Better together: Synergy in nanocellulose blends

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Summary

Cellulose nanopapers have gained significant attention in recent years as large-scale reinforcement for high-loading cellulose nanocomposites, substrates for printed electronics and filter nanopapers for water treatment. The mechanical properties of nanopapers are of fundamental importance for all these applications. Cellulose nanopapers can simply be prepared by filtering a suspension of nanocellulose, followed by heat consolidation. It was already demonstrated that the mechanical properties of cellulose nanopapers can be tailored by the fineness of the fibrils used or by modifying nanocellulose fibrils for instance by polymer adsorption but nanocellulose blends remain underexplored. In this work, we show that the mechanical and physical properties of cellulose nanopapers can be tuned by creating nanopapers from blends of various grades of nanocellulose, i.e. (mechanically refined) bacterial cellulose or cellulose nanofibrils extracted from never-dried bleached softwood pulp by chemical and mechanical pre-treatments. We found that nanopapers made from blends of two or three nanocellulose grades show synergistic effects resulting in improved stiffness, strength, ductility, toughness and physical properties.

1. Introduction

Nanocellulose is a class of biobased materials made of individualised cellulose fibrils with diameters in the nanometer range that possess interesting mechanical [1, 2] and chemical properties [3-5]. To produce nanocellulose (NC), cellulosic biomass, such as wood pulp, is disintegrated using for instance strong ultrasound [6], high-pressure homogenisers/microfluidisers [7] or stone grinders [8] to liberate elementary cellulose microfibrils from the starting material. The nanocellulose produced using this top-down approach is often called microfibrillated cellulose (MFC), nanofibrillated cellulose (NFC) or cellulose nanofibrils (CNF) in the literature. In addition to cellulosic-biomass, nanocellulose can also be produced bottom-up by the fermentation of low-molecular weight carbon sources using cellulose-producing Acetobacter, later renamed to Komagataeaibacter [9]. The cellulose synthesised by cellulose-producing bacteria, more commonly known as microbial cellulose or bacterial cellulose (BC), is secreted as nanocellulose directly. The biosynthesis of BC was described by Brown already in 1886 [10].

While both cellulosic biomass-derived NFC and BC possess similar fibril diameters (< 100 nm), the two types of nanocellulose differ in terms of purity and crystal structure. BC is pure cellulose while NFC is usually a composite consisting of cellulose and hemicellulose [11] and residual lignin. Crystallographically, NFC is cellulose-I dominant and BC is cellulose-I dominant [12]. Single nanocellulose fibrils were shown to possess tensile moduli of between 100 and 160 GPa based on Raman spectroscopy and X-ray diffraction experiments [13-16]. The tensile strength of single nanocellulose fibrils were estimated to be 2-6 GPa [17] based on the critical length of single nanocellulose fibrils, fragmented using high intensity ultrasound. Thus, nanocellulose is often explored as reinforcement for polymers [1, 18-20]. The application of nanocellulose as reinforcement for polymers often requires the nanocellulose to be used in the form of single nanofibrils. In fact, it has been
shown that the reinforcement efficiency can be explored effectively when nanocellulose is impregnated at a single fibre level [1]. However, it is difficult to exploit the properties of single cellulose nanofibrils fully in composites. Thus, in order to produce nanocellulose composites with promising and predictable mechanical properties high loading fractions have to be realised [1]. This can effectively be achieved following a paper based laminated composite route [21-24]. Besides using nanopapers for 2D reinforcement in composites [25, 26], nanocellulose in the form dense fibre networks, e.g. cellulose nanopapers [27], is also an important material for various advanced applications, including filtration membranes [28-30], packaging [31] and substrates for flexible, printed electronics [32]. Thereby, the ability to design [33], tailor and engineer the physical properties of nanopapers is of upmost importance.

The mechanical properties of nanopapers can be controlled by controlling the porosity of nanopapers, which is possible by producing them from dispersion media other than water [34, 35]. Nanopapers produced using water as dispersion medium for nanocellulose fibrils have porosities of around 20% while using tert-butanol allows to produce nanopapers with a porosity of up to 74%. Accordingly, this resulted in nanopapers with low tensile moduli, strength and strain-to-failure of 1.0 GPa, 23.2 MPa and 5.7%, respectively. While these results are exciting, it should be noted that the authors have not suggested any mechanisms as to how changing the dispersion medium affected the porosity of the resulting nanopapers. Gustafsson et al. [36] showed that the porosity of nanopapers depends on the drying conditions used during nanopaper preparation; slower water removal favours smaller pore sizes. During water evaporation the capillary contraction forces pull the nanofibres closer to each other. The duration of these forces acting on the fibrils is dependent on the drying temperature and hence it is responsible for the pore size.

The chemical nature of the nanofibrils will also affect the physical properties of the resulting nanopapers. Benitez et al. [37] showed that the nature of the counter-ion of the carboxylate groups, alkali metal or alkyl ammonium group, strongly influenced the mechanical properties of TEMPO-oxidised CNF. TEMPO-CNCF nanopapers without ion-exchange exhibited a tensile strength of 210 MPa and a Young’s modulus of 10 GPa, respectively. Upon ion-exchange, the smaller the alkali ion-diameters and the longer the alkyl chains, the higher the strength and strain to failure of the resulting TEMPO-oxidised CNF nanopapers. Thus, via the introduction of e.g. Li-ions tensile strengths of up to 300 MPa were achieved with a tensile modulus of up to 18 GPa. The mechanism was explained to be based on introduction of multiple hydrogen bonds.

Besides controlling the porosity of nanopapers and the surface properties of the nanofibrils used to make nanopapers, their mechanical properties can also be tailored improving the alignment of the nanofibrils in the nanopaper in the direction of load [38, 39]. Sehaqui et al. [40] have modified the physical properties of (2,2,6,6-tetramethylpiperidin-1-yl)oxy (TEMPO)-oxidised NFC nanopaper by aligning the nanocellulose fibrils in the TEMPO-oxidised nanocellulose paper using wet stretching. At a draw ratio of 1.6, the resulting tensile modulus and strength of TEMPO-oxidised NFC nanopaper possessed tensile modulus and strength of 33 GPa and 400 MPa, respectively. This represents a 3-fold increase in the tensile stiffness and two-fold increase in strength of the TEMPO-oxidised NFC nanopaper compared to neat and undrawn TEMPO-oxidised CNF, with a measured tensile modulus and strength of 10 GPa and 185 MPa, respectively.

Some authors explored the concept of hybridisation to modify the physical properties of (nano)papers. Xu et al. [41] hybridised CNF and cellulose nanocrysalts (CNC) and investigated this hybridisation effect on the optical properties of (hybridised) cellulose nanopapers. CNC were produced by sulphuric acid hydrolysis of bleached kraft pulp. It was found that the transparency of cellulose nanopapers increased with increasing CNC-to-CNCF ratios. This was attributed to an increase of the surface smoothness, decreased porosity and pore sizes of the resulting hybridised cellulose nanopapers. The CNC effectively filled the space between CNF fibrils in the nanopapers. Similar observations were made by Sehaqui et al. and Gonzalez et al., who studied the effect of hybridisation of (TEMPO-oxidised [42]) CNF [43] and conventional pulp fibres to produce multi-scale papers [44]. Increasing the ratio between CNF and pulp fibres within the hybridised multi-scale papers resulted in reduced porosity, opacity and surface roughness. Furthermore, the tensile properties of these multi-scale papers increased with increasing CNF-to-pulp fibre ratios. These studies show the potential of hybridisation of various cellulose fibres of different fibre diameters to enhance the physical and mechanical properties of (multi-scale) papers. Moreover, these works exploited the use of the smaller nanofibrils as effective nanofillers for the larger nanofibrils or pulp fibres.

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Inspired by the concept of hybridisation of pulp fibres with nanocellulose, we further postulate that hybridising various nanocellulose fibrils with different diameters will have synergistic effects beyond the conventional “void-filling”, if both the dimensions and the surface chemistry of the different nanofibrils are varied. Therefore, in this work, we report the effect of hybridising various types of nanocellulose; namely BC, mechanically refined BC (r-BC) and TEMPO-oxidised CNF (herein termed TEMPO-CNf) on the mechanical performance of the resulting hybridised cellulose nanopapers. It can be anticipated that by changing the porosity, surface smoothness and porosity of the cellulose nanopapers but also the surface interaction between nanofibrils, the mechanical properties of the resulting hybridised cellulose nanopapers can be tailored and engineered for various applications.

2. Materials and Methods

(a) Materials

Bacterial cellulose (BC) was kindly provided by fumib GmbH (Bad Langensalza, Germany) in the form of wet pellicles containing 92 wt.% water. The diameter of these BC nanofibrils was estimated to be 50 nm and fibril lengths of up to several micrometers [35, 45]. Never-dried elemental chlorine free bleached softwood (Picea abies and Pinus spp.) pulp, obtained from Stendal (Berlin, Germany), was used for the production of CNF. The chemical composition of the pulp was: 81.3% cellulose, 12.6% hemicellulose and 0.3% ash [46], (2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO, 98%), NaOCl (10-15% active chlorine), NaBr (99%), NaOH (99.6%) and HCl (37%) were purchased from Sigma-Aldrich.

Preparation of TEMPO-oxidised CNF (TEMPO-CNf)

TEMPO-CNf were produced from softwood pulp adapting the traditional route established by Saito et al. [47, 48] in order to limit the degree of oxidation whilst still allowing to effectively produce nanocellulose. Firstly, never dried softwood pulp (20.2 g dry cellulose content) was blended (Braun Multiquick 5 MX 2050, Kronberg/Taunus, Germany) in 1.5 L of H2O for 5 min to produce a well-dispersed slurry. The slurry was then transferred to a glass beaker and magnetically stirred. To this slurry, NaBr (3.6 g, 35 mmol) and TEMPO (0.38 g, 2.4 mmol) were added and the slurry stirred until TEMPO was fully dissolved (approximately 20 min). 23 g (37 mmol) of 12 wt.-% NaClO solution was added to start the cellulose oxidation. During the reaction, the pH of the slurry was kept between 10 and 10.5 by the addition of 0.1 M NaOH solution, as needed. When the pH of the cellulose slurry was observed to remain constant, the reaction mixture was left to stir for another 20 min before neutralising it with 0.1 M HCl. The TEMPO-oxidised pulp fibres were transferred into a Büchner funnel and flushed with approximately 4 L of deionised water. The washed TEMPO-oxidised pulp fibres were then recovered and diluted to a consistency of 1.5% prior to disintegrating them into TEMPO-CNf by blending (Braun Multiquick 5 MX 2050, Kronberg/Taunus, Germany). The blender was operated at maximum power for 40 min. The TEMPO-CNf were stored at 6°C until further use and analysis.

Preparation of mechanically refined BC (r-BC)

Prior to mechanical refinement of BC, the BC pellicles were cut into small pieces (5-10 mm in length and width) and blended to produce a BC slurry with a consistency of 0.3 wt.-% before passing the BC suspension 20 times through a disc mill (MKCA6-23, Fuchs disc mill Granomat JP 150, Granges-Paccot, Switzerland) to mechanically refine BC. The resulting r-BC in water suspension was then filtered and concentrated to a consistency of 1.5% using gravity filtration onto a filtration cloth.

(b) Preparation of various types of cellulose nanopapers

Nanopapers (50 g m⁻²; 50 µm in thickness) were prepared from BC, r-BC and TEMPO-CNf, as well as mixtures of the three materials. Binary blends (1:1 by weight) of nanofibrils were prepared from BC/r-BC, BC/TEMPO-CNf and r-BC/TEMPO-CNf combinations. A ternary blend of nanofibrils was prepared by blending all three types of nanocellulose in the ratio of 1:1:1 by weight. For the preparation of neat BC nanopapers, the BC pellicles were first cut into small pieces (5-10 mm in length and width) and blended for 2 min at a consistency of 0.2 wt.-% to produce a homogenous BC in water suspension. For the preparation of neat r-BC and neat TEMPO-CNf nanopapers, the prepared r-BC and TEMPO-CNf gels were diluted to 0.2 wt.-% consistency and

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blended for 2 min to create homogeneous r-BC in water and TEMPO-CNf in water suspensions. To prepare binary and ternary mixtures of nanofibrils, the different nanofibrils were blended together for 2 min in deionised water at a consistency of 0.2 wt-% to create homogenous BC/r-BC, BC/TEMPO-CNf, r-BC/TEMPO-CNf as well as BC/r-BC/TEMPO-CNf in water suspensions.

To fabricate (hybridised) cellulose nanopapers, the nanocellulose-in-water suspensions were vacuum-filtered onto a cellulose filter paper (VWR 413, 5-13μm pore size, Lutterworth, UK). The filter cake was then wet-pressed under 100 N-force between blotting papers (3MM Chr VWR, Lutterworth, UK) for 5 min at room temperature to remove excess water. The compressed wet filter cakes (with a moisture content of approximately 85%) were then further consolidated by sandwiching the filter cakes between fresh blotting papers and metal plates, followed by a hot pressing step using 10 kN-force for 1 h at 120°C (type 25-12-2H, Carver Inc., Wabash, USA).

(c) Characterisation of TEMPO-CNf and (hybridised) cellulose nanopapers

**Charge content of nanocelluloses**

The charge content of nanocelluloses was analysed by conductometric titration. A sample of the nanocellulose (0.15 g dry weight) was diluted to 60 mL with deionized water and 5 mL of a 0.01 M NaCl solution was added. The resulting nanocellulose dispersion was then stirred for 15 min. The pH of the dispersion was then adjusted to a value between 2.5 and 3 by the addition of 0.1 M HCl and titrated with 0.04 M NaOH solution at a rate of 0.05 mL min⁻¹ to pH 11. Both the evolution of pH and conductivity of the dispersion were recorded. The carboxylate content of nanocellulose was determined from the conductivity as function of the volume of NaOH (V(NaOH)) curves [47]. Nanocelluloses were also analysed using Fourier Transformation Infrared Spectroscopy (Carry 630, Agilent Technology, Austria) and elemental analysis (EA 1108 CHNS-O, Carlo Erba, Italy).

**Morphology of the (hybridised) cellulose nanopapers**

The morphology of the (hybridised) cellulose nanopapers was characterised using scanning electron microscopy (SEM) (Supra 55VP, Zeiss, Wien, Austria) operated at an accelerating voltage of 2 kV and a working distance of 7.2 mm. The specimens were mounted onto aluminium sample holders using carbon tape and gold coated (Leica SCD 050/EM QSG 100) at 60 mA for 75 s prior to SEM.

**Determination of the surface charge of (hybridised) cellulose nanopapers using ζ-potential**

The electrokinetic behaviour of (hybridised) cellulose nanopapers was determined using ζ-potential measurements using an Electrokinetic Analyzer (SurPASS, Anton Paar, Graz, Austria) equipped with an adjustable gap cell. The gap width of the cell was set to be 100 μm. The ζ-potential was determined from the streaming current measured between pH 2 and 10. For the measurement, the pH of the supporting electrolyte (1 mM KCl) was first adjusted to pH 10 by the addition of 0.05 M KOH aqueous solution and subsequently lowered by addition of 0.05 M HCl solution. The testing solution was pumped through the cell while the pressure drop across the cell steadily increased up to 300 mbar.

**Tensile properties of (hybridised) cellulose nanopapers**

Tensile tests were performed on a minimum of 5 test specimens for each of the prepared (hybridised) cellulose nanopapers at 25 °C and 50% relative humidity using an Instron universal test frame (Model 5969 Dual Column Universal Testing System, Instron, Darmstadt, Germany) equipped with 1 kN load cell. The strain of the test specimens was monitored using a non-contact video extensometer (Gig ProE, iMETRIUM, Bristol, UK). The tests were performed on tensile test specimens punched from the fabricated (hybridised) cellulose nanopapers using a punch die (Zwick ZCP 020 Manual Cutting Press, Zwick, Ulm, Germany). The test specimens were dog bone shaped with an overall length of 30 mm and the narrowest part being 2 mm.
specimens had a thickness of approximately 50 μm. Prior to every test, the thickness of each test specimen was measured at 5 different spots using a digital micrometer (705-1229, RS components, Corby, UK). The gauge length of the test specimen was set to be 15 mm and the crosshead displacement speed used was 1 mm min⁻¹. The Young’s modulus was determined from the linear elastic region of the stress-strain curve as secant between strength values separated by 0.2% strain. The absorbed energy during tensile fracture (J m⁻¹) was determined from the area under the stress-strain curve.

Fracture resistance of the (hybridised) cellulose nanopapers

The critical stress intensity factor (K₁) of the (hybridised) cellulose nanopapers was evaluated from single edge-notched specimens of 25 mm in overall length (L) and 15 mm in width (w). A sharp scalpel blade was used to introduce an initial crack on the edge of the centreline of the test specimen. This initial crack length (a) was maintained between 2.6 and 4.2 mm to ensure that the ratio between the initial crack length and the test specimen width (a/w) remained below 0.29 in order to maximise the efficiency of the function Y (see equation 1) [49]. The single edge-notched nanopaper test specimens were then loaded in uniaxial tension using a micro-tensile tester (Model MT-200, Deben UK Ltd, Woolpit, UK) equipped with 200 N load cell. A crosshead displacement speed of 1 mm min⁻¹ was used. The distance between the grips was set to be 15 mm. K. (see equation 2) of the (hybridised) cellulose nanopapers was calculated from the maximum stress (σmax) when the crack propagates:

\[ Y = 1.99 - 0.41 \frac{a}{w} + 18.7 \left( \frac{a}{w} \right)^2 - 38.48 \left( \frac{a}{w} \right)^3 + 53.85 \left( \frac{a}{w} \right)^4 \]  

(1)

\[ K_{1c} = Y \sigma_{max} a^{0.5} \]  

(2)

Based on K₁, the work of fracture (G.) was calculated (equation 3):

\[ G_{1c} = \frac{K_{1c}^2}{E} \]  

(3)

3. Results and discussion

TEMPO-CNf were synthesised by us from TEMPO-oxidised pulp with a purposely moderate charge content in order to facilitate easy processing but still allow for defibrillation. TEMPO-CNf possessed a charge content of 0.54 mmol g⁻¹, which arises from the carboxylate groups introduced during the TEMPO-oxidation. The successful oxidation of cellulose was further confirmed by FT-IR spectroscopy. An additional absorption band around 1605 cm⁻¹ was observed for TEMPO-CNf. This absorption band corresponds to the stretching of the carbonyl (C=O) functionality of the carboxylate groups [50]. Elemental analysis showed that TEMPO-CNf possessed an oxygen content of 51.56 ± 0.09 wt.-% compared to 50.87 ± 0.16 wt.-% for non-oxidised pulp fibres used as starting material. This further proved the success of the TEMPO-oxidation of pulp fibres to produce TEMPO-CNf. TEM images of TEMPO-CNf revealed a fibril diameter of around 5 nm (see Supplementary material), which is consistent with the fibril diameters of TEMPO-CNf produced by various others [48, 51, 52].

(a) Drainage times of nanocellulose blends

The drainage times of single, binary and ternary nanocellulose suspensions at a suction pressure of 16 hPa were recorded during nanopaper preparation. This was done in order to gain better understanding of the influence of various nanocelluloses on the preparation time of (hybridised) cellulose nanopapers. A dependency of the drainage times on the size of the nanocellulose used can be observed (Table 1). As expected, the biggest cellulose nanofibrils (BC) had a drainage time of only 3 min, slightly smaller r-BC 5 min and the very fine TEMPO-CNf 360 min. Concerning nanocellulose blends, it was observed that for binary and ternary nanocellulose blends the drainage time depended on the fraction of smaller (r-BC and TEMPO-CNf) nanofibrils present in the suspension. Thus, the drainage time for nanopapers containing TEMPO-CNf could be tremendously reduced to 150 min (r-BC/TEMPO-CNf), 120 min (BC/TEMPO-CNf) and even 60 min (BC/r-BC/TEMPO-CNf), which was attributed to the lower fraction of TEMPO-CNf of only 50% and 33%, respectively. These results showed that by hybridisation of TEMPO-CNf with (r-)BC the filtration efficiency could be significantly improved.

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(b) Morphology of neat (unrefined) BC, r-BC and TEMPO-CNFnanopapers
Figure 1 shows the scanning electron micrographs of nanocellulose of neat (unrefined) BC, r-BC and TEMPO-CNFnanopapers. A network structure consisting of nanofibrils, with a fibril diameter of ~50 nm, can be observed in neat BC nanopapers (Figure 1 B, C). The uniformity of the diameter of neat BC nanofibrils is a direct result of the biosynthesis of nanocellulose by cellulose-producing bacteria [53, 54]. The SEM images showing the morphology of r-BC nanofibrils within the r-BC nanopaper, on the other hand, showed regions containing a nanofibrillar structure, as well as regions where no nanofibrils can be observed (Figure 1 F). When BC was mechanically refined (Figure 1 D, E), the nanofibril diameter and length is anticipated to decrease due to the shearing action caused by the refinement process. As a result of the small fibril diameter of r-BC, no individual nanofibrils can be distinguished using SEM (Figure 1 F) on large parts of r-BC nanopapers. A similar morphology (Figure 1 G, H, I) was also observed for nanopapers made from TEMPO-CNFnanosheets, which have fibril diameters of < 5 nm. It is worth mentioning that even though r-BC had been subjected to 20 passes through a disc mill the remaining typical BC fibrils, observable as nanofibrillar structure (Figure 1 E), still were not completely disrupted. This suggests that the fibrillar structure of neat BC is strongly bound; so much so that even 20 passes through the grinder are not sufficient to fully disintegrate this network.

(c) Density and porosity of (hybridised) cellulose nanopapers
The thickness (h) and grammage (G) of the (hybridised) cellulose nanopapers, along with their envelope density (ρe) are summarised in Table 1. The envelope density, ρe, of neat (unrefined) BC was found to be 1030 kg m−3, corresponding to a nanopaper porosity of 31%. The observed high porosity of neat BC nanopapers is hypothesised to be a result of inhomogeneous dispersion of BC in water, which arises from difficulties in disrupting the fibrillar BC network of as-synthesised BC using a low energy blender [55]. r-BC nanopapers had slightly higher ρe and lower porosity compared to the neat BC nanopapers, which we attribute to the smaller fibril diameter of r-BC as a result of the mechanical refinement of neat BC (see Figure 1). This subsequently led to better packing efficiency of r-BC nanofibrils in the nanopaper [56]. This is also consistent with the observed high ρe and lower porosity of TEMPO-CNFnanopapers compared to both neat and r-BC nanopapers.

Hybridised cellulose nanopapers consisting of binary blends of nanocellulose fibrils (e.g. BC/r-BC, BC/TEMPO-CNFnanosheets and r-BC/TEMPO-CNFnanopapers) also possessed a higher ρe and lower porosity compared to non-hybridised cellulose nanopapers. This can again be attributed to the “void-filling” effect. The smaller fibrils of the two types of nanocellulose in the binary blends will fill the pore space formed in between the bigger BC fibrils. In the BC/r-BC nanopapers for example, the combination of fibrils of different sizes allowed for a more efficient packing of the fibrils, which resulted in a denser network structure as evidenced by increased nanopaper density. Hence, nanopapers made from a mixture of small, but differently sized, TEMPO-CNFnanosheets and r-BC fibrils had the highest density. As expected, ρe and the porosity of hybridised cellulose nanopapers produced from a ternary blend of nanocellulose (BC/r-BC/TEMPO-CNFnanosheets) was not significantly different to the blends nanopapers consisting of only BC/r-BC and BC/TEMPO-CNFnanosheets. The ternary blend consists of BC, r-BC and TEMPO-CNFnanosheets in a 1:1:1 ratio, implying that the resulting hybridised cellulose nanopaper contains a higher weight fraction of the smaller r-BC and TEMPO-CNFnanosheets and less of the bigger nanofibrils from BC. A void filling effect was also observed by Sehaqui et al. [42] and Gonzalez et al. [43] when blending micrometer sized pulp fibres with CNF and TEMPO-CNFnanosheets. In both cases, the ratio of cellulose fibre diameters was ~200-2000. In our work, we show that the “void-filling” effect still occurs even if both types of cellulose fibres are nanofibrils and the nanofibre diameter ratio is only ~10.

(d) Electrokinetic behaviour of (hybridised) cellulose nanopapers
ζ-potential provides information about the formation and composition of the double layer when the material is in contact with an electrolyte solution, and therefore, the amount of dissociable functional groups present on the surface of a material [57]. The ζ-potential is generally assumed to approximate the electrochemical potential at the boundary between the immobile (Stern) layer and the diffusive (Gouy) part of the double layer [58]. The formation of the electrochemical double layer is due to: (i) the adsorption of electrolyte ions onto the surface of the substrate and (ii) the dissociation of the Brønsted acidic/basic groups. The ζ-potential as a function of pH of the (hybridised) cellulose nanopapers in a supporting electrolyte of 1 mM KCl is shown in Figure 2. In the case of cellulose nanomaterials, the groups determining the surface charge are usually
carboxylic groups, i.e. glucuronic acids, which form by oxidation reactions, e.g. during pulping, mechanical disintegration or already occurring during exposure to air.

A negative $\zeta$ at high pH can be observed for neat BC, r-BC and TEMPO-CNF nanopapers as well as the BC/r-BC/TEMPO-CNF nanopapers made from a ternary blend of all types of nanocellulose studied in this work. This indicates that the (hybridised) cellulose nanopapers possessed an acidic surface character. At high pH, the Brønsted acidic groups on the surfaces of the (hybridised) cellulose nanopapers are fully dissociated. Lowering the pH causes protonation of these acidic groups, leading to an increase of the magnitude of the $\zeta$-potential until the isoelectric point (iep), at which $\zeta = 0$, is reached. The position of the iep depends on the concentration and pK, values of all accessible acidic groups present on the surface of the (hybridised) cellulose nanopapers, while $\zeta$ depends on the concentration of these dissociable groups present. BC has the highest $\zeta$ among the single-sourced cellulose nanopapers, with a plateau at high pH of $-19$ mV, showing that it contains the lowest amount of carboxylic groups. TEMPO-CNF nanopapers, on the other hand, contain much more dissociable carboxylic acid groups and, therefore, have a $\zeta$ at high pH of $-26$ mV. This value is rather low compared to values for TEMPO-CNF reported in literature [28, 29] but can be explained by the moderate charge content of our TEMPO-CNF of $0.54$ mmol g$^{-1}$. r-BC nanopapers also have a lower $\zeta$ ($-26$ mV) compared to neat (unrefined) BC nanopapers. The iep was around $3.3$ for BC, r-BC and the mixture of these two. For TEMPO-CNF, the iep was around $2.7$, indicating the presence of stronger acidic groups on TEMPO-CNF compared to (r-)BC.

Hybridised cellulose nanopapers made from the ternary mixture of BC/r-BC/TEMPO-CNF nanofibrils exhibited a $\zeta = f(pH)$ curve averaging those of the three constituents it consists of. The iep was closer to the TEMPO-CNF nanopapers. This demonstrated the stronger influence of TEMPO-CNF carboxylic groups within the ternary mixture as compared to BC. The binary mixtures (see Table 1) showed the expected behaviour (BC/r-BC) or slightly different $\zeta$-potentials than expected, with BC/TEMPO-CNF nanopapers having a lower and r-BC/TEMPO-CNF a higher $\zeta$-potential as predicted by averaging the $\zeta$-potentials of those materials. These deviations can be explained by the inhomogeneous composition of the surface of the blend nanopapers.

(e) Tensile properties of (hybridised) cellulose nanopapers

Representative stress-strain curves of (hybridised) cellulose nanopapers are shown in Figure 3. The tensile properties of the (hybridised) cellulose nanopapers were calculated following paper test standards (ASTM D828); tensile strength ($TS$), evaluated by taking the maximum load divided by the width at the narrowest part of the test specimen, and tensile index ($TI$), which is the nanopaper tensile strength, $TS$, normalised by the grammage of the nanopaper are summarised in Table 2.

All (hybridised) cellulose nanopapers exhibited an initial elastic deformation, followed by yielding and inelastic deformation before catastrophic failure of the nanopapers. Neat BC nanopaper possessed an average tensile modulus ($E$) and strength ($\sigma$) of $13.3$ GPa and $161$ MPa, respectively. These values corroborate the tensile properties of BC nanopapers reported by various researchers [55]. Cellulose nanopapers produced from mechanically refined BC (r-BC nanopaper), however, possessed lower tensile properties ($E = 8.9$ GPa and $\sigma = 138$ MPa) than neat BC nanopapers. This can be attributed to the damage of BC nanofibrils caused by the mechanical refining process. The weight average molecular weight ($M_w$, see Supplementare Material) of neat (unrefined) BC was determined to be $819$ kDa while that of the mechanical refined r-BC was $431$ kDa, which indicates that mechanical refining caused chain scissoring.

The TEMPO-CNF nanopapers manufactured in this work are very brittle (Figure 3). These nanopapers failed in the linear elastic region with a strain-to-failure ($\varepsilon$) of only $1.3\%$. BC nanopapers were significantly less stiff and exhibited a yield point at a strain of $0.7\%$, i.e. they deformed plastically probably due to fibril reorientation. The tensile modulus and strength of TEMPO-CNF nanopapers were $16.7$ GPa and $160$ MPa, respectively. These values are on par with reported tensile properties of TEMPO-CNF nanopapers [40, 59] although some authors reported higher tensile properties [27, 37, 52].

The Young’s modulus of hybridised cellulose nanopapers (BC/r-BC, BC/TEMPO-CNF, r-BC/TEMPO-CNF and BC/r-BC/TEMPO-CNF) was similar to those of neat BC nanopapers (Table 2), indicating that there are no synergistic effects when blending different types of nanocellulose together to enhance the stiffness of cellulose nanopapers. In terms of strength of hybridised cellulose nanopapers, papers containing binary blends with Phil. Trans. R. Soc. A.
TEMPO-CNFS as well as the nanopapers made from the ternary blend had much higher strength compared to neat (unrefined) BC nanopaper, as well as TEMPO-CNFS nanopapers. The tensile strength of these hybridised cellulose nanopapers was between 180-200 MPa. This can be attributed to the lower porosity of BC/TEMPO-CNFS, r-BC/TEMPO-CNFS and BC/r-BC/TEMPO-CNFS nanopapers due to the “void-filling” of smaller TEMPO-CNFS in between the bigger BC and r-BC nanofibrils. In addition to this, we also further postulate that the interaction (e.g. hydrogen bonding) between TEMPO-CNFS and BC as well as r-BC nanofibrils is greater than just between BC and r-BC nanofibrils due to the presence of carboxylate groups on TEMPO-CNFS. This hypothesis is consistent with the lack of significant improvements in the tensile properties of BC/r-BC blend nanopapers compared to neat (unrefined) BC nanopapers.

While the tensile strength of the hybridised cellulose nanopapers containing TEMPO-CNFS showed synergistic effects, their strain-to-failure (ε) was observed to be lower than that of neat BC and r-BC nanopapers. A material structure will fail at its weakest point. In this case, the weakest point in these hybridised cellulose nanopapers is the network of TEMPO-CNFS (ε = 1.3%). During tensile deformation, the network of TEMPO-CNFS within the hybridised cellulose nanopapers is expected to fail at a lower strain. This creates flaws within the hybridised cellulose nanopapers. Upon further tensile deformation (e.g. higher test specimen strain), the cellulose network of the bigger BC and/or r-BC nanofibrils still carries the majority of the load while the flaws arising from the TEMPO-CNFS network propagate until they coalesce, leading to the observed catastrophic failure of the hybridised cellulose nanopapers at a strain to failure of only ~2%. Nevertheless, blending TEMPO-CNFS with bigger nanocellulose fibrils, produces a hybrid effect [60]: when the lower strain fibril network formed by TEMPO-CNFS starts to fail the intact higher strain network formed by the bigger (r-)BC fibril network remains intact and carries the load, resulting in an improved tensile strength, higher strain to failure (as compared to the low strain to failure TEMPO-CNFS network) but reduced modulus. This allows exploiting the properties of the TEMPO-CNFS network to a much higher extent than possible in the nanopaper just formed from TEMPO-CNFS. Furthermore, a denser structure is formed in which the differently sized fibrils are intimately commingled as shown in the SEM images, which is what we should have given our manufacturing process (see Figure 1).

As expected, the nanopapers made from the blend of the three nanocelluloses had a lower strength than the binary mixtures because the content of TEMPO-CNFS in this mixture was lower. Still, the tensile strength of the ternary blend was higher than the one of each single constituent nanopaper, thus showing a synergistic behaviour. The blend of r-BC with BC produced nanopapers with the average properties of the fibril networks the paper was formed from. Eventually, the optimisation of the nanocellulose ratios within the blend nanopapers could produce even better tensile properties as compared to the binary mixtures.

The absorbed energy during tensile fracture of TEMPO-CNFS nanopapers revealed their very brittle nature (1.16 J m⁻³), whereas BC (2.56 J m⁻³) and r-BC (2.89 J m⁻³) required more energy, indicating that they are tougher. Hybridisation of TEMPO-CNFS with (r-)BC resulted in significantly improved absorbed energies that were almost on par with BC nanopapers and twice (2.12 to 2.29 J m⁻³) as high as pure TEMPO-CNFS nanopapers. Moreover, by blending BC with r-BC the highest absorbed energies (8 J m⁻³) in this study were obtained, clearly showing synergistic effects.

(f) Fracture resistance of (hybridised) cellulose nanopapers

The critical stress intensity factors (Kc) of the (hybridised) cellulose nanopapers tested on single edge notched test specimens are tabulated in Table 2. The representative load-displacement curves of single edge-notched test specimens are shown in Figure 4. Load-displacement instead of stress-strain curves were plotted as the stress field in a single-edge notched test specimen is not uniform. The stress at the crack tip tends towards infinity while the remote stress (e.g. stress away from the crack tip) would be much lower. It should be noted that due to the brittleness of TEMPO-CNFS nanopaper an initial crack could not be introduced into these test specimens. Therefore, the fracture resistance of TEMPO-CNFS could not be determined. The initial linear part of the load-displacement curves corresponds to the strain energy stored in the (hybridised) cellulose nanopapers during uniaxial tensile loading. When the applied load was high enough to create new surface area, the introduced crack started to propagate until the single-edge notched test specimens failed catastrophically.

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The fracture resistance, i.e. the energy required propagating a crack as expressed by $K_{\text{1c}}$, of BC and r-BC was 7.4 and 6.1 MPa m$^{0.5}$, respectively. Thus, mechanically refining BC resulted in a reduced fracture resistance meaning less energy is required to overcome the work of cohesion, which can be attributed to the lower tensile strength of r-BC nanopapers. The work of fracture, as quantified by $G_{\text{1c}}$, however, was almost identical with 4.2 and 4.1 kJ m$^{-2}$ for r-BC and BC, respectively. Concerning BC blends, a synergy in fracture resistance and work of fracture was clearly observed; the BC/r-BC nanopapers possessed a higher $K_{\text{1c}}$ (8 MPa·m$^{0.5}$) and work of fracture (5.5 kJ m$^{-2}$) than the BC and r-BC nanopapers.

Most importantly, hybridisation of TEMPO-CNf with (r-)BC resulted in tremendously improved fracture resistance, as displayed by $K_{\text{1c}}$ values close to those of (r-)BC. The crack propagates through the path of least resistance. In the context of the hybridised cellulose nanopapers, the path of least resistance is likely to be through the brittle TEMPO-CNf network within these hybridised cellulose nanopapers. Thus, the toughness in hybridised nanopapers was much higher than for TEMPO-CNf nanopapers. Our results are broadly in line with data reported by Mao et al. [61].

4. Conclusions
The mechanical properties of nanocellulose papers are of fundamental importance for their applications. Cellulose nanopapers were prepared by filtration of nanocellulose suspensions of bacterial cellulose, mechanically refined bacterial cellulose and TEMPO-oxidised CNf. Binary and ternary blends of these nanocellulose materials were prepared and used for nanopaper production. We showed that the mechanical and physical properties of nanopapers can be tuned by creating nanopaper blends by combining nanocellulose fibrils of different dimensions and charge. Pure TEMPO-CNf nanopapers were very brittle having the lowest strain to failure among all samples but the highest Young's modulus. Binary mixtures of the various single constituents had Young's moduli in between those of the one-component nanopapers. Interestingly, the measured ultimate tensile strength showed synergistic effects. By blending various types of nanocellulose with TEMPO-oxidised CNf it was possible to exploit the outstanding properties of these fibrils to a much higher extent because the presence of bigger fibrils and heterogeneities in the network structure seemed to reduce the flaw sensitivity in these papers. Thus it was proven that nanopaper properties can be tailored simply by blending various nanocellulose grades prior to nanopaper fabrication.

Additional Information

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Data Accessibility
The datasets supporting this article have been uploaded as part of the Supplementary Material.

Competing Interests
We have no competing interests.
Authors’ Contributions
A.M.: 1) substantial contributions to conception and design and acquisition, analysis and interpretation of data; 2) drafting the article
F.M.: 1) acquisition and analysis of data; 2) revising the article critically for important intellectual content
M.H.: 1) acquisition and analysis of data; 2) revising the article critically for important intellectual content
K.Y.L.: 1) substantial contributions to conception and design and acquisition, analysis and interpretation of data; 2) revising the article critically for important intellectual content
A.B.: 1) substantial contributions to conception and design and interpretation of data; 2) revising the article critically for important intellectual content
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References

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Table 1. Thickness ($h$), grammage ($G$), envelope density ($\rho$) porosity ($P$), iep and $\zeta_{\text{m}}$ of BC, r-BC and TEMPO-CNFi nanopapers and nanocellulose blend nanopapers.

<table>
<thead>
<tr>
<th>Type of nanocellulose suspension</th>
<th>Nanopaper</th>
<th>Filtration time</th>
<th>$h$ (µm)</th>
<th>$G$ (g m$^{-2}$)</th>
<th>$\rho$ (kg m$^{-3}$)</th>
<th>$P$ (%)</th>
<th>iep</th>
<th>$\zeta_{\text{m}}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single source</td>
<td>BC</td>
<td>3 min</td>
<td>50.9 ± 1.4</td>
<td>52.4 ± 1.4</td>
<td>1030 ± 30</td>
<td>31.3 ± 0.9</td>
<td>3.4</td>
<td>-19</td>
</tr>
<tr>
<td></td>
<td>r-BC</td>
<td>5 min</td>
<td>47.0 ± 3.2</td>
<td>51.4 ± 4.3</td>
<td>1090 ± 30</td>
<td>27.1 ± 0.7</td>
<td>3.4</td>
<td>-26</td>
</tr>
<tr>
<td></td>
<td>TEMPO-CNFi</td>
<td>360 min</td>
<td>44.5 ± 0.9</td>
<td>48.9 ± 0.6</td>
<td>1100 ± 30</td>
<td>26.7 ± 0.7</td>
<td>2.7</td>
<td>-26</td>
</tr>
<tr>
<td>Binary blends</td>
<td>BC/r-BC</td>
<td>4 min</td>
<td>51.3 ± 2.5</td>
<td>55.7 ± 2.4</td>
<td>1090 ± 10</td>
<td>27.6 ± 0.3</td>
<td>3.3</td>
<td>-23</td>
</tr>
<tr>
<td></td>
<td>BC/TEMPO-CNFi</td>
<td>120 min</td>
<td>46.5 ± 1.1</td>
<td>52.6 ± 0.9</td>
<td>1130 ± 30</td>
<td>24.6 ± 0.7</td>
<td>2.6</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>r-BC/TEMPO-CNFi</td>
<td>150 min</td>
<td>44.8 ± 1.8</td>
<td>52.1 ± 1.6</td>
<td>1160 ± 30</td>
<td>22.5 ± 0.6</td>
<td>2.7</td>
<td>-22</td>
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<tr>
<td>Ternary blend</td>
<td>BC/r-BC/TEMPO-CNFi</td>
<td>60 min</td>
<td>46.5 ± 1.0</td>
<td>53.6 ± 0.9</td>
<td>1150 ± 10</td>
<td>23.2 ± 0.2</td>
<td>2.7</td>
<td>-23</td>
</tr>
</tbody>
</table>

The porosity ($P$) of the (hybridised) cellulose nanopapers was calculated using: $P = 1 - \frac{\rho_c}{\rho}$, where $\rho$ corresponds to the skeletal density of cellulose, taken to be 1500 kg m$^{-3}$ [34].

Table 2. Ultimate tensile strength $\sigma$, strain to failure $\epsilon$, Young’s modulus $E$, paper tensile strength $TS$, paper tensile index $TI$, absorbed energy, fracture toughness $K_c$ and work of fracture $G_c$ of all pure and blend nanopapers.

<table>
<thead>
<tr>
<th>Type of nanocellulose suspension</th>
<th>Nanopaper</th>
<th>$\sigma$ (MPa)</th>
<th>$\epsilon$ (%)</th>
<th>$E$ (GPa)</th>
<th>$TS$ (kN/m)</th>
<th>$TI$ (Nm/g)</th>
<th>Absorbed energy (J/m$^2$)</th>
<th>$K_c$ (MPa·m$^{0.5}$)</th>
<th>$G_c$ (kJ·m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single source</td>
<td>BC</td>
<td>161 ± 3</td>
<td>2.7 ± 0.1</td>
<td>13.3 ± 0.7</td>
<td>7.7 ± 0.5</td>
<td>147 ± 6</td>
<td>2.56 ± 0.08</td>
<td>7.4 ± 0.5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>r-BC</td>
<td>138 ± 4</td>
<td>3.7 ± 0.3</td>
<td>8.9 ± 0.4</td>
<td>6.1 ± 0.3</td>
<td>113 ± 5</td>
<td>2.89 ± 0.26</td>
<td>6.1 ± 0.3</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>TEMPO-CNFi</td>
<td>160 ± 14</td>
<td>1.3 ± 0.1</td>
<td>16.7 ± 0.5</td>
<td>6.9 ± 0.5</td>
<td>140 ± 10</td>
<td>1.16 ± 0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Binary blends</td>
<td>BC/r-BC</td>
<td>157 ± 4</td>
<td>3.3 ± 0.2</td>
<td>11.6 ± 0.8</td>
<td>7.5 ± 0.4</td>
<td>133 ± 4</td>
<td>3.0 ± 0.20</td>
<td>8.0 ± 0.4</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>BC/TEMPO-CNFi</td>
<td>197 ± 6</td>
<td>2.0 ± 0.7</td>
<td>14.9 ± 0.8</td>
<td>8.8 ± 0.3</td>
<td>168 ± 7</td>
<td>2.29 ± 0.30</td>
<td>4.7 ± 0.6</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>r-BC/TEMPO-CNFi</td>
<td>190 ± 10</td>
<td>2.1 ± 0.2</td>
<td>13.7 ± 0.8</td>
<td>8.1 ± 0.5</td>
<td>160 ± 8</td>
<td>2.12 ± 0.53</td>
<td>4.6 ± 1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

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| Ternary blend | BC/r-BC/TEMPO-CNFS | 182 ± 9 | 2.2 ± 0.3 | 13.9 ± 0.6 | 8.2 ± 0.3 | 153 ± 6 | 2.23 ± 0.37 | 5.0 ± 0.9 | 1.8 |

**Figure 1.** SEM images of BC (A: 1k, B: 10k, C: 50k), r-BC (D: 1k, E: 10k, F: 50k) and TEMPO-CNFS (G: 1k, H: 10k, I: 50k) nanopapers.

**Figure 2.** ζ-potential as function of pH of BC (square), r-BC (diamond) and TEMPO-CNFS (circle) nanopapers and of a ternary mixture (1:1:1) of these three (triangle). (Online version in colour.)
Figure 3. Representative stress-strain curves of TEMPO-CN, BC/TEMPO-CN, r-BC/TEMPO-CN, BC/r-BC/TEMPO-CN, BC, BC/r-BC and r-BC nanopapers. (Online version in colour.)

Figure 4. Representative force-displacement curves obtained during fracture toughness tests for BC/TEMPO-CN, BC/r-BC/TEMPO-CN, r-BC/TEMPO-CN, r-BC, BC/r-BC and BC nanopapers. (Online version in colour.)