PAPER

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# A mathematical description of a growing cell colony based on the mechanical bidomain model

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**Abstract**

The mechanical bidomain model is used to describe a colony of cells growing on a substrate. Analytical expressions are derived for the intracellular and extracellular displacements. Mechanotransduction events are driven by the difference between the displacements in the two spaces, corresponding to the force acting on integrins. The equation for the displacement consists of two terms: one proportional to the radius that is the same in the intracellular and extracellular spaces (the monodomain term) and one that is proportional to a modified Bessel function that is responsible for mechanotransduction (the bidomain term). The model predicts that mechanotransduction occurs within a few length constants of the colony's edge, and an expression for the length constant contains the intracellular and extracellular shear moduli and the spring constant of the integrins coupling the two spaces. The model predictions are qualitatively consistent with experiments on human embryonic stem cell colonies, in which differentiation is localized near the edge.

Keywords: biomechanics, mechanotransduction, stem cell, mechanical bidomain model

(Some figures may appear in colour only in the online journal)

## 1. Introduction

Mechanical forces play a key role in controlling tissue growth (Sun *et al* 2012). In a colony of growing cells, tissue properties are often different at the periphery than in the interior (Nelson *et al* 2005, Ruiz and Chen 2008, Mertz *et al* 2012, Warmflash *et al* 2014, Rosowski *et al* 2015). For example, in colonies of growing human stem cells, traction forces and differentiation occur primarily at the edge of the colony. This behavior may be caused by mechanotransduction via integrin proteins in the cell membrane that couple the cytoskeleton to the extracellular matrix (Yim and Sheetz 2012).

Over the last five years, we have developed the mechanical bidomain model to predict where mechanotransduction occurs (Puwal and Roth 2010, Roth 2013, 2015, Sharma *et al* 2015). This macroscopic continuum model accounts for stresses and strains in both the intracellular and extracellular spaces, and their coupling. The fundamental hypothesis of this model is that mechanotransduction effects—such as mechanically induced stem cell differentiation—occur where the displacements in the intracellular and extracellular spaces differ, resulting in forces acting on integrins. Our model is different than other models in that it is macroscopic (applicable to cell colonies or intact tissue rather than single cells), it accounts for the elastic properties of the intracellular and extracellular spaces separately, and it locates mechanotransduction effects where the intracellular and extracellular displacements differ rather than where stresses or strains may be large. In previous analyses, we have applied this model to cardiac tissue, and analyzed, for example, where remodeling occurs in the area around an ischemic region in the heart (Gandhi and Roth 2016).

The goal of this paper is to apply the mechanical bidomain model to a colony of human embryonic stem cells

 

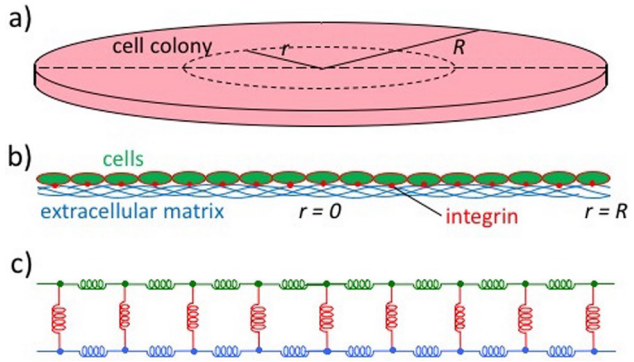


**Figure 1.** (a) A macroscopic view of a circular cell colony, of radius $R$. The distance from the center of the colony is $r$. The $z$ direction is perpendicular to the colony. (b) A microscopic, schematic view of the cells along the dashed line in panel (a). The green ovals are individual cells, the blue mesh is the extracellular matrix, and the red dots are integrin proteins. (c) The 1D mechanical bidomain model. The intracellular cytoskeleton is represented by the line of green springs, the extracellular matrix by the line of blue springs, and the integrins by the red springs. The bidomain is a macroscopic model, so the green, blue, and red springs do not represent individual cells but instead the behavior averaged over many cells. The stiffness of the green springs is represented by the intracellular shear modulus $\nu$, the stiffness of the blue springs by the extracellular shear modulus $\mu$, and the stiffness of the red springs by the integrin spring constant density $K$.

(figure 1(a)). The model, which is similar to that derived by Edwards and Schwarz (2011) and by Banerjee and Marchetti (2012), predicts that differentiation and traction forces occur within a few length constants of the colony edge. Moreover, it provides insight into how mechanical properties of the intracellular and extracellular spaces affect differentiation. One key prediction is that the difference between the intracellular and extracellular displacements has a different distribution than the intracellular and extracellular displacements themselves.

## 2. Methods

Our derivation of the mechanical bidomain model is similar to that given previously (Sharma *et al* 2015), except we use cylindrical coordinates $(r, \theta, z)$. The intracellular relationships between stress $(\tau)$ and strain $(\varepsilon)$ are

$$\tau_{irr} = -p + 2\nu\varepsilon_{irr} + T \quad \tau_{i\theta\theta} = -p + 2\nu\varepsilon_{i\theta\theta} + T \quad \tau_{izz} = -p + 2\nu\varepsilon_{izz}$$
$$\tau_{ir\theta} = 2\nu\varepsilon_{ir\theta} \quad \tau_{i\theta z} = 2\nu\varepsilon_{i\theta z} \quad \tau_{izr} = 2\nu\varepsilon_{izr}. \tag{1}$$

The stress consists of three parts: a hydrostatic pressure $p$ because the cells are mostly water, an isotropic term proportional to the strain with shear modulus $\nu$, and a term $T$ that represents a uniform stress caused by the growth and crowding of cells. Because we treat the colony as a continuum, $\nu$ contains contributions from both the intracellular cytoskeleton and intercellular adhesions (figure 1(b)). The intracellular stress-strain relationship is illustrated schematically by the line of green springs in figure 1(c); a stretching of a spring, or strain, causes a restoring force, or stress (terms in (1) containing $p$ and $T$ are not illustrated in the spring analogy of figure 1(c)). The extracellular space has similar stress–strain relationships

$$\tau_{err} = -q + 2\mu\varepsilon_{err} \quad \tau_{e\theta\theta} = -q + 2\mu\varepsilon_{e\theta\theta} \quad \tau_{ezz} = -q + 2\mu\varepsilon_{ezz}$$
$$\tau_{er\theta} = 2\mu\varepsilon_{er\theta} \quad \tau_{e\theta z} = 2\mu\varepsilon_{e\theta z} \quad \tau_{ezr} = 2\mu\varepsilon_{ezr}, \tag{2}$$

where $q$ is the extracellular pressure and $\mu$ is the extracellular shear modulus. The extracellular stress-strain relationship (2) is illustrated schematically by the line of blue springs in figure 1(c).

Previous analyses of the mechanical bidomain model assumed plane strain: no strain in the $z$ direction of figure 1(a) (Roth 2013, 2015). Here we consider a thin layer of cells and assume plane stress: no stress in the $z$ direction, $\tau_{izz} = \tau_{i\theta z} = \tau_{izr} = \tau_{ezz} = \tau_{e\theta z} = \tau_{ezr} = 0$. If the tissue is incompressible, $\varepsilon_{irr} + \varepsilon_{i\theta\theta} + \varepsilon_{izz} = 0$, then $\tau_{izz} = 0$ implies that $p = -2\nu(\varepsilon_{irr} + \varepsilon_{i\theta\theta})$. A similar relationship exists in the extracellular space.

We assume that the colony grows radially outward and looks the same in all directions, so the displacement has no $\theta$ component and all $\theta$ derivatives vanish. In that case, the strain is related to the intracellular displacement $u_r$ and extracellular displacement $w_r$ by

$$\varepsilon_{irr} = \frac{\partial u_r}{\partial r}, \ \varepsilon_{i\theta\theta} = \frac{u_r}{r}, \ \varepsilon_{ir\theta} = 0,$$
$$\varepsilon_{err} = \frac{\partial w_r}{\partial r}, \ \varepsilon_{e\theta\theta} = \frac{w_r}{r}, \ \varepsilon_{er\theta} = 0. \tag{3}$$

The equations of mechanical equilibrium are

$$\frac{\partial \tau_{irr}}{\partial r} + \frac{\tau_{irr} - \tau_{i\theta\theta}}{r} = K(u_r - w_r)$$
$$\frac{\partial \tau_{err}}{\partial r} + \frac{\tau_{err} - \tau_{e\theta\theta}}{r} = -K(u_r - w_r). \tag{4}$$

The terms on the right-hand-sides of (4) represent the coupling of the intra- and extracellular spaces by integrins (when we write 'integrins' this is short for 'transmembrane molecules mechanically coupling the intracellular and extracellular spaces' and may include other molecules located at focal adhesions), where $K$ is their spring constant density (Sharma *et al* 2015). The red springs in figure 1(c) represent the integrins. The fundamental assumption of the mechanical bidomain model is that mechanotransduction occurs because forces on integrins initiate a cascade of biological responses. Therefore, in colonies of stem cells we expect differentiation to occur not where the intra- or extracellular stresses or strains are large, but instead where the difference $u_r - w_r$ is large.

When we combine (1)–(4), we obtain the equations governing the intracellular and extracellular displacements

$$4\nu\left(\frac{\partial^2 u_r}{\partial r^2} + \frac{1}{r}\frac{\partial u_r}{\partial r} - \frac{u_r}{r^2}\right) = K(u_r - w_r),$$
$$4\mu\left(\frac{\partial^2 w_r}{\partial r^2} + \frac{1}{r}\frac{\partial w_r}{\partial r} - \frac{w_r}{r^2}\right) = -K(u_r - w_r). \tag{5}$$

The second derivative of the displacement implies that if the stretching of one intracellular (green) spring is greater than the

stretching of the adjacent intracellular spring in figure 1(c), then there is a net force on the tissue between them that must be balanced by a force from the integrins (red spring). A force on the integrins is caused by the intracellular and extracellular ends of the integrin moving radially by different amounts, thereby stretching it. This picture is similar to the 'tug-of-war' mechanism proposed by Trepat and Fredberg (2011), except that in our model the extracellular space is also elastic and can move. The second and third terms on the left-hand-sides of (5) arise because of the cylindrical geometry.

## 3. Results

To solve (5), we guess a solution of the form

$$u_r = Ar + BI_1\left(\frac{r}{\sigma}\right) \qquad w_r = Cr + DI_1\left(\frac{r}{\sigma}\right), \tag{6}$$

where $A$, $B$, $C$, $D$, and $\sigma$ are unknown constants and $I_1$ is a modified Bessel function (Abramowitz and Stegun 1970). If these solutions obey (5) then $C = A$, $D = -\frac{\nu}{\mu}B$, and $\sigma = \sqrt{\frac{4\nu\mu}{K(\nu+\mu)}}$. The parameter $\sigma$ is a length constant that arises naturally in the mechanical bidomain model (Sharma *et al* 2015).

To determine $A$ and $B$, we assume the intracellular and extracellular spaces are each stress free, $\tau_{irr} = \tau_{err} = 0$, at the outer edge of the colony, $r = R$. The resulting expressions for the displacements are

$$u_r = -\frac{T}{6(\nu+\mu)}\left[r + \frac{3}{2}\left(\frac{\mu}{\nu}\right)\frac{I_1\left(\frac{r}{\sigma}\right)}{\frac{dI_1\left(\frac{R}{\sigma}\right)}{dr} + \frac{I_1\left(\frac{R}{\sigma}\right)}{2R}}\right],$$
$$w_r = -\frac{T}{6(\nu+\mu)}\left[r - \frac{3}{2}\frac{I_1\left(\frac{r}{\sigma}\right)}{\frac{dI_1\left(\frac{R}{\sigma}\right)}{dr} + \frac{I_1\left(\frac{R}{\sigma}\right)}{2R}}\right]. \tag{7}$$

This solution has features in common with other analytical solutions of the mechanical bidomain model (Roth 2013, 2015, Sharma *et al* 2015). Each displacement consists of two terms. The first (monodomain) term is proportional to $r$. It is identical in the intra- and extracellular spaces so it does not contribute to the difference $u_r - w_r$ and therefore does not affect the integrins and does not trigger stem cell differentiation. The second (bidomain) term is different in the two spaces and does impact differentiation. If the length constant $\sigma$ is small compared to the colony radius $R$, the Bessel function behaves similarly to an exponential (Abramowitz and Stegun 1970) and is significant only within a few length constants of the edge

$$u_r = -\frac{T}{6(\nu+\mu)}\left[r + \frac{3}{2}\left(\frac{\mu}{\nu}\right)\sigma e^{\frac{r-R}{\sigma}}\right],$$
$$w_r = -\frac{T}{6(\nu+\mu)}\left[r - \frac{3}{2}\sigma e^{\frac{r-R}{\sigma}}\right]. \tag{8}$$

In this case, the bidomain term is small compared to the monodomain term and the difference in displacements is

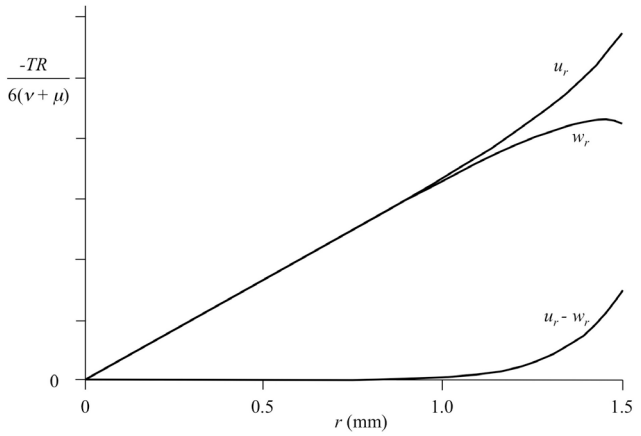


**Figure 2.** The intracellular ($u_r$) and extracellular ($w_r$) displacements, and their difference ($u_r - w_r$), in a cell colony with radius $R = 1.5$ mm, $\sigma = R/10$, $\nu = \mu$, and a negative value of $T$. The calculation is based on (7).

$$u_r - w_r = -\frac{T}{4\nu}\sigma e^{\frac{r-R}{\sigma}}. \tag{9}$$

Figure 2 shows plots of $u_r$, $w_r$, and $u_r - w_r$ as functions of radius.

The stress and strain distributions are shown in figure 3. Throughout most of the colony, including near the center, the stress and strain are nearly constant (a linearly increasing displacement corresponds to a constant strain). They vary near the edge of the colony in such a way that $\tau_{irr}$ and $\tau_{err}$ go to zero at the boundary ($r = R$). If the stress or strain were responsible for mechanotransduction, then large effects would be seen at the center of the colony. Because the stress and strain are tensors, their distribution depends on direction: the radial stress in the $r$ direction, $\tau_{rr}$, or the hoop stress in the $\theta$ direction, $\tau_{\theta\theta}$.

## 4. Discussion

The mechanical bidomain model makes three novel predictions about the elastic properties of cell colonies. First, the difference between the intracellular and extracellular displacements has a very different distribution than the two displacements individually (figure 2). There are large displacements in the interior of the colony but they are nearly the same in both spaces. Second, large stresses and strains exist in the interior of the colony (figure 3), whereas large differences in displacement exist primarily at the boundary (figure 2). The mechanical bidomain model is based on the hypothesis that differences in displacement are responsible for mechanotransduction. The gist of this hypothesis is that integrins (red springs in figure 1(c)) respond when they are stretched, and they are only stretched when the displacements in the intracellular and extracellular spaces are different (Sharma *et al* 2015). If the two spaces each undergo complicated displacements, but the displacements are the same in both spaces, then the integrins are not stretched. The predictions in figures 2 and 3 provide a way to test this hypothesis experimentally by measuring if mechanotranduction occurs where the stress

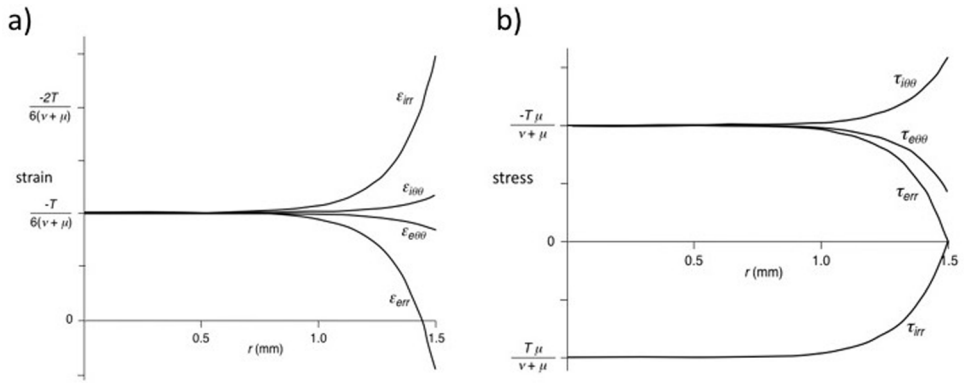


**Figure 3.** The intracellular and extracellular (a) strain, and (b) stress, in a cell colony with radius $R = 1.5\,\text{mm}$, $\sigma = R/10$, $\nu = \mu$, and a negative value of $T$. The calculation is based on (1)–(3) and (7).

and strain are large, or where the difference in displacements is large. Third, the model predicts how the length constant $\sigma$ depends on the shear moduli $\nu$ and $\mu$, and the integrin spring constant density $K$ (figure 4).

Our analysis of biomechanical forces in a colony of stem cells has many similarities with those presented by Edwards and Schwarz (2011), Banerjee and Marchetti (2012), and He *et al* (2014). Edwards and Schwarz's spring constant $k$ is analogous to our constant $K$, their localization length $l$ is similar to our length constant $\sigma$, and both their solution and ours contain the modified Bessel function $I_1$. There are, however, differences in the two calculations. Edwards and Schwarz considered only the intracellular space (a monodomain model), whereas we account for both the intracellular and extracellular spaces (a bidomain model). As a result, their model did not predict the monodomain term proportional to $r$ in (7), even though the monodomain term is often larger than the bidomain term (figure 2). Moreover, both Edwards and Schwarz (2011) and Banerjee and Marchetti (2012) interpreted the coupling term in (5) as representing cells attached to a microstructured surface consisting of an array of flexible elastomeric pillars, as are often used in traction force experiments (Style *et al* 2014). Our model, on the other hand, interprets this coupling as occurring via integrins. Therefore our coupling term takes on a different role than that in several previous models: in our model it is the signal that drives mechanotransduction. Our model could be applied to tissue colonies growing *in vivo*, as well as to cell monolayers cultured on a planar substrate. As $\mu$ goes to infinity in (7) only the intracellular bidomain term survives and we recover Edwards and Schwarz's result. He *et al* (2014) interpreted the integrin coupling like we do in our model, and included elastic intracellular and extracellular spaces. However, they applied their model to single cells interacting with a substrate, whereas our macroscopic model is applied to larger cell colonies.

Our model is consistent with the experimental observations of Rosowski *et al* (2015). Their colonies of human stem cells contain a band of differentiation at the colony edge, consistent with our (9), which predicts mechanotransduction acting through integrins should be largest at the edge (figure 2). They also found that this band of differentiated cells had a constant width regardless of the colony radius. Equation (9) predicts that differentiation should occur within a few length constants of the edge, where $\sigma$ is independent of $R$. Finally, they observed larger traction forces near the edge of the colony compared to the interior. If we take our integrin force, $K(u_r - w_r)$, as analogous to the traction force, then we too predict a larger force near the periphery.

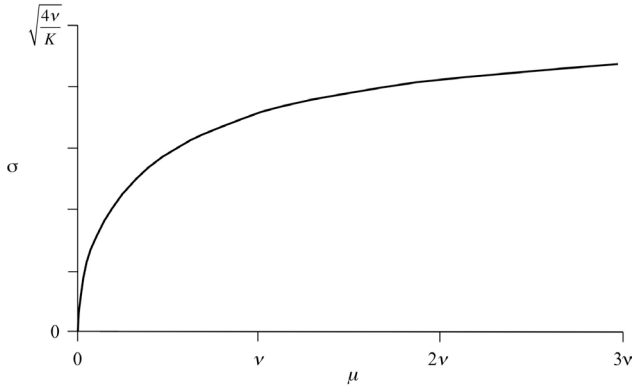


**Figure 4.** The length constant $\sigma$ as a function of the extracellular shear modulus $\mu$.

Our results are consistent with previous analyses based on the mechanical bidomain model. For instance, when a tissue sheet is sheared the resulting expressions for the intracellular and extracellular displacements each contain two terms: a monodomain term that is common to both spaces and a bidomain term that is responsible for forces on integrins (Sharma *et al* 2015). If $\sigma \ll R$, then the monodomain term is larger than the bidomain term, so measurements of tissue displacement are dominated by the monodomain term (figure 2). Experimental recordings of intracellular and extracellular displacements would need to be extremely precise in order to measure accurately their small difference. Moreover, the monodomain term implies that both the intracellular and extracellular spaces experience large, nearly uniform strains (figure 3). If one adopted the hypothesis that the intracellular stress or strain causes stem cell differentiation, one would expect differentiation to be distributed throughout the colony. However, if one adopts the hypothesis that the difference between the intracellular and extracellular displacements drives differentiation, then the model predicts that differentiation is confined to the edge of the colony.

One difference of this analysis and our previous calculations based on the mechanical bidomain model (Roth 2013, 2015, Sharma *et al* 2015) is that we assume plane stress rather than plane strain. Interestingly, if this example is solved using plane strain then $p = T$ and $u_r = w_r = 0$, so there is no mechanotransduction. The assumption of plane stress is therefore crucial for application of the model to monolayers of cells.

Our model provides insight into how the displacements depend on the model parameters such as the intracellular and extracellular shear moduli $\nu$ and $\mu$. For instance, if the cells are grown on a stiff substrate so that $\mu \gg \nu$, the length constant $\sigma$ governing the width of the differentiated layer becomes $\sigma = \sqrt{4\nu/K}$, independent of $\mu$. On the other hand, if cells are grown on a flexible substrate so $\mu \ll \nu$, then $\sigma = \sqrt{4\mu/K}$ and $\sigma$ decreases as $\mu$ decreases. Figure 4 plots $\sigma$ versus $\mu$, assuming constant values of $\nu$ and $K$. Analyzing the behavior of the model as a function of $\mu$ may be important, as stem cells differentiate into various cell types depending on the extracellular matrix stiffness (Engler *et al* 2006).

In our continuum model $\nu$ is a macroscopic parameter found by averaging over many cells and reflects both the cytoskeleton and cell–cell junctions. To understand this, conduct a thought experiment in which you dissolve away the extracellular matrix and then pull on the outer cells of the colony. How well this force is transmitted to the interior cells determines $\nu$. If there were no cell–cell junctions $\nu$ would vanish, as in the case of a sparse culture where cells are not in contact with each other (Engler *et al* 2006). Cell–cell junctions can be modulated by modifying the number of cadherin-based intercellular adhesions (Mertz *et al* 2013, Schlüter *et al* 2015, Gonzalez-Valverde *et al* 2016). Therefore, modifications of cadherins should impact the width of the differentiation layer unless $\nu \gg \mu$, in which case $\sigma$ is insensitive to the strength of cadherin coupling. We assume the distribution of cadherins is static, but it may change dynamically (Schlüter *et al* 2015).

If fewer integrins are present (or if their ability to couple the intracellular and extracellular spaces is compromised) their macroscopic spring constant density $K$ will decrease, widening the layer of differentiated cells. In previous studies using the mechanical bidomain model, the value of the parameter $K$ was not known. The data of Rosowski *et al* (2015) allows us to estimate $K$ using $\sigma = \sqrt{\frac{4\nu\mu}{K(\nu+\mu)}}$ with $\sigma = 150$ $\mu$m. In order to calculate $K$ accurately, we would need values of $\nu$ and $\mu$ for the specific stem cell colonies grown by Rosowski *et al*. Because these values were not measured, we must settle for an order-of-magnitude estimate. Shear moduli in soft tissues are on the order of 1000 Pa (Rehfeldt *et al* 2007). If we assume this value applies to both $\nu$ and $\mu$, then $K$ is on the order of $10^{11}$ Pa m$^{-2}$. The units of $K$ are not the units of a spring constant because $K$ is a macroscopic density that depends on the product of three factors: the spring constant of an individual integrin (N m$^{-1}$, or Pa m), the density of integrins in the membrane (1/m$^2$), and the ratio of membrane surface area to tissue volume (1/m). In other words, $K$ is a spring constant per unit volume (N/m/m$^3$ = Pa m$^{-2}$).

The parameter $T$ in our model represents the growth and crowding of cells. A positive value of $T$ corresponds to an active tension like in contractile muscle. For a growing colony, $T$ is negative and corresponds to 'growing pressure': As cells divide and enlarge they exert an outward stress on their neighbors. In (1) we include $T$ in only the intracellular space, although if the cells are also producing additional extracellular matrix we may need a term like $T$ in the extracellular space too.

Although our model is useful, it is based on several assumptions that limit its applicability.

1. We assume the strains are small and linear. Most biomechanical models consider finite, nonlinear strains (Fung 1981). Our assumption may be reasonable for a stem cell colony, but nevertheless does represent an approximation.
2. We assume plane stress, which implies that there are no stresses in the plane perpendicular to the colony (the $z$ direction). This should be a good approximation for a monolayer, but in a thicker tissue sample or for a thick extracellular substrate this assumption may break down.
3. We assume that the integrin spring constant density is linear, Hookean, and isotropic. We have no evidence about the spring constant properties and adopt this assumption as the simplest case. Furthermore, we assume the distribution of integrins is static, although it may be dynamic and respond to mechanotransduction signals (Wolfenson *et al* 2014).
4. We assume the cell colony is circular with no dependence on angle, allowing us to obtain an analytical solution to the model equations. More complicated colony geometries, such as those studied by Ruiz and Chen (2008), will require numerical analysis. Numerical methods for solving the equations of the mechanical bidomain model are being developed (Sharma *et al* 2015, Gandhi and Roth 2016).
5. We assume that cell growth, represented in our model as tension $T$, occurs uniformly and isotropically throughout the tissue ($\partial T/\partial r = 0$). This may be one of our weaker assumptions, as a feedback loop may exist between forces on integrins and cell growth, so that $T$ could be a function of $u_r - w_r$. Rosowski *et al* (2015) observed distinct actin organization and greater myosin activity near the edge of the colony, implying that $T$ could be nonuniform. An interesting area of future research would be to analyze such a feedback loop using the mechanical bidomain model.
6. We assume $T$ is isotropic in the $r$–$\theta$ plane, which is different from muscle where $T$ acts along the myofiber axis (Roth 2013). The data in Rosowski *et al* (2015) do not suggest that the cell colony is anisotropic. Feedback may cause the long axis of cells (and presumably $T$) to align with the direction of greatest intracellular stress (Bischofs and Schwarz 2003, Bischofs *et al* 2004, Zemel *et al* 2010), an effect not included in our model.
7. We assume that the passive elastic shear moduli of the cells and matrix are uniform and isotropic. Mechanosensing cells can align with the direction of large stiffness

(Bischofs and Schwarz 2003, Bischofs *et al* 2004, Zemel *et al* 2010). Our model does not include such an effect. Moreover, cells can respond to gradients in extracellular matrix stiffness (Sunyer *et al* 2016), but our model and the experiments of Rosowski *et al* (2015) do not include this mechanism. A stiffness gradient could be incorporated into the model by making $\mu$ a function of position.

8. We do not include $T$ in the expression for the stress in the $z$-direction, $\tau_{izz}$, because we assume cells grow as a monolayer adherent on a 2D substrate. A growing sphere of cells would require $T$ to act in all directions.
9. We assume that the tissue is in steady-state mechanical equilibrium, whereas cell colonies are constantly growing. Growth is slow enough that inertial terms need not be included in the mechanical equations, but processes may exist that lower membrane stress over time. For instance, bonds between integrins and the extracellular matrix may break and then reform after the extracellular stress is relieved. Our model does not contain such viscoelastic behavior that may be necessary to describe growth over hours, days, or weeks. For example, Kim *et al* (2013) have studied advancing monolayer sheets of Madin-Darby canine kidney (MDCK) epithelia cells. The mechanical bidomain model cannot be used to represent such systems unless this dynamic growth process is included in the mathematical description.
10. The mechanical bidomain model is a continuum model that averages over the discrete cellular properties. The assumption of a continuum should be valid as long as all the length scales of the problem are large compared to the size of individual cells. The length constant $\sigma$ is a few hundred microns, which is larger than the cell size but not dramatically so. Thus, the assumption of a continuum may provide a useful macroscopic prediction of the overall distribution of stress and strain, but microscopic cellular or even molecular effects may modulate our results (Bischofs and Schwarz 2003, Bischofs *et al* 2004, Engler *et al* 2006, Vermolen and Gefen 2012, Gonzalez-Valverde *et al* 2016).
11. The fundamental hypothesis of the mechanical bidomain model is that mechanotransduction effects, such as stem cell differentiation, are driven by forces on integrin proteins in the membrane. The alternative hypothesis—that mechanotransduction is caused by stress or strain—results in a very different prediction of where differentiation occurs. Yet another hypothesis is that cells respond to strain energy (Vermolen and Gefen 2012). We interpret the experimental observation of edge effects in cell colonies by Rosowski *et al* (2015) and others (Nelson *et al* 2005, Ruiz and Chen 2008, Mertz *et al* 2012, Warmflash *et al* 2014) as support for our hypothesis that forces on integrins cause mechanotransduction.

Human embryonic stem cell colonies are an *in vitro* model used to study the mechanisms of development. If the mechanical bidomain model accurately predicts the behavior of these colonies, it may similarly provide insight into the far more complex process of human development.

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