

# Cellular Physiology of Articular Cartilage in Health and Disease

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## 1. Introduction

Articular chondrocytes live in an unusual and constantly changing physicochemical environment. Due to the structure of the extracellular matrix, adult cartilage is avascular, relatively hypoxic and acidic compared to other tissues (Wilkins et al., 2000). In this challenging environment the maintenance and regulation of extracellular matrix by chondrocytes is dependent on signals received through this milieu (Lai et al., 2002). In joint disease, such as osteoarthritis, the extracellular environment is altered and the cellular physiology of the chondrocyte will change to reflect this, leading to alterations in its key role of regulating matrix turnover and hence contributing to the pathophysiology of joint disease (Goldring 2006).

This chapter will discuss the challenges to the chondrocyte and how cellular physiology is affected in both health and disease. We will discuss how the structure of the matrix confers its biomechanical properties to cartilage and how this translates to physiological sensing by the cartilage during static and dynamic loading with particular emphasis on effects on membrane transporters and cell signalling pathways. We will also consider how other features of cartilage in the adult influence the chondrocyte, such as oxygen tension, osmolarity and pH. Finally we will consider the changes that occur in osteoarthritis and how these translate to alterations in cellular physiology and hence matrix integrity, the loss of which is the key feature of osteoarthritis, and how these events may be new targets for treatment of this condition.

## 2. Structure of articular cartilage

Articular cartilage is a highly specialised tissue that provides a resilient, smooth, almost frictionless surface for joints to function efficiently and pain-free (Morris et al., 2002). In the adult, articular cartilage is avascular and predominately composed of extracellular matrix with a low density of resident cells, articular chondrocytes, which are responsible for the maintenance of the matrix in the healthy joint (Palmer & Bertone, 1994). Chondrocytes are embedded within a structurally organised matrix consisting of water, collagens, proteoglycans, glycosaminoglycans and non-collagenous proteins (Huber et al., 2000). The biochemical composition and structural alignment of these components within cartilage is responsible for the mechanical properties of this tissue and the cellular responses of the chondrocyte (Jeffery et al., 1991; Kuettner et al., 1991).

## 2.1 Articular chondrocytes and zonal organisation

Articular cartilage is organised to allow its main role to occur, that is, providing a smooth almost frictionless surface for pain-free mobility but also as a biomaterial that can also withstand compressive and shear forces. As well as the actual biochemical content of articular cartilage, the biomechanical properties rely on the structural organisation of the extracellular matrix and the cells embedded within them. The structure and organisation of articular cartilage not only varies with depth from the articular surface (divided into zones) but also the location within the joint (for example, weightbearing versus non-weightbearing surfaces).

Articular chondrocytes occupy 2-5% of the tissue volume and are sometimes considered relatively inactive metabolically due to an absence of a vascular supply but are responsible for maintaining the integrity of the extracellular matrix and can respond to mechanical stimuli, growth factors and cytokines. Articular cartilage has four distinct histological and biochemical zones (I-IV): superficial (tangential), intermediate (transitional), deep (radial) and calcified. The superficial zone is the thinnest along the articular surface and merges with the perichondrium at the articular margin. Type II collagen in the superficial zone is orientated tangentially to the articular surface to provide resistance to tensile forces. Proteoglycan composition in this zone acts as a non-selective barrier to diffusion of oxygen and water and a selective barrier to the diffusion of nutrients and hormones. This is largely due to the large amount of negatively charged anionic groups on the sulphated side chains on proteoglycans which allows smaller, non-ionic molecules through the matrix more readily than larger charged molecules. The pericellular matrix in the chondron (the chondrocyte and its pericellular microenvironment) consists of high levels of collagen type VI and aggregating proteoglycans and defines the physicochemical environment of the chondrocyte (Wang et al, 2008) and biochemical or mechanical signals perceived by the chondrocyte are therefore influenced by the structural and functional composition of the chondron (Guilak et al., 2006). Proteoglycans in the pericellular zone are thought to have a role in binding the chondrocyte to the matrix rather than the direct biomechanical role seen in the interterritorial matrix. Within the matrix surrounding the chondrocytes (territorial matrix) are thin type II and VI collagen fibrils, organised in a "basket-weave" formation and these fibrils extend out in a parallel arrangement to bind with larger type II collagen fibrils in the interterritorial matrix. In the intermediate zone the chondron structure is more typical. In this zone the collagen fibrils appear more widely spaced and their orientation is more random and there are increased amounts of proteoglycan compared to the superficial zone. In the deep zone the chondrocytes start to align themselves in columns perpendicularly to the joint surface along with thicker collagen fibrils. The collagen fibrils are orientated radially between these chondrocytic columns. The abundant proteoglycan within the interterritorial matrix with higher amounts of keratan sulphate side chains increases the permeability of the matrix in the deep zone and may be important in allowing diffusion of nutrients to the deeper layers of cartilage. Between the deep and calcified zones, there is a demarcation consisting of mineral associated with matrix vesicles within the interterritorial matrix and this is known as the tidemark.

## 2.2 The extracellular matrix

The extracellular matrix is a mechanically resilient structure comprising of collagens, proteoglycans and other non-collagenous proteins (Wilkins et al., 2000). Hydrated

proteoglycans confer resistance to compression and are constrained by the collagen fibrillar meshwork (thought of as a “string-and-balloon” model). Proteoglycans, with highly sulphated glycosaminoglycan (GAG) side chains and fixed negative charges, attract free cations and osmotically obliged water, leading to a hydrated matrix of raised osmolarity and lowered pH. Avascularity of matrix means that movement of hormones, cytokines, nutrients and metabolites occurs over relatively large distances along steep gradients. The low partial pressures of oxygen denote that cells undergo predominately anaerobic glycolysis and must endure high concentrations of lactic acid. Added to these challenges, normal mechanical loading causes profound fluctuations in the physiochemical environment.

### 2.2.1 Collagen

There are a number of collagen types recognised in articular cartilage, but type II collagen is the primary collagen of articular cartilage, comprising 80-90% of the total collagen content (Becerra et al., 2010). Type II collagen acts primarily to provide tensile stiffness in cartilage (Kaab et al., 1998). Other collagens are formed due to different gene expression, translational splicing and post-translational modification and many have important regulatory and structural roles and may be associated with type II collagen (e.g. functional binding) or other components of the matrix (for example binding and interactions with proteoglycans and the chondrocyte).

Collagen fibrils extend out from the pericellular envelope into the territorial matrix (Morris et al., 2002). Further collagen fibrils extend out into the interterritorial matrix, intimately involved with proteoglycans. Numerous contacts are present between the plasma membrane, collagens and proteoglycans through the extracellular matrix. Pericellular matrix contains little or no fibrillar collagen but type VI collagen microfibrils that interact with hyaluronic acid (HA), small proteoglycans and cell surface molecules. Type IX collagen is found throughout cartilage matrix and type XI collagen is mainly localised to the territorial matrix interacting with type II collagen, adding to tensile strength. Type IX collagen appears to localise with type II collagen fibrils in particular regions and covalent cross-linking may alter size and stability and hence mechanical properties of the type II collagen fibrils.

### 2.2.2 Proteoglycans

Proteoglycans and glycosaminoglycans contribute compressive stiffness to articular cartilage (Hardingham & Forsang, 1992). There are a number of different types of these macromolecules present throughout cartilage and they can also function as regulatory proteins and binding sites for other matrix components. Aggrecan, one of the most common proteoglycans in cartilage, is a high molecular weight proteoglycan ( $1-2 \times 10^6$  kDa) that binds HA in the matrix. Proteoglycans and proteoglycan link proteins are present throughout the extracellular matrix including the pericellular matrix and have structural relationships with collagens. Proteoglycans act as a selective permeability barrier and the structure of the matrix will dampen kinetic responses as diffusion through the matrix is slow. As well as contributing important mechanical properties to cartilage, proteoglycans are also important modulators of cell signalling and function.

### 2.2.3 Glycosaminoglycans

Glycosaminoglycans contain highly negatively charged polyanionic sulphate groups. It is this, as well as the large molecular weight of the proteoglycan aggrecan, that attracts cations,

such as  $\text{Na}^+$  and thereby water into the cartilage matrix and thus increasing tissue osmotic pressure (Wilkins et al., 2000). The resistance of the collagen fibrillar network to expansion therefore provides cartilage with an ability to resist compressive forces. The main glycosaminoglycans identified in articular cartilage are chondroitin-4-sulphate, chondroitin-6-sulphate, keratin sulphate and hyaluronic acid (Morris et al., 2002).

In a typical aggrecan molecule there can be up to 100 chondroitin sulphate side chains attached to the core protein (via xyulose-serine bond), each with up to 1000 repeating disaccharide units. Keratan sulphate is a smaller polysaccharide and there are usually around 50 keratin sulphate side chains linked to the aggrecan core protein (via a galactose-*N*-acetyl-threonine or -serine bond). Hyaluronic acid ( $1 \times 10^4$  kDa) is also classified as a glycosaminoglycan although it lacks the sulphated groups on its D-glucosamine and D-glucuronic acid disaccharide chains. Each HA can bind up to 100 aggrecan proteins. Early release of HA of the cell during synthesis may be important in articular cartilage structure since the length of HA influences proteoglycan binding and may affect proteoglycan aggregation and function (Palmer & Bertone 1994).

#### **2.2.4 Water and water flow in cartilage**

Water makes up approximately 70% of cartilage weight. Negatively charged proteoglycans attract cations and water follows leading to swelling of proteoglycans, resisting tension and shear forces. Since the macromolecular composition of extracellular matrix of cartilage determines matrix hydration and tissue volume it therefore determines the space for molecular transport and offers compressive resistance (as water is essentially incompressible). The hydrodynamic processes controlling the water content include osmosis, filtration, swelling and diffusion.

Osmotic flow of water occurs up gradients of osmotic pressure and cartilage can be thought of as a gel consisting of cross-linked non-ideal macromolecules (i.e. yield parameters which vary nonlinearly with concentration, a feature of a number of biological systems). It is thought that within the proteoglycan network, an ensemble of segments interacting with each other may form “pores” through which the flow resistance for water is lowered (Comper 1996).

### **3. Cellular physiology of articular chondrocytes**

The unusual biochemical structure of articular cartilage results in particular biomechanical properties that strongly influence the cellular physiology of the articular chondrocyte (Hall et al., 1996a). Due to the presence of fixed, highly negatively-charged polysulphated proteoglycans, there is an increase in cation ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{H}^+$ ) concentration in articular cartilage, compared to other tissues (e.g. plasma) leading to cartilage having raised osmolality ( $350\text{--}450\text{mOsm.kg}^{-1}$ ) compared to synovial fluid (around  $300\text{mOsm.kg}^{-1}$ ). Under load, the physical and ionic environment of cartilage alters. Dynamic load leads to increased hydrostatic pressure causing cartilage deformation/ membrane stretch and fluid flows (Urban, 1994). On removal of load the matrix regains its steady-state conformation. If loading continues, though, these dynamic components are followed by slower osmotic consequences. Under static loading conditions, fluid expression results in changes to the extracellular environment, raising fixed negative charge of glycosaminoglycans and hence increases osmotic pressure. These dynamic changes result in direct effects on articular

chondrocyte function since not only does the extracellular environment change, intracellular cation concentrations fluctuate with load and altered membrane transport activities occur due to mechanical deformation of membranes and changes in pressure, osmolarity and pH. Additionally, this environment is altered in joint disease such as osteoarthritis since biochemical and biomechanical changes occur which will directly influence the chondrocyte.

### 3.1 Membrane transport in articular chondrocytes

Chondrocytes possess many of the membrane transport systems found in other cell types (Wilkins et al., 2000). Active membrane transport systems exchange cations whose intracellular concentrations fluctuate with load not only to maintain cellular homeostasis but these mechanisms can be linked to solute transport and intracellular signalling events and mechanotransduction events, important in the articular chondrocyte to maintain cartilage integrity through extracellular matrix synthesis.

#### 3.1.1 Electrophysiology of articular chondrocytes

The resting membrane potential of articular chondrocytes is thought to be between -15mV and -44mV, maintained by  $\text{Na}^+/\text{K}^+$  ATPase and is influenced by cyclical pressure (Clarke et al., 2010; Funabashi et al., 2010; Hall et al., 1996a). Potassium channels are integral membrane proteins, participating in cell membrane potential and belong to a large superfamily including voltage-activated potassium channels ( $\text{K}_v$ ),  $\text{Ca}^{2+}$ -activated potassium channels ( $\text{K}_{\text{Ca}}$ ) and inward rectifier potassium channels ( $\text{K}_{\text{ir}}$ ). Using whole cell-patch clamp techniques, a voltage-dependent,  $\text{Ca}^{2+}$ -independent  $\text{K}^+$  current with rapid activation and very slow inactivation has been described in isolated canine articular chondrocytes (Wilson et al., 2004). ATP-sensitive  $\text{K}_{\text{ATP}}$  channels have also been demonstrated in articular cartilage (Mobasher et al., 2007).  $\text{K}_{\text{ATP}}$  channels may couple metabolic events (i.e. intracellular ATP levels) to membrane electrical activity and potentially their activity may be important in low oxygen conditions since hypoxia is known to lead to activation of  $\text{K}_{\text{ATP}}$  channels in other systems (Miki & Seino, 2005). Additionally, electrophysiological responses of chondrocytes from osteoarthritic cartilage appears to differ from healthy cartilage (Sanchez & Lopez-Zapata 2010).

#### 3.1.2 Volume regulation

The maintenance of cell volume in the face of alterations in the extracellular environment is an important cellular function (Hoffmann et al., 2009). Chondrocyte cell volume, as with other cell types, is determined by a pump-leak model where a double Donnan equilibrium exists between intracellular compartments and the matrix (Wilkins et al., 2000). Exclusion of  $\text{Na}^+$  ions from the cell is maintained by  $\text{Na}^+/\text{K}^+$  ATPase and cell volume is maintained by altered balance of leaks and pumps to hold cell water constant.

In articular chondrocytes, hypertonicity leads to regulatory volume increase (RVI) and raises intracellular potassium ( $[\text{K}^+]_i$ ) via  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  co-transporter (Hall et al., 1996b).  $\text{Na}^+/\text{H}^+$  exchange (NHE), unlike in other cell systems in the body, does not appear to play a role in volume regulation in cartilage due to the lack of  $\text{Cl}^-/\text{HCO}_3^-$  exchange activity which is required for RVI. During RVI,  $[\text{Na}^+]_i$  is increased and the  $\text{Na}^+/\text{K}^+$  pump is stimulated to keep  $[\text{K}^+]_i: [\text{Na}^+]_i$  ratio optimal for protein and enzyme function. Removal of static load in cartilage results in cell swelling and the activation of regulatory volume decrease (RVD) processes. Cell swelling following hypotonic challenge leads to RVD in many cells via  $\text{Cl}^-$

dependent  $K^+$  transporter,  $Ca^{2+}$ -activated  $K^+$  (with associated  $Cl^-$  ions) channel or an “osmolyte” channel (e.g. taurine, sorbitol and myo-inositol) (Hoffmann et al., 2009). In chondrocytes, loss of osmolytes appears to occur via “osmolyte” channel and volume activated  $K^+$  transport may also occur by this route (Hall & Bush 2001). Hypotonic challenge also leads to depolarisation via  $Na^+$  influx through stretch activated cation channels (SACC) (Sanchez et al., 2003).

In cartilage, cells lysis is prevented by the ECM (akin to plant cells and cell wall) and thus avoiding the effects extremes of hyposmolarity (although static loading in normal joints only leads to fluid losses and decrease in cartilage hydration of only around 5% per day and these losses are restored when load is removed). However, in osteoarthritis (OA), proteoglycans are lost and reduced  $Na^+$  and water content affects joint function. This increase in cartilage hydration under load is an early event in OA and could lead to changes in chondrocyte volume regulation (Bush & Hall 2005). Indeed the first changes in osteoarthritis are cell swelling suggesting the mechanisms for regulating cell volume are either lost or impaired.

### 3.1.3 Intracellular pH ( $pH_i$ ) regulation

The acidic extracellular environment ( $pH$  6.8) promotes inward leak of  $H^+$  ions so chondrocytes are subjected to chronic acid loading. With low  $O_2$  and anaerobic glycolysis as the primary source of metabolism resulting in lactate production, additional intracellular loading is also encountered. Articular chondrocytes have resting  $pH_i$  of around 7.1 and a relatively high intracellular buffering capacity of around  $30\text{mmol.l}^{-1}$  ( $pH_i$ ) (Wilkins & Hall 1992). Intracellular pH ( $pH_i$ ) regulation in chondrocytes predominately occurs through the amiloride-sensitive sodium-dependent  $Na^+/H^+$  exchanger (NHE). As discussed previously the extracellular matrix is rich in  $Na^+$  but poor in anions and therefore it appears that anion-dependent pH regulation been sacrificed in favour of  $SO_4^-$  uptake; an essential precursor for proteoglycan synthesis.

Extracellular acidity is an important regulator of cartilage matrix metabolism and activity of degradative enzymes. Changes in extra-and intracellular pH both elicit a bi-modal response of matrix synthesis (Wilkins & Hall, 1995). Small changes in extracellular pH ( $pH_o$ ) quickly and significantly (up to 50%) inhibit synthesis rates (particularly below  $pH$  6.9). It is possible, in normal cartilage, that matrix acidification could provide a means of regulating proteoglycan synthesis by a negative feedback system such that increased proteoglycan content raises  $H^+$ , thereby inhibiting synthesis.

There are a number of NHE isoforms characterised but the main “housekeeping” form is NHE-1 (Pedersen & Cala, 2004). Static loading leads to hyperosmolarity and hyperosmosis results in increased acid efflux in chondrocytes through the activation of NHE (Yamazaki et al., 2000). Enhanced  $H^+$  extrusion under conditions of loading may allow a defence versus cellular acidosis and a mechanism whereby effects of this loading can be transduced into changes in cartilage turnover. Hypotonic shock, however, leads to an increase in  $pH_i$  (alkalosis) via the opening of voltage-activated  $H^+$  channels (VAHC) (Sanchez & Wilkins, 2003).

Serum leads to increased acid extrusion on response to intracellular acidosis. NHE3 is expressed following exposure to serum and cytokines (Tattersall et al., 2003), particularly IGF-1 (Tattersall et al., 2008). In contrast to NHE1, NHE3 is inhibited by hypertonicity and by PKA pathways but activated by hypotonicity. Exposure to serum factors occurring in

osteoarthritic cartilage (damaged tissue more likely to be exposed to serum factors and IGF-1 is elevated in arthritic cartilage - van der Kraan & van den Berg, 2000) could therefore result in a differential response of NHE1 and 3 to hyperosmotic shock. Additionally this may have consequences for matrix synthesis which are dictated by pH. In addition to IGF-1, EGF has been shown to stimulate proton efflux by increasing activity of NHE involving PI3-kinase pathway (Lui et al., 2002).

Despite the chondrocyte already residing in an acidic extracellular matrix, further acidosis occurs in joint disease due to hypoxia and production of inflammatory cytokines altering blood flow. Since extracellular pH has a potent influence on cellular function (Das et al., 2010) any effect on the ability of the cell to regulate intracellular pH is likely to result in alteration in chondrocyte function, including matrix synthesis. Very low levels of oxygen, likely to be experienced in joint disease, reduce the activity of NHE resulting in intracellular acidosis in articular chondrocytes (Milner et al., 2006).

### 3.1.4 Intracellular calcium regulation

Intracellular calcium  $[Ca^{2+}]_i$  in chondrocytes, as in many other cells has numerous physiological functions (Berridge et al., 1998). In articular chondrocytes,  $[Ca^{2+}]_i$  is maintained at low levels (around 80nM) and  $Ca^{2+}$ -ATPase and  $Na^+$ - $Ca^{2+}$  exchanger appear to be the dominant mediators of calcium homeostasis in these cells (Sanchez et al., 2003). The maintenance of calcium is a balance between  $Ca^{2+}$  extrusion, influx via membrane channels and  $Ca^{2+}$  release from intracellular stores, such as endoplasmic reticulum and mitochondria (Duchen, 2004; Sanchez et al., 2006).

Alterations in intracellular calcium can affect matrix synthesis (Wilkins et al., 2000) and calcium signaling has been implicated in mechanotransduction in articular chondrocytes (Guilak et al., 1999). There are a number of studies showing that intracellular calcium in chondrocytes can be altered by hydrostatic pressure, osmotic stress and fluid flow (Kerrigan & Hall, 2008; Yellowley et al., 2002). Increased pressure and cell swelling induces a  $Gd^{3+}$ -sensitive  $[Ca^{2+}]_i$  increase (Wilkins et al., 2003) and it has been shown that intracellular  $Ca^{2+}$  levels can also be modulated by pH (Sanchez and Wilkins, 2003).

### 3.1.5 Metabolite transport

Transport of metabolites across the plasma membrane has an important role in maintaining chondrocytic biosynthetic output and matrix integrity. The uptake of sulphate ( $SO_4^{2-}$ ) is an important step in the synthesis of glycosaminoglycans and appears to occur in articular chondrocytes via a carrier-mediated mechanism that is  $Na^+$ -independent and sensitive to transmembrane  $H^+$  gradient (stimulated by acidic extracellular pH) (Meredith et al., 2007). Probable candidates include  $SO_4^{2-} \times Cl^-$  and  $SO_4^{2-} \times OH^-$  exchanger (anion exchanger). Amino acid uptake occurs via  $Na^+$ -dependent (proline, glycine and glutamine) and independent (leucine) transporters (Wilkins et al., 2000).

Inorganic phosphate ( $P_i$ ) uptake in chondrocytes appears to have both  $Na^+$ -dependent and -independent components and shows pH- sensitivity (Solomon et al., 2007). Transport of  $P_i$  across the cell membrane is an important component of the calcification process, particularly in the growth plate and the inappropriate formation of calcium-phosphate (hydroxyapatite) crystals in osteoarthritis could involve dysfunction of  $P_i$ -transporters.

Glucose provides energy source and is an essential precursor for glycosaminoglycan synthesis. GLUT transporters (e.g. IGF-1 modulated GLUT4) are mainly responsible for

glucose uptake (Shikhman et al., 2004; Windhaber et al., 2003) whereas lactic acid transport appears via monocarboxylate family of transporters (MCT) including MCT1 (“housekeeper”) and MCT4 (Meredith et al., 2002). MCT4 appears to be the main isoform in articular chondrocytes whose kinetics favour lactate export thereby allowing pyruvate conversion back to lactate to assist in NAD<sup>+</sup> regeneration and continued glycolysis.

#### **4. Mechanotransduction in articular cartilage**

The main functions of articular cartilage are concerned with load-bearing (Urban, 1994). During normal activity, pressures within cartilage may rise to 100-200 atmospheres (10-20 MPa) within milliseconds. The mechanical failure of extracellular matrix is a key event in the progression of degenerative joint disease since not only direct loss of function of the tissue occurs but detrimental effects on cellular activity and the potential repair process ensues. The ability of the chondrocyte to sense and respond appropriately to mechanical signals (mechanotransduction) is vital in maintaining cartilage integrity.

##### **4.1 Mechano-electrochemical properties of cartilage and signal transduction**

Physical environmental factors such as shear stress, fluid flow and electrical field alterations are known to be strong biologic factors in regulating cellular activities (Lai et al., 2002; Mow et al., 1999). During loading, a number of changes occur within cartilage, including increased hydrostatic pressure, cartilage/chondrocyte cell deformation, fluid flow and streaming potentials, changes in chondrocyte cell membrane and fluid loss resulting in changes to interstitial fluid osmolality/ionic content (Urban, 2000). Transducers of mechanotransduction in cells include activation of stretch activated channels allowing ingress of external Ca<sup>2+</sup>, alteration of membrane transporter activity (eg Na<sup>+</sup>/H<sup>+</sup> exchange) and activation of mechanosensitive ion channels and transporters such as transient receptor potential (TRP) channels and purinergic receptors. The ECM is directly linked to the cytoskeleton and nucleus of the cell via integrins. Integrins are central to many mechanotransduction pathways since they integrate a number of important intracellular signalling pathways, for example, focal adhesion kinase signaling via integrin-ECM (involving G-protein signaling) and other pathways (involving, for example MAPK and PI-3 kinases) (Loeser, 2002).

##### **4.1.1 Streaming potential and diffusion potential**

Streaming potentials and diffusion potentials can be used to describe the electrical forces generated during ionic species movement and these are thought to be important in mechanical signal transduction in cartilage. The potential induced by convection current (mechano-chemical force generated by cation and anion movement) in the presence of a pressure gradient gives the streaming potential of cartilage, whereas the potential induced by the diffusion in the presence concentration gradient is the diffusion potential and have been shown to be important modulators of chondrocyte metabolism (Kim et al., 1995).

##### **4.1.2 Effects of mechanical load on chondrocyte function and matrix synthesis**

Mechanical load is required to maintain cartilage integrity (Hasler et al., 1999). Matrix proteoglycan is lost from cartilage in immobilised joints and there is variation within a normal joint between unloaded and loaded regions. Regions subjected to load are often

thicker and have higher proteoglycan content and therefore likely to be mechanically stronger. Dynamic or cyclic loading stimulates proteoglycan and protein synthesis whereas static loading is associated with decreased synthesis and in addition, load-induced solute movement can also influence rates at which growth factors or cytokines reach the cells and alter cellular metabolism.

When load is applied there is an increase in hydrostatic pressure, extracellular pH decreases and there is an increase in extracellular free cation concentration and osmolality. Alterations in the osmotic balance occurs as fluid is expressed to try to restore the hydrostatic equilibrium and this increases the concentrations of proteoglycans and hence cations, resulting in osmotic consequences. The changes in hydrostatic pressure and osmotic alteration lead to cellular deformation and change in volume resulting in changes in  $[Na^+]_i$ ,  $[K^+]_i$ , pH<sub>i</sub> and  $[Ca^{2+}]_i$  due to altered transporter activity and therefore this can result in alterations in macromolecular synthesis.

During loading, cartilage from osteoarthritic joints will deform more than cartilage from non-diseased joints, since both the rate and amount of fluid loss are sensitive to proteoglycan concentrations. Therefore cartilage from degenerate joints will lose fluid faster than healthy cartilage and this is likely to alter the stimulus and hence the response of the chondrocyte in diseased tissue.

#### 4.1.3 Hydrostatic pressure

During normal walking, articular cartilage cycles between a resting hydrostatic pressure of 0.2MPa and pressures of 4-5 MPa (2-50atm). It is known that pressures in the 5-50MPa range can alter cellular morphology, reduce exocytosis, dissociate cytoskeletal elements, reduce protein synthesis and inhibit membrane transport. The timing of the cycles is also important - application of cyclical pressures ( $>0.5\text{Hz}$ ) have stimulatory effects on cartilage matrix synthesis. It also appears that isolated chondrocytes are more sensitive to pressure than *in situ* within the matrix and that the cytoskeleton and Golgi apparatus are involved in this response.

Physiological levels of hydrostatic pressure can affect membrane permeability to ions and amino acids and thus affect intracellular solute concentrations. Increase in hydrostatic pressure leads to increased rate of synthesis of matrix components and this may be exerted via alteration in intracellular pH. Browning et al., (1999) showed that application of 20-300atm to isolated cells led to NHE stimulation via phosphorylation-dependent processes. Additionally, hydrostatic pressure has been shown to inhibit membrane transport pathways (such as  $Na^+/K^+$ -pump,  $Na^+/K^+/2Cl^-$  cotransporter) (Hall et al., 1999). Conformational alterations by cell deformation may be responsible for change in membrane transport activity as well as changes in their phosphorylation status. For example, pressure may uncouple ATP hydrolysis or alter lipid environment as to retard  $Na^+$  binding or constrain conformational changes leading to altered activity of the  $Na^+/K^+$  pump. Alteration in ion channel activity is therefore likely to be intimately linked to matrix synthesis.

#### 4.1.4 Osmotic sensitivity of chondrocytes

Static loading leads to fluid expression and increased interstitial fluid osmolality. The link between ECM hydration and chondrocyte metabolism appears to be via volume regulation. Cells respond to unequal tonicity by water movement across plasma membrane and this is usually rapid leading to cell volume changes within seconds. The osmometric behaviour of

chondrocytes in situ and isolated from matrix appears to be similar although some differences in layers occur in situ (Bush & Hall, 2001). Superficial zone chondrocytes appear to swell more than middle or deeper zone cells and this may reflect less proteoglycan present in this zone. Additionally deeper zone chondrocytes may take longer to respond to water changes in cartilage so the response may depend on zone and local osmotic environment. Potentially, zone-specific alterations in physico-chemical signals may lead to differences in chondrocyte matrix biosynthesis.

Water can flux through membranes via aquaporins. Aquaporins (AQP) are water channel proteins that allow water to move in the direction of osmotic gradient and may also allow small solutes to pass, for example glycerol and urea. A role in cell volume regulation and mechanotransduction in chondrocytes has been proposed (Mobasheri et al., 2004). AQP1 and AQP3 are expressed in cartilage resulting in water permeability and may respond to environment changes since changes in aquaporin expression may be important in pathology.

Hyperosmotic stress induces a transient alteration in cellular volume and  $[Ca^{2+}]_i$  (Erickson et al., 2001) but a latency appears to exist between minimum cell volume reached and peak  $Ca^{2+}$  levels. This may be explained by  $Na^+$  entering the cell (possibly via voltage-gated sodium channels, VGSC, or epithelial sodium channels, ENaC), leading to depolarisation and subsequent increase in intracellular calcium levels. This then results in membrane hyperpolarisation and  $Ca^{2+}$  activated  $K^+$  channels open causing  $K^+$  efflux. Hypotonic shock also results in increased intracellular  $Ca^{2+}$  levels (Wilkins et al., 2003). Mechanosensitive  $Ca^{2+}$  channels appear to open in response to hypotonicity as well as calcium release from intracellular stores. Prolonged increase of intracellular calcium, however, is detrimental to the cell so mechanisms such as  $Na^+/Ca^{2+}$  exchanger are in operation to regulate this  $Ca^{2+}$  rise.

These stretch-activated ion channels may act as putative mechanical signal transducers since they lead to fluctuations in intracellular calcium levels that may affect gene expression. Potential mechanosensitive ion channels in chondrocytes could include VGSC, ENaC and N/L-type voltage gated calcium channels (VGCC). In epithelial cells, ENaC is linked to the actin cytoskeleton and integrin. In osteoarthritic cartilage, ENaC is absent and the lack of ENaC means that chondrocytes may have lost the ability to transduce mechanical signals effectively.

#### 4.1.5 Integrins

Integrins play a key role in the interactions between the cell and the extracellular matrix including cell anchorage, growth, differentiation, migration and matrix synthesis and degradation (Loeser, 1993). Integrins are cell surface receptors that recognise and bind to an Arg-Gly-Asp sequence on ECM proteins and are heterodimeric (one  $\alpha$  and one  $\beta$  subunit) transmembrane glycoproteins (Loeser, 2000). The importance of integrins, as well as being cell adhesion molecules, is that they may function as transmitters of information and be able to mediate intracellular responses to extracellular stimuli. The pericellular matrix and chondrocytes in the chondron contain collagen types II, VI and IV, aggrecan and fibronectin and integrins are known to interact with these proteins found in pericellular matrix. Immunoprecipitation and immunofluorescence experiments show co-localisation and association of integrin with ENaC and VGCC and therefore integrins may functionally activate ion transporters following deformation of the pericellular matrix.

Extracellular protein binding to the cell leads to receptor clustering and activates integrin. Integrins however, have no inherent kinase activity but will often complex with Shc, Crk, paxillin, vinculin, caveolin and/or FAK. Many of these proteins in the complex are activated by tyrosine phosphorylation which then leads to activation of other kinases such as Src, RhoA, Rac1, Ras, Raf1, Sos, Grb2, MEK kinase and member of the MAP kinase family (including ERK1/2, JNK and p38). This then leads to downstream signalling that regulate gene expression, for example MAP kinase, that lead to activation of transcription factors such as AP-1 and NF- $\kappa$ B.

The regulation of chondrocyte integrin function is important in the homeostasis of cartilage as well as in disease states in which interactions between chondrocytes and their ECM are altered. Factors that modulate chondrocyte ECM synthesis, such as IGF-1 and TGF- $\beta$ , also appear to modulate integrin-mediated attachment of chondrocytes to ECM proteins (Loeser 1994, 1997). The effects of IGF-1 and TGF- $\beta$  on chondrocyte integrin expression and function, however, *in vivo* may depend on the relative levels of each growth factor present and thereby providing a means for sophisticated control of cell-matrix interactions in cartilage. Growth factor receptor phosphorylation leads to increased integrin aggregation, possibly via MAP kinase activation. There appears to be co-localisation of IGF-1 receptor and  $\beta_1$  integrin subunit in chondrocytes. Cross-talk exists between integrins and growth factors/cytokines and as well as integrin activity being affected by growth factors, growth factor activity itself is dependent on integrin binding. Therefore a two-way signalling process occurs with integrin occupying a central role in this system. Increased expression of IGF-1 has been noted in osteoarthritic cartilage and could act in an autocrine manner to increased  $\alpha_1\beta_1$  possibly as part of a repair response mediating signals important for cell survival/proliferation.

Integrins have a central role in cell survival and inhibition of integrin function results in apoptosis (Loeser, 2002; Mobasheri et al., 2002). The Ras-MAPK pathway is important to chondrocyte survival and integrins are linked to Ras-MAPK pathway by downstream signaling factors including the docking protein Shc. Interruption of the Ras-MAPK pathway produces apoptosis (via increased expression of pro-apoptotic proteins or repression of anti-apoptotic proteins). Therefore disruption of the interactions between chondrocytes and the ECM (via integrins) may induce apoptotic cell death and may contribute to pathogenesis of osteoarthritis.

#### 4.1.6 Purinergic signaling

The potential role of purinergic signalling in mechanotransduction in cartilage was postulated following the finding that compressive loading of bovine chondrocytes in chondrons or in agarose pellets leads to ATP release (Chowdhury & Knight, 2006). ATP is an important mediator involved in autocrine/paracrine signalling and it can be released following cell damage and as well as being directly involved in signalling via release.

Chondrocytes have been shown to express P2Y2 receptors (Millward-Sadler et al., 2004) and normal chondrocytes release ATP after mechanical stimulation involving calcium signaling. Recently, Varani et al., (2008) characterised the expression of P2X<sub>1</sub> and P2X<sub>3</sub> receptors in bovine chondrocytes. Unlike P2Y receptors that are G-protein coupled, P2X receptors are membrane ligand-gated ion channels that open in response to binding of extracellular ATP. Stimulation of purinergic pathways (via P2X receptors) may be important in the response to joint inflammation since ATP further stimulates NO and PGE<sub>2</sub> production in chondrocytes following IL-1 $\beta$  stimulation.

The link between P2 receptors and cell signalling may involve connexin hemichannel expression (Knight et al., 2009). Connexins are membrane proteins that form hemichannels and hemichannels are one of the potential ways of releasing ATP (as well as through anion channels and via exocytosis of ATP-filled vesicles). Cyclic loading leads to hemichannel opening and ATP release in chondrocyte constructs (Garcia & Knight, 2010). In human cartilage, connexin 43 has been found in cells in the superficial region. The presence of these potential mechanosensitive cells primarily in the superficial/middle zones may indicate different mechanotransduction pathways than deeper zone cells. Since hypoxia is known to regulate connexin 43 dephosphorylation, translocation and proteosomal degradation in other cells the response to mechanical stimulation may be related to the oxygen environment of cartilage.

The primary cilium, a membrane-coated axoneme that projects from the cell surface into the extracellular microenvironment could also be involved in chondrocyte mechanotransduction. The function of primary cilium in chondrocytes has not established but in the study by Knight et al., (2009) approximately 50% of primary cilia had co-expression of connexin 43. It is postulated that deflection of the cilium may activate ATP release via hemichannels and once released, ATP may activate P2 receptors, triggering intracellular  $\text{Ca}^{2+}$  signalling cascades and mediate effects on proteoglycan and collagen synthesis and MMP expression and NO release.

In osteoarthritic chondrocytes, a reduction in purinergic signalling following mechanical stimulation has been reported. This could be due to desensitisation by ATP released into synovial fluid (increased ATP levels in synovial fluid are reported in OA patients) or by receptor down regulation (since reduction in receptor numbers has been described at the cell surface in OA chondrocytes). The changes in ATP-mediated signalling in OA cartilage is of importance since ATP is normally chondroprotective against proteoglycan loss.

#### **4.1.7 Transient receptor potential (TRP) channels**

Transient receptor potential (TRP) channels comprise a superfamily of more than 50 different ion channels with a preference of  $\text{Ca}^{2+}$ , playing a role in the transduction of several physical stimuli such as temperature, osmotic and mechanical stimuli. TRP channel opening induces membrane depolarisation while increasing cytosolic  $\text{Ca}^{2+}$  and/or  $\text{Na}^{+}$  concentrations. Most, but not all TRPC members act as store-operated  $\text{Ca}^{2+}$  channels whereas TRPV channels may be involved in a nonselective conductance of cations with a preference for  $\text{Ca}^{2+}$ . Since calcium entry through plasma membrane channels is recognised as a cellular signalling event per se, TRP channels provide an ideal candidate to link between mechanical stimuli and cellular response.

In human osteoarthritic chondrocytes, the majority of the investigated TRP genes are expressed (Gavenis et al., 2009) and a correlation appears between the degree of differentiation of chondrocytes and the expression of various members of the TRP family. Their role in cartilage health and disease is, as yet, unknown (Mobasheri & Barrett-Jolley, 2011).

### **5. Oxygen, mitochondria and reactive oxygen species in articular cartilage**

Adult articular cartilage is avascular and hence long diffusion pathways exist for nutrients solutes and oxygen to cross. Synovial fluid has low oxygen tension (6-10%) and articular

chondrocytes experience relatively low levels of oxygen, compared to other cell-types, with chondrocytes operating at oxygen tensions ranging from 6-10% at the articular surface to around 2% in the deep zones (Zhou et al., 2004). Despite this, articular chondrocytes not only survive but regulate extracellular matrix synthesis. Although energy production appears to be primarily via a glycolysis in this low oxygen environment, it is being recognised that mitochondria might play an important role in the health and disease of the joint through their involvement in reactive oxygen species generation, calcium regulation and the intimate role in cell death/survival pathways in cartilage.

### 5.1 Cartilage oxygen tension and cell metabolism

The oxygen tension gradient in cartilage is determined by cell density and distribution, cartilage thickness, oxygen tension in synovial fluid, oxygen supply from subchondral surface and oxygen consumption rate per cell (Zhou et al., 2004). Articular chondrocytes have a characteristic morphology and metabolism depending on their position in cartilage and part of this may be due to the oxygen gradients that exist. Although the majority of diffusion of oxygen appears to come from the articular surface facing the synovial fluid, there is thought to be a component of diffusion from vessels in the subchondral bone plate and therefore extreme levels of hypoxia (i.e. 1% or less) may not exist *in situ* in cartilage (but could do in disease where subchondral bone plate thickening is a feature of osteoarthritis).

Despite these low oxygen conditions, articular chondrocytes do survive and are able to maintain their cartilage phenotype (Grimshaw & Mason, 2000; Pfander & Gelse, 2007). To survive low oxygen conditions cells possess highly conserved adaptive mechanisms. The most important component is mediated by transcriptional activation involving binding of the transcription factor hypoxia-inducible factor-1 (HIF-1). In cartilage, during physiological hypoxia, HIF-1 $\alpha$  is expressed (Lin et al., 2004) and it appears to act as a survival factor since necrotic cartilage occurs in HIF-1 knock-out mice (Gelse et al., 2008).

Although articular chondrocytes reside in low oxygen levels, they are not unresponsive to hypoxia since changes in oxygen tension can have significant effects on matrix synthesis and cell growth. Indeed, matrix synthesis by articular chondrocytes may be optimal at lower tissue oxygen tensions, for example at 5% O<sub>2</sub>. Sox9, type II collagen and aggrecan expression is higher than at 21% O<sub>2</sub> (Marty-Hartert et al., 2005). Oxygen diffusion and movement through cartilage may occur at differential rates in response to biochemical and loading differences in different regions and this could lead to local oxygen gradients within pockets of cartilage which could influence cell metabolism as well as differential gene expression.

#### 5.1.1 Articular cartilage metabolism

Within the hypoxic environment of cartilage, articular chondrocytes predominately undergo glycolytic metabolism. Lactate is the major end-product of this process and this adds to the already acidic load experienced by these cells. ATP is generated by substrate level phosphorylation, whereas, apart from superficial layers where relatively higher oxygen levels can exist, oxidative phosphorylation appears to be a lesser component of ATP production.

Reduction of oxygen levels in other cells results in an increase in glucose usage and lactate production, thereby increasing ATP production - this is commonly known as the Pasteur effect. Articular chondrocytes, however, appear to display a negative Pasteur effect where

reductions in oxygen levels result in suppression of carbohydrate breakdown (Lee & Urban, 1997). This effect appears to be peculiar to articular cartilage since in fibrocartilaginous intervertebral disc, glucose uptake and lactate production increases under lowered oxygen levels.

### 5.1.2 Changes in oxygen tension in joint disease

Despite increased blood vessel formation in the synovial membrane and neoangiogenesis from the underlying bone into the deep zone of osteoarthritic cartilage, the hypoxic environment of cartilage appears more pronounced in osteoarthritis. Synovial fluid from osteoarthritic joints contains less oxygen than synovial fluids from healthy joints (Pflander & Gelse, 2007). Reductions in oxygen tension in joint disease could be due to increased oxygen usage by the synovial membrane, alterations in blood flow and gas exchange by fibrosis in the joint capsule and subchondral bone sclerosis (Svalastoga & Kiaet, 1989). Additionally, alterations in diffusion gradients caused by changes in matrix structure, altered biomechanical forces through the cartilage and alterations in oxygen consumption in inflammation (e.g. reactive oxygen species generation) contribute to the reduction in cartilage oxygen levels.

### 5.1.3 Hypoxia and HIF-1 in osteoarthritis

Chronic hypoxia in the osteoarthritic joint is associated with increased levels of HIF-1 in both synoviocytes and chondrocytes and related HIF-1 targeted genes, such as VEGF and iNOS. Additionally, HIF-1 $\alpha$  accumulation can also be increased by other factors such as pro-inflammatory cytokines and changes in mechanical loading, as well as hypoxia (Pflander & Gelse, 2007). HIF-1 $\alpha$  is important for anaerobic energy production and matrix synthesis by chondrocytes and appears to have a pivotal role for maintaining chondrocytic phenotype.

As well as the increased synthesis of matrix destructive enzymes, osteoarthritic chondrocytes show enhanced gene expression of type II collagen. This latter feature may be related to oxygen levels since increased accumulation of type II collagen induced by 1% oxygen is accompanied by stabilisation, nuclear translocation and increased activity of HIF-1 $\alpha$ . The increase in posttranslational modification of type II collagen may contribute to the increased synthesis of collagen type II seen during osteoarthritis as an effort to restore extracellular matrix.

## 5.2 The role of mitochondria in articular chondrocytes

Mitochondria are extremely important cellular organelles traditionally seen as the source of cellular energy production (Duchen, 2004). Articular chondrocytes contain fewer mitochondria compared to other, more metabolically active cell types, and this difference may reflect the cellular environment (i.e. hypoxia) and reliance on glycolytic metabolism rather than oxidative phosphorylation for energy production. Despite this, mitochondrial physiology and function in the chondrocyte is still critical to cellular function and they are involved in many important aspects of cell physiology in both health and disease such as ROS generation, Ca<sup>2+</sup> homeostasis and cell death and survival pathways. Indeed, mitochondrial dysfunction is a key component of a number of diseases, such as diabetes and cancer, and the role of the mitochondrion in osteoarthritis is now beginning to be more fully appreciated (Terkeltaub et al., 2002).

### 5.2.1 Mitochondria and the chemiosmotic principle of energy production

Mitochondria contain two membrane systems, an outer and inner mitochondrial membrane (Duchen, 2004). The inner mitochondrial membrane is folded into cristae and it is here that the membrane bound enzymes (a series of complexes) of the respiratory chain are located. The chemiosmotic principle of energy production involves the oxidation of cellular substrates to produce ATP. The reductants NADH and FADH<sub>2</sub>, generated from the tricarboxylic acid (TCA) cycle, enter the mitochondrial electron transport chain. NADH is oxidised to NAD<sup>+</sup> at complex I and FADH<sub>2</sub> is oxidised to FAD<sup>2+</sup> at complex II to provide electrons for ubiquinone at complex III. The electron chain complexes catalyse a series of redox reactions creating an electrochemical drive to transfer H<sup>+</sup> from the mitochondrial matrix into the intermembrane space across the inner mitochondrial membrane. This results in a large mitochondrial transmembrane potential of around -150 to -200mV and it is this membrane potential that provides the “protonmotive force” to cause H<sup>+</sup> influx through F<sub>1</sub>-F<sub>0</sub> ATP synthase and drive the ATPase “backwards” thus phosphorylating ADP to release ATP. The respiratory rate is regulated by this proton gradient which in turn is dependent on substrate availability, inhibitors of respiration (for example anoxia) and any mechanism that results in the uncoupling of the enzyme complexes.

In articular chondrocytes, both mitochondrial density and activity appears to be lower than other cell types with mitochondrial density significantly reduced in the deep zones compared with the upper zones of articular cartilage, likely to reflect oxygen levels in these zones. There is also evidence that the cytochrome component of the electron transport chain in articular chondrocytes may be incomplete *in situ* and provides further evidence that ATP derived from mitochondrial oxidative phosphorylation is not a major component of energy production in cartilage. Interestingly though, following transfer of cells to a relatively “oxygen-rich” environment (for example during culturing of cartilage explants or isolated cells in ambient conditions), mitochondrial biogenesis occurs (Mignotte et al., 1991). This change within the chondrocyte appears to result in a switch to oxidative phosphorylation. It has to be noted, therefore, that these conditions may not represent *in vivo* conditions of the chondrocyte and interpretation of data on cartilage metabolism requires an appreciation of these potential changes.

### 5.2.2 Mitochondria and reactive oxygen/nitrogen species

The process of electron transfer along the electron transport chain in mitochondria is not completely efficient and electrons may be “lost” during the redox reactions, resulting in the transfer of electrons to oxygen and the generation of oxygen radicals (reactive oxygen species, ROS). These highly reactive species can result in cellular damage due to lipid peroxidation and DNA damage so efficient mechanisms in the mitochondrion (for example superoxide dismutase) and cytoplasm (for example catalase) exist to reduce the risk of this occurring. In mitochondria of articular chondrocytes, it seems that the main site of ROS generation is complex III (Milner et al., 2007).

As well as being a potential source of cellular damage if left unchecked, reactive oxygen species are now thought to be important mediators of cell signalling. A large number of intracellular signalling pathways are regulated by ROS including cytokine receptors, receptor tyrosine kinases, receptor serine/threonine kinases and p38 MAPK cascades. This can be through the redox status of component proteins. Oxidation and reduction of -SH groups on amino acids can result in conformational change and alteration in enzyme

activity. ROS may also directly regulate activity of transcription factors through oxidative modifications of conserved cysteines. Redox-sensitive transcription factors include NF- $\kappa$ B, AP-1, sp-1, c-myb, p53, egr-1, HIF-1 $\alpha$  and c-fos (Lo & Cruz, 1995). DNA-binding by AP-1 is also regulated by post-translational modifications which are redox-sensitive and this is also seen with GTP-binding protein Ras.

Nitric oxide appears to have an important role in mitochondrial function (Duchen, 2004). Complex IV has a high affinity for NO and at low O<sub>2</sub> competes with oxygen to inhibit mitochondrial respiration and this may be of relevance in a low oxygen system. Mitochondria may also generate NO themselves and a specific NOS has been shown to be expressed by the mitochondrion. It appears that an intricate feedback mechanism involving NO, calcium, mitochondrial electron chain activity and ROS levels may exist in the mitochondrion that may be of particular importance in low-oxygen environments such as cartilage.

Cellular antioxidant mechanisms exist though, and it is seen as a balance between ROS production and removal that determine the difference between physiological and pathological ROS levels within the cell. As with ROS, the balance between physiological and pathological NO levels are also likely to be an important factor since high NO levels react with ROS resulting in peroxynitrite production and damage to the electron chain - a feature present in disease such as osteoarthritis.

### 5.2.3 Mitochondria and calcium uptake

Mitochondrial calcium handling is an important component of cellular calcium homeostasis since calcium “overload” is thought to be implicated in a number of pathological states including osteoarthritis. Mitochondrial calcium uptake is driven primarily by the electrochemical gradient established by the mitochondrial potential and the relatively low Ca<sup>2+</sup> concentration (Duchen, 2004). When cytosolic calcium increases, calcium moves into the mitochondrial matrix. Intramitochondrial calcium concentration is kept low under “resting” conditions by the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger that results in calcium efflux. Ca<sup>2+</sup> appears to be taken up into the matrix through the IMM by a uniporter. Additionally, voltage-dependent anion channels (VDAC) permeant to calcium exist in the outer mitochondrial membrane and may affect inner mitochondrial membrane calcium uptake by acting as a fast filter. The VDAC also appears to form part of the mitochondrial membrane permeability pore in initiating apoptosis. Calcium microdomains can exist within cells and the proximity of mitochondria to endoplasmic reticulum calcium release sites may result in mitochondria experiencing relatively high local concentrations, promoting rapid calcium uptake to allow direct transfer of calcium between mitochondria and ER (Contreras et al., 2010). In addition, the proximity to the plasma membrane by mitochondria also could allow regulation of calcium influx and therefore mitochondrial positioning could be important regulators of signalling pathways involving calcium.

### 5.2.4 Mitochondria and cell death

Mitochondria are intimately involved in cell death pathways. In many cells a reduction in mitochondrially derived ATP leads to loss of maintenance of ion gradients and regulation of calcium and intracellular osmolarity causing cell swelling and death. Cell swelling is an early feature of osteoarthritis and mitochondrial dysfunction is likely to be a significant component of cell death in cartilage disease.

The opening of a large conductance pore (mPTP) occurs through a conformational change of several proteins of the mitochondrial membrane due to a number of conditions such as high  $[Ca^{2+}]_m$ , oxidative stress, ATP depletion, high inorganic phosphate ( $P_i$ ) and mitochondrial depolarisation (Duchen 2004). This irreversible high conductance opening causes mitochondrial swelling, cytochrome c release, caspase activation and apoptotic cell death. Apoptotic cell death may be a normal feature of cartilage growth and development, particularly in the hypertrophic zone of the growth plate but factors resulting in abnormal activation are important causes of cellular death and subsequent loss of cartilage integrity in joint disease.

### 5.2.5 Mitochondria and osteoarthritis

Mitochondria are implicated in the pathogenesis of many diseases, including osteoarthritis and mitochondrial mediated diseases are often due to hypoxic cell stress or aging – relevant factors in joint disease. In diabetes mellitus, defects in the electron chain are described (especially Complexes I and IV) and in neuronal injury (ischaemia/reperfusion injury), mitochondrial injury leads to impaired intracellular  $Ca^{2+}$  buffering, increased ROS generation and promotion of apoptosis via release of cytochrome c. Additionally, ETC complex defects are present in Alzheimer's, Parkinson's and Huntington's disease and peroxynitrite-mediated nitration of tyrosines in Alzheimer's disease neurons are due to increased NO.

In osteoarthritis, mitochondrial content increases in number and size and mitochondrial swelling has been noted (Terkeltaub et al., 2002). Mitochondrial numbers increase at sites of crystal formation and matrix calcification is a feature of osteoarthritis. It appears that calcification is stimulated by NO/peroxynitrite and chondrocyte apoptosis and this in turn is modulated by ATP metabolism. Mitochondrial energy reserve is required for matrix synthesis and crystal suppression and therefore altered mitochondrial energy metabolism may lead to crystal formation.

Mitochondrial dysfunction of the electron chain (particularly complexes II and III) has been described in osteoarthritic chondrocytes and this will alter the respiratory state and mitochondrial membrane potential of the mitochondrion (Maniero et al., 2003). A collapse of the mitochondrial membrane potential results in mitochondrial swelling, disruption of the outer mitochondrial membrane and release of pro-apoptotic factors such as cytochrome c, AIF and procaspases from the intermembrane space and hence cell death.

### 5.3 Oxidative stress and reactive oxygen/nitrogen species in joint disease

When ROS levels exceed the cellular defence mechanisms, cellular damage can occur. This is known as oxidative stress. Increased oxygen consumption by the synovium during inflammation and the exposure to inflammatory mediators can lead to increase in ROS and RNS generation to levels that can induce cellular damage. In the inflamed joint synoviocytes appear to be the key cell driving this response, as opposed to chondrocytes, although it is the effect on the chondrocyte that will lead to compromise in cartilage integrity and hence disease (Schneider et al., 2005). In synoviocytes there are a number of sources of ROS generation, as well as mitochondrial derived ROS including xanthine oxidoreductase and membrane-bound NADPH oxidase (Henroitin et al., 2003).

Oxidative stress results in protein, lipid membrane, DNA damage and therefore cell injury and death (Finkl 2003). Lipid peroxyl radical formation can result in lipid bond cross-

linking and alteration in membrane properties as well as forming products, such as aldehydes and saturated hydrocarbons that are toxic to cells. Fragmentation of hyaluronic acid is reported following oxidative damage to the glycosidic bonds. Since hyaluronic acid is a key cartilage biomolecule both in structure and cell signalling, alterations to HA structure will lead to alterations in cytoskeletal polymerisation, for example and affect cell adhesive properties. Oxidative damage to other extracellular components and ROS-induced activation of matrix metalloproteinases can add to the degradative element of these molecules and additionally, the action of IL-1 on proteoglycan loss appears to be mediated by ROS and NO (Henroitan et al., 2003). The direct degradation of proteoglycans and collagen by ROS is due to upregulation of collagenases and other MMPs as well as decreased production of TIMPs. Additionally, NO is implicated in cartilage insensitivity to IGF-1 by inhibiting IGF-1 receptor autophosphorylation. Therefore the use of antioxidant therapy has justifiable support in joint disease.

## 6. Conclusion

Our knowledge of the cellular processes occurring in articular chondrocytes has grown immensely over recent years but it is the appreciation of the interaction and response of these cells to their unusual and challenging environment and how these change in diseases such as osteoarthritis that will open up new exciting opportunities for potential therapeutic modulation in joint disease. How the chondrocyte senses and adapts to the dynamic nature of the extracellular matrix in health and disease makes us realise the complexity of signals involved and the multiplicity of the component parts, such as, for example, the roles of cell volume regulation, intracellular pH homeostasis and mitochondrial function on cell function in cartilage. The challenge for the future then will be to tie all these elements together and be able to paint the big picture that reveals the many complex interactions occurring within the joint.

## 7. List of abbreviations

AQP	<i>Aquaporin</i>
ATP	<i>Adenosine triphosphate</i>
ECM	<i>Extracellular matrix</i>
ENaC	<i>Epithelial sodium channel</i>
ERK1/2	<i>Extracellular signal-regulated kinase 1/2</i>
ETC	<i>Electron chain transport</i>
FADH <sub>2</sub>	<i>Flavin adenine dinucleotide H<sub>2</sub></i>
GAG	<i>Glycosaminoglycan</i>
GLUT	<i>Glucose transporter</i>
HA	<i>Hyaluronic acid</i>
HIF-1	<i>Hypoxia-inducible factor-1</i>
Hz	<i>Hertz</i>
kDa	<i>kiloDaltons</i>
IGF-1	<i>Insulin-like growth factor-1</i>
IL-1	<i>Interleukin-1</i>
IMM	<i>Inner mitochondrial membrane</i>

MAPK	Mitogen-activated protein kinase
MCT	Monocarboxylate transporter
MEK	Mitogen-activated protein kinase kinase
MMP	Matrix metalloproteinase
MPa	Megapascals
mPTP	mitochondrial permeability transition pore
NADH	Nicotinamide adenine dinucleotide H
NHE	Na <sup>+</sup> /H <sup>+</sup> exchange
NO	Nitric oxide
OA	Osteoarthritis
OMM	Outer mitochondrial membrane
PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
PKA	Protein kinase A
TCA	Tricarboxylic acid cycle
TGF-β	Transforming growth factor-β
TRP	Transient receptor potential
VAHC	Voltage-activated H <sup>+</sup> channel
VDAC	Voltage-dependent anion channel
VGCC	Voltage-gated calcium channel
VGSC	Voltage-gated sodium channel

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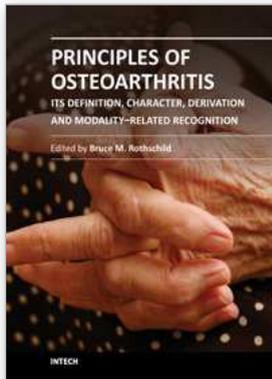
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## **Principles of Osteoarthritis- Its Definition, Character, Derivation and Modality-Related Recognition**

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This volume addresses the nature of the most common form of arthritis in humans. If osteoarthritis is inevitable (only premature death prevents all of us from being afflicted), it seems essential to facilitate its recognition, prevention, options, and indications for treatment. Progress in understanding this disease has occurred with recognition that it is not simply a degenerative joint disease. Causative factors, such as joint malalignment, ligamentous abnormalities, overuse, and biomechanical and metabolic factors have been recognized as amenable to intervention; genetic factors, less so; with metabolic diseases, intermediate. Its diagnosis is based on recognition of overgrowth of bone at joint margins. This contrasts with overgrowth of bone at vertebral margins, which is not a symptomatic phenomenon and has been renamed spondylosis deformans. Osteoarthritis describes an abnormality of joints, but the severity does not necessarily produce pain. The patient and his/her symptoms need to be treated, not the x-ray.

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