Probing the mechanical contributions of the pectin matrix
Insights for cell growth

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The plant cell wall has a somewhat
paradoxical mechanical role in the
plant: it must be strong enough to resist
the high turgor of the cell contents, but
at the right moment it must yield to
that pressure to allow cell growth. The
control of the cell wall’s mechanical
properties underlies its ability to regu-
late growth correctly. Recently, we have
reported on changes in cell wall elastic-
ity associated with organ formation at
the shoot apical meristem in Arabidopsis
thaliana. These changes in cell wall
elasticity were strongly correlated with
changes in pectin matrix chemistry, and
we have previously shown that changes
in pectin chemistry can dramatically
effect organ formation. These findings
point to a important role of the cell wall
pectin matrix in cell growth control
of higher plants. In this addendum we
will discuss the biological significance
of these new observations, and will
place the scientific advances made pos-
sible through Atomic Force Microscopy-
based nano-indentations in a relatable
context with past experiments on cell
wall mechanics.

The mechanical aspects of cell movement,
shape and growth are of interest across
kingdoms; from the effects of mechanical
stresses on cell morphologies and motil-
ity in cancer,1,2 to the shaping of plant
cells and organs.3-6 Here we focus on the
plant kingdom, and the special control
of cell shape and growth exerted by the
cell wall. The primary plant cell wall is
capable of growth, and can be thought
of as a mechanical composite, comprised
of rigid and strong cellulose microfibrils,
embedded in a pectin gel/matrix, and
both surrounded and connected by inter-
woven hemi-cellulose fibers.7,8 As new cell
wall is produced during growth, an indi-
vidual wall may also have a multilamellate
structure.

It is of vital importance that the reader
remembers that the cell wall structure just
described is idealized and generalized;
truth, we still know very little about
the exact structural arrangement of wall
components, and we certainly know that
cell wall composition varies extensively
between cell types, cell ages, tissues and
species.9-11 But if this is the case, how can
we ever hope to understand the mechani-
ical behavior of such a structure as it relates
to growth? In this addendum to Peaucelle
et al.4 we will present some discussion
about the data obtained therein and its
biological implications, but also place the
methodology in a context with respect
to past and future studies of cell wall
mechanics.

The Mechanical Properties
of Plant Cell Growth and their
Relation to Rheology

Scientists have long been interested in the
mechanics of cell growth and its regula-
tion by the cell wall, and subsequently by
wall mechanical properties. Mechanically,
the cell wall is thought of as viscoelas-
tic. It will behave as an elastic solid, dis-
playing, under a certain stress threshold,
instantaneous and reversible deformation

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The studies using plants have focused on large-scale behaviors as they relate to growth dynamics, but have been limited in their resolution with respect to individual cells within a tissue or the individual components of a single cell wall. In general these studies all involve the application of force to a tissue, by manipulating turgor or by adding physical weight and monitoring the resulting deformation (Fig. 2 and for a methods review see ref. 14). Examining the relationship between force and deformation over time provides information on elasticity and viscoelasticity. In 1877, Hugo de Vries devised a method to look at turgor induced elastic expansion in cell walls, with which he correlated an increase in bulk elastic wall behavior with increased growth rates in sunflower hypocotyls. The growth promoting hormone auxin (in aerial tissues) induced increases in wall viscoelasticity as shown by Heyn and later decomposed by Cleland into both plastic and elastic components. Cessation of growth in rye coleoptiles was correlated with a decrease in plasticity, although Nolte and Schopfer argue that in fact this plasticity is viscoelasticity. Recently, cell wall mutants have enabled a closer look at the mechanical roles of wall components. As an example, mutants lacking xyloglucan display growth defects such as root stunting and dwarfism, which is reflected in the reduced creep of isolated cell walls; however, paradoxically, mutant walls also exhibited increased elastic and plastic deformation.

Mechanical studies of cell wall analogs allow for insights into the contributions of various wall components to mechanical behavior without compensation in composition by the plant. Non-plant cellulose networks, produced by Gluconacetobacter xylinus (Acetabacter xylinus), exhibit extreme strength in tensile tests, and this strength is lessened by the addition of pectins or hemicellulose into the structure. Interestingly, the strength of the composite remained lesser after removal of all pectin by enzymatic digestion. It is possible that this tempering of strength by the pectin matrix and the interwoven hemicellulose is required for a material to be permissive of growth. Indeed, in this analogous material, viscoelastic behavior (as creep) was only seen when hemicellulose was included in the composite. Pectin gels also exhibit interesting material properties on their own, such as strain-stiffening, a characteristic of biological gels. The more you deform them the more force you need to apply to keep the deformation increasing. A similar phenomenon may occur in viscoelastic materials, shear thickening, where the viscosity increases with increasing deformation. It has recently been suggested that strain-stiffening of...
cell walls may contribute to regulation of growth rates in plant tissue.5

New Methods and New Scales

So far, the experiments introduced have dealt with composite behaviors of both wall components and multiple cells within tissues. In order to examine the cell wall properties of individual cells and composite tissues newer methods of rheological testing were required. Microindentors have been used to study cell mechanics in pollen tubes24 and onion epidermal cells26 but these equipments are limited in their force range and spatial resolution. In a recent study, we have presented an Atomic Force Microscopy (AFM)-based method for measuring cell wall elastic and viscoelastic behavior at cellular and tissue levels.4 This study was immediately preceded by a complementary AFM-based method developed by Milani et al. Together these two methods provide a unique look at the mechanical properties of cell walls, with the potential for unprecedented mechanostructural resolution.

Here we must introduce a vitally important concept: when examining the relationship between force and deformation, everything is relative! The data obtained will be relative to the time of the experiments, the scale of deformation, and the magnitude of forces applied (Fig. 2). As an illustration, the rapid indentations of 0.2 s in Peaucelle et al.4 provide purely elastic information, whereas indentations held for 10 sec provided information on stress-relaxation i.e., viscoelastic behavior. This can also be seen when contemplating the deformation scales used in Milani et al. (~50 nm), compared with those in Peaucelle et al.4 (250–500 nm) and the micrometer and millimeter deformations measured in previous methods (Fig. 2).17,21 One of the exciting implications of this physical reality is that by altering the type of indentation, one could gather data from different layers of a composite tissue and also from within a multi-lamellate cell wall itself.4,28 For the rest of this addendum, we will focus on the information obtained in Peaucelle et al.4 its scale and implications for cell wall behavior, and its relevance to the mechanics of growth.

Figure 2. Different scales in mechanical experimentation. (A) A classic extensometer where a piece of tissue (grey) is fixed between a rigid arm and a deforming load. The deformation of the tissue sample can be measured over time with constant load, or with changing loads. Deformation of whole tissue ranges from millimeters. Adapted from Cosgrove.27 (B) A diagrammatic representation of the AFM-based experimental methods of Milani et al.27 and Peacuelle et al.4 as applied to shoot apical meristems (i, M=meristem). The indentations of Milani (ii) were performed with a 40 nm pyramid indenter, to a depth of ~50 nm. This method is thought to provide information on a small section of the cell wall only. In contrast, the methods of Peaucelle (iii) with 1μm or 5 μm diameter spheres and ~500 nm indentation depths, provide information on larger cell wall sections and several tissue layers, respectively. Indenters are colored grey. Predicted ‘depth of information’ in B colored by grey-black gradient.

What is being Measured, and What does it Mean?

In Peaucelle et al.4 the elasticity measured for cell walls was strongly influenced by the pectin matrix. Manipulations of the pectin matrix chemistry were shown to alter organ outgrowth patterning at the shoot apical meristem,29 and we were able to correlate these chemical changes in pectin methylesterification levels with changes in the coefficient of elasticity (herein referred to as E, the apparent Young’s modulus) of the cell wall. Because of the time delay between the induction of chemical modification and measurement of E (12+ hours), it is possible that the alteration of pectin structure could have led to mechanical changes in other wall components, which also contributed to decreases in E; however, pectin modification is either a major contributor to the E measured, or acts as a trigger for this mechanical change. Future work aims to discover how much of these measurements can be directly attributed to which wall component. For now, we will focus on what we know about pectins and growth.

Mutants in pectin amount or composition display changes in rheology.30 In addition, changes in pectin chemistry are correlated with growth ability in hypocotyls.31,32 So how could changes in pectin structure, and resulting changes in elastic rheological properties, be affecting growth? There are several possible ways for pectin chemistry to affect growth. First, the pectin matrix may mechanically affect the movement of other cell wall polymers, as suggested by Abasolo et al. The authors
put forth a model whereby the stiffness/density/elasticity of the pectin matrix alters the ability of hemicellulose chains to unravel under extension forces.30 This may be extended to a conceivable effect of cellulose microfibril movement within the wall as well. Second, the pectin matrix itself may provide mechanical strength to the cell wall composite. As mentioned earlier Ca2+-cross-linked pectin gels exhibit strain stiffening.23,24 Strain stiffening may be a mechanism through which growth is controlled, although at this point it is unclear whether the load born by the pectic matrix would be biologically relevant in this context given the presence of major-load-bearing cellulose fibers, which would also exhibit strain stiffening. Third, alterations in pectin chemistry would lead to changes in the porosity of the pectin matrix.33 This may be exhibited in either the elasticity of the wall, or in its viscoelastic behavior if water movement within the pectin gel contributes to this parameter. Because the pectin gel matrix is not a solid, but a porous material, changes in its porosity would affect water movement and conceivably the movement of wall-modifying agents such as expansin, XTH or endoglucanases. Fourth, the hydration and swelling of the cell wall matrix, which is sensitive to pectin methylation status,34 could also affect the mechanical properties of the cell wall,35 perhaps by altering the interactions of other cell wall polymers.36 The hydration status of the cell wall may also conceivably affect enzyme activity and movement. And lastly, demethylsterification of pectins releases hydrogen ions into the cell wall, which cause an acidification which in turn may activate other wall modifying agents.

Conclusions

It is apparent from the literature, and from our recent work, that there can be a strong correlation between rheological elasticity and growth; however, it remains to be seen what components of the wall contribute to these changes and how they mechanistically control these behaviors. It is obvious that changes in pectin matrix chemistry have a more profound effect on growth than generally assumed,4,30,31 but we should not neglect the known importance of hemicellulose and cellulose as well.

The recent introduction of AFM-based micro and nanindentation methods4,27 have allowed higher spatial resolution that ever before—we can now examine single cell wall behavior and localized tissue behavior in planta, and are no longer limited to whole organ data. But as introduced here, when examining the relationship between force and deformation: everything is relative! This means that while new methods increase our understanding of the mechanical cell wall and add more questions, they provide a different and complementary set of data to more established methods. We must utilize mechanical data obtained at all length and time scales, if we truly wish to understand the mechanics of the cell wall with respect to growth.

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