Silicon cantilever functionalization for cellulose-specific chemical force imaging of switchgrass

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ARTICLE

Silicon cantilever functionalization for cellulose-specific chemical force imaging of switchgrass†‡

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A method for direct functionalization of silicon and silicon nitride cantilevers with bifunctional silanes was tested with model surfaces to determine adhesive forces for different hydrogen-bonding chemistries. Application for biomass surface characterization was tested by mapping switchgrass and isolated switchgrass cellulose in topographic and force-volume mode using a cellulose-specific cantilever.

Introduction

Atomic force microscopy carried out in tapping mode provides a high resolution surface map by measuring deflection of a cantilever probe moving across the surface of a sample. Chemical force microscopy (CFM) expands this method to detect chemical bonding forces between chemical groups on the sample surface by attachment of ligands, the sensing molecules, to the probe and measuring the pulling force required to move the cantilever across the sample surface. These force-distance measurements examine attractive and repulsive forces on the cantilever tip as it approaches and retracts from the sample surface. The intermolecular forces are mapped and related to surface structures detected by topographical imaging for identification[1]. Biological materials that have been studied by CFM include DNA[2], cellulose[3], toxins[4], and selectins.[5] The determination of the pulling force variation across the surface provides a high resolution map of specific polymers.

Lignocellulosic biomass is an abundant and renewable feedstock for both biofuels and other co-products, but cost-effective production is hampered by its recalcitrant composite structure and heterogeneous components. The association of cellulose with the other component polymers, lignin and hemicellulose in the hierarchical structure of the cell wall limits its accessibility to solvents and enzymes. Atomic force microscopy carried out in tapping mode has provided important topographical information on cell wall structure and hierarchial associations of cellulose fibrils[6,7], morphological effects of enzymatic degradation[8], and visualization of nanoparticles on cellulose microfibrils.[9] Chemical force mapping can potentially provide important insights into the chemical and enzymatic processes of biomass deconstruction by providing visualization of relevant surface properties. Although maps of adhesion forces between an unmodified silicon cantilever and nanocellulose crystals have been extracted computationally from tapping mode data to compare surface properties[9], specific differentiation between lignocellulose components requires probe functionalization.

Typical functionalization schemes are based on gold-thiol self-assembly (SAM), which requires coating of the cantilever probes with metals such as chromium, followed by gold, using thermal high-vacuum evaporators. Bifunctional alkanethiols are then used to functionalize the gold surfaces. The crystalline, polymeric nature of cellulose complicates attachment by alkanethiol chemistry. Simple dip-coating in soluble cellulose derivatives has been used to derivatize probes, as reported in a recent study which compared binding of cellulase enzymes to probes terminated with 4.5-µm polystyrene beads coated with hydroxypropyl cellulose[11]. However, the chemical structure and crystalline nature of native cellulose, which define the relative orientation of the hydroxyl groups on its surface, are altered by derivatization.

Here we describe an alternative, direct functionalization of silicon and silicon nitride cantilevers with bifunctional silanes to specifically detect cellulose by hydrogen bonding to the hydroxyl groups on the surface of cellulose microfibrils.

Experimental

Silicon nitride cantilevers and silicon chips were functionalized with commercial bifunctional silanes purchased from Gelest, Inc. (Morrisville, Pennsylvania, USA). The bifunctional silanes selected for the tests had the following organofunctional groups with the indicated chemical characteristics: ethyltrimethoxysilane (hydrophobic), bis(2-hydroxyethyl)-3-aminopropyl triethoxysilane (randomly oriented, non-specific hydroxyl groups), and N-(triethoxysilylpropyl) gluconamide (glucose-specific hydroxyl groups) (Figure 1).

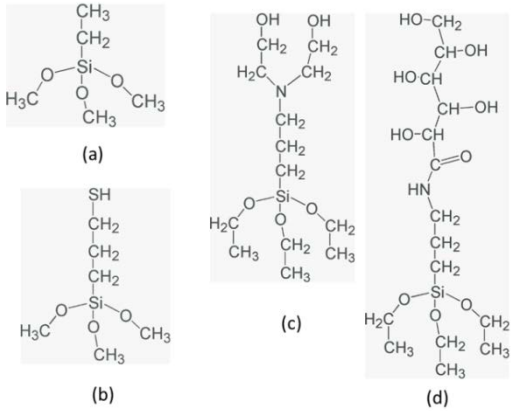

Fig. 1. The differences in the chemical structures of the bifunctional silanes that were used for functionalization determine their selectivity: (a) ethoxytrimethoxysilane; (b) 3-mercaptopropyltrimethoxysilane; (c) bis-hydroxyethyl aminopropyltriethoxysilane; (d) triethoxysilyl N-propylgluconamide (technical product information from Gelest, Inc.). Each biofunctional silane was precisely selected to probe the hydrogen-bonding profile of select biomass samples.

A commercial MultiMode Scanning Probe Microscope (MMSPM NanoScope IIIa, Digital Instruments, Santa Barbara, CA) was used to measure topography and adhesion forces. The MMSPM scan-head was housed in a custom-made environmental control system with humidity control and multiple gas delivery system. Ultrapure nitrogen (99.9997%) with 3% humidity was used for the experiments. Before functionalization, the cantilever probes and silicon chips were cleaned with a UV-ozone cleaner (Bioforce Nanosciences, Ames, Iowa). Tapping mode images were obtained using a silicon cantilever in ultrapure nitrogen gas with scan rates ranging from 1.2 to 1.5 Hz, frequencies from 320 to 30 kHz, and amplitudes from 135 to 300 mV, as described previously for imaging of cotton fibers[8] and bacterial cellulose.[9] Chemical force measurements were carried out using the following parameters: scan rates 1.507 Hz, scan size 3.000 um, number of samples 256, image data type phase, data scale 5.000 degrees.[12]

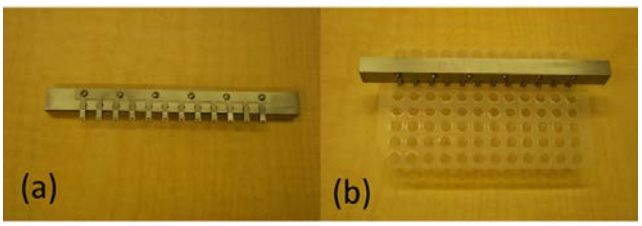

Fig. 2. A holder with twelve clips to hold commercial silicon cantilever chips was designed and fabricated (a). The position of the clips fits the wells of standard 96-well 7.5 x 12 cm microplates (b).

A special holder with twelve spring-loaded clips was designed and fabricated from stainless steel in-house at Oak Ridge National Laboratory to enable rapid and reproducible functionalization of multiple cantilevers using standard 96-well plates with dimensions 7.5 x 12 cm (Figure 2). Each clip holds a commercial silicon chip inserted with the attached cantilever extending out 6 mm from the holder to enable submersion in the wells.[12] A 96-well plate was prepared for the modification procedure by preloading the wells with modification solutions and sterile distilled water for washes, 0.250 ml per well. Twelve silicon nitride cantilevers were modified simultaneously. Three cantilevers were used for each set of modifications as follows: control (ethanol only), 20% ethyltrimethoxysilane in ethanol, 10% bis(hydroxyethy)-aminopropyltriethoxysilane, and 10% trimethoxysilyl N-propyl gluconamide. Residence time in the silane reagents was 2 min. Following functionalization and four water washes, the cantilevers were dried under vacuum. Functionalization of cantilevers and silicon chips was verified by contact angle measurements, a standard method for measuring changes in surface hydrophobicity. Procedure and results for contact angle measurements are detailed in the Electronic Supplementary Information (ESI†).

Results

The adhesive forces for each of the cantilever functionalization types were calibrated using silicon chips that had been coated with ethyltrimethoxysilane (ETMS), bis(hydroxyethyl)-aminopropyltriethoxysilane (BHEA-TMS), triethoxysilyl N-propyl gluconamide (TES-NPG), and 3-mercaptopropyltrimethoxysilane (MPTMS). Forces are reported as phase angles, which are directly proportional to pulling force (Table 1). The strength of the measured adhesion forces corresponded to hydrogen bond formation between the hydroxyl groups. The highest force, with a phase angle of 2, was observed when both the silicon chip and cantilever were modified with TES-NPG, which has five hydroxyl groups in glucose-specific steric positions. The pendant gluconamide group of TES-NPG would be able to assume the glucopyranoside ring conformation, which would match the hydrogen-bonding pattern of native cellulose I.

Table 1. Adhesive forces between cantilevers (N = 3) and silicon chips coated with bifunctional silanes[a] were determined by phase angle measurements.

Cantilever functionalization	Silicon chip functionalization			
	ETMS	MPTMS	BHEA-TMS	TES-NPG
No treatment	0	0	0	0
Solvent only	0	0	0	0
ETMS	0.4	0	0.1	0.1
BHEA-TMS	0.1	0.2	0.7	0.8
TMS-NPG	0	0.2	1.1	2

[a]Ethyltrimethoxysilane (ETMS), bis(hydroxyethyl)-aminopropyltriethoxysilane (BHEA-TMS), triethoxysilyl N-propyl gluconamide (TES-NPG), and 3-mercaptopropyltrimethoxysilane (MPTMS).

Following successful calibration with the model surfaces, the glucose-specific cantilevers that had been modified with N-(triethoxysilylpropyl) gluconamide were used to image switchgrass samples with differing amounts of surface-accessible cellulose. Switchgrass (*Panicum virgatum*, Alamo cultivar) was obtained through the BioEnergy Science Center at Oak Ridge National Laboratory and prepared at the Georgia Institute of Technology as described previously[13]. Extractive-free switchgrass was prepared by extraction of size-reduced switchgrass with benzene:ethanol, a treatment that removes waxes, proteins, lipids, and chlorophyll while leaving the lignocellulosic cell wall structure intact. Cellulose was isolated by treatment of extractive-free switchgrass with sodium

hypochlorite to remove lignin followed by treatment with hydrochloric acid to remove hemicellulose.

AFM images of cellulose and extractive-free lignocellulose (i.e., switchgrass) were carried out in tapping mode to provide topographical identification of surface features. Dispersed microfibrils with diameters corresponding to 5-10 nm as typically observed in AFM imaging of cellulose[6,7] are visible over the entire surface of the cellulose sample (Figure 3). The lignocellulose sample is more heterogeneous, exhibiting both aligned fibrillar structures and less defined globular masses consistent with the presence of cellulose microfibrils embedded in a matrix of hemicellulose and lignin (Figure 4). Small areas appear to correspond to aligned cellulose microfibrils exposed after removal of membrane lipids and proteins by the solvent extraction. These surface features resemble those reported for maize primary cell walls, which included 50 – 250 nm diameter cellulose macrofibrils composed of microfibrils not encased by lignin and hemicelluloses.[6]

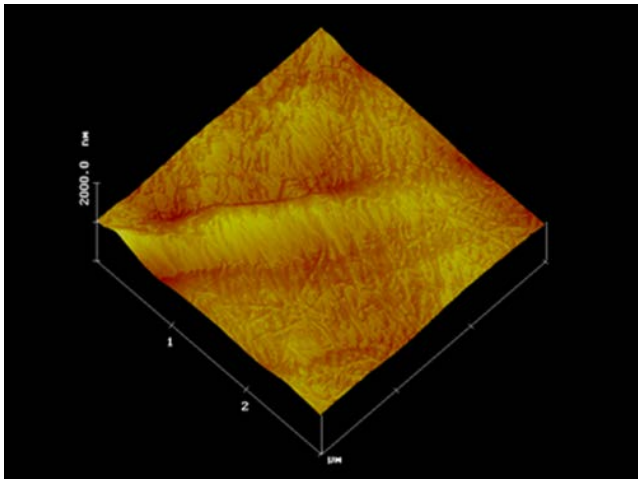

Fig. 3. Tapping mode image of cellulose isolated from switchgrass shows its microfibrillar structure.

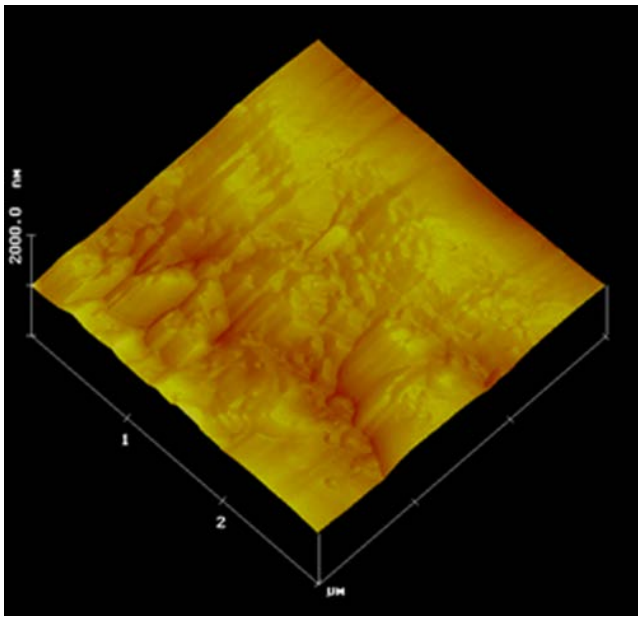

Fig. 4. Tapping mode imaging of extractives-free switchgrass shows heterogeneous laminate surface.

Chemical force mapping was then carried out using a silicon nitride cantilever functionalized with triethoxysilyl N-propyl gluconamide in force volume mode, which provides both topographical and adhesive force mapping for the specific area scanned. Topographic force volume mode, which has lower spatial resolution than tapping mode, showed parallel microfibril bundles (Figure 5). The adhesion force strengths are depicted using a false-color scheme (Figure 6) based on the calibrations described in Table 1 in the corresponding adhesion image (Figure 7). The adhesion forces were fairly uniform across the sample surface and corresponded in magnitude to the force observed in the calibration for the glucose hydroxyl matching between triethoxysilyl N-propyl gluconamide-modified cantilevers and silicon chips (Table 1).

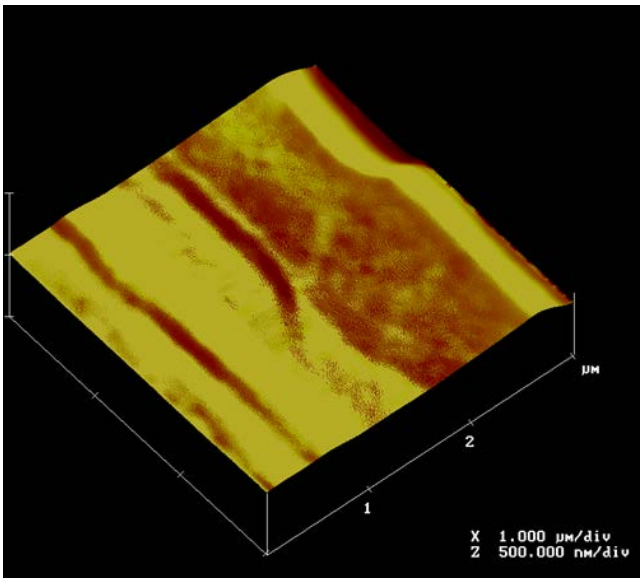

Fig. 5. Topographic image of switchgrass cellulose obtained in force-volume mode.

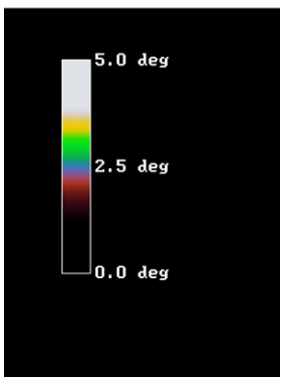

Fig. 6. A false color scheme based on the calibrations was used for depiction of the adhesion forces measured as angles of cantilever deflection in the images in figures 7 and 9.

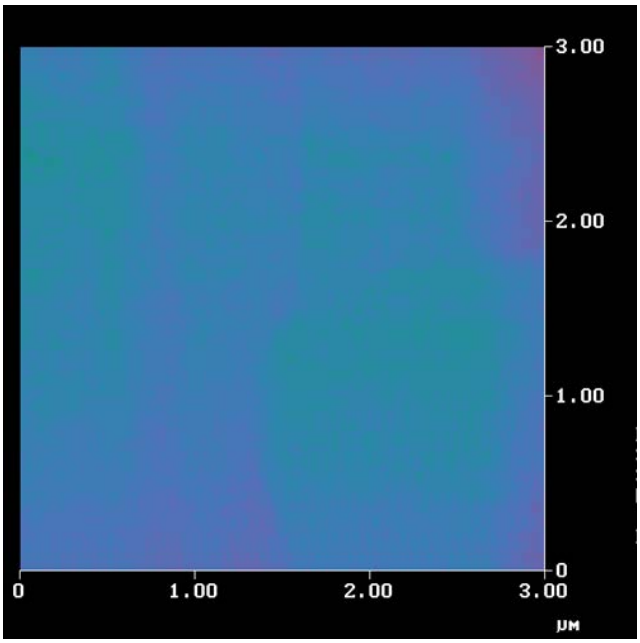

Fig. 7. Adhesion image of switchgrass cellulose obtained in force-volume mode.

The force-volume mode topographic image of the extractive-free lignocellulose resolved few surface features compared to tapping mode (Figure 3) and only showed general contours of the sample (Figure 8). In contrast, the adhesion image shows distinct areas with pulling force similar to that observed for cellulose flanked by regions of low adhesion (Figure 9). This pattern corresponds to the striated area of exposed cellulose microfibrils flanked by regions probably covered with hemicellulose and lignin observed in tapping mode (Figure 3).

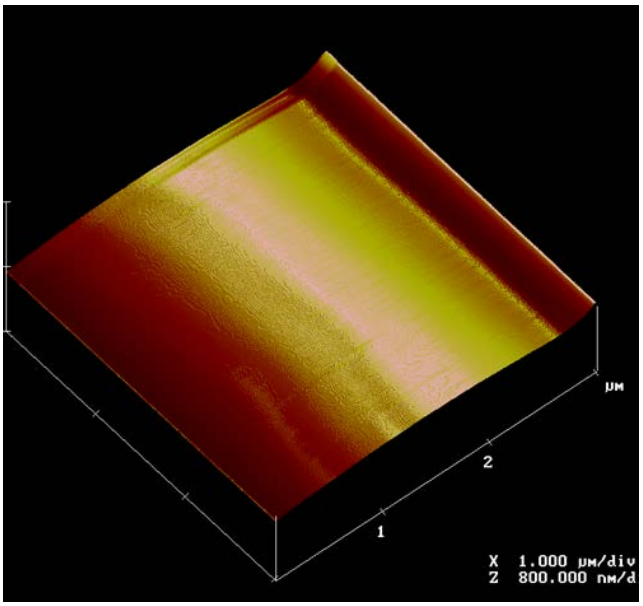

Fig. 8. Topographic image of extractives-free switchgrass obtained in force-volume mode.

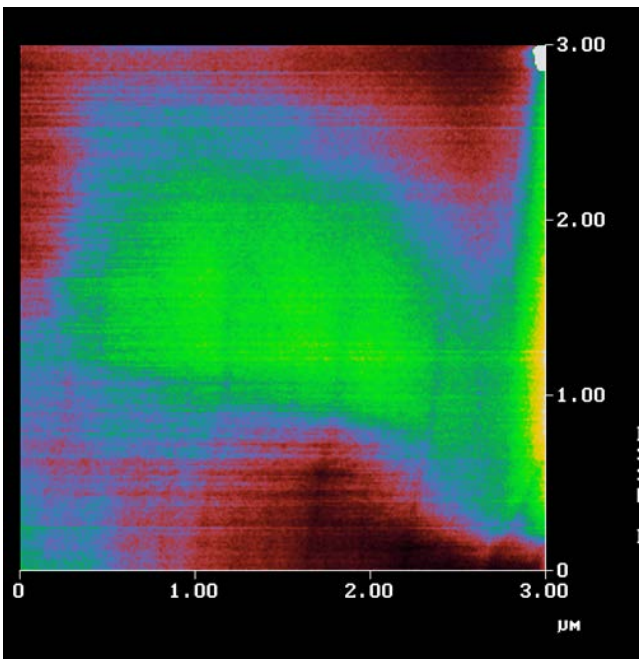

Fig. 9. Adhesion image of extractives-free switchgrass obtained in force-volume mode.

Conclusions

In conclusion, we have developed and demonstrated a technique for functionalization of cantilever probes for chemical force detection of surface accessible cellulose in biomass samples. Correlation of measured pulling forces with expected hydrogen bonding strengths was established by measurements on model silicon substrates functionalized with a range of bifunctional

silanes. Surfaces modified with triethoxysilane N-propyl gluconamide exhibited the highest adhesive force as predicted based on their glucose-specific hydroxyl group orientation. Tests of a gluconamide-functionalized cantilever on cellulose and lignocellulosic biomass mapped corresponding pulling forces to surface accessible cellulose microfibrils detected in tapping mode. The direct treatment of silicon and silicon nitride cantilevers with bifunctional silanes appears to offer a number of advantages compared to gold-thiol functionalization. Functionalization was faster (2 min residence time) than attachment of thiol-terminated hexasaccharides using the gold-thiol self-assembled monolayer (SAM) method (3 h residence time[14]). Comparison of prices for commercially available cantilever probes indicates that gold-chromium coating increases cantilever price 20 - 40% for silicon and silicon nitride probes, respectively (BudgetSensors 2012 price list http://www.budgetsensors.com). Following hydrolysis of the ethoxy or methoxy groups on the silicon of the silanes, the functional groups are attached through relatively stable siloxyl-silicon bonding, as is used commercially for production of aminopropyl glass for chromatography and many other products. The potential applications of the concept can be expanded by the wide range of bifunctional silanes that are currently commercially available (http://www.gelest.com).

Notes and references

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