Biomechanics-driven chondrogenesis: from embryo to adult

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ABSTRACT **Biomechanics plays a pivotal role in articular cartilage development, pathophysiology, and regeneration. During embryogenesis and cartilage maturation, mechanical stimuli promote chondrogenesis and limb formation. Mechanical loading, which has been characterized using computer modeling and *in vivo* studies, is crucial for maintaining the phenotype of cartilage. However, excessive or insufficient loading has deleterious effects and promotes the onset of cartilage degeneration. Informed by the prominent role of biomechanics, mechanical stimuli have been harnessed to enhance redifferentiation of chondrocytes and chondroinduction of other cell types, thus providing new chondrocyte cell sources. Biomechanical stimuli, such as hydrostatic pressure or compression, have been used to enhance the functional properties of neocartilage. By identifying pathways involved in mechanical stimulation, chemical equivalents that mimic mechanical signaling are beginning to offer exciting new methods for improving neocartilage. Harnessing biomechanics to improve differentiation, maintenance, and regeneration is emerging as pivotal toward producing functional neocartilage that could eventually be used to treat cartilage degeneration.—Responte, D. J., Lee, J. K., Hu, J. C., Athanasiou, K. A. Biomechanics-driven chondrogenesis: from embryo to adult. *FASEB J.* 26, 3614–3624 (2012). www.fasebj.org**

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Cartilage lines the articulating surfaces of long bones, functioning in the mechanically demanding environment of the joint space. The extracellular matrix of cartilage primarily includes collagen and proteoglycans. Cartilage is relatively acellular, with chondrocytes only comprising 1–5% of the tissue by volume (1). Although chondrocytes represent a small fraction of articular cartilage, they are crucial because they synthesize the matrix that imparts mechanical integrity to the tissue.

The significance of biomechanical stimuli has been well established for cartilage. Joint loading results in direct compression of chondrocytes inside a relatively impermeable matrix. Following tissue loading, hydrostatic pressure initially develops in the interstitial fluid, which is followed by fluid flow-induced shear. However, in time scales > 10 μs, the solid matrix begins to bear the applied load, resulting in deformation. Consequently, the cells residing in the matrix experience hydrostatic pressure, shear, compression, and, to a lesser extent, tension. This mechanical stimulation produces a signaling cascade, resulting in increased gene expression (2), matrix protein production (3), and intracellular ion influx (4). Understanding how mechanics influences chondrocytes is crucial because the resulting signaling cascades can alter matrix production and ultimately influence how well the tissue can function in the rigorous joint environment.

As summarized in **Fig. 1**, biomechanics plays a significant role throughout life. Biomechanics contributes to development during embryogenesis and cartilage formation, and it maintains the chondrocyte phenotype in adult tissues. Alternatively, abnormal loading can promote the onset of cartilage degeneration. Informed by these biomechanics studies, various mechanical stimuli have been employed to enhance stem cell differentiation and the development of more robust regeneration strategies. This perspective article highlights the importance of biomechanics in chondrogenesis from the early stages of neocartilage formation to pathophysiology of adult tissue. In particular, it describes the role of biomechanics in cartilage development, maintenance, disease, adult cell chondrogenesis, and cartilage regeneration.

BIOMECHANICS IN EMBRYOGENESIS AND FETAL DEVELOPMENT

Mechanical stimuli during embryonic and fetal development are crucial for cartilage differentiation and limb morphogenesis. The mechanical microenvironment acts as a potent regulator of stem cell fate and contributes to chondrogenesis in the early embryo. Biomechanics also plays a key role in skeletogenesis,

Abbreviations: GAG, glycosaminoglycan; HP, hydrostatic pressure; OA, osteoarthritis; DAH, differential adhesion hypothesis

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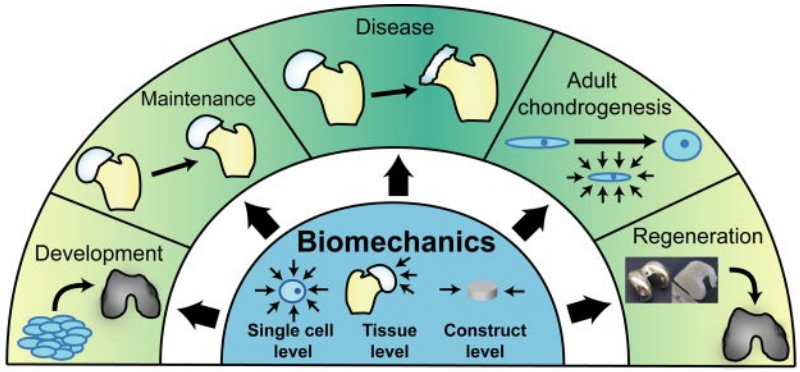

Figure 1. Multilevel role of biomechanics in chondrogenesis. Biomechanics plays a key role in cartilage development by promoting differentiation of stem cells and limb formation. After cartilage forms, normal loading is crucial for maintaining cartilage phenotype and preventing pathogenesis. Biomechanical stimuli can also be applied to enhance cartilage regeneration.

which begins with a primary cartilaginous matrix that later calcifies and forms bone *via* a process known as endochondral ossification (5). Immobilization experiments *in vivo* and computer models of chondrogenesis indicate the central role of mechanical stimuli in proper fetal cartilage development (6–14).

Stem cell response to changing mechanical microenvironment

Much of embryogenesis relies on resident stem cell differentiation into the needed cell types. Likewise, chondrogenesis involves the condensation of precartilaginous progenitor cells to form tightly packed cellular aggregates followed by differentiation into early chondrocytes (5). The contributions of mechanics to this process are currently unknown, as most studies focus on chemical pathways leading to embryonic cartilage formation. Some groups postulate that mechanical forces contribute to *de novo* chondrogenesis from early stem cells (15, 16); however, this hypothesis is difficult to test and current studies use *in vitro* culture platforms or computer simulations. The probable role of mechanics during development has prompted the use of biomechanics to characterize single stem cell populations, control differentiation, and further understand development.

At the single cell level, differentiating stem cells exhibit altered mechanical properties. For example, mouse stem cells demonstrate increased stiffness after just 6 d of chemical differentiation, with up to 3-fold higher stiffness values compared to undifferentiated cells (17). Similarly, the mechanical properties of single cells can be employed to evaluate differentiation states of embryonic (18) and marrow-derived (19) stem cells. Because of the relationship between mechanical properties and differentiation, biomechanics could provide a novel method of stem cell phenotyping and validation of isolation techniques.

The control of stem cell fate *via* cell-exerted forces (by modulating substrate stiffness) and exogenous mechanical stimulation is increasingly being explored as a differentiation strategy. By changing the substrate stiffness, the resultant cell-exerted forces can be altered to control stem cell lineage commitment. Growing embryonic stem cells on 2-dimensional substrates of various stiffnesses modulates differentiation gene expression (20) and growth rate kinetics (20) and can differentiate stem cells into all 3 germ layers, recapitulative of their native environments (21). Alternatively, applying compressive loading in 3 dimensions enhances chondrogenesis of progenitor cells, generating up to 3-fold increases in matrix protein synthesis (15). As evidenced, the ability of both cell-exerted and exogenous forces to chondrodifferentiate stem cells suggests that biomechanics is a key stimulus during cartilage development.

Though the mechanisms underlying precartilaginous condensation require elucidation, the differential adhesion hypothesis (DAH) offers insight into the segregation of cellular subpopulations (22). Spatiotemporal changes in progenitor cell adhesion molecule expression cause similar cells to transiently associate during chondrogenesis (23). However, cell-cell adhesion strength correlates linearly with cellular surface tension, irrespective of a homogeneous or heterogeneous interaction, suggesting surface tension as the primary driver of differential adhesion (24). Therefore, precartilaginous condensation may be the result of mesenchymal progenitor cells exhibiting similar surface tensions rather than similar biomarkers. Furthermore, disruption of surface tension inhibits differential adhesion (25). These findings indicate that cellular biomechanics is essential to the DAH and initiation of chondrogenesis. Gravity, the most basic of mechanical forces, also contributes to chondrogenesis: the absence of gravitational force reduces precartilaginous condensations in mesenchymal limb bud cells (26). Although these hypotheses for precartilaginous condensation remain untested at the early stages of chondrogenesis, the DAH and gravitational hypothesis allow extrapolation from mesenchymal stem cell studies to *in vivo* biomechanics-based embryonic development.

Studying chondrogenesis in embryonic stem cells can inform the mechanisms of *in vivo* cartilage development. As depicted in **Fig. 2**, *in vivo* chondrogenesis occurs in 5 stages, each subject to various biomechanical forces (27): *1*) mesenchymal progenitor cell migration to limb buds and progenitor cell condensation, *2*) chondrocyte differentiation and scaffold formation, *3*) continued extracellular matrix synthesis, *4*) chondrocyte maturation, and *5*) chondrocyte hypertrophy and tissue maturation. While the biomechanical influ-

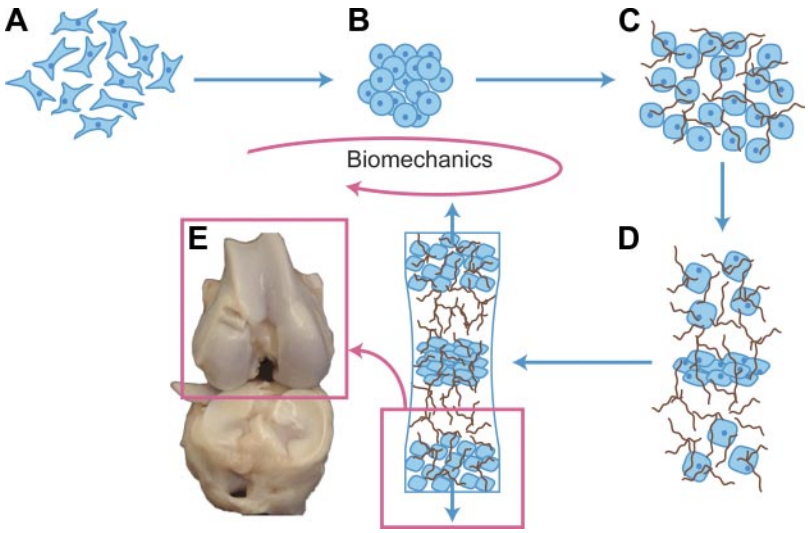

Figure 2. Biomechanics-driven development of cartilage from embryo to fetal stages and beyond. *A*, *B*) Progenitor cells migrate from the early mesoderm to sites of skeletogenesis (*A*), where they undergo precartilaginous condensations (*B*). *C*) Chondrocyte progenitors secrete cartilage-specific matrix and decrease expression of cell-cell interaction proteins. *D*) Proliferation continues at the subchondral growth front, while endochondral ossification occurs throughout the juvenile stages to transform cartilage into bone. *E*) Ends of long bones remain capped with a layer of articular cartilage throughout adulthood.

ence in the suggested progression is studied extensively *in vitro*, *in vivo* validation of the role of biomechanics requires further experimentation.

In summary, stem cell chondrogenesis not only provides a platform for which to study embryonic cartilage tissue formation, it can also be used to study mechanical forces as differentiation stimuli. The significance of biomechanics in stem cell chondrogenesis has been demonstrated for single-cell mechanics, cell-exerted forces, and condensation. Elucidating how specific forces can be employed to promote differentiation into a chondrocyte lineage could advance *de novo* cartilage formation efforts and inform how biomechanics can influence chondrogenesis *in vivo*.

Mechanical stimulation promotes cartilage formation in animal models

Studies of vertebrate limb development reveal that mechanical stimulation promotes chondrogenesis: compression of embryonic limb bud mesenchymal cells triggers chondrogenic marker expression—most notably, SOX9, a master gene responsible for activating many of the cartilage genes expressed in terminally differentiated cells (15, 16). These results indicate mechanical stimulation as a genetic regulator of chondrogenesis in progenitor cells.

Because of the difficulties associated with experimentally controlling biomechanics during embryogenesis, embryos with impaired limb mobility are used to determine the effects of mechanics on limb development (6, 7). Strategies, such as chemically paralyzing limbs (28) or using animals with skeletal muscle defects, create abnormal joint-loading conditions to subsequently disrupt skeletogenesis. For instance, early chemical immobilization of chick limbs results in abnormal limb shape and complete failure to form elements of the synovial joint (7), while later immobilization decreases cartilage matrix production and mechanical properties (9). In addition, muscle removal hinders articular cartilage development (8). These studies demonstrate that joint mechanics are vital at various stages of cartilage formation and development.

Models corroborate animal studies

While joint immobilization provides insight into *in vivo* cartilage development, the difficulties associated with this procedure have led to modeling of joint development (10), which can help elucidate the stages of chondrogenesis during limb development. Ultimately, comparing models to *in vivo* studies demonstrates the accuracy of these simulations.

The modeling approaches to simulate chondrogenesis under mechanical stimulation vary widely, incorporating stimuli based on muscle contraction (7), hydrostatic pressure (11), and shear (11). Intermittent and cyclic hydrostatic pressure and strain both help regulate matrix protein synthesis to affect macromolecular organization of collagen fibers, which, in turn, leads to changes in the mechanical properties of the tissue (11). When applied to a mesenchymal tissue model, a simulation can estimate the mechanical properties of different cartilage types. These results reveal a successful cartilage differentiation algorithm based on mechanical forces recapitulative of the native chondrogenic environment.

Alternatively, models predict that intermittent hydrostatic pressure inhibits degeneration and ossification of cartilage, while intermittent strain or shear stresses accelerate ossification and degeneration (13). Balance of such mechanical forces dictates the progression of what is known as the ossification front, or the line at which cartilage begins to calcify. These forces, thus, generate cartilage of suitable thickness during development. These models can predict the extent of cartilage formation based on chondrocyte proliferation and hypertrophy in response to biomechanical forces. Ultimately, algorithms can estimate an anatomically correct long bone shape based on cartilage growth and ossification (14), thus validating the simulation.

Despite having models of *in vivo* chondrogenesis and their contributions to understanding cartilage development, the role of biomechanics still requires validation *via* animal studies. In addition, models focusing on the interplay between solute transport and mechanical loading (29) will need to be refined to help distinguish the effects of biotransport and biomechanical stimuli. Animal and computer models must build on each other to provide insight into the effects of mechanical stimulation during *in vivo* chondrogenesis.

Tissue mechanics during maturation

In addition to exploring how mechanics drives chondrogenesis, it is important to investigate how these stimuli promote cartilage maturation from fetus to newborn. As skeletal tissues mature, mechanical forces help determine their intrinsic mechanical properties *via* matrix enhancement and organization.

Studies have examined tissue mechanics at different maturation stages (30, 31). For example, human fetal articular cartilage at 20 to 36 wk old exhibits an age-dependent increase in cartilage tissue compressive stiffness by a factor of 2.5 and in collagen content by 3-fold; proteoglycan decreases by 18% (30). Similarly, comparing the mechanical properties of fetal and newborn bovine tissue reveals a correlation between tissue strength and specimen age (31). These studies illustrate how biomechanical properties progress with age.

Mechanical stimulation also plays a vital role in generating regional variation in cartilage. Although fetal cartilage does not demonstrate regional variation, newborn and adult cartilage exhibits stiffer tissues in regions bearing the greatest static or dynamic loads (32). Similarly, the compressive and tensile properties of bovine fetal, newborn, and adult tissues exhibit age-dependent and regional variation (33). These results suggest that joint loading is a potent regulator of regional chondrogenesis.

As described above, during cartilage maturation, the tissue exhibits increases in mechanical properties and concomitant development of regional variation. While the underlying mechanisms of biomechanics-based chondrogenesis remain unclear, it appears that regional variation stems from joint loading. Beginning at the embryo stage, biomechanics undoubtedly influences chondrogenesis, as demonstrated by inhibiting embryo movement *in utero*. By understanding the potential role of biomechanics from a developmental biology standpoint, mechanical stimuli may be rationally designed to differentiate stem cells.

MECHANICAL STIMULATION PROMOTES MAINTENANCE OF CARTILAGE HOMEOSTASIS

In addition to playing a vital role during development, biomechanics is integral for maintaining cartilage homeostasis. In particular, cartilage homeostasis depends on the maintenance of chondrocyte phenotype and production of cartilage matrix molecules without the expression of inflammatory cytokines or catabolic factors. The effects of different forces on cartilage maintenance are assessed using models of load distribution (34–37), *in vivo* imaging techniques (38–40), and *in vitro* experiments on cartilage explants (41–45). The physiological magnitude of stresses present in articular cartilage range from 3 to 10 MPa (46, 47), typically at a frequency of 1 Hz (48). The role of forces, such as hydrostatic pressure and compression to maintain cartilage phenotype *in vivo*, has been confirmed experimentally *in vitro* using biomechanical stimuli to promote cartilage maintenance.

Assessing native cartilage mechanical forces

Modeling studies are employed to identify forces that maintain tissue properties. Using the biphasic theory of cartilage (*i.e.*, cartilage is composed of a solid and liquid phase that each contribute mechanical properties to the tissue; ref. 49) and applying appropriate assumptions in a finite element model, investigators have found that simulations, including compression of 1 MPa and hydrostatic pressure (HP), ranging from 2.8 to 10 MPa, show that mechanical forces exhibit depth dependence and can influence cellular phenotype: forces preserving the upper layers of cartilage are inherently different from those sustaining the lower layers (34). Simulations are also used to model the collagen network at strains up to 4% (35), and to model the pericellular matrix (36) at 10% strain, to illustrate how the matrix microenvironment is responsible for transducing tissue-level mechanical force to cells. Models also delineate how static compression (0.1 MPa) promotes matrix protein synthesis (37). A major shortcoming of these models lies in their simplification of a complex tissue. For example, models that follow the biphasic theory separate the solid and fluid phases of cartilage and may not include the solid-fluid interaction. Despite these limitations, modeling represents a useful approach for understanding force transduction at the cellular and tissue levels.

In vivo imaging techniques can noninvasively elucidate *in vivo* biomechanical forces during joint movement. For example, 2-photon laser microscopy and magnetic resonance imaging are used to reveal chondrocyte deformation at the single-cell level in response to muscle-induced mechanical loading (38), and at the tissue level during physiological loading (39). Imaging of articulating surfaces indicates that proper contact kinematics—that is, the spatial relationship of cartilage contact points within a joint during motion—is vital to preventing cartilage degeneration (40). In general, imaging methods noninvasively examine *in vivo* cartilage biomechanics, which could have important implications for studying cartilage disease.

Mechanical stimulation promotes cartilage homeostasis

Both *in silico* and *in vivo* work demonstrate that biomechanics plays a key role in cartilage maintenance. To verify the role of mechanics, the effects of stimuli, such as HP (41, 43, 50) and compression (37, 44, 45, 51–53), have been examined *in vitro*. HP does not result in deformation of incompressible media, so it is not expected to deform cells. Direct compression results in deformation of matrix and cells, which will also create fluid flow that is not observed with HP. These stimuli, which are described mechanistically in the section on chemically induced stimulation, have been studied with respect to cartilage homeostasis.

At the cellular level, applying HP to chondrocytes at 0.25–10 MPa (1 Hz, 4 h) up-regulates the expression of genes for cartilage matrix proteins (50). HP, thus, promotes and maintains appropriate expression levels of cartilage genes, which results in the synthesis of matrix proteins. Excessive HP stimulation (50 MPa, 12 h) of cells in damaged matrices leads to further degeneration of the surrounding matrix, indicating that HP can be detrimental in abnormal microenvironments (43). These results suggest that HP, especially in the range of 0.5-10 MPa, could act *in vivo* to increase the synthesis of cartilage extracellular matrix components.

Compression also promotes cartilage homeostasis. For example, cyclic compression has been shown to increase both collagen and proteoglycan content (54). Cartilage response to these effects is depth-dependent (45). In addition, studies demonstrate that passaging cells modulates their response to dynamic compression (5% strain, 0.1 Hz), suggesting a differentiation-dependent response to mechanical loading (53). Other factors, such as compressive load duration (37) and magnitude, are shown to alter the cellular response to stimulation, indicating that different compression regimens have differential effects on cartilage homeostasis.

Various biomechanical stimuli, including HP and compression, influence cartilage homeostasis. Modeling and imaging have been used to identify native mechanical forces, which have also been shown to promote *in vitro* homeostasis. As discussed below, when these forces are outside the range of physiological magnitudes, durations, or frequencies, they result in disuse or overuse regimens with detrimental effects.

ABNORMAL LOADING CAN PROMOTE DISEASE

Although mechanical loading can promote cartilage maintenance, insufficient or excessive mechanical stimulation, resulting in disuse or overuse, respectively, can induce degeneration (**Fig. 3**). These degenerative changes parallel osteoarthritis (OA), highlighting how abnormal biomechanical stimuli may initiate cartilage disease.

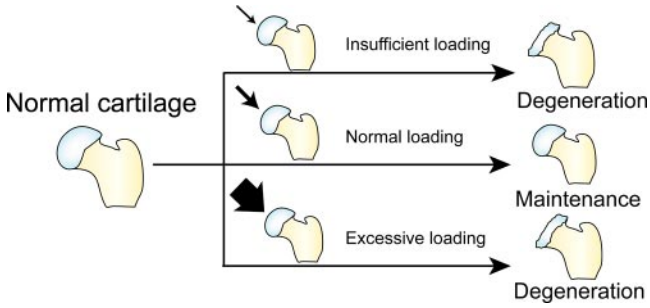

Figure 3. Role of biomechanics in cartilage pathophysiology. Physiological loading promotes the maintenance of cartilage phenotype. Abnormal loading, whether insufficient or excessive, can induce disease progression and eventually lead to degenerative conditions like osteoarthritis.

Excessive loading leads to cartilage degeneration

Excessive cartilage loading can promote the onset of cartilage degradation and OA. Cartilage trauma produces various degenerative effects, including matrix degradation and decreased mechanical properties (55). Cases in which the meniscus is removed, increasing loading on the tibia, also result in articular cartilage degeneration (56). Similarly, transecting the anterior cruciate ligament produces hypertrophic cartilage repair and increases bone volume (57), which is characteristic of OA. In contrast to the dramatic cases above, even slight changes in load distribution during daily activities, such as walking, may initiate early osteoarthritic effects in healthy knee joints (58). Collectively, these studies illustrate how elevated loading leads to osteoarthritic responses.

Cartilage disuse promotes degeneration

A lack of loading can also produce deleterious effects, resulting in changes that parallel the development of OA. Immobilizing joints externally or surgically allows observation of how reduced loading influences cartilage properties. In general, cartilage disuse for several weeks results in a more hydrated tissue (59, 60), with decreased proteoglycan content (59–62). Disuse also produces gross changes, such as thinner cartilage (61, 62). These effects parallel the swelling that occurs during OA (63), suggesting that a lack of joint motion could produce effects similar to those of OA. In addition, these changes in cartilage morphology and biochemical content do not return to control values after discontinuing the immobilization (62). The detrimental effects of disuse *via* immobilization have prompted the design of clinical modalities, *e.g.*, continuous passive motion (64), to introduce biomechanics soon after joint surgeries.

HARNESSING BIOMECHANICS TO PROMOTE CHONDROGENESIS OF ADULT CELLS

In addition to promoting chondrogenesis during development, various efforts have focused on harnessing

biomechanics to promote the chondrogenesis of adult cells (**Fig. 4**). These efforts aim to combat the detrimental effects of monolayer culture and expand cell sources that can be used for *in vitro* cartilage formation. In particular, biomechanical stimulation proves to be an exciting new strategy for effecting adult stem cell migration and differentiation, chondroinduction, and redifferentiation of chondrocytes.

Mechanical loading influences adult stem cell migration and chondrodifferentiation

To a limited degree, adult stem cells also exhibit an ability to respond *in vivo* to heal cartilage defects. In addition to chemical factors that promote adult stem cell migration, the mechanical microenvironment influences stem cell recruitment. A rabbit study demonstrates the effects of mechanics by grafting periosteal tissue—a source of progenitor cells—in an environment subject to mechanical loading (65). The loaded environment stimulates stem cell migration and subsequent *in vivo* chondrogenesis of stem cells present in the periosteum, demonstrating how biomechanics can initiate adult cell chondrodifferentiation. Similarly, simulations of joint motion show that loading and fluid flow can prompt stem cell release to heal cartilage defects (66). Mechanical loading [10% strain, 1 Hz (67) or HP between 3–10 MPa (68)] of cultured mesenchymal stem cells can also promote chondrodifferentiation. These studies illustrate how biomechanics can alter the behavior of adult stem cells and thus influence *in vivo* cartilage repair.

Effects of mechanical stimulation on redifferentiation

Chondrocyte dedifferentiation in monolayer limits the potential for cell expansion and large-scale experiments. Chondrocytes exhibit signs of phenotype loss (reduced gene and protein expression of molecules, such as collagen II and aggrecan), even after the first passage (69). Expansion also alters cellular mechanical properties (70) and changes the chondrocyte response to mechanical stimulation (71).

Biomechanical stimulation can be employed to enhance redifferentiation, particularly by using HP. Applying static 0.3 MPa HP for 6 h/d during redifferentiation increases synthesis of cartilage-specific matrix molecules up to 65% (72). Similarly, applying HP (5 MPa, 0.5 Hz) to dedifferentiated chondrocytes in pellet culture up-regulates chondrogenic gene expression and matrix production up to 5-fold (73). Certain regimens of HP have also been shown to decrease collagen II production (74), illustrating that more work needs to be done to understand how HP can be used as a redifferentiation agent.

Compression has also been used to redifferentiate chondrocytes. Applying compression (10% strain, 0.1 Hz) to passaged chondrocytes seeded on a collagen II scaffold has been shown to increase collagen and proteoglycan production (75). Another study has demonstrated that compression (5% strain, 0.1 Hz) increases glycosaminoglycan (GAG) production of expanded chondrocytes but does not alter collagen II expression (53). The beneficial responses to compression and HP indicate that mechanical stimuli can be effective redifferentiation agents.

Biomechanics-driven chondroinduction

Biomechanical stimuli are potent chondroinductive agents for various cell types. For example, applying HP (5 MPa, 1 Hz) to murine embryonic fibroblasts results in 2-fold increases in collagen synthesis and GAG production (76). Similarly, HP (5 MPa, 1 Hz) increases chondrogenic gene expression in neonatal human dermal fibroblasts (77). Mechanical forces have also been postulated to induce chondrogenic gene and protein expression in smooth muscle cells following atherosclerotic calcification (78). These studies illustrate that biomechanics can drive the chondroinduction of various cell types and thus can act as a differentiation agent.

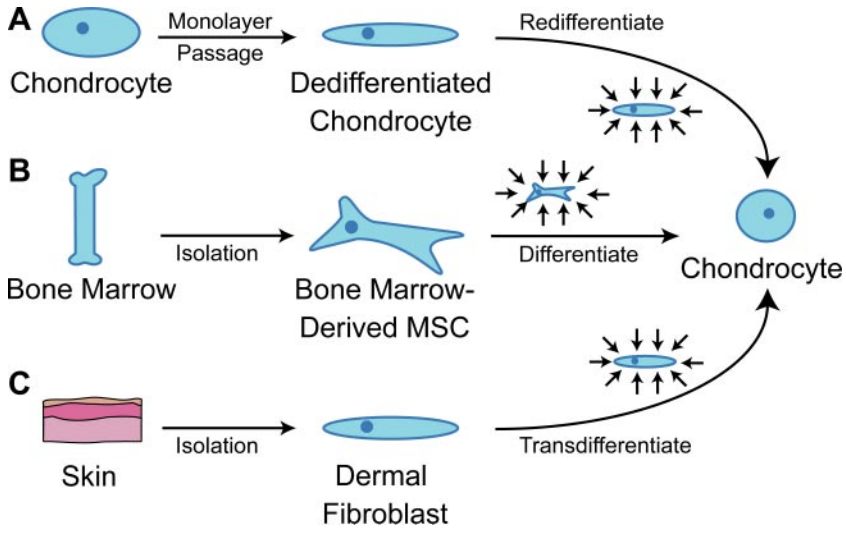

Figure 4. Harnessing biomechanics to drive adult cell chondrogenesis. *A*) Following monolayer culture, chondrocytes rapidly dedifferentiate. Biomechanical stimuli, such as HP, can promote redifferentiation. *B*) Under mechanical stimulation, mesenchymal stem cells migrate and chondrodifferentiate. *C*) Mechanical stimulation can be used to induce transdifferentiation into chondrocytes.

Although several studies have shown promising results for using HP to promote chondroinduction, the effects of other forms of mechanical stimulation need to be investigated. Applying biomechanical stimuli on differentiated cells for chondroinduction could eventually provide new cell sources to supplement stem cell efforts. Additional refinement of mechanical stimuli for adult cell chondrogenesis will increase the availability of cell sources for applications like tissue engineering.

BIOMECHANICS PROMOTES TISSUE ENGINEERING AND REGENERATION

Biomechanical stimuli have been widely employed to enhance tissue-level chondrogenesis and subsequently promote *de novo* cartilage formation. Several methods of mechanical stimulation, including HP, compression, and even shear have been used to improve the biomechanical properties of neocartilage, which could enable it to function *in vivo*. In addition, mechanotransduction research is used to rationally select chemical agents that can reproduce the effects of direct mechanical stimulation (**Fig. 5**).

Direct mechanical stimulation

HP has been applied to various cartilage engineering systems to improve neocartilage properties. Differences in the response to HP may be attributed to the variety of magnitudes and regimens utilized (79). Applying 3.44 MPa HP to chondrocyte-seeded polyglycolic acid meshes increases GAG production 10-fold (80). HP at 10 MPa increases collagen production but reduces GAG content in self-assembled cartilage constructs (3). Finally, HP above physiological levels at 50 MPa produces harmful effects when applied to HCS-2/8 cells (a chondrosarcoma cell line often used to model cartilage), resulting in decreased matrix production and increased expression of inflammatory cytokines (81). Differences in HP effects can also be attributed to different chondrocyte culture techniques: *e.g.*, the anabolic response to dynamic HP is evident for chondrocytes in pellet culture but not for cells cultured in alginate (82). The widespread use of HP to successfully enhance neotissue suggests that, with the appropriate identification of stimulation regimens, HP is a potent stimulus for *in vitro* cartilage formation.

Direct compression is also used to modulate matrix composition and influence neocartilage mechanical properties. Early work with cartilage explants demonstrates that compression can act as a catabolic (83) or anabolic (84) stimulus. In general, dynamic compression is more beneficial than static regimens, which tend to produce catabolic effects (75). More recent efforts, many focusing on tissue-engineering applications, demonstrate that compression can be used to improve matrix production and subsequently improve the functional properties of neocartilage. For example, compression at 1 Hz increases matrix deposition and consequently increases the equilibrium aggregate modulus 6-fold (85). The beneficial role of dynamic compression and the deleterious effects of static compression highlight the importance of selecting an appropriate compression regimen.

Although most biomechanical stimulation work focuses on HP and compression, shear forces are also investigated. Treating cartilage explants with direct dynamic shear (0.01–1 Hz) increases both collagen and proteoglycan synthesis (86). Similar results are observed for neocartilage exposed to shear, which show 40% more collagen, 35% more proteoglycans, and 3- and 6-fold increases in compressive strength and stiffness, respectively, over static controls (87). Fluid flow-induced shear increases the expression of proinflammatory cytokines (88), suggesting that direct shear application is more beneficial. There is comparatively little work on the effects of shear alone, since its application is often coupled with either compression or fluid flow.

Chemically induced mechanical stimulation

Various studies have elucidated pathways underlying the responses to mechanical stimuli, particularly regarding how these stimuli modulate ion transport. For example, cellular deformation increases intracellular concentrations of Ca^{2+} and Na^{+} by enhancing Na^{+}/H^{+} exchanger activity and stimulating stretch-activated ion channels (89, 90). The influx of Ca^{2+} leads to the production of intermediate signaling molecules, such as inositol triphosphate and diacylglycerol, which activate kinase cascades that are crucial for cartilage homeostasis (91). Applying agents like histamine, which increase intracellular Ca^{2+} levels, has also been shown to modulate signaling intermediates like cyclic AMP (92). These findings have spurred recent investigations of agents, including ionophores and hyperosmotic media, that recapitulate mechanotransduction pathways.

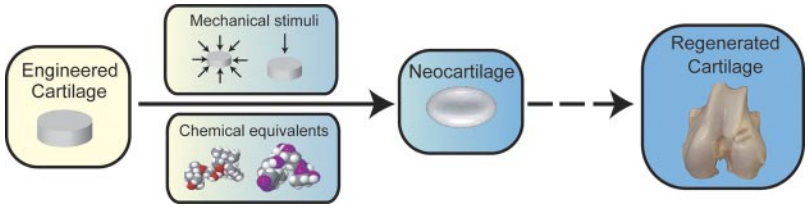

Figure 5. Direct biomechanical stimulation and mechanical equivalents. Biomechanical stimuli, such as compression and HP, can be applied to enhance neotissue formation, improving the biochemical and biomechanical properties of neocartilage. Analogously, application of chemical equivalents, including ionophores and hyperosmotic media, mimic mechanotransduction and produce effects similar to those of direct stimulation. Mechanical equivalents offer opportunities for rationally designing stimulation regimens based on known biomechanics pathways.

Exogenous chemicals can be applied to modulate intracellular ion levels, which are known to vary following compression (4), shear (93), and HP (90, 94). To recapitulate these signaling events, one study uses ouabain (a Na^+/K^+-ATPase inhibitor) and ionomycin (a Ca^{2+} ionophore) or a combination of the two to increase the tensile modulus of cartilage constructs by 40–95% (95). Electromagnetic fields, which can also affect ion channels, are also applied to increase matrix synthesis (96). These studies suggest that it is possible to apply exogenous agents to modulate ion influx, which mimics biomechanical stimulation, and subsequently enhance functional tissue-level properties.

Applying agents that increase osmolarity mimics the hyperosmotic environment that is created when compressive loading forces fluid out of cartilage. Hyperosmolarity modulates intracellular ion levels (97), suggesting that it could be employed like ion channel modulators to alter tissue properties. Studies with native cartilage demonstrate that hyperosmolarity upregulates key cartilage genes, such as SOX9 and aggrecan (98). Although applying hyperosmolarity to neocartilage has not been studied extensively, expanding chondrocytes in hypertonic medium enhances construct mechanical properties (99). This promising result indicates that hyperosmotic environments need to be investigated further in the context of cartilage regeneration.

FUTURE DIRECTIONS

Elucidating the role of biomechanics improves the study of cartilage development, pathophysiology, and regeneration. Despite exciting recent advances, several areas need further examination to more fully understand biomechanics and develop biomechanics-driven strategies for improving cartilage differentiation and regeneration.

To refine a biomechanics-driven approach, it will be necessary to develop a better understanding of how mechanics drives chondrogenesis. The load-bearing environment of cartilage, which influences chondrocyte differentiation and biomechanics, should, therefore, be a central part of cell efforts focusing on chondrogenesis. A more thorough understanding of how mechanics influences embryogenesis could improve stem cell biology, chondroinduction, and redifferentiation methods. Although there is strong evidence for biomechanics influencing cartilage development, further *in vivo* work is needed to clarify the role of mechanics in chondrogenesis.

As knowledge of biomechanical stimuli and their downstream pathways becomes more developed, it will be possible to rationally apply chemical agents that mimic mechanotransduction. These exogenous agents are considerably easier to administer than direct mechanical stimulation and will make it simpler to apply regimens involving multiple stimuli. Because the native joint environment presents a complex combination of mechanical and biochemical stimuli, it will be advantageous to combine agents such as growth factors with mechanical equivalents. This multistimulus approach will enable researchers to leverage biomechanics knowledge to improve cartilage regeneration.

An appreciation of the influence of biomechanics on development, maintenance, disease, adult cell chondrogenesis, and regeneration, plays a crucial role in disease prevention and therapy development. Future therapies, probably those based on tissue engineering efforts, will be solidly based on biomechanics due to its multifaceted role in driving chondrodifferentiation and synthesis of new tissue. The outcome of future therapies is also biomechanical as *de novo* cartilage has to be able to withstand the strenuous environment of the diarthrodial joint. Biomechanics research has driven recent developments in musculoskeletal medicine and will continue to be at the frontiers of cartilage biology and regeneration. FJ

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