Chapter 3

Biomechanics of Cartilage and Osteoarthritis

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Additional information is available at the end of the chapter

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1. Introduction

The etiology of osteoarthritis (OA) is not known with certainty. Undoubtedly, many factors contribute to articular cartilage failure but consistently abnormal biomechanics appear central to the condition. Indeed it is recognized that mechanical loading that is either below or in excess of the physiology range leads cartilage degeneration. There are currently no cures for OA and no effective pharmacological treatments that slow or halt disease progression. Physical activity is one of the most widely prescribed non-pharmacological therapies for OA management, based on its ability to limit pain and improve physical function. The detailed mechanisms underlying these beneficial effects of exercise and physical therapy are largely unknown. Structural integrity is important for joint function and can be lost as a consequence of a range of physical, biomechanical and inflammatory factors. This chapter will overview how joint loading influences cartilage structure and how mechanical loading is perceived by chondrocytes resulting in cellular responses that are either chondroprotective or promoting inflammatory and catabolic responses initiating and progressing OA.

2. Mechanical integrity of joint structure

The synovial or diarthrodial joint allows movement between bones and permits transmission of mechanical loads. The mechanical integrity of the joint elements including articular cartilage, synovium, subchondral bone, joint capsule, ligaments and periarticular connective tissues cooperates to provide optimal function. Loss of mechanical integrity results in a range of pathological changes within the joint recognized as osteoarthritis.

In a synovial joint the articulating bone ends are covered by a thin, highly hydrated specialized connective tissue, articular cartilage. A high interstitial fluid content distinguishes cartilage from most other connective tissues and contributes to the mechanical properties of the tissue

[1,2]. The major components of articular cartilage are type II collagen, proteoglycans, noncollagenous proteins and glycoproteins. Type II collagen forms the fibrillar meshwork which provides tensile strength [3-6] and entraps aggregating hydrophilic proteoglycans which help maintain the high tissue hydration [7-9]. Disruption of the fibrillar meshwork or loss of proteoglycan results in an in-ability of cartilage to distribute loads and its contribution to near frictionless joint movement leading, in time, to progressive structural damage and pathological features of OA.

3. Articular cartilage responses to mechanical loading *in vivo*

Mechanical loading within a physiological range is necessary to maintain joints in a healthy state. During normal daily activity articular cartilage is exposed to a range of mechanical forces during joint movement. Peak forces across the human hip and knee joints have been shown to reach 4 and 7 times body weight, respectively, during normal walking [10,11]. In vivo, mechanical loading is applied cyclically and the cells within cartilage, chondrocytes, are exposed to a composite of radial, tangential and shear stresses [12]. The effects of mechanical load bearing on the development and microscopic structure of the articular cartilage have been studied in some detail [13]. Elevated loading increases cartilage thickness, causes hypertrophy of the superficial zone chondrocytes, and increases the amount of intercellular matrix [14-17]. In normal human joints, load-bearing areas of the cartilage are thicker with a higher proteoglycan concentration and are mechanically stronger than non-load-bearing regions of the same joint [18-20]. Increasing weight-bearing of joints, in a variety of animal models, leads to elevation of proteoglycan content within articular cartilage [15,16,21-23]. In contrast, removal of load bearing leads to a reduction in proteoglycan content [13]. In a dog model, immobilization of a joint by placing a leg in a cast leads to cartilage atrophy, loss of Safranin O staining, and a decrease in its uronic acid content [21]. These changes are reversible on remobilization. Mechanical regulation is also an important factor for chondrogenesis and has been involved in the development of cell-based therapies for cartilage degeneration and disease [24].

3.1. Mechanical stress within articular cartilage

Articular cartilage is exposed to surprisingly large mechanical loads during joint movement. Using an instrumented hip prosthesis mechanical stresses have been measured in a 74-year-old female [25]. Rising from a chair, pressures in the hip joint cartilage can reach nearly 20 MPa and during walking, pressures cycle between atmospheric and 3-4 MPa at a frequency of around 1 Hz. With walking or running forces at the joint surface may vary from near zero to several times the whole body weight within a period of 1 second [10,11]. Loading of articular cartilage generates a combination of tensile, compressive and shear stress in the material. The tensile modulus of healthy human articular cartilage varies from 5-25 MPa, depending on the site of movement on the joint surface (i.e., high or low weight bearing regions), and the depth and orientation of the test specimen relative to the joint surface [4,26]. The compressive modulus varies from 0.4-2.0 MPa [27,28]. Articular cartilage responds to shearing forces by both stretching and deformation of the solid matrix. The dynamic shear modulus is within the range of 0.2-2.0 MPa for healthy bovine or canine cartilage [29-31]. These physiological stresses

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are important regulators of cartilage metabolism and integrity as mechanical loading serves to maintain fluid flow and ion phase function within the tissue and act to stimulate chondrocyte metabolism [32].

4. Articular cartilage explant and chondrocyte responses to mechanical loading *in vitro*

Rodan et al. [33] studied the effects of application of compressive forces (80 g/cm^2) to chick tibial epiphyses (16-day-old embryos) in culture and found that glucose consumption reduced to half of controls. Twenty four hours after the release of pressure, glucose utilization again increased, approaching control levels. The same pressure also stimulated thymidine incorporation into DNA. Exposing chick tibial epiphyses to continuous compressive forces (60 g/cm^2, equal to 5.865 kPa) caused a reduction of both cAMP and cGMP [34]. An equivalent hydrostatic pressure applied directly to cells isolated from chick tibial epiphyses also affects cyclic nucleotide accumulation [34]. Veldhuijzen et al. developed a model system that exposed cultured monolayer chondrocytes on the walls of tissue culture tubes to intermittent compressive forces of 12.8 kPa for 6 hours at a frequency of 0.3 Hz [35]. Contrary to the effect of continuous compressive forces, intermittent compressive forces caused a rise in levels of cAMP and a reduction in DNA synthesis. Palmoski and Brandt [36] studied the effects of both static and intermittent mechanical stress on full-thickness plugs of canine articular cartilage. When the plugs were exposed to compressive force using a regime of 60 sec on/60 sec off, glycosaminoglycan synthesis was reduced to 30-60% of controls. However, when a regime of 4 sec on/11 sec off was employed, the glycosaminoglycan synthesis increased by 34%, although protein synthesis and DNA, uronic acid, and water content remained unaltered indicating that different frequencies of cyclical strain produce differences in metabolic activity within chondrocytes.

Some models designed to test the effects of mechanical force on chondrocytes in vitro have focused on the effects of cell stretching. In these models there is usually deformation of a cell-laden, flexible membrane which can be regulated according to (1) the method of deformation of the membrane - by control of either the displacement or the force, and (2) the shape and mounting of the deformable membrane - either a circular membrane held at its periphery or a rectangular strip held at the two ends [37]. The devices utilized in the production of the displacement include (a) a vacuum driven diaphragm (silicone elastomer membrane, 2.5 mm in thickness) [38,39], (b) pin shaped displacement (silicone elastomer membrane, 0.254 mm in thickness) [40], (c) glass dome displacement (polytetrafluoroethylene membrane, 0.025 mm in thickness) [41,42], (d) air or fluid displacement (polyurethane membrane, 0.094 mm in thickness) [43-45], and (e) a circular groove displacement (silicone elastomer membrane, 0.076 mm in thickness; polyurethane membrane, 0.094 mm in thickness) [46,47]. The Flexercell™ strain unit [38] consists of a computer-controlled vacuum unit and a baseplate on which are held the culture dishes. These dishes have a flexible base. A vacuum is applied to the dishes via the baseplate. When a precise vacuum level is applied to the system, the bases of the culture plates are deformed by known percentage elongation that is maximal at the edge of the culture dish, but decreases towards the center. Using the system, straining the base of a culture dish

leads to strain of the attached cultured cells. When the vacuum is released, the bases of the dishes return to their original conformation.

By stretching a supportive flexible membrane on which chondrocytes were cultured, Lee et al. [48] found that a cyclic 10% mechanical stretch for 8 hours increased glycosaminoglycan synthesis and decreased protein and collagen synthesis. DeWitt et al. [49] showed increased radiosulphate and ^{14}C-glucosamine incorporation into glycosaminoglycans by chick epiphyseal chondrocytes in high density cultures subjected to a 5.5% strain at a frequency of 0.2 Hz. Protein synthesis after 24 hours mechanical strain remained unchanged. Using the Flexercell™ strain system, Fujisawa et al. [50] investigated the influence of cyclic tension force on the metabolism of cultured chondrocytes. Two levels of force (5 kPa or 15 kPa) and three frequencies 30 cycles/min (1 sec on/1 sec off), 0.5 cycles/min (1 sec on/119 sec off) and 0.25 cycles/min (1 sec on/239 sec off) were used. Both 5 and 15 kPa of high frequency cyclic mechanical stress for 48 hours significantly inhibited the syntheses of DNA, proteoglycan, collagen, and protein. The expression of interleukin-1, matrix metalloproteinase (MMP)-2 and MMP–9 mRNA were induced by 15 kPa of high frequency force. The production of pro- and active-MMP-9 which would lead to cartilage breakdown in vivo was also increased at this pressure and frequency of stimulation. Reducing the applied frequency decreased the inhibition of proteoglycan synthesis. Mechanical stretch producing 25% maximal elongation at a frequency of 0.05 Hz for 48 hours also induces the expression of high molecular weight heat shock protein (HSP) 105 kDa in the human chondrocytic cell line CS-OKB [51]. These findings suggest that the frequency of cyclic tension force is one of the key determinants of chondrocyte metabolism.

Using confocal microscopy and fluorescent techniques it is possible to monitor and measure intracellular calcium ion concentration in isolated articular chondrocytes subjected to controlled deformation with the edge of a glass micropipette [52]. Intracellular calcium ion concentration reaches a peak within 5 sec following 25% deformation of the cells and returns to baseline levels in 3-5 minutes. The immediate and transient increase of intracellular calcium waves is abolished by removing Ca^{2+} from the culture medium and is significantly reduced by the presence of gadolinium and amiloride, agents known to block mechanosensitive ion channels [53-56]. Inhibitors of intracellular Ca^{2+} release or agents known to cause cytoskeletal disruption including cytochalasin D and colchicine had no significant effect on the Ca^{2+} waves. The results indicate that mechanosensitive ion channels are upstream in the mechanotransduction signaling pathway, consistent with results obtained using electrophysiological parameters in the assessment of the cell response to cyclical mechanical strain [56].

The effects of fluid-induced shear stress on articular chondrocyte morphology and metabolism in vitro have also been investigated [57]. Fluid-induced shear stress (1.6 Pa = 16 dynes/cm^2) was applied by cone viscometer to both normal human and bovine articular chondrocytes. Shear stress for 48 and 72 hours caused individual chondrocytes to elongate and align tangential to the direction of cone rotation. Glycosaminoglycan synthesis was increased 2-fold. After 48 hours of shear stress, the release of prostaglandin E2 (PGE2) was increased 10 to 20-fold. In human articular chondrocytes, mRNA levels for tissue inhibitor of metalloproteinase (TIMP) increased 9-fold in response to shear stress compared to controls. In contrast, mRNA levels for the neutral metalloproteinases, collagenase, stromelysin, and gelatinase, did not show significant changes [57].

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5. Chondrocyte mechanoreceptors

The mechanisms by which chondrocytes recognize and respond to the various mechanical stresses encountered in mechanically loaded cartilage continue to be elaborated. A number of potential mechanoreceptors, sensory receptors that respond to vibration, stretching, pressure, or other mechanical stimuli have been identified in chondrocytes. In cartilage the extracellular matrix (ECM) transmits mechanical signals to the cell interior through changes in tension on the cell membrane. Integrins, stretch-activated ion channels, connexins, and primary cilia have each been identified as candidate mechanoreceptors [58-61].

5.1. Integrins

Integrins were first isolated, characterized, and sequenced from chick embryo fibroblast cDNA clones which encoded one subunit of the complex of membrane glycoproteins [62]. The name 'integrin' was proposed as the consequence of its role as an integral membrane complex involved in the transmembrane association between the ECM and the cytoskeleton. Integrins are a large family of α/β heterodimeric cell surface adhesion receptors that can bind a wide variety of ECM and cell surface ligands [63-69]. Most integrins bind ligands that are components of ECM, e.g. fibronectin, collagen, and vitronectin [70]; certain integrins can bind to soluble ligands (such as fibrinogen) or to counter receptors (such as intercellular adhesion molecules) on adjacent cells, leading to homo- or heterotypic aggregation [71,72]. Some of the integrin recognition sites in the ligands and counter receptors have been defined [73,74]. The first binding site to be defined was the Arg-Gly-Asp (RGD) containing sequence present in fibronectin, vitronectin, and a variety of other adhesive proteins [75,76].

Integrin expression by human chondrocytes has been investigated utilizing several techniques including immunohistochemistry, flow cytometry, immunoprecipitation, and northern blotting [77-79]. Normal adult human articular chondrocytes express $\alpha1\beta1$, $\alpha3\beta1$, $\alpha5\beta1$, $\alpha V\beta5$, $\alpha V\beta3$, $\alpha6\beta1$, $\alpha10\beta1$, and $\alpha2\beta1$, in which the $\alpha1\beta1$ (receptor for collagen), $\alpha5\beta1$ (receptor for fibronectin) and the $\alpha V\beta5$ (receptor for vitronectin) heterodimers are consistently expressed. In cartilage, integrin-ECM interactions are thought to be important in many aspects, physiological and pathological, of chondrocyte function, including adhesion, spreading, proliferation, signal transduction, biomechanical regulation, chondrogenesis, and gene expression [80]. Chondrocyte $\beta1$ integrin-ECM interactions are required for chondrocyte survival, matrix deposition and differentiation in models of chondrocyte development [81]. Integrins mediate chondrocyte adhesion to many ECM proteins including type II collagen, type VI collagen, fibronectin, laminin, and osteopontin [82-85]. Chondrocyte spreading and migration on type II collagen, type VI collagen, or fibronectin substrates in vitro is mediated by interactions with $\beta1$ integrins [86,87]. The interaction of the $\alpha5\beta1$ integrin with fibronectin is necessary for adhesion, spreading, and proliferation of both chicken and rabbit chondrocytes [88,89]. *In vitro* chondrogenesis (the differentiation of blastemal cells to chondroblasts and the formation of cartilage matrix) is inhibited by the function blocking anti-$\beta1$ integrin antibodies [90]. Laminin-$\alpha3\beta1/\alpha6\beta1$ interactions are regulated by the ligand trend (depletion/reconstitution or competition experiments) during early chondrocyte differentiation [89]. Other integrin-mediated chondrocyte-matrix interactions include $\beta1$-matrix Gla protein, $\beta3$-bone sialoprotein II, $\beta3$-osteopontin, and $\alpha2\beta1$-chondroadherin associations [84,86].

Integrins are involved in regulation of both cartilage matrix synthesis and integrity. Loss of integrin function inhibits type II collagen synthesis by chondrocytes in culture [91]. However, integrins are also involved in cartilage breakdown processes. Fibronectin fragments stimulate chondrolysis and decrease proteoglycan synthesis in cartilage explants through fibronectin-integrin dependent interactions [92]. Ligation of $\alpha 5\beta 1$ integrin with fibronectin in cultured chondrocytes results in the formation of focal adhesion complexes comprising actin, focal adhesion kinase (FAK) and the G protein Rho [93]. Nitric oxide (NO), a potential mediator of events occurring in osteoarthritis, can inhibit the assembly of the intracellular activation complex and the subsequent upregulation of proteoglycan synthesis that occurs following ligation of $\alpha 5\beta 1$ integrin to fibronectin [93].

Integrins also act as mechanoreceptors and transmit mechanical signals from the extracellular environment to the cytoskeleton [94-96]. Integrins can provide a gating function for signal transduction, by either supporting or prohibiting force transmission between ECM and the cytoskeleton [94]. Wang et al. [97], using a magnetic twisting device applied mechanical forces directly to cell surface receptors. They showed that the integrin subunit $\beta 1$ induced focal adhesion formation and supported a force-dependent stiffening response, whereas nonadhesion receptors did not [97]. Maniotis et al. [98] reported that living cells and nuclei are hard-wired. When integrins were stimulated by micromanipulating bound microbeads or micropipettes, cytoskeletal filaments reoriented, nuclei distorted, and nucleoli redistributed along the axis of the applied tension field. These effects were specific for integrins, independent of cortical membrane distortion, and were mediated by direct linkages between the cytoskeleton and nucleus [98]. Using a similar magnetic drag force device, intracellular Ca^{2+} concentration was shown to increase when the $\alpha 2$ or the $\beta 1$ integrin subunits were stressed, whereas mechanical loading of the transferrin receptor produced a significantly reduced effect [99]. An increase in tyrosine phosphorylation was observed as a reaction to mechanical stress on the $\beta 1$-subunits of the integrin family, whilst stress to the transferrin or low density lipoprotein receptors which have no connection to the cytoskeleton did not produce this reaction [100,101].

Integrins are also modulated by mechanical stress [102]. When chondrosarcoma cells were exposed to mechanical stimulation, mRNA expression of the $\alpha 5$ integrin subunit was found to increase whilst expression of the $\beta 1$, $\alpha 2$, and αV did not increase significantly [102]. The effect of mechanical stress on integrin subunit expression has also been investigated in cells cultured on type II collagen-coated dishes with a flexible base. Mechanical stress increased mRNA expression of the $\alpha 2$ integrin subunit whilst the levels of mRNA for integrin subunits $\beta 1$, $\alpha 1$, $\alpha 5$, and αV showed no or only small changes [102]. It is likely that mechanical induced regulation of integrins is closely regulated and may be dependent on the nature of the mechanical force acting on the cell and specific mechanoreceptor stimulated.

5.2. Stretch-activated Ion channels

Stretch-activated or stretch-sensitive ion channels (SACs) open as a consequence of mechanical deformation of the cell membrane [103]. SACs are directly activated by mechanical forces applied along the plane of the cell membrane that induce membrane tension and distortion of the lipid bilayer. These result in conformational changes which alter opening or closing rates of the channels permitting ion flux [104]. Application of mechanical forces perpendicular to

the cell membrane, as seen with hydrostatic pressure, appears to be less effective in activating SACs [103]. Activation of calcium permeable SACs leads to local increase in intracellular calcium levels and stimulation of downstream calcium-dependent intracellular signal cascades. SACs sensitive to gadolinium are necessary for load and fluid flow related cellular responses in both chondrocytes and bone cells.

5.3. Connexins

Connexins are widely expressed in connective tissue where networks of cells are seen such as in bone, tendon and, meniscus. They probably act to allow propagation of a mechanical stimulus through a tissue. They are a superfamily of twenty-one transmembrane proteins that form gap junctions and hemichannels [105]. Gap junctions allow continuity between cells permitting diffusion of ions, metabolites and small signaling molecules such as cyclic nucleotides and inositol derivatives. Cx43 is the most abundant connexin present in skeletal tissue. Conditional deletion of Cx43 reduces mineral apposition rate to mechanical loading and Cx43 hemichannels are important for fluid shear induced PGE2 and ATP release in osteocytic cells [106]. Connexins and gap junctions are present at the tip of osteocyte dendritic processes and between these processes and osteoblasts indicating their potential importance in permitting cell–cell communication among the osteocytic network although propagation may be only directed from osteocytes to osteoblasts [107]. Cx43 hemichannels are activated and mediate small molecule exchange between cells and matrix under mechanical stimulation in rat temporomandibular joint (TMJ) chondrocytes [108]. Cx32 and Cx43 are important in tenocyte mechanotransduction [109]. Cx32 junctions form a communication network arranged along the line of principal loading and stimulate collagen production in response to strain. Connexin dependent mechanotransducation may be important in adaptation of subchondral bone to mechanical loading of joints rather than having a major role in chondrocyte dependent mechanotransduction. Nevertheless recent studies suggest that primary cilia associated connexins may be functional in responses of chondrocytes to mechanical loading.

5.4. Primary Cilia

Primary cilia are solitary, immotile cilium present in most cells including chondrocytes and bone cells. They are microtubule-based organelles, growing from the centrosome to extend from the cell surface and contain large concentrations of cell membrane receptors, including integrins [110]. They function both as chemosensors and mechanosensors [111,112]. Bending of the cilium upon matrix deformation or with fluid flow is thought to cause cilium bending, pulling on associated matrix receptors and activation of the mechanoreceptors [113]. In addition to integrins, Cx43 hemichannels are also present on primary cilia and by regulating ATP release cilia and activation of purine receptors cilia-associated connexins may also be involved in mechanotransduction.

6. Chondrocyte mechanotransduction

Mechanoresponsiveness is a fundamental feature of all living cells [94,114,115]. Studies with cultured cells confirm that mechanical stresses can directly alter many cellular processes,

including signal transduction, gene expression, growth, differentiation, and survival [116]. Wright et al. [117] investigated the effects of applied hydrostatic pressure on the transmembrane potentials of articular chondrocytes. These studies have been pivotal in identifying potential mechanotransduction pathways in both normal and osteoarthritic human chondrocytes. In this system cells in monolayer culture were exposed to an increase in hydrostatic pressure by placing culture dishes in a sealed perspex pressure chamber with a gas inlet and outlet. Nitrogen or helium gas was used to pressurize the cultures. A hyperpolarization of the chondrocyte plasma membrane was induced by cyclic pressurization (0.33 Hz, 120 mmHg for 20 min) whilst depolarization was induced by continuous pressure (120 mmHg, 20 min). For the frequencies tested, the maximum values for chondrocyte hyperpolarization occurred at approximately 0.3-0.4 Hz. The mechanical stimulation regime (0.33 Hz, 120 mmHg, 20 min), similar to that used by Veldhuijzen et al. [35], allowed identification of a number of integral components of the electrophysiological response providing insight into molecules and pathways activated in chondrocyte upon mechanical stimulation. By the use of pharmacological inhibitors, it was shown that the hyperpolarization response in cultured human chondrocytes induced by cyclic pressurization involved Ca^{2+} activated K^+ channels and L-type calcium channels. Hyperpolarization was also produced by addition of the calcium ionophore A23187 to the culture medium showing that a rise in intracellular Ca^{2+} concentration within the cell could induce the response. Plasma membrane histamine H1 and H2 receptors, and β-adrenoreceptors did not appear to be involved in the hyperpolarization response. The studies also showed that the actin cytoskeleton, but not microtubules, was involved in the chondrocyte hyperpolarization response [117].

Subsequent studies identified that the electrophysiological response to cyclical pressurization was the result of deformation of the base of the culture dishes to which the chondrocytes were attached and therefore deformation (strain) on the chondrocytes rather than a direct effect of the increased hydrostatic pressure on chondrocytes [56]. The hyperpolarization response was proportional to the microstrain to which cells were subjected and did not occur when chondrocytes were subjected to cyclical pressurization in rigid glass culture dishes or when the plastic dishes were positioned in the pressurization chamber so as to avoid deformation of the base of the culture dish [56].

Experiments undertaken to identify the source of intracellular calcium that activated the SK channels leading to hyperpolarization demonstrated a requirement for extracellular calcium and activity of L-type calcium channels [118-120]. Thapsigargin which raises intracellular Ca^{2+} by inhibition of Ca^{2+}-ATPase in endoplasmic reticulum [121-123] caused hyperpolarization independent of mechanical strain but further hyperpolarization of the cells occurred after cyclical pressurization further supporting the idea that mechanically induced chondrocyte hyperpolarization is dependent on intracellular free Ca^{2+} levels [56]. In addition, TRPV4-mediated Ca^{2+} signaling has been demonstrated to play a central role in the transduction of mechanical signals to support cartilage extracellular matrix maintenance [124].

6.1. Intracellular signal cascades activated by mechanical stimulation

Stimulation of connective tissue cell mechanoreceptors is followed by generation of the secondary messenger molecules and activation of a cascade of downstream signaling events that regulate gene expression and cell function. Many intracellular signaling pathways are

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known to be activated by mechanical forces applied to tissues and cells including heterotrimeric guanine nucleotide binding proteins (G-proteins), protein kinases and transcription factors. These pathways that regulate tissue modelling/remodelling may be activated directly as a consequence of mechanoreceptor signaling or indirectly following production of autocrine/paracrine acting molecules.

PKB/Akt is a protein family of serine/threonine kinases that have multiple roles including inhibition of apoptosis by phosphorylation and inactivation of pro-apoptotic factors. Integrin-dependent activation of phosphoinositide3 kinase (PI3 kinase) by mechanical forces regulates PKB activity and can inhibit cell death. Inactivation of the PI3-K/PKB pathway may be important in deleterious effects of mechanical overloading of cartilage and bone loss in response to withdrawal of loading [125]. The activity of mammalian target of rapamycin (mTOR) may be an essential mechanotransduction component modulated by SH2-containing protein tyrosine phosphatase 2 and is required for cartilage development [126].

Mitogen-activated protein kinases (MAPKs) regulate multiple cellular activities, such as gene expression, mitosis, differentiation, and cell survival/apoptosis. ERK1/2, JNK and p38, of critical importance in regulation of matrix protein and protease gene expression have each been shown to be activated in chondrocytes following mechanical stimulation [127]. Mechanical stimuli may activate different MAPKs and through this mechanism differential cellular responses may occur. MAPK responses may also be cell type dependent. Mechanical stimulation induced ERK1/2 activation in bone cells requires calcium-dependent ATP release whilst in cartilage activation, under certain circumstances, is dependent on FGF-2 rather than through integrin mechanoreceptors [128]. Tyrosine phosphorylation of focal adhesion kinase (pp125FAK), beta-catenin, and paxillin following mechanical stimulation is also recognized in human articular chondrocytes [129].

In bone cells NF-κB, a protein complex that acts as a transcription factor, is directly stimulated by mechanical stimulation is dependent on intracellular calcium release [130]. In chondrocytes biomechanical signals within the physiological range block NF-κB activity and proinflammatory chondrocyte responses [131]. Mechanical stimuli that induce catabolic rather than anabolic responses in chondrocytes induce rapid nuclear translocation of NF-κB subunits p65 and p50 in a similar manner to IL-1β [132].

6.2. Growth factors and autocrine/paracrine signaling in mechanotransduction

As part of the cellular response to mechanical stimulation mechanosensitive connective tissue cells release a range of soluble mediators. These may be present in the cell and available for immediate release, or secretion may depend de novo synthesis by enzymatic activity or transcriptional activation and protein production. These mediators, including prostaglandins, nitric oxide, cytokines, growth factors, and neuropeptides are involved in downstream tissue modelling and remodelling responses initiated by the mechanosensitive cells or other effector cells. Production of soluble mediators by connective tissue cells in response to mechanical stimulation however may also be intrinsic to mechanotransduction pathways. Autocrine and paracrine activity allows increased regulation of the cellular response to mechanical stimuli by permitting cross talk between different components of a mechanotransduction cascade. As

the cellular responses to mechanical stimuli and soluble mediators activate similar signal cascades inducing either anabolic or catabolic responses, it would be expected that they may be antagonistic, additive or synergistic. Anabolic cytokines and growth factors enhance production of matrix under mechanical loading conditions whilst anabolic mechanical stimuli antagonize the effects of catabolic cytokines such as IL-1β [133].

Prostaglandins, predominantly PGE2, NO and ATP are produced when bone cells and chondrocytes are mechanically stimulated. Prostaglandin production is integrin dependent requiring an intact cytoskeleton and activation of SACs, PKC, and PLA2. In cartilage PGE2 induced by mechanical loads is catabolic. Mechanical loading of chondrocytes by physiological stimuli inhibits production of PGE2 and NO whereas damaging loading induces PGE2 release [134]. Following mechanical stimulation bone cells and chondrocytes release ATP which can bind and activate purinergic receptors on these and adjacent cells. Both metabotropic P2Y receptors and ionotropic P2X receptors, have been shown to be involved in mechanical load activated signal cascades in chondrocytes and bone cells and may have physiological roles [135].

IL-4 and IL-1β autocrine/paracrine activity is seen in the integrin-dependent mechanotransduction cascade of chondrocytes (IL-4 and IL-1β) and bone cells (IL-1β) to mechanical stimulation [136,137]. These molecules are secreted within 20 minutes of the onset of mechanical stimulation, suggesting release from preformed stores. IL-4 release relies on secretion of the neuropeptide substance P which binds to its NK1 receptor. Both IL-4 and substance P are necessary but not sufficient for the increased expression of aggrecan mRNA and decrease in MMP3 mRNA induced by the mechanical stimulus suggesting cross talk with other mechanosensitive signaling pathways. IL-1β is involved in the early mechanotransduction pathway of both osteoarthritic chondrocytes and human trabecular bone derived cells [138]. Mechanical loading may also induce release or activation of sequestered growth factors in extracellular matrix which will then act on near-by resident connective tissue cells. Basic fibroblast growth factor (FGF2) is a possible mediator of mechanical signaling in cartilage through such a mechanism [128]. Dynamic compression of porcine cartilage induces release of FGF2 with activation of ERK MAP kinase, synthesis and secretion of TIMP-1. In contrast FGF2 production by bovine cartilage is inhibited by 1 hour of compressive stress of 20 MPa [139]. This mechanical induced suppression of FGF2 is blocked by IL-4 indicating further roles for this pleiotropic cytokine in the regulation of chondrocyte responses to mechanical stimulation.

7. Mechanical loading and osteoarthritis

Abnormal mechanical loading is associated with osteoarthritis [140]. Most animal models of OA are mechanically induced, for example, by introducing joint instability by anterior cruciate ligament section [22] or by altering the loading across the joint by menisectomy [141]. These changes in joint loading affect cartilage structure and chondrocyte activity within days of the procedure, and may eventually result in complete loss of cartilage [142]. When cartilage matrix is lost or made deficient as a consequence of direct physical effects or proteolytic digestion the articular cartilage loses its mechanical function. The tensile modulus has been shown to decrease by as much as 90%, reflecting damage to the cartilage matrix network [26]. Animal

studies, for example, have shown that the tensile modulus of canine knee articular cartilage was reduced after one month of immobilization [143]. In the dog, severe OA lesions in the knee joint have been produced by treadmill exercise after the limb was immobilized for several weeks [144]. The compressive modulus also decreases with increasing severity of degeneration [27]. Other joint tissues (e.g., anterior cruciate ligament) also undergo similar changes of tensile and compressive modulus in an experimental OA model [145].

Mechanical loading out with that which joint tissues can normally withstand or are required to maintain a healthy state is central to the development of OA. OA arises when there is an imbalance between the mechanical forces within a joint and the ability of the cartilage to withstand these forces. This arises in two situations. In the first normal articular cartilage is exposed to abnormal mechanical loads whereas in the other the articular cartilage is fundamentally defective with biomaterial properties that are insufficient to withstand normal load bearing. Risk factors associated with development of OA may have influences in either one or both of these scenarios. The accumulation of advanced glycation end products (AGEs) in ECM with age results in a more brittle collagen network that is less able to withstand normal loads, again leading to cartilage degeneration.

The mechanisms by which abnormal mechanical loading may influence chondrocyte function to promote cartilage breakdown are beginning to be understood. Chondrocytes from osteoarthritic cartilage share mechanoreceptors with chondrocytes from normal chondrocytes [146,147]. Whilst activation of these receptors and downstream signaling cascades such as FAK, PKC, JAK/STAT and MAP kinases[148,149] result in pro-anabolic activity in normal chondrocytes activity through release of locally acting mediators that include the anti-inflammatory cytokine IL-4 and the neuropeptide substance P [136,150]. However the response of chondrocytes is aberrant with production of proinflammatory cytokines such as IL-1 and TNF-α which increase production of MMPs and aggrecanases, further accelerating disease progression and attenuating cartilage repair [151-156].

7.1. Altered responses to mechanical stimulation in osteoarthritic chondrocytes

Chondrocytes from osteoarthritic cartilage show a membrane depolarization response to IL-4 that is inhibited by functional receptor antibodies. It is unclear why chondrocytes from osteoarthritic cartilage should show differences in their response to 0.33 Hz mechanical stimulation and recombinant IL-4. This may be result of a general phenotypic change seen in OA chondrocytes in which the cells are resident in a pro-inflammatory, catabolic environment. Indeed the observation that mechanical stimulation in osteoarthritis may result in production of proinflammatory mediators is supported by the findings that α5β1 integrin ligation increases production of IL-1β by osteoarthritic human chondrocytes with subsequent induction of nitric oxide, PGE2, IL-6, and IL-8 [157]. These cytokines will inhibit anabolic responses and increase cartilage matrix breakdown by MMPs. This may be through direct mechanisms or by interfering with integrin signaling. Expression and function of molecules such as members of the SOCS (suppressors of cytokine signaling) which regulate cytokine signaling pathways [158] may also be implicated. These molecules modulate intracellular signals stimulated by IL-4 including JAK/STAT activation. SOCS-1 has been shown to bind to and inhibit kinase activity of JAK family members and inhibit IL-4 induced activation of JAK1 and STAT6. SOCS-3 has been shown mediate IL-1β inhibition of STAT5 activity. These and other

regulators of STAT transcription factor signaling may be responsible for modulation of IL-4 dependent responses of chondrocytes in osteoarthritic cartilage to mechanical stimulation.

8. Summary

A healthy synovial joint requires exposure to mechanical loads within a physiological range. Osteoarthritis develops when joints are subjected to mechanical loads which they are not biomechanically conditioned to withstand. This may be because the loads are excessive due to obesity or joint malalignment or a consequence of intrinsic or acquired biomechanical weakness of joint tissues such as seen when cartilage proteoglycans are depleted secondary to synovial inflammation. Abnormal mechanical loads may have direct physical effects on joint tissues including cartilage but increasingly knowledge of the pathological process within the osteoarthritic joint indicate that chondrocytes regulated catabolic processes are of prime importance in cartilage degradation. The mechanisms by which chondrocytes recognize mechanical loads and how these mechanical stimuli are transduced into biochemical responses which subsequently lead to altered gene expression and cell function is being increasing understood (Figure 1). Knowledge of how anabolic and catabolic signaling cascades are differentially regulated in response to physiological and pathological mechanical stimuli will enable future strategies to be developed to prevent and treat the progression of cartilage pathology in osteoarthritis.

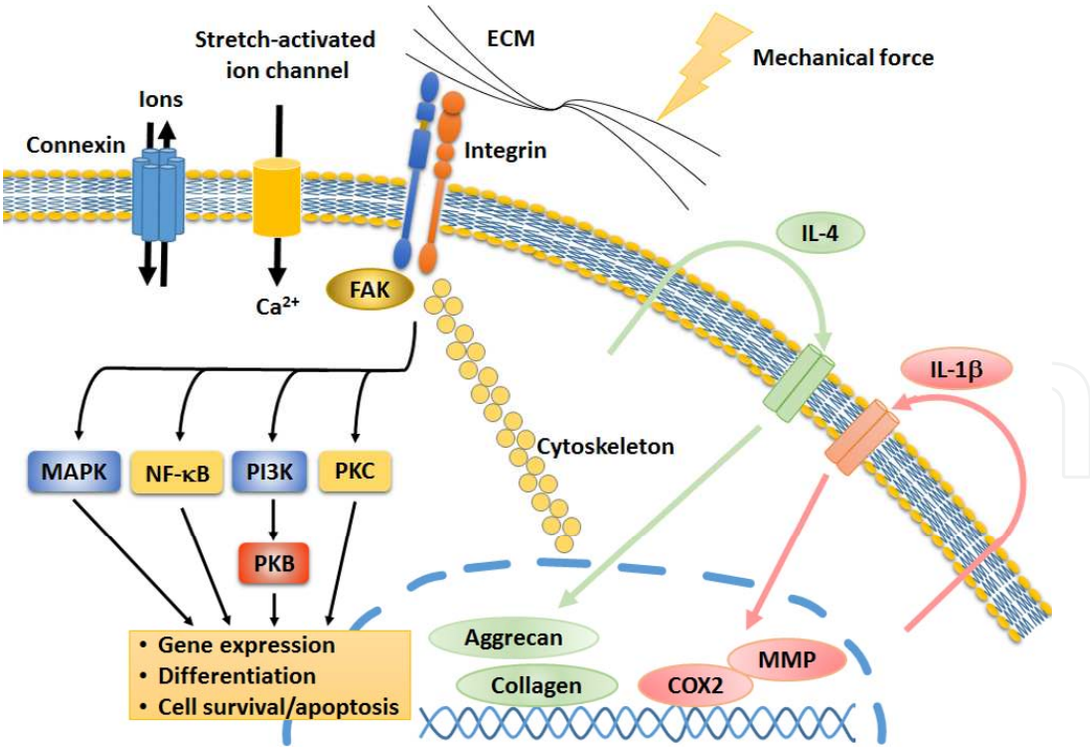

Figure 1. The major mechanotransduction components in chondrocytes. The integrin, connexin, and stretch-activated ion channel mechanoreceptors are stimulated by the mechanical forces transduced via the extracellular matrix (ECM). Downstream transduction pathways involve the cytoskeleton and signaling molecules, including FAK, PKC, PI3K,

PKB, NF-kB, and MAPK, which act to regulate gene expression, cell function and survival/apoptosis. The release and paracrine/autocrine activity of the anti-inflammatory cytokine IL-4 has beneficial effects in regulating an anabolic response with enhanced expression of aggrecan and inhibition of MMP expression. In contrast production of IL-1β, as seen in OA, has a catabolic outcome with activation of pathways resulting in increased expression of COX2 and MMPs.

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