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CARTILAGE TISSUE REMODELING IN RESPONSE TO MECHANICAL FORCES

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■ **Abstract** Recent studies suggest that there are multiple regulatory pathways by which chondrocytes in articular cartilage sense and respond to mechanical stimuli, including upstream signaling pathways and mechanisms that may lead to direct changes at the level of transcription, translation, post-translational modifications, and cell-mediated extracellular assembly and degradation of the tissue matrix. This review focuses on the effects of mechanical loading on cartilage and the resulting chondrocyte-mediated biosynthesis, remodeling, degradation, and repair of this tissue. The effects of compression and tissue shear deformation are compared, and approaches to the study of mechanical regulation of gene expression are described. Of particular interest regarding dense connective tissues, recent experiments have shown that mechanotransduction is critically important in vivo in the cell-mediated feedback between physical stimuli, the molecular structure of newly synthesized matrix molecules, and the resulting macroscopic biomechanical properties of the tissue.

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INTRODUCTION

During the past decade, increasing attention has focused on the ability of cells and tissues to respond to mechanical forces and other physical stimuli in their environment. Investigators have studied cellular mechanotransduction in a broad range of soft (1–3) and hard (4, 5) connective tissues, epithelial and endothelial tissues (6), and muscle (7). Significant advances have been made in the initial understanding of transduction mechanisms (e.g. see 8 for review). However, many of the unique mechanisms and associated responses to physical forces that are observed in different cell types remain to be elucidated.

This review focuses on the effects of mechanical loading on cartilage, and the resulting chondrocyte-mediated biosynthesis, remodeling, degradation, and repair of this tissue. In addition, cartilage can be viewed as model biological tissue in which cells within a dense extracellular matrix (ECM) are presented with a complex combination of physical forces and flows (9–12) as well as biological signaling factors. These physical stimuli and the resulting cellular responses should be studied at the molecular, cellular, and tissue levels to fully understand the feedback between applied macroscopic forces, ECM molecular structure, and the resulting macrocontinuum tissue material properties (9, 13)—a feedback process that is orchestrated by cells in vivo (e.g. 1).

Articular cartilage is subjected to a wide range of static and dynamic mechanical loads in human synovial joints (14–16), with peak stress amplitudes reaching 10–20 MPa (100–200 atm) during activities such as stair climbing (17). Experimental and theoretical studies show that cartilage compression of $\leq$15%–45% may occur in response to long-term or static loads within the physiological range (14, 18, 19). In contrast, compressions of only a few percent occur during short-duration (high-frequency) loading. The ability of cartilage to withstand physiological compressive, tensile, and shear forces depends on the composition and structural integrity of its ECM. In turn, the maintenance of a functionally intact ECM requires chondrocyte-mediated synthesis, assembly, and degradation of proteoglycans (PGs), collagens, noncollagenous proteins and glycoproteins, and other matrix molecules (20).

It is now well accepted that mechanical stimuli in the microenvironment of the chondrocytes can significantly affect the synthesis and degradation of matrix macromolecules. However, the cellular transduction mechanisms that govern

chondrocyte response to mechanical stimuli are not well understood. Recent data suggest that there are multiple regulatory pathways by which chondrocytes sense and respond to mechanical stimuli, including upstream signaling pathways (6, 21–25) and mechanisms that may lead to direct changes at the level of transcription (26–29), translation, and post-translational modifications (30–32) and cell-mediated extracellular assembly and degradation of matrix (33–36). Correspondingly, there may be multiple pathways by which physical stimuli can alter not only the rate of matrix production, but the quality and functionality of newly synthesized PGs, collagens, and other molecules. In this manner, specific mechanical loading regimens may either enhance or compromise the ultimate biomechanical function of cartilage.

SYSTEMS FOR THE STUDY OF CELLULAR MECHANISMS

The mechanisms by which chondrocytes respond to mechanical stimuli are difficult to study in vivo. As a result, in vitro models such as cartilage explant and three-dimensional chondrocyte/gel culture systems have become increasingly important for two reasons: (*a*) these systems can preserve or emulate native tissue structure and thereby enable quantitative correlations between mechanical and biological parameters; and (*b*) cell-matrix interactions and chondrocyte gene expression can be preserved in these systems (37–39). Geometrically defined explants can attain steady-state levels of matrix synthesis and turnover, suitable for studying perturbations caused by applied mechanical stimuli. Muir (40) recently emphasized the important but complex role of the native ECM and chondrocyte-ECM interactions in understanding the mechanisms of chondrocyte response to load. Of course, the coupling between mechanical, electrical, and chemical forces and flows within ECM may complicate the identification of specific physical stimuli, necessitating specialized experimental approaches. However, Muir (40), Parkkinen et al (31), and others have cautioned that the use of isolated chondrocytes that are depleted of natural matrix must be approached with care regarding the physiological interpretation of such tests and the potential for chondrocyte dedifferentiation. Therefore, three-dimensional agarose (37, 41, 42) and alginate (43–45) gel culture systems have been used to study phenomena such as chondrocyte phenotypic expression, proliferation, and accumulation of a PG-rich ECM during long-term culture.

Using such tissue and cell systems, investigators have studied the effects of applied mechanical compression (load or displacement control), hydrostatic pressure, physicochemical stimuli (pH and osmolarity), and electrical currents (for recent reviews, see 46, 47). Recently, a versatile apparatus for application of shear and compression to tissue specimens has been designed and fabricated (48). The instrument is housed in a standard incubator for long-term stimulation studies, and it can be used with closed-loop feedback control of displacement, load, shear angle, or torque (the shear modes incorporating a rotational platform). In such experiments, explant or gel culture disks are tested within autoclavable polysulfone

chambers such as those used previously (49, 50), which can be clamped into the jaws of the apparatus.

EFFECTS OF STATIC AND DYNAMIC COMPRESSION

The application of mechanical compression directly to cartilage explants, by using a range of amplitudes and frequencies, has been motivated by physiologically relevant loading parameters. The metabolic response to compression in vitro shows similar trends to those seen in animal studies: (*a*) static compression significantly inhibits synthesis of PGs and proteins (49, 51–55), whereas (*b*) dynamic compression can markedly stimulate matrix production (49, 56–61). The response to dynamic compression, however, depends on compression frequency and amplitude. For example, biosynthesis in 3-mm-diameter explants was not affected by low-strain-amplitude (1%–4%), unconfined compression at low frequency (<0.001 Hz), whereas aggrecan and protein synthesis in these same explants was stimulated by low strain amplitudes at higher frequencies [0.01–1 Hz (49)]. Most studies have used bovine, canine, or other animal cartilages, although a few experiments have demonstrated similar trends with human cartilage (52, 62).

Cellular and Intracellular Correlates

Several biophysical mechanisms may regulate the chondrocyte metabolic response to these static and dynamic compression regimens. Static compression has been shown to reduce the rate of transport of macromolecules from reduced average ECM pore size (51), change local ion concentrations, including pH, in the pericellular matrix via the Donnan effect (53, 63–65), and alter cell and nucleus structure (66–68). In addition, the effects of tissue compression on the deformation of the the matrix, chondrocytes, and nuclei have been studied to better understand the possible role of cell shape/deformation on chondrocyte signal transduction. Using Nomarski imaging (69, 70), confocal microscopy (67), and stereology of explant specimens fixed after static and dynamic compression (68), investigators have found that compression applied to the surfaces of cartilage specimens causes a corresponding compression of the pericellular (70), as well as territorial and interterritorial (67–69), matrix near and around the cells. In adult cartilage, columns of chondrocyte-containing chondrons appear to be compacted at all depths (69, 70), with accompanying loss of pericellular matrix volume and water content. These observations are consistent with other studies (71) that demonstrate that the equilibrium modulus of cartilage ECM is ~1000-fold larger than that of the chondrocyte; thus, cell deformation follows the imposed tissue deformation. Recent studies (67, 68, 72) have shown that distinct changes in cell and nucleus shape are produced by compression that is imposed at tissue surfaces. In general, compression caused flattening of the cells in the direction of loading and a decrease in cell volume (66–68) and could also cause changes in cell surface area (68), nucleus volume and height (68, 72), and nucleus surface area (68). These changes vary

with depth, the degree of anisotropy and inhomogeneity of the collagen network, and the age of the tissue (73).

Kinetics of Chondrocyte Response: Clues to Mechanisms

Measurements of the rate at which chondrocytes can sense and respond to mechanical stimuli can give valuable insight into intracellular regulatory mechanisms. Previous studies have revealed that the inhibition of biosynthesis during static compression can occur as rapidly as 1 h after application of compression (49, 74). For example, newborn calf cartilage disks subjected to 35% compression from their 1-mm-cut thickness showed an ~45% decrease in PG synthesis (sulfate radiolabel incorporation) within 2 h, compared with uncompressed controls (75; see Figure 3*A* below). In contrast, insulin-like growth factor 1 (IGF-1) at 300 ng/ml stimulated PG synthesis by twofold in these uncompressed calf cartilage disks after 24–48 h of exposure (see Figure 3*A* below), consistent with many previous studies (76). However, disks simultaneously compressed and treated with IGF-1 at time $t = 0$ showed an initial rapid decrease in biosynthesis by 2 h, followed by a dramatic increase above baseline after 24 h. This intriguing bimodal response kinetics suggests that the transduction mechanisms underlying the response to mechanical compression and the signaling factor IGF-1 are regulated via independent metabolic pathways. Thus, compression may regulate tissue response to biological factors and vice versa (75). Recovery of biosynthesis after release of static compression can be much slower than 1–2 h, and it depends on the duration and amplitude of the static compression before release (49, 54, 55, 74). After a 2-h static compression followed by release, aggrecan synthesis recovered fully in another 2 h (49, 74). In contrast, after release of a 12-h, 50%-static compression, 60 h were necessary for biosynthesis to return to free-swelling levels. Over time, the concentration of intracellular enzymes necessary for glycosylation and sulfation may decrease, hence the longer time necessary for recovery of aggrecan synthesis with increased duration of compression.

SPATIAL PATTERNS OF EXTRACELLULAR-MATRIX SYNTHESIS: Relationship to Physical Stimuli

Fields, Forces, and Flows: Poroelastic/Electromechanical Models

During the past decades, much progress has been made in understanding the magnitude and distribution of physical forces and flows in the neighborhood of the chondrocyte within loaded cartilage. Experiments have shown that compression of cartilage causes deformation of cells and matrix (50, 67–70, 77, 78), hydrostatic pressure gradients, fluid flow, streaming potentials and currents (9, 10, 79–81; Figure 1), and physicochemical changes including altered matrix water content, fixed charge density, mobile ion concentrations, and osmotic pressure (9, 53, 63, 64,

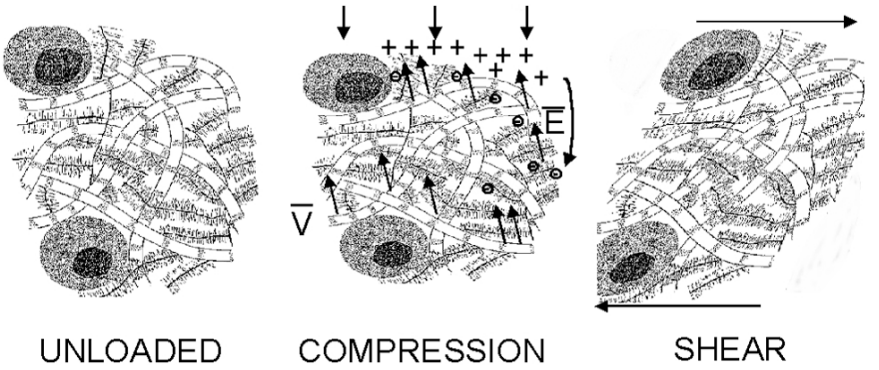

Figure 1 Schematic representation of loading regimes of articular cartilage. Dynamic compression of the extracellular matrix induces deformation of cells and matrix, hydrostatic pressure gradients, and interstitial fluid flow. Fluid convection and separation of counterions from the fixed charge groups of the proteoglycan constituents gives rise to electrical streaming potentials and currents. In contrast, tissue shear deformation of a poroelastic tissue does not induce volumetric changes, intratissue fluid flow, or pressure gradients.

82). Any of these mechanical, chemical, or electrical signals may modulate matrix metabolism. An understanding of the spatial distribution of these forces and flows at the tissue and even cellular length scales during compression of cartilage has been aided by the development of theoretical models for mechanical, physicochemical, and electromechanical behavior of ECM (79, 83–86; for reviews, see 46, 47, 87). Such models can provide a useful framework for correlating the observed spatial distributions of matrix synthesis with profiles of physical stimuli that occur within cartilage explants during static and dynamic compression (e.g. 50, 78, 80, 88).

Levenston et al recently developed a variational framework for describing coupled mechanical, electrical, and chemical/osmotic phenomena in hydrated tissues experiencing finite deformations (89–91), solved numerically by using the finite-element method. Depending on the requirements of a given experiment, versions of this formulation have been implemented that consider only mechanical behavior (90), coupled mechanical and electrical behavior (89), and a general form that considers intratissue flux of individual ionic and neutral solute species (91). This approach includes consideration of tissue inhomogeneity and anisotropy in a self-consistent manner; all material properties in the constitutive laws (modulus, permeability, conductivity, etc) are expressed as functions of both matrix composition and deformation. Thus, material inhomogeneity may result from matrix composition as well as strain-induced inhomogeneities caused by nonuniform deformation fields.

As an example, the unconfined compression geometry (Figure 2*A*) that is often used in experiments involving mechanotransduction in cylindrical cartilage disks that are subjected to static/dynamic compression has been modeled with an

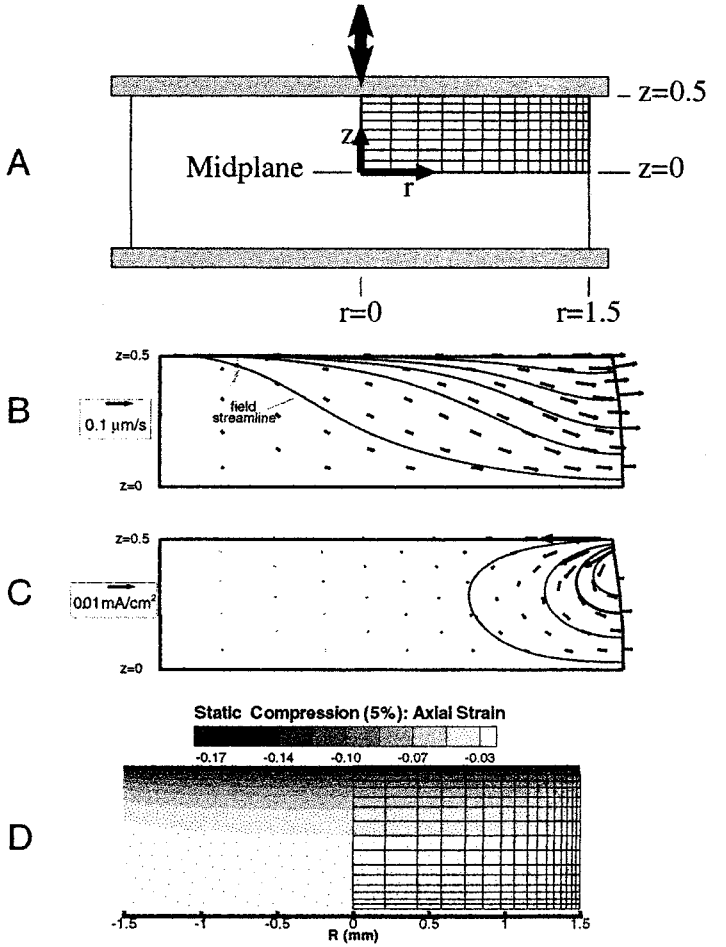

Figure 2 (*A*) Unconfined compression of a cylindrical disk specimen, which has been modeled by an axisymmetric finite-element implementation (89). (*B*) The relative fluid velocity profile in the upper quadrant of *A* at the end of a 60-sec-constant-velocity, 25 μm ramp compression of a 1-mm-thick disk that is 3 mm in diameter. (*C*) Current density profile at the end of the ramp compression (see 89 for details). (*D*) Axial strain profile predicted (89) for a statically compressed disk with depth-dependent equilibrium modulus (e.g. 92).

axisymmetric finite element implementation (Figure 2*A*). The predicted intratissue profiles of compression-induced physical phenomena such as fluid pressure, strain, relative fluid velocity (Figure 2*B*), and compression-induced current density (Figure 2*C*) were compared to observed patterns of altered biosynthetic activity in response to dynamic compression of cultured bovine cylindrical disks. Such an approach was also used by Soulhat et al (86), who developed an analytical fibril-reinforced composite poroelastic model of cartilage. Although the details

of these theoretical models differ, the qualitative trends are similar; the fluid velocity compared with the ECM is zero on axis (at $r = 0$, by symmetry) and generally increases with increasing distance toward the outer radial periphery of the disks. Furthermore, at higher compression frequencies, the region of maximal fluid velocity becomes increasingly localized near the outer edge of the disk specimen.

Spatial Patterns of Biosynthesis

To visualize the spatial profile of biosynthetic response to compression, Buschmann et al (88) and Quinn et al (50) developed methods of quantitative autoradiography that are applied at the tissue and cellular levels, respectively, with 1 μm length scale resolution. At the tissue level, they discovered that stimulation of PG synthesis in cartilage explants and cell/gel disks appeared with a spatial profile that most closely matched the profile of intratissue fluid flow and matrix deformation within the specimens. Thus, these physical stimuli appear to be critically important in regulating chondrocyte response to dynamic compression. Quinn et al further discovered that the most dramatic stimulation of PG synthesis occurred in the pericellular matrix region (50), which appears to be a very sensitive region in which mechanical stimuli can rapidly and directionally affect synthesis of aggrecan.

The model of Levenston et al (90, 91) also predicted the patterns of induced electrical-streaming potentials, which were similar to those of the fluid pressure, indicating that the macroscopic electrical potential is an unlikely tissue-level stimulus in this system, although the electric field or potential gradient may be important. Thus, cyclic compression gave rise to an induced current density near the edge of the explant (Figure 2*C*), which is proportional to the electric field strength. This current density is a direct consequence of deformation-induced inhomogeneities in the material properties, and it is not predicted by the homogeneous, infinitesimal-strain theory. Substantial depth-dependent variations in the biosynthetic response to static compression have also been found to correlate with local variations in tissue strains (78). Based on known depth-dependent material properties (77, 92), finite-element models (89) that represent static compression of a 1-mm full-thickness disk of inhomogeneous adult articular cartilage can exhibit local strains that vary by an order of magnitude through the thickness (Figure 2*D*).

COMPRESSION-INDUCED FLUID FLOW AND SOLUTE TRANSPORT

If convective transport is an operative metabolic stimulant during dynamic compression of cartilage, it might act (*a*) by directly stimulating chondrocytes [e.g. fluid shear at the cell surface (93)] or (*b*) by altering the pericellular concentrations

of macromolecular cytokines, growth factors, degradative enzymes, endogenous enzyme inhibitors, newly synthesized matrix macromolecules, or other nutrients. Cohen et al (94) and O'Hara et al (95) measured the effects of static and dynamic compression, respectively, on the partitioning and absorption of large and small molecules into cartilage, including radiolabeled bovine serum albumin, IGF-1, urea, and sodium. They concluded that static compression affected the transport of large solutes more than that of small solutes and that dynamic compression enhanced the desorption of large solutes much more than that of small solutes. The removal of PG could also increase the transport of large solutes into cartilage (96). Recently, Garcia et al developed a new approach to quantify the individual contributions of diffusion, convection, and electrical migration in the transport within cartilage of neutral and charged proteins and lower-molecular-weight solutes (97, 98). This approach enables quantification of the effects of solute binding of transport within tissue (e.g. the binding of IGF-1 to specific IGF binding proteins within cartilage matrix (98a). This approach also allows direct measurement of radiolabeled solute diffusion, convection (by application of an electric current to induce electroosmotic fluid flow within the tissue), and electrical migration of charged solutes (which would occur in the presence of streaming potential fields), by using fluid velocities of $\sim$1 μm/s [which corresponds to velocities obtained during normal walking frequencies (99)]. The results confirmed that convective enhancement of transport is particularly important for larger solutes (97) and that protein flux within cartilage could be greatly enhanced by fluid velocities that are relevant to physiologic mechanical compression. For example, transport of ^{125}I-labeled IGF-1 and ^{125}I-labeled recombinant human–tissue inhibitor of metalloproteinase-1 were enhanced by $\sim$20- and 70-fold, respectively, above diffusion alone by fluid velocities of $\sim$1–2 μm/s.

The implications of these studies were further explored by Bonassar et al (100), who found that 300 ng/ml of IGF-1 alone increased protein synthesis by 90% in calf cartilage disks and that dynamic compression alone increased protein synthesis by 40% (Figure 3*B*). Once again, when applied together, these two different classes of stimuli enhanced protein synthesis by 180%—2–4-fold greater than that achieved by either stimulus alone. IGF-1 augmented protein synthesis with a time constant of 12.2 h, whereas dynamic compression increased protein synthesis with a time constant of 2.9 h, a rate significantly faster than that of IGF-1. Indeed, in separate experiments with iodinated IGF-1, dynamic compression was found directly to accelerate the transport of labeled IGF-1 into the tissue (100). Together, these findings suggest that these signals act via distinct cell activation pathways. When used in concert with IGF-1, dynamic compression accelerated the biosynthetic response and the transport of the growth factor. This suggests that, in addition to independently stimulating articular chondrocytes, cyclic compression may improve the access of soluble growth factors to these relatively isolated cells.

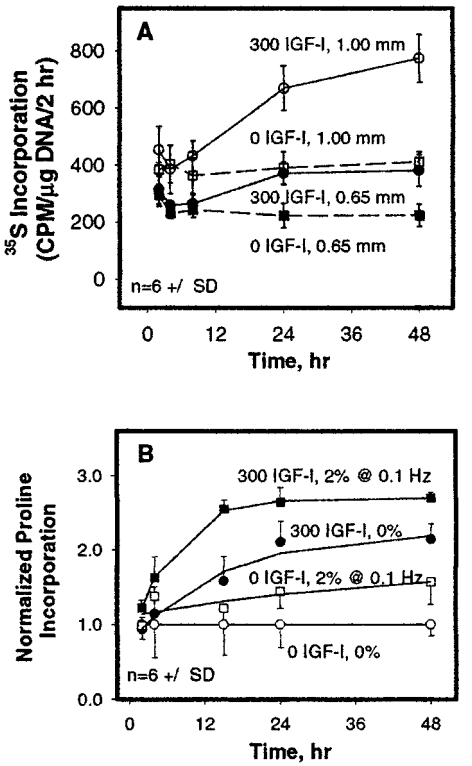

Figure 3 (*A*) ^{35}S-Sulfate incorporation as a measure of proteoglycan synthesis in cartilage explants that were removed from culture at 2, 4, 8, 24, or 48 h after simultaneous exposure to 0 or 300 ng/ml of IGF-1 and were held at either 1.0-mm cut thickness or 0.65-mm compressed thickness (reproduced from 75, by permission of the publisher). (*B*) ^{3}H-Proline radiolabel incorporation as a measure of total protein synthesis in cartilage disks during a 2-h label terminating at 2, 4, 16, 24, and 48 h after treatment with IGF-1 and/or imposition of a 2% sinusoidal strain at 0.1 Hz. Values are normalized to those of control disks [0 ng/ml IGF-1 with no compression (reproduced from 100, by permission of the publisher)].

TISSUE SHEAR

Joint loading in vivo results in a complex combination of compressive, tensile, and shear deformations in cartilage. Although strong evidence suggests the importance of compression-induced fluid flow in the stimulation of chondrocyte biosynthesis (50, 88, 100), it is difficult to separate the effects of fluid flow from the associated matrix deformation and cell/matrix interactions when using intact tissue explants. Dynamic compression is particularly complex, inducing volumetric changes, shear

stresses, and gradients in intratissue pressure and fluid flow (80; Figure 2). In contrast, macroscopic shear deformation of a poroelastic tissue such as articular cartilage should not induce volumetric changes, intratissue fluid flow, or pressure gradients (Figure 1). Therefore, Jin et al (101) and Frank et al (48) applied direct shear to explants to determine whether dynamic matrix and cell deformation (without fluid flow) could stimulate cell metabolism. Such studies are directly aimed at distinguishing the role of intratissue fluid flow and hydrostatic pressure gradients from matrix and cell deformation.

With the incubator-housed tissue shear instrument described above, cartilage explants maintained at their 1-mm cut thickness were subjected to a continuous dynamic-shear deformation of 0.1 Hz and 1% shear strain amplitude for 24 h. Matched control disks also maintained at 1-mm thickness for 24 h had no dynamic shear deformation. During the entire 24-h loading period, disks were incubated with 10 μCi/ml ^{35}S-sulfate and 20 μCi/ml ^{3}H-proline as measures of proteoglycan and total protein synthesis. Both ^{35}S-sulfate and ^{3}H-proline incorporation in dynamically sheared disks were significantly higher ($P < 0.002$), by 25% and 41%, respectively, than in control disks held at the same static offset compression (48, 101; Figure 4). In separate experiments, the spatial distribution of radiolabel was analyzed by separating an inner core from an outer annular ring in each explant by the methods of Kim et al (61). There was no significant difference in biosynthesis between the inner core and annular rings of the dynamically sheared samples, suggesting that matrix shear deformation, not fluid flow, was responsible for metabolic stimulation. While investigators have examined the effects of fluid-induced shear stress in monolayer cell culture (93, 102), the applied fluid velocities

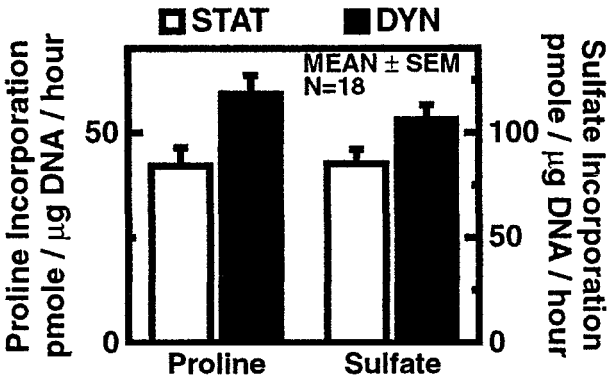

Figure 4 Sulfate and proline radiolabel incorporation (for proteoglycan and total protein synthesis) in cartilage disks subjected to dynamic tissue shear strain of 1% amplitude at 0.1 Hz, compared with control disks maintained at the same static-offset compressed thickness but with no dynamic shear (reproduced from 101, by permission of the publisher).

typically used in those studies have been many orders of magnitude higher than the fluid velocities that are estimated to occur within cartilage in vivo. Experiments such as those of Figure 4 can directly address the stimulatory potential of macroscopic matrix shear (without associated fluid flow), and it appears that such tissue shear can, indeed, regulate matrix biosynthesis. Ongoing studies by several groups continue to explore the possibility that fluid shear can also affect the chondrocytes.

MECHANOTRANSDUCTION: Intracellular Pathways and Molecular Mechanisms

Gene Expression

In recent studies (26, 28, 103), the effects of various mechanical forces on gene expression were investigated by using chondrocytes in monolayer culture. For example, static and intermittent hydrostatic pressure increased the expression of transforming growth factor β, as well as aggrecan and type II collagen mRNA, in high-density monolayer cultures (26, 28). In addition, constant fluid shear forces stimulated expression of mRNA for tissue inhibitor of metalloproteinase-1 in isolated human chondrocytes grown in monolayer (93). Dynamic mechanical forces have also been shown to influence matrix gene expression. When isolated bovine and human chondrocytes are cyclically stretched on flexible membranes, aggrecan and type II collagen mRNA expression is increased (103). Isolated chondrocyte systems are useful models for investigating chondrocyte response to mechanical load. However, extrapolating information obtained from isolated cells in monolayer culture to chondroctyes maintained in their native tissue is difficult owing to the complex physicochemical interactions that exist between the chondrocyte and the ECM in vivo (40).

Currently, there is limited information regarding the effects of compression on chondrocyte gene expression within native articular cartilage. One study reported that constant loads of 0.1 MPa applied for 1 h can transiently increase levels of aggrecan mRNA (27) during creep compression of explants. Ragan et al (104) recently found that chondrocyte expression of aggrecan and type-II collagen decreased with increasing magnitude of a 24-h static compression (in displacement control). However, total mRNA levels increased during the initial 0.5 h after application of the step compression (Figure 5*A*), whereas rates of synthesis of proteoglycans and collagen (Figure 5*B*) have been observed to decrease within 0.3 to 0.6 h after static compression (49). Thus, although mechanical compression can rapidly alter expression of these molecules, the observed decrease in synthesis caused by static compression appears not to be related solely to changes in mRNA expression. Using the techniques of Buschmann et al (105), Ragan et al (106) also discovered that dynamic sinusoidal compression could up-regulate aggrecan and type II collagen gene expression, using cartilage explants as well as isolated chondrocytes that are cultured in alginate cylindrical disks.

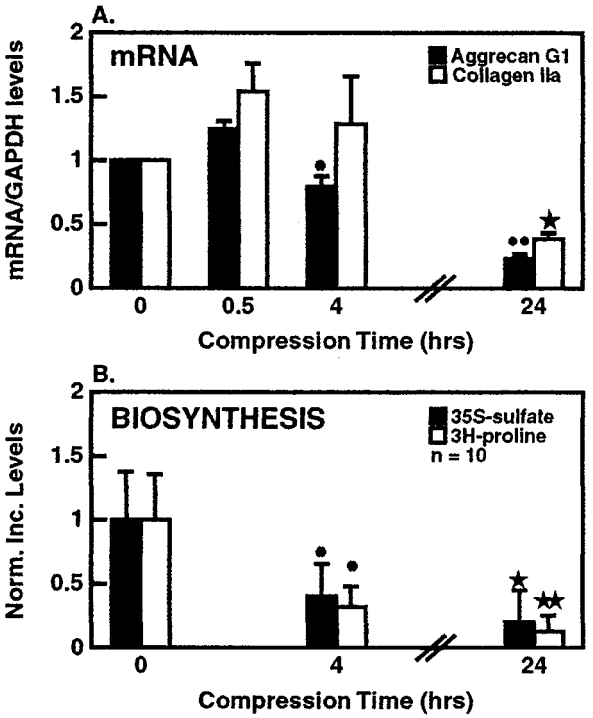

Figure 5 Aggrecan and type-II collagen gene expression and biosynthesis in response to a static compression to 50% of cut thickness (reproduced from 92, by permission of the publisher). (*A*) mRNA levels were quantified and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a function of time after step compression. (*B*) Sulfate and proline radiolabel incorporation for PG and total protein synthesis as a function of time after step compression, normalized to values of free-swelling controls at 24 h (see 104 for details).

Compression Affects Translation and Post-Translational Modifications of Extracellular Matrix Molecules

The rapid changes in synthesis of aggrecan after compression suggest that post-transcriptional processes and not transcription alone are rate limiting. One approach to identifying mechanotransduction mechanisms that involve translation and post-translational events is to study the differential effects of compression on synthesis of specific matrix molecules (32, 54, 107). The three molecular components of the proteoglycan aggregate, for example, involve very different intracellular biosynthetic pathways. Link protein, a typical glycoprotein, undergoes a set of well-defined post-translational steps before its secretion from the cell. On the other hand, the post-translational processing of aggrecan core protein is spatially and temporally much more elaborate, requiring the sequential addition

of *N*-linked oligosaccharides and the addition of chondroitin sulfate and keratan sulfate glycosaminoglycan (GAG) chains (108). Synthesis of chondroitin sulfate is initiated in the late endoplasmic reticulum and continued in the proximal regions of the Golgi complex (108). In marked contrast to both aggrecan and link protein, hyaluronan does not involve a protein precursor. Rather, hyaluronan is synthesized at the plasma membrane by hyaluronate synthase and secreted directly into the ECM (109).

Therefore, compression-induced alterations in the morphology and structure of the rough endoplasmic reticulum and Golgi apparatus could have marked effects on the form and function of newly synthesized matrix molecules such as aggrecan. For example, Kim et al (32) found marked changes in GAG chain length, charge density (sulfation), and spacing along the aggrecan core protein, which could be induced by static compression. The ability for subtle changes in GAG charge density or spacing along the core to cause significant changes in PG swelling pressure and cartilage modulus has also been suggested by a recent micromechanical model for GAG electromechanical interactions (110).

Ultrastructure and Morphology of Intracellular Organelles

Previous studies have shown that high pressure can cause changes in cell morphology, disorganization of the Golgi and microtubules (30), and disappearance of stress fibers (31) in chondrocytes that had spread on glass coverslips. Recently we initiated a study of the effects of compression on the structure and morphology of intracellular organelles including the rough endoplasmic reticulum, Golgi complex, mitochondria, liposomes, glycogen granules, and lipid droplets. Calf cartilage explants, which had been subjected to graded levels of static mechanical compression, and specimens were fixed during static compression, embedded, sectioned, and visualized by electron microscopy. The micrographs of Figures 6*A* and *B* (see color insert) (low and high magnification) are from a cell within tissue that was subjected to 20% static compression for 12 h (i.e. within the physiological range of static comppression). Qualitatively, it is clear that static compression can dramatically alter the morphology of organelles to the highly oriented anisotropic ultrastructure shown in Figure 6*B* (see color insert) (111). The possibility that such changes in organelle morphology could alter the location and activity of intracellular enzymes, such as sulfotransferases (which are resposible for sulfation of chondroitin sulfate-GAG in the Golgi apparatus) is now being explored by visualization with 2-photon microscopy (Figure 6*C*; see color insert).

INJURIOUS MECHANICAL LOADING AFFECTS CELL VIABILITY AND MATRIX DEGRADATION

In osteoarthritis, cartilage matrix composition is altered substantially, which considerably weakens the tissue to the extent that mechanical wear from joint motion can result in erosion of cartilage down to the bone surface (112). Cartilage matrix

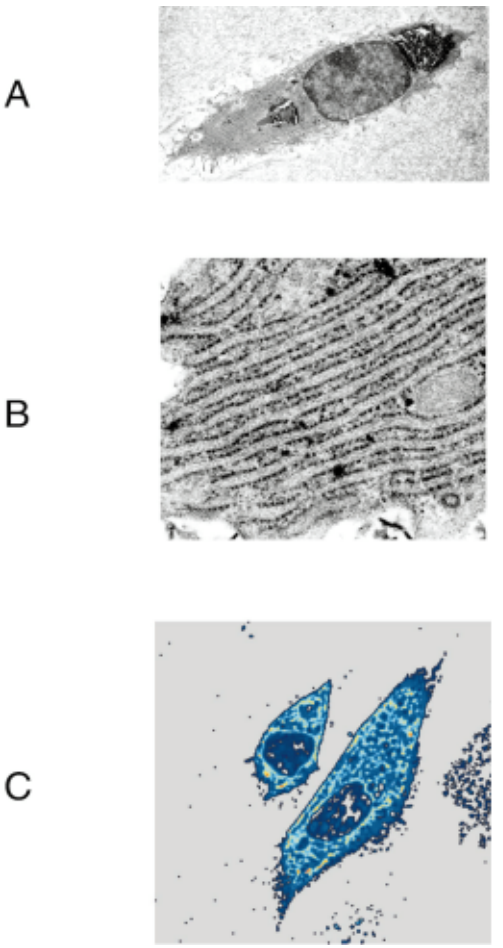

Figure 6 (*A*) Low and (*B*) high magnification of chondrocyte within a native cartilage tissue disk subjected to 20% axial static unconfined compression, fixed, and visualized by electron microscope. (*B*) Ordered, anisotropic morphology or rough endoplasmic reticulum during compression, compared with the more istropic, randomly ordered distribution in uncompressed controls (see 111 for details). (*C*) CHO cells showing green fluorescence protein-6-sulfotransferase fusion protein transfected into cells and localized within rough endoplasmic reticulum and Golgi apparatus, visualized by using two-photon fluorescence microscopy.

molecules are susceptible to degradation by several families of proteinases (113), including metalloproteinases (114), serine proteinases (115), and the recently reported aggrecanase family (116, 117). Acute mechanical overloads in vivo can cause severe cartilage damage (118, 119). Recent studies in vitro have simulated the effects of controlled impact loads on cartilage explants to assess matrix fissuring, chondrocyte viability, and damage to the collagen and proteoglycan constituents (35, 36, 120–124). Other than acute destruction, the mechanisms by which mechanical forces in the joint may contribute to specific catabolic pathways for matrix degradation remain to be elucidated. It is possible that mechanical compression could alter enzymatic pathways and thereby the forms of the catabolic fragments of aggrecan, link protein, hyaluronan, and collagen. For this reason, significant effort has centered on the discovery of inhibitors of enzymatic degradation that may preserve the biomechanical properties of cartilage ECM (125–127).

We recently developed models for controlled cell and matrix injury in vitro (36, 124, 128) in which the strain, strain rate, or peak stress of compressive loads onto cartilage explant disks could be servocontrolled. Induction of apoptosis at theshhold levels of peak stress and strain rates were identified (124) by the terminal deoxynucleotidyltransferase-mediated UTP end-labeling assay and measures of cell and nucleus morphology. Injurious compression caused mechanical failure of the collagen network (128), resulting in an $\sim$30% increase in explant water content and decreased unconfined compression stiffness (36, 124). However, confined compression stiffness was not markedly diminished, indicating that the acute effect of injurious compression was a partial disruption of the ECM, with the mechanical role of the collagen meshwork being most severely affected, whereas the compressive strength of the PG gel remained largely intact. Cell-level quantitative autoradiography revealed that the pericellular matrix surrounding cells in uncompressed control disks exhibited the highest rates of PG assembly and turnover, but the lowest rates of collagen deposition (36, 129). However, the remaining viable cells in injuriously compressed disks appeared to mediate a more rapid loss of PGs compared with controls. This increased release included aggregating species in addition to a spectrum of degradation fragments, which were also present in controls. Thus, mechanical injury to the cartilage ECM appeared to involve acute compromise of collagen function followed by accelerated cell-mediated PG degradation and release. The induction of chondrocyte apoptosis by threshold levels of injurious compression is a novel finding, with implications for the ability of such a damaged tissue and cell population to initiate a repair response. Apoptosis was observed at peak stresses below the levels at which macroscopic ECM damage and loss occurred. Follow-on studies are focusing on the effects of injurious loading on gene expression on enzymes and ECM molecules.

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