Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

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The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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Effects of age and mechanical loading on articular cartilage metabolism and corresponding consequences for tissue health

By

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Chapter A: Maturation & Metabolism of Articular Cartilage: A User's Guide to Aging Joints

Shannon K. Walsh

A1. Setting the Stage

A1.1. What's in a joint?

When you think of skeletal joints, you probably think of synovial joints. These are the connections between long bones, such as the tibia and the femur in the leg, that allow range of motion. Knees, hips, shoulders, elbows, wrists, and ankles are all examples of synovial joints (Figure 1A). Although their geometries differ and allow variable types of motion, synovial joints all share the same major anatomical components (Figure 1B). Of course, there are the long bone endings, the surfaces of which are each lined with a layer of articular cartilage. Unlike the cartilage found in the ears and nose, articular cartilage is specifically designed to provide cushion for bones by withstanding and distributing mechanical loads across the joint surface, ultimately protecting the underlying bone from pain and wear. Tendons, which connect muscle to bone, and ligaments, which connect bone to bone, provide joint stabilization and restrict motion to certain planes. Lastly, a soft fatty tissue called synovium (a.k.a. synovial membrane) produces synovial fluid, the viscous lubricant which fills the remaining space within the joint and provides nearly frictionless joint motion.

A1.2. Cartilage is tough, but aging is tougher

Articular cartilage, also referred to as joint cartilage or cartilage from this point forward, is composed of a cage-like mesh of collagen fibers. These fibers provide a scaffold for large, branched molecules called proteoglycans to lay within. In addition to providing extra structural support, proteoglycans play the very important role of keeping cartilage hydrated. Since proteoglycans are negatively charged, they keep positively charged water molecules bound within the tissue. Collagen, proteoglycans, and water give cartilage the ability to endure heavy and consistent compression. Embedded within this dense framework are cells called chondrocytes. These cells are responsible for maintaining cartilage health by breaking down old tissue and creating new tissue as necessary.

Like any body part, joint cartilage undergoes structural and biological changes throughout the aging process. From birth to adolescence, the cartilage thins, becoming more compact. As this occurs, the molecular components within the tissue take a more sophisticated form for enhanced resiliency to repetitive loading (Figure 1C). This structural advantage comes at a price, however, as the residing cell population becomes sparser and the remaining cells lose some of their important stem cell-like properties, which will be discussed later on in the chapter. *Unlike* most tissues, by the time the body reaches skeletal maturity and ceases growing, cartilage loses the ability to repair itself if injured.

Beyond adolescence, cartilage continues experiencing aging effects. The nature and severity of these changes vary from person to person, but often include additional tissue thinning, cartilage damage, and decreased proteoglycan concentration. Losing proteoglycan content is consequential for tissue health and integrity, as this causes cartilage to become dehydrated and therefore less capable of distributing mechanical loads to prevent its own fracture or that of the underlying bone. Proteoglycan-deficient cartilage can be thought of much like a waterbed that has been drained and is therefore less capable of providing sufficient cushion or properly distributing loads across the material. Additionally, the cell population continues to decrease throughout aging, and the very sparse remaining cells often lose the ability to perform key functions for tissue maintenance.

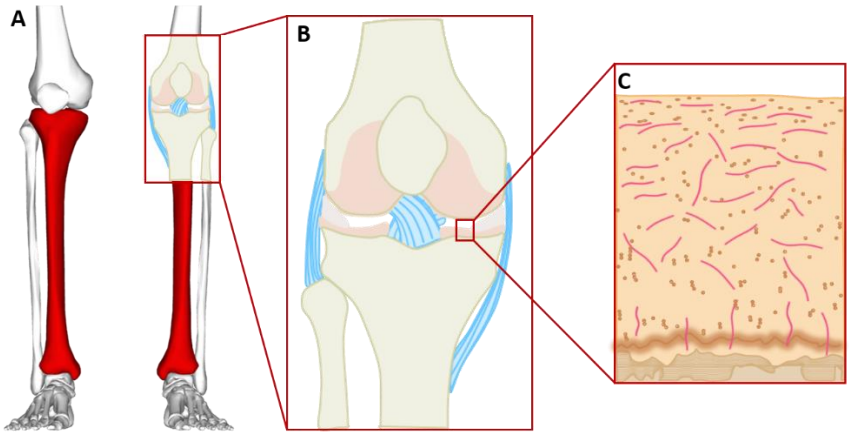

Figure 1. Schematic of multi-scale anatomy. **(A)** Human skeleton depicting tibia (shin bones) in red and the location of the femorotibial (knee) joint, one example of a synovial joint[1]. **(B)** Expanded schematic of a knee joint depicting bones (tan), articular cartilage (pink), and ligaments (blue). **(C)** Expanded schematic of fully developed articular cartilage cross section, depicting the tissue from the joint surface (top) to the underlying bone (bottom). Orientations of collagen fibers are represented by pink lines; chondrocytes are represented as brown dots.

Given the effects of aging described above, it is unsurprising that so many people eventually experience cartilage breakdown over time. As cartilage wears down, the underlying bone is exposed to friction, grinding, and impact that it is not designed to withstand. This is a condition known as osteoarthritis (OA), and it is a very common and painful disease that can become debilitating to those affected. Although OA differs from rheumatoid arthritis, an autoimmune condition in which antibodies attack the body's own cartilage causing inflammation and eventual tissue breakdown, these diseases share many of the same symptoms, including joint swelling, pain, and reduced range of motion. The global burden of OA is expected to rise in the coming decades as average life expectancy continues to increase. Simply put, we are outliving our joints. With artificial joint replacements currently only able to last approximately a decade, alternative therapies for joint preservation are necessary to maintain quality of life in the aged population. Thus, cartilage regeneration (the re-growth of tissue that has degraded) is a *very* active field of study, as researchers are constantly in search of novel ways to treat or prevent OA!

A1.3. Injuries: they don't help

Although OA can develop as a result of consistent joint wear over a lifetime, as is often the case, injuries can certainly initiate and expedite this degenerative (tissue breakdown) process. Athletic injuries, auto accidents, and other traumas which directly injure joint cartilage can initiate an unfortunate cascade of events. First, the impacted cells die, and, if untreated, a combination of subsequent mechanical stress and joint inflammation progressively break down the cartilage until severe OA has developed. Alternatively, injury to another joint-stabilizing tissue such as a ligament can result in secondhand cartilage damage over time. This occurs when the joint geometry is compromised such that the joint is now loaded in a different conformation than it was pre-injury, and previously non-weight-bearing cartilage is now being exposed to heavy loading that it is ill-equipped to handle. Anterior cruciate ligament (ACL) or meniscus tears within the knee often lead to long-term cartilage damage in this manner, as the knee becomes destabilized and more vulnerable regions of cartilage are subjected to concentrated mechanical stresses during ambulation (walking).

Orthopedic (skeletal system) injuries such as ligament tears often warrant surgery, and this procedure can also lead to cartilage damage post-operation in several ways. First, the surgical procedure itself can inflict direct damage upon the cartilage attached to or near the tissue being

repaired. Following surgery, the joint experiences significant inflammation throughout the first stage of recovery and rehabilitation, a condition which can also take a toll on the health of surrounding tissues including cartilage. Additionally, although such surgeries are intended to restore joint biomechanics to their original state, matching pre-injury mechanics perfectly is nearly impossible given current surgical limitations, resulting in subtle abnormalities to the joint's geometry and eventual secondary damage in many cases (Figure 2). Finally, orthopedic injuries and subsequent surgeries often require patients to completely immobilize the affected joint for some period of time to prevent further injury and to allow time for tissue healing. While this practice prevents injured joints from excessive loading, a total lack of motion is not healthy for articular cartilage either. This tissue is designed to experience frequent compression and requires some degree of loading for tissue health. Cartilage cells fail to act properly with respect to maintaining tissue buildup and breakdown in the total absence of mechanical loading signals. In fact, whether cartilage cells are loaded too much or too little, the consequence is ultimately the same; the rate of cartilage breakdown exceeds the rate of cartilage buildup, and the tissue degenerates. In this regard, one can imagine chondrocytes as factory workers, where both an excessive workload (excessive mechanical burden) or a complete lack of instruction (insufficient mechanical loading) result in underperformance and minimal factory output. As previously mentioned, aging can *also* cause cartilage cells to stop functioning properly in this same manner. With all these biological and mechanical challenges leading to cartilage breakdown, it is no wonder that OA is so common.

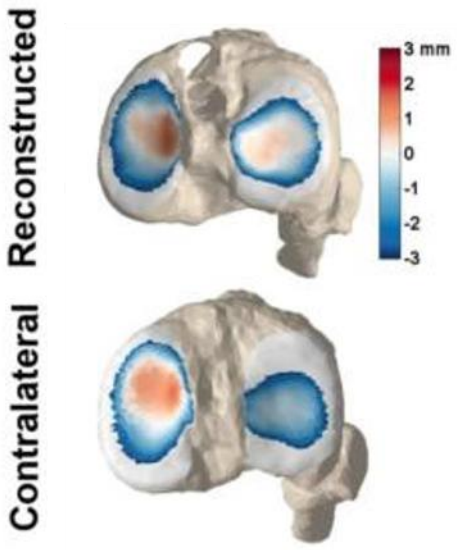

Figure 2. Representative map of tibia cartilage contact in reconstructed (operated) knee compared to the contralateral (unoperated knee) in a patient who has undergone ACL reconstruction surgery, as determined by magnetic resonance imaging (MRI)[2]. Red hue indicates contact between the joint surfaces and blue indicates space.

A2. My Research

A2.1. Mechanics + Biology = Mechanobiology

Articular cartilage is notoriously challenging to study. Relative to other tissues, including juvenile cartilage, adult cartilage is essentially a biological desert; chondrocytes are sparsely distributed throughout a very dense network of structural molecules and demonstrate much lower rates of biological activity than most other cell types (Figure 3). Given these limitations, many methods of measuring cell activity within cartilage tissue aren't powerful or sensitive enough to detect subtle changes in chondrocyte behavior within live tissue samples and are therefore inadequate for many cartilage research applications.

For this reason, lots of cartilage research happens on chondrocytes that have been chemically isolated from the network of collagen fibers and proteoglycans they reside within. These studies can give us great insights into specific chondrocyte functions on a fundamental level. For instance, researchers can simulate tissue injury by artificially damaging isolated cells, typically via hazardous chemicals, and can then observe chondrocytes' response to injury. This experimental "injury" model can also be used to test potential treatments, allowing researchers to determine which ones have the most potent therapeutic effect on the cells, thereby indicating which treatments are worth investigating further. However, isolated cell studies are insufficient to fully understand natural mechanisms of cartilage function within the body. Articular cartilage is unique in that it is avascular (lacking blood vessels) and aneural (lacking nerves); hence the "biological desert" reference. Without receiving chemical signals from blood or nerves like most cells do, chondrocytes rely heavily on mechanical signals from tissue compression to know what is going on in their environment and how to respond, much like a person's working senses often sharpen when one has been compromised. Therefore, taking these cells out of their native three-dimensional environment and observing them in the absence of the mechanical cues they receive in the body gives an incomplete look at how they operate on a holistic level.

The overarching goal of my work was to develop ways to observe and understand cartilage biology in the context of the mechanical loading experienced by this tissue; a research field known as mechanobiology. To be clear, this is already an established and well-occupied field with many scientists producing high-caliber research that analyzes numerous aspects of cartilage biology and various types of mechanical loading. I specifically aimed to analyze the short-term cartilage

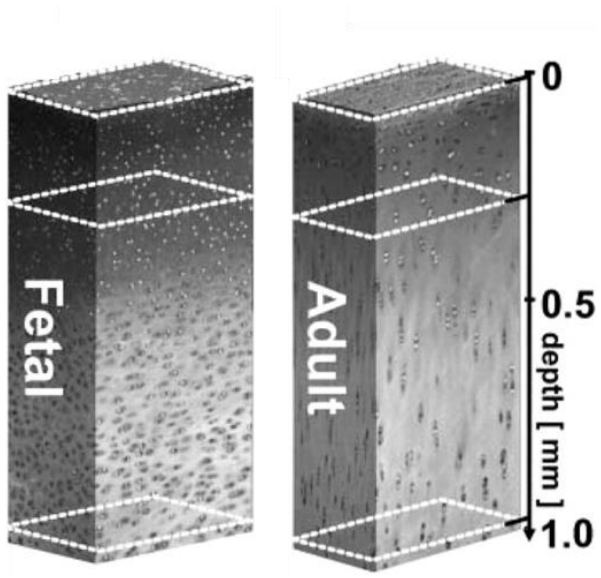

Figure 3. Three-dimensional representation of adult cartilage compared to fetal cartilage, depicting the volume and distribution of cells throughout the tissue from the joint surface (depth = 0 mm) toward the underlying bone surface (> 1 mm)[3].

response to injury-inducing mechanical compression with respect to chondrocyte metabolism, progenitor status, and anabolic gene expression—biological factors suspected to be critically involved in cartilage injury and degeneration—which will be discussed further in the following sections.

A2.2. Metabolic imaging: cancer research comes in clutch

Motive: When most people think of metabolism, they probably think of whole-body metabolism: food intake, exercise, body composition. Broadly, metabolism is the combination of processes by which biological energy is created, stored, and used. These processes can be observed on a cellular level as well. Mammalian cells derive energy from a couple of biochemical processes: glycolysis, the quick chemical breakdown of glucose which yields a relatively small amount of energy, and oxidative phosphorylation (oxphos), a slower chemical process in which electrons are transferred between molecules to create a lot of energy. A growing body of recent literature has indicated that metabolic dysfunction, typically demonstrated by a reduction in one or both of these processes, is a common feature of cartilage breakdown and osteoarthritis progression.

Challenge: Previous cartilage metabolism studies have been limited to either observing a snapshot of metabolic activity at a specific point in time, monitoring generic metabolic activity that is not specific to these individual metabolic processes, or studying cells that have been isolated from the tissue as previously discussed.

Research goals:

(1) Develop a method to observe specific chondrocyte metabolism over time in native, three-dimensional cartilage samples

(2) Use this method to determine the immediate effects of mechanical loading on cartilage metabolism

Approach: We borrowed a technique previously used in cancer research and adapted it for use in cartilage. This technique, known as optical redox imaging, can detect relative levels of glycolysis and oxidative phosphorylation activity by measuring the accumulation of chemical byproducts given off by each of these processes, respectively (Figure 4). Conveniently, these byproducts are fluorescent, meaning the sample doesn't need to be stained with any artificial elements and can therefore be imaged repeatedly over time.

Key outcomes:

- It can be done! Optical redox imaging is capable of detecting metabolic changes in cartilage samples over time without needing to isolate cells from their tissue matrix.
- Slow, mild compression of cartilage, such as what the tissue experiences during walking, appears to induce a proportionately mild and temporary response in glycolysis activity but not oxphos activity.
- Fast, damaging compression of cartilage, simulating an injury, appears to induce a more drastic and long-term response in both metabolic processes.
- Taken together, these results corroborate previous suggestions that oxphos is a particularly important component of the cartilage breakdown process *and* validate our experimental model as an effective tool for studying cartilage metabolism in a way previous studies have not been able to.

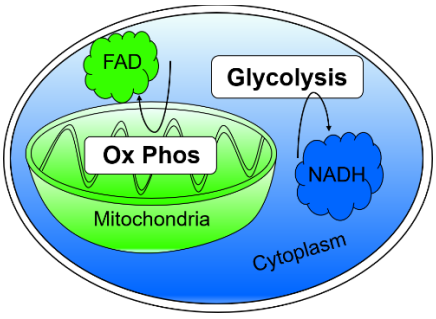

Figure 4. Schematic of a generic cell, depicting intracellular locations of the two main metabolic processes (oxidative phosphorylation and glycolysis) and their respective autofluorescent byproducts, FAD and NADH. Since these byproducts fluoresce on their own, relative amounts of green and blue fluorescence can be imaged and interpreted as relative activity of each of these metabolic processes.

A2.3. Cartilage aging: the (scientific) solution is in the (clinical) problem

Motive: In addition to cell metabolism, recent studies have implicated progenitor cell activity as another potentially important component of cartilage regeneration. Progenitor cells can be thought of as stem cells that are specific to a certain tissue. They are not truly stem cells, for they cannot give rise to multiple types of tissue, but they share certain features with stem cells such as the ability to multiply, migrate, and differentiate into the "terminal" cells which produce and/or maintain a specific biological tissue. Essentially, progenitors are the interns of the cell population; they know what they'd like to become, but haven't yet reached their final stage of development, and are working very hard in the meantime. Researchers have shown that cartilage in juveniles contains a greater proportion of certain progenitor cells than adult cartilage does, suggesting the lack of progenitor cells may be involved in adult cartilage degradation.

Gene expression serves as a good indicator of cells' intentions, which makes gene expression analysis an attractive approach for evaluating cellular activity. Every mammalian cell (excluding sperm or eggs) has a complete copy of our DNA in its nucleus. Our DNA serves as a blueprint for all the proteins in our entire body. However, not every cell needs to be making every protein all the time. Heart cells need to make cardiac tissue, liver cells need to make hepatic tissue, etc. Therefore, each cell only expresses (or "turns on") the genes necessary for the tissue it belongs to, and only when the corresponding protein needs to be produced. We wanted to determine the effects of aging on cartilage cells' gene expression to know if adult cells were attempting to make more or less of certain cartilage-specific proteins than juvenile cells.

Challenge: Cartilage degradation is notoriously difficult to study because it is inherently difficult to make observations about a process that's *not* occurring—in this case, regeneration.

Approach: To circumvent this challenge, we joined the abundant community of researchers who compare adult cartilage to pre-adolescent cartilage, the only type of cartilage that naturally regenerates, to understand where things are going wrong mechanistically in mature cartilage.

Research goals:

(1) Determine the effects of aging on chondrocyte metabolism, progenitor status, and expression of anabolic genes (those geared toward building the tissue up, vs. catabolic genes which result in tissue breakdown and turnover)

(2) Determine the consequences of aging on cartilage *response* to mechanical loading and tissue culture with respect to the biological factors listed above. Rapid mechanical loading is generally expected to incite a damage-oriented response from cells, and tissue culture (simulating the body environment by incubating samples in warm nutrient-rich fluid) has been shown to inspire some cells to become more progenitor-like.

Key results:

- Old cartilage appears to be trying to revert to a more juvenile biological state! We anticipated shifts in cartilage metabolism, progenitor cells, and gene expression throughout aging, but we expected it to happen in a consistent, linear pattern. That is, we expected changes to occur throughout aging in *one direction.* We did not expect to see aged cartilage attempting to mimic the growth-oriented biological activity demonstrated in young samples by reverting to pre-adolescent levels of metabolic activity, gene expression, and progenitor cell activity.
- Mechanical loading and tissue culture caused quite a few fluctuations in progenitor cells and gene expression patterns in juvenile samples, less so in adolescent samples, and hardly any in adult samples. In other words, despite the promising attempt to reverse inherent aging changes demonstrated in aged samples, old cartilage seems to be "set in its ways" when it comes to *actually* responding to environmental disturbances. It's as if the cells in aged samples have acknowledged their shortcomings but lack the tools to make actual improvements. This may help explain the unsuccessful development of effective therapies for aged cartilage. As they say, "you can lead an old chondrocyte to water, but you can't make it drink."

A2.4. Why does this matter?

Much like adding a pebble to the top of a mountain, these studies (like all studies) add a small bit of knowledge to the existing body of wisdom built up by previous researchers in the field. All this work serves to push the boundary of our collective understanding, occasionally culminating in scientific or medical breakthroughs which surge the field forward thanks to all who contributed. My work, outlined above, has both short- and long-term implications for cartilage research:

Short-term: We know that adult cartilage is unable to repair itself and therefore cannot prevent the accumulation of damage. However, this research provides evidence that aged cartilage is *trying* to combat aging effects. While this intention clearly does not translate into capability, these findings can help to guide our research moving forward by steering us toward specific aspects of tissue regeneration which demonstrate some promise in aged cartilage.

Long-term: Optical redox imaging (ORI) holds great promise for cartilage research and medicine. Given that this method can accommodate longitudinal (time series), informative metabolic monitoring during and after environmental changes such as mechanical loading or exposure to treatments, ORI opens many doors for cartilage researchers. We have demonstrated a couple of possible applications of this technique (imaging pre- and post-mechanical loading, across aging spectrum), but there are countless more that this method could be applied to. Simply put, ORI can be used to aid our understanding of tissue physiology as well as to test novel potential therapeutics from a metabolic perspective. Additionally, and perhaps most excitingly, because ORI is a relatively non-invasive and non-destructive technique there is some potential for eventually adapting this technique for clinical use in diagnosing cartilage damage in the body long before any macroscopic injury is present, letting us get ahead of osteoarthritis with early treatment and prevention.

Beyond the act of doing the research itself, *communicating* this work to the public is also very important! Ideally, the scientific and non-scientific community should be engaged in an ongoing, two-way dialogue in which the public is able to express their needs (particularly where research can help), scientists can express what type of community support they need to conduct the necessary work, and the fruits of this collaborative effort are communicated and celebrated by all. On that note I would like to sincerely thank the Wisconsin Initiative for Science Literacy at UW-Madison for sponsoring and supporting the creation of this chapter. I sincerely commend the sentiment behind the program's mission and encourage all researchers to embrace science communication as a fundamental component of our responsibilities as scientists.

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