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New Syncrotron-Based Technique to Map Adhesive Infiltration in Wood Cell Walls

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Introduction

Wood is a much more difficult material to bond than plastics and metals because of its structural, chemical, and mechanical complexities. These complexities give rise to numerous potential interactions between adhesives and wood on length scales ranging from bulk to molecular. Efforts to improve the durability of wood-adhesive bondlines are hindered by a lack of methods to probe interactions across these length scales, especially at the cell wall level.

Softwood is an anisotropic cellular material with numerous levels of structure, ranging from tree to polymer [1,2]. Figure 1a shows growth rings in a tree, which define the transverse, radial-longitudinal, and tangential-longitudinal planes of wood. The corresponding cellular structure in softwood is illustrated in Figure 1b. Within a growth ring, latewood cells typically have smaller lumina and thicker walls than earlywood cells. Tracheids aligned with the longitudinal axis are the most common type of cell in softwood. Because in a living tree liquid transport is one of the primary wood functions, ray cells are present for radial liquid transport, and small openings called pits between cells facilitate intercellular transport. Tracheid walls consist of three secondary cell wall layers (S1, S2, and S3), the primary wall layer, and the middle lamella (Figure 1c). The secondary cell walls are nano-fiber-reinforced composites of highly oriented, semicrystalline cellulose microfibrils embedded in a matrix of amorphous cellulose, hemicelluloses, and lignin. The lumen is the hollow space in the center of a tracheid. The middle lamella fills in between and holds together cells (Figure 1c). It consists of about 20% hemicelluloses embedded as an irregular, interconnecting network in a matrix of lignin.

Because of wood's structure, the surface of wood is never smooth at the sub-millimeter scale. During wood bonding, adhesive flows into the cellular structure of wood, filling lumina near the bondline, flowing through rays, and sometimes through pits into neighboring cell lumina [3]. Certain wood adhesives can also infiltrate wood cell walls with the potential to change the cell wall properties. Adhesives such as PF, PRF, MF, MUF, and UF (phenol-, phenol-resorcinol-, melamine-, melamine-urea-, and urea- formaldehyde, respectively) typically contain a low molecular weight component that can infiltrate cell walls and modify their properties [4]. In contrast, highly pre-polymerized adhesives, typically with higher molecular weights, such as PUR and PVAc (polyurethane and poly(vinyl acetate), respectively) have little effect on cell wall properties, and in general pre-polymerized adhesives have not been observed infiltrating wood cell walls.

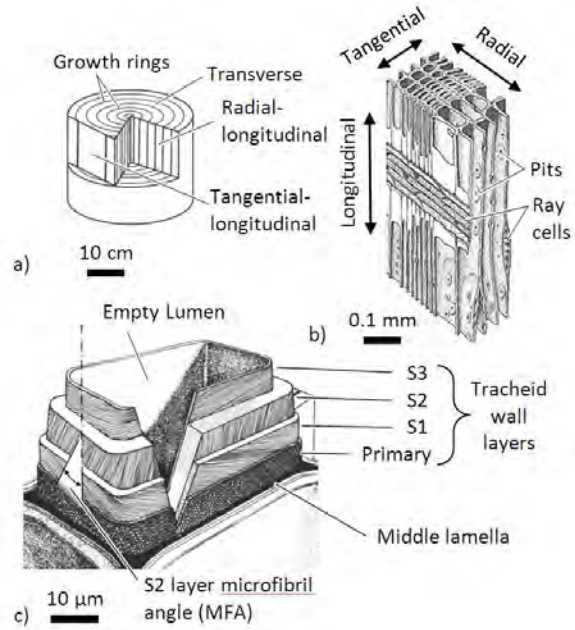

Figure 1. Breakdown of wood structure from a) bulk, to b) cellular, to c) cell wall layers.

Much remains to learn about the effect of infiltration on the properties of cell walls. Thus, researchers require tools that precisely quantify adhesive infiltration into wood cell walls. Here, we present a new approach using synchrotron-based x-ray fluorescence microscopy (XFM). Briefly, hard x-rays are monochromatized to an energy above the absorption edges of the elements of interest and focused onto the sample. X-rays knock out inner-shell electrons, and these vacancies are filled by relaxation of outer-shell electrons. For medium- to high-Z material, the excess energy is largely given off as fluorescence photons, which in turn are detected via an energy dispersive detector system. Because the energy of fluorescence photons is characteristic for each element, the elemental content and amount of element present at the sample spot can be determined. By raster scanning the sample through the focused beam, elemental maps are built up point by point (see, *e.g,* [5–7]). We demonstrate that XFM imaging has both the spatial resolution to map out adhesive infiltration

within individual cell walls and the sensitivity to detect adhesive infiltration down to trace quantities.

Experimental Work

Bromine-labeled PF adhesive was prepared by charging 3-bromophenol (10.7 g) and 37% formaldehyde (10.04 g) from an ice bath into a 25-ml reactor containing water (5.2 g) and a stirbar. For the initial methylolation step, 50% sodium hydroxide (0.7 g) was added and the reaction heated to 80°C with stirring. The reaction was monitored using thin layer chromatography (TLC) at various time points. TLC was performed using 1:1 ethyl acetate:hexane as the elution solvent on spot plates. Using 3-bromophenol, standard PF resin, and previously prepared Br-PF resins as references on the TLC plate, the reaction was monitored for the disappearance of 3-bromophenol and appearance of one methylol, two methylols, and three methylols. After 160 min, additional 50% sodium hydroxide (0.31 g) was added and the temperature increased to 95°C for 115 min.

To test Br ion contamination, or the possibility of Br separation from the aromatic ring during resin production, the 3-bromophenol (control) and adhesive were repeatedly dissolved in alkali, precipitated in phosphoric acid solution, centrifuged, and the top aqueous layer tested for Br. Br ions, if present, should stay with aqueous phase, which was then tested for Br by inductively coupled plasma mass spectroscopy (ICP-MS). We found no difference in Br concentration in the aqueous phase of the first, second, or third sequential wash, indicating that no significant amount of Br ion was present.

For wood adherends, a disposable microtome blade in a sledge microtome was used to prepare pristine tangential-longitudinal surfaces in loblolly pine (*Pinus taeda*). The surface measured approximately 10 mm longitudinal x 5 mm tangential and was about 10 mm in the radial direction. The prepared surface had a centered latewood growth ring. Bondlines were made by applying PF adhesive to the pristine surfaces of two loblolly pine adherends. Enough adhesive was added to ensure abundant squeeze-out. After 5 min open assembly time, they were clamped with a large binder clip. After 5 min more, they were placed, still clamped, in a 155°C oven, and cured there for 45 min.

X-ray fluorescence microscopy (XFM) of a 2-µm-thick transverse section of a wood-adhesive bondline was performed at beamline 2-ID-E at the Advanced Photon Source (APS) at Argonne National Laboratory (Argonne, IL, USA). The 2-µm-thick section was cut with a diamond knife in a Sorvall (Norwalk, Connecticut, USA) MT-2 ultramicrotome. The boat of the diamond knife was filled with deionized water. Using eyelash tools, the section was carefully lifted from the water, placed in a water drop on a glass slide under a dissecting light microscope, uncurled, and held flat while the water drop evaporated. After drying, the section could be handled with fine tweezers, and it was clamped in a copper StrataTek™ 1/1 mm double folding hole TEM grid obtained from Ted Pella, Inc. (Redding, CA, USA). The clamped 2-µm-thick section spanned the 1-mm hole with the bondline centered. The folded TEM grid was then taped to the beamline 2-ID-E aluminum stick sample holder. The beam had an incident energy of 15.0 keV and spot size of approximately 0.8 by 0.5 µm. A 160- by 380-µm image was created using 0.3-µm steps with a 5-ms dwell time at each step. Data analysis was carried out using the MAPS software package [8]. Briefly, full spectra at each scan position were fit to modified Gaussian, background was iteratively determined and subtracted, and the results compared to standard reference material (NBS 1832 and 1833, NIST). XFM images were exported as 32 bit float-tif images from MAPS and further analyzed in ImageJ (http://rsb.nih.gov/ij/) image analysis software.

Results and Discussion

An XFM image of a Br fluorescence signal in a wood-adhesive bondline is shown in Figures 2a and b in linear and logarithmic scales, respectively. Because Br is not naturally present in wood and the Br should be covalently bonded to the aromatic ring in the PF adhesive, the Br signal directly correlates to adhesive concentration. Near the bondline, adhesive is observed to flow into and fill lumina, coat lumina, and infiltrate cell walls.

The Br signal profile analysis (Figure 2c) shows the quantification of adhesive across six tracheids. The amount of Br measured in the bulk adhesive in the filled lumen and bondline was about 40,000 µg/cm^2. The tracheid walls immediately adjacent to the bondline have the largest amount of infiltration, with greater than 3,000 µg/cm^2 of Br. In contrast, the tracheid walls between the two coated lumina only have approximately 500 µg/cm^2 of Br. In the tracheid walls to the left of the filled lumen in the profile, adhesive infiltrates not only into the wall of the tracheid whose lumen is filled, but also past the middle lamella into the neighboring tracheid wall. In future analyses we will convert the mass of Br per unit area to weight percentage of adhesive.

We plan to use XFM images like those in Figure 2 to better understand how adhesives infiltrate cell walls and to quantify the amount of adhesive infiltration in cell walls. However, issues surrounding the spatial resolution of the XFM imaging must first be addressed. During XFM, a focused beam of x-rays illuminates a small spot approximately 0.8 by 0.5 µm and the detector characterizes the number and energy of fluoresced photons. To a first approximation, the fluorescence signal arises only from the volume of material illuminated by the beam. Because during imaging the 2-µm-thick section is held 15° from perpendicular to the x-ray beam, some spatial resolution will be lost at a cell wall-adhesive interface because the interface will not be parallel to the beam (Figure 3). When the beam moves from the cell wall, to the overlapping interface, then to the adhesive (locations #1-3 in Figure 3, respectively), a gradient will be observed in the Br signal

irrespective of whether or not the adhesive infiltrates the cell wall. This is a possible explanation of why in Figure 2c the Br signal of the adhesive coating the lumina is lower (~10,000 µg/cm^2) than the Br signal in the filled lumen of bondline (~40,000 µg/cm^2). If there is an adhesive coating and it is less than approximately 1 µm thick, the volume illuminated by the x-ray beam would never be completely filled with adhesive. This interface effect will need to be taken into consideration when characterizing the gradient of infiltrated adhesive near the cell wall-adhesive interface.

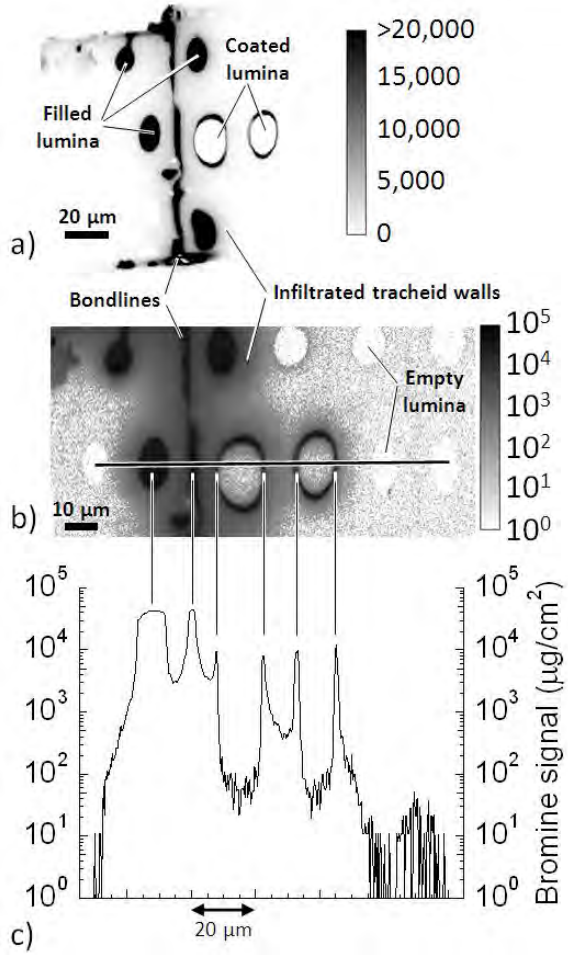

Figure 2. XFM image of Br signal in a Br-labeled PF adhesive-wood bondline as viewed on transverse plane using a) linear scale and b) logarithmic scale. In c) the profile across the bondline and multiple cells is displayed. Each point in the profile is the average of a 5 pixel column perpendicular to the profile line. All units are in µg/cm^2.

In Figure 2b the Br fluorescence image clearly shows the tracheid walls even far from the bondline. The original image from which Figure 2 was taken extended to 13 tracheids from the bondline, and throughout the image an even ~50 µg/cm^2 Br signal was observed clearly distinguishing the wood cells. In contrast, a control wood section showed no detectable Br signal. It could be that given enough time, unreacted 3-bromophenol, PF oligomers, or possibly even Br that separated during curing could diffuse far into the wood. Or, more likely, it is possible that while the section is being unfolded in the drop of water during preparation, trace amounts of unreacted 3-bromophenol or Br-labeled PF oligomers were carried into the aqueous phase and deposited evenly throughout the section. Finally, inside the coated lumina a substantial Br signal is detected even though the coated lumen edges are about 5 µm away. This must be an artifact, and we are investigating how best to account for it.

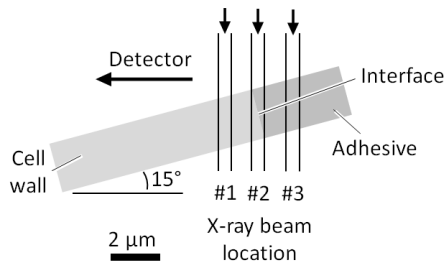

Figure 3. Schematic of 0.5-µm x-ray beam going through a 2-µm-thick section at three locations in the vicinity of a cell wall-adhesive interface.

Conclusions

The penetration of Br-labeled PF adhesive into wood can be studied with XFM imaging. Both the adhesive flow into lumina and the infiltration of adhesive into cell walls can be visualized and quantified. The cell walls nearest the bondline had the most infiltrated adhesive.

References

1. A.C. Wiedenhoeft, Structure and Function of Wood, 2013, In: Rowell RM, editor. Handbook of Wood Chemistry and Wood Composites. CRC, Boca Raton, pp 9–32.
2. R.M. Rowell, R. Petterson, and M.A. Tshabalala, Cell Wall Chemistry, 2013, In: Rowell RM, editor. Handbook of Wood Chemistry and Wood Composites. CRC, Boca Raton, pp 33-72.
3. F.A. Kamke and J.N. Lee, *Wood and Fiber Science*, 2007, 39, pp 205–20.
4. C.R. Frihart, *Journal of Adhesion Science and Technology*, 2009, 23, pp 611–27.
5. B.S. Twining, S.B. Baines, N.S. Fisher, J. Maser, S. Vogt, et al., *Analytical Chemistry*, 2003, 75, pp 3806–16.
6. T. Paunesku, S. Vogt, J. Maser, B. Lai, and G. Woloschak , *Journal of cellular biochemistry*, 2006, 99, pp 1489–502.
7. U.E.A. Fittschen and G. Falkenberg, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2011, 66 pp 567–80.
8. S. Vogt, *Journal de physique. IV,* 2003, 104 pp 635–8.