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GREEN AND LOW COST PRODUCTION OF THERMAL-STABLE AND CARBOXYLATED CELLULOSE NANOCRYSTALS AND NANOFIBRILS USING FULLY RECYCLABLE DI-CARBOXYLIC ACIDS

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Abstract:	Here we demonstrate potentially low cost and green production of high thermally stable and carboxylated cellulose nanocrystals (CNCs) and nanofibrils (CNF) from bleached eucalyptus pulp (BEP) and unbleached mixed hardwood kraft pulp (UMHP) fibers using highly recyclable di-carboxylic solid acids. The operating conditions were acid concentrations of 60 wt% at 100 °C for 60 min and 120 °C (no boiling at atmospheric pressure) for 120 min, for BEP and UMHP, respectively. The resultant CNCs had a higher thermal degradation temperature than their corresponding feed fibers and carboxylic acid group content from 0.2 - 0.4 mmol/g. The low strength (high pKa of 1.0-3.0) of organic acids also resulted in CNCs with both longer lengths of approximately 239 - 336 nm and higher crystallinity than CNCs produced using mineral acids. Cellulose loss to sugar was minimal. Fibrous cellulosic solid residue (FCSR) from the di-carboxylic acid hydrolysis was used to produce carboxylated CNFs through subsequent mechanical fibrillation with low energy input.
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TITLE:

Green and low cost production of thermally-stable and carboxylated cellulose nanocrystals and nanofibrils using highly recyclable di-carboxylic acids

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KEYWORDS:

Cellulose Nanomaterials, Cellulose nanocrystals (CNC), Cellulose nanofibrils (CNF), Di-carboxylic acid hydrolysis, Acid recovery, Thermal stability, Dispersion, Surface carboxylation

SHORT ABSTRACT:

Here we demonstrate a novel method for green and sustainable productions of highly thermally stable and carboxylated cellulose nanocrystals (CNC) and nanofibrils (CNF) using highly recyclable solid di-carboxylic acids.

LONG ABSTRACT:

Here we demonstrate potentially low cost and green productions of high thermally stable and carboxylated cellulose nanocrystals (CNCs) and nanofibrils (CNF) from bleached eucalyptus pulp (BEP) and unbleached mixed hardwood kraft pulp (UMHP) fibers using highly recyclable di-carboxylic solid acids. Typical operating conditions were acid concentrations of 50 – 70 wt% at 100 °C for 60 min and 120 °C (no boiling at atmospheric pressure) for 120 min, for BEP and

UMHP, respectively. The resultant CNCs have a higher thermal degradation temperature than their corresponding feed fibers and carboxylic acid group content from 0.2 – 0.4 mmol/g. The low strength (high pKa of 1.0-3.0) of organic acids also resulted in CNCs with both longer lengths of approximately 239 – 336 nm and higher crystallinity than CNCs produced using mineral acids. Cellulose loss to sugar was minimal. Fibrous cellulosic solid residue (FCSR) from the di-carboxylic acid hydrolysis was used to produce carboxylated CNFs through subsequent mechanical fibrillation with low energy input.

INTRODUCTION:

Sustainable economic development requires not only using feedstocks that are renewable and biodegradable but also uses green and environmental friendly manufacturing technologies to produce a variety of bioproducts and biochemicals from these renewable feedstocks. Cellulose nanomaterials, such as cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF), produced from renewable lignocelluloses are biodegradable and have unique mechanical and optical properties suitable for developing a range of bioproducts ^{1, 2}. Unfortunately, existing technologies for producing cellulose nanomaterials are either energy intensive when using pure mechanical fibrillation or environmentally unsustainable due to non-recycling or insufficient recycling of processing chemicals, such as when using the concentrated mineral acid hydrolysis process ³⁻⁸ or oxidation methods ⁹⁻¹¹. Furthermore, oxidation methods may also produce environmentally toxic compounds by reacting with lignocelluloses. Therefore, developing green manufacturing technologies for producing cellulose nanomaterials is critically important to make full use of the abundant and renewable material - lignocelluloses.

Using acid hydrolysis to dissolve hemicellulose and depolymerize cellulose is an effective approach for producing cellulose nanomaterials. Solid acids have been used for sugar production from cellulose with the advantage of easing acid recovery ^{12, 13}. Previous studies using concentrated mineral acids indicated that a lower acid concentration improved CNC yield and crystallinity ^{3, 5}. This suggests that a strong acid may damage cellulose crystals while a milder acid hydrolysis might improve the properties and yield of cellulose nanomaterials through the approach of integrated production and CNC with CNF ^{3, 14}. Here we document a method using concentrated solid di-carboxylic acids hydrolysis to produce CNC along with CNF ¹⁵. These di-carboxylic acids have low solubility at low or ambient temperatures, and therefore they can be easily recovered through the mature crystallization technology. They also have good solubility at elevated temperatures which facilitates concentrated acid hydrolysis without boiling or using pressure vessels. Since these acids also have a higher pKa than typical mineral acids used for CNC production, their use results in good CNC crystallinity, and despite lower CNC yields, with a substantial amount of fibrous cellulosic solid residue (FCSR or partially hydrolyzed fibers) remaining due to incomplete cellulose depolymerization. The FCSR can be used to produce CNF through subsequent mechanical fibrillation using low energy inputs. Therefore, cellulose loss to sugars is minimal as compared to using mineral acids.

It is well known that carboxylic acids can esterify cellulose through Fisher-Speier esterification ¹⁶. Applying di-carboxylic acids to cellulose can result in semi-acid un-crosslinked esters ¹⁷ (or carboxylation), to produce carboxylated CNC and CNF as we demonstrated ¹⁵ previously. The

method documented here can produce carboxylated and thermally-stable CNF and CNC that is also highly crystalline from either bleached or unbleached pulps while having relatively simple and high chemical recovery and using low energy inputs.

PROTOCOL:

Note: Bleached eucalyptus kraft pulp (BEP) and unbleached mixed hardwood kraft pulp (UMHP) fibers from commercial sources were used as feedstock for producing CNC and CNF.

Commercial maleic acids purchased were used for hydrolysis. Hydrolysis conditions were acid concentrations of 60 wt% at 100 °C for 60 min and 120 °C (no boiling at atmospheric pressure) for 120 min, for BEP and UMHP, respectively.

1. Preparation of concentrated di-carboxylic acid solution:

1.1. Heat 38 mL deionized (DI) water in a multiple-neck flask in a liquid glycerol bath on a heating plate to approximately 85 °C.

1.2. Add 38 g anhydrous maleic acid into the flask to make a 60 wt% solution with magnetic stirring. Using the densities of the acid solutions reported previously¹⁵, calculate the required amount of water and acid for making the acid solution at the specified mass concentration.

1.3. Heat the solution to the desired hydrolysis temperature of 100 or 120 °C (no boiling due to high di-carboxylic acid concentrations).

2. Hydrolysis reaction:

2.1. Once the acid solution is at temperature, add 10 g oven dried (OD) of BEP or UMHP fibers into 80 mL di-carboxylic acid solution (1.1) with continuous stirring.

2.2. Take an aliquot of the acid hydrolysate (approximately 2 mL) at the end of the predetermined reaction time of 60 min before terminating hydrolysis by adding 160 mL of 80 °C DI water.

2.2.1. Dilute 0.5 mL of the sampled hydrolysate for sugar and acid concentration analyses.¹⁵ Observe the remaining hydrolysate sample for crystallization taking place while cooling down to room temperature.

2.3. Separate the hydrolysate from the hydrolyzed pulp by vacuum filtration using a filter paper in a Buchner funnel.

Note: This separation needs to occur rather quickly before the temperature drops and the acid begins to crystallize out of solution. Due to high ionic strength from the acid solution, the CNC's generated in hydrolysis agglomerate and stay with the FCSR residuals. Approximately 80-90% of the acid will be removed with the filtrate.

3. CNC separation:

3.1. Wash the filtered solids from section 2 using DI water and dilute to 1% total solids with DI water. Centrifuge the filtrate at 11960 x g for 10 min.

3.2. Decant off the supernatant. Repeat the washing and filtration process using fresh DI water until the supernatant is turbid. The turbidity indicates that the ionic strength of the solution has fallen enough for the CNC to disperse and start to become colloidal.

3.3. Mix the turbid supernatant with the settled hydrolyzed pulp (2.3). Dialyze the mixture in a dialysis bag (MWCO 14 kDa) using DI water until the conductivity of the liquid approaches that of DI water. Measure the conductivity using a conductance meter.

3.4. Centrifuge the dialyzed sample at 3500 x g for 10 min to obtain a CNC dispersion in the aqueous phase. Retain the precipitate phase, i.e., FCSR, for CNF production.

3.5. Determine CNC yield from the measured amount of CNC in the dispersion using a COD method described previously^{3, 18}.

4. CNF production:

4.1. Determine the yield of precipitated FCSR by gravimetric measurements after separating the CNC dispersion. Dry the FCSR at 105 °C and measure the oven dry weight of the FCSR relative to the initial oven dry weight of BEP or UMHP fibers used.

4.2. Mechanically fibrillate the FCSR at fiber suspension of 0.5% by consecutively passing the suspension 3 times through a 200 µm orifice chamber followed by 2 times through an 87 µm orifice chamber, all at 100 MPa.

5. Atomic Force Microscopy (AFM) imaging:

5.1. Sonicate approximately 0.01 wt% CNC or CNF suspensions for 2 min. Deposit a drop of the dispersed suspension on a mica substrate. Air-dry the deposited suspension at ambient temperature.

5.2. Take AFM images of the air-dried CNCs and CNFs in vibrating tapping mode using manufacturer's protocol. Analyze the AFM images of approximately 100 individual CNCs or CNFs using commercial software to obtain diameter and length distributions.

6. Fourier Transform Infrared (FTIR) measurements:

6.1. Use a commercial FTIR spectrophotometer with a universal attenuated-total-reflection (ATR) probe to analyze the resultant CNC and CNF samples along with the original BEP and UMHP fibers to identify ester groups.

6.2. Record the absorption spectra of the samples in a wavelength range between 450 - 4000 cm⁻¹ with a resolution of 4 cm⁻¹ and 4 scans for each sample.

7. Conductometric titration:

7.1. Use conductometric titration to quantify the carboxyl group contents of samples resulted from esterification by the dicarboxylic acid.

7.1.1. Add CNC or CNF suspension with 50 mg (OD) of CNCs or CNFs into 120 mL of 1 mM NaCl solution. Titrate the mixture by adding approximately 0.2 mL of 2 mM NaOH solution at 30 s intervals.

7.1.2. Measure the conductivity using a conductance meter. Find the inflection point (the lowest point) on the conductivity curve during the course of adding NaOH.

7.2. Calculate the amount of carboxyl groups (mmol/L) based on the consumed NaOH relative to the inflection point using the following equation in which c is the concentration of NaOH solution (mol/L), v is the volume of added NaOH solution (mL), m is the mass of CNCs or CNFs in OD weight (g).

$$C(\text{mmol/g}) = \frac{c \times v}{m} (\text{mmol/g cellulose})$$

8. CNC and CNF thermal stability determination:

8.1. Conduct thermal degradation measurements of the CNC and CNF samples by thermogravimetric analysis (TGA).

8.1.1. Purge the furnace using a high purity nitrogen flow at 20 mL/min to prevent any unwanted oxidative decomposition. Dry the samples at 50 °C for 4 h before testing. Use a sample size of 5 mg in dry weight.

8.1.2. Record the weight of the sample as the furnace temperature is increased from ambient to 600 °C at a heating rate of 10 °C/min.

8.2. Normalize the measured weight loss by the initial weight.

8.3. Conduct separate thermal stability tests of CNC and CNF samples in an oven at 105°C. Record the color change of the samples after 4 and 24 h by conventional photography.

9. X-ray diffraction:

9.1. Press freeze-dried CNC or CNF samples at 180 MPa to make pellets as described previously.⁵ Conduct wide angle X-ray diffraction measurements of the pellet using Cu-K α radiation on an X-ray diffractometer in the 2θ range of 10 – 38° in steps of 0.02°.

9.2. Calculate the crystallinity index (CrI) of a pellet using the Segal method¹⁹ (without base line subtraction).

REPRESENTATIVE RESULTS:

Typical AFM images of the CNC and CNF from BEP and UMHP along with corresponding SEM

images of the feed acid hydrolyzed fibers are shown in Figures 1 and 2. The images clearly show the substantial reductions in fiber length by acid hydrolysis with minimal change in fiber diameters (comparing Figure 1a with 1b, and 2a with 2b). The shortened fiber length was also reflected by the measured cellulose degree of polymerization (DP) of the hydrolyzed fibers. DP was reduced from 1021 and 806 to 319 and 342, for the BEP and UMHP, respectively. CNCs isolated from the hydrolyzed fiber had relatively longer lengths and thicker diameters compared with the typical length and diameter of concentrated sulfuric acid produced CNCs cited in the literature ⁵; this is perhaps due to the weak strength of maleic acid. The mean CNC lengths and diameters measured by AFM image were 239 and 33 nm, 336 and 39 nm for the two samples shown in Figures 1c and 2c, respectively.

The weak strength of maleic acid also resulted in a substantially low CNC yield of 1.8% and 5.5% from BEP and UMHP, respectively. However, the remaining solids, i.e., FCSR, still have utility and were used to produce CNF through subsequent mechanical fibrillation to achieve the integrated production of CNC with CNF. CNC yield can be increased by using more severe reaction conditions as demonstrated previously.¹⁵ Depending on the application and economics, the severity can be adjusted to accommodate the desired CNC to CNF ratio.

The CNFs had a very long lengths based on AFM image measurements while actually having thinner diameters than their corresponding CNCs as (comparing Figure 1c with 1d, and 2c with 2d). It is felt these CNF's would be ideal for polymer reinforcement in composite applications.

Figure 1 SEM and AFM images of the bleached kraft Eucalyptus pulp (BEP) fibers and cellulose nanomaterials produced.

(1a) SEM image of BEP fibers; (1b) SEM image of the acid hydrolyzed BEP fibrous cellulosic solid residues (FCSR); (1c) AFM image of the BEP nanocrystals (CNCs); (1d) AFM image of the BEP cellulose nanofibrils (CNFs).

Figure 2 SEM and AFM images of the unbleached kraft mixed hardwood Pulp (UMHP) fibers and cellulose nanomaterials produced.

(2a) SEM image of UMHP fibers; (2b) SEM image of the acid hydrolyzed UMHP fibrous cellulosic solid residues (FCSR); (2c) AFM image of the UMHP nanocrystals (CNCs); (2d) AFM image of the UMHP cellulose nanofibrils (CNFs).

DISCUSSION:

The thicker CNC diameters of the CNC samples from maleic acid hydrolysis resulted in a moderate average aspect ratio 7.24 and 8.53, for the CNCs from BEP and UMHP, respectively, despite their long lengths as discussed above. The CNFs had a longer length and a thinner diameter, which resulted in a large aspect ratio of 13.9 and 19.0, for the CNCs from BEP and UMHP, respectively, both greater than their respective CNCs. It is possible to use severe mechanical fibrillation to reduce CNF diameter to improve the aspect ratio as the pressure used in microfluidization in the present study was quite low.

Lignin particles were visible on the CNC sample from UMHP. Lignin should also chemically

bound to CNC particles. It will be interesting to see the effect of lignin on surface hydrophobicity in future studies for a variety of applications.

Due to the presence of carboxyl group, the CNC samples were easily dispersible as shown in Figures 1c and 2c, which facilitates aqueous processing. The surface charges measured by zeta potential were -13 and -34 mV, for the CNCs from BEP and UMHP, respectively.

Thermal stability of the CNC and CNF samples were similar to those found in the feed fibers. This is important for applications requiring thermal processing at elevated temperatures such as extrusion for composite production. The improved thermal stability was attributed to the improved crystallinity.

Critical steps in the protocol are as follows. With the preparation of acid solution of desired concentration (section 1), one needs to use the density data presented in our early work¹⁵ to calculate the amount of salt needed to make the acid solution of desired concentration. For CNC separation (section 3), multiple steps of centrifugation and filtration may be needed. Dialysis is needed to separate CNC particles.

The presented process for producing cellulose nanomaterials is also suitable for nonwoody lignocellulosic materials. The method is relatively straightforward. Troubleshooting should be focused on making sure that the critical steps described above are carried out correctly.

The significance of the present process compared with traditional mineral acid hydrolysis or oxidation processes are (1) the resultant CNC and CNF are thermally stable and (2) the minimal loss of cellulose top sugars. Also, the acid can be easily recycled to achieve environmental sustainability and reduce production cost. There are no potential harmful products to be produced as the acid is used as a catalyst when compared with oxidation method.

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DISCLOSURES:

Chen and Zhu are co-inventors of a US patent application using di-carboxylic acids for CNC and CNF production.

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Figure 1a

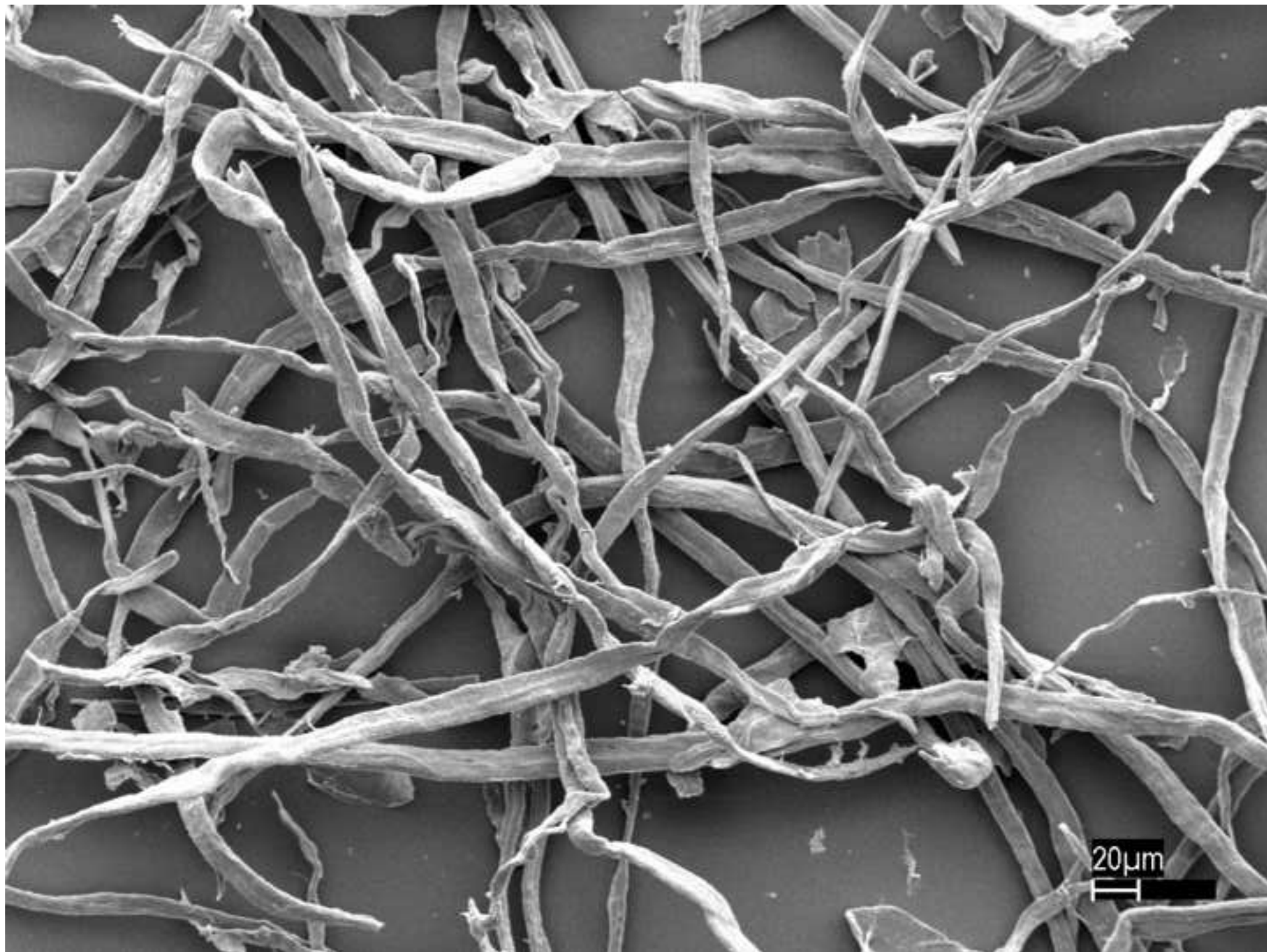


Figure 1b

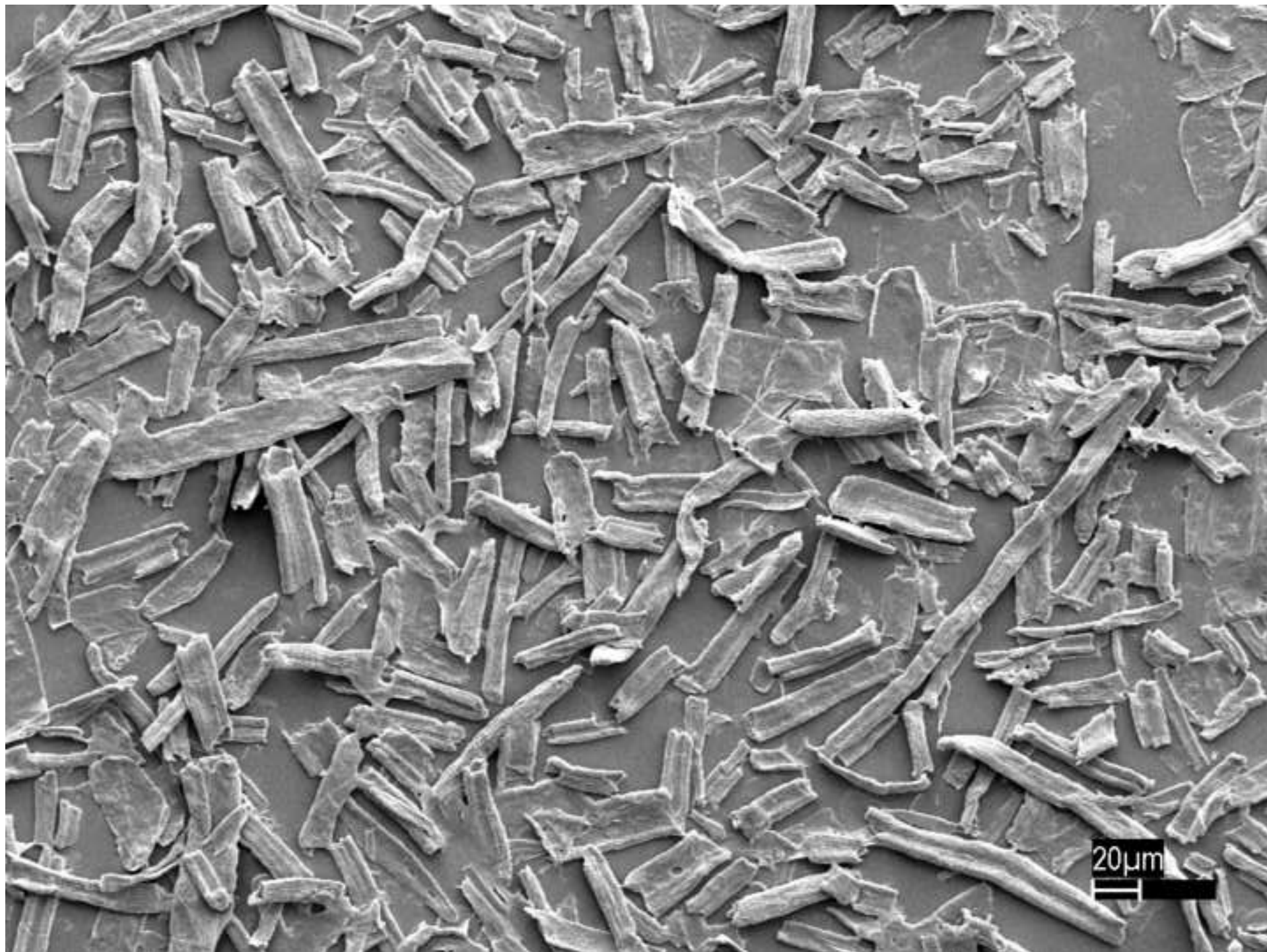
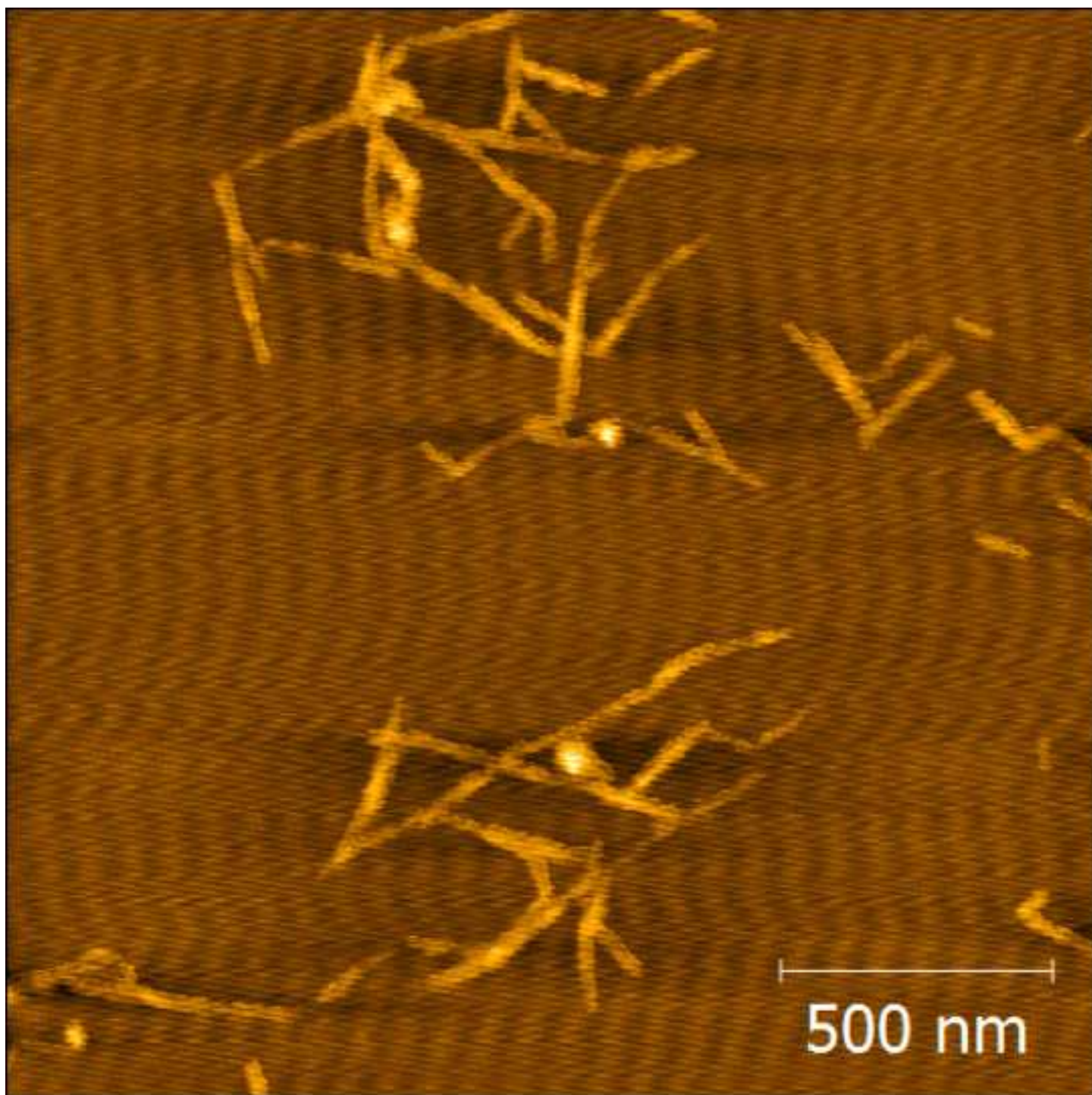


Figure 1c

[Click here to download Figure Fig 1a3 - 55-100-60-CNC-1, 2um, 512 pixels, Left.tif](#)



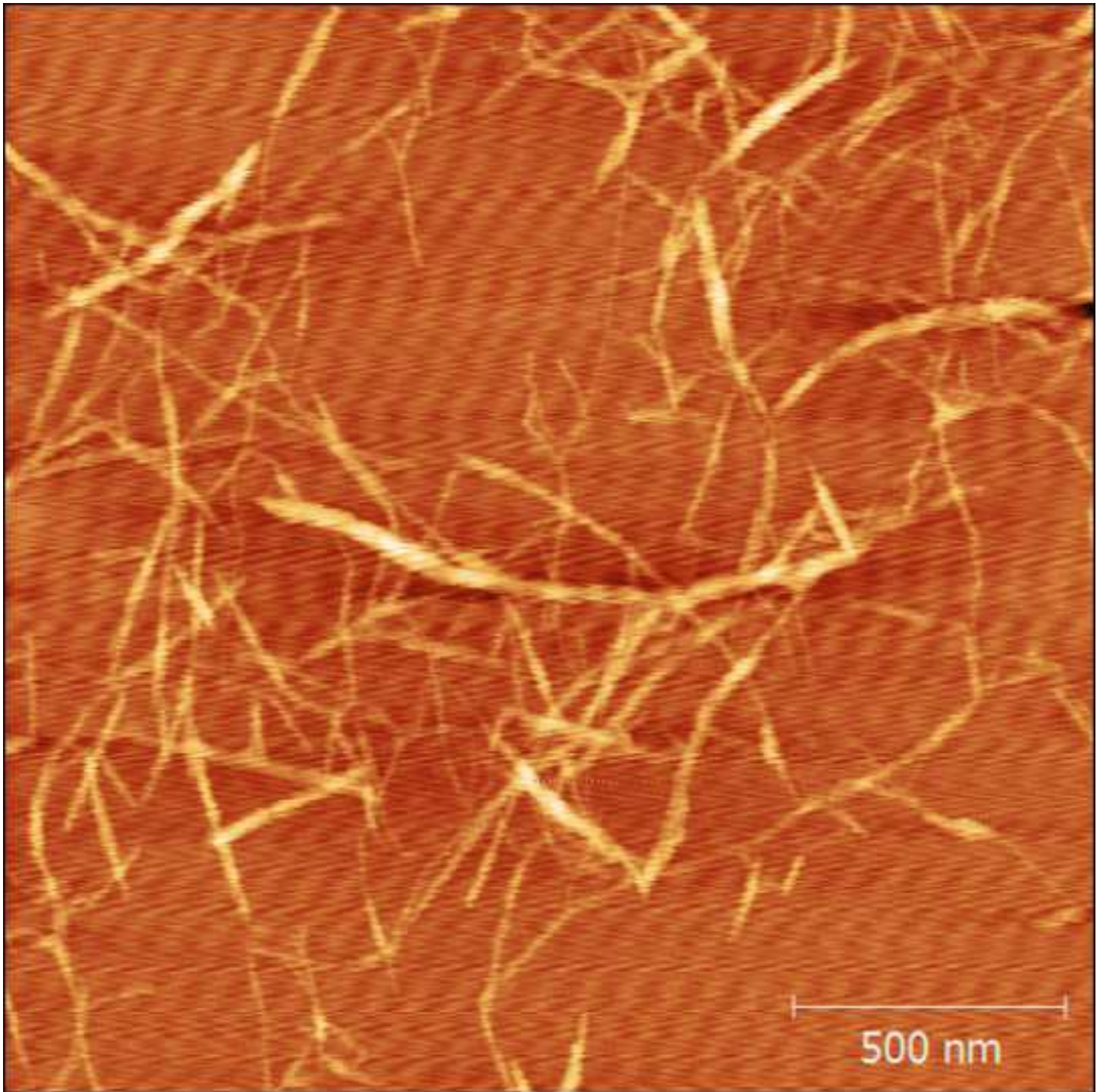


Figure 2a

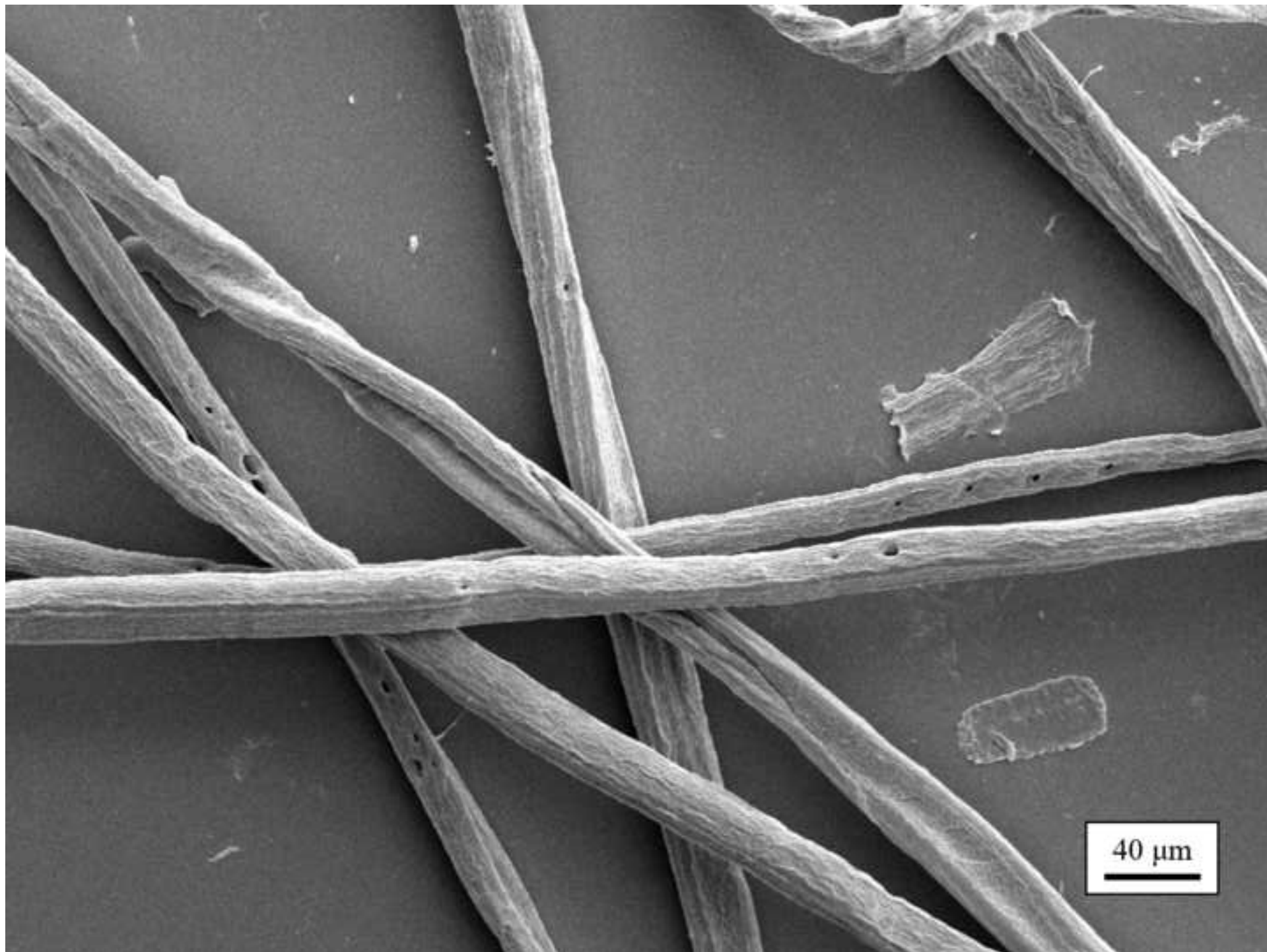


Figure 2b

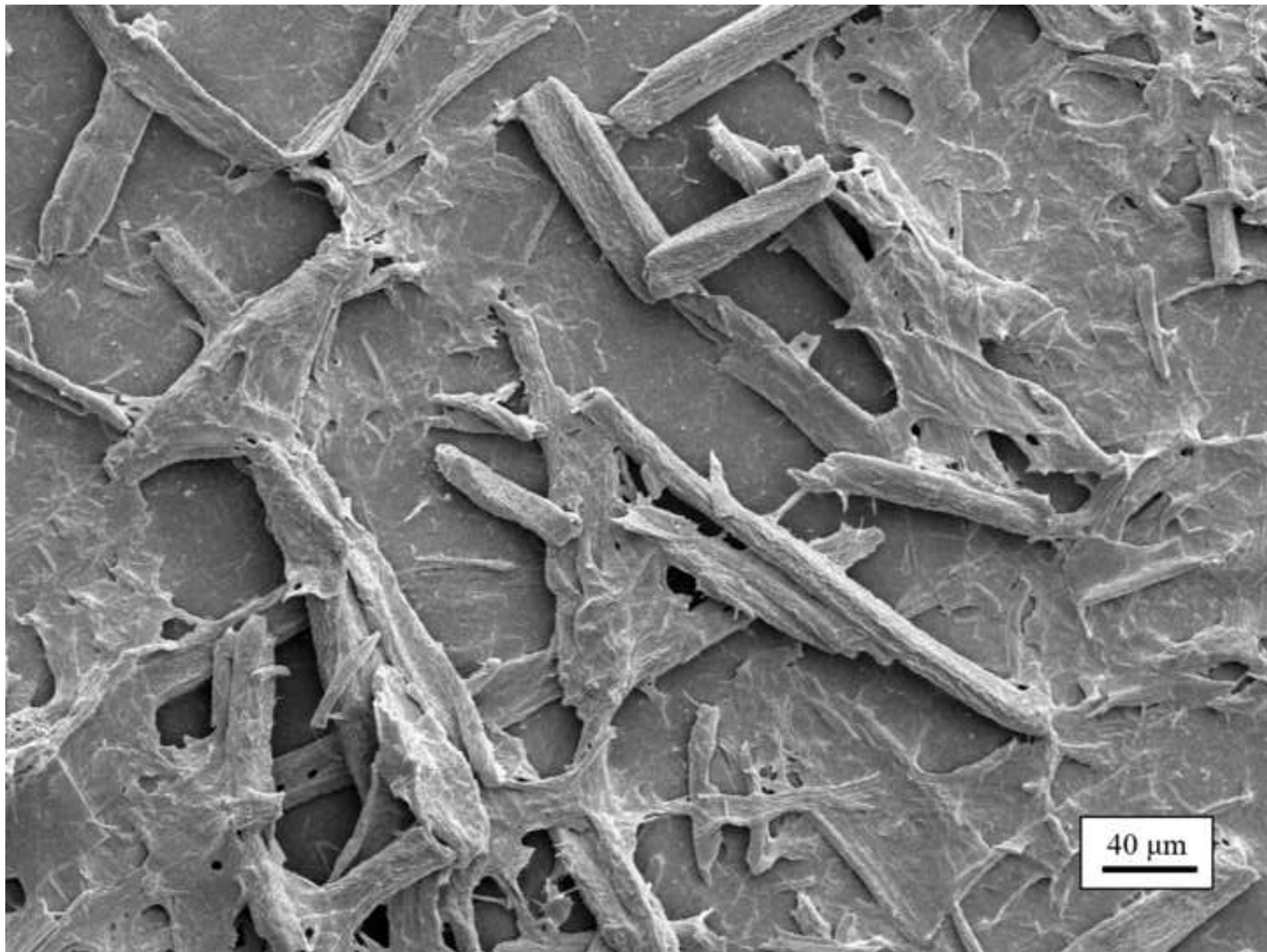
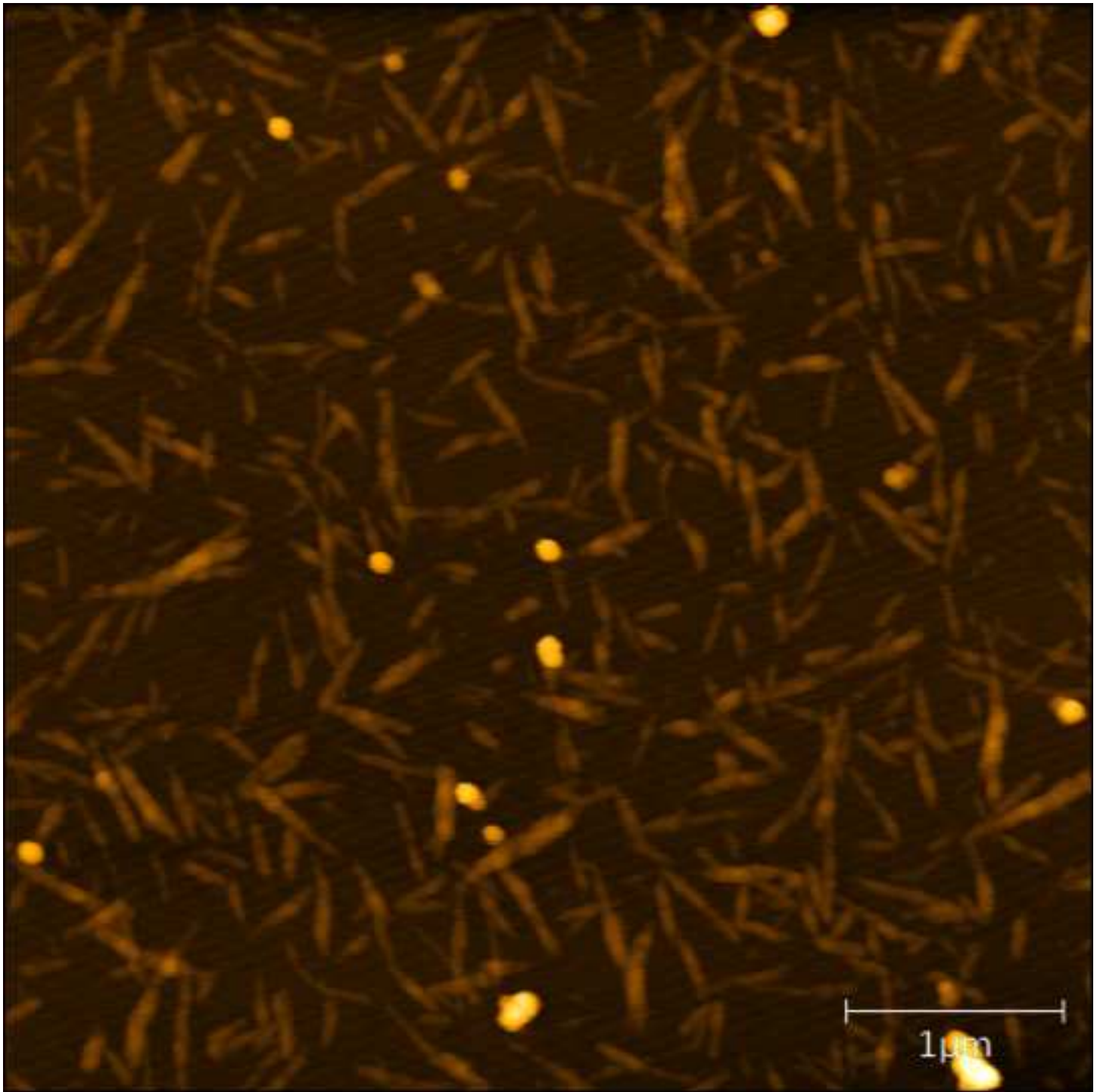


Figure 2c

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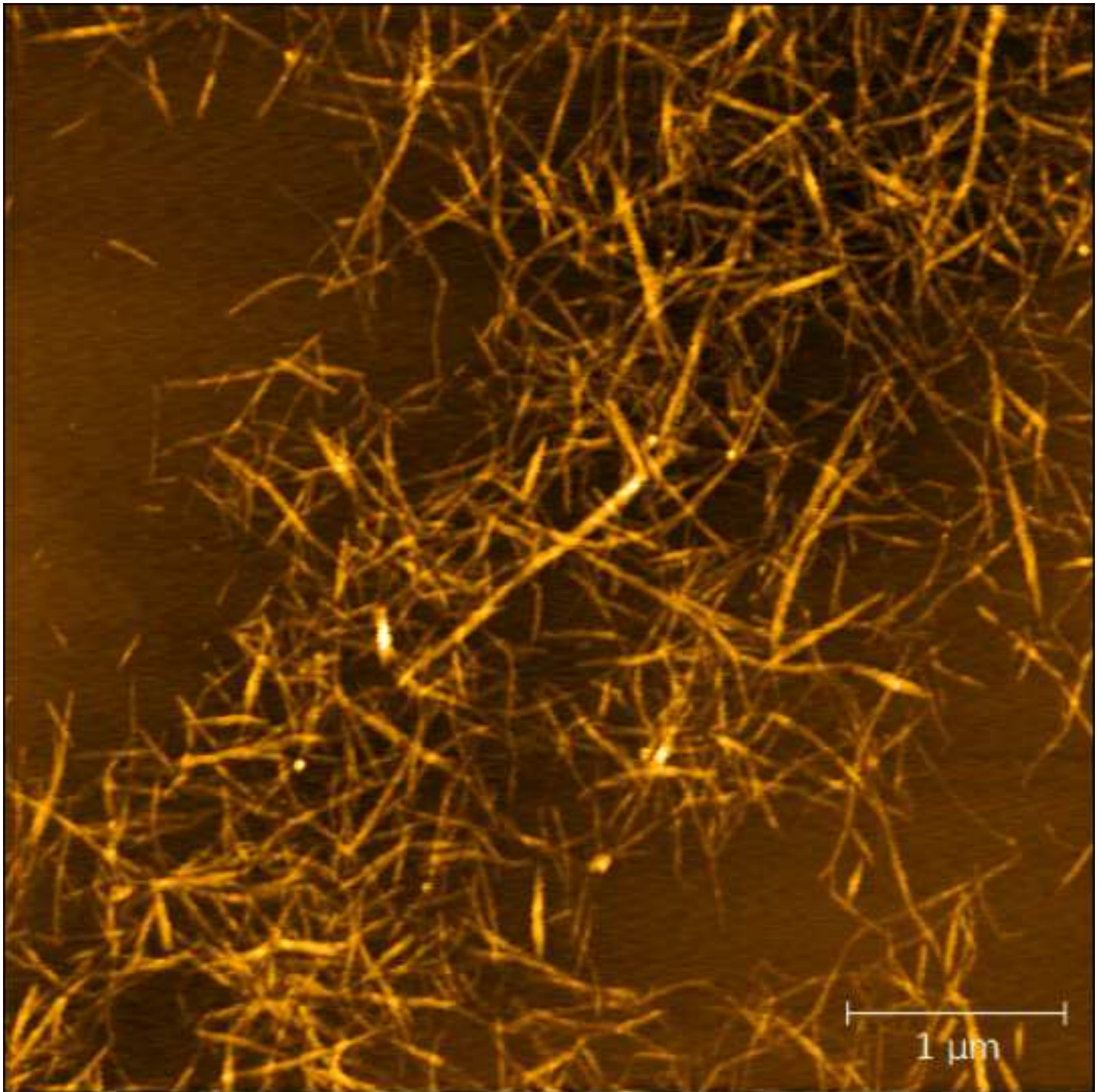


Table of Material and Equipment

Bleached eucalyptus pulp
Unbleached mixed hardwood kraft pulp
Maleic acid
Glycerol
Sodium hydroxide
Sodium chloride
Cupriethylenediamine solution
Acetone
Accu-Test™ Vials for COD Testing
Heating plate
Magnetic stir bar
Pyrex three-neck round-bottom flask
Dialysis tubing cellulose membrane
Disposable aluminum dishes
Disintegrator
Microfluidizer
Sonicator
Zeta potential analyzer
FTIR
Conductometric titrator
TGA analyzer
X-ray diffractometer
AFM imaging
SEM imaging

Company

Aracruz Cellulose
International Paper
Sigma-Aldrich
Sigma-Aldrich
Fisher Scientific
Mallinckrodt
GFS Chemicals
Fisher Scientific
Bioscience, Inc.
IKA
ACE Glass
Sigma-Aldrich
Sigma-Aldrich
Sigma-Aldrich
Testing Machines Inc.(TMI)
Microfluidics Corporation
Qsonica LLC.
Brookhaven Instruments Corporation
PerkinElmer
Yellow Springs Instrument (YSI)
PerkinElmer
Bruker Corporation
AFM Workshop
Carl Zeiss

Catalog Number	Comments
M0375-1KG/CAS110-16-7	Powder;assay:99.0%(HPLC)
G5516-4L/CAS56-81-5	
S318-500/CAS1310-73-2, 497-19-8	Certified ACS
7581-12/CAS7647-14-5	Crystal,AR
E32103-1L/CAS14552-35-3	1M,for determination of solution viscosity of pulps
A18-500/CAS67-64-1	Certified ACS
01-215-28	COD testing for 20 to 900mg/L standard range concentration Mode:C-MAD HS7 digital
CLS4965B500-1EA	
D9402-100FT	Typical molecular weight cut-off = 14000
Z154857-1PAK	Circles,60mm
	Mode:3510R-MT,50-60 Hz,180 W



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Replies to Editorial comments:

The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55079_R2_062816.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

1. Please combine all panels of one figure into a single image file.

Please combine the a figures from Figure 1 into one single image file. Please combine the b images from Figure 1 into one single image file. This would create two figures for clarity. Alternatively, you can combines 1a1 and 1b1 into a side-by-side image file, etc.

Despite your rebuttals and statements otherwise, the image files have not changed in the last two revisions.

RESPONSE: We did made into two Figures. The label Fig. 1a1 and 1b1 do not exist anymore. The editor must read the old version. However, we loaded the individual images, such as 1a, 1b, 1c, 1d for the consideration to preserve the resolution of the Figure. I am sure the Journal has better software to combine these individual images into one Figure.

2. Please employ professional copy-editing services. Despite statements otherwise, the language is still very difficult and awkward to read and understand.

RESPONSE: Proof read by our lab editorial service again.

3. Formatting:

-Figures – For all multipanel figures, the panels should appear in a single file, on a single page, with all panels clearly labeled.

RESPONSE: This is usually done by the Journal, not by the authors. Because Journal has better software to preserve resolution. We combined in the revised version per request.

-Please define all abbreviations at first occurrence (ie, AFM, etc.).

RESPONSE: Done

-For all equipment, please include the company and product number in the materials table.

RESPONSE: Very strange requirement. But done per request.

-9.1 – Please use “-” instead of “~” to indicate a range.

RESPONSE: Done

4. Please copyedit the manuscript for numerous grammatical errors, some of which are indicated below. Such editing is required prior to acceptance and should be performed by a native English speaker.

-4.1 – “after separated the CNC dispersion”

-Line 255 – “charge measured by zeta potential were”

-Line 258 – “Thermal stability of the CNC and CNF samples were”

-Line 263 – “Critical steps on the protocol”

-Line 267 – “that the resultant CNC and CNF are thermally stable and the minimal loss of cellulose” – what about the minimal loss of cellulose?

RESPONSE: Done

5. Additional detail is required:

-2.9 – Are the fibers dried in the lab? If so, please describe the process.

RESPONSE: The editor must read the old version, No. 2.9

-3.1 – How much water is used? Which filtered solids? Is this the hydrolyzed pulp or hydrolysate?

RESPONSE: This is not critical. When we say wash, it usually 10:1 dilution. Depending what washing method is used. The amount of water usage are different.

-3.3 – What is the molecular weight cutoff?

RESPONSE: Dialysis bags are commercially purchased.

-4.1 – How is this done? Is the FCSR dried first? If so, how?

RESPONSE: This is clearly described. Of course one need to dry to determine solids. We modified to reflect this comment.

-5.1 – How much is placed on the mica substrate?

RESPONSE: Just a droplet (amended). Any person skills in AFM will know this

-5.2 – How are the measurements/analyses performed? Please provide stepwise detail if this is to be filmed in detail or a citation if this is not to be filmed in detail.

RESPONSE: This is not necessary. This paper is not about how to do AFM. Exact operation procedures differs for different AFM systems

-6.1, 9.1 – How is this done? Please provide a citation.

RESPONSE: This is not necessary. This paper is not about how to do FTIR and X-Ray diffraction. These are standard lab equipment.

-8.1.2 – How is weight determined?

RESPONSE: It is automatically determined by the instrument.

6. Branding: 5.2 – Image-Pro Plus

RESPONSE: Not really, we added the vendor name in revision.

7. Discussion: Please discuss the critical steps in more detail. Please discuss the limitations and future applications of the protocol. Please also discuss any troubleshooting/modifications that can be performed.

RESPONSE: We amended the text to reflect this comment

Replies Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors investigated the properties of nanocellulose produced from bleached eucalyptus pulp (BEP) and an unbleached mixed hardwood kraft pulp (UMHP) fibers using fully recyclable di-carboxylic solid acids. The results obtained are quite interesting and the authors have fully and perfectly addressed the points raised by the previous reviewers. Therefore, I recommend that this manuscript be accepted for the publication in the Journal of Visualized Experiments.

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

The paper by Bian et al. contains a novel method to produce cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF) by a weak acid hydrolysis at elevated temperatures.

Major Concerns:

The produced nanocelluloses are inferior to CNC and CNF produced by other methods, as they have much larger diameters and much lower aspect ratios. The authors claim that the main advantages of their method are that it is "green" and produces nanocellulose which is thermally more stable. How more green their method is can be debated, as acids or oxidants used in the production of CNC can be recovered and re-used. It seems to me that CNC and CNF produced by this new method is inferior in many respects, compared to conventional CNC and CNF, because of their small aspect ratios. Hence the

uses of this nanocellulose will be limited. The authors should put more effort in reducing the aspect ratio to values comparable with other nanocelluloses, if this method is going to be adapted by others.

RESPONSE: Aspect ratio is only one measure of the cellulose nanomaterials. It can be tuned as we demonstrated previously. This paper is a method paper to show people how this method can be implemented.

Minor Concerns:

The authors provide insufficient and conflicting data, regarding the dimensions of the CNC and CNF produced. In the abstract it is mentioned that CNC lengths vary from 239-485 nm, whereas mean lengths of BEC and UMHP are given as 239 and 336 nm (line 219). Surely if the mean is 239 nm, a large fraction is smaller than 239 nm, contradicting the range given in the abstract. Why are no standard deviations given for length and width? For CNF no diameters and length are given at all, just average aspect ratios for the two pulps.

RESPONSE: We have corrected in the abstract.

It is claimed that the improved thermal stability is due to improved crystallinity lines (261-262). This is surprising as the crystallinity of conventional CNC is pretty large. The authors should back up their claim with data and/or references to the literature. Has CNF also an improved thermal stability? Surely CNF contains amorphous regions and it would be difficult to ascribe improved thermal stability to crystallinity for CNF.

RESPONSE: We disagree with the reviewer on the crystallinity issue. The present authors have done a substantial amount of work on the subject. The current understanding on cellulose crystallinity is still very limited due to incapable of accurately measure cellulose crystallinity. All measurements are ensemble measurement of a cellulose pellets. The reviewer is advised to check our early work (Chen et al., Cellulose 23:1753), CNC crystallinity by sulfuric acid hydrolysis is very low. The reviewer is also advised to check our recent review paper on subject (Chem Rev. DOI: [10.1021/acs.chemrev.6b00225](https://doi.org/10.1021/acs.chemrev.6b00225), 2016)

Additional Comments to Authors:

N/A

Reviewer #3:

Manuscript Summary:

I do not know JOVE as a journal, so please take any comments with a grain of salt. However, I think the topic of the manuscript is timely, needed and interesting. Scalable methods for highly dispersible acid functionalized CNC and CNF is a topic of current interest. The authors describe a unique and easy way to produce them.

Major Concerns:

More characterization of the materials is needed, although this is probably best suited for a followup paper.

RESPONSE: The comment is well taken. Agree that this is a method paper.

Minor Concerns:

The procedure was written in a non-standard method. It came off as a recipe or "directions" as opposed to the more standard "description". I suggest rewriting it to a more standard tone. However, this may be the manner of JOVE, I do not know.

RESPONSE: Yes. We have to rewrite the description according to Journal language. We have spent so much time doing that and continuing doing that.

Additional Comments to Authors:

N/A