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ORIGINAL ARTICLE

Morphological changes of Japanese beech treated with the ionic liquid, 1-ethyl-3-methylimidazolium chloride

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Abstract The morphological changes in wood tissues of Japanese beech (*Fagus crenata*) upon treatment with the ionic liquid, 1-ethyl-3-methylimidazolium chloride ([C2mim][Cl]), which can dissolve cellulose, were investigated. Treatment with [C2mim][Cl] induced significant swelling of all wood tissues. However, the swelling behavior of wood fibers was different from that of vessels. Intervascular pits were occluded, and pit membranes in ray-vessel pits were broken after treatment with [C2mim][Cl]. No significant differences in swelling behavior were found between latewood and earlywood, although different morphological changes for latewood and earlywood during [C2mim][Cl] treatment were seen in our previous studies on sugi (*Cryptomeria japonica*). We have found that the effects of [C2mim][Cl] on Japanese beech tissues are inhomogeneous and different from those found for other wood species.

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

Introduction

In recent years, the efficient use of biomass has been one of the most important approaches to mitigate exhaustion of fossil resources and global warming caused by increased emissions of greenhouse gases such as carbon dioxide. Biomass resources have potential as an alternative to fossil resources. Among various types of biomass, wood is a promising resource because it is carbon neutral, available in very large amounts, and does not conflict with food resources. Although many conversion methods such as acid hydrolysis [1], enzymatic saccharification [2], pyrolysis [3, 4] and supercritical fluid treatment [5] have been developed, and effective techniques have not yet been established.

Ionic liquid treatment has been suggested as a new conversion technology for biomass. Ionic liquids are organic salts that exist as liquids at ambient temperature. They have many unique characteristics such as non-volatility, chemical and thermal stability, non-flammability, reusability and so on. Since it was reported that some ionic liquids can dissolve cellulose [6], many studies on application of ionic liquids to cellulose have been carried out [7]. It has been also reported that wood can be liquefied in some ionic liquids [8, 9]. The main wood components, such as cellulose, hemicellulose and lignin, are reported to be liquefied during treatment with ionic liquids, with preferential liquefaction of cellulose and hemicellulose [8, 10]. The crystalline structures of cellulose are destroyed as the reaction proceeds in ionic liquids [10]. In addition, the reaction behavior of wood in ionic liquid is different for each wood species. Japanese beech is more readily liquefied than Western red cedar [11]. A study on the influence of reaction atmosphere revealed that the presence of oxygen and humidity enhances wood liquefaction [12].

Our previous observations [using light microscopy and scanning electron microscopy (SEM)] of morphological changes in wood after ionic liquid treatment showed that wood tissues in latewood of sugi (*Cryptomeria japonica*, a softwood) were distorted and dissociated but tissues in earlywood were not [13, 14]. However, morphological changes of wood tissues from species other than sugi upon

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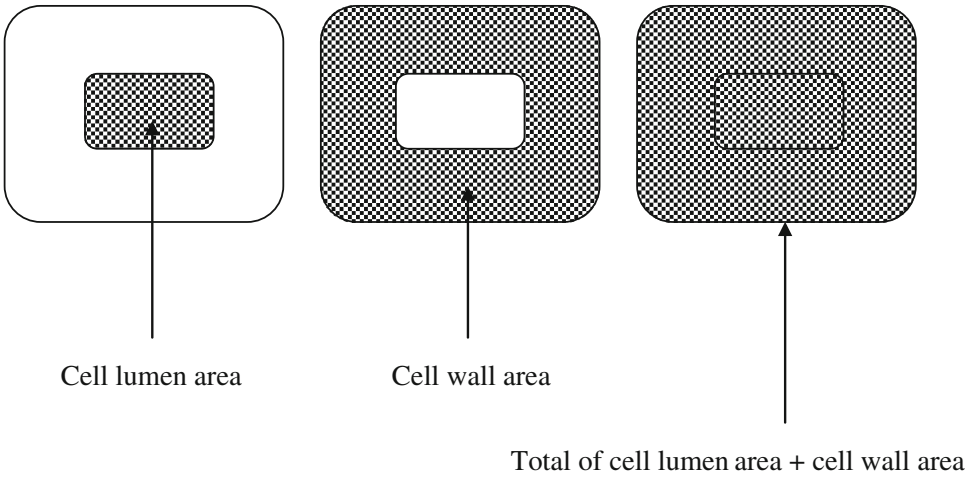

Fig. 1 Definition of cell lumen area, cell wall area and total of cell lumen area + cell wall area

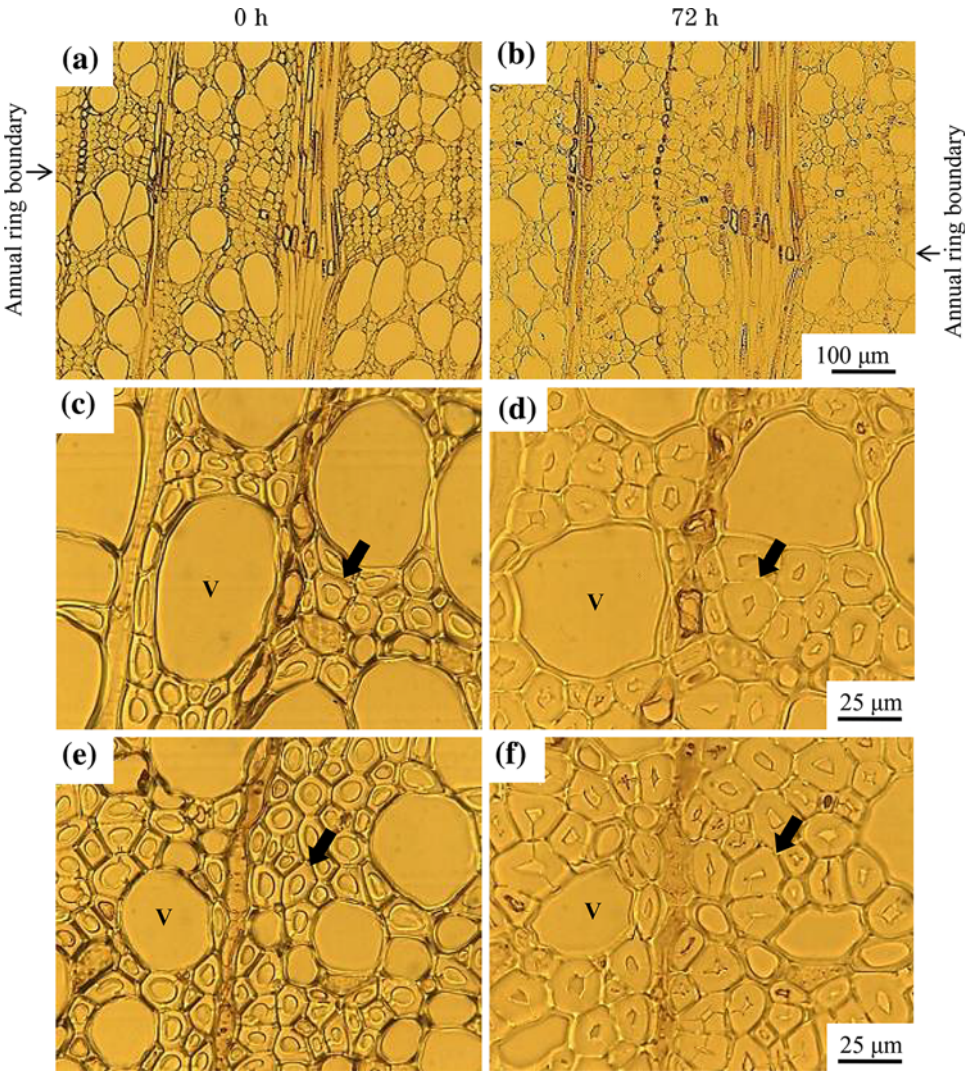

Fig. 2 Light microscopy images of transverse sections before and after treatment with [C2mim][Cl] at 120 °C for 72 h. *Upper*: around annual ring boundary. *Middle*: magnified view of earlywood. *Lower*: magnified view of latewood. *Left*: 0 h; *Right*: 72 h, *V* vessel, *Arrow*: wood fiber

treatment with ionic liquids have not yet been studied. Hardwood is anatomically quite different from softwood. Although the former has various tissues such as vessels and wood fibers, the latter is mainly composed of tracheids. The sizes of these wood tissues differ. These differences are expected to influence the reactions of ionic liquids with

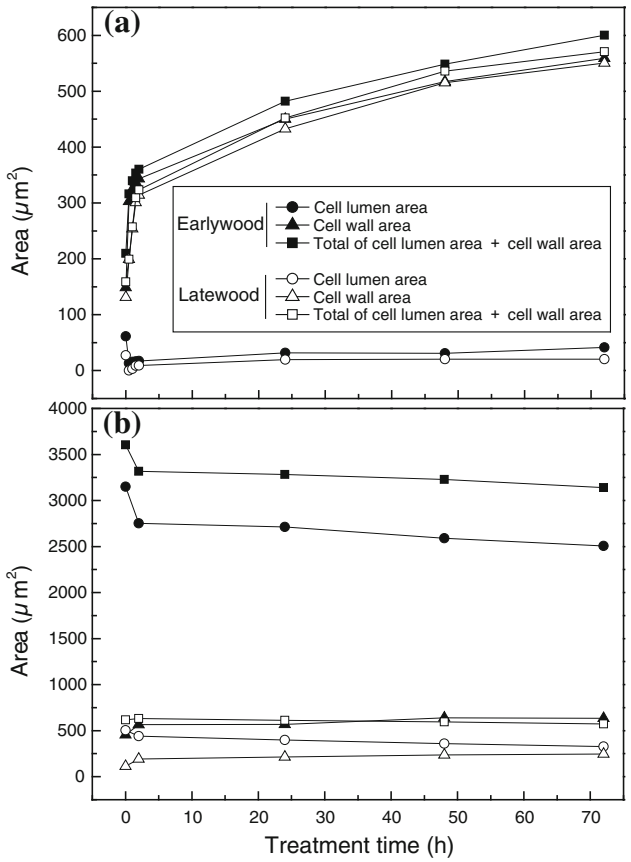

Fig. 3 Changes in cell wall area, cell lumen area and total of cell lumen area + cell wall area during [C2mim][Cl] treatment. **a** Wood fiber. **b** Vessel

the wood. In this paper, therefore, we have investigated detailed morphological changes in wood tissues of the hardwood, Japanese beech (*Fagus crenata*), after treatment with the ionic liquid, 1-ethyl-3-methylimidazolium chloride. To study these morphological changes, we used light microscopy and SEM.

Materials and methods

Samples and chemicals

Japanese beech samples (approx. 5(R) × 5(T) × 5(L) mm) were 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., Japan, Ltd.

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

Extracted wood samples were cut with a sliding microtome into 15-μm thick sections, which were mounted in a 20-μm deep hemocytometer (Sunlead Glass Corp.). Each mounted section was dried for 2 h at 105 °C, then 100 μL of [C2mim][Cl], heated to 120 °C, was dropped onto the mounted section. The hemocytometer was immediately closed with a cover glass; 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 both latewood and earlywood, using image analysis software (Motic Image Plus 2.2S), and the average was calculated for each 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 then the surfaced area was treated by dipping into [C2mim][Cl], heated 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 (JEOL JFC-1600) at an accelerating voltage of 25 kV. Any morphological changes of wood cells due to this high accelerating voltage were not observed.

Results and discussion

Figure 2 shows the light micrographs of the transverse sections treated with [C2mim][Cl] at 120 °C for 0 and 72 h. Significant morphological changes such as dissociation and collapse of wood cells were not observed after 72 h of treatment (Fig. 2b). In our previous study on liquefaction of Japanese beech by [C2mim][Cl] treatment, 95 % of wood were found to be liquefied after treatment at

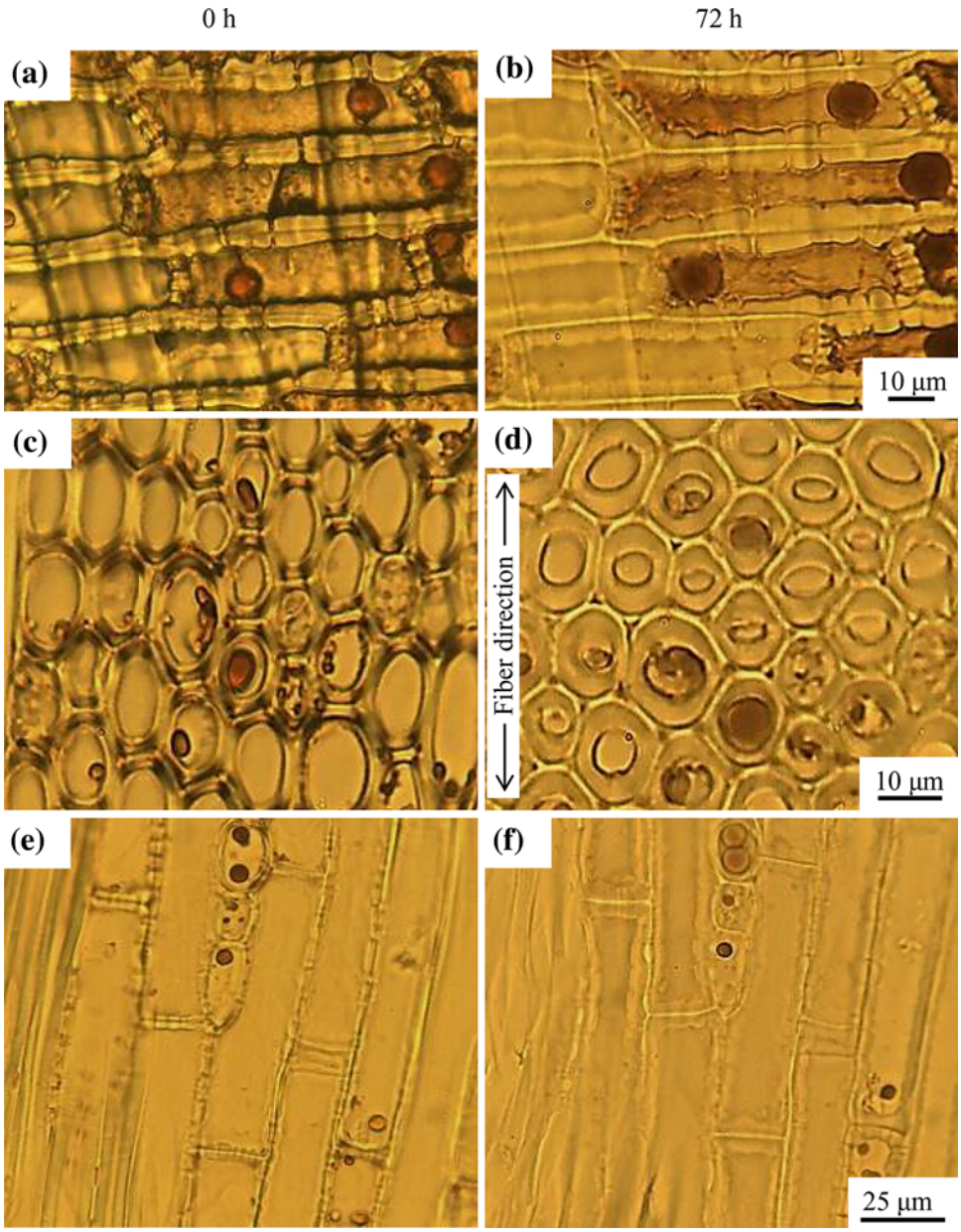

Fig. 4 Light microscopy images of parenchyma cells before and after treatment with [C2mim][Cl] at 120 °C for 72 h. *Top*: ray parenchyma in radial section; *Middle*: ray parenchyma in tangential section; *Bottom*: axial parenchyma. *Left*: 0 h; *Right*: 72 h

120 °C for 24 h. Although the reaction was processed by stirring in our previous experiment, the samples in this study were only soaked in [C2mim][Cl] without stirring. This is reason why wood cells were not liquefied completely as shown in Fig. 2 even after 72 h of treatment. In our previous study on morphological changes produced by [C2mim][Cl] treatment of wood tissues of sugi [14], wood cells in latewood were dissociated, while such dissociation was not seen in earlywood. Thus, the morphological changes of wood cells are different for each wood species. Closer investigation shows that for Japanese beech, the cell walls of wood fibers in both latewood and earlywood (indicated by arrows) swelled significantly (Fig. 2d, f). Although the cell walls of vessels in both latewood and earlywood (indicated by "V") were round in shape at 0 h (Fig. 2c, e), they became distorted after treatment (Fig. 2d, f), but did not show the significant swelling observed for wood fibers. The vessels were compressed by many other swollen tissues, such as the surrounding wood fibers.

To study the swelling behavior of wood fibers and vessels upon [C2mim][Cl] treatment in detail, we used image analysis software to determine the cell lumen area, cell wall area, and total of cell lumen area + cell wall area (defined in Fig. 1). The results obtained are shown in

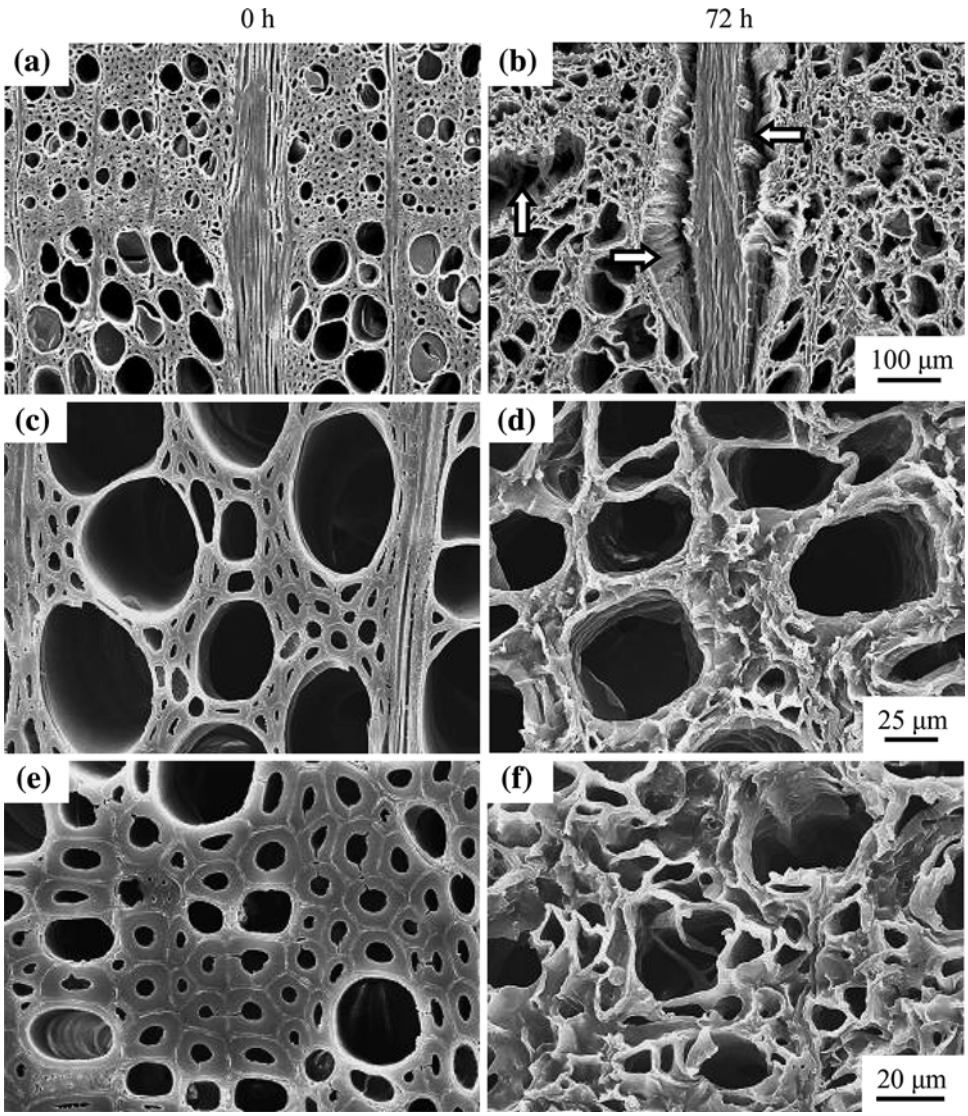

Fig. 5 SEM images of transverse section before and after treatment with [C2mim][Cl] at 120 °C for 72 h. *Top*: boundary of earlywood and latewood; *Middle*: earlywood; *Lower*: latewood. *Left*: 0 h; *Right*: 72 h

Fig. 3. In both latewood and earlywood, the cell lumen area of wood fibers decreases sharply to nearly zero. The cell wall area and the total of cell lumen area + cell wall area increase significantly in the initial stages of [C2mim][Cl] treatment (Fig. 3a). After these changes, the cell wall area and the total of cell lumen area + cell wall area increase much more gradually with prolonged treatment time. At the same time, the cell lumen area increased slightly. After 72 h of treatment, the cell wall areas in both latewood and earlywood had increased by a factor of approximately 4.

For vessels in earlywood, the cell lumen area and the total of cell lumen area + cell wall area initially decrease, while cell wall area increases, during [C2mim][Cl] treatment. After these initial changes, both the cell lumen area and the total of cell lumen area + cell wall area decrease gradually during further [C2mim][Cl] treatment. Meanwhile, the cell wall area increases gradually. These results indicate that cell walls of vessels in earlywood swelled towards the cell lumen without swelling outwards. Similar trends are observed for swelling of vessels in latewood, although the areas are smaller than those for vessels in earlywood. That is, the cell walls swelled toward the cell lumen, and the vessels were compressed by surrounding swollen tissues.

Overall, wood fibers swell much more than vessels. The increase in the total of cell lumen area + cell wall area of wood fibers counterbalances the decrease in the total cell lumen area + cell wall area of vessels that are distributed between wood fibers. This balance prevents dissociation and collapse of wood tissues.

Figure 4 shows light micrographs of ray parenchyma cells (a–d) and axial parenchyma cells (e, f) before and

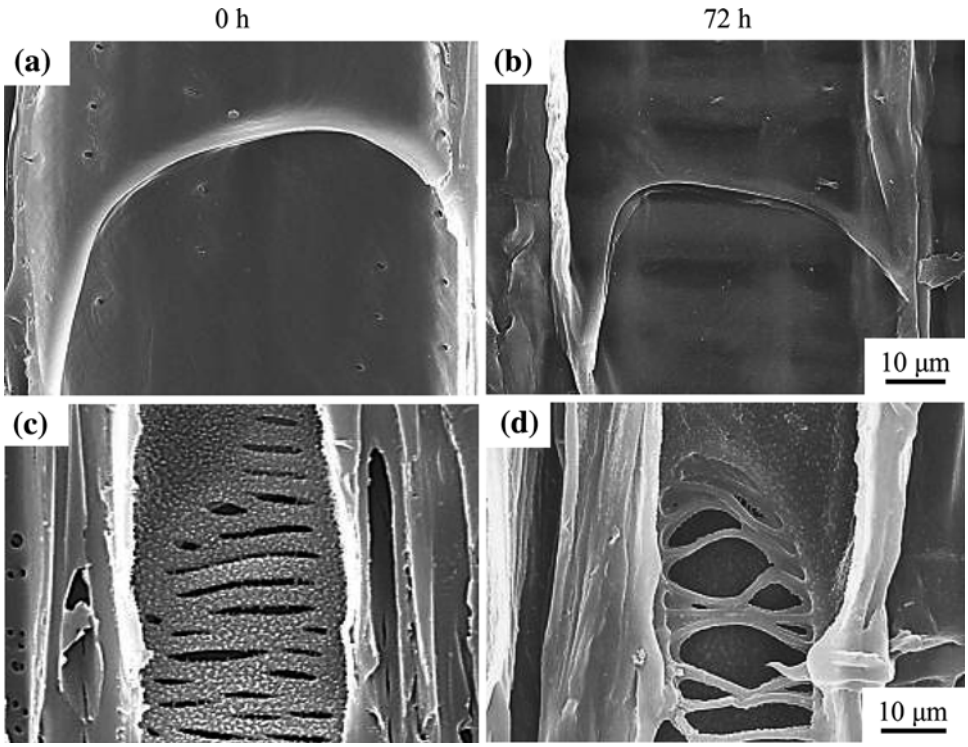

Fig. 6 SEM images of vessel perforations before and after treatment with [C2mim][Cl] at 120 °C for 72 h. *Top*: perforation rim; *Bottom*: scalariform perforation plate. *Left*: 0 h; *Right*: 72 h

after treatment with [C2mim][Cl] at 120 °C for 72 h. We found that parenchyma cells swelled towards the cell lumen. After 72 h of treatment, the cell wall area of parenchyma cells increased by a factor of not more than 1.5, whereas the cell wall area of wood fibers increased by a factor of 4. Therefore, compared with the wood fibers shown in Fig. 2b, d, the degree of swelling for parenchyma cells was slight. After 72 h of treatment, the cell wall thickness along fiber direction increased by 2 times, whereas the cell wall thickness along tangential direction increased by 1.4 times. Thus, ray parenchyma cell walls (as observed from the tangential section) greatly swelled along the fiber direction (Fig. 4d). Compared with the wood fibers shown in Fig. 2b, d, the degree of swelling was slight. In addition, ray parenchyma cell walls (as observed from the tangential section) greatly swelled along the fiber direction (Fig. 4d). Some resinoids in the parenchyma cells can still be seen after treatment with [C2mim][Cl]. Thus, these resinoids are not dissolved in [C2mim][Cl].

Figure 5 shows SEM images of transverse sections before and after treatment with [C2mim][Cl] at 120 °C for 72 h. Cracks are observed at the boundary of the annual ring and between ray parenchyma and peripheral tissues, as indicated by arrows (Fig. 5b), although such cracks were not seen in light microscope images of the transverse section (Fig. 2). This difference is most likely because the bonds between ray parenchyma and tissue adjacent to ray parenchyma, and at boundary of earlywood and latewood, were weakened by [C2mim][Cl] treatment, allowing significant cracking during the drying process required to prepare the samples for SEM. From the magnified views of earlywood and latewood, we observed that the cell walls of various tissues are distorted after 72 h of treatment (Fig. 5d, f). As shown in Figs. 2, 3 and 5, there are no significant differences between the morphological changes of latewood and earlywood.

Figure 6 shows SEM images of vessel perforations before and after treatment with [C2mim][Cl]. Although the perforation rim was unchanged after 72 h of treatment (Fig. 6b), the scalariform perforation plate was distorted (Fig. 6d).

Figure 7 shows SEM images of two kinds of parenchyma cells before and after treatment with [C2mim][Cl]. As shown in Fig. 5, wood fibers were distorted by [C2mim][Cl] treatment. In contrast, ray and axial parenchyma cells retained their original shapes, although their surfaces were smoothed down (Fig. 7b, d, f). In addition, the pits of ray parenchyma cells were occluded after 72 h of treatment, as indicated by arrows and circles (Fig. 7b). These morphological changes occur because the parenchyma cells start to be liquefied by [C2mim][Cl] treatment.

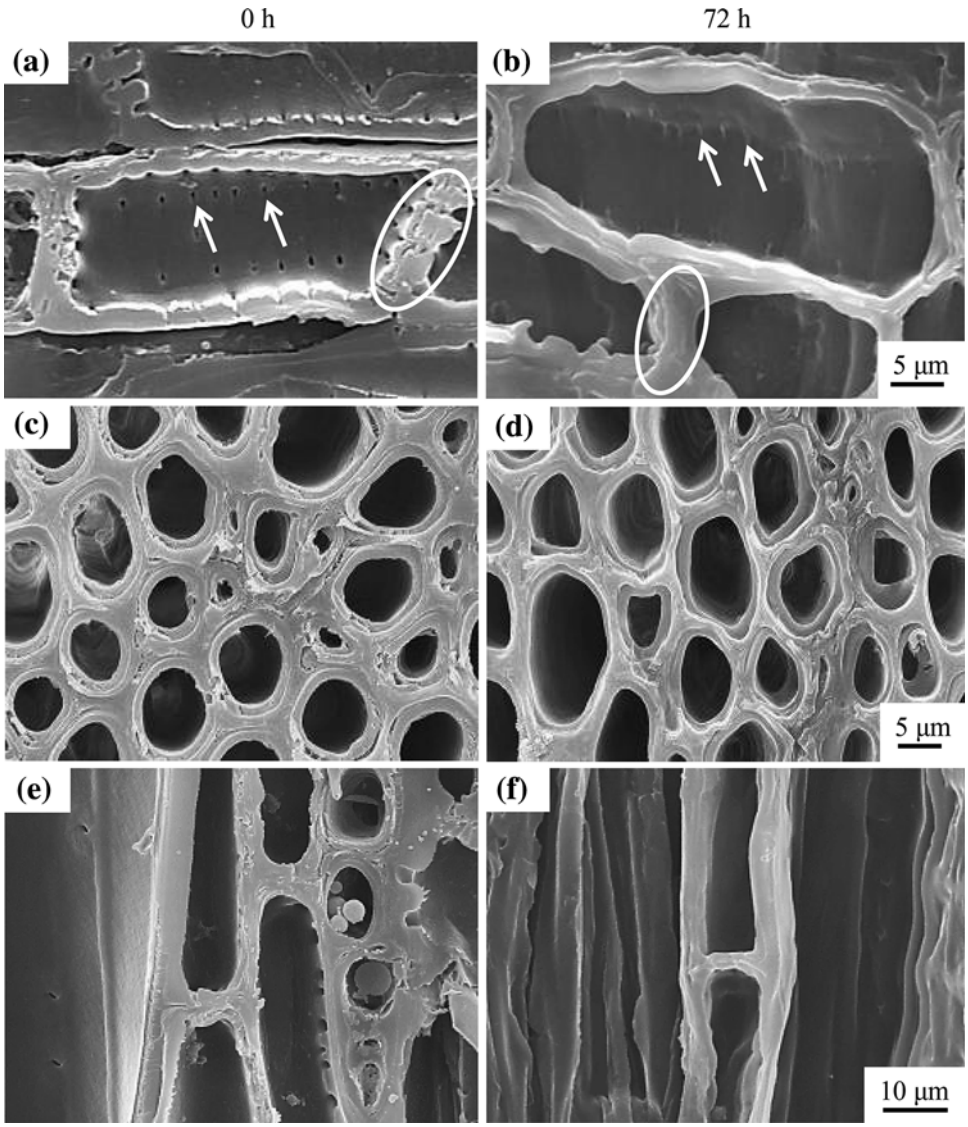

Fig. 7 SEM images of parenchyma before and after treatment with [C2mim][Cl] at 120 °C for 72 h. *Top*: ray parenchyma in radial section; *Middle*: ray parenchyma in tangential section; *Bottom*: axial parenchyma. *Left*: 0 h; *Right*: 72 h

Figure 8 shows SEM images of vessel pits. Intervascular pits of both vessels (Fig. 8b) and small vessels (Fig. 8d) were occluded after 72 h of treatment, as indicated by arrows. However, ray-vessel pits were not occluded, instead, large holes can be seen in ray-vessel pits because the pit membranes are broken after 72 h of treatment (Fig. 8f). These differences between the morphological changes of the two kinds of pits may be because of the difference in their diameters. The diameter of intervascular pits is smaller than that of ray-vessel pits. Small pits are thought to be easily occluded when wood cell walls are swollen during [C2mim][Cl] treatment. Simultaneously, they then become stuck shut by the liquefaction reaction with [C2mim][Cl]. However, large pits such as ray-vessel pits are not completely occluded, even after [C2mim][Cl] treatment. Instead, only the pit membranes were liquefied by [C2mim][Cl]. In the small vessels, many warts existed on the surface of cell lumen area; however, they became fewer and smaller after 72 h of treatment (Fig. 8d). In our previous paper on liquefaction of wood [10, 11], lignin and hemicelluloses are found to be liquefied more slowly than cellulose. Because the warts consist of hemicelluloses and lignin [15], they partly retain their original shapes after [C2mim][Cl] treatment, as shown in Fig. 8d).

Conclusion

In our previous studies on morphological changes in sugi wood after treatment with [C2mim][Cl], distortions and dissociations of wood cells because of swelling were seen in latewood, but not in earlywood. However, in this study

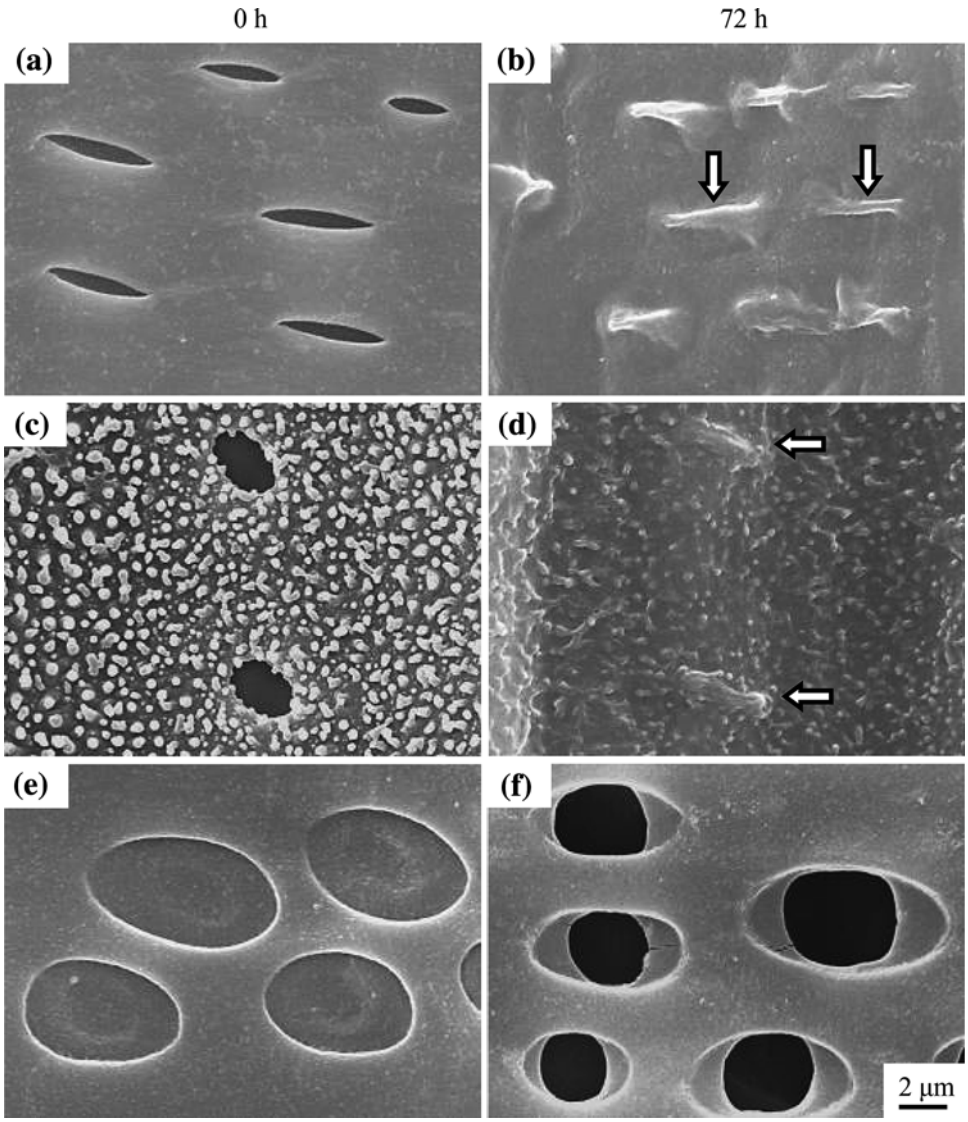

Fig. 8 SEM images of vessel pits treated with [C2mim][Cl] at 120 °C for 72 h. *Top*: intervascular pits; *Middle*: intervascular pits of small vessel; *Bottom*: ray-vessel pits. *Left*: 0 h; *Right*: 72 h

on morphological changes in Japanese beech after treatment with [C2mim][Cl], morphological changes in latewood are generally identical with those in earlywood, without dissociation of wood cells. This is because that vessels between wood fibers counteract the swelling of wood fibers upon [C2mim][Cl] treatment. SEM revealed that perforations and pits show quite different changes in morphology. Consequently, the reactivity of [C2mim][Cl] with wood is inhomogeneous, and is quite different for different wood species.

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