[Frontiers in Bioscience, Landmark, 21, 1222-1232, June 1, 2016]

The function of mechanical loading on chondrogenesis

Zhe Chen[1], Fuhua Yan[1], Yong Lu[1]

[1]Department of Radiology, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200025, China

TABLE OF CONTENTS

1. ABSTRACT

Articular cartilage is exquisitely sensitive to mechanical loading, one of the most important external factors that regulates its development, integrity and long-term maintenance. Cartilage undergoes degradation by its misuse or overuse. In this review, we elaborate on this role and discuss the application of mechanical stress on chondrocytes and mesenchymal stem cells in order to foster chondrogenesis.

2. INTRODUCTION

Articular cartilage is a highly specialized connective tissue that provides a nearly frictionless bearing surface, and can absorb and transmit compressive, tensile, and shear forces. These forces are crucial to its healthy development and maintenance, as cells play an important role in transducing mechanical stimuli into biochemical output, known as mechanochemical signaling or mechanotransduction. However, both excessive and insufficient force could promote the onset of cartilage degeneration. In this review, we will discuss both normal and abnormal types of mechanical forces and their effects. Furthermore, we will highlight recent progress in understanding the effects of mechanical loading (i.e. dynamic compression, fluid shear, tissue shear, and hydrostatic pressure) on chondrocytes and mesenchymal stem cells used in the development engineered cartilage, and explore the mechanism of mechanical stress transduction into biochemical signals that regulate and synergize with signaling cascades induced by other stimuli.

3. THE ROLE OF MECHANICAL STRESS IN THE DEVELOPMENT AND MAINTENANCE OF ARTICULAR CARTILAGE

3.1. The characteristics of mechanical stress in articular cartilage

The need of mechanical stimuli to control chondrogenesis has been well established. During embryonic and fetal development, compression of embryonic limb bud mesenchymal cells triggers the expression of chondrogenic markers, most notably the master gene Sox9, which is responsible for activating many other genes to promote differentiation of the cells (1, 2). Meanwhile, mechanical stimulus also promotes growth and organization of the extra cellular matrix (ECM) during maturation of fetal cartilage for many species. For example, at 20-to-36 weeks gestation human fetal articular cartilage exhibits a 2.5. fold increase in compressive stiffness, and a 3-fold increase in collagen content and integrity (3). Similarly, fetal and newborn bovine tissue reveals a correlation between tissue strength and specimen age (4).

In adults, articular cartilage is subjected to various mechanical stresses, including compressive, pressure, tensile, and fluid (shear flow) forces, which activate chondrocyte synthesis of aggrecan and collagen macromolecules that govern mechanical properties. Under normally active physiological conditions, peak dynamic mechanical stresses can reach 18 megapascals (MPa) (5). And this kind of moderate exercise can stimulate ECM synthesis (6-8). Additionally, static physiological stresses applied to knee joints for 5-30 min, as generated by standing, can result in approximately

40% compressive strains in knee cartilages (9). Opposingly, joint immobilization or reduction of loading can result in rapid loss and degradation of ECM content.

As we all know, articular cartilage is the primarily load-bearing tissue in the joint. In the human hip, contact pressure between cartilaginous surfaces is 1 MPa while standing (static loading), 0.1. to 4 MPa while walking (dynamic loading), and can reach 20 MPa when going from sitting to standing or while jumping (10).

There are conflicting results regarding the loading effects on chondrocyte macromolecule synthesis. Hydrostatic pressure (5 MPa) applied to agarose gel embedded with bovine chondrocytes was found to upregulate mRNA of aggrecan (4-fold) and type II collagen (50%) (11), and also increased proteoglycan biosynthesis (12). However, a similar level of static pressure (5-10 MPa) applied to bovine cartilage explants was found to suppress proteoglycan synthesis (13). Intermittent hydrostatic pressure (10 MPa, 1 Hz for 6-24 hours) has been reported to have protective effects by downregulating the release of matrix metalloproteinase (MMP) and pro-inflammatory mediators (14). But for monolayer-cultured human osteoarthritic chondrocytes, intermittent hydrostatic pressure (5 MPa, 1 Hz for 4 hours) can trigger apoptosis, increase mRNA expression of tumor necrosis factor-α (TNF-α), and induce production of nitric oxide synthase (15).

When cells proliferate and/or large amounts of ECM deposit in regions, compressive pressure will typically arise in order to resist rigidly external boundaries or prevent tissue expansion. This kind of compression plays a key role in controlling cell proliferation, growth, and differentiation, which directly affects cartilage ECM formation. For example, in the developing skeleton, immature cartilage tissue is always encapsulated by a perichondrium, a rigid connective tissue, which exerts a compressive force on the immature ECM. Removal of the mechanical constraint of the perichondrium can result in accelerate hypertrophic process of chondrocytes, which could affect the growth of articular cartilage and bone (16, 17). Chondro-progenitor cells continue to grow rather than differentiate into cartilage and lead to ectopic cartilage formation.

In articular cartilage, interstitial fluid flow generates shear stresses, which trigger physiological responses (18). In compression, the induced fluid flow may increase the transportation of nutrients and growth factors into or out of the tissue. Many studies have shown that flow induced shear has both positive and negative effects on chondrocyte metabolism. During vitro cultivation, monolayer chondrocytes exposed to flow-induced shear stresses that range from 0.2. to 1.6. Pa showed an increase of proteoglycan, prostaglandin E2, and nitric oxide synthesis, and a downregulation of collagen II and aggrecan mRNA expression (19, 20).

Tensional forces, such as the flexion of tendon or muscle contraction pulling on bone, are prominent in the skeletal system, and remarkably, also derive from the highly directional and asymmetric growth of articulating joint tissues. For example, there is stretching of periosteal tissue that is anchored to the bone shaft and the epiphyses, which expands tangentially (21). Tensional forces can profoundly affect the development of skeletal system (22). Severe osteogenic defects occur when the periosteal tension is reduced after resection of the epiphyses (21), and ectopically applied tensional forces transform cartilaginous tissue into bone (23, 24). In addition, loss of muscle-induced tension via experimentally induced paralysis or limb-tissue transplantation shows limited effects. These changes could alter the formation of specific sesamoid bones, such as the plantar tarsal sesamoid and patella, and may reduce the formation of bony ridges for tendon attachment (25).

3.2. Mechanical stress in cartilage disease

As mentioned above, chondrocyte response to moderate mechanical loading is necessary for normal cartilage homeostasis (26). Consistently, *in vivo* experiments on articular cartilage have shown that cellular responses (catabolism or anabolism) depend on frequency, duration and magnitude of loading (27). Moderate exercise and dynamic loading at specific frequencies in young rodents can produce an anabolic response in chondrocytes, which increases proteoglycan content and decreases proteoglycan degradation (26, 27). Conversely, high-intensity exercise, long-term immobilization, abnormally static loading and even a sudden increase in joint loading can lead to osteoarthritis (OA)-like matrix catabolism, which damages the collagen fiber network, degrades proteoglycans, and reduces cartilage stiffness (26-28). In addition to these homeostatic effects, normal joint loading may be an important regulator of developmental and postnatal growth of cartilage. Recently published data indicate that paralyze the shoulder via injecting botulinum toxin A into supraspinatus muscles of newborn mice can delay the development of tendon-bone insertions (29). Although these results pertain to tendon and/or attachment intervention, it is possible that a similar dysregulation of articular cartilage development and/or growth can occur in joints when suffering abnormal mechanical loading.

Injury to articular cartilage or supporting structures, such as the meniscus, would presumably lead to altered biomechanics, cytokine production, and eventual cartilage catabolism (26). Specifically, cytokines such as Interlukin-1 (IL-1) and TNF-α are the inducers of cartilage matrix degradation by inducing the expression of genes to encode matrix catabolic proteins, such as

MMPs, collagenases and aggrecanases (30, 31). On the other hand, both IL-1 and TNF-a can induce PGE2 production and nitric oxide (NO) metabolism, which act as strong catabolic signals by promoting injuries and enhancing apoptotic potential in chondrocytes (32).

4. TAKING ADVANTAGE OF MECHANICAL STRESS TO PROMOTE THE ACTIVITY OF CHONDROCYTES

4.1. Tissue engineering factors of chondrocytes

Because of the crucial role of mechanical stimuli in the development and maintenance of articular cartilage, more attention has been drawn to the use of exogenous mechanical stimulation of engineered cartilage. Functional tissue engineering has focused on dynamic compression, fluid flow-induced shear, and hydrostatic pressure, paying special attention to the magnitudes and frequencies of normal physiologic ranges. The effectiveness of the mechanical stimuli is usually assessed by evaluating ECM quality and production, gene expression, and tissue functionality (i.e. mechanical stiffness and perviousness). Accordingly, it is considered that mechanical stress plays an important role in culturing of engineered cartilage, and lack of appropriate mechanical stimulus may cause inappropriate function.

It is well-known that compressive loading on cartilage explants can modulate chondrocyte viability (33), gene expression (34-37), and biosynthesis of various ECM molecules (33, 37-40). For example, dynamic compression at moderate conditions (2-10% strain (37, 38), 0.5.-1.0. MPa (33, 40) and physiological frequencies (0.1. to 1.0. Hz)) can stimulate the biosynthesis of collagen (38), proteoglycan (37, 38, 40) and fibronectin (33). In the literatures, numerous short- and long-term studies have used unconfined dynamic compression protocols, spanning a wide range of frequencies (0.1. to 1.0. Hz), strains (3-15%) and stresses (0.5.-2.5. MPa) to a variety of engineered tissue types. Of those types, hydrogels or macroporous scaffolds and differentiated, undifferentiated, or de-differentiated cells were used to stimulate cell differentiation, proliferation and biosynthetic activity, and to promote the development of a functional ECM.

Although dynamic compression at physiological levels generally has positive effects on ECM biosynthesis, several studies have demonstrated the negative effects of abnormal dynamic compression on cartilage development. Although various investigations have reported that cyclic loading can lead to an increased release of matrix molecules such as proteoglycan and glycosaminoglycan (GAG) (41-46), prolonged continuous loading (± 4% strain, 0.1. or 1.0. Hz, 10 or 20 days) will cause inferior mechanical and biochemical properties in chondrocyte-seeded fibrin hydrogels (44). 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) could suppress sulfate incorporation, whereas alternate day loading (4×45 minute loading cycles applied every other day) could stimulate sulfate incorporation in chondrocyte-agarose constructs (41).

In order to detect the effects of fluid flow, Frank *et al.* (47) and Jin *et al.* (48) applied direct shear to cartilage explants. Dynamic shear deformation (1-3% strain, 0.1.-1.0. Hz) was shown to stimulate collagen and proteoglycan biosynthesis up to 50% and 25%, respectively (48). Waldman *et al.* showed that chondrocytes cultured in porous calcium phosphate scaffolds for four weeks with daily dynamic shear strain (2% shear strain at 1 Hz, superimposed on a 5% compressive tare strain for six or thirty minutes per day) had higher synthetic ratios of collagen (40%) and proteoglycan (35%) and significantly higher equilibrium modulus and maximum stress (six- and three-fold increases, respectively) than chondrocytes cultured under free-swelling for four weeks (49). These findings are similar to the changes from dynamic compression (50).

The benefits of intermittent hydrostatic pressure on the development of engineered tissues have also been explored. Intermittent hydrostatic pressure at 3.4.4 and 6.8.7 MPa (5 seconds pressurized/15 seconds nonpressurized, applied for 20 minute intervals every 4 hours for 5 weeks) was found to increase the glycosaminoglycan concentration in equine chondrocyte-seeded polyglycolic acid meshes; 6.8.7 MPa also increased collagen production (51). Furthermore, Mizuno *et al.* found cyclic hydrostatic pressure (2.8. MPa, 0.0.15 Hz) increased proteoglycan production over a 15-day culture period in bovine chondrocyte-seeded porous collagen scaffolds (52).

4.2. The mechanotransduction of cells from mechanical stress

Cellular response to mechanical stress is an important modulator of chondrocyte function. Pressure applied to cartilage deforms the ECM and chondrocytes, and increases hydrostatic pressure, which expels fluid from the tissue. However, the degree of these changes depends on the rate of applied pressure. Cyclic loading can rapidly increase pressure, momentarily deform cells, and cause short peaks of intratissue fluid flow (no tissue fluid loss), all which stimulates biosynthesis. Static loading, which generally depresses biosynthesis, causes fluid exudation and provokes an increase in proteoglycan concentration and osmolarity, and a reduction in pH, gradually leading to tissue degeneration.

Researchers have elucidated the biomechanical pathways that stimulate and regulate chondrocyte metabolism and physiology. It has been

reported that chondrocytes do not respond directly to the mechanical signals, but to the biochemical signals produced by mechanical stimulation, a process known as mechanotransduction. In mechanotransduction, mechanical stresses activate the intracellular signaling pathways, such as mechanoreceptors (e.g. integrins) (53), ion channels (slow conductance Ca^{2+}, sensitive K^{+} and stretch-activated ion channels) (54), soluble mediators (basic fibroblast growth factor, IL-4) (55, 56), and intracellular protein kinases (mitogen-activated protein kinase (MAPK) family) (57, 58), and then modulate chondrocyte biochemical activities.

One of the central signal transduction pathways involves integrin receptors in the chondrocyte membrane, which act as a bridge between the cytoskeleton and ECM. The major integrin receptors, $\alpha 1\alpha 1$, $\alpha 5\alpha 1$, $\alpha 10\alpha 1$, and $\alpha V\alpha 5$ (59, 60), bind to ECM components, transmit information to the chondrocyte cytoplasm and lead to activation of cytoskeleton and intracellular signaling proteins, such as focal adhesion kinase (61) and MAPK signaling molecules (62). Integrin $\alpha 5\alpha 1$, a primary chondrocyte receptor for fibronectin, is the most commonly implicated in mechanotransduction pathways. Cyclic pressurization has been shown to activate integrin $\alpha 5\alpha 1$, which hyperpolarizes chondrocyte membranes (63, 64), and stimulates GAG synthesis and proliferation through a TGF-$\alpha 3$-dependent pathway (65). The downstream activation of MAPK and MEK-Erk1 signaling pathway leads to a downregulation of Agc gene expression in bovine articular chondrocytes (66). Furthermore, the association of integrin complexes with IGF receptor I can facilitate the activation of MAPK signaling pathway (62). Evidence has shown that following exposure to fibronectin fragments, the activation of proline-rich tyrosine kinase 2 contributes to the upregulation of collagenase III expression via protein Kinase C (67). In addition to these signaling responses, it is important to note that the abrogation of cell-ECM interactions (anoikis) mediated by integrins leads to chondrocyte apoptosis (68).

It is widely recognized that the transduction of mechanical stress can also be facilitated via stress-activated ion channels located in plasma membrane. Among the numerous well-characterized ion channels, N- and L-type voltage-gated calcium channels (VGCCs) are the most relevant channels in chondrocytes (69, 70). Because cytoskeletal elements control the opening and closing of neuronal cell VGCCs, a similar regulatory paradigm might also exist in chondrocytes. As a result, the transfer of mechanical stress through the cytoskeleton could induce the opening of ion channels, the propagation of intracellular calcium waves, and the subsequent induction of phenotypic effects in cells (71). Furthermore, calcium transients activate signals via both calmodulin kinase and calcineurin/NFAT pathways (72, 73). Although these pathways have known importance in the modulation of chondrogenesis and chondrocyte differentiation (74, 75), further investigations are needed to fully characterize how calcium signaling in chondrocytes contributes to the anabolic or catabolic effects caused by mechanical stress.

5. HARNESSING MECHANICAL STRESS FOR MESENCHYMAL STEM CELLS (MSCs) DIFFERENTIATION

Controlled mechanical stress is not only useful for proper production of engineered cartilage, but may provide an exciting new strategy in harnessing control of stem cell chondrogenesis. Stem cells are a driving force in functional tissue engineering due to their capacity for self-renewal and pluripotency. Self-renewal enables the extensive ex-vivo (and *in vivo*) expansion of progenitor cells in a target tissue, which is a key feature to generate sufficient cells to meet the potential demand of tissue replacement. Pluripotency, the ability of stem cells to differentiate into multiple cell types, allows the possibility of generating multiple tissues (i.e. bone, cartilage, adipose, tendon, muscle, neural and other connective tissues) (76-81) from a single source cell, and promotes the reconstitution of complex multicellular interactions required for function of a single tissue. More attention has been focused on the potential of using human mesenchymal stem cells (MSCs) regenerative medicine for the treatments of musculoskeletal trauma and diseases (81, 82, 83).

However, harnessing the potential of MSCs is very challenging, as the time point and proper control of multi-lineage differentiation would affect the fate of cells, possibly leading to a pathological or a non-functional tissue. Biologists have appreciated the role of soluble factors (e.g. growth factors and cytokines), explicitly used to control stem cell differentiation through their own specific pathways activated by adhesive and mechanical means.

In multiple species, mechanical stress also regulates bone mass and strength (84, 85). Among the theories of mechanical signal responses, strain-induced fluid shear stress has received greater experimental support (86, 87). During repetitive loading and unloading, fluid shear stress occurs in the interstitial spaces around bone cells in bone marrow cavities (88) and can regulate the differential functions of cells by stimulating multiple intracellular signal pathways (89). Accordingly, there is growing interest for using mechanical stress to regulate osteoprogenitor cell differentiation, since recent studies have shown that mechanical stimulation can be used to initiate the osteogenic differentiation of bone marrow MSCs on both 2D planar substrates and 3D scaffolds (90, 91), greatly reducing the time required for cultured cell differentiation. In a 2D culture, rat MSCs exposed to shear stress showed an increase in gene expression and alkaline phosphatase (ALP)

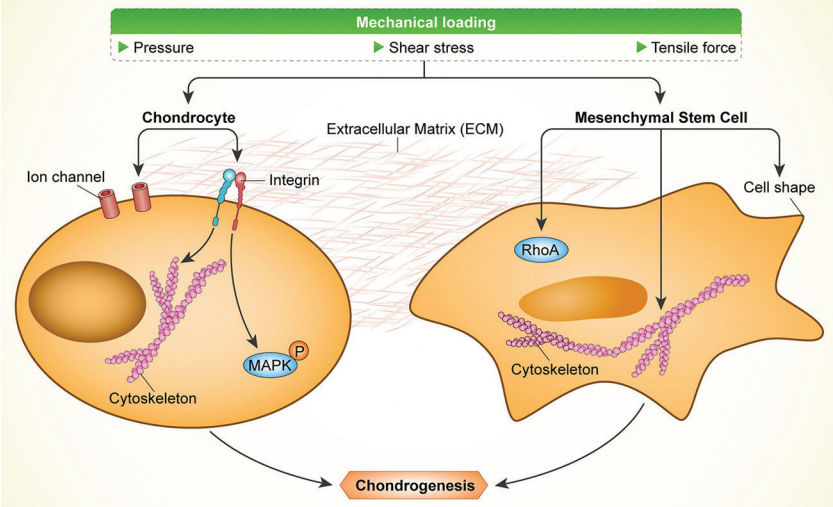

Figure 1. Mechanical loading influences chondrogenesis through multiple aspects. Articular cartilage possesses the characteristics of load-bearing environment with pressure, shear stress and tensile force. As the central component of articular cartilage, chondrocytes could perceive the mechanical signals through integrins, ion channels and cytoskeleton reshape pathway. And these researches have been applied in tissue engineering for chondrogenesis. In Addition, mechanical loading could regulate the differential potency of MSCs through cell shape, actin cytoskeleton, and the RhoA pathway, thus enhancing chondrogenic differentiation of mesenchymal stem cell to providing new chondrocyte cell sources.

activity of bone sialoprotein and osteopontin (92). And in a 3D scaffold, cell proliferation and osteogenic marker production, including ALP and calcium, were increased when MSCs were mechanically stimulated (93). However, it is still difficult to calculate the shear stress magnitudes applied to the 3D scaffold (94).

An important discovery showed that the differentiation of MSCs could be governed by substrate stiffness and the control of lineage switching is associated with cell distribution and intracellular tension. Engler *et al.* demonstrated that planting MSCs on polyacrylamide gels with different stiffness are sufficient to regulate the expression of neuronal, skeletal muscle, or osteogenic markers in the absence of exogenous soluble cues (95). Cell-generated tensional forces exist in equilibrium with the underlying substrates, but when weak counterbalance forces are present, like in a soft gel, the cellular contractility will undergo a compensatory decrease. Consequently, it is reasonable to attribute stiffness-dependent changes in stem cell differentiation to altered intracellular tension. Indeed, the addition of blebbistatin to block intracellular tension generation in MSCs obliterates the stiffness-driven differentiation (95). Consistent with the hypothesis that cells upregulate intracellular tension when the matrix stiffens and provides higher resistance forces, MSCs progressively assemble actin stress fibers and focal adhesions (tension-dependent structures) in response to the increasing stiffness of substrates.

Several studies directly examined the association among mechanical forces, gene expression and cell differentiation, and provide a better understanding of how mechanical signals regulate stem cell differentiation and lineage switching, and it has been indicated that cell shape, actin cytoskeleton and the RhoA pathway play important roles in the mechanical control of MSC differentiation. In the case of embryonic MSCs, a morphological change from round to elongated is sufficient to drive smooth muscle myogenesis, akin to the effect of mechanical stretch (96). Using micro-patterned islands of ECM (fibronectin) to control cell spreading, McBeath *et al.* demonstrated that cell shape could control the lineage of MSCs (97). In this system, MSCs can differentiate into either adipocytes or osteoblasts in response to a bipotential differentiation medium, which can induce either lineage. However, MSCs confined to small ECM islands (1024 μm^2) selectively underwent adipogenesis, whereas MSCs cells on large ECM islands (10000 μm^2) tended toward osteogenesis (97). This osteogenic-adipogenic switch in well-spread MSCs versus poorly-spread MSCs requires the generation of tension through RhoA-dependent actomyosin contractility. RhoA stimulates tension by its effector, Rho kinase, which indirectly elevates the level of active phosphorylated myosin light chains (98). Inhibition of tension, either cytochalasin D (an actin depolymerization agent) or Y-27632 (a Rho kinase inhibitor), promoted adipogenesis and mimic the phenotype of poorly spread cells. Moreover, manipulation of the RhoA pathway could override the effects of soluble differentiation factors such that dominant-negative RhoA could induce adipogenesis even in the context of pure osteogenic medium. On the other hand, constitutively active RhoA can trigger osteogenesis in a pure adipogenic medium. In control of stem cell differentiation, these findings highlight RhoA activity as a potential convergence point for mechanical and soluble factor signaling. Importantly, McBeath *et al.*

also demonstrated that the expression of constitutively-active Rho kinase rescued osteogenic differentiation of poorly-spread MSCs, which require myosin II activity, indicating that both cell shape and RhoA regulate osteogenic-adipogenic switching during the development of cytoskeletal tension (97).

Despite the established link between MSC differentiation and mechanical stimuli, it is important to acknowledge that changes in applied forces and stresses were not extensively measured in these experiments. It is possible that the employed mechanical manipulations also perturb paracrine signaling and/or adhesive cues. Further investigation to clarify the precise mechanisms of signaling pathways activated by mechanical stress will contribute to the achievement of a 'better' engineered cartilage.

6. CONCLUSIONS

As summarized in Figure 1, the load-bearing environment of articular cartilage, which influences the differentiation and biomechanics of chondrocyte, has been a central focus on chondrogenesis. A more thorough understanding of the effects of mechanical stimuli and their downstream pathways could improve stem cell biology, chondro-induction, and redifferentiation methods. Future therapies, including tissue engineering, will be solidly based on biomechanics due to its multifaceted role in driving chondro-differentiation and cartilage regeneration. Despite exciting recent advances, further examination is in urgent need to fully understand the biomechanics mechanism and develop biomechanics-driven strategies.

7. ACKNOWLEDGEMENTS

The authors thank Mr. David Kahn for critical reading and editing to improve the manuscript. This work was supported by Project of the National Natural Science Foundation of China (81372000); Project of Shanghai Jiao Tong University "Medical-Engineering Cross Fund" (YG2013MS25).

8. REFERENCES

1. Takahashi I, Nuckolls GH, Takahashi K, Tanaka O, Semba I, Dashner R, Shum L, Slavkin HC. Compressive force promotes sox9, type II collagen and aggrecan and inhibits IL-1beta expression resulting in chondrogenesis in mouse embryonic limb bud mesenchymal cells. *J Cell Sci* 111 (Pt 14): 2067-76 (1998) Doi not found
2. Elder SH, Kimura JH, Soslowsky LJ, Lavagnino M, Goldstein SA. Effect of compressive loading on chondrocyte differentiation in agarose cultures of chick limb-bud cells. *J Orthop Res* 18: 78-86 (2000) DOI: 10.1002/jor.1100180112
3. Mahmoodian R, Leasure J, Philip P, Pleshko N, Capaldi F, Siegler S. Changes in mechanics and composition of human talar cartilage anlagen during fetal development. *Osteoarthritis Cartilage* 19: 1199-209 (2011) DOI: 10.1016/j.joca.2011.07.013
4. Klein TJ, Chaudhry M, Bae WC, Sah RL. Depth-dependent biomechanical and biochemical properties of fetal, newborn, and tissue-engineered articular cartilage. *J Biomech* 40: 182-90 (2007) DOI: 10.1016/j.jbiomech.2005.11.002
5. Hodge WA, Fijan RS, Carlson KL, Burgess RG, Harris WH, Mann RW. Contact pressures in the human hip joint measured *in vivo*. *Proc Natl Acad Sci U S A* 83: 2879-83 (1986) DOI: 10.1073/pnas.83.9.2879
6. Palmoski MJ, Colyer RA, Brandt KD. Joint motion in the absence of normal loading does not maintain normal articular cartilage. *Arthritis Rheum* 23: 325-34 (1980) DOI: 10.1002/art.1780230310
7. Behrens F, Kraft EL, Oegema TR, Jr. Biochemical changes in articular cartilage after joint immobilization by casting or external fixation. *J Orthop Res* 7: 335-43 (1989) DOI: 10.1002/jor.1100070305
8. Saamanen AM, Tammi M, Jurvelin J, Kiviranta I, Helminen HJ. Proteoglycan alterations following immobilization and remobilization in the articular cartilage of young canine knee (stifle) joint. *J Orthop Res* 8: 863-73 (1990) DOI: 10.1002/jor.1100080612
9. Herberhold C, Faber S, Stammberger T, Steinlechner M, Putz R, Englmeier KH, Reiser M, Eckstein F. *In situ* measurement of articular cartilage deformation in intact femoropatellar joints under static loading. *J Biomech* 32: 1287-95 (1999) DOI: 10.1016/S0021-9290(99)00130-X
10. Urban JP. The chondrocyte: a cell under pressure. *Br J Rheumatol* 33: 901-8 (1994) DOI: 10.1093/rheumatology/33.10.901
11. Toyoda T, Seedhom BB, Kirkham J, Bonass WA. Upregulation of aggrecan and type II collagen mRNA expression in bovine chondrocytes by the application of hydrostatic pressure. *Biorheology* 40: 79-85 (2003)

Doi not found.

12. Toyoda T, Seedhom BB, Yao JQ, Kirkham J, Brookes S, Bonass WA. Hydrostatic pressure modulates proteoglycan metabolism in chondrocytes seeded in agarose. *Arthritis Rheum* 48: 2865-72 (2003)
DOI: 10.1002/art.11250

13. Hall AC, Urban JP, Gehl KA. The effects of hydrostatic pressure on matrix synthesis in articular cartilage. *J Orthop Res* 9: 1-10 (1991)
DOI: 10.1002/jor.1100090102

14. Trindade MC, Shida J, Ikenoue T, Lee MS, Lin EY, Yaszay B. Intermittent hydrostatic pressure inhibits matrix metalloproteinase and pro-inflammatory mediator release from human osteoarthritic chondrocytes *in vitro*. *Osteoarthritis Cartilage* 12: 729-35 (2004)
DOI: 10.1016/j.joca.2004.05.008

15. Islam N, Haqqi TM, Jepsen KJ, Kraay M. Hydrostatic pressure induces apoptosis in human chondrocytes from osteoarthritic cartilage through up-regulation of tumor necrosis factor-alpha, inducible nitric oxide synthase, p53, c-myc, and bax-alpha, and suppression of bcl-2. *J Cell Biochem* 87: 266-78 (2002)
DOI: 10.1002/jcb.10317

16. Rooney P, Archer CW. The development of the perichondrium in the avian ulna. *J Anat* 181 (Pt 3): 393-401 (1992)
Doi not found

17. Long F, Linsenmayer TF. Regulation of growth region cartilage proliferation and differentiation by perichondrium. *Development* 125: 1067-73 (1998)
Doi not found.

18. Burger EH, Klein-Nulend J. Mechanotransduction in bone--role of the lacuno-canalicular network. *FASEB J* 13 Suppl: S101-12 (1999)
Doi not found

19. Smith RL, Donlon BS, Gupta MK, Mohtai M. Effects of fluid-induced shear on articular chondrocyte morphology and metabolism *in vitro*. *J Orthop Res* 13: 824-31 (1995)
DOI: 10.1002/jor.1100130604

20. Smith RL, Carter DR, Schurman DJ. Pressure and shear differentially alter human articular chondrocyte metabolism: a review. *Clin Orthop Relat Res*: S89-95 (2004)
Doi not found

21. Glucksmann A. The role of mechanical stresses in bone formation *in vitro*. *J Anat* 76: 231-9 (1942)
Doi not found

22. Taber LA. Biomechanical growth laws for muscle tissue. *J Theor Biol* 193: 201-13 (1998)
DOI: 10.1006/jtbi.1997.0618

23. Takahashi I, Mizoguchi I, Nakamura M, Sasano Y. Effects of expansive force on the differentiation of midpalatal suture cartilage in rats. *Bone* 18: 341-8 (1996)
DOI: 10.1016/8756-3282(96)00012-9

24. Takahashi I, Onodera K, Sasano Y, Mizoguchi I. Effect of stretching on gene expression of beta1 integrin and focal adhesion kinase and on chondrogenesis through cell-extracellular matrix interactions. *Eur J Cell Biol* 82: 182-92 (2003)
DOI: 10.1078/0171-9335-00307

25. Hosseini A, Hogg DA. The effects of paralysis on skeletal development in the chick embryo. I. General effects. *J Anat* 177: 159-68 (1991)
Doi not found

26. Griffin TM, Guilak F. The role of mechanical loading in the onset and progression of osteoarthritis. *Exerc Sport Sci Rev* 33: 195-200 (2005)
DOI: 10.1097/00003677-200510000-00008

27. Arokoski JP, Jurvelin JS, Vaatainen U, Helminen HJ. Normal and pathological adaptations of articular cartilage to joint loading. *Scand J Med Sci Sports* 10: 186-98 (2000)
DOI: 10.1034/j.1600-0838.2000.010004186.x

28. Buschmann MD, Hunziker EB, Kim YJ, Grodzinsky AJ. Altered aggrecan synthesis correlates with cell and nucleus structure in statically compressed cartilage. *J Cell Sci* 109 (Pt 2): 499-508 (1996)
Doi not found

29. Thomopoulos S, Kim HM, Rothermich SY, Biederstadt C, Das R, Galatz LM. Decreased muscle loading delays maturation of the tendon enthesis during postnatal development. *J Orthop Res* 25: 1154-63 (2007)
DOI: 10.1002/jor.20418

30. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin Orthop Relat Res:* S27-36 (2004)
DOI: 10.1097/01.blo.0000144854.66565.8f

31. Goldring MB, Goldring SR. Osteoarthritis. *J Cell Physiol* 213: 626-34 (2007) DOI: 10.1002/jcp.21258

32. Goldring MB, Berenbaum F. The regulation of chondrocyte function by proinflammatory mediators: prostaglandins and nitric oxide. *Clin Orthop Relat Res*: S37-46 (2004) DOI: 10.1097/01.blo.0000144484.69656.e4

33. Steinmeyer J, Ackermann B, Raiss RX. Intermittent cyclic loading of cartilage explants modulates fibronectin metabolism. *Osteoarthritis Cartilage* 5: 331-41 (1997) DOI: 10.1016/S1063-4584(97)80037-4

34. Fehrenbacher A, Steck E, Rickert M, Roth W. Rapid regulation of collagen but not metalloproteinase 1, 3, 13, 14 and tissue inhibitor of metalloproteinase 1, 2, 3 expression in response to mechanical loading of cartilage explants *in vitro*. *Arch Biochem Biophys* 410: 39-47 (2003) DOI: 10.1016/S0003-9861(02)00658-6

35. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage oligomeric matrix protein (COMP). *Biorheology* 40: 101-9 (2003) Doi not found

36. Fitzgerald JB, Jin M, Dean D, Wood DJ. Mechanical compression of cartilage explants induces multiple time-dependent gene expression patterns and involves intracellular calcium and cyclic AMP. *J Biol Chem* 279: 19502-11 (2004) DOI: 10.1074/jbc.M400437200

37. Wong M, Siegrist M, Cao X. Cyclic compression of articular cartilage explants is associated with progressive consolidation and altered expression pattern of extracellular matrix proteins. *Matrix Biol* 18: 391-9 (1999) DOI: 10.1016/S0945-053X(99)00029-3

38. Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD. Biosynthetic response of cartilage explants to dynamic compression. *J Orthop Res* 7: 619-36 (1989) DOI: 10.1002/jor.1100070502

39. Larsson T, Aspden RM, Heinegard D. Effects of mechanical load on cartilage matrix biosynthesis *in vitro*. *Matrix* 11: 388-94 (1991) DOI: 10.1016/S0934-8832(11)80193-9

40. Parkkinen JJ, Ikonen J, Lammi MJ, Laakkonen J, Tammi M, Helminen HJ. Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. *Arch Biochem Biophys* 300: 458-65 (1993) DOI: 10.1006/abbi.1993.1062

41. Kisiday JD, Jin M, DiMicco MA, Kurz B, Grodzinsky AJ. Effects of dynamic compressive loading on chondrocyte biosynthesis in self-assembling peptide scaffolds. *J Biomech* 37: 595-604 (2004) DOI: 10.1016/j.jbiomech.2003.10.005

42. Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. *J Orthop Res* 20: 842-8 (2002) DOI: 10.1016/S0736-0266(01)00160-7

43. Lee CR, Grodzinsky AJ, Spector M. Biosynthetic response of passaged chondrocytes in a type II collagen scaffold to mechanical compression. *J Biomed Mater Res* A 64: 560-9 (2003) DOI: 10.1002/jbm.a.10443

44. Hunter CJ, Mouw JK, Levenston ME. Dynamic compression of chondrocyte-seeded fibrin gels: effects on matrix accumulation and mechanical stiffness. *Osteoarthritis Cartilage* 12: 117-30 (2004) DOI: 10.1016/j.joca.2003.08.009

45. Lee CR, Grad S, Gorna K, Gogolewski S. Fibrin-polyurethane composites for articular cartilage tissue engineering: a preliminary analysis. *Tissue Eng* 11: 1562-73 (2005) DOI: 10.1089/ten.2005.11.1562

46. Seidel JO, Pei M, Gray ML, Langer R, Freed LE. Long-term culture of tissue engineered cartilage in a perfused chamber with mechanical stimulation. *Biorheology* 41: 445-58 (2004) Doi not found.

47. Frank EH, Jin M, Loening AM, Levenston ME, Grodzinsky AJ. A versatile shear and compression apparatus for mechanical stimulation of tissue culture explants. *J Biomech* 33: 1523-7 (2000) DOI: 10.1016/S0021-9290(00)00100-7

48. Jin M, Frank EH, Quinn TM, Hunziker EB, Grodzinsky AJ. Tissue shear deformation stimulates proteoglycan and protein biosynthesis in bovine cartilage explants. *Arch Biochem Biophys* 395: 41-8 (2001)

DOI: 10.1006/abbi.2001.2543

49. Waldman SD, Spiteri CG, Grynpas MD. Long-term intermittent shear deformation improves the quality of cartilaginous tissue formed *in vitro*. *J Orthop Res* 21: 590-6 (2003) DOI: 10.1016/S0736-0266(03)00009-3
50. Waldman SD, Spiteri CG, Grynpas MD. Long-term intermittent compressive stimulation improves the composition and mechanical properties of tissue-engineered cartilage. *Tissue Eng* 10: 1323-31 (2004) DOI: 10.1089/ten.2004.10.1323
51. Carver SE, Heath CA. Increasing extracellular matrix production in regenerating cartilage with intermittent physiological pressure. *Biotechnol Bioeng* 62: 166-74 (1999) DOI: 10.1002/(SICI)1097-0290(19990120)62:2 <166:AID-BIT6>3.0.CO;2-K
52. Mizuno S, Tateishi T, Ushida T, Glowacki J. Hydrostatic fluid pressure enhances matrix synthesis and accumulation by bovine chondrocytes in three-dimensional culture. *J Cell Physiol* 193: 319-27 (2002) DOI: 10.1002/jcp.10180
53. Salter DM, Hughes DE, Simpson R, Gardner DL. Integrin expression by human articular chondrocytes. *Br J Rheumatol* 31: 231-4 (1992) DOI: 10.1093/rheumatology/31.4.231
54. Wright M, Jobanputra P, Bavington C, Salter DM, Nuki G. Effects of intermittent pressure-induced strain on the electrophysiology of cultured human chondrocytes: evidence for the presence of stretch-activated membrane ion channels. *Clin Sci (Lond)* 90: 61-71 (1996) DOI: 10.1042/cs0900061
55. Millward-Sadler SJ, Wright MO, Lee H, Nishida K. Integrin-regulated secretion of interleukin 4: A novel pathway of mechanotransduction in human articular chondrocytes. *J Cell Biol* 145: 183-9 (1999) DOI: 10.1083/jcb.145.1.183
56. Vincent T, Hermansson M, Bolton M, Wait R, Saklatvala J. Basic FGF mediates an immediate response of articular cartilage to mechanical injury. *Proc Natl Acad Sci U S A* 99: 8259-64 (2002) DOI: 10.1073/pnas.122033199
57. Li KW, Wang AS, Sah RL. Microenvironment regulation of extracellular signal-regulated kinase activity in chondrocytes: effects of culture configuration, interleukin-1, and compressive stress. *Arthritis Rheum* 48: 689-99 (2003) DOI: 10.1002/art.10849
58. Vincent TL, Hermansson MA, Hansen UN. Basic fibroblast growth factor mediates transduction of mechanical signals when articular cartilage is loaded. *Arthritis Rheum* 50: 526-33 (2004) DOI: 10.1002/art.20047
59. Ostergaard K, Salter DM. Immunohistochemistry in the study of normal and osteoarthritic articular cartilage. *Prog Histochem Cytochem* 33: 93-165 (1998) DOI: 10.1016/S0079-6336(98)80004-1
60. Loeser RF. Chondrocyte integrin expression and function. *Biorheology* 37: 109-16 (2000) Doi not found
61. Schaller MD, Otey CA, Hildebrand JD, Parsons JT. Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. *J Cell Biol* 130: 1181-7 (1995) DOI: 10.1083/jcb.130.5.1181
62. Shakibaei M, John T, De Souza P. Signal transduction by beta1 integrin receptors in human chondrocytes *in vitro*: collaboration with the insulin-like growth factor-I receptor. *Biochem J* 342 Pt 3: 615-23 (1999) DOI: 10.1042/bj3420615
63. Wright MO, Nishida K, Bavington C, Godolphin JL. Hyperpolarisation of cultured human chondrocytes following cyclical pressure-induced strain: evidence of a role for alpha 5 beta 1 integrin as a chondrocyte mechanoreceptor. *J Orthop Res* 15: 742-7 (1997) DOI: 10.1002/jor.1100150517
64. Millward-Sadler SJ, Wright MO, Lee H. Altered electrophysiological responses to mechanical stimulation and abnormal signalling through alpha5beta1 integrin in chondrocytes from osteoarthritic cartilage. *Osteoarthritis Cartilage* 8: 272-8 (2000) DOI: 10.1053/joca.1999.0301
65. Chowdhury TT, Salter DM, Bader DL, Lee DA. Integrin-mediated mechanotransduction processes in TGFbeta-stimulated monolayer-expanded chondrocytes. *Biochem Biophys Res Commun* 318: 873-81 (2004)

DOI: 10.1016/j.bbrc.2004.04.107

66. Hung CT, Henshaw DR, Wang CC, Mauck RL, Raia F. Mitogen-activated protein kinase signaling in bovine articular chondrocytes in response to fluid flow does not require calcium mobilization. *J Biomech* 33: 73-80 (2000) DOI: 10.1016/S0021-9290(99)00176-1

67. Loeser RF, Forsyth CB, Samarel AM, Im HJ. Fibronectin fragment activation of proline-rich tyrosine kinase PYK2 mediates integrin signals regulating collagenase-3 expression by human chondrocytes through a protein kinase C-dependent pathway. *J Biol Chem* 278: 24577-85 (2003) DOI: 10.1074/jbc.M304530200

68. Cao L, Lee V, Adams ME, Kiani C, Zhang Y, Hu W, Yang BB. beta-Integrin-collagen interaction reduces chondrocyte apoptosis. *Matrix Biol* 18: 343-55 (1999) DOI: 10.1016/S0945-053X(99)00027-X

69. Guilak F, Zell RA, Erickson GR, Grande DA. Mechanically induced calcium waves in articular chondrocytes are inhibited by gadolinium and amiloride. *J Orthop Res* 17: 421-9 (1999) DOI: 10.1002/jor.1100170319

70. Wang XT, Nagaba S, Nagaba Y, Leung SW. Cardiac L-type calcium channel alpha 1-subunit is increased by cyclic adenosine monophosphate: messenger RNA and protein expression in intact bone. *J Bone Miner Res* 15: 1275-85 (2000) DOI: 10.1359/jbmr.2000.15.7.1275

71. Chao PH, West AC, Hung CT. Chondrocyte intracellular calcium, cytoskeletal organization, and gene expression responses to dynamic osmotic loading. *Am J Physiol Cell Physiol* 291: C718-25 (2006) DOI: 10.1152/ajpcell.00127.2005

72. Zayzafoon M. Calcium/calmodulin signaling controls osteoblast growth and differentiation. *J Cell Biochem* 97: 56-70 (2006) DOI: 10.1002/jcb.20675

73. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 15: 707-47 (1997) DOI: 10.1146/annurev.immunol.15.1.707

74. Huser CA, Davies ME. Calcium signaling leads to mitochondrial depolarization in impact-induced chondrocyte death in equine articular cartilage explants. *Arthritis Rheum* 56: 2322-34 (2007) DOI: 10.1002/art.22717

75. Tomita M, Reinhold MI, Molkentin JD, Naski MC. Calcineurin and NFAT4 induce chondrogenesis. *J Biol Chem* 277: 42214-8 (2002) DOI: 10.1074/jbc.C200504200

76. Bennett JH, Joyner CJ, Triffitt JT, Owen ME. Adipocytic cells cultured from marrow have osteogenic potential. *J Cell Sci* 99 (Pt 1): 131-9 (1991) Doi not found

77. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats--similarities to astrocyte grafts. *Proc Natl Acad Sci U S A* 95: 3908-13 (1998) DOI: 10.1073/pnas.95.7.3908

78. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279: 1528-30 (1998) DOI: 10.1126/science.279.5356.1528

79. Young RG, Butler DL, Weber W, Caplan AI. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res* 16: 406-13 (1998) DOI: 10.1002/jor.1100160403

80. Alhadlaq A, Elisseeff JH, Hong L, Williams CG, Caplan AI, Sharma B, Kopher RA. Adult stem cell driven genesis of human-shaped articular condyle. *Ann Biomed Eng* 32: 911-23 (2004) DOI: 10.1023/B: ABME.0000032454.53116.ee

81. Marion NW, Mao JJ. Mesenchymal stem cells and tissue engineering. *Methods Enzymol* 420: 339-61 (2006) DOI: 10.1016/S0076-6879(06)20016-8

82. Lee CH, Marion NW, Hollister S, Mao JJ. Tissue formation and vascularization in anatomically shaped human joint condyle ectopically *in vivo*. *Tissue Eng Part A* 15: 3923-30 (2009) DOI: 10.1089/ten.tea.2008.0653

83. Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* 7: 259-64 (2001)

DOI: 10.1016/S1471-4914(01)02016-0

84. Batson EL, Reilly GC, Currey JD, Balderson DS. Postexercise and positional variation in mechanical properties of the radius in young horses. *Equine Vet J* 32: 95-100 (2000) DOI: 10.2746/042516400777591570

85. Kopher RA, Mao JJ. Suture growth modulated by the oscillatory component of micromechanical strain. *J Bone Miner Res* 18: 521-8 (2003) DOI: 10.1359/jbmr.2003.18.3.521

86. You J, Yellowley CE, Donahue HJ. Substrate deformation levels associated with routine physical activity are less stimulatory to bone cells relative to loading-induced oscillatory fluid flow. *J Biomech Eng* 122: 387-93 (2000) DOI: 10.1115/1.1287161

87. McGarry JG, Klein-Nulend J, Mullender MG. A comparison of strain and fluid shear stress in stimulating bone cell responses--a computational and experimental study. *FASEB J* 19: 482-4 (2005) Doi not found.

88. Tami AE, Schaffler MB, Knothe Tate ML. Probing the tissue to subcellular level structure underlying bone's molecular sieving function. *Biorheology* 40: 577-90 (2003) Doi not found

89. Reich KM, Gay CV, Frangos JA. Fluid shear stress as a mediator of osteoblast cyclic adenosine monophosphate production. *J Cell Physiol* 143: 100-4 (1990) DOI: 10.1002/jcp.1041430113

90. Sittichokechaiwut A, Edwards JH, Scutt AM. Short bouts of mechanical loading are as effective as dexamethasone at inducing matrix production by human bone marrow mesenchymal stem cell. *Eur Cell Mater* 20: 45-57 (2010) Doi not found

91. Friedl G, Schmidt H, Rehak I, Kostner G. Undifferentiated human mesenchymal stem cells (hMSCs) are highly sensitive to mechanical strain: transcriptionally controlled early osteo-chondrogenic response *in vitro*. *Osteoarthritis Cartilage* 15: 1293-300 (2007) DOI: 10.1016/j.joca.2007.04.002

92. Kreke MR, Huckle WR, Goldstein AS. Fluid flow stimulates expression of osteopontin and bone sialoprotein by bone marrow stromal cells in a temporally dependent manner. *Bone* 36: 1047-55 (2005) DOI: 10.1016/j.bone.2005.03.008

93. Datta N, Pham QP, Sharma U, Sikavitsas VI. *In vitro* generated extracellular matrix and fluid shear stress synergistically enhance 3D osteoblastic differentiation. *Proc Natl Acad Sci U S A* 103: 2488-93 (2006) DOI: 10.1073/pnas.0505661103

94. Jungreuthmayer C, Donahue SW, Jaasma MJ, Al-Munajjed AA. A comparative study of shear stresses in collagen-glycosaminoglycan and calcium phosphate scaffolds in bone tissue-engineering bioreactors. *Tissue Eng Part A* 15: 1141-9 (2009) DOI: 10.1089/ten.tea.2008.0204

95. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 126: 677-89 (2006) DOI: 10.1016/j.cell.2006.06.044

96. Yang Y, Beqaj S, Kemp P, Ariel I, Schuger L. 2000. Stretch-induced alternative splicing of serum response factor promotes bronchial myogenesis and is defective in lung hypoplasia. *J Clin Invest* 106: 1321-30 () DOI: 10.1172/JCI8893

97. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell* 6: 483-95 (2004) DOI: 10.1016/S1534-5807(04)00075-9

98. Kimura K, Ito M, Amano M, Chihara K, Fukata Y. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 273: 245-8 (1996) DOI: 10.1126/science.273.5272.245

Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; TNF-α, tumor necrosis factor-α; OA, osteoarthritis; IL-1, Interlukin-1; GAG, glycosaminoglycan; MSCs, mesenchymal stem cells; ALP, alkaline phosphatase

Key Words: Articular Cartilage, Mechanical Loading, Chondrocyte, Mesenchymal Stem Cell, Chondrogenesis, Review

Send correspondence to: Yong Lu, Department of Radiology, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200025, China. Tel: 86-021-64370045, Fax: 86-021-64370045, E-mail: ly10936@yahoo.com