MECHANICAL PROPERTIES OF SILICA CELLS IN BAMBOO MEASURED USING IN SITU IMAGING NANOINDENTATION

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Abstract. An in situ imaging nanoindentation technique was applied to measure the cell wall mechanical properties of silica cells, as well as pure biogenic silica, in Moso bamboo (*Phyllostachys pubescens* Mazel). For comparison, the mechanical properties of thick-walled epidermal cells close to these silica cells, as well as bamboo fibers, were also measured. The silica cells were found to have a high cell wall hardness of about 1 GPa, nearly two times that of the values observed for the other two types of cells. Furthermore, we found that biogenic silica had hardness as high as 2.68 GPa. This explains why silica cells have such high hardness. Conversely, bamboo fibers showed the highest values for indentation modulus, nearly two times greater than that of the other two cell types. This implies silica cells and epidermal thick-walled cells had a large microfibrillar angle compared with bamboo fibers. The results of this study imply that silica cells with exceptionally high hardness may be regarded as a model nanocomposite with cell wall polymers reinforced with SiO_2 nanoparticles. More research is needed on silica cells because they might provide inspiration for the development of innovative SiO_2-wood composite products with both high hardness and low production costs.

Keywords: Bamboo, biogenic silica, cell wall, nanoindentation, silica cells.

INTRODUCTION

Silica is commonly present in many plants, especially in gramineous plants such as rice, barley, and bamboo (Li et al 2006; Ma 2010). Silicic acid or silicates are taken up from soils and then deposited in the lumen and/or cell wall as amorphous silica (Marafon and Endres 2013). The presence of silica in plant tissues has several positive impacts on plants, such as imparting them with improved fungal resistance and antiherbivore defenses (Yoshida et al 1962; Massey et al 2006; Marafon and Endres 2013). The formation of silica in plants can be considered as a kind of biomineralization that occurs commonly in nature.

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In recent years, because of its mild synthesis process and lack of need for high temperature, high pressure, and harsh reaction conditions, biomineralization has aroused great interest among researchers (Mann and Ozin 1996; Gröger et al 2008). Although the deposition, distribution, and physiological functions of silica in plants have been extensively investigated (Motomura et al 2004) and the extraction of silica from natural sources has also been examined (Kow et al 2014; Vaibhav et al 2015), their potential significance for use in bionic applications has yet to be fully explored.

Bamboo is well known for its high silicon content compared with wood species. Most of this silicon is deposited in the epidermis in the form of silica cells (Liese 1985; Lux et al 2003). More recently, Wang et al (2007) observed nanostructured column-like silica in bamboo epidermis. This led us to believe that the walls of silica cells may be able to serve as a model for nanocomposites, in which the biopolymer is reinforced with nanoscale silica. However, no information is available on the mechanical properties of silica cell walls, nor biogenic silica, which is found in the cavities of these cells.

Nanoindentation is a powerful technique that can directly characterize the mechanical properties of materials at the micron and even nanoscale. Although this technique is theoretically most suitable for isotropic materials, it has also found valuable applications for several anisotropic plant cell walls, including softwood tracheids (Wimmer et al 1997), bamboo fibers (Yu et al 2011), and crop stalks (Wu et al 2010). However, we found that no studies had been conducted on the silica cells in bamboo. This is primarily because it is difficult to locate the silica cells in bamboo under the stereo light microscope attached to a nanoindenter. Second, it is very hard to get a very smooth cross section of silica cells for nanoindentatuon because silica cells are located in the hardest epidermal region of bamboo. Therefore, in this study, we used in situ imaging nanoindentation to locate and measure the indentation modulus and nanohardness of silica cells in mature Moso bamboo (*Phyllostachys pubescens* Mazel ex H. de Lebaie). For comparison, bamboo fibers, the most important cell component in bamboo, as well as the epidermal elongated thick-walled cells that are close to the silica cells, were also tested with the same technique.

EXPERIMENTAL PROCEDURES

Material and Methods

Sample preparation. Four-yr-old Moso bamboo, harvested from a bamboo plantation located in Zhejiang Province, China, was used to prepare the test samples. Matchstick-sized samples (2 $\times$ 2 mm in cross section) were randomly prepared from a culm section located approximately 2 m above ground level. The stick samples contained all main cell types found in bamboo, including fibers, parenchyma cells, silica cells, and epidermal elongated thick-walled cells. Wimmer et al (1997) gives more details on the preparation procedure of nanoindentation samples. An ultramicrotome (EM UC6, Leica, Germany) equipped with a diamond knife was used to obtain a very smooth surface for indenting.

In situ nanoindentation test. It is difficult to locate silica cells in bamboo with a conventional nanoindenter that only relies on an inbuilt reflected light microscope to find and locate indenting positions. In this study, the nanoindentation test was performed by an in situ imaging nanoindenter (Triboindenter, Hysitron, Eden Prairie, MN). During the test, an epidermal region that might contain silica cells was first selected with a light microscope attached to the instrument and then raster scanned with a Berkovich tip with a radius of less than 100 nm to obtain a high magnification image of the selected region. Silica cells and the biogenic silica can be located if enough locations in the epidermal region were imaged. The target peak load and loading-unloading rate were 250 μN and 50 $\mu N \cdot s^{-1}$, respectively. During all the tests, the sample chamber was kept at 23 $\pm$ 1°C and 40% RH. Two bamboo samples were tested and more than 15 indents were performed for silica cells, biogenic silica, and epidermal cells, whereas more than 50 indents for bamboo fibers were performed. The theoretical basis of nanoindentation was well described in several classic references (Oliver and Pharr 1992; Pharr et al 1992; Wimmer et al 1997).

SEM and EDAX. The morphologies of fibers, silica cells, and epidermal cells in Moso bamboo were observed through an environmental scanning electron microscope (SEM) coupled with X-ray microanalysis (ESEM XL 30, FEI, Hillsboro, OR). Energy-dispersive X-ray analysis (EDAX) was also performed to confirm the existence of silica cells.

RESULTS AND DISCUSSION

Figure 1 shows the optical micrograph of bamboo and SEM micrographs of the fibers, silica cells, and epidermal thick-walled cells found in Moso bamboo, as well as the EDAX analysis of a silica cell. These cells belong to two different systems, namely the epidermis system and the vascular bundle system. The vascular bundle system contains fibers, vessels, and sieve tubes, whereas the epidermal system, which is the outmost layer of bamboo, comprises several different cell types, including elongated thick-walled cells and silica cells. There are major anatomical differences among these cell types. The bamboo fibers are long thick-walled polyamellate cells with tapered ends, whereas silica cells are short and located close to elongated cells. The high Si signal in the energy-dispersive X-ray spectroscopy (EDS) analysis indicated the high silica content in the epidermal zone of bamboo.

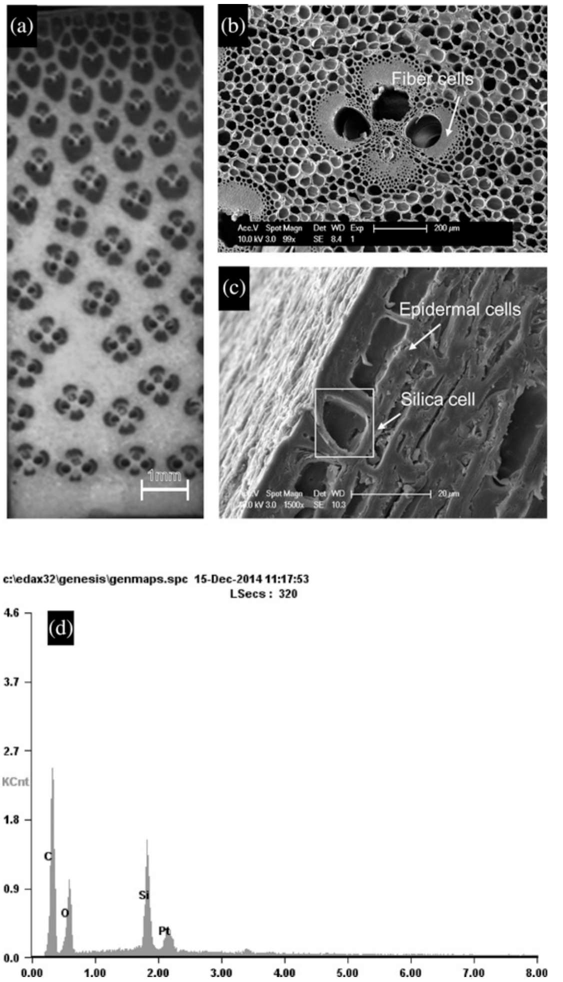

Figure 1. Cross section of (a) Moso bamboo, (b) its main cell type bamboo fibers, and (c) longitudinal sections of silica cells and epidermal elongated thick-walled cells located at the outmost layer of the bamboo. (d) Energy-dispersive X-ray analysis indicates the high silicon content of silica cells.

The typical load-displacement curves of the nanoindenation tests conducted on the three selected cell types from bamboo are shown in Fig 2. The nanoindenation curve of the silica cell wall displayed great elastic recovery, with more than 75% deformation elastically recovered. In contrast, the fiber and epidermal elongated cells displayed a high ratio of residual plastic deformation after load removal. That is to say, more than 60% indentation deformation for both bamboo fiber and epidermis cell remained. These differences in performance can be attributed to the deposition of silica in the silica cell wall. Figure 3 presents the load-displacement curves of biogenic silica deposited in the cell cavity of silica cells. A great elastic recovery after loading removal was clearly observed for biogenic silica. Similar behaviors were also observed in the unloading curve of fused silica or silica glass (Shorey et al 1998; Suzuki et al 2002). Therefore, the existence of silica in the cell wall significantly changes its elastic-plastic response during an indentation test.

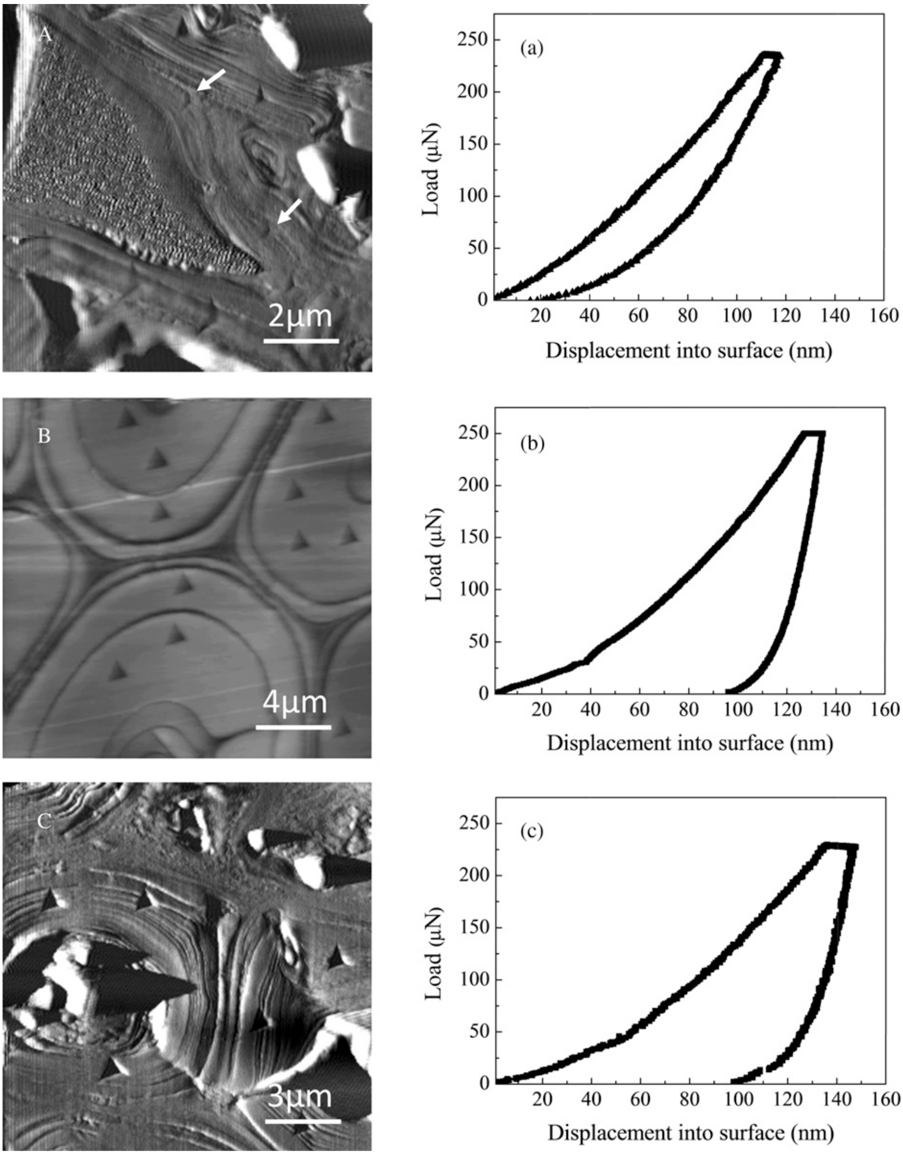

Figure 2. Residual indentations in the walls of different cell types ([A] silica cell, [B] bamboo fiber, [C] epidermal elongated cell) and the corresponding typical load-depth curves ([a] silica cell, [b] bamboo fiber [c] epidermal elongated cell).

The indentation modulus and hardness of the three types of cell walls and biogenic silica in bamboo are given in Table 1. The average hardness of bamboo fiber cell walls was found to be 0.49 GPa, whereas the epidermal elongated cell walls had an even lower hardness of 0.38 GPa. Interestingly, the silica cell walls had a hardness as high as 0.96 GPa, more than two times that of the adjacent epidermal cells. In terms of the indentation modulus, bamboo fibers exhibited an average value of 20.9 GPa, which agreed well with the previous results of 22.8 GPa (Meisam and Yang 2014) and roughly doubled the values recorded for silica cells (9.60 GPa) and the epidermal elongated cell walls (10.70 GPa). The previous results indicate that the deposition of

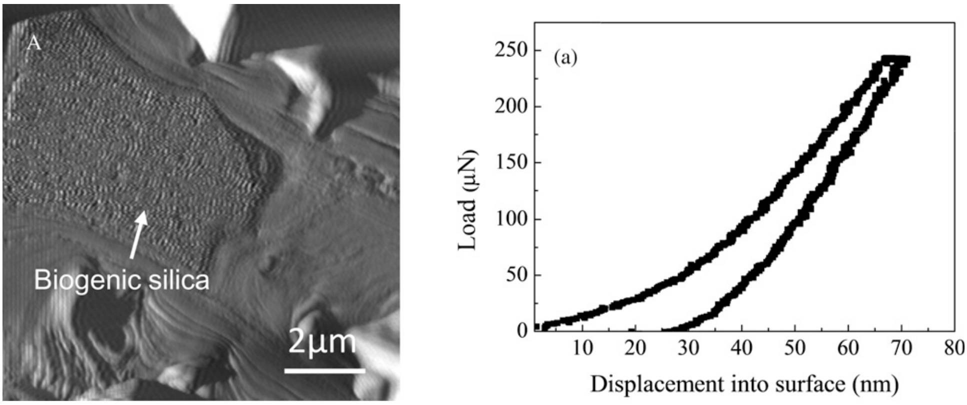

Figure 3. (A) Residual indentations in the pure biogenic silica and (a) the corresponding typical load-depth curves.

silica can significantly improve the hardness of plant cell walls, whereas elastic modulus is hardly affected. This difference can be explained by the nanoindentation results of biogenic silica, which have an average elastic modulus of 25.2 GPa and a hardness of 2.86 GPa, values that are respectively two and seven times those of the epidermal elongated cells. This explains why silica cells have an exceptionally high value in hardness but not in modulus. Conversely, the longitudinal indentation modulus of bamboo fibers was about twice as large as those of the other two tested cells. It is well known that the longitudinal elastic modulus of plant cell walls is mainly dependent on their microfibril angle (MFA) (Cave 1969; Page et al 1977). This could imply that bamboo fibers have much lower MFA than those found in the other two types of cells. Additionally, both the modulus and hardness of the biogenic silica in bamboo are much lower than those of fused quartz.

The formation of silica in bamboo can be considered as a kind of biomineralization that occurs commonly in nature. The chemical mechanism of biomineralization is of significant value for the preparation of nanostructured composites. This study demonstrates that silica cells in bamboo can act as a model composite, which may provide inspiration for the fabrication of new, advanced wood-SiO_2 composites at ambient temperatures.

CONCLUSION

The mechanical properties of silica cells and biogenic silica in bamboo were determined using a nanoindentation technique. Silica cell walls were found to exhibit excellent hardness and high levels of elastic recovery. They had a hardness of about 1 GPa, nearly two times the value of that recorded for the other two types of tested cells. Furthermore, biogenic silica was found to have a hardness of as high as 2.68 GPa, which explains the high level of hardness typically observed for silica cells. Significantly, this study highlights that silica cells can be regarded as a model nanocomposite, in which cell wall polymers are reinforced by SiO_2 nanoparticles. Therefore, more

Table 1. Indentation modulus and hardness of three types of cell walls and biogenic silica in Moso bamboo.

	MOE (GPa)		Hardness (GPa)	
Cell types	Mean	SD	Mean	SD
Silica cells	9.60	0.75	0.96	0.15
Fiber cells	20.90	1.46	0.49	0.03
Epidermal cells	10.70	2.30	0.38	0.08
Biogenic silica	25.20	2.68	2.86	0.33
Fused quartz[a]	69.90	—	9.25	—

[a] The data of fused quartz quoted technical manuals of Hysitron tribolndenter.

research is needed on silica cells in plants, because these might hold the key to the development of novel SiO_2-wood composites that combine high performance with low production costs.

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