Enhancing strength and toughness of cellulose nanofibril network structures with an adhesive peptide

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A B S T R A C T

The mechanical properties of cellulose nanofibrils network structure are essential for their applications in functional materials. In this work, an adhesive peptide consisting of just 11 amino acid residues with a hydrophobic core sequence of FLIVI (F – phenylalanine, L – leucine, I – isoleucine, V – valine) flanked by three lysine (K) residues was adsorbed to 2,2,6,6-Tetramethyl-1-piperidinyloxy radical (TEMPO) oxidized cellulose nanofibrils (TO-CNF). Composite films were prepared by solution casting from water suspensions of TO-CNF adsorbed with the adhesive peptide. The nanofibrils network structure of the composite was characterized by atomic force microscopy (AFM). The structure of the peptide in the composites and the interactions between TO-CNF and the peptide were studied by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The mechanical properties of the composites were characterized by tensile tests and dynamic mechanical analysis (DMA). With 6.3 wt.% adhesive peptide adsorbed onto TO-CNF, the composite showed a modulus of 12.5 ± 1.4 GPa, a tensile strength of $344.5 \pm (15.3)$ MPa, and a strain to failure of $7.8 \pm 0.4\%$, which are 34.4%, 48.8%, and 23.8% higher than those for neat TO-CNF, respectively. This resulted in significantly improved toughness (work to fracture) for the composite, 77% higher than that for the neat TO-CNF.

1. Introduction

Environmental issues and the growing demand for sustainable and energy efficient materials have inspired increased research activities on basic and applied aspects of nanomaterials from renewable resources. Cellulose nanopaper is a new type of paper made of cellulose fibers of nanometer scale dimensions, i.e. cellulose nanofibrils (CNFs), constituting a network of fibers similar to conventional paper. (Henriksson, Berglund, Isaksson, Lindstrom, & Nishino, 2008) Cellulose nanopaper has unique properties such as high strength and work to fracture, (Henriksson et al., 2008) high transparency, (Nogi, Iwamoto, Nakagaito, & Yano, 2009) low thermal expansion, (Nogi & Yano, 2008; Yano et al., 2005) large specific surface area, (Sehaqui, Zhou, Ikkala, & Berglund, 2011) and low gas permeability (Fukuzumi, Saito, Wata, Kumamoto, & Isogai, 2009). This is due to the network structure of intertwined cellulose nanofibrils oriented random-in-the-plane, with low porosity and nanoscale voids distributed homogeneously. (Henriksson et al., 2008) Applications of cellulose nanopaper in flexible displays, foldable electronics, biosensors, optoelectronics, and energy-storage devices have been demonstrated. (Nogi et al., 2009; Nogi, Karakawa, Komoda, Yagyu, & Nge, 2015; Nogi & Yano, 2008; Zhu, Fang, Preston, Li, & Hu, 2014) Improving the tensile strength and modulus of CNFs network structure without the loss of high toughness, transparency, and thermal stability is desired for cellulose nanopaper, since it is a candidate substrate for flexible electronics and as reinforcement in functional nanocomposites.

Inspired by plant cell wall biosynthesis, hemicelluloses and cellulose derivatives such as xylan, xyloglucan, hydroxyethyl cellulose, and carboxymethylcellulose (CMC) have been coated on CNFs to enhance the formation of fibril network, resulting stronger and stiffer films. (Arola, Malho, Laaksonen, Lille, & Linder, 2013; Pahimanolis et al., 2013; Prakobna et al., 2015; Sehaqui, Zhou, & Berglund, 2011; Zhou et al., 2009) By using processing techniques such as cold drawing, tape casting, and wet-stretching, CNFs films with high modulus and specific strength owing to the alignment of CNFs have also been prepared. (Baez, Considine, & Rowlands, 2014; Pahimanolis et al., 2013; Sehaqui

et al., 2012; Tang, Butchosa, & Zhou, 2015; Torres-Rendon, Schacher, Ifuku, & Walther, 2014) Chemical and ionic crosslinking has been used in order to enhance the mechanical performance of CNF network nancomposites. This has been used with polymer matrices such as CMC, (Pahimanolis et al., 2013) poly(vinyl alcohol) (PVOH), (Spoljaric, Salminen, Luong, & Seppala, 2014) and poly(acrylic acid) (PAA). (Spoljaric, Salminen, Luong, & Seppala, 2013) However, it remains challenging to simultaneously improve modulus, yield strength, ultimate strength and strain-to-failure. One reason is that improved interfibril interaction tends to increase modulus and yield strength, but reduce strain to failure.

Proteins, such as silk fibroin, (Fan, Zhang, Shao, & Hu, 2013) collagen, (Fratzl et al., 1998) elastin, (Zou & Zhang, 2009) and resilin, (Elvin et al., 2005; Kappiyoor, Balasubramanian, Dudek, & Puri, 2011) show excellent mechanical properties owing to hydrogen bonds between β-strands of β-sheet structures. (Cheng et al., 2014; Lamour et al., 2017) Peptides or proteins rich in β-sheet structure normally generate long fibrils, called amyloid fibrils. In such proteins, the strands are stacked along the fiber axis, forming sheets that keep hold by hydrogen bonds, (Chiti et al., 1999) in which the N−H of the amide group interacts with C=O of the carboxylic groups of the protein backbones. β-sheet structure is very rigid, conferring superior mechanical properties to amyloid fibrils, reaching a Young's modulus close to that of silk and an ultimate strength similar to steel. (Knowles et al., 2007) Because of their unique mechanical properties, amyloid protein and/or peptides are potential building blocks for the preparation of composites and copolymers. (Johnson, Wanasekara, & Korley, 2012) However, studies on mechanical properties of composites from CNFs and protein or peptides are rather limited in literature. CNFs have been previously used to reinforce soy protein (SP) to obtain aerogels with compressive modulus similar to neat CNFs at high SP loading (ca. 70%). (Arboleda et al., 2013) Recently, the seminal work of Linder et al. demonstrated that the introduction of a recombinant engineered fusion protein based on cellulose-binding modules (CBMs) affects the stiffness and strength of CNF composites owing to an interlinking architecture through the coupling of protein molecules. (Malho et al., 2015) CBMs have strong adhesiveness to cellulose through a set of aromatic residues and hydrogen bonding. (Varnai et al., 2014)

Inspired by the design of interface interactions using adhesive proteins, we report the preparation and characterization of a composite of CNFs and an adhesive peptide. The peptide With a sequence of KKKFLIVIKKK (K – lysine, F – phenylalanine, L – leucine, I − isoleucine, V − valine) is made up of just eleven amino acid residues (Fig. 1a), with a tendency to spontaneously form β-sheet structure. This peptide has previously demonstrated strong adhesion to wood. (Mo et al., 2008) The peptide contains a hydrophobic core sequence of FLIVI flanked by charged amino acid sequence, i.e. three lysine residues. The strategy was to enhance mechanical properties of the composites through the interaction between side chains of the peptides with CNFs and the formation β-sheet structures of the peptide in the CNF network structure (Fig. 1b). The structure of the composites was characterized by FTIR and XRD, and the morphology was observed by AFM. The mechanical properties of the composites were studied by tensile tests and DMA.

2. Experimental section

2.1. Materials

2,2,6,6-Tetramethyl-1-piperidinyloxy radical (TEMPO), sodium bromide, and sodium hypochlorite were purchased from Sigma-Aldrich and used without further purification. The adhesive peptide (AP) with an amino acid sequence of KKKFLIVIKKK was purchased from GenScript USA Inc., Piscataway, NJ. Commercial never-dried softwood sulphite pulp was provided by Nordic Paper. The pulp contained ca. 86% cellulose (degree of polymerization, 1200), 13.8% hemicellulose,

and traces of residual lignin (0.7%). Ultrapure water (resistivity ≥ 18.2 MΩ cm) was used in all experiments.

2.2. Preparation of TEMPO-oxidized cellulose nanofibrils (TO-CNF)

The CNF was prepared by using TEMPO-mediated oxidation of wood pulp fibers according to the method reported by Saito et al. (Saito, Kimura, Nishiyama, & Isogai, 2007) Briefly, the pulp fibers were dispersed in water at 2 wt.%. Sodium bromide (1 mmol/g dry pulp) and TEMPO (0.1 mmol/g dry pulp) were added, followed by the slow addition of sodium hypochlorite (NaClO) (10 mmol/g dry pulp) and the pH of the suspension was maintained at a constant value of 10 by adding 0.1 M sodium hydroxide (NaOH) until all NaClO was consumed. The oxidized pulp was washed with ultrapure water to neutral pH and dispersed in water to afford a 0.2 wt.% suspension, followed by disintegration using a high speed kitchen blender (Vita-Prep 3 model, Vita-Mix Corp., USA) for 5 min. The suspension was further sonicated using Branson S-250A (USA) at 70% output control (probe tip diameter, 13 mm) for 2 min, and a transparent aqueous suspension of cellulose nanofibrils was obtained. The carboxylate content of the modified pulp fibers was 1.6 mmol/g, as determined by conductometric titration. (Perez, Montanari, & Vignon, 2003; Salajkova, Berglund, & Zhou, 2012)

2.3. Adsorption of AP to TO-CNF

A stock solution of the AP in water (30 mg/mL) was prepared. Solutions with different AP concentrations were made from the stock solution by diluting with water. 250 μL of each AP solution was mixed with 750 μL 0.2 wt.% TO-CNF water suspensions in polypropylene Eppendorf tubes and incubated at room temperature with orbital shaking for 24 h. The suspensions were filtered through centrifuge filters (Amicon® Ultra, Ultracel® – 10 K, Merck Millipore Ltd.). The amount of free AP was determined by the bicinchoninic Acid (BCA) protein assay (Smith et al., 1985) from the adsorption at 562 nm of the filtrate using a standard curve derived from AP solutions of increasing concentrations. Neat AP solutions were also filtered through centrifuge filters and used as the control of nonspecific binding of AP to the filters. Filtrate of neat TO-CNF suspension through the centrifuge filter was used as the blank. Experiments were carried out at least in triplicates.

2.4. Preparation of TO-CNF/AP composite films

The solutions of AP at different concentrations were prepared by dissolving 5, 10, and 20 mg peptide in 25 mL water, respectively. The AP solution was added slowly to a 75 mL 0.2 wt.% TO-CNF water suspension with magnetic stirring at 250 rpm. After stirring for 24 h, the suspension solution was degassed under vacuum and casted in a polystyrene Petri dish with a diameter of 8.5 cm, then dried in the air at room temperature. A transparent film with a thickness of ca. 20 μm was formed on the Petri dish and peeled off. Thus, TO-CNF/AP composite films with an AP content of 3.2, 6.3, and 11.8 wt.% were prepared, respectively. The neat TO-CNF film was prepared in the same way but without the addition of AP.

2.5. Characterizations

Electrophoretic mobility of TO-CNF and TO-CNF adsorbed with AP was measured on Malvern Instruments Zetasizer ZEN3600 (Worcestershire, UK). All the samples were diluted to 0.02 wt% with water and measured. The reported ζ–potential values are obtained by converting measured mobility values with the Smoluchowski equation. The FTIR spectra were collected using a Perkin-Elmer Spectrum 100 FT-IR Spectrometer (Waltham, MA) equipped with a single horizontal Golden Gate ATR cell. 32 scans were co-added over the range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1}. X-ray diffraction (XRD) diffractograms were recorded with a Philips X'Pert Pro diffractometer