Review

Development of completely dispersed cellulose nanofibers

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(Communicated by Kenji MORI, M.J.A.)

Abstract: Plant cellulose fibers of width and length ~0.03 mm and ~3 mm, respectively, can be completely converted to individual cellulose nanofibers of width and length ~3 nm and ~1 µm, respectively, by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation under aqueous conditions and subsequent gentle mechanical disintegration of the oxidized cellulose in water. The obtained TEMPO-oxidized cellulose nanofibers (TOCNs) are new bio-based, crystalline nanomaterials with applications in the high-tech and commodity product industries. Sodium carboxylate groups, which are densely, regularly, and position-selectively present on the crystalline TOCN surfaces, can be efficiently ion-exchanged with other metal and alkylammonium carboxylate groups in water to control the biodegradable/stable and hydrophilic/hydrophobic properties of the TOCNs. TOCNs are therefore promising nanomaterials that can be prepared from the abundant wood biomass resources present in Japan. Increased production and use of TOCNs would stimulate a new material stream from forestry to industries, helping to establish a sustainable society based on wood biomass resources.

Keywords: cellulose nanofiber, TEMPO-mediated oxidation, cellulose microfibril, bio-based nanomaterials, surface modification, sustainable society

Introduction

Vascular plants, including trees, have hollow fiber tissues, and those fibers tightly adhere to one another with hydrophobic and nearly non-biodegradable lignin. Plant cell walls consist of crystalline cellulose microfibrils of width ~3 nm, each of which consists of 20–40 fully extended cellulose chains. These cellulose microfibrils behave like "reinforcing steel" in plant cell walls and are surrounded by non-crystalline polysaccharides, i.e., hemicelluloses, and non-crystalline and hydrophobic lignin. These three main components, i.e., cellulose, hemicelluloses, and lignin, form natural composites in plant cell walls at the molecular and nanosized levels. This contributes to tough, water-resistant, stable, durable, and anti-microbial properties, resulting in long-term resistance of trees to weather, gravity, rain, and biological attack. Cellulose microfibrils are therefore bio-based nanofibers that are abundantly present on earth and are biosynthesized in large quantities annually through photosynthesis by plants.1–3

Developments in pulping and bleaching technologies have enabled the isolation and purification of plant cellulosics. Energy- and cost-effective, and environmentally friendly processes for pulping and bleaching have been established for obtaining pulps or wood cellulosics for producing materials such as paper and board, viscose rayon fibers, and various cellulose derivatives. The Kraft or sulfate pulping process, which is the main technique used globally in pulping mills, enables simultaneous production of purified wood cellulosics and biomass energy. Biomass energy is produced by electric power generation systems through the reductive incineration of lignin-rich pulping effluent after condensation. It is also possible to recycle and reuse pulping chemicals containing sodium and sulfur elements by using pulping effluent recycling systems. However, annual paper consumption has been decreasing, particularly in developed countries, because populations are decreasing, and the use of printing/wiring paper including news print, which were major paper products in the 20th century, is decreasing because...
of the increasing use of various electronic media. New applications of paper pulp or wood cellulosics are therefore being investigated globally.

Cellulose nanofiber production has been regarded as a promising new use for wood cellulosics since the beginning of the 21st century.\textsuperscript{4–9} Wood cellulose fibers prepared by isolation and purification (or pulping and bleaching) of wood chips are 0.02–0.04 mm and 1–3 mm in width and length, respectively, and consist of numerous cellulose microfibrils. Cellulose nanofibers are defined as those of width <100 nm and originate from cellulose microfibrils or their bundles. The preparation of cellulose nanofibers from wood and other plant cellulosics, their characterization, and applications have been intensively investigated in this decade through industry-government-academia collaborations.

The high consumption of fossil resources for energy and materials in the 20th century has caused environmental problems, global warming, and climate change as a result of increases in atmospheric CO\textsubscript{2} concentrations in this and previous centuries. The establishment of a sustainable and resource-circulating society based on renewable biomass resources for energy and materials, partly in place of fossil resources, is an urgent issue for humanity. Cellulose nanofibers have potential applications in new bio-based nanomaterials. If a material stream can be created from unutilized wood biomass resources in domestic forests to CO\textsubscript{2}-accumulated cellulose nanofibers in new materials, together with continuous tree planting and cultivation, Japan could be a leading and model country in contributing to the reduction of atmospheric CO\textsubscript{2} and the establishment of a sustainable society.

Recent developments in nanocellulose science and technology

Cellulose molecules have hierarchical structures in plant cell walls; crystalline cellulose microfibrils are the second smallest elements formed from cellulose molecules (Fig. 1).\textsuperscript{6,9} In the commercial production of aqueous slurries of microfibrillated cellulose (MFC), which was developed in the 1980s, wood cellulose fibers with high cellulose contents are suspended in water and repeatedly disintegrated by a high-pressure homogenizer. MFC is a mixture of both nano- and micron-sized fibers. However, MFC production requires high energy consumption because the cellulose microfibrils in wood cellulose
fibers are tightly bound to one another through numerous hydrogen bonds. Applications of MFC have therefore been limited.

In this century, some breakthrough techniques have been developed in nanocellulose fields. New machines, such as grinder-type homogenizers, high-pressure homogenizers, and aqueous counter-collision apparatuses, have been developed to improve the efficiency of nanofibrillation of wood cellulose fibers and reduce energy consumption. Various pretreatments of wood cellulose fibers, e.g., mild chemical and endo-type cellulase treatments, have also been proposed for improving nanofibrillation efficiency and reducing energy consumption. Position-selective chemical pretreatments of hydroxy groups on cellulose microfibril surfaces have been investigated, e.g., carboxymethylation, acetylation, 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation (details of which are described later), periodate oxidation/post NaClO₂-oxidation, phosphorylation, and introduction of cationic groups through etherification. These diverse pretreatments of wood cellulose fibers provide various nanocelluloses that differ in properties such as morphology, nanostructure, size, surface chemical structure, and reactivity.⁹) Nanotechnology, new nanomaterials (such as carbon nanotubes, fullerenes, graphenes, metal nanowires, inorganic nanoparticles, and nanocatalysts), and related analytical techniques have developed rapidly since the 1990s, and this has led to progress in nanocellulose science and technology. Because nanocelluloses prepared from abundant biomass resources and inexpensive wood celluloses (e.g., the price of commercial bleached kraft pulp is ~60 Japanese yen/dry kg) are available, scientists and engineers in various academic and industrial fields have investigated the development of more detailed and efficient procedures for the preparation and characterizations of nanocelluloses. The nanocellulose bulk materials (i.e., nanocellulose films, foams, aerogels, and hydrogels) and nanocellulose-containing composite materials with various organic and inorganic materials have been investigated for nanocellulose applications in various material fields.⁴) As a consequence, diverse nanocellulose-containing composite materials with unexpectedly excellent properties have been developed, e.g., light-weight, strong materials, transparent and thermally stable films, oxygen-barrier films, thermal insulation aerogels/foams, and recyclable catalyst supports.

**TEMPO-mediated oxidation of cellulose and other polysaccharides**

Cellulose is a homogeneous, linear polysaccharide consisting of D-glucosyl units linked through β-(1→4) glucoside bonds. Although each glucosyl unit in cellulose has three hydroxy groups, which are convertible to various groups such as esters and ethers, crystalline native celluloses are stable and intrinsically unsuitable for chemical reactions or dissolution/ regeneration in terms of environmental aspects and reaction efficiencies. The preparation of cellulose derivatives with high degrees of substitution generally requires large amounts of organic solvents, reagents, promoters, high temperatures, and long reaction times. Washing and purification processes that need large amounts of water and alcohol are also used to isolate the reaction products, resulting in the formation of a large amount of washing effluents containing by-products, solvents, and other chemicals, which need to be treated.¹⁰)–¹³)

Breakthrough chemical or biological techniques for efficient conversion of native celluloses to modified celluloses therefore need to be developed. In living bodies, numerous catalytic or enzymatic reactions always proceed at room temperature under aqueous and atmospheric conditions to selectively produce specific compounds and the energy necessary for life. Such enzymatic reactions or enzyme-mimicking reactions are ideal for efficient and environmentally friendly modifications of cellulose.

In 1995, de Nooy et al. in the Netherlands first reported TEMPO-mediated oxidation of watersoluble starch, amylopectin, and pullulan, and achieved position-selective oxidation of C₆-primary hydroxy groups to sodium C₆-carboxylate groups with a TEMPO/NaBr/NaClO system in water at pH 10.¹⁴)–¹⁶) TEMPO is a water-soluble and stable nitroxy radical, and commercially available for laboratory and industrial use. The reaction mechanism of TEMPO-mediated oxidation is shown in Fig. 2 with the glucosyl unit of cellulose as the substrate. This oxidation proceeds position-selectively at C₆-OH groups with catalytic amounts of TEMPO and NaBr at room temperature under aqueous and atmospheric conditions, because of steric hindrance caused by the four methyl groups attached to one TEMPO molecule. Only inexpensive NaClO, used as the primary oxidant, is consumed during the oxidation, and forms NaCl. A small amount of NaOH is consumed for neutralization of C₆-carboxy groups formed during the oxidation to maintain the reaction
mixture at pH 10. This TEMPO-mediated oxidation is complete within 2 h (Fig. 3).\(^6\)

In 1996, we started to study the scientific aspects of TEMPO-mediated oxidation of cellulose, chitin, and other water-insoluble polysaccharides to prepare new chemically modified polysaccharide derivatives.\(^{17-20}\) In particular, we anticipated the production of new water-soluble polysaccharides from water-insoluble cellulose and chitin by TEMPO-mediated oxidation because of the formation of abundant hydrophilic sodium C6-carboxylate groups in the oxidized polysaccharides. However, the results showed that none of the native celluloses, such as wood celluloses, microcrystalline cellulose powders, cotton lint and linters, and bacterial, tunicate, and algal celluloses, with the native cellulose I crystal structure became water soluble after TEMPO-mediated oxidation, even under harsh conditions, but maintained their original water-insoluble and fibrous morphologies.\(^{19,20}\)

In contrast, when regenerated celluloses, such as viscose rayon and cuprammonium rayon fibers, and ball-milled decrystallized native celluloses were used as starting materials, the water-insoluble celluloses became water-soluble through almost complete conversion of C6-OH groups to sodium C6-carboxylate groups by TEMPO-mediated oxidation (Fig. 4).\(^{17,20}\) The regenerated celluloses have the cellulose II crystal structure, with low crystallinities. Almost quantitative preparation of new water-soluble sodium \(\beta\)-(1→4)-polyglucuronates from these celluloses was achieved. Water-insoluble chitin, curdlan, paramylon, and starches also became water soluble through complete oxidation of C6-OH groups to sodium C6-carboxylate groups via this oxidation. We then investigated the properties of these new water-soluble sodium polyuronates.

Most of these artificially prepared sodium polyuronates are biodegradable and can be metabolized to CO\(_2\) and water by some microorganisms in natural environments.\(^{21,22}\) We isolated and identified a lyase-type degradation enzyme for sodium \(\beta\)-(1→4)-polyglucuronates from microorganisms. The filamentous fungus *Trichoderma reesei* was found to produce a similar lyase when it was grown on \(\beta\)-(1→4)-polyglucuronate as the sole carbon source. The cDNA encoding enzyme was cloned, and the recombinant enzyme was heterologously expressed in *Pichia pastoris*. The amino acid sequence showed no similarity to the amino acid sequences of previously described functional proteins. The crystal structure of the lyase was established; it showed that a calcium
ion was bound to a site far from the cleft and appeared to contribute to the stability (Fig. 5).23)–25)

These results indicate that, in the case of cellulose, a new biodegradation route other than the well-known cellulases or enzymatic hydrolysis processes is present in nature. Some of the C6-OH groups of water-insoluble cellulose molecules are initially oxidized to C6-carboxy groups by some enzymes, and this makes the cellulose more hydrophilic. The lyase-type enzymes present in fungi and microorganisms degrade the C6-carboxy-group-containing molecules to CO2 and water. However, this hypothesis is speculative at present because no enzyme for oxidation of C6-OH groups of cellulose to C6-carboxy groups has yet been found.

Although some interesting new water-soluble sodium polyuronates have been prepared from water-insoluble polysaccharides by TEMPO-mediated oxidation and related scientific data have been accumulated, these new polyuronates have not been used as functional polysaccharides in food, materials, or medical products at the industrial or commercial level to date. In 2002, we restarted TEMPO-mediated oxidation of native celluloses. There was little interest in this research at the time because no water-soluble oxidized celluloses were obtained from native celluloses by TEMPO-mediated oxidation under any conditions.19),20)

**Preparation of TEMPO-oxidized cellulose nanofibers (TOCNs)**

A typical procedure for the preparation of fibrous TEMPO-oxidized celluloses (TOCs) from wood cellulose is as follows. Papermaking-grade softwood bleached kraft pulp (SBKP, average fiber length and width ~3 mm and 0.03 mm, respectively)
is used as the wood cellulose. The SBKP is suspended in water containing catalytic amounts of TEMPO and NaBr. A solution containing NaClO at 3.8–10 mmol/g-SBKP is added to the slurry at room temperature and pH 10 to start the TEMPO-mediated oxidation, with continuous addition of 0.5 M NaOH to maintain the mixture at pH 10. After stirring the mixture for approximately 1.5 h, no NaOH consumption is observed. This point is regarded as completion of oxidation. Because TEMPO-oxidized SBKP has the original fibrous morphology without any strong swelling, it is smoothly separated from TEMPO, salts, and other compounds present in the oxidation mixture by filtration with water, without clogging. Purified TEMPO-oxidized cellulose fibers with sodium counterions (TOC-COONa) are obtained in yields of ~90%. The SBKP consists of ~90% cellulose and ~10% hemicelluloses; most of the hemicelluloses are degraded to water-soluble compounds during oxidation and removed from the fibrous TOC-COONa by filtration with water (Fig. 6).6),20),26)–28)

The sodium carboxylate contents of TOCs prepared under various conditions are determined by conductivity titration.20),28) The carboxy content increases from ~0.01 mmol/g for the original SBKP up to ~1.7 mmol/g with increasing the amount of NaClO added in the oxidation (Fig. 7); the carboxy content increases approximately 170-fold compared with that of the original SBKP. However, the original cellulose I crystal structure, crystallinity, and crystal size are unchanged by oxidation. This indicates that oxidation occurs at C6-OH groups exposed on the crystalline cellulose microfibril surfaces (Fig. 8).6)

Based on this hypothesis, an attempt was made to prepare glucose/glucuronic acid alternating copolymers linked with β-(1→4)-glycoside bonds by surface-peeling of TEMPO-oxidized crystalline native celluloses with a 20% NaOH aqueous solution. Because cellulose molecules formed by surface peaking are insoluble in water, only the glucose/glucuronic acid alternating copolysaccharide molecules should be obtained as a water-soluble fraction from the 20% aqueous NaOH extract after neutralization with acid (Fig. 9A). The TEMPO/NaBr/NaClO oxidation was performed to highly crystalline tunicate cellulose and SBKP in water at pH 10. When all the C6 primary hydroxy groups in cellulose molecules are oxidized to sodium carboxylate groups, the oxidized products, i.e., \( \beta-(1\rightarrow4)-D\text{-polyglucuronate Na salt} \) (Fig. 4), should have a carboxylate content of 5.05 mmol/g. Because the carboxylate contents of the water-soluble products obtained from TEMPO-oxidized SBKP and tunicate cellulose by surface peeling were 2.7 and 2.6 mmol/g, respectively, these values were approximately half that of cellouronic acid Na salt; these water-soluble products had glucosyl and sodium glucuronosyl residues at a molar ratio of approximately 1:1.

The \( ^{13}C \) NMR spectrum of the water-soluble product prepared from TEMPO-oxidized tunicate cellulose by surface peeling and the corresponding signal assignments is shown in Fig. 9B. These assignments were achieved from \( ^{1}H \) and \( ^{13}C \) NMR,
double quantum filter-correlation spectroscopy, hetero-nuclear single quantum coherence, and constant time hetero-nuclear multiple-bond connectivity (CT-HMBC) spectra of the product. The $^{13}$C signal intensities due to $C_4$ and $C_{4a}$ are quite similar to each other, which also indicates that the obtained water-soluble product consists of glucosyl and sodium glucuronosyl residues alone with a molar ratio of approximately 1:1 linked with O-(1→4)-glycoside bonds. The question is whether these two different sugar residues form a regularly alternating, a block, or a random copolysaccharide structure. The CT-HMBC spectrum revealed that the $C_{4a}$ signal of the sodium glucuronosyl residue had no correlation with $H_{1a}$ but had a clear correlation with $H1$ of the glucosyl residue. These long-range interactions clearly showed that the residue adjacent to the glucosyl residue was always the sodium glucuronosyl residue. It was therefore concluded that the glucose/
glucuronic acid alternating co-polysaccharide linked with \( \beta-(1\rightarrow4) \)-glycoside bonds can be prepared by surface peeling of the TEMPO-oxidized tunicate cellulose microfibrils with 20% aqueous NaOH, according to the scheme in Fig. 9A. Similar results were obtained for water-soluble products prepared from TEMPO-oxidized SBKP by surface peeling.

These results support that TEMPO-mediated oxidation of native celluloses with the cellulose I crystal structure is significantly position-selective and specific, forming sodium C6-carboxylate groups densely, regularly, and position-selectively at every one of two glucosyl units along cellulose chains present on crystalline cellulose microfibril surfaces (Fig. 8).

When fibrous TOC-COO\( \text{Na} \) with sodium carboxylate contents \( >1 \text{ mmol/g} \) are suspended in water and mild mechanical disintegration treatment is applied to the TOC-COO\( \text{Na} \)/water slurries (using a domestic blender or ultrasonic homogenizer), highly viscous and transparent gels are obtained. The gel is diluted with water, dried on a transmission electron microscopy (TEM) grid, and observed by TEM after staining. The TEM images show completely individual TEMPO-oxidized cellulose nanofibers (TOCNs) with homogeneous widths of \( \sim 3 \text{ nm} \) and lengths
Atomic force microscopy (AFM) images show similar structures. TEMPO-mediated oxidation of wood cellulose and subsequent mild mechanical disintegration of the fibrous TOC-COONa in water therefore enables complete separation of wood cellulose fibers into individual cellulose microfibrils of width ~3 nm; these nanofibers are the smallest crystalline elements in cellulose fibers (Fig. 1).6)

TOCNs are different from other reported nanocelluloses. They have the smallest and homogeneous widths, and consist of completely individual nanofibers, without formation of any network structures. They have abundant sodium C6-carboxylate groups on the nanofiber surfaces,6) and these groups are ion-exchangeable with other metal and alkylammonium carboxylate groups. The sodium C6-carboxylate groups are densely formed on the crystalline wood cellulose microfibril surfaces by TEMPO-mediated oxidation. Osmotic effects and electrostatic repulsions between the cellulose microfibril result in complete individualization to give long TOCNs (because of less damage to the TOCNs during mechanical disintegration), with lower energy consumption during gentle mechanical disintegration of TOC-COONa in water. The TOCN widths are sufficiently smaller than the wavelengths of visible light for TOCN/water dispersions to be transparent, but they show birefringence when observed between cross-polarizers (Fig. 10).

**Preparation and characterization of TOCNs from various native cellulosess**

TEMPO-mediated oxidation has been applied to commercially available paper pulps such as SBKP and hardwood bleached kraft pulp, and high-purity...
celluloses such as cotton lint and linters, bacterial, tunicate, and algal celluloses. The highest carboxylate contents introduced into TEMPO-oxidized products prepared from these celluloses vary, depending on the cellulose I crystal size or the corresponding specific surface area of the sample cellulose microfibrils (Fig. 11), based on the oxidation mechanism of crystalline native cellulose microfibrils (Fig. 8). Accordingly, TEMPO-oxidized celluloses prepared from different cellulose sources are converted to TOCNs with various nanofibril widths and lengths.

Plant cell wall samples consisting of crystalline cellulose microfibrils, hemicelluloses, and lignin are delignified under mild conditions by repeated oxidation with NaClO₂ in water at pH 4–5 and 70 °C. Delignified samples consisting of cellulose and hemicelluloses are called holocelluloses. Plant holocelluloses were prepared from ginkgo (gymnosperm),

Fig. 10. Conversion of wood cellulose fibers to TEMPO-oxidized cellulose nanofibers of widths ~3 nm through TEMPO-mediated oxidation and subsequent gentle mechanical disintegration of oxidized cellulose in water.
Japanese cedar (softwood), birch and eucalyptus (hardwood), and rice straw and kenaf (grass). These holocelluloses were oxidized using the TEMPO/NaBr/NaClO system in water at pH 10 to introduce the maximum amounts of sodium carboxylate groups, i.e., 9.17 mmol/g. Sugar composition analysis showed that all the TOCs prepared from different plant holocelluloses consisted of glucose and oxidized glucoses with only small amounts of mannose and xylose originating from hemicelluloses. Most of the hemicelluloses present in the holocelluloses were therefore degraded to water-soluble compounds during oxidation and removed from the water-insoluble TOCs by washing with water.

TOCNs were prepared from these TOCs by mechanical disintegration in water. The average widths of all these TOCNs were ~3 nm, irrespective of the plant species used as the starting material (Table 1). The crystalline cellulose microfibrils have the same width, regardless of the plant holocellulose source. This is advantageous in industrial applications of TOCNs because any plant biomass resource provides TOCNs of almost the same width when the TOCNs are prepared under appropriate conditions. However, SBKP is the most suitable resource for TOCN preparation in terms of oxidation efficiency and complete nanofibrillation in water with low disintegration energy consumption. SBKP can be produced in domestic pulping mills from softwood chips from abundant and unutilized forest thinnings. It is therefore possible to create a new material stream from domestic softwood forests to industries through TOCNs, and this enables establishment of a sustainable society and reduction of the CO2 concentration in atmospheric air.

Surface modifications of TOCNs through ion-exchange in water

The sodium C6-carboxylate groups densely, regularly, and position-selectively present on crystalline cellulose microfibril surfaces in TOCs and TOCNs can be converted to protonated carboxy groups (TOC-COOH and TOCN-COOH, respectively), and other metal and alkylammonium carboxylate groups through ion-exchange in water (Fig. 12). The biodegradability of fibrous TOC-COOM (M: proton or metal ion) and TOCN-COOM films can be controlled on the basis of the counterions. Cast-dried TOCN-COONa films are disrupted in water because of their highly hydrophilic properties. However, when TOCN-COONa films are soaked in aqueous CaCl2, MgCl2, AlCl3, and FeCl3 solutions, followed by washing with water and drying, the obtained TOCN-COOM films have sufficient wet-strengths, depending on the metal counterions (Fig. 13A). Cast-dried TOCN-COONa films have significantly better oxygen-barrier properties or low oxygen permeabilities under dry conditions. However, the oxygen-barrier properties of TOCN-COONa films deteriorate significantly at high relative humidity (RH) conditions because of the hydrophilic properties of TOCN-COONa. When the counterions of TOCN-COONa films are converted to Ca2+ and Al3+, the TOCN-COOM films have good oxygen-barrier properties, even under high RH conditions (Fig. 13B). The hydrophilic properties of TOCN-COONa films can therefore be controlled by simple counterion

Table 1. Average widths (nm) of TOCNs, prepared from various plant holocelluloses, determined from atomic force microscopy height images

| Plant          | Ginkgo | Japanese cedar | Birch | Eucalyptus | Rice straw | Kenaf stalk |
|----------------|--------|----------------|-------|------------|------------|-------------|
| Number-average width | 3.12 ± 0.38 | 3.10 ± 0.39 | 3.11 ± 0.42 | 3.11 ± 0.36 | 3.08 ± 0.32 | 3.13 ± 0.30 |
| Width-average width  | 3.16   | 3.14           | 3.16  | 3.15       | 3.11       | 3.16        |

![Graph showing relationship between cellulose I crystal sizes or cellulose microfibril widths of various native celluloses](image-url)
exchange of sodium C6-carboxylate groups with other metal ions. TOCN-COOG and TOCN-(COO)₂Cu films are good deodorants for H₂S and CH₃SH gases, which cause serious odor problems in our daily lives.³⁵)

Almost complete ion-exchange from fibrous TOC-COONa to TOC-COO⁻/+NR₄ is possible by neutralization of protonated TOCs (TOC-COOH) with quaternary alkylammonium hydroxides (R₄N⁺/OH⁻) in water. These counterion exchanges proceed stoichiometrically at a 1:1 molar ratio (Fig. 12).³⁶) The obtained TOC-COO⁻/⁺NR₄ can be converted to transparent and highly viscous TOC-COO⁻/⁺NR₄ dispersions by mechanical disintegration in water or an organic solvent. Cast-dried TOC-COO⁻/⁺NR₄ films are transparent, flexible, and hydrophobic, depending on the length of the introduced alkyl chain.³⁷) The biodegradable/stable and hydrophilic/hydrophobic properties of TOCs and TOCNs can therefore be controlled by simple counterion exchange in water. This enables advantageous surface modifications for applications of TOCs and TOCNs in various material fields.

Properties of TOCN bulk and composite materials

Because TOCNs have sodium C6-carboxylate groups densely positioned on crystalline cellulose microfibril surfaces, the TOCN elements form self-assembled, nematic-ordered structures in water or organic solvents through efficient electric repulsion between TOCN elements (without application of external forces or energy). This self-assembly of TOCNs in water or organic solvents leads to the formation of various TOCN bulk materials with different properties and functions (Fig. 14). When TOCN/water dispersions are cast and dried on a plate, transparent, flexible TOCN films with high mechanical strengths, high oxygen-barriers, and low coefficients of thermal expansion are obtained.³⁸) These TOCN films have these properties because nanolayered, highly dense structures are formed from the nematic-ordered TOCN/water dispersions during drying.³⁹) When the sodium carboxylate groups of TOCNs (TOCN-COONa) are protonated with dilute HCl in TOCN/water dispersions, followed by
thorough washing with water, self-standing TOCN-COOH hydrogels with high stiffness are obtained, even at low TOCN concentrations of ~0.1% (i.e., ~99.9% water). These TOCN-COOH hydrogels can be converted to transparent, high-strength, bendable aerogels with good thermal insulation properties by solvent exchange and subsequent critical point drying. When TOCN-COOH/water/tert-butyl alcohol dispersions are freeze-dried, spider-web-like network structures with nanosized pores can be obtained, which can be used as high-performance air filters.

One of the most promising applications of TOCNs is in light-weight, high-strength composite materials with polymer or inorganic matrices because the tensile strengths and Young’s moduli of single TOCN elements are ~3 GPa and ~140 GPa, respectively, despite their low densities of ~1.6 g/cm³. However, TOCNs are highly hydrophilic because of the abundant sodium C6-carboxylate groups on the TOCN surfaces, therefore it is generally difficult to prepare TOCN/polymer composite films in which the hydrophilic TOCN elements are individually dispersed in hydrophobic polymer matrices without agglomeration. The mechanical properties of fiber-reinforced polymer composites are reflected by three factors: Young’s moduli of the incorporated fibers, and their volume fractions and aspect ratios (i.e., length/width ratios). When the TOCN elements are individually dispersed in polymer matrices, with retention of their high aspect ratios, TOCN/polymer composites with high mechanical strengths can be obtained at low TOCN addition levels, i.e., 1–5%. These light-weight, strong TOCN/polymer composites are promising materials for reducing automotive weights and improving the efficiencies of gasoline or electric power consumption to reduce CO₂ emissions.

The preparation and characterization of TOCN/polymer or inorganic filler/TOCN composite films have been investigated in our laboratory. Poly(vinyl alcohol), hydrogenated butadiene-acrylonitrile latex (submitted for publication), poly(acrylamide), carbon nanotubes, and nanoclays were mixed with TOCN-COONa under aqueous conditions. The properties of the obtained composite films were investigated for TOCN contents, distribution of TOCN elements in matrices, and related theoretical calculations. The prepared transparent and flexible composite films have good mechanical and oxygen-barrier properties at TOCN contents of only 1–5% TOCN because the TOCN elements are individually nanodispersed in the matrices without agglomeration, as shown by TEM observations of film cross-sections.

When TOCNs are composited with hydrophobic polymers such as poly(styrene), poly(lactic acid), and cellulose triacetate to prepare TOCN/polymer composite films, the TOCN-COOH counterions are converted to TOCN-COOH, hydrophobic TOCN-COO⁻/⁺NR₃ or TOCN-COO⁻/⁺NH₃⁺ CH₂CH₂(OCH₂CH₂)₉-OCH₃. These counterion-exchanged TOCNs can be nanodispersed in some organic solvents such as N,N-dimethylformamide, N,N-dimethylacetamide, CH₃Cl₂, or toluene, which dissolve various hydrophobic polymers. The counterion-exchanged TOCN/organic solvent dispersions and polymer/organic solvent solutions were mixed at various TOCN/polymer weight ratios, and TOCN/polymer composite films were prepared by
casting and drying. These composite films showed significantly improved mechanical properties at low TOCN contents. These methods for preparation of TOCN/polymer composite films using organic solvents were performed as model experiments to prepare ideal composite films consisting of individually nanodispersed TOCN elements in polymer matrices (Fig. 15).

Versatile and diverse surface modifications of TOCN elements can be achieved through counterion exchanges of sodium C6-carboxylate groups densely present on the TOCN surfaces. However, it is difficult to prepare TOCN/polymer composite films consisting of individually nanodispersed TOCN elements by general melt-molding or melt-extrusion processes. The TOCN elements initially nanodispersed in water form large agglomerates through water removal during thermal molding, resulting in almost no improvement in the mechanical properties of the TOCN/polymer composites. This issue must be resolved to enable industrial-level preparation of high-strength composite materials from TOCNs. Another shortcoming of TOCNs is the decrease in the thermal degradation temperature from ~300 °C for the original cellulose to ~200 °C, which initially occurs through decarboxylation of the abundant sodium carboxylate groups present in TOCNs.55)

Conclusions and future prospects

Research aimed at the development of practical applications of nanocelluloses has been intensively conducted worldwide through industry-government-academia collaborations. This is because nanocelluloses, including TOCNs, are new bio-based nanomaterials that can be produced from abundant biomass resources and have potential applications in various material fields. Since 2013, some of the TOCNs and related materials developed in our laboratory have been commercialized, such as an ink dispersant component for ballpoint pens with
smooth writing performances, developed by Daiichi Kogyo Seiyaku and Mitsubishi Pencil (http://d.material-expo.jp/en/Exhibitors/2523701/DKS-CO-LTD/Products/1107877/RHEOCRYSTA, 2018). In the same period, Nippon Paper Industries and Nippon Paper Crecia commercialized TOCN-containing disposable diapers for adults with super-deodorizing functions endowed by metal carboxylate group-containing TOCNs, which alleviates the burden for care assistants (http://www.nipponpapergroup.com/english/news/year/2015/news150421003061.html, 2018). The world’s largest plant for TOCN production with a capacity of 500 t/y was installed in the Ishinomaki Mill of Nippon Paper Industries, Miyagi, Japan, and started TOCN production in 2017 (Fig. 16) (http://www.nipponpapergroup.com/english/news/year/2017/news170425003763.html, 2018). Two additional plants produce TOCNs in Finland and the United States (https://www.fpl.fs.fed.us/research/facilities/nanocellulose.pilot-plant.php; http://betulium.com/, 2018).

Numerous fundamental and applied studies of TOCNs have been performed worldwide in both academia and industry to enable commercialization of TOCN-containing materials as soon as possible. A sustainable society based on renewable wood biomass

Fig. 15. Preparation of representative TOCN-containing composite materials with or without surface modification of TOCNs before compositing.
resources is expected to be established through the use of TOCNs in high-tech and commodity materials (Fig. 17). However, only ten years have passed since TOCNs were developed as new bio-based nanomaterials through fundamental research on TEMPO-mediated oxidation of polysaccharides in our laboratory. We therefore need to accumulate more fundamental, experimental, theoretical, and analytical data to improve the reliability of TOCNs in practical applications at the industrial level. The establishment of methods for determining the length/length distributions of TOCNs is an example of the fundamental research topics that need to be tackled in the near future.

Acknowledgements

I would like to express my sincere thanks to Dr. Kenji Mori, Professor Emeritus at The University of Tokyo and Member of the Japan Academy, for giving me the opportunity to contribute this review to the Proceedings of the Japan Academy, Series B, and for many suggestions for improving this manuscript. I would like to thank my colleagues at The University of Tokyo, and all of the present and past collabo-

Fig. 17. Concept of material stream from forestry to high-tech material fields through TOCNs prepared from abundant and unutilized softwood biomass resources.
rators and graduate students in Japan and overseas. This work has been primarily supported by the Japan Society for the Promotion of Science (JSPS), New Energy and Industrial Technology Development Organization (NEDO), and Japan Science and Technology Agency (JST), continuously since 1996. I thank Helen McPherson, Ph.D., from the Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

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(Received Jan. 16, 2018; accepted Feb. 14, 2018)
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