

Imaging of ink jet penetration in uncoated paper using microscopic techniques

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ABSTRACT: Ink penetration in paper is influenced by the structure of the interfiber and intrafiber void spaces and the surface characteristics of the fibers. This report describes new techniques to determine the influence of the fiber surfaces and the cell wall internal structure on ink spreading and penetration. The location and penetration of ink is demonstrated by optical, scanning electron, confocal laser scanning, and transmission electron (TEM) microscopy methods. Ink penetration, as determined by each of these methods, is compared. The hemicellulose of the fiber's internal void surfaces can be determined by immunochemical labeling in conjunction with TEM imaging. We demonstrate this by using primary monoclonal antibodies with specificity for hemicellulose with a secondary colloidal gold marker. This technique provides a way to visualize the location of hemicelluloses inside the cell wall and on the surfaces of nanopores. Combining paper structure with fluid spreading and wicking models can identify the influence of fiber surfaces and the cell wall on drop absorption.

Application: Microscopic techniques used to determine biological and physical locations in plants on a fibrous level also can be used to study ink diffusion, water uptake, and other characteristics of fibrous material.

Ink drop imbibition within the paper sheet structure is a complex process. Pore spaces in the paper exist between the fibers (interfiber or macropores of a few micrometers) and within the cell walls themselves, on a nanometer scale. Imbibition of ink drops can occur by capillary action in the interfiber pores, wicking into the intrafiber and fiber cell wall nanopores and spreading along the surface of the fibers. Ink jet drops are in the picoliter range and, as a consequence, intrafiber wicking is the most significant absorption phenomenon coupled to the spreading along the fibers. Earlier studies of ink penetration have focused mostly on the penetration of the interfiber pore space [1-4]. Picoliter inkjet-printed droplets penetrate the interfiber pores and tend to spread along the fibers [1]. However, capillary action by the fiber cell walls tends to retard this spreading. This capillary action depends on the nanocapillary sizes, network topology, and fiber surface energetics. In this work, we demonstrate techniques that are suitable for locating and quantifying ink spreading and penetration into the fiber cell walls by various microscopic techniques. These include immunoelectron microscopy to show the chemical constitution of the nanopore surfaces and their location inside the cell wall. Our technique uses ink drops laden with 50 nm silver nanoparticles for imaging and detection by scanning electron microscopy (SEM). Ink penetration into fibers was further displayed using transmission electron microscopy (TEM) of gold nanoparticles tagged to xylan antibodies, and 50 nm silver and 10-30 nm gold particles in the ink drops [5].

MATERIAL AND METHODS

Hemicelluloses are a key component in determining the energy of the paper surface; hence, techniques that can identify the topochemical nature of the intrafiber pore surfaces are necessary. The chemical nature can be determined by combining immunochemical techniques with electron microscopy. This is demonstrated using hemicellulose-specific primary monoclonal antibodies (mAbs) coupled with a secondary nanogold marker. Interfiber pores have been determined to be in the range of 1-5 μm [9]. Additionally, cell wall nanopore sizes have been determined to be 10-100 nm [10]. Quantification of the ink penetration into nanopores helps to elucidate drop dynamics and to show the relationship between the surface chemistry and micropore structure of the fiber [11].

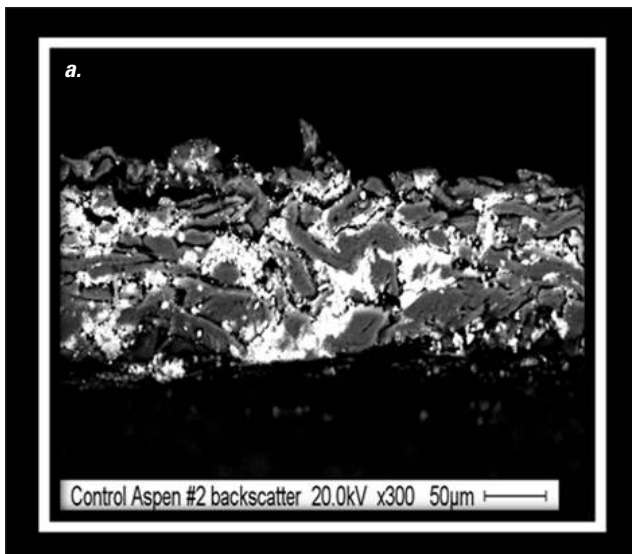
Substrate

Handsheets of various fiber contents were used in this study, as was a commercial store brand recycled copy paper. All handsheets were formed according to TAPPI T 205 sp-06 "Forming handsheets for physical tests of pulp," using the same circular mold. The samples of commercial store brand recycled paper were taken from the same ream.

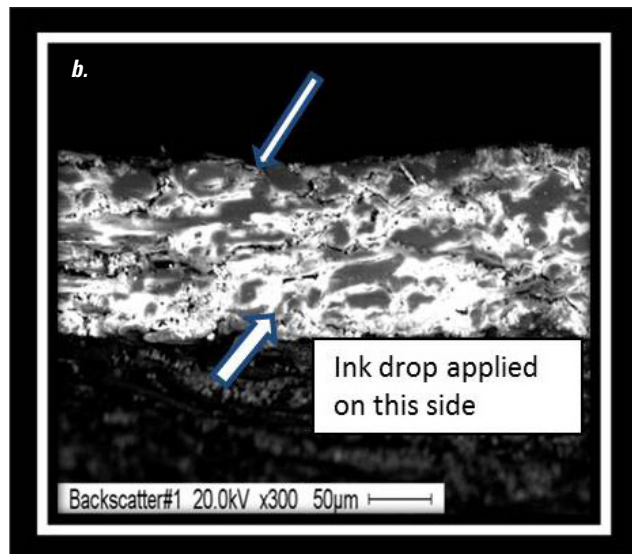
Optical microscopy

The experiments used a commercial store brand water-based black ink for ink-jet printers. The ink drops (0.2 μL) were applied to one side of the paper samples via pipette. Paper samples were clamped into the sliding microtome and sliced using a steel knife. The paper was sliced in the z-direction through

INK PENETRATION



1a. Scanning electron microscopy (SEM) image in the z direction of control paper (generic recycled content copy paper) using SEM atomic number backscatter. No Ag tracer is present. Paper filler is bright white.



1b. SEM image of silver/ink tracer in z direction of paper (generic recycled content copy paper) using SEM atomic number backscatter. Ag tracer appears right along the ink pathway and includes paper filler. Large arrow shows AgNP tracer.

the width of the ink drops. Optical light micrographs of the inked paper samples were taken to measure ink penetration and drop width in paper after each slice (Fig. 2).

Scanning electron microscopy

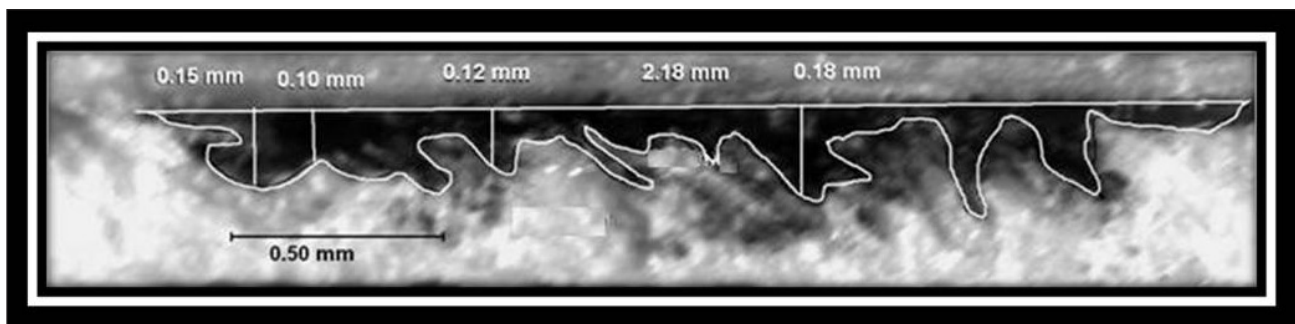
Unaided ink colorants were too low in electron density to render them visible under electron or confocal laser illuminations [12]. To solve this problem, we introduced silver nanoparticles (AgNP) (range: 50-250 nm) mixed in the ink to trace its voyage into the paper and aid its imaging by SEM and confocal laser scanning microscopy (CLSM) techniques [13]. Ink drops were applied via pipette (0.2 μ L) to the paper samples. These were sectioned to a fresh face using a sliding microtome with a steel knife and imaged in the z-direction by SEM (Figs. 1 and 4) in atomic number backscatter mode to locate the AgNPs. An SEM operating at 20 kV was used in both secondary and backscatter mode to image samples.

Confocal laser scanning microscopy

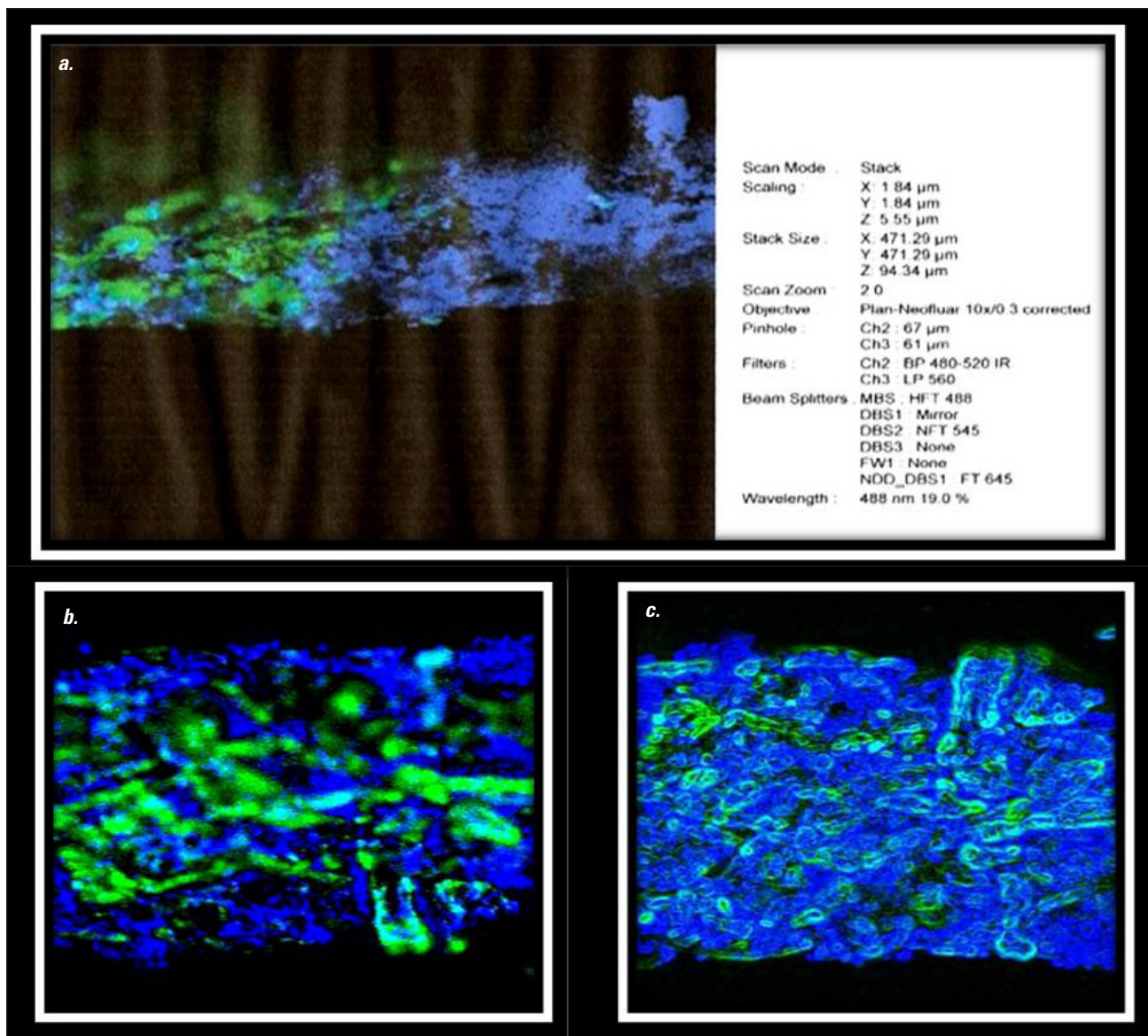
Paper and ink visualization using a CLSM [14] was used to obtain similar images along the z-directional thickness. A CLSM with a 488 nm laser was used. Fluorescent filters separate the AgNP areas and appear green against a blue background (Fig. 3).

Transmission electron microscopy

After successful imaging with these methods, we introduced AgNP (range: 50-250 nm) mixed in the ink to trace its voyage into the paper and aid its imaging by TEM. Additionally, we used smaller gold nanoparticles (10-30 nm) in the ink that would conceptually let liquid penetrate through the paper voids, on and into the fiber surfaces and cell wall nanopores for TEM imaging. The gold nanoparticles were mixed in the ink in the manner described previously. Ink drops (0.2 μ L) were applied via pipette to the paper samples. These were placed directly into epoxy resin and polymerized overnight at 60°C into blocks. Ultrathin sectioning was performed on a



2. Optical images taken of microtomed sections of dry inked paper in the z direction showing measured ink drop penetration at 50x magnification with 2.19 mm ink spread in the cross direction along the sample side of ink application.



3a-c. Confocal laser scanning microscopy (CLSM) Z-stack image of AgNP tracer using Plan Neofluar at 10x objective. Ink shows green, un-inked paper fibers are blue using two filters, BP480-520IR and LP 560 at 488 nm wavelength at 19% stacked image. Figures 3b and 3c are enlarged images of sections of Figure 2 for additional detail.

microtome using a 35° angle diamond knife. The sections were stained with uranyl acetate and viewed on a TEM at 80-100 keV.

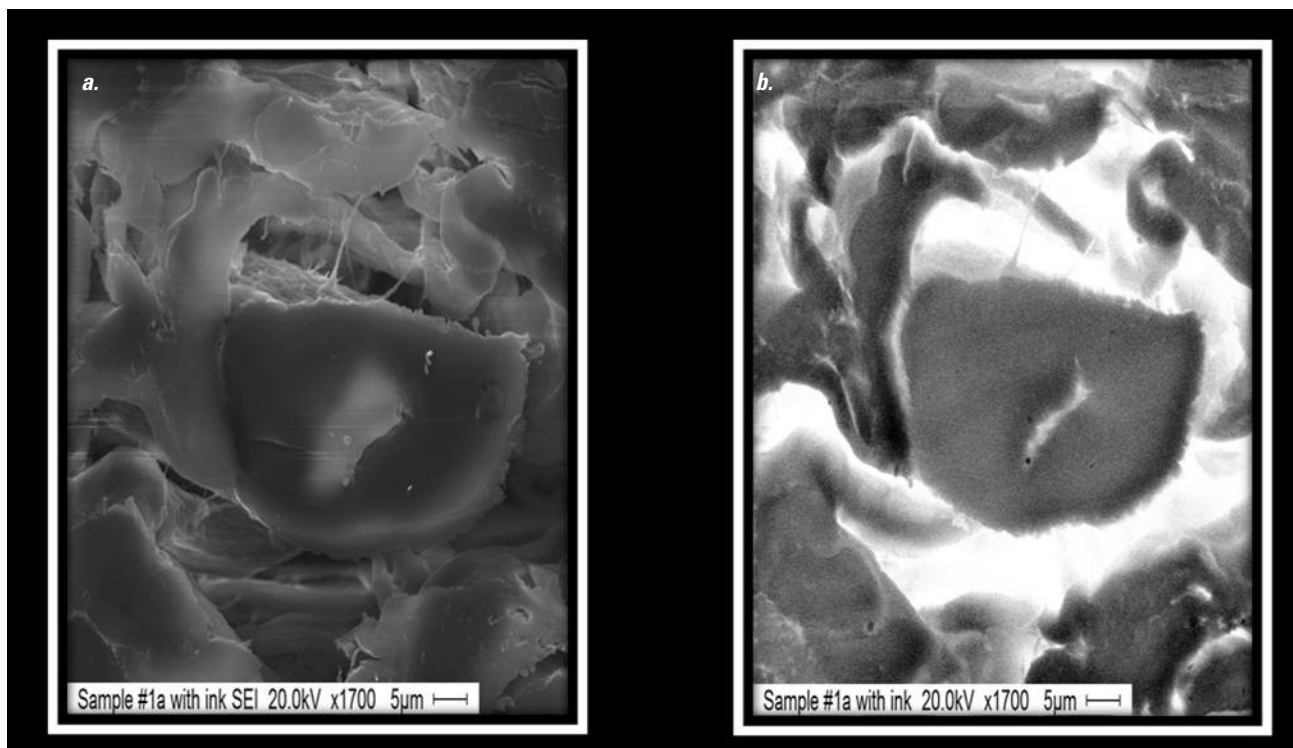
Antibody staining for TEM

A wide range of molecular probes, such as mAbs, have proven very effective in locating plant cell wall components, especially polysaccharides [15,16]. Two mAbs, LM 10 and LM11, have been shown to bind to polysaccharides and were used in this study. LM 10, in particular, bound only to unsubstituted or low substituted xylans and did not bind to arabinoxylan [17]. LM11, in contrast, bound strongly to arabinoxylan and to low-substituted xylans [17]. Although mAbs can bind to hemicelluloses, the specific location can be seen only

under electron microscopic observation. Because the mAbs and other components cannot be directly detected under electron beam illumination, suitable inert tags must be attached to ensure their visibility. Secondary gold nanoparticle tags were used with mAbs for this purpose.

Immuno-gold labeling TEM

A review by Ruel [18] identified immunochemical labeling as a precise way for locating and visualizing hemicelluloses in plant cell walls. This post-embedding technique [19] consisted of floating ultrathin sections of paper on a series of drops of blocking agents (phosphate-buffered saline [PBS] containing 1% bovine serum albumin [BSA] and PBS containing 1% cold water fish gelatin) and then a 1:20 μ L



4a-b. SEM images of Ag tracer. Figure 4a is an SEM image of inked paper under secondary conditions. Figure 4b is an SEM image of AgNP inked paper under atomic number backscatter conditions. AgNP ink is bright white.

dilution of the mAbs in PBS BSA for 15 min each. The samples were then washed and subsequently floated in a dilution of 1:20 μ L of 18 nm gold in goat anti-rat immunoglobulin G (IgG) (secondary marker). The sections were fixed with glutaraldehyde, rinsed in water, stained with uranyl acetate, and visualized by TEM.

RESULTS AND DISCUSSION

SEM, optical, and CLSM results

Figure 1a shows an SEM image of the cross-section of the paper without the AgNPs. The paper filler containing calcium carbonate and trace titanium shows as bright white in atomic number backscatter mode. **Figure 1b** shows an SEM image of the same sample with ink laced with AgNPs. The silver nanoparticles show as bright white regions comingled with some of the filler particles. Furthermore, the micrographs show clear evidence of the ink following the inter-fiber voids.

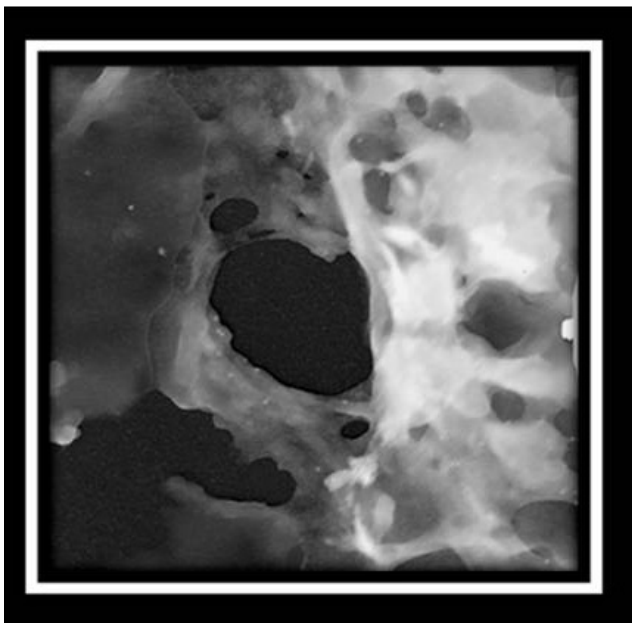
Figure 2 shows a cross-section of a paper sample with an ink drop. The sample was sectioned in the z-direction using a microtome and imaged in an optical microscope at 50x magnification. Images clearly indicate the location and route of the ink drop and were correlated with stacked serial sections of the same ink drop.

Paper samples with and without AgNPs were imaged in the CLSM using XYZ and XZY scan modes. **Figure 3a** is a series of sequenced XY images stacked at an interval of 1 μ m in the z-direction to provide a reconstruction of the ink drop in the paper. The CLSM pictures were compared with like z-

direction optical images of inked paper applied from the upper surface. **Figures 3b** and **3c** show two sample CLSM images taken from Figure 3a. The blue regions indicate the uninked portions within the paper structure. The bright green regions show the AgNP-labeled ink was attributed to the strong fluorescence the silver underwent because of the green filter. This technique allows for visualization of the relative amounts of ink onto and around the paper components, particularly in comparing varying fiber types and fillers.

The optical and CLSM images each showed the same drop quite clearly in terms of drop size, boundary, and location. We also compared the drop images to those obtained by secondary SEM imaging and SEM imaging using the backscatter mode shown in **Fig. 4**. The ink spread observed in the samples under optical condition spread (2.2 mm) and depth (0.15 mm) compared with CLSM imaging (2.35 mm) and depth (0.18 mm) verified the two techniques. The structure of the AgNP inked papers was identified by CLSM. Differentiating the liquid from the fiber/void areas can help quantify ink penetration into the structures, because the AgNP tracer showed clearly under the green filter. Additionally, the same position can be imaged for correlative purposes by first using the CLSM and then the SEM using this nondestructive technique.

Figure 4 shows two SEM micrographs obtained under normal and backscatter conditions. Normal imaging (Fig 4a) shows a fiber cross section with its lumen directly visible. Backscatter (Fig. 4b), however, results in illumination of the



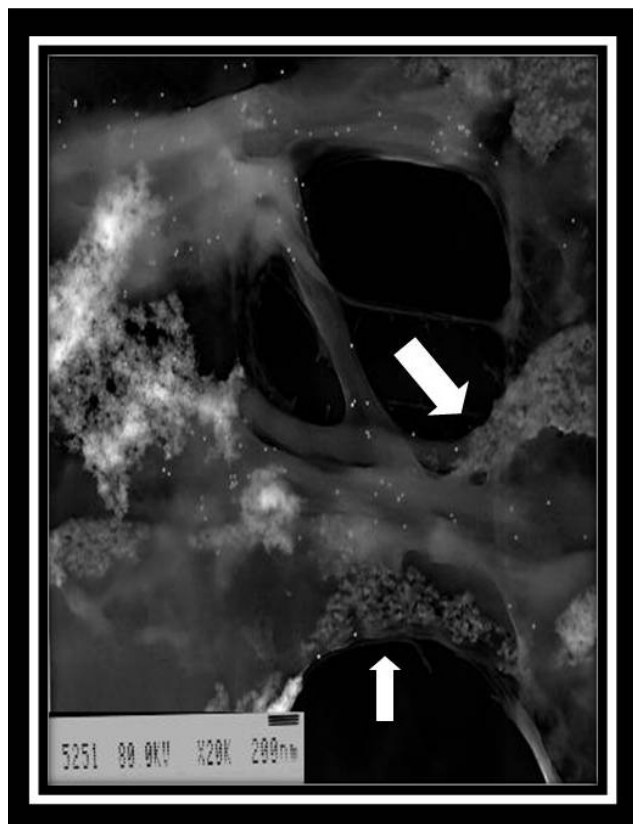
5. Transmission electron microscopy (TEM) image of thin sectioned monoclonal antibody LM 10 labeled paper with secondary 18 nm gold label showing hemicelluloses presence within the paper fiber and around a hardwood vessel at 30,000x magnification.

AgNPs (because of the high atomic mass of Ag) surrounding the fiber.

TEM results

Figures 5 and 6 show TEM images obtained after conducting the immunolabeling with nanoparticle gold. The xylan antibody-tagged gold nanoparticles appear as bright white spheres in these images. The gold nanoparticles attach themselves to the appropriate hemicellulose components (unsubstituted or low-substituted xylans), depending on the antibody used.

Figure 6 shows an ultrathin section of paper with the fiber structure (lumen, pits, and fibrous matter). The secondary gold label shows the location of the hemicelluloses in the fiber and is especially heavy in regions bordering the lumen and inside the fibrous structures. The gold label can only be taken to be representative and proportional to the number of hemicellulose epitopes exposed above the plastic. Normally, only 10%-20% of these epitopes are available for labeling [19]. Labels around the edges of the fibers likely represent hemicellulose that is on the fiber surface but is separated by the length of the IgG molecules on the primary and secondary antibody. The TEM image (Fig. 6) shows two different types of bright spots. The hemicellulose tag is similar to that in Fig. 5, and is concentrated around the edge of the fiber cavity. The larger clustered spots correspond to the AgNPs within the fiber and near the hemicellulose tags. The AgNPs have clearly penetrated the cell wall carried by the ink. Future experiments will determine if the AgNP



6. TEM image of AgNP traced, thin sectioned LM 10 monoclonal antibody labeled paper. Tracer within the fiber sporadically overlaps the hemicellulose tag. Large arrows show AgNP tracer inside the fiber. Smaller brighter spheres are the hemicelluloses gold label at 20,000x magnification.

tracers have a preference for particular components of the cell wall nanostructure and help in the development of diffusion and wicking parameters for modeling.

CONCLUSION

This study demonstrates a viable method of locating paper fibers and their subcellular components (Fig. 6). The ink path can be quantified using AgNPs. Bound primary mAbs with secondary gold labels were used to identify the location of hemicellulose components of the paper fibers in a semiquantitative manner. Correlation of the AgNPs to the hemicellulose label clearly shows penetration into the cell walls. Labeling with antibodies possessing high specificity hemicellulosic polysaccharides will further allow identification of xylan and other fiber components. **TJ**

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ABOUT THE AUTHORS

We chose this topic because it compliments previous research done by my major professor utilizing my industrial experience in digital imaging papers and papermaking. It was a challenge that intrigued me because there are still many unknowns in ink spread and diffusion in paper.

This research compliments many mathematical models and theories on spreading and diffusion, including cell wall pores research and fiber wicking. The idea was to use antibodies to correlate the fiber characteristics with the visual location of the ink penetration due to the nanoparticles.

The research differs in that it takes ink spreading to a visual nano-level and allows the imaging of ink (which is colorless under the following conditions) using confocal, scanning, and transmission electron microscopic techniques as opposed to optical viewing.

The most difficult part of this research was finding the picture that described the theory without words. This was addressed by imaging numerous ink droplets on paper until the picture told the story.

Ink penetration and wicking into and around the fibers can be understood on a nanoscale. This will lead the way for enhanced understanding on diffusion and wicking into fibers and other materials. The most interesting finding was that the idea to trace the ink with

nanoparticles for visibility actually worked.

The more in-depth the understanding of ink penetration into fiber and cell wall components of paper, the better chance papermakers and printers have to simplify deinking, improve printability, and develop ink formulations, perhaps without the need for costly additives.

Our next step is to keep looking at ink/liquid penetration to try to understand any correlations with wood morphology, topography, and chemistry using smaller nanoparticles and differing liquid and paper mediums.



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