Light, Confocal and Scanning Electron Microscopy of Wood-Adhesive Interface

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INTRODUCTION

Microscopical techniques which provide complementary information, such as correlated light, confocal and electron microscopy, have been very useful in our work on the characterization of wood products at the cellular and tissue levels and the assessment of their in-service performance. The work presented in this article is part of an investigation aimed to understand interfacial interactions between the components of biomaterials based products, and is based on a technical approach involving combined use of light microscopy (LM), fluorescence confocal laser scanning microscopy (CLSM) and then field-emission scanning electron microscopy (FE-SEM) to probe the interface between wood and adhesive in Pinus radiata plywood.

Plywood is a wood based product that is strong, durable and versatile, being used in both interior and exterior applications from wall panel and furniture making to caravan and bus construction. Plywood is produced by gluing together a number of layers (plies) of wood with the grain directions at right angles to each other in successive plies. Thus, in the cross-sections through two successive plies, the grain in one ply is cut transversely and in the other longitudinally, as can be seen in the illustrations presented in this article.

Commercially, adhesives have been used to produce a range of wood products of high value, such as laminated beams, plywood, and strand, chip and particle boards. The performance of adhesives depends on many factors, and the physical and chemical characteristics of both wood surfaces and adhesives determine the quality of bonding. Wood-adhesive interactions that take place at the interface, i.e., the place of contact of adhesive with wood, involve chemical bonding as well as physical factors, such as mechanical interlocking (entanglement) of adhesive into wood tissues. Collectively, these interactions determine the bond strength. Understanding factors that determine the quality of adhesive bonds can potentially lead to the production of high performance wood products using minimal adhesive amounts needed to adequately bond wood surfaces, which can result in significant savings in production costs.

Techniques for characterizing wood-adhesive interface are continually evolving. Microscopy has been used before to characterize the adhesive line, but the main aim has been to examine the depth of adhesive penetration into contact tissue layers. In this article we describe a novel microscopy approach that we have developed to probe the wood-adhesive interface in order to more clearly understand the physical nature of wood-adhesive interactions in a commercial plywood product which had been bonded with a phenol-formaldehyde adhesive. The technique involved correlative microscopy of 90-µm thick, microtome-cut sections through...
the wood-adhesive interface, after appropriate treatments of sections. The same sections were sequentially examined by LM, CLSM and FE-SEM. This is a new approach in wood-adhesive research which has provided valuable new information, particularly on the intricate nature of the adhesive penetration pathways within the surface tissues of the bonded wood plies.

MATERIALS AND METHODS
The correlative microscopy approach undertaken in our work involved the development of specific staining and imaging techniques, which enabled the same sections to be examined sequentially by LM, CLSM and FE-SEM and gather information that was not possible to obtain using any one of these microscope types alone.

Light and Confocal Microscopy
The sections cut through the wood-adhesive interface using a sliding microtome at a thickness of 90 µm were stained with aqueous toluidine blue stain and mounted in glycerol on a glass slide prior to examination with LM. After viewing and photographing with LM, the same sections were examined with a Leica TCS/NT CLSM under fluorescence mode at excitation wavelengths of 488 and 568 nm and emission wavelengths of 530 and 600 nm, as described in an earlier work [5].

Scanning Electron Microscopy
The toluidine blue stained sections, which had been examined with LM and subsequently with CLSM, were removed from the glass slides by immersing in water in a Petri dish and then processed for the FE-SEM work according to a method described earlier [6]. Briefly, the sections were treated with 1% aqueous OsO₄ (osmium tetroxide) for 2 hours at room temperature, washed in water, briefly rinsed in ethanol, clamped between two glass slides and air-dried.

The sections were then mounted on carbon tapes applied to stubs, coated with chromium in a sputter coater and examined with a JEOL 6700F FE-SEM.

RESULTS AND DISCUSSION
In the light microscope the toluidine blue stain, which imparts a bluish green colour to lignified wood cell walls, proved effective in clearly differentiating wood cell walls from the phenol-formaldehyde adhesive, which kept its original brownish colour. Light microscopy proved useful in following ways:

1. It provided evidence that the adhesive had penetrated into the lumens of rays and also of axial tracheids up to four cells deep from the adhesive line (Figures 1, 2).

2. Light microscopy indicated that the adhesive may also have penetrated into the cell walls particularly of tracheids in contact with and in vicinity to the adhesive line, as the cell walls in these tissue regions displayed an orangeish and not the bluish green colour typical of the cell walls of tissues that were distant from the adhesive line and out of direct reach of the adhesive.

3. Light microscopy enabled a large number of sections to be examined in a relatively short time, and thus large segments of the wood-adhesive interface. However, the inability of light microscopy to bring particularly the distant wood tissues in the same focal plane as the wood-adhesive interface in a section was a severe limitation of this type of microscopy.

The combination of toluidine blue staining of sections and use of suitable operating conditions of CLSM enabled the adhesive to be sharply differentiated from wood cell walls based on bright contrasting colours of the adhesive (appearing greenish) and wood cell walls.

Figure 2:
Light micrograph showing a higher magnification view of the wood-adhesive interface. The adhesive penetration into tracheids is up to several cells deep from the adhesive line (arrow). Scale bar = 35 µm.

Figure 3:
Confocal fluorescence micrograph of the adhesive line (greenish) between two plies, showing deep penetration of adhesive into the rays. The adhesive has also penetrated into tracheids in the surface layers of the glued plies all along the adhesive line as well as into cracks within the tissues (arrow). Scale bar = 50 µm.
walls (appearing reddish). It was also possible to visualize adhesive penetration into wood tissues more clearly than with LM, as the unique capabilities of CLSM in optical sectioning through an object and then obtaining a composite image of sequential sections through a considerable depth enabled large tissue areas to be brought in the same focal plane as the adhesive line, which was not possible with light microscopy. Furthermore, a combination of superior colour differentiation between the adhesive (greenish) and wood cell walls (reddish) and image sharpness made it possible to visualize wood-adhesive interface more clearly than was possible with light microscopy, which greatly enhanced the value of information obtainable on wood-adhesive interactions.

In Figure 3, the entire view in field is in the same focal plane and thus sharply defined, enabling the intricate pathways of adhesive penetration into wood tissues of the plies flanking the adhesive line to be clearly resolved. The adhesive has penetrated into the lumens of ray tissues and axial tracheids, as well as into cracks present within the ply face exposed to the adhesive line that are likely to have formed during the peeling of veneers from logs. The light green to orangeish colour (not reddish) of the surface tissues embedded in the adhesive suggests that the adhesive has also penetrated wood cell walls. Penetration of the adhesive into cell walls is more obvious in Figure 4, a higher magnification view of a wood-adhesive region shown in Figure 3.

Comparison of tracheids which are farthest from the adhesive line, where cell walls are reddish coloured with the middle lamella showing greatest brightness and thus clearly distinguishable from the secondary wall, with those nearest the adhesive line, where cell walls appear light green, suggests that the adhesive may have completely penetrated cell walls in addition to penetrating cell lumens. In the transition zone, cell walls appear to be only partly impregnated with the adhesive, as the patchiness of cell wall colours (cell walls not uniformly coloured) would suggest.

Figure 4 also displays an intricate pattern of adhesive distribution within the tissues nearest the adhesive line, which appear to have been damaged probably during veneer peeling, with some cells severely compressed and cracks within cell walls present. The adhesive has penetrated all accessible spaces and is present in cell lumens, cell wall cracks and cell walls, thus creating a wood-adhesive polymer composite in the wood-adhesive interface region.

SEM added another important dimension to visualizing adhesive penetration into wood tissues. Use of FE-SEM in our work in combination with BEI imaging provided remarkably high definition of the wood-adhesive interface, made possible by the high resolution capability of this instrument and the enhancement of differentiation between the adhesive and wood cell walls based on a special technique that we developed to increase the contrast of the adhesive relative to cell walls.

The technique involved treating the toluidine blue-stained sections (which we had examined with light microscopy and subsequently with CLSM) with OsO₄ prior to examination with the FE-SEM in combination with BEI. BEI, which enables contrast differentiation between two or more components in a composite material to be obtained based on atomic number differences, has been widely used to examine both biological and non-biological objects [7,8]. Higher atomic number components appear brighter than lower atomic number components under BEI because of greater yield of backscattered electrons. For this reason, differentiation between the components differing in their atomic number is based on brightness intensities, with higher atomic number components appearing brighter than the lower atomic number components.

External applications, as stains or tracers, of high atomic number substances have also been employed to enhance the yield of backscattered signals and thus contrast differentiation based on atomic number differences [9,10]. Osmium is a high atomic number substance, and therefore it enhances the emission of backscattered electrons from the materials.
with which it can physically or chemically react, such as the phenol-formaldehyde adhesive used in our work, thus increasing their brightness under SEM-BEI viewing.

The micrograph in Figure 6, which was taken with the FE-SEM in the BEI mode illustrates the value that BEI adds to imaging osmium tetroxide-reacted phenol-formaldehyde adhesive. In comparison to the rather poor differentiation between wood cell walls and the adhesive achieved when imaged with the FE-SEM in the secondary electron imaging (SEI) mode, which is the standard imaging mode in the SEM work (Figure 5), excellent differentiation between wood cell walls and the adhesive was achieved when the same sections were subsequently examined in the BEI mode (Figure 6) for the reason mentioned above. This enabled adhesive distribution within wood tissues to be clearly visualized, as shown in Figures 6 and 7.

In Figure 6, a low magnification FE-SEM-BEI image, the pathway of adhesive penetration into the damaged surface tissues of a ply is clearly visible. The adhesive is present within the lumens of tracheids and also in the damaged tissue regions containing cracks.

The rather intricate pattern of adhesive distribution within surface wood tissues is observable in the high resolution image shown in Figure 7, which is a high magnification view of a region of the wood-adhesive interface in Figure 6. The presence of adhesive is detectable even in very small dimension cell wall cracks, which light microscopy and CLSM could not resolve.

CONCLUSIONS

The unique imaging approach that we employed, involving sequential viewing of the same sections taken through wood-adhesive interface, by correlative LM, CLSM and FE-SEM-BEI microscopy, provided new fundamental information on wood-adhesive interaction, which forms the basis for understanding why the mechanical interlocking of adhesive into wood tissues is considered important in adhesive performance.

Effective interlocking of wood tissues by an adhesive involving adhesive penetration into all accessible micro- and nano-pores along the adhesive line is even more crucial for optimal adhesive performance where mechanically weakened wood surfaces, such as in wood plies produced commercially by mechanical peeling of logs, have to be stabilized and strengthened.

REFERENCES