kushner, I. and Somerville, J.A. Permeability of human synovial membrane to plasma proteins. Relationship to molecular size and inflammation. *Arthritis and Rheumatism* **14**, 560-570 (1971).
28. Pejovic, M., *et al.* Determination of the apparent synovial permeability in the knee joint of patients suffering from osteoarthritis and rheumatoid arthritis. *British Journal of Rheumatology* **34**, 520-524 (1995).
29. Hashimoto, S., *et al.* Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. *Arthritis and Rheumatism* **41**, 1632-1638 (1998).
30. Hashimoto, S., *et al.* Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. *Arthritis and Rheumatism* **41**, 1266-1274 (1998).
31. Arokoski, J.P., *et al.* Normal and pathological adaptations of articular cartilage to joint loading. *Scandinavian Journal of Medicine & Science in Sports* **10**, 186-198 (2000).
32. Vanwanseele, B., *et al.* Longitudinal analysis of cartilage atrophy in the knees of patients with spinal cord injury. *Arthritis and Rheumatism* **48**, 3377-3381 (2003).
33. Smith, R.L., *et al.* Rabbit knee immobilization: bone remodeling precedes cartilage degradation. *Journal of Orthopaedic Research* **10**, 88-95 (1992).
34. Ytterberg, S.R., *et al.* Exercise for arthritis. *Bailliere's Clinical Rheumatology* **8**, 161-189 (1994).
35. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. *Arthritis and Rheumatism* **43**, 1905-1915 (2000).
36. Arokoski, J.P., *et al.* Biomechanical and structural characteristics of canine femoral and tibial cartilage. *Journal of Biomedical Materials Research* **48**, 99-107 (1999).
37. Roberts, S., *et al.* Mechanical and biochemical properties of human articular cartilage in osteoarthritic femoral heads and in autopsy specimens. *The Journal of Bone and Joint Surgery. British Volume* **68**, 278-288 (1986).
38. Larsson, T., Aspden, R.M. & Heinegard, D. Effects of mechanical load on cartilage matrix biosynthesis in vitro. *Matrix* **11**, 388-394 (1991).
39. Roemhildt, M.L., *et al.* Effects of increased chronic loading on articular cartilage material properties in the lapine tibiofemoral joint. *Journal of Biomechanics* **43**, 2301-2308 (2011).
40. Kim, Y.J., *et al.* Compression of cartilage results in differential effects on biosynthetic pathways for aggrecan, link protein, and hyaluronan. *Archives of Biochemistry and Biophysics* **328**, 331-340 (1996).
41. Ragan, P.M., *et al.* Down-regulation of chondrocyte aggrecan and type-II collagen gene expression correlates with increases in static compression magnitude and duration. *Journal of Orthopaedic Research* **17**, 836-842 (1999).
42. De Croos, J.N., *et al.* AP-1 DNA binding activity regulates the cartilage tissue remodeling process following cyclic compression in vitro. *Biorheology* **45**, 459-469 (2008).
43. Lin, P.M., *et al.* Increased stromelysin-1 (MMP-3), proteoglycan degradation (3B3- and 7D4) and collagen damage in cyclically load-injured articular cartilage. *Osteoarthritis and Cartilage* **12**, 485-496 (2004).
44. Smith, R.L., *et al.* Pressure and shear differentially alter human articular chondrocyte metabolism: a review. *Clinical Orthopaedics and Related Research*, S89-95 (2004).
45. Fanning, P.J., *et al.* Mechanical regulation of mitogen-activated protein kinase signaling in articular cartilage. *The Journal of Biological Chemistry* **278**, 50940-50948 (2003).
46. Tibbles, L.A. and Woodgett, J.R. The stress-activated protein kinase pathways. *Cellular and Molecular Life Sciences* **55**, 1230-1254 (1999).
47. Hung, C.T., *et al.* Mitogen-activated protein kinase signaling in bovine articular chondrocytes in response to fluid flow does not require calcium mobilization. *Journal of Biomechanics* **33**, 73-80 (2000).
48. Smith, R.L., *et al.* In vitro stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure. *Journal of Orthopaedic Research* **14**, 53-60 (1995).
49. Bonassar, L.J., *et al.* Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. *Archives of Biochemistry and Biophysics* **379**, 57-63 (2000).
50. Liu, G.Z., *et al.* Nitric oxide mediates the change of proteoglycan synthesis in the human lumbar intervertebral disc in response to hydrostatic pressure. *Spine* **26**, 134-141 (2001).
51. Taskiran, D., *et al.* Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. *Biochemical and Biophysical Research Communications* **200**, 142-148 (1994).
52. Loeser, R.F. Integrins and cell signaling in chondrocytes. *Biorheology* **39**, 119-124 (2002).
53. Dürr, J., *et al.* Localization of beta 1-integrins in human cartilage and their role in chondrocyte adhesion to collagen and fibronectin. *Experimental Cell Research* **207**, 235-244 (1993).
54. Enomoto, M., *et al.* Beta 1 integrins mediate chondrocyte interaction with type I collagen, type II collagen, and fibronectin. *Experimental Cell Research* **205**, 276-285 (1993).
55. Jalali, S., *et al.* Shear stress activates p60src-Ras-MAPK signaling pathways in vascular endothelial cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **18**, 227-234 (1998).
56. Takahashi, M. and Berk, B.C. Mitogen-activated protein kinase (ERK1/2) activation by shear stress and adhesion in endothelial cells. Essential role for a herbimycin-sensitive kinase. *The Journal of Clinical Investigation* **98**, 2623-2631 (1996).
57. Traub, O., *et al.* PKC-epsilon is required for mechano-sensitive activation of ERK1/2 in endothelial cells. *The Journal of Biological Chemistry* **272**, 31251-31257 (1997).
58. Wright, M.O., *et al.* Hyperpolarisation of cultured human chondrocytes following cyclical pressure-induced strain: evidence of a role for alpha 5 beta 1 integrin as a chondrocyte mechanoreceptor. *Journal of Orthopaedic Research* **15**, 742-747 (1997).
59. Wright, M., *et al.* 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* **90**, 61-71 (1996).
60. Millward-Sadler, S.J., *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).
61. Millward-Sadler, S.J., *et al.* 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 and Rheumatism* **43**, 2091-2099 (2000).
62. Sen, B., *et al.* Mechanical loading regulates NFATc1 and beta-catenin signaling through a GSK3beta control node. *The Journal of Biological Chemistry* **284**, 34607-34617 (2009).
63. Takeuchi, R., *et al.* Low-intensity pulsed ultrasound activates the phosphatidylinositol 3 kinase/Akt pathway and stimulates the growth of chondrocytes in three-dimensional cultures: a basic science study. *Arthritis Research & Therapy* **10**, R77 (2008).
64. Thomas, R.S., *et al.* Effects of Wnt3A and mechanical load on cartilage chondrocyte homeostasis. *Arthritis Research & Therapy* **13**, R203 (2011).
65. Kerr, B.A., *et al.* Small GTPase protein Rac-1 is activated with maturation and regulates cell morphology and function in chondrocytes. *Experimental Cell Research* **314**, 1301-1312 (2008).
66. Clancy, R.M., *et al.* Outside-in signaling in the chondrocyte. Nitric oxide disrupts fibronectin-induced assembly of a subplasmalemmal actin/rho A/focal adhesion kinase signaling complex. *The Journal of Clinical Investigation* **100**, 1789-1796 (1997).

67. Enomoto-Iwamoto, M., *et al.* Involvement of alpha5beta1 integrin in matrix interactions and proliferation of chondrocytes. *Journal of Bone and Mineral Research* **12**, 1124-1132 (1997).
68. Shakibaei, M., *et al.* Signal transduction by beta1 integrin receptors in human chondrocytes in vitro: collaboration with the insulin-like growth factor-I receptor. *The Biochemical Journal* **342 Part 3**, 615-623 (1999).
69. Pulai, J.I., *et al.* The alpha5beta1 integrin provides matrix survival signals for normal and osteoarthritic human articular chondrocytes in vitro. *Arthritis and Rheumatism* **46**, 1528-1535 (2002).
70. Bonassar, L.J., *et al.* The effect of dynamic compression on the response of articular cartilage to insulin-like growth factor-I. *Journal of Orthopaedic Research* **19**, 11-17 (2001).
71. Schneiderman, R., *et al.* Concentration and size distribution of insulin-like growth factor-I in human normal and osteoarthritic synovial fluid and cartilage. *Archives of Biochemistry and Biophysics* **324**, 173-188 (1995).
72. Arner, E.C. and Tortorella, M.D. Signal transduction through chondrocyte integrin receptors induces matrix metalloproteinase synthesis and synergizes with interleukin-1. *Arthritis and Rheumatism* **38**, 1304-1314 (1995).
73. Lo, Y.Y., *et al.* Requirements of focal adhesions and calcium fluxes for interleukin-1-induced ERK kinase activation and c-fos expression in fibroblasts. *The Journal of Biological Chemistry* **273**, 7059-7065 (1998).
74. Ikeda, M., *et al.* Calcium-independent activation of extracellular signal-regulated kinases 1 and 2 by cyclic strain. *Biochemical and Biophysical Research Communications* **247**, 462-465 (1998).
75. Yonezawa, I., *et al.* VLA-5-mediated interaction with fibronectin induces cytokine production by human chondrocytes. *Biochemical and Biophysical Research Communications* **219**, 261-265 (1996).
76. Gray, M.L., *et al.* Mechanical and physiochemical determinants of the chondrocyte biosynthetic response. *Journal of Orthopaedic Research* **6**, 777-792 (1988).
77. Boustany, N.N., *et al.* Time-dependent changes in the response of cartilage to static compression suggest interstitial pH is not the only signaling mechanism. *Journal of Orthopaedic Research* **13**, 740-750 (1995).
78. Wright, M.O., *et al.* Response of plasma membrane to applied hydrostatic pressure in chondrocytes and fibroblasts. *Connective Tissue Research* **28**, 49-70 (1992).
79. Aldegheri, R., *et al.* Articulated distraction of the hip. Conservative surgery for arthritis in young patients. *Clinical Orthopaedics and Related Research*, 94-101 (1994).
80. Nishino, T., *et al.* Joint distraction and movement for repair of articular cartilage in a rabbit model with subsequent weight-bearing. *The Journal of Bone and Joint Surgery. American Volume* **92**, 1033-1040 (2010).
81. Nishino, T., *et al.* Effect of gradual weight-bearing on regenerated articular cartilage after joint distraction and motion in a rabbit model. *Journal of Orthopaedic Research* **28**, 600-606 (2010).
82. van Valburg, A.A., *et al.* Joint distraction in treatment of osteoarthritis (II): effects on cartilage in a canine model. *Osteoarthritis and Cartilage* **8**, 1-8 (2000).

Abbreviations

ADAMTS: a distintegrin and metalloproteinase with thrombospondin motifs
AP-1: activator protein-1
AS: ankylosing spondylitis
BMI: body mass index
CS: chondroitin sulphate
ECM: extracellular matrix
ERK: extracellular signal-regulated kinase
GAG: glycosaminoglycan
HA: hyaluronan
IGFBP: IGF binding protein
JNK: c-Jun N-terminal kinase
KS: keratan sulphate
MAPK: mitogen-activated protein kinase
MMP: matrix metalloproteinase
NO: nitric oxide
OA: osteoarthritis
PI3K: phosphatidyl inositol-3 kinase
PKC: protein kinase C
PLC: phospholipase C
PRG-4: proteoglycan-4
RA: rheumatoid arthritis
SAPK: stress-activated protein kinase
SK-channel: small-conductance apamin-sensitive Ca^{2+}-activated K^+ channel
SZP: superficial zone protein
TIMP: tissue inhibitor of metalloproteinases