Multiscale Models in the Biomechanics of Plant Growth

Plant growth occurs through the coordinated expansion of tightly adherent cells, driven by regulated softening of cell walls. It is an intrinsically multiscale process, with the integrated properties of multiple cell walls shaping the whole tissue. Multiscale models encode physical relationships to bring new understanding to plant physiology and development.

Unlike animals, plants are unable to propel themselves over great distances in search of nutrients. Rooted to one spot, they instead explore and harvest their local environment. To facilitate this, their growth and development has a high degree of plasticity, whereby environmental signals drive morphological changes. The associated movements, which include responses to light, gravity, physical obstructions, water, and soil-bound nutrients, are regulated by numerous competing signals that are transmitted by hormones. Exquisitely controlled variations in growth rate enable a growing shoot to turn toward the sun or a root to navigate a stony soil in search of water.

For most cells in a green plant, growth has a direct mechanical interpretation (66). Cells are under remarkably high turgor pressure (often in excess of that in a car tire) generated by osmosis through the semipermeable plasma membrane. The cell’s internal turgor is resisted by tensile forces in the stiff cell wall, which sits outside the plasma membrane, giving each cell an intrinsic rigidity. Regulated softening of the cell wall allows turgor pressure to drive cell expansion. The cell wall comprises a network of cellulose microfibrils embedded in a pectin matrix and cross-linked by hemicellulose polymers. This composite structure endows the wall with important mechanical properties (18). When the microfibrils are locally aligned, the wall becomes mechanically anisotropic (its resistance to in-plane extension parallel to the fibers is much greater than its resistance to extension perpendicular to the fibers) (6). The direction in which an individual cell grows is therefore influenced by the microfibril orientation. The biomechanical properties of the matrix and cross-links (together, potentially, with the availability of water to maintain turgor and the rate of production of new cell wall material) determine how rapidly a cell wall elongates or whether it elongates at all. The arrangement of aligned, adherent cells within an organ, with each expanding cell acting as a microscopic piston, endows aerial organs with rigidity and roots with the capacity to penetrate stiff soil.

The plant cell wall lies at the heart of the growth process and therefore figures prominently in this brief review, with our aim being to provide an introduction to the biomechanics of plant cell growth for animal physiologists and others who may be unfamiliar with plant biology. In contrast to diffuse growth, in which a cell expands uniformly along its length, specialized cells such as pollen tubes and root hairs exhibit tip growth, involving localized vesicular trafficking of wall material; this is not addressed here. Many additional factors are encountered across the half-dozen orders of magnitude between the thickness of a cell wall and the height of a mature plant. Individual cells adhere tightly to one another, transmitting the rigidity of individual cells to the tissue. Plant tissues have a high degree of spatial organization, often being formed by concentric layers of cells of different types that perform different functions and respond to different hormones. Cell division and elongation can occur in distinct spatial zones, with division confined to meristematic regions near root and shoot tips and elongation confined to proximal zones; thus division and differentiation lay down a template that is then amplified by cell elongation.

This survey addresses the primary cell wall of green plants; for comparison with the stiffer secondary cell wall of woody plants, see Ref. 19. As an example, we focus on growth of the root of the model species Arabidopsis thaliana (FIGURE 1A). Its geometric simplicity and abundant imaging and genetic data facilitate the development of mathematical and computational models that integrate biomechanical and biochemical processes (although its diminutive size makes Arabidopsis awkward for biomechanical experiments). Techniques of multiscale modeling provide efficient ways to embed the plant’s hierarchical geometric structure in computational simulations of growth. Studies of Arabidopsis provide a valuable test-bed for other plant species and address more general challenges in modeling biomechanics and morphogenesis in multicellular organisms.
Constitutive Models

A biomechanical description of a growing organism must be based around a constitutive model relating the stress (force per unit area) acting on a cell or tissue to the resulting deformation (a strain or strain rate). The machinery of tensor calculus is required to express such relationships in three dimensions. However, for slender elongating organs, it can be sufficient to relate the axial component of stress (e.g., the turgor pressure \( P \) that drives elongation) to the axial strain rate \( \left[ \text{the relative elongation rate or RER, represented mathematically as } (1/L)\frac{dL}{dt} \text{ for a short length } L(t) \text{ of tissue} \right] \). Within its elongation zone, a growing Arabidopsis root can have an RER as large as 40% per hour (72), with individual cell lengths increasing by over a factor of 10 (FIGURE 1B).

Homogeneous engineering materials (such as steel or water) are typically described by a small number of intrinsic material parameters (Young’s modulus, viscosity, etc.) that are insensitive to the size of the sample (provided, of course, that it exceeds molecular dimensions). In contrast, plant tissues, like most biological materials, are heterogeneous at multiple length scales, making material characterization much more delicate. For example, measurements of the “effective” parameters characterizing a piece of tissue may vary with the size of the sample (a bulk parameter characterizing a piece of cell wall is likely to differ from that describing a multicellular block of tissue) and time-scale [a material can exhibit recoverable (elastic) responses over short times but irreversible (viscous) responses over longer timescales]. This calls for a suite of biomechanical models (representing cell wall, cell, tissue, organ, etc.) targeting processes operating at distinct spatial and temporal scales. Multiscale methods are important in translating the properties of a model from one spatial or temporal scale to the next. Moving up spatial scales, geometric factors describing the organism’s internal architecture can be expected to contribute increasingly to its overall biomechanical properties.

Theoretical models of the growth of biological tissues have traditionally followed an elegant formalism (32, 60) that fits naturally into a mathematical description of the material as primarily elastic. This has been successful in explaining the development of the hidden (residual) stresses that can arise in intact growing tissue (71) and are released when the material is excised or cut. The characterization of a material as predominantly viscous, elastic, or plastic is, however, a modeling assumption that should be considered carefully on a case-by-case basis, depending on the question at hand and the level of complexity sought in the model. In describing plant tissues, for example, it may be sufficient to focus directly on the slow irreversible deformations of cell wall material, allowing growth to have a predominantly viscous character. Such approaches can be simpler than, but need not be incompatible with, the more general traditional framework that also incorporates elastic effects.

Plant growth involves transport of water to expanding cells combined with targeted softening of...
cell walls. Although water transport can be rate-limiting in some instances (54), it is generally sufficiently rapid for cells to regulate $P$ as they elongate (17). In a popular mathematical model of growth due to Lockhart (47), plant tissue is represented as a viscoplastic material. In this framework, the rate of axial elongation of the primary root, represented mathematically using the RER, is taken to be proportional to the difference between the stress $S$ driving growth and a yield stress $Y$ provided $S$ exceeds $Y$, the constant of proportionality being the extensibility $\phi$: thus $\text{RER} = \phi(S - Y)$ for $S > Y$; if $S < Y$, then no elongation takes place ($\text{RER} = 0$). The deformation of the cell wall is here treated as slow and irreversible, being associated with the breakage of chemical bonds, and does not account for elastic deformations that arise (for example) when the tissue is gently prodded.

Although remarkably simple, Lockhart’s model for axial elongation has proved effective in describing growth both of isolated cell wall and intact tissue; following pioneering studies in large algal cells (33), the Lockhart model is now widely recognized and adopted in the plant physiology community. However, although it is possible to measure the RER quite accurately (8, 9), $S$, $Y$, and $\phi$ need careful interpretation. For an isolated segment of cell wall, $S$ is an intramural stress, and $Y$ and $\phi$ characterize the properties of the wall alone. For a multicellular tissue, $S$ is determined by $P$ averaged over multiple cells, and $Y$ and $\phi$ reflect the integrated properties of numerous adherent cells (28). We therefore distinguish $Y_{\text{wall}}$ from $Y_{\text{cell}}$ and $Y_{\text{tissue}}$, etc. These material parameters vary as the cell walls soften or stiffen under the action of enzymes. Furthermore, the Lockhart equation can be insufficient to describe more complex motions such as twisting or coiling, which involve three-dimensional deformations and competition between viscous and elastic effects.

We now briefly summarize representations of plant growth at three distinct length scales, highlighting developments in multiscale models that integrate mechanics and biological regulation.

**From Molecule to Cell Wall**

The plant cell wall is a composite material that undergoes mechanical, chemical, and structural modification during growth (14). According to the “multinet” hypothesis, microfibrils are passively reoriented as the surrounding matrix deforms (55). Cell elongation can lead to an increase in the distance between neighboring microfibrils (6), stretching and breaking the polymers that connect them.

Stretching a sheet of material typically causes it to thin. To maintain the integrity of the wall of an elongating cell, new material must be continually deposited on its inner face. Microfibrils are laid down in the cell wall by cellulose synthase complexes, which move within the plasma membrane along a network of cortical microtubules (12). Thus the mechanical anisotropy of the cell wall, and therefore of the tissue as a whole, is sensitive to the orientation of this network. Studies combining live imaging with computational models have shown that microtubule orientation can be regulated by the local stress field (34, 46, 65), possibly via the action of the severing protein katanin (73) and its regulators (78). This allows the cell wall to respond to its mechanical environment via reinforcements that accommodate elevated tensile stress. The cell wall has its own sensing and signaling capacity (79), regulating the rate of deposition of new wall material (11). Although growth rates and wall deposition rates can be independently regulated (22), the deposition rate may be an important determinant of the overall elongation rate (24).

To describe the growth of an individual cell, it is necessary to formulate a constitutive model of the cell wall. This can be achieved either by adopting an empirical formulation (such as the Lockhart equation) or by deriving relationships based on descriptions of underlying molecular processes. Models in the latter class fall into three categories: computations that simulate large numbers of molecular interactions, chemo-rheological theories of polymer networks, and models based on principles of thermodynamics.

In the first category, an idealized cell wall is constructed by stochastic self-assembly of microfibrils and hemicellulose cross-links; stress-strain characteristics are then determined by numerical simulation. Such models account for wall anisotropy and make reasonable ab initio predictions of elastic moduli (41); this approach suggests that the dominant contribution to the Young’s modulus arises from hemicellulose/microfibril interactions (80). At present, such approaches fail to account for the pectin matrix and restrict attention to small elastic deformations.

Models in the second category track the breakage of polymers that have been stretched by the relative motion of microfibrils during wall elongation. These may be either hemicellulose cross-links or the interpenetrating pectin network; their relative importance in supporting the load exerted on an elongating cell wall remains a subject of debate (51, 53). As the wall elongates, an individual polymer connecting two microfibrils will be stretched and carried toward the outer face of the cell wall as new material is deposited on the inner face (26, 52). If the rate at which a cross-link breaks under load increases very rapidly once the cross-link is sufficiently elongated, the wall is predicted to
reach a near-saturated state under high strain rates in which most cross-links remain intact until they are within a short distance of the outer face of the wall (26). As a consequence, the predicted stress/strain-rate relation recovers yielding behavior characteristic of Lockhart’s model. Homogalacturonan, a major component of the pectin matrix, is a polymer formed from galacturonic acid, which has carboxyl residues that are esterified with methanol (53). The enzyme pectin methylesterase demethylsterifies the residues, enabling them to cross-link with each other by associating with Ca$^{2+}$ (in an “egg-box” structure), allowing pectin to stiffen into a gel. During wall elongation, cross-links are tightened by stretching, break, and then reform in a relaxed state. Growth rates may therefore be regulated by calcium levels and the rate at which polygalacturonic acid is supplied to the inner face of the cell wall (57). This conceptual picture has been used to derive a pectin-focused mathematical model of the elongating cell wall, in which the strain rate is linked to the average cross-link dissociation rate (61).

The third category of wall model uses non-equilibrium thermodynamics and encompasses interactions between mechanical, chemical, and thermal effects, all of which contribute to the wall’s internal energy (5, 76). Chemical and mechanical factors interact, for example, via turgor influencing the rate at which free pectate is incorporated into the wall (56, 58). Similar interactions arise in models of active gels, where Onsager relationships link biochemistry and mechanics (43).

Significant further effort is needed to identify a constitutive model of the cell wall that unifies these different approaches and that integrates mechanical and metabolic processes. Scaling up from descriptions of individual molecules to the whole wall is itself a major challenge in multiscale modeling. Even recent computational models of individual cells as fiber-reinforced hyperelastic (65) or hyperelastic-viscoplastic materials (38) incorporate only a subset of key features while demanding an array of mechanical tests to identify independent parameters. Fortunately, valuable biomechanical data are emerging from multiscale measurements using novel indentation and imaging techniques (13, 49, 59, 65), notably high-resolution atomic force microscopy. Meanwhile, for multicellular growth simulations, the Lockhart equation continues to provide a reasonably compact description of wall properties, requiring only a small number of biomechanical parameters.

**From Cell Wall to Tissue**

For an isolated and perfectly spherical cell, the turgor-induced tension in the cell wall can be expected to be uniform and isotropic. For a cylindrical cell, however, the axial tension in the curved cell wall is half the hoop tension (6). Growth anisotropy is therefore regulated not only by the structure of the cell wall but also by the cell’s shape and its external environment.

Microfibrils oriented in planes orthogonal to the axis of a cylindrical cell wall (like hoops around a barrel; [FIGURE 1C](http://physiologyonline.physiology.org/)) will inhibit radial expansion while providing minimal resistance to axial elongation (6). However, this is not a stable arrangement. Any deviation of the fiber direction will be amplified by elongation; the fiber will rotate as the wall stretches, providing an increasingly large resistance to elongation. The combination of stretching (leading to wall thinning) and deposition of new fibers and matrix on the inner face of the wall (compensating for thinning) results in fibers being carried toward the outer face of the cell wall. This leads to a distribution of fiber age and orientation across the cell wall, with newer untitled fibers near the inner face and older tilted fibers near the outer face. This configuration has been observed experimentally (3) and modeled by representing a growing cell wall as cylinder, with its curved walls a fiber-reinforced viscous fluid (27). The axial extensibility $\Phi_{cell}$ of an individual cell is predicted to depend on the cell radius, the cell wall thickness, and a matrix viscosity; fiber reorientation can reduce $\Phi_{cell}$ sufficiently to suppress cell elongation. For a cell with a strongly anisotropic wall, there can be complex coupling between axial, radial, and azimuthal deformations and between the associated components of stress; the twisting motions and torques arising in individual cells may underpin coiling and waving motions of some roots and tendrils.

To understand the mechanical interactions between a cell and its neighbors, it is helpful to consider the differences between the “inner” cells (within a transverse cross-section of a root or stem) and the epidermal cells at the periphery. If the turgor pressure $P$ does not vary significantly from cell to cell, then interior cells can adopt a polygonal cross-section with straight-sided walls. In contrast, the outer walls of epidermal cells support a large jump between $P$ and the lower external pressure. Accordingly, such walls are curved, thicker, and structurally distinct: in hypocotyls, internal walls have transversely oriented microfibrils, whereas the outward-facing walls of epidermal cells are polylamellate with relatively weak anisotropy [in *Arabidopsis* (20, 31)] or with microfibrils oriented along the growth direction [in *Helianthus* (44)].

A further important distinction between epidermal and internal cells arises from two observations: distinct growth-regulating hormones target distinct cell layers, and cells located nearer the
periphery of the root or stem have a greater length of cell wall intersecting the transverse cross-section. With a simple viscoplastic constitutive model, the mechanical properties of the whole cross-section of a root can be estimated by integrating the properties of the individual cell walls that intersect it (28). If each cell wall is ascribed a yield stress $Y_{wall}$ and extensibility $\Phi_{wall}$, for example, the corresponding parameters $Y_{tissue}$ and $\Phi_{tissue}$ describing the whole cross-section may be calculated (28), upscaling the Lockhart model from cell wall to tissue level. $Y_{tissue}$ and $\Phi_{tissue}$ turn out to be more strongly influenced by epidermal cells than any of the interior cell layers, simply by virtue of geometry. It is therefore natural that the hormone auxin, a primary growth regulator and the major signal driving gravitropic bending, targets the root epidermis (72).

The role of epidermal cells as a “pacemaker” of tissue expansion is well established (45), arising from observations that a transversely cut stem will contract at its periphery and swell at its center. Thus, in stems at least, epidermal cells are under tension and inner tissues are under compression, although these residual stresses are hidden in the intact plant. The axial stress considered here is a tissue stress obtained by averaging over multiple cells, yielding the net difference between the compressive turgor force within the cytoplasm of each cell and the tensile force within each wall (7, 35). The concept of tissue stress is related to the mathematical notion of homogenization, whereby the fine details of a structure are integrated to yield a description of the average properties over a larger length scale (21). This is a well developed concept for engineering materials with periodic microstructure. For biological materials, having irregular microstructure, a set of statistical samples can be used to build up a representative distribution of the tissue properties (29, 69).

Integration of mechanical properties over the transverse cross-section of a slender organ such as a root enables growth to be described compactly in terms of the shape of the evolving root centerline. The temporal evolution of individual cells can be related to the spatial pattern of growth along the root using simple kinematic relationships (4, 9, 68). For a steadily growing root, measurements of cell length as a function of distance from the root tip can be used to infer both the RER and the speed at which cells move relative to the root tip (FIGURE 1B). This description can be extended to account for root bending. Asymmetries in mechanical properties across a cross-section will cause a root to bend as it elongates, with softer cells expanding quicker on the outside of a bend (an example of differential growth causing a change in morphology). However, gradients in yield and extensibility have distinct characteristics, with the latter but not the former predicted to have curvature growing in proportion to the RER (28); experimental comparison of the distributions of RER and curvature growth rate supports the notion of distinct mechanisms of curvature generation operating at different phases of a gravitropic bend (15). By incorporating elastic effects, centerline models can also be used to account for twisting, coiling, and buckling deformations (50, 70, 77) that may arise as a root penetrates stiff soil or a tendril entwines a branch.

**From Tissue to Organ**

In many instances, it is possible to treat plant tissue as a continuous three-dimensional material, using concepts from continuum mechanics. Thin multicellular structures such as the shoot apical meristem (the domed apex of the plant that specifies the patterning of side branches) can be described in engineering terms as a pressure vessel (34, 42); likewise, leaves or petals have been represented as so-called elastic shells (23). In the framework of elastic growth models, growth is typically simulated by prescribing shape changes of isolated tissue elements and then computing the shape that results when the elements are combined together, ensuring the tissue is continuous and non-overlapping (32, 40, 60, 71). Finite-element methods are commonly used to solve the resulting equations of nonlinear elasticity (34, 40), taking small incremental steps forward in time and allowing elastic deformations to maintain the integrity of the tissue as some of its component parts expand.

Many biological processes demand an explicit representation of individual cells, for which a continuum description is inappropriate. This is particularly important when the tissue deformation has cell-scale heterogeneity, such as primordium development in the shoot apical meristem or in the emergence of a new lateral root. Multicellular models incorporate the geometry and mechanical properties of individual cells. Popular model classes include vertex-based and meshless models [for the latter, cell walls and the cell interior are represented by an interacting system of particles (39, 74)]. Finite-element methods have been applied to multicellular structures (10, 34, 62); these models currently treat walls as elastic materials, but most models do not yet incorporate growth, cell division, or gene regulatory networks.

For plant growth, vertex-based models, which exploit the polygonal or polyhedral geometry of closely packed cells (37), are an appealing approach, given that the key structural element is the cell wall. Each cell is represented by mechanical relationships defining the forces acting along the
edges of each polygon (or within the faces of each polyhedron), with \( P \) acting normally to each boundary. The tissue geometry evolves through movement of the vertices, ensuring that forces balance at each vertex (inertial acceleration being negligible for slow growth). Vertex-based models of animal tissues often incorporate target volumes and surface areas for each cell, treating cells as elastic objects (37), and topological transformations occur when cells change neighbors; in plants, growth is regulated by the local properties of the cell wall (which effectively changes the cell’s target surface area and volume), and topological transformations are normally inhibited by strong cell-to-cell adhesion. Vertex-based approaches have been applied to leaves (48), fruit in two (1) and three dimensions (36), three-dimensional growth of the embryonic root following germination (8), and roots (30, 48) (in Ref. 30, diffusible growth regulators were coupled to models of cell wall properties derived from microscale models; see FIGURE 1B). Even when a three-dimensional tissue is represented by a two-dimensional cross-section (FIGURE 1B), simulations can be expensive, and particular care is required to describe highly elongated cells accurately (16, 30).

Vertex-based approaches have been extensively applied to the shoot apical meristem, where the outermost cell layer (which plays a dominant role in the patterning of primordia) has been treated alone in a two-dimensional representation. Microfibril and microtubule orientations are assigned to each cell, allowing elastic properties to be anisotropic (34, 67), the rate of relaxation of the rest length of cell walls to be angle-dependent (63), or assigning cells an elliptical target shape (2, 73). Such models capture cell-scale growth heterogeneity and provide powerful tools with which to investigate feedback mechanisms between stress and microtubule directions (10, 34) or growth rates (73), the effect of different cell division rules on tissue growth (2, 64), and the effect of stress on cell division patterns and shape (25). Such studies tackle central questions of morphogenesis in plant and animal systems by coupling mechanisms of mechanotransduction, operating at the molecular level, to tissue-level biomechanics; theoretical models are essential in testing potential mechanisms [addressing questions such as whether molecular sensors respond to stress rather than strain (10)] against experimental data.

Conclusion

As is the case for animal systems, the structural complexity of plant tissue is reflected by a diversity of modeling approaches. Questions that link molecular biology to whole-organ physiology demand multiscale techniques, which allow the integration of different models at distinct levels of spatial organization. Computational complexity, coupled to epistemic uncertainties (such as poor knowledge of parameter values, or factors neglected or poorly described in sub-models), make it challenging simply to bolt models together. Judicious approximations that summarize key features of a model at a given level allow essential information to be passed between levels. It is valuable to test different schemes of varying complexity to assess the usefulness of simpler methods (e.g., Ref. 10). The final computational implementation of a multiscale model should make robust predictions that are not obscured by numerical error and that are sufficiently efficient to allow different parameter values to be explored.

Unlike many biochemical or gene network models, biomechanical models make extensive use of geometric information and spatial relationships. The equations of mechanics—of partial rather than ordinary differential type—can require sophisticated numerical algorithms (such as the finite-element method) for their solution. Although powerful commercial packages are available, these often need careful tailoring to specific applications, whereas bespoke methods developed for individual problems may not be generalizable; leveraging open-source finite-element packages is instead a popular compromise.

Multicellular models of plant growth and development are presently moving to three-dimensional representations of plant tissues, incorporating more realistic details of cell wall mechanics and addressing complex topological changes such as arise in lateral root emergence, where an underlying primordium must burst through overlying tissue layers (75). Future years should see the development of models that capture the full architecture of rooting and branching systems, while preserving the cross-talk with underlying molecular processes. [1]

This work was supported by Biotechnology and Biological Sciences Research Council Grant BB/J009717/1.

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions: O.E.J. and J.A.F. prepared figures; O.E.J. and J.A.F. drafted manuscript; O.E.J. and J.A.F. edited and revised manuscript; O.E.J. and J.A.F. approved final version of manuscript.

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