Size and arrangement of elementary fibrils in crystalline cellulose studied with scanning tunneling microscopy

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Scanning tunneling microscopy (STM) was used to investigate the ultrastructure of cellulose. The materials used in the experiments were cotton fiber, dewaxed cotton fiber, and microcrystalline cellulose. The results showed that the elementary fibrils in all these kinds of cellulose could be directly observed from the surface and cross-sectional view with high resolution. The elementary fibrils assembled together in parallel, and their lateral dimension showed a great variability in different kinds of cellulose, but was uniform in the same kind of cellulose. Elementary fibrils were the smallest structural units of cellulose, and they further aggregated into microfibrils, and the microfibrils constituted fibrils. In each gradation, the fibers piled up in parallel. STM was useful in studying the fine structure of cellulose. © *1997 American Vacuum Society.*
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I. INTRODUCTION

Cellulose is the major polysaccharide component of plant cell wall and is the most abundant organic compound on the planet. It plays a very important role in the carbon and energy cycles of the biosphere. Thus, more and more attention has been paid to studying its structure, function, and biodegradation.[1] The existence of an elementary cellulose fibril (ECF), as the structural units of cellulose, was proposed a long time ago.[2,3] Since then, the size of the smallest crystalline unit has been measured in cellulose from valonia,[4–8] bacteria,[4–8] cotton,[2,7,9] flax,[2] jute,[2] and ramie,[2,4–8,10,11] by transmission electron microscopy (TEM), wide-angle x-ray scattering (WAXS), and small-angle x-ray scattering (SAXS). It is evident from the reported data that the size of these crystalline regions showed a great variability. In some cases, this was interpreted as being due to the agglomeration of elementary subunits of about 35 A in size,[11] and in other cases this interpretation was refuted and the existence of an ECF was questioned altogether.[8] However, Jakob *et al.* reported that the wood cell wall was built with elementary cellulose fibrils having a uniform thickness of 25 ± 2 A, which was shown by investigating the same sample independently with three different experimental techniques, TEM, WAXS, and SAXS.[9] But to date, to the best of our knowledge, the ultrastructure of cellulose has not yet been known completely.

The appearance of the scanning probe microscopy (SPM) technique has principally opened new possibilities for the studying of biological objects, such as macromolecules. Compared with other structure analytical tools, SPM can be performed in ambient condition, and its resolution is very high. It has been widely used to image the topography of DNA, protein, and membranes.[12] In the past, atomic force microscopy has been used to study the structure of algal cellulose.[13,14] Here, scanning tunneling microscopy (STM) was used to investigate the ultrastructure of three kinds of cellulose, and high-resolution STM images have been obtained.

II. MATERIALS AND METHODS

Cotton fiber, dewaxed cotton fiber, and microcrystalline cellulose were used as the materials. The cotton fiber was from the cultivated cotton variety H123 A. The dewaxed fibers and microcrystalline cellulose Sigma cell type 50 were purchased as commercial reagents. The cotton fiber and dewaxed cotton fiber were made into powder by smashing, washed with distilled water, and then heated to dry at 50 °C. For the STM experiments, the cellulose powder was suspended in anhydrous ethanol, 20 μl of suspension was dropped on newly cleaved highly oriented pyrolytic graphite, and then dried in air. The STM experiments were carried out in ambient environment with a domestic STM setup CSPM-930a (manufactured by the Institute of Chemistry, Academia Sinica). The normal STM constant current mode was used, with tungsten tips made by electrochemical etching. Tunneling current and bias voltage were indicated in the relevant legends of the photographs.

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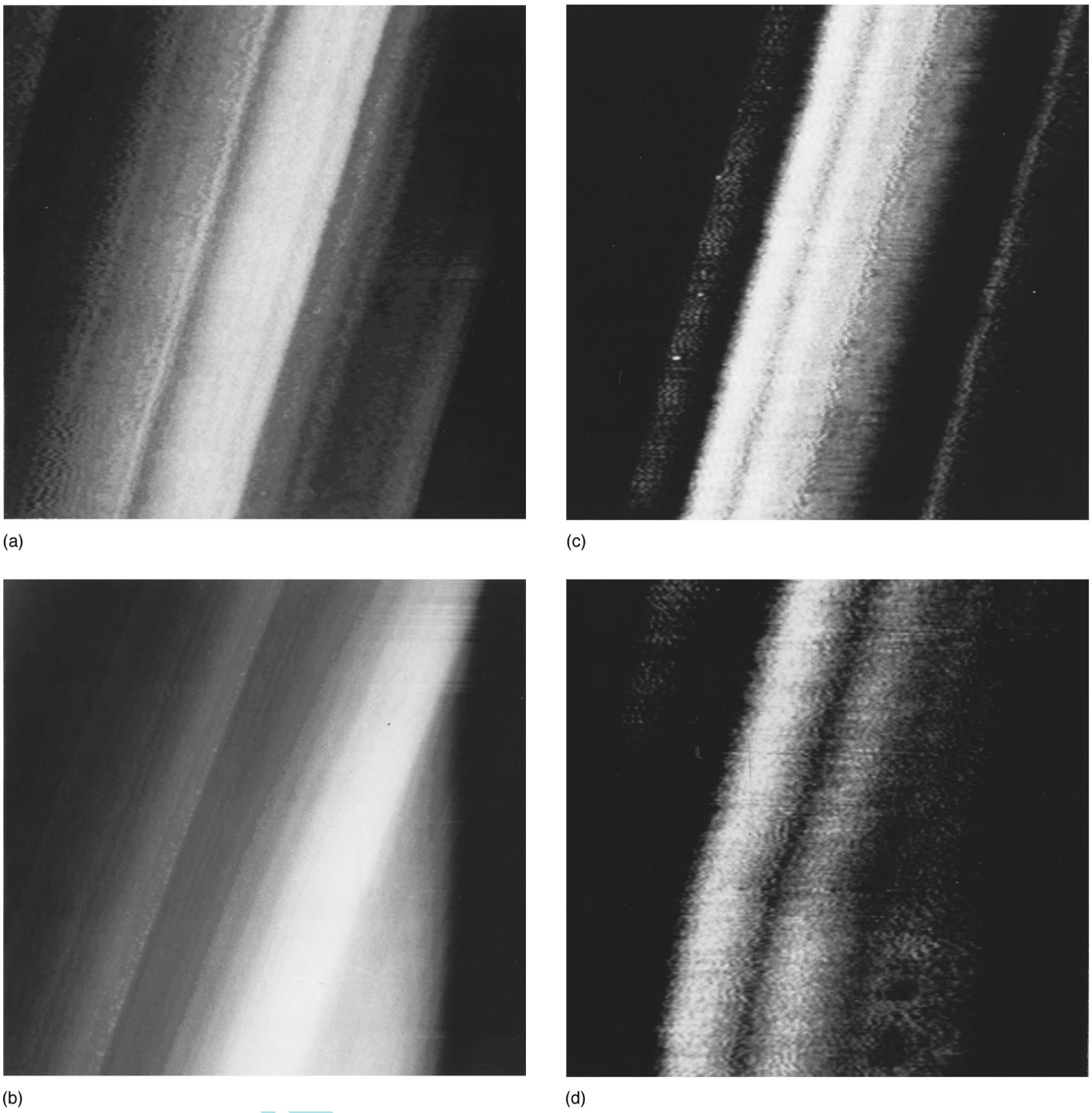

FIG. 1. The STM images of cotton fiber of the variety H123 A. V_{bias} = 740 mV, I_{ref}=0.18 nA, scan area: (a) 320 nm×320 nm, (b) 64 nm×64 nm, (c) 32 nm×32 nm, and (d) 16 nm×16 nm.

III. RESULTS AND DISCUSSION

Cotton fiber is the only native pure cellulose material with an amount of cellulose up to 95%–97%. After being dewaxed, it is a good kind of substrate for structure and biodegradation researchers.

Because the dimension of the cotton fiber with 10–100 μm in diameter was greatly beyond the STM maximum scanning scale, the STM tip could only scan in a part area of the cotton fiber surface. Figure 1 shows the STM images of the H123 A cotton fiber. The cellulose fibrils were clearly observed [Fig. 1(a)]. The lateral diameter ranged from 90 to 120 nm, and their length was greatly beyond the scanning scale. It could be also seen that the cellulose fibrils were composed of thinner fibers, which was called microfibril, with 25–35 nm in diameter. With higher resolution, the microfibrils could be seen more clearly [Fig. 1(b)]. It was also shown that the microfibrils consisted of much thinner fibers with a 25 ± 3 Å diam. The thinner fibers were elementary fibrils, which were made up of cellulose molecules. Their structures were shown in Figs. 1(c) and 1(d). Some of the

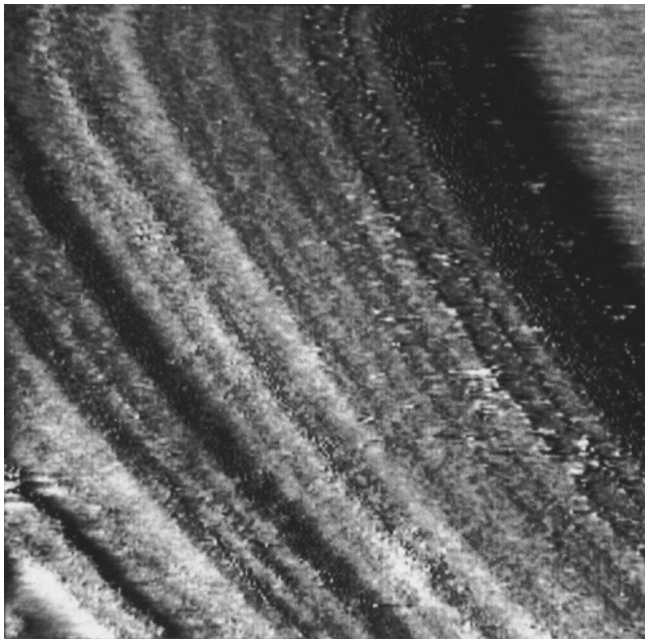

FIG. 2. The STM image of the dewaxed cotton fiber, V_{bias}=830 mV, I_{ref}=0.18 nA, and scan area, 32 nm×32 nm.

FIG. 3. The STM image of microcrystalline cellulose Sigma cell type 50. V_{bias}=475 mV, I_{ref}=0.81 nA. Scan area 40 nm×40 nm.

elementary fibrils were agglomerated tightly, some of them were dissociated from others. It could be concluded that the cotton fiber was built with elementary fibrils. Cellulose, as the native macromolecule, was constituted in the stepping mode. The cellulose molecule was β-1, four-linked *D*-glucose monomers with cellobiose as repeat units; they aggregated together to form elementary fibrils depending on intra- and intermolecular hydrogen bonds. Elementary fibrils were the smallest structural units of cellulose. Elementary fibrils further assembled into microfibrils, and microfibrils constituted fibrils. In each gradation, the fibers were parallel to each other.

Figure 2 shows the STM image of dewaxed cotton fiber with high resolution. The surface structure of the dewaxed cotton fiber could be observed, and was similar to that of the H123 A cotton fiber; the elementary fibrils could be seen directly. The thinnest elementary fibril assembled in parallel with a diameter of of 30 ± 3 Å, the chains in the elementary fibrils with a 7–10 Å diam might be the cellulose molecule.

In order to understand the size and arrangement of the elementary fibrils in the crystalline cellulose comprehensively, the surface and transverse section structures of the highly crystallized microcrystalline cellulose were studied with STM. The STM image is shown in the Fig. 3. It could be seen from the cross-sectional view that the elementary fibrils piled up, which was consistent with that observed from the surface view. The cross section of the elementary fibril was oval in shape, the diameters of the elementary fibrils were 18 ± 2 Å.

The elementary fibril diameters of these three kinds of cellulose used above are summarized in Table I. The results suggested that the diameter of the elementary fibrils in the same kinds of cellulose was uniform. This was consistent with the results reported by Jakob *et al*. Otherwise, its actual value varied with the materials. It is evident from the reported data that the size of these crystalline regions showed a great variability. This might be due to the agglomeration of elementary subunits,[11] just like the observed results in this article.

The ultrastructure of cellulose has been studied with electron microscopy, and a lot of information about this complex biological system was gained. However, to date, to the best of our knowledge relatively little is known about its detailed structure at the microscopic level. This is, in part, due to the fiber structure of the cellulose with crystalline and amorphous domains at the microscopic level. Transmission electron microscopy requires tedious sample preparation procedures and staining methods, which contain the danger of altering the structure of the sample, it is difficult to directly observe the structure of the elementary fibrils with higher resolution. Small-angle x-ray scattering has widely been applied to the study of cellulose fibril, and some parameters, such as the diameter of elementary fibril, might be determined with higher accuracy.[15] However, SAXS does not provide direct images of the structure, and the information gained with SAXS is averages. Compared with TEM and

TABLE I. The elementary fibril diameter measured in three types of celluloses.

Types of cellulose	Diameter of the elementary fibrils (Å)
Cotton fiber of H123 A	25± 3 Å
Dewaxed cotton fiber	30± 3 Å
Microcyrstalline cellulose Sigma cell type 50	18± 2 Å

SAXS, the sample preparation for STM is very simple, and the STM now available can process data almost immediately using relatively compact equipment and can provide three-dimensional structural information with atomic resolution, so STM could be used to investigate the surface structure from real time and real space. From the results above, we can concluded that it is possible to directly observe the fine structure of cellulose of a crystalline region, such as the arrangement of microfibrils and elementary fibrils; it is especially possible to observe the structure and size of the individual elementary fibril and compare the differences between them. More over, STM is able to directly investigate the structure of biomolecules under physiological conditions; it can be applied to observing the dynamic process of biodegradation of cellulose by cellulase, such as the adsorption of cellulase on a cellulose surface.

IV. CONCLUSION

To sum up, STM was used to investigate the ultrastructure of cellulose of cotton fiber, dewaxed cotton fiber, and microcrystalline cellulose. The existence of an elementary fibril could be demonstrated by STM with high resolution. Moreover, the size and arrangement of the elementary fibrils in the cellulose could also be clearly observed. All the results showed that STM is a useful technique for investigating the fine structure of cellulose.

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