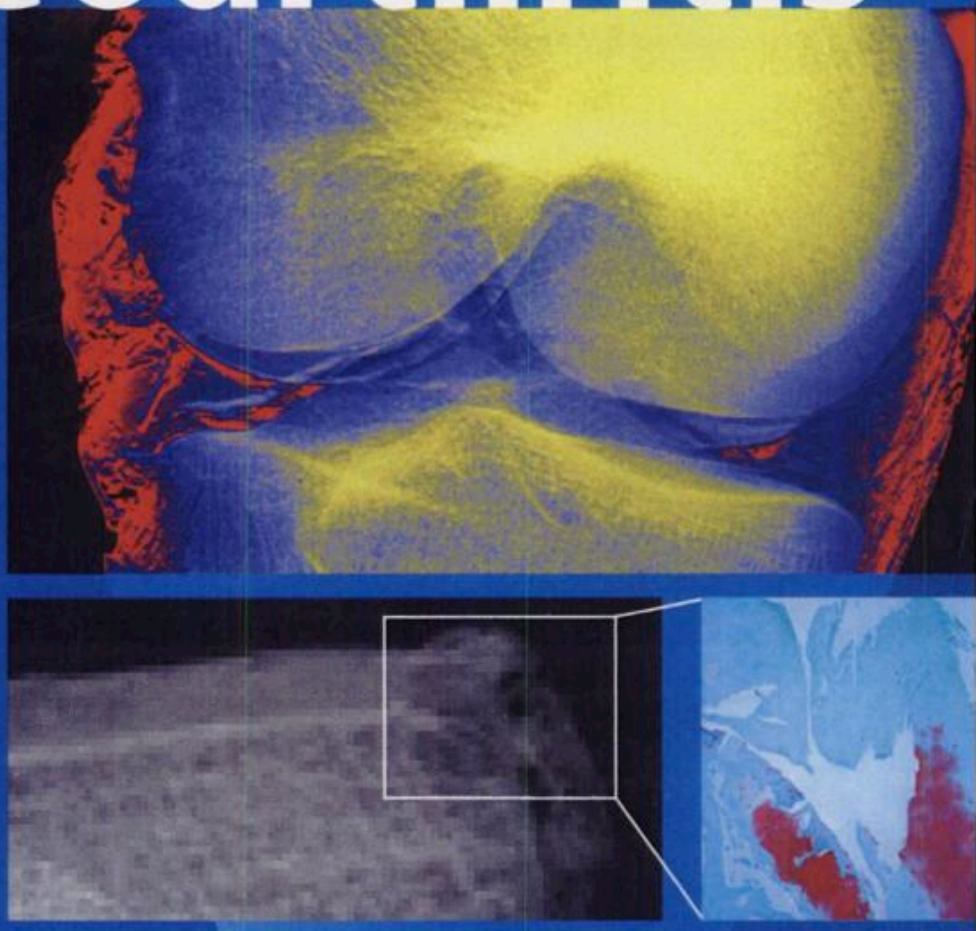
Vincent C. Hascall
Klaus E. Kuettner

Editors

The Many Faces of Osteoarthritis



Birkhäuser

The Many Faces of Osteoarthritis

Vincent C. Hascall
Klaus E. Kuettner

Editors

Birkhäuser Verlag
Basel · Boston · Berlin

Editors

Vincent C. Hascall
Orthopaedic Research Center
Department of Biomedical Engineering
The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195
USA

Klaus E. Kuettner
Department of Biochemistry
Rush Medical College at Rush Presbyterian-St. Luke's
Medical Center
1653 West Congress Parkway
Chicago, IL 60612-3864
USA

A CIP catalogue record for this book is available from the Library of Congress, Washington D.C., USA

Deutsche Bibliothek Cataloging-in-Publication Data
The many faces of osteoarthritis / Vincent C. Hascall ; Klaus E. Kuettner ed.. -
Basel ; Boston ; Berlin : Birkhäuser, 2002
ISBN 3-7643-6581-1

ISBN 3-7643-6581-1 Birkhäuser Verlag, Basel – Boston – Berlin

Printed on acid-free paper produced from chlorine-free pulp. TCF ∞
Cover design: Micha Lotrovsky, CH-4106 Therwil, Switzerland
Cover illustration: Diffraction enhanced x-ray imaging (DEI) of human synovial joint cartilage. The top image is one of an intact human knee joint taken with the DEI technique at the National Synchrotron Light Source at Brookhaven National Laboratory. The bottom two pictures are a DE image of a portion of a talar dome (left) and its histological profile stained with Safranin-O/fast green (right). In both DE images, the articular cartilage is clearly visible. Furthermore, the contrast heterogeneity that gives the appearance of a lesion in the lower DE image is histologically validated. (The top image with friendly permission of Carol A. Muehleman and Matthias E. Aurich, the bottom pictures reprinted from *Osteoarthritis and Cartilage*, volume 10, Mollenhauer J, Aurich ME, Zhong Z, Muehleman C, Cole AA, Masnah M. Oltulu O, Kuettner KE, Margulis A, Chapman LD. Diffraction-enhanced x-ray imaging of articular cartilage. pp. 163–171 (2002) by permission of the publisher WB Saunders.)

Printed in Germany
ISBN 3-7643-6581-1

9 8 7 6 5 4 3 2 1

www.birkhauser.ch

Preface

The conference "The Many Faces of Osteoarthritis", convened at Lake Tahoe, June 23–27, 2001, was held in my honor to acknowledge and to pay tribute to my contributions in the field and also to celebrate my birthday, which happened to fall in that time frame. The meeting was one of the happiest events in my professional life, and I am much indebted to the organizers and to all my colleagues and friends who contributed to its success. On a personal note, I was particularly pleased to meet again so many former trainees. The resulting book reflects the scientific presentations and discussions between a select group of invited investigators, all experts in this field. As will be evident, the meeting is a logical continuation of scientific workshops in the field, two of which were originally sponsored by Hoechst Pharmaceutical Company of Germany. This scientific meeting is now sponsored by two pharmaceutical companies, again Hoechst (now Aventis), and also Glaxo-SmithKline. The latter is a scientific partner for members of the Department of Biochemistry, which I have chaired since 1980 at Rush Medical College in Chicago.

In 1987, the Department of Biochemistry was awarded one of only three national grants in osteoarthritis as a Specialized Center of Research (SCOR) from the National Institute of Arthritis, Musculoskeletal and Skin Diseases of the National Institutes of Health. These Specialized Centers of Research support a cluster of individual, but interrelated, basic and clinical research projects. The SCOR at Rush entitled "Osteoarthritis: A Continuum (From Cartilage Metabolism to Early Detection and Treatment)" involved investigators from basic science and clinical departments, and I served as Program Director. In 1992, and again in 1997, Special Study Sections evaluated all SCOR grants in osteoarthritis and the program at Rush was approved each time for an additional five years, and is currently the only SCOR on osteoarthritis in the country.

To guarantee the best possible progress and research direction, the SCOR grant investigators are assessed and evaluated on an annual basis by a Scientific Advisory Committee composed of eight internationally renowned scientists (nicknamed "Scoriers") which not only came up with the idea of this meeting, but also put it together. In addition, they also evaluated the manuscripts for this book (not an easy task) and wrote a brief overview to reflect the general discussions during the meeting. The strength, however, lies in the fact that the meeting was set up in the familiar Gordon Research Conference style.

The conference "The Many Faces of Osteoarthritis" reflects the current state of knowledge and will help elucidate the etiopathology of osteoarthritis (OA), hope-

fully leading to early detection of the disease and novel treatment modalities. OA is the most common joint pathology and primarily affects the older population. Extended research, however, has shown substantial dissimilarities between OA and the aging processes. OA can be envisioned as a group of overlapping distinct diseases that may have different etiologies but show similar biologic, morphologic and clinical outcomes. The disease is characterized by unique pathological changes in some synovial joints, predominantly affecting the articular cartilage, but also the entire joint, including the synovial tissue and subchondral bone. It is only in later stages that it can be diagnosed indirectly by loss of articular cartilage as revealed in radiography, a method used to diagnose the disease in clinical practice and in epidemiological studies. Not all individuals with radiographic evidence of OA have clinical symptoms. However, the probability of symptoms increases with the severity of radiographic changes. It is hypothesized that both mechanical and biological events destabilize the normal coupling of synthesis and degradation of the matrix of the articular cartilage by its chondrocytes, with modulation also in the subchondral bone. The disease may be initiated by multiple factors including genetic, developmental, metabolic and especially traumatic ones that may have occurred much earlier in life.

In the early 1980s, when research in articular cartilage biochemistry was still in its infancy, I was asked, together with Dr. Vincent Hascall, to organize in September 1985 a Workshop Conference (sponsored by Hoechst-Werk Albert, Wiesbaden, Germany) entitled "Articular Cartilage Biochemistry." At this meeting, the results of ongoing research on the structure and metabolism, both of normal and osteoarthritic articular cartilage, and related arthritic disorders were presented. Through a detailed analysis of the matrix macromolecules, the biosynthesis and the normal and pathological metabolism of cartilage components by chondrocytes were reported. The questions asked were, for example: How do cells communicate in order to synchronize macromolecular synthesis and secretion with degradation in the different micro-regions of the extracellular matrix? What are the signals and the receptors in the intercellular transduction mechanisms? What are the critical interactions among intercellular macromolecules that infer tissue specificity upon extracellular processes? The participants of this meeting advanced the understanding of the mechanisms underlying cartilage degeneration in arthritic diseases. In 1986, the presentations and lively discussions of the workshop were published by Raven Press (New York) with Drs. Klaus Kuettner, Rudolf Schleyerbach and Vincent Hascall as co-editors.

The rapid growth of knowledge and methodologies in cartilage research resulted in an increase in diverse methods used to study cartilage biology and biochemistry. Therefore, in order to achieve a standardization of methods, I was asked by Dr. Alice Maroudas to conjointly organize an international seminar entitled "The Bat-Sheva Seminar on Methods Used in Research on Cartilaginous Tissues", which was held at the Nof Ginossar Kibbutz, Israel, in March 1989. This seminar was sponsored by the Bat-Sheva de Rothschild Foundation for the Advancement for Sci-

ence in Israel. The proceedings of the meeting were published in a book by Academic Press (London and San Diego) in 1990 under the title "Methods in Cartilage Research" with Drs. Alice Maroudas and Klaus Kuettner as co-editors. The time was right for assembling the various methodologies into a single volume that reflected the sophistication of each aspect in this field and also provided a comprehensive source for investigators from other disciplines. The book describes for example: Qualitative and quantitation techniques for the tissue specimens, extraction methods, chondrocyte and explant cultures, and the tissue composition and organization. Furthermore, physical and mechanical properties as well as their relevance to physiological processes were delineated. Different approaches were described, compared, discussed and assessed. The major aim was to show investigators the various choices and possibilities in research and to discuss the appropriateness of study designs without ignoring their inherent shortcomings, limitations and difficulties.

Three years later another workshop conference (again sponsored by Hoechst-Werk Albert) entitled "Articular Cartilage and Osteoarthritis" highlighted current basic scientific and clinical research efforts to further advance the understanding of articular cartilage physiology and pathophysiology and also the etiopathology of osteoarthritis. Among the questions asked were: How does the microenvironment, containing collagens and proteoglycans, serve as a prerequisite for the maintenance of cell differentiation? What regulates the biosynthesis of proteoglycans and glycosaminoglycans? Does secretion and diffusion of recently identified "morphogens" play a role in remodeling and/or repair? What are the influences of mechanical stresses upon the biosynthesis of extracellular macromolecules? What is the role of non-collagenous macromolecules upon extracellular matrix specificity? How does a degradation enzyme contact a collagen fibril (in the case of collagenolysis) when the latter is normally encased in a matrix of other macromolecules? Clinical research findings on potential diagnostic markers of early OA and investigational, potential therapeutic interventions were presented. They provided invaluable sources of information, illuminating observations and promising new approaches for all orthopedic surgeons, rheumatologists and basic research scientists investigating joint diseases. This workshop symposium was afterwards published, again by Ravens Press (New York) in 1992, with Drs. Klaus Kuettner, Rudolf Schleyerbach, Jacques Peyron and Vincent Hascall as co-editors.

In April 1994, Dr. Victor Goldberg and I were asked to organize a workshop entitled "New Horizons in Osteoarthritis." Our task was to bring together an international, interdisciplinary group of leading scientists and clinicians to define the present knowledge and delineate future research directions on the etiopathogenesis of OA, as well as to develop new strategies in research for the understanding of the etiopathology of OA. This workshop was sponsored by the American Academy of Orthopedic Surgeons, the National Institute of Arthritis and Musculoskeletal and Skin Diseases, the National Institute on Aging, the National Arthritis Foundation, and the Orthopedic Research and Education Foundation. During this workshop,

groups of investigators developed concepts and specific plans on various topics, which were presented, modified and endorsed by all participants. A book entitled "Osteoarthritic Disorders" was published by the American Academy of Orthopaedic Surgeons (Rosemont) in 1995, edited by Drs. Klaus Kuettner and Victor Goldberg. It contains invited summary manuscripts describing the current state of research in specific areas. Each summary is followed by a recapitulation of the extensive discussions. This covers the definition (including epidemiology) and classification of OA, cartilage changes in aging and changes in the osteoarthritic joint as an organ, role of mediators and inflammation in the degradative mechanisms, repair of cartilage, and the monitoring of preclinical and clinical progression and treatment of OA. At the time of the workshop the group identified gaps in factual knowledge and stated that more information was needed before questions addressed during the meeting could be answered. The book also contains selected reports, descriptions of the brainstorming discussions of future investigations, an overview, and consensus opinions and recommendations for new research directions in OA.

The group of investigators in the field of cartilage and osteoarthritis research is relatively small. Thus, most of the "Scoriers" were present at the four meeting/workshops/symposiums, mentioned above. Thus, the meeting in Lake Tahoe was a logical consequence of the numerous interactions that we had at these scientific "state-of-the-art" meetings. The remarkable growth of the field and the rapid increase in diversity of advancements, including the application of novel molecular biological approaches and new imaging techniques, was certainly reflected at this meeting at Lake Tahoe. The progress made since the first meeting about 16 years ago is enormous. Investigators now focus on receptors, transduction mechanisms, and specific cytokines that may regulate both the degradation and the repair/regeneration of the tissue. Still, significant research will be necessary in defining and clarifying questions about the basic etiopathology of OA. Clinical and epidemiological investigators will have to continue to interact with the basic scientists to identify the pathogenic characteristics of OA, in order to develop and assess new therapeutic interventions.

Special recognition is due to Dr. Hari Reddi, who was the local organizer of the meeting, and together with his administrative and well experienced staff (most of all Ms. Lana Rich), were responsible for inducing and maintaining an interaction between the scientists. Their time, energy and effort is highly appreciated. Special thanks go to Dr. Vincent Hascall, who was "volunteered" by his "Co-Scoriers" to be the "mastermind" of the scientific program of this meeting at Granlibakken/Lake Tahoe. His commitment and pursuit guaranteed its success. In this task he was invaluably supported by his secretary Ms. Kathy Vukovich. The meeting was dedicated, by Vince, to the friendship among scientists.

Spring 2002

Klaus E. Kuettner

Influence of tissue shear deformation on chondrocyte biosynthesis and matrix nano-electromechanics

Moonsoo Jin[1], Alan J. Grodzinsky[1], Thomas H. Wuerz[1], Gregory R. Emkey[1], Marcy Wong[2], and Ernst B. Hunziker[2]

[1]Continuum Electromechanics Group, Center for Biomedical Engineering, Massachusetts Institute of Technology, M.I.T. Room 38-377, Cambridge, MA 02139, USA; [2]M.E. Mueller Institute for Biomechanics, University of Bern, Bern, Switzerland

Introduction

Articular cartilage provides lubrication and load bearing functions during the motion of synovial joints. Such a specialized biomechanical function is enabled by the mechanical and electromechanical properties of cartilage extracellular matrix (ECM) and the interaction between cartilage and synovial fluid. Within cartilage matrix, highly charged aggrecan molecules are embedded within a dense collagen fibrillar network. The proteoglycan-associated repulsive forces are restrained by tensile forces within the collagen network. At the molecular level, these repulsive or swelling stresses are mostly due to electrical double layer repulsion associated with the negative fixed charges on glycosaminoglycan (GAG) chains, in addition to the elastic and entropic interactions between GAG macromolecules.

In vivo loading of cartilage causes coupled electromechanical and physicochemical changes that are known to modulate chondrocyte metabolism *via* multiple pathways including upstream signaling, transcriptional and translational regulation, post-translational modification, intracellular vesicular transport, and extracellular processing [1, 2]. Low levels of oscillatory strain (1–5% dynamic strain amplitude at frequencies ranging from 0.01–1.0 Hz) can stimulate chondrocyte biosynthesis of proteoglycans, collagens, and other important matrix proteins [1, 3–6]. Higher levels of strain and strain rate can cause cell death and matrix damage [7–9]. As the magnitude of strain [10] and strain rate in response to physiological loading depend in part on the mechanical properties of cartilage tissue, it is important to understand both the biophysical and biological stimuli that regulate the coordinated synthesis and degradation of ECM by the chondrocytes.

In this chapter we first review the regulation of cartilage metabolism by mechanical loading, especially the effect of tissue shear deformation on mRNA regulation and matrix synthesis. We then describe experimental and theoretical studies of certain nano-electromechanical interactions within cartilage ECM that govern the macroscopic mechanical behavior of cartilage.

The Many Faces of Osteoarthritis, edited by Vincent C. Hascall and Klaus E. Kuettner

Physical regulation of cartilage metabolism

The development and maintenance of cartilage *in vivo* is regulated in part by mechanical loading, including compressive and shear deformations, and concomitant mechanical and physicochemical forces and flows [1]. Soluble factors including vitamins, hormones, growth factors, and cytokines are known to be important regulators of chondrocyte biosynthesis and differentiation [2, 11]. Therefore, investigators have studied the regulation of cartilage metabolism *in vitro*, by utilizing soluble factors combined with mechanical or physicochemical stimuli [11–14]: static and cyclic hydrostatic pressure, fluid-induced shear, dynamic tissue deformation, changes in osmolarity and pH. The cellular mechanisms that underly mechanotransduction responses are not well understood. Signaling mechanisms may include stretch-activated ion channels [15], ligand-cell surface receptor interactions, and integrin-cytoskeleton machinery [16] that can trigger kinase cascades leading to changes in transcriptional activity. Mechanical stresses may also affect chondrocyte biosynthesis at the level of translation and post-translational modification by changing the structure of organelles such as endoplasmic reticulum and Golgi apparatus [1].

The influence of tissue shear loading on chondrocyte biosynthesis

Dynamic compression of cartilage explants causes cell and matrix deformation as well as fluid flow within the extracellular matrix (ECM) in the environment of the cells, mimicking these aspects of loading *in vivo*. Previous studies suggested that the increase in proteoglycan and protein synthesis caused by dynamic compression *in vitro* was associated with intratissue fluid flow, streaming potential, and cell deformation [17, 18]. These studies modeled the spatial profiles of biophysical phenomena within cylindrical explants, including fluid flow and hydrostatic pressure, and compared them to the measured spatial profiles of newly synthesized proteoglycans using quantitative autoradiography. Possible effects of fluid flow on cartilage metabolism include (1) increased availability of nutrients and growth factors due to the convective transport, (2) streaming potentials, and (3) flow-induced shear stress. It has been speculated that each of these possible mechanisms can regulate chondrocyte biosynthesis.

In a recent study [19], we examined the effect of cell and matrix deformation (in the absence of significant fluid flow) on mRNA regulation and matrix biosynthesis using applied tissue shear deformation. Since tissue shear causes little or no volumetric deformation or intratissue pressure gradients, there is minimal intratissue fluid flow. Cartilage disks (3 mm diameter by 1 mm thick) were obtained from the femoropatellar groove of 1–2-week-old calves. Sinusoidal shear deformation of 0.5–6% dynamic strain amplitude at frequencies between 0.01–1.0 Hz was applied

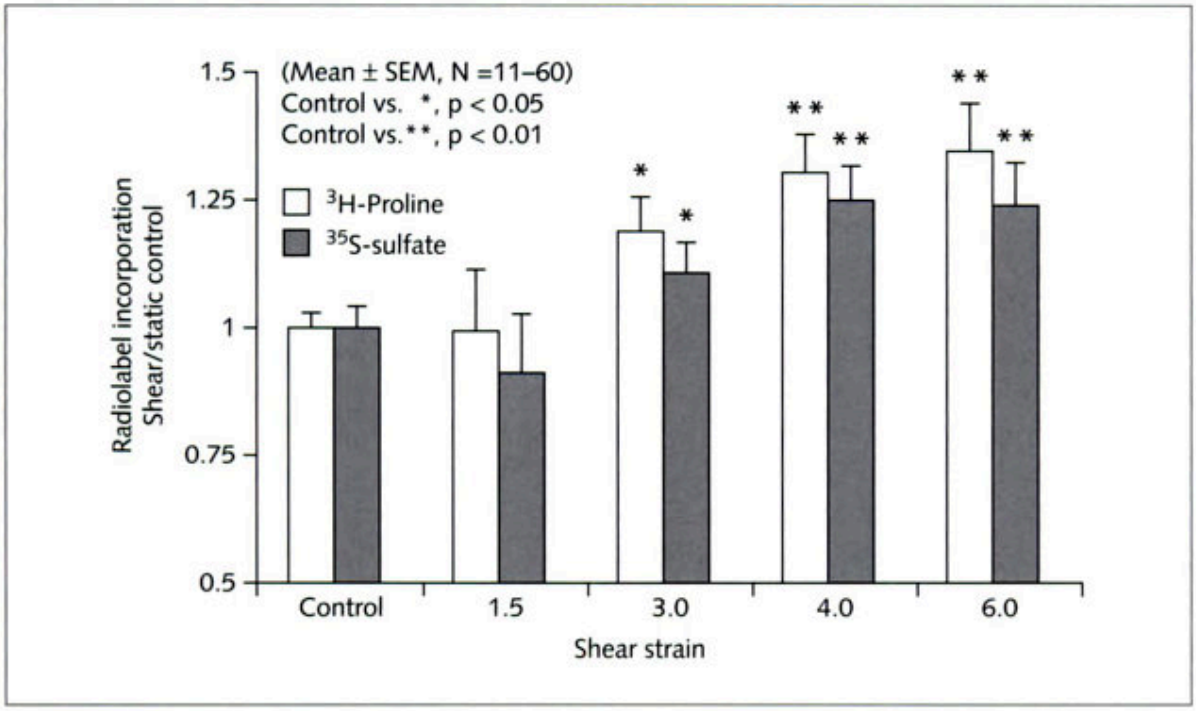


Figure 1

Radiolabel incorporation (pmol/μg DNA/h) in dynamically sheared disks was normalized to that in control disks where disks were maintained within tissue culture media without FBS. Synthesis of total protein and proteoglycans was significantly increased by shear deformation above 1.5% dynamic strain amplitude at a frequency of 0.1 Hz. The increase at 6% shear was similar to that observed previously in disks incubated in the presence of 10% FBS and subjected to 3% shear strain [19].

to groups of disks using an incubator-housed biaxial apparatus [39]; anatomically matched control disks were maintained at the same static offset compression (i.e., the cut thickness of the disks) but not subjected to shear. During the entire loading period, disks were incubated in DMEM ± 10% FBS, with ^{35}S-sulfate and ^{3}H-proline incorporation used as measures of proteoglycan and total protein synthesis, respectively [19].

Shear loading above 1.5% strain amplitude applied for 24 h at a frequency of 0.1 Hz with no FBS caused significant stimulation of total protein and proteoglycan synthesis over static controls (Fig. 1). By 6% strain amplitude, proline and sulfate incorporation had increased by ~40% and ~25%, respectively (Fig. 1), which was close to the stimulatory level caused by 3% shear strain in the presence of 10% FBS in our previous study [19]. In the presence of 10% FBS [19], tissue shear deformation stimulated biosynthesis at strain amplitudes as low as 1%.

In a separate series of experiments (using 10% FBS), the spatial profiles of radiolabel incorporation into newly synthesized matrix molecules were further analyzed

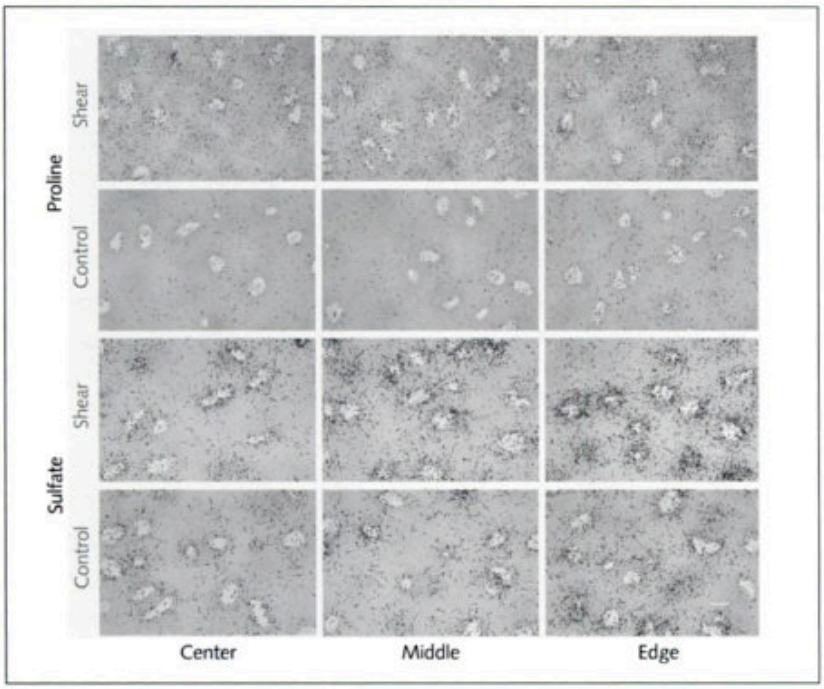
by quantitative autoradiography. Cartilage disks were distributed into four groups (n = 6 each): a dynamic shear and control group maintained in medium containing 10 μCi/ml ^{35}S-sulfate, and another dynamic shear and control group with 20 μCi/ml ^{3}H-proline. Both shear groups were subjected to 3% dynamic shear strain amplitude at 0.1 Hz for 24 h. After loading in the presence of label, the disks were prepared for the quantitative autoradiography analysis [19]. Sub-sectional images (100 μm × 80 μm) selected from center (r = 0 to 0.3 mm), middle (r = 0.6 to 0.9 mm), and edge regions (r = 1.2 to 1.5 mm) of the cross-section (3 mm wide by 1 mm high by 1 μm thick) of cylindrical cartilage explants (Fig. 2A) were used to analyze radial variations in proline and sulfate grain densities.

Grain densities of dynamically sheared disks were normalized to the static control disks (Fig. 2B). Overall, the proline grain density increased significantly by 50% due to shear loading ($p < 0.001$) and was relatively uniform with increasing radius from the center of the disk ($p > 0.6$, by two-way ANOVA). In contrast, the sulfate grain densities increased with a trend towards significance ($p = 0.076$), and there was a significant trend in the radial variation of sulfate grains ($p < 0.05$). In previous studies of the effects of dynamic compression on cartilage explants [17, 18], the relative increase in sulfate grains was higher near the peripheral edges of the disk where chondrocytes were subjected to a higher level of intratissue fluid flow. Therefore it is interesting to note that the increase in sulfate incorporation in response to tissue shear deformation was found to be greater in the center region. Together, these results may be associated with the influence of tissue shear deformation on local (cell-level) facilitated transport or diffusion of growth factors and other macromolecules throughout the entire region of cylindrical disks, in the absence of significant fluid flow.

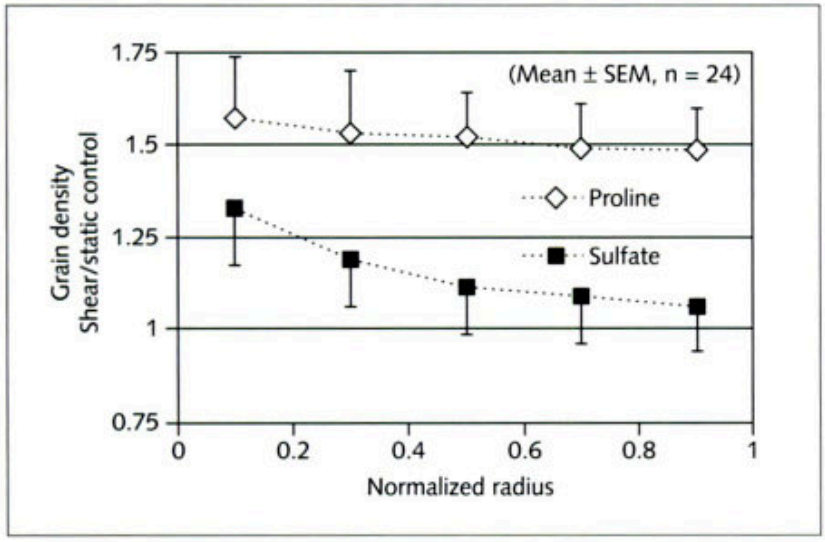
The effect of tissue shear loading on changes in mRNA expression of type II collagen and aggrecan core protein were further investigated using RT-PCR (data not shown). For these experiments, cartilage disks were subjected to 3% shear strain amplitude at 0.1 Hz for 0.5, 2, 6, and 24 h. Compared to the level of matched static controls, the expression of type II collagen mRNA in response to tissue shear increased ~50% by 0.5 h. This level of stimulation was maintained over 24 h. However, there was no significant increase in the expression of aggrecan core protein mRNA by shear deformation.

Matrix nano-electromechanics

Experimental and theoretical studies of the mechanical and electromechanical properties of cartilage can be classified into macroscopic-tissue-scale *versus* nano-molecular-scale approaches. Macroscopic approaches focus on the material properties of cartilage tissue and the ability of cartilage to respond to complex joint loading *in vivo*. Measurements of tissue properties have utilized testing configurations to quan-



a



b

Figure 2

(A) Autoradiographic appearance of chondrocytes with proline and sulfate grains from center, middle, and edge regions of cartilage explants. Proline and sulfate grain density appeared higher in dynamically sheared disks than in static controls (white bar at the bottom right = 10 μm). (B) The radial variation in proline and sulfate grain densities of the shear group was normalized to that of the control group. Overall proline grain density increased by 50% due to shear loading ($p < 0.001$) and was relatively uniform with disk radius ($p < 0.6$, two-way ANOVA). In contrast, the relative increase in the sulfate grain densities due to shear was marginally significant ($p < 0.076$) and there was a significant trend in the radial variation of sulfate grains ($p < 0.05$) with higher stimulation near center region of cylindrical disk.

tify the compressive, shear, and tensile behavior of the ECM [20–27]. The tensile strength of the collagen network within native tissue has been determined using the force balance between an applied stress, the proteoglycan (PG) swelling pressure, and the collagen tensile stiffness [23]. This study showed that the ability of collagen fibrils to limit the hydration of tissue and, thus, maintain a high PG concentration in normal cartilage was significantly compromised in osteoarthritic tissue. Measurements of tissue-level mechanical properties have also been performed before and after enzymatic degradation of proteoglycan consituents [21], and after changes in bath ionic concentration and pH [21, 22, 24]. These studies have revealed the critically important contribution of GAG-associated electrostatic interactions to the compressive and shear stiffness of cartilage under equilibrium as well as time varying conditions.

Theoretical models have been developed to describe the macroscopic behavior of cartilage tissue, addressing the interaction between fluid and solid matrix, and the intrinsic properties of the constituent macromolecules [28]. Poroelasticity and mixture theories have predicted the observed stress relaxation, creep, and electrokinetic behavior of cartilage under volumetric deformation [25, 26]. The intrinsic viscoelastic properties of the solid matrix have been incorporated into a poroviscoelasticity theory, thus addressing combined relaxation from the intrinsic properties of macromolecules and the interaction between solid and fluid phases [27].

The investigation of nano-molecular interactions and biophysical properties of constituents of the ECM has enhanced our understanding of the origin of the macroscopic properties of cartilage. For example, aggrecan is non-covalently bound to hyaluronan, stabilized by link protein. The viscosity and shear modulus of solutions of aggrecan with link protein were found to be higher than those of link-free aggrecan solutions, highlighting the stabilizing effect of link protein in PG aggregates [29]. Nano-scale structural visualization of aggrecan and collagen fibrils has been performed by electron microscopy [30, 31] and, recently, by atomic force microscopy (AFM), which enables the imaging of macromolecules in ambient air or in near physiological fluids [32, 33]. In addition, intermolecular and intramolecular electrostatic repulsion forces between GAG chains end grafted onto a gold-coated silicon wafer were quantified using high resolution force spectroscopy [34]. The resulting force per area occupied by a single GAG-chain was found to be on the order of the known macrocopic Donnan swelling stress of cartilage tissue [20, 23], giving further support to the molecular level origin of electrostatic swelling forces within the tissue. The Poisson-Boltzmann (PB) mean field theory has been used to model such electrostatic repulsion interactions between charged polyelectrolyte molecules in colloidal systems [35]. Of relevance to our study, the contribution of GAG electrostatic interactions to the osmotic swelling pressure of proteoglycan solutions and the equilibrium compressive modulus of cartilage was predicted quantitatively using the PB theory applied to the unit cell model of GAG [36].

Effect of electrostatic interactions between GAGs on the shear modulus of cartilage

The mechanical properties of cartilage derive from distinct electrical (charge-dependent) and non-electrical (charge-independent) contributions. The electrical contributions are mainly associated GAG electrostatic interactions, while the non-electrical contributions are associated with the electrically neutral collagen fibrils as well as with the elastic forces due to the steric and entropic effects within the ECM. As previously described, GAG molecules play an important role in the shear behavior of cartilage by inflating the collagen network, causing a tensile prestress that enables the collagen-aggrecan matrix to resist shear deformation [21, 23]. The shear modulus of cartilage changed significantly after extraction of aggrecan [21]. In addition, it has been inferred from measurement of the confined compression modulus of cartilage tissue [37] that increased ionic strength could decrease the shear modulus of cartilage. However, we know of no previous studies of the possible mechanisms at the nano-molecular scale by which GAG electrostatic interactions may contribute directly to the tissue shear stiffness.

To explore this, we measured the dynamic and equilibrium shear modulus of cartilage disks (9.65 mm diameter by 1 mm thick) in a torsional configuration (at 10% compressive offset) and varied the NaCl concentration at neutral pH to modulate the electrostatic interactions between GAGs [22]. At physiological pH, the properties of the collagen fibrils do not change significantly with ionic strength in the range of 0.01–1.0 M [38]. For measurement of the equilibrium shear modulus, a ramp-and-hold shear strain of 1.5% was applied, resulting in an initial increase and subsequent relaxation of the shear stress. This sequence was repeated four times, and the slope of the relaxed equilibrium stress and strain was used to compute the equilibrium modulus. After returning the specimen to 0% shear strain, a 0.8% amplitude sinusoidal shear strain was applied at 0.5 Hz. This sequence of equilibrium and dynamic shear tests at 0.15 M NaCl was then repeated sequentially after re-equilibration in 0.05 M, 0.01 M, 0.5 M, and 1.0 M NaCl, and corresponding moduli were calculated at each concentration.

The equilibrium shear stress recorded after 10–30 min of relaxation increased linearly with applied shear strain at all ionic concentrations. At each shear strain, the equilibrium shear stress decreased monotonically with increasing NaCl due to the shielding of electrostatic repulsive forces between GAGs [19]. The equilibrium shear modulus, computed by the linear regression from the stress-strain curves, decreased monotonically with increasing NaCl concentration (Fig. 3). The dynamic shear modulus also decreased with increasing NaCl concentration in a similar manner (Fig. 3). These data suggest that electrostatic interactions between GAG chains contribute significantly to the shear properties of cartilage.

We then hypothesized that cartilage's resistance to shear deformation was provided, in part, by changes in the electrostatic forces between neighboring GAG

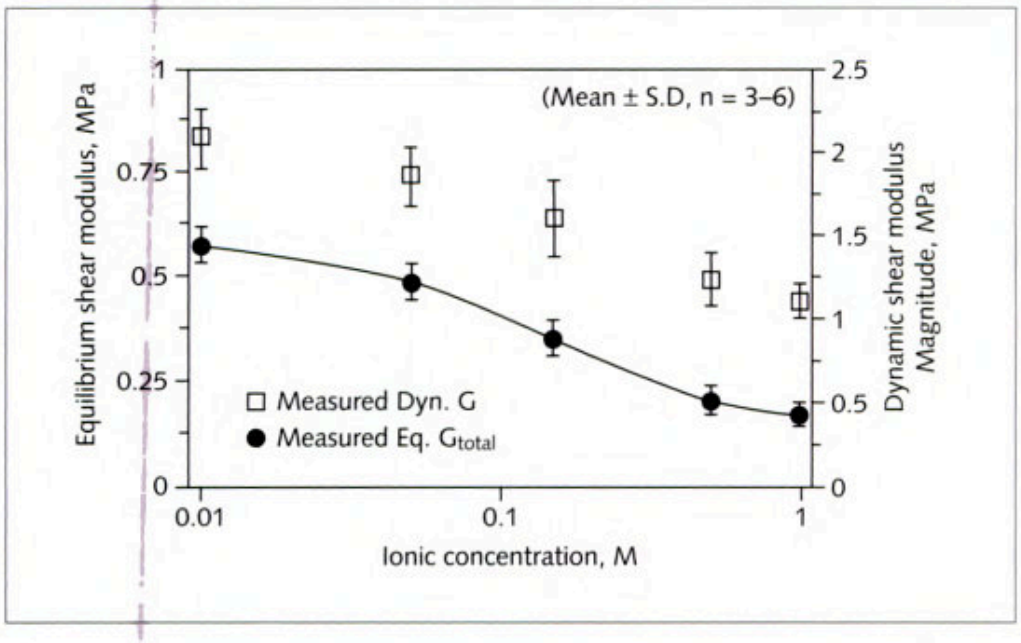


Figure 3
The equilibrium and dynamic shear modulus increased monotonically as the ionic concentration decreased, suggesting the important role of GAG electrostatic interactions in the shear properties of cartilage. The solid line is the predicted electrical component of equilibrium shear modulus (G_e) using two fitting parameters: the GAG concentration (C_{GAG}) and ionic strength-independent shear modulus (G_{ne}). The fitting parameters were determined as C_{GAG} = 5.7% by wet weight and G_{ne} = 173 kPa which are similar to values from independent measurements.

chains caused by the nano-scale rearrangement of GAGs that occurs during macroscopic tissue shear deformation. To test this hypothesis, we modeled GAG segments as charged rods with the outer boundary representing the average distance between neighboring GAGs (unit cell model). This unit cell approach has been used previously to model streaming potentials [40], compression-induced changes in hydraulic permeability [41], and GAG electrostatic interactions in compression [36]. The orientation of the unit cell axis and the vectors describing the unit cell boundary, motivated by the model of Quinn et al. [41], were varied in a manner consistent with the applied macroscopic shear deformation. Theoretical predictions of the electrical contribution to the equilibrium shear modulus (G_e) were obtained using an energy method by differentiating the changes in the electrostatic free energy with respect to the applied macroscopic deformation [22]. The Poisson-Boltzmann equation was incorporated into the unit cell model (PB unit cell) to predict the nano-scale electric potential and mobile ion distribution, which enabled the calculation of the free energy. The overall change in the free energy in response to macroscopic shear defor-

mation was calculated by probabilistically averaging over all GAG orientations, using the approach of Quinn et al. [41]. The shear modulus (G_{total}) was modeled as the sum of an electrical contribution, G_e, and an ionic strength-independent (non-electrical) contribution, G_{ne}, e.g., associated with the collagen network ($G_{total} = G_e + G_{ne}$). The solid line of Figure 3 is the predicted electrical component G_e based on the best fit of two adjustable parameters: the GAG concentration ($C_{GAG} = 5.7\%$ by wet weight at 0% compressive offset) and $G_{ne} = 173$ kPa, where the chi-square function was minimized. For comparison, the measured GAG concentration in newborn calf femoropatellar groove cartilage has been reported previously to be $5.6 \pm 0.6\%$ by wet weight [3] which is very close to the best fit value obtained from the present model. The value of G_{ne} is close to the value obtained by extrapolating the measured equilibrium shear modulus to that obtained at 1.0 M NaCl concentration, by which electrostatic interactions are essentially screened (Fig. 3). The good comparison between experimental measurements and theoretical predictions (Fig. 3) strongly suggests that the nano-structural rearrangement of GAG molecules during shear deformation is an important determinant in the shear properties of cartilage.

Conclusions

Articular cartilage is a unique material that can support high loads and deformations during joint loading and simultaneously exhibit extremely low levels of friction. Biomechanical studies of cartilage have focused on native tissue as well as individual molecular components of the ECM. Various macroscopic continuum theories have been developed to describe the material behavior of cartilage during compression, shear, and tensile deformation. At the molecular scale, the properties of aggrecan and collagen fibrils have been studied with attention to electromechanical, physicochemical, and rheological characteristics of these components. Here we described the contribution of nano-scale GAG electrostatic interactions to the shear properties of cartilage, and we suggested that nano-scale rearrangement of GAG molecules is an important mechanism underlying the shear stiffness of tissue during *in vivo* shear loading.

At the same time, mechanical loading forces concomitant with biophysical changes appear to regulate matrix biosynthesis and turnover across multiple pathways including upstream signaling, transcription, translation, post-translational modification, and intracellular vesicular transport. Tissue shear deformation, which induces little or no fluid flow, was found to stimulate protein and proteoglycan synthesis, and the increase in protein synthesis was accompanied by an increase in expression of type II collagen mRNA. The specific mechanisms by which the multiple regulatory pathways interact and lead to the changes in chondrocyte metabolism are under continued study.

Acknowledgments
This research was supported by NIH Grant AR 33236. The authors are indebted to the contributions of Drs. Eliot Frank and Thomas Quinn, and thank Elke Berger, Veronique Gaschen, and Prasanna Perumbuli for technical contributions.

References

1 Grodzinsky AJ, Levenston ME, Jin M, Frank EH (2000) Cartilage tissue remodeling in response to mechanical forces. *Annu Rev Biomed Eng* 2: 691–713
2 Hering TM (1999) Regulation of chondrocyte gene expression. *Front Biosci* 4: D743–761
3 Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD (1989) Biosynthetic response of cartilage explants to dynamic compression. *J Orthop Res* 7(5): 619–636
4 Kim YJ, Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD (1994) Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. *Arch Biochem Biophys* 311(1): 1–12
5 Parkkinen JJ, Lammi MJ, Helminen HJ, Tammi M (1992) Local stimulation of proteoglycan synthesis in articular cartilage explants by dynamic compression *in vitro*. *J Orthop Res* 10(5): 610–620
6 Wong M, Siegrist M, Cao X (1999) Cyclic compression of articular cartilage explants is associated with progressive consolidation and altered expression pattern of extracellular matrix proteins. *Matrix Biol* 18(4): 391–399
7 Newberry WN, Garcia JJ, Mackenzie CD, Decamp CE, Haut RC (1998) Analysis of acute mechanical insult in an animal model of post-traumatic osteoartrosis. *J Biomech* Eng 120: 704–709
8 Loening AM, James IE, Levenston ME, Badger AM, Frank EH, Kurz B, Nuttall ME, Hung HK, Blake SM, Grodzinsky AJ, Lark MW (2000) Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. *Arch Biochem Biophys* 381: 205–212
9 Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ (2001) Biosynthetic response and mechanical properties of articular cartilage after injurious compression. *J Orthop Res* 19(6): 1140–1146
10 Wong M, Wuethrich P, Buschmann MD, Eggli P, Hunziker E (1997) Chondrocyte biosynthesis correlates with local tissue strain in staticallycompressed adult articular cartilage. *J Orthop Res* 15(2): 189–196
11 Bonassar LJ, Grodzinsky AJ, Srinivasan A, Davila SG, Trippel SB (2000) Mechanical and physicochemical regulation of the action of insulin-like growth factor-I on articular cartilage. *Arch Biochem Biophys* 1379(1): 57–63
12 Urban JP, Hall AC, Gehl KA (1993) Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. *J Cell Physiol* 154(2): 262–270
13 Parkkinen JJ, Ikonen J, Lammi MJ, Laakkonen J, Tammi M, Helminen HJ (1993)

Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. *Arch Biochem Biophys* 300(1): 458–465

14 Sah RL, Trippel SB, Grodzinsky AJ (1996) Differential effects of serum, insulin-like growth factor-I, and fibroblast growth factor-2 on the maintenance of cartilage physical properties during long-term culture. *J Orthop Res* 14(1): 44–52

15 Wright M, Jobanputra P, Bavington C, Salter DM, Nuki G (1996) 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(1): 61–71

16 Millward-Sadler SJ, Wright MO, Davies LW, Nuki G, Salter DM (2000) Mechanotransduction *via* integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes. *Arthritis Rheum* 43(9): 2091–2099

17 Kim YJ, Bonassar LJ, Grodzinsky AJ (1995) The role of cartilage streaming potential, fluid flow and pressure in the stimulation of chondrocyte biosynthesis during dynamic compression. *J Biomech* 28(9): 1055–1066

18 Buschmann MD, Kim YJ, Wong M, Frank E, Hunziker EB, Grodzinsky AJ (1999) Stimulation of aggrecan synthesis in cartilage explants by cyclic loading is localized to regions of high interstitial fluid flow. *Arch Biochem Biophys* 366(1): 1–7

19 Jin M, Frank EH, Quinn TM, Hunziker EB, Grodzinsky AJ (2001) Tissue shear deformation stimulates proteoglycan and protein biosynthesis inbovine cartilage explants. *Arch Biochem Biophys*. 395(1): 41–48

20 Eisenberg SR, Grodzinsky AJ (1987) The kinetics of chemically induced nonequilibrium swelling of articular cartilage and corneal stroma. *J Biomech* Eng 109(1): 79–89

21 Zhu W, Mow VC, Koob TJ, Eyre DR (1993) Viscoelastic shear properties of articular cartilage and the effects of glycosidase treatments. *J Orthop Res* 11(6): 771–781

22 Jin M, Grodzinsky AJ (2001) Effect of electrostatic interactions between glycosaminoglycans on the shear stiffness of cartilage: A molecular model and experiments. *Macromolecules* 34(23): 8330–8339

23 Basser PJ, Schneiderman R, Bank RA, Wachtel E, Maroudas A (1998) Mechanical properties of the collagen network in human articular cartilage as measured by osmotic stress technique. *Arch Biochem Biophys* 351(2): 207–219

24 Frank EH, Grodzinsky AJ (1987) Cartilage electromechanics – II. A continuum model of cartilage electrokinetics and correlation with experiments. *J Biomech* 20(6): 629–639

25 Frank EH, Grodzinsky AJ (1987) Cartilage electromechanics – I. Electrokinetic transduction and the effects of electrolyte pH and ionic strength. *J Biomech* 20(6): 615–627

26 Mow VC, Kuei SC, Lai WM, Armstrong CG (1980) Biphasic creep and stress relaxation of articular cartilage in compression: Theory and experiments. *J Biomech Eng* 102(1): 73–84

27 Setton LA, Zhu W, Mow VC (1993) The biphasic poroviscoelastic behavior of articular cartilage: role of the surface zone in governing the compressive behavior. *J Biomech* 26(4–5): 581–592

28 Li LP, Buschmann MD, Shirazi-Adl (2000) A fibril reinforced nonhomogeneous poroelastic model for articular cartilage: inhomogeneous response in unconfined compression. *J Biomech* 33(12): 1533–1541
29 Zhu W, Lai WM, Mow VC (1991) The density and strength of proteoglycan-proteoglycan interaction sites in concentrated solutions. *J Biomech* 24(11): 1007–1018
30 Buckwalter JA, Roughley PJ, Rosenberg LC (1994) Age-related changes in cartilage proteoglycans: quantitative electron microscopic studies. *Microsc Res Tech* 28(5): 398–408
31 Holmes DF, Gilpin CJ, Baldock C, Ziese U, Koster AJ, Kadler KE (2001) Corneal collagen fibril structure in three dimensions: Structural insights into fibril assembly, mechanical properties, and tissue organization. *Proc Natl Acad Sci USA* 98(13): 7307–7312
32 Chen CH, Hansma HG (2001) Basement membrane macromolecules: insights from atomic force microscopy. *J Struct Biol* 131(1): 44–55
33 Sun HB, Smith GN, Hasty KA, Yokota H (2000) Atomic force microscopy-based detection of binding and cleavage site of matrix metalloproteinase on individual type II collagen helices. *Anal Biochem* 283(2): 153–158
34 Seog J, Dean D, Plaas A, Wong-Palms S, Grodzinsky AJ, Ortiz C (2002) Direct measurement of glycosaminoglycan intermolecular interactions via high-resolution force spectroscopy. *Macromolecules* 35(14): 5601–5615
35 Katchalsky A (1971) *Pure Applied Chemistry* 26: 327–373
36 Buschmann MD, Grodzinsky AJ (1995) A molecular model of proteoglycan-associated electrostatic forces in cartilage mechanics. *J Biomech Eng* 117(2): 179–192
37 Bursac P, McGrath CV, Eisenberg SR, Stamenovic D (2000) A microstructural model of elastostatic properties of articular cartilage in confined compression. *J Biomech Eng* 122(4): 347–353
38 Ripamonti A, Roveri N, Braga D, Hulmes DJ, Miller A, Timmins PA (1980) Effects of pH and ionic strength on the structure of collagen fibrils. *Biopolymers* 19(5): 965–975
39 Frank EH, Jin M, Loening AM, Levenston ME, Grodzinsky AJ (2000) A versatile shear and compression apparatus for mechanical stimulation of tissue explants. *J Biomech* 33: 1523–1527
40 Eisenberg S, Grodzinsky AJ (1988) Electrokinetic micromodel of extracellular matrix and other polyelectrolyte networks. *Physicochemical Hydrodynamics* 10(4): 517–539
41 Quinn TM, Dierickx P, Grodzinsky AJ (2001) Glycosaminoglycan network geometry may contribute to anisotropic hydraulic permeability in cartilage under compression. *J Biomech* 34: 1483–1490

Cartilage tissue engineering using a new self-assembling peptide gel scaffold

John D. Kisiday[1], Moonsoo Jin[2], Bodo Kurz[4], Han-Hwa Hung[4], Carlos Semino[4], Shuguang Zhang[4] and Alan J. Grodzinsky[1,2,3,4]

[1]Division of Biological Engineering, Departments of Mechanical[2] and Electrical[3] Engineering, [4]Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139-4307, USA

Introduction

Emerging therapies for repair of articular cartilage include delivery of cells or cell-seeded scaffolds to a defect site to initiate de novo tissue regeneration. Biocompatible scaffolds assist in providing a template for cell distribution and extracellular matrix accumulation in a three-dimensional defect geometry. A variety of scaffolds have been investigated for cartilage repair in tissue culture and/or in animals. However, no scaffold-based cartilage construct is yet available for clinical application. In this study, we have explored the use of a novel self-assembling peptide hydrogel as a three-dimensional tissue engineering scaffold for cartilage repair [1].

Self-assembling peptides are characterized by amino acid sequences of alternating hydrophobic and hydrophilic side groups, with sequences of charged amino acid residues including alternating positive and negative charges [2–4]. The proposed model for self-assembly is outlined in Figure 1 [5]. When dissolved in water, the peptides assemble into stable beta sheets one peptide molecule thick containing two distinct surfaces of either hydrophobic or hydrophilic side chains. Hydrophobic bonding orients the beta sheet into a twisted tape configuration, creating nanofibers 10–20 nm thick. Exposure to electrolyte solution then initiates beta sheet assembly into an interwoven nanofiber network, creating the hydrogel structure (Fig. 1).

Previous cell culture studies have been conducted using self-assembling peptide material by seeding cells onto the surface of pre-assembled peptide hydrogel. Diverse mammalian cell types were found to attach and proliferate on the peptide membrane-like surface [2–4]. In particular, neuronal cells were found to attach, differentiate, and undergo extensive neurite outgrowth and synapse formation on the surface of the 16 amino acid peptide $(RADA)_4$ gel [4]. Additionally, two peptides were tested for immunogenicity in rats. Injection of $(EAKA)_4$ and $(RADA)_4$ into leg muscle of Fisher 344 rats resulted in no detectable toxic reaction after 9 days and 5 weeks, respectively [4]. These studies suggested that self-assembling peptide hydro-

The Many Faces of Osteoarthritis, edited by Vincent C. Hascall and Klaus E. Kuettner

gels are suitable for cell and animal compatibility studies relevant to cartilage tissue repair.

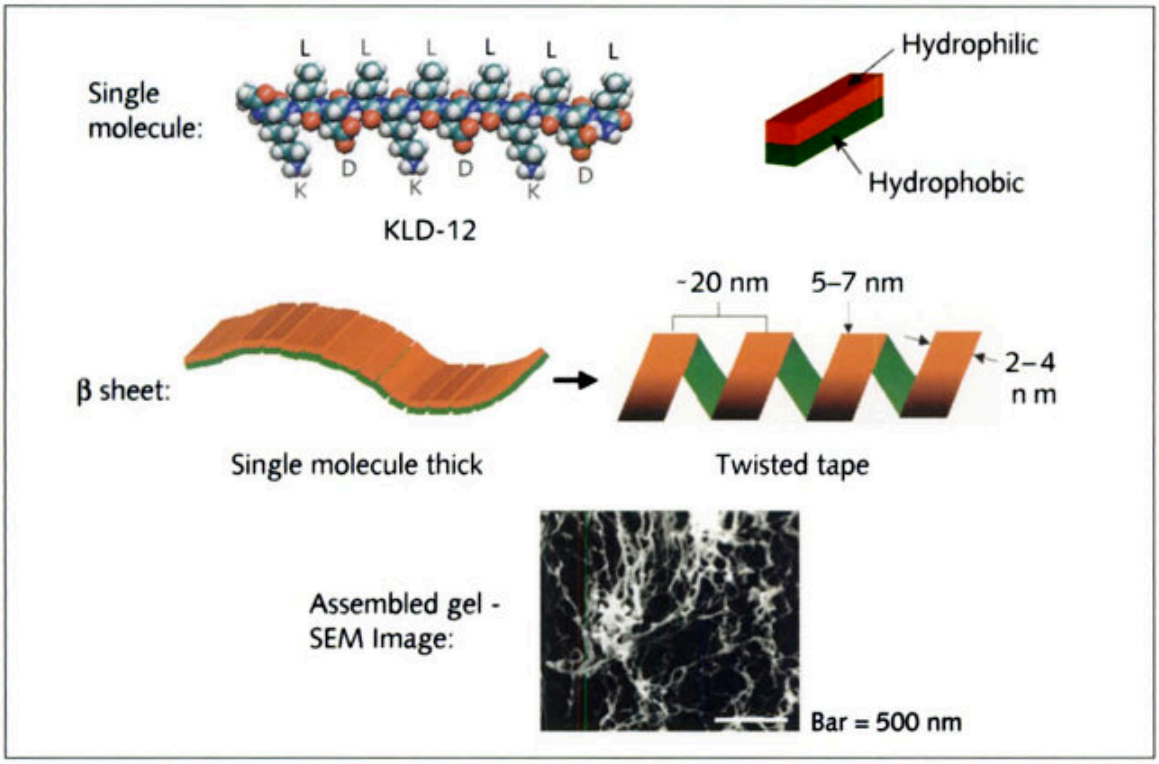
Practical applications to cartilage repair require the distribution of chondrocytes in a three-dimensional geometry for implantation into chondral defects. We hypothesized that a self-assembling peptide hydrogel would provide an appropriate environment for retention of chondrocyte phenotype and synthesis of cartilage extracellular matrix (ECM). In this study a method was developed to encapsulate chondrocytes within the peptide hydrogel during self-assembly. The time-dependent evolution of ECM biosynthesis, accumulation (including identification of collagens as an indicator of phenotypic expression) and mechanical functionality was quantified during subsequent *in vitro* culture. Chondrocyte proliferation was evaluated during early timepoints in culture. We also compared chondrocyte behavior in self-assembling peptide scaffolds to that in agarose hydrogel culture as a reference for chondrogenic potential in a well-established gel culture system [6, 7].

Methods

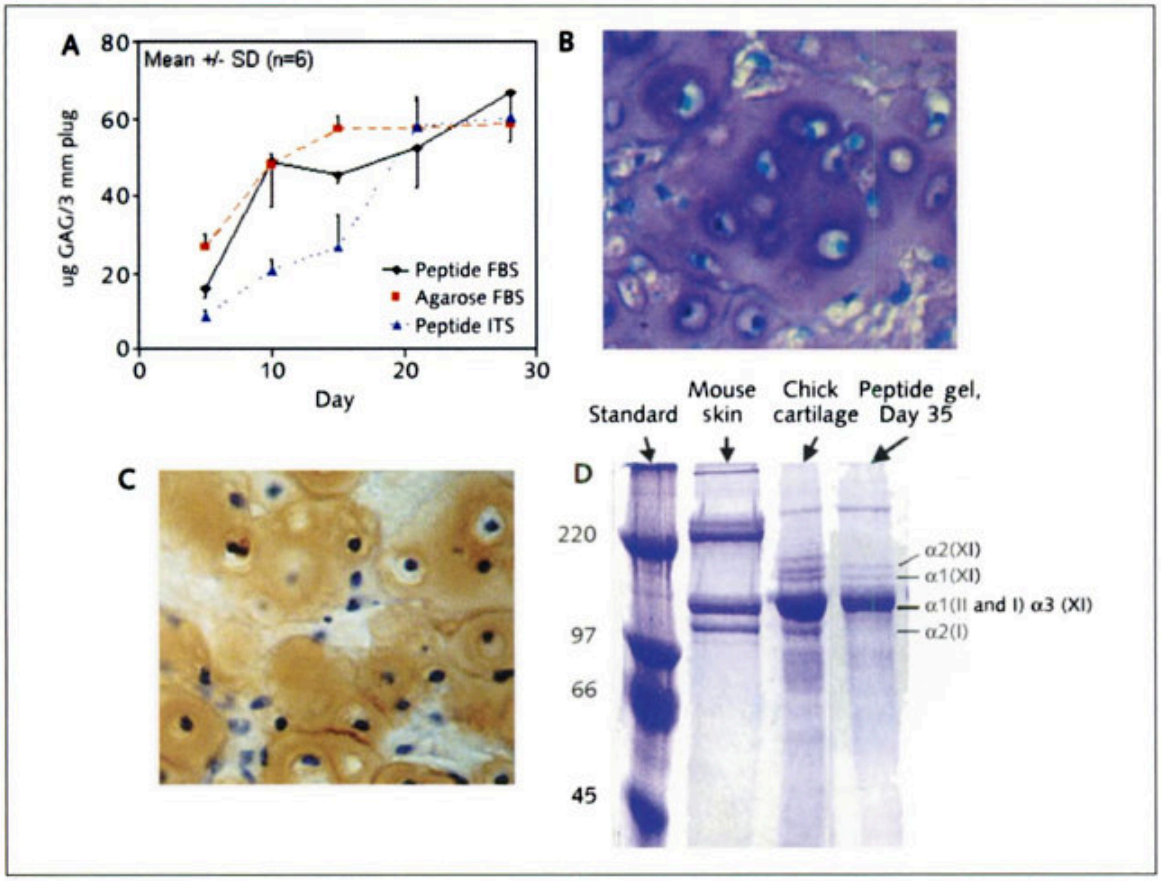
The amino acid sequence AcN-KLDLKLDLKLDL-CNH_2 (KLD12) was synthesized and lyophilized to a powder. A 0.5% peptide casting solution was obtained by dissolving KLD12 in an aqueous solution of 295 mM sucrose. Primary chondrocytes from 1–2 week-old bovine femoropatellar groove cartilage were re-suspended in the casting solution at a concentration of 15×10^6 cells/ml. The suspension was injected into a casting frame and placed into a 1X PBS bath for 20 min to initiate self-assembly into a 1.6-mm-thick slab [1]. A control agarose hydrogel was seeded in a similar manner. Peptide and agarose gels were cultured in DMEM supplemented

Figure 1 (top of next page)
Model of peptide self-assembly (adapted from [5]).

Figure 2 (bottom of next page)
Matrix accumulation in chondrocyte-seeded peptide hydrogel. (A) Total glycosaminoglycan (GAG) accumulation in cell-seeded peptide hydrogel cultured in FBS and ITS/FBS medium. Chondrocyte-seeded agarose hydrogel analyzed in parallel as a well-defined reference chondrocyte culture system. (B) Toluidine blue staining of chondrocyte-seeded peptide hydrogel, day 15. (C) Immunohistochemical staining for type II collagen in cell-seeded peptide hydrogel, day 15. D) SDS-PAGE of collagens extracted from chondrocyte-seeded peptide hydrogel, day 35. Standards (kindly donated by Prof. P. Bruckner, Münster): Chick cartilage for collagen II and XI banding pattern; mouse skin for collagen I alpha helix 2, indicative of collagen expression of a de-differentiated, fibroblastic phenotype.


Single molecule:
L L L L L L
K D K D K D
KLD-12
Hydrophilic
Hydrophobic
β sheet:
~20 nm
5–7 nm
2–4 n m
Single molecule thick
Twisted tape
Assembled gel - SEM Image:
Bar = 500 nm

1


A
Mean +/- SD (n=6)
ug GAG/3 mm plug
Day
Peptide FBS
Agarose FBS
Peptide ITS
B
C
D
Standard
Mouse skin
Chick cartilage
Peptide gel, Day 35
220
97
66
45
α2(XI)
α1(XI)
α1(II and I) α3 (XI)
α2(I)

2

with 10% FBS (FBS). A second peptide gel preparation was seeded similarly and maintained in DMEM plus 1% ITS (insulin, transferrin and selenium) and 0.2% FBS (ITS). The following measurements were performed on both chondrocyte seeded peptide and agarose hydrogels: (1) quantification of the rates of biosynthesis of proteoglycans and proteins *via* ^{35}S-sulfate and ^{3}H-proline radiolabel incorporation, respectively, (2) GAG accumulation (DMMB dye binding), and (3) cell proliferation as determined by viable cell content (MTS viable cell assay, Promega). In order to interpret MTS values, a calibration curve for 3-D cultures was first established using agarose cultures. Agarose gels were seeded at various cell concentrations and analyzed during the first 5 days of culture. Groups of plugs were punched and analyzed for MTS output, or digested and evaluated for DNA content *via* Hoechst dye analysis [8]. Mean MTS output was plotted against mean DNA content to establish the calibration curve. MTS data were then obtained for seeded peptide plugs, and viable cell counts were determined using the calibration curve. BrdU incorporation in chondrocyte seeded peptide hydrogels was used to further explore cell proliferation. Chondrocyte seeded peptide hydrogels were also characterized for GAG accumulation (histological sections stained with toluidine blue), collagen type (immunohistochemical techniques and SDS-PAGE of extracted collagen), and mechanical stiffness (evaluated in uniaxial confined compression).

Results

Quantification of ECM biosynthesis and accumulation was evaluated during 4 weeks of culture. Radiolabel incorporation and total GAG accumulation (Fig. 2A) in chondrocyte seeded peptide and agarose hydrogels cultured in FBS were similar at all timepoints. Values in agarose were comparable to previously reported data showing increasing GAG accumulation and decreasing biosynthetic rates with time in culture [6]. Total GAG accumulation was significantly lower in ITS medium during the first 2 weeks in culture. However, by day 21 there was no significant difference between the ITS and 10% FBS peptide gels. Toluidine blue staining of chondrocyte seeded hydrogels on day 15 (Fig. 2B) showed GAG deposition throughout the gel, with highest intensity in the pericellular regions. Consistent with the accumulation of a continuous GAG matrix, the equilibrium confined compression modulus increased to ~27 kPa on day 28, a ~30-fold increase over day 0 (~0.6 kPa). Immunostaining for collagen II at day 15 showed strong positive staining throughout the gel, forming a continuous matrix as seen with GAG deposition (Fig. 2C). Collagen I staining was light background only. Electrophoresis of extracted collagens showed the presence of type II and IX collagen, and no α2-chain band characteristic of type I collagen expression by de-differentiated chondrocytes (Fig. 2D). Chondrocytes encapsulated in peptide hydrogels were found to proliferate significantly over 9 days of culture. Viable cell density on day 9 increased ~80% relative

to day 2 values. BrdU incorporation was consistent with MTS results, showing significant populations of proliferating cells on day 3 and 7. In contrast, viable cell densities in chondrocyte-seeded agarose hydrogels increased by only ~20% on day 7 relative to day 2, consistent with previously reported data [6].

Discussion

The data of Fig 2, along with the observed increase in compressive stiffness, demonstrate the potential of a self-assembling peptide gel for hosting a chondrogenic repair response with seeded primary chondrocytes *in vitro*. The ultimate utility of the scaffold will be determined by long-term development of functional, integrated repair tissue *in vivo*. However, *in vitro* conditioning may also be utilized to stimulate the development of optimal cell/scaffold/ECM constructs prior to implantation. Therefore, in addition to the free swelling *in vitro* cultures typified by the data of Figure 2, we also investigated the effects of dynamic mechanical compression on ECM biosynthesis by primary chondrocytes seeded in peptide gels. Groups of six 12-mm diameter seeded peptide disks were placed into the six wells of a custom chamber, one disk per well, for use with an incubator-housed loading system [9]. Compression was applied to seeded peptide samples with porous platens. A center mounted spring was used to create a 400–800 μm gap between the platens and gel disks when the lid was unloaded. In this manner, samples may alternate between periods of cyclic compression and "free-swell" culture. Seeded peptide gels were subjected to sinusoidal dynamic compression of 2.5% strain amplitude superimposed on 5% static offset strain at a frequency of 1.0 Hz starting on day 22 of culture. Loading was applied for 45 min, followed by 5.25 h of free-swell culture. Loading was applied 4×/day, every other day. Samples were radiolabeled with ^{35}S-sulfate on days 27 (non-loading period), 32 (loading period), and 33 (non-loading), and the results were normalized to the incorporation in samples maintained in static, free-swelling culture. ^{35}S-sulfate incorporation (measured to be ~98% macromolecular in all samples) in loaded samples was significantly higher than that in free-swelling culture at all timepoints. ^{35}S-Sulfate incorporation was higher on day 33 than day 32, indicating proteoglycan synthesis was greater during periods of loading. Total GAG content was measured on day 32 and 33. In both cases, GAG content was 9% higher in loaded samples. This represents a ~20–30% increase in GAG content during the 12 and 13 day loading periods. Therefore, mechanical loading may be used to accelerate proteoglycan accumulation during *in vitro* culture prior to implantation.

Flexibility in peptide design may also be advantageous to stimulating a complete repair response. Peptide sequences may potentially be designed for cell attachment to guide tissue regeneration, and for enzymatic degradation to enable spatially and temporally controlled biodegradability. Peptide sequences may also allow for tethering of growth factors to peptides for direct delivery to encapsulated cells. Such

flexibility may be combined with *in vitro* conditioning, such as mechanical loading or ITS-supplemented medium, to generate multiple approaches towards complete tissue regeneration.

Acknowledgements
Funded by NIH Grant AR33236 and the DuPont-MIT Alliance.

References

1 Kisiday J, Jin M, Kurz B, Hung HH, Semino C, Zhang S, Grodzinsky AJ (2002) Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: Implications for cartilage tissue repair. *Proc Natl Acad Sci USA* 99(15): 9996–10001

2 Zhang S, Holmes T, Lockshin C, Rich A (1993) Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc Natl Acad Sci USA* 90 (8): 3334–3338

3 Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A (1995) Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials* 16(18): 1385–1393

4 Holmes TC, de Lacalle S, Su X, Liu G, Rich A, Zhang S (2000) Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. *Proc Natl Acad Sci USA* 97(12): 6728–6733

5 Marini DM, Hwang W, Lauffenburger DA, Zhang S, Kamm RD (2002) Left-handed helican ribbon intermediates in the self-assembly of a β-sheet peptide. *Nano Letters* 2: 295–299

6 Benya PD, Shaffer JD (1982) Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell* 30(1): 215–224

7 Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB (1992) Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. *J Orthop Res* 10(6): 745–758

8 Kim YJ, Sah RL, Doong JY, Grodzinsky AJ (1988) Fluorometric assay of DNA in cartilage explants using Hoechst 33258. *Anal Biochem* 174(1): 168–176.

9 Frank EH, Jin M, Loening AM, Levenston ME, Grodzinsky AJ (2000) A versatile shear and compression apparatus for mechanical stimulation of tissue culture explants. *J Biomech* 33(11): 1523–1527