Surface modification of forest biomaterials

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Nanotechnology in Forest Biomaterials
Outline

• Background

• Cellulose functionalization
  – Traditional methods (esterification, etherification)
  – Click chemistry (selected examples)
  – TEMPO-mediated oxidation
  – EDC/NHS chemistry

• Characterization (FTIR, AFM, QCM-D, TEM, XPS)

• Summary
Why to modify cellulose?

- Demand for value-added cellulose-based products

- The physical/chemical properties of cellulose need to meet the specific requirements of given application

- Gain/alter specific property/properties (strength, hydrophilicity/hydrophobicity, antibacterial, magnetic, conductivity, barrier properties etc.)

Remember: Trade-off between the strength loss (disrupted hydrogen bonding) and gaining new properties
Challenge

- In order to preserve the native cellulose structure, the chemical reactions cannot be made in the dissolved homogeneous state.

- Fibers are prone to aggregation if nonaqueous reaction medium is used.

- Therefore, heterogeneous chemical reactions of cellulose and in particular native cellulose nanofibers (CNF) in aqueous medium allowing surface modifications are called for.
Cellulose reactivity

- Reactive sites: 3 x OH-groups + 1 x reducing end aldehyde group

Accessibility (dissolved state vs. surface functionalization)

Regioselectivity often challenging; However, position 6 appear to be the most reactive
Typical etherification/esterification reagents

Cellulose OH-groups react with:

- Carboxylic acids
- Acyl halides
- Chloroacetic acid
- Anhydrides
- Epoxides

\[ RCOOH \]
\[ H_3C\text{Cl} \]
\[ \text{Cl-CH}_2\text{COOH} \]
\[ R_1\text{CO}_2\text{C}-R_2 \]
\[ O\text{CH}_2\text{OH} \]
Commercial cellulose derivatives

- Due to the nature of polysaccharides esterification and etherification reactions are the most common approaches for their chemical modifications.

Schematic presentation of cellulose esters (top) and ethers (bottom) commercially produced.

Properties & applications of some commercial cellulose esters and ethers

<table>
<thead>
<tr>
<th>Cellulose derivatives</th>
<th>DS range</th>
<th>Solubility</th>
<th>Product applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose esters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>1.5–3.0</td>
<td>MeOH, PhNO₂,</td>
<td>Films, fibers, explosives</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ethanol-ether</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>1.0–3.0</td>
<td>Acetone</td>
<td>Films, fibers, coatings, heat and rot resistant fabrics</td>
</tr>
<tr>
<td>Cellulose ethers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl</td>
<td>1.5–2.4</td>
<td>Hot H₂O</td>
<td>Food additives, films, cosmetics, greaseproof paper</td>
</tr>
<tr>
<td>Carboxymethyl</td>
<td>0.5–1.2</td>
<td>H₂O</td>
<td>Food additives, fibers, coatings, oil-well drilling muds, paper size, paints, detergents</td>
</tr>
<tr>
<td>Ethyl</td>
<td>2.3–2.6</td>
<td>Organic solvents</td>
<td>Plastics, lacquers</td>
</tr>
<tr>
<td>Hydroxyethyl</td>
<td>Low DS</td>
<td>H₂O</td>
<td>Films</td>
</tr>
<tr>
<td>Hydroxypropyl</td>
<td>1.5–2.0</td>
<td>H₂O</td>
<td>Paints</td>
</tr>
<tr>
<td>Hydroxypropylmethyl</td>
<td>1.5–2.0</td>
<td>H₂O</td>
<td>Paints</td>
</tr>
<tr>
<td>Cyanoethyl</td>
<td>2.0</td>
<td>Organic solvents</td>
<td>Products with high dielectric constants, fabrics with heat and rot resistances</td>
</tr>
</tbody>
</table>
Click chemistry concept

- Reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by non-chromatographic methods, and be stereospecific

- Simple reaction conditions (*i.e.* tolerate oxygen and water), readily available starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation

**Click reactions**

- **Azide-Alkyne Huisgen Cycloaddition:**

- **Thiol-ene & thiol-yne:**

- **Diels-Alder:**

http://www.scripps.edu/chem/sharpless/click.html (1000+ articles)

Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC)

Widely applied for the generation of carbohydrate mimetics and derivatives

Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Green, M. G.; Fokin, V. V.; Sharpless. K. B. Angew. Chem., Int. Ed. 2002, 41, 1053-1057.
Azidation via nucleophilic displacement followed by CuAAC (direct route)

Tosyl group is a good leaving group

Nucleophilic displacement
Functionalization of cellulose using polysaccharides (indirect route)

- Modifications in aqueous media (an industrially attractive method)
- Raw materials from biorefineries, completely renewable materials

Modified cellulose for various applications
Certain polysaccharides have a natural affinity to cellulose surfaces

I) CMC (and other water-based cellulose derivatives)

II) Guar gum (galactomannan)

III) Xyloglucan

Neutral and even anionic polysaccharides can be irreversibly adsorbed on cellulose (CMC, Laine et al. 2000; Xyloglucan, Christiernin et al. 2003, Glucomannan, Hannuksela et al. 2003)
1. Surface modification of cellulosic materials by irreversible adsorption of polysaccharides

- Carboxymethyl cellulose (CMC) can be used to modify cellulose surfaces (Laine et al. 2000)

![Graph showing the attached amount of CMC (mg/g) vs. treatment time (min)]

- Neutral and even anionic polysaccharides can be irreversibly adsorbed on cellulose (CMC, Laine et al. 2000; Xyloglucan, Christiernin et al. 2003, Glucomannan, Hannuksela et al. 2003)
Generic method for modular functionalization of cellulose via sequential adsorption & click chemistry

(A): Introduction of “clickable” functionalities on the oxidized galactose-containing hetero-polysaccharides using reductive amination

(B): Introduction of “clickable” functionalities on carboxymethylcellulose (CMC) via EDC/NHS assisted amidation

Virtually any functionality can be attached!


Azide-functionalized carboxymethyl cellulose (CMC)

Reaction is carried out in a test tube in prior to the adsorption onto the cellulose model surface.

Characteristic azide stretching band at about 2120 cm$^{-1}$

EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

NHS = N-Hydroxysuccinimide

Aalto University

Reaction is carried out in a test tube in prior to the adsorption onto the cellulose model surface.
Alkyne-functionalized CMC

Reaction is carried out in a test tube in prior to the adsorption onto the cellulose model surface.
Elemental Analysis of Modified CMCs

<table>
<thead>
<tr>
<th>Sample</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>O%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC</td>
<td>37.6</td>
<td>5.2</td>
<td>0.2</td>
<td>57.0</td>
</tr>
<tr>
<td>CMC-azido</td>
<td>44.5</td>
<td>7.2</td>
<td>8.2</td>
<td>40.2</td>
</tr>
<tr>
<td>CMC-alkyne</td>
<td>46.0</td>
<td>6.9</td>
<td>6.5</td>
<td>40.7</td>
</tr>
</tbody>
</table>

The amount of nitrogen corresponds to a grafting density of 85-90% of the available carboxyl groups of starting CMC (DS=0.7)
Adsorption of activated CMC onto cellulosic model surface

Preparation of model films – spin coating

_dispersing of nanofibril suspension with an ultrasound microtip

Ultra-centrifugation

The finest fraction is spin-coated on substrate

NFC model film
Quartz crystal microbalance with dissipation (QCM-D)

Combined mass and viscoelastic properties

In situ adsorption monitoring:
decrease in frequency = increase in adsorbed mass

Cellulose model film
(AFM image, 5x5µm²)
Atomic force microscopy (AFM)

AFM image of nanofibrillar cellulose (NFC)

Method for investigating the surface topography of a sample
Adsorption of modified CMCs

Functionalized CMCs adsorb onto cellulose surface very similarly than unmodified CMC
Synthesis of BSA-alkyne click-precursor (weight marker for QCM analysis)

Bovine Serum Albumin, 66 kDa

Click reaction

BSA do not adsorb onto surface of cellulose

Covalent linking needed for the surface decoration
QCM-D experiments with BSA-weight marker

The positive frequency shifts during rinsing with the buffer-solution and H$_2$O can be due to the pH changes and/or the removal of covalently unbound protein (layers above covalently linked BSA)

AFM image of control sample

AFM image of surface modified sample
X-ray photoelectron spectroscopy (XPS)

• Surface analysis technique; Analysis depth less than 10 nm

• Information on chemical compounds and elemental depth distributions
XPS of cellulose surfaces

- Covalently bound BSA shows higher amount of nitrogen
- Oxygen/Carbon ratio is changed due to the covalent attachment of BSA

<table>
<thead>
<tr>
<th>Sample</th>
<th>O 1s</th>
<th>C 1s</th>
<th>N 1s</th>
<th>S 2p</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC-azido/BSA-alkyne/Cu(I)</td>
<td>22.8</td>
<td>64.2</td>
<td>12.6</td>
<td>0.4</td>
<td>0.36</td>
</tr>
<tr>
<td>CMC-azido/BSA-alkyne (control)</td>
<td>42.6</td>
<td>56.5</td>
<td>0.9</td>
<td>0</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Fluorescent labeling of cellulose

(a) Photograph of unmodified filter paper under UV-light (wavelengths 254 and 366 nm),

(b) photograph of the filter paper after the reaction with dansyl probe without Cu¹ (negative control) and

(c) photograph of the filter paper after Cu¹-catalyzed reaction with dansyl probe.

Surface modification of microfibrillated cellulose (MFC)

- Adsorption of azide-modified CMC on pulp fibres
- Fluidization
- ”Click” reaction with alkyne-functionalized fluorescent probe (dansyl)

- Washing of the sample several times by centrifugation to remove unattached molecules

The alkyne-functionalized fluorescent probe attached only on the azide-CMC functionalized MFC.
XPS of the modified MFC

- MFC-CMC-dansyl
- MFC
- Cellulose reference

<table>
<thead>
<tr>
<th>sample</th>
<th>O 1s</th>
<th>C 1s</th>
<th>N 1s</th>
<th>S 2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose reference</td>
<td>42.1%</td>
<td>57.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFC</td>
<td>42.0%</td>
<td>58.0%</td>
<td>0.05%</td>
<td>0.03%</td>
</tr>
<tr>
<td>MFC-CMC-dansyl</td>
<td>40.6%</td>
<td>58.9%</td>
<td>0.46%</td>
<td>0.14%</td>
</tr>
</tbody>
</table>
Surface modification of cellulose nanocrystals (CNCs)

TEMPO-mediated oxidation selectively oxidizes the primary hydroxyl groups while leaving the secondary hydroxyl groups unaffected.

Cross-linking: gel formation

Gel formation:

- Vigorous stirring (1 min)
- CuSO₄/Ascorbic acid

Precursors in H₂O → Gel

Elemental analysis revealed the presence of nitrogen

<table>
<thead>
<tr>
<th>Sample</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>% O²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tempo-ox. CNC</td>
<td>41.75</td>
<td>5.76</td>
<td>0.08</td>
<td>52.41</td>
</tr>
<tr>
<td>CNC</td>
<td>43.55</td>
<td>6.11</td>
<td>0.04</td>
<td>50.30</td>
</tr>
<tr>
<td>CNC-AZ</td>
<td>42.87</td>
<td>5.20</td>
<td>1.90</td>
<td>50.03</td>
</tr>
<tr>
<td>CNC-PR</td>
<td>43.20</td>
<td>5.29</td>
<td>0.79</td>
<td>50.72</td>
</tr>
<tr>
<td>CNC-Click</td>
<td>43.28</td>
<td>6.03</td>
<td>1.51</td>
<td>49.18</td>
</tr>
</tbody>
</table>

- Rapid formation of a gel
Transmission Electron Microscopy (TEM)

TEM images of TEMPO-oxidized cellulose nanocrystals (a) and cellulose nano-platelet gel (b). Scale bar 500 nm

The average dimensions of the crystals were found to be approximately 10-20 nm width and 100-200 nm long.
Click Modified Cellulose for Introducing New Chemistry onto Cellulose Surfaces

**GRAFTING**
- a) bottlebrush polymers
- b) rubber/silk mimics
- c) self-assembly
- d) ionic interactions
- e) thickness / persistent length
- f) conductivity

**DENDRONIZATION**
- a) hydrophobic/philic
- b) solubility
- c) functionality/reactivity
- d) multivalency
- e) self-assembly
- f) "fiber" thickness
- g) persistent length
- h) cross-linking

**IONIC INTERACTIONS**
- a) charge
- b) ionic interactions (LbL)
- c) counter ion exchange
- d) surfactants
- e) cross-linking

**BIOFUNCTIONALIZATION**
- a) Bovine serum albumin (BSA)
  - biotin, DNA, RNA, proteins etc.
- b) wet strenght properties
- c) lubrication

**nano particle**
- Imaging
- Fluorescence
- Antimicrobial properties
- Magnetic properties
- Dendron

**dye**
- Imaging
- Sensing

"chitosan" mimics
- Radiolabeling
- Imaging
- Side chain LC
- Self-assembly
Traditional chemistry vs. click chemistry

**Traditional:**
- Well established/optimized
- Often demand organic solvents and/or rather harsh reaction conditions (non-selective and detrimental for the cellulosic structure)
- Each functionalization requires a distinct reaction

**Click:**
- Activated platform for desired functionalization (easy to perform)
- Aqueous media/mild reaction conditions
- Copper (I) catalyst troublesome in biological applications (CuAAC)
Summary

• Esterification and etherification are the most common reactions for the functionalization of biomaterials (OH- and COOH- groups in starting material)

• Click chemistry allows for a generic platform in which the desired counterpart molecule can be installed (the amount of chemical compounds in commercial click libraries is heavily increasing)

• Increasing demand of novel biomaterials requires a wide toolbox of functionalization reactions
2. Carboxymethylated CNF: Attachment of luminescent carbon dots on CNF

- Carboxymethylated CNF: -0.3 mmol/g
- CDs: a) AFM image
  b) phase image
  c) TEM image
- CDs: contain NH$_3$: +0.5 mmol/g at pH 4.5
- EDC/NHS assisted coupling reaction:
  - covalent attachment of CDs on carboxymethylated CNF
Bulk modification of CNF gel

- EDC/NHS activation of carboxymethylated CNF gel
- CD attachment
- Dialysis
- CD dosages:
  - 3 and 30 mg/g
Application: Cellulose nanopaper

- Translucent nanopaper (~8.4 mg/cm²) was made of CNF and CD-CNFI
- The CM-CNFI nanopaper is very moisture sensitive (wrinkles) but does not brake in water. Can be modified by dipping.
- The filtration time decreases 20% when the CNF is CD-modified.
Fluorescent cellulose nanopaper

- CNF film (a, d, g,): not fluorescent
- Dipped film (b, e, h): CDs on the surface
  - A thin fluorescent layer
- CD-CNf film (c, f, i): 30 mg/g CDs
  - Fluorescent film
- AFM images:
  - Roughness of the film is lower when the film has been modified with CDs (a:46 nm, b:30 nm, c:31 nm)
3. Immobilization of sensing molecules on cellulose

NFC-films were modified using sequential TEMPO-mediated oxidation and EDC/NHS activation. Antibodies were immobilized on the activated NFC-films by either inkjet printing or physical adsorption.

Macroscale topography of NFC film

Confocal laser scanning microscopy (CLSM) image of unmodified NFC film (a). Digital photograph (4.5 x 2.5 cm²) of an unmodified NFC film (b).

Contact angle measurements (CAM) of oxidized NFC films

- Contact angle of the NFC film decreased and the total charge increased along the oxidation reaction.
- Spreading out of the water droplet was observed at contact angles below 15 degrees.

The effect of TEMPO-mediated oxidation on contact angle (blue line) and total charge (green line) of NFC films as a function of the oxidation time.
Immobilization of antibodies on EDC/NHS-activated NFC films

CLSM intensity images of adsorbed FITC-stained antihuman IgG (0.1 mg/mL) in 10 mM phosphate buffer (pH 7.4) on unmodified NFC film (a) and EDC/NHS activated NFC-film (b).

Films were rinsed with 10 mM NaCl (pH 10) to remove electrostatically bound antibodies.
Inkjet-printing of antibodies on EDC/NHS activated NFC films

Printed dansylated antihuman IgG (1 mg/mL in 10 mM phosphate buffer, pH 7.4) on EDC/NHS-activated NFC film under UV-light (366 nm) (a). CLSM intensity image of printed FITC-stained antihuman IgG (1 mg/mL in 10 mM phosphate buffer, pH 7.4) on activated NFC film (b).
Summary

• Carboxylic groups were introduced on (nano)cellulosic materials either directly or indirectly and utilized as anchor groups for the subsequent modification reactions.

• Generic concepts in attaching biomolecules (or any other molecules) on the (nano)cellulosic substrates.

• Possible applications include anti-counterfeiting, biosensing, fluorescent imaging, sensors etc.
Questions