Published in final edited form as:

Soft Matter. 2012 July 28; 8(28): 7446–7451. doi:10.1039/C2SM25452B.

Growth of curved and helical bacterial cells

Hongyuan Jiang and Sean X. Sun

Department of Mechanical Engineering and Whitaker Biomedical Engineering Institute, Baltimore, MD 21218, USA

Sean X. Sun: ssun@jhu.edu

Abstract

A combination of cell wall growth and cytoskeletal protein action gives rise to the observed bacterial cell shape. Aside from the common rod-like and spherical shapes, bacterial cells can also adopt curved or helical geometries. To understand how curvature in bacteria is developed or maintained, we examine how *Caulobacter crescentus* obtains its crescent-like shape. *Caulobacter* cells with or without the cytoskeletal bundle crescentin, an intermediate filament-like protein, exhibit two distinct growth modes, curvature maintenance that preserves the radius of curvature and curvature relaxation that straightens the cell (Fig. 1). Using a proposed mechanochemical model, we show that bending and twisting of the crescentin bundle can influence the stress distribution in the cell wall, and lead to the growth of curved cells. In contrast, after crescentin bundle is disrupted, originally curved cells will slowly relax towards a straight rod over time. The model is able to quantitatively capture experimentally observed curvature dynamics. Furthermore, we show that the shape anisotropy of the cross-section of a curved cell is never greater than 4%, even in the presence of crescentin.

1. Introduction

Bacterial cell walls are built through a complex biochemical process under high internal turgor pressure; therefore mechanical forces and biochemistry are both important in determining the overall cell shape. Mechanisms of growth and shape control have been extensively discussed in the literature.^{1–4} Recently, a number of cytoplasmic cytoskeletal proteins have been implicated in bacterial cell shape maintenance.^{4–7} For example, MreB, a prokaryotic actin homologue, is the primary cytoskeletal protein in rod-like bacteria for maintaining the cylindrical shape.^{4–9} Disassembly of MreB filaments in bacteria leads to a morphological transformation from a rod to a sphere.^{8–13} Crescentin is an intermediate filament-like protein found in *Caulobacter crescentus*. Crescentin forms a bundle on the concave side of the crescent-shaped *C. crescentus* (Fig. 1). Experiments^{14,15} have shown if the crescentin bundle is detached from the cell wall, it collapses into a helix and appears to move freely in the cell. After crescentin is disrupted, initially curved cells gradually lose their characteristic curved shapes and eventually become straight rods.^{14,15} Remarkably, artificially producing crescentin in *Escherichia coli* leads to helical cells.¹⁵ Not only crescentin but also physical forces applied by external constraints can change the cell shape.

Experiments showed that straight rod-like *E. coli* cells grown in circular micro-chambers become curved or even helical due to the confinement of micro-chambers.^{15,16} Moreover, these cells slowly return to their native straight rod-like shape after escaping from these chambers.^{15,16} This indicates that both the crescentin bundle and the mechanical forces applied by external constraints generate curved cells. Strikingly, the growth modes of the cell with or without crescentin are different^{17,18} as shown in Fig. 1. Here, based on a mechanochemical model of cell wall growth, we provide quantitative explanations of how crescentin controls cell curvature.

2. Mechanical stresses in the cell wall

Due to a high internal concentration of osmolytes, bacterial cells maintain a high turgor pressure. This implies that the growing cell wall is under tension, and the growth dynamics is strongly influenced by the mechanical stress.¹⁹ Here, we can compute the stress field in the cell wall for curved and helical cell shapes using a mathematical model. Since the cell wall is a stretchable network of polysaccharides, it can be modeled as a thin elastic shell. The undeformed mid-plane of a cell wall can be described by a three dimensional surface $\mathbf{r} = \mathbf{r}(\xi_1, \xi_2)$, where (ξ_1, ξ_2) is a curvilinear coordinate system.²⁰ The tangential vectors of the surface at any point are given by $\mathbf{m}_a = \partial \mathbf{r} / \partial \xi_a$, where a ranges from 1 to 2. The unit vector normal to the surface can be defined as $\mathbf{m}_3 = \mathbf{m}_1 \times \mathbf{m}_2 / |\mathbf{m}_1 \times \mathbf{m}_2|$. Therefore, the orientation of an arbitrary point is characterized by the co-variant basis $(\mathbf{m}_1, \mathbf{m}_2, \mathbf{m}_3)$. Notice that for certain geometry, like a helical cell wall, $\mathbf{m}_1$ and $\mathbf{m}_2$ are not orthogonal. So it is convenient to introduce a second set of basis vectors, *i.e.*, the contravariant basis $(\mathbf{m}^1, \mathbf{m}^2, \mathbf{m}^3)$, defined so that $\mathbf{m}^i \cdot \mathbf{m}_j = \delta_j^i$. Here δ_j^i is the Kronecker delta symbol, *i.e.* $\delta_j^i = 1$ for $i = j$ and zero otherwise. The metric tensor is defined by $\bar{g}_{ij} = \mathbf{m}_i \cdot \mathbf{m}_j$ and $\bar{g}^{ij} = \mathbf{m}^i \cdot \mathbf{m}^j$. In this paper, we use Greek letters to indicate index 1 or 2 and use English letters to indicate index 1, 2 or 3.

The deformed mid-plane of the cell wall can be described by another three dimensional surface $\mathbf{r}(\xi_1, \xi_2)$. In the same way, we introduce two sets of basis $(\mathbf{m}_1, \mathbf{m}_2, \mathbf{m}_3)$ and $(\mathbf{m}^1, \mathbf{m}^2, \mathbf{m}^3)$. And the metric tensor can be introduced similarly.

The mechanical equilibrium equations of a thin shell are

$$\frac{\partial T^{\alpha\beta}}{\partial \xi_\alpha} + T^{\alpha\beta} \Gamma_{\alpha\gamma}^\gamma + T^{\alpha\gamma} \Gamma_{\alpha\gamma}^\beta + p^\beta = 0, \quad (1)$$

$$T^{\alpha\beta} \kappa_{\alpha\beta} = p^3, \quad (2)$$

where $\mathbf{p} = p^i \mathbf{m}_i$ is the external force per unit area, $\mathbf{T} = T^{\alpha\beta} \mathbf{m}_\alpha \otimes \mathbf{m}_\beta$ is the stress resultant

tensor and $T^{\alpha\beta}$ is symmetric. $\Gamma_{i\alpha\beta} = \mathbf{m}_i \cdot \frac{\partial^2 \mathbf{r}}{\partial \xi_\alpha \partial \xi_\beta}$ and $\Gamma_{\alpha\beta}^i = \mathbf{m}^i \cdot \frac{\partial^2 \mathbf{r}}{\partial \xi_\alpha \partial \xi_\beta}$ are the Christoffel

symbols of the first kind and second kind. $\kappa_{\alpha\beta} = \mathbf{m}_\alpha \cdot \frac{\partial \mathbf{m}_\beta}{\partial \xi_\beta}$ is the curvature tensor.

2.1. Stress field in curved rod-like cells with a circular cross-section

For a bacterial cell wall without any internal or external constraints, the only non-zero external loading is the turgor pressure $p^3 = P$. If we neglect the cell poles and assume that the shape of the cross-section is a circle, the shape of a curved rodlike cell can be described as one part of a torus:

$$\mathbf{r} = [(R+r\cos\varphi)\cos\theta, (R+r\cos\varphi)\sin\theta, r\sin\varphi] \quad (3)$$

where R is the curvature radius of the centerline, r is the radius of the cross-section, θ is the span angle of the torus and φ is the rotational angle along the circumferential direction of the torus (see Fig. 1). The solution to eqn (1) and (2) is

$$T^{11} = \frac{P(2R+r\cos\varphi)}{2r(R+r\cos\varphi)}, \quad (4)$$

$$T^{22} = \frac{Pr}{2(R+r\cos\varphi)^2}, \quad (5)$$

$$T^{12} = T^{21} = 0. \quad (6)$$

The mixed components of the stress resultant tensor can be given by $T_d^\beta = g_{\alpha\beta} T^{\alpha\beta}$ and $T^\beta_\alpha = T^{\beta\rho} g_{\rho\alpha}$. In this case, $T^1_1 = T_1^1 = \frac{Pr(2R+r\cos\varphi)}{2(R+r\cos\varphi)}$, $T^2_2 = T_2^2 = \frac{Pr}{2}$ and other components are zero. We give the mixed component of the stress resultant tensor T_d^β and T^α_β since they are more convenient to use for the energy expression. As R goes to infinity, the two resultant stresses reduce to Pr and $Pr/2$, which are resultant stresses of a cylindrical cell wall.

2.2. Stress field in curved rod-like cells with an elliptic cross-section

In the previous section, we assumed that the cross-section of a curved rod-like cell is a circle. However, the shape of the cell cross-section may deviate from a circle if other mechanical elements such as the crescentin bundle are attached to the cell wall. To simplify the problem, we can assume that the cross-section is an ellipse and examine any possible shape anisotropy. In this case, the shape of a curved rod-like cell with elliptic cross-section can be described as

$$\mathbf{r} = [(R+a\cos\varphi)\cos\theta, (R+a\cos\varphi)\sin\theta, b\sin\varphi] \quad (7)$$

where a and b are the major and minor semi-axes of the cross-section. R , θ and φ share the same definition as in the last section. The solution to eqn (1) and (2) is

$$T^{11} = \frac{Pa(2R+a\cos\varphi)}{\sqrt{2b(R+a\cos\varphi)} \sqrt{(a^2+b^2)-(a^2-b^2)\cos 2\varphi}}, \quad (8)$$

$$T^{22} = \frac{P[ab^2 - (a^2 - b^2)(2R\cos\varphi + a\cos 2\varphi)]}{\sqrt{2}b(R + a\cos\varphi)^2 \sqrt{(a^2 + b^2) - (a^2 - b^2)\cos 2\varphi}}, \quad (9)$$

$$T^{12} = T^{21} = 0. \quad (10)$$

It is easy to verify that the above solution can be reduced to eqn (4)–(6) when $a = b = r$.

2.3. Stress field in a helical cell wall

For a helical cell, we can describe the cell shape using

$$\mathbf{r} = \left[(R + r\cos\varphi)\cos\theta + \frac{Lr\sin\theta\sin\varphi}{\sqrt{R^2 + L^2}}, (R + r\cos\varphi)\sin\theta - \frac{Lr\cos\theta\sin\varphi}{\sqrt{R^2 + L^2}}, L\theta + \frac{Rr\sin\varphi}{\sqrt{R^2 + L^2}} \right]. \quad (11)$$

Here R is the radius of the centerline, r is the radius of the cross-section, θ is the span angle of the helix, φ is the rotational angle along the circumferential direction of the helix, and $p = 2\pi L$ is the pitch of the helix (see Fig. 1). In this case, the two basis vectors $\mathbf{m}_1$ and $\mathbf{m}_2$ are not orthogonal and the resultant shear stress is not zero anymore. It is not possible to compute the stress resultant tensor by solving eqn (1) and (2) directly. However, we can assume $L/R \ll 1$ and examine leading order expansions of the stress field as a function of the small parameter:

$$T^{11} = T_a^{11} + T_b^{11}L + T_c^{11}L^2 + T_d^{11}L^3 + \dots \quad (12)$$

$$T^{22} = T_a^{22} + T_b^{22}L + T_c^{22}L^2 + T_d^{22}L^3 + \dots \quad (13)$$

$$T^{12} = T_a^{12} + T_b^{12}L + T_c^{12}L^2 + T_d^{12}L^3 + \dots \quad (14)$$

By substituting the above expansions to the equilibrium eqn (1) and (2) and requiring that the coefficients of each power of L sum to zero, we obtain a series of recurrence ODEs. The series solution of eqn (1) and (2) is

$$T^{11} = \frac{P(2R + r\cos\varphi)}{2r(R + r\cos\varphi)} + \frac{P(R\cos\varphi - r\sin^2\varphi)}{2R(R + r\cos\varphi)^3}L^2 - \frac{P(r^3 - 2rR^2 + R(r^2 + R^2)\cos\varphi + rR^2\cos^2\varphi)}{2R^4(R + r\cos\varphi)^4}L^4 + O(L^5) \quad (15)$$

$$T^{22} = \frac{Pr}{2(R + r\cos\varphi)^2} + \frac{Pr(3r^2 - 2R^2 + r^2\cos 2\varphi)}{4R^2(R + r\cos\varphi)^4}L^2 + \frac{Pr[2(R^2 - r^2) - R^4 + Rr(r^2 - R^2)\cos\varphi - r^2R^2\cos 2\varphi]}{2R^5(R + r\cos\varphi)^5}L^4 + O(L^5) \quad (16)$$

$$T^{12} = \frac{Pr^2\cos\varphi}{2R(R + r\cos\varphi)^3}L + \frac{Pr^2\cos\varphi(2r^2 - 5R^2 + rR\cos\varphi)}{4R^4(R + r\cos\varphi)^4}L^3 + O(L^5) \quad (17)$$

The above solution can be reduced to eqn (8)–(10) when $L = 0$.

3. Free energy of the cell wall

The bacterial cell wall is a network of peptidoglycan (PG) strands. PG strands are constantly inserted into or deleted from the network by enzymes. Computational modeling²¹ showed that removal of peptide bonds in a static PG network can lead to curved cells. Although changes in peptidoglycan (PG) cross-linking seem to promote *Helicobacter pylori*'s helical shape,²² there are no discernible variations in crosslinking, thickness or composition in curved *C. crescentus* cell wall.¹⁵ Therefore, although the microstructure of the cell wall may influence the cell shape, the helical shape of *C. crescentus* is mainly created and maintained by differential cell wall growth induced by crescentin.¹⁵ Previous models have considered the elastic interaction of the crescentin bundle with the cell wall.^{21,23,24} In these models, the cell wall is treated as a static elastic structure. However, in living cells, the wall is constantly growing and reorganizing; thus a non-equilibrium theory must be the starting point of analysis.

Such a dynamic growth model that includes both mechanics and some elements of biochemistry for the bacterial cell wall has been proposed for rod-like cells.^{8,9,25} The model considers an elastic PG network undergoing subunit turnover from the action of transpeptidases, transglycosylases and hydrolases. Chemically active PG monomers are inserted into the cell wall, making new bonds and releasing stored chemical energy. At the same time, initially stress-free PG subunits are stretched by the cell turgor pressure, P . Therefore, the growth of cell wall is determined by the competition between the increased strain energy and the released chemical energy.^{8,9,25} The total free energy without considering crescentin can be written as

$$G_1 = \int (f - \varepsilon) dA, \quad (18)$$

where ε is the chemical bond energy per unit area during the growth process. f is the strain energy density of the cell wall, which can be given by

$f = \frac{h}{2} D^{\alpha\beta\rho\mu} \gamma_{\alpha\beta} \gamma_{\rho\mu} + \frac{h^3}{24} D^{\alpha\beta\rho\mu} \Delta \kappa_{\alpha\beta} \Delta \kappa_{\rho\mu}$. Here, $\gamma_{\alpha\beta}$ and $\kappa_{\alpha\beta}$ are the mid-plane Lagrange strain tensor and the curvature change tensor. h is the thickness of the cell wall. $D^{\alpha\beta\rho\mu} = \lambda \bar{g}^{\alpha\beta} \bar{g}^{\rho\mu} + \mu (\bar{g}^{\alpha\rho} \bar{g}^{\beta\mu} + \bar{g}^{\alpha\mu} \bar{g}^{\beta\rho})$ is the elastic tensor.²⁸ Here, $\lambda = \nu E / (1 - \nu^2)$ and $\mu = E / 2(1 + \nu)$ are two constants, where E and ν are Young's modulus and Poisson ratio of the cell wall. The stress resultant tensor is related to the mid-plane Lagrange strain tensor by the constitutive relation $T^{\alpha\beta} = h D^{\alpha\beta\rho\mu} \gamma_{\rho\mu}$. We can use the results from the previous section to explicitly compute the strain tensor and the strain energy density.

If the deformation in the cell wall is small, and the deformed shape is close to the reference shape $\mathbf{r}(\xi_1, \xi_2)$, the strain energy density can be explicitly computed:

$$f = \frac{2(\lambda + \mu) T^{\alpha}_{\beta} T^{\beta}_{\alpha} - \lambda (T^{\alpha}_{\alpha})^2}{8\mu(\lambda + \mu)/h} + \frac{\kappa_1}{2} (2H)^2 + \kappa_2 K \quad (19)$$

The first term of eqn (19) is the stretch energy and the other terms are the bending energy. T^a_β is the mixed component of the stress resultant tensor. H and K are the mean curvature and Gaussian curvature, respectively. Here, we assume that the spontaneous curvature of the cell wall is zero. Two constants $\kappa_1 = h^3(\lambda + 2\mu)/12$ and $\kappa_2 = -h^3\mu/6$ are similar to the bending modulus and the Gaussian rigidity of a lipid membrane. For a thin shell, the bending energy is generally small compared to the stretch energy and is therefore negligible. Note that the strain energy now only depends on the reference shape $\mathbf{r}(\xi_1, \xi_2)$. Therefore the free energy of the cell wall is simplified. Also, it should be noted that the energy of the cell membrane is neglected here since it is significantly softer than the cell wall.

If we further consider the energy contribution of pressure and volume changes, the total free energy without considering crescentin can be modified to read

$$G_1 = \int (f - \varepsilon) dA - \int P dV, \quad (20)$$

where $-\int P dV$ is the energy associated with internal osmotic pressure. We found that the addition of $-\int P dV$ only changes the simulation results quantitatively for a helical cell. The similar conclusion can be drawn for spherical and cylindrical bacterial cell walls.^{8,25} For example, for a spherical cell, the free energy is $G_1 = 4\pi[P^2R^4/8h(\lambda + \mu) - \varepsilon R^2]$, which yields

a steady radius $R_s = \sqrt{4h\varepsilon(\lambda + \mu)}/P$ if we do not include the contribution of pressure and volume changes.²⁵ In contrast, if the term $-\int P dV$ is considered, the free energy is modified to $G_1 = 4\pi[P^2R^4/8h(\lambda + \mu) - \varepsilon R^2 - PR^3/3]$. In this case, the steady radius still exists and it is $R_s = \left[1 + \sqrt{1 + 4\varepsilon/(\lambda + \mu)h}\right] (\lambda + \mu)h/P$.

Crescentin is an intermediate filament-like protein that assembles into stiff and stable filamentous structures *in vitro*,^{14,26} and adheres to the concave side of the cell wall *in vivo*^{14,15} (Fig. 1). By replacing the wild-type crescentin with non-functional CreS-GFP,¹⁴ or inhibiting peptidoglycan cross-linking with β -lactam antibiotic mecillinam in *bla* cells,¹⁵ the crescentin bundle can be detached from the cell wall. The detached crescentin bundle adopts a left-handed helical shape with a preferred helical pitch, $p_0 = 1.4\text{--}1.6\ \mu\text{m}$.^{14,15} The preferred radius of the crescentin helix R_0 is not measured in experiments, but the radius of the helix is close to the radius of *C. crescentus* after it is detached from the cell wall.^{14,15} Therefore, the preferred radius is assumed to be similar to the radius of *C. crescentus* cells. The width of crescentin helix is estimated to be 100–300 nm. The mechanical properties of crescentin are unknown. However, crescentin is an intermediate filament-like protein. Therefore, we assume that Young's modulus of the crescentin filament is similar to that of intermediate filament, *i.e.*, $E_c = 300\text{--}900\ \text{MPa}$.²⁷

To understand how crescentin influences cell wall growth, we model the crescentin bundle as a helical elastic rod adhered to the inner side of the elastic cell wall (Fig. 1). So the shape of the crescentin is given by eqn (11) when $\phi = \pi$, *i.e.*, $\mathbf{r} = [(R - r)\cos \theta, (R - r)\sin \theta, L\theta]$. Assuming that the bundle can slide freely on the cell wall, bending and twisting are more important than bundle stretch. The strain energy of the helical bundle^{28,29} is

$$G_2 = \int_0^L \left[\frac{E_c I}{2} (\kappa - \kappa_0)^2 + \frac{\mu_c J}{2} (\tau - \tau_0)^2 \right] ds \quad (21)$$

where $\kappa_0 = \frac{R_0}{R_0^2 + p_0^2 / (2\pi)^2}$ and $\tau_0 = \frac{p_0 / (2\pi)}{R_0^2 + p_0^2 / (2\pi)^2}$ are the curvature and twist of the spring in the relaxed state. R_0 and p_0 are the preferred radius and helical pitch of the spring.

$\kappa = \frac{R-r}{(R-r)^2 + p^2 / (2\pi)^2}$ and $\tau = \frac{p / (2\pi)}{(R-r)^2 + p^2 / (2\pi)^2}$ are the curvature and the twist in the deformed state, respectively. R is the curvature radius of the cell centerline and r is the radius of the cross-section (Fig. 1). $R - r$ and p are the radius and helical pitch of the spring in deformed state. E_c and μ_c are Young's modulus and shear modulus of the helix. $I = \pi d^4 / 64$ and $J = \pi d^4 / 32$ are the area and polar moments of inertia of a circular cross-section with diameter d . The total energy of the cell wall-crescentin system is then a sum of the individual contributions, i.e., $G = G_1 + G_2$. Therefore, the cell wall and crescentin constitute a composite material. In this paper, we study the growth of this composite material, not just the cell wall itself.

4. Growth laws

Having obtained an explicit expression for the cell wall free energy as a function of the cell reference shape, we now can compute the evolution and growth of the reference shape driven by this free energy. To simply characterize the shape and growth of a curved bacteria cell (Fig. 1), we need three dynamic parameters: the radius of the cross-section r , the curvature radius of the centerline R and the span angle of the torus θ . Therefore, the free energy can be written as $G = G(r, R, \theta)$ from eqn (20) and (21). For the helical shape, one additional parameter, the helical pitch p , is required (Fig. 1). In this case, the free energy can be written as $G = G(r, R, \theta, p)$. The poles of *E. coli* cells are rigid and inert.³⁰ Therefore, we assume that they are also rigid and inert in *C. crescentus* cells and neglect the cell poles in the growth calculation. The change of the total energy can be written as $dG = \sum_i F_i da_i$, where a_i are shape parameters of the cell, i.e., r, R, θ or p ; $F_i = -G / a_i$ is defined as the driving force corresponding to the parameter a_i . The growth velocity can be defined as⁸

$$\frac{1}{a_i} \frac{da_i}{dt} = M_i F_i. \quad (22)$$

where M_i is a phenomenological growth constant corresponding to parameter a_i . At the molecular scale, cell wall growth is regulated by the spatio-temporal activity of cell wall synthesis and lytic enzymes. At the continuum level, the spatio-temporal activity of the enzymes is characterized as the growth constants. In our calculation, the growth constants are assumed to be independent of time and space. In general, however, we can use spatio-temporally varying growth constants to model the spatio-temporal regulation of cell wall growth. Indeed, recently we used a spatially varying growth constant to examine shape instabilities in growing *E. coli* cells.⁸

5. Results

We first consider curved rod-like cells without the influence of crescentin. Similar to rod-like cells, curved rod-like cells can maintain a constant radius of cross-section (Fig. 2a).^{8,25} In this case, the resultant stresses along the circumferential direction and axial direction are $Pr(2R + r\cos\phi)/[2(R + r\cos\phi)]$ and $Pr/2$, respectively (see Section 2.1). ϕ is the rotational angle along the circumferential direction (see Fig. 1). On the concave side of the cell ($\phi = \pi$), the resultant stress along the circumferential direction is $Pr(2R - r)/[2(R - r)]$, which is larger than the resultant stress $Pr(2R + r)/[2(R + r)]$ on the convex side ($\phi = 0$). Since the cell wall growth is stress-dependent (eqn (1)), the effect of the stress-dependent growth is that the curvature radius R always increases with time (Fig. 2b) no matter what M_θ is used (Fig. 2c). Therefore, cells tend to straighten and the curvature relaxes during growth, which is consistent with the experimental observations.^{15,16} Our model predicts that the curvature declines with time exponentially (Fig. 3a) which can be compared with the experimental measurement for *C. crescentus* cells with disrupted crescentin bundle.^{15,18} The value of the growth constant M_R is fitted to experimental data from ref. 15 and 18 (see Fig. 3a). From the experimental data, we know how the curvature of cell centerline $1/R$ evolves with time. In the simulation, the growth dynamics of R is mainly determined by the value of M_R and we found an appropriate M_R to reproduce the curvature relaxation curve found in the experiment (see Fig. 3a). The growth dynamics of r and θ is unavailable in the experiment. Therefore, M_r and M_θ are unknown, and their values are estimated in this calculation.

When the crescentin bundle is attached to the cell wall, curvature still tends to relax in a growing cell. However, the tendency is counterbalanced by the bending and twist of the crescentin bundle. Therefore, there is a steady curvature radius for the cell centerline R (Fig. 2e). Thus, in *C. crescentus*, the cell wall and the crescentin bundle should be regarded as a composite material. The steady curvature radius is determined by the mechanical properties of this composite. The stiffness of crescentin bundle $E_c I$ is proportional to the 4th power of the cross-section diameter of bundle since $I = \pi d^4/64$. Therefore, thicker crescentin bundles lead to smaller steady radius R as shown in Fig. 3b. We predict that the steady radius can be altered by changing the number of filaments in the crescentin bundle, which can be achieved by changing the expression level of crescentin.¹⁵ Strains lacking crescentin are straight rods ($R \rightarrow \infty$), while overproducing of crescentin results in smaller curvature radius.¹⁵ This observation is consistent with our prediction. Furthermore, the crescentin bundle does not have to be the same length as the cell. In fact, if the cell becomes very long, the crescentin bundle might grow slower than the cell wall, and is shorter than the cell. In this case, the cell is straight at the two ends, but remains helical in the middle of the cell (Fig. 3D and E in the ref. 31). All these findings are in accord with our predictions.

It is also interesting to ask whether the crescentin bundle has an influence on cross-sectional shape of the cell. One might expect that the cell cross-section is not circular if crescentin exerts significant forces. In our model, we can investigate an elliptical cross-section (see Section 2.2) and ask the equilibrium values of elliptical axes, a and b . We predict that the circular shape is quite robust, and the shape anisotropy, $1 - a/b$, is never greater than 4% (Fig. 4). Interestingly, the long axis of the elliptical cross-section is predicted to be perpendicular to the plane of curvature of the cell, although in this calculation, MreB is not

considered and may further stabilize the circular cross-sectional shape.⁸ Including MreB in the model would only quantitatively change the results.

The model can be extended to helical curved cells where the helical pitch of the cell is described by p . The growth of this helical cell can be characterized by R , θ , r and p (Fig. 1). In this case, material basis vectors are not orthogonal and the resultant shear stress is not zero. We can only obtain a series solution if we assume $p/(2\pi) \ll R$ (see eqn (15)–(17) in Section 2.3). Fig. 5b and c show that the curvature radius R and the helical pitch p increase with time if crescentin bundle is disrupted in *C. crescentus*. Therefore, similar to Fig. 2, the cell always straightens no matter what M_θ is used. In contrast, if crescentin is intact (Fig. 5e–h), R and p approach fixed values during cell growth so that the cell can maintain a spiral shape. In both cases, cells always develop a constant cross-sectional radius r (Fig. 5a and e).

6. Concluding remarks

We have described a mechanochemical explanation of curved bacterial cells with a stiff PG cell wall. The model shows that *C. crescentus* cells can develop curvature in the presence of the crescentin bundle. When the bundle is disrupted, cell curvature relaxes exponentially with time. Two distinct growth modes observed in experiments are fully explained by a single model. The model provides an explicit relationship between mechanical stress and growth of the PG network.

The continuum model presented here, however, does not have molecular details, which requires further *in vivo* elucidation. For example, it was found recently that PBP enzymes add new cell wall material in a processive MreB-dependent manner.^{32–34} Also, the cell wall probably grows by creating PG defects using hydrolases and filling up the defects using PBPs. The concentration of molecular defects may also depend on the mechanical stress. This implies that the cell wall mechanical properties are not constant, and should depend on the dynamics of molecular scale defects. These molecular-level details can be incorporated into the present continuum framework by considering a dynamics constitutive relationship for the cell wall, and a more complex model for the growth constants M_i in eqn (22). However, these molecular details still must obey the energy balance criterion discussed in this paper. Indeed, the growth law of eqn (22) is a statement on the kinetics of cell wall addition and how this kinetics is influenced by the global stress in the cell wall. Refinement of the present model combined with new experimental data will provide further connections between the molecular level and the cell level to explain the bacterial cell shape.

References

1. Holtje JV. Microbiol Mol Biol Rev. 1998; 62:181–203. [PubMed: 9529891]
2. Young KD. Microbiol Mol Biol Rev. 2006; 70:660–703. [PubMed: 16959965]
3. Vollmer W, Bertsche U. Biochim Biophys Acta, Biomembr. 2008; 1778:1714–1734.
4. Cabeen MT, Jacobs-Wagner C. Nat Rev Microbiol. 2005; 3:601–610. [PubMed: 16012516]
5. Moller-Jensen J, Lowe J. Curr Opin Cell Biol. 2005; 17:75–81. [PubMed: 15661522]
6. Shih YL, Rothfield L. Microbiol Mol Biol Rev. 2006; 70:729–754. [PubMed: 16959967]
7. Cabeen MT, Jacobs-Wagner C. J Cell Biol. 2007; 179:381–387. [PubMed: 17967949]
8. Jiang HY, Si FW, Margolin W, Sun SX. Biophys J. 2011; 101:327–335. [PubMed: 21767484]

9. Jiang HY, Sun SX. *Microbiol Mol Biol Rev.* 2011; 75:543–565. [PubMed: 22126993]
10. Kruse T, Møller-Jensen J, Løbner-Olesen A, Gerdes K. *EMBO J.* 2003; 22:5283–5292. [PubMed: 14517265]
11. Iwai N, Nagai K, Wachi M. *Biosci, Biotechnol, Biochem.* 2002; 66:2658–2662. [PubMed: 12596863]
12. Figge RM, Divakaruni AV, Gober JW. *Mol Microbiol.* 2004; 51:1321–1332. [PubMed: 14982627]
13. Gitai Z, Dye N, Shapiro L. *Proc Natl Acad Sci U S A.* 2004; 101:8643–8648. [PubMed: 15159537]
14. Ausmees N, Kuhn JR, Jacobs-Wagner C. *Cell.* 2003; 115:705–713. [PubMed: 14675535]
15. Cabeen MT, Charbon G, Vollmer W, Born P, Ausmees N, Weibel DB, Jacobs-Wagner C. *EMBO J.* 2009; 28:1208–1219. [PubMed: 19279668]
16. Takeuchi S, DiLuzio WR, Weibel DB, Whitesides GM. *Nano Lett.* 2005; 5:1819–1823. [PubMed: 16159230]
17. Mukhopadhyay R, Wingreen NS. *Phys Rev E: Stat, Nonlinear, Soft Matter Phys.* 2009; 80:062901.
18. Sliusarenko O, Cabeen MT, Wolgemuth CW, Jacobs-Wagner C, Emonet T. *Proc Natl Acad Sci U S A.* 2010; 107:10086–10091. [PubMed: 20479277]
19. Typas A, Banzhaf M, Gross CA, Vollmer W. *Nat Rev Microbiol.* 2012; 10:123–136. [PubMed: 22203377]
20. Nelson, DR.; Piran, T.; Weinberg, S. *Statistical Mechanics of Membranes and Surfaces.* World Scientific; Singapore: 2004.
21. Huang KC, Mukhopadhyay R, Wen B, Gitai Z, Wingreen NS. *Proc Natl Acad Sci U S A.* 2008; 105:19282–19287. [PubMed: 19050072]
22. Sycuro LK, Pincus Z, Gutierrez KD, Biboy J, Stern CA, Vollmer W, Salama NR. *Cell.* 2010; 141:822–833. [PubMed: 20510929]
23. Kim JS, Sun SX. *Biophys J.* 2009; 96:L47–L49. [PubMed: 19383443]
24. Wolgemuth CW, Inclan YF, Quan J, Mukherjee S, Oster G, Koehl MA. *Phys Biol.* 2005; 2:189–199. [PubMed: 16224124]
25. Jiang HY, Sun SX. *Phys Rev Lett.* 2010; 105:028101. [PubMed: 20867742]
26. Esue O, Rupprecht L, Sun SX, Wirtz D. *PLoS One.* 2010; 5:e8855. [PubMed: 20140233]
27. Guzmán C, Jeney S, Kreplak L, Kasas S, Kulik AJ, Aebi U, Forró L. *J Mol Biol.* 2006; 360:623–630. [PubMed: 16765985]
28. Bower, AF. *Applied Mechanics of Solids.* CRC Press; 2009.
29. Antman, SS. *Nonlinear Problems of Elasticity.* Springer; 2005.
30. Thwaites JJ, Mendelson NH. *Adv Microb Physiol.* 1991; 32:173–222. [PubMed: 1882728]
31. Charbon G, Cabeen MT, Jacobs-Wagner C. *Genes Dev.* 2009; 23:1131–1144. [PubMed: 19417107]
32. Dominguez-Escobar J, et al. *Science.* 2011; 333:225–228. [PubMed: 21636744]
33. Garner EC, et al. *Science.* 2011; 333:222–225. [PubMed: 21636745]
34. van Teeffelen S, et al. *Proc Natl Acad Sci U S A.* 2011; 108:15822–15827. [PubMed: 21903929]

Fig. 1.

Growth of bacterial cell walls (green surface) is influenced by cytoskeletal bundles such as crescentin (red bundle). Curved bacterial cells can have two different growth modes: (I) detachment of crescentin in *C. crescentus* cells,^{14,15} or the release of curved *E. coli* cells from circular micro-chambers,^{15,16} leads to curvature relaxation (cell straightening). The curvature radius of the cell centerline, R , increases with time, but the span angle θ increases slowly or remains constant. (II) When crescentin is attached to the cell wall, a curved rod-like shape develops if cells are short, but a spiral shape is obtained if cells grow longer.^{14,15} In this case, the curvature radius of the cell centerline R and helical pitch p remain unchanged, but the span angle θ increases with time.

Fig. 2.

Time evolution of (a) the radius of the cross-section r , (b) the curvature radius of the centerline R and (c) the span angle of the torus θ in a curved rod-like cell after crescentin bundle is disrupted ($E_c I = 0$). In the calculation, the crescentin is removed at time $t = 0$. The same variables are plotted in (d), (e) and (f) for curvature maintenance in normal cells ($E_c I = 100 \text{ nN } \mu\text{m}^2$). The parameters used are $P = 0.3 \text{ MPa}$, $E = 30 \text{ MPa}$, $\nu = 0.3$, $h = 3 \text{ nm}$, $\varepsilon = 0.05 \text{ J m}^{-2}$, $R_0 = 0.5 \text{ } \mu\text{m}$, $M_r M_R = 90 \text{ mJ}^{-1} \text{ s}^{-1}$, $M_\theta = 0$ (red), 9×10^6 (green) and 2.7×10^7 (blue) $\text{J}^{-1} \text{ s}^{-1}$.

Fig. 3.

(a) The curvature of cell centerline, $1/R$, decreases exponentially with time for *C. crescentus* cells with disrupted crescentin bundle. The simulation result is compared with experimental data from ref. 15 and 18. (b) The static curvature radius R as the function of crescentin bundle stiffness. The same parameters are the same as in Fig. 2 except $M_\theta = 0$.

Fig. 4.

The shape anisotropy of the cross-section of a curved cell. When the crescentin bundle is present, the cell cross-section is more elliptical in shape. The shape anisotropy is defined as the ratio of the two semi-axes of the ellipse a and b . As the crescentin bundle stiffness increases, the cross-section becomes more anisotropic, but the ratio a/b is never smaller than 0.96.

Fig. 5.

Time evolution of (a) the radius of the cross-section r , (b) the curvature radius of the centerline R , (c) the helical pitch and (d) the span angle of the helix θ in a helical cell after crescentin bundle is disrupted ($E_c I = 0$). In the calculation, the crescentin is removed at time $t = 0$. The same variables are plotted in (e), (f), (g) and (h) for curvature maintenance in normal cells ($E_c I = 100 \text{ nN } \mu\text{m}^2$). In the simulation, $p_0 = 1.6 \text{ } \mu\text{m}$ and other parameters are the same here as in Fig. 2.