

Wood Cell Wall Structure and Organisation in Relation to Mechanics



Lennart Salmén

Abstract The wood cell wall, as well as the entire wood structure, is a highly intermixed assembly of biopolymers building up various structural elements. The understanding of the organisation of these wood polymers and their interaction is a key to be able to better utilise wood materials. The complexity of the wood cell wall is here discussed regarding the cellulose fibrillar network, the cellulose aggregate structure and the arrangement of the matrix polymers of hemicelluloses and lignin. The ability to model the wood cell wall properties, based on the structural organisation within different cell wall structures, and the difficulties in relating predictions to actual measurements of cell wall properties are described. The deficiencies regarding our structural knowledge in relation to mechanical properties are also being defined.

Keywords Cell wall · Cellulose · Hemicellulose · Lignin · Microfibrils
Microfibril angle · Micromechanics · Humidity · Temperature
Secondary cell wall

Introduction

Wood is a material that has been utilised by mankind since the beginning of civilization, not only as an energy resource but also as a building material and as a source for creating various utilities. The advantages of this material come to a high degree from its availability and excellent mechanical performance in relation to its density, which relates to the structural organisation of the material. Plants have a remarkable organisation with a highly hierarchical structure from the level of cellulose microfibrils to the cell wall organisation and the tissue level (Fratzl 2003; Gibson 2012).

Wood is the secondary xylem of trees, i.e. dicotyledons, and provides both strength and water transport for the plant. In gymnosperms, softwood, tracheids are the dominating cell type providing both for transport and strength. Parenchyma cells in radial

L. Salmén (✉)
RISE Bioeconomy, Stockholm, Sweden
e-mail: lennart.salmen@ri.se

rays functioning for storage for gums, starch, resin, tannins, latex and other materials. In angiosperms, hardwood, fibres provide the mechanical rigidity while larger thin-walled vessel elements, joined end-to-end function as water-transporting channels also here with parenchyma cells in rays for storage. The mechanical features of wood fibres or tracheids are dominated by the structure of the secondary cell wall. Understanding the cell wall structure and its properties are the key not only to properties of wood or plant materials themselves, but also to the way these materials may be utilised in modern material applications and how to dismantle them in order to utilise individual polymeric components. In addressing questions of the relationships between structure and mechanics of a biological material like wood, one should consider the hierarchical levels involved. In many cases where an engineering property of the material is requested, a homogenisation of properties at a higher hierarchical level may be adequate (Hofstetter and Gamstedt 2009; de Borst and Bader 2014). When it comes to questions regarding the roles of different components of the cell wall and the underlying phenomena governing structure/property relations, knowledge of the structural organisation on the molecular level may be necessary. This issue is here addressed primarily with regard to the structure of the wood cell wall, as well as the properties of the constituent biopolymers building up this structure.

The Fibre Cell Wall

At the wood cell wall level, the structural organisation is a composite involving primarily cellulose microfibrils, amorphous hemicelluloses and lignin. This is illustrated in Fig. 1, which shows the build-up of the main secondary wall, the S_2 wall, with the cellulose aggregates undulating in the length direction of the fibre. The variability is still large on this nanoscale, and makes it difficult to get a deeper insight into the structural arrangement, as the variability is at the limit of resolution of imaging techniques presently available. Thus, understanding of the arrangement of the wood-polymer components of the cell wall still relies on indirect observations of their response to specific techniques or methods. Here, modelling plays an important role as a way of testing different theories against each other to assess the importance of different factors. At the present time, too many of the variables are nevertheless not sufficiently well determined to permit a complete understanding of the detailed structure of the cell wall.

It is well known that cellulose dominates many of the properties of the plant cell wall, especially its strength properties. For example, the microfibril angle (MFA), relative to the long axis of the secondary S_2 wall (which constitutes 80% of the fibre volume) to a high extent determines the properties in the longitudinal fibre direction such as stiffness, strength and toughness. The fact that the MFA is usually less than 30° and is often in the range of $5\text{--}10^\circ$ with fibrils having a right-handed chirality, makes the fibre highly anisotropic. Numerous modelling approaches have been carried out based on estimated properties of the wood components and have shown a sufficiently good correlation with the measured longitudinal fibre strength

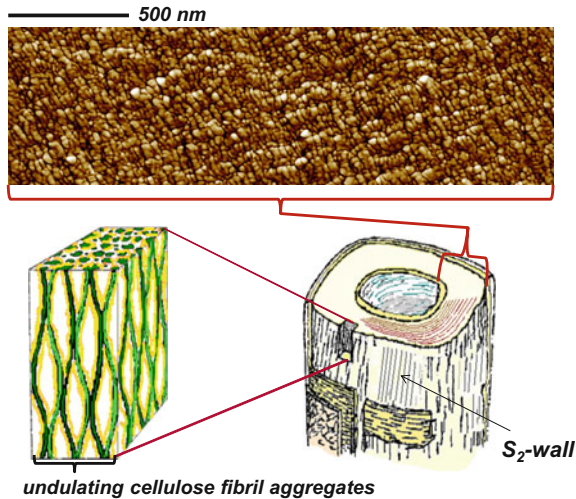
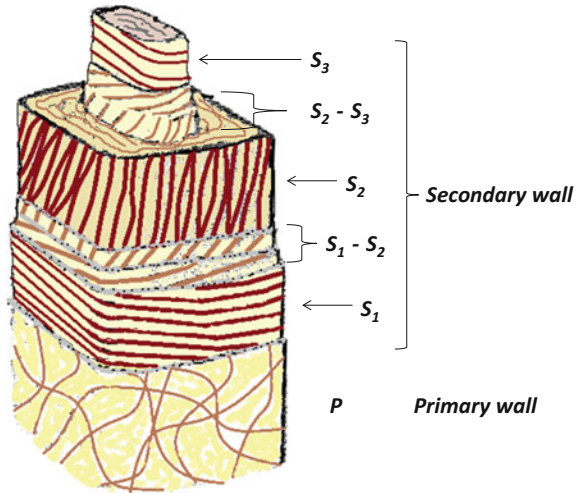


Fig. 1 Schematic representation of the complex organisation of the tracheid cell wall polymers with cellulose microfibrils arranged in undulating aggregates (after Boyd 1982) with hemicelluloses and lignin interspaced. The structure across the secondary S_2 wall (as viewed by Atomic Force Microscopy, AFM Fahlén and Salmén 2005) shows a clear variability of aggregate sizes equally dispersed over the entire cross section. An indication of a lamellar structure may be seen

properties. However, modelling transverse fibre properties is still far from satisfactory (Bergander and Salmén 2002; Wang et al. 2014a, b). The problem relates both to the fact that measurement of transverse fibre properties in itself is extremely difficult and to the fact that the properties of the constituent polymers in the transverse direction are much more uncertain. In early models of the cell wall, only the main layers, the primary wall, P, and the three secondary walls, S_1 , S_2 and S_3 (with different microfibril angles), were considered (Cave 1968; Mark 1972; Salmén and de Ruvo 1985; Bergander and Salmén 2002). Later models increased the complexity by adding transition layers between S_1 and S_2 and between S_2 and S_3 (Wang et al. 2013, 2014a, b). The existence of such layers has been confirmed in a number of studies, although the variation of the MFA and extent of each layer has not been fully quantified; there may even be large variations from fibre to fibre. However, it seems clear that there is no cross-laminar structure of the S_1 wall (Brändström et al. 2003; Reza et al. 2014) but rather that it forms a gradual transition towards the S_2 (Reza et al. 2014; Reza 2016) as indicated in Fig. 2.

Incorporating such a structure into the modelling of the cell wall properties has to some extent improved the modelling accuracy. However, despite this progress, there are still rather substantial uncertainties with respect to an overestimated transverse rigidity (Wang et al. 2013). The difficulties in measurements of transverse fibre properties (Bergander and Salmén 2000a, b), may have led to an underestimation of these properties. The experimentally determined stiffness values are lower than modelled values (Wang et al. 2013, 2014a, b) indicating that both structural information and a

Fig. 2 Schematic representation of the structure of a wood fibre indicating the orientation of the cellulose microfibril aggregates in the different layers. The existence of transition layers between the S_1 and S_2 layers as well as between the S_2 and S_3 is indicated (Wang et al. 2013; Reza et al. 2014)



precise knowledge of the properties of the constituents and their interaction are still lacking.

The following information is most urgently required for a better understanding of the secondary wall of wood cells:

- The three-dimensional structure of the cellulose fibrillar structure
- The elastic, viscoelastic and expansion properties of the cellulose fibril aggregate (considering the structural arrangement of non-crystalline areas)
- The degree of interaction between the wood components.

The Cellulose Fibrillary Network

The cellulose microfibrils of the secondary wall are generally regarded as consisting of concentric lamellae (Kerr and Goring 1975) as indicated in Figs. 1 and 2. However, such a strict organisation has been questioned, using modelling and Transmission Electron Microscopy, TEM, studies of the cell wall. Instead, it has been proposed that microfibrils are arranged more randomly in clusters, with varying degrees of tangential or radial organisation (Donalson 2001). Such a more radial arrangement of microfibril aggregates was also suggested by Sell and Zimmermann (1993) based on fracture analysis. This fractured structure was later disputed as being only a reflection of the process of dissipation of fracture energy (Fahlén and Salmén 2002). All of these studies have been based on transverse sections of the fibre wall whereas in reality a three-dimensional imaging is necessary to be able to resolve the structure. An attempt to perform such measurements was recently made by using TEM images taken at angles to the cell wall (Reza et al. 2014) and using image processing to construct tomograms (Reza 2016). The data suggest that microfibril aggregates run transverse

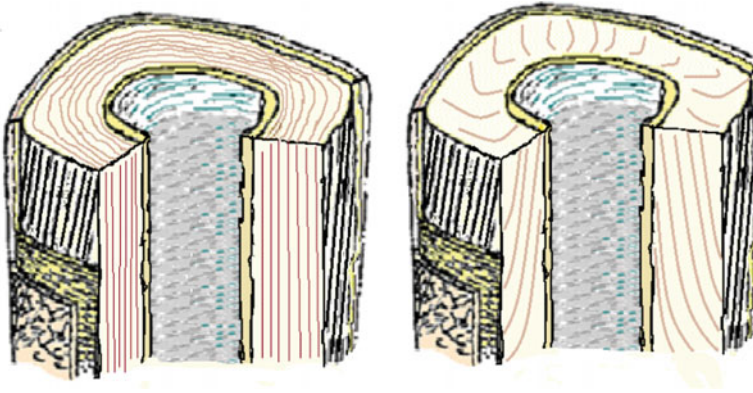


Fig. 3 Schematic illustration of the organisation of the lamellar structure of the secondary S_2 cell wall. Left, the conventional concentric lamellar structure (Kerr and Goring 1975). Right, a radial lamellar structure proposed based on TEM tomographs (Reza et al. 2014; Reza 2016). The latter structure is difficult to reconcile with the mechanism of the cell wall assembly process

to the fibre axis in the S_2 -wall layer going from the outer S_1 -wall continuously towards the inner S_3 -wall as illustrated in Fig. 3. This configuration is very different from the conventional concept. At present times, it is difficult to understand how such a fibrillar structure could be developed during cell growth, considering that the microfibrils are continuously produced from the plasma membrane in a layer-by-layer deposition during the formation of the cell wall. The lamellar structure of the cell wall is also corroborated by studies of the shrinkage of the cell wall when lignin is removed (Stone et al. 1971). Lignin removal results in a distinct shrinkage of the thickness of the cell wall suggesting that the lignin is organised mostly as tangential lamellae within the S_2 -wall layer.

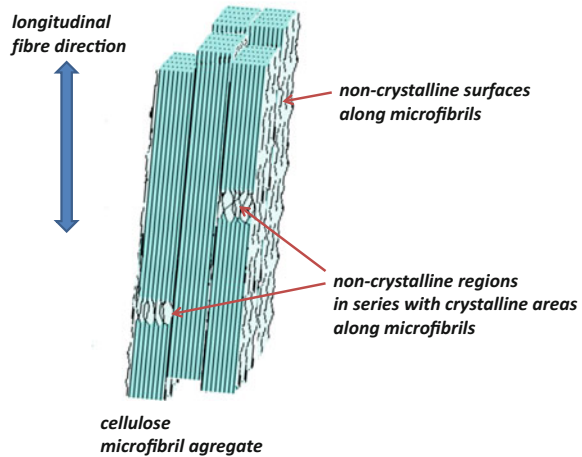
In the longitudinal direction, images clearly show that the cellulose microfibrils are not straight but are undulating at regular intervals. They apparently connect to each other forming a network with differently sized aggregates (Bardage et al. 2004), as schematically indicated in Fig. 1. The size distribution is large with a mean aggregate size of around 16 nm (Bardage et al. 2004; Fahlén and Salmén 2005). Lenticular spaces, with a larger longitudinal axis where matrix material of hemicellulose and lignin are deposited, are clearly visible in Scanning Electron Microscopy, SEM, images (Bardage et al. 2004). This structure resembles that of the models of an undulating cellulose network originally suggested by Boyd (1982). This undulating structure may be the reason for the puzzling orientation interpreted from tomography by Reza et al (2014). Indeed, in order to fully answer the question of the three-dimensional organisation of the cellulose microfibrils and aggregates, more accurate imaging of the cell wall is needed, probably requiring synchrotron facilities to provide the necessary resolution.

Cellulose Aggregate Properties

The cellulose microfibrils are clearly the cell wall units that have the largest impact on mechanical properties of the cell wall, but the exact properties of these nanostructured filaments are still the subject of discussion. Most modelling and experimental data converge towards a longitudinal elasticity of crystalline cellulose of 140–150 GPa (Sakurada et al. 1962; Kroon-Batenburg et al. 1986; Iwamoto et al. 2009). However, there is a large range in moduli determined from molecular modelling and experiments with values as low as 120 and as high as 170 GPa (Matsuo et al. 1990; Tashiro and Kobayashi 1991; Nishino et al. 1995; Tanaka and Iwata 2006; Bergensträhle et al. 2007; Iwamoto et al. 2009). Considering that the cellulose microfibril is not composed of 100% crystalline cellulose, one must take into account the organisation of disturbed, non-crystalline portions of the cellulose. A large part of these non-crystalline areas have been assigned to the surfaces of the microfibrils (Wickholm et al. 1998) but the presence of non-crystalline parts along the length of the microfibril in the form of periodical defects have been noticed using various methods (Nishiyama 2009). The existence of non-crystalline portions combined with the observation of undulations (Fig. 1) explains why cellulose aggregates have a lower effective elasticity than an entirely crystalline structure. The possible twisting of the microfibrils (Fernandes et al. 2011) could also contribute to non-regular structures in the length direction. The influence on the longitudinal cellulose stiffness may though be rather small (Fernandes et al. 2011). Most of the non-crystalline regions may be associated with the surface areas of the microfibrils rather than interrupting the crystalline regions along the length of the microfibril (Salmén and Bergström 2009). The effects of moisture on the cellulose stiffness indicates, together with the fact that moisture affects only non-crystalline structures of the cellulose (Nishiyama et al. 2002), that the deformation follows a relation of parallel amorphous and crystalline structures (Salmén and Bergström 2009). This is corroborated by the fact that for low MFA ($<10^\circ$) the strain of small wood elements exposed to a tensile stress is to more than 95% given by the strain of the crystalline cellulose (Nakai et al. 2006). From this, it can be concluded that non-crystalline structures arranged in series with crystalline sections must have a rather limited extension, possibly because of adjacent crystalline microfibrils which prevent them from being highly strained (Fig. 4). Due to the uncertainty of the estimated values for the cellulose aggregate stiffness as well as of the experimental cell wall data it is presently not possible to back calculate the true value of the cellulose microfibril stiffness. However, given that the transverse properties seem to be highly overestimated, it seems to be unlikely that the tensile elasticity of the cellulose aggregates exceeds 140–150 GPa.

It is even more difficult to assess the cellulose properties in the transverse direction of the cellulose aggregates. Two factors may be considered; the effect of the undulations and the connections between microfibrils in aggregates. The cellulose crystals are inaccessible to moisture but it is now clear that the surfaces, between the fibrils in the aggregates, do adsorb moisture (Lindh and Salmén 2017;

Fig. 4 Schematic representation of the organisation of non-crystalline cellulose areas in a cellulose microfibril aggregate. The largest part of the non-crystalline areas is associated with the surface of the microfibrils while a smaller portion is indicated as disturbances along the microfibrils



Lindh et al. 2017), and cellulose microfibrils have both hydrophilic and hydrophobic surfaces (Matthews et al. 2006; Fernandes et al. 2011).

Due to the asymmetry of the building block of cellulose, the glucose unit, the cellulose molecule has two chain ends of different characters, where one end may form an aldehyde function, the reducing end. During its synthesis at the plasma membrane, by the rosette terminal complex, several cellulose chains are extruded at the same time thus having similar direction. It has also been shown that the formed microfibril is composed of molecules pointing in the same direction, i.e. that the reducing end of the cellulose is pointing only upwards or downwards in the same microfibril (Hieta et al. 1984; Chanzy and Henrissat 1985). The fact that the rosettes during cellulose synthesis are travelling both up- and downwards in relation to the fibre direction implies that cellulose microfibrils may be pointing in different directions with the reducing end either up or down in relation to the microfibril axis, a fact verified for the cell wall of *Valonia* (Revol and Goring 1983). Conversion of cellulose, in the cell wall, to cellulose II involves a blending of microfibrils with opposite orientations of the chains forming a regular crystal structure with alternating chains with up- and downward reducing end groups. In order that this may be possible microfibrils close to each other must expose opposite molecular directions. However, if these opposite microfibrils exist in the same aggregate or in different aggregates close to each other, see Fig. 5, is an unsolved question. How the connections within the aggregate should be mediated by the presence of hemicellulose molecules, glucomannan in the case of softwood (Tokoh et al. 1998), is also unclear. The implications with regard to the forces holding together the fibrils in aggregates are thus highly uncertain.

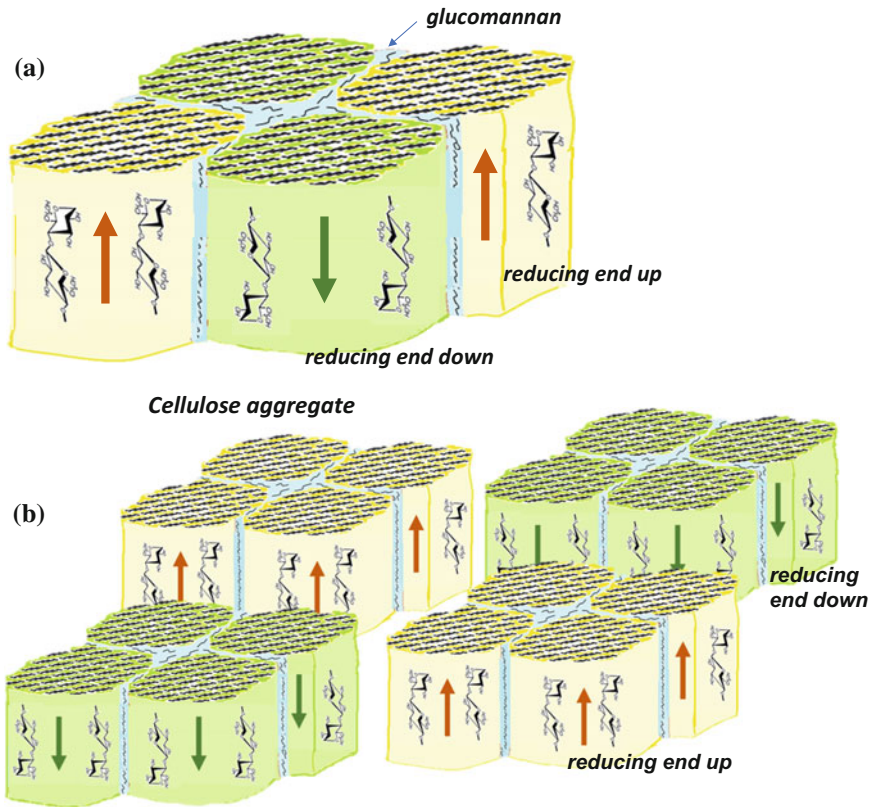


Fig. 5 Schematic representation of arrangement of cellulose microfibrils in **a** aggregate composed of microfibrils with different cellulose chain directions, the reducing end pointing upwards (marked yellow) or downwards (marked green) **b** uniform aggregates with different cellulose chain direction in different aggregates. Hemicellulose, glucomannan in softwood, separates the individual microfibril surfaces within the aggregate

The Matrix Polymers

The matrix polymers consist of hemicelluloses (xylan and glucomannan) and lignin. The properties of these polymers are difficult to determine precisely since they are intimately mixed within the structure of the cell wall. Furthermore, extraction of any of the components probably alters the properties of the component of interest or at least alters its environment. Since all of these matrix polymers are amorphous, the approximate range of properties can be estimated based on their glass transition temperature (Salmén 1982; Salmén et al. 1985). Many questions remain though to be answered concerning interrelation between lignin and hemicelluloses and their organisation within the cell wall. It is unclear, for example, whether or not lignin and xylan are intermixed so that they act as a single component. Measurements based on

lignin softening tend to indicate that at least some part of the lignin acts as a separate component (Olsson and Salmén 1997).

Recent data indicate that all of the matrix polymers are to some extent aligned in relation to the fibre axis. In softwood, the glucomannan is closely associated with the cellulose microfibrils (Åkerholm and Salmén 2001) and also has a preferred orientation, albeit less prominently than the microfibrils. It may be that the presence of glucomannan controls the organisation of the microfibrils and prevents the formation of larger ribbon structures (Tokoh et al. 1998) Similarly, xylans in both softwood and hardwood exhibit a preferred orientation in the direction parallel to the cellulose microfibrils (Simonovic et al. 2011).

In the case of lignin, it is difficult to talk about a real orientation. In the middle lamella, the lignin is clearly arranged in an isotropic manner (Salmén et al. 2012). In the secondary wall of both hardwoods and softwoods, a weakly detectable preferred direction parallel to the cellulose seems to exist (Simonovic et al. 2011; Salmén et al. 2012). It has also been shown that the phenylpropane units of the lignin molecule have a tangential orientation i.e. following the tangential lamellae of the S_2 wall (Atalla and Agarwal 1985).

The anisotropy of the amorphous biopolymers (hemicelluloses and lignin) means that the transverse elasticity will be somewhat lower than the elasticity in the polymer chain direction. Since they have a preferred orientation along the microfibrils, the matrix will also display a slightly reduced stiffness in the transverse fibre direction. The tight interaction between the wood components is also reflected by the fact that during moisture absorption the cellulose crystalline structure of the microfibrils has been observed to be compressed, presumably as a result of the swelling of the matrix substances (Abe and Yamamoto 2005).

The impact of lignin on the stiffness of the wood fibre structure has also been discussed at the molecular deformation level, since no molecular deformation of lignin has been detected using spectroscopic techniques (Gierlinger et al. 2006; Salmén and Bergström 2009). Recent studies indicate, however, that such deformations of lignin may be recorded but only when wood fibres are highly deformed (Salmén et al. 2016). The conclusion drawn by the authors is that in native wood lignin contributes only marginally to the tensile strength properties in line with earlier suggestions based on Raman-spectroscopy studies on single wood fibres (Burgert 2006; Gierlinger et al. 2006).

The amorphous wood polymers are highly hygroscopic, and even native lignin absorbs sufficient moisture to affect its properties although not at ambient temperatures. Consequently, the properties of the cell wall depend on the environment and it is essential to control testing conditions. Transient changes in the testing environment such as variations in humidity or temperature, therefore, compromise measurement of fibre properties.

At higher degrees of deformation, especially for fibres with a larger MFA, sliding of the matrix material along microfibrils probably contributes to plastic flow (Spatz et al. 1999; Keckes et al. 2003). The involvement of hemicelluloses in the stick-slip mechanism of the deformation is highly plausible (Altaner and Jarvis 2008). However, the role of hemicellulose in the sliding mechanisms may not be essential,

as it has been demonstrated that during mechano-sorptive creep the contents of neither lignin nor of the two types of hemicelluloses, glucomannan and xylan in softwood, play a role (Olsson and Salmén 2014). Indeed, the mechano-sorptive creep of fibres seems to be governed exclusively by the MFA of the cellulose microfibrils (Dong et al. 2010). Given that moisture may access the interfaces between microfibrils (Lindh and Salmén 2017; Lindh et al. 2017) it is reasonable to hypothesize that water molecules may act as a lubricant promoting sliding between microfibrils.

Modelling Cell Wall and Fibre Properties

Modelling structure–property relations may serve several purposes from that of predicting the service properties of a construction down to understanding molecular phenomena of the material. In the concept here addressed, modelling serves the purpose of increasing our understanding of the role of the different wood-polymer constituents and their arrangement for the mechanical properties of the cell wall. These models rely on knowledge of thermomechanical properties of the wood polymers and the structural arrangement of them in different cell wall elements. The fact that most data, as well as the more theoretically simpler relations, refer to elastic properties has led to that most models are focused on various aspects of cell wall elasticity. Thus, the elastic properties under both dry and moist conditions, the hygroexpansive properties and the relation to the MFA have been modelled. Both angle-ply models and concentric cylinder models of the cell wall have been used (Hofstetter and Gamstedt 2009), where both types of models give good agreement with regard to the sensitivity of the longitudinal stiffness variation to the MFA and moisture (Yamamoto and Kojima 2002; Joffre et al. 2014; Wang et al. 2014a, b). Regarding hygroexpansivity, the models estimate values in the proper range of data (Joffre et al. 2014; Wang et al. 2014a, b) but there are not enough measurements under critical conditions to validate the predictions. Data for the transverse elasticity, are also scarce, but so far it seems that all models overestimate the stiffness (Bergander and Salmén 2002; Wang et al. 2014), see Fig. 6. However, it is well demonstrated that the extent of softening is well captured by the assumed softening of the hemicelluloses, indicating the essential role of hemicellulose properties for the transverse cell wall properties (Salmén 2004).

It could be noted that modelling, outgoing from wood polymer properties, utilising homogenisation techniques in several steps may provide reasonable stiffness data on the tissue level for all directions (de Borst and Bader 2014). However, in this case the influence of the structural organisation at the level of the cell wall layer may not be easily assessed. On the other hand, it is shown that also by utilising the measured transversal fibre stiffness (Bergander and Salmén 2000a, b) incorporated into a tissue structure containing ray cells transverse stiffness values of the wood tissue may be reasonably estimated (Salmén 2007).

With the development of molecular dynamic (MD) simulations, the possibility of predicting cell wall properties based on atomistic properties of the constituent

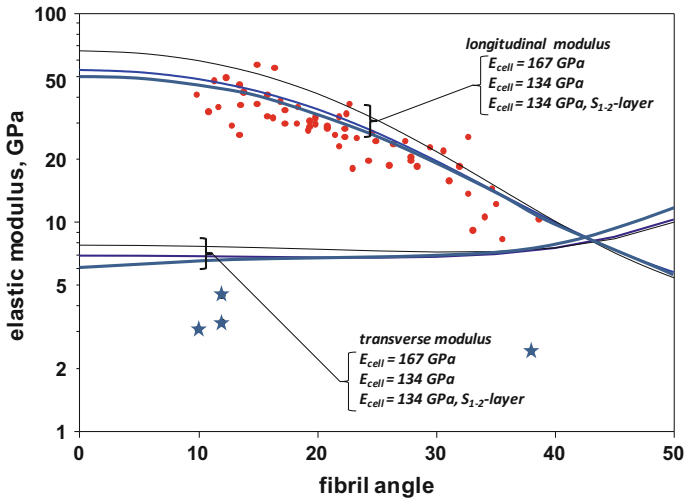


Fig. 6 The elastic modulus in the longitudinal and transverse direction of a softwood fibre cell wall. Calculations are based on micromechanics and laminate theory considering oriented hemicelluloses and lignin in the S_2 wall (Bergander and Salmén 2002), and in one case the incorporation of a transition layer (Wang et al. 2014a, b). Curves are displayed from the top and down with cellulose modulus = 167, GPa; = 134 GPa; = 134 GPa + S_{1-2} layer. Measured data in the longitudinal direction (dots) are those of Cave (1969) and in the transverse direction (stars) those of Bergander and Salmén (2000a, b)

polymers increases. Such MD simulations of the action of moisture on cellulose clearly demonstrate that it is possible to model the loss of stiffness and hygroexpansive properties of amorphous cellulose (Keckes et al. 2003). Consequently, hemicelluloses may not be the exclusive agent responsible for softening of the wood fibre. Knowledge of the distribution and organisation of the non-crystalline cellulose portion is, therefore, essential for understanding how the properties are affected by moisture changes in the cell wall. Because of the dominant role of the cellulose, particular interest should be dedicated to the effect of moisture on microfibrils.

Cell Wall Property Measurements

Measurements of the mechanical properties at the fibre or cell wall level represent a challenge, not only because of the small dimensions but also because of biological variation. Any measured property has to be related to the appropriate hierarchy level. In general, due to the dominance in the volume of the fibre in relation to the connecting middle lamella, there is a good agreement between the longitudinal elastic modulus at the fibre level and that at the tissue level (Wang et al. 2014a, b). However, when it comes to failure properties, the ultimate strain and breaking stress are lower in the

tissue compared to the fibre level because fibres are debonded at high stresses. These levels are probably also highly dependent on the environmental conditions, as these conditions influence the properties of the middle lamella.

One of the much-used experimental strategies to assess and understand cell wall properties is based on a nanoindentation technique. For large MFA, the elastic modulus values are close to those measured in tensile fibre testing, but for low MFA, the modulus measured through indentation often greatly underestimates the properties (Gindl et al. 2004; Eder et al. 2013).

The contribution of the different wood polymers to the properties of the cell wall has often been measured by selective removal of part of the component. Nanoindentation techniques typically yield lower moduli and lower hardness values than the native cell wall when lignin and/or hemicelluloses are removed (Wang et al. 2016; Zhang et al. 2016). However, this result may to some extent reflect the drop in material density rather than truly lower material properties of the remaining polymers.

To assess the ability of different parameters to predict structure–property relations of the wood fibre, it is not enough to be able to estimate a single value by some specific measurement. The model and structure may only be considered reliable if it is possible to predict some variation in important cell wall parameters. The most important parameters include MFA, wood-polymer composition and the effect of moisture. Differences in the effect of moisture on the stiffness of the S₂ wall of different wood samples were noted by nanoindentation measurements (Wagner et al. 2015), which could perhaps be related to variations in chemical composition and MFA differences. However, as indicated long ago, the relative degree of moisture softening is independent of the amount of lignin and hemicellulose (Salmén et al. 1985; Kolseth and Ehrnrooth 1986; Zhang et al. 2016). Nanoindentation has shown that the effect of moisture on the hardness of the cell wall is greater than the effect on the elastic modulus (Yu et al. 2011). To some extent, this could be related to the complex deformation of the nanoindenter. However, if one considers the wood fibre to be a more ductile material, at least at the higher moisture contents, the result is highly plausible. The influence of the MFA was insignificant for hardness (Li et al. 2014).

Another interesting approach for mechanical testing is micropillar compression. In this technique micrometre-sized pillars are eroded from the cell wall using a focused ion-beam. The pillar is then uniaxially compressed with a flat indenter with a diameter of a few micrometres (Adusumalli et al. 2010; Raghavan et al. 2012) thus deforming a somewhat larger area as compared to the nanoindentation technique. This gives a more uniaxial deformation of the material and less controversial data may be obtained. It has been possible to determine the yield point in compression as a function of MFA, and it appears to be in accordance with theoretical predictions (Schwiedrzik et al. 2016). At higher strains, buckling affects the results but this may, on the other hand, give information regarding the integrity of the cell wall.

In order to obtain stiffness values on an even higher level of resolution, i.e. for the in-situ wood polymers, a variety of AFM techniques may be promising. These include contact resonance atomic force (CR-AFM) (Arnould and Arinero 2015). However, the measured properties are generally lower than those obtained from

nanoindentation, and they are not yet fully correlated to the structure and polymers of the cell wall.

X-ray tomography tools offer increasingly greater resolution, allowing structures and properties to be monitored. Current technology allows resolution at the fibre level, but hopefully in the future subcellular levels can be reached. Synchrotron X-ray tomography has been used to measure the hygroexpansion coefficients of a single wood fibre in different directions (Joffre et al. 2016). As an example, the values of a longitudinal hygroexpansivity of 0.014/RH and a transverse hygroexpansivity of 0.17/RH can be regarded as material properties, but they are in the lower range of previous estimates which emphasises the difficulties in obtaining reliable experimental data. So far this is a rather exclusive technique, but future studies are certain to benefit from technological progress in this field.

Future Perspective

From the picture of cell wall properties presented here, it is clear that the principal gap in knowledge is related to the difficulty in making precise measurements of properties at the cell wall level. In order better to evaluate the importance of various parameters for modelling, critical measurements particularly with regard to transverse fibre properties must be made. To enhance our understanding of the cell wall the following structural information is necessary;

- The three-dimensional structure of the cellulose aggregates within the different cell wall layers. This would presumably require synchrotron X-ray tomography investigations in order to reach the proper level of resolution.
- Elastic properties of the cellulose microfibril or aggregate structure including the non-crystalline regions. Due to the dominance of the cellulose, an unambiguous data set for cellulose would provide a solid base for all calculations.
- Transverse cell wall properties, elastic and hygroexpansional as a function of MFA. With data as a function of MFA, it would be possible better to match different modelling approaches to assess the influence of different variables.
- A better knowledge of the interactions of the cell wall components in the transverse direction. This may be a way in order to clarify the reasons for the discrepancy between modelled and measured transverse fibre properties.

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