Silica Nanocasts of Wood Fibers: A Study of Cell-Wall Accessibility and Structure
Per Valdemar Persson,† Jonas Hafré,‡ Andrew Fogden,† Geoffrey Daniel,‡ and Tommy Iversen*†
STFI, Swedish Pulp and Paper Research Institute, Box 5604, SE-114 86 Stockholm, Sweden, and SLU,
Department of Wood Science, Swedish University of Agricultural Sciences, WURC, Box 7008,
SE-750 07 Uppsala, Sweden

Received December 17, 2003; Revised Manuscript Received February 6, 2004

The porosity and the available surface area of a lignocellulosic fiber can influence the accessibility and reactivity in derivatization and modification reactions because the porous cell-wall network determines the upper size limit for molecules that can penetrate and react with the interior of the wall. To obtain information concerning the accessibility of the porous cell wall of wood fibers, surfactant-templated sol–gel mineralization has been examined. Wood and kraft pulp samples of Norway spruce were impregnated with a silica sol–gel and subsequently heated (calcined) and transformed into structured mesoporous silica. Microscopy studies (environmental scanning electron microscopy, transmission electron microscopy, TEM) on the silica casts showed that the three-dimensional architecture of the wood and pulp fiber cell wall was revealed down to the nanometer level. Image analysis of TEM micrographs of silica fragments from the never-dried pulp revealed complete infiltration of the cell-wall voids and microcavities (mean pore width 4.7 ± 2 nm) by the sol–gel and the presence of cellulose fibrils with a width of 3.6 ± 1 nm. Cellulose fibrils of the same width as that shown by image analysis were also identified by nitrogen adsorption measurements of the pore size distribution in the replicas.

Introduction

The network of cellulose fibrils, hemicelluloses, and lignin that make up the cell walls of wood fibers forms a three-dimensional architecture in which the spacing between the various constituents defines the porous structure. The porous structure determines the internal accessible surface area and the upper size limit for molecules that the cell wall may exchange with an external medium. This determines the penetrability, accessibility, and reactivity of wood fibers when, for example, macromolecules such as enzymes or high-molecular-weight polymers are used for fiber modification.

Electron microscopy is an important tool that has been extensively used to study the cell-wall structure of plant fibers and the morphological changes that occur during the processing of lignocellulosic materials. Depending on the techniques used, the samples are often required to pass through different preparation steps before being studied by electron microscopy. Drying steps are often required in the preparation of biological samples, and these can cause artifacts or distortions of the native structure, especially the porous structure. For example, in a chemical pulp, water removal closes most of the cell-wall pores.† This has initiated the development of casting methods, which enable the ultrastructure of sample surfaces of moisture-containing structures to be visualized in great detail. Rapid freeze replica techniques, in particular, have been successfully used for studies of wood and pulp samples.2–5

Instead of investigating a soft water-swollen biological material directly, it is also possible to examine a replica as the pore structure of a fixed stable mesoporous silica material obtained by nanocasting.6 Mineralization of biological tissue followed by subsequent removal of the organic matrix has previously been the method of choice in the materials science field to transfer structural information to a hard inorganic framework.7–12 Recently, we reported a method for three-dimensional morphology studies that utilizes silica sol–gel as a casting medium and can be used on water-swollen wood and pulp samples.13,14 The organic material is subsequently removed by calcination, resulting in a mesoporous silica replica whose three-dimensional structure can be analyzed down to the nanometer level using transmission electron microscopy (TEM).

In this study, image analysis of TEM micrographs and nitrogen adsorption measurements on silica replicas have been used to reveal details of the accessible internal surfaces and porous structure of the cell wall of wood and pulp fibers.

Materials and Methods

Preparation and Characterization of Wood and Pulp Samples. Tetraethyl orthosilicate (TEOS, 98%) and hexa-
decyltrimethylammonium chloride (CTAC, 98%) were purchased from Merck and Fluka, respectively, and used as received. Air-dried wood chips from Norway spruce (Picea abies) were used for impregnation. The kraft pulp sample was prepared by delignification of wood chips at 170 °C (0.22 M SH–, 0.40 M OH–, liquor-to-wood ratio 20:1) to kappa number 18, corresponding to a residual lignin content of 3% (standard method SCAN-C 1:00). The yield was determined as 44.8%. The monosaccharide yield was determined by hydrolysis of untreated and sol–gel-impregnated wood and pulp samples. After reduction with potassium borohydride, the samples were acetylated using 1-methyimidazole. The monosaccharides were finally determined by anion exchange chromatography with pulsed amperometric detection. The Klason lignin was determined using the standard method TAPPI T 222 om-88.

Preparation of the Surfactant-Templated Sol–Gel. A total of 3.7 g of CTAC was dissolved in 11 mL of 99.5% ethanol and 1.8 mL of HCl (aq, 1.39 M). A total of 10 mL of TEOS was added, whereby heat was generated with the formation of polysilicic acid. The sol–gel was cooled to room temperature before use. Poly(tetrafluoroethylene) vessels were used for all TEOS-containing mixtures.

Preparation of Silica Nanocasts. Wood and never-dried kraft pulp (1–2 g) were stepwise solvent-exchanged to 99.5% ethanol and placed under a vacuum to remove air. The samples were immersed in the sol–gel and kept at 60 °C for 72 h. The impregnated chips were finally subjected to high-temperature treatment in air (575 °C, 6 h) for calcination and removal of all organic matter. A reference silica sample was prepared by treating a sol–gel at 60 °C for 72 h, without wood or kraft pulp present, followed by drying and calcination.

Electron Microscopy and Energy-Dispersive X-ray Analysis. The carbon and silicon contents of the impregnated samples before calcination were determined using energy-dispersive X-ray analysis (EDXA) in combination with field-emission environmental scanning electron microscopy (FE-ESEM) on a Philips XL30 ESEM-FEG using an acceleration voltage of 10–15 kV, a pressure of 67 Pa, and a working distance 10 mm and imaging with a backscatter detector. X-ray analysis was performed on 50 locations using line scans. The high acceleration voltage was necessary to achieve an acceptable signal-to-noise ratio. Semiquantification of the carbon and silicon contents across the cell walls was performed, by comparing the cell walls with the corresponding filled lumina. The silica casts formed in the calcination process were analyzed by ESEM and TEM. ESEM images were taken using an Electroscan ESEM 2020, at an acceleration voltage of 10–15 kV. TEM observations were performed on a Philips CM12, at an acceleration voltage of 60 kV.

Image Analysis. The paper-printed TEM micrograph of the pulp cast was digitized and magnified to 500 000×. The light-shaded structures, corresponding to cellulose structures in the pulp, were analyzed manually using a millimeter scale along four arbitrarily chosen sections adding up to a total length of 1 µm containing approximately 140 fibril units.

Nitrogen Adsorption. Nitrogen adsorption measurements on the calcined silica samples were performed using a Micromeritics ASAP 2010. The surface area was calculated from the Brunauer–Emmet–Teller (BET) isotherm, and the pore size distribution was obtained using the Barrett–Joyner–Halenda algorithm.

Results and Discussion

Silica Impregnation of Wood and Kraft Pulp. The high affinity of the surfactant-templated silica sol–gel toward the polar groups of the fiber cell-wall constituents combined with the high degree of structural integrity after removal of the organic matrix make this method suitable for studies of cell-wall pore systems and the changes that take place during chemical treatment, exemplified in this paper by the delignification process that occurs during kraft pulping. Figures 1 and 2 show ESEM micrographs of silica-impregnated wood and kraft pulp samples, respectively, before calcination. The relative amounts of carbon and silicon introduced into the cell walls have been measured along the dashed lines using the EDXA technique.

The dark structures, corresponding to pores in the pulp, were measured in the same manner after inversion of the image.
The ratio $a / b$ enables different samples to be compared. Calculations on the wood and pulp samples ($a_w / b_w \approx 0.2$ and $a_p / b_p \approx 0.4$, respectively) show that about twice the amount of silica has penetrated the cell wall of the pulp fiber than the wood fiber. It is also worth noting that the surfactant (CTAC) of the sol–gel gives rise to a carbon signal in the filled lumen, as seen in both spectra. The increase in weights of the wood and pulp samples as a result of the impregnation (dry weight) before calcination were found to be 50 and 104%, respectively, which is in agreement with the results from EDXA. These values correspond to wood polymer contents in the impregnated samples of 66 and 49%, respectively, which agrees with the determined total lignin and monosaccharide yield (wood 67%, pulp 54%, w/w, dry weight). The difference reflects the change in cell-wall pore volume caused by delignification. The cumulative pore volume as determined by the solute exclusion technique has, for example, been reported to increase from 0.5 cm$^3$ g$^{-1}$ to 1.4 cm$^3$ g$^{-1}$ when spruce wood is converted into a kraft pulp of 45% yield.\(^1\)

**Silica Nanocasts of Wood and Kraft Pulp.** A total of 1 g each of the wood and pulp samples resulted in respectively 0.18 and 0.37 g of silica after impregnation and subsequent calcination. These results show that a quantitative hydrolysis of TEOS [into silica in the form of $\text{Si(OH)}_4\text{Si(OH)}_2\text{Si(OH)}_4$] took place during impregnation and that all the organic content was removed during calcination. The silica casts were studied by ESEM, TEM, and nitrogen adsorption. As seen in Figure 3, ultrastructural features such as tracheids, ray cells, and bordered pits were well reproduced with respect to both structure and dimensions in the wood and pulp samples.

The kraft pulping process removes lignin and hemicellulose from the wall and leads to a substantial increase in pore volume.\(^1\) This treatment also induces ultrastructural changes, some of which are visible in Figure 3. The most obvious difference is the loss of pit membranes and sometimes pit chambers in the kraft pulp fibers, which thereby increases the accessibility of the fiber lumen.

Figure 4 shows TEM images of nanocasts of the wood and kraft pulp cell walls. In contrast to the featureless structure of the lignified cell wall (Figure 4a), the kraft pulp...
nanocast exhibits a regular structure (Figure 4b,c). It is expected that the silica distribution on the surface and not inside the compact solid cellulose fibrils gives rise to pores of corresponding size after calcination.

The original cell-wall porous structure is, thus, represented by the dark regions (i.e., those filled with silica), whereas the light (unfilled) structures, oriented and uniform in size, probably originate from the cellulose fibrils. The nanocasting reveals that the porous structure of the pulp fiber cell wall on the nanometer level can be described as an anisotropic ordered structure oriented in the direction of the cellulose fibrils. These pores were rather long compared to their width; seemingly continuous structures of up to 1 μm in length were observed in micrographs of the pulp cast. Pore lengths of this size have been reported previously from NMR relaxation measurements. The accessibility of the never-dried kraft pulp is high because the silica sol–gel has completely infiltrated the cell-wall network. However, questions remain concerning the behavior of the lignin and hemicelluloses. From the appearance of the regular fibrillar structures in the pulp nanocast, it seems that these noncrystalline components, when partially degraded, are integrated into the mesoporous silica structure, in contrast to the wood where the native lignin-hemicellulose matrix appears to be much less permeable.

**Image Analysis.** A digitized image of the never-dried kraft pulp nanocast was subjected to direct measurements (Figure 4b, parallel lines) to quantify the widths of both the light and dark structures. The result is shown in Figure 5. The mean value (± standard deviation) of the width of the light-shaded structures assigned to cellulose fibrils was determined as 3.6 (±1; n = 150) nm. Electron microscopic observations of rapid-freeze deep-etched softwood and pulp samples have reported similar sizes for cellulose fibril and fibril aggregate surfaces. The width of the cellulose structures is also in agreement with fibril dimensions from electron microscopy studies of embedded and stained samples and from NMR measurements. The mean width of the dark elongated structures, that is, the pores of the original pulp, was determined to be 4.7 (±2; n = 145) nm, which is in accordance with earlier studies using the solute exclusion technique. NMR relaxometry, and inverse size exclusion chromatography.

**Nitrogen Adsorption.** The preparation of silica nanocasts of fiber materials with moisture-sensitive structures makes it possible to apply methods such as porosimetry. Accordingly, the pore distribution of the silica casts was measured using the nitrogen adsorption technique. The results from

![Figure 5. Analysis of the pulp cast structure (measured along the white lines of Figure 4b magnified to 500 000×). (3) Cellulose structures (light-shaded structures in TEM image); (■) pores (dark structures). All measurements were made to the nearest integer value.](Image)

The mean value of the width of the light-shaded structures assigned to cellulose fibrils was determined as 3.6 (±1; n = 150) nm. Electron microscopic observations of rapid-freeze deep-etched softwood and pulp samples have reported similar sizes for cellulose fibril and fibril aggregate surfaces. The width of the cellulose structures is also in agreement with fibril dimensions from electron microscopy studies of embedded and stained samples and from NMR measurements. The mean width of the dark elongated structures, that is, the pores of the original pulp, was determined to be 4.7 (±2; n = 145) nm, which is in accordance with earlier studies using the solute exclusion technique. NMR relaxometry, and inverse size exclusion chromatography.

**Nitrogen Adsorption.** The preparation of silica nanocasts of fiber materials with moisture-sensitive structures makes it possible to apply methods such as porosimetry. Accordingly, the pore distribution of the silica casts was measured using the nitrogen adsorption technique. The results from

![Figure 6. Pore diameter distribution calculated from nitrogen adsorption measurements on silica casts; expansion of 3–5 nm pore diameter shown in the inset. (■) Reference; (●) wood; (■) kraft pulp.](Image)

The differences in porosity and surface area are significant and can probably, at least partly, be ascribed to the chemistry of the casting process. The surfactant of the impregnation mixture (CTAC) is reported to contribute to the surface area and porosity through the formation of a micellar mesophase, which gives rise to micro- or mesopores during the calcination process, depending on the choice of surfactant and reaction conditions. As seen in Table 1, the porosity as well as the total surface area of the silica decreases when templated by wood or kraft pulp. One possible explanation is that lignin, hemicellulose, or wood extractives interact with the sol–gel and thereby inhibit the formation of micropores. The association of polysaccharides, such as polyuronides and glycosaminoglycans, with silica has been reported previously. Even though the total surface area of the pulp cast is lower than that of the reference (873 and 1224 m² g⁻¹, respectively), the region in which the contribution from the cellulose fibril and fibril aggregate surfaces is expected (d > 3.5 nm) exhibits a much higher surface area (128 and 24 m² g⁻¹, respectively). This difference is clearly visible in Figure 6, which shows the pore size distributions of calcined wood, pulp, and reference silica samples.
in the case of the wood sample (at a slightly smaller diameter, see inset).

**Conclusion**

The beneficial structural stability and chemical robustness of silica permit a detailed structural analysis to be made on casts from wood and pulp. By analyzing the silica casts using scanning electron microscopy, it was shown that the three-dimensional anatomical features of wood and pulp were well reproduced. The uptake of silica by the pulp was different from that by wood, which can be ascribed to the increased accessibility as a result of the removal of cell-wall components, such as lignin, hemicelluloses, and extractives, that takes place during the kraft cook, leading to increased accessibility and a different chemical environment. The image analysis of transmission electron micrographs revealed cellulose fibril structures at a mean diameter of 3.6 (± 1 nm and elongated micrometer-long pores of 4.7 (± 2 nm. In conclusion, the overall results suggest that the transfer of information regarding structure and cell-wall accessibility from native tissue to the silica nanocasts is successful down to the width of single cellulose fibrils, that is, 3 – 4 nm.

**Acknowledgment.** This work was carried out within the framework of the Wood Ultrastructure Research Centre (WURC) at Uppsala, Sweden, which is financed by VINNOVA, the Nordic pulp and paper industry, and the Swedish University of Agricultural Sciences. Joanna Hornatowska, Owe Lidbrandt, and Marianne Ahlbom are thanked for technical assistance on ESEM/FE-ESEM. IRECO is gratefully acknowledged for financial support.

**References and Notes**