CELL-WALL MECHANICAL PROPERTIES OF BAMBOO INVESTIGATED BY IN-SITU IMAGING NANOINDENTATION

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ABSTRACT

A novel in-situ imaging nanoindentation technique was used to investigate the cell-wall mechanical properties of bamboo fibers and parenchyma cells. In-situ imaging confirmed neither "piling up" nor "sinking in" occurred around the indentations in the cell walls. The load-displacement curves revealed different deformation mechanisms of the cell walls when indented, respectively, in the longitudinal and transverse direction of bamboo fibers. There existed significant differences in MOE between longitudinal (16.1 GPa) and transverse direction (5.91 GPa) for the cell walls of bamboo fibers, while no differences were significant in hardness. Furthermore, the measured longitudinal MOE and hardness of parenchyma cell walls were 5.8 GPa and 0.23 GPa. This corresponds to 33% and 63% of the corresponding value of bamboo fibers. It was found that the longitudinal MOE of the cells of bamboo fibers remained almost constant from the outer portion to the inner portion of bamboo culms, while hardness showed a decreasing tendency. It was concluded that the nanoindentation technique was capable of effectively characterizing the mechanical properties of bamboo at the cellular level, though it might underestimate the real longitudinal MOE of the cell walls. The results highlighted the extreme importance of locating indentations at the nano scale for the mechanical characterization of complicated natural biomaterials such as wood and bamboo.

Keywords: Bamboo fibers, cell wall, hardness, in-situ imaging nanoindentation, MOE.

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INTRODUCTION

Nanoindentation, also called Depth Sensing Indentation, is becoming increasingly popular for mechanical characterization of thin films and block materials with extremely small volume. In this technique, a nano-scale diamond tip is indented into the surface of materials, meanwhile recording load and displacement continuously during a complete cycle from loading to unloading. Modulus of elasticity (MOE) and hardness can be calculated from analyzing the load-displacement data according to the method proposed by Oliver and Pharr (1992). Some typical applications of nanoindentation include mechanical characterization of trabecular and cortical bone (Turner et al. 1999), human dental enamel (Habelitz et al. 2001), and various animal bone tissues (Rho and Pharr 1999).

Wimmer et al. (1997) and Wimmer and Lucas (1997) introduced nanoindentation to wood science by estimating the mechanical properties of the secondary wall and the cell corner middle lamella of spruce tracheids. Their investigations showed the promise of nanoindentation in probing the mechanical properties of wood at cellular level. The subsequent investigations conducted by Gindl et al. (2002) and Gindl and Schöberl (2004) focused on microfibril angle (MFA) and lignification related to longitudinal hardness and elastic modulus of the secondary cell wall of tracheids. A more recent study used the newly developed "Continuous Stiffness Measurement" technique to investigate the difference in MOE and hardness between the cell walls of earlywood and latewood tracheids (Jiang et al. 2004). Conventional nanoindentation technique has two major shortcomings when being applied to the heterogeneous natural porous biomaterial like wood and bamboo. The first is the relatively complicated sample preparation procedure, similar to that for transmission electron microscopy. The second is the accurate determination of indentation position on the specific zone of the samples. It is possible to place indentations on the edge of or in the cell cavity, which would bring about significant errors or even invalid results. This is especially the case for thin-walled cells.

It was well known that bamboo fibers play the most important role in determining the macro-mechanical properties of bamboo (Tommy et al. 2004). In addition, recent interest in bamboo fiber-reinforced composites has attracted more attention in the mechanical properties of bamboo fibers (Takagi et al. 2003). As the first part of a series of investigations, this paper focused on the mechanical properties of bamboo fibers and the parenchyma cells surrounding them. The nanoindenter used in this study incorporates a unique in-situ imaging function, allowing us to greatly simplify the procedure of sample preparation and improve the reliability of results.

MATERIALS AND METHODS

Specimen preparation and MFA measurement

Moso bamboo (*Phyllostachys edulis* (Carr.) H. De Lehaie) was selected for this study, as it is the most widely utilized in China. It was six years old and was taken from a bamboo plantation located in Xiaoshang District, Hangzhou City, China. Bamboo disks 4 cm thick were cut from the 2-m height of bamboo. Bamboo blocks were cut from disks with the final dimensions T (radial) × 8 mm (tangential) × 5 mm (longitudinal). These blocks were then softened in 80°C water for 3–4 days. A sliding microtome (SM2000R, Leica, Germany) was used to prepare the specimens for nanoindentation. The step size was less than 5 μm and knife marks should not be observed visually. All the samples were conditioned at 20°C and 65% relative humidity for a week before testing. Meanwhile, nine bamboo blocks were cut out from three different heights (1 m, 3 m, and 5 m) of 3 bamboo culms. They were used to investigate the radial variation of microfibril angle (MFA) of bamboo fibers with a powder X-ray diffractometer (X'Pert Pro, Panalytical, USA). The specimens were continuously cut out in tangential direction from the outer portion to the inner portion of bamboo culms with the final dimensions of 1 mm thick, 10 mm wide, and 40 mm long.

The radiation source was a CuKa (λ = 0.154 nm), tube voltage 40 kV, tube current 40 mA, 2 × 2-mm aperture incident beam. The mean microfibrillar angle was determined according to the method developed by Cave (1966).

Nanoindentation test

Elastic punch theory states that the elastic modulus of materials and hardness can be inferred from load-displacement curves of nanoindentation. According to the method of Oliver and Pharr (1992), the unloading segment can be fitted very well with a power-law function, from which the initial slope of the unloading curve, namely elastic contact stiffness (S), can be determined. Based on S, the reduced elastic modulus E_r can also be obtained. Then the MOE and hardness of materials can be calculated from the following formulae:

$$\frac{1}{E_r} = \frac{1 - v^2}{E} + \frac{1 - v_i^2}{E_i} \tag{1}$$

$$H = \frac{P}{A} \tag{2}$$

Where P is peak load; A, that can be calculated from an empirical formula, is the projected area at peak load; E_r is called the reduced elastic modulus; E_i and v_i are respectively the elastic modulus and Poisson ratio of the tips. For diamond tips, E_i is 1141 GPa, and v_i is 0.07. E and v are, respectively, the elastic modulus and Poisson ratio of samples. It should be pointed out that the modulus E and E_r are almost identical for soft materials like bamboo and wood, which eliminated the need to obtain the Poisson ratio of the cell wall of bamboo fibers.

Microtomed bamboo blocks were directly adhered to metal stubs with fast cure adhesive, and then fixed on a motorized sample stage by magnetic force. A nanoindenter (Triboindenter, Hysitron Inc. USA) was chosen to conduct nanoindentation tests because of its unique in-situ imaging function. The set peak load and loading-unloading rate were 300 μN and 50 μN/s for longitudinal indentation. A load holding segment of two seconds was added between loading and unloading segments to remove the effect of creep. The peak load for transverse indentation was 150 μN with the same unloading rate as the longitudinal direction. A Berkovich diamond indenter with a tip radius of less than 100 nm was adopted for indentation and scanning. A target region was first selected with a light microscope attached to the instrument, and then raster-scanned with the indenter tip to obtain a high magnification image. A smooth micro-zone (roughness is less than 20 nm) was selected from the raster-scanned image for nanoindentation testing. After indentation, the same zone was again raster-scanned to determine the actual positions of indentations.

RESULTS AND DISCUSSION

In-situ imaging nanoindentation and topography of indentations

Conventional nanoindenters rely on a reflected light microscope combined with motorized mechanical stage to find and locate indenting positions, which is only suitable for homogeneous materials that normally have no strict requirements for the indenting locations. Furthermore, the whole sample surface usually needs to be very flat and smooth to perform a shallow indentation test, because it is impossible to select smooth micro-zones from a relatively rough surface with the help of a light microscope. Wimmer et al. (1997) suggested using an ultramicrotome with a diamond knife to prepare the surface of wood samples. Though this method can get a high quality surface for nanoindentation, much time is required. However, in-situ imaging nanoindentation integrates the functions of imaging and indenting to one nanoscale diamond tip. A piezoceramics tube other than a motorized sample stage is used to precisely locate the positions of indentations (spatial resolution less than 10 nm).

Figure 1 is a detailed schematic illustration for the operating procedure of an in-situ imaging

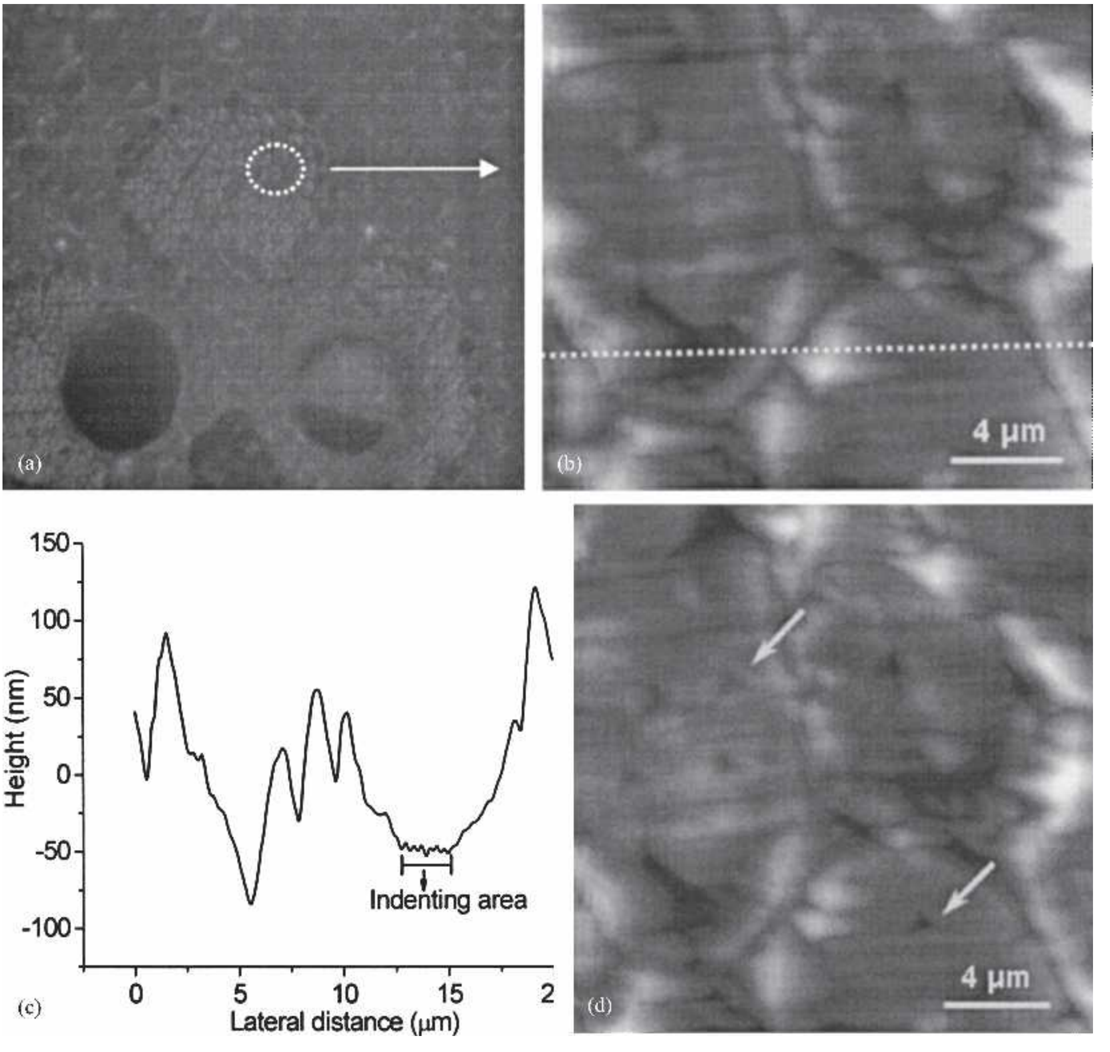

FIG. 1. Schematic illustration for the operation procedure of an in-situ imaging nanoindentation test. A: the light micrograph of the cross-section of bamboo. B: the cross-section of bamboo fibers obtained by scanning the zone enclosed with the white dot line in image A with a Berkovich tip. C: the surface profile along the while dot line in image B. D: image obtained by scanning the same zone after indenting with the same tip. The indentations are indicated with the white arrows.

nanoindentation test. A target region enclosed with the dot line in Fig. 1A was first selected with a light microscope attached to the instrument, and then raster-scanned with an indenter tip to obtain a high magnification image (Fig. 1B). This function is similar to that of an atomic force microscope (AFM). A smooth micro-zone then can be easily selected from the image for nanoindentation test. The roughness of the selected indenting area can be confirmed through a surface profile analysis shown in Fig. 1C. It can be found that the average roughness of the indenting area is less than 20 nm. This is sufficient for a nanoindentation test, though the surface of the whole zone is rather coarse. The same zone was again raster-scanned to determine the actual positions of the indentations (Fig. 1D), which will help us to select valid indentations.

Furthermore, with in-situ imaging, the topography of the indentations could be directly ob-

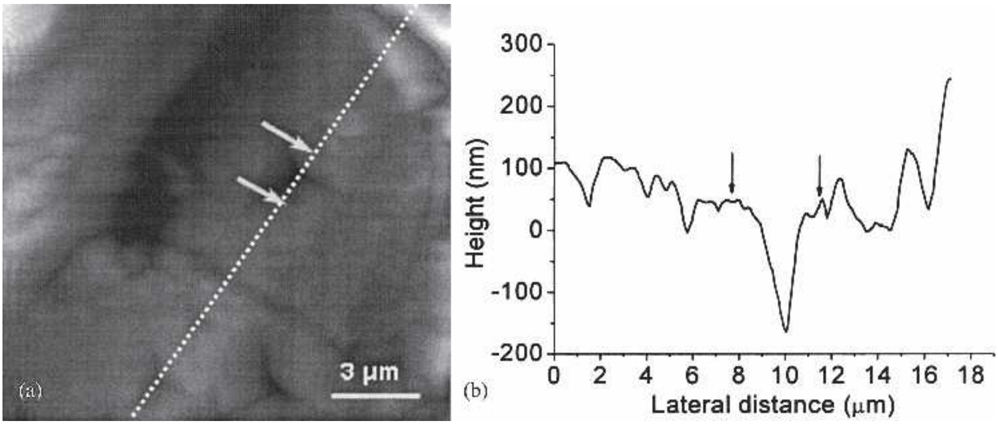

FIG. 2. Topography of indentations with different depth. B is the surface profile along the white dot line in image A. The indentation is indicated with white arrows.

served to obtain some important mechanical information about the cell walls. It is very important to determine if the "piling-up" or "sinking-in" will appear around the indentations. Some materials tend to be displaced far away from the indentation, resulting in a sink-in of material against the sides of the indenter, while piling-up means some materials tend to deform more locally, creating a pile-up of material against the sides of the indenter. Piling-up (or sinking-in) leads to contact areas that are higher than (or less than) the cross-sectional area of the indenter at a given depth, which will make the measured value larger than (or less than) the actual value (Cheng and Cheng 1998). In a previous paper (Jiang et al. 2004), it had been assumed "sinking-in" was one of the reasons for the underestimation of cell-wall MOE of softwood tracheids measured by nanoindenter with no in-situ imaging function. But the assumption was now denied as neither of them was found through a profile analysis as shown in Fig. 2B.

Mechanical characteristics of bamboo fibers cell wall

The two load-displacement curves in Fig. 3 reveal different deformation mechanisms of the cell walls when indented in the longitudinal and transverse directions. Only about 25% of the deformation recovers elastically for the longitudinal indentation, compared with 75% elastic recovery for indenting in the transverse direction. This demonstrates that plastic deformation is the main mechanism for indenting the cell wall of bamboo fibers in the longitudinal direction, while elastic deformation dominates in the transverse direction. It is assumed that the deformation mechanism of the cell walls during indenting depends on the interaction between tips and cellulose microfibrils. For the longitudinal indentation, the tip tends to cause slip dislocation between microfibrils. The permanent deformation caused will contrarily restrain the longitudinal elastic recovery of matrix as stress is re-

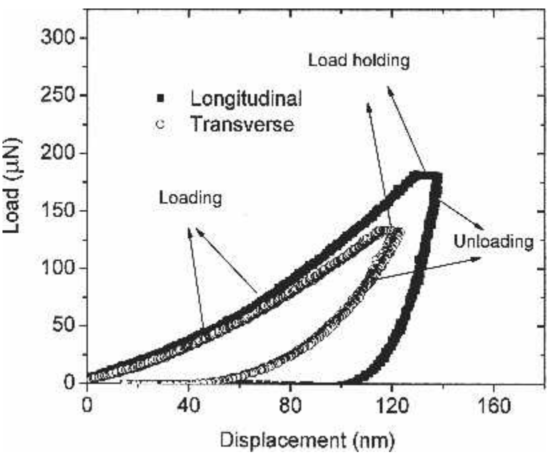

FIG. 3. Load-displacement curves, indented respectively in the longitudinal and transverse directions.

leased because of the prohibition of microfibrils arranged nearly parallel to the long axis of bamboo fibers. For the transverse indentation, the tip tends to bend the microfibrils rather than cause slip dislocation; thus the restraint effect from the microfibrils is much less.

High dependence of mechanical properties of bamboo fibers cell wall on MFA

Figure 4 indicates that the longitudinal MOE of bamboo fiber remains nearly constant from the outer portion to the inner portion of bamboo culms. This implies that the MFA of bamboo fibers should remain relatively constant along the radial direction of the bamboo culms. This deduction is based on the generally accepted opinion that the longitudinal MOE of cell wall is highly dependent on MFA (Cave 1968, 1969; Page et al. 1977). Figure 5 shows the radial variation of MFA for bamboo fibers from the outer portion to the inner portion of bamboo culms, and validates our deduction. It was found that the MFA of bamboo fibers remained almost constant in the radial direction.

Figure 4 also shows a slight decreasing tendency in longitudinal hardness of bamboo fibers from the outer portion to the inner portion of bamboo culms. This can be explained by the radial variation of chemical compositions of bamboo fibers. Unlike the longitudinal MOE of

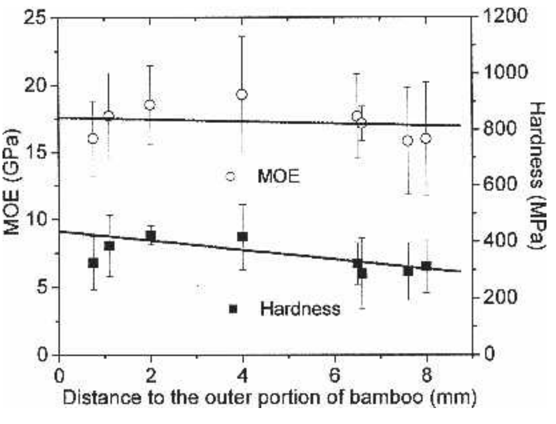

FIG. 4. Radial variation of the MOE and hardness of the cell walls of bamboo fibers from the outer portion to the inner portion of bamboo culms.

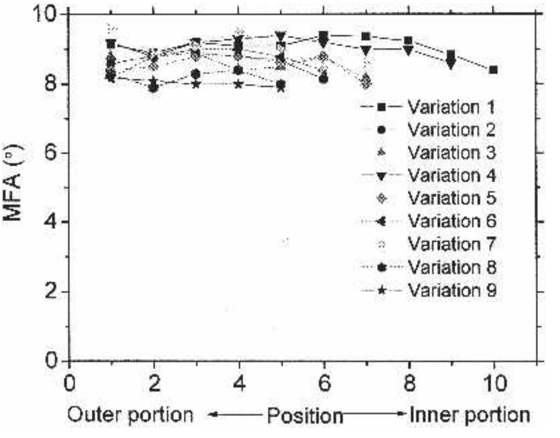

FIG. 5. Radial variation of the MFA of bamboo fibers from the outer portion to the inner portion of bamboo culms. Here X axis is relative position.

the cell wall that is determined almost by MFA, the longitudinal hardness of the cell wall is assumed to positively correlate to the content of cellulose and lignin. It is well known that the mass fractions of cellulose and lignin normally decrease from the outer portion to the inner portion of bamboo culms (Wang 2001); thus hardness should correspondingly decrease.

The transverse mechanical properties of natural fibers are very important for designing an energy efficient thermo-mechanical pulping process. However, the relevant data are scarce because of the difficulties involved in measurement. Bergander and Salmén (2000a, 2000b) combined transverse tension on wood microtome sections in the sample chamber of SEM with digital image correlation technique to estimate the transverse elastic modulus of the cell walls of Norway spruce (*Picea abies* (L.) karst.) tracheids. The measured value from very limited experiments showed great variation ranging from 0.7 GPa to 4.5 GPa, lower than the average value 5.91 GPa measured in this study for the cell walls of bamboo fibers. The longitudinal MOE of bamboo fibers is much higher than the corresponding transverse MOE (Table 1). This is reasonable as the average MFA of bamboo measured with X-ray diffraction is only about 9° (Fig. 5). But it should be noted that there is no significant difference in hardness between the

TABLE 1. *MOE and hardness of bamboo fiber cell walls in longitudinal and transverse direction (SD = Standard deviation, CV% = coefficient of variation, n = number of measurements).*

	Longitudinal		Transverse	
	MOE (GPa)	Hardness (MPa)	MOE (GPa)	Hardness (MPa)
Max	25.83	692.73	8.63	507.20
Min	10.85	233.98	4.52	290.89
Average	16.01	359.99	5.91	391.00
SD	3.15	104.29	1.27	63.31
CV(%)	19.66	28.97	21.44	16.19
n	61	61	20	20

longitudinal and transverse directions. Wimmer et al. (1997) found there was no significant difference in the longitudinal hardness between the S_2 layer of the secondary wall and the intercellular lamella of Norway spruce tracheids, though the former was much higher in MOE. This finding was confirmed by Gindl and Schöberl (2004). Cell-wall hardness was thought to be independent of MFA according to their findings. However, in this study a high positive linear relationship between MOE and hardness both in the longitudinal and transverse directions was observed (Fig. 6). This is somewhat different from the result of Wimmer and Lucas (1997), which indicated that hardness and MOE of the corner of the compound middle lamella (CCML)

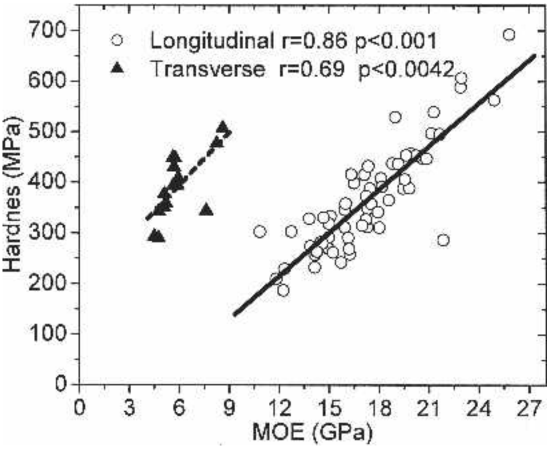

FIG. 6. Correlation between the MOE and hardness of the cell walls of bamboo fibers, respectively for longitudinal and transverse direction.

were highly correlated (r = 0.74) to each other, but no such correlation existed for the S_2 layer. The S_2 layer showed a constant hardness over its range of MOE. Considering the high dependence of longitudinal MOE on MFA, as well as the highly positive linear relation between MOE and hardness, it seems unreasonable to fully ignore the effect of MFA on longitudinal hardness. It is assumed that both MFA and lignin are correlated to hardness. The influence of MFA on hardness will decrease with the increase of MFA. When MFA reaches a certain value, the composition and packing density of the cell walls will act as the determinant of cell-wall hardness.

The average longitudinal MOE of bamboo fibers measured by nanoindentation in this study was 16.0 GPa with a maximum value of 25.8 GPa. Though reliable experimental data directly measured with other methods have not yet been reported, a preliminary estimation based on volume ratio of bamboo fibers and the macro longitudinal MOE of bamboo was about 39.2 GPa for the cell wall of bamboo fibers (Huang et al. 2005). Furthermore, a theoretically calculated value of 48 GPa (Yu 2003) was obtained for the cell walls of softwood tracheids. In that study, the MFA used for calculation was similar to that of the bamboo in this study. So it can be concluded that nanoindentation significantly underestimates the longitudinal MOE of the cell walls of bamboo fibers. This phenomenon has also been noted by Wimmer and Lucas (1997) and Gindl et al. (2002). According to the analysis of Swadener et al. (2001), the determined elastic modulus by nanoindentation of an anisotropic material is a mixture of the modulus among all axes, which leads to an underestimation of the higher moduli. Gindl and Schöberl (2004) further pointed out that the underestimation of cell-wall longitudinal MOE decreases with the increase of MFA. Therefore, the transverse cell-wall MOE measured with nanoindentation will more closely represent the actual value. According to a theoretical calculation, the cell-wall transverse MOE is nearly 9 GPa (Yu 2003), which is relatively closer to the measured value of 5.9 GPa in this study. Thus the present theory of nanoindentation based on the assumption of isotropy and homogeneity should be revised for better application to the anisotropic biomaterials such as wood and bamboo.

Mechanical difference between bamboo fibers and parenchyma cells

Many scholars regard bamboo as a typical natural composite in which bamboo fibers act as a reinforcement phase encased in a matrix phase of parenchyma cells (Amada and Untao 2001; Tommy et al. 2004). For this analogy to be correct, bamboo fibers must possess mechanical properties much higher than the parenchyma cells. Figure 7 shows significant differences in longitudinal MOE and hardness between bamboo fibers and parenchyma cells. The longitudinal MOE and hardness of the cell walls of parenchyma cells determined by nanoindentation are only 5.8 GPa and 230 MPa, respectively. These values are 33% and 63% of the corresponding values for bamboo fibers. Considering the random orientation of microfibrils in the parenchyma cells, this demonstrates again the higher dependence of MOE on MFA than on hardness. Additionally, it can be inferred that the MOE of parenchyma cells, as determined by nanoindentation, is much closer to the actual value than bamboo fibers.

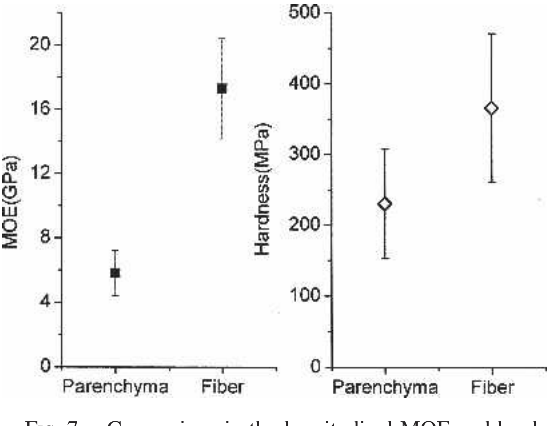

FIG. 7. Comparison in the longitudinal MOE and hardness of the cell walls between bamboo fibers and parenchyma cells.

SUMMARY AND CONCLUSIONS

In this study, a novel in-situ imaging nanoindentation technique was used to investigate the cell-wall mechanical properties of bamboo fibers and parenchyma cells. It was found in-situ imaging enabled the user to find local microzones suitable for nanoindentation though the macro roughness of the sample may be too high. This allows the user to greatly simplify sample preparation. Another advantage of in-situ imaging is that the user can readily identify the indentation location.

The following conclusions were obtained with in-situ imaging nanoindentation. First, neither "sinking-in" nor "piling-up" was found around the indentations in the bamboo fiber cell walls. Second, plastic deformation was the main component when a tip was indented into the cell wall of bamboo fibers in the longitudinal direction. Conversely, elastic deformation was dominant in the transverse direction. Third, the longitudinal MOE of bamboo fibers was higher than the transverse MOE. But no significant difference was found between longitudinal and transverse hardness. Finally, the longitudinal MOE and hardness of parenchyma cell were 5.8 GPa and 230 MPa. This corresponds to 33% and 63% of the corresponding values for bamboo fibers. It is concluded that nanoindentation is capable of effectively characterizing the mechanical difference of bamboo at the cellular level though it may significantly underestimate the cell-wall longitudinal MOE of bamboo fibers. It is suggested that the present theory of nanoindentation based on the assumptions of isotropy and homogeneity be revised for better application to anisotropic biomaterials such as wood and bamboo.

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