Published in final edited form as:

J Nanotechnol Eng Med. 2010 November 1; 1(4): . doi:10.1115/1.4002461.

Periodic Nanomechanical Stimulation in a Biokinetics Model Identifying Anabolic and Catabolic Pathways Associated With Cartilage Matrix Homeostasis

Asit K. Saha and

Department of Mathematics and Computer Science and Center for Allaying Health Disparities Through Research and Education (CADRE), Central State University, Wilberforce, OH 45384

Sean S. Kohles

Department of Mechanical and Materials Engineering, Reparative Bioengineering Laboratory, Portland State University, Portland, OR 97201; Department of Surgery, Oregon Health and Science University, Portland, OR 97239

Asit K. Saha: asaha@centralstate.edu; Sean S. Kohles: kohles@cecs.pdx.edu

Abstract

Enhancing the available nanotechnology to describe physicochemical interactions during biokinetic regulation will strongly support cellular and molecular engineering efforts. In a recent mathematical model developed to extend the applicability of a statically loaded, single-cell biomechanical analysis, a biokinetic regulatory threshold was presented (Saha and Kohles, 2010, "A Distinct Catabolic to Anabolic Threshold Due to Single-Cell Static Nanomechanical Stimulation in a Cartilage Biokinetics Model," *J. Nanotechnol. Eng. Med.*, **1**(3), p. 031005). Results described multiscale mechanobiology in terms of catabolic to anabolic pathways. In the present study, we expand the mathematical model to continue exploring the nanoscale biomolecular response within a controlled microenvironment. Here, we introduce a dynamic mechanical stimulus for regulating cartilage molecule synthesis. Model iterations indicate the identification of a biomathematical mechanism balancing the harmony between catabolic and anabolic states. Relative load limits were defined to distinguish between "healthy" and "injurious" biomolecule accumulations. The presented mathematical framework provides a specific algorithm from which to explore biokinetic regulation.

Keywords

biomathematics; extracellular matrix; chondrocytes; biodynamics; biophysics

1 Introduction

Recent research in systems biology at the nanoscale has emerged as a frontier in biological science. Nanobioscience combines many branches of science such as mathematics, computer science, physics, molecular engineering, biology, biotechnology, and medicine. Knowledge about the dynamics of biomolecules at the nanolevel provides a better understanding of every living system. This information can help develop new drugs and their targeted delivery, regenerative medicine, tissue engineering methods, and tissue remodeling procedures [1]. Researches in nanobioscience and nanobiotechnology are

considered to be preferential fields in many countries with its relevance expected to increase heavily in the near future. The design of complex dynamics of nano/biomolecular functions is a goal extremely difficult to achieve with classical approaches. Design and construction of such complex nanomolecules can be adapted toward the demands of technical application for both metalloproteins and other proteins [2].

Knowledge-based tissue engineering is an emerging interdisciplinary field that seeks to address these needs by applying the principles of nanobioscience and nanobioengineering to the development of viable tissue substitutes that restore and maintain the function of human tissues such as cartilage. Articular cartilage is a heterogeneous tissue with a very complex composition of biomolecules with high structural stability. Mature articular cartilage contains approximately 5% of its volume as cells (chondrocytes) and 95% as extracellular matrix (ECM). Out of this 95% of the ECM, 70% is water. There is normally no trace of minerals in articular cartilage. Within the ECM, 60% is collagen, 25% is proteoglycans, and 15% is a range of miscellaneous matrix proteins. The structural molecules of type-II collagen and proteoglycan create a highly hydrated tissue with unique tribological and compressive/tensile properties.

The distribution and architecture of different biomolecules within articular cartilage are not uniform. Instead, articular cartilage is divided into four histologic layers. These are superficial, middle, deep, and calcified. In the superficial layer, chondrocytes are flattened with a low density of proteoglycan and a high density of collagen fibrils arranged parallel to the articular surface. In the middle layer, chondrocytes are spherical in shape with proteoglycan concentration being very high among the four layers with an unsystematic arrangement of collagen fibers. In the deep layer, collagen fibers are perpendicular to the underlying bone and columns of chondrocytes arrayed along the axis of fibril. The calcified layer is a transitional section between the cartilage and the underlying subchondral bone [3]. The overall nanoscale architecture of articular cartilage components is highly dependent on nanolevel fluid and solid biomechanics. An understanding of the natural organization and biologic allocation of cartilage mechanics will allow a better understanding of cartilage biology.

Unfortunately, articular cartilage has a limited capacity to repair itself. Under normal conditions, its thickness is 2–4 mm depending on the joint location [3]. The tissue is susceptible to osteoarthritis, a degeneration of the articular tissue. This is the most common form of arthritis in the world, affecting 10% of the total population in Australia [4] and 100,000 diagnosed Canadians each year [5]. In the United States alone, more than 1×10^6 people annually suffer some form of osteoarthritis [6]. Damage to the articulating surface can occur when acute or fatigue compression and/or shear forces are applied; thus, it is a disease with biomechanical mechanisms.

There are typically two ways to repair damaged articular cartilage including nonoperative and operative approaches. Current treatments have had minimal success stimulating the regeneration of cartilage tissue [7–10]. Tissue engineering offers a new option in modern medicine by using a patient's own cells to produce bioengineered tissues and organs that function like their natural counterparts. There are many approaches to cartilage tissue engineering but all involve one or more of the following key factors: harvested cells, signaling molecules, and 3D scaffold matrices. In most cases, chondrocytes are seeded onto a scaffold made of a hydrogel (alginate and agarose), a biodegradable polymer (polylactic acid or polyglycolic acid (PGA)), or a biopolymer (collagen). The constructs are then cultured and embedded into the defect to persuade the growth of new tissue [11–15]. The overriding goal is for the cells to attach to the scaffold, multiply, differentiate, and organize into normal, healthy articular cartilage as the scaffold degrades. The signaling molecules can

be adhered to the scaffold or incorporated directly into the scaffold as a means to control the cell-matrix biology.

The current challenges in cartilage tissue engineering are understanding and controlling the cartilage biology. Without a clear understanding of the physiologic regulation of cartilage biomolecules, a successful engineered tissue is impossible. Cell-cell and cell-ECM interactions are two main fundamental nanoscale mechanisms responsible for tissue regeneration and/or tissue remodeling as well as ECM homeostasis. From experimental observations, one can justifiably argue that there exist two basic pathways, anabolic and catabolic, controlled by chondrocytes that lead to the matrix homeostasis [16,17]. In the case of cartilage matrix growth, repair, and remodeling, anabolic processes exceed the catabolic activities. Alternatively, catabolic processes exceed the anabolic activities when disease and degeneration occur. These relationships have been observed in controlled osteoarthritic culture models, where experimental results show that the combination of anabolic growth factors and protective catabolic blockers may be a means for partial restoration of the cartilage matrix [18].

It is now clear that the interplay between growth factor and cytokine biomolecules maintains regulatory balance due to their catabolic and anabolic interplay. Identification of these two molecules and their roles is a significant advancement in cartilage biology; however, there is still limited knowledge as to how these molecules are functionally active. Based on a system biological approach, we have recently predicted that anabolic actions of different growth factors are essentially dose and time dependent [19,20]. Extending that work to a system dynamics mechanistic model, results strongly indicate that a balanced network of anabolic and catabolic pathways emerges out of cytokine and growth factor interactions, which helps the ECM to reach homeostasis [21]. As such, this article further investigates the role of mechanical stress on influencing matrix homeostasis in the context of tissue engineering. More precisely, our primary objective is to investigate the influence of nanomechanical stress on the anabolic and catabolic pathways of matrix homeostasis in the articular cartilage through a systems biology approach. Mechanical stresses are defined as static or dynamic, a constant load acting continuously on the tissue or through variable loading, respectively. We previously identified a catabolic to anabolic threshold due to static nanomechanical loading [22]. In the present study, we describe a biokinetic balance achieved through a periodic loading with respect to time.

2 Materials and Methods

2.1 Mathematical Model

The important anabolic proteins are the various growth factors, while the catabolic proteins include the cytokines. During a healthy balanced regulation, growth factors such as transforming growth factor- β (TGF- β), insulin-like growth factor-1 (IGF-1), and osteogenic protein-1 stimulate the chondrocytes to synthesize the structural macromolecules, while cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- α stimulate chondrocyte secretion of proteinases causing ECM degradation.

The foundation of our model involves anabolic/catabolic pathways that maintain ECM structural molecule depositions [21], as briefly summarized here. Let $[Cy]$, $[Gf]$, and $[Ec]$ represent the concentration levels of cytokines, growth factors, and ECM, respectively, at any particular time t . According to the schematic network (Fig. 1), the mathematical model is defined as follows:

$$\begin{aligned}
\frac{d[Cy]}{dt} &= \eta_1 [Cy] \{ \mathfrak{R}_2 - [Gf] \} - \mu_1 [Cy] \\
\frac{d[Gf]}{dt} &= \eta_2 [Gf] \{ [Cy] - \mathfrak{R}_1 \} - \mu_2 [Gf] \\
\frac{d[Ec]}{dt} &= \frac{v[Gf]}{K+[Cy]} - \sigma [Ec]
\end{aligned} \tag{1}$$

where $i=1$ (cytokines) or 2 (growth factors), η_i is the synthesis rate, μ_i is the degradation rate, v is the synthesis rate for overall ECM, σ is the degradation rate of overall ECM, K is the saturation parameter, and $\mathfrak{R}_i$ is the pivotal concentration.

The dimensionless form of the system can be written as

$$\begin{aligned}
\frac{d}{dT} \begin{pmatrix} C \\ G \end{pmatrix} &= \begin{pmatrix} \alpha_1 C (\Omega_2 - G) - \beta_1 C \\ \alpha_2 G (C - \Omega_1) - \beta_2 G \end{pmatrix} \\
\frac{d}{dT} E &= \frac{\lambda G}{1+C} - \delta E
\end{aligned} \tag{2}$$

where

$$\begin{aligned}
C &= K^{-1} [Cy], \quad G = K^{-1} [Gf], \quad E = K [Ec], \quad \text{and} \quad T = K^2 [t] \\
\alpha_1 &= K^{-1} \eta_1, \quad \alpha_2 = K^{-1} \eta_2, \quad \beta_1 = K^{-2} \mu_1, \quad \beta_2 = K^{-2} \mu_2 \\
\lambda &= K^{-1} v, \quad \delta = K^{-2} \sigma, \quad \Omega_1 = K^{-1} \mathfrak{R}_1, \quad \text{and} \quad \Omega_2 = K^{-1} \mathfrak{R}_2
\end{aligned}$$

The system characterized in Eq. (2) has been classified into two parts. The first part only involves growth factor and cytokine dynamics independent of other ECM biomolecules, and the second part describes the ECM dynamics as dependent on anabolic and catabolic pathways controlled by growth factor/cytokine dynamics.

It is evident from experimental work that mechanical stress induces complex changes at the nanoscale. These influences may result in structural changes to the chondrocytes and other ECM biomolecules and/or they can also change the dynamics of different biomolecules, all resulting in cell-mediated biosynthesis [23]. The percentage of growth factors and cytokines in intact ECM is vanishingly small, but these biomolecules act as potent modulators of chondrocyte behavior [24]. The ECM of native articular cartilage acts as a reservoir of IGF-1, TGF- β , and IL-1. The communication between cells and ECM biomolecules is mostly done by releasing, receiving, and detecting extracellular signaling molecules such as growth factors and cytokines. The modeling approach described above follows the observations that a number of selective growth factors remarkably increased the collagen accumulation in ECM [25]. Both in vitro and in vivo experiments have also reported that some growth factor accumulation can either enhance or inhibit the production of biomolecules [26–29].

As an enhancement to this model, we now introduce a mechanical stimulus in order to mimic the functional physiologic pressures in the cartilage (typically 3–18 MPa) [30,31]. Application of these forces is essential for the maintenance of the phenotype and for the production of new tissue [32]. Alternatively, abnormal mechanical loading leads to altered chondrocyte behavior, resulting in pathological matrix synthesis and ultimately osteoarthritis or osteoporosis (cell apoptosis) [33,34]. To clarify the influence of nanomechanical stresses on matrix molecule homeostasis, we now modify our fundamental system (Eq. (2)) by incorporating mathematical components representing a periodic mechanical loading at the same molecular scale

$$\frac{d}{dT} \begin{pmatrix} C \\ G \end{pmatrix} = \begin{pmatrix} \alpha_1 C(\Omega_2 - G) - \beta_1 C \\ \alpha_2 G(C - \Omega_1) - \beta_2 G \end{pmatrix} + \begin{pmatrix} \rho_{11} & \rho_{12} \\ \rho_{21} & \rho_{22} \end{pmatrix} \begin{pmatrix} 1 + \cos T \\ 1 + \cos T \end{pmatrix}$$

$$\frac{d}{dT} E = \frac{\lambda C}{1+C} - \delta E \quad (3)$$

where

$$\begin{pmatrix} \rho_{11} & \rho_{12} \\ \rho_{21} & \rho_{22} \end{pmatrix}$$

is the scaling matrix of the mechanical stress on the individual regulatory pathways. Here, ρ_{11} is the scaling factor when mechanical stress is directly acting on catabolic pathways, ρ_{12} is the cross-scaling factor when mechanical stress is directly acting on anabolic pathways that influence catabolic pathways, ρ_{21} is the cross-scaling factor when mechanical stress is directly acting on catabolic pathways that influence anabolic pathways, and ρ_{22} is the scaling factor when mechanical stress is directly acting on anabolic pathways.

If it is assumed that the main structural molecules within the articular cartilage, collagen and glycosaminoglycan (GAG), follow independent growth kinetics, then we can write $E \approx \text{collagen} + \text{GAG}$. Hence, system (3) can be rewritten as

$$\frac{d}{dT} \begin{pmatrix} C \\ G \end{pmatrix} = \begin{pmatrix} \alpha_1 C(\Omega_2 - G) - \beta_1 C \\ \alpha_2 G(C - \Omega_1) - \beta_2 G \end{pmatrix} + \begin{pmatrix} \rho_{11} & \rho_{12} \\ \rho_{21} & \rho_{22} \end{pmatrix} \begin{pmatrix} 1 + \cos T \\ 1 + \cos T \end{pmatrix}$$

$$\frac{d}{dT} \text{collagen} = \frac{\lambda_1 G}{1+C} - \delta_1(\text{collagen})$$

$$\frac{d}{dT} \text{GAG} = \frac{\lambda_2 G}{1+C} - \delta_2(\text{GAG}) \quad (4)$$

2.2 Steady State Condition

The steady state conditions (C_{ss} , G_{ss} , collagen_{ss} , and GAG_{ss}) within the system, as described by Eq. (4), can be obtained from the following relationship [22]:

$$(C_{ss}, G_{ss}, \text{collagen}_{ss}, \text{and } \text{GAG}_{ss}) = \left(\left\{ \Omega_1 + \frac{\beta_2}{\alpha_2} \right\}, \left\{ \Omega_2 - \frac{\beta_1}{\alpha_1} \right\}, \left\{ \frac{\lambda_1 G_{ss}}{\delta_1(1+C_{ss})} \right\}, \text{and } \left\{ \frac{\lambda_2 G_{ss}}{\delta_2(1+C_{ss})} \right\} \right) \quad (5)$$

This nontrivial steady state is obtained when no mechanical loading occurs. A phase-space hypothetical kinetic relationship of growth factor and cytokines has been discussed previously as an interpretation of these relationships [21].

3 Biomolecular Simulation

3.1 Parameter Estimation

Normalized parameter estimations associated with growth factor and cytokine kinetics within an engineered tissue environment are limited in availability. Therefore, a mathematical association between growth factor and cytokine abundance was drawn from data characterizing the development of sweat glands in human fetal skin [35]. This tissue source facilitated an initial parametric relationship as a pilot modeling approach [21].

The parameters related to ECM structural molecules and proteoglycans were determined previously through a deterministic approach, where kinetic rate ratios of synthesis per decay contribute to steady state levels of ECM accumulation [20]. Experimental and theoretical stresses were based on a microfluidic environment designed for biological cell investigations [36], where stresses applied to a suspended cell range from 0.02 Pa to 0.04 Pa. The overall parameter estimations now include biokinetic and biomechanical factors (Tables 1 and 2).

3.2 Modeling Iterations

In this modeling perspective, three different loading phases have been considered to demonstrate our system dynamics model. All three phases include simulations of the (a) dynamics of anabolic and catabolic pathways regulated by growth factors and cytokines and (b) growth kinetics of different matrix biomolecules such as GAG and collagen. All simulations were run using commercial software (MATHCAD 14.0, Parametric Technology Corp., Needham, MA). Phase one described the dynamics of anabolic and catabolic pathways and the consequent ECM molecule deposition with and without a static mechanical stress [21,22]. In phase two presented here, the results of the biokinetics of the anabolic and catabolic networks and the consequent ECM molecule deposition are shown due to an imposed dynamic stress (periodic loading). In phase three, also presented here, the case of an injurious dynamic loading is explored. In all cases, the stress-free system is in a transient state (between time zero and the steady state) when the dynamic loading is applied ($\sim 10,000$ th dimensionless time step).

For simplicity, the cross-scaling factors are assumed to be zero ($\rho_{12}=\rho_{21}=0$). Normalized periodic load components were stepping up through the factor $\rho_{ii}(1 + \cos T)$, where $i=1,2$, as noted above. Again, the periodic mechanical loading was applied at the 10,000th dimensionless time step while running without any imposed mechanical stress before the application. The relative magnitude of the mechanical loading was increased from $\rho=0.02$, 1.0, 10.0, <86.2 (considered healthy) and >86.2 (considered injurious), as applied to a previous experimental data set (Table 2) [15]. Biomolecule concentrations through time were modeled as relative units.

4 Results

4.1 Biodynamics With Healthy Periodic Loading

It has previously been observed that this modeled system can reach the steady state around the 50,000th dimensionless time step iteration without any kind of mechanical loading. It was also observed that although the collagen molecule accumulation is very high at about the 1,000th dimensionless time step, its abundance is reduced to a much lower value by the steady state condition. In the case of the other modeled structural molecule GAG, no dramatic change is observed between its peak concentration at the 10,000th dimensionless time step and its steady state values [22].

The initial simulation was of the low load condition $\rho=0.02$ applied at the 10,000th time step. A minimal difference in the steady state values of the ECM accumulation is observed (Fig. 2). The collagen molecule reaches a much lower value than previously modeled [21]. The anabolic and catabolic pathways are also similarly oscillatory compared with the no-load condition, heading toward fixed values in a longer time frame (Fig. 3). When the periodic mechanical loading is increased from $\rho=0.02$ to $\rho=1.0$ with all other conditions remaining the same, some differences may be indicated in the steady state values of the ECM molecule deposition. Here, both GAG and collagen steady state concentrations are slightly higher than those observed in the situation with less loading (Fig. 4). The anabolic and catabolic pathways are oscillatory in nature, but the amplitude of the oscillation

becomes very small over time (Fig. 5). Finally, with an increase in the periodic loading level from $\rho=1.0$ to $\rho=10.0$, we find a dramatic difference in the steady state values of the ECM molecules (Fig. 6). Both GAG and collagen concentrations at the steady state are now much higher than those observed in the situations without any loading [22] and with the lowest amount of loading $\rho=0.02$ (Figs. 2 and 3). The anabolic and catabolic pathways remain oscillatory in nature and with high amplitudes (Fig. 7). From the computational analysis, the system appears to behave optimally (in terms of maximum accumulation) when $\rho \leq 86.2$ (Fig. 8) with high turnover of the ECM biomolecules and the anabolic/catabolic pathways following a perfect oscillation (Fig. 9).

4.2 Biodynamics With Injurious Periodic Loading

When the applied periodic mechanical loading is made to be greater than 86.2, a catastrophic behavior is observed in the ECM molecule accumulation dynamics (Fig. 10). We define this value of $\rho \approx 86.2$ as a threshold load beyond which mechanical loading may become injurious or deleterious to engineered tissue development. This threshold loading may change its value if we consider a different data set for the anabolic and catabolic networks. A similar imbalance was observed for the highest loading condition when applied to other experimental data sets (Table 2) [37–39]. The reason for getting the same catastrophic nature in all four data sets is due to the mathematical assumption that only the anabolic and catabolic pathways are responsible for matrix homeostasis, as similarly developed for all data sets.

Another interesting feature we observed is that if the amplitude of the oscillatory (limit) cycle increases, then the ECM molecule accumulation tends toward higher values in the steady state. Failed ECM development occurs when the locus of the limit cycle veers closer to either of the axes (growth factor axis or cytokine axis). In those cases, either of the concentrations (growth factors or cytokines) becomes vanishingly small in amount and cannot compete with the rival components (anabolic versus catabolic behavior). In the simulation, the cytokine concentration at one time point becomes very close to zero, whereby the modeled system responded abruptly.

5 Discussion

To maintain the integrity of articular cartilage tissue, positive interactions between the chondrocytes and the surrounding ECM are absolutely necessary. The presented work indicates the significant role that mechanical stimuli maintain when acting on chondrocytes alone, ECM alone, or both. Previous authors have described mechanical signals as transmitted to chondrocytes via the ECM [40]. It was also suggested that mechanical stimuli actually provoke the chondrocytes into triggering the anabolic and catabolic pathways for tissue remodeling. Whether it is direct or indirect (via ECM), any mechanical stress at the tissue level results in either deformation of chondrocytes and ECM biomolecules [41] and/or aggravation of cellular kinetics or biochemical activity at the nanoscale [42]. Previous studies suggest that mechanical force plays an important role in regulating chondrocyte behavior, subsequently guiding ECM structural molecule homeostasis [43,44]. However, the mechanotransduction pathways by which chondrocytes and other biomolecules respond to mechanical forces are not fully understood. Any applied stress at the mesoscale (tissue level) could result in complex changes in the nanoscale. Overall, these structural and biochemical changes are detected by mechanoreceptors on the cell surface, which include mechanosensitive ion channels and integrins [45,46].

Model results presented here suggest that a balance between anabolic and catabolic pathways is the key relationship for matrix homeostasis and healthy tissue remodeling, specifically in an engineered tissue culture setting. Any mechanical stress physically

influences the fundamental biokinetics as directly initiated through either of the modeled constituents: chondrocytes or ECM. Our system dynamics model characterized the qualitative behaviors of these fundamental anabolic and catabolic networks and the resulting matrix molecule accumulation under the influence of a bounded periodic mechanical loading.

Translating the anabolic and catabolic mechanisms (growth factor/cytokine dynamics) as a controllable trigger within the in vitro and in vivo environments represents a limitation in the modeling approach. In vitro, one can administer growth factors and cytokines externally in the cell-polymer construct, thus directly assessing the proposed mathematics [25]. In vivo, the biologic processes are complex and may be difficult to directly compare with the mathematics. Biologic triggers can be derived from many sources such as a feedback signal from the ECM, a destabilization in the concentration gradients of growth factors and cytokines in the ECM, or by the mechanical stimuli. It has been suggested that chondrocyte mechanoreceptors, a kind of sensory receptor, such as mechanosensitive ion channels and integrins, could be involved in the recognition of these physiochemical changes [47,48]. However, there is no clear evidence available at this stage to fully describe the catabolic/anabolic mechanisms.

Experimentalists and clinicians are unified in their conclusions that physical movements and induced mechanical forces can maintain the healthy status of a cartilage tissue [49,50]. Of note is the heterogeneous structural organization of collagen and proteoglycan through the depth of the tissue. In this conjecture, there may be site-specific dependencies of mechanical stimuli guiding the localized health or degeneration of cartilage. Many experimental works have investigated the mechanical loading effects at the tissue, cellular, and molecular levels [51–65].

In our previous studies, we observed that the anabolic/catabolic networks are necessary for matrix homeostasis but that this homeostasis has limited sustenance [21,22]. We identified a need for an additional mechanism to sustain homeostasis. In the model presented here, we clarify the importance and justification of mechanical loading as a discrete mathematical component. This was previously demonstrated for the static loading case [22]. In this article, we investigated the effects of dynamics via a periodic loading influence on matrix homeostasis. Our findings suggest that load levels exist, which have zero, positive, or negative influences on homeostasis. With a specific mechanical loading regime, a high turnover in the biomolecules is present and concomitant with a harmonic balance between the anabolic and catabolic pathways. It is also evident from our investigation that there are mechanical limits that may produce catastrophic outcomes of matrix biomolecule accumulation leading to injury or disease. Future studies will attempt to validate these models as well as investigate the role of mechanical stimuli in cellular migration during native and engineered tissue remodeling.

Acknowledgments

The support provided by the National Institutes of Health (NIH) as a Research Infrastructure for Minority Institutions (RIMI) Exploratory Program Grant (No. P20 MD003350) established the CSU Center for Allaying Health Disparities Through Research and Education (CADRE). Support was also provided by an NIH Academic Research Enhancement Award (No. R15 EB007077) to PSU as well as from the Collins Medical Trust and a Portland State University Faculty Enhancement Grant.

References

1. Roco MC. Nanotechnology: Convergence With Modern Biology and Medicine. *Curr Opin Biotechnol* 2003;14:337–46. [PubMed: 12849790]

2. Nicolini, C. Nanobiotechnology and Nanobiosciences (Pan Stanford Series on Nanobiotechnology). Vol. 1. World Scientific Publishing, Inc; Singapore: 2008. p. 1-380.
3. Chun AL, Jomha NM, Webster TJ, Fenniri H. Localized Articular Cartilage Defects—A Review on Current Modes of Treatment and How Nanotechnology Can Play a Role. *NanoBiotechnology* 2005;1:43–63.
4. Patterson, K. Media Release: Innovative Community Project to Combat Bone and Joint Diseases. Australian Department of Health and Ageing; 2003.
www.health.gov.au/internet/wcms/Publishing.nsf/Content/health-mediarel-yr2003-kp-kp03123.htm
5. Arthritis Society of Canada. Media Release. Arthroscopy: Facts and Figures. 2004 Oct 20.
<http://www.arthritis.ca>
6. Langer R, Vacanti JP. Tissue Engineering. *Science* 1993;260:920–926. [PubMed: 8493529]
7. Minas T, Nehrer S. Current Concepts in the Treatment of Articular Cartilage Defects. *Orthopedics* 1997;20(6):525–538. [PubMed: 9195635]
8. Buckwalter JA, Mankin HJ. Articular Cartilage Repair and Transplantation. *Arthritis Rheum* 1998;41:1331–1342. [PubMed: 9704631]
9. Gilbert JE. Current Treatment Option for the Restoration of Articular Cartilage. *Am J Knee Surg* 1998;11:42–46. [PubMed: 9533054]
10. Gross AE. Cartilage Resurfacing: Filling Defects. *J Arthroplasty* 2003;18(3):14–17. [PubMed: 12730921]
11. Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB. Chondrocytes in Agarose Culture Synthesize a Mechanically Functional Extracellular Matrix. *J Orthop Res* 1992;10:745–758. [PubMed: 1403287]
12. Freed LE, Marquis JC, Nohria A, Emmanual J, Mikos AG, Langer R. Neocartilage Formation In Vitro and In Vivo Using Cells Cultured on Synthetic Biodegradable Polymers. *J Biomed Mater Res* 1993;27:11–23. [PubMed: 8380593]
13. Lee CR, Grodzinsky AJ, Spector M. The Effects of Cross-Linking of Collagen-Glycosaminoglycan Scaffolds on Compressive Stiffness, Chondrocyte-Mediated Contraction, Proliferation and Biosynthesis. *Biomaterials* 2001;22:3145–3154. [PubMed: 11603587]
14. Mooney DJ, Mazzoni CL, Breuer C, McNamara K, Hern D, Vacanti JP, Langer R. Stabilized Polyglycolic Acid Fiber-Based Tubes for Tissue Engineering. *Biomaterials* 1996;17:115–124. [PubMed: 8624388]
15. Wilson CG, Bonassar LJ, Kohles SS. Modeling the Dynamic Composition of Engineered Cartilage. *Arch Biochem Biophys* 2002;408:246–254. [PubMed: 12464278]
16. Chambers MG, Bayliss MT, Mason RM. Chondrocyte Cytokine and Growth Factor Expression in Murine Osteoarthritis. *Osteoarthritis Cartilage* 1997;5:301–308. [PubMed: 9497937]
17. Moos V, Fickert S, Muller B, Weber U, Slepner J. Immuno-histological Analysis of Cytokine Expression in Human Osteoarthritic and Healthy Cartilage. *J Rheumatol* 1999;26:870–879. [PubMed: 10229409]
18. Haupt JL, Frisbie DD, McIlwraith CW, Robbins PD, Ghivizzini S, Evans CH, Nixon AJ. Dual Transduction of Insulin-Like Growth Factor-I and Interleukin-1 Receptor Antagonist Protein Controls Cartilage Degradation in an Osteoarthritic Culture Model. *J Orthop Res* 2005;23:118–126. [PubMed: 15607883]
19. Saha AK, Mazumdar JN, Kohles SS. Prediction of Growth Factor Effects on Engineered Cartilage Composition Using Deterministic and Stochastic Modeling. *Ann Biomed Eng* 2004;32:871–879. [PubMed: 15255217]
20. Saha AK, Mazumdar JN, Kohles SS. Dynamic Matrix Composition in Engineered Cartilage With Stochastic Supplementation of Growth Factors. *Australas Phys Eng Sci Med* 2005;28:97–104. [PubMed: 16060315]
21. Saha AK, Kohles SS. A Regulatory Cell-Matrix Interaction Model Describing Anabolic and Catabolic Influences on Cartilage Biomolecule Homeostasis. *Int J Comp Healthcare*. 2010 unpublished.
22. Saha AK, Kohles SS. A Distinct Catabolic to Anabolic Threshold Due to Single-Cell Static Nanomechanical Stimulation in a Cartilage Biokinetics Model. *Journal of Nanotechnology in Engineering and Medicine* 2010;1(3):031005.

23. Kohles SS, Wilson CG, Bonassar LJ. A Mechanical Composite Spheres Analysis of Engineered Cartilage Dynamics. *ASME J Biomech Eng* 2007;129:473–480.
24. Badylak SF. The Extracellular Matrix as a Scaffold for Tissue Engineering. *Semin Cell Dev Biol* 2002;13:377–383. [PubMed: 12324220]
25. Meaney Murray M, Rice K, Wright RJ, Spector M. The Effect of Selected Growth Factors on Human Anterior Cruciate Ligament Cell Interactions With a Three-Dimensional Collagen-GAG Scaffold. *J Orthop Res* 2003;21:238–244. [PubMed: 12568954]
26. Cuevas P, Burgos J, Baird A. Basic Fibroblast Growth Factor (FGF) Promotes Cartilage Repair In Vivo. *Biochem Biophys Res Commun* 1988;156:611–618. [PubMed: 3190672]
27. Pei M, Seidel J, Vunjak-Novakovic G, Freed LE. Growth Factors for Sequential Cellular De- and Re-Differentiation in Tissue Engineering. *Biochem Biophys Res Commun* 2002;294:149–154. [PubMed: 12054755]
28. Sah RL, Chen AC, Grodzinsky AJ, Trippel SB. Differential Effects of bFGF and IGF-1 on Matrix Metabolism in Calf and Adult Bovine Cartilage Explants. *Arch Biochem Biophys* 1994;308:137–147. [PubMed: 8311446]
29. Sah RL, Trippel SB, Grodzinsky AJ. Differential Effects of Serum Insulin-Like Growth Factor-1, and Fibroblast Growth Factor-2 on the Maintenance of Cartilage Physical Properties During Long Term Culture. *J Orthop Res* 1996;14:44–52. [PubMed: 8618165]
30. Afoke NYP, Byers PD, Hutton WC. Contact Pressures in the Human Hip Joint. *J Bone Jt Surg, Br* 1987;69-B:536–541.
31. Hodge WA, Carlson KL, Fijan RS, Burgess RG, Riley PO, Harris WH, Mann RW. Contact Pressures From an Instrumented Hip Endoprosthesis. *J Bone Jt Surg, Am* 1989;71(9):1378–1386.
32. Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB. Mechanical Compression Modulates Matrix Biosynthesis in Chondrocyte/Agarose Culture. *J Cell Sci* 1995;108:1497–1508. [PubMed: 7615670]
33. Ehrlich MG, Armstrong AL, Treadwell BV, Mankin HJ. The Role of Proteases in the Pathogenesis of Osteoarthritis. *J Rheumatol* 1987;14:30–32. [PubMed: 3040991]
34. Buckwalter JA, Martin JA, Brown TD. Perspectives on Chondrocyte Mechanobiology and Osteoarthritis. *Biorheology* 2006;43:603–609. [PubMed: 16912432]
35. Li J, Fu X, Sun X, Sun T, Sheng Z. The Interaction Between Epidermal Growth Factor and Metalloproteinase Induces the Development of Sweat Glands in Human Fetal Skin. *J Surg Res* 2002;106:258–263. [PubMed: 12175976]
36. Wilson ZD, Kohles SS. Two-Dimensional Modeling of Nanomechanical Strains in Healthy and Diseased Single-Cells During Microfluidic Stress Applications. *J Nanotechnol Eng Med* 2010;1(2):021005.
37. Freed LE, Hollander AP, Martin I, Barry JR, Langer R, Vunjak-Novakovic G. Chondrogenesis in a Cell-Polymer-Bioreactor System. *Exp Cell Res* 1998;240:58–65. [PubMed: 9570921]
38. Vunjak-Novakovic G, Freed LE, Biron RJ, Langer R. Effects of Mixing on the Composition and Morphology of Tissue Engineered Cartilage. *AIChE J* 1996;42:850–860.
39. Vunjak-Novakovic G, Obradovic B, Martin I, Bursac PM, Langer R, Freed LE. Dynamic Cell Seeding of Polymer Scaffolds for Cartilage Tissue Engineering. *Biotechnol Prog* 1998;14:193–202. [PubMed: 9548769]
40. Ramage L, Nuki G, Salter DM. Signalling Cascades in Mechanotransduction: Cell-Matrix Interactions and Mechanical Loading. *Scand J Med Sci Sports* 2009;19:457–469. [PubMed: 19538538]
41. Urban JP. The Chondrocyte—A Cell Under Pressure. *Br J Rheumatol* 1994;33:901–908. [PubMed: 7921748]
42. Li C, Wernig F, Leitges M, Hu Y, Xu Q. Mechanical Stress-Activated PKC δ Regulates Smooth Muscle Cell Migration. *FASEB J* 2003;17:2106–2108. [PubMed: 12958154]
43. Lammi MJ. Current Perspectives on Cartilage and Chondrocyte Mechanobiology. *Biorheology* 2004;41:593–596. [PubMed: 15299289]
44. Wheeler CA, Fitzgerald JB, Grodzinsky AJ. Cartilage Mechanobiology: The Response of Chondrocyte to Mechanical Force. *Curr Opin Orthop* 2005;16:346–353.

45. Peggian-Murphy B, Lee DA, Bader DL, Knight MM. Activation of Chondrocytes Calcium Signalling by Dynamic Compression is Independent of Number of Cycles. *Arch Biochem Biophys* 2005;444:45–51. [PubMed: 16289021]
46. Wright M, Jobanputra P, Bavington C, Salter DM, Nuki G. Effects of Intermittent Pressure-Induced Strain on the Electrophysiology of Cultured Human Chondrocytes: Evidence for the Presence of Stretch-Activated Membrane Ion Channels. *Clin Sci* 1996;90:61–71. [PubMed: 8697707]
47. Martinac B. Mechanosensitive Ion Channels: Molecules of Mechanotransduction. *J Cell Sci* 2004;117:2449–2460. [PubMed: 15159450]
48. Ingber D. Integrins as Mechanochemical Transducers. *Curr Opin Cell Biol* 1991;3:841–848. [PubMed: 1931084]
49. Kiviranta I, Jurvelin J, Tammi M, Säämänen AM, Helminen HJ. Weight Bearing Controls Glycosaminoglycan Concentration and Articular Cartilage Thickness in the Knee Joints of Young Beagle Dogs. *Arthritis Rheum* 1987;30:801–809. [PubMed: 3619962]
50. Helminen, HJ.; Kiviranta, I.; Säämänen, AM.; Jurvelin, JS.; Arokoski, J.; Oettmeier, R.; Abendroth, K.; Roth, AJ.; Tammi, MI. *Articular Cartilage and Osteoarthritis*. Kuettner, KE.; Hascall, VC.; Schleyerbach, R., editors. Raven; New York: 1992. p. 501-510.
51. Quinn TM, Grodzinsky AJ, Hunziker EB, Sandy JD. Effects of Injurious Compression on Matrix Turnover Around Individual Cells in Calf Articular Cartilage Explants. *J Orthop Res* 1998;16:490–499. [PubMed: 9747792]
52. Kim Y-J, Grodzinsky AJ, Plaas AHK. Compression of Cartilage Results in Differential Effects on Biosynthetic Pathways for Aggrecan Link Protein, and Hyaluronan. *Arch Biochem Biophys* 1996;328:331–340. [PubMed: 8645012]
53. Lee HS, Millward-Sadler SJ, Wright MO, Nuki G, Salter DM. Integrin and Mechanosensitive Ion Channel-Dependent Tyrosine Phosphorylation of Focal Adhesion Proteins and β -Catenin in Human Articular Chondrocytes After Mechanical Stimulation. *J Bone Miner Res* 2000;15:1501–1509. [PubMed: 10934648]
54. Millward-Sadler SJ, Wright MO, Davies LW, Nuki G, Salter DM. 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* 2000;43:2091–2099. [PubMed: 11014361]
55. Wu QQ, Chen Q. Mechanoregulation of Chondrocyte Proliferation, Maturation, and Hypertrophy: Ion-Channel Dependent Transduction of Matrix Deformation Signals. *Exp Cell Res* 2000;256:383–391. [PubMed: 10772811]
56. Fanning PJ, Emkey G, Smith RJ, Grodzinsky AJ, Szasz N, Trippel SB. Mechanical Regulation of Mitogen-Activated Protein Kinase Signaling in Articular Cartilage. *J Biol Chem* 2003;278:50940–50948. [PubMed: 12952976]
57. Murata M, Bonassar LJ, Wright M, Mankin HJ, Towle CA. A Role for the Interleukin-1 Receptor in the Pathway Linking Static Mechanical Compression to Decreased Proteoglycan Synthesis in Surface Articular Cartilage. *Arch Biochem Biophys* 2003;413:229–235. [PubMed: 12729621]
58. Szafranski JD, Grodzinsky AJ, Burger E, Gaschen V, Hung HH, Hunziker EB. Chondrocyte Mechanotransduction: Effects of Compression on Deformation of Intracellular Organelles and Relevance to Cellular Biosynthesis. *Osteoarthritis Cartilage* 2004;12:937–946. [PubMed: 15564060]
59. Fitzgerald JB, Jin M, Dean D, Wood DJ, Zheng MH, Grodzinsky AJ. Mechanical Compression of Cartilage Explants Induces Multiple Time Dependent Gene Expression Patterns and Involves Intracellular Calcium and Cyclic AMP. *J Biol Chem* 2004;279:19502–19511. [PubMed: 14960571]
60. Vincent TL, Hermansson MA, Hansen UN, Amis AA, Saklatvala J. Basic Fibroblast Growth Factor Mediates Transduction of Mechanical Signals When Articular Cartilage is Loaded. *Arthritis Rheum* 2004;50:526–533. [PubMed: 14872495]
61. Perkins GL, Derfoul A, Ast A, Hall DJ. An Inhibitor of the Stretch-Activated Cation Receptor Exerts a Potent Effect on Chondrocyte Phenotype. *Differentiation* 2005;73:199–211. [PubMed: 16026542]

62. Fitzgerald JB, Jim M, Grodzinsky AJ. Shear and Compression Differentially Regulate Clusters of Functionally Related Temporal Transcription Patterns in Cartilage Tissue. *J Biol Chem* 2006;281:24095–24103. [PubMed: 16782710]
63. Mouw JK, Imler SM, Levenston ME. Ion-Channel Regulation of Chondrocyte Matrix Synthesis in 3D Culture Under Static and Dynamic Compression. *Biomech Model Mechanobiol* 2007;6:33–41. [PubMed: 16767453]
64. Niehoff A, Offermann M, Dargel J, Schmidt A, Brüggemann GP, Bloch W. Dynamic and Static Mechanical Compression Affects Akt Phosphorylation in Porcine Patellofemoral Joint Cartilage. *J Orthop Res* 2008;26:616–623. [PubMed: 18050339]
65. Vincent TL, McLean CJ, Full LE, Peston D, Saklatvala J. FGF-2 is Bound to Perlecan in the Pericellular Matrix of Articular Cartilage, Where It Acts as a Chondrocyte Mechanotransducer. *Osteoarthritis Cartilage* 2007;15:752–763. [PubMed: 17368052]

Fig. 1.
The schematic network of growth factors and cytokines on a representative chondrocyte. Both growth factors and cytokines stimulate the chondrocyte positively, whereas the resulting effect acts on the ECM in two different pathways.

Fig. 2. ECM molecule accumulation when a periodic mechanical loading of 0.02 is applied at the 10,000th time step. The system is load-free from 0 to <10,000 time steps. Model parameters were derived from previous experiments [15].

Fig. 3. The phase-space dynamics of growth factors and cytokines (anabolic and catabolic actions) after an application of dynamic loading of 0.02 magnitude at the 10,000th time step. The limit cycle here moves clockwise and limits at an internal fixed point. This behavior can be construed as a “dump” oscillation.

Fig. 4. ECM molecule accumulation when an intermediate periodic mechanical loading level of 1.0 is applied at the 10,000th time step. Again, the system is free from loading up to the application of the mechanical stimulation.

Fig. 5. Phase-space dynamics of the growth factor and cytokine response (anabolic and catabolic balances) with a dynamic loading of 1.0 applied at the 10,000th time step. The limit cycle here also moves clockwise toward an internal fixed point. This also shows a dump oscillation although trending toward the steady state in a longer time frame. The system is moving toward a vanishingly small amplitude oscillation.

Fig. 6. ECM molecule accumulation when the highest healthy or maintenance mechanical load of 10.0 is applied at the 10,000th time step

Fig. 7.

The steady state balance between anabolic and catabolic pathways with dynamic loading of 10.0 applied after the 10,000th time step. The limit cycle still moves clockwise with a clear “back and forth” oscillation.

Fig. 8. ECM molecule accumulation when an “optimal” or growth periodic mechanical load of 86.2 is applied at the 10,000th time step when initially free from any loading

Fig. 9.
The steady state dynamics of anabolic and catabolic pathways with the dynamic load of 86.2 applied at the 10,000th time step. Again, the limit cycle here moves clockwise with a clear oscillation.

Fig. 10. An imbalanced or “catastrophic” response of the ECM molecule accumulations when a mechanical load greater than 86.2 was applied. Here, the steady state condition is never reached with dramatic ECM synthesis and loss exhibited.

Table 1

Mathematical relationships as developed previously for the biokinetic algorithm [21,22]

Parameter	Description	Relationship
α_1	Cytokine synthesis rate	$\alpha_1 = 0.048 + \beta_1$
α_2	Growth factor synthesis rate	$\alpha_2 = 0.044 + \beta_2$
β_1	Cytokine decay rate	$\beta_1 = \frac{1}{\tau_1} \left\{ \frac{\Omega_2 - G_{ss}}{G_{ss} + 1 - \Omega_2} \right\}$
β_2	Growth factor decay rate	$\beta_2 = \frac{1}{\tau_2} \left\{ \frac{C_{ss} - \Omega_1}{\Omega_1 - C_{ss} - 1} \right\}$
Ω_1	Growth factor pivotal parameter	$C_{ss} - 1 < \Omega_1 < C_{ss}$
Ω_2	Cytokine pivotal parameter	$G_{ss} < \Omega_2 < G_{ss} + 1$

Experimental input parameters developed from representative cartilage engineering scenarios [20]. Here, PGA is the polyglycolic acid scaffold, BAC is the bovine articular cartilage, τ_{ECM} is the matrix biomolecule characteristic time constants, dw is the dry weight, and ww is the wet weight.

Table 2

Scaffold	Cell type	Ref.	GAGss	$\tau_{GAG}(\text{day})$	Collagens	$\tau_{collagen}(\text{day})$	α_{GAG} β_{GAG}	$\alpha_{collagen}$ $\beta_{collagen}$
PGA	BAC	[37]	6.8% ww	31.3	3.7% ww	15.9	3.256×10^{-2} 6.139×10^{-4}	6.672×10^{-2} 3.837×10^{-3}
PGA	BAC	[38]	11.7% dw	18.6	50.4% dw	131.5	5.441×10^{-2} 3.662×10^{-4}	7.607×10^{-3} 2.937×10^{-6}
PGA	BAC	[15]	6.1% dw	187	6.5% dw	18.9	5.474×10^{-3} 1.264×10^{-4}	5.400×10^{-2} 1.108×10^{-3}
PGA	BAC	[39]	1.6% ww	22.2	2.4% ww	16.7	5.900×10^{-2} 1.400×10^{-2}	6.800×10^{-2} 8.363×10^{-3}