The Influence of Mechanical Stimuli on Articular Cartilage Tissue Engineering

C. Lee, S. Grad, M. Wimmer and M. Alini*

Summary

I t has been well-established that mechanical stimuli are crucial to the healthy development and maintenance of native articular cartilage. Consequently, physical factors have become an important element in articular cartilage tissue engineering. This article reviews how mechanical loading (i.e. dynamic compression, fluid shear, tissue shear, and hydrostatic pressure) has been used to direct the development of tissue engineered articular cartilage. The effects of mechanical stimuli are assessed with respect to extracellular matrix (ECM) production, gene expression, and the development of tissue functionality (i.e. mechanical stiffness and surface lubrication). In the context of this discussion, the modes of mechanotransduction from the tissue level to the cellular level and the generation of the cellular response are also considered. Finally, the role of theoretical modeling to predict the influence of mechanical stimuli is discussed.

*Correspondence to: M. Alini, Biomaterials and Tissue Engineering, AO Research Institute, Davos Platz, Switzerland.
E-mail: mauro.alini@aofoundation.org

Introduction

Articular cartilage tissue engineering has been a rapidly growing area of research for bioengineers over the past one and half decades. Over the past five years, the evolving field of "functional tissue engineering" has become increasingly popular. In functional tissue engineering, controlled mechanical loadings are applied to developing constructs in an attempt to direct the formation of a more biomechanically-competent tissue. The purpose of this article is to summarize how various mechanical stimuli have been used to influence tissue engineering of articular cartilage. After a brief review of the structural and functional biology of articular cartilage, we evaluate the recent literature on the effects of mechanical loading on native and tissue engineered articular cartilage. In the development of a functional tissue engineering protocol, it is essential to understand how mechanical forces stimulate the desired response. Therefore, we review here some of the proposed mechanotransduction pathways. Finally, in looking towards the future of articular cartilage tissue engineering, we discuss the role of computer modeling in predicting mechanically-driven tissue development.

Articular cartilage biology

Structure

Articular cartilage is a highly specialized connective tissue that provides a nearly frictionless bearing surface, while also absorbing and transmitting compressive, tensile, and shear forces across diarthrodial joints (i.e. knee, hip, knuckles, etc.). Articular cartilage is 65-80% by weight water with the solid components of the matrix being predominately type II collagen (50-90% of the dry weight and lesser amounts of types VI, IX, X and XI collagens) and large proteoglycans (5-10% dry weight) (1). The relative

amounts and organization of collagen and proteoglycan vary through the depth of the
tissue, reflecting the variable distribution of load through the tissue. The collagenous
components of the matrix provide tensile and shear strength to the tissue. The
orientation of the collagen network transitions from parallel to the joint surface in the
superficial zone to perpendicular to the subchondral bone in the deep zones (2). This
arcuate structure is optimal for resisting shear forces along the surface and compressive
forces in the deep zones. The highly negatively-charged proteoglycans draw water into
the tissue, creating a swelling pressure which, coupled with the charge repulsion, allows
articular cartilage to resist high compressive forces. The distribution of proteoglycans
also reflects the zonal functions of articular cartilage, with the superficial layer having a
relatively high concentration of the lubricating protein SZP (superficial zone protein,
also known as lubricin, megakaryocyte stimulating factor, or proteoglycan 4) (3) and the
deeper zones having a higher concentration of large aggregating proteoglycans
(aggrecan).

Damage/disease of articular cartilage

Normal joint function depends on the low joint friction and absorption and transmission
of loads afforded by healthy articular cartilage. Even relatively small changes in the
integrity, composition, or organization of the cartilage matrix will alter its mechanical
properties and compromise its functionality. Unfortunately, adult articular cartilage has
a limited capacity for natural regeneration, making osteoarthritis, commonly referred to
as the "wear-and-tear" disease of articular cartilage, one of the ten most disabling
diseases in developed countries (4). Osteoarthritis disables approximately 10% of
individuals over the age of 60, with an estimated 20.7 million Americans suffering from
osteoarthritis (5), at a cost to the American economy of an estimated $60 billion per year
in direct and indirect costs. The etiology of osteoarthritis is multi-factorial, but it is
commonly believed that unrepaired focal lesions (i.e. due to injury) can predispose the
joint to degenerative changes, characteristic of osteoarthritis. Thus, the aim of articular

cartilage tissue engineering is to repair these focal defects before they progress to wide-spread joint degeneration.

Tissue engineering of articular cartilage

The seemingly simple nature of articular cartilage – there is a single cell type, the chondrocyte, and the tissue is avascular and aneural – coupled with the huge clinical problem of chondral injury and osteoarthritis, has made articular cartilage a prime target for tissue engineers.

The aim of tissue engineering is to develop a viable replacement for damaged tissue. Traditionally, tissue engineering has been a three-pillar system, based on the biological components of healthy tissues: scaffold (i.e. extracellular matrix), cells, and signaling factors. For recent reviews of various scaffolds, cells, and growth factors that have been used in articular cartilage tissue engineering, see (6-9). Signaling factors traditionally include cytokines, growth factors, and other regulatory molecules that modulate cellular metabolism, but for load-bearing tissues, such as articular cartilage, physical forces are also important signaling factors for the development and maintenance of a functional tissue.

Effects of mechanical loading

At the joint level, articular cartilage is loaded primarily in compression. In the human hip joint, contact pressures have been measured to be 1 MPa during static standing, ranging from 0.1 to 4 MPa while walking, and up to 20 MPa while standing up from a chair or jumping (10, 11). Compressive loading at the joint level affects many different physical, electrical, and biochemical phenomena at the cellular level, including deformation, fluid flow, hydrostatic pressure, streaming potentials, osmotic pressure,

nutrient and ion concentration gradients, and pH changes (12, 13). Functional tissue engineering has typically focused on four types of physical stimuli – tissue level dynamic compression and the associated phenomena of fluid flow-induced shear, tissue shear, and hydrostatic pressure.

Compression

It has been well-established that compressive loading of cartilage explants can modulate chondrocyte viability (14), gene expression (15-18), and biosynthesis of various ECM molecules (14, 18-21). For example, dynamic compression at moderate levels (2-10% strain (18, 21); or 0.5-1.0 MPa (14, 20)) and physiological frequencies (0.01 to 1.0 Hz) can stimulate the biosynthesis of collagen (21), proteoglycan (18, 20, 21) and fibronectin (14).

Based on this knowledge, numerous short- and long-term studies have used unconfined dynamic compression protocols, spanning a wide range of frequencies (0.001 to 1.0 Hz), strains (3-15%) and stresses (0.5-2.5 MPa), to a variety of tissue engineering systems using hydrogels or macroporous scaffolds and differentiated, undifferentiated, or de-differentiated cells to stimulate cell differentiation, proliferation, biosynthetic activity, and the development of a functional ECM.

Short-term (hours to days) studies have demonstrated the potential benefits of applying dynamic compression on ECM biosynthesis rates by chondrocytes seeded in a range of scaffolds, including agarose hydrogels (22, 24), self-assembling peptide hydrogels (25), and macroporous PLA/PGA scaffolds (26). Using a chondrocyte-agarose gel system, Buschmann *et al.* found that dynamic loading (3%, 0.01-1 Hz over 10 hours) increased proteoglycan synthesis 6-25% and protein synthesis by 10-35%, depending on the amount of time in culture prior to loading and the frequency of loading (22). Lee *et al.* also used an agarose system and found that dynamic compression at 15% strain, 1 Hz led to a 50% increase in glycosaminoglycan synthesis by deep-zone chondrocytes (23, 24) and a 40% increase in proliferation of superficial-zone chondrocytes (24).

Dynamic compression can also positively affect de-differentiated and undifferentiated chondrocytes. Protein and proteoglycan biosynthesis rates by de-differentiated canine chondrocytes seeded in type II collagen scaffolds increased two-fold in response to dynamic compression (±3% strain, 0.1 Hz) (27). Additionally, monolayer-expanded human articular chondrocytes seeded in three-dimensional PEGT/PBT scaffolds showed increased rates of glycosaminoglycan synthesis when dynamically compressed (±5% strain, 0.1 Hz), provided that there was sufficient ECM deposition prior to mechanical loading (28). Dynamic compression has also been shown to promote chondrogenic differentiation, as indicated by cartilage nodule formation (29, 30), glycosaminoglycan synthesis (31, 32), and gene expression (33) of chick limb-bud mesenchymal cells (0-9 kPa stress, 0.15-0.33 Hz) (34, 35) and rabbit bone marrow mesenchymal cells (±10% strain, 1 Hz) (33) seeded in agarose gels.

Interestingly, several recent studies investigating the effects of mechanical loading on gene expression of the cells in tissue engineering systems have failed to show a change in gene expression with similar dynamic compression loadings that have stimulated protein biosynthesis. Hunter *et al.* found no change in collagen I, collagen II, or aggrecan gene expression by bovine chondrocytes seeded in collagen gels when subjected to dynamic compressions of ±4% strain at 1 Hz (36). Similarly, although dynamic compression stimulated protein biosynthesis by expanded human articular chondrocytes in PEGT/PBT foams, there were no changes in aggrecan, versican, collagen I, collagen II, or the transcription factor SOX9 (Sex determining region Y-box 9) mRNA levels (37). The lack of gene response suggests that mechanical compression affects the synthesis of these matrix proteins and activation of the SOX9 transcription factor post-transcriptionally. This may be an evolutionary adaptation to conserve the cells' resources – as long as the mechanical loads remain within a physiological range, if transcription were to be up- or down-regulated with every perturbation in the mechanical environment, then cells would be expending a great deal of energy to adjust to the frequently changing conditions.

The benefits of dynamic compression on the development of a functional ECM can be more fully appreciated only in longer-term studies. Mauck *et al.* found a 2-fold increase in proteoglycan and a 3-fold increase in collagen accumulation in agarose constructs subjected to intermittent compressive strains (±10% strain at 1 Hz, for 3 consecutive cycles per day of 1 hour on/1 hour off, 5 days per week) for four weeks (38). This loading protocol also resulted in a more uniform distribution of type II collagen and a more biomechanically-functional construct, with the aggregate modulus of 24.5 kPa being approximately two-fold higher than that of free-swelling controls. Over long-term culture, it appears that very little stimulation is required to produce significant changes in biochemical and biomechanical properties. Chondrocyte-seeded peptide gels subjected to loading every second day (45 minutes of ± 2.5% strain, 1 Hz loading, followed by 5.25 hours of free-swelling culture, repeated 4 times/day every other day) contained 22% more glycosaminoglycan, had an 18% increase in equilibrium compressive modulus, and had a 60-70% increase in dynamic compressive stiffness compared to unloaded controls after 39 days of culture (25). In another study using chondrocytes seeded in porous calcium phosphate scaffolds, it was found that only six minutes of dynamic compression at ±5% strain, 1 Hz per day were needed to stimulate a 40% increase in collagen content, a 30% increase in proteoglycan content, and a three-fold increase in equilibrium modulus over a four-week period (39).

Although dynamic compression at physiological levels generally has positive effects on ECM biosynthesis, there are also several studies that demonstrated negative effects of dynamic compression on cartilage development. Various investigations have shown that cyclic loading leads to an increased release of matrix molecules (25-27, 40-42). Additionally, prolonged continuous loading has been shown to lead to inferior mechanical and biochemical properties in chondrocyte-seeded fibrin hydrogels subjected to continuous compression (±4% strain, 0.1 or 1.0 Hz) for 10 or 20 days (40). Similarly, Kisiday *et al.* found that daily intermittent compression (0.5 hours loading/0.5 hours free-swelling or 1 hour loading/1-7 hours free-swelling) suppressed sulfate

incorporation whereas alternate day loading (4 x 45 minute loading cycles applied every other day) stimulated sulfate incorporation in chondrocyte-agarose constructs (25).

Fluid flow

The effects of dynamic compression may be due to a number of factors including fluid flow, tissue and cell deformation, and/or hydrostatic pressurization. *In vitro* studies have shown both positive and negative effects of fluid flow-induced shear on chondrocyte metabolism. Monolayer-cultures of chondrocytes that were exposed to flow-induced shear stresses ranging from 0.16 to 0.6 Pa had elevated rates of proteoglycan synthesis but also had increased prostaglandin E2 and nitric oxide synthesis, higher rates of apoptosis, and a down-regulation of collagen II and aggrecan mRNA expression (43, 44). At the tissue level, compression-induced fluid flow may be stimulatory by increasing the rate of transport of nutrients and growth factors. The potential importance of such convective transport has been demonstrated in a study showing that dynamic compression acts synergistically with IGF-1 to increase biosynthetic activity (45).

In the context of cartilage tissue engineering, the effects of fluid flow-induced convective transport and shear have been exploited using spinner flask, rotating-wall, and perfusion culture systems. Spinner flasks, in which constructs are suspended in the midst of mechanically stirred culture media, create turbulent flow fields around the constructs. This system increases mass transfer to the constructs, but the turbulent flow generated at the construct surface induced the formation of a fibrous outer capsule and inferior mechanical properties of chondrocyte-seeded polymer constructs (46). Rotating wall bioreactors, in contrast, produce laminar flow fields with shear stresses of approximately 0.08 Pa along the construct surface (47). Cultivation in rotating wall bioreactors has been shown to lead to increased mechanical and biochemical properties in polyglycolic acid (PGA) scaffolds (46). Using a concentric cylinder bioreactor, Saini and Wick found that high shear stresses created by steady fluid flow over chondrocyte-seeded polylactic acid (PLA) scaffolds suppressed glycosaminoglycan accumulation and

favored collagen deposition, but dynamic flow promoted extensive glycosaminoglycan and collagen accumulation (48). These three systems increase mass transfer to the constructs and create shear forces at the surface of the constructs, with little influence on the interior of the constructs. Raimondi *et al.* developed a perfusion system which produces a median shear stress of 0.003 Pa (as estimated by a computational fluid dynamics model) through the entire construct and found that this system led to increased cellularity and better structural integrity of constructs (47).

As with bioreactors that modulate chondrocyte behavior by cyclic compression, the effects seen in these studies applying fluid shear may be due to more than the effect of physical stimuli of the fluid-induced shear. Fluid flow through and around tissue engineered constructs also undoubtedly increases nutrient transport. That IGF-1 and cultivation in a rotating wall bioreactor synergistically increase the mechanical and biochemical properties of PGA scaffolds indicates the role of mechanical stimulation on nutrient transport (49).

Electrical fields

Interstitial fluid flow also generates streaming potentials and currents as the cations of the fluid phase move past the fixed negative charge of the proteoglycan network (50, 51). These induced electrical effects can also directly influence chondrocyte metabolism and differentiation. Electrical currents have been shown to stimulate protein synthesis in articular cartilage explants (52). Additionally, monolayer chondrocyte cultures exposed to pulsed electric fields had higher collagen II and aggrecan mRNA levels and protein synthesis that varied in a time-, frequency- and voltage-dependent manner compared to non-pulsed cultures (53). Furthermore, low frequency pulsed electric-magnetic fields have been shown to increase the rate of chondrogenic differentiation during endochondral ossification (54). In contrast to those studies indicating a positive chondrogenic effect of electrical fields, however, an earlier study with growth plate chondrocytes cultured in monolayer reported a negative effect of electric fields on the

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rate of protein biosynthesis (55). It is unclear whether the differences in results are due to the source of chondrocytes or the specific nature of the applied electrical field.

Despite these known effects of electrical fields on chondrocyte behavior, to our knowledge, the role of electrical fields in articular tissue engineering has not yet been explored. It is important to note that fluid-flow induced streaming potentials that are generated in mature tissue upon compression would not occur (or occur in a much more limited manner) upon compression of developing tissue engineering constructs which lack the fixed-charge density (i.e. proteoglycan-dense ECM) of mature tissue. Thus, to realize the potential benefits of electrical stimulation, electrical fields would likely have to be directly applied to the constructs.

Tissue shear

To isolate the effects of tissue deformation from phenomena related to fluid flow, Frank *et al.* (56) and Jin *et al.* (57) applied direct shear to cartilage explants. Dynamic shear deformation (1-3% strain, 0.01-1.0 Hz) was shown to stimulate collagen and proteoglycan biosynthesis 50% and 25%, respectively (57). Interestingly, shear loading preferentially up-regulated collagen biosynthesis over proteoglycan biosynthesis (2:1), whereas dynamic compression leads to a nearly identical up-regulation of collagen and proteoglycan biosynthesis for the same bovine cartilage explant system (21).

Thus far, only one group has attempted to directly exploit tissue shear strain to modulate the *in vitro* development of cartilage constructs. Waldman *et al.* cultured chondrocytes in porous calcium phosphate scaffolds for four weeks under free-swelling culture followed by four weeks of culture with daily dynamic shear strain (58). Constructs subjected to six or 30 minutes of cyclic shear strain (2% shear strain at 1 Hz, superimposed on a 5% compressive tare strain) per day had higher rates of collagen and proteoglycan synthesis (58). After four weeks of daily six minute loadings, loaded constructs contained 40% more collagen and 35% more proteoglycan than free-swelling controls (58). Constructs exposed to shear forces also had a significantly higher

equilibrium modulus and maximum stress (six- and three-fold increases, respectively) (58). These increases over statically-cultured constructs are similar to the increases reported for similar constructs under dynamic compression (39).

Hydrostatic pressure

When cartilage is loaded, there is an initial resistance to fluid flow that results in the generation of hydrostatic pressure within the tissue. Although the effects of hydrostatic pressure would seem to be more straight-forward, since the application of hydrostatic pressure produces a single stimulus – pressurization, there are conflicting results regarding the effects of this loading on chondrocyte behavior. Static hydrostatic pressure (5 MPa) applied to agarose gel-embedded bovine chondrocytes can up-regulate aggrecan (4-fold) and type II collagen (50%) mRNA levels (59) and proteoglycan biosynthesis (60). Similar levels of static pressure, however, have been shown to suppress proteoglycan biosynthesis in bovine cartilage explants (61). Cyclic hydrostatic pressurization has been shown to stimulate proteoglycan synthesis in both bovine (5-15 MPa) (20,61) and human (0.2 MPa, 30 min on/2 min off) cartilage explants (62) and normal human (63) and bovine (64, 65) monolayer chondrocyte cultures. Another study, however, found that monolayer-cultured bovine chondrocytes had lower rates of proteoglycan synthesis when subjected to cyclic hydrostatic pressure (5 MPa, 0.003-0.5 Hz, 1.5-20 hours) (20). Chondrocytes isolated from osteoarthritic cartilage are also responsive to hydrostatic pressure, but it is unclear whether it is a positive or a negative response. Cyclic hydrostatic pressure has been shown to have protective effects, down-regulating the release of matrix metalloproteinase and pro-inflammatory mediator (10 MPa, 1 Hz for 6-24 hours) (66), but also triggered apoptosis and increased the mRNA expression of TNF-α (tumor necrosis factor-α) and iNOS (inducible nitric oxide synthase) (5 MPa, 1 Hz for 4 hours) (67) of monolayer-cultured human osteoarthritic chondrocytes.

The benefits of cyclic hydrostatic pressure on regulation of the development of tissue engineered constructs have also been explored. Intermittent hydrostatic pressure at 3.44

and 6.87 MPa (5 seconds pressurized/15 seconds depressurized, applied for 20 minute intervals every 4 hours for 5 weeks) was found to increase the amount of glycosaminoglycan accumulation in equine chondrocyte-seeded PGA meshes (68). In the same system, the 6.87 MPa stimulation also increased collagen production (68). Mizuno *et al.* have also used cyclic hydrostatic pressure (2.8 MPa, 0.015 Hz) to increase proteoglycan accumulation in bovine chondrocyte-seeded porous collagen scaffolds over a 15-day culture period (69).

Complex loading

Loading of articular cartilage *in vivo* is much more complicated than simple compression, shear, or pressurization. To address the complex interaction of joint-level compression, shear, and articulating motion, we have developed a bioreactor that allows for simultaneous compression, shear, and articular fluid transport of developing constructs (70). By adapting motion trajectories from human locomotion, most notably, we have shown that the articulation of a ceramic hip ball along the surface of chondrocyte-seeded polyurethane constructs resulted in an increase in the mRNA and protein levels of SZP, mRNA levels of hyaluronan synthase (HAS), and in the release of hyaluronan (71).
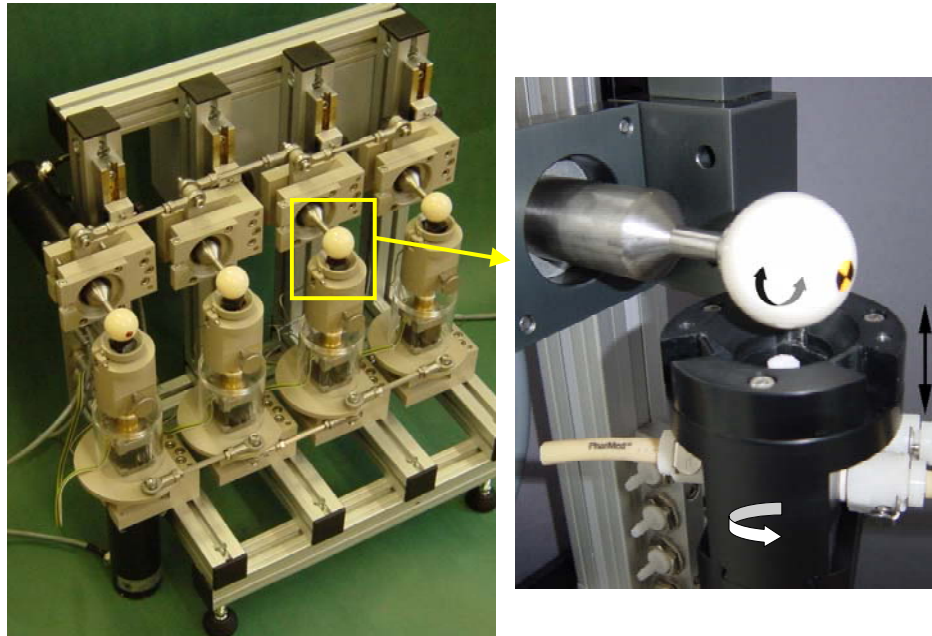
Fig. 1: Bioreactor designed to apply complex articular motion to developing articular cartilage constructs. The bioreactor can simultaneously load up to 4 constructs with computer-controlled compression, torsional shear, and articulating surface motion.
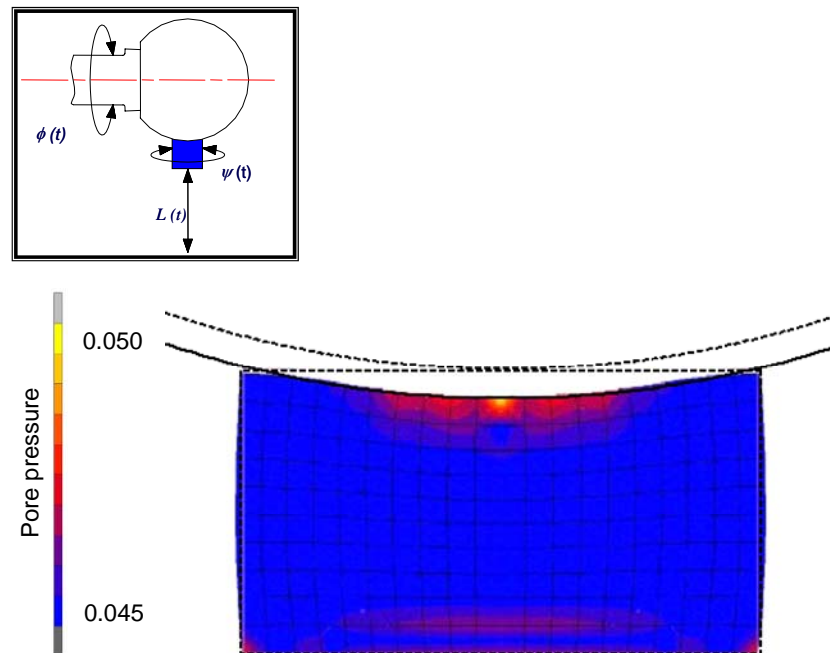
Fig. 2: Finite element modeling predicting distribution and magnitude of the pore pressure that develops inside a polyurethane scaffold subjected to dynamic axial compression by the articulating ball of the bioreactor shown in Figure 1.

Mechanotransduction pathways

As mentioned previously, loading of native and developing articular cartilage leads to the generation of various physical stimuli, including cell/tissue strain, fluid pressurization and flow, electrokinetic phenomena, and convective transport (72-74). To better understand which load-induced physicochemical changes are most important, various researchers have attempted to define the cellular pathways that are responsible for generating an appropriate biosynthetic response.
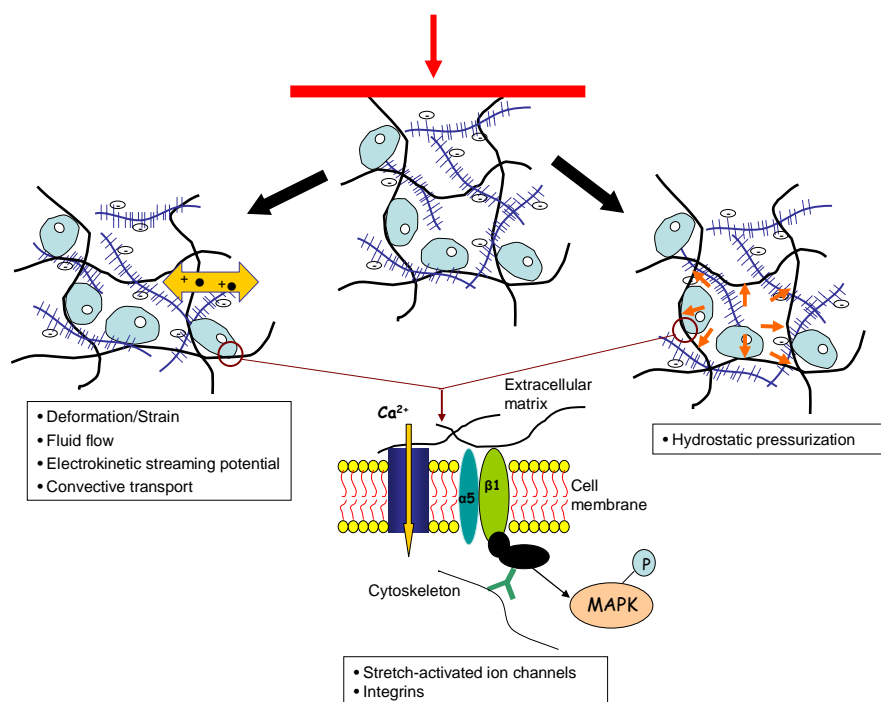

Fig. 3: Schematic illustration of the proposed mechano-transduction pathways showing the different mechanical and physicochemical phenomena that develop when articular cartilage is subjected to compressive loading and possible mechanisms of physical-to-chemical signal transduction.

Complex loading

The most direct pathway for load-induced changes is deformation of the cell and/or nucleus. The equilibrium modulus of the chondrocyte has been estimated to be approximately 1000-fold lower than the cartilage matrix, implying that as the tissue deforms, the chondrocytes also deform (75). Early work correlated load-induced changes in cell shape (76) and nuclear structure (13, 77) with the biosynthetic response to mechanical stimuli. It has been proposed that deformation of the nucleus can change the accessibility of genomic DNA for transcription (78). Deformation of chondrocyte cytoskeletal components such as microtubules and vimentin (79) has also been implicated in the mechanotransduction pathway (80).

Integrins

The position of integrins as a bridge between the cytoskeleton and the ECM suggests their likely role as a mediator in the mechanotransduction pathway. Major integrins in normal articular cartilage are α1β1, α5β1, α10β1, and αVβ5 (81, 82). Of these, the α5β1 integrin, the primary chondrocyte receptor for fibronectin, is most commonly implicated in mechanotransduction pathways. For example, it has been reported that membrane hyperpolarization following cyclical pressurization occurs via the α5β1 integrin (83, 84). Additionally, enhanced chondrocyte GAG synthesis and proliferation in response to dynamic compression appears to be mediated by α5β1 through a TGF-β3 dependent pathway (85).

Membrane channels, pumps, and receptors

Mechanical stimuli may also be transduced to the cell by activating various cell membrane ion channels and transporters. Several investigations have shown that mechanical strain affects ion channels of the chondrocyte membrane, thereby converting mechanical signals to various chemical signals that can affect chondrocyte differentiation, proliferation, and ECM synthesis. For example, there is the influx of Ca2+ (a second-messenger in numerous signaling pathways) following cell deformation

(86), exposure to fluid shear (87), and hydrostatic pressurization (88). Furthermore, the presence of nifedipine, a calcium channel-specific blocker, has been shown to suppress the stretch-induced proliferation and maturation of chondrocytes (89) and the stimulation of parathyroid related protein (PTHrP), a growth factor known to affect chondrocyte proliferation and ECM biosynthesis (90). Calcium ions have also been implicated as an activator of potassium channels that are involved in membrane hyperpolarization following loading (91).

In addition to calcium channels, there are a number of other ion channels and transporters that may be involved in mechanotransduction pathways. By using channel-specific blockers, Wu and Chen found that sodium channels are involved in stretch-induced chondrocyte proliferation (89). Additionally, hydrostatic pressures of 16 kPa have also been shown to activate sodium ion channels, leading to membrane depolarization (92). Hydrostatic pressure (20-30 MPa) has also been shown to induce an increase in intracellular pH by phosphorylation of the Na^+/H^+ pump, thus allowing an efflux of H^+ (93). Furthermore, hydrostatic pressure can regulate Na^+/K^+ and $Na^+/K^+/Cl^-$ pumps, with 20 seconds of pressure at 7.5 MPa having been shown to inhibit the $Na^+/K^+/Cl^-$ co-transporter and 10 minutes of 2.5-50 MPa pressure reversibly inhibiting the Na^+/K^+ pump (94).

Stress-induced activation of the glutamate receptor N-methyl-D-aspartate (NMDA) can also trigger a cellular response by stimulating membrane hyperpolarization (95). It has also been shown that glucose transporters can be affected by mechanical loading with 1-30 MPa of hydrostatic pressure reducing glucose uptake up to 30% (96).

Downstream signaling

Activation of integrins and membrane channels leads to a complex chain of events that can modulate cellular activity by triggering the release of various autocrine/paracrine molecules or the activation of other pathways. Stretch of the α5β1 integrin can trigger tyrosine phosphorylation of various regulatory proteins, leading to secretion of

substance P and IL-4, two soluble autocrine/paracrine signaling molecules (97). Cell strain has been shown to trigger adenosine tri-phosphate (ATP) release which can act as an autocrine/paracrine signaling molecule and affect membrane hyperpolarization (98). Stress-activated channels have also been implicated in the strain-induced synthesis of Indian Hedgehog (Ihh) protein, an important regulator of chondrocyte proliferation and differentiation (99).

Mechanical activation of integrins can also trigger a cellular response through activation of the mitogen-activated protein kinases (MAPKs). Activated MAPKs are believed to be involved in the activation of various transcription factors, including AP-1 and NFκB (100).

Theoretical modeling

There is an obvious plethora of data demonstrating the positive effects of mechanical stimuli on the functional development of articular cartilage. The optimal loading protocols, however, remain to be identified. It is unlikely that a single loading regime will be applicable across the wide range of systems (i.e. scaffold and cell types). Rather, each system will require a unique loading regime that considers scaffold composition and architecture, cell-scaffold interaction, cell density and distribution, and the rate and spatial patterning of ECM development. Furthermore, since the material properties change with matrix development, the loading regime needs to change with time to maintain consistent stimuli at the cell level. To help predict the type and magnitude of mechanical stimuli that are most beneficial for developing constructs, several research groups have turned to computer models (47, 101-107). Theoretical modeling, predicting flow and stress fields, for example, can be used in synergy with biological experiments to predict how tissue level loading will influence cell behavior and tissue development. For example, Raimondi *et al.* have used a computational fluid dynamics model to predict fluid flow-induced shear in perfused chondrocyte-Hyalograft 3D (a non-woven scaffold made from hyaluronic acid) constructs (47). Wimmer *et al.* have also developed a finite

element model that incorporates macro- and micro-scale models to predict the pore pressure gradients and stresses in porous polyurethane scaffolds loaded in their multi-component loading bioreactor (101, 105). An incorporated stochastic "tissue growth function" (108) should help to determine the time course as well as the spatial distribution of matrix development. In addition to incorporating physical factors and predicting the mechanical stimuli that arise from various loading conditions, theoretical models have also incorporated a predicted cellular response to try to model biosynthesis and development of engineered tissues (104). Of course, all of these models must be validated by biological experiments, but they can have predictive value and they can be used to reduce the nearly infinite number of combinations of possible loading-scaffold-cell-growth factor to a reasonable set of the most promising conditions that can be evaluated experimentally.

Future outlook

Mechanical stimulation will undoubtedly continue to play a large role in articular cartilage tissue engineering. In order to maximize the benefits from mechanical loading, however, there are a number of issues that must be considered. First, the source of the cells to be used must be considered. The state of differentiation (109, 110) and the region (i.e. superficial or deep layers) of origin (24, 88, 111) of the chondrocytes appear to influence the cellular response to mechanical stimuli. Additionally, there may be high donor-to-donor variability (112), which may in part be due to age (113) and/or health of the cartilage (83, 114). Second, the interaction of mechanical loading with other environmental factors such as oxygen tension (62, 115) and growth factors (38, 49, 116) must be considered.

So far, the majority of studies are short-term, especially with respect to the time-scale of normal human tissue development and degeneration. These studies have focused on the

biosynthetic rates of the major matrix molecules such as proteoglycan and collagen and, to a lesser extent, the accumulation of these molecules and the macroscopic mechanical properties of the engineered constructs. In order to better predict the long-term outcome, the specific type (i.e. short-chain proteoglycan or aggrecan), organization, and interactions (i.e. cross-linking of the collagen and proteoglycan network) of ECM components must also be considered.

The human body is the ultimate "bioreactor" and should provide the most optimal conditions for articular cartilage maturation. Thus, the most efficient tissue engineering approach may be to use *in vitro* cultivation only to direct the initial development of the implant, with further development to take place *in vivo*, possibly using physical therapy regimes stemming from the knowledge gained from *in vitro* bioreactor studies. Finally, the true success of any tissue engineering protocol is the functional integration of the engineered tissue at the host site and can only be determined by long-term *in vivo* studies.

Acknowledgements

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