Mechanosensing and Mechanochemical Transduction in Extracellular Matrix
Frederick H. Silver

Mechanosensing and Mechanochemical Transduction in Extracellular Matrix

Biological, Chemical, Engineering, and Physiological Aspects

Foreword by Stephen C. Cowin
Cover illustration: Diagram illustrating binding of a cell to a collagen fibril through specific integrins attached to the cell membrane. The integrin subunits are \( \alpha \) and \( \beta \).
This volume presents a lucid description and timely organization of our present understanding of how the various tissues and organs of the body sense their mechanical environment and pass on this sensory information. In our early education one learns of our five senses (seeing, hearing, smelling, tasting, and touching). Each of these senses is associated with a specialized type of cell that is a transducer of environmental signals and which also transmits this sensory information to the brain. Beyond these five senses there is a larger community of transducer cells in our tissues that communicate their sensory readings to local tissue- or organ-based control systems rather than to the brain. These sensory cells are usually components of multiply connected cellular networks. These networks maintain the health and function of the tissues and organs and serve as local control systems for the tissue or organ’s function. Most collagenous tissues, including bone and tendon, are examples of such tissues; organ examples include the kidney and the digestive system. There are many more examples described in this volume. The multiply connected cellular networks in these tissues and organs control the remodeling of the tissues to accommodate changes in their mechanical environment.

The production of the materials for the construction of tissues is one of the better-understood topics concerning tissue formation. Cell and molecular biologists have documented the manufacturing of most of the tissue constituents by cells within or near the tissue. Cells not only manufacture these tissue constituents, but they also have the sensory apparatus for maintaining the tissue and adapting the tissue structure to changed environments, including mechanical load environments. We know that tissues and organs are created in fairly repeatable structural patterns, patterns that are due to both the genetic information and the mechanical environment, but we do not know exactly what percentages of a particular pattern are due to either one of these two factors. We do not know much about the beginning of tissue construction (morphogenesis), but we do know something about the self-assembly methods by which tissues construct themselves and they are described in this volume. When the tissue adapts its structure to
accommodate a new mechanical environment, we do not know how the
decision is made for the structural adjustment. We do know that tissues and
organs grow or reconstruct themselves simultaneously with their active per-
formance of their normal function, but we do not understand the processes
that permit tissues and organs to accomplish this. This volume provides a
knowledge base for the people who will explore these and other related
issues. These are people who think mechanistically about the relations
between cell activity and tissue structure. These people generally have a
background in engineering or biophysics, but also there is a growing com-
munity of biologically educated people who think mechanistically, rather
than descriptively, about biological processes.

Stephen C. Cowin
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Many years ago I was told by a sage in the field of collagen that we knew all that we needed to know about the physical nature of collagen. I was surprised at the response and still to this day wonder why anyone would go into research who thought there was nothing new to learn. The purpose of this book is to tell biologists, chemists, physiologists, engineers, and anyone who listens, that just the opposite exists. There is a rich world of new information that is waiting to be explored on mechanotransduction. To hope to achieve an understanding of the processes involved we need scientists of all backgrounds and experiences. I hope this book acts as a stimulus to attract any and all that can contribute to this field. I also urge that those who are interested not let all the terminology scare them from contributing to this field. We need more self-motivated people to answer the fundamental physical questions about mechanotransduction. This only will be achieved by promoting better interaction among individuals in life sciences, chemistry, physiology, and engineering. Although there has been at lot of discussion of interdisciplinary education and research we need more emphasis on problem solution rather than adding to the unbounded data that exist in the literature. Much of the information on mechanotransduction cannot be compared because the model systems used are different in many studies. We need to stop emphasizing the funding of interesting observations and pay more attention to the development of more generalized studies that emphasize how systems work. This is essential to prolongation of life and the amelioration of diseases. We have reached a limit in successfully applying trial-and-error solutions to complex healthcare problems.

We spend our whole lives working against a gravitational field that contributes to the failure of our host systems. However, we really don’t understand how the gravitational field affects biological systems. The purpose of the approach used is to attempt to illustrate how basic chemistry and physics can be applied to try to understand some basic phenomena that are observed. At no point in the book do I mean to imply that the subject is simple. The simple approach used only is meant to be a starting point for more complex analyses. It is my hope that this text will stimulate new thinking about how man has evolved over a period of several million years and the role of gravitational forces in the development and maturation of the human host.
I would like to take this opportunity to thank all my students and colleagues who have provided inspiration and guidance to pursue this project. I had the opportunity to be part of the Harvard and MIT educational systems during the 1970s and early 1980s at a time when much of the emphasis was on learning and thinking. Much of the philosophy of that period has influenced my continued interest in the interface between the biochemical and physical aspects of life. I am particularly thankful to my last group of graduate students at Rutgers, Pat Snowhill, Paul Seehra, Istvan Horvath, Gino Bradica, and Joseph Freeman, for helping me explore the final pieces to the puzzle that has fascinated me for two decades. I would also like to thank them for helping me prepare many of the diagrams on mechanochemical transduction and to thank Dr. David Christiansen for preparing many of the other diagrams used in this text. They have inspired me to complete this book under conditions that would challenge any academic. I hope that at least one reader will be as stimulated by this material to continue the pursuit of knowledge in this field as I have been.
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1
Introduction to Mechanochemical Transduction in Tissues

1.1 Background to Tissue Structure and Mechanochemical Transduction

Life as we know it on earth reflects the influence of gravity on our development, maturation, and aging. Although the molecular biological revolution has attracted an enormous amount of attention in the press and in the scientific literature, with the hope that gene manipulation will cure many diseases, we have only recently learned of the influence of gravitational and other external forces on expression of genes and cellular and tissue function. As we show later in this book, the cellular control mechanisms by which environmental influences affect cell behavior are similar for mechanical forces as well as other external influences. This suggests that altering the external forces acting on tissues may modify gene expression. We indirectly manipulate gene expression every day through exercise and space flight, yet we fail to consider in depth the potential power of this approach in modulating growth and development, wound healing, and aging. When I was a young scientist, just a few years ago, one of my colleagues told me that we knew everything about fibrous collagen that was important. This was long before we had a suspicion that fibrous collagen is one of the key players in transmitting forces between the external environments and cells. The goal of this text is to introduce to the young scientist and engineer the concepts necessary to begin to understand how external and internal forces modulate cellular processes in tissues.

Mechanical forces play a role in the development and evolution of tissues found in the human body. Gravitational forces acting on mammalian tissues increase the net muscle forces required for movement of vertebrates. As body mass increases during development, musculoskeletal and other tissues are able to adapt their size to meet the increased mechanical requirements. However, the control mechanisms that allow for rapid growth in tissue size during development are altered during maturation and aging. The role of mechanical forces in controlling tissue growth, development, aging, and disease processes is an important subject in the design and development
of implants that are needed to restore the function of damaged tissues. Without an understanding of the influence of these forces on tissue and cellular metabolism, the design of synthetic implants becomes an art as opposed to a science.

From the point of view of biomedical engineering, the human body is composed of a structural framework to which the various organs and tissues are attached. The functional units include the heart, lungs, gastrointestinal (GI) system, brain, and other organs and the structural framework includes the musculoskeleton and connective tissue matrix that holds the organs together and allows for locomotion. The organs must work in concert to ensure the proper metabolite levels and blood flow are maintained whereas the musculoskeleton allows for packaging these organ units into a system that can move itself from place to place. Without the organ units acting in a cooperative fashion, life as we know it could not be sustained; in the absence of a movable framework we would be dependent on our environment for transportation to other locations. The structural framework is necessary to perform a number of functions including: (1) supporting the organ systems against destruction, (2) organizing individual units of cells into tissues and organs, (3) forming connections and conduits between different tissues and organs, (4) storing elastic energy produced during locomotion, and (5) transducing external and internal mechanical loads into changes in gene expression and protein synthesis. The structural framework is termed connective tissue or extracellular matrix and is found in either soft (non-mineralized) or hard (mineralized) forms. One purpose of this text is to study the structure of the extracellular matrix that is found in soft and hard connective tissue, and to examine the mechanisms by which mechanical forces are transduced into changes in cellular function. Understanding the relationship between external and internal mechanical loading and the resulting turning on and off of genes during development, aging, and disease is key to improving healthcare and prolonging life. We show later that all cells appear to be under the influence of both internal and external forces and that the balance of these forces, the net force, may be important in dictating whether tissue is formed or destroyed.

Extracellular matrix (ECM), composed of collagen and elastic fibers, proteoglycans, is the ubiquitous substrate for cell adhesion found throughout the human body. It serves as the stimulus for cell growth and differentiation, and it adapts to changes in external mechanical loading to provide mechanical support to tissue. It is well known that connective tissue cells adapt their ECM to changes in externally applied mechanical loads during wound healing and development. For this response to occur, a feedback mechanism must exist by which cells that sense mechanical stress via their substrate respond by altering patterns of protein expression, thus remodeling their ECM to meet changing mechanical requirements. In addition to their ability to adapt to externally applied loads, cells have the ability to generate their own internal forces through the production of cytoskeletal
tension. These externally applied forces and internal cytoskeletal forces appear to be integrated with other environmental signals, which are then transduced into a biochemical response in the cell cytoplasm and nucleus. Thus both developmental biology and human homeostasis involve constant integration of external and internal mechanical signals into biochemical processes that take place in cells. It is the balance between these mechanical stimuli and biochemical processes that is maintained during normal homeostasis.

1.1.1 Significance

Development of new healthcare technologies as well as the rapid diagnosis of disease requires an integrated understanding of chemistry, biology, medicine, and engineering, because the various biochemical pathways involved in homeostasis are linked to tissue structure and function. Mechanical and biochemical feedback loops exist that integrate mechanical and chemical events that are required for development and homeostasis. These mechanical and chemical feedback loops are altered during aging and maturation and along with preprogrammed cell death lead to initiation of disease processes. For example, using echocardiography, the reflection of ultrasonic waves at interfaces between the inner and outer walls of blood vessels or the heart is used to diagnose aortic dilatation, progression of atherosclerotic lesions, cardiac hypertrophy, and the potential need for drug or device intervention. Mechanical forces play a major role in progressive changes seen in blood vessels that are involved in the disease process. Mechanical forces play an important role in tissue homeostasis of musculoskeletal tissues as evidenced by the bone resorption and muscle atrophy experienced by astronauts, as well as in disease processes such as the deposition of lipids and the progression of atherosclerosis. Therefore, it is not only important to be able to measure the structure and normal function of tissues, but it is important to be able to understand the relationship between external mechanical loading to tissues and the resultant changes to gene expression and protein synthesis.

Almost every phase of biomedical engineering involves the measurement, replacement, or modification of tissues and organs. For this reason it is essential that workers in biology, medicine, and biomedical engineering understand the relationship among biochemical pathways, mechanical loading, tissue structure, and normal function. The purpose of this text is to integrate this material in order to provide a conceptual framework needed to understand how external forces affect tissue metabolism.

It is now recognized that epithelial, endothelial, and a variety of other “parenchymal” cell types respond to external mechanical loading by changing expression of certain genes and regulating synthesis of several types of macromolecules. Although many biological products are affected by mechanical loading we limit our discussion to the effects of external mechanical loading on changes in cell cytoplasmic structure and changes to
the extracellular matrix surrounding these cells. This requires that we have an in-depth understanding of cell and ECM macromolecular structure.

1.1.2 Background Definitions

There are a number of terms that must be defined in order to proceed with introducing the concepts explored in this book. The human body is composed of a variety of tissues that includes organs such as the lungs, heart, liver, and spleen which provide both biochemical and biophysical functions such as detoxifying chemicals found in the blood (liver) and filtering expired cells from the blood (spleen), providing exchange of CO$_2$ and O$_2$ (lungs), and circulating blood through the cardiovascular system (heart). Although each of these organs contains different cell types with different organizations, the extracellular matrix that is composed of cells, collagen, and elastic fibers, proteoglycans and cell attachment factors form the structural support. Thus, the human body is composed of extracellular matrix (ECM) containing macromolecules that form a continuous interface with the surrounding cells. The ECM can be highly aligned with large collagen fibers such as those found in tendon; in this case it is termed dense regular connective tissue. It can also be loosely woven as is found in the top layer of skin and termed loose connective tissue. In organs, the ECM forms what is termed the parenchyma, the scaffold of the tissue, and the cells form the functional units. ECM is also found in conduits such as blood and lymphatic vessels, planar sheets such as the pleural and peritoneal membranes that separate different anatomical structures, and in dense and loose connective tissue found in musculoskeletal tissue and in surface lining structures (epithelial and mucosal tissue line the inner and outer surfaces of body).

Fifty years ago, ECM was considered to be a passive scaffold for cells; today we are aware that ECM-cellular communication occurs through mechanical force transduction that affects gene expression and ECM synthesis and that mechanochemical transduction is a key element in modern human physiology and molecular biology.

Connective tissue and components of the ECM provide more than structural support to cells that form organs and other tissues of the body. The interactions between these tissues that occur during development and growth can only be understood after we analyze the structure and properties of each of the components. There are numerous types of cells found in the ECM that continuously form and remodel the material found in their extracellular matrix. Material outside the cell, termed extracellular matrix, consists of collagen fibrils and fibers that form a continuous network (see Table 1.1). Collagen fibers contain other nonfibrous materials that not only form bridges between collagen fibers but also form attachments to the cell membrane. As diagrammed in Figure 1.1, collagen fibrils bind directly to cells via specific attachment molecules termed integrins. In addition, cells have binding sites for other macromolecules such as fibronectin that is
found in the ECM loosely associated with collagen fibers. Different cell types manufacture a number of integrins; each type of integrin is specific to a particular macromolecule found in ECM.

The fibrous components of tissues are analogous to nylon fibers used to construct fabrics in the clothing industry or the steel wire that is an integral part of the belt that prevents blowouts in automobile tires. In ECM the fibers are composed of collagen, a protein containing three polypeptide chains, elastic tissue containing an amorphous polypeptide termed elastin, and a fibrillar component termed microfibrillar protein or fibrillins. Collagen fibers in ECM prevent overexpansion and failure as does the steel wire in tires; however, in addition, collagen fibers transmit stresses in tissues to cells that are transduced into chemical signals that help maintain the homeostasis of these tissues. Elastic fibers are found primarily in tissues including vessel wall and skin where they contribute to tissue shape recovery after tissue unloading.

ECMs contain a variety of cell types (see Table 1.2) such as fibroblasts in the skin, chondrocytes in cartilage, and osteoblasts in bone. The role of these cells is to synthesize and deposit the ECM surrounding the cell and to change the amount and location of the ECM in response to trauma and changes in external loading. For instance, collagen synthesis in these cells is typically up-regulated by the application tensile forces and is down-

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition</th>
<th>Building blocks (repeat unit)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Protein</td>
<td>Amino acids</td>
<td>Found in banded extracellular fibrils, basement membranes and thin fibrils extracellularly</td>
</tr>
<tr>
<td>Elastin</td>
<td>Protein</td>
<td>Amino acids</td>
<td>Structureless component of elastic fibers</td>
</tr>
<tr>
<td>Microfibrillar protein Proteoglycans</td>
<td>Protein core &amp; large side chains (glycosaminoglycans)</td>
<td>Amino acids and disaccharides</td>
<td>Associated with collagen fibers</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>Polysaccharide</td>
<td>Disaccharides</td>
<td>Found between collagen fibers</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Glycoprotein</td>
<td>Amino acids and monosaccharides</td>
<td>Attachment factor between collagen fibers and fibroblasts</td>
</tr>
<tr>
<td>Laminin</td>
<td>Glycoprotein</td>
<td>Amino acids and disaccharides</td>
<td>Attachment factor, component of basement membrane</td>
</tr>
</tbody>
</table>
regulated by the application of compression. These cells form attachments to collagen and elastic fibers as well as to cell attachment factors such as fibronectin and hyaluronan. External forces borne by collagen and elastic fibers found in ECM are transferred to cells via cell surface integrins; some of the external loads are dissipated via connections among the cell surface and fibronectin and hyaluronan located between collagen and elastic fibers. Fibronectin is a glycoprotein that is involved in mediating the attachment to collagen fibers; cells can directly bind to collagen via integrin receptors. Laminin is a component of basement membranes and facilitates binding of epithelial cells to type IV collagen networks that are also present in

![Diagram illustrating binding of a cell to a collagen fibril through specific integrins attached to the cell membrane. The integrin subunits are α and β.](image)

**Figure 1.1.** Diagram illustrating binding of a cell to a collagen fibril through specific integrins attached to the cell membrane. The integrin subunits are α and β.

<table>
<thead>
<tr>
<th>Table 1.2. Cells found in connective tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell</strong></td>
</tr>
<tr>
<td>Chondrocytes</td>
</tr>
<tr>
<td>Epithelial</td>
</tr>
<tr>
<td>Endothelial</td>
</tr>
<tr>
<td>Smooth muscle</td>
</tr>
<tr>
<td>Fibroblasts</td>
</tr>
</tbody>
</table>
basement membranes form the interface between epithelial and endothelial cells and the surrounding ECM. To complicate matters even more, there are about twenty types of collagen found in ECM and several types of proteoglycans. The fiber-forming collagens consist of types I, II, and III and form the structural support for most tissues (see Table 1.3). Other classifications of collagens include the fibril-associated collagen with interrupted triple helices (FACIT) and the nonfibril-forming collagens. The specific roles of these collagens are the subject of much speculation.

Historically the material between collagen fibers in tissues has been referred to as interfibrillar matrix. The major components of the interfibrillar matrix include proteoglycans, hyaluronan, and water (see Table 1.1) and serve to bind the fibers together and prevent interfiber friction. In skin, the collagen fibers undergo alignment and slippage during mechanical loading that allows skin to bend over joints and absorb impact loads. The proteoglycans that are found between the collagen fibers are important in supporting elastic energy storage as well as in dissipating applied loads. Proteoglycan degradation in the ECM is the first change seen that is associated with the development of osteoarthritis and loss of articular cartilage. This loss precedes wearing away of the cartilage surface that leads to limited locomotion due to excessive pain.

Throughout this text we refer to the relationship between external mechanical loading and cellular response that affects the physiology of ECM. Inasmuch as mechanical terms are used repeatedly, some of these terms are listed in Table 1.4. These terms refer to the ability of tissue to change its shape and to deform. Strain refers to the changes in tissue dimensions with respect to the initial dimensions, and extensibility refers to the strain a tissue can undergo before failure. The force per unit cross-sectional area of a tissue is referred to as stress; the stress at which a tissue tears is

<table>
<thead>
<tr>
<th>Collagen type</th>
<th>Tissue or organ</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tendon, skin, bone and fascia</td>
<td>Thick extracellular fibrils and fibers</td>
</tr>
<tr>
<td>II</td>
<td>Cartilage</td>
<td>Thin fibrils around cartilage cells</td>
</tr>
<tr>
<td>III</td>
<td>Cardiovascular tissue</td>
<td>Intermediate size extracellular fibrils</td>
</tr>
<tr>
<td>IV</td>
<td>Basement membranes</td>
<td>Network forming component</td>
</tr>
<tr>
<td>V</td>
<td>Tendon, skin and cardiovascular tissue</td>
<td>Pericellular matrix around cells</td>
</tr>
<tr>
<td>VI</td>
<td>Cardiovascular tissue, placenta, uterus, liver, kidney, skin, ligament, and cornea</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>VII</td>
<td>Skin</td>
<td>Anchoring fibrils</td>
</tr>
<tr>
<td>VIII</td>
<td>Cardiovascular tissue</td>
<td>Around endothelial cells</td>
</tr>
<tr>
<td>IX</td>
<td>Cartilage</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>X</td>
<td>Cartilage</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>XI</td>
<td>Cartilage</td>
<td>Extracellular matrix</td>
</tr>
</tbody>
</table>
referred to as the ultimate strength. Stiffness is a measure of the ratio of change in stress divided by the change in strain and is the resistance a material offers when a strain is applied.

1.2 Cell and ECM Macromolecular Structure

All cells found in ECM are complex organizations of macromolecules. They are arranged in such a manner as to allow small molecules and ions to diffuse into the cell and for these components to be synthesized into macromolecules used in forming structural materials within and outside the cell. Macromolecules are made up of long chains of repeat units that are connected end to end in the form of proteins, nucleic acids, lipids, and polysaccharides.

1.2.1 Cellular Components

Cells are composed of proteins, nucleic acids, lipids, ions, and water. These materials are discussed further in Section 2.1 and are organized into functional compartments that include the cell and nuclear membranes, cytoplasm, nucleus, and organelles (Figure 1.2). The organelles that are important in this book include endoplasmic reticulum (ER), mitochondria, ribosomes, Golgi apparatus, and lysosomes (Table 1.5). Cellular changes associated with mechanical loading or due to the presence of an implant are studied by evaluation of the cell structure before and after loading or after contact with a surface. Changes in cellular components such as changes in the staining pattern or swelling are the first indication that cell injury is occurring and that cell death may follow.

The normal cell has an outer layer with a chemical structure similar to fat that has been esterified. It is found in the form of a cell membrane that separates the fluid and tissue outside a cell from the organelles inside the cell. The cell or plasma membrane is a lipid bilayer that contains proteins on the inner and outer surfaces (Figure 1.3). The function of the cell membrane is to: (1) maintain ionic and chemical concentration gradients; (2) carry specific surface markers and receptors such the human leukocyte anti-

<table>
<thead>
<tr>
<th>Terms</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>change in length/initial length</td>
</tr>
<tr>
<td>Extensibility</td>
<td>strain at failure</td>
</tr>
<tr>
<td>Stress</td>
<td>force/cross-sectional area</td>
</tr>
<tr>
<td>Stiffness</td>
<td>change in stress/change in strain</td>
</tr>
<tr>
<td>Toughness</td>
<td>strain energy absorption: area beneath stress-strain curve</td>
</tr>
</tbody>
</table>
Figure 1.2. Diagram of cell components. Generalized diagram showing components of cell including ER, mitochondria, ribosomes, Golgi apparatus, lysosomes, and other components.
### Table 1.5. Components of the normal cell and their functions

<table>
<thead>
<tr>
<th>Cell component</th>
<th>Composition</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell membrane</td>
<td>Lipid bilayer, containing surface proteins (peripheral proteins), proteins totally embedded in the membrane (integral proteins), and glycoproteins partially embedded in the membrane</td>
<td>Maintains ionic and chemical concentration gradients, cell-specific markers, intercellular communication, regulates cell growth and proliferation</td>
</tr>
<tr>
<td>Cytosol or cytoplasm</td>
<td>Water, ions, soluble proteins</td>
<td>Contains enzymes and structures for generation of energy (ATP)(^a) in the absence of oxygen (TCA(^b) cycle), activation of amino acids, carrying out specialized cell functions</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>Membrane-enclosed channels</td>
<td>Involved in transport of proteins for extracellular secretion and modification or detoxification of chemicals</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Contains membrane-lined channels to which enzymes are attached that generate ATP from glucose</td>
<td>Involved in TCA cycle, respiratory chain, and oxidative phosphorylation</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>Small and large subunits composed of ribosomal RNA; strands of messenger RNA form a complex with large subunits</td>
<td>Involved in synthesis of enzymes and structural components and proteins for extracellular release</td>
</tr>
<tr>
<td>Golgi apparatus</td>
<td>Membrane-lined tubular system that forms stacks</td>
<td>Packaging of proteins in vesicles for extracellular release</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>Membrane-lined vesicles containing hydrolytic enzymes</td>
<td>Involved in breakdown to intracellular and extracellular material</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Membrane-limited area of cell containing nucleolus and chromatin</td>
<td>Site of synthesis of RNA and chromatin, involved in cell division</td>
</tr>
</tbody>
</table>

\(^a\) ATP = adenosine triphosphate.  
\(^b\) TCA = tricarboxylic acid cycle.

---

**Figure 1.3.** Diagram of cell membrane. The plasma membrane is a lipid bilayer containing peripheral proteins, including HLA antigens, proteoglycans, and integral proteins, such as pore-forming proteins that transverse the cell membrane.
The contacts between two adjoining cell membranes (see the gap junctions in Figure 1.2) are stabilized by specific cell adhesion molecules (CAMs), which include the Ca\(^{2+}\) dependent cadherins in adherence junctions, and by connexins in gap junctions. The connexins are components of the connexon channels between cells. Spontaneous contraction of neighboring cells leads to cellular tension and mechanical transmission of tensile forces through adherens junctions. Results of recent studies suggest that N-cadherins in fibroblasts transmit mechanical forces applied to adherens junctions by activating stretch-sensitive calcium-permeable channels, leading to an increased actin polymerization. Analogously, connexin 43 is present in gap junctions between cells including chondrocytes of knee cartilage, in growth plate, and between fibrocartilage-like cells at tendon and ligament insertions. Mechanical forces exerted at cell junctions can affect intercellular communications.

Typically, the integrity of the cell membrane is evaluated by identifying the percentage of cells taking up a vital dye termed trypan blue. Cells that take up trypan blue have defective cell membranes and therefore external loading or contact with materials that cause cells to have faulty membranes are likely to cause cell injury and death. Cell cytotoxicity is evaluated by contacting a surface of a material or the extract of a material or implant with cells in culture. If more than five percent of the cell population stains with dye after contact with the material or an extract then it is considered cytotoxic.

Cells normally exhibit polarity; that is, they have a top, bottom, left, and right sides. Most cells such as the epithelium present an apical pole that is characterized by many ruffles in the cell membrane termed microvilli and a basal pole that is in contact with a basement membrane. This polarity is very important because normal cell function can only be expressed if the cell has the correct orientation.

The material within the cell membrane is gellike and is termed the cytoplasm or cytosol. It is composed of ions, water, soluble proteins, and enzymes that are involved in generation of energy in the form of ATP by a process termed the tricarboxylic acid cycle (TCA) or Krebs cycle in the absence of oxygen (Figure 1.4). It is also involved in activation of amino acids for protein synthesis (Table 1.5 and Figure 1.4).

In addition to organelles, the cell cytoplasm contains actin filaments that make up the cellular cytoskeleton that controls shape. Myosin and α-actinin are also found in the cytoplasm and are believed to be involved in cell contraction. Other filaments including intermediate filaments, tubulin, calmodulin, and spectrin form networks within the cytoplasm that modify cell and organelle mobility and shape.

Endoplasmic reticulum (ER) is a branching system of membrane-limited channels that are found within the cytoplasm (Table 1.5 and Figure 1.2). These channels are 40 to 70 nm wide and are enclosed by a membrane similar to the plasma membrane. In some cases the channels are covered
with spheres 20 nm in diameter; this is termed rough ER. The spheres are ribosomes and are the site of the synthesis of proteins. The ER serves as a conduit for export extracellularly for the proteins synthesized within the cell cytoplasm. In the absence of ribosomes, the ER is termed smooth (SER). Proteins such as collagen are synthesized on the ribosomes, pass into the center of these channels, and are transported into the Golgi apparatus where they are packaged into vesicles for release from the cell. Membranes that line the SER are involved in modification and detoxification of low molecular weight materials that are released by implants. Implants or bio-

**Figure 1.4.** Krebs cycle and protein synthesis. This diagram illustrates the generation of ATP through the Krebs cycle and the protein synthesis that occurs within the cell cytoplasm.
materials that stimulate proliferation of the SER result in its proliferation leading to an increase in cell size. Increased cell size in the presence of an implant is an indication that the implant may cause irreversible cell changes. Active proliferation of the ER suggests that a cell is responding to increased mechanical loading.

Mitochondria are cigar-shaped organelles (Figure 1.2) that are separated from material found in the cell cytoplasm by a double membrane. Both inner and outer mitochondrial membranes are similar to plasma membranes. The inner membrane is connected to a series of folded channels (cristae) upon which the enzymatic reactions of the acid cycle (TCA), respiratory chain, and oxidative phosphorylation occur (Figure 1.4). These reactions are required to generate ATP from glucose in the presence of oxygen. Mitochondria are prevalent in cells that are actively secreting proteins such as collagen in response to increased external mechanical loading and in cells that are synthesizing enzymes required for modification or detoxification of chemicals.

Proteins are synthesized on ribosomes, which are made of small and large subunits containing nucleic acids. The small subunit (see Figure 1.4) is shaped like a donut that has been cut in half. The large subunit is spherical and contains a notched groove on the top surface. A strand of messenger ribonucleic acid (mRNA) is found between the notched groove of the large subunit and the hole in the center of the small subunit; mRNA in association with the large and small ribosomal subunits acts as a template for protein synthesis. Synthesis of enzymes and structural proteins used within the cell occurs on free ribosomes found within the cytoplasm. Proteins that are synthesized for release from the cell are synthesized on ribosomes attached to endoplasmic reticulum.

The Golgi apparatus (Figure 1.2) is a membrane-bound system of tubes that is connected to the endoplasmic reticulum. The tubular system is in the form of individual tubules that make up a winding system of cisternae that form stacks termed a dictyosome. Proteins synthesized on ribosomes attached to the inner membrane of the endoplasmic reticulum are transported into the Golgi apparatus where they are packaged into vesicles. Vesicles 40 to 80 nm in diameter at the outside of the cisternae are used to release these proteins extracellularly during wound healing and during bone formation. Extracellular matrix cells synthesize large quantities of collagen and other proteins and therefore the Golgi apparatus is prominent. Coincidental with synthesis of new proteins is the removal of old proteins via a process termed phagocytosis.

Phagocytosis is a process by which foreign or old autologous proteins are ingested by the cell and then removed by fusion with lysosomes; these structures are vesicles containing hydrolytic enzymes. Lysosomes (Figure 1.5) are membrane-bound vesicles that are used to break down proteins, nucleic acids, sugar polymers, and other materials that are either extracellular or
intracellular. Lysosomes are 0.1 to 0.8 µm in diameter and contain a variety of hydrolytic enzymes. Cells rich in lysosomes include polymorphonuclear leukocytes (neutrophils), monocytes, and macrophages. These white cells are involved in the inflammatory process to remove dead cellular debris and damaged extracellular matrix. Excessive external mechanical loading or implantation of a medical device leads to trauma and cell death that stimulates migration of inflammatory cells and phagocytosis. Inflammatory cells clean up the debris by attempting to eat the material including the damaged collagen or an implant. When tissues are unloaded some of the cellular and noncellular materials are removed by phagocytosis.

The nucleus of the cell (Figure 1.2) is composed of a porous nuclear membrane, the nucleolus, and soluble materials. The nucleolus contains ribonucleic acids (RNA) and genetic materials also termed chromatin that code for the proteins synthesized upon the ribosomes in the cell cytoplasm. The nuclear membrane is continuous with the outer membrane of the endoplasmic reticulum. Messenger RNA synthesized in the nucleus is transported across the nuclear membrane and is involved in protein synthesis. It fits into the groove between the large and small rRNA subunits (Figure 1.2)
where it acts as a template for tRNA (transfer RNA) to add the appropriate amino acids to the growing protein chain. mRNA is synthesized from a DNA template that is in the form of a double helix (Figure 1.6). DNA in turn is associated with proteins termed histones and the complex makes up the chromosomes or genetic material. There are 23 sets of chromosomes containing all the genetic material (DNA) required to synthesize all the proteins found within the human body. Chromosomes within the

Figure 1.6. DNA, histones, and cell division. Shown are (1) the structure of DNA, histones and genetic material; (2) changes in genetic material via radiation or free radical induced damage; and (3) the cell cycle, synthesis of DNA and mitosis.
cell are characterized by the size and shape of their long and short legs. They all look like the letter x with varying lengths and geometries. External mechanical forces are transduced into changes in expression of genes found on chromosomes located in the nucleus and lead to a variation in the expression of mRNA that is synthesized into proteins in the cytoplasm.

Ribosomal RNA is synthesized and packaged within the nucleolus of the cell. The nucleic acids that are synthesized within the nucleus pass through pores in the nuclear membrane into the cytoplasm. The nucleolus appears as a dense spot in the nucleus under the light microscope after staining with special dyes. The staining characteristic of the chromatin, which contains the genetic material of the cell, is somewhat different. Chromatin is acidic and therefore stains darkly with basic dyes. Loosely coiled chromatin (euchromatin) stains lightly with basic dyes whereas tightly coiled DNA (heterochromatin) stains darkly with basic dyes. Increases in external mechanical loading result in increased amounts of heterochromatin. Resting cells exhibit increased amounts of euchromatin. The nuclear staining characteristics can be useful in determining the activity of cells that are exposed to changes in mechanical loading. Division of cells within the capsule surrounding an implant indicates that the capsule thickness is likely to increase and that the implant is causing a reaction. Contraction of the tissue that surrounds an implant appears to be stimulated by mechanochemical transduction by fibroblasts found in the capsule.

During cell division (see M phase in Figure 1.6) heterochromatin is separated and arranged into distinct clumps of genetic material, the chromosomes. The karyotype is a fingerprint of the genetic material and is obtained by preventing termination of cell division. This allows for observation of the size and shape of the genetic material found in any cell that is useful to determine if contact with an implant or external mechanical loading causes changes in the genetic material termed a mutation. Cellular replication can be associated with an implant in both positive and negative senses. In a positive sense wound healing is associated with cell replication and synthesis of nucleic acids and proteins. In a negative sense cell replication in wounds after remodeling has occurred is reflective of an abnormal scarring process. Therefore, the analysis of cell replication around an implant must be done in conjunction with other information concerning the cell to be correctly interpreted.

1.2.2 Macromolecular Structure

The principal building blocks of tissues are large molecules referred to as macromolecules or polymers. Without these large molecules life as we know it would not be possible because these moieties are responsible for the
completion of most biological processes. Biological macromolecules can be broken down into four classes of large molecules, namely, proteins, polysaccharides (sugar polymers), nucleic acids, and lipids.

The differentiation among these classes of large molecules is a result of differences in the repeat unit, that is, the chemical structure that is repeated over and over again to make a large chain. Another way of stating this fact is that the properties of long chains of repeat units linked together are very dependent on the chemistry of the chain. The physical properties of long chained molecules also depend on the rotational freedom about the backbone as diagrammed in Figure 1.7. It is very interesting to note that regardless of the exact chemistry of the backbone of a macromolecule the physical behavior is fixed. What this boils down to is that the modulus (stiffness) or resistance of a polymer to deformation is independent of the backbone chemistry. What is dependent on the backbone chemistry is the temperature at which a particular behavior is observed; that is, all polymers behave at some temperature like a rubber band (easily reversibly stretched). The temperature at which a polymer behaves like a rubbery material is termed the glass transition temperature. The glass transition temperature is affected by the chemistry of the repeat unit. The glass transition temperature is dependent on the chemistry of the repeat unit by virtue of how it influences the backbone flexibility. The relationship between the chemistry of the backbone of a polymer and its rubberiness is a bit more complex than just analysis of the backbone rotational freedom; however, that discussion is beyond the scope of this book.

Now if we consider a chain of carbon atoms similar to that observed in lipids and poly(ethylene) we can next ask the question about how this chain exists in three dimensions. We know from general chemistry that a chain of

![Figure 1.7. Mobility of hydrocarbon chain. The diagram shows mobility of a polymer chain composed of carbon atoms attached by single bonds with hydrogen side chains. Rotational freedom of the single carbon-to-carbon bonds allows hydrogen atoms to rotate freely about the backbone.](image-url)