Morphology of native cellulose: the microfibril

Eero Kontturi

Department of Forest Products Technology, School of Chemical Technology

CHEM-E2140 Cellulose-based fibres
Learning outcomes

• Knowledge on how cellulose occurs in nature (in microfibrils)
• Command on basic facts about microfibrils
• Awareness of the gaps in knowledge of cellulose microfibrils
• Awareness of the disputes concerning microfibrils
Outline

(1) Facts about native cellulose microfibrils (CMFs)
   • Quick view on biosynthesis
   • Relatively undisputed facts about CMFs

(2) Disputed issues about CMFs
   • Width of CMFs
   • Number of chains in a CMF
   • Twist along the CMF
   • Longitudinal disorder: fringed fibrillar model and levelling off DP

(3) Bundling of CMFs
What do we know about the cellulose microfibril?
Cellulose microfibril in the cell wall

The cell walls of all plant fibres are reinforced by cellulose microfibrils.

Wood fibre consists of a layered cell wall matrix.

Cellulose microfibril
Diameter: 2-20 nm
(In wood: 3-4 nm)
Appearance of microfibrils

Algal microfibrils ~20 nm width
Ramie microfibrils ~6-7 nm width
Biosynthesis of cellulose microfibril

*Ligand for cellulose synthase

UDP-Glucose

Aalto University
School of Chemical Technology

Cellulose synthase: a rosette

- Cellulose synthase (CesA) complex is called a *rosette*
- 6 CesA units form a rosette subunit, 6 subunits form a complete rosette
- Each CesA synthesizes one cellulose chain
- $6 \times 6$ rosette is held as circumstantial evidence for $6 \times 6$ chain model for the cellulose microfibril
Major implication of cellulose biosynthesis

- Cellulose crystallizes as it synthesizes
- Native cellulose is always in the form of microfibrils
- There are no individual chains of cellulose in nature
- There is no amorphous cellulose in nature
Relatively undisputed facts on native microfibrils

- Smallest supramolecular unit of cellulose in the plant cell wall
- Monodisperse width (nm range)
- The width depends on the botanical source
- Very long (µm range, owing to high DP of native cellulose)
Disputed issues about cellulose microfibril (CMF)
Cellulose microfibril (CMF) – major controversies

- Width of the CMF
- How many chains make up the CMF
- Twist (chirality) along the CMF
- Longitudinal disorder: the fringed-fibrillar model
Unit cell vs. crystallite width

In general, the unit cell of crystalline cellulose is recognized and agreed upon but the width of the crystallite and general morphology of the microfibril is still elusive.

Crystallographic details in 1Å resolution (cellulose Iα ja Iβ):

Nishiyama et al.

Iα: one chain triclinic  Iβ: two chain monoclinic
Terminological note

- 36 and 24 chain models refer to the smallest CMFs, such as those present in wood cells
- Often these smallest CMFs are referred to as elementary fibrils
- In many species, the CMFs are larger but they are multiplicates of the elementary fibrils
- Often the CMFs (or elementary fibrils) aggregate, forming larger CMF bundles
# Measured CMF widths

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of crystallinity</th>
<th>Microfibril width*</th>
<th>Microfibril width**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal cellulose</td>
<td>&gt;80 %</td>
<td>10 nm</td>
<td>10-35 nm</td>
</tr>
<tr>
<td>Bacterial cellulose</td>
<td>65-79%</td>
<td>5 nm</td>
<td>4-7 nm</td>
</tr>
<tr>
<td>Cotton linters</td>
<td>56-65%</td>
<td>5 nm</td>
<td>7-9 nm</td>
</tr>
<tr>
<td>Ramie</td>
<td>44-47%</td>
<td>5 nm</td>
<td>3-12 nm</td>
</tr>
<tr>
<td>Hemp</td>
<td>60%</td>
<td>3-5 nm</td>
<td>3-18 nm</td>
</tr>
<tr>
<td>Flax</td>
<td>56%</td>
<td>4-5 nm</td>
<td>3-18 nm</td>
</tr>
<tr>
<td>Dissolving pulp</td>
<td>43-56%</td>
<td>4-5 nm</td>
<td>10-30 nm</td>
</tr>
</tbody>
</table>

*) Deduced from X-ray diffraction (reflection from 110 lattice plane)

**) Deduced from transmission electron microscopy images

Models for CMF
Models for cellulose crystallite / microfibril: the 6×6 model

The 6×6 model (rectangular)
- Most common model for CMF
- Based on circumstantial evidence on the 6×6 organisation of the rosette in biosynthesis
- Roughly fits the evidence (XRD and microscopy data)
- Much of the molecular modelling of CMFs is performed with this model

Figure taken from: Okita et al. *Biomacromolecules* 2010, 11, 1696.

Alternative 6×6 model

The 6×6 model (irregular hexagon)
- Based entirely on atomic force microscopy (AFM) data
- Widely used despite the fairly weak experimental evidence
- Used in some molecular modellings of CMFs

Ding and Himmel *J. Agric. Food Chem.* **2006**, *54*, 597
24 chain model

Two possibilities: rectangle or “diamond” model

24 chain model

- Based on several techniques: FTIR, NMR, and diffraction
- A credible alternative
- A disputed model but has the most substantial experimental data of all CMF models
- Suggests that only 4 of the 6 rosettes are simultaneously active during biosynthesis

18-24 chain model

• Based on molecular dynamics simulations in aqueous environment
• Comparison with previously published experimental data suggests that a 36 chain model is highly unlikely
• Proposes that CMF is made of either 18 or 24 chains
• Endorsed by the researchers who originally came up with the 24 chain model

Twist along CMF
Simulations suggest twist

- Computational models of very small cellulose crystals are twisting
- The periodicity of the twist is longer than with individual chains
- The twist is right-handed
CMF twist: experimental evidence

Electron tomography on a cellulose nanocrystal

- Experimental evidence on CMF twist is not unambiguous
- Many images abound in literature but quantitative data is missing
CMF twist: experimental evidence

- Experimental evidence on CMF twist is not unambiguous
- Many images abound in literature but quantitative data is missing

Proposed alteration of CMF twist

- Some accounts suggest that the twist is altered and “localized” upon drying

CMFs of *M. denticulata* alga after drying

Suggestion on what happens to a pristine CMF (a) upon drying (b) and in a totally dried state (c)

Implication of the CMF twist

Cellulose nanocrystals spontaneously forms a liquid crystal phase in solution

Photograph of liquid crystal suspension of cellulose nanocrystals

Chiral nematic phase formed by cellulose crystallites

Tight packing by the chiral interaction of screwlike rods

Fringed fibrillar model
Crystallographic data presents evidence that cellulose within microfibrils is not totally crystalline.

Proposition: cellulose runs through alternating crystalline and “amorphous” regions.
Fringed-fibrillar model of CMFs

According to various models, **disordered** cellulose segments coexists with crystalline cellulose in native cellulose microfibrils.

Hearle 1958  
Dolmetsch 1968  
Hess and Kiessig 1953
Semicrystallinity of microfibrils

- Original models were designed for all polymeric fibrils: synthetic, regenerated and native alike


Synthetic polymers vs. cellulose

- High resolution morphology by AFM

Polyethylene

Cellulose microfibrils in bleached kraft pulp

The original fringed fibrillar model

- Modern TEM images of microfibrils isolated by TEMPO-mediated oxidation do not support the Hearle model where fibrils are branched and polydisperse in width.


More realistic picture of a microfibril

- “Amorphous” regions are more like defects between the crystallites
Implications of fringed-fibrillar model

Alternating crystalline-amorphous regions explain well the macroscopic mechanical properties of cellulosic materials.

The length and width of the crystalline domains depend on the native source of the material.

Elastic properties of isolated cellulose nanofibrils depend on their native source.
Reservations with fringed fibrillar model

• When you see data of the “degree of crystallinity” of cellulose, its physical meaning is unclear
• If the degree of crystallinity is, e.g., 64%, it does not mean that 64% of the cellulose is crystalline and 36% is “amorphous”
• Probably much of the material responsible for the “amorphous” response resides on the microfibril surface
• Cellulose I and cellulose II degrees of crystallinity should not be compared with each other
• Systematic sets of data can be compared with each other if the crystalline forms, the analytical method, and the raw materials are similar
Leveling-off degree of polymerization (LODP)
Acid hydrolysis of cellulose

- Acid hydrolysis involves the breakage of glycosidic bond by addition of water, catalyzed by acid
- High concentrations are required for complete degradation (e.g., 72% (w/w) H$_2$SO$_4$)
Kinetics of acid hydrolysis of cellulose

Traditionally LODP is determined with 2-3 M HCl at around 100ºC.

Common explanation for LODP: “amorphous” regions are hydrolysed and crystallites are left intact.
## LODP of different cellulose sources

<table>
<thead>
<tr>
<th>Material</th>
<th>LODP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood pulp</td>
<td>100-250</td>
</tr>
<tr>
<td>Cotton linters</td>
<td>100-250</td>
</tr>
<tr>
<td>Ramie</td>
<td>200-350</td>
</tr>
<tr>
<td>Valonia</td>
<td>7000</td>
</tr>
</tbody>
</table>
Molecular weight distribution at LODP

Cotton linters LODP ~150
Discrepancies with LODP

<table>
<thead>
<tr>
<th>Cellulose substrate and reference</th>
<th>LODP</th>
<th>Yield loss (%)</th>
<th>Conditions for determining LODP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton linters[44]</td>
<td>200-250</td>
<td>n.a.</td>
<td>2.5 N HCl, 105°C, 15 min</td>
</tr>
<tr>
<td>Cotton linters[50]</td>
<td>187</td>
<td>7</td>
<td>2.5 N H₂SO₄, 96°C, 6 h</td>
</tr>
<tr>
<td>Cotton linters[44]</td>
<td>253</td>
<td>2</td>
<td>2.5 N H₂SO₄, 100°C, 30 min</td>
</tr>
<tr>
<td>Cotton linters[53]</td>
<td>190</td>
<td>4.4</td>
<td>2.4 N HCl, 100°C, 1 h</td>
</tr>
<tr>
<td>Cotton linters[54]</td>
<td>100</td>
<td>6</td>
<td>6.5 N HCl, 108°C</td>
</tr>
<tr>
<td>Cotton linters[55]</td>
<td>200</td>
<td>3.5</td>
<td>2.5 N HCl, 100°C, 30 min</td>
</tr>
<tr>
<td>Cotton linters[56]</td>
<td>162</td>
<td>5</td>
<td>5% HCl, 95°C, 1 h</td>
</tr>
</tbody>
</table>

- No standard method to measure LODP exists
- Many different values for similar cellulose grades have been reported
- Amount of material lost during hydrolysis (yield loss) also varies a great deal
Does LODP represent the length of the crystalline region?

Cellobiose length in cellulose I crystal: 1.03 nm

- Length calculated from LODP should correspond to crystallite length measured by XRD or NMR
Crystallite length vs. LODP

<table>
<thead>
<tr>
<th>Material</th>
<th>LODP</th>
<th>Crystal length by XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood pulp</td>
<td>100-250</td>
<td>23 nm</td>
</tr>
<tr>
<td>Cotton linters</td>
<td>100-250</td>
<td>35 nm</td>
</tr>
</tbody>
</table>

- Crystal length determined from CMFs does not correlate with LODP
- Possible reason: diffraction and/or spectroscopy cannot detect the CMF twist and interprets it for a shorter crystallite
Careful comparison of LODP and small angle neutron scattering

DP of ramie fibres after HCl hydrolysis

Small angle neutron scattering pattern of untreated ramie

Crystallite length (i.e. length of crystalline domains) by SAXS agrees with the level-off degree of polymerization (LODP).

Careful comparison of LODP and small angle neutron scattering

- The yield loss upon controlled acid hydrolysis is very small (~1%)
- This implies a very short disordered region (4-5 anhydroglucose units)
- Disordered – *not* amorphous

**DP of ramie fibres after HCl hydrolysis**

Cellulose nanocrystals, LODP and crystallite length
Acid hydrolysis targets the disordered regions in a cellulose microfibril. Result: cellulose nanocrystals
Does the LODP correlate with cellulose nanocrystal length?

- Length calculated from LODP should correspond to the length of cellulose nanocrystals
Does the LODP correlate with cellulose nanocrystal length?

From wood pulp, the nanocrystal length correlates with LODP (~100)

Does the LODP correlate with cellulose nanocrystal length?

- From cotton linters, the nanocrystal length is slightly longer than LODP (~150)

Mean length: 103 nm

CNC length – comprehensive treatise

<table>
<thead>
<tr>
<th>Cellulose source and reference</th>
<th>Hydrolysis conditions</th>
<th>Mean length of CNCs (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton linters(^{[71]})</td>
<td>65 wt% H(_2)SO(_4), 45(^\circ)C, 30 min</td>
<td>163</td>
</tr>
<tr>
<td>Cotton linters(^{[71]})</td>
<td>65 wt% H(_2)SO(_4), 72(^\circ)C, 30 min</td>
<td>128</td>
</tr>
<tr>
<td>Cotton linters(^{[70]})</td>
<td>64 wt% H(_2)SO(_4), 45(^\circ)C, 45 min</td>
<td>226</td>
</tr>
<tr>
<td>Cotton linters(^{[70]})</td>
<td>64 wt% H(_2)SO(_4), 45(^\circ)C, 240 min</td>
<td>177</td>
</tr>
<tr>
<td>Cotton linters(^{[76]})</td>
<td>64 wt% H(_2)SO(_4), 45(^\circ)C, 45 min</td>
<td>103</td>
</tr>
</tbody>
</table>

LODP of cotton linters: 100-250 (50-125 nm)

- LODP and CNC length do not match
- Yet systematic studies on the issue are lacking
Implications of longer than LODP nanocrystals

If LODP and nanocrystal lengths do not match:
• Nanocrystal hydrolysis is interrupted before LODP is reached
• Nanocrystals are probably not single crystals of cellulose
Bundling of CMFs
Appearance of CMFs

Aggregates: 12-20 nm

AFM image of a surface of bleached birch kraft pulp; sample untreated.

Individual microfibrils: ~3.5 nm

TEM image of longitudinal cross-section of chlorite delignified pine cell wall; freeze-dried and stained

Heyn J. Ultrastructure Res. 1969, 26, 52.
Appearance of CMFs

Aggregates: 12-20 nm

Individual microfibrils: ~3.5 nm

TEM image of radial cross-section of wood cell wall.

TEM image of longitudinal cross-section of chlorite delignified pine cell wall; freeze-dried and stained


Heyn J. Ultrastructure Res. 1969, 26, 52.
When does CMF bundling occur?

**Hornification**
- Well-known phenomenon with chemical pulp fibres
- Water swells the fibres by penetrating between CMFs
  → Fibres are porous in water
- When dried, the pores disappear
- Upon rewetting, the swelling is not restored to the same level
  → Porosity is irreversibly decreased upon drying
  → CMFs have bundled
When does CMF bundling occur?

- Just removing a plant from its native growth environment causes CMF bundling (aggregation)
Implications of CMF bundling

Why is this important?
- Reduced surface area
- Reduced accessibility

Fewer reaction sites

Difficulties to extract cellulose nanofibrils
Summary

• Native cellulose resides exclusively in cellulose microfibrils (CMFs)

• Width of CMFs is monodisperse but difficult to analyse unambiguously

• Number of cellulose chains in a CMF is not agreed upon

• Longitudinal disorder in CMF (fringed fibrillar model) does exist but the disordered regions are rather dislocations than bulky amorphous regions

• Levelling-off degree of polymerization, cellulose nanocrystal length, and the measured length of CMF crystalline regions do not match together perfectly

• CMFs have a tendency to bundle together upon drying