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Editors

International Centre for Mechanical Sciences

Biomechanical Modelling at the Molecular, Cellular and Tissue Levels

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INTERNATIONAL CENTRE FOR MECHANICAL SCIENCES

COURSES AND LECTURES - No. 508

BIOMECHANICAL MODELLING AT THE MOLECULAR, CELLULAR AND TISSUE LEVELS

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PREFACE

This volume consists of Lecture Notes based on lectures delivered at the Advanced School on "Biomechanical Modelling at the Molecular, Cellular and Tissue Levels" held at the International Centre for Mechanical Sciences (CISM) in Udine, Italy, in the period September 11–15, 2006. The course was presented by 6 lecturers, 2 from Europe, 3 from the USA and 1 from New Zealand, and was attended by more than 100 participants from 23 countries.

The mechanics of biological structures at the molecular, cellular and tissue levels is a multidisciplinary area of research that is expanding rapidly and brings together researchers in biology, medicine, engineering, physics, chemistry, materials science and applied mathematics. Against this background, the aim of the course was to present a state-of-the-art overview of biomechanical modelling at the molecular, cellular and tissue levels, with particular reference to nanostructures, cells, growth and remodelling, and the cardiovascular system, including experimental, continuum mechanical, computational and simulation aspects. This provides a rational basis for applications to, for example, (i) tissue engineering, which aims to identify the critical structural and mechanical requirements needed for each tissue construct, (ii) the design and development of implants, and (iii) the improvement of diagnostics and therapeutical procedures that involve tissue mechanics.

The lectures pointed out that diverse soft tissues and cells exhibit complex adaptations (and maladaptations) in response to changes in their chemomechanical environment for which mathematical models are needed to integrate information from the databases on such adaptations. In particular, a theoretical (mixture) framework for the description of growth and remodeling in biological tissues was presented with examples on organ culture, fusiform aneurysms and cell mechanics. The course also included treatment of passive and active cardiac mechanics with emphasis on the histological structure of the heart wall, on an orthotropic microstructurally-based constitutive model for the myocardium, on remodelling phenomena and on a three-dimensional finite element model of the heart, the aim here being to accurately predict the mechanical changes in the left and right ventricular myocardia during the cardiac cycle.

An outline of the nonlinear theory of elasticity provided a framework for analyzing the mechanical properties of soft biological tissue, with special emphasis on the properties of arterial wall tissue and the fibrous structure of its constituent layers. Specific constitutive laws were introduced, and the theory was illustrated by application to the problem of extension and inflation of an artery. Attention was also given to questions of convexity and strong ellipticity of the constitutive laws that are important for assessing the suitability of the models from the mathematical, mechanical and computational points of view.

A section of the course dealt with the mechanical function of human arterial tissue in health and disease and focused on providing data on their general mechanical characteristics. Explicit expressions for the arterial stress response were presented in a form appropriate for the implementation in nonlinear finite element programs. Aortic dissections were discussed, in particular the failure properties of the aorta. In addition, dissection-type failure following balloon angioplasty was investigated as a prototype example on a constitutive and numerical basis for particular stents, and for patient-specific stenoses.

We have pleasure in thanking our colleagues, Jay D. Humphrey and Peter J. Hunter, for presenting their lectures and for preparing chapters for this volume. We also thank Ming Dao (who stood in at short notice in place of Subra Suresh) and Gang Bao for contributing lectures to the course. To the participants, who contributed to lively discussions we offer our grateful thanks. Special thanks are due to the Rector of CISM, Professor Giulio Maier, for his encouragement, enthusiasm and hospitality, and to Professor Paolo Serafini, Executive Editor of CISM, for his encouragement to publish these lecture notes. The assistance of the office staff at CISM was also much appreciated.

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CONTENTS

Need for a Continuum Biochemomechanical Theory of Soft Tissue and Cellular Growth and Remodeling

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Abstract. This chapter is motivated by the observation that diverse soft tissues and cells exhibit remarkable adaptations in response to changes in their chemomechanical environment, and that there is a pressing need for mathematical models to integrate information from the rapidly expanding data bases on such adaptations. Although both the biological motivation and the theoretical framework presented herein apply generally to soft tissues and cells, ideas are illustrated by focusing on the vasculature. Toward this end, note that it has been said by many in different ways, but it was said particularly well by Malek and Izumo (1992): 'The blood vessel is no longer considered to be simply a non-thrombogenic passive conduit for blood flow. Rather, it is increasingly viewed as a continually-adapting, physically and chemically interdependent network of elements with the common goal of maintaining optimal function in response to continually changing hemodynamic and metabolic conditions.' I submit that mathematical models can help us to understand better the complex adaptations (and maladaptations) manifested by vascular tissues and cells, for such models can build intuition via simulations that contrast the effects of competing hypotheses, they can help guide the design and interpretation of revealing experiments, and they can be used to design improved clinical interventions and medical devices.

1 Introduction

The last four decades have seen tremendous advances in the continuum biomechanics of soft tissues and cells (see, for example, Fung, 1993; Mow et al., 1994; Zhu et al., 2000; Humphrey, 2002, 2003a; Holzapfel and Ogden, 2006; Cowin and Doty, 2007). Nevertheless, two conspicuous shortcomings

remain. Most constitutive relations and stress analyses have focused on conditions at a single instant rather than how the material properties and stress fields evolve due to normal development as well as in response to perturbed loads, disease, injury, or clinical treatment; and, biomechanical analyses have been based on the assumption that tissues and cells are materially uniform rather than consisting of many different constituents that collectively define the whole. The primary goal herein is to encourage a new direction in soft tissue and cell mechanics whereby one can model time-dependent changes in composition, structure, geometry, and properties that occur in response to changes in the chemomechanical environment. Although it is not yet possible to identify most of the underlying mechanisms that are responsible for such growth and remodeling of tissues and cells, expanding data bases provide tremendous guidance on salient aspects of development, adaptation, and disease progression, thus it is appropriate that we begin to interpret these data within mathematical frameworks. Toward this end, our approach will focus on the development, extension, and application of continuum mechanical theories to model biochemical processes that manifest at the tissue or cell level as changes in structure, function, and biomechanical properties.

Although the ideas presented here are developed in general and thereby are meant to apply to various soft tissues and cell types in general, specific examples come primarily from vascular biology and mechanics. Cardiovascular disease remains the leading cause of disability and death in the industrialized world and its prevalence is growing in the developing world. There is, therefore, strong motivation to understand better both vascular biomechanics and mechanobiology, and their potential to contribute to the improvement of health care. Indeed, although advances in mechanobiology are being realized for many different cell types, there is an extensive literature on the three primary cell types that reside in the vascular wall, hence providing another strong motivation to focus on the vasculature. It is hoped that this brief chapter will promote multi-disciplinary research amongst basic scientists, engineers, mathematicians, and physicians so that together we can advance the diagnosis and treatment of vascular diseases and injuries and likewise those affecting other organ systems and tissues.

2 A Brief on Arterial Structure

From the perspective of mechanics, arteries are thick-walled, layered, composite tubes (Figure 1). In particular, the three primary layers are called the intima (inner), media (middle), and adventitia (outer). The intima consists primarily of a monolayer of endothelial cells and an associated base-

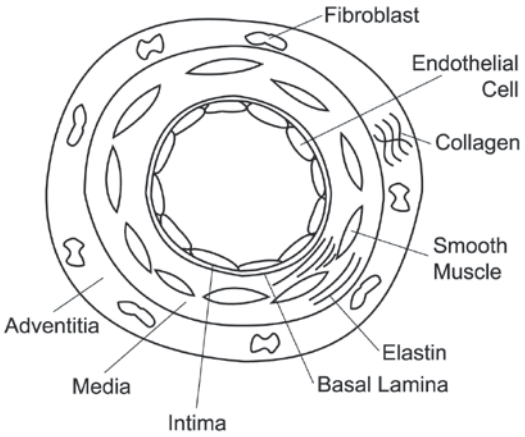

Figure 1. Schema of the three primary layers (intima, media, and adventitia) of the arterial wall and some of the many constituents that endow the wall with its structural integrity.

ment membrane (or, basal lamina) that is composed primarily of type IV collagen and laminin. The endothelial cells render the luminal surface nonthrombogenic and thus facilitate the free flow of blood. Indeed, it was long thought that the primary purpose of the endothelium was to provide a selective diffusive barrier between the flowing blood and the contents of the vascular wall. It is now known, however, that the endothelium is biologically very active, capable of producing a host of molecules (adhesion, vasoactive, proteolytic, growth promoting, etc.) in response to altered chemomechanical stimuli. The media is the parenchymal (or, functional) layer of the arterial wall. It consists primarily of smooth muscle cells embedded in an abundant extracellular matrix that consists of elastin, elastin-associated proteins (fibrillins and fibulins), fibrillar and reticular collagens, and proteoglycans.

Whereas smooth muscle cells are primarily synthetic in development, they are primarily contractile in maturity and thereby are responsible for controlling acute changes in vascular caliber in response to various chemomechanical signals. For example, smooth muscle cells typically exist in a partially contracted state (i.e., they have a basal tone), which allows them to either relax or contract further in response to additional stimulation by the endothelial-derived vasodilator nitric oxide (NO) or the vasoconstrictor endothelin-1 (ET-1), respectively. Many other molecules similarly affect smooth muscle tone, including angiotensin (particularly important in hy-

pertension), thrombin (important in cases of blood clots), and even potent smooth muscle mitogens such as PDGF (platelet-derived growth factor). The adventitia consists primarily of type I collagen with admixed elastin, proteoglycans, and embedded fibroblasts. It is thought by many that the adventitia serves mechanically as a type of protective sheath, preventing acute over-distension of the arterial wall that could damage the medial smooth muscle cells. Again, however, recent data suggest further that the adventitia plays many important biological roles (Strauss and Rabinovitch, 2000). For more details on the basic structure of the arterial wall, and how it varies throughout the arterial tree, see Rhodin (1979) and Humphrey (2002).

3 Clinical Manifestations

Abdominal aortic aneurysms, aortic dissections, arteriovenous malformations, atherosclerosis, cerebral vasospasm, hypertension, intracranial aneurysms, primary pulmonary hypertension, and stroke – these are but a few of the many vascular diseases that result in significant morbidity and mortality worldwide, typically by affecting other organs and organ systems such as the brain, heart, lungs, kidneys, and even musculoskeletal system. Although each of these areas, and indeed many more, deserve focused attention, herein we briefly review as examples two particular vascular adaptations/diseases to motivate our study below. In particular, because of the ubiquitous processes of cell and matrix turnover that underlie all of these different manifestations of vascular growth and remodeling (cf. Figure 2), focusing on a few examples will allow us to observe general characteristics of importance.

3.1 Altered Flow

It appears that Thoma was the first, in 1893, to report that during early development the caliber of the arterial lumen increases in response to sustained increases in flow, and conversely, it decreases in response to sustained decreases in flow (see Taber, 1995). Many years later, Rodbard (1975) speculated, "Let us assume that each endothelial cell is equipped with receptors which are sensitive to the magnitude of the drag force that impinges on it .... Deviations of drag from this set-point initiate negative feedback mechanisms that return the magnitude of the drag to its set-point. In blood vessels, these effects appear to operate through two related mechanisms: an immediate physiological adjustment in vascular tone induced by the change in flow, and a delayed anatomical change that develops when the changed flow rate persists." This basic hypothesis has been largely confirmed over

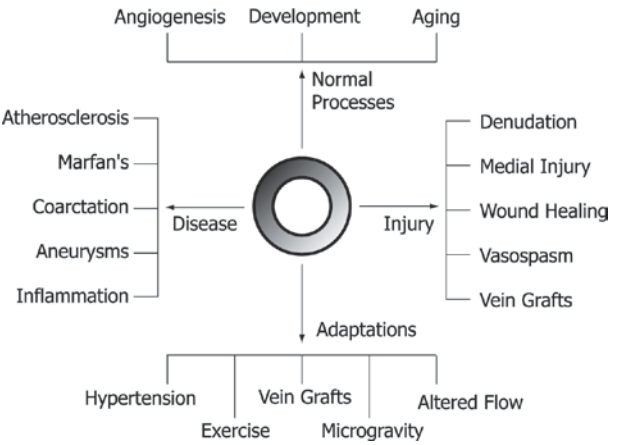

Figure 2. Diagrammatic indication of some of the many different manifestations of cell and matrix turnover (i.e., growth and remodeling) in the vasculature, emphasizing that normal processes, adaptations, disease progression, and responses to injury share common mechanisms (from Humphrey, 2002, with permission).

the decades. For example, Holtz et al. (1984) showed in vivo that vasodilatation (or vasoconstriction) in response to acute increases (or decreases) in blood flow requires an intact endothelium whereas Kamiya and Togawa (1980) reported that sustained increases in flow in maturity cause a 'permanent' enlargement of the vascular lumen. Langille et al. (1989) subsequently demonstrated the steps by which acute and chronic changes in caliber occur. They reported that in response to a sustained reduction in blood flow, the acute decrease in caliber was due entirely to vasoactivity during an early period (3 days), due partly to vasoactivity at intermediate times (7 days), and due to growth and remodeling at later times (14 days) that resulted in a 'permanent' alteration in the wall.

These and many complementary studies thus revealed the importance of the endothelial cell as a chemomechanical receptor and the smooth muscle cell as a key effector of change via both short-term changes in vasoactivity and long-term changes in extracellular matrix turnover. In other words, despite its primarily contractile phenotype in maturity, vascular smooth muscle retains its synthetic ability and thereby can control both acute and chronic adaptations. Although we did not mention the adventitial fibroblasts here, it is becoming increasingly apparent that the ability of these

cells to migrate, proliferate, and turnover collagen also plays a critical role in many cases of vascular growth and remodeling (cf. Strauss and Rabinovitch, 2000).

Finally, note that in flow-induced alterations, it appears that there are parallel shifts in both the pressure-diameter and the active length-tension curves, which is to say that the growth and remodeling induced changes in geometry and structure are reflected by changes in mechanical behavior (Kamiya and Togawa, 1980; Langille et al., 1989). Indeed, it appears that most adaptive responses to altered flow help restore various mechanical parameters toward normal (i.e., homeostatic) values, including flow-induced wall shear stress and pressure-induced intramural circumferential stress. Although cells cannot sense directly stress or strain, which are merely convenient continuum metrics, it is becoming increasingly clear that these metrics can serve as very useful correlates to model the mechanobiology (Humphrey, 2001).

3.2 Aneurysms

Intracranial saccular aneurysms are focal dilatations of the arterial wall that occur at or near bifurcations in the circle of Willis, the primary network of arteries that supplies blood to the brain. The natural history of these lesions consists of three primary phases: pathogenesis, enlargement, and potential rupture. Although the pathogenesis is not well understood, it is clear that mechanical factors play key roles (Humphrey and Canham, 2000). It is thought, for example, that the sparseness of medial elastin in intracranial arteries, the lack of an external elastic lamina, and the lack of firm perivascular support render intracranial vessels more susceptible to the formation of aneurysms. Indeed, it appears that the medial elastin must be absent or markedly fragmented in order for an aneurysm to form; loss of elastin not only reduces the structural integrity of the wall, it also eliminates strong biological signals to the medial smooth muscle cells, including anti-migratory and anti-apoptotic (Brooke et al., 2003; Karnik et al., 2003). In summary then, intracranial saccular aneurysms are typically very thin-walled, consisting largely of collagen and fibroblasts due to the loss of elastin and smooth muscle cells.

Reasons for, and time courses of, the enlargement of saccular aneurysms also remain debated. Whereas the initiating event may yield a small dilatation, these lesions can enlarge to have diameters in excess of 25 mm, although most lesions tend to be 5-10 mm in diameter when discovered and treated (note: for reference, intracranial arteries are on the order of 4 mm or less in diameter). Over the years, various hypotheses have been offered

to explain why these lesions enlarge. For example, it has been suggested that aneurysms enlarge via a limit point instability (i.e., a bifurcation in a quasi-static equilibrium distension), much like the observation that inflated rubber balloons can experience a period of rapid expansion despite a diminishing pressure. Using what we felt were more appropriate analyses based on nonlinear elasticity and constitutive relations commonly used to model soft tissues, however, we have suggested that it is unlikely that these lesions experience a limit point instability (Kyriacou and Humphrey, 1996). It has also been suggested that saccular aneurysms may enlarge via a dynamic instability, or resonance, in response to the pulsatility of the blood pressure. Again based on more appropriate nonlinear analyses, it appears that at least particular sub-classes of lesions are dynamically stable (Shah and Humphrey, 1999). Clearly, there must be another reason why these lesions enlarge.

Canham et al. (1999) noted that if saccular aneurysms consist primarily of collagen fibers, which when straight are very stiff and nearly inextensible, then it is not possible for the lesion to enlarge significantly without collagen turnover. Indeed, among others, Bruno et al. (1998) reported significant upregulation of various matrix metalloproteinases (MMPs) in aneurysms, which degrade multiple components of the extracellular matrix. Turnover is defined, of course, by a delicate balance between degradation and synthesis of material. Based on polarized light microscopic examination of aneurysmal collagen, Canham et al. (1999) suggested that the wall of the lesion does indeed consist of fibers that were formed at different times as well as in different directions. Heightened collagen synthesis has subsequently been suggested by others (cf. Kassam et al., 2004), and Peters et al. (2001) said it well: "aneurysmal dilation results in a highly dynamic cellular environment in which extensive wound healing and tissue/extracellular matrix remodeling are taking place." In other words, it now appears that saccular aneurysms enlarge via a process of collagen turnover (Figure 3), a hallmark of vascular growth and remodeling in development, in adaptation, and in disease.

Finally, it is well accepted that saccular aneurysms rupture when wall stress exceeds wall strength, again emphasizing the importance of continuum mechanical analyses. Fortunately, the rupture-potential for these lesions is very low, with an apparently less than a 0.1% chance of rupture per year. This suggests that these lesions tend to be biologically as well as mechanically stable, or in other words that the growth and remodeling response to the initial insult (pathogenesis) is typically very effective. Hence, in addition to the need to understand better the biomechanics and mechanobiology of these lesions for purposes of improving clinical treat-

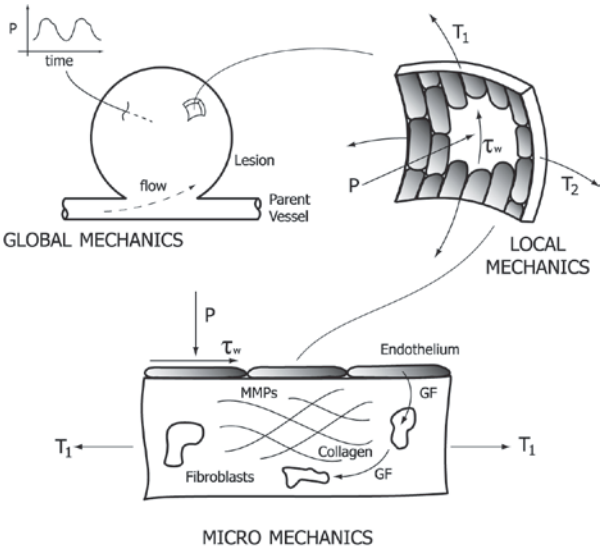

Figure 3. Interrelations between mechanics and the enlargement and potential rupture of intracranial saccular aneurysms, emphasizing the importance of mechanics at multiple length and time scales (from Humphrey, 2002, with permission).

ment, there is probably much that we can learn about effective growth and remodeling processes in general.

4 Mechanobiological Motivation

Cells are the fundamental units of life and thereby are the effectors of change – from morphogenesis to subsequent growth and remodeling of tissues in maintenance (i.e., a continual, balanced turnover of cells and matrix in unchanging mechanical conditions), adaptation, repair, disease, and aging. One of the most important discoveries of the 1970s was that different cell types are very sensitive to their mechanical as well as their chemical environment. That is, cells can change their gene expression, and thus fundamental activities, in response to epigenetic cues as well as genetic cues, including responses to changes in altered mechanical and chemical stimuli (Figure 4). Let us now consider a few specific examples.

One of the earliest direct indications that vascular cells are mechanosensitive came from the work of Rosen et al. (1974). Their working hypothesis

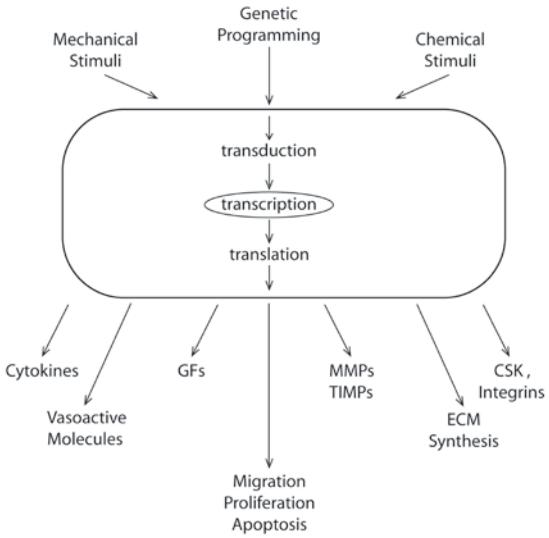

Figure 4. Schematic of genetic and epigenetic (chemomechanical) controlled cellular activities (for example, migration, proliferation, apoptosis), including the production of diverse molecules (for example, extracellular matrix (ECM) proteins, intracellular cytoskeletal (CSK) proteins, growth factors (GFs), matrix metalloproteinases (MMPs), tissue-inhibitors of metalloproteinases (TIMPs), etc.).

was "the interaction of chronically elevated shearing stresses imposed on the endothelium by blood flow can induce subsequent permeability alternations through a mechanism similar to that of the delayed-prolonged inflammatory response . . .". To test this hypothesis, they cultured bovine endothelial cells on cover slips and subjected the cells to different levels of flow-induced shear stresses in a parallel plate experimental system. They found 2.3 to 3.7 fold increases (relative to no-flow controls) in cellular production of the enzyme histidine deoxycarboxylase in response to a 1.5 hour exposure to mean shear stresses from $\sim$0.3 to 0.6 Pa (note: the decarboxylation of the amino acid histidine results in the formation of histamine, which in turn can affect endothelial permeability and smooth muscle contractility). Hence, it became clear that a change in mechanical stimulus could affect directly the cellular production of an important molecule - vascular mechanobiology thus became an important area of study.

Soon thereafter, Leung et al. (1976) were motivated by the following ob-

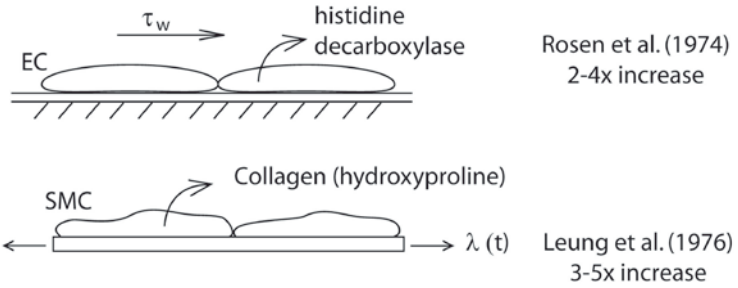

Figure 5. Schema recalling two of the earliest studies that demonstrated mechanobiological responses by vascular endothelial and smooth muscle cells. Rosen et al. (1974) subjected cultured endothelial cells to well controlled shear stresses in a parallel plate experiment whereas Leung et al. (1976) subjected smooth muscle cells cultured on deformable membranes to cyclic stretching.

servation: "Recent investigations have revealed consistent qualitative and quantitative relationships between the composition of arterial walls and estimates of medial stress. These findings suggest that physical forces related to pressure and flow direct medial cell biosynthesis, thereby modulating structural adaptations to hemodynamic changes." To test this hypothesis, they cultured vascular smooth muscle cells on sheets of elastin and subjected the cells to 2 days of 10% cyclic uniaxial stretching. They found that cyclic strain increased the production of extracellular matrix proteins (collagen I and III) and glycosaminogylcans (hyaluronate and chodroitin sulfate) by 3 to 5 fold relative to the unstretched controls. Hence, both an altered stress (shear) and an altered strain (stretch) correlated well with cellular changes in the production of important soluble and insoluble constituents (Figure 5). That is, as noted above, although not sensed directly by the cell, continuum metrics such as stress and strain can be very useful for correlating altered mechanical stimuli to changes in cellular activity as shown by Rosen et al. (1974) and Leung et al. (1976). The interested reader should also consult other early papers, as, for example, Wolinsky (1970) and Flaherty et al. (1972), which provided important motivation for these two studies.

Since these seminal studies in the mid-1970s, many subsequent studies have confirmed the importance of and broadened the scope of mechanosensitive cellular responses (for example, see, Resnick and Gimbrone, 1995; Williams, 1998; McCarty and Bennett, 2000; Romer et al., 2006). Note, too,

that Davies (1995) suggested that the many different types of mechanosensitve responses can be classified according to three types: Immediate Responses (seconds to minutes) include ion channel activation, cytosolic calcium changes, and second messengers; Intermediate Responses (minutes to hours) include cell replication and gene regulation; and Delayed Responses (hours to days) include changes in cell shape and alignment as well as reorganization of the extracellular matrix. For example, the aforementioned acute flow-induced dilatation in arteries could result from immediate responses whereas the chronic flow-induced enlargement would result from a combination of immediate, intermediate, and delayed responses. For more information on the details of such responses, as well as more general issues of growth and remodeling, see the following reviews: Langille (1993), Gibbons and Dzau (1994), and Taber (1995).

Based on this brief review of vascular mechanobiology, it should be evident that as we continue to learn more about the molecular and cell biology, there is an increasingly greater need to understand better the biomechanics of individual cells and the individual sub-cellular components. Of course, many cell types act cooperatively (for example, via paracrine signaling) and they exhibit strong interactions with the extracellular matrix in which they reside (for example, anti-apoptotic signals to smooth muscle by elastin), hence cells cannot be studied only in isolation. Indeed, it is important to emphasize that the extracellular matrix plays many different roles: it endows a tissue or organ with its structural integrity and it provides important biological signals to cells both directly and indirectly by sequestering and releasing various biomolecules. After we discuss some basic cell and matrix biology, we will return to these cooperative functions as we begin to build a continuum framework for analysis of tissue and cells.

5 A Brief on Vascular Cell and Matrix Biology

5.1 Cell Biology

Cells consist of three primary components: the cell membrane, the cytoplasm, and the nucleus (Figure 6). Actually, the nucleus is the most conspicuous of the many organelles that are distributed throughout the cytoplasm, others of which include the mitrochodria (which are sources of cellular energy, primarily via the production of adenosine triphosphate, ATP), the smooth and rough endoplasmic reticulum (for example, where proteins are made), and the Golgi apparatus (for example, where assembled proteins are sorted and prepared for secretion). Remembering that turnover typically entails a delicate balance between production and removal, note that the lysosomes within the cytoplasm provide one means for intracellular degra-

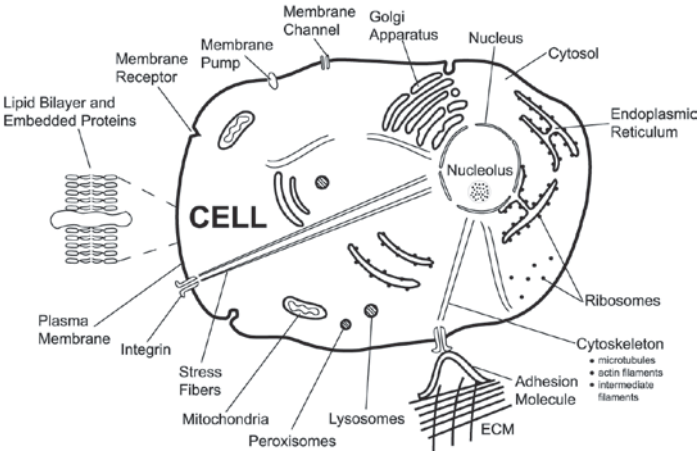

Figure 6. Schema of a typical cell showing various organelles and structural constituents (from Humphrey, 2002, with permission). For more on cell structure and function, see Lodish et al. (2000) and Alberts et al. (2002).

dation of material. The plasma membrane plays a key role in separating the intracellular and extracellular environments, but also in coordinating transport of different molecules across the membrane. In addition, the membrane enables the cell to interact chemically (via receptors) and mechanically (via integrins) with the extracellular environment. Of particular interest mechanically, the cytoplasm also includes the cytoskeleton that provides the cell with most of its structural integrity and the cytosolic fluid (70% of cell is water) that allows the cell to exhibit viscoelastic responses as well as providing a means for diffusive transport of soluble molecules.

The cytoskeleton consists of three primary types of filaments: actin ($\sim$8 nm in diameter), intermediate filaments ($\sim$10 nm in diameter), and microtubles ($\sim$25 nm in diameter). Although each of these proteins is very important mechanically and biologically, let us consider the actin; it can account for up to 10% of cell mass in muscle cells and 1 to 5% of cell mass in other cell types (Lodish et al., 2000). Actin can exist as a globular monomer (G-actin) or a filamentous polymer (F-actin). The filamentous actin can form orthogonal networks or parallel structures depending on which actin-binding protein links the filaments (for example, filamin and fimbrin, respectively). It is well known that actin can also interact with the protein myosin, and thereby form the actomyosin interactions that enable muscle cells to contract. With regard to turnover, actin filaments can be

disassembled and reassembled within minutes, often exploiting significant cytosolic stores of G-actin, which thus enables a highly dynamic growth and remodeling within the cell. Finally, note that the cytoskeleton in general, and actin in particular, can be coupled directly to extracellular matrix proteins via so-called focal adhesion complexes. These structures include transmembrane proteins called integrins (see Figure 6) as well as intracellular linker proteins that include paxillin, vinculin, and α-actinin.

Collectively, the cytoskeletal-integrin-extracellular matrix interactions are thought to be essential to many, although certainly not all, mechanotransduction pathways (Figure 4). Indeed, a very interesting observation is that the size of the focal adhesion complexes tend to increase proportionately with the load borne at the complex (Balaban et al., 2001). In other words, just as the whole vessel appears to grow and remodel to maintain constant some mechanical factor (for example, wall shear stress or intramural hoop stress), so too the focal adhesion complexes appear to maintain constant the local stress ($\sim$3 to 5 kPa). Because of the direct connection between the focal adhesion complexes and bundles of F-actin and myosin, called stress fibers, it is interesting that this finding on the constancy of stress is consistent with observations that stress fibers also tend to have a preferred pre-stretch ($\sim$15 to 25%) or pre-stress in quiescent cells (Costa et al., 2002; Deguchi et al., 2006). In other words, it appears that the cell attempts to maintain constant diverse mechanical parameters intracellularly, across the cell membrane, and extracellularly.

Let us now consider in more detail the vascular smooth muscle cell (VSMC). As noted above, vascular smooth muscle cells are highly proliferative and synthetic during development; specifically, their turnover rates are $\sim$80% per day in the embryo and $\sim$40% per day in the fetus (Stenmark and Mecham, 1997) and they synthesize most of the proteins and proteoglycans that constitute the extracellular matrix of the developing wall. In contrast, in healthy conditions in maturity, smooth muscle cells are typically quiescent (turnover rates are $\sim$0.05% per day) and contractile; thus, they endow the vascular wall with its ability to dilate or contract in response to various chemical, mechanical, and neural signals and thereby to regulate local blood flow, pressure, temperature, and chemical compositions. Consistent with their role of controlling lumen diameter, VSMCs are oriented primarily in the circumferential direction in most vessels and they tend to have a spindle-like shape (typically about 100 microns long and 5 microns in width, except at the nucleus where they are wider). Although they do not have the sarcomeric microstructure found in skeletal and cardiac muscle, their contractility is achieved nonetheless via actomyosin interactions with the filaments oriented largely along the long axis of the cell. Just as

for other cells, the integrity of the smooth muscle depends on a delicate balance between replication (mitosis and cytokinesis) and death (apoptosis or necrosis) as well as intricate interactions between cells and between cells and the extracellular matrix.

5.2 Matrix Biology

As noted above, the primary structural proteins in the arterial wall are elastin, collagen I, and collagen III. Vascular elastin consists of ∼786 amino acid residues (223 Gly, 100 Pro, 167 Ala); it is stabilized by unique desmosine and isodesmosine cross-links; it is highly hydrophobic; and it associates with important affiliated proteins such as the fibulins and fibrillins. The aorta, for example, can contain up to 50% elastin by dry weight and the common carotid artery up to 30%. It appears that most of the elastin is produced early in development (i.e., during fetal and postnatal periods), prior to the vessel achieving its final geometry (for example, luminal radius and length; see Davis, 1995). It also appears that elastin is the most elastic protein in the body as well as the most biologically and thermally stable protein. Collectively, these observations suggest that vascular elastin in maturity 'remembers' configurations from development, which is likely responsible in part for the residual stress (Chuong and Fung, 1983; Vaishnav and Vossoughi, 1983) and the axial pre-stretch (Dobrin et al., 1975; Han and Fung, 1995) that exist in normal arteries. Consistent with this idea, Zeller and Skalak (1998) showed that elastin is under tension in the unloaded wall and that this contributes significantly to the measured residual stresses; this 'constituent specific' contribution to wall mechanics will prove important below.

Collagen is the most abundant protein in the body and the most important structurally; it is a significant constituent in bone, tendons, muscle, skin, the eye, and of course arteries. There are 20+ members of the collagen family, each characterized by a triple helix structure of the form $(G\text{-}X\text{-}Y)_{\mathrm{n}}$ where G is glycine and X and Y are other amino acids but often proline or hydroxyproline. Of the various types of collagen, the fibrillar forms (type I and III) are the most important in vascular mechanics. Depending on the type of artery (elastic versus muscular), 20 to 50% of the wall is collagen by dry weight. Collagen III typically constitutes ∼20 to 30% of the arterial collagen (Kucharz, 1992) and it precedes collagen I in wound healing responses (Doillon et al., 1985). It consists of three α1(III) helices containing ∼1466 amino acid residues (413 Gly, 281 Pro, 62 Lys), with cross-link sites at residues 161, 263, and 1106 and interchain disulfide bonding sites at 141, 144, 1196, and 1197. Collagen I is the main constituent of the adventitial

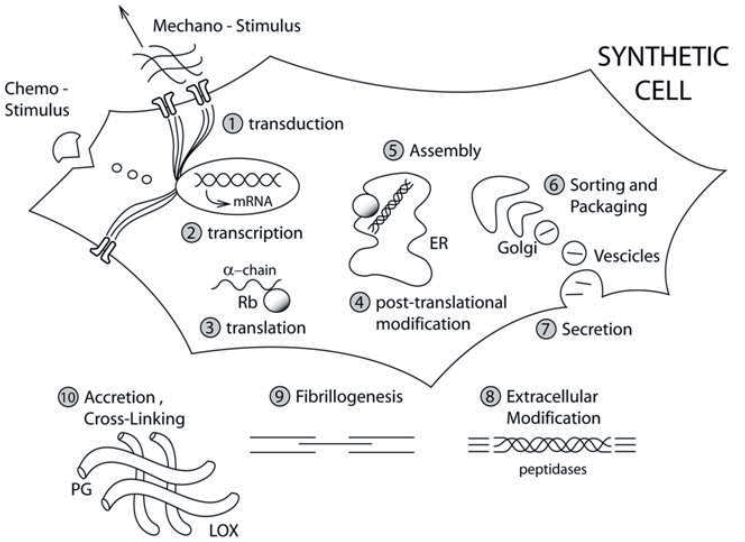

Figure 7. Schema of the chemo-mechano-stimulated production of collagen by a smooth muscle cell. Highlighted are transduction, transcription, translation, and post-translational modifications that lead up to secretion in the extracellular space, where fibrillogenesis and cross-linking yield the structurally important extracellular collagen. Although not shown, it appears that the collagen is organized within extant matrix with a preferred pre-stretch (or pre-tension) and orientation. PG denotes proteoglycans and LOX denotes the enzymatic cross-linker lysyl oxidase.

layer of an artery (where it is produced by fibroblasts) and it is found in abundance in the media, often associated closely with the smooth muscle cells that produce it (Kucharz, 1992). The two α1(I) chains consist of $\sim$1462 amino acid residues (391 Gly, 278 Prol, 58 Lys), with cross-link sites at 170, 265, 1108, and 1208, whereas the α2(I) chain consists of 1366 residues (382 Gly, 230 Pro, 50 Lys), with cross-link sites at 84, 177, 1023 (Ayad et al., 1994).

As an example of protein synthesis, consider the detailed process whereby type I collagen is synthesized and incorporated within extant extracellular matrix (Nimni, 1992; Bishop, 1998; Rodriguez-Feo et al., 2005; see Figure 7). In response to various genetic or epigenetic stimuli (including altered mechanical loads as shown in 1976 by Leung et al., 1976), the genes for the α-helices specific to type I collagen (COL1A1, COL1A2) are upregulated

on different chromosomes. Associated mRNAs are produced within the nucleus and translocated to cytoplasmic ribosomes, on which the appropriate amino acids are then sequenced in the $(G\text{-}X\text{-}Y)_{\mathrm{n}}$ motif. At this point, the resulting α-helices contain propeptide end groups that disallow interchain interactions. Next, the α-helices are transported through the endoplasmic reticulum (ER) where some propeptides are cleaved, specific prolines and lysines are hydroxylated, and specific hydroxylysines are glycosalated. Copious hydrogen bonds form as two $\alpha 1$ chains and one $\alpha 2$ chain form a triple (super) helix molecule called procollagen; note again that propeptides at each end of the molecule prevent intracellular reactions with other procollagen molecules.

Prepared in the Golgi apparatus, the procollagen molecules are transported in vesicles that can coalesce at and fuse to the plasma membrane to create channels in which to secrete the procollagen. Indeed, cells appear to align these channels (called fibropositers in development) to control the orientation of the newly deposited procollagen (Canty et al., 2004; Canty and Kadler, 2005). Once in the extracellular space, the propetides are cleaved and the resulting collagen molecules ($\sim$1.5 nm in diameter and 300 nm long, with a MW $\sim$100,000) begin to self-assemble in a quarter staggered fashion to form microfibrils (4 to 8 nm in diameter), which combine with other microfibrils to form fibrils (10 to 200 nm in diameter). Continued assembly of fibrils by accretion, which can be aided by the incorporation of reticular collagens (for example, type VI or XII; Lodish et al., 2000) and select proteoglycans (for example, decorin; Orgel et al., 2006), yield long, strong fibers (0.5 to 20 μm in diameter) that form lysine based cross-links via the action of the enzyme lysyl oxidase.

It appears that the formation of individual α-helices, assembly into a triple helix motif, and packaging and secretion to the extracellular space take on the order of 7 min, 8 min, and 20 min, respectively (Nimni, 1992). Whereas extracellular, enzymatic-mediated cross-linking can likewise occur quickly (for example, within 30 minutes via the action of lysyl oxidase; Sodek and Ferrier, 1988), the overall degree of cross-linking can increase over longer periods as molecules 'age' or succumb to the effects of diseases such as diabetes (characterized by extensive non-enzymatic, glycation-based cross-links). Increased cross-linking stiffens the tissue and can delay subsequent degradation. Finally, note that there appears to be a significant intracellular degradation of procollagen, perhaps as high as 30-50% of that which is produced. It may be that such degradation serves as a type of 'quality control' to ensure that secreted molecules contain the precise amino acid sequence and post-translational modifications to ensure effective incorporation into structural fibers. Such degradation again occurs on the scale of

minutes. Overall, promoters of collagen synthesis include TGF-β, PDGF, ET-1, ANG-II, and mechanical stimuli; inhibitors of collagen synthesis include NO, FGF, IFN-γ and TGF-α (Rodriguez-Feo et al., 2005).

Although often ignored in biomechanical papers, the integrity of the three primary structural proteins of the arterial wall depends directly on their interactions with many other constituents. For elastin this includes interactions with fibrillins and fibulins. For example, fibrillin-1 is a large glycoprotein that binds to elastin and is essential to the formation of elastic fibers; mutations in the fibrillin gene can result in Marfan's syndrome (cf. Figure 2), which can render the aorta susceptible to dissection. Type I collagen has important interactions with many reticular collagens, adhesion molecules such as fibronectin, and multiple proteoglycans. Unfortunately, detailed information on these interactions and their effects on chemical, mechanical, and thermal stability is not complete, a situation similar to that with regard to cytoskeletal proteins and their interactions via the various binding proteins. There is clearly a need for much more research on fiber-fiber and filament-filament interactions.

Finally, it should be noted that the basement membrane of the vascular wall, on which the endothelial cells reside and through which they resist the shearing stresses due to blood flow, consists largely of fibronectin in development and collagen IV and laminin in maturity. In addition to its structural role, the basement membrane also serves as a selective barrier for diffusive transport and it provides multiple biological cues to the endothelium (for example, influencing cell survival, migration, differentiation). Fibronectin is an adhesive glycoprotein, laminin is large flexible glycoprotein having a cruciform shape and held together by disulfide bonds, and type IV collagen is a network forming collagen that is more flexible than the fibrillar types of collagen. It appears that the laminin and collagen IV are bound together via various proteoglycans, including perlecan. It should be noted that monocytes/macrophages migrate across the basement membrane in atherosclerosis and other situations, which requires a focal degradation of the membrane.

Extracellular matrix is typically degraded either via phagocytosis or the action of proteases. As noted by Parks and Mecham (1998), "The spatially and temporally precise removal and remodeling of connective tissue are critical to several developmental, homeostatic, and reparative processes." Matrix metalloproteinases (MMPs) represent the primary family of proteases that degrade arterial wall constituents such as elastin and collagen. MMPs are secreted in a latent, or pro-form, that can be activated by different means, including the action of other proteases (for example, serine proteases) or mechanical loading. MMPs can also be inactivated by tissue

inhibitors of matrix metalloproteinases (TIMPs). There are 16+ members of the MMP family, but here we consider only three of the key ones. MMP-1 cleaves the fibrillar collagens, which enables MMP-9 to degrade the resulting fragments; MMP-2 degrades types IV, V, X, etc. collagen; and MMP-9 degrades elastin, fibronectin, laminin, and gelatin. Note, therefore, that many MMPs work together, as, for example, MMP-2 (degrading type IV collagen) and MMP-9 (degrading laminin) would be involved in the degradation of the basement membrane that allows migration of monocytes into the arterial wall where they can differentiate into macrophages.

The process by which each MMPs works is also complex. For example, MMP-1 cleaves collagen I by binding to the triple helix molecule at a location about 3/4 of the distance from the N-terminal, by changing its conformation, and by unwinding the triple helix before hydrolyzing hydrogen bonds (Chung et al., 2004). Strauss et al. (1994a) showed that if MMP activity is inhibited, there are comparable decreases in both collagen degradation and synthesis. In other words, as might be expected of a system that typically relies on a delicate balance between continual degradation and synthesis, the two appear to be highly coupled. Indeed, altered rates of synthesis and degradation appear to be very similar both in fold-change and time-course in responses to injury (Strauss et al., 1994b). Overall, the normal half-life of fibrillar collagens varies tremendously from tissue to tissue; it is perhaps longest in bone and shortest in the periodontal ligament[1] (TenCate and Deporter, 1975; Sodek and Ferrier, 1988; Gineyts et al., 2000). As noted earlier, it appears that the half-life of vascular collagen is on the order of 70 days (Nissen et al., 1978), which can decrease 4- to 10-fold in cases of disease and injury (Bashey et al., 1989). Note that a 10 fold decrease yields a half-life of 7 days, which is to say that remodeling could be completed within 2 weeks or nearly so. This is consistent with a recent report on time-dependent changes in the mechanical properties of the basilar artery in hypertension (Hu et al., 2007). For quick reference, note that half-lives of 2500, 1000, 100, 70, 50, 10, and 5 days correspond to turnover rates of 0.02% (basal EC turnover), 0.05% (basal SMC turnover), 0.5%, 0.7% (basal vascular collagen turnover), 1%, 5%, and 10% (basal periodontal ligament turnover) per day.

Ruberti and Hallab (2005) asked the provocative question, "how could a fibroblast 'select' which fibrils are removed and which are left intact?" Toward this end, they subjected a corneal specimen (which consists primarily of orthogonally arranged collagen I) to a fixed uniaxial strain and

[1] Scurvy, which results from vitamin C deficiency, inhibits the replacement of degraded collagen. Because collagen in the periodontal ligament has such a short half-life (3-10 days), loose teeth are often the first symptom of scurvy.

then exposed the specimen to a collagenase. They observed that the fibers that were strained highly did not degrade as quickly as those that were unstrained. In other words, mechanical strain (or stress) correlated inversely with the rate of degradation. They wrote, "Strain stabilization of collagen would explain how the observed response of a mechanically activated fibroblast can result in a matrix morphological change that is optimized for the applied load." Based on anecdotal evidence, one may speculate further that strains well above or well below homeostatic values could promote an increased rate of degradation, whether by increasing binding affinities or by increasing protease synthesis/activation.

In summary, it is emphasized that the structural integrity of the extracellular matrix depends upon the mass fractions, orientations, and interactions (for example, cross-links) associated with the individual fibers, but also on a delicate balance between production (synthesis and deposition) and removal (degradation), each of which can be stimulated by chemomechanical factors. Recall from Figure 5, for example, that Leung et al. (1976) showed a direct correlation between cyclic stretching of smooth muscle cells and collagen production, a clear mechano-stimulus. Li et al. (1998) showed further that there is a strong coupling in vascular smooth muscle cells between mechanical stretch and the production of angiotensin-II (ANG-II) and transforming growth factor-beta (TGF-β), both of which are promoters of collagen synthesis. For example, basal values of TGF-β1 and ANG-II production were 13 pg/ml and 0.05 ng/ml before stretch, but 55 pg/ml (4.2 fold increase) and 0.65 ng/ml (13 fold increase) when stretched. Similar findings have been reported for platelet derived growth factor (PDGF) and mechanical loading (Bishop, 1998). Hence, although mechanical stimuli clearly mediate matrix turnover, the associated mechanisms may be both direct and indirect. As it can be seen, even if we consider only a few steps in the processes of collagen production (contrast Figures 7 and 8) and degradation, control of the biochemical processes involved in ensuring structural integrity is extremely complex.

6 Utility of Continuum Mechanics

According to Truesdell and Noll (1965; pp. 5), "Whether the continuum approach is justified, in any particular case, is a matter, not for the philosophy or methodology of science, but for the experimental test ...". The last four decades have revealed many successes wherein continuum biomechanics has been used to describe the mechanical behavior of tissues and cells subjected to diverse conditions of interest as well as to estimate the stresses and strains that they experience. Regarding arteries, for exam-

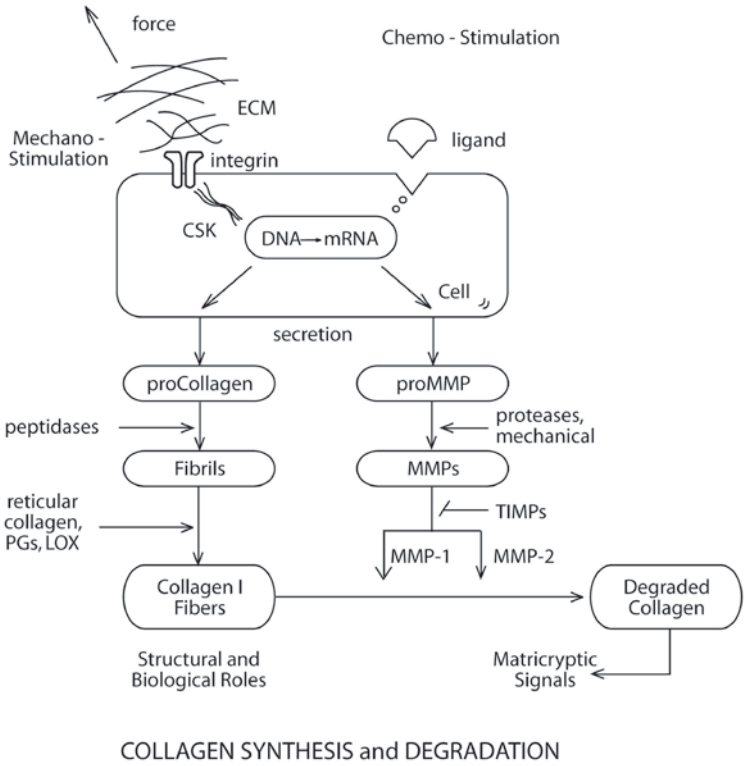

Figure 8. Highly simplified schema of the delicate balance between collagen synthesis and degradation, each of which can be stimulated by chemical and mechanical factors. Until, and even after, the details of collagen and matrix metalloproteinase (MMP) synthesis and action are well understood, simplified descriptions of the kinetics can provide significant information for purposes of understanding and modeling the associated growth and remodeling. Note: ECM is extracellular matrix, PGs is proteoglycans, LOX is lysyl oxidase, TIMPs is tissue inhibitors of matrix metalloproteinases (MMPs). Matricryptic signals refer to latent chemical signals (e.g., sprecific amino acid sequences) that can be exposed following degradaton.

ple, increasingly sophisticated models over the years have suggested that the structural complexity of the wall, including the inherent anisotropy, residual stress, basal tone, and heterogeneity, appears to work together to

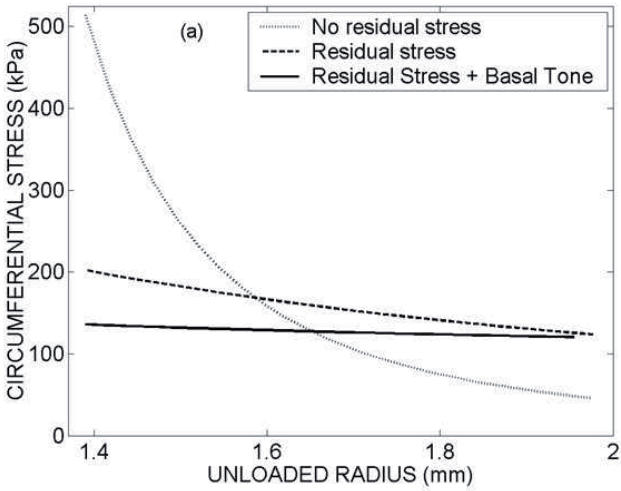

Figure 9. Illustrative results from nonlinear elasticity for the predicted circumferential stress in an artery subjected to in vivo pressures and axial loads. Note the strong gradient predicted (∼1979) when residual stress and basal tone are ignored; such gradients would be otherwise expected for the finite inflation and extension of a thick-walled nonlinearly elastic tube. Inclusion of the effects of residual stress (∼1986) and basal smooth muscle tone (∼1999) suggest, however, that the transmural distribution of the circumferential Cauchy stress may be nearly uniform. Although not shown, results are similar for the axial stress.

yield a nearly uniform, equibiaxial stress field within the major layers of the wall (cf. Figure 9). This computational result is both teleologically reasonable and consistent with observations by many, including Clark and Glagov (1985) who suggested, based on data from different species, that the aorta thickens in order to maintain constant the mean circumferential tension in each musculo-elastic (elastin-collagen-smooth muscle) layer within the wall. In other words, it appears that arteries develop in such a way to achieve a preferred, homeostatic, mechanical state. Indeed, this appears to be consistent with studies on cell-matrix constructs (Brown et al., 1998) as well as sub-cellular structures such as stress fibers within cells that tend to have a preferred pre-stretch (Costa et al., 2002; Deguchi et al., 2006) or pre-stress (Ingber, 1993; Wang et al., 2002) and focal adhesions complexes that increase or decrease in size in response to increases and decreases in

loads so as to maintain constant the stress (Balaban et al., 2001; Goffin et al., 2006). Hence, predictions by state of the art continuum models are consistent with observations at tissue, cellular, and sub-cellular levels, which together suggest strongly that there exists an underlying 'biomechanical homeostasis'.

Despite manifold successes of continuum biomechanics (cf. Fung, 1993; Mow et al., 1994; Humphrey, 2003a; Cowin and Doty, 2007), increasingly precise detail on the structure and function of tissues and cells has caused some to suggest that there is a need to move to other approaches of modeling. Here we consider two such examples. First, Ingber wrote (see Ingber et al., 2000) "Because they ignore microstructural features, continuum models cannot provide specific predictions that relate to the functional contribution of distinct cytoskeletal filaments to cell mechanics. Furthermore, although these models can provide empirical fits to measured mechanical properties in cells under specific experimental conditions, they cannot predict how these properties alter under new challenges to the cell." This is not true, of course, but perhaps came from a narrow perspective of continuum biomechanics. Indeed, consider further comments from this paper, namely "many biologists fail to recognize the important difference between engineering models that can describe ('curve-fit') a complex cell behavior vs. one, like tensegrity, that can explain and predict multiple behaviors at many different size scales from mechanistic principles." After noting that spring-dashpot and even liquid droplet models can mimic certain behaviors in living cells, it was suggested further "However, we know that these biological structures are not constructed in this manner, and, indeed, the ... model does not mesh with the microarchitectural complexity that is observed within the cytoplasm of living cells.

Essentially, these are all ad hoc models, and, as such, they do not provide a means to explain these behaviors in mechanistic or molecular terms and do not lead to specific predictions that are independent of the experimental system." The tensegrity model of the cytoskeleton assumes that tensile (for example, actin and intermediate filaments) and compressive (microtubules and thick, cross-linked actin bundles) elements form a precise self-equilibrating structure (Stamenović and Ingber, 2002), an idea that is important and conceptually correct. Yet, tensegrity has relied on very specific representative structural units (for example, 6-struts); it has not modeled the complex interactions between filaments and the many linker proteins (for example, filamin, fimbrin, myosin) that dictate structural responses, and it has not incorporated details on the kinetics of cytoskeletal turnover (including actin polymerization and depolymerization). In other words, tensegrity models, often employed within the context of linearized

elasticity and small strains despite considerable evidence to the contrary, are but simple mechanical analog models, not unlike spring and dashpot models used in muscle mechanics and tissue viscoelasticity. It is suggested, therefore, that despite criticisms by some, the continued development and extension of continuum models (including mixture theories) can play important roles in our understanding of the complexities of cell mechanics as well as those of whole tissues and organs.

Second, with regard to cell-mediated growth and remodeling, Cowin (2006) recently wrote: "most continuum models assume smooth bijective mappings of the reference state of an object onto a subsequently deformed state of the same object" and "the real cell level mapping will not be a continuum one-to-one mapping." To illustrate his point, he wrote further that "Cells move around like guests circulating at a cocktail party, they replicate themselves with some ease and they produce new material for the tissue of which they are a part. If one takes a picture of the reference state of a tissue at the cellular level and then examines a picture of a subsequent state of the tissue one sees regions that were once neighbors now separated by other regions, that there now exist regions that did not exist before and there are regions that existed before that do not exist now." It was then suggested that cellular automata models do not suffer these limitations (restrictions of one-to-one mappings) of the continuum theory. As will be addressed more below, this concern can be avoided and is being addressed by many, including Cowin. Indeed, very successful continuum theories have long described situations wherein material particles move relative to each other in a single constituent media (for example, Poiseuille flow in a tube), in multi-constituent media (for example, flow through porous media or a solid-fluid mixture), and in cases wherein different constituents are produced and removed (for example, reaction-diffusion equations that result from continuum mass balance). Notwithstanding the algorithmic simplicity of some cellular automata models, we must continue to exploit the power and potential of continuum biomechanics.

Perhaps the best question to ask, therefore, is: How should we exploit the rapidly increasing information in molecular and cell biology? Ultimately, of course, cell and matrix biology results from the complex interactions of diverse molecules – from simple but essential molecules such as glucose and oxygen to complex proteins having large molecular weights (for example, collagen and MMPs). Indeed, it is clear that much will be gained by studying molecular biomechanics. Yet, injuries occur and surgeries are performed at the level of tissues and organs. Likewise, many pathologies occur at a tissue level, whether it be the development of a occlusive plaque within an artery or a large dilatation of the arterial wall that we call an aneurysm. Clearly,

molecular, cellular, and tissue level biomechanics are each important; we should not argue which is most important. Rather, the focus should be on how we can, and when we should, integrate such information via multi-scale models. It goes without saying, of course, that biology is not special in this regard for all gross material behaviors ultimately depend on behaviors at atomic or molecular levels. Hence, it is interesting to note the following (Truesdell and Toupin, 1960; pp. 226–228): "Thus, in the physics of today, corpuscles [discrete particles] are supreme. It might seem mandatory ... that we begin with the laws governing the elementary particles and derive from them, as mere corollaries, the laws governing apparently continuous bodies. Such a program is triply impractical: A. The laws of the elementary particles are not yet fully established. B. The mathematical difficulties are at present insuperable. C. In such special cases as have actually been treated, the mathematical 'approximations' committed in order to get to an answer are so drastic that the results obtained are not fair trials of what the basic laws may imply." Indeed, Truesdell and Noll (1965; pp. 5–8) went on to say, "It should not be thought that the results of the continuum approach are necessarily either less or more accurate than those from a structural approach. The two approaches are different, and they have different uses ... continuum physics serves to correlate the results of measurements on materials and to isolate aspects of their response. It neither conflicts with structural theories nor is rendered unnecessary by them." With regard to systems physiology, pathophysiology, and many clinical interventions, we can likewise expect that over appropriate length scales, continuum biomechanics will serve well our need to correlate cell-mediated responses with the associated chemomechanical stimuli.

7 Basics of Continuum Biomechanics

Consider a configuration of a body that serves as a convenient reference, and then assume that we can define at each point (or location) within this configuration a 'continuum average' of properties or physical quantities of interest. That is, we assign at each point and each time that value of the property or quantity of interest that equals its average within a neighborhood about that point (often called a representative volume element), namely

$$\wp \equiv \langle \wp \rangle = \frac{1}{\Omega} \int \wp \mathrm{d}\Omega, \tag{1}$$

where the region Ω over which the averaging is done can define a line, a surface, or a volume depending on whether the associated theory is 1-D, 2-D, or 3-D (where D represents spatial dimension). In classical continuum

mechanics, it is typically assumed that a body is materially uniform (i.e., it consists of a single constituent), which is to say that each point is endowed with but a 'single' material behavior. In many cases, the Helmholtz potential ψ is a convenient descriptor of such behaviors. One then postulates and exploits five basic balance relations (for example, Bowen, 1989): balance of mass, linear momentum, energy, angular momentum, and entropy. In a referential description, that is with all field variables defined at points $\mathbf{X}$ in the reference configuration and each time t, the associated initial-boundary value problem (I-BVP) can be formulated in terms of 'three' equations of motion (mass, linear momentum, and energy)

$$\det\mathbf{F} - \rho_o/\rho = 0, \tag{2}$$

$$\mathrm{Div}(\mathbf{T}_o) + \rho_o\mathbf{b} - \rho_o\dot{\mathbf{v}} = \mathbf{0}, \tag{3}$$

$$\rho_o\dot{\varepsilon} - \mathrm{tr}(\mathbf{T}_o^T\dot{\mathbf{F}}) + \ \mathrm{Div}(\mathbf{q}_o) - \rho_o g = 0, \tag{4}$$

which are subject to two restrictions (angular momentum, entropy),

$$\mathbf{F}\mathbf{T}_o^T = \mathbf{T}_o\mathbf{F}^T, \tag{5}$$

$$\xi_o \equiv -\rho_o(\dot{\psi} + \eta\dot{T}) + \mathrm{tr}(\mathbf{T}_o^T\dot{\mathbf{F}}) - \mathbf{q}_o \cdot \mathrm{Grad}(T)/T \geq 0. \tag{6}$$

Here, ρ is the mass density per current volume and ρ_o is the mass density per original volume, $\mathbf{F}\,(= \partial\mathbf{x}/\partial\mathbf{X})$ the deformation gradient (actually a gradient of the motion, which contains information on both deformation and rigid body motion), $\mathbf{T}_o$ the first Piola-Kirchhoff stress (called the nominal stress by some), $\mathbf{b}$ the body force vector, $\mathbf{v}\,(= \mathrm{d}\mathbf{x}/\mathrm{d}t)$ the velocity, ε the internal energy density, $\mathbf{q}_o$ the referential heat flux vector, g a heat supply density (essentially a volumetric source or sink), $\psi(= \varepsilon - \eta T)$ the Helmholtz potential, η the entropy density, T the absolute temperature, and ξ_o the rate of dissipation. Note, too, that the over-dot implies a total derivative with respect to time $(\mathrm{d}(\ )/\mathrm{d}t)$. In each case, these quantities are allowed to vary with position $\mathbf{X}$ (in the reference configuration) and time t, and the subscript o reminds us that the quantity is referred to that 'original' reference. For a derivation of these basic postulates see any standard textbook on continuum mechanics (for example, Chadwick, 1976; Bowen, 1989; Holzapfel, 2000).

Via Nanson's relation and accepted definitions for various measures of stress and deformation, this I-BVP can be formulated similarly in a spatial description, that is, with all field variables defined at points $\mathbf{x}$ in the current configuration and each time t, as

$$\dot{\rho} + \rho\mathrm{div}\mathbf{v} = 0, \tag{7}$$

$$\mathrm{div}(\mathbf{t}) + \rho\mathbf{b} - \rho\dot{\mathbf{v}} = \mathbf{0}, \tag{8}$$

$$\rho\dot{\varepsilon} - \mathrm{tr}(\mathbf{tD}) + \mathrm{div}\mathbf{q} - \rho g = 0, \tag{9}$$

subject to the two restrictions,

$$\mathbf{t} = \mathbf{t}^T, \tag{10}$$

$$\xi \equiv -\rho(\dot{\psi} + \eta\dot{T}) + \mathrm{tr}(\mathbf{tD}) - \frac{1}{T}\mathbf{q}\cdot\mathrm{grad}(T) \geq 0. \tag{11}$$

Here, we note that quantities that are convenient in the spatial formulation are related directly to those that are convenient in the referential formulation, as, for example, the Cauchy stress $\mathbf{t} = \mathbf{T}_o\mathbf{F}^T/\det\mathbf{F}$, the stretching tensor $\mathbf{D} = (\dot{\mathbf{F}}\mathbf{F}^{-1} + \mathbf{F}^{-T}\dot{\mathbf{F}}^T)/2$, and the heat flux vector $\mathbf{q} = \mathbf{F}\mathbf{q}_o/\det\mathbf{F}$. Clearly, the deformation gradient plays the key role in relating these different quantities, with the motion $\mathbf{x} = \hat{\mathbf{x}}(\mathbf{X}, t)$ assumed to be sufficiently smooth and hence invertible.

Regardless of description, referential or spatial, with independent variables $(\mathbf{X}, t)$ and $(\mathbf{x}, t)$, respectively, we have five equations of motion when written relative to a convenient Frame of Reference (i.e., a specific coordinate system and a clock): one equation from mass balance, three equations from linear momentum balance, and one equation from energy balance. Hence, if the motion is prescribed (say $\mathbf{x} = \hat{\mathbf{x}}(\mathbf{X}, t)$ as in the semi-inverse approach common in finite elasticity), then the differential equation for mass balance allows us to determine the mass density field, the differential equations for linear momentum balance allow us to determine the three components of the body force vector field, and the differential equation for energy balance allows us to determine the heat source/sink field. In order to close this system mathematically, however, we need additional equations for the stress (six to nine components), the internal energy (a scalar), and the heat flux (three components) at each point, with each equation subject to restrictions imposed by angular momentum balance (three equations) and entropy balance (i.e., the second law of thermodynamics, which is a scalar equation).

The additional requisite equations are called constitutive relations, which are defined point-wise. In brief, *a constitutive relation describes the response of a material to an applied load under conditions of interest, with this response specific to the internal constitution of the material.* As we shall discuss further below, it is important to emphasize that constitutive relations describe behaviors of materials under particular conditions of interest, not the materials themselves. It is for this reason that different constitutive relations are needed for the same material subject to different conditions

(for example, for water in its solid, liquid, and gaseous forms depending on the temperature).

Although there exist many classes of problems wherein cells, tissues, or organs are heated or cooled well beyond the physiologic range (see, for example, Diller and Ryan, 1998; Humphrey, 2003b), we focus herein on uniformly isothermal problems wherein $T \equiv T_o \; \forall \; (\mathbf{x}, t)$, with $\mathbf{q} = \mathbf{0}$, $\dot{T} = 0$, $\text{grad}(T) = \mathbf{0}$. Hence, the spatial energy equation reduces to a simple expression for stress power, $\text{tr}(\mathbf{tD}) = \rho\dot{\varepsilon}$, and the second law requires that $-\rho\dot{\psi} + \text{tr}(\mathbf{tD}) \geq 0$ or $\rho\dot{\eta}T \geq 0$. The second law of thermodynamics thus provides restrictions even on purely mechanical constitutive relations in isothermal problems. Thus, the key equations of motion for an isothermal process reduce, in spatial form, to

$$\dot{\rho} + \rho \text{div}\mathbf{v} = 0 \qquad \text{and} \qquad \text{div}(\mathbf{t}) + \rho\mathbf{b} - \rho\mathbf{a} = \mathbf{0}. \tag{12}$$

Similar to before, there are more unknowns (ten) than equations (four) given a prescribed motion (i.e., a semi-inverse approach for solution), hence we must still introduce constitutive relations to close the system of equations.

If we further confine our attention to processes that are elastic, or nearly so, then the stress responses are conveniently modeled using $\rho_o\psi = W$, where W is a so-called strain-energy function (defined per reference volume). The second law thus requires that, in isothermal processes, the Cauchy stress $\mathbf{t}$ is determined from the Helmholtz potential, or strain energy, via

$$\mathbf{t} = \frac{1}{\det \mathbf{F}} \mathbf{F} \left(\rho_o \frac{\partial \psi}{\partial \mathbf{F}^T} \right) = \rho \mathbf{F} \frac{\partial \psi}{\partial \mathbf{F}^T} = \frac{\rho}{\rho_o} 2\mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T = \frac{2}{\det \mathbf{F}} \mathbf{F} \frac{\partial W}{\partial \mathbf{C}} \mathbf{F}^T, \tag{13}$$

subject to possible kinematic constraints such as incompressibility (note: we typically use the referential form of the second law and relate the resulting first Piola-Kirchhoff stress to the Cauchy stress to arrive at this important relation). Note, too, that $\mathbf{C} = \mathbf{F}^T\mathbf{F}$ is the right Cauchy-Green tensor, which via the Polar Decomposition Theorem ($\mathbf{F} = \mathbf{RU} = \mathbf{VR}$), is shown easily to be independent of rigid body motion $\mathbf{R}$ and thus is a convenient measure of deformation. Finally, note that the Green strain $\mathbf{E} = (\mathbf{F}^T\mathbf{F} - \mathbf{I})/2$ is also commonly used in biomechanics, with $2\partial(\;)/\partial\mathbf{C} = \partial(\;)/\partial\mathbf{E}$. For purposes below, equations (12) and (13) will be our focus. Of course, this discussion has necessarily been brief. The interested reader should see Holzapfel (2000) and Taber (2004) for further details on nonlinear solid mechanics appropriate for biomechanics and refer to the excellent chapters in this book by G.A. Holzapfel and P.J. Hunter for methods of solving problems in weak form (for example, using finite element methods). Here, however, let us begin to explore possible extensions that will allow us to describe and predict evolving changes in geometry, structure, and properties.

8 Prior Studies on Mechanics of Growth

Two of the primary contributors to establishing the modern field of biomechanics are Y.C. Fung and R. Skalak (see pp. 12 and 13 in Humphrey, 2002, for pictures), and both contributed significantly to biomechanical modeling of growth and remodeling. Skalak (1981) brought the topic of soft tissue growth within the purview of continuum biomechanics by introducing the concept of 'kinematic growth'. Extension of this basic concept by Rodriguez et al. (1994) subsequently motivated others to model aspects of arterial growth ranging from development to adaptations to altered flows and pressure (Taber, 1995, 1998; Rachev et al., 1998; Rachev, 2000; Fridez et al., 2001; Kuhl et al., 2007). Briefly, this theory is built upon the assumption that an original intact stress-free body can be cut fictitiously into small stress-free elements that, in turn, can grow independently (i.e., change in size or shape). Because the grown elements need not be compatible, they must then be assembled to produce an intact, possibly residually stressed, traction-free configuration (for example, unloaded vessel). These two actions are described by a growth tensor $\mathbf{F}_g$ and an elastic assembly tensor $\mathbf{F}_e$ (Figure 10), with $\mathbf{F}_1 = \mathbf{F}_e\mathbf{F}_g$ and the total deformation $\mathbf{F} = \mathbf{F}_2\mathbf{F}_1$ where $\mathbf{F}_2$ represents usual elastic deformations from a traction-free configuration due to applied loads (for example, in vivo pressures). Other key features of the theory are that stresses depend only on $\mathbf{F}_2\mathbf{F}_e$, since the growth $\mathbf{F}_g$ is assumed to occur in stress-free configurations, and the rate of growth $\mathrm{d}\mathbf{F}_g/\mathrm{d}t$ is prescribed via evolution equations to depend on differences between current and homeostatic values of stress (i.e., growth must continue until stress returns to its target value; it is interesting that growth is assumed to be controlled by stress and yet to occur in stress-free states).

Although the theory of kinematic growth yields many reasonable predictions, as revealed in the many papers, Humphrey and Rajagopal (2002) suggested that it models the consequences of growth, not processes by which growth actually occurs. Consistent with Figures 7 and 8, growth/atrophy necessarily result from the production/removal of material: extracellular matrix is synthesized by cells or degraded by proteases and likewise cell mass changes either by cellular proliferation (increase in number) and death (apoptosis/necrosis) or by cellular hypertrophy and atrophy (increase or decrease in cell mass via the synthesis or degradation of intracellular proteins).

Our suggestion that modeling growth should focus on mass production and removal is consistent with ideas of Fung (1993), who suggested that there was a need for 'mass-stress relations'. He hypothesized that "the remodeling of the blood vessel involving growth or resorption of cells and extracellular materials is linked to the stress in the vessel." Specifically, he