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Hierarchical Assembly of Nanocellulose based Filaments by Interfacial Complexation

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In the present study, interfacial complexation spinning of oppositely charged cellulose-materials is applied to fabricate hierarchical and continuous nanocellulose based filaments under aqueous conditions by using cationic cellulose nanocrystals (CCNCs) with different anionic celluloses including soluble sodium carboxymethyl cellulose (CMC) and insoluble TEMPO-oxidized nanofibers (TO-CNF) and dicarboxylated cellulose nanocrystals (DC-CNC). The morphologies of the wet and dry nanocellulose based filaments are further investigated by optical and electron microscopy. All fabricated continuous nanocellulose based filaments display a hierarchical structure similar to the natural cellulose fibers in plant cells. To the best of our knowledge, this is not only the first report about the fabrication of nanocellulose based filaments by interfacial complexation of cationic CNCs with anionic celluloses but also the first demonstration of fabricating continuous fibers directly from oppositely charged nanoparticles by interfacial nanoparticle complexation

(INC). This INC approach may provide a new route to design continuous filaments from many other oppositely charged nanoparticles with tailored characteristics.

1. Introduction

Cellulose-based nanomaterials, i.e., cellulose nanocrystals, cellulose nanofibers, bacterial nanocellulose and cellulose spheres, which are collectively referred to as nanocellulose (NC), can be produced from different cellulose sources such as plant biomass, algae and tunicate by various mechanical, chemical or enzymatic treatments.^[1-4] These nanoscale cellulosic entities have attracted tremendous attention owing to their unique and appealing features such as high aspect ratios, facile chemical modification, low density and excellent mechanical properties.^[5, 6] Moreover, depending on preparation and processing conditions, different types of functional groups, such as sulfate, carboxyl,^[7-11] sulfonate,^[12, 13] quaternary ammonium^[14] and phosphonate groups,^[15-17] can be introduced on the NC surface. The charged surface of NC has been demonstrated to be highly beneficial in enhancing the interaction between NC and other nanoparticles or small molecules,^[18] which, in turn, endows NC versatile performance for wide range of applications, e.g., catalysis,^[19] water purification,^[20-22] pharmaceuticals and biomedical products.^[2, 3, 23-25]

To date, various forms of nanocellulose based materials, such as membranes, hydrogels, aerogels and nanopapers, have been manufactured in lab scale.^[26-33] Nanocellulose based cellulosic filaments has also attracted growing interest in recent years to be used in composite reinforcement, textiles, biomedical product, etc.^[34-36] Compared to regenerated cellulosic filaments, the NC based continuous fibers retain the one-dimensional nature and very fine dimensions of NC while preserving the native cellulose I structure.^[37-40]

So far, a variety of techniques has been explored to prepare continuous NC filaments, such as wet-, dry-, or electrospinning, microfluidics and flow focusing.^[37, 40-42] Recently, an approach called interfacial polyelectrolyte complexation (IPC) spinning was successfully applied in the fabrication of NC based filaments using 2,2,6,6-tetramethylpiperidiny-1-oxyl radical (TEMPO)-oxidized nanofibers (TO-CNF) and soluble chitosan as raw materials.^[43, 44] The IPC fiber drawing method is a process based on the spontaneous self-assembly of filament upon drawing of the insoluble polyelectrolyte complex formed at interface of two oppositely charged polyelectrolytes by tweezers or pipette tips.^[45] Since the self-assembled fibers were firstly reported in 1998,^[46] a wide range of stable bi- or multi-component fibers from different soluble polycation-polyanion pairs has been manufactured and tested.^[47-50] This environmentally friendly fiber drawing process, which can be conducted under aqueous, neutral pH and room temperature conditions, is in many ways more desirable and favorable compared to many other fiber fabrication processes that typically require volatile organic solvents.^[37, 45] Inspired by the IPC method, a flame-retardant continuous cellulosic fibers fabricated by extruding anionic cellulose nanofibers into cationic silica nanoparticle coagulation bath in acidic condition was reported recently. These filaments were formed through interfacial complexation (IC) as mentioned by the authors, but actually were not fully created by interfacial complexation, because of the gelation of the negatively charged CNF in the acid solutions also facilitated the formation of cellulosic filament.^[51]

In present study, continuous filament drawing by interfacial complexation of cationic cellulose nanocrystals (GT-CNC, prepared by nanofibrillation of cationized dialdehyde cellulose with Girard's reagent T) with three different anionic celluloses, including soluble sodium carboxymethyl

cellulose (CMC), and insoluble TO-CNF and dicarboxylated cellulose nanocrystals (DC-CNC), was investigated. As a result, three types of hierarchical nanocellulose based filaments were successfully fabricated by simple interfacial complexation of oppositely charged celluloses in aqueous suspensions. The morphologies of the wet and dry nanocellulose based filaments were characterized by optical and electron microscopy. The mechanical properties of the formed filaments were also tested and compared. To the best of our knowledge, this is the first report about the fabrication of nanocellulose based filaments by interfacial complexation of cationic CNCs with anionic celluloses and among the first reports to fabricate continuous fibers directly from nanoparticles (without any soluble matrix).

2. Result and Discussion

Three different types of nanocelluloses were firstly synthesized (GT-CNC, TO-CNF and DC-CNC), of which GT-CNC was positively charged while TO-CNF and DC-CNC were both negatively charged. In addition, a soluble anionic derivative of cellulose, carboxymethyl cellulose, was also tested as one constituent to form nanocellulose based filaments. The nanoparticle dimensions, charge density and DP_v of samples are summarized in **Table 1**. Both GT-CNC and DC-CNC were rod-like nanocrystals having similar dimensions (see Figure S1, 100 ± 60 nm in length and 10 ± 4 nm in width for GT-CNC, and 133 ± 65 nm in length and 8 ± 2 nm in width for DC-CNC). In contrast, TO-CNFs exhibited flexible elongated nanofibrillar morphology with an average length ranging from 172 to 958 nm and a lateral dimension of 5 ± 2 nm (Figure S1 c). The charge density of each nanocellulose was determined to be 1.1 mmol/g (GT-CNC), 3.9 mmol/g (CMC), 1.0 mmol/g (TO-CNF) and 1.8 mmol/g (DC-CNC), respectively. For cellulose nanocrystals (CNC), GT-CNC

and DC-CNC had a similar DP_v value, which was 53 and 60, respectively, while the DP_v of TO-CNF showed a high value of 176 probably due to less harsh conditions during the synthesis.

When cationic GT-CNC suspension was simply mixed with anionic counterpart (i.e. CMC, TO-CNF or DC-CNC) by shaking manually for couple of seconds, precipitated complexes generated immediately (Figure S2). The release of counterions **is the driving force for** the complexation **between** oppositely charged cellulose **nanoparticles**.^[52] Similarly, stable and insoluble continuous cellulosic macrofibers could be successfully drawn from the suspensions interface using interfacial complexation (IC) method as shown in **Figure 1**. Herein, three types of nanocellulose based filaments were prepared by combining cationic GT-CNC with different anionic celluloses (videos of the preparations of different of nanocellulose based filaments can be found in supporting information, videos S1-3). Firstly, droplets of two oppositely charged cellulose solutions (GT-CNC with CMC, TO-CNF or DC-CNC) were placed adjacent to each other on a plastic Petri dish. Then, a pair of tweezers was plunged into the two droplets to make the droplets into contact, which resulted in the instantaneous formation of insoluble film on the interface. The nanocellulose based filament was fabricated by picking the interface film up and continuously dragging vertically upwards by tweezers (Figure 1b-g). When the fiber was drawn by hand with a quick speed (e.g. 200 mm/min without breaking the formed wet fiber, see Figure 1b, c), small beads appeared along the fiber axis. These beads were formed by the release of salt counter-ions and water during the complexation of the two oppositely charged solutions, which happened in the drawn wet fibers but not in the droplet due to the high drawing speed.^[43, 49] However, **once the beaded fiber was** dried, **inhomogeneous and thicker section was observed in the bead area** (Figure S3 in Supporting

information), which may be unfavorable to the mechanical properties of fibers. In contrast, if the fiber was drawn as slowly as possible (e.g. lower than 40 mm/min, Figure 1d,e), homogeneous and beadless filaments with even thickness were successfully formed because the release of counterions and water occurred already at the droplet-air interface before they left the solution (see dried uniform nanocellulose based filaments in Figure S4 in supporting information).^[45, 53] Consequently, in order to engineer homogeneous continuous cellulosic filament, the drawing process was conducted by a tensile testing machine with a constant rate of 40 mm/min (Figure 1f, g; See video S4 in supporting information). If no otherwise specified, all the cellulosic filaments prepared below were drawn mechanically. The effects of concentrations of GT-CNC and anionic counterparts on the formation of these continuous cellulosic fibers and their corresponding fiber diameters are presented in Table S1. It was found that all combinations of 100 μ L of 0.6 to 0.8 wt% GT-CNC with 100 μ L of 0.3 to 0.6 wt% anionic celluloses solutions could easily be drawn to produce filaments up to a length of 70 cm.

Figure 2 shows the typical optical microscopy and SEM images of dried nanocellulose based filaments (concentration of each constituent used here was 0.6 wt%). All drawn fibers showed compact structures and had different diameters depending on the constituents. These formed filaments were flexible and could be tied to form knot (Figure 2b). As shown in the magnification images (Figure 2d), all the drawn cellulosic filaments displayed a nervation/veining pattern similar to the reported typical structure of IPC spun fibers,^[45] in which conglomerated and parallel sub-micron **fibers (S-MFs)** aligned along the fibers longitudinal axis. However, **these S-MFs** were densely aligned and packed together, which make it difficult to determine the dimensions of

individual fibrils. As reported by several researchers,^[53, 54] when the manually drawn nascent beaded fiber was placed in contact with a glass slide before drying, the bead regions would spread out resulting in a flat fibers with thicker regions (**Figure 3a**, beaded fibers were drawn manually). Thus, in order to probe topographical details of these individual **S-MFs**, SEM observation of dried “fanning out” fibers were performed (Figure 3, higher magnified images see Figure S5). It can clearly be seen from the dried specimens (Figure 3b and c), that all the primary nanocellulose based filaments consisted of multiple thinner fibrous sub-structures with a diameter of $\leq 1 \mu\text{m}$. Typical diameters of the aligned **S-MFs** are summarized in **Table 2**. Generally, the diameters of aligned **S-MFs** of GT-CNC/CMC fibers were larger than those of GT-CNC/TO-CNF and GT-CNC/DC-CNC. The difference in the diameters might be attributed to the different mechanisms of filament formation, i.e., GT-CNC/CMC fibers was formed by soluble CMC polyelectrolyte with insoluble cationic cellulose nanocrystals while GT-CNC/TO-CNF and GT-CNC/DC-CNC filaments were formed by two oppositely charged cellulose nanoparticles. This process is called as interfacial nanoparticle complexation (INC) here. The possible mechanism of the interfacial nanoparticle complexation of oppositely charged nanocelluloses will be further discussed in following part.

Figure 4 displays the nearly circular cross-sections of different nanocellulose based filaments. Even though **S-MFs** would be bundled together when the fabricated wet fibers were dried. Some outlines of **S-MFs** could be observed in the edge area of fracture filaments (Figure 4b). For the magnified images (Figure 4c), it can be clearly observed that these **S-MFs** were composed of ordered nano-sized fibers. The average diameter of these aligned nanofibers in each specimen varied (Table

2), and was $94\pm 24\text{ nm}$ (GT-CNC/CMC), $14\pm 5\text{ nm}$ (GT-CNC/TO-CNF) and $20\pm 8\text{ nm}$ (GT-CNC/DC-CNC), respectively. Obviously, these nanofibers observed in cross-section area were much smaller than **S-MFs** observed along the filament longitudinal axis (Table 2). It can be inferred that it was the subunit nanofibers (S-NFs) comprised the **S-MFs**. It could be noted that showed larger average diameter than that of individual constituted nanocellulose particles (Table 1). Therefore, we deduced that the S-NFs were formed by combination of two oppositely charged celluloses via interfacial complexation, in which ionic interaction acted as the major driving force. The difference in the diameter of S-NFs in each sample was likely attributed to the different combination mechanism of the two oppositely charged celluloses. The S-NFs in GT-CNC/CMC were formed by coating a sleeve of soluble anionic CMC on the surface of cationic GT-CNC, which is quite similar with the filament formed by chitosan and TO-CNF,^[43] while the S-NFs in GT-CNC/TO-CNF and GT-CNC/DC-CNC were generated by combination of two oppositely charged nanocelluloses via INC method. Smaller S-NF dimension of GT-CNC/TO-CNF than that of GT-CNC/DC-CNC further supported this combination hypothesis as pristine TO-CNF ($5\pm 2\text{ nm}$) had a smaller diameter compared to that of DC-CNC ($8\pm 2\text{ nm}$). Overall, it can be concluded that hierarchical nanocellulose based fibers were successfully fabricated using two oppositely charged cellulosic components by interfacial complexation method. Firstly, ionic self-assembly of cationic GT-CNC with different anionic celluloses resulted in the formation of nano-sized S-NF. Subsequently, S-NFs were bonded together forming **S-MFs**. By packing the **S-MFs** in parallel with each other, cellulosic filaments were finally obtained (Figure 4e). Schematic hierarchical structure of fabricated continuous nanocellulose based filaments is shown in Figure 4e, showing a similar

fibrillar structure with natural cellulose fibers in plant cell (more SEM image of these hierarchical nanocellulose based filaments can be found in Figure S6-8 in supporting information).

The obtained continuous cellulose filaments were characterized by elemental analysis (concentration of each constituents used to fabricate the filament was 0.6 wt%). The nitrogen content in each sample was listed in Table 2. All the charged cellulose samples were regarded as fully charged at their corresponding pH conditions (see pH conditions in experimental section), which had been demonstrated in other literatures.^[55-58] Nitrogen was detected in all cellulosic filaments, which indicated that GT-CNC was successfully incorporated into the cellulosic filament. Besides, the N content of fabricated filament increased with the increase of the charged density of the constituted anionic cellulose (see charge density in Table 1), i.e., more GT-CNC were bound into the cellulosic filaments with higher anionic group content by ionic interaction. GT-CNC content in each nanocellulose based filament was 66.7 (GT-CNC/CMC), 52.1 (GT-CNC/TO-CNF) and 62.5 wt% (GT-CNC/DC-CNC), respectively, which was calculated by dividing the N content in the cellulosic filament by N content in GT-CNC (4.8 wt%). Interestingly, the GT-CNC contents in GT-CNC/TO-CNF and GT-CNC/DC-CNC were quite close to the theoretical values. This result indicates that the charges on the surface of each nanocellulose were almost fully compensated by interfacial nanoparticle complexation. However, GT-CNC content in the drawn GT-CNC/CMC fiber showed a lower value compared to the theoretical GT-CNC content, which means that less GT-CNC was incorporated into the GT-CNC/CMC filament. On one hand, this might be attributed to the different fiber formation mechanism of GT-CNC/soluble CMC compared to the interfacial nanoparticle complexation of two oppositely charged nanocellulose. On the other hand, it is also

related to the optimum ionic association conditions (such as pH and ionic strength) between CMC and GT-CNC, which needs further investigation in future.^[59]

The mechanical properties of fabricated nanocellulose based filaments formed by using 0.8 wt% GT-CNC with 0.3 wt% anionic cellulose were characterized and compared. The Young's modulus, ultimate tensile strength and strain at break of these fibers are listed in **Table 3**. The entire set of measurements for each sample is presented in Figure S11 in supporting information. It was observed that GT-CNC/TO-CNF fibers exhibited the highest values in both Young's modulus (8.4 ± 1.5 GPa) and tensile strength (153 ± 11 MPa), even though the negatively charged group content in the pristine TO-CNF was the lowest. We attributed this phenomenon to the high aspect ratio of TO-CNF. Compared to the tensile strength of IPC fiber (220 MPa) prepared by 0.4 wt% TO-CNF with 1 wt% chitosan via the same method,^[43] GT-CNC/TO-CNF fibers formed by 0.3 wt% TO-CNF with 0.8 wt% GT-CNC here showed a lower ultimate tensile strength (153 ± 11 MPa). The difference in the tensile strength may be attributed to different fabrication conditions (such as concentrations and pH), and charge densities of the components (TO-CNF with 1.8 mmol/g carboxylated group was used in their work). With higher concentrations of the constituted components, an increased tensile strength could be achieved (Figure S12). By comparing of the strains at break, GT-CNC/CMC fibers showed the highest elongation ($9.0 \pm 1.6\%$) while GT-CNC/DC-CNC fibers had the lowest strain ($2.6 \pm 0.5\%$). The difference of strain at break in these samples was probably caused by the lack of crystallinity of CMC compared to the higher-order crystallinity in DC-CNC (see XRD crystalline patterns of CMC and DC-CNC in Figure S13 in supporting information). However, due to the higher anionic group content in CMC,

GT-CNC/CMC fibers had an ultimate tensile strength (115 ± 13 MPa) similar to that of GT-CNC/DC-CNC fibers (121 ± 7 MPa). Overall, it can be concluded that not only the charge density but also the structure of nanocellulose could influence the mechanical properties of the fabricated nanocellulose based filaments. Further investigations about the effects of fabrication conditions (such as drawing rate, concentrations and pH) and charge densities of the constituted components on the mechanical properties will be conducted in future. It is believed that by using suitable pairs of oppositely charged nanocelluloses together with an improved fabrication process, even better mechanical properties of cellulosic filaments can be achieved.

3. Conclusion

In this work, we demonstrated fabrication of bio-based nanocellulose based filaments via a simple interfacial complexation method under aqueous conditions. Three types of nanocellulose based filaments were successfully prepared via interfacial complexation by using cationic cellulose nanocrystals (GT-CNC) with anionic soluble CMC or insoluble negatively charged nanocellulose (i.e., TO-CNF or DC-CNC). All these cellulosic filaments exhibited a hierarchical structure with aligned **sub-micron** fibers (**S-MFs**) along their axes and ionically bonded subunit nanofibers (S-NFs) in their cross-section area. The average diameter of S-NFs in each specimen was 94 ± 24 nm (GT-CNC/CMC), 14 ± 5 nm (GT-CNC/TO-CNF) and 20 ± 8 nm (GT-CNC/DC-CNC), respectively. The S-NFs in GT-CNC/CMC were formed by coating a surrounding sleeve of CMC on the surface of GT-CNC while the S-NFs in GT-CNC/TO-CNF and GT-CNC/DC-CNC were generated by combination of two oppositely charged nanocelluloses via interfacial nanoparticle complexation (INC). Elemental analysis suggested that cationic GT-CNC was successfully incorporated into the

nanocellulose based filament. GT-CNC/TO-CNF fibers exhibited the highest values in both Young's modulus (8.4 ± 1.5 GPa) and tensile strength (153 ± 11 MPa) compared to GT-CNC/CMC and GT-CNC/DC-CNC fibers. It is believed that these formed nanocellulose based filaments have potential applications e.g. in tissue engineering, drug delivery and composite reinforcement. The combination of the cationic cellulose nanocrystals with the other polyanions (e.g. alginate, gellan, DNA, etc.) to form interfacial complexation filaments would be also possible. Moreover, the feasibility of the fiber drawing by INC method using oppositely charged nanocelluloses was further demonstrated here, which provides the possibility to engineer new nanocellulose based filaments from the other types of oppositely charged nanocelluloses pairs. The INC method broadens the scope of IPC fibers fabrication method to insoluble oppositely charged nanoparticles, which might provide a new route to fabricate continuous fibers from the other oppositely charged nanoparticles pairs (e.g. cationic chitin nanocrystals with anionic graphene oxide).

4. Experimental Section

4.1 Materials

Bleached birch (*Betula pendula*) chemical wood pulp obtained in dry sheets was used as a cellulose raw material after disintegration in deionized water. The properties of birch pulp were determined in a previous study.^[60] Sodium carboxymethyl cellulose (CMC, $M_w=25,000$, degree of substitution, $DS=1.2$), 2,2,6,6-tetramethylpiperidiny-1-oxy radical (TEMPO), sodium bromide (NaBr), lithium chloride (LiCl), sodium hypochlorite solution (NaClO, 15 wt%), sodium chlorite (NaClO₂), sodium periodate (NaIO₄) and Girard's reagent T (2-hydrazinyl-2-oxoethyl)-trimethylazanium chloride, GT) were obtained as p.a. grade from Sigma–Aldrich and used without further purification. A solution

of 1 wt% CMC was prepared by adding 1 g CMC in 100 g deionized water and stirring up to complete dissolution (pH=7.1). Deionized water was used in all experiments.

4.2 Preparation of 2,3-Dialdehyde Cellulose (DAC) by Periodate Oxidation

15.0 g of bleached birch pulp was torn by hand into 1 cm × 1 cm pieces and stirred in 1500 ml deionized water in a beaker overnight. Then, 12.3 g of NaIO₄ and 27 g of LiCl were added into the suspension and reacted at 65°C for 3 h. After reaction, oxidized cellulose pulp was washed by filtration using deionized water for three times and stored at 4 °C for further use. The aldehyde content of DAC was determined to be 3.86 mmol/g based on an oxime reaction between the aldehyde group and NH₂OH·HCl.^[61]

4.3 Preparation of 2,3-Dicarboxylated Cellulose Nanocrystals (DC-CNC)

2,3-dicarboxylated cellulose was produced according to a previously reported method.^[62, 63] In brief, a 6.0 g portion of DAC prepared as described above was dispersed in 133.2 ml of deionized water by mixing for 1 min at 11,000 RPM with an Ultra-Turrax mixer (Germany). 5.24 g of sodium chlorite (2.5 times compared to aldehyde groups, 21.6 mmol) dissolved in 12 wt% aqueous solution of acetic acid was added in the dispersed DAC solution. At the same time, 6.12 g of 30 wt% hydrogen peroxide (54 mmol) was also added dropwise into this oxidation system. The reaction mixture was stirred for 48 h at room temperature. After that, carboxylated cellulose fibers were washed by filtration until the conductivity of the filtrate was below 20 μS cm⁻¹. After adjusting the pH to 8 using NaOH, the modified cellulose material (solid content of 0.7 wt%) suspension was passed through a double-chamber system (400 and 200 μm) of a microfluidizer (Microfluidics

M-110EH-30, USA) at 1000 bar pressure for two times to obtain anionic 2,3-dicarboxylated nanocrystals (DC-CNC). The concentration of obtained DC-CNC was 0.65 wt% (pH=7.1).

4.4 Preparation of Cationic Cellulose Nanocrystals (GT-CNC) Using Girard's Reagent T

Cationic cellulose nanocrystals were prepared based on our previously reported method with some modifications.^[56, 64, 65] 24.32 g of Girard's reagent T was firstly dissolved in 600 ml of deionized water in a beaker (The GT/aldehyde molar ratio was 7.8). The pH of suspension was adjusted to 4.5 with dilute HCl, 6.0 g of non-dried DAC was added and the reaction was continued under stirring for 72 h at room temperature.^[65] The reaction was stopped by removing the reactive chemicals from the solution by filtration and washing with water until the conductivity of the filtrate was below 20 $\mu\text{S cm}^{-1}$. The cationized cellulose was individualized to nanocrystals using a microfluidizer similarly with DC-CNC. The concentration of obtained GT-CNC was 0.82 wt% (pH=5 was adjusted to 5 using dilute HCl).

4.5 Preparation of TEMPO-oxidized Cellulose Nanofibers (TO-CNF)

TO-CNF was prepared according to a previously reported method.^[5, 66] 1 g of bleached birch pulp was suspended in water (100 mL) containing TEMPO (0.016 g, 0.1 mmol) and sodium bromide (0.1 g, 1 mmol). 7.0 mmol of NaClO solution was added drop wise to the cellulose suspension, and the mixture was stirred at room temperature and kept at pH 10 for 5 h using dilute NaOH. The oxidation was stopped by adding 10 mL of ethanol, and oxidized fibers were completely washed by filtration with deionized water. The oxidized cellulose fiber suspension with a solid content of 0.6 wt% (pH=7.0) was sonicated for 30 min using an ultrasonic homogenizer (output power of 450 W, 6 mm probe tip diameter) to obtain TEMPO-oxidized cellulose nanofibers (TO-CNF).

4.6 Preparations of Nanocellulose based Filaments from CMC and Charged nanocelluloses

The filaments drawing was performed either manually by a previously reported method or by using a universal testing machine (Zwick D0724587, Switzerland). One droplet (100 μ L) of GT-CNC suspension was placed on a polystyrene Petri dish and another droplet of 100 μ L anionic cellulose solution (CMC, TO-CNF or DC-CNC) was pipetted next to it without the two droplets coming into contact. Subsequently, a pair of tweezers was plunged into the two droplets to make them into contact and then continuous filaments were drawn from the formed interface manually or mechanically (universal testing machine). For mechanical drawing, the plunged tweezer was clamped to the universal testing machine and dragged upwards with a constant rate of 40 mm/min until the supply of cellulose suspension was exhausted, which resulted in the formation of a continuous, homogeneous filaments. Finally, the nanocellulose based filaments were wrapped on the glass rod and dried at room temperature.

4.7. Characterization

4.7.1 *Measurement of Carboxyl Content*

The carboxyl contents of the DC-CNC, TO-CNF and CMC samples were determined by the electric conductivity titration method using a protocol described by Rattaz et al.^[67]

4.7.2 *Degree of Polymerization (DP_v) of Nanocellulose*

The degree of polymerization was estimated using the limiting viscosity. Freeze-dried nanocellulose sample (0.15-0.2 g) was dissolved in 50 mL of 0.5 M cupriethylene diamine (CED) and their intrinsic viscosities were determined using a capillary viscometer. Viscosity average DP_v value was calculated according to a previously reported equation:^[68]

$$(\text{DPv}^{0.905} = 0.75[\eta]) \quad (1)$$

4.7.3 Elemental Analysis

Both the cationic group content of GT-CNC and the composition of the nanocellulose based filaments were calculated directly from the nitrogen content of the product as determined by an elemental analyzer (CHNS/O 2400 Series II, PerkinElmer, USA).

4.7.4 Optical Microscopy

A Zeiss LSM 710 NLO confocal microscope was used to examine the formed wet or dried drawn fibers. Optical microscopy images were taken using optical microscope (Leica MZ6) equipped with a camera (Leica DFC420, Germany).

4.7.5 Field Emission Scanning Electron Microscopy

SEM images of the formed nanocellulose based filaments were obtained using a field emission scanning electron microscopy (FESEM, Zeiss Sigma HD VP, Oberkochen, Germany) at 0.5 kV acceleration voltage. Fractured cross-sections of the fibers were obtained from samples frozen in liquid nitrogen. All samples were sputtered with platinum before observing.

4.7.6 Characterization of Mechanical Properties

The mechanical properties of the drawn fibers were studied using a universal testing machine (Zwick D0724587, Switzerland) equipped with a 200 N load cell. The fabricated cellulose filaments were glued on paper frames before being tested to avoid fiber slippage, and were then mounted on the clamps. All the samples were tested at a strain rate of 5 mm/min at room temperature and a gauge length of 20 mm using a pre-force of 0.01 N until breakage. Five replicates for each material

were tested and all samples were placed at a relative humidity of 50% at a temperature of 22°C for at least one day prior to the testing. The diameters of drawn fibers were measured with an optical microscope (Leica MZ6 equipped with a Leica DFC420 camera) by assuming the cross-section to be circular. The average values and standard deviations of the diameter, tensile strength, strain and Young's modulus are reported.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure 1. (a) Drawing process of nanocellulose based filaments by IC method (The blue droplet represents the cationic cellulose constituent and the red one is anionic cellulose constituent; the green arrows indicate the direction of drawing motion); (b-g) photographs of manual or mechanical drawing process of the nanocellulose based filament; (h) an example of manually drawn wet nanocellulose based filament.

Figure 2. Optical microscopy images (a) and scanning electron micrographs (b-d) of different dried nanocellulose based filaments (concentration of each constituent suspension was 0.6 wt%): (b) knotted filaments; (d) high magnified filament surfaces.

Figure 3. The optical microscopy images (a) of the nascent wet beaded fibers using a microscope equipped with a beam splitter mirror and scanning electron micrographs (b, c) of dried beaded cellulosic fibers fabricated by different cellulose constituents.

Figure 4. SEM images (a-d) of cross-sectional fractures of nanocellulose based filaments prepared by GT-CNC with different anionic cellulose constituents at different magnification (concentration of each constituents suspension was 0.6 wt%) and (e) schematic hierarchical structure of fabricated nanocellulose based filament.

Table 1. Characteristics of different charged cellulose samples.

Sample	Length × width [nm] ^{a)}	Charge	Charge density [mmol/g]	DP _v
GT-CNC	100±60 × 10±4	Positive	1.1	53
CMC ^{b)}	–	Negative	3.9	97
TO-CNF	172-958 × 5±2	Negative	1.0	176
DC-CNC	133±65 × 8±2	Negative	1.8	60

^{a)} (The length and width of nanocelluloses were measured from TEM images of >100 individual nanoparticles); ^{b)} (The DP value of CMC were calculated from the Mw provided by Sigma–Aldrich)

Table 2. Average diameters of individual sub-micron fibers and their subunit nanofibers, and GT-CNC content in each drawn nanocellulose based filament.

Sample name	Average diameter of individual S-MFs [nm]	Average diameter of the S-NFs [nm]	Nitrogen (N) content [wt%]	GT-CNC content [wt%]	GT-CNC content in theory [wt%] ^{a)}
GT-CNC/CMC	360-1000	94±24	3.2	66.7	78.0
GT-CNC/TO-CNF	120-700	14±5	2.5	52.1	47.6
GT-CNC/DC-CNC	150-450	20±8	3.0	62.5	62.1

^{a)} (The value was calculated by dividing the theoretical absorbed GT-CNC weight by the theoretical formed nanocellulose based filament weight in total based on the charge ratio of cationic group and anionic group content in each constituted pairs, for example, 1.1 g 1.8 mmol/g DC-CNC could assembled with 1.8 g 1.1 mmol/g GT-CNC)

Table 3. Mechanical properties of the fabricated nanocellulose based filaments.

Sample	Diameter [μm]	Ultimate tensile strength [MPa]	Strain at break [%]	Young's modulus [GPa]
GT-CNC/CMC	37 $\pm$ 8	115 $\pm$ 13	8.9 $\pm$ 1.8	5.3 $\pm$ 1.3
GT-CNC/TO-CNF	25 $\pm$ 5	153 $\pm$ 11	4.9 $\pm$ 1.4	8.4 $\pm$ 2.0
GT-CNC/DC-CNC	29 $\pm$ 6	121 $\pm$ 7	2.6 $\pm$ 0.5	7.5 $\pm$ 0.6

Table of contents

Nanocellulose based filaments are fabricated by interfacial complexation using cationic cellulose nanocrystals with soluble anionic celluloses or insoluble negatively charged nanocellulose under aqueous conditions. The fabricated continuous nanocellulose based filaments display a hierarchical structure similar to the natural cellulose fibers in plant cells, which may have potential applications e.g. in tissue engineering, drug delivery and composite reinforcement.

Keyword cationic nanocellulose, TEMPO-oxidized nanofiber, filament, interfacial complexation

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Hierarchical Assembly of Nanocellulose based Filaments by Interfacial Complexation