8 Mechanical Articular Cartilage Injury Models and Their Relevance in Advancing Therapeutic Strategies

Bodo Kurz, Melanie L. Hart, and Bernd Rolauffs

Abstract

This chapter details how Alan Grodzinsky and his team unraveled the complex electromechanobiological structure-function relationships of articular cartilage and used these insights to develop an impressively versatile shear and compression model. In this context, this chapter focuses (i) on the effects of mechanical compressive injury on multiple articular cartilage properties for (ii) better understanding the molecular concept of mechanical injury, by studying gene expression, signal transduction and the release of potential injury biomarkers. Furthermore, we detail how (iii) this was used to combine mechanical injury with cytokine exposure or co-culture systems for generating a more realistic trauma model to (iv) investigate the therapeutic modulation of the injurious response of articular cartilage. Impressively, Alan Grodzinsky's research has been and will remain to be instrumental in understanding the proinflammatory response to injury and in developing effective therapies that are based on an in-depth understanding of complex structure-function relationships that underlay articular cartilage function and degeneration.

Keywords

Cartilage · Injury · Compression · Structure-function

8.1 Introduction

As a tribute to the tremendously important work of Grodzinsky and colleagues in the context of mechanical articular cartilage injury models, the

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B. K. Connizzo et al. (eds.), *Electromechanobiology of Cartilage and Osteoarthritis*, Advances in Experimental Medicine and Biology 1402, https://doi.org/10.1007/978-3-031-25588-5_8

following text sections detail how Grodzinsky and colleagues have set out to unravel the complex and, at that time, unknown electrokinetic, biomechanical and biosynthetic characteristics of articular cartilage, implementing the cartilage injury machine as the go-to model to develop structure-function relationships. Over time, this led to model-based insights and an in-depth understanding of mechanical injury mechanisms and therapeutic strategies with fundamental clinical relevance.

8.2 From Electromechanobiological Structure-Function Relationships to Developing a Versatile Shear and Compression Model for Understanding the Injurious Response of Articular Cartilage

8.2.1 Unraveling Central Electrokinetic and Biomechanical Properties of Articular Cartilage – The Basis for Understanding Tissue Failure Under Injurious Compressive Loads

In earlier works, which began in the 1980s and preceded the arrival of the worldwide famous cartilage "injury machine", Grodzinsky and colleagues examined the compressive stiffness of articular cartilage in oscillatory (sinusoidal) confined compression over a wide frequency range including high frequencies relevant to impact loading. Interestingly, the currently well-established non-linear behavior of cartilage under load was initially found in this early study, which related this non-linear behavior of cartilage to a compression amplitude that exceeds a threshold value, which, in turn, is frequency-dependent. For linear viscoelastic behavior, stiffness defined in the usual sense was shown to depend on ionic strength and proteoglycan content, as well as the electrostatic forces between matrix charge groups over a frequency range of 0.001 to 20 Hz. Extending these findings, Grodzinsky and colleagues used the observed sinusoidal streaming potentials generated by oscillatory compression to relate the streaming potential field to the fluid velocity field [1]. These studies showed that interstitial fluid flow is significant to cartilage behavior over this entire frequency range.

Based on the knowledge that oscillatory compression of cartilage using physiological loads produces electrical potentials resulting from an electrokinetic streaming transduction mechanism, Grodzinsky and Frank reported in two parallel studies two electromechanical phenomena, namely, 'streaming current' and 'current-generated stress' [2], and subsequently formulated a continuum model for linear electrokinetic transduction in cartilage [3]. In another study, Grodzinsky and colleagues developed an electromechanical model that focused on ionic transport as the rate limiting step in chemically modulating electrical interactions between the charged macromolecules of the extracellular matrix (ECM). This aided in predicting the kinetics of changes in swelling and isometric compressive stress that occur in charged, hydrated tissues, including articular cartilage and corneal stroma, due to changes in salt concentration [4]. Not surprisingly, Grodzinsky and colleagues further advanced this topic and revealed that the modulation of ^{3}H-proline (collagen synthesis marker) incorporation by both loading and load release is faster than that of ^{35}S-sulfate (sulfated glycosaminoglycans (sGAG) synthesis marker) incorporation, and that the response to dynamic loading is not determined simply by the time average component of the dynamic load, as the response to unloading is not just the inverse of the response to loading and is characterized by an overshooting response [5]. Subsequently, this team developed an organ culture system to study the effects of static compression and physico-chemical changes [6]. Subjecting cartilage explants from the epiphyseal plate of 1 to 2-week-old calves to static compressive stresses of 0–3 MPa in unconfined compression, the Grodzinsky team demonstrated, as it is well-known today, that the ^{3}H-proline and ^{35}S-sulfate incorporation

decreases monotonically with increasing stress, which suggested in conjunction with later studies the beneficial, regenerative effects of dynamic compression over static compression. Perhaps less known today is that this study also demonstrated that ^{3}H-proline and ^{35}S-sulfate incorporation independently of mechanical compression strongly depended on pH, but was independent of $SO4^{2-}$ and K^{+} in the range studied, suggesting that compression-induced changes in local, interstitial pH may contribute to the biosynthetic response to static compression.

Using atomic force microscopy (AFM), Grodzinsky and colleagues in 2015 investigated the dynamic nanomechanical properties of murine cartilage over a wide frequency range of 1 Hz to 10 kHz [7]. Specifically, they studied the role of GAGs on the dynamic modulus and poroelastic properties of murine femoral cartilage by inducing GAG deletion. Interestingly, this study showed that poroelastic (i.e., fluid-flow-dependent) properties such as the hydraulic permeability, which is related to the resistance of the ECM matrix to fluid flow, and the high frequency modulus, which is related to fluid pressurization and the fibrillar network of the ECM, are more sensitive indicators of GAG loss induced by loss of mechanical function, compared to the equilibrium properties in which fluid flow is negligible. From this work, a fibril-reinforced finite element model was developed to estimate the poroelastic properties of mouse cartilage over a wide range of loading rates, which may be useful for understanding early cartilage aggrecan degradation relevant to mouse models of OA.

8.2.2 The Invention of a Successful In Vitro Cartilage Injury Model

In 1989, Grodzinsky, Robert Sah and colleagues designed two culture chambers for the uniaxial radially unconfined compression and mechanical testing of live cartilage explants [8]. They used one chamber inside a standard incubator and equipped the other chamber with a mechanical spectrometer to record load and displacement during compression. To the best of the knowledge of the authors, this design represents the initial prototype of Alan Grodzinsky's so-called cartilage "injury machine", which contributed to, and to no small extent, the overall understanding of tissue and cellular responses to compressive injury.

In the beginning, the focus was not injury per se. The authors used dynamic stiffness measurements of cartilage explants cut into standardized 3-mm diameter explants and identified a characteristic frequency of 0.001 Hz (cycles/s) that separated low- and high-frequency regimes [8]. At 0.0001–0.001 Hz, significant fluid was exuded from the explants, but at a frequency range of 0.01–1 Hz, the hydrostatic fluid pressure increased within explants, illustrating a frequency-dependent flow and deformation phenomena. Although the authors reported deformation of chondrocytes and matrix at all frequencies, this important early study demonstrated differential effects on dynamic compression on chondrocyte biosynthesis. Interestingly, the currently well-known effects of dynamic compression of stimulating cellular biosynthesis were shown to be present at the higher frequencies even at relatively low amplitudes of 1–5% with ^{3}H-proline and ^{35}S-sulfate incorporation increasing by ~20% and ~40%, respectively, with tissue volume remaining almost constant. In contrast, at lower frequencies of <0.001 Hz, low amplitudes of 1–5% had negligible effects and higher amplitudes were needed to induce increased biosynthesis with collagen (^{3}H-proline) exceeding sGAG (^{35}S-sulfate) incorporation. These insights are today perhaps even more relevant than they were at publication in 1989, as a rapidly growing body of literature documents the fundamental importance of biomechanical forces from the nanometer to the macroscopic scales. From today's perspective, another exciting point is that the authors noted that the reported *in vitro* findings were in general agreement with the *in vivo* studies on joint loading and motion of that time, which helped establish that *in vitro* studies on cartilage compression might aid in testing and optimizing therapeutic strategies to combat diseases of cartilage [8]. In a subsequent study, Grodzinsky and colleagues reported on the

effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants [9]. Interestingly, they demonstrated, to the best of our knowledge for the first time, that high amplitude dynamic cyclic compression (20%, 40%, and 60%) at a slow frequency (2 h of compression and 2 h of release for 24 h) induced convective fluid flow, which thereby enhanced the loss of ^{35}S- and ^{3}H-labeled macromolecules (sGAG and collagen) from the tissue into medium. In contrast, prolonged static compression induced matrix consolidation, which hindered the diffusional transport and loss of sGAG and collagen macromolecules. Thus, both early studies [8, 9] together demonstrated that the effects of dynamic compression on induced biosynthesis vs. ECM component loss from the tissue are subject to complex time-, frequency, and amplitude-dependent effects, and, importantly, that higher frequencies of 0.01–1 Hz even at low amplitudes of 1–5% induce anabolic, biosynthetic effects in articular cartilage tissue.

In the late 1990s, Grodzinsky and colleagues focused on the metabolic effects of mechanical injury, as those were and continue to be relevant to the development of strategies for cartilage repair [10, 11]. In healthy tissue, matrix deposition and turnover were spatially dependent, with the highest rates of proteoglycan deposition, turnover and the lowest rates of collagen deposition (^{3}H-proline autoradiography) occurring in the pericellular matrix. Interestingly, many of the well-known effects of injurious compression today were already reported in these studies. Hence mechanical injury of calf explants resulted in macroscopic tissue damage, led to mechanical failure, a subtotal decrease in cell viability with the emergence of an apparently inactive cell population but also containing catabolically active, abnormally large cells, and sustained, elevated rates of proteoglycan turnover in the cell-associated matrices of viable cells. The authors also formulated the idea of using the mechanical injury model as an *in vitro* model for understanding the responses of chondrocytes and the cartilage extracellular matrix to mechanical injury, which led to a range of studies using the well-known cartilage "injury machine", which was further developed and described by EH Frank et al. [12], as described below.

8.2.3 The Effects of Mechanical Compressive Injury on Articular Cartilage Biomechanics, Metabolic Behavior and Cell Viability and Their Strain-, Strain Rate- and Peak Stress-Dependency

Based on the initial studies by Sah et al. [8, 9] and Quinn et al. [10, 11], the impact of injurious compression on relevant parameters of articular cartilage integrity were studied in more detail by Grodzinsky and colleagues, using the injury machine, a specially designed computer-controlled and incubator-housed shear- and compression-device, described in [12]. In the original setup, cartilage disks of 3 mm diameter and approximately 1 mm thickness (obtained from the femoropatellar groove of 1–2 week old calves) were held between impermeable platens in an unconfined culture medium-filled chamber (Fig. 8.1). Uniaxial movement or rotation of the upper platen induced either compression or shear forces to the tissue, with displacement and load being monitored and controlled by the software.

The nature of injury-related cell death was of interest since programmed cell death might be a target for therapeutic approaches and repair mechanisms. Grodzinsky's group used an injurious compression protocol that consisted of six repetitive on/off cycles of displacement-controlled strain, ranging from 30–50%, applied at a strain rate of 1000 mm/s (=1/s). They reported that injury-induced apoptosis is maximal by 24 hours after injury and occurs at peak stresses as low as 4.5 MPa and increases dose-dependently with injurious peak stress. Moreover, a peak stress-dependent increase in tissue swelling, which was significant at 13 MPa, and GAG release, which was significant from 6 to 13 MPa peak stress, together with a decreased equilibrium and dynamic tissue stiffness, which was significant at 12 and 7 MPa peak stress, suggested

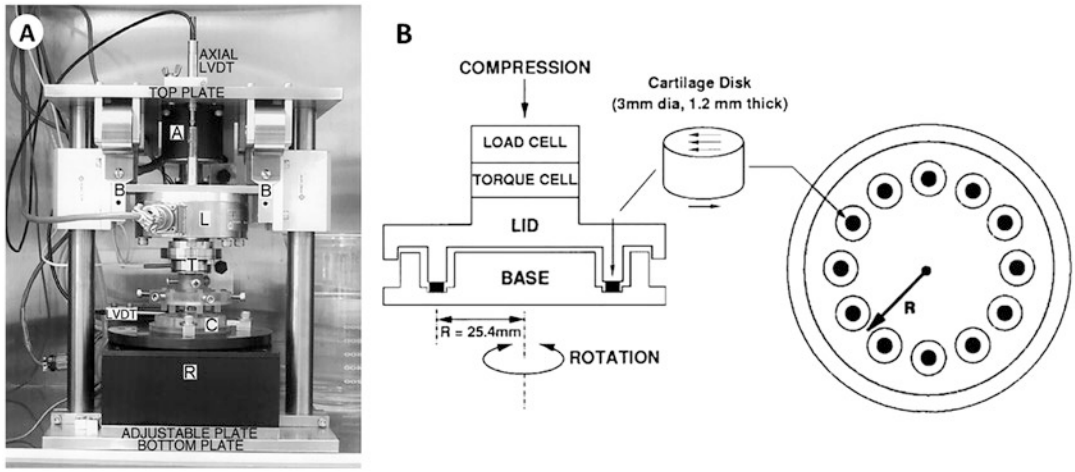

Fig. 8.1 A versatile shear and compression apparatus design by Grodzinsky and colleagues. Frank et al. described in 2000 [12], in detail, the setup of the shear/compression apparatus, which was used for the majority of the injury studies described in this chapter. Fig. **a** (left): Image of the incubator-housed loading device. An axial linear stepper motor (A) in a bearing/carriage assembly (B) applies axial compression to tissue explants located in a sample chamber (C), which is positioned on a rotary position table (R; driven by stepper motor behind the table) for application of shear forces. Load and shear stress are measured by a load (L) and torque cell (T). The adjustable plate may be moved to accommodate other fixtures. The "Linear Variable Differential Transformer" (LVDT), an electromechanical transducer that converts its displacement into a corresponding electrical signal, is placed on the left of the sample chamber (C). Fig. **b** (right): Design of the autoclavable polysulphone sample chamber with a lid and base. Cartilage explants are placed in medium-filled wells in the chamber base. The platens of the nonrotating lid compress the cartilage and rotation of the base induces shear stress to the cartilage disks/explants. The design of the sample chamber allows stimulation of up to 12 explants and single explant chambers were also designed (not shown). (Figs. **a** and **b** are reprinted from [12] with permission from Elsevier)

damage to or degradation of the collagen fibril network as well as GAG release in this range of peak stresses [13]. Thus, the peak stresses causing matrix damage and degradation were higher than those that induced apoptosis. Cell death was further investigated using a single impact of compression. While TUNEL-positive cell rates increased from 7% in unloaded controls to 33% after injury, in electron microscopy (EM) data the apoptosis rate increased from 5% in unloaded controls to 62% in injured cartilage and proved that the dead cells in injured tissue were 97% apoptotic based on cellular morphology [14].

Kurz et al. [15] investigated the effects of strain rate on cell viability, cartilage matrix biosynthesis and mechanical properties after 50% strain using a single injurious compression. A strain rate of 0.01/s resulted in no measured effect on the cells or on the ECM, although peak stresses reached levels of about 12 MPa, whereas faster strain rates of 0.1 and 1/s induced peak stresses of ~18 and ~24 MPa, increased cell death, and significantly decreased both proteoglycan and total protein biosynthesis. Comparably, increasing strain rate was associated with impaired mechanical properties and the remaining viable cells had lost their ability to have their biosynthesis stimulated by low-amplitude sinusoidal compression, suggesting an impaired reparative capability of the surviving population, in agreement with the emergence of an apparently inactive cell population in Quinn et al. [10], discussed above. This clinically relevant inability to exhibit a reparative response to dynamic compressive stimulation was most extensive after injury was applied with the highest strain rates suggesting that strain rate as well as peak stress, or strain are important parameters that define the post-injurious fate of injured cartilage.

Grodzinsky and colleagues then investigated the relationship between injurious peak stress and post-injurious proteoglycan loss in bovine

cartilage, and also in human knee and ankle cartilage. In bovine cartilage, the injury-related GAG release was highest during the first 4 h after injury, but remained higher than that in controls during the first 24 hours post-injury [16]. For experiments on human knee and ankle cartilage with no history of OA, the team applied a uniaxial unconfined injurious compression of 65% strain at 2/s, which was quicker than the bovine injurious compression model. Increased injurious peak stress (at a constant final strain and compression rate) was associated with less proteoglycan loss after injury [17], corroborating studies on bovine articular cartilage [13, 16]. When injured, fewer human ankle vs. knee cartilage explants suffered macroscopic damage and neither a post-injurious increase in proteoglycan loss from injured ankle cartilage relative to controls nor a relationship between peak stress and proteoglycan loss was observed as opposed to knee cartilage explants. Besides uncovering differences in the response of human knee and ankle cartilage to injury, this study suggested that peak stress itself did not appear to be an important cause of proteoglycan loss from human cartilage.

8.2.4 Understanding the Molecular Concept of Mechanical Injury by Studying Gene Expression, Signal Transduction and the Release of Potential Injury Biomarkers

Several studies of Grodzinsky and colleagues have used the injury model to investigate signaling pathways and gene expression patterns after mechanical overload. The first demonstrated that the angiogenesis factor VEGF (vascular endothelial growth factor) might play an important autocrine or paracrine role in the progression of post-traumatic OA (PTOA) [18]. Mechanical injury induced the expression of the transcription factor hypoxia-inducible factor-1 (HIF-1), a known promoter of VEGF expression. The subsequent expression of VEGF activated autocrine production of MMPs (MMP-1, -3 and -13) in chondrocytes, whereas tissue inhibitor of metalloproteinases-1 and -2 (TIMP-1 and -2), the inhibitors of MMPs, were reduced. Motivated by these interesting results, a more detailed study of injury-related gene expression followed [19]. mRNA levels in non-injured, free swelling bovine cartilage varied over five orders of magnitude with matrix molecules being the most highly expressed, while cytokines, MMPs (except MMP-3), aggrecanases (ADAMTS-5), and transcription factors showed lower expression levels. Specifically, the matrix molecules fibronectin and type I collagen, as well as TNF, GAPDH, and β-actin and finally IGF-1, IGF-2, and ADAMTS-4 as well as type II collagen, aggrecan, fibromodulin, link protein, and IL-1 showed little change in expression after injury vs. non-injured cartilage, whereas MMP-3 increased 250-fold, ADAMTS-5 increased 40-fold, and TIMP-1 increased 12-fold. The MMP-activating transcription factors c-fos and c-jun showed an immediate transient upregulation followed by a rapid decline within hours and a slowly increasing expression pattern was seen for most other MMPs and their inhibitors [19].

Two other studies on bovine cartilage characterized proteins lost to the medium from cartilage explant cultures after either injurious mechanical compression or treatment with IL-1β or TNFα, using mass spectrometry [20, 21]. While cytokines predominantly promoted the release of proteins that are involved in inflammation and a stress response including acute-phase and complement proteins, injury caused the release of intracellular proteins, including Grp58, Grp78, 4-actinin, pyruvate kinase, and vimentin and also caused increased release and evidence of proteolysis of type VI collagen subunits, cartilage oligomeric matrix protein, and fibronectin. These data suggested loss of cartilage integrity such as matrix damage primarily of the pericellular matrix (PCM), supporting the idea of a high turnover in the PCM or increased damage to the PCM with injury. The data also suggested cell membrane disruption, which could be responsible for reported decreases in tissue compression and shear stiffness or cell apoptosis, changes in gene expression, or for the decrease in the ability of

the remaining viable cells to up-regulate biosynthesis in response to anabolic loading as described in the above section. Although MMP-2 appeared to decrease overall in that study, mechanical injury but not cytokines increased the release of MMP-14 (MT1-MMP) and TIMP-2, which are known to interact together to activate pro-MMP-2, and many of the proteins identified as being increased in the medium in that study are in fact substrates of MMP-2 including osteopontin, galectin 1, HSP-90, and CTGF, all of which are shown to be elevated with injury or cytokine treatment. Therefore, the authors suggest a possible role for MMP-2 in overall regulation of cell surface-associated molecules in cartilage. Of the aggrecanases, only a single ADAMTS-4 peptide was identified likely because of the enzymes ADAMTS-4 and -5 being present at a very low concentration. An observed decrease in the release of C-terminal telopeptides of several collagen types following both cytokine- and injury-treatment was interpreted as decreased collagen synthesis. Another study used a targeted proteomics approach to follow the progression of matrix degradation in response to mechanical damage and cytokine treatment of human knee cartilage explants in order to study the kinetics of cartilage degradation (IL-6 and TNFα). They identified candidate proteases, including MMP-1, MMP-3, MMP-10 and MMP-13, and the absence of collagen pro-peptides and elevated levels of specific cartilage oligomeric matrix protein (COMP) and COL3A1 neo-epitopes as potential biomarkers for the earliest events in PTOA [22]. Together these studies show the differential effects of cytokines vs mechanical damage on pro-inflammatory and stress-related vs. damage-associated protein release.

8.2.5 Elucidating the Zonal, Age and Species-Dependency of Injurious Compression

Next to the impact of strain, strain rate and peak stress as described above, Grodzinsky and our two groups investigated the zonal dependence of biomechanical, biochemical, and matrix-associated changes caused by compressive injury [23]. Our teams biomechanically characterized, injured (strain: 50%, strain rate 1/s) and re-characterized cartilage explants from the superficial and deeper zones of bovine calves. Having added histology, diffraction-enhanced x-ray imaging, and texture analysis to biochemical and biomechanical methods, the study elucidated that injured superficial zone explants showed surface disruption, compaction, and importantly, immediate biomechanical impairment after injury, whereas injured deeper zone explants showed collagen crimping but remained undamaged and biomechanically intact. Moreover, superficial zone explants that appeared intact on histology exhibited textural alterations, whereas deeper zone explants showed collagen crimping but were otherwise histologically and biomechanically intact. Overall this showed that the softer superficial zone was more vulnerable to compressive injury than the deeper zones, which, in conjunction with delayed superficial proteoglycan loss, may predispose the injured articular surface to further softening and tissue damage, thus increasing the risk of development of PTOA.

In another study our groups injured bovine cartilage explants with or without the superficial zone being present. Neither the peak stresses during compression nor the rate of apoptotic cell death specifically in deeper zones were significantly different in the two groups. It was speculated that the superficial zone might be too thin and soft, and that its relative contribution to the effects measured on the total tissue in a full area-loaded and unconfined 50% compression model are negligible. However, explants with an intact superficial zone showed a different macroscopic appearance, with the lower ends showing larger swelling laterally than the upper end of the explants, probably due to the fact that superficially the fibrils are oriented parallel to the platen which may stabilize the integrity of that particular side of the explant. However, the overall release of GAG was up to five-fold lower in explants containing the superficial zone [24]. On a side note, the superficial zone harbors the majority of chondrocytes, which suggests a significant role of the superficial zone in mediating

post-injurious effects related to the tissue's cells. Another study by Grodzinsky and colleagues investigated injured superficial zone tissue alone in comparison to tissue from deeper cartilage layers and found increased lubricin biosynthesis to be an early transient response of the superficial layer of cartilage, whereas the deeper layers exhibited reduced expression after injury. Histologic and immunohistochemical analyses revealed that superficial zone explants exhibited marked cellular depletion and displayed an amorphous/swollen surface architecture with diminished GAG and collagen content after injury, whereas deeper zone explants, injured without the superficial layer, displayed some loss in GAG and collagen content, but the effect was not as prominent as for the superficial tissue alone [25]. Together these studies demonstrate a significant role of the superficial zone in mediating the effects of injury.

Together with our groups Grodzinsky also investigated age and maturation of the articular cartilage as a factor of the injurious response by using tissue from newborn calves compared to cartilage from more mature animals [26, 27]. Injurious compression induced significantly more apoptosis in newborn calves (22% of cells) than in cartilage from adult cows (2–6%), and there was less GAG loss and no significant reduction in ^{3}H-proline and ^{35}S-sulfate incorporation in cartilage from 2-year-old animals in contrast to the data from Kurz et al. [15], where a single compression induced significant GAG loss and reduction in biosynthetic activity in tissue from 2-week-old animals suggesting that immature cartilage tissue might be more vulnerable to matrix destruction after cartilage injury, which could be of clinical importance, since joint injuries in the younger, more active population are increasing. Since load stresses during compression increase with maturation of the tissue (a single axial compression of strain of 50% with a strain rate of 1 s induces mean peak stresses of 17–23 MPa in newborn tissue vs. 25 MPa in younger (6–16-month-old) tissue vs. approximately 29 MPa in 22–23-month-old tissue [26]), peak stresses do not seem to be responsible for the maturation-dependent differences in tissue response to injury, since most parameters of tissue damage increase with increasing peak stress in general.

Grodzinsky and colleagues also demonstrated a species dependency of the effects of injury by transferring the bovine *in vitro* model, whose parameters were at that time well established, to tissues of human [28] or horses [29]. The team screened specimens cultured for 28 days with subsequent histological analysis [29]. At a strain rate of 1/s the threshold strain necessary for inducing morphological and biochemical ECM changes was 60% and, thus, higher than in bovine cartilage. Patwari et al. [28] needed a uniaxial unconfined injurious compression of 65% strain at 4/s in human knee and ankle cartilage in order to induce comparable tissue damage. Both studies demonstrate that the established injury model is applicable to different species but that the strain, strain rate and peak stress leading to "injured" cartilage is species-dependent.

8.2.6 Combining Mechanical Injury with Cytokine Exposure or Co-culture Systems for Generating a More Realistic Trauma Model

A further study of Grodzinsky and colleagues investigated the effects of injury alone vs. in combination with IL-1α or TNFα on the amount of proteoglycan loss using newborn bovine as well as matched knee and ankle tissues from adult healthy human donors. The team demonstrated that in bovine cartilage MMP-3 but not MMP-13 mRNA levels increased. The proteoglycan loss, which was at that time well-known to occur after injury, was significantly increased, although its extent of only 2% of the total content and loss only over the first 3 days following injury was surely surprising. Importantly, the combination of injury with either IL-1α (1 ng/ml) or TNFα (100 ng/ml) caused, during the same time frame, substantial increases of 35% and 54% in proteoglycan loss. In human knee cartilage, comparable interactions between cytokine and injury effects were observed after injury but

with lower magnitude than in bovine cartilage. Consistent with current knowledge, there was no significant interaction between injury and IL-1α in human ankle cartilage [28]. Overall, incorporating cytokines into the *in vitro* mechanical injury model was successful and helpful for studying the interactions between mechanical forces and pro-inflammatory cytokines that may be persistently present after joint trauma, adding insight into subsequent degradative pathways of PTOA progression.

A further study demonstrated that interactions between injured cartilage and other joint tissues are important in matrix catabolism and gain more complexity into the system [30]. The authors found that mechanically injured cartilage co-cultured with the joint capsule tissue alters chondrocyte expression patterns and increases ADAMTS-5 production and subsequent GAG loss. In a related study Swärd et al. [31] found additional aggrecan fragment types released at an earlier time after injury when synovial joint tissue was present, indicative of different proteolytic pathways for aggrecan degradation under co-culture conditions, with increased aggrecanase and MMP activity toward aggrecan. On the other hand, Lee et al. [29] demonstrated that synoviocytes protect cartilage from the effects of injury *in vitro* under certain circumstances. Thus, synoviocytes extracted from normal equine synovium exerted both positive and negative effects on injured equine cartilage, but ultimately protected injured cartilage from progressing toward an OA phenotype. Co-culture of synoviocytes and injured cartilage significantly reduced the expression of ADAMTS-4 and -5, but also increased the expression of MMP-1 and reduced the expression of TIMP-1 in synoviocytes. In contrast, injured cartilage cultured with synoviocytes increased the expression of both collagen type 2 and ADAMTS-5. Moreover, an additional protective effect of synoviocytes on injured cartilage was the reduction of both focal cell loss and chondrocyte cluster formation, two major hallmarks of OA. This is supported by an early study by Kurz et al. [32] showing that articular chondrocytes are protected against the negative effects of reactive oxygen species-induced cytotoxicity and lipid peroxidation under co-culture conditions with synoviocytes indicating that more research is needed to understand the interaction between different joint cell types.

8.2.7 Predicting Articular Cartilage Properties and Injurious Damage on the Structural, Biochemical and Biomechanical Level

Throughout the years Grodzinsky and colleagues have developed several models for predicting the properties and injurious damage of articular cartilage on the structural, biochemical as well as biomechanical level**.** This began as early as 1987, as briefly discussed above, when Grodzinsky and colleagues developed an electromechanical model for predicting the kinetics of changes in swelling and isometric compressive stress that can be induced by changes in salt concentration in charged, hydrated tissues [4]. In 2015, Grodzinsky and colleagues developed a fibril-reinforced finite element model to estimate the poroelastic properties of mouse cartilage over a wide range of loading rates [7]. In 2013, Grodzinsky and our two groups demonstrated that biomechanical stress, which occurs during compressive injury, predetermines the biomechanical, biochemical, and structural consequences of articular cartilage as well as the structural and functional damage that occurs when the tissue fractures [33]. Interestingly, damage prediction in a blinded experiment using stress-vs-time grades was 100% correct and also sensitive enough to differentiate the complexity of cartilage matrix disruptions. Moreover, the injuriously dissipated energy and the maximum stress rise during injury correlated with the extent of biomechanical and biochemical damage in zonal analyses. Thus, we introduced a novel method based on the interpretation of compressive yielding for accurately predicting the extent of structural damage during injury [33].

In 2018, Orozco et al. [34] investigated the fixed charge density of proteoglycans in injured immature cartilage and subsequently dynami-

cally compressed cartilage for up to 12 days to induce biosynthesis. Based on these data they introduced a novel model that implemented deviatoric and maximum shear strain and also fluid velocity-controlled algorithms with the goal of simulating the loss of the fixed charge density of proteoglycans over time. Interestingly, the homogeneity and localization of the predicted loss of the fixed charge density depended on the degeneration algorithm being driven by fluid velocity vs. shear strain. Using a novel finite element model that incorporates (1) diffusion of the pro-inflammatory cytokine IL-1 into tissue, and (2) the effect of excessive levels of shear strain near chondral defects during physiologically relevant loading, Grodzinsky and colleagues developed this further to a computational model which simulates spatial and temporal changes of fixed charge densities in injured cartilage in order to predict the simultaneous effect of tissue inflammation and abnormal biomechanical loading on loss of cartilage proteoglycans [35]. Their data suggests that the presence of lesions plays a role in cytokine diffusion-driven degradation and also predisposes cartilage for further biomechanical degradation. These models are promising *in silico* tools for predicting disease progression, recognizing lesions at high risk, simulating treatments, and ultimately optimizing treatments to postpone the development of PTOA.

8.3 Therapeutic Modulation of the Injurious Response

Throughout the years, Grodzinsky and colleagues extended the mechanical articular cartilage injury model to test a spectrum of disease-modifying agents, which will be discussed in detail below, and have proven that an array of therapeutics can protect against injury-related responses (dexamethasone, IL-10, IGF-1, MnTMPyP antioxidant MnTMPyP, E2 estrogen, and 10–20% dynamic compressive loading) and sometimes even promote a pro-regenerative response to injury or inflammatory insult of healthy cartilage (dexamethasone, IL-10), OA-injured cartilage (IL-10) and chondrocyte-containing collagen ACI grafts (IL-10, BMP-2). Moreover, Grodzinsky and associates developed charged-nanoscale sized carrier systems to efficiently transport therapeutics (dexamethasone or IGF-1) into the cartilage, offering cartilage-targeting therapies. Some of the therapeutics advanced to clinical testing such as dexamethasone in prevention of PTOA (ClinicalTrials.gov Identifier: NCT02318433). These promising targets remain at the horizon of advancing cartilage injury-related therapeutic strategies and could pave the way forward for the development of clinical therapies that will enhance the repair of cartilage after injury (Fig. 8.2).

8.3.1 Dexamethasone and 17b-Estradiol – Steroid Hormone Treatment of Mechanically-Injured Articular Cartilage and in an In Vivo PTOA Model Lead to Clinical Assessment

A large body of work by Grodzinksy and colleagues has focused on use of dexamethasone, a corticosteroid used to treat a wide-spectrum of conditions, in preventing degenerative responses in cartilage and the onset of PTOA after injury [36–45]. In both healthy human and bovine cartilage explant mechanical injury models of injury alone or in combination with subsequent inflammatory (TNF-α alone or in combination with IL-6 and sIL-6R) insult, continuous dexamethasone treatment inhibited the production of pro-inflammatory cytokines, MMPs and nitric oxide (NO), prevented GAG loss, reduced the release of aggrecan and COMP fragments, and promoted proteoglycan synthesis [36, 38, 39, 44] demonstrating that dexamethasone protects against injury-related changes. Moreover, in non-injured IL-α stimulated bovine cartilage, dexamethasone significantly increased the mRNA expression of ACAN and COL2A1 and decreased IL-6, caspase-3, ADAMTS-4, MMP-3 and -13, and COX2 4 days after treatment [37]. These studies show that dexamethasone provides protection against not only injury-related effects but

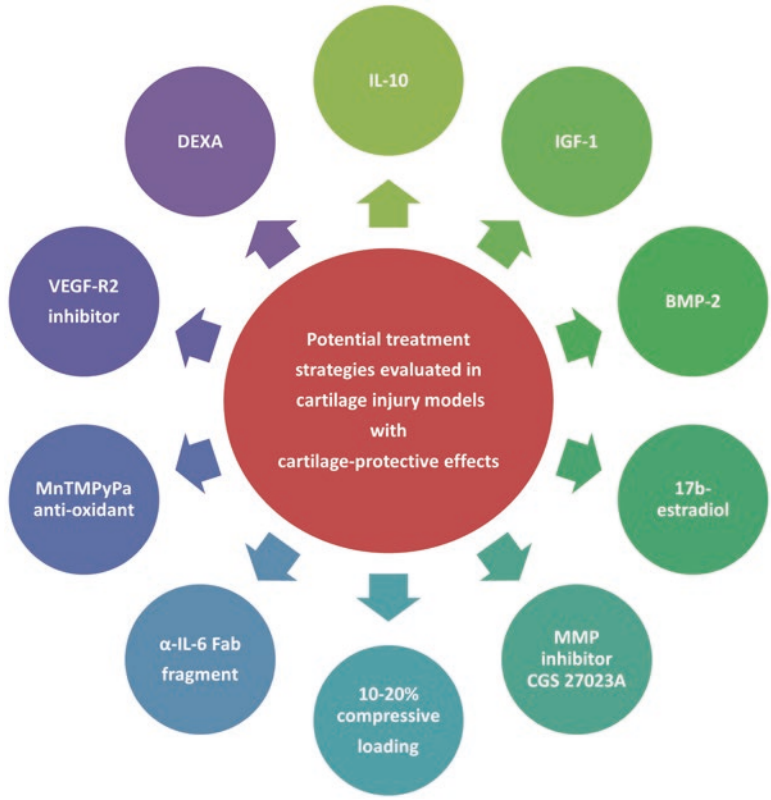

Fig. 8.2 Summary of the potential treatment strategies investigated by Grodzinsky and colleagues in cartilage injury models with cartilage-protective effects

also pro-inflammatory cytokines that may be persistently present after joint trauma.

While these studies clearly show that dexamethasone is protective against injury-related trauma and that dexamethasone could be a potential treatment to regulate many early cartilage degradative changes associated with joint injury, as reviewed by Grodzinsky and Black [46], some studies suggest that dexamethasone may have catabolic effects on the cartilage tissue by promoting apoptosis and reducing proliferation of healthy chondrocytes. However, these effects have been attributed to high doses or non-localized long-term treatment. Adverse effects have also associated with long-term systemic dexamethasone use, including stunting the growth of developing cartilage and bone and causing bone density loss thereby decreasing load potential. Therefore, Grodzinsky and other groups started to engineer biomaterial-based strategies to improve and extend the residence time of dexamethasone by preventing its joint clearance and allowing penetration of the cartilage as a means of delivering a low dose and more localized treatment strategy [43]. One such strategy developed by Grodzinsky and colleagues involved covalently linking a low dose of dexamethasone to the small, highly cationic molecule avidin. Due to avidin's net charge (+20), electrostatic interactions between the cationic avidin and anionic cartilage allow dexamethasone-nanosized carriers [45] to penetrate the full depth of the cartilage within 24 hours of application. Moreover, within thicker cartilage explants such as rabbits, as opposed to thinner rat cartilage, which better resembles the human cartilage thickness, the dexamethasone-carriers were retained within the cartilage tissue for up to 3 weeks offering a prolonged intra-articular localized treatment strategy [39–42]. Compared to a single bolus treatment, prolonged dexamethasone treatment was more effective in reducing synovial joint inflammation in rabbits by half and, whereas prolonged treatment did not prevent

MMP-3 and -13 mRNA expression and GAG loss, it was capable of significantly decreasing the mRNA expression of *IL-1β*, *MMP-1*, and *ADAMTS-5* and it restored *ACAN* to normal expression levels 3 weeks after anterior cruciate ligament transection (ACLT) injury [39, 42].

Another study investigated the effects the E2 estrogen hormone 17b-estradiol, which is the most widely clinically used estrogen in oral contraceptive pills and in hormone replacement therapy in the treatment of symptoms related to menopause, in mechanically-injured mature bovine articular cartilage. Physiological concentrations of E2 prevented mechanical injury-related cell death (nuclear blebbing and TUNEL staining; effect reversable by addition of fulvestrant, an E2 antagonist) and reduced GAG release [24] suggesting that therapeutic compounds containing the E2 estrogen may regulate and protect against joint-related trauma. Since dexamethasone and E2 both are steroid hormones, it might be speculated that higher concentrations of one or the other might trigger effects through cross-binding to different subtypes of steroid hormones.

Collectively, these studies and the work of others as summarized [46], show that dexamethasone inhibits the early processes involved in PTOA development. In view of all of this data, a pilot clinical study at the Mayo Clinic (ClinicalTrials.gov Identifier: NCT02318433) was initiated to test whether a single, intra-articular injection (4 mg) of dexamethasone given soon after intra-articular fracture of the distal radius reduces the incidence or severity of PTOA.

8.3.2 Interleukin 10 (IL-10) Treatment of Mechanically-Injured Articular Cartilage and Cell-Laden ACI Grafts

Together with our two groups, Grodzinsky investigated the therapeutic effects of the anti-inflammatory IL-10 cytokine on injured cartilage using a pre-injury [47] and post-injury [48, 49] treatment approach. In the pre-injurious treatment study, a single (10 ng/ml) dose of IL-10 significantly decreased injury-related cell death, release of GAG and NO and the mRNA expression of *NOS2*, *MMP-3* and *-13* and *ADAMT-S4* 4 days after injury of mature bovine articular cartilage [47]. In the post-injurious treatment study, continuous low doses of IL-10 were applied to mature bovine cartilage directly after injury and post-injurious effects were assessed up to 3 weeks after injury. In both non-injured and injured cartilage, IL-10 was capable of inducing the mRNA expression of *COL2A1*, *ACAN*, and *SOX9* 3 days after treatment. In injured cartilage, IL-10 treatment additionally significantly inhibited the expression of mechanical injury-induced *COL1A1* and *COL10A1*. Moreover, continuous post-injurious IL-10 treatment inhibited injury-related apoptosis, restored type 2 collagen in the ECM, and inhibited the loss of aggrecan, hyaluronic acid, and GAG 1 to 3 weeks after injury. These studies show that pre- and post-treatment of articular cartilage with low doses of IL-10 (e.g., 100 pg/ml) is highly protective against injury-related damage [48].

The effects of continuous low-dose (100 pg/ml) IL-10 treatment alone or in combination with the growth factor bone morphogenetic protein 2 (BMP-2) of post-operative material containing human chondrocytes seeded in type I/III collagen was also measured to assess the potential of IL-10 to support graft maturation in this clinically applied autologous chondrocyte implantation (ACI) transplant material (Novocart 3D®). Three weeks after injury, IL-10 significantly increased the GAG content within the grafts vs. non-treated grafts. The combination of continuous IL-10 + BMP-2 also significantly up-regulated *COL2A1*, *ACAN*, and *SOX9* and reduced injury-related *COL1A1* mRNA expression and the *COL1A1/COL2A1* ratio compared to IL-10 or BMP-2 treatment alone 3 days post-injury [48] suggesting that the combination of IL-10 and BMP-2 may enhance the repair of autologous transplanted chondrocytes after cartilage injury.

The chondro-regenerative effects of post-injurious application of IL-10 alone or in combination with lysed platelet concentrate (PC) was

additionally assessed in the treatment of mechanically-injured human OA articular cartilage and chondrocyte-containing ACI grafts from patients undergoing ACI treatment. In OA injured explants, IL-10 and PC similarly reduced apoptosis 4 days after injury. Whereas IL-10 treatment did not modulate the gene expression in OA injured cartilage explants, PC significantly increased *COL2A1* and *ACAN* expression and decreased *COL10A1* expression 3 days after injury. However, continuous IL-10 treatment had better ECM preserving effects in sGAG retention and reduction of type 1 collagen in the ECM after cartilage injury compared to PC treatment, which was less protective. Moreover, PC did not recover the loss of type 2 collagen in the superficial zone of the cartilage explants, and in fact, treatment increased type 1 collagen deposition, indicative of fibro-cartilage [49]. In the ACI samples, the combination of continuous PC and IL-10 was most effective in enhancing *COL2A1* mRNA expression but had no effect on *ACAN* expression. The combination treatment also enhanced sGAG and collagen 2 neosynthesis in the ECM. However, similar to the injured OA cartilage, PC induced *COL1A1* and *COL10*A*1* mRNA expression, which was reduced by co-treatment with IL-10 [49]. Thus, IL-10 was more potent in preserving ECM integrity and mitigating the potentially negative effects of PC suggesting that IL-10 is better in controlling injury-induced degenerative pathways.

Together these studies show that IL-10 treatment can control the post-traumatic environment when applied pre- or post-injury and that IL-10 can additionally support neo-cartilage formation, graft integration and maturation thereby enhancing cartilage repair following ACI treatment.

8.3.3 IGF-1 in Treatment of Mechanically-Injured Articular Cartilage and in an In Vivo PTOA Model

Several studies by Grodzinsky and colleagues have shown that the growth factor insulin-like growth factor 1 (IGF-1) is another potential therapeutic that protects against cartilage injury-related effects [37, 43, 50, 51]. As interleukins, such as IL-1α are typically present in the joint following joint trauma, one study investigated whether IGF-1 alone or in combination with dexamethasone could modulate moderately aggressive (high dose) cytokine IL-1α effects in young healthy bovine cartilage explants and an adult human healthy articular cartilage sample. In young bovine non-injured cartilage, continuous dexamethasone treatment more favorably reversed IL-1α-mediated effects on the mRNA level of *ACAN, COL2A1*, *IL-6*, *caspase-3*, *ADAMTS4*, *MMP-3* and *-13*, and *COX2* 4 days after treatment. However, the combination of IGF-1 and dexamethasone significantly inhibited the loss of sGAG and type II collagen, rescued the suppression of matrix (proteoglycan) biosynthesis, and inhibited the loss of chondrocyte viability caused by IL-1α treatment 1–2 weeks after continuous treatment. In adult healthy human cartilage, only IGF-1 rescued matrix biosynthesis, while dexamethasone alone inhibited sGAG loss and improved cell viability within the cartilage explants [37].

To improve the pharmacokinetics of IGF-1, nanoscale-sized cartilage-penetrating nanocarriers were developed by Grodzinsky and the Hammond group that enable the encapsulation and delivery of IGF-1 throughout the full depth of cartilage tissue [43, 50, 51]. These nanocarriers allow ionic complexation of cationic IGF-1 with anionic poly (L-glutamic acid), which has clinically been used in other FDA-approved polymer-drug conjugate systems. The surface is further modified with an excess of positive charge using cationic poly (L-arginine) that allows transport of the therapeutic growth factor across cell membranes and transport through the negatively charged cartilage ECM and full depth of cartilage [50]. Their groups further developed the nanocarriers by covalently conjugating some of the cationic side groups with polyethylene glycol (PEG) oligomers, creating a small library of nanoscale molecules with varying surface charge. With increasing surface charge and a corresponding decreasing PEGylation, increased cartilage binding was observed. Compared to free IGF-1,

which was cleared within 7 days, a single dose of the IGF-1 via the nanoscale carrier enhanced the joint residence time to 4 weeks in an in vivo rat knee PTOA model of cartilage injury (anterior cruciate ligament (ACL) transection and medial meniscus resection (ACLT1MMx)) when administered within 48 hours of injury. Moreover, a single injection of PEG-containing-IGF-1 carriers reduced synovial inflammation, the width of cartilage degeneration by 60% and volumetric osteophyte burden by 80% vs. untreated rats at 4 weeks post-surgery and was far better than free IGF-1 [51].

The results indicate the potential of a charged cartilage-targeting approach that enables delivery of IGF-1 to target cells within cartilage and over an extended period of time. Moreover, these studies show that IGF-1 is another potential early interventional therapy that could delay or prevent the onset of PTOA following joint injury.

8.3.4 Anti-IL-6 Fab-Fragment in Treatment of Mechanically-Injured Articular Cartilage

IL-6 is highly present after joint trauma, making it a relevant target for controlling injury-related responses. Since full-sized antibodies are too large to penetrate beyond the cartilage surface due to steric hindrance of the dense matrix, Grodzinsky's group investigated the transport of smaller (48 kDa) anti-IL-6 antigen Fab-fragments in healthy human and bovine cartilage [52, 53]. Uptake of the anti-IL-6 Fab significantly increased following mechanical injury, and an additional increase in uptake was observed in response to combined mechanical injury and inflammatory insult with TNFα. This may be due to a combined increase in injury-related tissue swelling which causes an increase in tissue hydration and water content and a decrease in GAG density following injury allowing the Fab-fragment to move with less hindrance within the cartilage, resulting in an increased uptake ratio [52]. While pre-treatment with the anti-IL-6 Fab-fragment had no effect on sGAG loss after injury alone or by TNFα treatment alone, the anti–IL-6 Fab-fragment partially (by approximately 20%) reduced sGAG loss due to the combination of injury plus TNFα treatment in bovine and human explants [53]. This may be attributed to the incomplete non-uniform penetration and slow diffusion of the anti–IL-6 Fab into the cartilage tissue [52]. However, this data nonetheless supports that joint trauma and the inflammatory response following joint injury play a critical role in altering the transport properties of damaged cartilage, especially if the molecules or therapeutics are smaller than 42 kDa.

8.3.5 Antioxidant Treatment of Mechanically-Injured Articular Cartilage

Inhibition of reactive oxygen species has also been explored. Apoptotic cell death due to mechanical injury was almost completely inhibited when mature bovine cartilage was either pre-treated or treated immediately after injury with a compound (manganese(III)tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride; MnTMPyP) that mimics native superoxide dismutase (SOD) and acts as a peroxynitrite and hydrogen peroxide scavenger [26]. Vitamin E (α-tocopherol) was also tested but had no effect on reducing the number of post-injury apoptotic cells. This data suggests that therapies having an antioxidant component or diets enriched in antioxidants may help decrease mechanically-induced cell death in articular cartilage.

8.3.6 MMP Inhibitors and a VEGFR-2 Kinase Inhibitor in Treatment of Mechanically-Injured Articular Cartilage

Several MMP inhibitors have also been tested. Injury-related GAG release from bovine tissue 1 to 7 days post-injury was reduced by the MMP inhibitor CGS 27023A whereas the biosynthesis inhibitor cycloheximide, MMP inhibitor GM 6001 and aggrecanase activity inhibitor SB 703704 had no effect [16]. A VEGF receptor 2

(VEGFR-2) kinase inhibitor was able to reduce the injury-dependent expression of the MMPs (MMP-1, -3, and -13), whereas TIMP-1 and -2, the inhibitors of MMPs, were reduced, which might make the HIF-1α/VEGF pathway a potential target for therapeutic approaches of PTOA [18].

8.3.7 Moderate vs. High Dynamic Compressive Loading of Mechanically-Injured Articular Cartilage

Grodzinksy's research also indirectly showed that dynamic loading of the joint following a joint trauma may be a beneficial physical therapy regime to promote healing of cartilage tissue since moderate (10% and 20% strain) but not high (30%) dynamic compression inhibited the pro-catabolic response of combined mechanical injury and subsequent persistent inflammation (TNF-α, IL-6, sIL-6R). Thus, 10% and 20% strain prevented GAG loss, diminished aggrecanase activity and decreased apoptosis in injured bovine cartilage explants. Moreover, in the presence of cytokines alone, 10% and 20% strain significantly upregulated *COL2A1* expression levels. Importantly, this study also showed that, compared to 10% and 20% strain, loading cartilage with 30% strain amplitudes significantly increased apoptosis and induced the upregulation of inflammatory (*COX-2*) and *ADAMTS-5*, the main aggrecanase involved in articular cartilage breakdown and the loss of ECM [54]. Together, this suggests that appropriate moderate loading of the joint in post-injury rehabilitation may improve cell and tissue function and generate stronger hyaline cartilage and that higher loads may be detrimental to cartilage.

8.4 Final Remarks

Alan Grodzinsky's research on the electromechanobiology of articular cartilage began more than 40 years ago with a groundbreaking publication on the compression-induced electrical potential differences between the surface and deepest regions of cartilage [55]. This important publication related the magnitude-, sign- and time-dependence of the induced electrical potentials to the, at that time, known features of cartilage mechanics and fluid flow and, effectively, 'explained' how mechanically induced electric fields *in vivo* may help regulate the transport of ions and interstitial fluid in charged, hydrated tissues. In the opinion of the authors of this book chapter, two additional key points among the many relevant contributions during 40 years of research are not only outstanding but truly relevant. Grodzinsky and colleagues have transformed our understanding of how complex structure-function relationships govern the tissue's behavior, define the tissue's response to injury, and can be utilized to overcome injury to the tissue by dynamic stimulatory loading. Moreover, his research has been instrumental in understanding the proinflammatory response to injury and in developing treatment strategies that are based on an in-depth understanding of the structure and function of articular cartilage.

Collectively, Alan Grodzinsky's work is not just highly impressive in content, quality, and significance, it also went full circle from uncovering groundbreaking electromechanobiological characteristics of articular cartilage to 'translating' them into a therapeutic strategy. As a prime example, the use of dexamethasone for preventing PTOA (ClinicalTrials.gov Identifier: NCT02318433) and linking dexamethasone to the small, highly cationic molecule avidin for full-thickness penetration and increased duration of stay. On a personal note, Alan's work ethics, quality of science, and motivational nature were instrumental in achieving these accomplishments and the authors are grateful for having played a small part in Alan's scientific success.

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