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ORIGINAL ARTICLE

Comparative study of morphological changes in hardwoods treated with the ionic liquid, 1-ethyl-3-methylimidazolium chloride

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Abstract Three hardwoods of varying vessel arrangement were treated with the ionic liquid, 1-ethyl-3-methylimidazolium chloride ([C2mim][Cl]), which can dissolve cellulose, to investigate its influence on wood tissue morphology. Characterization was carried out by light and scanning electron microscopy. The wood fibers of all species swelled significantly during [C2mim][Cl] treatment. The swelling behavior varied according to wood species and differed from other cell types such as ray parenchyma cells. Morphological changes of the pits also varied between wood species. Treatment with [C2mim][Cl] affects wood tissues differently depending on wood species and cell type. These differences are not due to the vessel arrangement and its presence, but possibly from the chemical component and the microfibril angle of various wood tissues.

Keywords Hardwood · Ionic liquid · Light microscopy · Scanning electron microscopy · 1-Ethyl-3-methylimidazolium chloride

Introduction

Great benefits and significant developments have been derived from science and technology in recent years. However, energy and environmental problems such as global warming from greenhouse gas emissions and fossil resource exhaustion are of concern. To help resolve these issues, woody biomass is being considered as a substitute to fossil resources. Woody biomass is carbon neutral, available in massive amounts, and is inedible so it does not conflict with food resources. A number of conversion technologies such as acid hydrolysis [1], enzymatic saccharification [2], fast pyrolysis [3], and the use of supercritical fluids [4] have been studied. However, improvements are required to establish these as effective technologies.

Recently, ionic liquid treatment has been investigated as a new biomass conversion technology. Ionic liquid is the general term for organic salts that exists as liquid at a relatively low temperature (<100 °C). They have many unique characteristics, such as non-volatility, non-flammability, and chemical and thermal stability. They are also highly soluble and reusable [5] and have therefore raised worldwide interest as a green solvent.

A number of studies on application of ionic liquids to cellulose [6] have been reported after the discovery that some ionic liquids can dissolve cellulose [7]. Recent studies have revealed that wood can also be liquefied in some ionic liquids [5, 8, 9] and the chemical and morphological reaction behaviors of wood in ionic liquid have been researched. Wood components are depolymerized during ionic liquid treatment [10]. Softwood and hardwood show different liquefaction behavior in ionic liquids because of differences in the lignin chemical structure [11]. The reaction atmosphere influences wood liquefaction, that is, oxygen and humidity enhance the depolymerization of wood components [12].

Observations of morphological changes in wood tissues during ionic liquid treatment have been carried out. Laser scanning fluorescence microscopy revealed that the cell walls swell toward the lumen side during ionic liquid 1-*n*-ethyl-3-methylimidazolium acetate treatment at room

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temperature [13, 14]. In our previous paper where observations were conducted by polarizing microscopy, the ionic liquid, 1-ethyl-3-methylimidazolium chloride ([C2mim][Cl]), was found to destroy the crystalline structure of cellulose in wood with treatment time although the wood tissue shape was retained [15]. Light and scanning electron microscopy (SEM) studies have shown that the wood tissue of the latewood of *Cryptomeria japonica* became distorted and dissociated significantly compared with those in earlywood [15, 16]. Furthermore, the morphological changes in wood after ionic liquid treatment were different between soft- and hardwoods [17]. Hardwood morphology, such as vessel arrangement and cell wall thickness, varies between species, and hardwoods contain many types of cells compared with softwoods. The reactivity of ionic liquids with wood must be affected by anatomical differences. In this paper, therefore, we conducted a comparative study on the morphological changes in hardwood tissues of various species with characteristic tissue forms after treatment with the ionic liquid, 1-ethyl-3-methylimidazolium chloride.

Materials and methods

Samples and chemicals

Three hardwood samples, *Quercus glauca* (radial-porous wood), *Quercus mongolica* (ring-porous wood) and *Trochodendron aralioides* (non-porous wood), were cut into block samples [approximately 5(R) × 5(T) × 5(L) mm]. These samples were then extracted with ethanol/benzene (1:2, v/v) for 8 h in a Soxhlet apparatus. The extracted wood was oven dried at 105 °C for 24 h prior to use. 1-Ethyl-3-methylimidazolium chloride ([C2mim][Cl]) was purchased from Tokyo Kasei Kogyo Co. Ltd, Japan.

Treatment with [C2mim][Cl] for light microscopy analyses

Extracted wood samples were cut with a sliding microtome into 15-μm-thick sections and mounted in a 20-μm-deep hemocytometer (Sunlead Glass Corp.). Each mounted section was dried for 2 h at 105 °C before 100 μL of [C2mim][Cl], heated to 120 °C, was dropped onto the mounted section. The hemocytometer was closed with a cover glass immediately thereafter. This was designated as the beginning of the treatment. The hemocytometer was placed in an oven at 120 °C for various time periods and then removed from the oven to examine the anatomical changes in the wood section using light microscopy (BH-2; Olympus). Three areas (cell lumen area, cell wall area, and total of cell lumen area + cell wall area; defined as shown in Fig. 1) were measured for five neighboring cells in late- and earlywood, using image analysis software (Motic Image Plus 2.2S) and the average was calculated for each area.

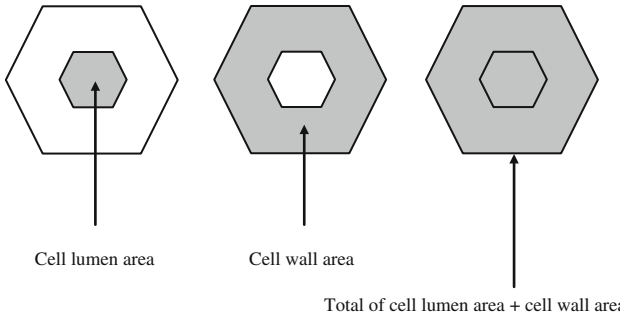

Fig. 1 Definition of cell lumen area, cell wall area and total of cell lumen area + cell wall area

Treatment with [C2mim][Cl] for SEM observations

For SEM observations, extracted wood samples were surfaced with a sliding microtome. The surfaced samples were dried for 24 h at 105 °C and the surfaced area was then treated by dipping into [C2mim][Cl] and heating to 120 °C, for various periods of time. During the dipping treatment, the [C2mim][Cl] was stirred gently with a magnetic stirrer. The treated specimens were dipped into dimethylsulfoxide (DMSO) to remove the [C2mim][Cl] and then washed with distilled water to remove the DMSO. After drying for 24 h at 105 °C, each specimen was mounted on a specimen holder and then Pt-coated. The exposed surface was examined by SEM (JFC-1600; JEOL) at an accelerating voltage of 25 kV. Morphological changes in the wood cells resulting from this high accelerating voltage were not observed.

Results and discussion

Light micrographs of the transverse sections treated with [C2mim][Cl] at 120 °C for 0 and 72 h are shown in Fig. 2. The cell walls of all wood samples (indicated by open arrows or asterisks) swelled significantly after 72 h of treatment (Fig. 2D–F). The cell walls of *Quercus glauca* and *Quercus mongolica* in latewood became slightly disordered and dissociated partially, but in earlywood, were well ordered after 72 h of treatment (Fig. 2d, e). Cracks (indicated by filled arrow) occurred in the vessels of *Quercus mongolica*. On the other hand, the cell walls of *Trochodendron aralioides* in both late- and earlywood were well ordered after 72 h of treatment (Fig. 2f). In our previous studies on the morphological changes by

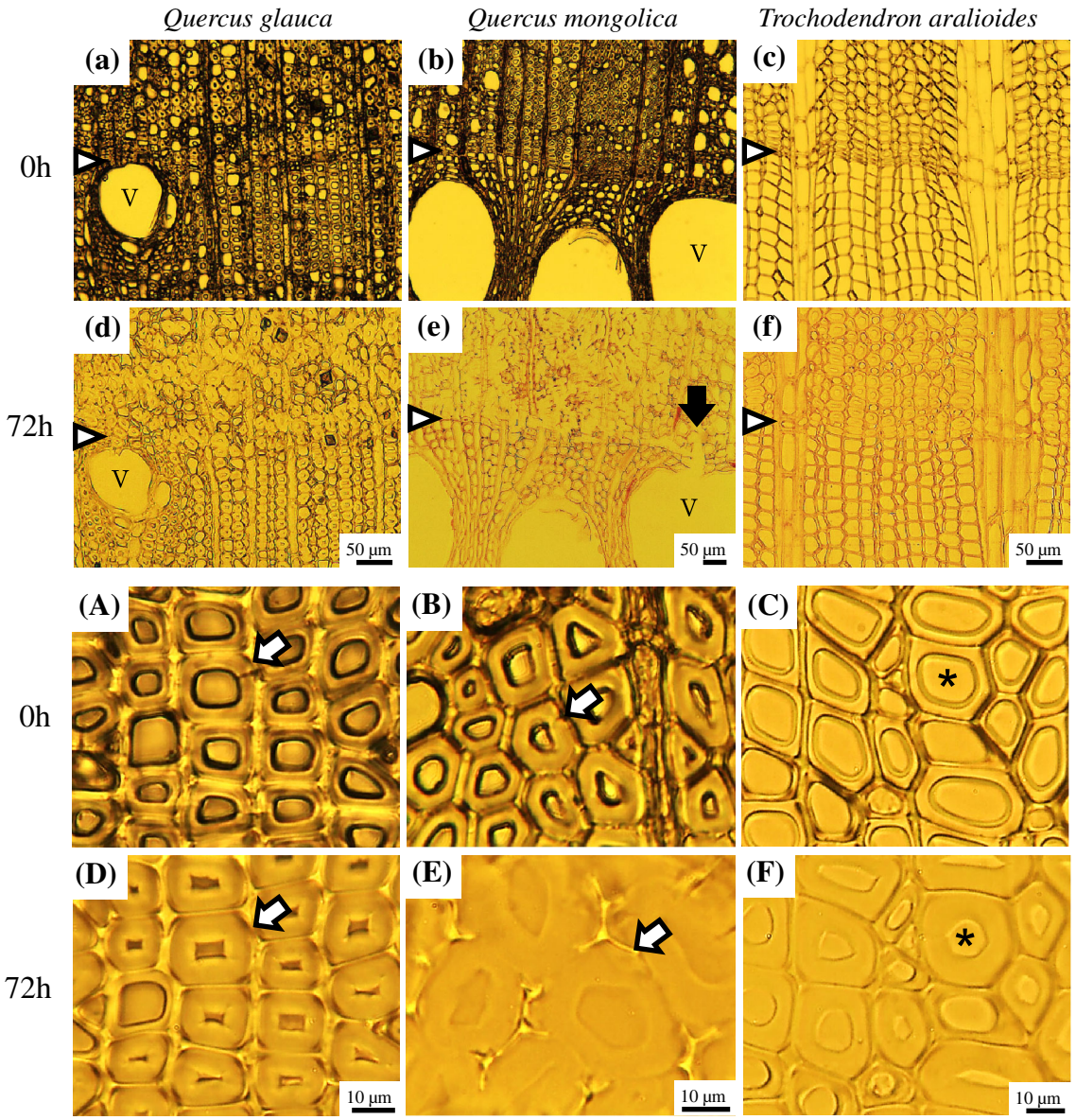

Fig. 2 Light microscopy images of transverse sections before and after treatment for 72 h with [C2mim][Cl] at 120 °C. **a–f** Around annual ring boundary. **A–F** Magnified view of **a–f**, respectively. **a–c**, **A–C** 0 h, **d–f**, **D–F** 72 h. *V* vessel, *open arrowhead* annual ring boundary, *open arrow* wood fiber, *asterisk* tracheid, *filled arrow* crack

[C2mim][Cl] treatment, the cell walls of the tracheids of *Cryptomeria japonica* that is softwood were dissociated in latewood but not in earlywood [15, 16], whereas the wood fibers of *Fagus crenata* that is hardwood only swelled significantly and were not dissociated in both late- and earlywood [17].

To analyze the swelling behavior of wood fibers during [C2mim][Cl] treatment in detail, we used image analysis software to determine the cell lumen area, cell wall area, and total cell lumen area + cell wall area (defined in Fig. 1). Figures 3 and 4 show results obtained for early- and latewood, respectively (Figs. 3c, 4c refer to data in a previous paper [17]). Because *Trochodendron aralioides* is composed of tracheid instead of wood fiber, Figs. 3d and 4d show tracheid swelling behavior. The cell lumen area of wood fibers of all species decreases rapidly, while the cell wall area and the total of cell lumen area + cell wall area of all species except *Trochodendron aralioides* increase significantly in the initial stages of [C2mim][Cl] treatment. Although cell wall area of tracheids of *Trochodendron aralioides* increases significantly, the total of cell lumen area + cell wall area increase to some extent. Thereafter, the cell wall area and total of cell lumen area + cell wall area of the *Quercus mongolica* in both late- and earlywood

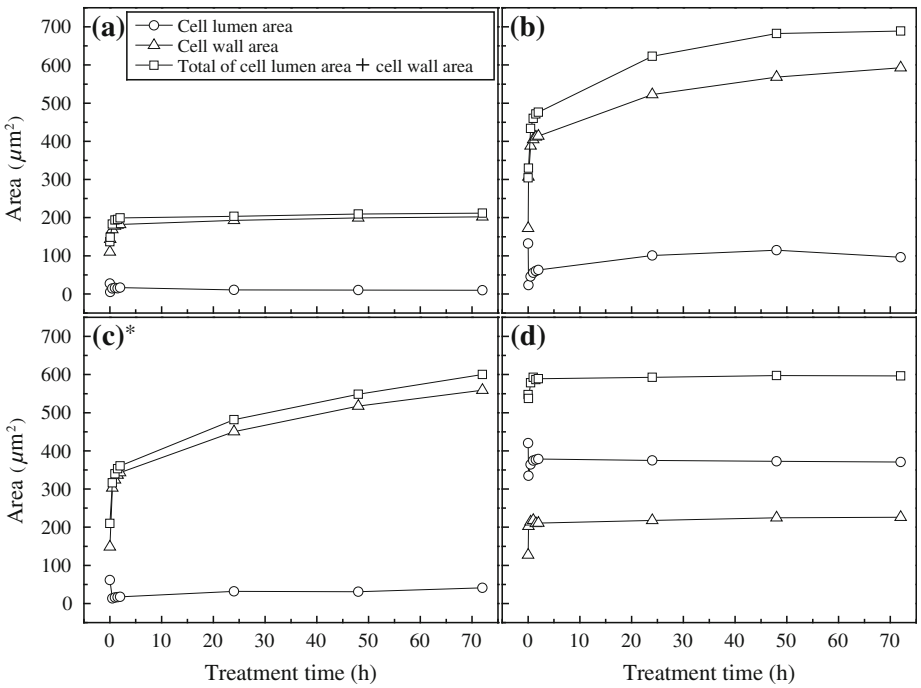

Fig. 3 Changes in cell wall area, cell lumen area, and total of cell lumen area + cell wall area for wood fiber of *Quercus glauca* (**a**), *Quercus mongolica* (**b**), *Fagus crenata* (**c**), and tracheid of *Trochodendron aralioides* (**d**) in earlywood during [C2mim][Cl] treatment. *Asterisk* refers to data in previous paper [17]

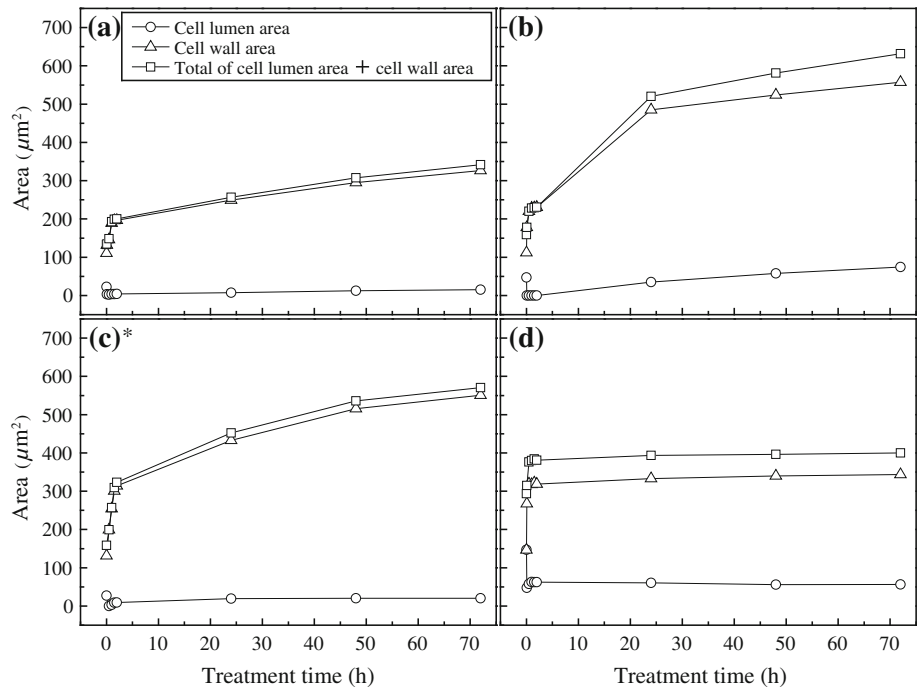

Fig. 4 Changes in cell wall area, cell lumen area, and total of cell lumen area + cell wall area for wood fiber of *Quercus glauca* (**a**), *Quercus mongolica* (**b**), *Fagus crenata* (**c**), and tracheid of *Trochodendron aralioides* (**d**) in latewood during [C2mim][Cl] treatment. *Asterisk* refers to data in previous paper [17]

increase gradually with prolonged treatment time like *Fagus crenata*. After 72 h of treatment, the cell wall areas in late- and earlywood had increased by 5 and 3.5 times, respectively. The swelling behavior of the other two species differs from *Quercus mongolica* and *Fagus crenata*. After changes in the initial stages, the cell wall area and total cell lumen area + cell wall area of *Quercus glauca* in the latewood increase gradually with prolonged treatment time, but level off in earlywood. Meanwhile, those of *Trochodendron aralioides* level off in both late- and earlywood. After 72 h of treatment, the cell wall areas of *Quercus glauca* in late- and earlywood had increased by 3 and 2 times, and that of *Trochodendron aralioides* in late- and earlywood had increased by 2 and 1.5 times, respectively. The swelling behavior such as swelling ratio and time-dependent change of wood fibers during [C2mim][Cl]

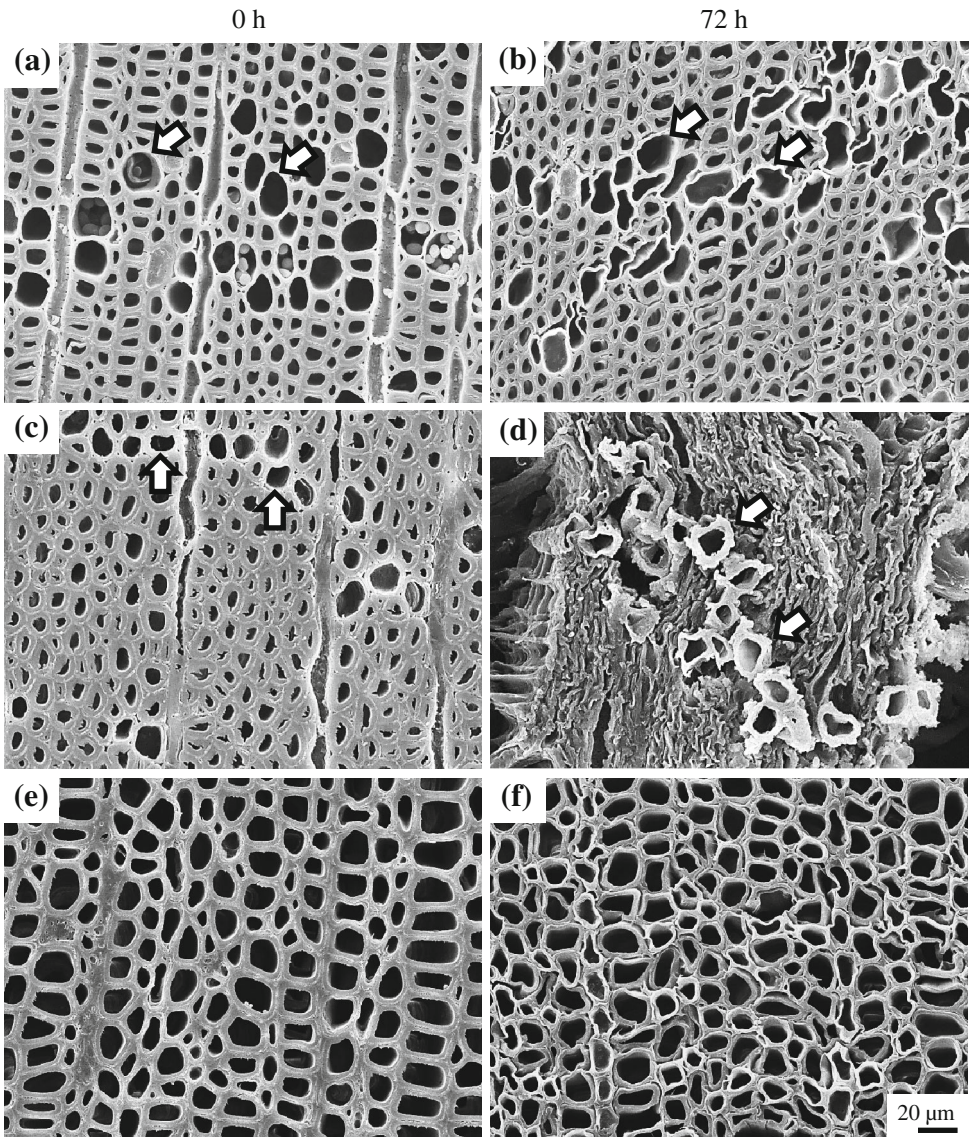

Fig. 5 SEM images of transverse section before and after treatment for 72 h with [C2mim][Cl] at 120 °C. *Top*: *Quercus glauca*, *middle*: *Quercus mongolica*, *bottom*: *Trochodendron aralioides*. *Left* 0 h, *right* 72 h, *arrow* axial parenchyma cell

treatment therefore differs according to wood species and region such as late- and earlywood.

Figure 5 shows SEM images of transverse sections before and after treatment for 72 h with [C2mim][Cl] at 120 °C. The adjacent cell walls of wood fiber of *Quercus glauca* and *Trochodendron aralioides* are somewhat dissociated, and the axial parenchyma cells of *Quercus glauca* are distorted (Fig. 5b, f). Axial parenchyma cells of *Trochodendron aralioides* could not be found after [C2mim][Cl] treatment, because they are present in small amounts only in latewood. Conversely, the cell walls of the wood fiber of *Quercus mongolica* were collapsed significantly, but the axial parenchyma cells (indicated by arrows) maintained their shape (Fig. 5d). These morphological changes could not be found in light microscopy images. It is necessary to dry the samples as pretreatment for SEM observation. The morphological changes such as dissociation and distortion in SEM images are due to shrinkage of the cell walls which swelled by [C2mim][Cl] during drying process. The swelling ratio of the wood fibers of *Quercus mongolica* was higher than the other two species as in Figs. 3 and 4. Therefore, the degree of cell wall shrinkage during drying increased and they showed remarkable deformation. The reasons for the difference in morphological changes between the wood fiber and axial parenchyma cells of *Quercus mongolica* are unclear but result from differences in chemical composition and tissue structure of the two kinds of cells. As the one reason, there is difference in chemical composition. The lignin concentration of the axial parenchyma cells is reported to be higher than that of the wood fibers [18]. In our previous studies on the liquefaction of wood [10, 11], we found that lignin has a higher resistance to [C2mim][Cl] than cellulose and hemicellulose. Thus, the reactivity of the axial

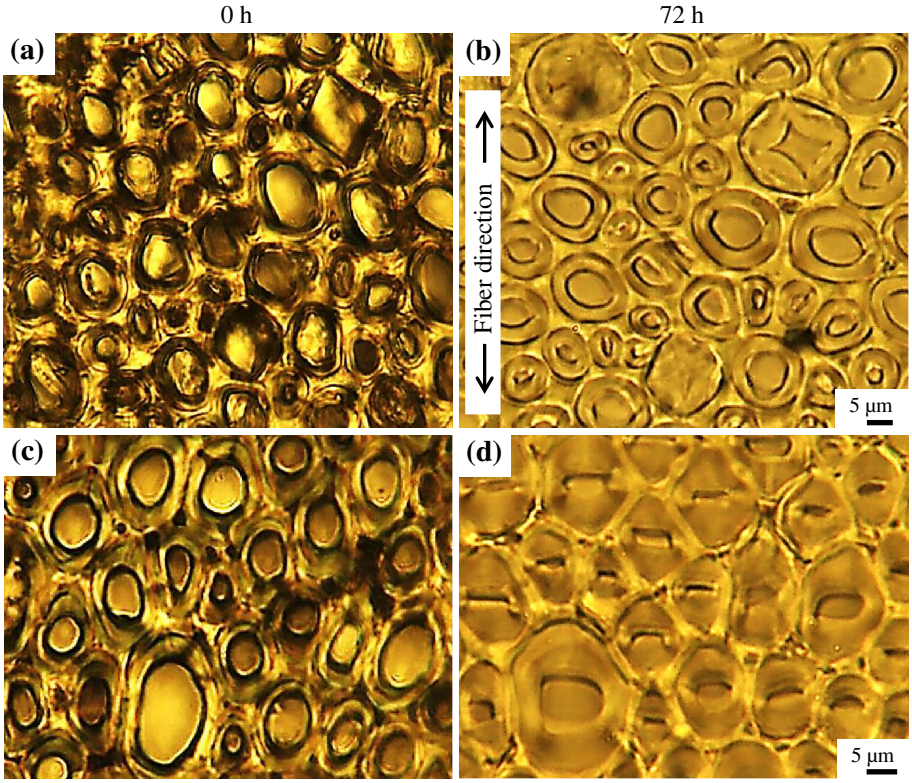

Fig. 6 Light microscopy images of ray parenchyma before and after treatment for 72 h with [C2mim][Cl] at 120 °C. *Top*: *Quercus glauca*, *bottom*: *Quercus mongolica*. *Left* 0 h, *right* 72 h

Table 1 Swelling ratio of cell wall area and thickness of ray parenchyma cells after treatment for 72 h with [C2mim][Cl] at 120 °C

Wood samples	Cell wall area	Cell wall thickness	
		Fiber direction	Tangential direction
Quercus glauca	1.32	1.21	1.20
Quercus mongolica	1.41	1.66	1.33
Trochodendron aralioides	1.39	1.34	1.39
Fagus crenata	1.49	2.02	1.42

Swelling ratio = cell wall area or cell wall thickness after treatment/cell wall area or cell wall thickness before treatment

parenchyma cells is thought to be lower than that of wood fibers. As another reason, there is difference in tissue structure. The axial parenchyma cells are separated into compartments by end walls. Because of the presence of the end walls, the cell walls of the axial parenchyma can resist the force perpendicular to the fiber axis. The morphological changes are therefore different from the wood fibers and the axial parenchyma cells.

Figure 6 shows light micrographs of ray parenchyma cells before and after treatment with [C2mim][Cl] at 120 °C for 0 and 72 h. The lumen of ray parenchyma cells of *Quercus glauca* (Fig. 6b) and *Trochodendron aralioides* (data not shown) maintained their round shape after treatment for 72 h, whereas that of *Quercus mongolica* became elliptical (Fig. 6d) like that of Japanese beech [17].

To study the swelling behavior of ray parenchyma cells during [C2mim][Cl] treatment in detail, we used image analysis software for determining the cell wall area and thickness in the fiber and tangential direction. Results obtained as a swelling ratio calculated from the following equation are shown in Table 1.

Swelling ratio = cell wall area or cell wall thickness after treatment/cell wall area or cell wall thickness before treatment.

After treatment for 72 h, the cell wall area of the ray parenchyma cells of all species increased by approximately 1.3–1.5. The cell wall area of the wood fibers increased significantly with the exception of the earlywood of *Trochodendron aralioides* as in Figs. 3 and 4. Therefore, in general, the degree of swelling for the ray parenchyma cells is lower than the wood fibers. The differential shrinkage and swelling by moisture are attributed mainly to the cell wall structure [19]. Swelling of the cell walls is mainly caused by swelling of the amorphous region between microfibrils, and bundles of the microfibrils swell extensively in the perpendicular direction. The lower the microfibril angle for the longitudinal cell axis, the higher the swelling perpendicular to the longitudinal cell axis. In wood fibers, the middle layer of the secondary wall (S_2) that is usually oriented 5–20° to the fiber axis occupies a

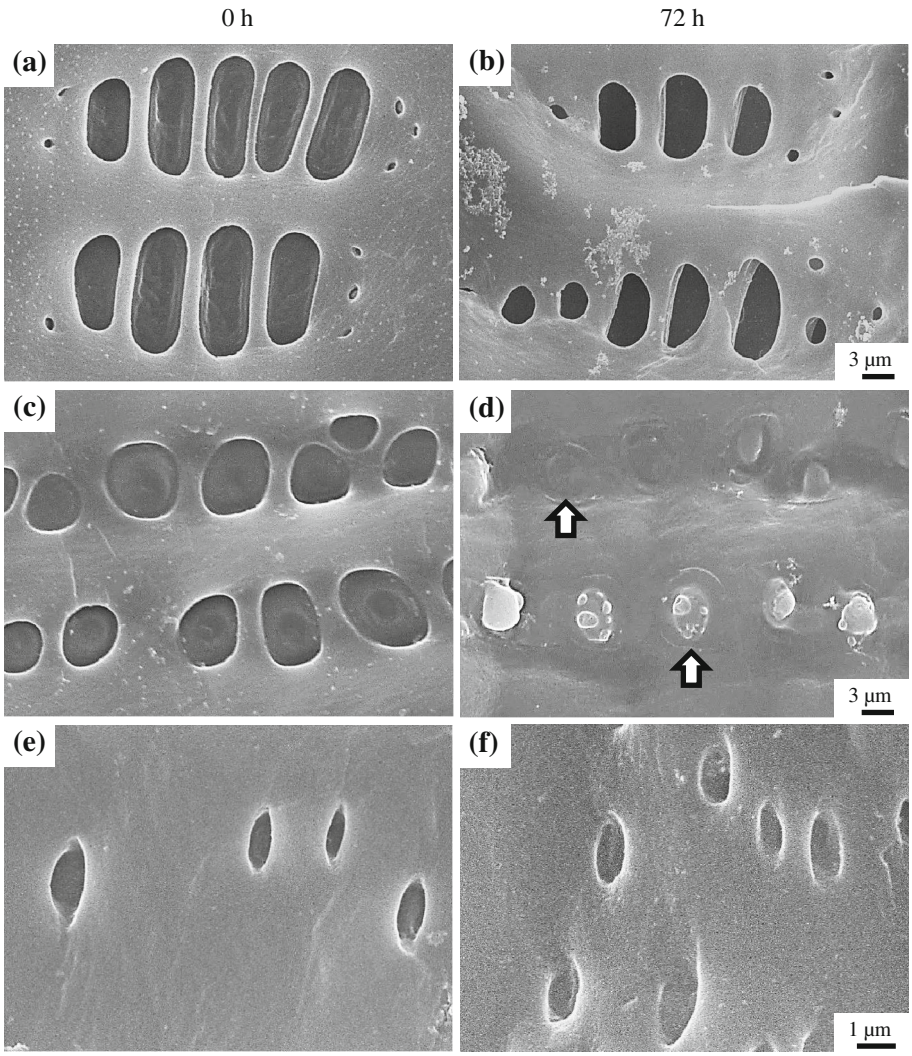

Fig. 7 SEM images of various pits before and after treatment for 72 h with [C2mim][Cl] at 120 °C. *Top* ray-vessel pits of *Quercus glauca*, *middle* ray-vessel pits of *Quercus mongolica*, *bottom* ray–ray pits of *Quercus glauca*. *Left* 0 h, *right* 72 h

large area [20]. In ray parenchyma cells, the inner (S_1) and outer (S_3) layers of the secondary wall that are oriented 30–60° to the longitudinal cell axis are thicker than the other layers [21]. Therefore, the occupancy rate and microfibril angle of their thickest layers affect their swelling behavior significantly and the wood fibers may swell more than the ray parenchyma cells during [C2mim][Cl] treatment. Other causes for the difference in swelling ratio may be the difference in chemical composition. The lignin concentration of ray parenchyma cells is reported to be 1.5 times higher than that of wood fibers [22]. Ray parenchyma cells react with difficulty with [C2mim][Cl]. The cell wall thickness of *Quercus glauca* and *Trochodendron aralioides* increased in the fiber and tangential direction in almost the same ratio while that of *Quercus mongolica* and *Fagus crenata* increased significantly in the fiber direction in comparison with the tangential direction after [C2mim][Cl] treatment.

Figure 7 shows SEM images of various pits before and after treatment for 72 h with [C2mim][Cl] at 120 °C. Ray-vessel pit membranes of *Quercus glauca* were broken after 72 h of treatment (Fig. 7b). This is similar to that of *Fagus crenata*, which has been reported previously [17]. However, the ray-vessel pit membranes of *Quercus mongolica* protrude towards the lumen side of the vessel without being broken (indicated by arrows in Fig. 7d). This suggests that the ray-vessel pit membranes of *Quercus mongolica* are hardly liquefied by [C2mim][Cl]. No change in ray–ray pits was observed for all species studied in this paper (results for *Quercus glauca* only are shown here Fig. 7f). However, in our previous study, the ray–ray pits of *Fagus crenata* were occluded after 72 h of treatment [17].

Conclusions

The results obtained in this study are summarized in Table 2. Previous studies showed that liquefaction behaviors during [C2mim][Cl] treatment are different between softwood and hardwood [11], and reaction behaviors in

Table 2 Characteristics of morphological change in various types of tissue after treatment for 72 h with [C2mim][Cl] at 120 °C

Wood samples	Wood fiber (swelling ratio*)		Vessel	Ray parenchyma cell	Ray-vessel pit (pit membrane)	Ray–ray pit
	Earlywood	Latewood				
Quercus glauca	2	3	Slightly swelled	Isotropic swelling	Disrupted	Unchanged
Quercus mongolica	3.5	5	Cracked	Anisotropic swelling	Undisrupted	Unchanged
Trochodendron aralioides	1.5**	2**	–	Isotropic swelling	–	Unchanged
Fagus crenata	4	4	Distorted	Anisotropic swelling	Disrupted	Occluded

* Swelling ratio = cell wall area after treatment/cell wall area before treatment

** Swelling ratio of tracheid

wood tissue are also different for each other [15, 17]. This morphological study of various hardwoods after [C2mim][Cl] treatment revealed that the swelling behavior of wood fibers varied among wood species of hardwoods and according to location such as late- and earlywood. The ray parenchyma cells of all species did not swell easily by [C2mim][Cl] treatment compared with wood fibers and two kinds of swelling behavior exist. Furthermore, the pit reaction behavior revealed various differences in wood species and pit type. These morphological changes are not due to the difference in vessel arrangement and its existence, but possibly from the differences in chemical composition and microfibril angle of various wood tissues. The effect of [C2mim][Cl] on wood tissue therefore depends on cell and pit type with characteristic reactions.

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