The middle lamella—more than a glue

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The middle lamella—more than a glue

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Abstract

In plant tissues, cells are glued to each other by a pectic polysaccharide rich material known as middle lamella (ML). Along with many biological functions, the ML plays a crucial role in maintaining the structural integrity of plant tissues and organs, as it prevents the cells from separating or sliding against each other. The macromolecular organization and the material properties of the ML are different from those of the adjacent primary cell walls that envelop all plant cells and provide them with a stiff casing. Due to its nanoscale dimensions and the extreme challenge to access the structure for material characterization, the ML is poorly characterized in terms of its distinct material properties. This review explores the ML beyond its functionality as a gluing agent. The putative molecular interactions of constituent macromolecules within the ML and at the interface between ML and primary cell wall are discussed. The correlation between the spatiotemporal distribution of pectic polysaccharides in the different portions of the ML and the subcellular distribution of mechanical stresses within the plant tissue are analyzed.

Introduction

The mechanical properties of plant tissues differ from animal tissues since plant cells are encased in a stiff wall and they are glued together preventing them from sliding against each other. These traits determine the unique biological and structural functionalities of plant tissues. The material gluing adjacent plant cells together is known as middle lamella (ML). The ML is a thin layer of approximately 50 nm thickness that is sandwiched between the primary cell walls of neighboring cells. It is distinguishable at light and electron microscopic levels and its material properties and chemical composition are different from those of the adjacent cell walls.

The walls of growing plant cells (primary cell walls) are composed of cellulose, hemicellulose, pectic-polysaccharides and structural proteins. Cellulose microfibrils are embedded into this network of non-cellulosic polysaccharides [1–4]. In general, the hemicellulose in primary walls comprise xyloglucan and arabinoxylan, the pectic polysaccharides include homogalcturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) [2]. Pectic polysaccharides are complex in nature and thought to form large complex macromolecules by covalent bonding [5, 6]. In young and growing plant tissues the ML is primarily composed of pectic polysaccharides with small amounts of protein. Cellulose and hemicellulose are absent from the ML [7, 8]. Immunolabel for these components allows for ready distinction of the ML from the primary walls in parenchymatous and collenchymatous cells.

Clear distinction of the ML in tissues with secondary walls is more difficult. After cessation of cellular growth, sclerenchymatous cells such as vessel elements and fibers deposit a thickened secondary wall on the inner face of the primary wall. The secondary walls are composed of cellulose and hemicellulose (xylan and glucomannan). Pectin is scarce and instead lignin rigidifies the wall [2, 9, 10]. In sclerenchyma fibers three distinct layers (figure 1(d)) are deposited that can be differentiated by their distinct arrangement of cellulose microfibrils [11, 12]. The thickness of the secondary wall (up to 13 μm) [13] typically dwarfs the dimensions of the primary wall (300 nm–1.2 μm) [14]. By consequence, the ML joining sclerenchymatous cells with thickened secondary walls is hardly distinguishable from the relatively thin primary walls. Therefore, the entire interface material layer connecting cells with thick secondary walls is termed compound middle lamella (CML). Even if not readily distinguishable, the CML corresponds to a tripartite layer of two primary wall layers and the true ML separating them. Lignification of secondary wall layers actually initiates in the ML and hence the entire CML is enriched with lignin [15].

The contribution of the ML to maintaining the structural integrity of a plant is crucial as it acts as a

cementing agent that prevents plant cells from sliding against or detaching from each other. The ML therefore controls cell adhesion as well as developmentally and environmentally triggered cell separation [16–20]. Tight regulation of cell separation is crucial for the genesis of specialized tissue architecture such as that of aerenchyma, or for senescence-based organ separation. Through controlled modification of ML chemistry, plants can initiate the abscission of flowers, leaves and roots [18, 21, 22], as well as the dehiscence of seed pods for seed dispersal [7], and of anthers for pollen release [23]. Similarly, the process of fruit softening during ripening and loss of tissue firmness involve the softening or partial dissolution of the ML [24, 25]. As an interface between cells, the ML also helps transferring and distributing the loads applied to a plant by external (such as wind and rain) or internal (such as turgor) agents. In addition, the ML interface accommodates microscopic intercellular channels, the plasmodesmata, which play an important role in intercellular communication and mass transport. Being part of the extracellular matrix, the ML has to yield and expand to accommodate cell growth, but how this in-plane expansion happens is virtually unknown. In the case of unevenly growing neighbouring cells the ML has the responsibility to ensure that cell adhesion is maintained despite differential growth and resulting changes in tissue geometry. The ML is also reported to play an important role in the modification of cell wall chemistry as a defence mechanism against pathogen invasion [26, 27].

Finally, the ML is also of interest in biotechnological applications since the modification of its mechanical properties has for example the potential to increase biofuel yield from lignocellulosic materials [28]. The pretreatment that is necessary for the procedure is a costly process [29, 30] and involves reduction of the recalcitrance of lignin and hemicellulose, the reduction of the crystallinity of cellulose and the increase in the porosity of the biomass [31–33]. The upstream pretreament is mainly accomplished by physical treatment such as comminution of woody biomass into smaller pieces and/or physicochemical processes such as steam explosion. For both procedures the cell to cell interface, or ML, is critical. Evidence is provided by the fact that shear forces are more efficient in reducing woody mass particle size than chipping comminution [34]. Shear forces are likely to separate cells at the cellulose poor ML, whereas the chipping acts by breaking the secondary walls. The mechanics of the shear based comminution can therefore be influenced by modifying the mechanical properties of the ML, as is also illustrated by the fact that a lower moisture content makes the process more efficient [35, 36]. The hygroscopic and amorphous nature of pectin suggests that it is the ML material that is responsible for this behavior. Rendering the ML material more brittle through modulation of its biochemistry will therefore be a desirable trait that would lower the cost of mechanical pretreatment for biofuel production.

The biological role of the ML has been described as early as 100 years ago [37], intercellular adhesion and separation have been studied extensively [19, 38–40] and excellent reviews summarize the biological aspects of this structure [7, 16, 18, 41, 42]. However, the fact that the ML represents a distinct physical layer with distinct material properties has rarely been covered in detail. Because of its nanoscale dimensions and the formidable technical challenges associated with accessing the ML directly, our understanding of this structure is largely unexplored. This review focuses on our current understanding (and the lack thereof) of the ML as a physical entity. A better understanding of the mechanical behavior of the ML and its ability to distribute stress within the tissue will improve our ability to analyze the concepts governing tissue morphogenesis in plant organs [43].

Architecture of the middle lamella

To analyse and predict how a physical structure responds to loads and forces, its exact shape, dimensions and material properties must be known. The ML has a reticulate geometry that consists of flat sheets where it joins two adjacent cells and 3D corner structures at the junctions formed by more than two cells. The geometry of the ML can therefore be characterized by the thickness of the flat sheet portions and by the shape of the corner structures (figure 1). At corner junctions the ML can either fill the available space and thus be typically thicker than the adjacent flat sheet, or the triangular space can be air or water filled with the ML lining the space (figure 1(e)).

Depending on the plant species and tissue type the ML can either be visible without further staining, or it has to be revealed by staining pectin (Ruthenium Red) or lignin ($KMnO_4$) or by immunolabel for pectins. Both light and fluorescence based techniques as well as transmission electron microscopy have been used to perform such labeling [44–47], but only the latter provides the spatial resolution necessary for measuring the exact dimensions of the ML. Analysis of published images in the primary tissues composing the tomato pericarp, sugar beet root and mature suspension cultured carrot cells showed that the flat sheets of the ML are typically 50–100 nm thick [46, 48–50].

In cells with secondary wall, it becomes essentially impossible to distinguish the thin primary wall from the ML. In such cases, physical parameterization typically uses the dimensions of the CML, i.e. the layer that comprises all three—the ML and the two layers of primary cell wall (figure 1(d)). In the spruce xylem the thickness of the CML was measured to be 200–400 nm in the flat sheets; whereas at the corners it measured up to 1200 nm [47, 51, 52].

Biochemical constituents of the middle lamella

With currently available technologies, it is essentially impossible to isolate the ML from the neighboring primary cell wall with the aim to perform biochemical

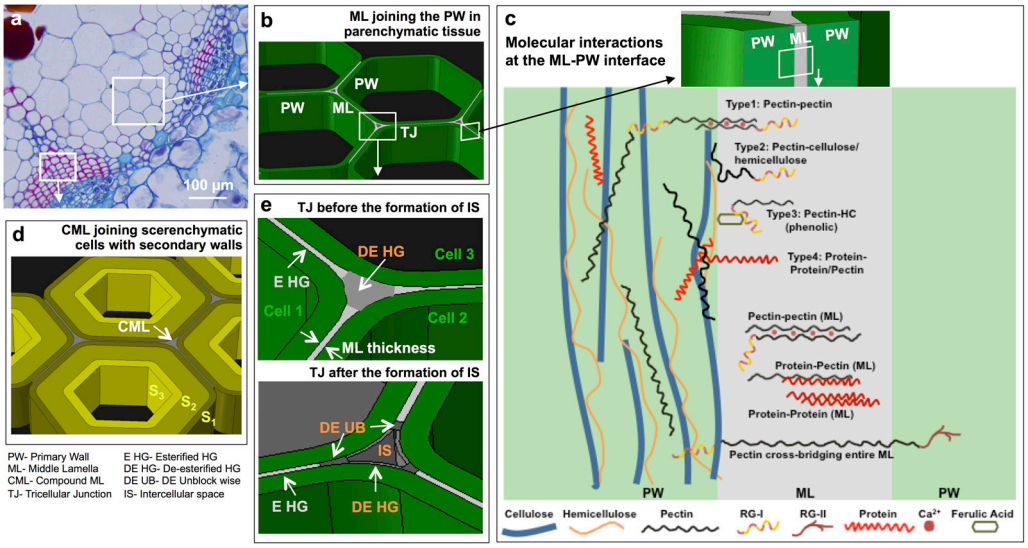

Figure 1. (a) Cross-section of a plant stem showing cells with primary and secondary walls. (b) The ML joining the primary cell walls is characterized by the thickness of the flat sheet portion linking two adjacent cells and by the geometry of multicellular junctions where the ML is expanded. In 2D sections the junctions are typically tricellular (TJ). (c) Different types of molecular interactions at the PW-ML interface and within the ML (d) The CML joining thick secondary walls in sclerenchymatous tissue. The secondary walls often display three distinct layers, S_1, S_2 and S_3. (e) Distribution of pectin at junctions before and after the formation of intercellular spaces (IS). Before formation of intercellular spaces, the ML in the junction is characterized by de-esterified HG (DE HG), while the flat sheet ML is characterized by esterified HG (E HG). After the formation of intercellular spaces, the ML lining the junction is characterized by DE HG whereas unblock-wise de-esterified HG (UB DE) are located at the transition zones between junction and flat sheet.

analyses. Even laser dissection microscopy does not provide the spatial resolution that would be necessary to do so. Therefore, our understanding of the biochemical composition of the ML is largely based on immunohistochemical studies. These studies have revealed that the ML consists predominantly of pectic polysaccharides [7, 53], which are typically synthesised in the Golgi apparatus and delivered to cell wall by exocytosis [5, 54, 55]. The use of antibodies such as JIM5 (specific to partially esterified HG), JIM7 (esterified HG), LM7 (non-blockwise partially esterified HG), and PAM1 (blockwise partially esterified HG), respectively, has allowed to determine that in the mature ML the pectins are partially esterified HG [5, 42, 56–59]. Secondary ion mass spectroscopy (SIMS) and electron-loss spectroscopy (EELS) have revealed that Ca^{2+} and calcium pectate are more concentrated at the locations where the ML lines cell corners with intercellular spaces, suggesting that low/partially methyl-esterified HG are enriched at these locations [58, 60]. Based on labeling with LM7 and PAM1 [61], it can be inferred that the de-esterification of HG in the ML can be a combination of blockwise and non-blockwise. This distinguishes the ML from the primary cell wall in which pectin de-esterification is primarily blockwise [61, 62]. Another feature of the chemical composition of the ML located in cell junctions is the presence of hydroxyproline rich glycoproteins (HRGPs) [63, 64], proteins that are common in the primary cell wall. Whether or not other cell wall proteins such as arabinogalactan proteins (AGPs), glycine-rich proteins (GRPs), proline-rich proteins (PRPs), etc [65] are present in the ML is largely unknown.

The presence of RG-I, recognized by anti-RG-I serum, in the ML is described by seemingly contradictory findings. In red clover leaf and root tissue, RG-I was found to be restricted to the ML lining junctions, with 80–90% label associated with the expanding portion of the middle lamella [8]. In the ML of suspension-cultured sycamore cells, RG-I was found both at the flat sheets and junctions [66]. However, studies on tomato pericarp and potato tuber indicate the presence of RG-I only at flat sheets but not in junctions [49, 58]. Interestingly, bast fiber development seems to be associated with significant presence of RG-I as suggested by presence of LM5 label specific to (1–4)-β-D galactan [67, 68] which is associated with RG-I [49]. Bast fibers intrude surrounding tissues requiring cell surfaces to slide against each other [69]. Therefore, while RG-I is clearly present in the ML, its spatial distribution may differ depending on plant species, developmental stage and cell type. Whether the ML contains RG-II is not clear [70] and the absence of label for borate-RG-II complex suggests that it may be entirely absent [71].

The CML in sclerenchymatous tissue has a polysaccharide composition similar to the ML in primary tissues, including the presence of HG and RG-I. However, in addition to these matrix polysaccharides, the CML also contains significant amounts of lignin [15]. The lignin concentration can be as high as 50% (w/w), much higher than that in the adjacent secondary walls were it rises only to approximately 20% [72].

Formation of the middle lamella during cytokinesis

The ML is formed during cytokinesis, when the cytoplasm of the mother cell is divided into two daughter cells by the newly forming cell plate. The cell plate is formed from HG pectin containing vesicles that fuse at the equatorial plane of the cell [41, 73, 74]. The vesicles are delivered to this region by the phragmoplast, a cytoskeletal array that controls the spatial arrangement of the initiating cell plate in the center of the cell and its subsequent radial expansion until it connects with the mother cell wall and separates the two portions of the cytoplasm [75]. The expanding cell plate is not immediately constructed as a solid structure but starts out as a fenestrated network that gradually fills any gaps until a smooth cell wall layer is formed [74]. When and how during this process the ML is differentiated and becomes biochemically distinct from the adjacent primary cell wall layers is not well understood [7, 12, 41, 74]. Studies on cell division in the root apical meristem suggest that the ML proper is only recognizable once the cell plate has expanded sufficiently to connect with the parental cell wall and cell wall polysaccharides are deposited on both sides of the mature cross-wall [76]. This is mainly based on electron microscopical studies of growing tissue stained by PATAg (periodic acid-thiocarbo-hydrazide-Ag proteinat), an agent that has non-specific affinity for polysaccharides. The differentiated ML is intensily marked by this stain, and a PATAg based distinction between the ML and the adjacent primary wall layers does typically not occur before the cell plate is inserted into lateral walls of the mother cell.

Immunohistochemistry for callose, cellulose, hemicellulose, pectin, and structural proteins has allowed to establish a more detailed timeline of cell plate differentiation [74]. Formation of the cell plate is accompanied by the appearance of methylesterified HG, and arabinogalactan protein throughout the plate [55, 76, 77]. During maturation of the cell plate, methyl-esterified HG is gradually de-esterified through the action of pectin methyl-esterase (PME). This process is either more rapid or more pronounced in the ML compared to the adjacent primary walls as evidenced by immunohistochemistry and PATAg stain. In presence of Ca^{2+}, the de-esterification of HG leads to stiffening of the pectin material, suggesting that this configurational change is an important process during ML differentiation. In the ML of mature cell walls there is also an abundance of RG-I, which is not yet present during the formation of developing cell plate and whose deposition must therefore occur during later stages of maturation [8]. Taken together, it is reasonable to propose that the newly forming ML material is initially soft and neutral in nature, but during maturation becomes negatively charged and stiffened through ion linkages.

Mechanical properties of the middle lamella

Because of its pectin rich nature, one might assume that the ML is a relatively weaker material compared to the cellulose rich, adjacent primary cell wall. However, in experimental tests in which living plant tissues were mechanically stretched, fracture upon material failure typically occurred within the cell wall material rather than at the ML [38, 78]. Zamil *et al* [79] speculated that the reason for this fracture pattern may be geometrical rather than mechanical. As the ML does not form a straight line across the tissue (figure 2(a)), the tensile load applied to a strip of epidermis tissue does not act perpendicular to most of the ML interface, but instead exposes most sections to shear forces that might less easily lead to fracture. Moreover, other structural features such as cell shape, size and tricellular junctions complicate the overall mechanical behavior. To avoid the complicating geometry of the multicellular tissue, a set of novel test protocols was designed in which material strips of subcellular size were excised. This geometrically simple test strip only consisted of the primary walls of two adjacent cells separated by a single straight line of middle lamella (figure 2(b)). To measure the behavior of the wall in dry state, samples were first precisely cut using a focused ion beam (FIB) and mounted onto a tensile testing device designed using microelectromechanical system (MEMS) technology. The samples were glued to the MEMS device by FIB-assisted platinum deposition and subsequently stretched till fractured (figure 2(b)). For samples in hydrated state, cryotome sectioning of the frozen sample was performed. The thawed samples were then mounted onto the MEMS device using cyanoacrylate glue and stretched till fracture (figure 2(c)). Remarkably, in both dry and hydrated state, the excised subcellular strips fractured within the primary wall region, not at the ML [79, 80]. Since geometrical complications were eliminated in this experimental set-up, the results confirm that the ML is as strong as, if not stronger than the primary cell wall material. This is consistent with the emerging role of pectin as a major load bearing network in plant cell walls [81, 82].

Taken together, biochemical and mechanical testing have clearly evidenced that the ML is a material that is distinct from the adjacent primary cell walls (PW). It is therefore interesting to zoom in and focus on the interaction between the two materials (figure 1(c)). In studies on ML mediated cell separation, generally no distinction is made whether the actual separation of two cells occurs through degradation of the ML layer proper or by detachment of the bonding at the PW-ML interface [16]. In cell-to-cell adhesion assays, the activity of cell wall modifying enzyme PME, which de-esterifies HG promoting Ca^{2+} mediated gelation/material stiffening, holds a dominant place [41, 61, 83, 84]. The downregulation of PME in tomato and the

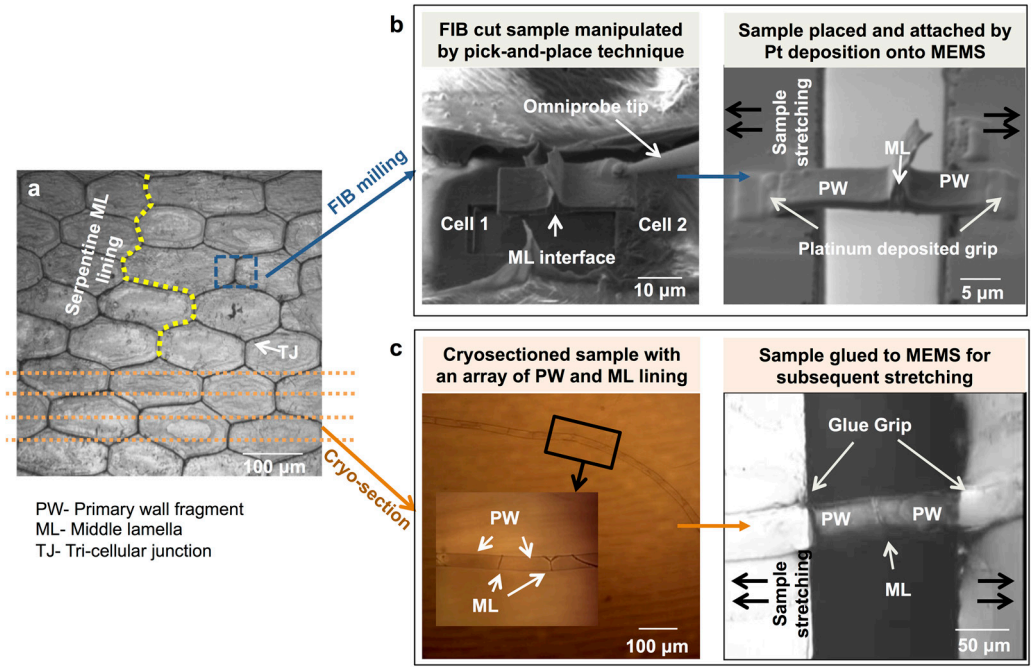

Figure 2. (a) Onion epidermal tissue showing serpentine lining of ML across the sample. (b) Fundamental steps of middle lamella sample preparation using focused ion beam (FIB) technique. (c) Major steps of ML sample preparation using cyrosectioning technique. Images of (b) are reproduced from figures 3(b) and (d) of Zamil *et al* [79], and the left image of 2C is from figure (a) of Zamil *et al* [80] and is reproduced with permission from Springer.

overexpression of PMEI (pectin methyl esterase inhibitor) in Arabidopsis are reported to affect cell-to-cell adhesion [85]. Furthermore, the contribution of polygalacturonases (PGs) in intercellular adhesion [86–88] and in cell separation [89–91] is well documented. In the context of the present review, it must be taken into consideration that both PME and PGs also affect the primary cell wall and a direct conclusion from a defect in cell-to-cell adhesion to potentially altered mechanical properties of the ML is therefore not possible.

The concept that separation at the interface rather than ML degradation is involved at least in some cases is supported by the observation that during cell separation endo-β-1,4-glucanase gets activated [92, 93]. This enzyme degrades cellulose and hemicellulose (xyloglucan) which are enriched in the PW and absent in the ML [8, 94]. In conclusion, cell separation can likely be accomplished either through dissolution of the pectin components of the ML or through the disintegration of the molecular interactions at the ML-PW interface. The challenge is to find out which of the two mechanisms is involved in any given case. While evidence for cellulose and hemicellulose digestion in cell separation suggests that bonds at the PW-ML interface are concerned, the inverse is harder to prove conclusively since pectins, the major component of the ML, are also present in the PW. A selective experimental treatment of the ML that leaves the PW unaltered is therefore more challenging. However, the abundance of non-blockwise de-esterified HG at the tricellular junction ML and its absence in the PW [61] may offer avenues for experimental strategies and so may the differing patterns of non-blockwise and blockwise de-esterification during developmental stages [58, 61].

Molecular interactions at the PW-ML interface and within the ML

At the PW side of the PW-ML interface the molecular composition comprises cellulose, hemicellulose, pectin and structural proteins. These molecules have to link to the ML, which is predominantly composed of pectin with small amounts of protein. Multiple types of molecular interactions are conceivable between these two layers (figure 1(c)). The first type are Ca^{2+} mediated ionic interactions that could link ML pectins to PW pectins. When the HG backbone of pectin is only partially or scarcely esterified, Ca^{2+} ions can cross-bridge the negatively charged polymers. This mechanism is considered to be the main contributor of strength to the pectin network in the PW and ML [7, 16, 39, 81]. From a structural point of view, the exact conformation of the pectins forming Ca^{2+} bridges is not well understood. Recent cell-to-cell adhesion models consider pectin to be a giant macromolecule, in which RG-I is the backbone and HG and RG-II are side chains [27, 95]. These authors propose that at the PW-ML interface the main backbone of pectin is strongly impregnated into or attached to the PW and the side chains branch into the ML to participate in Ca^{2+} mediated bridges. The second type of molecular linkages are hydrogen bonds that may mediate the interaction of ML pectin to the cellulose and hemicellulose of the PW. Pectin is reported to strongly

interact with both cellulose [96, 97] and xyloglucan [98]. The third type are ester linkages assisted by phenolic compound ferulic acid between ML pectin and PW xyloglucan. Cell separation studies on Chinese water chestnut, asparagus and sugar beet suggest that ferulic acid moieties crosslinking the arabinose of RG-I and the xylan backbone of arabinoxylan (hemicellulose) may play important roles in cell-to-cell adhesion [16, 26, 99]. The fourth possible type of interaction are non-covalent interactions between proteins of the ML and pectin and proteins of the PW [16].

Within the ML only two of the four types of molecular interactions mentioned above are likely to be present: type 1 (pectin–pectin interaction) and type 4 (protein–pectin and protein–protein interactions) (figure 1(c)). Ca^{2+} based cross-bridging of low esterified HG in the ML is well documented in the literature, and considered as the main mediator of its strength [19, 83, 100]. However, the nature of pectin-pectin interaction in the ML might be different from that at the PW-ML interface, since RG-II is absent in the ML, whereas it is present in the PW pectin [101–103]. The mechanical properties of the ML seem also to be influenced by the arabinan sidechain of RG-I [104]. The arabinan side chain might restrict HG cross-links [105], which is important to maintain fluidity of the constituent macromolecules in the ML. The protein–protein interactions in the ML might also be different from those at the PW-ML interface. In the ML, only the HRGPs can interact with each other. However, as the PW contains a wider variety of proteins and glycoproteins [106], the protein–protein interactions at the PW-ML interface might be quite diverse.

The strength of the ML may actually originate from the covalent bond strength of the pectic-polysaccharide backbone. The thickness of the ML is so small that a single pectin macromolecule might traverse the entire layer and directly couple the PW of two adjacent cells [16] (figure 1(c)). Should this be the case, the backbone of traversing pectin molecules determines the mechanical behavior. In other words the 'covalent bond' within the pectin backbone would play a vital role in strengthening the ML if both ends of the molecule are embedded into the respective PWs. This is a realistic possibility since at least some pectins are found to be covalently linked to the XG of the PW [98, 107]. Further support for the role of covalent bonds is provided by the observation that treatment with chelating agents rarely leads to full cell separation [7], and that sometimes calcium or ester based bonds may not be the main mediator of cell-to-cell adhesion [101].

Mechanical characterization of the middle lamella

To retain the structural integrity of the plant tissue when it is subjected to internal or external loading, the ML must possess normal (perpendicular to the direction of applied force) and shear (parallel to the direction of applied force) resistance. Understanding how the ML responds to these different types of forces is important. Given that the ML is a bio-polymeric material layer, the characterization of its mechanical properties also needs to consider time dependent behavior such as stress-relaxation (behavior under constant rate of deformation) and creep (behavior under constant load).

Because of extreme technological challenges associated with the thin dimensions of the ML, the mechanical characterization of this structure in isolation is essentially elusive. The most direct characterization has been done using the pioneering techniques described above [79, 80], in which the sample strip contains a single line of ML attaching two adjacent fragments of cell wall. The rationale behind the original design of this experimental test was that the primary cell wall fragments were expected to be much stiffer than the ML material given the presence of cellulose and xyloglucans in the former [108]. Application of tensile stress on the strip was therefore expected to primarily stretch the ML material and the measured mechanical properties would reflect those of the ML. Since this assumption turned out to be erroneous and fracture occurred within the PW material, not the ML, these experiments did not allow to quantitatively characterize the ML proper. However, it should be noted that the experiments described by Zamil *et al* were performed on onion epidermis, a tissue with extremely tight adherence between cells. A different type of tissue or plant species in which the PW is tougher than the ML will therefore have to be found to obtain meaningful values from these experiments.

To interpret the mechanical behavior of the ML based on the tensile testing of PW-ML-PW samples, mathematical or finite element analysis (FEA) based computation modeling is required. Only advanced modeling techniques can capture the complexity of the experimental data which comprise the combined behavior of the PW, the ML and the PW-ML interface and their 3D geometry. FEA is a numerical method that serves to find an approximate solution to a complex problem. FEA subdivides or discretizes a large, complex structure into smaller, simpler elements, which are connected to each other by nodes. The equations that represent these simple elements are then assembled into a larger system of equations that provides an approximate solution for the entire problem. The capability of discretizing a very complex geometry or domain makes FEA extremely powerful for systems for which an analytical solution is impractical or impossible. Originally developed for engineering disciplines, FEA has found application in remote disciplines such as plant biology [109–114]. In biological applications, FEA models can be employed to understand how an observable biological phenomenon, say morphology, can be explained by physical and mechanical behavior of tissues or cells and their interactions at different length scales [115]. The particular usefulness of these models is their

ability to make quantitative and experimentally verifiable predictions. In the context of the ML, one could envisage modeling the mechanical behavior of the PW-ML-PW structure using specific definition of material properties characterizing the PW and ML as well as the interaction between them. The predicted mechanical behavior under various types of load conditions can then be validated experimentally. This kind of inverse methodology has been used successfully for the structurally analogous system of a multilayer contruction material behavior glued by adhesive [116, 117]. FEA modeling would also allow calculating how the overall mechanical behavior of the tissue changes when the properties of selected locations such as tricellular junctions are altered. In other words, an FEA computational model allows to investigate the effect of a micron-scale spatial variation in mechanical properties on the entire tissue or organ.

Compared to the ML of tissues with primary walls, the CML in tissues with secondary wall might be more amenable to experimentation. One of the reasons is that cells with secondary walls can simply be sectioned as the cells are dead and the tissue is very stiff. Studies using atomic force microscopy on horizontally sectioned wood fibers revealed that the CML is not as stiff as the adjacent secondary cell wall material [118, 119]. However, it has to be noted that in these samples the ML cannot be distinguished from the PW and that both are lignified. The mechanical behavior of the CML in wood tissue is therefore likely to be very different from the ML in the onion epidermis. Also, it is important to note that indentation based techniques are conceptually different from tensile tests. The material is compressed by a small stylus, a force application that would be relevant to deduce the behavior of a wooden stem under the compressive load of its own weight, but not necessarily to understand the capacity of the ML to hold the cells of a tissue together against tensile and shear stress.

Relating the spatiotemporal distribution of pectin to mechanical stress distribution

The ML in plant tissues shows a very specific distribution of esterified and de-esterified HG both at tissue, cell and subcellular level [58, 120, 121]. This is considered to be developmentally important since cell and tissue morphogenesis are controlled by HG chemistry [84]. In the ML the spatial distribution is well defined and changes with the developmental stage of the tissue (figure 2(e)). Studies using monoclonal antibodies JIM7, JIM5, LM7 and PAM1 showed that esterified HG is ubiquitous throughout the whole ML domain, whereas de-esterified HG is concentrated at the tips of tricellular junctions displaying intercellular spaces and it is absent from flat sheets [48, 61, 121]. Electron energy loss spectroscopy (EELS) and secondary ion mass spectroscopy (SIMS) revealed similar trends for the abundance of Ca^{2+} with an enrichment at the ML in tricellular junctions [58, 60, 122]. This is consistent with the notion that in these locations Ca^{2+} mediates bridging of de-esterified HG, leading to stiffening of the material. Within the tricellular junctions the distribution of de-esterified pectins is quite intriguing. Blockwise and non-blockwise de-esterified pectins form a very specific pattern of arrangement, which differs before and after the formation of intercellular space [58, 61] (figure 1(e)). The difference in mode of de-esterification (blockwise versus non-blockwise) is known to influence the material and physical properties of cell wall material [123] and the same is likely to be true for the ML. Before the formation of intercellular space in cell junctions, non-blockwise de-esterified HGs are enriched in the center of the junction, the location where maximum stress would be expected in a tissue consisting of cells under hydrostatic pressure. As the intercellular space develops, the non-blockwise de-esterified HG gets concentrated at the tips of the wedge like regions joining the neighboring cells (figure 1(e)). In this tissue geometry the stress is indeed concentrated at these locations due to the formation of intercellular space [40]. The flat sheets on the other hand maintain their relatively high degree of methyl-esterification. This suggests that the ML in the flat sheets is less stiff, possibly to provide a cushioning effect for plasmodesmata exposed to shear, while the stiff ML at the junction tips ensure that the cells do not detach despite increased tensile stress in these regions. While this remains a hypothesis, it is well established that mechanical forces from internal and external loads and resulting stress distribution can lead to strategic subcellular and tissue scale stiffening or loosening of materials and that morphogenesis is influenced by mechanical load conditions [124–126]. The observed spatial distribution of pectins in the ML is therefore consistent with the role of this polymer as stress-bearing in certain portions of the apoplast [127].

Conclusion

From both biophysical and biochemical perspective the ML plays a crucial role in the plant's structure and function. To date, most of our understanding of the ML stems from the study of cell-to-cell adhesion and separation. From immunohistochemical studies, we have a rich set of data showing the presence and distribution of different types of pectin and other polysaccharides in the ML forming flat sheets and corner junctions. While it is known that these spatial variations are tightly regulated both in time and space, our understanding of how the macromolecular distribution and interactions lead to specific mechanical behavior within the ML material or at the PW-ML interface is very limited. Filling this knowledge gap will require monitoring agents able to change the degree of linkage between polysaccharides such as Ca^{2+} ions. Technologies such as SIMS and solid-state nuclear magnetic resonance (NMR) spectroscopy

will therefore likely serve as important tools in future research. Furthermore, cell adhesion assays will need to pay more attention to the specific action of the agents used, so that their effects on ML and/or PW-ML interface can be distinguished. More structural biochemical information will also help us understand the mechanisms that enable the in-plane expansion of the ML during cellular growth. The experimental characterization of the ML as a distinct layer will remain a challenge due to its nanoscale size and complex molecular composition. New experimental systems and protocols clearly need to be explored. Computational modeling will be an important tool to complement data obtained from molecular characterization and mechanical testing. Lastly, it is important that the ML is considered as a separate layer with distinct and precisely defined distribution of material properties, which will give us new insight into the manner in which cells coordinately grow in a multicellular system and how structural integrity is maintained at size and organizational scales ranging from cellular to tissue and organ.

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References

[1] Cosgrove D J 2005 Growth of the plant cell wall *Nat. Rev. Cell Biol.* **6** 850–61
[2] Cosgrove D and Jarvis M 2012 Comparative structure and biomechanics of plant primary and secondary cell walls *Frontiers Plant Sci.* **3** 6
[3] McCann M and Rose J 2010 Blueprints for building plant cell walls *Plant Physiol.* **153** 365
[4] Keegstra K, Albersheim P, Darvill A, Roberts K, Sederoff R and Staehelin A 2010 Plant cell walls *Plant Physiol.* **154** 483–6
[5] Willats W G, McCartney L, Mackie W and Knox J P 2001 Pectin: cell biology and prospects for functional analysis *Plant Mol. Biol.* **47** 9–27
[6] Burton R A, Gidley M J and Fincher G B 2010 Heterogeneity in the chemistry, structure and function of plant cell walls *Nat. Chem. Biol.* **6** 724–32
[7] Jarvis M C, Briggs S P H and Knox J P 2003 Intercellular adhesion and cell separation in plants *Plant Cell Environ.* **26** 977–89
[8] Moore P J and Staehelin L A 1988 Immunogold localization of the cell-wall-matrix polysaccharides rhamnogalacturonan I and xyloglucan during cell expansion and cytokinesis in Trifolium pratense L.; implication for secretory pathways *Planta* **174** 433–45
[9] Li X and Chapple C 2010 Understanding lignification: challenges beyond monolignol biosynthesis *Plant Physiol.* **154** 449–52
[10] Mellerowicz E J and Sundberg B 2008 Wood cell walls: biosynthesis, developmental dynamics and their implications for wood properties *Curr. Opin. Plant Biol.* **11** 293–300
[11] Zhong R and Ye Z-H 2014 Secondary cell walls: biosynthesis, patterned deposition and transcriptional regulation *Plant Cell Physiol.* **56** 195–214
[12] Rose J K C 2003 *The Plant Cell Wall Annual Plant Reviews* (Oxford: Blackwell) vol 8 p 381
[13] Plomion C, Leprovost G and Stokes A 2001 Wood formation in trees *Plant Physiol.* **127** 1513–23
[14] Moghaddam P R and Wilman D 1998 Cell wall thickness and cell dimensions in plant parts of eight forage species *J. Agric. Sci.* **131** 59–67
[15] Westermark U 1985 The occurrence of p-hydroxyphenylpropane units in the middle-lamella lignin of spruce (Picea abies) *Wood Sci. Technol.* **19** 223–32
[16] Waldron K W and Brett C T 2007 The role of polymer cross-linking in intercellular adhesion *Plant Cell Separation and Adhesion* ed J A Roberts and Z Gonzalez-Carranza (Ames: Blackwell) pp 183–204
[17] Roberts J A, Whitelaw C A, Gonzalez-Carranza Z H and McManus M T 2000 Cell separation processes in plants—models, mechanisms and manipulation *Ann. Bot.* **86** 223–35
[18] Roberts J A, Elliott K A and Gonzalez-Carranza Z H 2002 Abscission, dehiscence, and other cell separation processes *Annu. Rev. Plant Biol.* **53** 131–58
[19] Domozych D S *et al* 2014 Pectin metabolism and assembly in the cell wall of the charophyte green alga penium margaritaceum *Plant Physiol.* **165** 105–18
[20] Wolf S, Mouille G and Pelloux J 2009 Homogalacturonan methyl-esterification and plant development *Mol. Plant* **2** 851–60
[21] Patterson S E 2001 Cutting loose. Abscission and dehiscence in arabidopsis *Plant Physiol.* **126** 494–500
[22] Yamada Y, Koibuchi M, Miyamoto K, Ueda J and Uheda E 2015 Breakdown of middle lamella pectin by ·OH during rapid abscission in Azolla *Plant. Cell Environ.* **38** 1555–64
[23] Roberts J A and Gonzalez-Carranza Z 2007 *Plant Cell Separation and Adhesion Annual Plant Reviews* (Oxford: Blackwell) vol 25 p 232
[24] Brummell D A 2006 Cell wall disassembly in ripening fruit *Funct. Plant Biol.* **33** 103–19
[25] Harker F R, Redgwell R J, Hallett I C, Murray S H and Carter G 1997 Texture of fresh fruit *Horticultural Reviews* (New York: Wiley) pp 121–224
[26] Waldron K W, Smith A C, Parr A J, Ng A and Parker M L 1997 New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture *Trends Food Sci. Technol.* **8** 213–21
[27] Vorwerk S, Somerville S and Somerville C 2004 The role of plant cell wall polysaccharide composition in disease resistance *Trends Plant Sci.* **9** 203–9
[28] Xiao C and Anderson C T 2013 Roles of pectin in biomass yield and processing for biofuels *Frontiers Plant Sci.* **4** 67
[29] Singhvi M S, Chaudhari S and Gokhale D V 2014 Lignocellulose processing: a current challenge *RSC Adv.* **4** 8271–7
[30] Kumar P, Barrett D M, Delwiche M J and Stroeve P 2009 Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production *Ind. Eng. Chem. Res.* **48** 3713–29
[31] Limayem A and Ricke S C 2012 Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects *Prog. Energy Combust. Sci.* **38** 449–67
[32] Sun Y and Cheng J 2002 Hydrolysis of lignocellulosic materials for ethanol production: a review *Bioresour. Technol.* **83** 1–11
[33] Agbor V B, Cicek N, Sparling R, Berlin A and Levin D B 2011 Biomass pretreatment: fundamentals toward application *Biotechnol. Adv.* **29** 675–85
[34] Ortíz I and Quintero R 2014 Recent advancements in pretreatment technologies of biomass to produce bioenergy BT *Bioenergy Research: Advances and Applications* ed V Gupta, M Tuohy, C Kubicek, J Saddler and Xu F (Amsterdam: Elsevier) ch 4 pp 57–69
[35] Mani S, Tabil L G and Sokhansanj S 2004 Grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass *Biomass Bioenergy* **27** 339–52
[36] Oyedeji O, Fasina O, Adhikari S, McDonald T and Taylor S 2016 The effect of storage time and moisture content on grindability of loblolly pine (Pinus taeda L.) *Eur. J. Wood Wood Prod.* **74** 857–66
[37] Allen C E 1901 On the origin and nature of the middle lamella *Bot. Gaz.* **32** 1–34
[38] Parker C C, Parker M L, Smith A C and Waldron K W 2001 Pectin distribution at the surface of potato parenchyma cells in relation to cell–cell adhesion *J. Agric. Food Chem.* **49** 4364–71

[39] Marry M, Roberts K, Jopson S J, Huxham I M, Jarvis M C, Corsar J, Robertson E and McCann M C 2006 Cell–cell adhesion in fresh sugar-beet root parenchyma requires both pectin esters and calcium cross-links *Physiol. Plant.* **126** 243–56
[40] Jarvis M C 1998 Intercellular separation forces generated by intracellular pressure *Plant. Cell Environ.* **21** 1307–10
[41] Bou Daher F and Braybrook S A 2015 How to let go: pectin and plant cell adhesion *Frontiers Plant Sci.* **6** 523
[42] Knox J P 1992 Cell adhesion, cell separation and plant morphogenesis *Plant J.* **2** 137–41
[43] Moulia B 2013 Plant biomechanics and mechanobiology are convergent paths to flourishing interdisciplinary research *J. Exp. Bot.* **64** 4617–33
[44] Hao Z *et al* 2014 Loss of Arabidopsis GAUT12/IRX8 causes anther indehiscence and leads to reduced G lignin associated with altered matrix polysaccharide deposition *Frontiers Plant Sci.* **5** 397
[45] Wi S G, Singh A P, Lee K H and Kim Y S 2005 The pattern of distribution of pectin, peroxidase and lignin in the middle lamella of secondary xylem fibres in alfalfa (Medicago sativa) *Ann. Bot.* **95** 863–8
[46] Blumer J M, Clay R P, Bergmann C W, Albersheim P and Darvill A 2000 Characterization of changes in pectin methylesterase expression and pectin esterification during tomato fruit ripening *Can. J. Bot.* **78** 607–18
[47] Gao J, Sik K J, Nasko T, Ottaviano A and Geoffrey D 2014 Chemical and ultrastructural changes in compound middle lamella (CML) regions of softwoods thermally modified by the Termovuoto process *Holzforschung* **68** 849
[48] Guillemin F, Guillon F, Bonnin E, Devaux M-F, Chevalier T, Knox J P, Liners F and Thibault J-F 2005 Distribution of pectic epitopes in cell walls of the sugar beet root *Planta* **222** 355–71
[49] Jones L, Seymour G B and Knox J P 1997 Localization of pectic galactan in tomato cell walls using a monoclonal antibody specific to (1[-]4)-[beta]-D-Galactan *Plant Physiol.* **113** 1405–12
[50] Liners F and Cutsem P 1992 Distribution of pectic polysaccharides throughout walls of suspension-cultured carrot cells *Protoplasma* **170** 10–21
[51] Hafrén J, Daniel G and Westermark U 2000 The distribution of acidic and esterified pectin in cambium, developing xylem and mature xylem of Pinus sylvestris *IAWA J.* **21** 157–68
[52] Kim J S, Gao J and Daniel G 2015 Cytochemical and immunocytochemical characterization of wood decayed by the white rot fungus Pycnoporus sanguineus I. Preferential lignin degradation prior to hemicelluloses in Norway spruce wood *Int. Biodeterior. Biodegrad.* **105** 30–40
[53] Jarvis M C 1984 Structure and properties of pectin gels in plant cell walls *Plant. Cell Environ.* **7** 153–64
[54] Kim S J and Brandizzi F 2014 The plant secretory pathway: an essential factory for building the plant cell wall *Plant Cell Physiol.* **55** 687–93
[55] Toyooka K, Goto Y, Asatsuma S, Koizumi M, Mitsui T and Matsuoka K 2009 A mobile secretory vesicle cluster involved in mass transport from the golgi to the plant cell exterior *Plant Cell* **21** 1212–29
[56] Clausen M H, Willats W G T and Knox J P 2003 Synthetic methyl hexagalacturonate hapten inhibitors of anti-homogalacturonan monoclonal antibodies LM7, JIM5 and JIM7 *Carbohydr. Res.* **338** 1797–800
[57] Verhertbruggen Y, Marcus S E, Haeger A, Ordaz-Ortiz J J and Knox J P 2009 An extended set of monoclonal antibodies to pectic homogalacturonan *Carbohydr. Res.* **344** 1858–62
[58] Bush M S, Marry M, Huxham I M, Jarvis M C and McCann M C 2001 Developmental regulation of pectic epitopes during potato tuberisation *Planta* **213** 869–80
[59] Roy S, Jauneau A and Vian B 1994 Analytical detection of calcium ions and immunocytochemical visualization of homogalacturonic sequences in the ripe cherry tomato *Plant Physiol. Biochem.* **32** 633–40
[60] Roy S, Gillen G, Conway W S, Watada A E and Wergin W P 1995 Use of secondary ion mass spectrometry to image 44calcium uptake in the cell walls of apple fruit *Protoplasma* **189** 163–72
[61] Willats W G T *et al* 2001 Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls: implications for pectin methyl esterase action, matrix properties, and cell adhesion *J. Biol. Chem.* **276** 19404–13
[62] Limberg G, Korner R, Buchholt H C, Christensen T M, Roepstorff P and Mikkelsen J D 2000 Analysis of different de-esterification mechanisms for pectin by enzymatic fingerprinting using endopectin lyase and endopolygalacturonase II from A. niger *Carbohydr. Res.* **327** 293–307
[63] Smallwood M, Beven A, Donovan N, Neill S J, Peart J, Roberts K and Knox J P 1994 Localization of cell wall proteins in relation to the developmental anatomy of the carrot root apex *Plant J.* **5** 237–46
[64] Burlat V, Kwon M, Davin L B and Lewis N G 2001 Dirigent proteins and dirigent sites in lignifying tissues *Phytochemistry* **57** 883–97
[65] Cassab G I 1998 Plant cell wall proteins *Annu. Rev. Plant Biol.* **49** 281–309
[66] Moore P, Darvill A, Albersheim P and Staehelin L 1986 Immunogold localization of xyloglucan and rhamnogalacturonan I in the cell walls of suspension-cultured sycamore cells *Plant Physiol.* **82** 787–94
[67] Gorshkova T and Morvan C 2006 Secondary cell-wall assembly in flax phloem fibres: role of galactans *Planta* **223** 149–58
[68] Gorshkova T A, Chemikosova S B, Sal'nikov V V, Pavlencheva N V, Gur'janov O P, Stolle-Smits T and van Dam J E G 2004 Occurrence of cell-specific galactan is coinciding with bast fiber developmental transition in flax *Ind. Crops Prod.* **19** 217–24
[69] Pinzon-Latorre D and Deyholos M K 2014 Pectinmethylesterases (PME) and pectinmethylesterase inhibitors (PMEI) enriched during phloem fiber development in flax (*Linum usitatissimum*) *PLoS One* **9** e105386
[70] Williams M N, Freshour G, Darvill A G, Albersheim P and Hahn M G 1996 An antibody Fab selected from a recombinant phage display library detects deesterified pectic polysaccharide rhamnogalacturonan II in plant cells *Plant Cell* **8** 673–85
[71] Matoh T, Takasaki M, Takabe K and Kobayashi M 1998 Immunocytochemistry of rhamnogalacturonan II in cell walls of higher plants *Plant Cell Physiol.* **39** 483–91
[72] Donaldson L A 2001 Lignification and lignin topochemistry—an ultrastructural view *Phytochemistry* **57** 859–73
[73] Dhonukshe P, Baluska F, Schlicht M, Hlavacka A, Samaj J, Friml J and Gadella T W J J 2006 Endocytosis of cell surface material mediates cell plate formation during plant cytokinesis *Dev. Cell* **10** 137–50
[74] Drakakaki G 2015 Polysaccharide deposition during cytokinesis: challenges and future perspectives *Plant Sci.* **236** 177–84
[75] Seguí-Simarro J M, Austin J R, White E A and Staehelin L A 2004 Electron tomographic analysis of somatic cell plate formation in meristematic cells of arabidopsis preserved by high-pressure freezing *Plant Cell* **16** 836–56
[76] Matar D and Catesson A M 1988 Cell plate development and delayed formation of the pectic middle lamella in root meristems *Protoplasma* **146** 10–7
[77] Rybak K, Steiner A, Synek L, Klaeger S, Kulich I, Facher E, Wanner G, Kuster B, Zarsky V, Persson S and Assaad F F 2016 Plant cytokinesis is orchestrated by the sequential action of the TRAPPII and exocyst tethering complexes *Dev. Cell* **29** 607–20
[78] Ryden P, Sugimoto-Shirasu K, Smith A C, Findlay K, Reiter W-D and McCann M C 2003 Tensile properties of arabidopsis cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-borate complexes *Plant Physiol.* **132** 1033–40
[79] Zamil M S, Yi H and Puri V M 2014 Mechanical characterization of outer epidermal middle lamella of onion under tensile loading *Am. J. Bot.* **101** 778–87

[80] Zamil M S, Yi H and Puri V 2015 The mechanical properties of plant cell walls soft material at the subcellular scale: the implications of water and of the intercellular boundaries *J. Mater. Sci.* **50** 6608–23
[81] Peaucelle A, Braybrook S and Höfte H 2012 Cell wall mechanics and growth control in plants: the role of pectins revisited *Frontiers Plant Sci.* **3** 6
[82] Dick-Pérez M, Zhang Y, Hayes J, Salazar A, Zabotina O A and Hong M 2011 Structure and interactions of plant cell-wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR *Biochemistry* **50** 989–1000
[83] Micheli F 2001 Pectin methylesterases: cell wall enzymes with important roles in plant physiology *Trends Plant Sci.* **6** 414–9
[84] Peaucelle A, Braybrook S A, Le Guillou L, Bron E, Kuhlemeier C and Höfte H 2016 Pectin-induced changes in cell wall mechanics underlie organ initiation in arabidopsis *Curr. Biol.* **21** 1720–6
[85] Lionetti V, Cervone F and De Lorenzo G 2015 A lower content of de-methylesterified homogalacturonan improves enzymatic cell separation and isolation of mesophyll protoplasts in arabidopsis *Phytochemistry* **112** 188–94
[86] Babu Y and Bayer M 2014 Plant polygalacturonases involved in cell elongation and separation—the same but different? *Plants* **3** 613–23
[87] Atkinson R G, Sutherland P W, Johnston S L, Gunaseelan K, Hallett I C, Mitra D, Brummell D A, Schröder R, Johnston J W and Schaffer R J 2012 Down-regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water loss in apple (Malus × domestica) fruit *BMC Plant Biol.* **12** 129
[88] Posé S, Paniagua C, Cifuentes M, Blanco-Portales R, Quesada M A and Mercado J A 2013 Insights into the effects of polygalacturonase FaPG1 gene silencing on pectin matrix disassembly, enhanced tissue integrity, and firmness in ripe strawberry fruits *J. Exp. Bot.* **64** 3803–15
[89] Tucker G A, Schindler C B and Roberts J A 1984 Flower abscission in mutant tomato plants *Planta* **160** 164–7
[90] Gonzalez-Carranza Z H, Elliott K A and Roberts J A 2007 Expression of polygalacturonases and evidence to support their role during cell separation processes in Arabidopsis thaliana *J. Exp. Bot.* **58** 3719–30
[91] Petersen M, Sander L, Child R, van Onckelen H, Ulvskov P and Borkhardt B 1996 Isolation and characterisation of a pod dehiscence zone-specific polygalacturonase from Brassica napus *Plant Mol. Biol.* **31** 517–27
[92] Webb S T J, Taylor J E, Coupe S A, Ferrarese L and Roberts J A 1993 Purification of β1, 4 glucanase from ethylene-treated leaflet abscission zones of Sambucus nigra *Plant. Cell Environ.* **16** 329–33
[93] Trainotti L, Ferrarese L, Poznanski E and Vecchia F D 1998 Endo-β-1,4-glucanase activity is involved in the abscission of pepper flowers *J. Plant Physiol.* **152** 70–7
[94] Schnepf E 1983 The structure of cells (Prokaryotes, Eukaryotes) *Biophysics* ed W Hoppe *et al* (Berlin: Springer) pp 1–19
[95] Vincken J-P, Schols H A, Oomen R J F J, McCann M C, Ulvskov P, Voragen A G J and Visser R G F 2003 If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture *Plant Physiol.* **132** 1781–9
[96] Wang T, Zabotina O and Hong M 2012 Pectin–cellulose interactions in the arabidopsis primary cell wall from two-dimensional magic-angle-spinning solid-state nuclear magnetic resonance *Biochemistry* **51** 9846–56
[97] Wang T and Hong M 2016 Solid-state NMR investigations of cellulose structure and interactions with matrix polysaccharides in plant primary cell walls *J. Exp. Bot.* **67** 503–14
[98] Brett C T, Baydoun E-H and Abdel-Massih R M 2005 Pectin–xyloglucan linkages in type I primary cell walls of plants *Plant Biosyst.* **139** 54–9
[99] Ng A, Harvey A J, Parker M L, Smith A C and Waldron K W 1998 Effect of oxidative coupling on the thermal stability of texture and cell wall chemistry of beet root (Beta vulgaris) *J. Agric. Food Chem.* **46** 3365–70
[100] Braybrook S A, Hofte H and Peaucelle A 2012 Probing the mechanical contributions of the pectin matrix: insights for cell growth *Plant Signal Behav.* **7** 1037–41
[101] McCartney L and Knox J P 2002 Regulation of pectic polysaccharide domains in relation to cell development and cell properties in the pea testa *J. Exp. Bot.* **53** 707–13
[102] Chormova D, Messenger D J and Fry S C 2014 Rhamnogalacturonan-II cross-linking of plant pectins via boron bridges occurs during polysaccharide synthesis and/or secretion *Plant Signal. Behav.* **9** e28169
[103] O'Neill M A, Ishii T, Albersheim P and Darvill A G 2004 Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide *Annu. Rev. Plant Biol.* **55** 109–39
[104] Iwai H, Ishii T and Satoh S 2001 Absence of arabinan in the side chains of the pectic polysaccharides strongly associated with cell walls of Nicotiana plumbaginifolia non-organogenic callus with loosely attached constituent cells *Planta* **213** 907–15
[105] Jones L, Milne J L, Ashford D and McQueen-Mason S J 2003 Cell wall arabinan is essential for guard cell function *Proc. Natl Acad. Sci.* **100** 11783–8
[106] Rose J K C and Lee S-J 2010 Straying off the highway: trafficking of secreted plant proteins and complexity in the plant cell wall proteome *Plant Physiol.* **153** 433–6
[107] Cumming C M, Rizkallah H D, McKendrick K A, Abdel-Massih R M, Baydoun E A H and Brett C T 2005 Biosynthesis and cell-wall deposition of a pectin–xyloglucan complex in pea *Planta* **222** 546–55
[108] Zamil M S, Yi H, Haque A and Virendra M P 2013 Characterizing microscale biological samples under tensile loading: stress–strain behavior of cell wall fragment of onion outer epidermis *Am. J. Bot.* **100** 1105–15
[109] Panagiotopoulou O 2009 Finite element analysis (FEA): applying an engineering method to functional morphology in anthropology and human biology *Ann. Hum. Biol.* **36** 609–23
[110] Richmond B G, Wright B W, Grosse I, Dechow P C, Ross C F, Spencer M A and Strait D S 2005 Finite element analysis in functional morphology *Anat. Rec.* A **283** 259–74
[111] Madzvamuse A, Wathen A J and Maini P K 2003 A moving grid finite element method applied to a model biological pattern generator *J. Comput. Phys.* **190** 478–500
[112] Kolston P J 2000 Finite-element modelling: a new tool for the biologist *Phil. Trans. R. Soc. London.* A **358** 611
[113] Fayant P, Girlanda O, Chebli Y, Aubin C-E, Villemure I and Geitmann A 2010 Finite element model of polar growth in pollen tubes *Plant Cell* **22** 2579–93
[114] Weber A, Braybrook S, Huflejt M, Mosca G, Routier-Kierzkowska A-L and Smith R S 2015 Measuring the mechanical properties of plant cells by combining micro-indentation with osmotic treatments *J. Exp. Bot.* **66** 3229e3241
[115] Geitmann A 2010 Mechanical modeling and structural analysis of the primary plant cell wall *Curr. Opin. Plant Biol.* **13** 693–9
[116] da Silva L F M and Campilho R D S G 2012 Advances in numerical modelling of adhesive joints *Advances in Numerical Modeling of Adhesive Joints* (Berlin: Springer) pp 1–93
[117] Mustapha F, Sim N W and Shahrjerdi A 2011 Finite element analysis (FEA) modeling on adhesive joint for composite fuselage model *Int. J. Phys. Sci.* **6** 5153–65
[118] Clair B, Arinero R, Leveque G, Ramonda M and Thibaut B 2003 Imaging the mechanical properties of wood cell wall layers by atomic force modulation microscopy *IAWA J.* **24** 223–30
[119] Wimmer R and Lucas B 1997 Comparing mechanical properties of secondary wall and cell corner middle lamella in spruce wood *IAWA* **18** 77–88
[120] Palin R and Geitmann A 2012 The role of pectin in plant morphogenesis *Biosystems* **109** 397–402

[121] Knox J P, Linstead P J, King J, Cooper C and Roberts K 1990 Pectin esterification is spatially regulated both within cell walls and between developing tissues of root apices *Planta* **181** 512–21
[122] Huxham M I, Jarvis C M, Shakespeare L, Dover J C, Johnson D, Knox P J and Seymour B G 1999 Electron-energy-loss spectroscopic imaging of calcium and nitrogen in the cell walls of apple fruits *Planta* **208** 438–43
[123] Goldberg R, Pierron M, Bordenave M, Breton C, Morvan C and du Penhoat C H 2001 Control of mung bean pectinmethylesterase isoform activities: influence of pH and carboxyl group distribution along the pectic chains *J. Biol. Chem.* **276** 8841–7
[124] Mirabet V, Das P, Boudaoud A and Hamant O 2011 The role of mechanical forces in plant morphogenesis *Annu. Rev. Plant Biol.* **62** 365–85
[125] Bidhendi A J and Geitmann A 2015 Relating the mechanics of the primary plant cell wall to morphogenesis *J. Exp. Bot.* **167** 449–61
[126] Schopfer P 2006 Biomechanics of plant growth *Am. J. Bot.* **93** 1415–25
[127] Goldberg R, Morvan C, Jauneau A and Jarvis M C 1996 Methyl-esterification, de-esterification and gelation of pectins in the primary cell wall *Pectins and Pectinases Proc. of an Int. Symp.* vol 14 (Amsterdam: Elsevier) pp 151–72