Advanced Course on Tissue Remodeling
ACTR'04 – (pp.9–45) – Warsaw, 2004.

Reading Bones: Insights into the Modeling of Bone Morphogenesis, Growth and Adaptation

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The growth and remodeling of a tissue depends upon certain features in the history of its mechanical environment as well as its genetic makeup. The mechanical environment influences the tissue's developing morphology, the process of simply increasing the size of existing morphological structures, and the formation of the proteins of which the tissue is constructed. The relationships between genetic information, various epigenetic mechanisms and tissue development are discussed. The developmental growth and remodeling of most structural tissues is enhanced by the use of those tissues and retarded by their disuse. The mechanical or mathematical modeling of tissue growth and development using cellular automata models and continuum mechanical models is reviewed.

Key words: *tissue, growth, remodeling, simulation, modeling*

1. Introduction

A tissue is a composite material whose constituents, and therefore structure, are continually changing due to growth and response of the tissue to its physical and chemical environment. A tissue's physiochemical environment includes the environment it is presently experiencing and the recent history of that environment. A tissue itself is a collection of cells and extracellular matrices that perform specialized functions. The extracellular matrix (ECM) consists of fibers (e.g., the proteins collagen and elastin) and a ground substance (e.g., proteoglycans). Animal tissues are classified into four main groups: connective, epithelial, muscle and nerve. The main tissue examples considered in this chapter are connective tissues. Connective tissue "con-

nects" the body organs and tissues; it holds organs in place and provides the structure that gives the body shape. Compared with other types of tissue, connective tissue has relatively few cells and much extracellular substance. Connective tissues include cartilage, tendons, ligaments, the matrix of bone and the adipose (fatty) tissues as well as skin, blood and lymph.

Growth is the process of gradual increase in the net volume of a tissue, but it may also include some resorption. There is evidence that growth in length of an organism is proportional to the cube root of growth in volume [1]. A distinction is made between *appositional* growth and *interstitial* growth; that is the difference between growth on the surface and growth within a volume of tissue. Appositional growth occurs, for example, when a tree adds another layer to the outside of its trunk and interstitial growth occurs within the cavities or spaces in the tissues of animals or plants. Hard tissues, bone and teeth, grow by apposition and soft tissues grow interstitially. In this chapter the primary concern is with the influence of the recent history of mechanical loading on growth rate although growth rate may also be slowed or accelerated by hormones, vitamins, bioelectrical factors, surgical intervention, imbalances between deposition and resorption and other factors.

In addition to, and apart from, growth, mature tissues may remodel their extracellular matrix to adapt its structure to the mechanical loading environment it is and has been experiencing. As an illustration of the adaptation of tissues to a changed mechanical load environment, consider the data on the response to microgravity. Almost 1000 mature humans have accumulated almost 70 person-years of space flight. The direct and indirect effects of microgravity are numerous. The removal of gravity means that the limbs no longer have weight, and muscles are no longer required to maintain posture. The stretch sensors in the musculoskeletal system then receive a signal different from the one they receive on Earth. They no longer have to maintain the normal musculoskeletal structure of the body against gravity and the system begins to diminish its capacity. Bone mass is lost from key parts of the skeleton at the rate of about 1% per month and some muscles atrophy rapidly. The vestibular system in the inner ear, the system that allows us to control our balance and equilibrium, automatically compensates for gravity and some adaptation of this system is necessary when there is microgravity. A researcher studying the effect of microgravity on the vestibular system has indicated that we are not able to predict whether, after a yearlong trip from Earth, an astronaut would be able to stand up on Mars [2]. Much of the

body is fluid and therefore the body, designed for life on Earth, experiences a fluid shift to the head and away from the feet in microgravity. The fluid shift initiates a number of interacting renal, hormonal and mechanical effects that regulate fluid and electrolyte levels [3]. The kidney filtration rate, for example, is increased by twenty percent, and the skull may actually gain bone rather than lose it because of the increased fluid pressure within the skull due to the caudal shift of fluids in microgravity.

Morphogenesis refers to the processes that are responsible for producing the complex shapes of adults from the simple ball of cells that derives from division of the fertilized egg. Morphogenetic events include pattern and template formation in tissue development. Morphogenetic processes interact with the growth and remodeling processes and often all three process types occur simultaneously.

The subject of this chapter is the simulation and mathematical modeling of tissue growth and remodeling. These subjects are viewed from the perspective of how they are, or could be, mathematically modeled. In 1885 Wilhelm Roux stated the need for mechanical modeling in the study of tissue development in his statement of objective [4] for his new journal, the *Archiv für Entwickelungsmechanik der Organismen*, a journal that is still published. His statement of objective was contained in an introductory essay entitled "The problems, methods, and scope of developmental mechanics" [4]. Readers not familiar with Roux could see many of the same ideas in the first (1917) edition of D'Arcy Thompson's *On Growth and Form* [5], and almost all writers in this area indicate the influence of D'Arcy Thompson as a starting point. In spite of this perceptive start a century ago, innovative mechanics is only beginning to be applied to the study of tissue development. The biologist Albert Harris [6] wrote: "Systems of interacting forces and stimuli don't have to be very complicated before the unaided human intuition can no longer predict accurately what the net result should be. At this point computer simulations, or other mathematical models, become necessary. Without the aid of mechanicians, and others skilled in simulation and modeling, developmental biology will remain a prisoner of our inadequate and conflicting physical intuitions and metaphors." In 1990 the geneticist Jonathan Bard wrote: "I ... assert that the process of tissue formation is in many ways the cellular equivalent of molecular selfassembly and that the appropriate language in which to analyze morphogenesis is that of the differential equation ..." [7].

In the next section the relationship between genetic information and tissue development is discussed. In the section after that various epigenetic mechanisms for tissue patterning that have been suggested are described. After a short section on the supramolecular assembly of tissues, the mechanical or mathematical modeling of tissue growth and development occupies the remainder of the chapter. A section on modeling considerations is followed by four sections on continuum models, the first on kinematic models of growth, the second on bone remodeling, the third on soft tissue growth and remodeling and the fourth on heart and joint growth, remodeling and morphogenesis. The final section concerns cellular automata models for cell sorting.

Anthropologists and forensic experts study bone because, due to its mineralized character, it lasts many lifetimes and furnishes a readable record, while it is more difficult to discern structure in soft tissue and the soft tissue disappears relatively quickly, seldom leaving a record to be studied. The development of mechanical models for growth and remodeling of bone have preceded those for soft tissue because bone models are also easier to construct because bone deformations under load are small and because they grow by apposition rather than interstitially. It is for these reasons that there are so many examples associated with bone tissue in this chapter and not the fact that the author has studied bone tissue more than the other tissues.

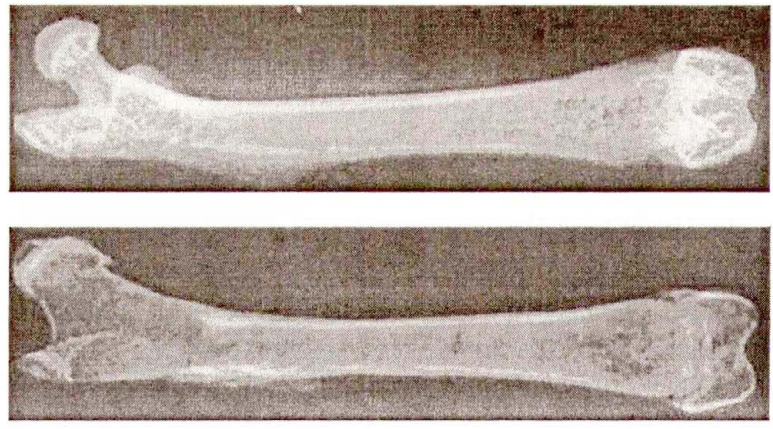
2. Genetic information and the development of a biological tissue

Biological tissues form distinctive and repeated patterns found throughout the animal kingdom. The relationship of genetic information to biological structure, and to the proteins that form the structure, is indirect and not yet fully understood. The three viewpoints are [7] the genetic view, the epigenetic view and the middle view. In the (strong) genetic view tissue structure is predetermined by precise information stored in the genome interpreted by cells as specific instructions. Tissue structure is directly determined by DNA-coded information laid down in the egg. In the epigenetic view tissue structure develops by a process of interaction of cells with their environment. Tissue structure arises from changes in the properties of the cells and the then existing tissue structure. In the middle view both genetic and epigenetic contribute, and the extent to which both contribute depends on the animal or

the tissue under consideration [4]. The answer most preferred is the middle route, as recommended by Daedalus to Icarus. The combination of genetic and epigenetic contributions also applies to the making of proteins. The folding of a protein is created by the interactions of the polypeptide chain within itself and, in the development of a tissue, there are also interactions of the developing tissue with the tissue's external environment that contribute to the determination of the resulting structure [8].

Separation of the influence of genetics and epigenetics was apparently achieved for a specific period in the development phase of a tissue in an animal experiment [9]. In this experiment the cartilaginous primordia or anlage (template) of the femur in a developing mouse was transplanted into the mouse's spleen, a site where the usual mechanical loading of the femur was absent. Cartilaginous primordia are themselves the result of genetic information and epigenetic developmental processes. After development, the bone was compared with that developed in the mouse hind limb under normal physiological loading. It is assumed that neither genetic information nor its usual mechanical loading is delivered to the developing femur for the period that it is in the spleen. The influence of genes and the influence of the combination of normal mechanical loading and the genes can be discerned by comparing these two mouse femora illustrated in Fig. 1. The morphologically normal mouse femur was developed within a normal functional loading environment (Fig. 1(a)). The morphology of a mouse femur deprived of its normal mechanical loading was produced by having the bone develop from its cartilage anlage, without normal mechanical loading, in the spleen (Fig. 1(b)). Although the bone developed as a recognizable femur, it lacked the refinements associated with the normal functional bone. The major features induced by normal mechanical loading were the organization of the internal trabecular structure, the width of the medullary cavity and thickness of the cortical wall, the wasting of the femoral neck, and the curvature of the femoral diaphysis [10].

A basic method of approach in science and engineering is reductionism. The philosophy of this approach is to decompose the object of study into its constituent parts, analyze each part separately, then reconstruct the object and predict its response to stimuli from a knowledge of response of the constituent parts to stimuli. The partial separation of the influence of the primordial template contained in the cartilage anlage and epigenetics in the animal experiment described in the previous paragraph is an illustration of



a)

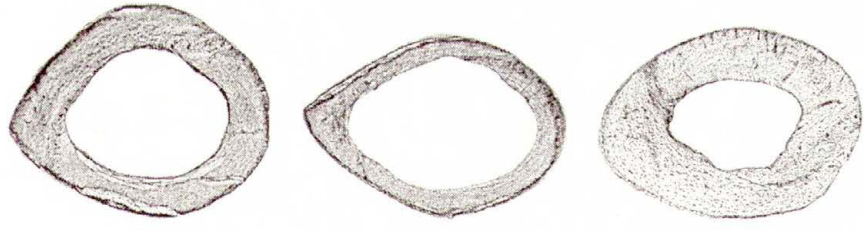
b)

FIGURE 1. Two mouse femora illustrate the effects of genetics and the effects of normal mechanical loading and genetics. (a) Normal morphology of a mouse femur developed within a normal functional loading environment. (b) Morphology of a mouse femur deprived of its normal mechanical loading. This femur was produced by transplanting the primordial template to the spleen and allowing it to develop in the spleen. The experiment thus provides insight into the nature of the primordial template for the mouse femur. From Chalmers and Ray [9].

the reductionist idea that the development of a biological structure can be separated into the effect of the primordial template and the effect of the history of mechanical loading on the biological structure. The idea underlying the primordial template is that, in the absence of mechanical loading, the form of a skeletal element will revert to a form determined by the information in the primordial template. As noted above, the information contained in the primordial template comes from both genetic and epigenetic sources. The experiment described in the paragraph above provides insight into the nature of the primordial template for the mouse femur. The reductionist hypothesis is that any decrease in the functional loading of a bone induces remodeling changes that reduce the bony mass and allow the form of the bone to revert to its primordial template. Studies of the development of cultured chick limb *in vitro* have shown that some of the prominences on specific bones developed in culture and some did not. This is interpreted as indicating that some bony features required functional loading for development whereas others were part of the primordial template. There is a caveat associated with the use of the concept of a primordial template because this reductionist idea does not easily allow for the interaction of genetic and epigenetic influences

that are now known to occur during development. It is now known that some development genes are only activated at the stage of development where they are to be effective and are inactive at other stages. In mathematical terms, the separation of genetic and epigenetic effects can be non-linear rather than linear and therefore not open to reductionism.

The information contained in the primordial template may be different for different strains of a species. Mice from different inbred strains have noticeable structural differences in their bones [11, 12]. The cross-sections of femurs at the mid diaphysis and the vertebrae of three inbred strains of 16-week-old female mice are shown in Figs. 2 and 3, respectively. The age of peak bone mass is 16 weeks in these animals. The names of the inbred strains in the two figures are (left) A/J, (center) C57BL/6J ("Black 6" or "B6") and (right) C3H/HeJ ("C3H"). The A/J strain have slender femurs, small cortical area, small polar moment of inertia, high ash content, low whole bone stiffness, low maximum strength and are brittle. The B6 femurs have small cortical area, large polar moment of inertia, low ash content, and low whole bone stiffness, low maximum strength and are ductile. The C3H femurs have large cortical area, large polar moment of inertia, intermediate ash content, and high whole bone stiffness, high max load and moderate brittleness. The A/J vertebrae have small cross-sectional area, small cortical area, intermediate bone mineral content, low stiffness, moderate brittleness, and low strength. The B6 vertebrae have large cross-sectional area, small cortical area, low bone mineral content, low stiffness, are ductile, and have high strength. The C3H vertebrae have large cross-sectional area, large cortical area, high bone



(a) (b) (c)

FIGURE 2. Images of femoral cross-sections at the mid diaphysis of three strains of 16-week-old female mice. The names of the inbred strains are (left) A/J, (center) C57BL/6J ("Black 6") and (right) C3H/HeJ ("C3H"). From Tommasini et al. [12].

(a) (b) (c)

FIGURE 3. Images of vertebral cross-sections of three strains of 16-week-old female mice. The names of the inbred strains are (left) A/J, (center) C57BL/6J ("Black 6") and (right) C3H/HeJ ("C3H"). From Tommasini et al. [12].

mineral content, high stiffness, high brittleness, and high strength. All three strains have similar vertebral solid volume fraction of bone matrix. Thus the primordial template for a species is not unique. If one views the development of a skeleton or a biological structure as the solution to a mathematical problem, one concludes that the solution is not unique, as well as being development-path dependent.

Fortunately, with the present rate of decoding the human genetic information, a great deal more will be understood in a decade. Certain basic features of the full organism are recorded in the primordial template, but during embryonic development and during early growth the structure of the organism is modified or molded by epigenetic factors. For example, the skin forming the soles of the feet is already thickened in the human fetus, but our common experience shows that walking barefoot or wearing poorly fitting footwear will contribute to the thickness and distribution of this tissue on the foot bottom.

In the following section the various proposals for epigenetic mechanisms that may contribute to the patterning of tissue development are considered.

3. Epigenetic mechanisms for tissue patterning

The most important epigenetic mechanisms that are considered as determinants of individual development are (1) interactions of cell metabolism with the physicochemical environment within and external to the organism, (2) interactions of tissue masses with the physical environment and (3) inte-

ractions among tissues themselves, according to a temporally evolving set of rules [8]. Different epigenetic processes have prevailed at different stages of morphological evolution, and the forms and characters assumed by metazoan organisms (i.e., the broad class of multicellular animals having cells differentiated into tissues and organs and usually a digestive cavity and nervous system) originated in large part by the action of such processes.

3.1. Epigenetic mechanisms associated with the interactions of cell metabolism with the physicochemical environment

Alan Turing, famous for his 1936 creation of the prototype plan for digital computers and for his effort in deciphering the German Enigma cipher during World War II, is also renowned for the chemical reaction mechanism hypothesis. In his 1952 paper entitled "The chemical basis of morphogenesis" he hypothesized that the diffusing patterns of reacting chemicals can form steady state heterogeneous spatial patterns, and this phenomenon could be a biological mechanism of pattern formation [13]. Turing introduced the term "morphogen" to describe the particular chemical substance that was the signal for structural change or development. The development and application of the Turing system to biological and mathematical problems is summarized in the book of Murray [14]. One particularly graphic use of the Turing system described by Murray is his application of the reaction-diffusion equations to mammalian coat patterns. A Turing related diffusion model to explain the development of skeletal pattern in the embryonic chick limb bud was presented in Newman and Frisch [15]. The pattern of development of the chick limb bud between 4 and 7 days of incubation is shown in Fig. 4. The solid black regions represent definitive cartilage; striped areas represent early cartilage. The predicted pattern of limb development using the diffusion model of Newman and Frisch [15] for the morphogen is shown in Fig. 5. The solid black regions in this figure represent cartilage or precartilage condensation; the striped regions represent the distribution of the morphogen preceding chondrogenesis.

There is another set of models in which diffusion-like equations are combined with equations describing mechanical activity. The model of mesenchymal morphogenesis gives an example of this sort where an active stress exerted by cells is taken into account. The mesenchyme is part of the embryonic mesoderm, consisting of loosely packed, unspecialized cells set in a gelati-

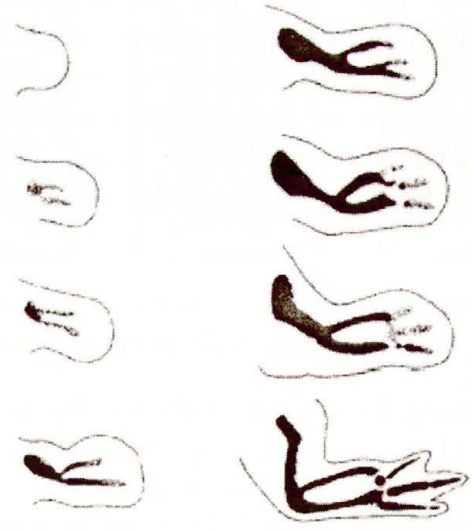


FIGURE 4. Development of the chick limb bud between 4 and 7 days of incubation. The solid black regions represent definitive cartilage; striped areas represent early cartilage. From Newman and Frisch [15].

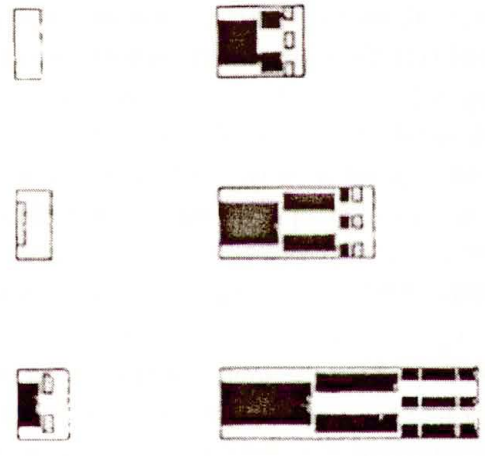
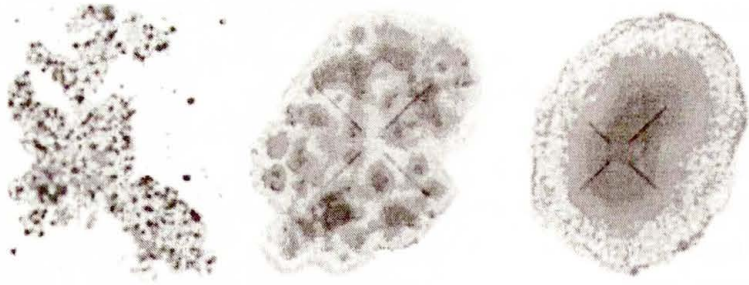


FIGURE 5. The predicted pattern of limb development using the Newman and Frisch [15] diffusion model. The solid black regions represent cartilage or pre- cartilage condensation; striped regions represent the distribution of the morphogen preceding chrondrogenesis. Elongation of the domain is based on empirical measurements; it represents growth superposed on the diffusion in the domain. From Newman and Frisch [15].

nous ground substance consisting of proteoglycans, from which connective tissue, bone, cartilage, and the circulatory and lymphatic systems develop. The condensation of cartilaginous skeletal rudiments in the developing vertebrate limb was also modeled in Oster et al. [16] with equations similar to those in Newman and Frisch [15], but with a model that incorporated the cumulative effects of cell-generated forces [17] in place of the purely chemical diffusive mechanism used in Newman and Frisch [15].

A second mechanism for the morphogenetic event of pattern formation in tissue development is that cells somehow sense the tissue in which they reside and make use of this "positional information" to trigger further steps in development. This concept is due to developmental biologist Lewis Wolpert who suggests that there is both a "weak" statement of the positional information mechanism and a "strong" statement [18, 19]. In the weak statement of the concept the operational mechanism of "positional information" is not specified. In this case a cell does something just by being in a certain tissue at a certain stage of its development; the cell knows where it is. In the "strong" statement of the positional information concept Wolpert suggests that the cell knows where it is because it can sense the concentration of some chemical or, to use Turing's term, morphogen. There is a gradient of the morphogen and the cell "knows" from the concentration of the morphogen that it "measures" where it is located along the gradient.

The idea underlying the differential adhesion hypothesis [20, 21] is that combinations of different cell types behave as immiscible liquids because of surface tension, but with the cell-to-cell adhesion forces playing the role of molecule-molecule attraction forces. Two fluids are said to be immiscible if they do not mix, miscible if they do mix. The most well known examples of immiscible fluids are oil and water. In immiscible liquids a liquid with a higher surface tension will form droplets enclosed by drops of another liquid with lower surface tension. In combinations of different cell types, cells with higher surface tension will band more tightly together forcing the cells with a lower surface tension to the outside. The last statement is the differential adhesion hypothesis. Fig. 6 shows experimental images from the sorting of chicken embryo cells in culture [22, 23]. In Fig. 6 the light cells are neural retinal cells and dark cells are pigmented retinal cells. The initial situation, shown in the first panel, is random mixture of light and dark cells. The second panel shows the dark clusters formed by 10 hours, and the third panel shows the dark cell core surrounded by a light cell shell at 72 hours. It has been experimentally



(a) (b) (c)

FIGURE 6. Experimental images from chicken embryo cells in culture: light cells are neural retinal cells and dark cells are pigmented retinal cells. An initial random mixture of light and dark cells (a) forms dark clusters after around 10 hours (b), and eventually sorts to produce a dark cell core surrounded by light cells after around 72 hours. From Alber et al. [23].

demonstrated [24] that the mean size of the interconnected cellular domains, like those in panel (b) of Fig. 6, increase linearly in time, consistent with the analogy between combinations of different cell types and immiscible fluids.

A mechanism other than surface tension-induced relative adhesiveness has been suggested to be consistent with the analogy between combinations of different cell types and immiscible fluids. Harris [25] suggested that the observations that support the surface tension-induced relative adhesiveness mechanism are equally consistent with an explanation based on the cell's own contractility of its cytoskeletal structure. The idea is that the contraction of the surface layer of a cell induced by the contraction of the underlying cytoskeleton simulates surface tension since surface tension is due to the mutual attraction of subunits in a surface layer.

3.2. Epigenetic mechanisms associated with interactions of tissue masses with the tissue's physical environment

Wolff's law is generally considered to be a statement on the epigenetic effect of mechanical loading on a tissue, generally to the effect that, over time, the mechanical load applied to a living tissue influences the structure of the tissue. The sensors of this mechanical load applied to the tissue are thought to be the tissue cells, e.g., the endothelial cells in blood vessels, the chondrocytes in articular cartilage and the osteocytes in bone. This "weak" or "loose" statement of Wolff's law represents the common usage of the term

"Wolff's law" today, although Wolff only concerned himself with bone tissue [26, 27]. Wolff's precise claim was that his "law" was a rigorous mathematical statement, namely that the pattern of trabecular architecture of cancellous bone coincided with stress trajectories in the bone. This "strong" form of Wolff's law is not valid, as many students of the subject over the last century have concluded [28]. The concept of functional adaptation to mechanical loading of a tissue ("Wolff's law") is more legitimately credited to Roux [29].

3.3. Epigenetic mechanisms associated with interactions among tissues themselves

Mechanical instability, or buckling, is another mechanism that might classify as a positional information mechanism of the "weak" type. Examples of mechanical instability are numerous and include column and plate buckling. Realistic models for some biological pattern-forming processes are the deflections associated with column and plate buckling when the beam or plate is on an elastic foundation under axial compression (like a column with lateral support from springs). The supporting springs are considered to be so close together that they can be treated as continuous elastic support. As the compressive axial load acting on this beam is increased it will deflect from its straight-line shape into the shape of $(N + 1)$ half-sine waves where $N = 0, 1, 2, 3$, etc., and where N depends upon the value of the compressive load. This is just one example of many mechanical instability, or buckling, situations that might be used by developing organisms to generate patterns for their structure. This particular example gives rise to a one-dimensional pattern, whereas plates compressed in two directions will give rise to a great variety of two-dimensional patterns. A fascinating buckling plate pattern formation hypothesis for the patterns associated with leaf and petal formation has been developed in Green [30, 31]. A pattern of leaf and petal formation is called a phyllotaxy or phyllotaxis. A buckling pattern formation hypothesis for spiral phyllotaxis was developed in Green [31] and [30]. The apical meristem, or growth tip, of a plant was modeled as an undulating shell of circular shape on an elastic foundation subjected to several types of compressive stress systems due to turgor and/or growth. The compressive stress systems included one in the tangent plane of the undulating shell of circular shape and one pushing the undulating shell of circular shape from below, that is to say on the plant tissue side of the growth tip. The existence of

compressive stresses at the appropriate shifting active region between two circles on the undulating surface of the shell representing the meristem surface is demonstrated in Dumais and Steele [32] using meristem experiments and numerical calculations of the stresses in the shell (tissue). The results of the numerically calculated stresses in the shell (tissue) produced a buckling pattern characterized by the same pattern as the spiral phyllotaxis. A great deal of similarity between the flower structure and the calculated buckling pattern was demonstrated. The buckling pattern of an undulating shell of circular shape on an elastic foundation is not the only model for the pattern displayed by the spiral phyllotaxis; there are a number of other models including reaction-diffusion models.

The proponents of buckling mechanisms suggest that these mechanisms are favored over the morphogen approach of Turing for a number of reasons. First, mechanical instabilities can create physical structures directly, in one step, in contrast to the two or more steps that would be required with morphogens if positional information first had to be specified by morphogen gradients and then only secondarily implemented in physical form. Second, physical forces act at much longer range (and more quickly) than can diffusing chemicals. A difficulty with the morphogen approach is the identification of morphogens and the fact that quite different morphogen systems can produce the same pattern. Some argue that the mechanical activities of cells can themselves accomplish the morphogenetic functions usually attributed to the diffusion and reactions of chemical morphogens. Systems of cells may also accomplish this by exerting traction forces by which they can propel themselves and rearrange extracellular materials, in particular the fibrous protein collagen. Because the compression and alignment created by these cellular forces can, in turn, affect cell behavior, positive feedback cycles of several kinds arise, and these cycles are capable of spontaneously generating regular geometric patterns of cells and matrix.

4. Supramolecular assembly

The question of how a tissue evolves from its primordial template to a mature, functioning tissue has not been fully answered. Exactly what is both known and conjectured about the mechanisms used to form one tissue, the tendon, from collagen molecules *in vivo* is described in Silver et al. [33]. It is clear from the fact that animals generally mature in repeatable fash-

ion that a tendon must grow in length so as to maintain its connection to both bone and muscle during the maturation process. It must also grow in cross-sectional area so that it can transmit increasingly greater forces between muscle and bone during the maturation process. The production of the collagen molecule by the cell is now well understood, and the structure of the collagen molecule has been deduced. The structural hierarchy of the collagen fibrils and the tendon is well understood. An excellent summary of both *in vivo* and *in vitro* supramolecular assembly of the tendon is recorded in Silver et al. [33]. If the author knew similar developments of other tissues, they would be noted here.

5. Some modeling considerations

Models to describe tissue growth and remodeling may be classified as phenomenological or mechanistic. The phenomenological models attempt to simulate cause and effect (e.g., changed mechanical loading leading to changed tissue architecture) without a consideration of the intermediary mechanical and biological mechanisms involved. Phenomenological models allow for conveniently testing the consequences of different hypotheses about tissue growth and adaptation. This approach is often useful for eliminating some assumptions that don't match experimental or clinical results and observations (e.g., only compressive static loading leads to a particular tissue formation) or stimulate further investigations (e.g., strain rates and spatial gradients may regulate adaptation). The theory of adaptive elasticity for bone, reviewed and critiqued in Section 7 is a phenomenological model. The basis concepts underlying phenomenological models for soft tissue growth and adaptation will be described in Section 8.

Mechanistic models, on the other hand, start instead with parameters (e.g., cell activities and microenvironment) that are linked to portions of the biological processes involved in tissue maintenance, turnover, and repair. These models, currently less developed than some of the phenomenological models because they are more complex, may lead to successfully linking mechanical and biological causes and effects. These models offer the promise of not only extending the descriptive and predictive capabilities of phenomenological models, but may offer insights into manipulation of the tissue response, and development of pharmacological therapeutic agents. A mechanistic model for bone adaptation is described by Cowin [34]. A mecha-

nistic model that relates the effect of mechanical load applied to a whole bone to the bone fluid flow around the cells buried in the bone and, most significantly, to the bone adaptation process has been presented in a series of papers over the last decade by Cowin, Weinbaum and associates: [35, 36, 37, 38, 39, 40, 41, 42, 43, 44]. Mechanistic bone models are not reviewed here; aspects of these models are reviewed by Cowin in [34] and [45].

In the creation of phenomenological mathematical models for tissue remodeling and growth, the modeler must select a parameter to represent the effect of the mechanical loading on the development and remodeling of the tissue. In a mechanistic model such a parameter would arise from biophysical considerations. Do biological tissues sense stress, strain, strain rate or some other parameter of the mechanical loading? This is equivalent to asking if a tissue has baroreceptors (stress receptors) or stretch receptors (strain receptors) or another type of receptors. One answer to the question is that tissues sense strain or stretch or strain rate or stretch rate and not stress [46]. The reason that tissues sense strain or strain rate and not stress is that strain is a primary, directly measurable, physical quantity whereas stress is not. Stress is an abstract concept, a creation of man that can be measured only indirectly. Baroreceptors are described as "a spraytype nerve ending lying in the walls of arteries that are stimulated when stretched" (cf., e.g., [47]). Clearly, baroreceptors sense stretch. From this perspective, the terminology "baroreceptor" is a misnomer; they are stretch receptors.

Strain is excluded as a possible growth stimulus for soft tissue [48] because of the difficulty in defining a reference configuration for its measurement in a growing material. The question is one of gage length. If the gage length is changing due to the addition of new tissue, how can one measure strain? Several studies have considered this question of the appropriate stimulus [49, 50] and some have developed models in which the growth of soft tissue is dependent upon applied stress [48, 51, 52, 53, 54]. The substantial and important structure of these studies are independent of the fact that it is assumed that the growth of soft tissue is dependent upon applied stress; the alternative structure necessary to convert the stimulus from applied stress to loading rate of deformation or loading strain is easily constructed. The exact stimulus for growth is presently unknown (in bone there is strong evidence to suggest that it is strain rate). However, the argument sometimes presented is that strain requires a reference configuration and stress does not. The problem is that reference configuration for strain will change in time due to tissue

growth and remodeling. There is a concern that this is really a problem and, if it is a problem, it can be addressed in another way.

First, is this really the problem? It is not possible that the gage length used for the sensing of strain does not change as the tissue grows, as noted by Cowin [55]. For example, the cell may sense the strain itself and not change its length on the remodeling time scale t. Numerous cell culture studies have shown that the key connective tissue building cells begin to respond to strains above about 0.5% with various signals that indicate the cells are aware that they are being excited. Generally cells in tissues at different stages of growth are the same size. Cells probably do not change their length on the time scale t as the tissue grows; the cells can however stiffen or relax their cytoskeleton on a time scale that is greater than the loading time scale τ and less than the remodeling time scale t. The tissue either adds or subtracts cells or ECM to achieve the volume change; it is unlikely that it changes the (sensor) cell size. One cell does not likely determine the response of a tissue; it is more likely that the response of the tissue is determined by averaging the response of numerous cells by intercellular communication. Thus it appears that it is possible that the gage length used for the sensing of strain does not change as the tissue grows. However this observation does not entirely solve the problem because it leaves open the question of how to construct a model with such a microstructural gage length.

Second, if it is a problem, can it be addressed in another way? Appropriate measures of the rate of deformation or strain upon which the growth stretch may depend may be constructed if the two-time scales in living tissue mechanics are recognized. These time scales differ by many orders of magnitude [55]. Let T_r denote the time that biological processes take to complete significant growth (or remodeling) associated with a mechanical loading, and let $T_L$denote the characteristic period of mechanical loading. Rough estimates of these numbers are two weeks and one second, respectively; thus T_L/T_r is a small number, of the order 10^{-6}. In order to keep these time scales separate let τ denote time on the loading time scale and t denote time on the remodeling time scale. Measures of tissue strain may be constructed using two facts, the fact that the tissue is strained on the time scale τ and the fact that the gage length changes on the time scale t, a million times longer.

The loading rate of deformation of an object is an instantaneous kinematic measure independent of reference configuration or gage length, and thus does not have the disadvantage that might exclude strain as a growth stimulus.

The rate of deformation is suggested as a remodeling stimulus for endothelial and bone cells in the sense that the shear stress due to fluid motion over these epithelial cells is a stimulus for their activity and shear stress is proportional to the rate of deformation of a fluid. It is an open question as to how much of the rate of deformation is actually transferred to the epithelial cells. The cell layer is compliant and the undulations of the cell contour are observed in laminar shear flows in which the cells are attached to a supporting plate. Adaptive remodeling of the vasculature has been observed so as to maintain nearly constant endothelial fluid shear stress (rate of deformation to the cell) throughout the entire arterial side of the circulation [56]. Explicit forms of appropriate or plausible measures of loading rate of deformation or loading strain upon which the growth stretch rate are presented in Cowin [55]; see also Luo et al. [57]. The main point is that the recognition of two time scales in the growth process, the loading time scale and the growth (or remodeling) time scale, scales that differ by many orders of magnitude, permit the consideration of strain and rate of deformation as growth stimuli in finite growth models of soft tissues.

6. Continuum kinematic models of growth

The growth and development of hard and soft tissues are kinematically different. The growth of hard tissues is appositional, that is to say the growth is by deposition and resorption at a surface. The growth of soft tissues is interstitial volumetric growth. Thus an increase in a hard tissue occurs by adding material on an existing surface while soft tissues grow by adding material internally. The kinematics of growth of an organism was a primary interest of D'Arcy Thompson and the subject is well illustrated in his famous book [5]. The classical ideas of growth kinematics were recast in the mathematics of contemporary continuum mechanics in Skalak et al. [58]. This included continuum kinematics models for volumetrically and surface distributed growth by deposition or resorption, the growth of horns and spiral shells (a favorite of D'Arcy Thompson), allometric growth and the initial idea for a kinematic model in which simultaneously occurring growth and deformation are considered as a sequence of two mappings, one representing stress free growth and the other representing the deformations of the tissue due to forces acting upon the tissue. This last topic will be discussed at the beginning of Section 9. However, the other topics on the modeling of the kinematics of

growth of an organism are not reviewed here because the review of Skalak et al. [58], with its refinements [59, 60], are still current and readable.

7. Continuum models for bone remodeling

The first continuum model for bone adaptation to mechanical loading was the (phenomenological) theory of adaptive elasticity [61, 62, 63]. The objective of these papers was the formulation of a model for the understanding and prediction of the strain-controlled remodeling properties of normal living bone. The strain adapting properties of living bone are represented by a strain-controlled chemical reaction that transfers mass, momentum, entropy and energy to and from the porous elastic solid. The addition of mass to the porous solid modifies its porosity. Bone adaptation to environmental strain is a collective phrase for the continual processes of growth, reinforcement and resorption that occur in living bone. The resulting theory describes an elastic material that adapts its structure to applied loading, hence the term *adaptive elasticity*. Seen from the perspective of a quarter of a century, there were major successes in the 1976 theory of adaptive elasticity and there were things that should be done differently if the theory is to be renovated. The largest success was the development of a thermodynamic open-system model of tissue adaptation. This model has been followed, or assumed as a starting point, by most subsequent models of tissue adaptation (a survey of these models appears in Hart [64]). The essence of the model was the assumption that the load adapting properties of living bone can be modeled by a chemically reacting porous medium in which the rate of reaction is strain controlled. The porous medium has two components: a porous elastic solid representing the matrix structure of bone including the bone cells and a perfusant that represents the extracellular fluid and the blood plasma which flow through the matrix structure. A schematic diagram of this model is shown in Fig. 7. The fact that living bone is encased in a living organism is reflected in the model by setting the porous structure in a bath of the perfusant. The perfusant bath is assumed to be an isothermal heat reservoir, an assumption that appears to be easily justified by common knowledge concerning living organisms. The mechanical load is applied directly to the porous structure across the walls of the perfusant bath as illustrated in Fig. 7. The system consisting of the porous structure and its perfusant bath is considered to be closed with respect to mass, heat energy, and entropy transfer, but open with

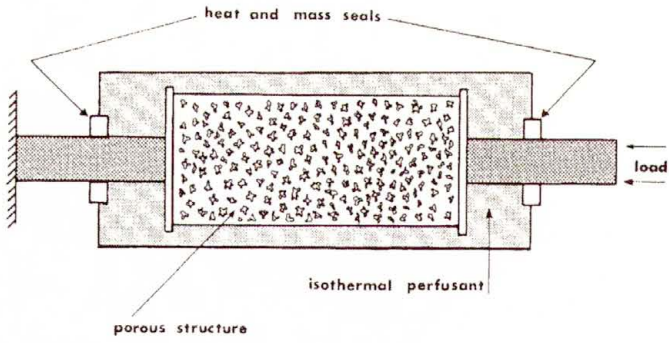


FIGURE 7. A schematic diagram of the conceptual model for bone adaptation to mechanical loading. From Cowin [61].

respect to momentum transfer from loading. The system consisting of only the porous structure without its entrained perfusant is open with respect to momentum transfer as well as mass, energy, and entropy transfer. The bone matrix was considered to be the control system since the mechanical properties of the bone matrix alone determine the mechanical properties of the bone. Balance and constitutive equations were developed only for the bone matrix. The perfusant was accounted for only insofar as it transfers mass, momentum, energy, or entropy to the bone matrix. The rate at which mass, momentum, energy and entropy transfers occur was assumed to depend on the local strain and the other independent variables. One of the strongest assumptions made was the assumption that the internal energy of the mass added to the porous tissue was equal to the internal energy of the porous tissue at the site where the mass is added. In retrospect this is a fairly easy assumption to justify if one recalls how bone tissue is deposited and if the bone adaptation is viewed on two time scales as discussed in Section 5 above. New bone deposition begins with a deposit of osteoid on an existing bone surface. The osteoid is of much lower modulus than the existing bone. Over a time period of a month the osteoid will become mineralized and its modulus will increase approximately linearly with the mineralization. Since the mechanical loading time scale is much, much shorter than the adaptation time scale the osteoid/bone will undergo many million cycles of mechanical loading in the course of the adaptation tissue deposition time period. It follows that the internal energy can be gradually increased with the mineralization in the tissue deposition time period so that, when viewed from the long-term adaptation time scale, it will be equivalent to having the new mass deposited at the same internal energy as the existing mass in the porous bone

structure. Other features common to most of the later models (these models are described by Hart [64]) of internal adaptation include the formulation of the theory in terms of a constitutive relation for the mass supply term introduced by the open system formulation of the mass balance equation; the porosity of the medium could be increased or decreased. The increased or decreased porosity is then assumed to influence the elastic constants through their constitutive dependence upon porosity.

There are changes recommended if the theory of adaptive elasticity were to be reformulated. A major change would be the introduction of two time scales discussed in Section 5 above. The reformulated theory of adaptive elasticity should allow the mass supply and the free energy to depend upon the remodeling time and the history of deformation; thus functions of deformation gradients in the original theory should be replaced by functionals of the history of reference solid volume fraction and deformation gradients. Such strain history dependent relationships are familiar from non-linear viscoelasticity. The reformulated theory would then consist of the usual equations for an elastic object and equations which change on the long time scale, very slowly adding or subtracting mass and free energy and, thereby, changing the elastic constants. Perhaps the point most in need of revision in the theory of adaptive elasticity is the stimulus for remodeling. The model pointed to time-averaged-strain and strain-energy as the first measures of stimulus. The experimental literature suggests however that it must be a stimulus that involves the rate-of-strain or the rate-of-loading. It has been shown that a moderate periodic strain applied to living bone is more effective in causing remodeling than a static strain of the same magnitude. It has also been shown that a constant load applied with fixed springs to isolated living bone caused it to resorb as if there were no load placed on the bone [65]. In other words, the living bone showed no effect from the constant, non-time varying loading. Further evidence that the effective stimulus was strain rate is provided in O'Connor et al. [66] and Rubin and Lanyon [67]. The mechanistic models (see Section 5) that relate the effect of mechanical load driven bone fluid flow around the cells buried in bone to the bone adaptation process strongly suggest strain rate as a strong mechanical stimulus [34, 36].

The theory of adaptive elasticity has been recently reviewed and critiqued in Cowin [68]. This model and other phenomenological models for cortical and cancellous bone adaptation have recently been reviewed in Hart [64]. These reviews are still current.

8. Continuum models for soft tissue growth and remodeling

8.1. The separability hypothesis

In the final section of the Skalak et al. paper on the analytical description of growth [59], discussed in Section 6, a kinematic model is described in which simultaneously occurring growth and deformation are considered as a sequence of two mappings, one representing stress free growth and the other representing the deformations of the tissue due to forces acting upon the tissue. This may be the first statement of the separability hypothesis that simultaneously occurring growth and deformation may be decomposed into a growth deformation and an elastic deformation. The validity of this hypothesis is easy to imagine in a thought experiment involving a whole bone without residual stress. One simply measures the size of the bone in a fixed, unstressed situation periodically over a significant growth period. Since the measurement is done in the unstressed situation, and there is no residual stress, the measurement represents the growth of the bone. The hypothesis is not valid if there is an inelastic deformation of the bone as there would be an Ilizarov lengthening procedure.

The hypothesis was extended [48] to a general three-dimensional theory of mechanically modulated volumetric growth for soft incompressible biological tissues. The mapping composition idea described in Skalak et al. [58] was rendered in Rodriguez et al. [48] as a composition of deformation gradient mappings. The overall growth deformation is represented by a mapping, denoted by $\mathbf{F}_{\mathrm{eg}}$, of the initial configuration of the object $B(t_0)$ into the instantaneous configuration $B'(t_1)$. The decomposition of the mapping suggested by Rodriguez et al. [48] is represented by

$$\mathbf{F}_{\mathrm{eg}} = \mathbf{F}_{\mathrm{e}} \cdot \mathbf{G}, \tag{8.1}$$

where $\mathbf{G}$ is a symmetric tensor representing the growth deformation gradient and $\mathbf{F}_{\mathrm{e}}$ represents an elastic deformation necessary to maintain overall compatibility of the mapping $\mathbf{F}_{\mathrm{eg}}$. The representation (1) follows from the fact that any deformation gradient can be decomposed into a product of deformation gradients as long as there is a definition of one of the elements of the decomposition that provides a method to calculate the other element of the decomposition. That is the case because of our ability to measure growth [69]. The symmetry of $\mathbf{G}$ follows from the fact that any deformation gradient may be decomposed into the product of an orthogonal tensor

representing the rigid object rotation and a symmetric positive tensor representing the shape changing deformation. When growth is measured any effect of rigid object rotation is easily removed, hence **G** is necessarily symmetric as pointed out by Skalak et al. [58]. The mapping decomposition (1) is illustrated in Fig. 8. Note that there is no mechanical loading applied to any of the objects in Fig. 8, but it is possible for the final configuration $B'(t_1)$ to have residual stresses due to incompatibilities induced by growth. It is interesting to compare Fig. 8 with the two photos of mouse femora shown in Fig. 1. It is reasonable to associate the normal morphology of a mouse femur developed within a normal functional loading environment (Fig. 1a) with the final configuration $B'(t_1)$ in Fig. 8 and the mouse femur deprived of its normal mechanical loading (Fig. 1b) with the configuration $B(t_1)$ in Fig. 8, the configuration resulting from growth without loading.

This decomposition (1) is analogous to the decomposition of the deformation gradient used in plasticity theory to separate finite elastic and plastic deformations since the 1960's [70, 71, 72, 73] and in the literature on polymeric swelling to separate elastic and swelling deformations since the 1940's [74]. Each biological situation in which the assumption (1) is to be applied must be carefully scrutinized to evaluate the justification of the separation of the growth and deformation effects. The separability hypothesis is an example of the reductionism problem discussed in Section 2. In the case where the growth is well mapped so that the growth tensor **G** is known, there should

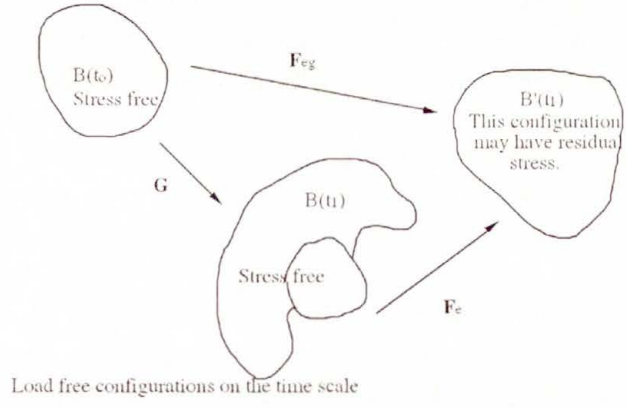


FIGURE 8. An illustration of the relationship between the various (unloaded) configurations considered and the deformation gradients that represent mappings between these configurations. This diagram is conceptually identical with Figure 1 of Rodriguez et al. [48]. Adapted from Cowin [55].

be no difficulty. An example of that might be the development and loading of the mouse femur suggested by the photos in Fig. 1. However if there is any morphological development event occurring that cannot be represented by the growth tensor $\mathbf{G}$, the decomposition (1) is not possible. This restriction is due to the definition of growth; if we were able to describe morphological events mathematically, the growth tensor could be replaced by a growth and morphogenesis tensor and the predictive capacity of the model increased.

Models of tissue growth in the cardiovascular system employing these ideas have been described by Taber and coworkers [52, 53, 54] and Rodriguez et al. [75, 76].

8.2. The loading time scale and the growth time scale

The recognition of two time scales in the growth process, the loading time scale and the growth (or remodeling) time scale, scales that differ by many orders of magnitude, permits the consideration of strain and rate of deformation as growth stimuli in finite growth models of soft tissues as noted in Section 5. Explicit forms of appropriate or plausible measures of loading rate of deformation or loading strain upon which the growth stretch rate may depend may be constructed using the two time scales, the loading time scale τ and the remodeling time scale t. An extension of the representation in Fig. 8 to include these two time scales and two loaded configurations is shown in Fig. 9; Figure 8 is repeated in the box outlined with dotted lines in Fig. 9. The growth tensor $\mathbf{G}$ is now a function of t, not τ thus $\mathbf{G} = \mathbf{G}(t)$. The deformation gradient associated with the loaded configuration from the unloaded, but not necessarily stress free, configuration is denoted by $\mathbf{F}_{\mathrm{L}}(\tau, t)$. The tissue is mechanically strained on the loading time scale τ and therefore the deformation gradient of loading $\mathbf{F}_{\mathrm{L}}$ is considered to be dependent on both time scales. If the residual elastic deformation $\mathbf{F}_{\mathrm{e}}$ is significant and thought to be a growth stimulus, then rather than employing the loading deformation $\mathbf{F}_{\mathrm{L}}$ as the basis for the independent constitutive variable in the growth stimulus, the basis could be provided by the composition $\mathbf{F}_{\mathrm{eL}}$, $\mathbf{F}_{\mathrm{eL}} = \mathbf{F}_{\mathrm{L}}\mathbf{F}_{\mathrm{e}}$, of the residual elastic deformation $\mathbf{F}_{\mathrm{e}}$ and the loading deformation $\mathbf{F}_{\mathrm{L}}$. This would make the growth stimulus dependent on both the loading and the residual strain.

$$\mathbf{F} = \mathbf{F}_{\mathrm{L}} \cdot \mathbf{F}_{\mathrm{eg}} = \mathbf{F}_{\mathrm{L}}(\tau, t) \cdot \mathbf{F}_{\mathrm{e}}(\tau, t) \cdot \mathbf{G}(t). \tag{8.2}$$

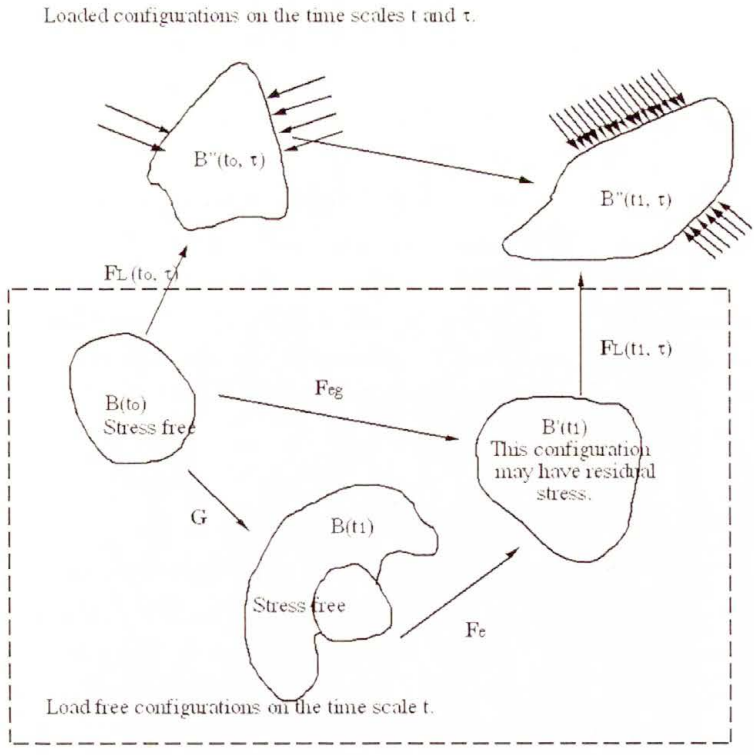


FIGURE 9. An illustration of the relationship between the various configurations considered. Broadly there are two classes of configurations, loaded and unloaded. The unloaded ones are within the portion of the diagram outlined with the dotted lines. This portion of the diagram is identical with Fig. 8. The loaded configurations are above and outside of the dotted lines. The deformation gradient associated with the deformation to the loaded configuration from the unloaded (but not necessarily stress free) configuration is denoted by $\mathbf{F}_{\mathrm{L}}(\tau, t)$. It is shown at times t_0 and t_1. Adapted from Cowin [55].

8.3. Residual stresses

The presence of residual stress in biological tissues has been widely reported in the last two decades. Residual stresses in the arteries were demonstrated by performing experiments on unloaded arterial rings dissected from blood vessels [77, 78, 79, 80]. Two transverse cuts of an artery yield an unloaded arterial ring. If that arterial ring is cut so that it is no longer an approximately circular ring but a sector of a circular ring, the unloaded sector of the arterial ring opens and displays an opening angle indicating that,

when it was a complete ring, it had internal stresses. In fact if one keeps cutting the sector of circular ring into smaller pieces, each cut produces a new opening angle indicating the presence of internal stresses in each progressively smaller piece. The presence of residual stress has been established in a number of other tissues, including veins [81], ventricular myocardium [82, 83] and the trachea [84]. The residual stress in biological organs is thought to be a consequence of non-uniform growth, resorption, and remodeling [85, 86, 58]. Residual stresses in the heart and arteries are thought to minimize the peak stresses experienced by these tissues *in vivo* [87, 88, 79] and thus improve their mechanical function. The inclusion of the residual stress in the stress constitutive equations used to describe these soft tissues has been the focus of Hoger and coworkers [89, 90, 91].

8.4. General continuum models of growth

A general constitutive theory of the stress-modulated growth of soft tissues is developed by Lubarda and Hoger [92]. The work provides an explicit representation of $\mathbf{G}$ for various material symmetries, and an incremental formulation for stress-modulated growth process. A theory of material growth (mass creation and resorption) is presented in Epstein and Maugin [93]. In this work growth is viewed as a local rearrangement of material inhomogeneities. The question of growth in continuum growth models is examined from a rigorous mathematical approach in DiCarlo and Quiligotti [73].

9. Continuum models for heart and joint growth, remodeling and morphogenesis

Modeling the development of an organ or an organism is an objective that is presently experiencing a pioneering exploration. Such models will necessarily involve the processes of growth, remodeling and morphogenesis. Two examples are briefly described here, the modeling of the embryonic chicken heart [94, 49, 95, 96] and a model for articular joint morphogenesis [97, 98].

The heart is the first functioning organ in the embryo and it continues to function without interruption although its morphology changes dramatically during development. Development consists of a coordinated, dynamic interaction between genetic and environmental factors that regulate the primary developmental processes of volume change (growth), tissue property change (remodeling), and shape change (morphogenesis). A mathematical model for

the embryonic heart development in the chicken as it transforms from a single tube into a four-chambered pump is given in Taber and Perucchio [94].

A mathematical model for joint morphogenesis encompassing the hypothesis that the stress distribution created in a functional joint will modulate the growth of the primoridal template and lead to the development of congruent articular surfaces has been described by Heegaard and coworkers [97, 98]. In a computational model the morphogenesis of a human finger joint (proximal interphalangeal joint) was simulated for the period between days 55 and 70 of fetal life. It was assumed the biological growth rate was proportional to the chondrocyte density in the growing tissue. Cyclic hydrostatic stress caused by joint motion was assumed to modulate the baseline biological growth, with compression slowing it and tension accelerating it. The model prediction was that the articular surfaces became more congruent, and the primordial template exhibited an asymmetric sagittal profile similar to that observed in adult phalangeal bones.

10. Cellular automata type immiscible fluid models for cell sorting

The models considered thus far in this chapter are continuum models based on the calculus. In a large and extensively illustrated book Wolfram [99] argues that the computational capabilities now available permit investigators to change their methods of calculation from calculus-based to computational algorithm-based. Thus equations are replaced by the simple step-by-step procedures of computer programs. In these algorithms time and space are no longer considered as continuous but as digital steps. The continuum is replaced by a grid or lattice. The algorithms for the accomplishment of this objective are called cellular automata.

When one first encounters cellular automata they appear similar to a game of chess or checkers because they involve arrays of squares that look like a chess or checker-board. The rules for any system, including chess or checkers, "can be viewed as corresponding to a program, so its behavior can be viewed as corresponding to a computation" ([99], p.165).

Recall from Section 3.1 that immiscible fluids are of interest in the mechanical modeling of biological tissue development because cell sorting is analogous to the separation of phases in the immiscible fluids. Lattice models of immiscible fluids have been found to be very effective calculational tools.

Recall also the differential adhesion hypothesis described in Section 3.1, the hypothesis that combinations of different cell types behave as immiscible liquids (for example, oil and water) because of surface tension, but with the cell-to-cell adhesion forces playing the role of molecule-molecule attraction forces. Recall that in immiscible liquids a liquid with a higher surface tension will form droplets enclosed by another liquid with lower surface tension. In combinations of different cell types, cells with higher surface tension will band more tightly together forcing the cells with a lower surface tension to the outside. The last statement is the differential adhesion hypothesis. This sorting was illustrated in Fig. 6; Figure 6 is repeated as the top set of three panels in Fig. 10. These three panels showed the experimental images from chicken embryo cells in culture. The light cells are neural retinal cells and dark cells are pigmented retinal cells. The initial situation, shown in the first

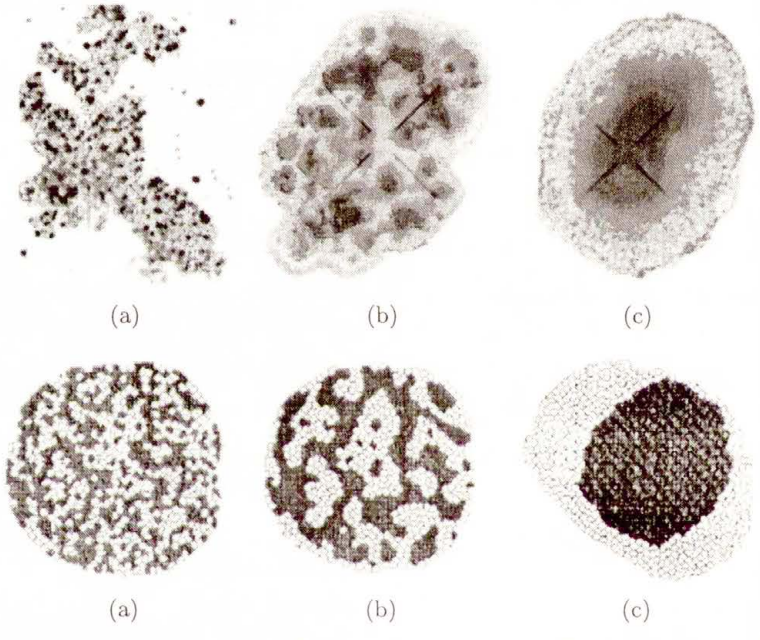


FIGURE 10. The top panel repeats Fig. 6 and represents experimental images from chicken embryo cells in culture: light cells are neural retinal cells and dark cells are pigmented retinal cells. An initial random mixture of light and dark cells (a) forms dark clusters after around 10 hours (b), and eventually sorts to produce a dark cell core surrounded by light cells after around 72 hours. The bottom panels show the corresponding images from a simulation with three cells types: light cells, dark cells and medium. From Alber et al. [23]

of the three panels, is a random mixture of light and dark cells. The second panel shows the dark clusters formed by 10 hours, and the third panel shows the dark cell core surrounded by a light cell shell at 72 hours. The bottom set of three panels in Fig. 10 shows the corresponding images from an immiscible lattice gas simulation with three cell types: light cells, dark cells and medium [22, 23].

There appears to be a great deal of potential for modeling the mechanics of biological tissue development using cellular automata. There is a challenge however to combine both deformation and tissue development in cellular automata models.

Acknowledgement

The Whitaker Foundation, NIH, NSF, and the PSC-CUNY Research Award Program of the City University of New York supported this work. A substantial portion of the material in this contribution is taken from Cowin [100].

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