1 Introduction

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2nd CLASS
LECTURE NO. 1

1.1 Point of Departure

Biology is the study of living things; mechanics is the study of motions and the applied loads that cause them. Biomechanics can be defined, therefore, as the study of the motions experienced by living things in response to applied loads. Herein, however, we consider that *biomechanics is the development, extension, and application of mechanics for the purposes of understanding better the influence of applied loads on the structure, properties, and function of living things and the structures with which they interact*. Thus, the domain of biomechanics is very broad. It includes, among many other things, studying the effects of wind loads or gravity on the growth of plants, the mechanical properties of foodstuffs, the flight of birds, the drag-reducing properties of the skin of dolphins, and human athletic performance. Additionally, biomechanics addresses many issues of health as well as disease, injury, and their treatment in both humans and animals. This shall be our primary motivation herein; thus, it is easy to see that biomechanics is fundamental to the rapidly growing field of biomedical engineering.

It is not possible to identify a true "father of biomechanics," but many point to either Leonardo da Vinci (1452–1519) or Galileo Galilei (1564–1642). Among many other things, da Vinci was interested in a means by which man could fly, and to this end, he studied the mechanics of the flight of birds. Mankind's attempt to base the *design* of engineering systems on nature's way of doing something (e.g., the honeycomb structure within a beehive or a bat's radar system) is called bionics, which remains a very important area within biomechanics. In contrast to da Vinci, Galileo was interested in the intrinsic strength of bones and, in particular, its relation to the structural design of bones. Based on a preliminary *analysis*, he suggested that bones are hollow, for this

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improves the strength-to-weight ratio. Clearly, then, biomechanics focuses on both design and analysis, each of which is fundamental to engineering.

Jumping forward to the late nineteenth century, Wilhelm Roux put forth the idea of a "quantitative self-regulating mechanism" that results in functional adaptation by tissues, organs, and organisms, an idea that was consistent with the concept of a stress-mediated organization of the microstructure of bone that was put forth by Julius Wolff in 1884. Briefly, Wolff suggested that the fine structure within bones (i.e., oriented trabeculae) is governed by lines of tension that result from the applied loads. Although his analysis was not correct, the basic idea was extremely important. For more on "Wolff's law of bone remodeling," see Chap. 4 as well as Roesler (1987). Indeed, we will return many times to this observation that mechanical loads control tissue structure and function, which has given rise to the very important area of research called *mechanobiology*.

Many other savants were interested in biomechanical applications. They include R. Hooke (1635–1703), L. Euler (1707–1783), T. Young (1773–1829), J.L.M. Poiseuille (1799–1869), and H. von Helmholtz (1821–1894). Despite the caliber of scientists who have sought answers in biomechanics over the centuries, our field did not truly come into its own until the mid-1960s. Although historians will likely argue over the reasons for this, it is suggested here that five nearly concurrent developments provided both increased motivation and increased capabilities in biomechanics. Recall that the 1960s was the decade of mankind's pursuit of the Moon. When faced with the question, "How will man respond to the altered loads associated with space travel, including a reduced gravitational load on the Moon?," clinical medicine could not provide the answers, for it is based largely on observations. There was a need, therefore, for a predictive science, one focusing on how the body responds to mechanical loads. In addition, note that much of biomechanics deals with the response of soft tissues (i.e., tissues other than bones and teeth). It has long been known that soft tissues exhibit complex nonlinear behaviors that could not be described by the classical mechanics of continua developed in the eighteenth and nineteenth centuries. Rather, biomechanics had to await the post-World War II renaissance in continuum mechanics (∼1948–1965) through which the nonlinear theories achieved a more complete and rational foundation. During this same period, 1950s–1960s, technological developments gave rise to the digital computer. Computers are essential in biomechanics for solving many important but complex boundary and initial value problems, for controlling complicated experiments, and for performing nonlinear analyses of the data. Paralleling the development of computers was the advancing of powerful numerical methods of analysis, including the finite element method, which was introduced in 1956 and has become a standard tool in the biomechanicist's arsenal for attacking basic and applied problems. Finally, it is not coincidental that biomechanics emerged at the time that modern biology was born, which

was due in large part to the identification in the 1950s of the basic structure of proteins (by L. Pauling) and DNA (by J. Watson and F. Crick). In summary then, *the 1950s and 1960s provided important new motivations as well as theoretical, experimental, and technological advances that allowed the emergence of biomechanics*. This is, of course, only a synopsis of some of the essential historical developments. The interested reader is encouraged to investigate further the history of our field.

Although biomechanics encompasses a broad range of topics, the purposes of this book are twofold: first, to introduce fundamental concepts and results from solid and fluid mechanics that can be applied to many different problems of importance in biology and medicine and, second, to illustrate some of the many possible applications by focusing on the mechanics of human health, disease, and injury. Hence, to motivate our study further, let us briefly review some of the many cases wherein biomechanics can and must contribute to the advancement of health care. Once we have sufficient motivation, we shall then briefly review results from Cell and Matrix Biology, results on which we shall build in Chaps. 2–11.

1.2 Health Care Applications

There are many obvious examples wherein biomechanics plays a central role in the delivery of health care, roles that literally span all levels from the molecule to the person. Beginning with the latter, a simple example of an important biomechanical contribution is the design of efficient wheel-chairs. By efficient, of course, we mean having sufficient strength with minimal weight, but also ease of maneuverability, ease of transport in a car or van, flexibility in the positioning of the patient, and even affordability. One does not realize the importance of what may seem to be such a simple device until a family member is incapacitated and in need. Selection of materials, design, experimentation, and stress analysis each play important roles in the engineering of an efficient wheelchair. Another common example at the level of the whole person is the design of transportation systems that improve occupant safety. Again, one only needs to see the devastation wrought on a family when someone is injured severely in a vehicular accident to appreciate the need for biomechanical solutions to improve safety in transportation.

Intracranial saccular aneurysms are balloonlike dilatations of the arterial wall that tend to form in or near bifurcations in the circle of Willis (Fig. 1.1), the primary network of arteries that supply blood to the brain. Although the natural history of saccular aneurysms is not well understood, it is generally accepted that mechanical factors play important roles. Hemodynamic forces may contribute to the initial local weakening of the wall, intramural forces that balance the distending blood pressure may contribute to the enlargement of the lesion

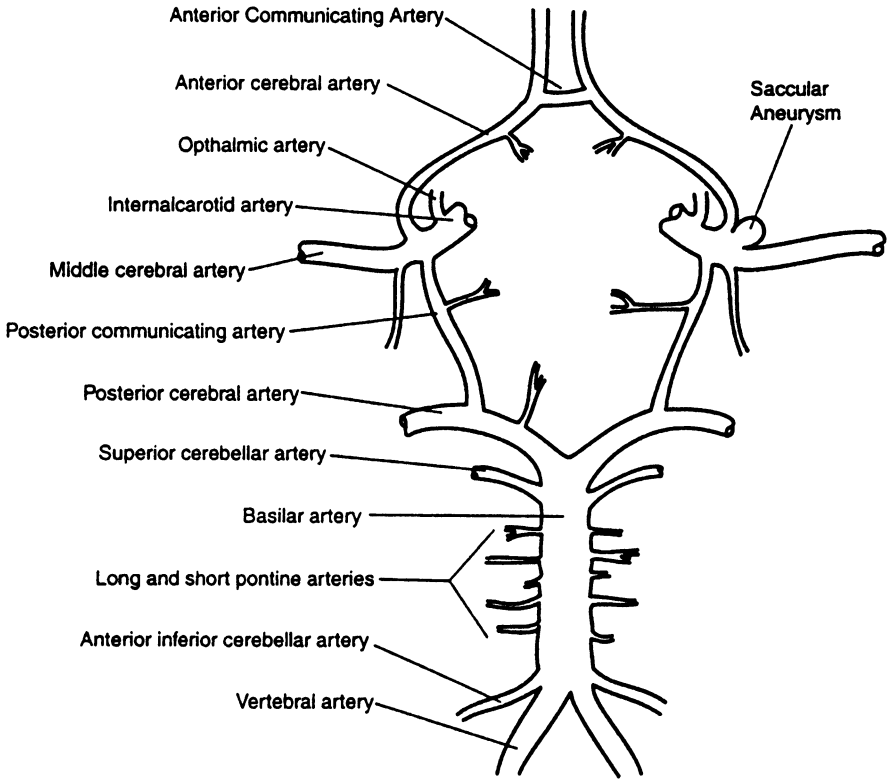

FIGURE 1.1 Schema of the circle of Willis, the primary network of arteries that supplies blood to the brain. Note the intracranial saccular aneurysm, which is a focal dilatation of the arterial wall on the left middle cerebral artery (with the circle viewed from the base of the brain). Such lesions tend to be thin-walled and susceptible to rupture. From Humphrey and Canham (2000), with permission from Kluwer Academic Publishers.

from a small bulge to a sac over 25 mm in diameter (*note*: the parent artery is often less than 4 mm in diameter), and it is thought that rupture occurs when the intramural forces exceed the strength of the wall. Ruptured saccular aneurysms are the primary cause of spontaneous subarachnoid hemorrhage (i.e., bleeding within the brain due to nontraumatic cause) and thus are responsible for significant morbidity and mortality. Understanding the biomechanics of aneurysms at the tissue level is thus potentially very important in neurosurgery.

On yet another scale, it was discovered around 1974 that endothelial cells, which line all blood vessels, are very sensitive and responsive to the forces imparted on them by the flowing blood. In particular, these cells express different genes, and thus produce different molecules, depending on the magnitude and direction of the blood-flow-induced forces (Fig. 1.2). Many different situations alter the flow of blood and thus the forces felt by the

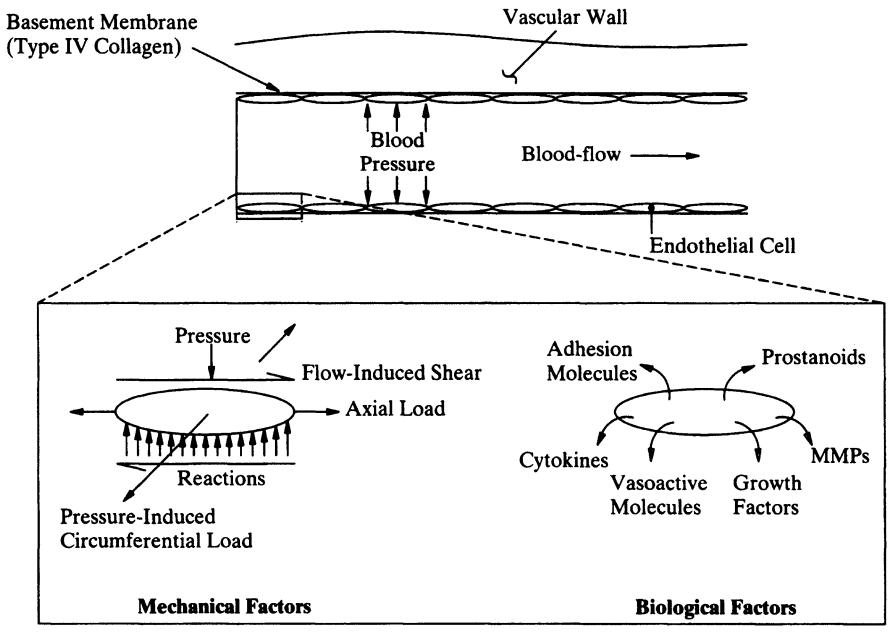

FIGURE 1.2 Schema of the monolayer of endothelial cells that lines the inner surface of a blood vessel, with a free-body diagram showing various mechanical loads that act on a single cell: flow-induced shear forces; radial forces due to the blood pressure; circumferential forces due to cell–cell contacts and the distension due to the pressure; and axial forces due to cell–cell contacts and the prestretch that appears to arise during development. Also shown are classes of molecules that are produced by endothelial cells in response to changes in these mechanical loads. MMPs denotes matrix metalloproteinases—molecules that degrade extracellular matrix.

endothelium: exercise or the lack thereof, diseases such as atherosclerosis and aneurysms, a microgravity environment on the space shuttle, the implantation of medical devices including artificial arteries or left ventricular assist devices, and even the surgical creation of arterio-venous fistulas for kidney dialysis. To understand and ultimately to control endothelial function, we must understand the associated biomechanics and mechanobiology—how the fluid-induced forces deform a cell, how the cell senses these forces, and how the transduction of these forces controls gene expression. It is thought, for example, that loads applied to the surface of a cell are transmitted to the proteins within the cell through membrane-bound protein receptors. Hence, from the wheelchair to individual proteins in the cell membrane, and everywhere in between, biomechanics has a vital role to play in analysis and design that seeks to improve health care.

Figure 1.3 is a rendition of the drawing of a man by da Vinci that emphasizes interesting symmetries of the body. Shown, too, are some of the many examples

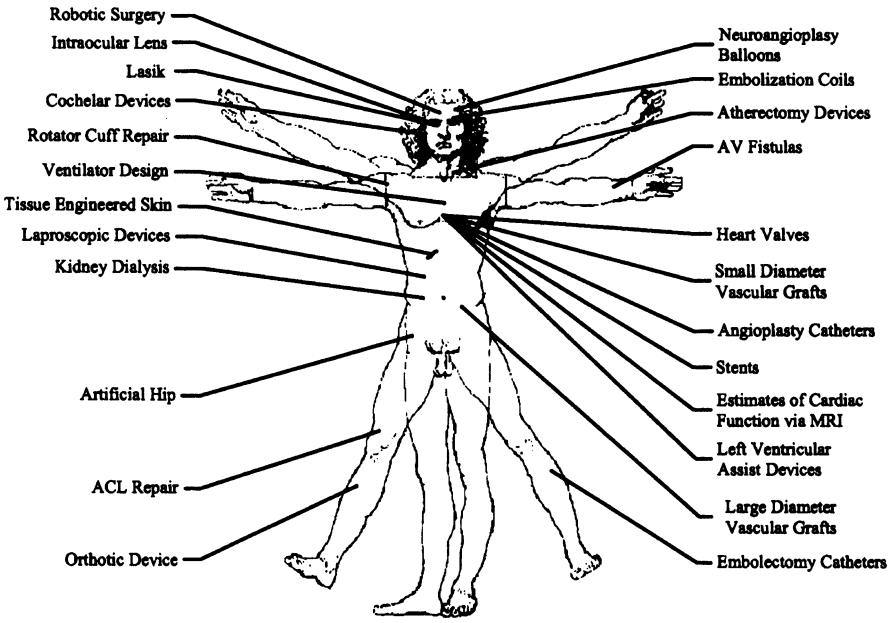

FIGURE 1.3 A schema of da Vinci's man showing a few of the many different aspects of human physiology, pathophysiology, and injury that can be addressed using biomechanics.

wherein mechanics plays a key role: from understanding why abdominal aortic aneurysms rupture, to identifying the failure strength of the anterior cruciate ligament (ACL) in an elite athlete, which must be protected during training and competition; from designing an artificial heart valve that must open and close over 30 million times per year, to understanding why artificial hip implants loosen over time and cause pain; from understanding what pressure must be applied to an angioplasty balloon to open a diseased artery, to understanding how deep and how many incisions should be made to modify the curvature of the cornea to correct for visual problems; from understanding the role of stresses in biological growth for the purpose of engineering tissue replacements, to designing a mechanical ventilator for those in respiratory distress; from using computer-aided modeling to guide robotic-assisted surgery, to designing needles that induce less damage to the arterial or venous wall; from designing an orthotic device for supporting an injured limb, to specifying a rehabilitation schedule that promotes tissue healing. In these and many, many other cases, biomechanics plays vital roles in the research laboratory, biomedical device industry, and hospital on a daily basis.

1.3 What Is Continuum Mechanics?

The functioning of the body and, likewise, the success of many clinical interventions depend on chemical, electrical, mechanical, and thermal processes. Nevertheless, we shall focus herein solely on the mechanics. Recall, therefore, that classical physics is typically thought to consist of a number of related areas of study: acoustics, electromagnetics, mechanics, optics, and thermodynamics. Thus, most of classical physics is concerned with the behavior of matter on a "natural" scale of observation or experience. Although its foundations and applications continue to be vibrant areas of research, the fundamental ideas upon which classical physics rests (due to Gibbs, Huygens, Maxwell, Newton, and others) were identified prior to the twentieth century. In contrast, modern physics is concerned primarily with phenomena at "extreme" scales of observation and thus includes atomic (or nuclear) physics, low-temperature physics, quantum mechanics, and relativity. Clearly, biomedical engineering is supported by, and relies on, both classical and modern physics. Without the latter, important diagnostic tools such as CAT (computerized axial tomography) scans and MRI (magnetic resonance imaging) would not be possible. In this introductory text, however, we shall rely solely upon classical mechanics.

Classical mechanics is typically thought to offer two basic approaches: continuum mechanics and statistical mechanics. Consider, for example, a simple glass of water at room temperature. On the natural scale of observation, we see and can think of the water as a continuous medium. In reality, however, we know that water is a collection of discrete, interacting molecules composed of hydrogen and oxygen atoms, and we know that there are gaps between the H_2O molecules and even gaps between the electrons and nucleus of each of the atoms. In statistical mechanics, we attempt to describe the (statistical) mean behavior of the individual molecules so as to understand gross behaviors on a natural scale of observation. In continuum mechanics, we also consider a volume-averaged mean behavior, but one that is independent of any consideration of the individual molecules. Perhaps a good example that illustrates when the continuum and statistical approaches are each useful is the analysis of drag on the Saturn V rocket that carried the *Apollo* spacecraft into space. When the rocket took off, the drag due to the frictional interaction between the surface of the rocket and the molecules of the air could be studied within a continuum context because there were so many closely spaced molecules that a gross, volume-averaged description of their properties was meaningful. In the upper atmosphere, however, the molecules of the air may be far enough apart that one should consider statistically their individual behaviors. In other words, *the continuum assumption (or hypothesis) tends to be reasonable when* $\delta/\lambda \ll 1$, where δ is a characteristic length scale of the microstructure and λ is a characteristic length scale of the physical problem of interest. For the rocket, δ may be the distance between the individual molecules of the air and λ the diameter of

the rocket. In this case, the ratio of δ/λ is much less than 1 near the ground but perhaps on the order of 1 in the upper atmosphere. With regard to biomechanics, consider the following. If one is interested, for example, in the forces felt by cells (on average) within the wall of a large artery due to the distending blood pressure, the characteristic length scales would be micrometers (μm) for the microstructure (e.g., size of the cell and diameters of the fibers in the extracellular matrix) and millimeters (mm) for the physical problem (wall thickness). Thus, $\delta/\lambda \sim \mu m/mm \sim 0.001$ which is much less than 1 and the continuum assumption would be expected to be reasonable. Similarly, if one is interested in the velocity of blood at the centerline of a large artery, the characteristic length scales would again be micrometers for the microstructure (diameter of a red blood cell) and millimeters for the physical problem (luminal diameter), and again $\delta/\lambda \ll 1$. The situation would be very different in a capillary, however, wherein $\delta/\lambda \sim 1$ because the diameter of the red blood cell and capillary are both about 5–8 μm. We shall see throughout this text that the continuum assumption tends to be very useful in a wide variety of problems of design and analysis in biomechanics; hence, it is adopted throughout. Nevertheless, we are well advised to remember the following: "Whether the continuum approach is justified, in any particular case, is a matter, not for the philosophy or methodology of science, but for experimental test" (Truesdell and Noll 1965, p. 5). In other words, the utility of any of our designs or analyses must first be checked in the laboratory.

Recall, too, that matter is typically thought to exist in one of three phases: solid, liquid, or gas. Mechanics tends to be divided along these lines into *solid mechanics* and *fluid mechanics*, where fluid mechanics includes the study of both liquids and gases. That is, one can define a fluid as a substance that assumes (within short times) the shape of the container in which it is placed, whereas a solid tends to resist such shape changes unless so forced. Referring to Fig. 1.4, therefore, note that solid and fluid mechanics are generally studied in the order of increasing complexity, which has (artificially) given rise to subfields of study. Although no solid is rigid, the assumption of a rigid body can lead to many useful designs and analyses, as, for example, in satellite dynamics. Likewise, all fluids resist the forces that cause them to deform, or flow. Again, however, neglecting this intrinsic resistance to flow (or, viscosity) can lead to many useful engineering solutions, particularly in aerodynamics. Hence, despite being based on unrealistic assumptions, rigid-body solid mechanics and inviscid fluid mechanics are both useful and convenient starting points for study.[1]

[1] It is assumed herein that the student has had an introduction to mechanics, which typically covers rigid body statics and sometimes dynamics. If not, a brief review of statics is found in Appendix 1.

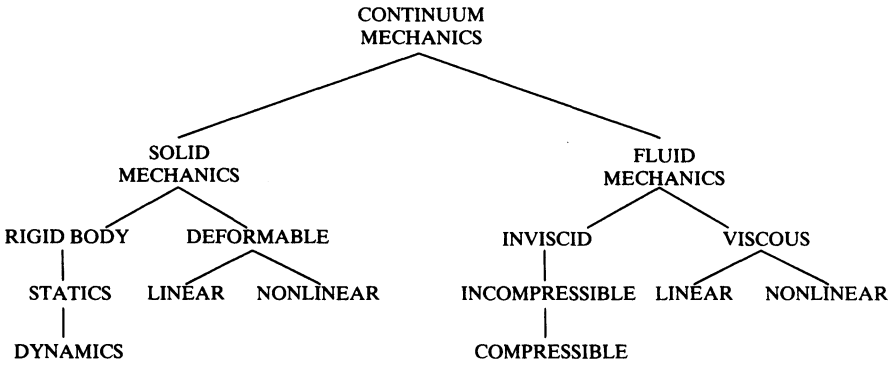

FIGURE 1.4 Flowchart of traditional divisions of study within continuum mechanics. Note that solid mechanics and fluid mechanics focus primarily on solidlike and fluidlike behaviors, not materials in their solid versus fluid/gaseous phases. Note, too, that linear and nonlinear refer to material behaviors, not the governing differential equations of motion. As we shall see in Chap. 11, many materials simultaneously exhibit solidlike (e.g., elastic) and fluidlike (e.g., viscous) behaviors, which gives rise to the study of viscoelasticity and the theory of mixtures, both of which are important areas within continuum biomechanics.

Our focus herein is on deformable solids and viscous fluids, for which it is often convenient to study separately the linear and nonlinear behaviors (Fig. 1.4), which give rise to additional subfields of study such as elasticity and plasticity (in solid mechanics) or Newtonian and non-Newtonian fluid mechanics. Although many problems in biomechanics necessitate dealing with the complexities associated with nonlinear behaviors (e.g., the stiffening response of soft tissues to increasing loads or the flow-dependent viscosity of blood), we shall focus primarily on the linear behavior of both solids and fluids. Not only do such problems serve as a natural preparation for the consideration of the more complex problems found in advanced courses, but many solutions to linear problems are fundamental to clinical and industrial applications as well as to basic research. For an introduction to nonlinear cardiovascular solid mechanics, see Humphrey (2002).

1.4 A Brief on Cell Biology[2]

The word "cell" comes from the Latin *cellulea*, meaning "little rooms." This terminology was coined by Hooke (1635–1703) who was perhaps the first to describe a cellular structure, which in his case was remnant cell walls in a thin

[2] Much of Sects. 1.4 and 1.5 are from Humphrey (2002).

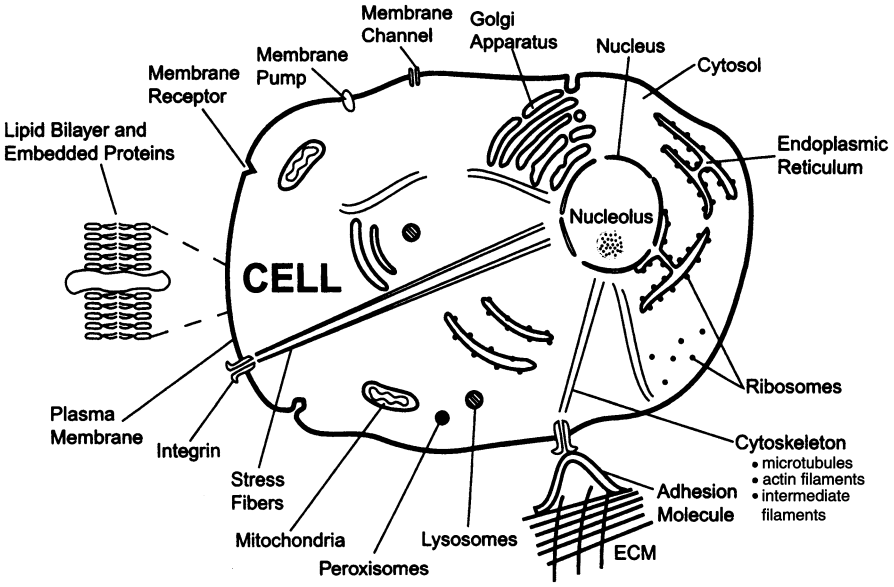

FIGURE 1.5 Schema of a mammalian cell showing its three primary constituents: the cell membrane (with various receptors, pumps, channels, and transmembrane proteins), the cytoplasm (including many different types of organelles, the cytoskeleton, and the cytosol), and the nucleus. From a mechanics perspective, the three primary proteins of the cytoskeleton (actin, intermediate filaments, and microtubules) are of particular importance. [From Humphrey (2002), with permission.].

slice of cork. Today, by the word "cell," we mean "the fundamental, structural, and functional unit of living organisms" (Dorland's Medical Dictionary 1988). For a detailed discussion of cell biology, see the wonderful work by Alberts et al. (2008) or similar texts; here, we simply offer a brief introduction.

Most cells consist of various organelles (i.e., organized structures having specific functions), the cytosol, the cytoskeleton, and an outer membrane (Fig. 1.5). The most conspicuous organelle is the nucleus, which contains the genetic information, chromosomal DNA. The nucleus consists of its own porous membrane or envelope, which mediates all transport into and out of the nucleus, a nucleoplasm that contains a fibrous scaffold, and a nucleolus that produces the ribosomes that are responsible for translating mRNA data for protein synthesis. The primary functions of the nucleus, therefore, are to archive and replicate the genetic code as needed to direct cellular activity. Whereas the cells in a given organism contain the same genetic information (the genotype), each cell does not "express" the same genes. The genes that are expressed define the phenotype; hence, skin cells are different from bone cells and so on. That cells are able to express different genes in response to changing external stimuli, particularly mechanical loads, will prove to be very important in biomechanics

and, thus, is discussed separately in Sect. 1.6. Other organelles within a cell include the mitochondria, endoplasmic reticulum (rough and smooth), and the Golgi apparatus. Mitochondria provide the cell with usable energy by oxidizing foodstuffs (e.g., sugars) to make adenosine triphosphate (ATP). A typical cell may have over 1,000 distributed mitochondria, which, together, may constitute up to one-fourth of the total cell volume. The rough endoplasmic reticulum represents an interconnected space that specializes in the synthesis of proteins; it connects to the outer portion of the nuclear membrane and is intimately associated with ribosomes—carriers of the RNA. The smooth endoplasmic reticulum is tubular in structure; although it aids in the packaging of proteins, it specializes in the synthesis of lipids and steroids. The Golgi apparatus plays a key role in the synthesis of polysaccharides as well as in the modification, packaging, and transport of various macromolecules; this transport includes secretion into the extracellular space. In addition to these organelles, which are responsible for the conversion of energy or processing of products, lysosomes and peroxisomes are responsible for the degradation of various substances within the cell. Lysosomes are capable of digesting proteins, carbohydrates, and fats and thereby aid in both the breakdown of foodstuffs and the removal of unnecessary cellular components. With an internal pH of about 5, lysosomes accomplish this degradation via various acidic enzymes, including nucleases, proteases, and lipases. Peroxisomes are capable of generating and degrading hydrogen peroxide, which is cytotoxic, and they assist in the detoxification of other compounds (e.g., formaldehyde). Of course, cells also ingest extracellular substances via a process called phagocytosis, which facilitates a controlled intracellular degradation by the lysosomes and peroxisomes. A controlled degradation of "old" constituents plays an important role in the biomechanics of tissue maintenance, adaptation, and wound healing.

The cytoplasm is defined as that part of the interior of the cell that does not include the nucleus. Thus, it consists of all the other organelles, the cytoskeleton, and the cytosol. The cytosol constitutes up to one-half of the total cell volume and consists primarily of water.[3] The cytoskeleton consists primarily of three classes of filamentous proteins: actin, which is often the most abundant protein in a cell; microtubules, which are formed from tubulin; and intermediate filaments, which include vimentin, lamins, and keratins. These cytoskeletal filaments have diameters of 5–25 nm and they can polymerize to form linear units that span distances between organelles or even over the entire length of a cell. Collectively, these filamentous proteins along with hundreds of different types of accessory proteins endow the cell with much of its internal structure, they aid in cell division, they enable cell mobility, and they maintain cell shape. The cytoskeleton is thus fundamental to cell mechanics. Moreover, much of the

[3] Note: 70 % of the total cell volume is due to water.

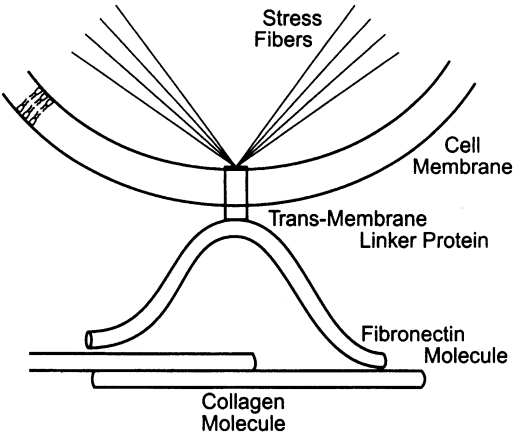

FIGURE 1.6 Schema of some of the constituents that participate in cell–matrix interactions that are important to the mechanobiology. The transmembrane protein that "links" the cytoskeletal (e.g., stress fibers) and extracellular (e.g., fibronectin and collagen) proteins is often a member of the family of integrins. [From Humphrey (2002), with permission.].

water and other proteins within the cytosol are bound to the cytoskeleton, which aids in the selective positioning or movement of components within the cell. The cytoskeleton is a dynamic structure, continually reorganizing to meet the needs of the cells. For example, the intermediate filaments can increase in density in response to increased mechanical stress. Likewise, stress fibers consisting of temporary bundles of actin often form within fibroblasts. They serve to connect the strong network of intermediate filaments that surround the nucleus to the plasma membrane at sites where it is connected to the extracellular matrix via transmembrane linker proteins (e.g., integrins). This arrangement (Fig. 1.6) may allow the stress fibers to transduce the level of tension in the extracellular matrix to the nucleus and thus to control gene expression (i.e., mechanotransduction). Conversely, stress fibers in fibroblasts also allow them to exert tension on the extracellular matrix, which is particularly useful during morphogenesis or repair in wound healing. Understanding the mechanics of growth and remodeling is one of the most important open problems in biomechanics at this time; this general area is discussed more in Sect. 1.6.

Note that striated muscle (e.g., that makes up the myocardium of the heart wall or skeletal muscle) contains an additional, specialized intracellular constituent—the myofibril. These contractile elements are approximately 1–2 μm in diameter, they span the length of the cell, and they consist of a chain of shorter (2.2 μm) units, called sarcomeres. According to the sliding filament model proposed in 1954, sarcomeres consist of overlapping thin (actin) and thick (myosin) filaments. It is thought that the myosin has tiny "cross-bridges"

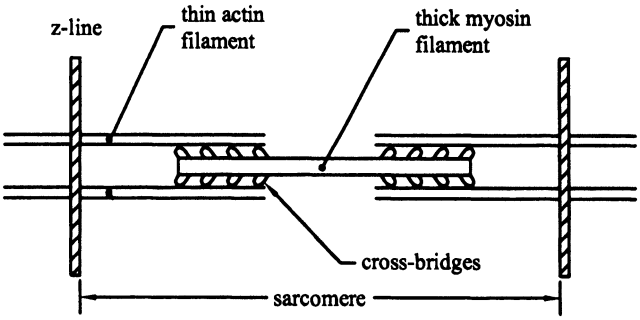

FIGURE 1.7 Schema of the cross-bridge mechanism that is thought to control the contraction and relaxation of muscle. In particular, the cross-bridges allow a ratcheting motion between the thick myosin filaments and the thin actin filaments. Calcium plays a key role in this process.

that attach, detach, and reattach in a ratcheting fashion with the actin, which thereby produces movement associated with the contraction of muscle (Fig. 1.7). Smooth muscle cells similarly rely on actin–myosin interactions although they do not have a sarcomere structure. Thus, studying the biomechanics of muscular organs such as the heart, blood vessels, diaphragm, or uterus as well as studying locomotion at the organism level all require an understanding of the associated cell biology.

The cell membrane separates the cellular contents from their surroundings. It consists primarily of a phospholipid bilayer with embedded proteins and is on the order of 5 nm thick (cf. Fig. 1.5). Held together by noncovalent bonds, this membrane is described in biology texts as having "fluidity"; that is, the lipid molecules can exhibit rapid lateral diffusion, which is to say that they can readily exchange places with each other. It appears that this fluidity endows the membrane with a self-sealing capability and it plays a role in some processes of transport across the membrane (e.g., ion transport facilitated by glycolipids). The embedded proteins likewise play many roles: they may participate in the conduction of electrical signals or the transport of various substances across the membrane by serving as selective channels, gates, and pumps. Alternatively, these proteins may serve as enzymes to catalyze specific reactions, they may act as selective receptors that bind extracellular substances to the cell membrane, or they may serve as anchors for the attachment of intracellular cytoskeletal filaments or extracellular proteins to the membrane (Fig. 1.6). The latter is accomplished primarily via a special class of transmembrane proteins, the integrins, which consist of two noncovalently associated glycoproteins referred to as α and β units (there are at least 14 different α units and 9 different β units). Some integrins bind to specific proteins (e.g., laminin or fibronectin), whereas others bind to multiple proteins by recognizing a

particular amino acid sequence (e.g., arginine–glycine–aspartic acid, or RGD). Integrins are found in large numbers, but their binding to a particular ligand tends to be weak. This would be advantageous in cell migration, for example, wherein local adhesion would be short-lived. Cells can regulate the activity of their integrins, and, conversely, gene expression can be mediated by the extracellular matrix via the integrins. Finally, note that some of the embedded membrane proteins are decorated with carbohydrates; this glycocalyx, or "sugar coat," appears to protect the cell from mechanical and chemical damage and may participate in certain transient adhesion processes.

Cells can be interconnected via three types of junctions: occluding, or tight, junctions seal cells together; anchoring junctions mechanically attach cells to other cells or extracellular matrix at specific sites; and communicating (e.g., gap) junctions allow cell-to-cell exchange of electrical or chemical signals. At any particular instant in the mature organism, most cells are simply performing their primary function (e.g., muscle cells are contracting and fibroblasts are synthesizing extracellular matrix). Nonetheless, normal tissue maintenance also typically requires a delicate balance between continuous cell replication and cell death; in the adult, for example, millions of cells are produced each minute to replace cells that are damaged, killed, or simply experience a normal cell death (apoptosis). Of course, cells reproduce by duplicating their contents and dividing in two. Although we will not consider the details of the cell cycle (see Alberts et al. 2008), note that it appears that cells require multiple external signals before they will divide. Growth factors, for example, are special proteins that bind to specific receptors on the cell membrane and encourage cell division. According to Gooch et al. (1998),

> Growth factors can stimulate or inhibit cell division, differentiation, and migration. They up- or down-regulate cellular processes such as gene expression, DNA and protein synthesis, and autocrine and paracrine factor expression. [They] ... can interact with one another in an additive, cooperative, synergistic, or antagonistic manner. They may cause dissimilar responses when applied to different cell types or tissues, and their effect on a certain type of cell or tissue may vary according to concentration or time of application.

Among the over 50 different growth factors in humans are the platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), and transforming growth factors (TGFs). Mechanical stresses and injuries have both been shown to modulate the secretion of growth factors; hence, tissues that normally have a slow turnover of cells (replication and death) can experience rapid increases in turnover in response to certain mechanical stimuli. Understanding and quantifying these homeostatic control mechanisms is a newly identified, important topic in biomechanics and mechanobiology.

This is but a cursory introduction to the general structure and function of the cell, yet it serves as sufficient motivation for our purposes. Of course,

in most cases, we will not be interested in a single cell, but rather large populations of communicating cells. In this regard, the role of the extracellular matrix, in which most cells are embedded, is of utmost importance. Let us now consider this important component in more detail.

1.5 The Extracellular Matrix

It is axiomatic in continuum mechanics that the properties of a material result from its internal constitution, including the *distributions, orientations,* and *interconnections* of the constituents. Examination of microstructure is essential, therefore, for quantifying the mechanical behavior and analyzing the internal distribution of forces. In most tissues and organs in the body, the microstructure depends largely on the extracellular matrix (ECM).

The ECM serves multiple functions: it endows a tissue with strength and resilience and thereby maintains its shape; it serves as a biologically active scaffolding on which cells can migrate or adhere; it may regulate the phenotype of the cells; it serves as an anchor for many proteins, including growth factors and enzymes such as proteases and their inhibitors; and it provides an aqueous environment for the diffusion of nutrients, ions, hormones, and metabolites between the cell and the capillary network. In many respects, therefore, it is the ECM that regulates cell shape, orientation, movement, and metabolic activity. It is the cells (e.g., fibroblasts), however, that fashion and maintain the ECM. Hence, the ECM and cells have a strong symbiotic relation.

The ECM consists primarily of proteins (e.g., collagen, elastin, fibronectin, and laminin), glycosaminoglycans (GAGs), and bound and unbound water (Fawcett 1986; Ayad et al. 1994; Ninomiya et al. 1998; Alberts et al. 2008). The GAGs are often bound covalently to protein cores, thus forming proteoglycans. Although collagen was long regarded to be a single protein, more than 25 distinct forms have been identified. Collectively, the collagens are the most abundant protein in the body (∼25–30 % of all protein), common forms being types I, II, III, and IV, as well as types V, VI, and VIII. Types I and III form fibers and provide structural support in tension; they are found in tendons, skin, bone, the heart, arteries, and cornea. Type II collagen occurs as fibrils; it is found largely in cartilage, which also contains significant proteoglycans. Type IV collagen forms as a porous network (basement membrane) that acts as a scaffolding for epithelial and endothelial cells (adhesion being aided by fibronectin or laminin); it is found, for example, in the lens capsule of the eye as well as in the inner layer of blood vessels. Types V and VI collagen tend to associate with smooth muscle cells, whereas type VIII tends to associate with endothelial cells. For more on the collagens, see Kucharz (1992).

Synthesized by various cells (Fig. 1.8), the collagen molecule consists of three polypeptide α chains, each containing 1,300–1,700 amino acid residues.

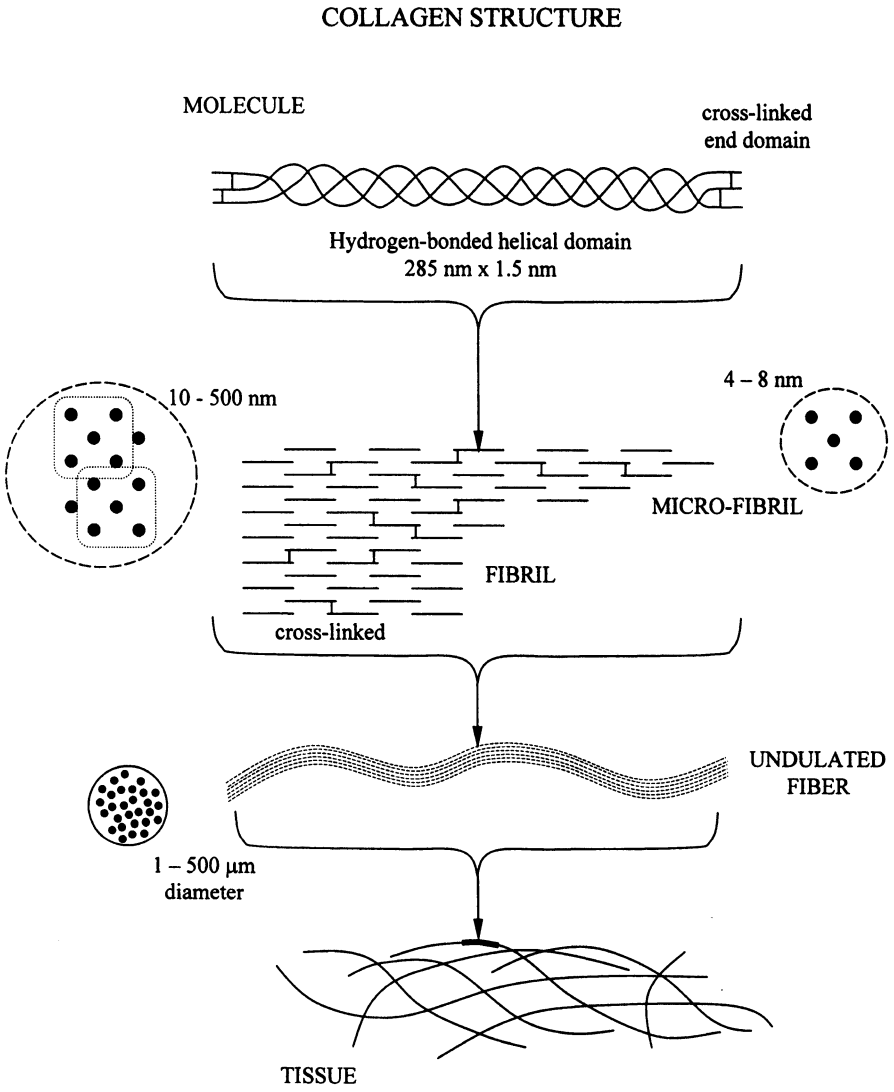

FIGURE 1.8 Schema of collagen at different levels of organization: from the triple-helix molecule consisting of three α-helices of repeating triplets of amino acids (G-X-Y), where G is glycine and X and Y are often proline or hydroxyproline, to an undulated fiber that could be found in arteries, cartilage, cornea, the heart, lungs, skin, tendons, and many other tissues. [From Humphrey (2002), with permission.].

The majority of these residues (~1,000–1,400) are organized into a central triple-helix motif (Ayad et al. 1994), which is on the order of 285 nm long and 1.5 nm in diameter. The triple helix results from the repetition of a triplet of amino acid residues of the form $(G\text{-}X\text{-}Y)_n$, where *G* stands for Glycine,

the simplest amino acid, and *X* and *Y* may be any of the other 19 common amino acids, although often proline or hydroxyproline. The triple-helix structure is stabilized by abundant interchain hydrogen bonds, many via the hydroxyprolines. Intramolecular covalent cross-links in or near the nonhelical ends of the molecule provide further structural stability, often via hydroxylysine. Type IV collagen also has extensive disulfide bonds. Details on the biosynthesis of collagen can be found in Nimni (1992) and Kucharz (1992); details on the chemical structure can be found in Ayad et al. (1994).

Vascular type IV collagen is synthesized, for example, by endothelial cells, whereas types I and III collagen are synthesized by fibroblasts and smooth muscle cells; it takes the cell on the order of 10–60 min to synthesize a complete intracellular collagen precursor, called procollagen (Nimni 1992). Following secretion by the cell, newly synthesized type I collagen molecules undergo extracellular modifications prior to assembly (polymerization) into 4–8-nm-diameter microfibrils consisting of repeating quarter-staggered (which gives the characteristic 67-nm periodicity) groups of four to five molecules in cross section. This assembly results from electrostatic and hydrophobic bonding (which liberates previously bound water) between molecules. The specific directional assembly may be aided by narrow extracellular channels within the plasma membrane of oriented cells [e.g., the fibroblast (Birk et al. 1989)]. Note, therefore, that the orientation of cells appears to be governed by the local force field (Carver et al. 1991) and so too for the orientation of the collagen [e.g., in tendons, the collagen tends to be oriented uniaxially, whereas in skin, it is distributed primarily in a two-dimensional (2-D) fashion]. The microfibrils, in turn, are organized into successively larger fibrils (∼10–500 nm in diameter) and ultimately fibers (1–500 μm in diameter), the specific diameter of which is also thought to be dictated largely by the mechanical force field in the ECM. The extracellularly organized fibrils and fibers are stabilized by interchain cross-links that occur primarily through the conversion of lysine and hydroxylysine (in the nonhelical portion of the molecule), via the enzyme lysyl oxidase, into peptide-bound aldehydes. Further aldehyde cross-linking of collagen is important industrially with regard to the engineering of bioprosthetic heart valves, which must exhibit sufficient biocompatibility, strength, and efficiency as a valve. The need to understand the microstructure, which governs these characteristics, is thus clear. Finally, note that additional cross-links also form in type III collagen via intermolecular disulfide bonds. Cross-links can be either reducible or nonreducible; reducible cross-links can be broken, for example, during thermal treatment.[4] Overall, the degree of cross-linking tends to increase with age, which results in concomitant stiffening;

[4] Advances in laser, microwave, and radio-frequency technologies continue to encourage new uses of thermal energy to treat disease and injury (Humphrey 2003b).

pathological stiffening, via the addition of glycosylated cross-links, occurs in diabetes. Note, too, that collagen fibers are usually undulated at physiologic loads; thus, they exhibit their true stiffness only when straightened under the action of applied loads. For example, the tensile strength of nearly straight uniaxially oriented type I collagen in tendons can be 100 MPa in mature tissue.

Finally, the half-life of collagen varies tremendously throughout the body: it is only a few days in the periodontal ligament but typically many months in tendons and possibly years in bones.[5] In the cardiovascular system, the half-life of collagen is on the order of 15–90 days. Regardless of the specific half-life, maintenance of physiologic levels of collagen depends on a delicate balance between continual synthesis and degradation, the kinetics of which is complex but may be assumed to be of first order (Niedermuller et al. 1977; Gelman et al. 1979). Degradation can be accomplished by blood-plasma-borne serine proteases, the extracellular release of matrix metalloproteinases (MMPs), as, for example, by macrophages or via intracellular lysosomal activity within phagocytotic fibroblasts (Ten Cate and Deporter 1975). As noted earlier, phagocytosis can be a highly selective mechanism of degradation. It also appears that much of the synthesized collagen is degraded prior to its incorporation into the ECM (McAnulty and Laurent 1987). The reason for this is not clear, but may simply reflect an internal mechanism for culling imperfectly synthesized molecules (i.e., a cellular quality control). *In response to disease or injury, however, the rates and control of the continual degradation and synthesis can change dramatically,* as needed. Wound healing in skin is a prime example of an accelerated turnover of collagen, which in this case may result in a collagenous scar.

Strictly speaking, elastic fibers in the ECM consist of two components—one microfibrillar (10 nm in diameter) and one amorphous. Whereas the former consists of multiple glycoproteins, the amorphous (major) portion is called elastin. It consists of a polypeptide chain of ∼786 amino acid residues, the majority of which are glycine, alanine, and proline. Elastin is synthesized in minutes, as the precursor tro proelastin, via normal pathways—mRNA, endoplasmic reticulum, Golgi apparatus, and so forth. Moreover, it appears that synthesis can be assumed to be a first-order process, one that is completed in less than 1 h (Davidson and Giro 1986). In the vasculature, this synthesis is accomplished primarily by smooth muscle, but also by specialized fibroblasts and, perhaps, endothelial cells. Once secreted into the extracellular space, the soluble tropoelastin is cross-linked to form the insoluble (stable) elastin meshwork. Two unique amino acids, desmosine and isodesmosine, are largely responsible for the formation of distributed covalent cross-links between the

[5] In contrast, many cellular proteins have half-lives of hours or days (Alberts et al. 2008).

relatively loose and unstructured chains. It is the loose, amorphous, but highly cross-linked structure of elastin which results in a meshwork that exhibits an elastic (i.e., nondissipative or recoverable) response over large deformations (indeed, elastin appears to be the most elastic protein in the body). Moreover, a high concentration of nonpolar hydrophobic amino acids renders elastin one of the most chemically, thermally, and protease-resistant proteins in the body. Indeed, in contrast to collagen, the turnover of elastin is much slower in the adult, perhaps on the order of years to decades (Lefevre and Rucker 1980). Much of the production of elastin occurs during development. The protease elastase, which can be secreted by macrophages, is capable of degrading elastin, however. Such degradation appears to play a role in the formation of aneurysms in the vasculature. For more details on elastin, see Robert and Hornebeck (1989).

Elastic fibers appear to consist of aggregated 10-nm-diameter microfibrils embedded in the amorphous elastin. These fibers can be from 0.2 to 5.0 μm in diameter, and they tend to branch and form networks or sheets. When straight, elastic fibers can experience uniaxial extensions of 150 % without breaking (compared to less than 10 % for collagen), and they return to their original configuration when unloaded. Indeed, it has been said that the primary role of elastic fibers is to store and then return mechanical energy.

Other important components of the ECM include the aforementioned fibronectin and laminin, both of which play important roles in cell adhesion (cf. Fig. 1.6). Fibronectin consists of ∼2,476 amino acid residues; it is a widely distributed glycoprotein—synthesized by fibroblasts, endothelial cells, and smooth muscle cells—that mediates cellular interactions and migration. For example, fibronectin binds fibroblasts to underlying collagen substrates, thereby playing an important role in normal development, growth, remodeling, and wound healing. It may likewise play a role in the aggregation of platelets. The ability of fibronectin to bind to different proteins and cells is due to the presence of different binding sites, which depend in part on the aforementioned RGD sequence. The laminins constitute a family of large glycoproteins (over 3,000 amino acid residues) that are associated with the basement membrane; they self-assemble into a feltlike sheet. Laminin, one of the first proteins produced in the embryo, has numerous functional binding domains, as, for example, for heparan sulfate, type IV collagen, and various cells. Hence, like fibronectin, this protein plays an important role in the migration and anchoring of cells.

Proteoglycans represent a relatively small portion of the ECM in most tissues and have no preferred structural organization; they play important roles nonetheless. Proteoglycans consist of a core protein to which is attached multiple glycosaminoglycan (GAG) chains via covalent bonds. GAGs are linear polymers that contain repeating disaccharide units, the principal ones being hyaluronan, chondroitin sulfates, dermatan sulfates, keratan sulfates, heparan

sulfates, and heparin. Because GAGs tend to occupy large volumes compared to their mass, and because they are highly negatively charged, they tend to imbibe considerable water into the ECM. Water, in turn, enables many of the necessary diffusive processes within the ECM and enables the tissue to withstand compressive loads (this is particularly important in cartilage). Moreover, hyaluronan, for example, gives the aqueous portion of the ECM its fluidlike consistency, or viscosity. It is for this reason that the nonfibrous portion of the ECM is often referred to as an amorphous ground substance or gel matrix.

Referring to Sect. 1.4, note that the core protein of the proteoglycan is made on membrane-bound ribosomes and transported to the endoplasmic reticulum. Upon passage to the Golgi apparatus, GAGs are affixed to the core and possibly modified (Alberts et al. 2008). By associating with the fibrous proteins in the ECM, proteoglycans and individual glycosaminoglycans create a highly complex 3-D structure embodied with chemical reactivity and intercellular signaling pathways. For example, fibroblast growth factor (FGF) binds to heparan sulfate, which may not only localize the FGF, it may also activate it. The ubiquitous transforming growth factor (TGF) likewise binds to numerous proteoglycans. Similarly, proteases and protease inhibitors may bind to proteoglycans, thus localizing activity, inhibiting activity, or providing a storage mechanism for later use.

In addition to the binding of specific cells to fibronectin and laminin, recall from Sect. 1.4 that cell–matrix interactions are often mediated by the integrins. For example, the integrins that are connected to intracellular actin can "pull" on extracellular proteins to which they are bound. Alternatively, tensions in the ECM may be sensed by the nucleus of a cell via the ECM–integrin–cytoskeletal connections. It is through the integrins, therefore, that cells influence the ECM and the ECM may provide inputs for cell growth.

Finally, when discussing the extracellular matrix in tissue and organs, the role of fibroblasts cannot be overemphasized. Fibroblasts belong to the differentiated cell family known as connective tissue cells [other members in this family include osteocytes, chondrocytes, adipocytes, and smooth muscle cells (Alberts et al. 2008)]. Fibroblasts are the least differentiated member of this family and are found throughout the body. Their primary responsibility is regulation of the collagen-rich ECM. For example, in response to tissue damage, fibroblasts will quickly migrate to the site of injury, proliferate, and then synthesize new collagen. Such activity is regulated in part by growth factors, in particular FGFs and TGF-β. Likewise, macrophages are essential in regulating the ECM: they dispose of dead cells and degrade unneeded matrix material. Macrophages are mononuclear phagocytes that arise from stem cells in the bone marrow, enter the bloodstream as monocytes, and eventually enter tissues wherein they increase in size and phagocytic activity. Macrophages secrete a wide variety of products in addition to proteases, including coagulation factors, prostaglandins, and cytokines.

1.6 Mechanotransduction in Cells

As noted earlier, one of the most exciting and important recent findings in cell biology is that mechanical stimuli have a direct influence on gene expression in many different cell types. Such cells have been classified as *mechanocytes*, which include chondrocytes, endothelial cells, epithelial cells, fibroblasts, macrophages, myocytes, and osteoblasts. Consider, for example, the endothelial cell. Endothelial cells form a contiguous monolayer throughout the vasculature (Fig. 1.2). Because the luminal surface of the endothelial cell is decorated with the glycosaminoglycan heparan sulfate, it was long thought that these cells serve primarily as a smooth, nonthrombogenic surface that minimizes blood clots and thus facilitates blood flow. We now know that this is but one of the many functions of the endothelium. In response to local increases in blood flow, endothelial cells increase their production of nitric oxide (NO), a potent vasodilator; conversely, in response to local decreases in blood flow, endothelial cells increase their production of endothelin-1 (ET-1), a potent vasoconstrictor. That is, by altering its production of vasoactive molecules that diffuse into the wall and cause vascular smooth muscle cell relaxation or contraction, the endothelium is able to help control the diameter of the blood vessel in response to changing hemodynamic demands. Of course, sympathetic and parasympathetic signals as well as circulating hormones also contribute to the control of blood vessel diameter.

In addition to its mechanosensitive control of the production of vasoactive molecules (e.g., NO, ET-1), growth regulatory molecules (e.g., PDGF, FGF), cytokines (e.g., IL-1,6), and adhesion molecules (e.g., vascular cell adhesion molecule VCAM-1, monocyte chemoattractant protein MCP-1), the endothelium also changes its shape and ultrastructure in response to changing hemodynamic loads. In vivo and in vitro studies both reveal that these cells realign to follow the direction of the blood flow and they realign perpendicular to an applied uniaxial stretching of a substrate on which they are adhered (*Note*: whereas the flow of blood along the axis of an artery causes cells to align in the axial direction, the distending blood pressure stretches the vessel circumferentially, which, being perpendicular to the axial direction, also causes the cells to align in the axial direction; hence, these two effects are complementary.) Additionally, an increased blood flow induces an increase in the density of flow-aligned stress fibers (i.e., specialized actin filaments). For beautiful time-lapse figures of these changes, see Galbraith et al. (1998).

One of the key questions facing biomechanics and mechanobiology, therefore, is how are these many different changes effected? In other words, how does a cell sense a changing mechanical environment and how is this signal transduced to the nucleus wherein different genes are expressed? This question becomes more acute when we realize, for example, that vascular smooth muscle cells independently express different genes in response to the changing

hemodynamics even though they are not in direct contact with the pressure-driven blood flow. As noted by Zhu et al. (2000), which is a very readable, nice review of cell mechanics, critical questions are as follows: How do forces applied to a tissue distribute around the surface of a cell? How are these forces balanced within the interior of the cell? How does this internal force field induce a biological response? See, too, the review by Stamenovic and Ingber (2002). Although we do not have answers to these and similarly important questions, competing hypotheses and theories are under consideration. The student is encouraged to read, for example, the provocative paper by Ingber et al. (2000), which contrasts two ideas on how the intracellular forces balance the externally applied loads. One idea is based on tensegrity (tensional integrity), an architectural concept advanced by Buckminster Fuller wherein a metastable structure is constructed from self-equilibrating tensional and compressive elements; the other idea focuses on the combined fluidlike and solidlike behaviors exhibited by cells under different conditions. Clearly, there is a pressing need for more data on the mechanical properties of cells; fortunately, experimental tools such as laser tweezers (see Chap. 3) and the atomic force microscope (Chap. 5) allow increased insight into cell mechanics and, indeed, the various intracellular constituents, which includes actin filaments, intermediate filaments, microtubules, the plasma membrane, and even the cytosol. The need to understand cellular responses also leads naturally to a focus on molecular biomechanics (i.e., how individual molecules respond to applied loads). Zhu et al. (2000) point out, for example, that in response to applied loads, a molecule may rotate/translate, it may deform, or it may unfold/refold. By changing the conformation of a molecule, one can change its biochemical character, as, for example, the availability of binding sites. In summary then, there is a need for mechanics at all scales in biology—from the molecule to the cell to the organ to the organism. Although much is known, much remains to be discovered.

1.7 General Method of Approach

The biomechanical behavior of biological tissues and organs results from the integrated manifestation of the many components that constitute the structure and their interactions. Although we may not always be directly interested in cellular- or molecular-level phenomena, as, for example, when calculating the forces within the wall of an aneurysm to evaluate its rupture potential or when designing a wheelchair, some knowledge of the associated cell and matrix biology can always provide important insight. In the case of an aneurysm, its fibroblasts regulate the continuous production and removal of intramural collagen in response to changes in the intramural forces; in the case of the wheelchair, the skin may break down at the cellular level (e.g., decubitus ulcer)

in response to frictional forces, which must be designed against. Throughout this book, therefore, we will continually refer to the biology that motivates the mechanics.

Whereas Issac Newton (1642–1727) developed a "discrete" mechanics in which his fundamental postulates were assumed to apply to individual mass points (whether the Earth or an apple), Leonard Euler (1707–1783) showed that these same postulates apply to every mathematical point within a body. We submit, therefore, that *every continuum biomechanics problem can be addressed via the five fundamental postulates of continuum mechanics by specifying three things*: the geometry (i.e., the domain of interest), the constitutive relations (i.e., how the material responds to applied loads under conditions of interest), and the applied loads (or associated boundary conditions). Moreover, we agree with Fung (1990) and others that the key to success in this approach is often the identification of robust constitutive relations. We discuss specific constitutive relations in Chaps. 2, 6, 7, and 11. Here, however, simply note that there are five steps in every constitutive formulation[6]:

Delineate general characteristic behaviors
Establish an appropriate theoretical framework
Identify specific functional forms of the constitutive relation
Calculate the values of the material parameters
Evaluate the predictive capability of the final constitutive relation

Specifically, the first step is to *observe* the particular behaviors of interest and then, by *induction*, to delineate general characteristics of the material's response to the applied loads. In practice, this step is as difficult as it is critical. In many cases, the biomechanicist must distill the results from tens to hundreds of papers in the biological and clinical literature to delineate the underlying mechanism or general characteristics of importance. Once accomplished, one then attempts to formulate a general hypothesis and establish a theoretical framework; robust theories should rely on the axiomatic and *deductive* foundations of mathematics and mechanics. Two frameworks that we will consider in detail in this book are the theories of the linearly elastic behavior of solids and the linearly viscous behavior of fluids. Next, one must perform *experiments* to test the hypothesis or theory, which includes identification of specific functional relationships between quantities of interest and calculation of the values of the associated material parameters. Because of the unique behaviors exhibited by living tissues, performing theoretically motivated experiments may necessitate the design and construction of a novel experimental system or transducer. Moreover, based on comparisons to experimental data, one will often need to refine

[6] A former student suggested that these five important steps in a constitutive formulation are remembered easily via the acrostic DEICE.

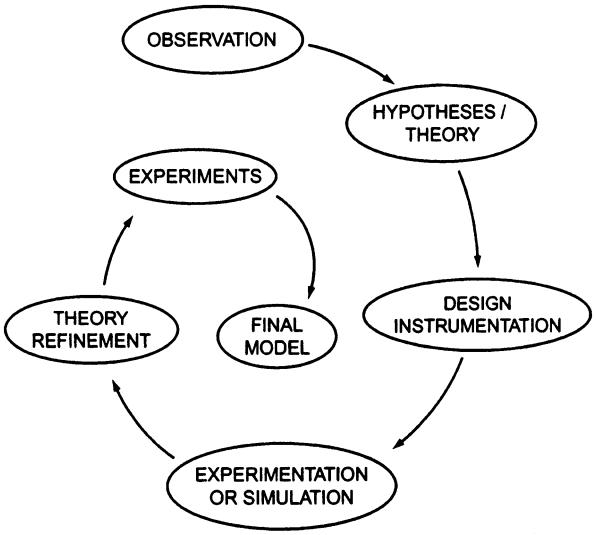

FIGURE 1.9 Illustration of the scientific approach employed by many in biomechanics. In particular, note that observations and experiments are equally important, but very different. The latter must be designed based on a hypothesis or theory for the purpose of testing an idea. Because science is "relative truth," we often need to iterate to improve our models of the physical and biological worlds.

the hypothesis or theory and then to perform additional experiments and data analysis. This iterative procedure continues until the associated constitutive relation has predictive capability, which must be verified against additional observations or experimental data. Only then can one begin to answer applied questions of interest, often via numerical simulations (i.e., computations) and then animal and clinical trials. See Fig. 1.9 for a summary of this overall approach, which is best appreciated via the examples that are provided in Chaps. 2–11. In conclusion, we emphasize that *a constitutive relation is but a mathematical descriptor of particular behaviors exhibited by a material under conditions of interest; it is not a descriptor of a material per se*. Hence, multiple theories will likely be needed to describe the myriad behaviors exhibited by a given molecule, cell, tissue, or organ under different conditions. Moreover, although we should always seek to understand and quantify the basic mechanisms by which responses to applied loads occur, this is often difficult or impossible; hence, we must sometimes rely on phenomenological descriptors or empirical correlations. Regardless of approach, the main goal of biomechanics must remain clear—to improve health care delivery via careful and appropriate design and analysis.