In vitro models for the study of osteoarthritis

Accepted Manuscript

Title: In vitro models for the study of osteoarthritis

Author: Craig I. Johnson, David J. Argyle, Dylan N. Clements

PII: S1090-0233(15)00302-0
DOI: http://dx.doi.org/doi:10.1016/j.tvjl.2015.07.011
Reference: YTVJL 4560

To appear in: *The Veterinary Journal*

Accepted date: 9-7-2015

Please cite this article as: Craig I. Johnson, David J. Argyle, Dylan N. Clements, In vitro models for the study of osteoarthritis, *The Veterinary Journal* (2015), http://dx.doi.org/doi:10.1016/j.tvjl.2015.07.011.

Review

In vitro models for the study of osteoarthritis

Craig I. Johnson *, David J. Argyle, Dylan N. Clements

The Roslin Institute and the Royal (Dick) School of Veterinary Studies, Division of Veterinary Clinical Sciences, The University of Edinburgh, Easter Bush Veterinary Centre, Edinburgh, EH25 9RG, UK.

* Corresponding author. Tel.: +44 131 6519164.
E-mail Address: Craig.Johnson@roslin.ed.ac.uk (C. I. Johnson)

Highlights

- No in vitro model of osteoarthritis has been validated against the native disease
- Cytokine and compression models are most commonly used
- Cytokine based models often use concentrations far greater than values measured in vivo
- Supraphysiological loads are also used often to exaggerate the response
- The development of an in vitro model might require a combinatorial, multi-modal approach

Abstract

Osteoarthritis (OA) is a prevalent disease of most mammalian species and is a significant cause of welfare and economic morbidity in affected individuals and populations. In vitro models of osteoarthritis are vital to advance research into the causes of the disease, and the subsequent design and testing of potential therapeutics. However, a plethora of in vitro models have been used by researchers but with no consensus on the most appropriate model. Models attempt to mimic factors and conditions which initiate OA, or dissect the pathways active in the disease. Underlying uncertainty as to the cause of OA and the different attributes of isolated cells and tissues used mean that similar models may produce differing results and can differ from the naturally occurring disease.

This review article assesses a selection of the in vitro models currently used in OA research, and considers the merits of each. Particular focus is placed on the more prevalent cytokine stimulation and load-based models. A brief review of the mechanism of these models is given, with their relevance to the naturally occurring disease. Most in vitro models have used supraphysiological loads or cytokine concentrations (compared with the natural disease) in order to impart a timely response from the cells or tissue assessed. Whilst models inducing OA-like pathology with a single stimulus can answer important biological questions about the behaviour of cells and tissues, the development of combinatorial models encompassing different physiological and molecular aspects of the disease should more accurately reflect the pathogenesis of the naturally occurring disease.

Keywords: Cytokine; In vitro; Loading; Model; Osteoarthritis

Introduction

Osteoarthritis (OA) is the most common form of arthritis and is one of the leading causes of disability worldwide. Most mammalian populations are affected, including humans and domesticated animal species including sheep (Vandeweerd et al., 2013), horses (Ireland et al., 2013), cats (Clarke et al., 2005) and dogs (Clements et al., 2009). Estimates in human populations suggest that 9.6% of men and 18% of women over the age of 60 years have symptomatic OA (Woolf and Pfleger, 2010). In 2006-2007 in the United Kingdom 94% of hip and 97% of knee replacements were carried out for relief of OA, costing an estimated £809 million[1]. Consequently OA is a major concern, particularly in ageing populations (Nguyen et al., 2011).

Symptoms of OA most commonly include pain, swelling and stiffness in the affected joint, resulting from the degradation of articular cartilage (Madry et al., 2012), changes in the composition of the subchondral bone (Sniekers et al., 2008; Saito et al., 2012) and synovitis (Goldhammer et al., 2010). Historically, OA was primarily observed in elderly individuals which led to the idea that OA was a 'wear-and-tear' type disease (Berenbaum, 2013). However, this idea is now less favoured because younger patients often display symptoms of OA secondary to injuries or because of a genetic predisposition to the disease (Da Silva et al., 2009).

OA is a multifactorial disorder and no single aetiological mechanism has been found common to all forms of the disease (Iliopoulos et al., 2007). Large genetic studies have identified numerous genetic risks for OA (Reynard and Loughlin, 2013), although the odds ratios for most single nucleotide polymorphism (SNP) associations are low, and rarely do

[1]See: http://www.arthritisresearchuk.org/arthritis-information/data-and-statistics/osteoarthritis.aspx (accessed 24 November, 2014)
£1 = approx. US$1.50, €1.36 at 06 April 2015.

they apply to more than one form of the disease. The mechanisms which underpin genetic risks are often unidentified, and in vitro models are critical if we are to expand our understanding of their role in disease progression.

Both in vivo and in vitro models of OA have been used in the past (Goldring et al., 2008; Grenier et al., 2014; Fang and Beier, 2014). Whilst models can be broadly grouped according to the primary mechanism by which the catabolic process is stimulated, each with their own strengths and weaknesses (Table 1), subtle variations mean that an almost infinite number of variations exist for a single model (Benam et al., 2015). Similarly, whilst in vivo models may give the most accurate reflection of the naturally-occurring whole-joint disease, the ease of manipulating an in vitro system, as well as a shift towards the 3R philosophy of refining, reducing and replacing the use of animals in animal science (Madden et al., 2012) makes in vitro modelling of the disease desirable. The observation that spontaneous OA in domestic animals has a similar pathogenesis to that observed in humans (Clements et al., 2006), and the availability of naturally-occurring, early-stage diseased tissue, for example at slaughter in food animal species or following a veterinary surgical intervention in companion animals (Clements et al., 2009) makes domestic animals an important source of clinical material for such models.

Additionally, models of OA offer the opportunity to study early features of the development of the disease, prior to the development of a fulminant catabolic process, which have been difficult to dissect because of the lack of available tissue from early disease and the limited molecular changes associated with it (Aigner et al., 2006). No consensus on the most appropriate model for the representation of particular features of OA has been made, as each model has its own mechanisms for the induction of a general catabolic process. Furthermore,

the molecular phenotypes of different forms of OA also show distinct differences (Xu et al., 2012), and such subtleties can be considered when designing models of OA rather than ignoring them when using more general in vitro models.

The relevance of in vitro models to clinical disease always needs to be interpreted with caution. For example, numerous publications report the chondroprotective effects of glucosamine and chondroitin sulphate on in vitro models (Dechant et al., 2005; Chan et al., 2007), but clinical trials have failed to show such effects in vivo (Wandel et al., 2010; Sawitzke et al., 2010). Whilst some effects of the differences can be ascribed to delivery, complexity, duration and variation of the phenotype, ultimately in vitro models should be designed to better reflect the natural in vivo disease. This particularly applies to the disease state, where almost all models are designed to replicate the symptoms of end-stage OA with little or no regard to the early disease where chondroprotection is likely to yield greater benefits.

This review summarises the two most commonly used in vitro models of OA, namely, cytokine-based models and load-based models, and then discusses their various merits and how they reflect the naturally-occurring processes.

Cytokine induction of OA-like processes

Classic research on OA has focused on cartilage, but other tissues such as bone, synovium, ligament, infrapatellar fat (Maccoux et al., 2007) and periarticular muscles (Geyer et al., 2009) are also involved (Fig. 1). The changes seen in these tissues are attributed to diffusible factors, including proteolytic enzymes, such as matrix metalloproteinases (MMPs) and members of the ‘a disintegrin and metalloproteinase with thrombospondin-motif’

(ADAMTS) family that are present in the joint environment during the disease (Little et al., 2005; Clements et al., 2006).

Cytokines in OA

During OA, synoviocytes, mononuclear cells or chondrocytes may increase their expression of catabolic proteins (Fernandes et al., 2002) following stimuli such as cytokine or chemokine exposure, including interleukin (IL)-1β and tumour necrosis factor (TNF)-α, which are present in the joint following synovial inflammation (Sohn et al., 2012). Pro-inflammatory cytokines make ideal candidates for the induction of OA-like biological changes in articular cells or tissues in culture, in which temporal and concentration effects can be explored.

Models of OA where cytokines are the primary method of induction are very common and are generally well understood. The model is usually inexpensive and is very easily manipulated. The ability to expand cells in vitro also means that many replicates are possible, allowing multiple hypotheses to be tested from single sources of tissue. Nevertheless, cells in culture (particularly chondrocytes) are prone to dedifferentiate to fibroblasts after only a small number of passages (Caron et al., 2012; Minegishi et al., 2013), and isolating cells from their matrix removes possible matrix-effects. Additionally, inter-tissue crosstalk is difficult to characterise in vitro and both time- and concentration-dependent effects are not well understood (Table 1).

Evidence for a role of IL-1β in OA is well established, and it has been used as a potential therapeutic target, for example through the design of vectors activated by IL-1 that protect against its catabolic effect (Campbell et al., 2005) or through the antagonism of the

IL-1 receptor (IL-1R) (Chevalier et al., 2009). Exposure to IL-1β stimulates chondrocytes and synovial cells to produce catabolic proteases (Maccoux et al., 2007) with apocrine signalling further enhancing MMP release and the resulting degradative cascade. The catabolic response can be blocked by the inhibition of IL-1β through antagonism with the IL1-R antagonist (IL-1Ra) (Bujak and Frangogiannis, 2009).

Inflammatory molecules produced by chondrocytes in response to IL-1β, include prostaglandin (PG)E_2, cyclooxygenase (COX)-2, IL-6, IL-8 and leukaemia inhibitory factor (LIF). IL-1β also leads to the accumulation of reactive oxygen species, through expression of inducible nitric oxide synthase (iNOS) by the transcription factor nuclear factor kappa B (NF-κB), ultimately leading to apoptosis (Fig. 2a). This mechanism can also be accelerated by IL-1β-mediated damage to mitochondrial DNA, leading to a further release of reactive oxygen species and enhancing apoptosis (Loeser, 2011).

IL-1 plays a role in bone pathophysiology relevant to OA, particularly IL-1α which is also known as osteoclast activating factor (Lee et al., 2010). In bone, there is an increase in the activity of PGE_2 in osteoblasts and stromal cells, as well as an increase in the expression of receptor activator of NF-κB Ligand (RANKL). RANKL is critically involved in the activation, maturation and survival of osteoclasts (Tanaka et al., 2005). IL-1 has also been shown to induce multinucleation of osteoclasts, thus potentiating the function of the cells. In vivo, when adult rats were injected with a moderate amount of IL-1β (1 μg/kg bodyweight), an increase in serum and urinary Ca^{2+} concentration was noted, as well as an increase in osteoclast number, implying an increase in bone resorption (Nguyen et al., 1991)

TNF-α has also been used to induce OA-like changes in in vitro experiments, because it is found in diseased synovial fluid (Horiuchi et al., 1999; Fujita et al., 2005), and is able to induce catabolism and inhibit anabolic pathways in joint tissues and cartilage cells (Liacini et al., 2003). While IL-1β and TNF-α are the most commonly used cytokines in modelling OA, other cytokines may also play important roles. Concentrations of IL-6, IL-8, vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein-1 (MCP-1) are all increased in the synovial fluid of OA joints (Sohn et al., 2012). Osteoclasts are recruited by IL-6, and thus it may be an important modular of the bone remodelling observed in OA (Silfverswärd et al., 2004). However, in model designs, these cytokines are rarely considered, possibly because they are characterised as chondrocyte-derived and thus they can be induced by other cytokines such as IL-1β or TNF-α (Bunning et al., 1990). Using cytokines in combination may allow for the induction of OA-like cell and tissue responses that more closely replicate the natural disease, particularly in lieu of synovial effects in the model design.

In vivo determination of cytokine concentrations

Cytokine-based models use a wide variety of concentrations and durations of cytokine stimulation, namely those which produce a measurable downstream effect, rather than a concentration that reflects that in naturally occurring disease. Besides, OA is a slowly-progressing disease, and relatively small increases in cytokine concentrations have been identified in naturally-affected joints.

When OA synovial fluid is assayed, the quantities of IL-1 (< 2 ng/mL) and TNF (almost 3 ng/mL) are highly variable between experiments, but are low in comparison with those used to exert an effect in vitro (Table 2). The variation in physiological concentrations

is evident and may be the result of several factors, including the method used to quantify the cytokines, or the phenotype of the disease. In contrast, the concentrations used in models are typically much higher at up to 100 ng/mL of IL-1β (Macrory et al., 2009), and up to 50 ng/mL of TNF-α (Gabriel et al., 2010).

Explant-based models of cytokine stimulation

Explant-based models are simple and easy to produce, and have the major advantage that they can be used to examine the response of cells in their natural extracellular matrix and, once removed from their extracellular matrix, the cell phenotype is altered (Zien et al., 2001). Using explanted tissue also allows features such as matrix degradation to be observed. However, the use of tissue explants creates new problems; for example, cells at the explant edge die (Hunziker and Quinn, 2003; Gilbert et al., 2009), there are limitations to the number of samples which can be obtained from the same source and more than one tissue might be required to maintain viability (Amin et al., 2009).

Cartilage is highly sensitive to TNF-α and physiologically relevant concentrations as low as 0.25 ng/mL (Westacott et al., 1990) are sufficient to increase the release of glycosaminoglycans (GAGs) from OA cartilage (human) when compared with healthy cartilage in a 14 day period (Westacott et al., 2000). Species-specific differences may exist in the stimulation required to elicit a particular response (such as GAG release); thus, GAG release from feline cartilage explants requires stimulation with both recombinant human IL-1β and oncostatin-M (OSM) in combination (Gabriel et al., 2010), although a feline-specific stimulus may have elicited a different response.

A possible autocrine network has been suggested because both IL-β and TNF-α show strong positive protein staining in the superficial zone of cartilage as well as in the synovial fluid in late-stage OA. Meanwhile, deep zone cells only demonstrate marginal staining in the most severe cases (Tetlow et al., 2001), illustrating the differential responses of chondrocytes in disease. Notably, the early stages of disease rarely demonstrate any chondrocyte expression of cytokines, implying that any inflammatory cytokines present in the joint at the early stage of the disease are most likely to be synovial in origin (Tetlow et al., 2001).

Chondrocyte culture-based cytokine models

The choice of whether to use a monolayer, a cell scaffold or intact tissue will influence the cells' response to the cytokine stimulus applied. The sensitivity of chondrocytes to their molecular and loading environment dictates that ideally they should not be isolated from their matrix, or if they are, the matrix in which they are embedded should closely match the behaviour of normal, healthy tissue. However, the low cellularity of cartilage tissue necessitates the demand for large explants, thereby reducing the number of replicates which can be obtained from a single tissue source. Monolayer cultures allow the expansion of the cellular resource, although this is finite for tissues such as cartilage (Nicholson et al., 2007), as the cell phenotype changes in monolayer culture (Zien et al., 2001).

The ease of using chondrocytes in monolayer combined with their rapid response to cytokine stimulation has resulted in this being the most widely used model. Numerous models that use cytokines added to cell or tissue culture medium have been shown to produce OA-like responses in chondrocytes in monolayer, such as a decrease in the expression of type 2 collagen and aggrecan, and an increase in the expression of MMP-13, across multiple species (Miyaki et al., 2009; Novakofski et al., 2012; Yang et al., 2014).

Alternatively, stimulating chondrocytes with the synovial fluid from OA patients (Hoff et al., 2013), a more physiologically-relevant stimulus, produces similar results, including the expression of the pro-inflammatory cytokines IL-6, IL-8, IFN-γ, MCP-1, granulocyte-colony stimulating factor (G-CSF) and VEGF. However, this method of stimulation is also limited by the imprecise understanding of the relative contribution of different mediators which are driving catabolism in this model, and the lack of repeatability because of the limited synovial fluid volume that can be obtained from a single source.

Co-culture-based cytokine models

Recognising that OA is a disease that affects and involves the interaction between multiple tissues are co-culture experiments that permit the study of these interactions in vitro. Cytokine or osmotic pressure stimuli can be easily applied to co-culture models, though the tissues might require different culture conditions, necessitating some compromise on the culture conditions used when cultured together. The co-culture of synovial membrane with chondrocytes is one method by which the complexity of the pro-inflammatory cascade can be reproduced in vitro, because synovium is the primary source of these mediators (Ushiyama et al., 2003).

Co-culturing synovium from OA patients with healthy cartilage explants produces an increase in the expression of IL-1, IL-4, IL-7, IL-8, IL-10, IL-13 and osteoprotegerin (OPG), similar to synovial fluid from OA joints, as well as reducing GAG production in the cartilage (Beekhuizen et al., 2011). Whilst it may be desirable to use synovium to model OA in vitro, it is composed of two different, but interacting, cell types and shows highly variable lesion patterns both across different OA joints, and within a single joint with clinical OA (Rhodes et

al., 2005; Goldhammer et al., 2010; Smith, 2011). Consequently, deconstructing the effects within the synovial co-culture model is complex, although characterisation of the factors responsible for the response will help to standardise across experiments. The use of bone in co-culture experiments is also important, because it appears to have a role in maintaining the long-term viability of chondrocytes in the superficial zone of articular cartilage (Amin et al., 2009).

Bone cytokine models

Evaluation of the response of bone to cytokine stimulation in models of OA is scarce, which is unsurprising because it is difficult to ascertain the precise cytokine environment to which bone cells are exposed to in OA. Most data on cytokine roles in bone focus on the specific roles of TNF-α, IL-6 and IL-1 release during osteocyte injury (Komori, 2013). Further, generation of RANKL is induced by IL-6 and IL-1, and mice lacking RANKL completely lack osteoclasts (Kong et al., 1999). Osteoclasts driven to apoptosis release soluble RANKL, and conditioned media from these cells further induce osteoclastogenesis (Al-Dujaili et al., 2011). Notably, bone plays a larger role than previously considered in cartilage health, and removal of cartilage explants from the underlying bone tissue leads to a higher percentage of cell death in chondrocytes than if chondrocytes were left attached to the bone (Amin et al., 2009).

Mouse calvarial cultures incubated with IL-1 showed bone resorption, demonstrating that cytokines act on cultured bone (Gowen et al., 1983), and cultured osteoblasts actively synthesised NO in response to IL-1α in a dose-dependent fashion, although IL-1β, TNF-α and IFN-γ failed to elicit a response (Ake et al., 1994). Evidently, cytokines play a role in bone turnover, and bone is responsive to inflammatory stimuli. IL-1β has been shown to induce the

293 release of PGE_2, MMP-3 and MMP-13 from osteoblasts (Pecchi et al., 2012), although this
294 can be inhibited with chondroitin sulphate.
295
296 **Load-based models of inducing OA**
297 Chondrocytes are sensitive to load, and must always be under sufficient force to
298 maintain extracellular matrix homeostasis, yet below that which induces apoptosis or
299 stimulates an inflammatory cascade within the tissue (Henrotin et al., 2012). Subchondral
300 bone is also mechanosensing, and responds in vivo by changing its thickness and reducing its
301 resorption when loading is increased (Murray et al., 2001). Identifying the load thresholds
302 that alter the balance from maintenance of homeostasis to injury is important to our
303 understanding of the magnitude of a beneficial or deleterious load.
304
305 Load models are easily manipulated and, as a result, high throughput experiments can
306 be performed. Signalling pathways associated with mechanotransduction are becoming well
307 understood (Millward-Sadler and Salter, 2004; Mobasheri et al., 2005), allowing better
308 appreciation of the processes associated with this model. Removing cells from their native
309 matrix and embedding them into an artificial scaffold alters, at least theoretically, the native
310 signalling network, and the force used in the experiment is innately dependent on the ability
311 of the scaffold to withstand that force.
312
313 Cell loading models require high cell numbers that might not be available from some
314 sources. Using entire tissues overcomes this problem, and allows cells to use natural cell-
315 matrix interactions and cross-tissue communication as well as much greater forces.
316 Conversely, native tissue experiments are limited to larger species and lower numbers of
317 replicates. Additionally, cells have been shown to undergo substantial cell death at the cut

edge (Huntley et al., 2005; Huntley et al., 2005a) so distorting observations at these sites (Table 1).

In vivo determination of cartilage load

Several in vivo studies have attempted to determine the physiological pressures experienced by articular cartilage during loading. The pressure passing through the load-bearing region of a human acetabular prosthesis has been determined to be approximately 3.5 MPa during locomotion (Hodge et al. 1989), and the articular contact pressure of the human knee does not exceed 8 MPa, even when the menisci have been removed (Fukubayashi and Kurosawa, 1980). Loads of a similar magnitude have been reported in both the medial and lateral compartments of canine elbow joints, with mean contact pressures between 3.0 and 4.0 MPa and peak pressures between 6.6 and 9.1 MPa (Cuddy et al., 2012). These data suggest that the articular loading experienced by different joints in different species are physiologically comparable, and concurs with estimates of the articular cartilage compressive stress in different mammalian species, which only vary within one order of magnitude from mice to cows (Simon, 1970).

Chondrocytes sense the loading of their environment through integrin receptors (Bader et al., 2011). When activated, the integrins stimulate stress pathways leading to cytoskeletal disruption and release of inflammatory cytokines, such as IL-1β and TNF-α (Valhmu et al., 1998; Durrant et al., 1999; Bader et al., 2011). Cytokine-induced proteolytic enzyme release is mediated by nitric oxide, PGE_2 and reactive oxygen species. The extracellular proteins cleaved by the activated proteases are then capable of further induction of both proinflammatory cytokines and matrix proteases, though the receptors activated by collagen fragments remain elusive (Klatt et al., 2009).

The signalling pathways induced by static loading and cytokine induction are similar and the mechanism that governs both is similar in both models (Fig. 2b). The compression pathway, however, appears reliant on the magnitude and duration of the stress (Fanning et al., 2003).

Explant based models of cartilage loading

The use of tissue explants, assessing the response of cells embedded in the natural matrix, is the simplest method for assessing the effects of load. Load is applied to tissue explants through various methods, based on the variable in question. Most typically, 'drop towers' in which a free weight is released from a predetermined height onto the tissue are used to impart a single impact load. This is believed to replicate the development of 'post traumatic' OA, which occurs following an injurious articular load. Load can also be applied in a cyclical manner with devices such as pneumatic or hydraulic loading chambers.

Static loading can induce similar deleterious changes in cartilage explants when applied at an appropriate magnitude. For example a compressive strain of 50% applied to a cartilage explant results in a decrease in the synthesis of collagen type II and proteoglycans (Chen et al., 2001). Static compression of calf patellofemoral cartilage to 25% or 50% strain for 24 h produces deleterious changes in cartilage metabolism, resulting in an increase in expression of *MMP3, 9* and *13* mRNA and decrease in *COL2A1* and aggrecan (*ACAN)* within 1-2 h post loading (Fitzgerald et al., 2004). IL-1 receptor activation and activation of the extracellular-signal related kinase 1/2 (ERK1/2), p38, mitogen-associated protein kinase (MAPK) family member pathways in a time-dependent manner meditate these changes (Fanning et al. 2003).

A load equivalent to 1.5 × bodyweight placed on a human knee joint caused only 10% strain in the patellofemoral cartilage following 10 min of static loading (Wong and Sah, 2010). Similarly, intact human femoral head cartilage loaded to the equivalent of a single leg stance (less than 2.3 × bodyweight) is subject to a strain of 33% (Greaves et al., 2010). Consequently the use of higher strains in in vitro models exaggerates the maximal normal physiological load experienced by a joint in vivo, although this reflects the requirement to induce an effect within a shorter timeframe. Furthermore, the elastic (Young's) modulus varies across cartilage within a joint (Shepherd and Seedhom, 1999), and thus the load required to induce a specific strain, or strain produced from a specific load will also differ across samples from the same joint.

In vitro studies have been used to determine the critical stress thresholds of cartilage explant, in which apoptosis, collagen degradation and nitrite accumulation are observed. Values range between 4.5 MPa for cyclic loading (six compressions to a final strain of 30-50%, held for 5 min rested for 25 min, Loening et al., 2000) and 15 MPa for a single impact load (Torzilli et al., 1999) for bovine cartilage, although the results between experiments are highly variable. Notably, bovine cartilage explants subjected to a 0.5 MPa cyclic loading increase proteoglycan synthesis across various cycle lengths (Parkkinen et al., 1992) supporting the hypothesis that moderate loading is beneficial to cartilage health.

Chondrocyte culture-based loading models

The response of cells to load can be dissected further by isolating cells in culture, embedding them in an artificial matrix, and compressing them using a bioreactor. This has the advantage of permitting very precise changes in loading parameters, as it is highly

reproducible, as well as looking at the effects of different matrices on the cellular response. However, a large caveat to such experiments is that the cell response observed in vitro may not represent that observed in vivo where the interaction of the matrix is critical to the effect produced.

In vitro loading of isolated chondrocytes seeded in a 3-dimensional (3-D) culture (typically agarose) results in both an increase in cell proliferation and proteoglycan synthesis when cyclically loaded at a physiological strain of 15% (Lee and Bader, 1997). 3-D culture also allows for the application of bi-axial cyclic loading (direct compression or tension, and shear) (Pingguan-Murphy and Nawi, 2012), and addresses the observation that in vivo several loads may simultaneously impact on a joint during normal activity. When subjected to biaxial loading (10% compressive strain with 1% sheer strain) for two 12 h periods, separated by a 12 h resting period, both cell proliferation and an increase in GAG production were observed, demonstrating the importance of closely representing the joint environment in in vitro experiments.

Cyclic loading of bovine chondrocyte-embedded calcium polyphosphate scaffolds at 1 kPa and 1 Hz for 30 min, activates Erk1/2 and c-Jun N-terminal kinase (JNK) as seen with static loading, and causes an increase in activator protein-1 (AP-1) binding. This stimulus induces *MMP-3* and *13* expression and results in MMP-13 mediated extracellular matrix (ECM) degradation. However, following the loss of functional tissue, collagen type II and aggrecan gene expression occurs after 12 h, and synthesis occurs by 24 h post loading (Fig. 2c) (De Croos et al., 2006).

Co-culture based loading models

Co-culture methods have been used to explore the effect of loading on articular tissues in parallel, although they are challenged by the necessity to use different culture conditions for each tissue, or to compromise the culture conditions. For example, alginate-embedded chondrocytes cultured on a porous filter above mechanically stretched osteoblasts become more hypertrophic during stimulation of the osteoblasts. This change was most pronounced when the osteoblasts were subjected to tensile loads, suggesting molecular 'cross-talk' occurs between the two cell types in response to mechanical stress in bone (Lin et al., 2010).

Co-culture of OA osteoblasts with healthy alginate bead-embedded chondrocytes result in a phenotypic shift to chondrocyte hypertrophy and matrix mineralisation, which does not occur with healthy osteoblasts stimulated with IL-1, IL-6 or OSM (Sanchez et al 2005). This demonstrates the limits of artificially stimulating cells and the phenotypic differences of naturally-diseased cells and highlights the need for better characterisation of the soluble factors released by these cells as well as better definition of the molecular stimulation required to induce the OA phenotype in healthy cells.

Bone loading models

Osteocytes are the major mechanosensors of bone, although they are rarely included in models of OA because they are notoriously difficult to culture in vitro. Analysis of the osteocyte (in contrast to osteoblast) response to compression has been hindered due to this challenge (Kato et al., 1997). Osteocyte cell lines seeded into type I collagen gels layered with osteoblasts on their surface respond to mechanical loading, with co-cultured constructs increasing type I collagen expression with loading, and osteocyte embedded gels expressing PGE_2 after mechanical stimulation (Vazquez et al., 2014).

Relevance of load: From in vivo to in vitro models

The validity of a loading model depends, at least to some extent, on its relationship with the natural environment of the joint and its loading in vivo. The loading parameters of a selection of cell and tissue loading models are presented in Table 3. The use of scaffolds can impart some structure to the cells for culture-based models, and permit cyclic loading of isolated cells, albeit within ranges that are governed by the strength of the matrix in which they are embedded.

The heterogeneity of the cartilage structure means that precise reconstruction of the tissue in vitro may not be possible, and so compromises must be made when constructing a load based model (Gannon et al., 2012). The individual phenotype of each chondrocyte is related to their location in the tissue (Fujioka et al., 2013; Schuurman et al., 2015) and therefore in homogeneous tissue models, chondrocytes may not behave in the same manner.

Future directions

The multifactorial nature of OA should be considered when designing a model to reproduce it, even if it is only testing a single parameter, such as the response to a load or catabolic stimulus. A deeper understanding of the pathways evoked in in vitro models, and their relevance to the changes seen in naturally-occurring OA phenotypes is important in order to improve the translational relevance of the conclusions drawn.

To date the vast majority of explant- and culture-based models have assessed cartilage in isolation. While this might reflect the ease of manipulating cartilage and the resilience of chondrocytes in cell culture, progress in tissue engineering and cell culture techniques will

allow for the development of more advanced models including other cell types. The responsiveness of bone to stimuli, and the cross talk that occurs between the different tissues in OA joints dictate that models should consider the role of multiple tissues when assessing the response to a given stimulus so as to enable more meaningful translation to the anticipated response in vivo.

There is no all-encompassing model that is suitable for all studies of OA, and no single model can be used to perfectly simulate naturally-occurring events. Whilst models seek to answer specific biological questions, more standardised end-points for the molecular and physiological parameters assessed are necessary, as at present it is impossible to directly compare the outcomes of the many in vitro OA models published in the scientific literature.

Other areas of articular health research, such as those looking at histological assessments of cartilage repair and damage[2] (Glasson et al., 2010), have developed guidelines upon which assessments should be made through the consensus of experts and the publication of their recommendations. In molecular biology, similar guidelines have been developed for performing microarray and quantitative polymerase chain reaction studies (Brazma et al., 2001; Bustin et al., 2009). Whilst the in vitro models of OA have many different functions and outcomes, guidelines could still be developed to determine the endpoints which are matrix- and chondro-protective, and the minimum number of features of a model (such as measures of matrix release and turnover, transcription changes and/or cellular morphometric changes) which are agreed to represent an 'OA-like' scenario. Similarly, models seeking to investigate the pathogenesis of OA must justify the nature of stimulatory conditions, relative to the in vivo disease, beyond simply that required to produce a response. At the very least in

[2] See: http://www.cartilage.org/_files/contentmanagement/ICRS_evaluation.pdf (accessed 7 April, 2015)

vitro models should be standardised to a particular disease phenotype, with reasoning for the source of the cells and/or tissues used and the outcomes to be measured, as there is no single OA phenotype which can be encompassed by all models.

Conclusions

The molecular pathways underpinning cytokine-stimulation and load-based in vitro models of disease are similar. The combination of different models types may permit the use of stimuli which are physiologically relevant, and which allow us to understand the development and progression of the disease, particularly the early phase, rather than simply the catastrophic downstream events after it has begun. Standardisation of the approaches, both within and between different species will allow the wider applicability of results between studies, which in turn will enhance our understanding of the disease.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

CIJ is in receipt of a studentship funded by the BBSRC and Zoetis Ltd.

References

Aigner, T., Fundel, K., Saas, J., Gebhard, P.M., Haag, J., Weiss, T., Zien, A., Obermayr, F., Zimmer, R., Bartnik, E., 2006. Large-scale gene expression profiling reveals major pathogenetic pathways of cartilage degeneration in osteoarthritis. Arthritis and Rheumatism 54, 3533–3544.

517 Ake, Y., Saegusa, Y., Matsubara, T., Mizuno, K., 1994. Cultured osteoblast synthesize nitric
518 oxide in response to cytokines and lipopolysaccharide. The Kobe Journal of Medical
519 Sciences 40, 125–37.

520 Al-Dujaili, S.A., Lau, E., Al-Dujaili, H., Tsang, K., Guenther, A., You, L., 2011. Apoptotic
521 osteocytes regulate osteoclast precursor recruitment and differentiation in vitro. Journal
522 of Cellular Biochemistry 112, 2412–2423.

523 Amin, A.K., Huntley, J.S., Simpson, A.H.R.W., Hall, A.C., 2009. Chondrocyte survival in
524 articular cartilage: the influence of subchondral bone in a bovine model. The Journal of
525 Bone and Joint Surgery 91, 691–699.

526 Bader, D.L., Salter, D.M., Chowdhury, T.T., 2011. Biomechanical influence of cartilage
527 homeostasis in health and disease. Arthritis 2011, Article ID 979032.

528 Beekhuizen, M., Bastiaansen-Jenniskens, Y.M., Koevoet, W., Saris, D.B.F., Dhert, Wouter J
529 A, Creemers, L.B., van Osch, G.J.V.M., 2011. Osteoarthritic synovial tissue inhibition
530 of proteoglycan production in human osteoarthritic knee cartilage: establishment and
531 characterization of a long-term cartilage-synovium coculture. Arthritis and Rheumatism
532 63, 1918–1927.

533 Benam, K.H., Dauth, S., Hassell, B., Herland, A., Jain, A., Jang, K.-J., Karalis, K., Kim, H.J.,
534 MacQueen, L., Mahmoodian, R., et al., 2015. Engineered In Vitro Disease Models.
535 Annual Review of Pathology 10, 195–262.

536 Berenbaum, F., 2013. Osteoarthritis as an inflammatory disease (Osteoarthritis is not
537 osteoarthrosis!). Osteoarthritis and Cartilage 21, 16–21.

538 Bougault, C., Aubert-Foucher, E., Paumier, A., Perrier-Groult, E., Huot, L., Hot, D.,
539 Duterque-Coquillaud, M., Mallein-Gerin, F., 2012. Dynamic compression of
540 chondrocyte-agarose constructs reveals new candidate mechanosensitive genes. PloS
541 One 7, e36964.

542 Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach,
543 J., Ansorge, W., Ball, C.A., Causton, H.C. et al., 2001. Minimum information about a
544 microarray experiment (MIAME)-toward standards for microarray data. Nature Genetics
545 29, 365–371.

546 Bujak, M., Frangogiannis, N.G., 2009. The role of IL-1 in the pathogenesis of heart disease.
547 Archivum Immunologiae et Therapiae Experimentalis 57, 165–176.

548 Bunning, R.A., Russell, R.G., Van Damme, J., 1990. Independent induction of interleukin 6
549 and prostaglandin E2 by interleukin 1 in human articular chondrocytes. Biochemical and
550 Biophysical Research Communications 166, 1163–1170.

551 Bush, P.G., Hodkinson, P.D., Hamilton, G.L., Hall, A.C., 2005. Viability and volume of in
552 situ bovine articular chondrocytes-changes following a single impact and effects of
553 medium osmolarity. Osteoarthritis and Cartilage 13, 54–65.

554 Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R.,
555 Nolan, T., Pfaffl, M.W., Shipley, G.L., et al., 2009. The MIQE guidelines: minimum
556 information for publication of quantitative real-time PCR experiments. Clinical
557 Chemistry 55, 611–622.

558 Campbell, S.E., Bennett, D, Nasir, L, Gault, E.A., Argyle, D.J., 2005. Disease- and cell-type-
559 specific transcriptional targeting of vectors for osteoarthritis gene therapy: further
560 development of a clinical canine model. Rheumatology 44, 735–743.

561 Caramés, B., López-Armada, M.J., Cillero-Pastor, B., Lires-Dean, M., Vaamonde, C., Galdo,
562 F., Blanco, F.J., 2008. Differential effects of tumor necrosis factor-alpha and interleukin-
563 1beta on cell death in human articular chondrocytes. Osteoarthritis and Cartilage 16,
564 715–722.

565 Caron, M.M.J., Emans, P.J., Coolsen, M.M.E., Voss, L., Surtel, D.A.M., Cremers, A., Van
566 Rhijn, L.W., Welting, T.J.M., 2012. Redifferentiation of dedifferentiated human
567 articular chondrocytes: comparison of 2D and 3D cultures. Osteoarthritis and Cartilage
568 20, 1070-1078.

569 Chan, P.-S., Caron, J.P., Orth, M.W., 2007. Effects of glucosamine and chondroitin sulfate on
570 bovine cartilage explants under long-term culture conditions. American Journal of
571 Veterinary Research 68, 709–715.

572 Chen, C.T., Burton-Wurster, N., Borden, C., Hueffer, K., Bloom, S.E., Lust, G., 2001.
573 Chondrocyte necrosis and apoptosis in impact damaged articular cartilage. Journal of
574 Orthopaedic Research 19, 703–711.

575 Chevalier, X., Goupille, P., Beaulieu, A.D., Burch, F.X., Bensen, W.G., Conrozier, T.,
576 Loeuille, D., Kivitz, A.J., Silver, D, Appleton, B.E., 2009. Intraarticular injection of
577 anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-
578 controlled study. Arthritis and Rheumatism 61, 344–352.

579 Clements, D.N., Carter, S.D., Innes, J.F., Ollier, W.E.R., Day, P.J.R., 2006. Analysis of
580 normal and osteoarthritic canine cartilage mRNA expression by quantitative polymerase
581 chain reaction. Arthritis Research and Therapy 8, R158.

582 Cook, J.L., Kuroki, K., Visco, D., Pelletier, J-P, Schulz, L., Lafeber, F.P.J.G., 2010. The
583 OARSI histopathology initiative - recommendations for histological assessments of
584 osteoarthritis in the dog. Osteoarthritis and Cartilage 18 Suppl. 3, S66–79.

585 Cuddy, L.C., Lewis, D.D., Kim, S.E., Conrad, B.P., Banks, S.A., Horodyski, M., Fitzpatrick,
586 N., Pozzi, A., 2012. Contact Mechanics and Three-Dimensional Alignment of Normal
587 Dog Elbows. Veterinary Surgery 41, 818–828.

588 D'Lima, D.D., Hashimoto, S., Chen, P.C., Colwell, C.W., Lotz, M.K., 2001. Human
589 chondrocyte apoptosis in response to mechanical injury. Osteoarthritis and Cartilage 9,
590 712–719.

591 Da Silva, M.A., Yamada, N., Clarke, N.M.P., Roach, H.I., 2009. Cellular and epigenetic
592 features of a young healthy and a young osteoarthritic cartilage compared with aged
593 control and OA cartilage. Journal of Orthopaedic Research 27, 593–601.

594 De Croos, J.N.A., Dhaliwal, S.S., Grynpas, M.D., Pilliar, R.M., Kandel, R.A., 2006. Cyclic
595 compressive mechanical stimulation induces sequential catabolic and anabolic gene
596 changes in chondrocytes resulting in increased extracellular matrix accumulation. Matrix
597 Biology 25, 323–331.

598 Dechant, J.E., Baxter, G.M., Frisbie, D.D., Trotter, G.W., McIlwraith, C.W., 2005. Effects of
599 glucosamine hydrochloride and chondroitin sulphate, alone and in combination, on
600 normal and interleukin-1 conditioned equine articular cartilage explant metabolism.
601 Equine Veterinary Journal 37, 227–231.

602 Durrant, L.A., Archer, C W, Benjamin, M., Ralphs, J.R., 1999. Organisation of the
603 chondrocyte cytoskeleton and its response to changing mechanical conditions in organ
604 culture. Journal of Anatomy 194, 343–353.

605 Fang, H., Beier, F., 2014. Mouse models of osteoarthritis: modelling risk factors and
606 assessing outcomes. Nature Reviews. Rheumatology 10, 413–421.

607 Fanning, P.J., Emkey, G., Smith, R.J., Grodzinsky, A.J., Szasz, N., Trippel, S.B., 2003.
608 Mechanical regulation of mitogen-activated protein kinase signaling in articular
609 cartilage. The Journal of Biological Chemistry 278, 50940–50948.

610 Fernandes, J.C., Martel-Pelletier, J., Pelletier, J.-P., 2002. The role of cytokines in
611 osteoarthritis pathophysiology. Biorheology 39, 237–246.

612 Fitzgerald, J.B., Jin, M., Dean, D., Wood, D.J., Zheng, M.H., Grodzinsky, A.J., 2004.
613 Mechanical compression of cartilage explants induces multiple time-dependent gene
614 expression patterns and involves intracellular calcium and cyclic AMP. The Journal of
615 Biological Chemistry 279, 19502–19511.

616 Fujioka, R., Aoyama, T., Takakuwa, T., 2013. The layered structure of the articular surface.
617 Osteoarthritis and Cartilage 21, 1092–1098.

618 Fujita, Y., Hara, Y., Nezu, Y., Yamaguchi, S., Schulz, K.S., Tagawa, M., 2005. Direct and
619 indirect markers of cartilage metabolism in synovial fluid obtained from dogs with hip
620 dysplasia and correlation with clinical and radiographic variables. American Journal of
621 Veterinary Research 66, 2028–2033.

622 Fukubayashi, T., Kurosawa, H., 1980. The contact area and pressure distribution pattern of
623 the knee. A study of normal and osteoarthrotic knee joints. Acta Orthopaedica
624 Scandinavica 51, 871879.

625 Gabriel, N., Innes, J.F., Caterson, B., Vaughan-Thomas, A., 2010. Development of an in vitro
626 model of feline cartilage degradation. Journal of Feline Medicine and Surgery 12, 614–
627 620.

628 Gannon, A.R., Nagel, T., Kelly, D.J., 2012. The role of the superficial region in determining
629 the dynamic properties of articular cartilage. Osteoarthritis and Cartilage 20, 1417–1425.

630 Geyer, M., Grässel, S., Straub, R.H., Schett, G., Dinser, R., Grifka, J., Gay, S., Neumann, E.,
631 Müller-Ladner, U., 2009. Differential transcriptome analysis of intraarticular lesional vs
632 intact cartilage reveals new candidate genes in osteoarthritis pathophysiology.
633 Osteoarthritis and Cartilage 17, 328–335.

634 Gilbert, S.J., Singhrao, S.K., Khan, I.M., Gonzalez, L.G., Thomson, B.M., Burdon, D.,
635 Duance, V.C., Archer, C.W., 2009. Enhanced tissue integration during cartilage repair in
636 vitro can be achieved by inhibiting chondrocyte death at the wound edge. Tissue
637 Engineering. Part A 15, 1739–1749.

638 Glasson, S.S., Chambers, M.G., Van Den Berg, W.B., Little, C.B., 2010. The OARSI
639 histopathology initiative - recommendations for histological assessments of
640 osteoarthritis in the mouse. Osteoarthritis and Cartilage 18 Suppl. 3, S17–23.

641 Goldhammer, M.A., Smith, S.H., Fitzpatrick, N., Clements, D.N., 2010. A comparison of
642 radiographic, arthroscopic and histological measures of articular pathology in the canine
643 elbow joint. The Veterinary Journal 186, 96–103.

644 Goldring, M.B., Otero, M, Tsuchimochi, K., Ijiri, K., Li, Y., 2008. Defining the roles of
645 inflammatory and anabolic cytokines in cartilage metabolism. Annals of the Rheumatic
646 Diseases 67 Suppl. 3, 75–82.

647 Gowen, M., Meikle, M.C., Reynolds, J.J., 1983. Stimulation of bone resorption in vitro by a
648 non-prostanoid factor released by human monocytes in culture. Biochimica et
649 Biophysica Acta 762, 471–474.

650 Greaves, L.L., Gilbart, M.K., Yung, A.C., Kozlowski, P., Wilson, D.R., 2010. Effect of
651 acetabular labral tears, repair and resection on hip cartilage strain: A 7T MR study.
652 Journal of Biomechanics 43, 858–863.

653 Grenier, S., Bhargava, M.M., Torzilli, P.A., 2014. An in vitro model for the pathological
654 degradation of articular cartilage in osteoarthritis. Journal of Biomechanics 47, 645–652.

655 Henrotin, Y., Pesesse, L., Sanchez, C., 2012. Identification of Mechanosensitive Genes in
656 Chondrocytes and Osteoblasts and Their Role in OA Pathogenesis, in: Kamkin, A.,
657 Kiseleva, I. (Eds.), Mechanical Stretch and Cytokines. Springer Netherlands, Dordrecht,
658 pp. 223–233.

659 Hodge, W.A., Carlson, K.L., Fijan, R.S., Burgess, R.G., Riley, P.O., Harris, W.H., Mann,
660 R.W., 1989. Contact pressures from an instrumented hip endoprosthesis. The Journal of
661 Bone and Joint Surgery 71, 1378–1386.

662 Hoff, P., Buttgereit, F., Burmester, G.-R., Jakstadt, M., Gaber, T., Andreas, K., Matziolis, G.,
663 Perka, C., Röhner, E., 2013. Osteoarthritis synovial fluid activates pro-inflammatory
664 cytokines in primary human chondrocytes. International Orthopaedics 37, 145–151.

Horiuchi, T., Yoshida, T., Koshihara, Y., Sakamoto, H., Kanai, H., Yamamoto, S., Ito, H., 1999. The increase of parathyroid hormone-related peptide and cytokine levels in synovial fluid of elderly rheumatoid arthritis and osteoarthritis. Endocrine journal 46, 643–649.

Huntley, J.S., Simpson, A., Hall, A.C., 2005. Use of non-degenerate human osteochondral tissue and confocal laser scanning microscopy for the study of chondrocyte death at cartilage surgery. European Cells and Materials 9, 13–22.

Huntley, J.S., McBirnie, J.M., Simpson, A.H., Hall, A.C., 2005. Cutting-edge design to improve cell viability in osteochondral grafts. Osteoarthritis and Cartilage 13, 665–671.

Hunziker, E.B., Quinn, T.M., 2003. Surgical removal of articular cartilage leads to loss of chondrocytes from cartilage bordering the wound edge. The Journal of Bone and Joint Surgery 85-A Suppl., 85–92.

Ikenoue, T., Trindade, M., Lee, M., 2003. Mechanoregulation of human articular chondrocyte aggrecan and type II collagen expression by intermittent hydrostatic pressure in vitro. Journal of Orthopaedic Research 21, 110–116.

Iliopoulos, D., Malizos, K.N., Tsezou, A., 2007. Epigenetic regulation of leptin affects MMP-13 expression in osteoarthritic chondrocytes: possible molecular target for osteoarthritis therapeutic intervention. Annals of the Rheumatic Diseases 66, 1616–1621.

Jeffrey, J.E., Aspden, R.M., 2006. The Biophysical Effects of a Single Impact Load on Human and Bovine Articular Cartilage. Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine 220, 677–686.

Jeffrey, J.E., Gregory, D.W., Aspden, R.M., 1995. Matrix damage and chondrocyte viability following a single impact load on articular cartilage. Archives of Biochemistry and Biophysics 322, 87–96.

Kahle, P., Saal, J.G., Schaudt, K., Zacher, J., Fritz, P., Pawelec, G., 1992. Determination of cytokines in synovial fluids: correlation with diagnosis and histomorphological characteristics of synovial tissue. Annals of the Rheumatic Diseases 51, 731–734.

Kato, Y, Windle, J., Koop, B., 1997. Establishment of an osteocyte-like cell line, MLO-Y4. Journal of Bone and Mineral Research 12, 2014–2023.

Kiviranta, I., Tammi, M., Jurvelin, J., Arokoski, J., Säämänen, A.M., Helminen, H.J., 1992. Articular cartilage thickness and glycosaminoglycan distribution in the canine knee joint after strenuous running exercise. Clinical Orthopaedics and Related Research 302–308.

Klatt, A.R., Paul-Klausch, B., Klinger, G., Kühn, G., Renno, J.H., Banerjee, M., Malchau, G., Wielckens, K., 2009. A critical role for collagen II in cartilage matrix degradation: collagen II induces pro-inflammatory cytokines and MMPs in primary human chondrocytes. Journal of Orthopaedic Research 27, 65–70.

Komori, T., 2013. Functions of the osteocyte network in the regulation of bone mass. Cell and Tissue Research 352, 191–198.

703 Kong, Y.-Y., Yoshida, H., Sarosi, I., Tan, H.-L., Timms, E., Capparelli, C., Morony, S.,
704 Oliveira-dos-Santos, A.J., Van, G., Itie, A., et al., 1999. OPGL is a key regulator of
705 osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature
706 397, 315–323.

707 Lee, D.A., Bader, D.L., 1997. Compressive strains at physiological frequencies influence the
708 metabolism of chondrocytes seeded in agarose. Journal of Orthopaedic Research 15,
709 181–188.

710 Lee, Y.-M., Fujikado, N., Manaka, H., Yasuda, H., Iwakura, Y., 2010. IL-1 plays an
711 important role in the bone metabolism under physiological conditions. International
712 Immunology 22, 805–816.

713 Levin, A., Burton-Wurster, N., Chen, C.T., Lust, G., 2001. Intercellular signaling as a cause
714 of cell death in cyclically impacted cartilage explants. Osteoarthritis and Cartilage 9,
715 702–711.

716 Liacini, A., Sylvester, J., Li, W.Q., Huang, W., Dehnade, F., Ahmad, M., Zafarullah, M.,
717 2003. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is
718 mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular
719 chondrocytes. Experimental Cell Research 288, 208–217.

720 Lin, Y.-Y., Tanaka, N., Ohkuma, S., Iwabuchi, Y., Tanne, Y., Kamiya, T., Kunimatsu, R.,
721 Huang, Y.-C., Yoshioka, M., Mitsuyoshi, T., et al., 2010. Applying an excessive
722 mechanical stress alters the effect of subchondral osteoblasts on chondrocytes in a co-
723 culture system. European Journal of Oral Sciences 118, 151–158.

724 Little, C.B., Flannery, C.R., Hughes, C.E., Goodship, A., Caterson, B., 2005. Cytokine
725 induced metalloproteinase expression and activity does not correlate with focal
726 susceptibility of articular cartilage to degeneration. Osteoarthritis and Cartilage 13, 162–
727 170.

728 Loening, A.M., James, I.E., Levenston, M.E., Badger, A.M., Frank, E.H., Kurz, B., Nuttall,
729 M.E., Hung, H.H., Blake, S.M., Grodzinsky et al., 2000. Injurious mechanical
730 compression of bovine articular cartilage induces chondrocyte apoptosis. Archives of
731 Biochemistry and Biophysics 381, 205–212.

732 Maccoux, L.J., Salway, F., Day, P.J.R., Clements, D.N., 2007. Expression profiling of select
733 cytokines in canine osteoarthritis tissues. Veterinary Immunology and
734 Immunopathology 118, 59–67.

735 Macrory, L., Vaughan-Thomas, A., Clegg, P.D., Innes, J.F., 2009. An exploration of the
736 ability of tepoxalin to ameliorate the degradation of articular cartilage in a canine in
737 vitro model. BMC Veterinary Research 5, 25.

738 Madden, J.C., Hewitt, M., Przybylak, K., Vandebriel, R.J., Piersma, A.H., Cronin, M.T.D.,
739 2012. Strategies for the optimisation of in vivo experiments in accordance with the 3Rs
740 philosophy. Regulatory Toxicology and Pharmacology 63, 140–154.

741 Madry, H., Luyten, F.P., Facchini, A., 2012. Biological aspects of early osteoarthritis. Knee
742 Surgery, Sports Traumatology, Arthroscopy 20, 407–422.

743 McNulty, A.L., Rothfusz, N.E., Leddy, H.A., Guilak, F., 2013. Synovial fluid concentrations
744 and relative potency of interleukin-1 alpha and beta in cartilage and meniscus
745 degradation. Journal of Orthopaedic Research 31, 1039–1045.

746 Mengshol, J.A., Vincenti, M.P., Coon, C.I., Barchowsky, A., Brinckerhoff, C.E., 2000.
747 Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression
748 in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappaB:
749 differential regulation of collagenase 1 and collagenase 3. Arthritis and Rheumatism 43,
750 801–811.

751 Millward-Sadler, S., Salter, D., 2004. Integrin-Dependent Signal Cascades in Chondrocyte
752 Mechanotransduction. Annals of Biomedical Engineering 32, 435–446.

753 Minegishi, Y., Hosokawa, K., Tsumaki, N., 2013. Time-lapse observation of the
754 dedifferentiation process in mouse chondrocytes using chondrocyte-specific reporters.
755 Osteoarthritis and Cartilage 1–8.

756 Miyaki, S., Nakasa, T., Otsuki, S., Grogan, S.P., Higashiyama, R., Inoue, A., Kato, Y., Sato,
757 T., Lotz, Martin K, Asahara, H., 2009. MicroRNA-140 is expressed in differentiated
758 human articular chondrocytes and modulates interleukin-1 responses. Arthritis and
759 Rheumatism 60, 2723–2730.

760 Mizuno, S., Ogawa, R., 2011. Using changes in hydrostatic and osmotic pressure to
761 manipulate metabolic function in chondrocytes. AJP: Cell Physiology 300, C1234–
762 C1245.

763 Mobasheri, A., Barrett-Jolley, R., Carter, S., Martín-Vasallo, P., Schulze-Tanzil, G.,
764 Shakibaei, M., 2005. Functional Roles of Mechanosensitive Ion Channels, ß1 Integrins
765 and Kinase Cascades in Chondrocyte Mechanotransduction. In: Kamkin, A., Kiseleva, I.
766 (Eds.), Mechanosensitivity in Cells and Tissues. Academia, Moscow.

767 Murray, R.C., Vedi, S., Birch, H.L., Lakhani, K.H., Goodship, A.E., 2001. Subchondral bone
768 thickness, hardness and remodelling are influenced by short-term exercise in a site-
769 specific manner. Journal of Orthopaedic Research 19, 1035–1042.

770 Nguyen, L., Dewhirst, F.E., Hauschka, P. V, Stashenko, P., 1991. Interleukin-1 beta
771 stimulates bone resorption and inhibits bone formation in vivo. Lymphokine and
772 Cytokine Research 10, 15–21.

773 Nguyen, U.D.T., Zhang, Y., Zhu, Y., Niu, J., Zhang, B., Felson, D.T., 2011. Increasing
774 prevalence of knee pain and symptomatic knee osteoarthritis: survey and cohort data.
775 Annals of Internal Medicine 155, 725–732.

776 Nicholson, I.P., Gault, E.A., Foote, C.G., Nasir, L., Bennett, D., 2007. Human telomerase
777 reverse transcriptase (hTERT) extends the lifespan of canine chondrocytes in vitro
778 without inducing neoplastic transformation. The Veterinary Journal 174, 570–576.

779 Novakofski, K.D., Torre, C.J., Fortier, L.A., 2012. Interleukin-1α, -6, and -8 decrease Cdc42
780 activity resulting in loss of articular chondrocyte phenotype. Journal of Orthopaedic
781 Research 30, 246–251.

782 Otero, M., Lago, R., Lago, F., Reino, J.J.G., Gualillo, O., 2005. Signalling pathway involved
783 in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin
784 with interleukin-1. Arthritis Research and Therapy 7, R581–591.

785 Parkkinen, J.J., Lammi, M.J., Helminen, H.J., Tammi, M., 1992. Local stimulation of
786 proteoglycan synthesis in articular cartilage explants by dynamic compression in vitro.
787 Journal of Orthopaedic Research 10, 610–620.

788 Pecchi, E., Priam, S., Mladenovic, Z., Gosset, M., Saurel, A.-S., Aguilar, L., Berenbaum, F.,
789 Jacques, C., 2012. A potential role of chondroitin sulfate on bone in osteoarthritis:
790 inhibition of prostaglandin E_2 and matrix metalloproteinases synthesis in interleukin-1β-
791 stimulated osteoblasts. Osteoarthritis and Cartilage 20, 127–135.

792 Pingguan-Murphy, B., Nawi, I., 2012. Upregulation of matrix synthesis in chondrocyte-
793 seeded agarose following sustained bi-axial cyclic loading. Clinics 67, 939–944.

794 Reynard, L.N., Loughlin, J., 2013. The genetics and functional analysis of primary
795 osteoarthritis susceptibility. Expert Reviews in Molecular Medicine 15, e2.

796 Rhodes, L.A., Grainger, A.J., Keenan, A.-M., Thomas, C., Emery, P., Conaghan, P.G., 2005.
797 The validation of simple scoring methods for evaluating compartment-specific synovitis
798 detected by MRI in knee osteoarthritis. Rheumatology 44, 1569–1573.

799 Rigoglou, S., Papavassiliou, A.G., 2013. The NF-κB signalling pathway in osteoarthritis. The
800 International Journal of Biochemistry and Cell Biology 45, 2580–2584.

801 Saito, M., Sasho, T., Yamaguchi, S, Ikegawa, N., Akagi, R., Muramatsu, Y., Mukoyama, S.,
802 Ochiai, N., Nakamura, J., Nakagawa, K., et al., 2012. Angiogenic activity of
803 subchondral bone during the progression of osteoarthritis in a rabbit anterior cruciate
804 ligament transection model. Osteoarthritis and Cartilage 20, 1574–1582.

805 Sanchez, C., Deberg, M.A., Piccardi, N., Msika, P., Reginster, J.-Y.L., Henrotin, Y.E., 2005.
806 Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic
807 chondrocytes. Osteoarthritis and Cartilage 13, 988–997.

808 Sawitzke, A.D., Shi, H., Finco, M.F., Dunlop, D.D., Harris, C.L., Singer, N.G., Bradley, J.D.,
809 Silver, D., Jackson, C.G., Lane, N.E., et al., 2010. Clinical efficacy and safety of
810 glucosamine, chondroitin sulphate, their combination, celecoxib or placebo taken to treat
811 osteoarthritis of the knee: 2-year results from GAIT. Annals of the Rheumatic Diseases
812 69, 1459–1464.

813 Schuurman, W., Klein, T.J., Dhert, W.J.A, Van Weeren, P.R., Hutmacher, D.W., Malda, J.,
814 2015. Cartilage regeneration using zonal chondrocyte subpopulations: a promising
815 approach or an overcomplicated strategy? Journal of Tissue Engineering and
816 Regenerative Medicine 9, 669-678.

817 Shepherd, D.E., Seedhom, B.B., 1999. The 'instantaneous' compressive modulus of human
818 articular cartilage in joints of the lower limb. Rheumatology 38, 124–132.

819 Silfverswärd, C.-J., Frost, A., Brändström, H., Nilsson, O., Ljunggren, O., 2004. Interleukin-4
820 and interleukin-13 potentiate interleukin-1 induced secretion of interleukin-6 in human
821 osteoblast-like cells. Journal of Orthopaedic Research 22, 1058–1062.

822 Simon, W.H., 1970. Scale effects in animal joints. I. Articular cartilage thickness and
823 compressive stress. Arthritis and Rheumatism 13, 244–256.

824 Smith, M.D., 2011. The normal synovium. The Open Rheumatology Journal 5, 100–106.

825 Sniekers, Y.H., Intema, F., Lafeber, Floris P J G, van Osch, G.J.V.M., Van Leeuwen,
826 J.P.T.M., Weinans, H., Mastbergen, S.C., 2008. A role for subchondral bone changes in
827 the process of osteoarthritis; a micro-CT study of two canine models. BMC
828 Musculoskeletal Disorders 9, 20.

829 Sohn, D.H., Sokolove, J., Sharpe, O., Erhart, J.C., Chandra, P.E., Lahey, L.J., Lindstrom,
830 T.M., Hwang, I., Boyer, K.A., Andriacchi, T.P., et al., 2012. Plasma proteins present in
831 osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4.
832 Arthritis Research and Therapy 14, R7.

833 Sylvester, J., El Mabrouk, M., Ahmad, R., Chaudry, A., Zafarullah, M., 2012. Interleukin-1
834 induction of aggrecanase gene expression in human articular chondrocytes is mediated
835 by mitogen-activated protein kinases. Cellular Physiology and Biochemistry 30, 563–
836 574.

837 Tanaka, S., Nakamura, K., Takahasi, N., Suda, T., 2005. Role of RANKL in physiological
838 and pathological bone resorption and therapeutics targeting the RANKL-RANK
839 signaling system. Immunological Reviews 208, 30–49.

840 Tetlow, L.C., Adlam, D.J., Woolley, D.E., 2001. Matrix metalloproteinase and
841 proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage:
842 associations with degenerative changes. Arthritis and Rheumatism 44, 585–594.

843 Torzilli, P.A., Grigiene, R., Borrelli, J., Helfet, D.L., 1999. Effect of impact load on articular
844 cartilage: cell metabolism and viability, and matrix water content. Journal of
845 Biomechanical Engineering 121, 433–441.

846 Ushiyama, T., Chano, T., Inoue, K., Matsusue, Y., 2003. Cytokine production in the
847 infrapatellar fat pad: another source of cytokines in knee synovial fluids. Annals of the
848 Rheumatic Diseases 62, 108–112.

849 Valhmu, W.B., Stazzone, E.J., Bachrach, N.M., Saed-Nejad, F., Fischer, S.G., Mow, V.C.,
850 Ratcliffe, A., 1998. Load-controlled compression of articular cartilage induces a
851 transient stimulation of aggrecan gene expression. Archives of Biochemistry and
852 Biophysics 353, 29–36.

853 Vazquez, M., Evans, B.A.J., Riccardi, D., Evans, S.L., Ralphs, J.R., Dillingham, C.M.,
854 Mason, D.J., 2014. A new method to investigate how mechanical loading of osteocytes
855 controls osteoblasts. Frontiers in Endocrinology 5, 208.

856 Walsh, D.A., McWilliams, D.F., Turley, M.J., Dixon, M.R., Fransès, R.E., Mapp, P.I.,
857 Wilson, D., 2010. Angiogenesis and nerve growth factor at the osteochondral junction in
858 rheumatoid arthritis and osteoarthritis. Rheumatology 49, 1852–1861.

859 Wandel, S., Jüni, P., Tendal, B., Nüesch, E., Villiger, P.M., Welton, N.J., Reichenbach, S.,
860 Trelle, S., 2010. Effects of glucosamine, chondroitin, or placebo in patients with
861 osteoarthritis of hip or knee: network meta-analysis. BMJ 341, c4675.

862 Westacott, C.I., Barakat, A.F., Wood, L., Perry, M.J., Neison, P., Bisbinas, I., Armstrong, L.,
863 Millar, A.B., Elson, C.J., 2000. Tumor necrosis factor alpha can contribute to focal loss
864 of cartilage in osteoarthritis. Osteoarthritis and Cartilage 8, 213–221.

865 Westacott, C.I., Whicher, J.T., Barnes, I.C., Thompson, D., Swan, A.J., Dieppe, P.A., 1990.
866 Synovial fluid concentration of five different cytokines in rheumatic diseases. Annals of
867 the Rheumatic Diseases 49, 676–681.

868 Wong, B.L., Sah, R.L., 2010. Effect of a focal articular defect on cartilage deformation
869 during patello-femoral articulation. Journal of Orthopaedic Research 28, 1554–1561.

870 Woolf, A.D., Pfleger, B., 2010. Burden of major musculoskeletal conditions. Bulletin of the
871 World Health Organisation 81, 646–656.

872 Xu, Y., Barter, M.J., Swan, D.C., Rankin, K.S., Rowan, A.D., Santibanez-Koref, M.,
873 Loughlin, J., Young, D.A., 2012. Identification of the pathogenic pathways in
874 osteoarthritic hip cartilage: commonality and discord between hip and knee OA.
875 Osteoarthritis and Cartilage 20, 1029–1038.

876 Yang, B., Kang, X., Xing, Y., Dou, C., Kang, F., Li, J., Quan, Y., Dong, S., 2014. Effect of
877 microRNA-145 on IL-1β-induced cartilage degradation in human chondrocytes. FEBS
878 letters 588, 2344–2352.

879 Yuan, X.L., Meng, H.Y., Wang, Y.C., Peng, J., Guo, Q.Y., Wang, A.Y., Lu, S.B., 2014.
880 Bone-cartilage interface crosstalk in osteoarthritis: potential pathways and future
881 therapeutic strategies. Osteoarthritis and Cartilage 22, 1077–1089.

882 Zien, A., Aigner, T., Zimmer, R., Lengauer, T., 2001. Centralization: a new method for the
883 normalization of gene expression data. Bioinformatics (Oxford, England) 17 Suppl. 1,
884 S323–331.

885

886 **Tables**

887 **Table 1**

888 Advantages and disadvantages of some of the most commonly used in vitro models

Model Type	Variables that can be applied	Advantages	Disadvantages	Examples
Monolayer culture	Cytokine stimulation, osmotic pressure	Allows expansion of cellular resource from a single sample, investigation of distinct pathways in isolation	Altered phenotype of isolated cells due to isolation from tissue and absence of normal extracellular matrix	(Sylvester et al., 2012; Novakofski et al., 2012)
Co-culture	Cytokine stimulation, osmotic pressure	Considers cross-talk between cell types	Altered phenotype of isolated cells Different cell types require different culture conditions, or compromise if culture together	(Lin et al., 2010; Beekhuizen et al., 2011; Vazquez et al., 2014)
3D-culture	Cytokine stimulation, osmotic pressure, physical injury and loading regimens.	Affords structure and force to sensitive cells.	Magnitude of force is scaffold dependent, and may not reflect that of the normal tissue Isolation and expansion of cell types first	(Mizuno and Ogawa, 2011; Bougault et al., 2012; Pingguan-Murphy and Nawi, 2012)
Explant	Cytokine stimulation, osmotic pressure, physical injury and loading regimens.	Inexpensive, easily produced, cells maintained in normal extracellular matrix.	Cell death at cut edge of tissue, few replicates available from same source, more than one tissue type may be required to maintain viability, physical attributes may change in culture	(Fitzgerald et al., 2004; Bush et al., 2005; Jeffrey and Aspden, 2006; Gabriel et al., 2010)

889

890 **Table 2**

891 Osteoarthritic synovial fluid interleukin (IL)-1β and tumour necrosis factor (TNF)-α

892 concentrations in vivo

Condition	Cytokine	
	IL-1β	TNF-α
Control (human) (Kahle et al., 1992)	<20 pg/mL	2890 pg/mL
Control (canine) (Fujita et al., 2005)	490 pg/mL	105.3 pg/mL
OA (human) (Kahle et al., 1992)	21 pg/mL	80 pg/mL
OA - Hip dysplasia (canine) (Fujita et al., 2005)	2010 pg/mL	600 pg/mL
OA - Mild (porcine) (McNulty et al., 2013)	109 pg/mL	-
OA - Moderate (porcine) (McNulty et al., 2013)	122 pg/mL	-

893

894 **Table 3**

895 A summary of load-induced changes in OA models

Species studied	Regimen tested	Duration of experiment	Findings of experiment	Citation
Dog (in vivo)	20-40 km/day	15 weeks	Increased water content. Decreased collagen content. Decreased proteoglycan content.	(Kiviranta et al., 1992)
Bovine explant	Six on-off cycles of 30-50% strain	5 min compression: 25 min release	Apoptosis of cells. Degradation of collagen network. Glycosaminoglycan release.	(Loening et al., 2000)
Bovine explant	100 g, 500 g and 1 kg dropped from 2, 5, 10 and 20 cm	N/A	Linear decrease in cell viability. Increased hydration of tissue. Partial depth fissures.	(Jeffrey et al., 1995)
Canine shoulder explant	5 MPa at 0.3 Hz	20 or 120 min	Cell death increased with increased loading time. Glycosaminoglycan and NO were not significantly altered.	(Levin et al., 2001)
Canine shoulder explant	5 MPa at 0.3 Hz	0, 2, 20, 120 min	Necrosis and apoptosis of cells increased with loading time. Proteoglycan increased in 2 and 20 minute groups but decreased at 120 minutes.	(Chen et al., 2001)
Agarose embedded equine chondrocytes	15% strain either statically or cyclically (0.3-3 Hz)	48 h	Dynamic strain increased cell proliferation. Static strain decreased glycosaminoglycan content, while cyclic strain increased glycosaminoglycan.	(Lee and Bader, 1997)
Bovine explant	25-50% strain over 3 min period	Maintained for 1, 2, 4, 8 and 24 h	Relative expression of matrix genes decreased. Relative expression of proteases increased.	(Fitzgerald et al., 2004)
Human chondrocyte monolayer	1, 5 or 10 MPa hydrostatic pressure at 1 Hz	4 h per day for either 1 or 4 days	mRNA and protein expression of aggrecan and collagen type 2 upregulated after 4 days. No difference at 1 day.	(Ikenoue et al., 2003)
Full thickness human cartilage explant	Single mechanical load of 14 MPa for 500 ms	Measurements taken 96 h after injury	DNA fragmentation in 34% of loaded chondrocytes (vs. 4% of control) GAG release increased in loaded explants (1.9% vs. 0.8% total GAG content)	(D'Lima et al., 2001)

896

Figure legends

Fig. 1. Healthy and diseased synovial joint showing the changes in the entire joint organ. Bone weakness and wearing has been reported, as well as synovial thickening and swelling, subchondral bone thickening, osteophyte formation and cartilage degradation. In addition, tendons can become weak and inflamed and ligaments can become lax.

Fig. 2. Mechanistic pathways of in vitro models of OA. Both cytokines (a) and injurious loading (b) combine to cause the typical OA-like phenotype, showing apoptosis of cells, tissue degradation and inflammatory gene expression. In these cases, feedback loops occur either through inflammation, causing further cytokine stimulation, or abnormal loading, caused by tissue degradation. However, normal homeostatic loading is vital to tissue health, and works through similar pathways, as shown by the green line in (c), leading to tissue growth.

MAPK, mitogen-activated protein-kinase; JNK, c-Jun N-terminal kinase; AP-1, activator protein-1; NFκB, nuclear factor kappa B; COX2, cyclooxygenase-2; PGE2, prostaglandin-E_2; MMP, matrix metalloproteinase; ADAMTS, a distintegrin and metalloproteinase with a thrombospondin motif; ROS, reactive oxygen species.