Nanoscience in nature: cellulose nanocrystals

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Nanoscience, the study of matter invisible to human eyes, seeks to unravel and understand the building blocks of our planet. Nature, perhaps the most talented nanoscientist, has already mastered the synthesis of biological nanomaterials including proteins, lipids and polysaccharides. This article will explore a very unique nanomaterial, derived from cellulose, that has received great academic and industrial interest over the last few years. Cellulose nanocrystals are shards of a very common polymer and possess a number of interesting properties including a high aspect ratio and large tension modulus. Cellulose nanocrystal structure can be manipulated during the extraction procedure to control size, degree of crystallinity and surface charge. Furthermore the crystals can be functionalized with surface functional groups, including sulfate esters, and successfully incorporated into polymer matrices. This article will explore physical and chemical extraction procedures, and characterization techniques including atomic force microscopy, transmission electron microscopy and x-ray diffraction. Finally, the future promise of cellulose nanocrystals will be discussed including potential applications in electronics, materials and medical industries.

Cellulose is the most abundant biological polymer on the planet and it is found in the cell walls of plant and bacterial cells. Composed of long chains of glucose molecules, cellulose fibers are arranged in an intricate web that provides both structure and support for the cell[1] Interestingly, within the jungle of fibers are regions which are very well ordered: chains are aligned parallel and are packed close together. Crystalline is the name given to these unique fiber regions which often measure between micrometers to nanometers in length[1]

Individual cellulose nanocrystals are produced by breaking down the cellulose fibers and isolating the crystalline regions. Strong acid hydrolysis, a process described nearly 60 years ago by Ranby et al., has been used to successfully isolate cellulose microcrystals (**Figure 1**).[2] Because of their small size, they cannot be imaged using conventional optical microscopy. It is only with the advent of higher resolution imaging techniques such as atomic force microscopy (AFM) and transmission electron microscopy (TEM) that many nanomaterials including cellulose nanocrystals have been successfully characterized[3]

Over the last few decades, a number of industrial uses for microcrystalline cellulose have been developed. Excellent moisture absorption and chemical inactivity has lead to its widespread use in the pharmaceutical industry as a tablet excipient[1] On the other hand, nanocrystalline cellulose has yet to make a significant industrial breakthrough and its properties continue to be investigated. Because of their large surface area-to-volume ratio and aspect ratio, nanocrystals are predicted to have many potential applications in fields including electronics, materials science and medicine[3]

STRUCTURE

Nanocrystals are derived from cellulose, a linear polymer consisting of β(1-4) linked glucose sugars. Individual cellulose strands are held together by hydrogen bonding in either parallel or antiparallel configurations (cellulose allomorphs I-α and I-β respectively)[4] Regions can contain either long segments of closely bundled cellulose fibres, or short segments exhibiting little parallel organization. The former structure is preferable, as it possesses crystalline properties instead of disordered or amorphous properties.

There is no absolute measure of crystallinity and it can only be investigated in relative terms between different cellulose fibers. The uptake of water molecules (swelling) can be used as a measure of relative crystallinity, as amorphous regions have been observed to absorb more water molecules than crystalline regions.[5]

EXTRACTION

Cellulose nanocrystals must be harvested from the cell walls. Although cellulose comprises approximately 33% of most plant cells,[6] the remainder is an assortment of lipids and proteins that must be removed prior to crystal extraction. To achieve this, researchers have established procedures that involve the use mechanical grinding techniques to grind bulk
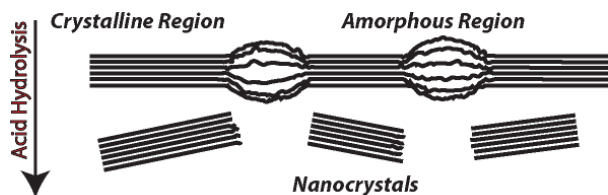
Figure 1: Acid hydrolysis breaks down dissordered (amorphous) regions and isolates nanocrystals.

cellulose followed by treatment with alkyl hydroxides and peroxides.[7]

Cellulose nanocrystal production frequently involves an additional chemical procedure. Strong acids such as sulfuric, nitric and hydrochloric acid have been shown to successfully degrade cellulose fibers. Sulfuric acid has been extensively investigated and appears to be the most effective. The current accepted explanation depicts this process of acid hydrolysis as a heterogeneous process that involves the diffusion of acid into the cellulose fibres, followed by cleavage of glycosidic bonds.[7]

Acid type, acid concentration, hydrolysis time and hydrolysis temperature are factors that have been shown to govern the products of the hydrolysis process.[1] It is believed that acid interacts mainly with the amorphous regions of cellulose, as they are the most easily accessible and have the greatest surface area. Therefore, the amorphous regions are the first to be targeted by the strong acid, followed by regions of increased crystallinity. A controlled hydrolysis can therefore extract regions of a specific crystallinity from a cellulose sample (**Figure 2**).

CHARACTERIZATION

Once isolated, crystals are often suspended in a solution. Evaporating the solution on a substrate will produce a film of nanocrystals that can be imaged and characterized using a number of techniques: 1) Optical Microscopy (OM) which is limited to imaging objects greater than about half of the wavelength of visible light (> 250 nm) and therefore can only image large crystal aggregates; 2) Atomic Force Microscopy (AFM) which involves rastering a cantilever with a very fine tip (tip radius ~ 20 nm) across the sample and obtaining an image by measuring the cantilever deflection; and 3) Transmission Electron Microscopy (TEM) in which electrons are accelerated to a high voltage and
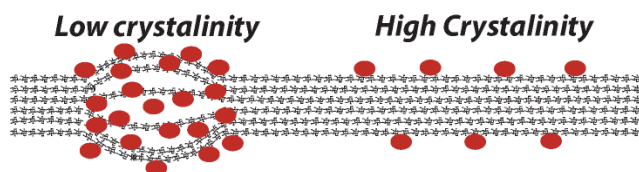

Figure 2: Red circles demonstrate potential sites of hydrolysis. Regions of high crystallinity possess fewer sites and therefore take longer to be broken down.
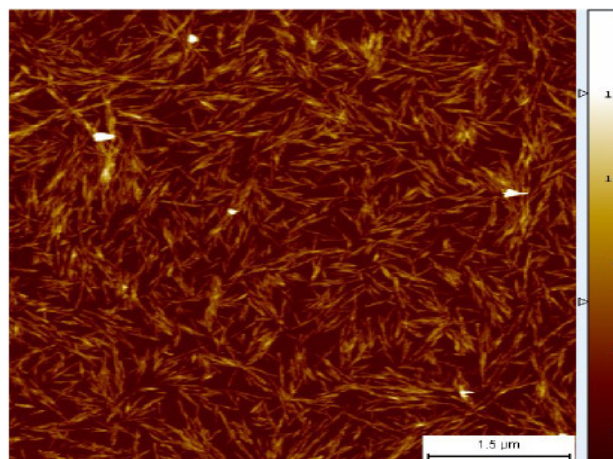

Figure 3: Cellulose nanocrystals deposited on silicon surface (Oke I. 2009)

detected after they pass through the sample.[3] Both AFM and TEM can achieve nanometer resolution and are therefore effective for imaging cellulose nanocrystals. However, flat samples with minimal topographical variations are required to obtain the best images (**Figure 3**).

Beyond imaging, chromatography can be used to determine the charge of the crystals and mass spectroscopy can be used to determine their composition. Nuclear magnetic resonance and x-ray diffraction have also been used to further investigate crystal structure.[1]

PROPERTIES

The physical dimensions that are obtained for cellulose nanocrystals are determined by both the source of cellulose and the hydrolysis conditions that are used during extraction. For example, Bondeson et al. performed a systematic analysis of nanocrystal properties produced using sulfuric acid hydrolysis, using microcrystalline cellulose as the initial reagent. With a sulfuric acid concentration of 63.5% (w/w) and a hydrolysis time of approximately 2 hours, it was possible to produce cellulose nanocrystals with a length that was between 200 and 400 nm and a width that was less than 10 nm. The overall yield of nanocrystalline cellulose was approximately 30% of the initial biomass. Although a pulp to acid ratio of approximately 1:10 appears to be consistent in the literature, temperature and hydrolysis time vary significantly between investigations[8].

There is a pattern that exists between all investigations: as both temperature and acid exposure time are increased, crystals decrease in length and the overall yield decreases. Prolonged exposure to acid results in extremely low yields which has been attributed to acid breakdown of entire cellulose fibers including the crystalline regions.

Furthermore, as a result of sulfate group interactions during sulfuric acid hydrolysis, nanocrystal surfaces display a net positive charge. This property creates a strong attractive
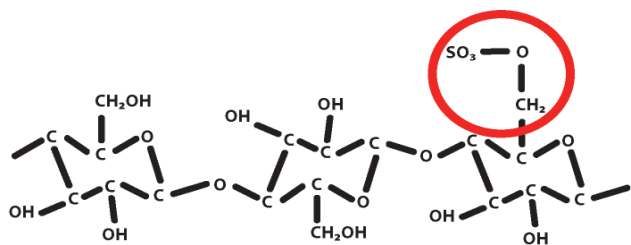
Figure 4: Red circle demonstrates how a sulfate ester group can functionalize the surface of a cellulose chain.

interaction between the individual crystallites which results in an increase in the suspension viscosity and birefringence properties. Sulfate ester groups displace the hydroxide groups and act as surface functional groups[9] Other functional groups can also be incorporated into the surface of cellulose nanocrystals (**Figure 4**).[10]

Finally, one of the most important properties of materials used in structural applications is the material's ability to resist breaking under tensile stress. Cellulose nanocrystals possess a tensile modulus of 143 GPa, a value that is about 100 times greater than that of a typical glassy polymer.[11]

APPLICATIONS

Much of current cellulose nanocrystal research is directed towards determining novel applications for the material.

Materials
The desirable mechanical properties of cellulose nanocrystals may soon lead to their incorporation as a reinforcing component in building materials to increase their strength and durability[12] Cellulose nanocrystals can be functionalized to be either hydrophobic or hydrophilic and can be incorporated into pre-existing structural polymers.[12] Such applications will require well-dispersed and oriented nanocrystals[13] Therefore, further investigation into the interactions between crystallites will help in validating their use in a number of industrial applications. Thermostability is an additional challenge in materials science since industrial polymer nanocomposite processing temperatures may often exceed 200 ^{0}C[14].

Electronics
Rigid nanocrystalline rods can be combined with flexible and lipophilic azo polymer chains to synthesize an amphotropic liquid crystal polymer[15] So far, this novel polymer has been shown to exhibit liquid crystalline behaviour above 135 ^{0}C. Such a temperature exceeds temperatures encountered in everyday use and future research will be directed towards lowering the functional temperature.

Catalysis
Combining gold nanoparticles with high surface area cellulose nanocrystals will produce a composite material capable of immobilizing enzymes and creating a very active biocatalyctic matrix. Industrial processes that rely on enzyme-catalyzed reactions could benefit from such a material with improved homogeneity, reusability and cost effectiveness[16]

Energy
One of the most promising recent initiatives of the biofuel industry involves breaking down cellulose with enzymes and fermenting the sugars to produce ethanol. Naturally, the goal is to harness as much energy as possible, and increasing the efficiency of breaking down crystalline cellulose will increase the overall yield. To carry out these experiments, it is desirable for researchers to develop methods of producing samples containing purely crystalline cellulose[17].

Biomedical
Cellulose nanocrystals may also be functionalized with a specific antigen and tagged with fluorescent CdSe quantum dots[18] During *in vitro* tests in the presence of an antigen, the functionalized nanocrystals have demonstrated rapid agglutination and accurate detection. Such techniques could be used in medical imaging to locate tumours or bacterial cells based on the expressed antigens[19] Furthermore, drugs may also be incorporated onto the cellulose nanocrystals which may act as vectors in targeted drug delivery[20].

CONCLUSION

Attainable from nearly every corner of the world, cellulose nanocrystals will prove to be a valuable resource in the near future. A well-established method for extraction exists, as do the tools to analyze and characterize this nanomaterial. Surface functionalization will very likely be the main focus of nanocrystal research over the next few years. Innovations may lead to versatile structural materials, efficient drug delivery and improved methods of targeting tumor cells. Although much more research will need to be performed, it is already clear that cellulose nanocrystals possess a number of desirable properties. Until further advances are achieved, researchers must look up to nature as the master of nanomaterials.

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