Comparison Between In Vitro and In Vivo Cartilage Overloading Studies Based on a Systematic Literature Review

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Received 8 August 2017; accepted 27 March 2018
Published online 12 April 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.23910

ABSTRACT: Methodological differences between in vitro and in vivo studies on cartilage overloading complicate the comparison of outcomes. The rationale of the current review was to (i) identify consistencies and inconsistencies between in vitro and in vivo studies on mechanically-induced structural damage in articular cartilage, such that variables worth investigating to further explore using either one of these approaches can be identified; and (ii) suggest how the methodologies of both approaches may be adjusted to facilitate easier comparison and therewith stimulate translation of results between in vivo and in vitro studies. This study is anticipated to enhance our understanding of the development of osteoarthritis, and to reduce the number of in vivo studies. Generally, results of in vitro and in vivo studies are not contradicting. Both show subchondral bone damage and intact cartilage above a threshold value of impact energy. At lower loading rates, excessive loads may cause cartilage fissuring, decreased cell viability, collagen network destructuring, decreased GAG content, an overall damage increase over time, and low ability to recover. This encourages further improvement of in vitro systems, to replace, reduce, and/or refine in vivo studies. However, differences in experimental set up and analyses complicate comparison of results. Ways to bridge the gap include (i) bringing in vitro set-ups closer to in vivo, for example, by aligning loading protocols and overlapping experimental timeframes; (ii) synchronizing analytical methods; and (iii) using computational models to translate conclusions from in vitro results to the in vivo environment and vice versa. © 2018 The Authors. Journal of Orthopaedic Research® Published by Wiley Periodicals, Inc. on behalf of the Orthopaedic Research Society. J Orthop Res 36:2076–2086, 2018.

Keywords: cartilage; mechanics; In vitro; In vivo; post-traumatic OA

In articular cartilage, the zonally differentiated collagen fibril structure and embedded hydrophilic proteoglycans (PGs) provide the tissue with its remarkable mechanical properties. A decreased structural integrity during osteoarthritis (OA) is associated with an impaired load-bearing functionality. These mechanical changes also occur within the micro-environment of the chondrocytes, which can affect their protein production and lead to additional structural changes. The structure-mechanics of articular cartilage are thus considered to play a crucial role in OA induction as well as progression.

Although inflammation can also play a major role in the onset of OA, biomechanical risk factors, such as body weight, joint alignment, and knee trauma, are well established. A widely used experimental model for the investigation of OA is therefore to induce cartilage damage by mechanical overloading. The ultimate goal of such research is to determine mechanical thresholds that would induce damage in a specific way to particular parts of the cartilage structure. Examples may be shear stress or impact energy that would cause damage to the cartilage matrix, or the tensile strain or strain rates that would rupture or de-structure the collagen fiber network. Understanding such thresholds for healthy and compromised cartilage provides an upper limit to the mechanical perturbations that cartilage can withstand. These may ultimately be taken into account for making decisions on treatment strategies or to advise on post-operative recovery.

In vitro overloading studies are conducted to assess damage initiation and/or short-term explant effects providing results within a timeframe of seconds to several weeks, whereas in vivo experiments normally last months up to a year. A major advantage of an in vitro study is the possibility to apply a specific loading protocol in a highly controlled fashion. Once a culture protocol has been established and bioreactors are validated, performing additional in vitro studies is generally cheaper and faster than in vivo studies, and there is no discomfort for test subjects or need for ethical approval. In addition, in vitro the biochemical conditions are controlled and typically kept constant, allowing for comparison between samples based solely on difference of loading. The main advantage of an in vivo study is that it provides a natural biomechanical and biological environment to study OA as a total joint disease. In vivo studies also include inflammation, bone adaptation, and other longer term processes. In vitro studies are thus used to answer more fundamental research questions, while in vivo studies can be used to investigate a response under natural conditions over longer time.

Unfortunately, methodological differences make it challenging to directly compare results between in vitro and in vivo studies. By taking the results from many in vivo and in vitro studies together and categorizing them by outcome parameters, we aim to identify consistent factors in the relationship between...
mechanical overloading and articular cartilage damage development. Inconsistencies between in vitro and in vivo studies may also be revealed. These may identify in vitro approaches that are not sufficiently representative for in vivo conditions, or they address aspects that can only be studied appropriately in vivo.

The aim of the current review therefore is to (i) identify consistencies and inconsistencies between in vitro and in vivo studies on mechanically-induced structural damage in articular cartilage, such that variables that would be interesting to further explore using either one of these approaches can be identified; and (ii) suggest how the methodologies of both approaches may be adjusted to facilitate easier comparison and therewith stimulate translation of results between in vivo and in vitro studies. This is anticipated to enhance our understanding of the development of osteoarthritis, and to help replacing part of our in vivo studies by in vitro approaches.

APPROACH

The PubMed database was searched for relevant papers using various forms and synonyms of the following terms in their title: Cartilage, damage, stress, strain, overload, load, impact, meniscectomy, transection, tear. This yielded 433 papers, from which only those relevant to this review were selected. Papers on in vitro cartilage synthesis (tissue engineering) and/or computational cartilage models were, in general, beyond the scope of this review and therefore largely excluded. However, the role of the latter in connecting in vitro to in vivo is discussed in chapter 5. In vivo studies did not include human clinical studies, because in those studies OA is not experimentally induced. Only publications written in English were considered. The reference lists of selected papers were also searched for additional relevant publications. The search was updated just before submission to the journal of orthopaedic research.

Papers were classified as reporting on either in vitro or in vivo overloading studies. Information on macrostructural cartilage damage, swelling behavior, chondrocyte viability, collagen network structure, proteoglycans, and tissue mechanics were documented and used to create a map of events following trauma (see Fig. 1 for an illustrative summary of the structural features that were reviewed). The reader is directed to alternative review papers for additional information on genetics, epigenetics, and the expression of smaller proteins which might also affect structure-mechanics during cartilage degeneration.

IN VITRO STUDIES; SHORT-TERM RESULTS

Mechanical overloading in vitro can lead to immediate crack formation in the cartilage layer.11,12 En face examination of cracked samples reveals that the rupture orientation is initially in the split line direction and that additional fissures are also oriented in this direction or at a constant angle to it.13–18 Fissures proceed to increasing depths into the cartilage matrix following the arcade-like architecture of the collagen network, that is, originally parallel to the articular surface and then transitioning into the radial orientation, until finally the fissures run along the calcified boundary and cartilage delamination occurs.19–21 The probability and severity of surface fissuring occurring following trauma generally increases with higher impact energy,22–24 applied stress,19,25–35 stress rate,14,26,28–30,36,37 frequency of dynamic loading,17 loading duration,18,28 and a period of prolonged creep prior to overloading.21,38 From a certain impact threshold, in this case an impact energy of 0.25 J imposed on 5 mm diameter cartilage explants, it has even been observed that there was damage to the subchondral bone without macroscopic damage to the cartilage.24,39 Increasing levels of macrostructural damage however can also be attributed to specimen-related characteristics, such as a higher degree of degeneration,40,46 higher stiffness,13 lower level of maturity,20 increased in-plane surface strain,15 or decreased thickness of subchondral bone.16,24 In contrast, cartilage is less prone to fracture when the most superficial layer is removed,13,19 or when a pre-strain of 10% or higher is applied prior to overloading.41 The amount and depth of surface fissures in cartilage decreases its surface strain-limiting abilities, induces tissue swelling,42 and alters the compressive load distribution,43 which may lead to further mechano-biological damage.44,45

Decreased cell viability following mechanical overloading indicates excessive local strain on a macrostructural level.46 Both the amount of necrotic and apoptotic cells can increase after injurious loading,47,48 in any overloaded area in the cartilage.47,48 Also,

Figure 1. Illustration of possible structural features in the intact (top) and excessively loaded (bottom) cartilage. A higher intensity of blue colour indicates a (locally) higher PG concentration. Viable cells are shown in purple while dead cells are shown in red. In the full-thickness cartilage constructs the black lines indicate primary fibrillar direction. The isolated boxes show the collagen network on the ultrastructural level.
fissures caused by mechanical overloading are always surrounded by dead cells.25,31,55–59 Following traumatic compression, the depth of non-viable cells from the articular surface increases with increasing contact stress,25,27,49–51,60–62 increasing loading duration,8,18,50,54,60 (up to a number of cycles27), lower maturity level,31,63 absence of the superficial layer,52 higher impact energy,24,31,58,64,65 the amount of cartilage preloading,58 and absence of subchondral bone.16 The viability response to varying strain rates is rather complex because cartilage behaves in a relatively stiff and incompressible manner under high strain rates, and is more compliant under slower or sustained loading when the water is given time to be expelled.2,39,62,66,67 At low compressive strain rates, chondrocyte death might be found throughout the entire cartilage depth, whereas at higher strain rates viability is only decreased in the superficial layer.14,29,36,56,62 This indicates that the most superficial layer experiences higher stresses than the underlying tissue at higher strain rates.68 If the strain rates are then further increased the superficial layer of dead cells becomes thicker.29,67 Cell viability in the superficial layer seems to be unaffected by the frequency of cyclic intermittent loading.69 Cell viability generally keeps decreasing over time when chondral explants are maintained in culture following a traumatic event29,49,52,64,70 and this time-dependent response is dependent on the loading protocol that is used.48,56,57,63 During extended culture times cell death also increases in control samples, which complicates the investigation of cell death as a result of trauma.65,71 A decreased cell viability leads to an even lower ability of tissue remodeling following trauma72 and the release of biochemical factors by perturbed, apoptotic, or necrotic cells.

Changes in bulk collagen content are not usually observed following trauma,73–75 however resulting structural changes alone can have a large effect on cartilage mechanics. These changes to the collagen network can be due to fibril denaturation,72,74 or fibril rupture as observed during, for example, surface fissuring, but can also be due to a reduced level of inter-fibrillar connections.76 Such a reduced interconnectivity has been termed network de-structuring, which is when the network is transformed from its normal highly interconnected “pseudo-random” appearance77 to a less interconnected structure with an overall increased aligned fibroisty.78 Associated with this de-structuring is an increased matrix tendency to soften and swell due to the decreased constraint of proteoglycan swelling.28,34,37,53,56,64,73,79–82 However, the extent of softening following injurious loading may be a combined effect of network de-structuring and proteoglycan alterations described in the following paragraph. It has further been shown that tissue softening due to excessive loading can precede collagen denaturation,75 which seems logical since the interconnections between the collagen fibrils are weaker in tension than the fibrils themselves.83 Cartilage exhibits increased swelling and softening with higher loads,24,53,56,67,75 reveals more microcracks within the collagen network with higher impact energy or applied stress or stress rate,84 and levels of degraded collagen coincide with or come at a later stage than cell death indicating that this is also caused by excessive microstrains.27,50,61 The increased compliance within the tissue as a result of collagen network de-structuring has the potential to affect chondrocyte metabolism and the microstructural response to compression.

In principle, PG release following overloading can be caused by de-structuring of the collagen network, fracturing of the PGs themselves and/or excessive pressure and diminished boundaries. The reduced constraint from a loosened collagen network on the PGs can also lead to a decreased PG density.44 An increased glycosaminoglycan (GAG) release25,53,72 and synthesis26 and decreased content11,67 in overloaded samples compared to controls are normally observed from certain threshold loads.32,34,36,83 However, GAG synthesis tends to decrease at higher loads34,37,85 as cell viability decreases.28,29,48 Changes in GAG content have also been shown to be zonally dependent. It has been hypothesized that GAG loss starts in the transition zone, and is mainly synthesized in the deep zone as a response.50 Higher contact stress and prolonged loading lead to increased GAG release and synthesis, and decreased GAG content.50,64,65 At similar strains, cyclic compression results in increased PG release when compared to static compression, and the lost PGs are then also smaller in size.86 Varying the frequency and duration of intermittent cyclic loading affects PG synthesis and release in a non-linear and irregular manner.69 Furthermore, an increased GAG release has been shown to be associated with cartilage fracture following injury.59 Depending on the mechanical protocol used, GAG release can be higher in injured samples compared to controls at varying time-points following loading.14,29,30,34,64,71,86 Because of the simultaneous GAG release and synthesis, it may be possible that no changes in GAG content are observed in some culture studies.74 A lower GAG concentration following injury reduces the hydrostatic pressure within cartilage, with the ability to affect both mechanical and metabolic properties.

In summary, in vitro overloading studies have shown that structural changes following trauma are highly dependent on the mechanical loading applied and the tissue used for experimentation. The resulting structural damage depends on the local stresses induced by the trauma. A reduced level of fibrillar interconnectivity is probably the first sign of excessive internal stress, since tissue weakening can be observed prior to viability changes.73 A further increased stress then leads to cell death, collagen denaturation, and eventually surface fissuring. The amount of GAG loss depends on the degree of collagen network de-structuring and GAG synthesis. Damage on macro-
micro-, and nano-structural level has the potential to alter the local mechanical environment and thus lead to further mechanobiological changes. The overall cartilage response to overloading in vitro has been schematically summarized in Figure 2.

**IN VIVO STUDIES; LONG-TERM RESULTS**

The aim of many in vivo studies on cartilage overloading is to induce post-traumatic OA using some form of mechanical insult to study OA progression. Such an experimental model can be used to test the effects of a novel treatment strategy following injury or to study individual components in relation to OA progression within a natural environment. Cartilage degeneration can be induced either in a direct fashion (impact or other overloading protocol) or indirectly, for instance by altering joint kinematics.

A way of investigating the effect of mechanical overloading on cartilage in vivo is by introducing a one-off trauma, such as an impact load, after which the test animal is allowed natural weight bearing without additional damage to any surrounding soft tissue. In a number of those studies, the overloading protocol caused immediate fissuring of the cartilage surface,

indicating GAG production but not containment.

In excessive loading of the whole joint has shown that this superficial fissuring and GAG loss was visible in the highest and not in the femur.

The rate of damage increase over time following a one-off trauma varies with animal model and applied trauma. However, there is a general trend that cartilage deteriorates further and does not recover after a trauma causing surface fissuring.

The tissue may gradually exhibit chondrocyte clusters, empty lacunae, and increasing GAG loss, and at some point GAG staining may only be visible in the direct vicinity of the cells indicating GAG production but not containment.

In terms of mechanical properties, an immediate reduction in cartilage thickness is associated with a decrease in stiffness, with both thickness and stiffness decreasing further over time.

A high impact which immediately damages the subchondral bone but not the cartilage can still result in cartilage degeneration over time, indicating either a delayed direct effect from the impact or a translated effect from the underlying bone.

Cartilage damage can also be induced in vivo by altering the animal model's joint kinematics, for example, by ACL transection, which induces joint instability, or meniscectomy, resulting in directly increased tibia-femoral contact stresses, or repeated long-term overloading by muscle stimulation.

ACL transection, meniscectomy, and repeated long-term overloading have been shown to induce cartilage degeneration over time in terms of surface fissuring, hypocellularity, loss of structural integrity, GAG increase followed by decrease, and overall tissue softening.

In these models, the tibia is generally more severely or equally damaged compared to the femur, and less degeneration has been observed in the patella compared to the femur, indicating a greater effect on areas that receive the most stress during locomotion. Fissuring and GAG loss tend to worsen over time, although occasionally the degenerative grade remains relatively stable between varying observation points.

The rate of cartilage degeneration also varies with injury modality, that is, when the magnitude and duration of abnormal loading are higher there is an increased rate.

Similarly, combining various damage modalities such as ACL transection, meniscectomy, and/or application of an impact increases the rate of cartilage degeneration.

Thus, in vivo overloading studies, whether impact-induced or induced by permanent alterations to joint kinematics, all seem to consistently lead to increasing joint degeneration over time (see Fig. 3). This was expected since many in vivo studies develop or use overloading models which lead to general OA-related effects. The damage further seems to be more severe with higher stresses. In the absence of intervention, these studies have not shown that spontaneous repair can counteract the drastic overloading protocols.

**COMPARISON BETWEEN IN VITRO AND IN VIVO OVERLOADING STUDIES**

A wide variety of experimental and analytical protocols was employed within the reviewed studies. An example of an experimental difference is the use of various indenter shapes to induce cartilage damage. The effects of such differences could not be elucidated from this review and would be best explored in a direct comparative experimental study. The current review can be interpreted as "multiple overloading pathways leading to degeneration," bearing in mind that these pathways may have similarities but are not necessarily the same. An overall challenge that remains is to identify which factors in these pathways play important roles, in vitro and/or in vivo.
In general, the results of in vitro and in vivo studies do not contradict each other. It has been shown with both study types that after an extremely high impact the subchondral bone is damaged, while the cartilage initially stays intact. At less high impacts, immediate cartilage fissuring, decreased cell viability, collagen network de-structuring, an overall decreased GAG content, and an overall damage increase over time are reported for both in vitro and in vivo. Both study types further reveal the low ability of cartilage to recover from cell death or structural damage to the collagen network as a result of overloading. Tissue viability and a functional collagen organization may therefore be important targets for novel therapeutic treatments of early cartilage degeneration. That the outcomes of in vitro and in vivo studies do not contradict each other, encourages further development and extension of in vitro systems to study in vivo effects, which may reduce and refine the use of animals. It would also be interesting to investigate whether specific parameters (e.g., stress or strain rate), which are shown to affect cartilage in vitro, can be controlled in vivo to prevent damage due to overloading. These rationales justify efforts to further align in vitro and in vivo study methodologies.

One way to increase resemblance between in vitro and in vivo experimental set-ups is by attempting to make in vitro loading protocols more physiological. To assess whether this is feasible one should consider the available experimental set-ups (see Table 1). Each of the four set-ups shown in Table 1 has its advantages and disadvantages making it suitable for specific research targets. Most of the in vitro experiments performed until today were explant studies, using either living (i.e., cultured) or dead tissue. Although these explant systems allow for an accurately defined loading protocol, the relatively small samples with their unconstrained boundaries likely affect tissue response and may therefore not be representative of loading within a total joint. Systems that are currently perhaps underexplored and could potentially bridge the gap between explant and in vivo studies are total joint motion simulators. The loading that can be applied with such systems is more physiological than loading regimes commonly used in explant studies, while it allows for more controlled loading than is possible in vivo. A major drawback of these systems is that it is still very challenging to accurately determine the exact loading within a joint. However, the joint motion simulator may for instance be employed to develop short-term post-operative recommendations for in vivo joint movement.

An alternative method to align loading protocols of in vitro and in vivo studies is to restrict motion of subjects in an in vivo study to investigate the effect of altered loading. It has been shown that interventions such as joint distraction, high tibial osteotomy, and bracing lead to decreased cartilage degeneration and improved patient-reported outcome. However, it is complicated to determine the isolated effect of loading since it is impossible to measure in vivo loads. For bracing, it has been shown that improvement in pain is small-to-moderate while improvement in gait mechanics was moderate-to-high. It has also been demonstrated that the placebo effect can play a role in patient experience. Thus, the exact contribution of load-reduction in motion-alternating treatments has not yet been elucidated and further research with an objective approach needs to be performed.

Another important difference between current in vitro and in vivo methods is the analytical timepoints. The maximum duration of explant incubation reported in the papers included in this review was 4 weeks. The number of reviewed papers on in vivo studies showing results of 4 weeks or less is small (7 out of 32). In vivo studies generally lasted several months up to a year for the larger species (lapine, murine, ovine, and primates). The number of measurement timepoints is usually limited to 2 or 3, because of ethical consider-
It is often required to sacrifice test subjects for each timepoint. However, with the recent advances on in vivo cartilage imaging and corresponding image analysis\textsuperscript{128} it will be possible in the future to add more timepoints for analysis of the same subject. This will help decreasing the number of subjects needed and increasing the accuracy of time-dependent measurements (see Fig. 4). Simultaneously, ex vivo incubation systems are vastly improving, as it has been shown that cartilage-on-bone explants can stay intact for up to 8 weeks.\textsuperscript{129} These explants can also be compressed and biochemically supplemented as required, and are thus increasingly resembling the in vivo environment.\textsuperscript{130} Such systems may in time provide an opportunity to omit short-term animal studies (see overlap between explant incubation time and in vivo studies in Fig. 4). Thus, increased overlap in timeframes of in vitro and in vivo studies may help elucidate the isolated effect of loading, particularly in the in vivo set-up where the effect of loading cannot be isolated, and it may help comparing results between in vivo and in vitro.

An additional option to further align in vitro and in vivo results is by making the analytical methods more comparable. The papers reviewed here showed a wide variety of reported results, both of structural and mechanical aspects. Also some papers, particularly those on in vivo studies, merely report Mankin score as an outcome, which is a widely accepted and validated cartilage damage score but does not specify the nature of this damage. This also complicates comparison with in vitro studies. Related to the lack of information of structural damage of these studies, the effect of individual factors observed following trauma, such as surface cracking, network de-structuring, or cell death, are currently underexplored in vivo. Increased reporting of such information would make in vitro and in vivo studies more comparable, thereby potentially increasing our understanding of how load affects degeneration.

The final way in which in vitro studies can be linked to in vivo studies is through computational modeling. A full review of the current state of available computational models is beyond the scope of this review, but it is acknowledged that these models can provide valuable support. Computer models, once thoroughly validated, may assist in translation from geometrically simple to complex conditions, from short to long-term effects, or across length-scales. First, geometrical complexity generally challenges the accuracy of the mechanical or

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**Table 1. Overview of Experimental Methods to Assess the Cartilage Response to Overloading**

| Whole joint ↓ | Explant ↓ |
|----------------|----------------|
| • Uncertainties about distribution of stresses in the joint | • Unphysiological boundary conditions |
| • Including repair | • Possible to use human cadaveric tissue |
| • Long-term | |

**Living tissue →**

- Includes repair
- Long-term

**Animal (in vivo) studies**

- Real-life response
- Low motion control
- Uncertainties about translation to human
- Need to get ethical approval

**Culture studies**

- Can investigate cell phenotype
- Simulated environment

**Dead tissue →**

- No repair
- Short-term
- Possible to use human cadaveric tissue

**Total joint movement simulator**

- Motion control
- Possible to use human cadaveric tissue

**Structure-mechanical testing**

- Highest controllability and repeatability
- Cheapest

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**Figure 4.** Timeline of previous overloading studies and proposed timeline for future overloading studies.
biological behavior that can be incorporated. For example, some recently developed models for studying patient-specific knee joint structure and personal gait mechanics to predict the tissue's degenerative behaviour\textsuperscript{131–133} are geometrically complex, and may be useful to compare to in vivo data. However, such models can only be computed with less sophisticated cartilage material models that cannot be related to damage mechanisms at the tissue scale. In contrast, advanced material models\textsuperscript{134} are too computationally intensive to be used in patient-specific whole joint models, but they are helpful when interpreting mechanical details of in vitro studies on explant materials. Second, translating from short-term (related to in vitro mechanical details of in vitro studies on explant materials) to long-term effects (related to in vivo studies) requires algorithms for damage progression related to repeated mechanical overloading.\textsuperscript{132,135} In addition, incorporation of biological or pathological effects such as inflammatory conditions are important. Capturing such effects in a quantitative manner, with mathematical equations, is challenging, yet important to make future computational models more versatile and applicable to predict long-term changes. Simulations of bone adaptation,\textsuperscript{136} and fracture healing (study comparing various theories by Isaksson et al.\textsuperscript{137}), demonstrate the feasibility of making long-term predictions of tissue changes. However, making such long-term predictions for cartilage is still in its infancy. Third, translations across length-scales (multi-scale or multi-level approaches; reviewed by Halloran et al.\textsuperscript{134}) allow connections between the level of the musculoskeletal system, the joint, the tissue or the cell. The larger two levels represent the level at which in vivo experiments are performed, whereas the smaller scales relate more to in vitro studies. Finally, another aspect unique to computer modeling is the ability to compute parameters that are difficult to assess experimentally. This may contribute to translating results from in vivo to in vitro studies and vice versa, as distributions of the same variable can be computed, and observed effects can be related to this distribution. It should be recognized that the validity of computational models generally depends on implementation accuracy, the level of validation, and the suitability of the model to address the pertaining question. The choice of the model to use depends on the particular research question, and care must be taken not to over-interpret results in the domain in which the model is weak. Yet, the advantage of modeling is that they can be tuned either way, and therefore form an intermediate between in vitro and the in vivo results.

CONCLUSIONS

In conclusion, comparison of in vitro and in vivo studies based on study outcome parameters is complicated by the underlying experimental differences. However, the outcomes of in vitro and in vivo studies do not contradict each other. This encourages further improvement of in vitro systems, where loading can be the only experimental variable, to study effects in the highly complex complete joint in vivo. Efforts to bridge the gap between in vitro and in vivo studies could include (i) bringing in vitro set-ups closer to in vivo or vice versa, for example, by modifying loading protocols and/or experimental timeframes; (ii) synchronizing the analytical methods of both study types; and (iii) using computational models as a tool to corroborate in vitro results against in vivo predictions. Although one aspiration is to create in vitro models that closely resemble the in vivo situation, it will be highly challenging, if at all possible, to simulate the complete complex in vivo environment over time as depicted by black box #2 in Figure 3. For the foreseeable future it will therefore, for ethical reasons, be unavoidable to perform animal testing prior to application in humans. Appropriate in vitro tests prior to animal studies however may reduce the amount of animal studies and should therefore be further developed.

AUTHORS’ CONTRIBUTIONS

Substantial contributions to research design, or the acquisition, analysis or interpretation of data by MN and AH. Drafting the paper or revising it critically by MN, AH, KI, and CCvD. Approval of the submitted and final versions: MN, AH, KI, and CCvD.

ACKNOWLEDGMENTS

This work was performed under the framework of Chemelot InSciTee. The authors are also grateful for the contributions of Dr. A. Thambyah and Dr. A.C. Vrancken during the preliminary stage of writing this review. There were no conflicts of interest.

REFERENCES

1. Broom ND, Marra DL. 1985. New structural concepts of articular cartilage demonstrated with a physical model. Connect Tissue Res 14:1–8.
2. Burgin LV, Edelsten L, Aspden RM. 2014. The mechanical and material properties of elderly human articular cartilage subject to impact and slow loading. Med Eng Phys 36:226–232.
3. Bader DL, Kempson GE, Egan J, et al. 1992. The effects of selective matrix degradation on the short-term compressive properties of adult human articular cartilage. Biochimica et Biophysica Acta (BBA)—General Subjects. 1116:147–154.
4. Kempson GE, Muir H, Pollard C, et al. 1973. The tensile properties of the cartilage of human femoral condyles related to the content of collagen and glycosaminoglycans. Biochimica et Biophysica Acta (BBA)—General Subjects 297:456–472.
5. Broom ND, Chen MINH, Hardy A. 2001. A degeneration-based hypothesis for interpreting fibrillar changes in the osteoarthritic cartilage matrix. J Anat 199:683–698.
6. Madej W, Van Caam A, Blanev Davidson EN, et al. 2016. Ageing is associated with reduction of mechanically-induced activation of Smad2/3P signaling in articular cartilage. Osteoarthritis Cartilage 24:146–157.
7. Berenbaum F. 2013. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage 21:16–21.
8. Felson DT. 2013. Osteoarthritis as a disease of mechanics. Osteoarthritis Cartilage 21:10–15.
9. Blagojevic M, Jinks C, Jeffery A, et al. 2010. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage 18:24–33.

10. Bennell KL, Bowles KA, Wang Y, et al. 2011. Higher dynamic medial knee load predicts greater cartilage loss over 12 months in medial knee osteoarthritis. Ann Rheum Dis 70:1770–1774.

11. Leucht F, Dürselin L, Hogrefe C, et al. 2012. Development of a new biomechanically defined single impact rabbit cartilage trauma model for in vivo studies. J Invest Surg 25:235–241.

12. Malekipour F, Whitton C, Oetomo D, et al. 2013. Shock absorbing ability of articular cartilage and subchondral bone under impact compression. J Mech Behav Biomed Mater 26:127–135.

13. Silyn-Roberts H, Broom ND. 1990. Fracture behaviour of cartilage-on-bone in response to repeated impact loading. Connect Tissue Res 24:143–156.

14. Evers BJ, Dvoracek-Driksna D, Orth MW, et al. 2001. The extent of matrix damage and chondrocyte death in mechanically traumatized articular cartilage explants depends on rate of loading. J Orthop Res 19:779–784.

15. Flachsmann R, Broom ND, Hardy AE. 2001. Deformation and rupture of the articular surface under dynamic and static compression. J Orthop Res 19:1131–1139.

16. Krueger JA, Thiase P, Evers BJ, et al. 2003. The extent and distribution of cell death and matrix damage in impacted chondral explants varies with the presence of underlying bone. J Biomech Eng 125:114–119.

17. Sudeghi H, Shepherd DE, Espino DM. 2015. Effect of the variation of loading frequency on surface failure of bovine articular cartilage. Osteoarthritis Cartilage 23:2252–2258.

18. Kerin AJ, Coleman A, Wisnom MR, et al. 2003. Propagation of surface fissures in articular cartilage in response to cyclic loading in vitro. Clin Biomech 18:960–968.

19. Zimmerman NB, Smith DG, Potteren LA, et al. 1988. Mechanical disruption of human patellar cartilage by repetitive load in vitro. Clin Orthop Relat Res 229:302–307.

20. Thambyah A, Broom ND. 2010. How subtle structural changes associated with maturity and mild degeneration influence the impact-induced failure modes of cartilage-on-bone. Clin Biomech 25:737–744.

21. Thambyah A, Zhang G, Kim W, et al. 2012. Impact induced failure of cartilage-on-bone following creep loading: a microstructural and fracture mechanics study. J Mech Behav Biomed Mater 14:239–247.

22. Vertetamo A, Seedhom BB. 2007. Effect of a single impact loading on the structure and mechanical properties of articular cartilage. J Biomech 40:3580–3589.

23. de Bont F, Bril N, Schmitt R, et al. 2015. Evaluation of single-impact induced cartilage degeneration by optical coherence tomography. BioMed Res Int 2015:486794.

24. Jeffreys JF, Gregory DW, Aspden RM. 1995. Matrix damage and chondrocyte viability following a single impact load on articular cartilage. Arch Biochem Biophys 322:87–96.

25. Alexander PG, Song Y, Taboas JM, et al. 2013. Development of a spring-loaded impact device to deliver injurious mechanical impacts to the articular cartilage surface. Cartilage 4:52–62.

26. Bonnevie ED, Delco ML, Fortier LA, et al. 2015. Characterization of tissue response to impact loads delivered using a hand-held instrument for studying articular cartilage injury. Cartilage 6:226–232.

27. Chen CT, Bhargava M, Lin PM, et al. 2003. Time, stress, and location dependent chondrocyte death and collagen damage in cyclically loaded articular cartilage. J Orthop Res 21:888–898.

28. Chen CT, Burton-Wurster N, Lust G, et al. 1999. Compositional and metabolic changes in damaged cartilage are peak-stress, stress-rate, and loading-duration dependent. J Orthop Res 17:870–879.

29. Quinn TM, Allen RG, Schalet BJ, et al. 2001. Matrix and cell injury due to sub-impact loading of adult bovine articular cartilage explants: effects of strain rate and peak stress. J Orthop Res 19:242–249.

30. D’Lima DD, Hashimoto S, Chen PC, et al. 2001. Impact of mechanical trauma on matrix and cells. Clin Orthop Relat Res 391S:S90–S99.

31. Repo RU, Finlay JB. 1977. Survival of articular cartilage after controlled impact. J Bone Joint Surg Am 59:1068–1076.

32. Lee CM, Kizis JD, McIlwraith CW, et al. 2013. Development of an in vitro model of injury-induced osteoarthrisis in cartilage explants from adult horses through application of single-impact compressive overload. Am J Vet Res 74:40–47.

33. Atkinson TS, Haut RC, Altiero NJ. 1998. Impact-induced fissuring of articular cartilage: an investigation of failure criteria. J Biomech Eng 120:181–187.

34. Farquhar T, Xia Y, Mann K, et al. 1996. Swelling and fibronectin accumulation in articular cartilage explants after cyclical impact. J Orthop Res 14:417–423.

35. Kaplan JT, Neu CP, Drissi H, et al. 2017. Cyclic loading of human articular cartilage: the transition from compaction to fatigue. J Mech Behav Biomed Mater 65:734–742.

36. Morel V, Quinn TM. 2004. Cartilage injury by ramp compression near the gel diffusion rate. J Orthop Res 22:145–151.

37. Tzortzilis PA, Grigioni R, Borrelli JJ, et al. 1999. Effect of impact load on articular cartilage: cell metabolism and viability, and matrix water content. J Biomech Eng 121:433–441.

38. Bourne DA, Moo EK, Herzog W. 2015. Cartilage and chondrocyte response to extreme muscular loading and impact loading: can in vivo pre-load decrease impact-induced cell death? Clin Biomech 30:537–545.

39. Burgin LV, Aspden RM. 2008. Impact testing to determine the mechanical properties of articular cartilage in isolation and on bone. J Mater Sci Mater Med 19:703–711.

40. Workman J, Thambyah A, Broom ND. 2017. The influence of early degenerative changes on the vulnerability of articular cartilage to impact-induced injury. Clin Biomech 43:40–49.

41. Morel V, Mercay A, Quinn TM. 2005. Prestrain decreases cartilage susceptibility to injury by ramp compression in vitro. Osteoarthritis Cartilage 13:964–970.

42. Morel V, Berutto C, Quinn TM. 2006. Effects of damage in the articular surface on the cartilage response to injurious compression in vitro. J Biomech 39:924–930.

43. Haut RC, Ide TM, De Camp CE. 1995. Mechanical responses of the rabbit patello-femoral joint to blunt impact. J Biomech Eng 117:402–408.

44. Rolauf B, Muehleman C, Li J, et al. 2010. Vulnerability of the superficial zone of immature articular cartilage to compressive injury. Arthritis Rheum 62:3016–3027.

45. Nickien M, Thambyah A, Broom ND. 2015. How a radial focal incision influences the internal shear distribution in articular cartilage with respect to its zonally differentiated microanatomy. J Anat 227:315–324.

46. Jang KW, Buckwalter JA, Martin JA. 2014. Inhibition of cell-matrix adhesions prevents cartilage chondrocyte death following impact injury. J Orthop Res 32:448–454.

47. Patwari P, Gaschen V, James IE, et al. 2004. Ultrastructural quantification of cell death after injurious compression of bovine calf articular cartilage. Osteoarthritis Cartilage 12:245–252.
48. Chen CT, Burton-Wurster N, Borden C, et al. 2001. Chondrocyte necrosis and apoptosis in impact damaged articular cartilage. J Orthop Res 19:703–711.

49. Clements KM, Bee ZC, Crossingham GV, et al. 2001. How severe must repetitive loading be to kill chondrocytes in articular cartilage? Osteoarthritis Cartilage 9:499–507.

50. Lin PM, Chen CT, Torzilli PA. 2004. Increased stromelysin-1 (MMP-3), proteoglycan degradation (3B3 and 7D4) and collagen damage in cyclically load-injured articular cartilage. Osteoarthritis Cartilage 12:485–496.

51. Torzilli PA, Deng XH, Ramcharan M. 2006. Effect of compressive strain on cell viability in statically loaded articular cartilage. Biomech Model Mechanobiol 5:125–132.

52. Bartell LR, Fortier LA, Bonassar LJ, et al. 2015. Measuring microscale strain fields in articular cartilage furing rapid impact reveal thresholds for chondrocyte death and a protective role for the superficial layer. J Biomech 48:3440–3446.

53. Loening AM, James IE, Levenston ME, et al. 2000. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys 381:205–212.

54. Lucchetti E, Adama CS, Horton WEJ, et al. 2002. Cartilage viability after repetitive loading: a preliminary report. Osteoarthritis Cartilage 10:71–87.

55. Lewis JL, Deloria LB, Oyen-Tiesma M, et al. 2003. Cell death after cartilage impact occurs around matrix cracks. J Orthop Res 21:881–887.

56. Morel V, Quinn TM. 2004. Short-term changes in cell and matrix damage following mechanical injury of articular cartilage explants and modelling of microphysical mediators. Biomech Biomed Eng 41:509–519.

57. Stolberg-Stolberg JA, Furman BD, Garriques NW, et al. 2013. Effects of cartilage impact with and without fracture on chondrocyte viability and the release of inflammatory markers. J Orthop Res 31:1283–1292.

58. Bush PG, Hodgkinson PD, Hamilton GL, et al. 2005. Viability and volume of in situ bovine articular chondrocytes—changes following a single impact and effects of medium osmolarity. Osteoarthritis Cartilage 13:54–65.

59. Backus JD, Furman BD, Swimner T, et al. 2011. Cartilage viability and catabolism in the intact porcine knee following transarticular impact loading with and without articular fracture. J Orthop Res 29:501–510.

60. Chahine NO, Ateshian GA, Hung CT. 2007. The effect of mechanical compression on the loss of newly synthesized proteoglycans compression of cartilage/bone explants in vitro leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. J Orthop Res 20:1265–1273.

61. Nickien M, Thambyah A, Broom ND. 2013. How changes in microscale strain fields in articular cartilage correlate with the macrolevel behavior of articular cartilage. WIREs Syst Biol Med 5:495–509.

62. Broom ND. 1986. Structural consequences of traumatizing articular cartilage. Ann Rheum Dis 45:225–234.

63. Bank RA, Soudry M, Maroudas A, et al. 2000. The increased swelling and instantaneous deformation of osteoarthritic cartilage is highly correlated with collagen degradation. Arthritis Rheum 43:2202–2210.

64. Maroudas A. 1976. Balance between swelling pressure and collagen tension in normal and degenerate cartilage. Nature 260:808–809.

65. Venn M, Maroudas A. 1977. Chemical composition and swelling of normal and osteoarthritic femoral head cartilage. J. chemical composition. Ann Rheum Dis 36:121–129.

66. Maroudas A, Venn M. 1977. Chemical composition and swelling of normal and osteoarthritic femoral head cartilage. II. swelling. Ann Rheum Dis 36:399–406.

67. Broom ND. 1984. Further insights into the structural principles governing the function of articular cartilage. J Anat 139:275–294.

68. Kaleem B, Maier F, Drissi H, et al. 2017. Low-energy impact of human cartilage: predictors for microcracking the network of collagen. Osteoarthritis Cartilage 25:544–553.

69. Waters NP, Stoker AM, Carson WL, et al. 2014. Biomarkers affected by impact velocity and maximum strain of cartilage during injury. J Biomech 47:3185–3195.

70. Bentle AM, Bean JS, Hulman JF. 1991. Passive role of articular chondrocytes in the pathogenesis of acute meniscectomy-induced cartilage degeneration. Vet Pathol 28:207–215.

71. Thibault M, Poole AR, Buschmann MD. 2002. Cyclic compression of cartilage/bone explants in vitro leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. J Orthop Res 20:1265–1273.

72. Stolberg-Stolberg JA, Furman BD, Garriques NW, et al. 2013. Effects of cartilage impact with and without fracture on chondrocyte viability and the release of inflammatory markers. J Orthop Res 31:1283–1292.
87. Alexander PG, McCarron JA, Levine MJ, et al. 2012. An in vivo lapine model for impact-induced injury and osteoarthritic degeneration of articular cartilage. Cartilage 3: 323–333.
88. Borrelli JJ, Silva MJ, Zaege MA, et al. 2009. Single high-energy impact load causes post-traumatic OA in young rabbits via a decrease in cellular metabolism. J Orthop Res 27:347–352.
89. Borrelli JJ, Zaege MA, Martinez MD, et al. 2010. Diminished cartilage creep properties and increased trabecular bone density following a single, sub-fracture impact of the rabbit femoral condyle. J Orthop Res 28:1307–1314.
90. Ewers BJ, Newberry WN, Haut RC. 2000. Chronic softening of cartilage without thickening of underlying bone in a joint trauma model. J Biomech 33:1689–1694.
91. Cho H, Pinkhassik E, David V, et al. 2015. Detection of early cartilage damage using targeted nanosomes in a post-traumatic osteoarthritic mouse model. Nanomedicine 11: 939–946.
92. Dekel S, Weissman SL. 1978. Joint changes after overuse and peak overloading of rabbit knees in vivo. Acta Orthopaedica Scandinavica 49:519–528.
93. Christiansen BA, Anderson MJ, Lee CA, et al. 2012. Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis. Osteoarthritis Cartilage 20:773–782.
94. Arunakul M, Tochigi Y, Goetz JE, et al. 2013. Replication of chronic abnormal cartilage loading by medial meniscus destabilization for modeling osteoarthritis in the rabbit knee in vivo. J Orthop Res 31:1555–1560.
95. Horisberger M, Fortuna R, Valderrabano V, et al. 2013. Long-term repetitive mechanical loading of the knee joint by in vivo muscle stimulation accelerates cartilage degeneration and increases chondrocyte death in a rabbit model. Clin Biomech 28:536–543.
96. Brophy RH, Martinez M, Borrelli JJ, et al. 2012. Effect of combined traumatic impact and radial transection of medial meniscus on knee articular cartilage in a rabbit in vivo model. Arthroscopy 28:1490–1496.
97. O'Byrne EM, Parker DT, Roberts ED, et al. 1995. Oral administration of a matrix metalloproteinase inhibitor, CGS 27023A, protects the cartilage proteoglycan matrix in a partial meniscectomy model of osteoarthritis in rabbits. Inflammation Res 44:S117–S118.
98. Cake MA, Read RA, Guille B, et al. 2000. Modelfication of articular cartilage and subchondral bone pathology in an ovine meniscectomy model of osteoarthritis by avocados and soya unsaponifiables (ASU). Osteoarthritis Cartilage 8:404–411.
99. Cake MA, Read RA, Appleyard RC, et al. 2004. The nitric oxide donor glyceryl trinitrate increases subchondral bone sclerosis and cartilage degeneration following ovine meniscectomy. Osteoarthritis Cartilage 12:974–981.
100. Fischenich KM, Button KD, Coatney GA, et al. 2015. Chronic changes in the articular cartilage and meniscus following traumatic impact to the lapine knee. J Biomech 48:246–252.
101. Fischenich KM, Button KD, DeCamp C, et al. 2016. Comparison of two models of post-traumatic osteoarthritis: temporal degradation of articular cartilage and meniscus. J Orthop Res 35:486–495.
102. Hanashi D, Koshino T, Usugi M, et al. 2002. Effect of femoral nerve resection on progression of cartilage degeneration induced by anterior cruciate ligament transection in rabbits. J Orthop Sci 7:672–676.
103. Amiable N, Martel-Pelletier J, Lussier B, et al. 2011. Proteinase-activated receptor-2 gene disruption limits the effect of osteoarthritis in mice: a novel target in joint degradation. J Rheumatol 38:911–920.
104. Coyle CH, Henry SE, Haleem AM, et al. 2012. Serum CTXii correlates with articular cartilage degeneration after anterior cruciate ligament transection or arthroplasty followed by standard exercise. Sports Health 4:510–517.
105. Elsaid KA, Zhang L, Shaman Z, et al. 2015. The impact of early intra-articular administration of interleukin-1 receptor antagonist on lubricin metabolism and cartilage degeneration in an anterior cruciate ligament transection model. Osteoarthritis Cartilage 23:114–121.
106. Hayami T, Pickarski M, Wesołowski GA, et al. 2004. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis Rheum 50:1193–1206.
107. Hayami T, Pickarski M, Zhuo Y, et al. 2006. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone 38:234–243.
108. Hayami T, Zhuo Y, Wesołowski GA, et al. 2012. Inhibition of cathepsin K reduced cartilage degeneration in the anterior cruciate ligament transection rabbit and murine models of osteoarthritis. Bone 50:1250–1259.
109. Appleyard RC, Ghosh P, Swain MV. 1999. Biomechanical, histological and immunohistological studies of patellar cartilage in an ovine model of osteoarthritis induced by lateral meniscectomy. Osteoarthritis Cartilage 7:281–294.
110. Burger C, Mueller M, Wlodarczyk P, et al. 2007. The sheep as a knee osteoarthritis model: early cartilage changes after meniscus injury and repair. Lab Anim 41:420–431.
111. Desando G, Giavaresi G, Cavallio C, et al. 2016. Autologous bone marrow concentrate in a sheep model of osteoarthritis: new perspectives for cartilage and meniscus repair. Tissue Eng Part C Methods 22:608–619.
112. Du G, Zhan H, Ding D, et al. 2016. Abnormal mechanical loading induces cartilage degeneration by accelerating meniscus hypertrophy and mineralization after ACL injuries in vivo. Am J Sports Med 44:652–663.
113. Berjon JJ, Munuera L, Calvo M. 1991. Degenerative lesions in the articular cartilage after meniscectomy: preliminary study in dogs. J Trauma 31:342–350.
114. Boileau C, Martel-Pelletier J, Jouzeau JY, et al. 2002. Licoférol (ML-3000), a dual inhibitor of 5-lipoxygenase and cyclooxygenase, reduces the level of cartilage chondrocyte death in vivo in experimental dog osteoarthritis: inhibition of the pro-aptotic factors. J Rheumatol 29:1446–1453.
115. Brandt KD, Thonar EJ. 1989. Lack of association between serum keratan sulfate concentrations and cartilage changes of osteoarthritis after transection of the anterior cruciate ligament in the dog. Arthritis Rheum 32:647–651.
116. Desrochers J, Amrein MA, Matyas JR. 2010. Structural and functional changes of the articular surface in a post-traumatic mode of early osteoarthrisis measured by atomic force microscopy. J Biomech 43:3091–3098.
117. Elliott DM, Guilak F, Vail TP, et al. 1999. Tensile properties of articular cartilage are altered by meniscectomy in a canine model of osteoarthritis. J Orthop Res 17:503–508.
118. Ghosh P, Sutherland JM, Taylor TK, et al. 1983. The role of subchondral bone remodeling in osteoarthritis: reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis Rheum 50:1193–1206.
119. Cho H, Pinkhassik E, David V, et al. 2015. Detection of early cartilage damage using targeted nanosomes in a post-traumatic osteoarthritic mouse model. Nanomedicine 11: 939–946.
120. Ploegmakers JJ, van Roermund PM, van Melkebeek J, et al. 2016. Joint distraction attenuates osteoarthritis by reducing secondary inflammation, cartilage degeneration and subchondral bone aberrant change. Osteoarthritis Cartilage 23:1728–1735.
121. Elliott DM, Guilak F, Vail TP, et al. 1999. Tensile properties of articular cartilage are altered by meniscectomy in a canine model of osteoarthritis. J Orthop Res 17:503–508.
121. van Valburg AA, van Roermund PM, Marijnissen AC, et al. 2000. Joint distraction in treatment of osteoarthritis (II): effects on cartilage in a canine model. Osteoarthritis Cartilage 8:1–8.

122. van Valburg AA, van Roermund PM, Marijnissen AC, et al. 1999. Joint distraction in treatment of osteoarthritis: a two-year follow-up of the ankle. Osteoarthritis Cartilage 7:474–479.

123. Wiegant K, Intema F, van Roermund PM, et al. 2015. Evidence of cartilage repair by joint distraction in a canine model of osteoarthritis. Arthritis Rheumatol 67:465–474.

124. Birmingham TB, Moyer R, Leitch K, et al. 2017. Changes in biomechanical risk factors for knee osteoarthritis and their association with 5-year clinically important improvement after limb realignment surgery. Osteoarthritis Cartilage 25:1999–2006.

125. Moyer R, Birmingham TB, Bryant D, et al. 2015. Valgus bracing for knee osteoarthritis: a meta-analysis of randomized trials. Arthritis Care Res 67:493–501.

126. Moyer R, Birmingham TB, Bryant D, et al. 2015. Biomechanical effects of valgus knee bracing: a systematic review and meta-analysis. Osteoarthritis Cartilage 23: 178–188.

127. Zhang W, Robertson J, Jones AC, et al. 2008. The placebo effect and its determinants in osteoarthritis: meta-analysis of randomised controlled trials. Ann Rheum Dis 67:1716–1723.

128. Guermazi A, Alizai H, Crema MD, et al. 2015. Compositional MRI techniques for evaluation of cartilage degeneration in osteoarthritis. Osteoarthritis Cartilage 23: 1639–1653.

129. Schwab A, Meeuwsen A, Ehlicke F, et al. 2017. Ex vivo culture platform for assessment of cartilage repair treatment strategies. Altern Anim Exp 34:267–277.

130. van Haafken EE, Ito K, van Donkelaar CC. 2017. The initial repair response of articular cartilage after mechanically induced damage. J Orthop Res 35:1265–1273.

131. Halloran JP, Sibole S, van Donkelaar CC, et al. 2012. Multiscale mechanics of articular cartilage: potentials and challenges of coupling musculoskeletal, joint, and microscale computational models. Ann Biomed Eng 40:2456–2474.

132. Mononen ME, Tanska P, Isakkson H, et al. 2016. A novel method to simulate the progression of collagen degeneration of cartilage in the knee: data from the osteoarthritis initiative. Sci Rep 6:21415.

133. Guo H, Santner TJ, Lerner AL, et al. 2017. Reducing uncertainty when using knee-specific finite element models by assessing the effect of input parameters. J Orthop Res 35:2233–2242.

134. Wilson W, Huyghe JM, van Donkelaar CC. 2006. A composition-based cartilage model for the assessment of compositional changes during cartilage damage and adaptation. Osteoarthritis Cartilage 14:554–560.

135. Hosseini SM, Wilson W, Ito K, et al. 2014. A numerical model to study mechanically induced initiation and progression of damage in articular cartilage. Osteoarthritis Cartilage 22:95–103.

136. Huiskes R, Ruimerman R, van Lenthe GH, et al. 2000. Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. Nature 405:704–706.

137. Isaksson H, Wilson W, van Donkelaar CC, et al. 2006. Comparison of biophysical stimuli for mechanado-regulation of tissue differentiation during fracture healing. J Biomech 39:1507–1516.