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FROM CELLULOSE TO CELL

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Summary

The cell wall is often pictured as a more-or-less random feltwork of cellulose microfibrils in association with other polysaccharide and protein complexes. There is evidence from morphology, morphogenesis and mechanics that the structures in the cell wall are far more regular and that their interactions are driven by their chemical and morphological properties. In particular, a model based on liquid crystal structures has more than morphological implications.

Key words: cellulose, cell wall, liquid crystal, lignin, model, mechanical properties, stiffness, strength, toughness.

Cellulose

Cellulose is a polysaccharide which, because of the β-1,4 links between the sugar units, produces a strongly linear ribbon structure that is very stiff and forms stable fibres. The theoretical modulus of the cellulose molecule is 250 GPa, but the best experimental estimate for the stiffness of cellulose (and, for that matter, for other linear polysaccharides in the cell walls) is approximately 130 GPa. The specific gravity of cellulose is approximately 1.5, so it is then possible to compare its mechanical (strength and stiffness) performance with those of other engineering materials. One concludes that cellulose is a high-performance material, comparable with the best fibres technology can produce.

The best model for the production of cellulose was proposed by R. D. Preston, originally to fill a gap in a talk he was giving at a conference dinner. Faced with the prospect of no novelties, he decided to introduce something controversial and proposed that cellulose is spun from an enzyme which more-or-less floats around in the cell membrane (Fig. 1). It was an idea for which he had no real evidence at the time, but it stimulated a search for a particular type of structure (life is always easier when you know what you are looking for – a rare luxury). Enzyme rosettes have been found arrayed hexagonally in rafts of 100 or more which wander around the cell membrane leaving behind them a trail of cellulose microfibrils each approximately 5 nm in diameter containing approximately 40 molecular chains. These aggregate into larger microfibrils, which can be up to 20 nm in diameter. The microfibril is not totally crystalline since it contains sugars other than glucose (usually mannose and xylose), appears to be thinner when observed using X-ray diffraction than when using the electron microscope (suggesting that the core of the microfibril is crystalline with an amorphous outer layer) and can be penetrated by some electron microscopy stains (suggesting that there are gaps between the molecular chains). These imperfections can account for the measured modulus being lower than the theoretical one.

Assembly of cellulose

The cellulose is assembled into a shell around the cell, thus forming the skeleton both for the cell and for the plant. The orientation of the cellulose microfibrils in the cell wall is influenced by several factors. They can be arranged in parallel throughout the thickness or change direction from layer to layer in a manner reminiscent of liquid crystals (Neville, 1993). The importance of this analogy lies in the intrinsic ability of liquid crystals to organise into defined structures, moving between liquid and solid phases as they do so. Liquid crystals were discovered by a botanist, Friedrich Reinitzer, who in 1988 published his observations showing that esters of cholesterol have two melting points. Between those two temperatures, the liquid showed iridescence and birefringence – sure signs of crystallinity and order. One hundred years later, we now know that liquid crystalline materials can ‘self-assemble’ into larger-scale structures, producing films and fibres without need for containment or moulding, since the molecular chains are stiffer than found in conventional polymers (Donald and Windle, 1992). In plant cell walls, the structures correspond to nematic (parallel) and cholesteric (screw-pile or helicoidal) liquid crystals (Neville, 1993). The liquid crystalline structures need to be assembled: in insect cuticle, a structure with many similarities with plant cell walls, this takes place in the Schmidt layer between the epidermal cells and the cuticle that they produce (Neville, 1975). The equivalent in plants is the periplasm, a narrow region confined between the most recently deposited cell wall layers (outer side) and the cell plasma membrane (inner side). This is such a thin layer that its existence is disputed, although it has been observed in the

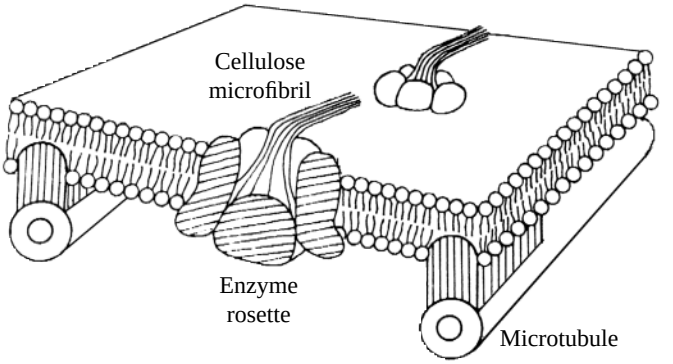

Fig. 1. Rosettes of cellulose synthetase in the membrane of a cell confined by microtubules inside the cell. The cellulose microfibrils have been cut short. Redrawn from Neville (1993).

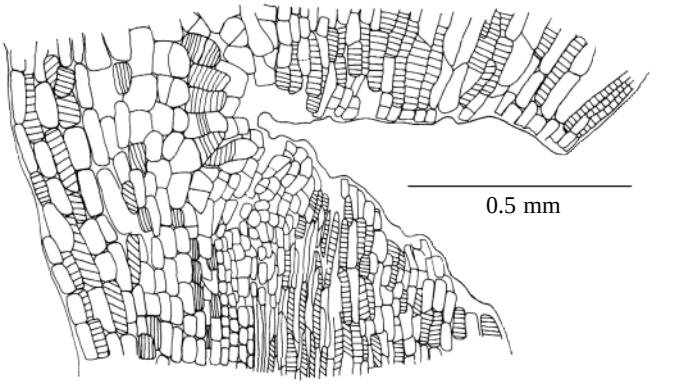

Fig. 2. Cellular structure of a pea root wounded 24 h previously. Lines in the cells mark the orientation of microfibrils just beneath the cellular cortex. At the tip of the crack, the microfibrils are orientated parallel to the main axis of the root, making those cells elongate orthogonally to the axis and invade the wound site. Modified from Hush et al. (1990).

epidermis of quince seeds (Willison and Abeysekera, 1989). Within this assembly layer, the molecules are orientated into liquid crystalline forms. The intrinsic stiffness of the cellulose molecule aids its self-assembly, as do the bulky side chains often found on hemicelluloses. However, cellulose itself cannot control this process since it does not form liquid crystals except in unphysiological conditions or when mixed with hemicelluloses, which are therefore the most likely candidates for controlling the system and can contribute up to 40 % of the cell wall. The asymmetry required for liquid crystal self-assembly is provided by the C-5 of the hemicellulose sugar ring. A credible model is that the cellulose microfibrils are surrounded by a sheath (however thick that needs to be) of hemicellulose which can then direct the self-assembly process (Neville, 1993).

There is another influence in the orientation of cellulose in the cell wall, and that is the orientation of microtubules arranged on the inner face of the cell cortex (Fig. 1). The orientation of the cortical microtubules can be changed by external stimuli such as light (amount, colour), auxin and mechanical strains, such as those due to bending (Fischer and Schopfer, 1997). These stimuli are additive, so a small amount of auxin makes the cells more sensitive to the other stimuli. At the same time, the growth rate changes, so it is the reorientation of cellulose microfibrils, mediated by changes in the cortical microtubules, that governs growth, both qualitatively and quantitatively. Blue light causes the microtubules to orientate longitudinally, red light makes them orientate transversely, allowing the plant to elongate (Zandomeni and Schopfer, 1993). How is the orientation of the microtubules controlled at the cellular level? The only mechanical signal to which the cell can respond is strain (i.e. changes in length), and the best and most likely site for this strain receptor is a component of the cytoskeletal tensegrity structure (Ingber, 1998).

Somehow, these two mechanisms must coexist. Clues are provided by the work of Overall on wound healing in pea roots (Hush et al., 1990). The wound was created by removing a wedge of tissue across the axis of the root of 3- to 4-day-old seedlings 3 mm from the tip. Sections were stained with fluorescent markers for the microtubules and examined in the confocal microscope. Cells from an intact root are long and thin, extending parallel to the main axis of the root because the cellulose fibres of the cell wall are orientated circumferentially. However, in cells taken from the vicinity of the wound approximately 24 h after wounding, the microtubules have rotated their orientation so that they are parallel to the wound surface, which is more-or-less orthogonal to the long axis of the root (Fig. 2). This is accompanied by elongation of the cells towards the wound, suggesting that the cellulose is being laid down in the new direction. The final step in the initiation and maintenance of this new cell polarity around a wound is the establishment of new planes of cell division which are again parallel to the contours of the wound. All these responses ensure that the plant tissue grows in towards the wound area and fills it in with new cellular material. The one thing Overall (Hush et al., 1990) does not mention is how the reorientation of the cellulose fibres is achieved. The implication is that it is simply due to changed orientation of the microtubules causing the newly laid down cellulose to have a different orientation. But this would be insufficient to account for the shape changes. The necessary change in the anisotropy of stiffening could occur only if the cellulose through the entire thickness of the cell wall changed its orientation, and this would be possible only if the cell wall is adaptively labile – or in a liquid crystalline state. The same has been postulated for the lability of orientations in insect cuticle, but never, as far as I know, demonstrated experimentally. Perhaps this would be easier in a cellular system in which the external state of strain can be changed more easily. The mechanism of change has been speculated upon (‘acid loosening’, etc.) but, as far as I know, never quantified. By analogy with other water-miscible composites such as paper and insect cuticle, the change in water content (driven, perhaps, by changes in pH) need be only a few per cent. The more directed the structural bonds in the system, the fewer the bonds that need

to be solvated, since these can be identified by the biochemistry of the system. Once again a liquid crystal system has distinct advantages.

Other cell wall components

Within the cell walls, the microfibrils are cross-linked and stabilised by shorter molecules which make the cellulose microfibrils into a network. Hemicelluloses are bonded non-covalently onto the cellulose so that, in order for the forces to be transmitted effectively, a significant length of the hemicellulose has to lie alongside the cellulose. For instance, xylans manage to get close enough by being a simple chain of xylose units, but push away from surface of the cellulose microfibril by having side chains that make this closeness impossible. The xylan can then bridge to another cellulose microfibril and bond to that with its other naked end.

Three other networks have been identified in the cell wall which can be considered more or less independently (Brett and Waldron, 1996). (1) Pectins form a network which can reform independently (giving rise to the jam industry) and leave the rest of the cell wall apparently unaffected when they are removed. They have many and large side chains which allow them to fill spaces between microfibrils and cells. Regions with no side chains occur at the ends of the molecules, allowing them to interact by means of complexes formed with Ca^{2+}. The pectin chains can be cross-linked with the other networks and are important for sticking cells together. (2) Another network is made of protein/polysaccharide complexes (glycoproteins), whose main component is extensin, a strange protein which contains approximately 40 % hydroxyproline (Hyp) which frequently occurs as part of the sequence -(-Ser-Hyp-Hyp-Hyp-Hyp-)-. Proline is well known for its effect on the conformation of the protein backbone – the side group curls back on the peptide link and greatly reduces the mobility of the polymer. In addition, it is an attachment point for short polysaccharides. So extensin looks like a very small, stiff, woolly piece of string. Little is known of its function, partly since it is so well cross-linked into the cell wall that it is very difficult to remove. Most of the available information on its chemistry comes from genetic studies. So at least one can say that it cross-links, and therefore stiffens and stabilises, the cell wall. (3) Depending on the plant and the stage of development, there may also be some lignin. This both limits the mobility of the cell wall fibres and makes the walls more hydrophobic and therefore stiffer, partly because water is a plasticiser or lubricant, partly because the charged groups which would interact with the water, were it present, interact with each other, increasing the degree of crosslinking further.

Mechanical properties

The stiffness of the cell wall varies according to the amount and orientation of the various components, including water. Cowdrey and Preston (1966) used two models to describe the mechanical properties of lignified cell wall. Their first – a composite model of cellulose fibres in a lignin matrix – proved to fit their measured data best. However, their second model, in which the cellulose microfibrils spiral helically around the cell, has proved more useful in predicting the properties of unlignified cells even though this model has a significant flaw: the matrix that binds cellulose microfibrils together is ignored. We have developed a model that takes a middle route, allowing for limited connectivity between the components which is expressed in a variable shear modulus. In addition, molecular chemistry very often imposes much more regular and precise structures than engineering theory demands. There is a much more hierarchical progression of molecular types rather than just crystalline fibres and rubbery matrix: the progression is from crystalline microfibrils to linear polysaccharides with side chains that are well orientated but not crystalline, down to random polymer networks such as lignin (Eda et al., 1984).

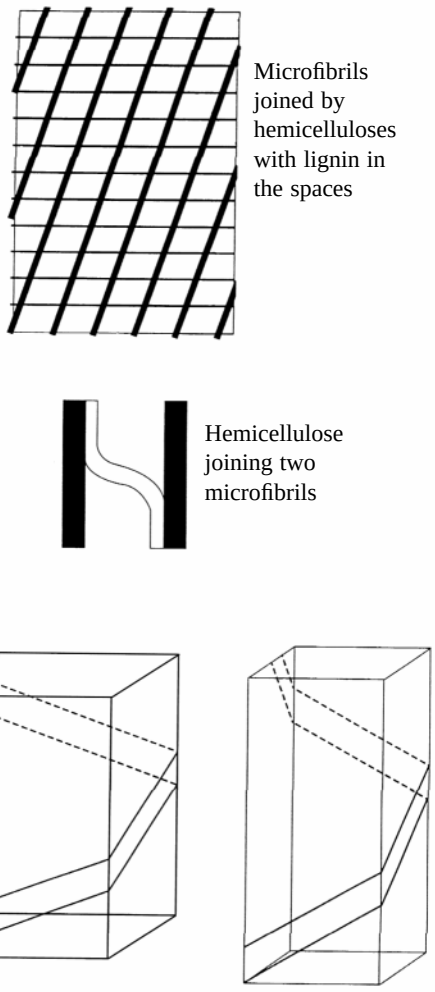

Fig. 3. Generalised structure of the secondary cell wall of xylem from *Nicotiana tabacum*. As the cell is stretched, its volume is reduced and the microfibrils in the cell wall move closer together. Modified from Hepworth and Vincent (1998).

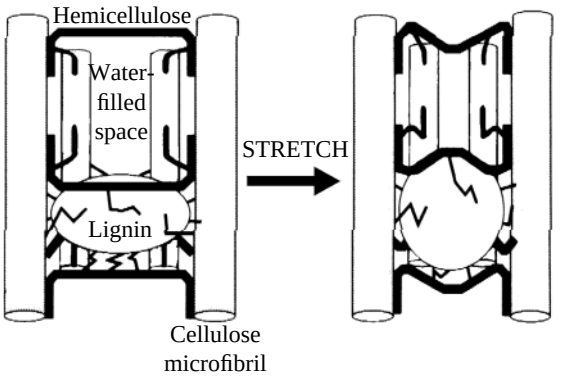

Fig. 4. Deformation of 'matrix' material between microfibrils of cellulose as they draw closer together when the cell wall is stretched. Modified from Hepworth and Vincent (1998).

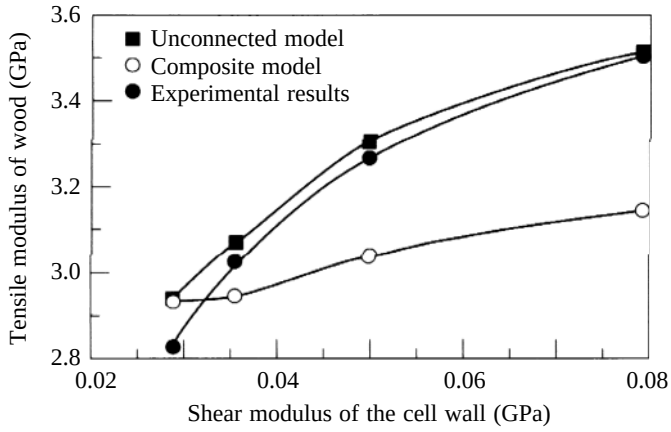

Fig. 5. The tensile modulus of *Nicotiana tabacum* xylem tissue as a function of the shear modulus of the cell wall. The 'unconnected model', in which the fibres are held together only by hemicelluloses, fits the data better than a model based on the theory of fibres in an adhering matrix (composite model). Modified from Hepworth and Vincent (1998).

In a new molecular model for the cell wall, developed using the partially lignified cell wall of the 'woody' tissue of tobacco *Nicotiana tabacum* (Hepworth and Vincent, 1998), the cellulose microfibrils are continuous along the length of the cell and are arranged at an angle of approximately 10° to the long axis (Fig. 3). The matrix molecules are organized at two levels. The hemicelluloses and pectins have a clear horizontal orientation with little or no interconnectivity. The lignins are randomly oriented and fill in some of the gaps in the structure, depending on how much lignin is present (Fig. 4). When the cell wall is stretched, the helix will open out and the wall surface area will be reduced. This reduction will result in the microfibrils being forced closer together because the surface area of the cell is being reduced. Thus, the matrix material between the microfibrils will be compressed. The reduction in wall area will lead to an increase in wall thickness as the lignin is squashed out radially. If the hemicelluloses and pectins are not oriented at 90° to the long axis of the cell, they will experience some direct tensile loading transmitted from the microfibrils, and the angle of the microfibril helix will not change as much. The tensile modulus is now dependent on the mechanical properties of the matrix chains in tension and compression and on the compressive properties of the lignin. There is no direct tensile stress transfer through the matrix from one microfibril to another as in a normal composite. Even with a large helical angle to the vertical, the microfibrils could still display their full tensile modulus if the gaps in the matrix were filled with a stiff incompressible material. The hemicelluloses and pectins compartmentalize the lignin and store elastic strain energy produced by the compression of lignin. This model accounts very well for experimental mechanical data from cells which are only partly lignified (Fig. 5).

The key to this model is specific internal matrix connectivity and molecular orientation. Where the hemicellulose and pectin chains are orientated at a large angle to the long axis, then the covalent connectivity of the lignin becomes crucial in determining the properties (Hepworth and Vincent, 1998). If lignin were strongly covalently linked to the matrix polysaccharides as well as to other lignin chains, then the matrix would become vertically connected and the mechanical behaviour would be described by ordinary composite theory (Preston, 1974).

These morphological characteristics now have to be accommodated in any explanation of the mechanical properties of plant cell walls. This is by no means an easy task, partly because the properties have often been badly measured and (for instance) viscoelastic and plastic responses have been confused (Hohl and Schopfer, 1992) and partly because it is extremely difficult to measure the mechanical properties of something as small and fragile as a cell wall. There are, of course, exceptions. There are many fibre cells which are several centimetres long; there are plants with very large cells (*Chara*, *Nitella*). However, there is no guarantee that these cells will be at all typical. It is also very difficult to decide how much material there is in the cell wall. In the sclerenchyma cells from leaves of New Zealand flax *Phormium tenax*, the microfibrils are orientated along the length of the fibre and the cell walls are fairly dense. The cell wall has a Young's modulus of the order of 70 GPa and a tensile strength of 1 GPa (King and Vincent, 1996). The cellulose content is not known.

The success of the micromechanical model suggests that there is sufficient free space for components to be able to change their geometrical relationships and that this has been factored into the mechanical design by natural selection, rather than being a mathematical inconvenience (the engineer's response!). Using a microphotograph of the wall of an onion parenchyma cell, Hiller et al. (1996) calculated that the area fraction of cell wall occupied by cellulose microfibres is only 0.055. In a simple-minded model of the way the cell wall works ($E_{cw}=E_f V_f$, where E_{cw} is stiffness of the cell wall, E_f is stiffness of the fibre (cellulose) and V_f is relative amount

of fibre), this would give a stiffness of 7.2–2.4 GPa depending on whether all or only a third of the cellulose is orientated in the direction of extension. A test based on pushing a microprobe through similar parenchymatous cell walls and modelling the deformation so resulting using the finite element method (Hiller et al., 1996) gave a modulus for the cell wall of potato parenchyma of 3 GPa (measured) and 7.7–16.5 GPa (calculated from independent data).

The probe technique can also be used to measure fracture properties. This suggests that the energy required to rupture the cellulose microfibrils (which are essentially crystalline and brittle) is only 0.001 % of the total energy required to fracture the cell wall. The rest is elastic (one-third) and plastic (two-thirds) energy, which implies that a lot of the energy is dissipated as heat. The figures obtained by experiment are 24.5 J m^{-2} (cutting), 12.7 J m^{-2} (microprobe) and 0.137 mJ m^{-2} (calculated from the bond energy in cellulose). Cutting on a larger scale gives values for work of 200 J m^{-2} (Vincent, 1990), and fracture energy values for similar tissue are higher still at 330–1350 J m^{-2}. These higher values must represent even larger contributions of plastic deformation. Clearly, any mechanical values are strongly dependent on boundary conditions and the tendency of the cell wall to reorganise itself in response to external forces.

Growth and development

Any review of the mechanical properties of plant cell walls has to show how these are related to biology – growth and development. This may not be so simple, since the mechanical properties that influence growth by definition include time as a variable and so take us into the realm of viscoelasticity. Certainly it is part of the nature of liquid crystals to exist in a partially liquid state and therefore to provide the necessary changes in mechanical properties, and this has been alluded to above. However, the viscoelastic properties of plant cell walls have not always been measured properly (Nolte and Schopfer, 1997). For instance, the cell walls of growing rye coleoptiles reportedly exhibit irreversible (plastic) extensibility (Kutschera, 1996). But basically similar measurements with cell walls of maize coleoptiles show that the apparent plastic extensibility is in reality slowly reversible, i.e. viscoelastic. Similarly, rye coleoptile walls also behave as a perfectly viscoelastic material if the test is made over a long enough time for the imposed extension to be recovered (Nolte and Schopfer, 1997). So plastic extensibility has not yet been convincingly demonstrated. Reported changes in the mechanical properties of cell walls produced by growth-controlling factors such as auxin or light may perhaps, therefore, be attributed to changes in viscoelasticity that are not directly related to the chemo-rheological processes controlling wall extension of growing cells.

Ultimately, however, growth must involve the addition of material to the cell wall. The advantage of a liquid crystal system is that the new material can be added and integrate itself seamlessly with the rest of the structure using the existing structure as its template.

Finale

The idea that plant cell walls are basically liquid crystalline is not new – the first pertinent observations were made over 20 years ago. But ideas of how the cell organises the world on the outer side of its membrane are still rather vague. Although much is known about the chemistry, very little is known about the control of morphology. Yet the morphology of the cell wall – the orientations of fibres and their interactions with other components – is crucial to the mechanical properties of the plant. Molecular biology has shown how intracellular shapes are derived from self-assembly driven by chemistry – the same must be true outside the cell. Perhaps the time is ripe for the experimental techniques and observations developed for and applied to liquid crystals to be applied to the cell wall.

One implication may be that the question that I posed to some colleagues recently ‘What is the largest liquid crystalline structure?’ has a simple answer. A tree.

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