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NOTE

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A novel preparation of microcast for wood micromorphology using polydimethylsiloxane without digesting cell wall

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Abstract We developed a novel method for preparation of microcasts of wood with silicone elastomer (polydimethylsiloxane; PDMS). PDMS was so flexible and elastic that it was possible to isolate the microcasts by simply pulling them out of the mold without digesting the cell wall after the resin was cured for 2 days at room temperature. The casts of some cell wall sculptures, such as spiral thickenings and bordered pits, had high fidelity. By contrast, the casts of distinctly bordered pits and tails of vessel elements were often deformed or broken. Bars of scalariform perforation plates were always torn and remained in the resin casts. The microcast preparation using PDMS is useful for easy investigation of cell wall sculptures. It might be also useful for microfractography of bars of scalariform perforation plates.

Key words Microcast · Silicon elastomer · PDMS · Cell wall sculpture

Introduction

Microcasting has been used for the examination of wood micromorphology because this technique can visualize the morphology of cavities in wood tissue. Accordingly, studies using this technique have provided valuable information about vessel networks[1] and the morphology of minute spaces, such as pit cavities.[2]

Resin materials for casts, to date, have been polystyrene,[2–4] polyester,[5] low density polyethylene,[1] polymethylmethacrylate,[6] and silicon elastomer.[7] In general, the corresponding monomers penetrated into the vacant spaces of wood as a mold, and were polymerized to yield resins. However, in any case, their use required the removal of the mold by digestion of the cell wall components. The complete removal of the mold is a key process in yielding a good replica with high fidelity. However, this process used hazardous chemicals, because woody cell walls are complex materials that are difficult to digest completely. Thus, it is desirable to develop a simple preparation method to take resin casts.

In this study, we developed a simple method of preparation of microcasts using polydimethylsiloxane (PDMS) that was used for making replicas or imprints on a nanoscale.[8–10] Although PDMS is a viscous liquid, it readily penetrates into cavities of wood cells from openings on transverse surfaces, together with curing agent. After curing, the material transforms into a flexible elastomer. Therefore, it is possible to isolate the microcasts by simply pulling them out of the mold without digesting the cell wall. It was expected that the resultant replica reflected the fine structure of the mold with high fidelity.

Materials and methods

Small sapwood blocks of *Pinus parviflora*, *Juglans ailanthifolia*, *Tilia japonica*, and *Betula platyphylla* var. *japonica* were used as molds. Transverse faces were microtomed to expose flat and clean surfaces. The mold was placed in liquid PDMS containing curing agent (184 Silicone Elastomer Kit, Dow Corning, USA) to a depth of about 2 mm for 2 days at room temperature. After curing, the resultant wood block with elastomer was immersed in chloroform for approximately 3 h. The resultant elastomer was pulled out of the block using forceps. Similarly, a replica of the microtomed radial face of *B. platyphylla* var. *japonica* was prepared by placing the mold in liquid PDMS containing the curing agent for 2 days without pressure, and then the replica was peeled off. After trimming by hand-cutting with a steel blade, the microcasts were fixed on specimen stubs with electron-conductive paste, coated with Au–Pd by vacuum evaporation, and observed under a scan-

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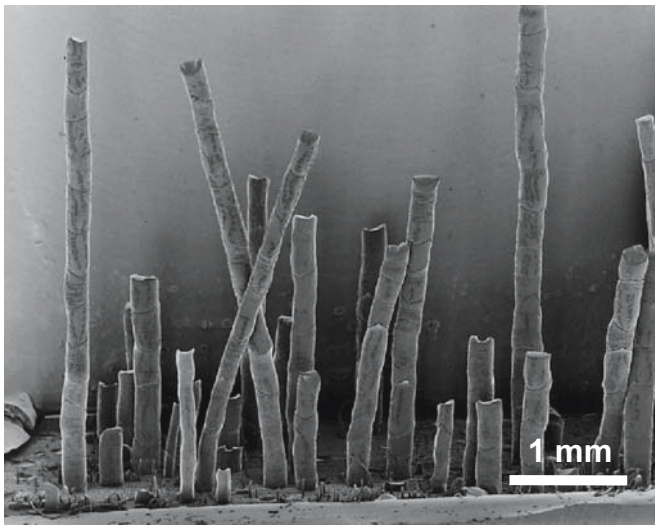

Fig. 1. Selective isolation of vessel casts of *Juglans ailanthifolia*

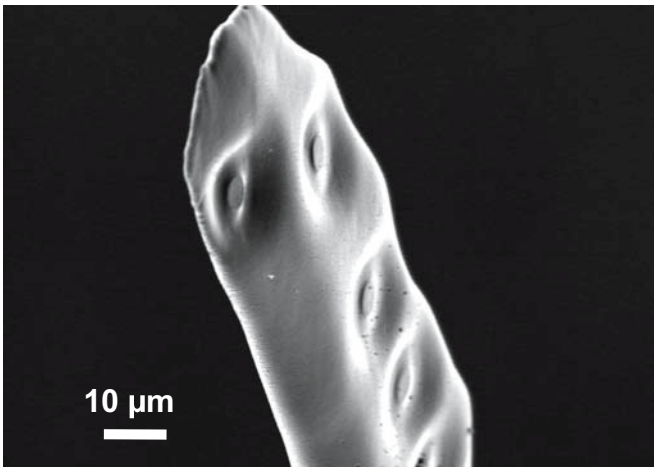

Fig. 2. Part of a microcast of *Pinus parviflora* showing the morphology of the lumen of a tracheid tip

ning electron microscope (SEM; JSM5300 or JSM6301F, Jeol, Japan).

Results and discussion

Preparation of microcasts

PDMS is a viscous liquid, and is transformed into elastomer after curing with a curing agent. When one transverse face of each wood block was placed on PDMS liquid containing the curing agent, the PDMS penetrated into the void spaces of the block, probably due to capillary action. The penetrated PDMS ascended up to 2 cm in height. In this procedure, it took 2 days for PDMS penetration and curing. When PDMS was cured within 45 min at 100°C according to the curing protocol of the PDMS kit, bubbles formed in the resultant elastomer. This bubble formation was thought to be due to the evaporation of water in the mold and it was expected to be overcome by degassing prior to curing. However, the treatment was not successful because PDMS absorbed moisture from the air during the penetration. In contrast, bubble formation did not occur at all or only slightly when the resin was cured at room temperature. Therefore, we allowed the penetration and curing to proceed at room temperature even though it took longer than at 100°C.

We sometimes failed to remove the cured elastomer because it was tightly fixed to the mold. The treatment with chloroform prior to the isolation of microcasts was effective in facilitating the removal of the elastomer from the mold, probably due to suppression of adhesion between the mold and the elastomer. It was possible to take vessel casts of several millimeters in length by using chloroform soaking (Fig. 1). Thus, the chloroform treatment is useful for preparing microcasts without digesting the cell wall components. In the process of casting of the radial face, the microcast was easily peeled off the mold without chloroform treatment, because the microcast did not penetrate and remained on the surface of the mold.

Replica images

A microcast of vessels was selectively prepared (Fig. 1). Such selective isolation of vessel casts has been attempted by the control of the viscosity of resin[1] or by removing other structural elements using sonication.[3] In our method, not only the viscosity but also the preparation procedure contribute to the selective isolation. It was most likely that casts of the other structural elements, such as wood fibers, were torn from the vessel cast and left in the mold when the casts were longitudinally pulled out from the transverse faces. Thus, the method using PDMS was useful to examine the structure of vessels. However, it was difficult to obtain long casts of vessels because the casts were often broken during the removal process. In addition, artificial deformation of vessel casts often took place even if long and intact casts were obtained (Fig. 1). It is most likely that the microcasts are too flexible to preserve the true three-dimensional arrangement of such long structures. Thus, this method is not adequate to examine the arrangement and networks of vessels, although it is useful to examine the micromorphology of each vessel.

PDMS showed high fidelity at the fine structural level. The casts clearly demonstrated the morphology of minute spaces, such as the lumina of tracheid tips (Fig. 2), and cell wall sculptures, such as spiral thickenings (Fig. 3) and pits (Fig. 4). PDMS replication indicated fidelity at the submicron level as previously reported.[9] Therefore, no microfibril replica was observed by this method.

There were also limitations in our microcast preparation using PDMS. Figure 5 shows bars of scalariform perforation plate of *Betula platyphylla* var. *japonica* that were torn and remained in the resin casts. It is impossible to prevent such artificial destruction of this type of minute structure, which

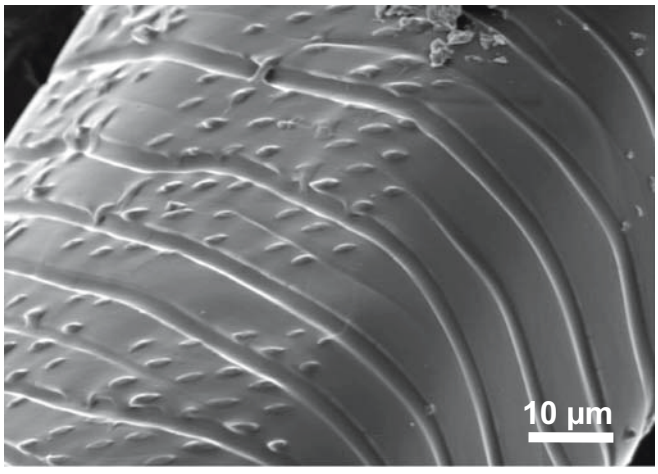

Fig. 3. Part of a vessel element cast of *Tilia japonica* showing imprints of spiral thickenings

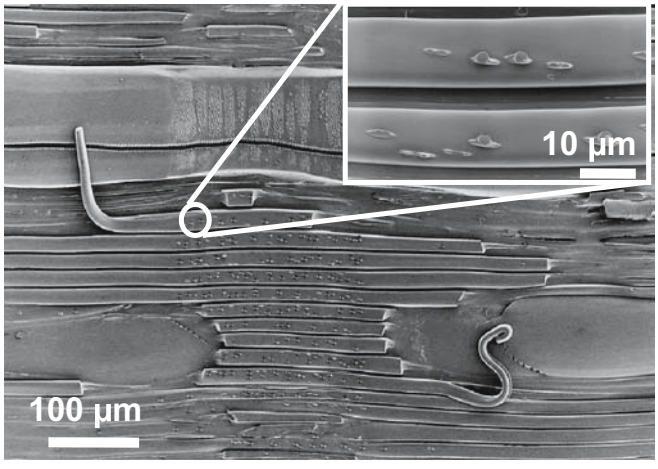

Fig. 4. Resin cast from a radial section of *Betula platyphylla* var. *japonica* showing casts of minutely bordered pits on wood fiber walls and deformed casts of wood fiber tips.

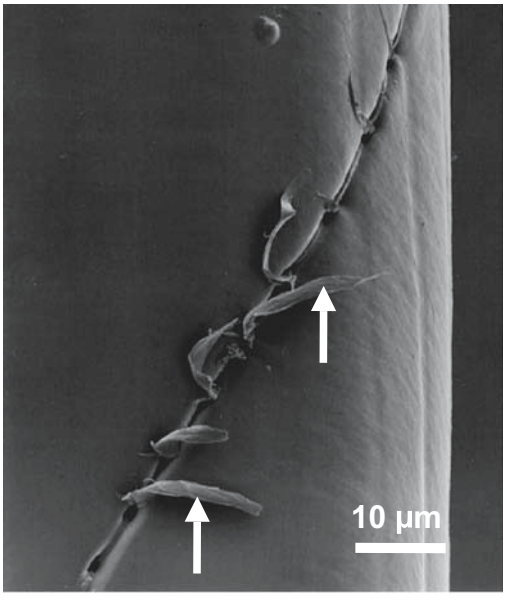

Fig. 5. Resin cast of a vessel of *Betula platyphylla* var. *japonica*. *Arrows* indicate bars of scalariform perforation plate that were torn and remained in the cast

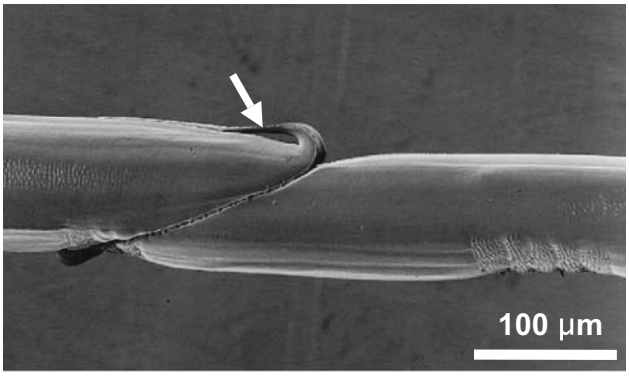

Fig. 6. Microcast of a vessel of *Betula platyphylla* var. *japonica*. *Arrow* indicates a tail of a vessel element that was turned up by pulling out

are present in larger cavities, during preparation. However, it is conversely possible to use our technique for microfractography of such minute structures.

In addition, it was also difficult to prevent deformation and destruction of the resin casts of some other structures. Casts of vessel element tails and wood fiber tips were often found to be deformed (see Figs. 4 and 6). Casts of the cavities of distinctly bordered pits were often also torn away (Fig. 4), because strong stress is applied to the casts during pulling out.

Our method of resin casting has some limitations and demerits in studies of wood micromorphology. However, this method has relevant characteristics that are quite different from those of previous methods. Cured PDMS, as a replica resin, is so flexible and elastic that we can isolate the casts by removal without the use of hazardous chemicals to digest of wood tissues. It is expected that this replica preparation is not only simple but is also unique and useful for some analyses, such as microfractography.

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