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# Mechanics of cell growth

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ABSTRACT

Cell growth describes an essential feature of biological tissues. This growth process may be modeled by using a set of relatively simple governing equations based on the axioms of mass and momentum balance, and using a continuum framework that describes cells and tissues as mixtures of a solid matrix, a solvent and multiple solutes. In this model the mechanics of cell growth is driven by osmotic effects, regulated by the cells' active uptake of solutes and passive uptake of solvent. By accounting for the anisotropy of the cells' cytoskeletal structures or extracellular matrix, as well as external constraints, a wide variety of growing shapes may be produced as illustrated in various examples.



## 1. Introduction

Growth is the process by which mass is added to, or removed from a solid material. In biological growth this mass supply is typically made available in soluble form, such as molecular building blocks that combine to produce a larger structure. These solutes are carried by a solvent, usually water, which together form a solution that must interface with the solid constituents of biological tissues to allow mass exchanges. Therefore, a natural starting point of theoretical frameworks for growth mechanics is the axiom of mass balance, which can describe exchanges between different constituents of a biological mixture. The material properties and structure of the solid may be altered as a result of these mass exchanges, leading to concomitant alterations in stresses. Consequently, growth mechanics also requires frameworks that accommodate evolving constitutive relations to describe these changes in material behavior.

There are multiple pathways for growth in biological tissues, as well as for non-living materials. In biological tissues the growth and division of cells is arguably the most distinguishing pathway. Cells may also synthesize and release matrix products that contribute to the growth of the extracellular matrix. Growth processes may also take place in the intracellular environment, such as the synthesis of a cytoskeleton from the building blocks of actin, microtubules, and intermediate filaments. More generally, growth processes may result from any set of chemical reactions that add mass to the solid content. A nice overview of various modes of biological growth and their relation to growth mechanics is provided in the review by Taber (1995).

Continuum mechanics provides a unifying framework that facilitates the combination of growth processes via enforcement of mass balance, with the analysis of stresses via enforcement of momentum balance. In this article we review our previously reported mixture theory formulation of the mechanics of cell growth (Ateshian et al., 2009) by outlining the main governing equations, solving simple canonical problems analytically, and illustrating more complex processes of growth using numerical simulations. Though it may seem at first that the cell growth process is far too complex to describe in the context of continuum mechanics, it is in fact possible to reduce it to a very fundamental mechanism involving osmotic gradients between the intracellular and extracellular fluid environments. The cell membrane plays a critical role as a barrier that allows passive transport of solvent and only some solute species, and active transport of others. Therefore, in a continuum framework of cell growth, it is necessary to account for these selective transport mechanisms in order to replicate the effects of osmotic gradients in the growth process, as reviewed in this article.

## 2. Overview of cell growth

In general, growth may occur either inside a body or on its surface (Skalak et al., 1982; Taber, 1995). The former is called

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interstitial (or volumetric) growth and the latter is called appositional (or surface) growth. Volumetric growth requires interstitial mass transport (Taber and Humphrey, 2001; Hsu, 1968; Cowin and Hegedus, 1976; Rodriguez et al., 1994; Humphrey and Rajagopal, 2002; Garikipati et al., 2004), whereas appositional growth relies on mass transport to and from the growth surface (Skalak et al., 1997; Ateshian, 2007, 2011). Interstitial growth requires that the molecular building blocks be able to transport through the solid in order to allow mass exchange within the solid volume. In biological growth this requirement typically implies that the growing solid material must be porous and permeable to the fluid solution containing the soluble building blocks. As will become more apparent in the remainder of this review, cell growth represents an interstitial growth process since it involves solute and solvent transport into the intracellular space.

### 2.1. Cell cycle and nutrients

A brief review of the cell cycle is instructive for understanding the process of cell division and growth (Alberts et al., 2002), as well as setting the stage for modeling such growth in the context of continuum mechanics.

The cell cycle is divided into four phases, of which the two major ones consist of the S phase (or synthesis phase), during which DNA is replicated, and the M phase (or mitosis phase), during which chromosome segregation and cell division occurs. The S phase is preceded by the G1 phase and followed by the G2 phase (also known as gap phases), during which the cell grows and doubles its mass; these three phases together comprise the interphase. The S phase typically occupies nearly half of the cell cycle time, as do the gap phases, whereas the M phase is much shorter. Depending on environmental conditions, cells may also enter a resting state, G0, which varies in duration but may persist for years, before returning to a proliferative state (Alberts et al., 2002).

Cell growth is dependent on the availability of nutrients and growth signals in the environment. Growth and division do not necessarily proceed in synchrony, as division may occur without growth, and growth without division. These two mechanisms are regulated independently and it is understood that cell growth does not depend on cell-cycle progression. Nevertheless, some coordination usually occurs, whereby proliferation and growth produce cells whose size remains unchanged over consecutive generations (Alberts et al., 2002).

Cell growth requires nutrients and is stimulated by growth factors that activate intracellular signaling pathways, leading to the accumulation of various proteins and other macromolecules. Cell division requires mitogens, which are generally produced by neighboring cells. Cells also require survival factors to suppress apoptosis. All of these extracellular controls combine to regulate organ growth (Alberts et al., 2002).

In addition to inorganic ions, cell nutrients derive from the digestion of carbohydrates, lipids, and proteins, to produce basic nutrient units such as sugars, fatty acids and glycerol, and amino acids, which are transported by blood circulation throughout the body. Uptake of nutrients by cells may take place via diffusion through the cell membrane, facilitated diffusion through specialized channels, or active transport via solute pumps. Larger macromolecules may be transported into the cell by endocytosis. Water transport occurs by permeation through the plasma membrane and specialized water channels called aquaporins (Alberts et al., 2002).

The growth of a cell consists of the uptake of nutrients as well as water across the cell membrane. Some of these nutrients become building blocks of macromolecules, such as proteins and nucleic acids, which form large complexes having negligible influence on the osmolarity of the intracellular environment, whereas other nutrients remain solvated. In animal cells, water uptake is principally controlled by the osmolarity of the intracellular fluid in relation to the external environment (Kedem and Katchalsky, 1958; Weiss, 1996). Therefore, uptake of soluble nutrients via passive or active mechanisms produces an obligatory uptake in water (Weiss, 1996), though cell volume expansion may be partially resisted by the mechanical stiffness of the cytoskeleton and the surrounding environment (Haider et al., 2006; Ateshian et al., 2009).

### 2.2. Continuum modeling of cell growth

Modeling of cell growth may be performed at various scales. For example, the growth of an individual cell may be modeled by using a continuum framework for a heterogeneous medium representing the principal cell components, such as the cell membrane and cytoplasm. At a higher scale, the effect of cell growth on organ growth may be modeled by using a homogenized representation that does not distinguish among individual cells. Conversely, at a lower scale, continuum modeling may describe the growth of a wide range of subcellular components, such as various organelles and their contents, cytoskeletal structures, etc. The principal requirements of a framework for modeling cell growth are the ability to account for nutrient and water uptake into the cell and the synthesis of various macromolecular structures within the cell. Therefore, the modeling framework should accommodate solutes (nutrients and osmolytes), solvent (water), and a solid matrix (representing macromolecular structures that contribute negligible osmolarity to the intracellular space).

The framework of mixture theory (Truesdell and Toupin, 1960; Eringen and Ingram, 1965; Bowen, 1968, 1969) is able to address modeling at these various scales, using either interstitial or appositional growth, depending on the specific structure or scale under consideration (Ateshian, 2007, 2011). In addition, when modeling at the scale of an individual cell, mixture theory can describe various trans-membrane transport mechanisms, such as passive and active transport of various nutrients (Ateshian et al., 2006, 2010). The driving force for passive transport of solvent and solutes across the cell membrane is the gradient in their mechano-chemical or mechano-electrochemical potential (Kedem and Katchalsky, 1958; Katzir-Katchalsky and Curran, 1965).

At the most fundamental level cell growth is driven by the addition of mass to the cell. This mass is transported across the plasma membrane in the form of solutes and solvent (water). The solutes may remain solvated inside the cell, or bind to the osmotically inactive constituents such as the cytoskeleton, which may be generically called the solid matrix of the cell. In either case the osmolarity of the intracellular environment increases transiently relative to the extracellular environment. Solvent will thus be driven into the cell, down its mechano-chemical potential gradient, until this gradient returns to zero. The transient active transport of solutes and passive transport of solutes and solvent into the cell may be modeled explicitly in a general mixture framework (Ateshian et al., 2006, 2010; Ateshian, 2011) and validated experimentally (Albro et al., 2007, 2009), extending the classical presentation based on irreversible thermodynamics (Weiss, 1996). However, for the purposes of growth modeling it may suffice to analyze the steady-state response when mechano-chemical potential gradients have returned to zero. Therefore, cell growth may be represented simply by defining the steady-state relationship between osmotic forces and the cell's solid and solute content.

## 3. Mass and momentum balance

Mass exchanges with the solid generally involve any number of soluble species, therefore a suitable framework for describing

growth processes is the theory of mixtures (Truesdell and Toupin, 1960; Eringen and Ingram, 1965; Bowen, 1968, 1969). In mixture theory, each constituent $\alpha$ of the mixture has its own motion, such that the current position of an elemental mixture region is represented by $\mathbf{x}=\boldsymbol{\chi}^{\alpha}(\mathbf{X}^{\alpha}, t)$, where $\mathbf{X}^{\alpha}$ is the reference position of constituent $\alpha$. Each constituent $\alpha$ of the elemental mixture region currently located at $\mathbf{x}$ may have originated from different reference locations $\mathbf{X}^{\alpha}$.

### 3.1. Mass balance for interstitial growth

Since mixtures may involve any number of constituents, it is useful to classify them into various categories based on their contribution to growth and the method by which they transport into the cell across its plasma membrane. (1) Solutes that enter the cell and bind to its solid matrix, thereby increasing the intracellular solid mass, may transport passively (down their mechano-electrochemical gradient), actively (via pumps or transporters), or via endocytosis. In any case, their transient presence in soluble form inside the cell is not considered in a steady-state growth theory, they are therefore associated directly with the intracellular solid matrix, denoted by $\alpha=s$. (2) Membrane-impermeant solutes that are actively transported into the cell, and remain in soluble form, regulate the intracellular osmolarity relative to the extracellular environment; they are denoted by $\alpha=i$.

Membrane-permeant solutes that transport passively and remain in soluble form may influence the balance between extracellular and intracellular osmolarity if they, and the cell solid matrix, are electrically charged. In this review the effects of charge will be neglected because it is assumed they have limited relevance to growth processes; therefore, the contribution of these membrane-permeant solutes will not be considered. Finally, the solvent transports passively into the cell during growth processes, via aquaporins or directly through the plasma membrane.

For the intracellular solid ($\alpha=s$) and membrane-impermeant solute ($\alpha=i$), the statement of mass balance in integral form is given by

$$\frac{d}{dt}\int_V \rho^{\alpha}\,dV=\int_V \hat{\rho}^{\alpha}\,dV,\quad \alpha=s,i, \tag{3.1}$$

where $\rho^{\alpha}$ is the apparent density of constituent $\alpha$ of the mixture (mass of $\alpha$ per volume of the mixture), and $\hat{\rho}^{\alpha}$ is the volume density of mass supply to constituent $\alpha$ due to growth. Here, $V$ represents a mixture region whose boundary is defined on constituent $\alpha$; for example, the smallest region $V$ may represent is a single cell, in which case the boundary of $V$ is the cell membrane that encloses both the intracellular solid and membrane-impermeant solute within the same region. This equation states that the net mass of constituent $\alpha$ in $V$, $\int_V \rho^{\alpha}\,dV$, changes only when there is net uptake (or loss) of $\alpha$ into $V$ as represented by $\hat{\rho}^{\alpha}\neq 0$.

Since it is generally more convenient to use the differential form of the axiom of mass balance, the time derivative on the left-hand-side of (3.1) may be conveniently brought inside the integral by performing the integration on the (time-invariant) referential volume $V_r$ of the mixture region, which represents the mixture volume in a stress-free state prior to growth. Since the mixture region is defined on the solid matrix, elemental mixture volumes $dV$ and $dV_r$ are related by $dV=J\,dV_r$, where $J=\det\mathbf{F}$ and $\mathbf{F}$ is the deformation gradient of the solid. Recognizing that the domain $V_r$ is arbitrary, the axiom of mass balance may be rewritten in differential form as

$$\dot{\rho}_r^{\alpha}=\hat{\rho}_r^{\alpha},\quad \alpha=s,i \tag{3.2}$$

where $\rho_r^{\alpha}=J\,\rho^{\alpha}$ is the apparent density, and $\hat{\rho}_r^{\alpha}=J\,\hat{\rho}^{\alpha}$ is the volume density of mass supply, relative to the mixture volume in the reference configuration. The superscripted dot on the left-hand side represents the material time derivative, which may be evaluated either in the spatial frame following constituent $\alpha$, when $\rho_r^{\alpha}=\rho_r^{\alpha}(\mathbf{x},t)$, or in the material frame when $\rho_r^{\alpha}=\rho_r^{\alpha}(\mathbf{X}^{\alpha},t)$. Based on its definition, any change in $\rho_r^{\alpha}$ can only represent a change in the mass of that constituent in the mixture. Therefore, the relation of (3.2) makes it clear that chemical reactions involving mass exchanges with constituent $\alpha$ ($\hat{\rho}_r^{\alpha}\neq 0$) produce a change in the apparent density $\rho_r^{\alpha}$, independently of changes in $\rho^{\alpha}$ that may also occur as a result of deformation. At a very fundamental level, interstitial growth mechanics is embodied in Eq. (3.2) (Ateshian, 2007, 2011).

Since $\rho_r^{\alpha}$ varies only as a result of chemical reactions, it is natural to include it as a state variable in growth mechanics (Ateshian, 2007; Ateshian and Ricken, 2010). Biological complexity arises from the fact that the number of constituents $\alpha$ in a biological mixture, such as the cell, may number in the thousands or tens of thousands. Therefore, constitutive relations for $\hat{\rho}_r^{\alpha}$, as a function of $\rho_r^{\beta}$'s of all constituents, may or may not be easy to formulate and validate in a complex system intended to describe whole cell responses. However, as in all modeling strategies, a range of assumptions may be adopted when examining a particular system under specific conditions, whereby only a few state variables are assumed to drive the system's response.

### 3.2. Mixture momentum balance

In any porous material whose individual constituents are intrinsically incompressible, the Cauchy stress tensor $\mathbf{T}$ for the mixture is given by

$$\mathbf{T}=-p\mathbf{I}+\mathbf{T}^{s}, \tag{3.3}$$

where $p$ is the interstitial fluid pressure and $\mathbf{T}^{s}$ is the effective stress in the porous solid matrix. The fluid pressure may represent a combination of mechanical and osmotic effects,

$$p=\tilde{p}+R\theta c, \tag{3.4}$$

where $\tilde{p}$ is the mechanical pressure arising from loading and deformation of the porous solid, as well as ambient pressure conditions; $R$ is the universal gas constant; $\theta$ is the absolute temperature (assumed uniform); and $c$ is the osmolarity of the fluid (solvent+solute) medium. This expression embodies a constitutive relation from physical chemistry whereby the osmotic contribution to the fluid pressure, $R\theta c$, is described by the ideal behavior known as the van't Hoff relation (Atkins, 1982).

In physical chemistry the osmolarity of a solution is evaluated as the number of moles of solute per volume of fluid. In a mixture that includes a porous solid, the osmolarity must be similarly evaluated as the number of moles of solute per volume of *interstitial fluid* in the mixture in the *current* configuration (since the amount of interstitial fluid inside a deformable porous solid may vary with deformation). Therefore, as described in our earlier study (Ateshian et al., 2009), the interstitial osmolarity may be related to the solute and solid contents according to

$$c=\frac{\rho_r^{i}}{M^{i}(J-(\rho_r^{s}/\rho_T^{s}))}, \tag{3.5}$$

where $\rho_r^{i}$ is the mass of interstitial solutes per volume of the mixture in the reference configuration; $M^{i}$ is the molecular weight of the solute, an invariant quantity; $\rho_r^{s}$ is the mass of the solid per volume of the mixture in the reference configuration; and $\rho_T^{s}$ is the true density of the solid (mass of solid per volume of solid), also an invariant quantity since the solid is intrinsically incompressible. Even though the material forming the skeleton of the porous solid matrix is considered to be intrinsically incompressible, the porous solid may change in volume as a result of compressibility of the

pore space, as fluid enters or leaves the pores. Thus, whereas $J = 1$ in the reference (stress-free) configuration of the porous solid matrix, $J$ is not in general equal to unity.

Since $\rho_T^s$ is invariant, it is notationally convenient to define

$$\varphi_r^s \equiv \frac{\rho_r^s}{\rho_T^s}, \tag{3.6}$$

which represents the ratio of solid volume in the current configuration to the mixture volume in the reference configuration. Both $\rho_r^s$ and $\varphi_r^s$ represent direct measures of the total content of the solid constituent in cells as it increases with growth; $\rho_r^s$ is the total mass of solid normalized to the referential volume of cells (the initial volume prior to growth, under a stress-free state); $\varphi_r^s$ is the total volume of solid normalized to the referential volume of cells. Even when accounting for growth, it can be shown that $\varphi_r^s \leq J$, since $J$ is the ratio of mixture volume in the current configuration to mixture volume in the reference configuration, and the solid volume cannot exceed the mixture volume in a common configuration (Ateshian et al., 2009). The variables $\varphi_r^s$ and $\rho_r^s$ may be used interchangeably to refer to the solid content of the mixture.

Similarly, since $M^i$ is invariant it is convenient to define

$$c_r \equiv \frac{\rho_r^i}{M^i}, \tag{3.7}$$

which represents the number of moles of solute per mixture volume in the reference configuration. Both $\rho_r^i$ and $c_r$ represent direct measures of the total content of solute in cells as it increases with growth; $\rho_r^i$ is the total mass of solute normalized to the referential volume of cells; $c_r$ is the total number of moles of solute normalized to the referential volume of cells. The variables $c_r$ and $\rho_r^i$ may be used interchangeably to refer to the solute content of the mixture. $c_r$ is a natural variable for describing the solute molar content in cells irrespective of cell size, whereas $c$ is a natural variable for describing the intracellular osmolarity, taking cell size into account. Therefore $c_r$ may be used to describe cell growth by solute uptake, whereas $c$ may be used to evaluate the osmotic contribution to the fluid pressure in (3.4). Using (3.5)-(3.7), it is evident that $c$ and $c_r$ are related by $c_r = (J - \varphi_r^s)c$.

Under quasi-static conditions, in the absence of external body forces, the momentum balance for the mixture is

$$\mathrm{div}\mathbf{T} = -\mathrm{grad}p + \mathrm{div}\mathbf{T}^s = \mathbf{0}\,. \tag{3.8}$$

Boundary conditions require that the mixture traction, $\mathbf{t} = \mathbf{T}\cdot\mathbf{n}$, be continuous, where $\mathbf{n}$ is the unit outward normal to the boundary surface. Similarly, the mechanical pressure $\tilde{p}$ must be continuous across the boundary (Ateshian et al., 2006; Ateshian, 2007).

## 4. Cell growth

### 4.1. Traction-free homogeneous growth

Consider a single cell, or an aggregate of cells, bathing in a fluid containing nutrients and osmolytes at normal levels for a biological environment. For simplicity consider that cell properties and composition are uniform. The cells are bathing in an extracellular fluid environment with a prescribed osmolarity $c^e$, subject only to an ambient pressure $\tilde{p}^e$. The boundary conditions between the intracellular and extracellular space require that

$$\mathbf{T}^s\cdot\mathbf{n} = R\theta(c - c^e)\mathbf{n}, \tag{4.1}$$

implying that any imbalance in osmotic pressure between the intracellular and extracellular environments must be compensated by solid matrix stresses. For a homogeneous state of stress under these boundary conditions it follows from (3.8) and (4.1) that

$$\mathbf{T}^s = R\theta(c - c^e)\mathbf{I} = R\theta\left[\frac{c_r(t)}{J - \varphi_r^s(t)} - c^e\right]\mathbf{I}. \tag{4.2}$$

Explicit dependence on spatial position $\mathbf{x}$ was dropped from the notation because of the assumption of homogeneity. In the reference, stress-free configuration of the solid matrix ($\mathbf{T}^s = \mathbf{0}$), the internal and external osmolarities must balance out, thus $c = c^e$ when $J = 1$. Equivalently, using (3.5),

$$\frac{c_r(t_0)}{1 - \varphi_r^s(t_0)} = c^e, \tag{4.3}$$

where $t_0$ represents a time prior to growth, when the solid is also in its reference configuration.

**Example 1.** When modeling cells, $\varphi_r^s(t_0)$ represents the volume fraction of solid (osmotically inactive) constituents in the intracellular space in the reference configuration; it must satisfy $0 \leq \varphi_r^s(t_0) \leq 1$. Depending on the cell type, this value typically ranges from 0.2 to 0.4 under normal physiological conditions. The osmolarity of the extracellular environment, $c^e$, is typically around 300 mM. $c_r(t_0)$ represents the molar concentration of membrane-impermeant solute on a mixture volume basis, in the reference configuration. Based on (4.3), $c_r(t_0)$ typically varies in the range 180–240 mM. Given that $R = 8.314\times10^{-6}$ mJ/nmol K and $\theta = 310$ K at body temperature, the osmotic pressure term $R\theta c$ is on the order of 1 MPa. Osmotic pressures are normally balanced across the cell boundary, $c \approx c^e$, therefore isolated cells do not experience osmotic pressure differences of that magnitude. Indeed, the typical modulus of a cell is on the order of $10^{-3}$ MPa and isolated cells do not have the strength to resist osmotic pressure differences on the order of 1 MPa. It is a well-known fact that when cells are transferred from a physiological environment ($c^e = 300$ mM) into distilled water ($c^e = 0$) they rapidly expand by taking up water, then burst (*lyse*) when their volume approximately doubles or triples. However, when surrounded by a sufficiently strong extracellular matrix, their resistance to lysis may increase considerably as suggested by (4.2), since the solid matrix may prevent excessive cell volume expansion.

If the extracellular osmolarity is kept constant, as is generally the case under physiological conditions, it follows from (4.2) and (4.3) that the state of stress may be rewritten as

$$\mathbf{T}^s = R\theta\left[\frac{c_r(t)}{J - \varphi_r^s(t)} - \frac{c_r(t_0)}{1 - \varphi_r^s(t_0)}\right]\mathbf{I}. \tag{4.4}$$

Cell growth (mass addition) may now be modeled by allowing $\rho_r^i(t)$ and $\rho_r^s(t)$ to increase over time according to the mass balance relation of (3.2). An increase in $\rho_r^i$ typically represents uptake of solutes from the extracellular environment (though it may also represent the release of ligands from intracellular substrates). An increase in $\rho_r^s$ typically represents the binding of a ligand to the intracellular solid matrix, leading to solid matrix growth. During cell growth it may be convenient to assume that the solute and solid contents, $\rho_r^i$ and $\rho_r^s$, increase proportionally since daughter cells are typically nearly identical to their progenitor, though not an obligatory relationship.

In the continuum representation of biological tissues adopted here, it is possible to consider that the extracellular matrix (ECM) also influences the solid matrix stress $\mathbf{T}^s$. If the volume fraction of ECM is negligible compared to the volume fraction of cells in the tissue, the above relations may be used identically, with the understanding that the solid matrix stiffness should account for ECM structures as well. When the ECM volume fraction is non-negligible, some homogenization scheme may be adopted to weigh

the contribution of cells and ECM to the overall tissue response (Ateshian et al., 2009).

**Example 2.** Consider the case when the solid matrix contributes negligible stiffness, $\mathbf{T}^s \approx \mathbf{0}$. In this case (4.4) produces

$$J = [1 - \varphi_r^s(t_0)]\frac{c_r(t)}{c_r(t_0)} + \varphi_r^s(t). \tag{4.5}$$

This linear relation shows that an increase in $\rho_r^i(t)$ and $\rho_r^s(t)$ produces a proportional increase in $J$, and thus the volume of the cells. This increase in volume occurs as a result of solvent uptake into the cells, driven by transient osmotic gradients, as well as the increases in solute and solid content. There are no constraints on the shape of the growing body in the limit when the solid matrix has no stiffness, as this solution only prescribes the change in volume. It is particularly noteworthy that there is no constraint on how large the volume of these growing cells may be. For example, with an extracellular osmolarity $c^e = 300$ mM and reference conditions $\varphi_r^s(t_0) = 0.3$ and $c_r(t_0) = 210$ mM, a tenfold increase in volume ($J = 10$) may be achieved by a tenfold increase in solid and solute content over time, such as $\varphi_r^s(t)$ increasing from 0.3 to 3 and $c_r(t)$ increasing from 210 mM to 2100 mM. (Since $\varphi_r^s(t)$ is the current cell solid volume content normalized to the initial cell volume, it can exceed unity with growth.) When there is negligible solid matrix resistance to growth, the actual intracellular osmolarity $c = c_r(t)/[J - \varphi_r^s(t)]$, as given in (3.5), remains constant and equal to $c^e$. Each cell need not increase tenfold in volume, as cells may in fact divide during the growth process.

The cytoskeletal structure of cells directs their growth. If the solid matrix exhibits material anisotropy, cell growth will occur preferentially along directions of least resistance to osmotically-driven expansion. Specific forms of growth may be modeled by adopting isotropic or anisotropic constitutive relations for $\mathbf{T}^s$.

**Example 3.** Consider the canonical example of growth of a collection of cells forming a tubular structure. If the cellular cytoskeleton elasticity is isotropic, suppose for simplicity that its constitutive relation is given by a special form of Saint-Venant's constitutive relation (Truesdell and Noll, 1992),

$$\mathbf{T}^s = \frac{\mu^s}{J}\mathbf{B}\cdot(\mathbf{B} - \mathbf{I}), \tag{4.6}$$

where $\mathbf{B}$ is the left Cauchy-green deformation tensor for the solid matrix and $\mu^s$ is its modulus. In this case the homogeneous state of strain will be isotropic, given by $\mathbf{B} = \lambda^2\mathbf{I}$ where $\lambda$ is the stretch ratio in all directions. Substituting this solution into (4.4) and recognizing that $J = \lambda^3$ produces a single equation for the unknown $\lambda$,

$$\mu^s\left(\lambda - \frac{1}{\lambda}\right) - R\theta\left[\frac{c_r(t)}{\lambda^3 - \varphi_r^s(t)} - \frac{c_r(t_0)}{1 - \varphi_r^s(t_0)}\right] = 0. \tag{4.7}$$

A solution for $\lambda$ may be obtained at any time $t$, given the growth-driven composition variables $\rho_r^i(t)$ and $\rho_r^s(t)$. In this case the tube grows isotropically, simply increasing in size (Fig. 4.1). The resulting homogeneous solid matrix residual stress is given by (4.6), though the total stress is zero. Consistent with Example 2, a solution to this equation can be found even in the limit as the stiffness reduces to zero, $\mu^s \to 0$. For example, when $\mu^s = 10^{-3}$ MPa, a tenfold increase in solute and solid content produces $J = 9.986$. This useful result demonstrates that having a non-zero cytoskeletal stiffness provides the necessary equations to predict the shape of a growing cell, even when the stiffness is very low. It also shows that residual stresses may not constrain growth significantly when the stiffness is very low.

**Example 4.** Let the cytoskeletal structure exhibit orthotropic symmetry, with planes of symmetry represented by the orthonormal vectors $\mathbf{a}_a^0$ ($a = 1, 2, 3$) in the stress-free configuration. For example,

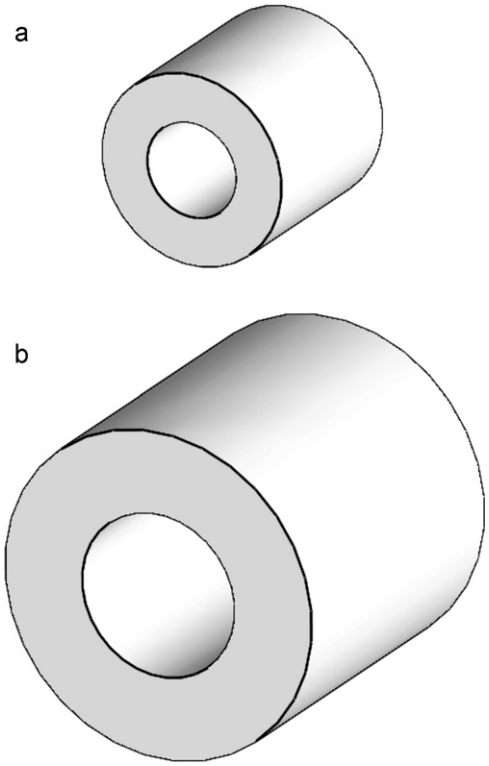


**Fig. 4.1.** Unconstrained growth of a tubular cellularized tissue with isotropic solid matrix. Growth is induced by increasing the cell solute and solid content fivefold. (a) Initial configuration and (b) final configuration.

assume that the solid matrix constitutive relation is given by a generalization of (4.6) to the orthotropic case (Tong and Fung, 1976; Ateshian and Costa, 2009),

$$\mathbf{T}^s = \frac{1}{2J}\sum_{a=1}^{3}\mu_a^s\lambda_a^2[\mathbf{A}_a\cdot(\mathbf{B} - \mathbf{I}) + (\mathbf{B} - \mathbf{I})\cdot\mathbf{A}_a], \tag{4.8}$$

where $\mu_a^s$ are moduli and $\mathbf{A}_a = \mathbf{a}_a \otimes \mathbf{a}_a$ are structural tensors in the current configuration, with $\mathbf{F}\cdot\mathbf{a}_a^0 = \lambda_a\mathbf{a}_a$ and $\lambda_a = |\mathbf{F}\cdot\mathbf{a}_a^0|$. In the case of homogeneous tube growth, let the planes of symmetry be orthogonal to the radial, circumferential and axial directions, $\{\mathbf{a}_a^0\} = \{\mathbf{e}_R, \mathbf{e}_\Theta, \mathbf{e}_Z\}$. Then, the deformation gradient is given by $\mathbf{F} = \sum_{a=1}^{3}\lambda_a\mathbf{a}_a^0 \otimes \mathbf{a}_a^0$, where $\{\lambda_a\} = \{\lambda_R, \lambda_\Theta, \lambda_Z\}$ are the radial, circumferential and axial stretches. It follows that

$$\mathbf{T}^s = \frac{1}{J}\sum_{a=1}^{3}\mu_a^s\lambda_a^2(\lambda_a^2 - 1)\mathbf{A}_a^0, \tag{4.9}$$

and the three stretch ratios can be obtained from the simultaneous solution of

$$\begin{aligned}
&\mu_R^s\lambda_R(\lambda_R^2 - 1) - \lambda_\Theta\lambda_Z R\theta\left[\frac{c_r(t)}{\lambda_R\lambda_\Theta\lambda_Z - \varphi_r^s(t)} - \frac{c_r(t_0)}{1 - \varphi_r^s(t_0)}\right] = 0,\\
&\mu_\Theta^s\lambda_\Theta(\lambda_\Theta^2 - 1) - \lambda_R\lambda_Z R\theta\left[\frac{c_r(t)}{\lambda_R\lambda_\Theta\lambda_Z - \varphi_r^s(t)} - \frac{c_r(t_0)}{1 - \varphi_r^s(t_0)}\right] = 0,\\
&\mu_Z^s\lambda_Z(\lambda_Z^2 - 1) - \lambda_R\lambda_\Theta R\theta\left[\frac{c_r(t)}{\lambda_R\lambda_\Theta\lambda_Z - \varphi_r^s(t)} - \frac{c_r(t_0)}{1 - \varphi_r^s(t_0)}\right] = 0.
\end{aligned} \tag{4.10}$$

The solution shows that growth will occur preferentially along directions of least stiffness (Fig. 4.2). An axial growth elongation of the tube will occur if $\mu_Z^s \ll \mu_R^s, \mu_\Theta^s$ (Fig. 4.2b); a radial and circumferential growth elongation will occur if $\mu_R^s = \mu_\Theta^s \ll \mu_Z^s$ (Fig. 4.2c). In each case, if the cytoskeletal stiffness is negligible along *any* direction ($\mu_a^s \to 0$), residual stresses in the cytoskeleton will be negligible for all components of $\mathbf{T}^s$, because impending osmotic

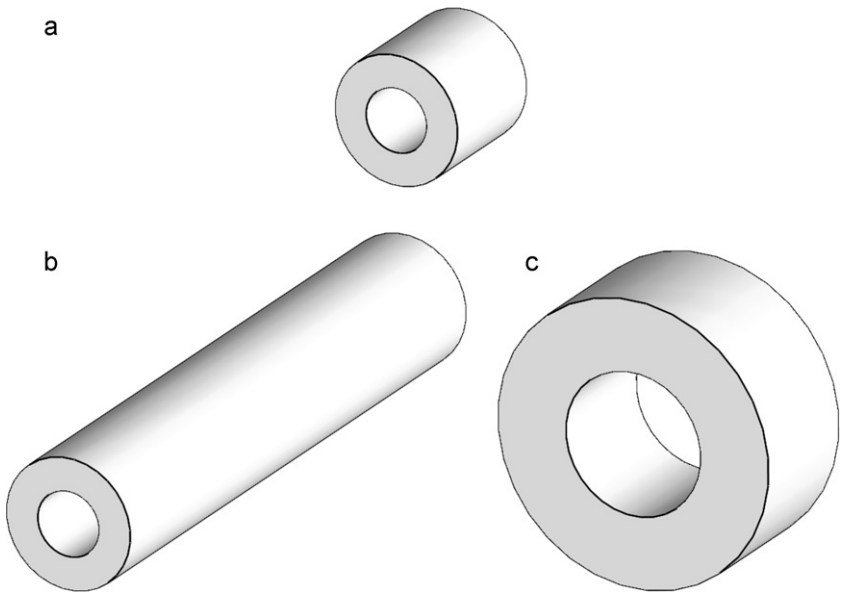


**Fig. 4.2.** Unconstrained growth of a tubular cellularized tissue with orthotropic solid matrix. Growth is induced by increasing the cell solute and solid content fivefold. (a) Initial configuration and (b) final configuration when axial stiffness is negligible, $\mu^s_Z \ll \mu^s_R$, $\mu^s_\Theta$. (c) Final configuration when radial and circumferential stiffnesses are negligible, $\mu^s_R = \mu^s_\Theta \ll \mu^s_Z$.

pressurization is relieved by growth along the direction with negligible stiffness. Even when cytoskeletal residual stresses do arise as a result of growth, cytoskeletal remodeling could attenuate or relieve these stresses during (or following) the growth process.

### 4.2. Externally constrained growth

External constraints can easily redirect the growth of cells. For example, cells and tissues consisting of cell aggregates may be constrained by surrounding structures such as ECM, basal membranes, or other types of substrates. These external constraints can be modeled in the continuum framework by prescribing suitable displacement and traction boundary conditions.

**Example 5.** In the case of isotropic growth described in Example 3, consider that the original tissue geometry is cuboidal, with its faces orthogonal to Cartesian coordinate axes. Consider that the faces orthogonal to the $Y$ and $Z$ axes are constrained, such that the corresponding stretch ratios are $\lambda_Y = \lambda_Z = 1$. In this case, growth can proceed only as an elongation along $X$ and the deformation gradient is given by $\mathbf{F} = \lambda_X \mathbf{e}_X \otimes \mathbf{e}_X + \mathbf{e}_Y \otimes \mathbf{e}_Y + \mathbf{e}_Z \otimes \mathbf{e}_Z$. Substituting this deformation into (4.6) and enforcing traction-free conditions on the $X$ faces using (4.1) produces an equation for the unknown $\lambda_X$,

$$\mu\left(\lambda_X - \frac{1}{\lambda_X}\right) - R\theta\left[\frac{c_r(t)}{\lambda_X - \varphi^s_r(t)} - \frac{c_r(t_0)}{1-\varphi^s_r(t_0)}\right] = 0. \tag{4.11}$$

This equation may be solved for $\lambda_X$. In the limit as the modulus reduces to zero, the solution is given by

$$\lim_{\mu\to 0}\lambda_X = \frac{c_r(t)}{c_r(t_0)}[1-\varphi^s_r(t_0)] + \varphi^s_r(t), \tag{4.12}$$

showing that the tissue grows in linear proportion to $\rho^i_r(t)$ and $\rho^s_r(t)$ when there is negligible solid matrix stiffness.

These illustrations demonstrate that directed growth may be easily modeled by internal and external constraints. They also show that residual stresses can be negligible during growth, even when large changes in tissue volume take place.

### 4.3. Inhomogeneous growth

External constraints may induce inhomogeneous growth even when growth rates and material properties are homogeneous. More generally, growth rates may vary across a population of cells, $\hat{\rho}^\alpha_r = \hat{\rho}^\alpha_r(\mathbf{x}, t)$, $\alpha = s, i$. Solid matrix anisotropy and moduli may also vary with location. In these general cases it is convenient to solve the governing equations described in Section 3.2 using numerical schemes such as the finite element method. An implementation of this growth process has been incorporated in the open source finite element code FEBio (http://www.febio.org), which was used to produce the illustrative examples presented here.

An example of inhomogeneous growth driven by external constraints is illustrated in Fig. 4.3, which shows the growth of a cell abutting against stiff structures that alter its shape as it grows. The stress tensor in the cell is given by the relations of (3.3)–(3.5), with an isotropic cytoskeleton having stiffness on the order of $10^{-3}$ MPa. Contact interfaces are introduced between the cell and the top and bottom rigid platens. As the cell grows its shape is constrained by the platens, as shown in the figure. In the absence of these

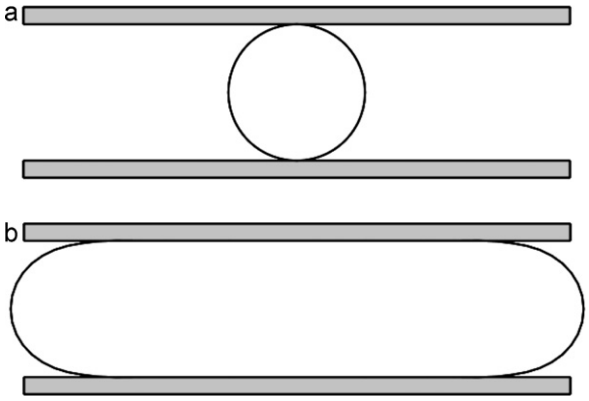


**Fig. 4.3.** Constrained growth of an initially round cell abutting against rigid platens. Growth is induced by increasing the cell solute and solid content fivefold. (a) Initial configuration and (b) final configuration.

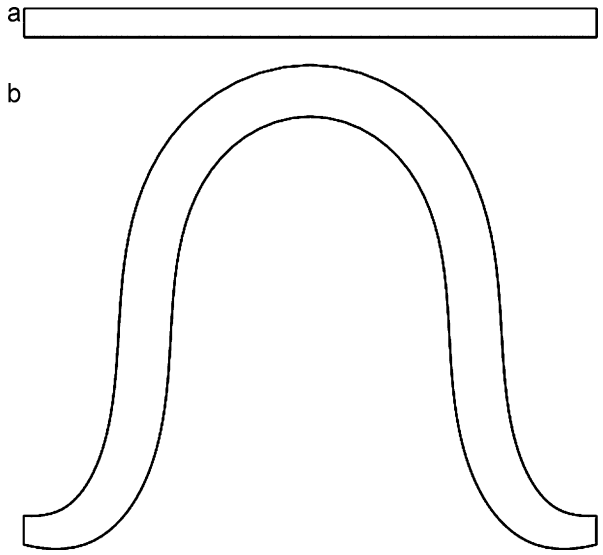


**Fig. 4.4.** Growth and buckling of a tissue layer constrained at lateral ends. Growth is induced by increasing the cell solute and solid content fivefold. The solid matrix is isotropic, with an elastic modulus of $10^{-3}$ MPa. (a) Layer in initial configuration and (b) layer in final configuration. Buckling is induced by nudging the structure with a small, transient vertical force at the center of the layer at the start of the growth process.

external constraints the growth would simply produce a larger circular shape, in analogy to the tube in Fig. 4.1.

External constraints can induce buckling to produce a wide range of growth outcomes. Consider a cellularized tissue layer which is externally constrained from expanding laterally (Fig. 4.4a). The growth of cells in the tissue may produce buckling of the layer in various modes, such as the case shown in Fig. 4.4b. This type of growth is reminiscent of the common process of invagination observed in morphogenesis.

An illustration of inhomogeneous growth rates is provided in Fig. 4.5, which shows a bilayered strip of tissue where growth occurs only in the top layer. Both layers have an isotropic solid matrix with moduli on the order of 0.1 MPa. This example demonstrates that inhomogeneous growth produces curling of the tissue layer into a nearly perfect cylindrical geometry, with the concave side corresponding to the region of no growth.

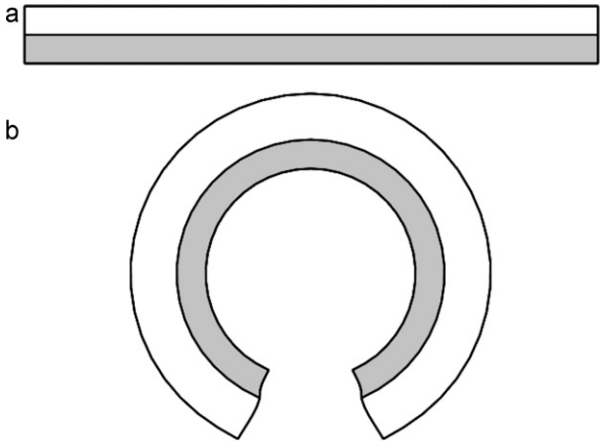


**Fig. 4.5.** Inhomogeneous growth in a bilayered strip. Growth is induced only in the top layer, by increasing the cell solute and solid content by a factor of 2.5. Both strips have a compressible neo-Hookean isotropic solid matrix with a Young's modulus of $10^{-1}$ MPa and Poisson's ratio of 0. (a) Initial configuration and (b) final configuration.

## 5. Summary

A dominant mechanism of growth in biological tissues is via cell growth and division. The growth of cells requires the active uptake of soluble mass that provides building blocks for various intracellular structures, such as the cytoskeleton or chromosomes, and contributes to the osmolarity of the intracellular space. The resulting mechano-chemical gradient drives solvent into the cell as well, contributing to its volumetric growth. The signaling mechanisms that trigger these processes remain a major research topic in biology, however the mechanics of cell growth may be reduced to a fundamental set of equations as summarized in this review.

By modeling cells as a mixture of solid, solvent and solutes, it is possible to describe the active uptake of solutes into the intracellular space by including a supply term in the axiom of mass balance as shown in (3.1). Standard manipulations from kinematics make it more explicitly evident that mass supply terms directly determine mass content in a mixture, as shown in (3.2), by using the referential volume of the mixture for normalizing the apparent density and mass supply. The volume density of mass supply $\hat{\rho}_r^\alpha$ must be described by a constitutive relation that establishes the conditions under which mass uptake is triggered. This type of constitutive relation would normally be informed by biological experiments. Therefore it is expected that state variables should include the local concentration, $\rho_r^\beta$, of various growth factors, mitogens and survival factors. However, even without explicit forms of constitutive relations for $\hat{\rho}_r^\alpha$, it is possible to model growth by letting intracellular solute and solid constituents increase in mass over time.

The intracellular solute and solid content control the osmolarity of the intracellular environment as shown in (3.5). An increase in solute or solid content during cell growth increases the intracellular osmolarity, leading to a reduction in the mechano-chemical potential of the solvent relative to the extracellular environment. Though boundary conditions require that the solvent mechano-chemical potential always be continuous across the cell boundary, transient processes arise because the intracellular (and possibly extracellular) value of this potential may be transiently non-uniform. Solvent then transports into the cell to raise the mechano-chemical potential until it becomes uniform throughout the mixture. In effect, the solvent uptake re-dilutes the intracellular environment toward an osmolarity value closer to the extracellular osmolarity.

Because all the constituents of the mixture are idealized as intrinsically incompressible, solvent uptake must be accompanied by an increase in cell volume. Indeed, solvent uptake is most responsible for producing cell growth, since the solvent volume fraction is typically greater than the solid and solute volume fractions in the cell. This volume increase is resisted by the solid matrix of the intracellular (cytoskeleton) and extracellular (matrix) environment, as indicated by (4.1). If the solid matrix produces negligible resistance to the osmotic forces being generated, the intracellular osmolarity will once again equal the extracellular osmolarity at steady-state, as illustrated in Example 2. The resistance offered by the solid matrix may also be transient, since cells may remodel their cytoskeleton or extracellular matrix by solubilizing their solid constituents and reforming new ones.

The anisotropy of the solid matrix plays an important role in guiding the shape of the growing cell, as illustrated in Examples 3 and 4. In these examples, the relative magnitudes of the solid matrix moduli along various preferred material directions play a more significant role than their absolute value, implying that the solid matrix need not meet a particular threshold of stiffness to guide the growth process. Similarly, external constraints, such as substrates that offer significant resistance to cell growth along certain directions, may guide the shape of growing cells as illustrated in Figs. 4.3–4.5. In some of these examples, such as the bilayered strip

of Fig. 4.5, the final shape of the growing tissue may vary according to the relative magnitude of osmotic forces and solid matrix stiffness.

The governing equations for cell growth presented in this review are also applicable to the related process of cell volume regulation (Hoffmann et al., 2009). Many cells are known to regulate their volume in a manner that counteracts changes in the osmolarity of the extracellular environment. They control their volume by selectively taking up or expelling solutes from the intracellular environment via active transport mechanisms. Regulatory volume increases may thus be produced by increasing $\rho_r^i$; conversely, regulatory volume decreases may also be produced by decreasing $\rho_r^i$. Since the magnitude of osmotic pressure that may be achieved in this manner can reach as high as $\sim$1 MPa, cell volume regulation may have a significant effect on the surrounding extracellular matrix. Thus, in addition to growth, osmotic effects may also produce contraction of cells and tissues, possibly contributing to some of the well-known observations in fibroblast-seeded collagen gels (Dallon and Ehrlich, 2008).

In conclusion, cell growth describes a mechanism of interstitial or volumetric growth characteristic of biological tissues. This growth process may be reduced to a set of relatively simple governing equations based on the axioms of mass and momentum balance for mixtures. The mechanics of cell growth must account for the presence of interstitial fluid, since the growth mechanism is driven by osmotic effects. By accounting for solid matrix anisotropy and external constraints, a wide variety of growing shapes may be produced, with some canonical examples illustrated above.

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