A DNA based method for directed self assembly of cellulose nanocrystals into advanced nanomaterials

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Outline

- Concept
- Background
- The experimental results
  - preliminary study
- Conclusions
The cellulose sub-elementary fibril in plants is the most abundant nanomaterial on Earth!

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CELLULOSE NANOCRYSTALS (CNXLs)

CELLULOSE WHISKERS

CELLULOSE NANOWHISKERS

CELLULOSE MICROCRYSTALLITES

NANOCRYSTALLINE CELLULOSE
concept

ssDNA + molecular coupler → DNA-coupler graft + CNXL → ssDNA/coupler/CNXL → Duplex DNA/coupler/CNXL Hybrid nanomaterial

c-ssDNA

concept
Applications

A need exists for biomedical implants that can match the mechanical properties of the tissue, then dissolve in the body at a predetermined rate to match the body’s regeneration of the tissue, e.g., bone, skin.

- Sensor technology incorporating DNA
- DNA microarrays
- Fabrication of 3D nano-structures could lead to hierarchical materials
SOURCES OF: CELLULOSE NANOCRYSTALS
Wood

© J. Harrington
Sugar Beets
Cotton
Barnacles (tunicin)
Bacterial Cellulose
CELLULOSE BIOSYNTHESIS

CELLULOSE NANOCRYSTAL PRODUCTION

Native cellulose - Semi crystalline Polymer (~70% crystalline).

Crystalline portion

Amorphous portion

CONTROLLED ACID HYDROLYSIS
TEM image of cellulose nanocrystals
Tip broadening artifacts

Tip shows CNXL to be wider than it really is

Accurate height measurement

“Flying” in travel direction
~ 7 nm
~150 nm
A bonus for the unwary cellulose chemist....
**Cellulose nanocrystal size and geometry depends upon source**

<table>
<thead>
<tr>
<th>Source</th>
<th>Length</th>
<th>Cross section</th>
<th>Aspect ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunicate</td>
<td>100 nm – microns</td>
<td>10-20 nm</td>
<td>5 to &gt; 100 (high)</td>
</tr>
<tr>
<td>Algal (Valonia)</td>
<td>&gt; 1000 nm</td>
<td>10 to 20 nm</td>
<td>50 to &gt; 10 nm (high)</td>
</tr>
<tr>
<td>Bacterial</td>
<td>100 nm – microns</td>
<td>5-10 x 30-50 nm</td>
<td>2 to &gt; 100 (medium)</td>
</tr>
<tr>
<td>Cotton</td>
<td>200-350 nm</td>
<td>5 nm</td>
<td>20 to 70 (low)</td>
</tr>
<tr>
<td>Wood</td>
<td>100–300 nm</td>
<td>3 – 5 nm</td>
<td>20 to 50 (low)</td>
</tr>
</tbody>
</table>

Cellulose nanocrystals have a high surface area

<table>
<thead>
<tr>
<th>Material</th>
<th>m²/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-glass fibers*</td>
<td>~1</td>
</tr>
<tr>
<td>Paper fibers</td>
<td>4</td>
</tr>
<tr>
<td>Graphite</td>
<td>25-300</td>
</tr>
<tr>
<td>Fumed silica</td>
<td>100-400</td>
</tr>
<tr>
<td>Fully exfoliated clay</td>
<td>~ 500</td>
</tr>
<tr>
<td><strong>Cellulose nanocrystals</strong></td>
<td>250</td>
</tr>
<tr>
<td>Carbon nanotubes***</td>
<td>~ 100 - ?</td>
</tr>
</tbody>
</table>

** Winter, W. presentation at ACS meeting, San Diego, March 2005
## Stronger than steel
## Stiffer than aluminum

<table>
<thead>
<tr>
<th>Material</th>
<th>Tensile strength (GPa)</th>
<th>Modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cellulose crystal</td>
<td>7.5(^1)</td>
<td>145(^2)</td>
</tr>
<tr>
<td>Glass fiber</td>
<td>4.8</td>
<td>86</td>
</tr>
<tr>
<td>Aluminum wire</td>
<td>0.62</td>
<td>73</td>
</tr>
<tr>
<td>Steel wire</td>
<td>4.1</td>
<td>207</td>
</tr>
<tr>
<td>Graphite whisker</td>
<td>21</td>
<td>410</td>
</tr>
<tr>
<td>Carbon nanotubes(^3)</td>
<td>11-63</td>
<td>270-970</td>
</tr>
</tbody>
</table>

1. Marks, *Cell wall mechanics of tracheids* 1967
2. Sturcova, et al. (2005) Biomacromol. 6, 1055
DNA

http://tigger.uic.edu/classes/phys/phys461/phys450/ANJUM04/DNA_helix.jpg
Branched structures

Seeman 2003
A. Form array from single-stranded DNA.

B. Hybridize 5-nm Au particles to tile D.

C. Hybridize 10-nm Au particles to tile B.

D. Pinto 2005
Figure 4. TEM image of the two-particle array. The pattern of alternating parallel rows of small and large gold particles is clearly visible. (Particles tend to aggregate on the mica surface outside the arrays, e.g., lower right corner of image.)
Fig. 1. AFM images of self-assembled SWNTs via hybridization of DNA, (a)–(d) are typical images from four different AFM samples.

Fig. 2. Typical AFM images of unfunctionalized SWNTs (a) and ssDNA-SWNTs (b).
Figure 2. Directional assembly of asymmetrically functionalized AuNPs into (A, B) cat paw, (C, D) satellite, and (E, F) dendrimer-like structures. Inset: scale bar = 20 nm.
CNXL-DNA experiment
Surface modification of CNXLs

- Titration of C.CNXLs indicated the presence of 1.4 mmols of acid/g CNXLs.

*Araki et.al, Langmuir, 17: 21-27, 2001*
AFM of Carboxy-CNXLs
Oligomers

72-mer, dodecyl linker:
- 5’-amino-C12-
  CAGTCAGATCAGGACATGAGATCATCAGTCAGATCAGGACATGAGATCAT
GCTAGTCAGCTACGGTCACTGCTAGTCCGTACGTACCATGTTCATAGTGTTAGGT-3’
- And compliment
- GC content = 49%
- $T_m = 70 \, ^\circ C$
- Purchased from IDT, Inc.
Oligomers

20-mer, hexamer linker:
- 5’-amino-C6-GCT CTA CCT GAC TAG CTC GT-3’ and compliment
- GC content = 55%
- \( T_m = 56 \, ^\circ C \)
- Purchased from Oligos, etc.
Classic EDC reaction

EDC = 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride

Voicu 2004
Araki 2001
Deen 1990
Optimization of grafting reaction

DNA/CNXL ratio

Molar ratio ssDNA/CO2H

umole ssDNA/g CNXL

Low CNXL concentration

Molar ratio ssDNA/CO2H
FTIR

1. Carboxy-CNXL, protonated
2. EDC-NHS activated carboxy-CNXL, pH 4.5
3. pH 7.5
Mix the complimentary strands together

ssDNA-CNXL  Duplex DNA-CNXL  Carboxy-CNXL
Dynamic light scattering (DLS)

- Laser
- Sample chamber
- Laser optics
- Scattered light (90°)
- Detector optics
- Avalanche photodiode detector
- Signal processing, mathematical modeling done by computer

- Spherical shape assumed by software!
DLS numbers are not accurate, but show increasing size with grafting, as expected.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Diameter ($D_h$) at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNXL</td>
<td>90-100 nm</td>
</tr>
<tr>
<td>Carboxy-CNXL</td>
<td>120-130 nm</td>
</tr>
<tr>
<td>ssDNA-CNXL</td>
<td>140-158 nm</td>
</tr>
<tr>
<td>dsDNA-CNXL duplex</td>
<td>548-620 nm</td>
</tr>
</tbody>
</table>
CNXLs disperse as DNA melts
CNXLs bond as DNA binds
UV melting curve supports DLS data

Temperature, °C

Absorbance

DNA-g-CNXL duplex

ssDNA-g-CNXL

Heating
Cooling

dA = 22%
AFM images – ssDNA-CNXL about the same size as carboxy-CNXLs
AFM image – duplex DNA-CNXL
AFM image – duplex DNA-CNXL
AFM image – duplex DNA-CNXL
AFM section analysis measures particle height
Particle height from section analysis

DNA graft adds ~2 nm

- Carboxy CNXL
- ssDNA-CNXL
- dsDNA-CNXL

Duplexed DNA-CNXL

Supports data from DLS

~ 20-30 measurements/sample
Patterning on silicon wafers

Proof of concept
Succinic anhydride solution

1. ssDNA (EDC)
2. ssDNA-g-CNXL

Amine surface
1/2 succinylated (carboxylated) surface

1. ssDNA (EDC)
2. ssDNA-g-CNXL

APTES modified Si wafer

1. co-ssDNA-g-CNXL
2. Clean

Succinic anhydride solution

No CNXLs

CNXLs
• Wafer sonicated in DI water after overnight hybridization with ssDNA-g-CNXL
Conclusions

- CNXLs were successfully carboxylated
- Carboxy-CNXLs were successfully grafted with DNA oligomers
- The DNA on the grafted CNXLs duplexed and bound the CNXLs together
- The duplex formation was reversible via raising the temperature above the DNA melting point
- While the applications of this novel material remain distant, we believe we have shown that this concept has potential for use in thermoplastically formable implants and other bio-based nanomaterials
Acknowledgements

This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2003-35103-13711.