Polymer Testing 74 (2019) 113–118

Contents lists available at ScienceDirect

Polymer Testing

journal homepage: www.elsevier.com/locate/polytest

Eco-friendly lignin nanofiber mat for protection of wood against attacks by environmentally hazardous fungi

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ARTICLE INFO

Keywords:
Electrospinning
Lignin
Sapstain fungi
Fungal infection
Global wood trade

ABSTRACT

There are multiple wood-attack fungi that deteriorate wood globally. Control of wood-attack fungi is costly and requires moisture sensors, application of different chemicals, and correct identification of the fungi. Wood-attack via fungi results in the loss of about one third of the annual timber (20 billion board feet) harvest. In particular, the environmentally hazardous fungal contamination of wood materials during shipping represents a serious threat to the biodiversity of native eco-systems with undesirable financial impacts. Thus far, various approaches have been attempted to prevent fungal invasion; however, the detrimental effect of fungal contamination on the environment and human health still remains as an issue to be resolved. Herein, we present an eco-friendly lignin-based nanofiber (NF) mat that effectively protects wood from fungi. Mat-covered wood specimens were fully protected against fungal infection over a two week incubation period. The NF mats with an inter-fiber pore size of several hundred nanometers impeded the penetration of fungi into the wood surfaces, thus preventing their growth in the wood. The experiment, which mimicked the real shipping process, demonstrated the excellent wood-protection ability of the eco-friendly lignin-based NF mat.

1. Introduction

Wood, which may be considered a renewable energy source and an environmentally friendly material, has been an important raw material throughout the history of mankind. With the growth of the world economy and population in recent decades [1], the global demand for wood as an energy source (especially for developing countries) and industrial resource has increased, resulting in a dramatic increase in the global transportation of wood and wood products [2].

With the rapid growth of the international wood trade, the adverse effects of the inadvertent introduction of plants and associated microorganisms into other countries via trade, where these organisms are often termed “alien species”, has attracted significant attention because the introduction such invasive alien species can cause significant damage to indigenous species and the local environment [3–6]. Environmentally hazardous alien fungi are considered as one of the major culprits [7] implicated in damage to the eco-system [8]. Some examples include the plane canker stain in Europe caused by *Ceratocystis platani* that was introduced from North America, the chestnut blight disease in USA and Europe caused by *Cryphonectria parasitica,* and the Dutch elm disease in North America and Europe caused by the sapstain fungi *Ophiostoma ulmi* and *O. novo-ulmi*, which originated from Asia.

More recently, the sapstain (or blue stain) fungal infection, which causes discoloration of the outermost younger part (or sapwood) of living wood, has become a source of deterioration of the aesthetic value of wood (Figs. 1a and 1b) and thus causes economic losses in the forestry and timber industry, and in particular in value-added wood products [9,10]. Various approaches have been used to protect sapwood against sapstain fungal infection, including desiccation of the lumber or the use of various diffusible chemical preservatives [11]. However, the desiccation requires time to achieve complete drying, as well as energy for heating or blowing. Chemical methods also have adverse effects on the environment and health as they involve somewhat toxic chemicals.

To address these issues, biological methods have been proposed and

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https://doi.org/10.1016/j.polymertesting.2018.12.023
Received 11 October 2018; Received in revised form 9 December 2018; Accepted 21 December 2018
Available online 23 December 2018
0142-9418/

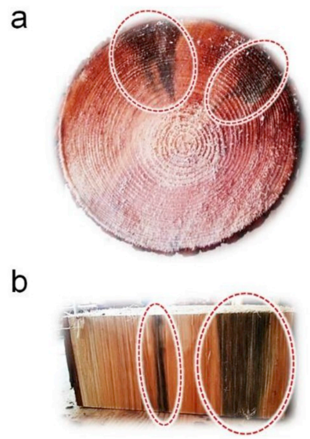

Fig. 1. Photographs of pine sapwood discolored by sapstain fungi. (a) Top-view and (b) cross-sectional images. Red circles indicate discolored area.

have attracted considerable attention as potential alternatives to chemical methods. In the framework of biological methods, the colorless mutants of the sapstain fungi (i.e., *O. piliferum*, *O. floccosum*, *O. piceae*, and *O. plurianulatum*) have been studied for preventing staining of sapwood [12,13]. Sapwood was pretreated with these albino strains as a means of inhibiting the growth of sapstain fungi. Although these biological control agents exhibit environmentally friendly characteristics, they suffer from issues such as a relatively short duration of effectiveness and low stability.

The proliferation of the invisible sapstain fungi during international shipping enables the fungi to spread (via sporulation) to the otherwise unpolluted clean wood materials placed adjacent to the polluted materials [2]. For this reason, novel environmentally friendly methods that can prevent the spread of sapstain fungi by inhibiting their growth are required. In other words, an approach that can physically block fungal access in a non-toxic way should be developed. In addition, since fiber-shaped hypha generated by fungi can readily penetrate a thin microporous mat or membrane, a thicker one is required to prevent penetration of hypha, thus delaying or completely eliminating fungal invasion [14]. In the present study, we develop a facile, simple, and low-cost method for the desired wood protection by using an eco-friendly, lignin-based nanofiber (NF) mat. Lignin, a well-known natural polymer extracted from wood, has attracted considerable attention as a biocompatible material because of its cost-effective and non-toxic properties [15,16]. In particular, it has been recently proposed as a carbon source in the energy industry, including for batteries [17–19] and supercapacitors [20,21]. However, only limited applications of lignin have been reported so far, and lignin is still largely considered as a worthless by-product of pulp and paper manufacturing.

Herein, we fabricate eco-friendly, lignin-based electrospun NF mats to protect wood against sapstain fungal infection. Not only the NF mat is sufficiently thick to prevent fungi from physically accessing the underlying wood, but also the stretchability of NF mat revealed in the tensile tests indicates a sufficient durability in spite of the periodic expansion and contraction of the protected wood. Pine sapwood specimens covered with such mats are perfectly protected against fungal infection during a two-week incubation period.

2. Experimental section

2.1. *Eco-friendly lignin-based nanofiber mat*

Low-sulfonate alkali lignin (M_w = 10 kDa), polycaprolactone (PCL, M_n = 80 kDa), formic acid (≥95%), and acetic acid (99.7%) purchased from Sigma-Aldrich were used to prepare the electrospinning solution used for fabrication of the lignin-based nanofibers (NFs). A 15 wt% lignin/PCL (1:1 w/w) mixture was dissolved in a formic acid/acetic acid (1:3 w/w) mixture and was magnetically stirred for 24 h at room temperature. The solution was supplied by two syringe pumps (Legato 100, KD Scientific) equipped with 10 mL syringes (SGE Analytical Science) and 18-gauge needle tips (Nordson EFD) at a fixed flow rate of Q = 200 μL h^{-1}. Simultaneously, a high DC voltage of V = 9–10 kV was applied to each syringe by using DC power supplies (EL20P2, Glassman High Voltage). It should be emphasized that the electrospun NFs were collected using a rotating drum collector designed in-house with a side-to-side motion for fabricating a well weaved mat with a large area at a fixed rotation speed of V_r = 200 rpm over the course of 20 h (cf. Fig. 2a). The needle tip-to-collector distance was 6 cm in all cases.

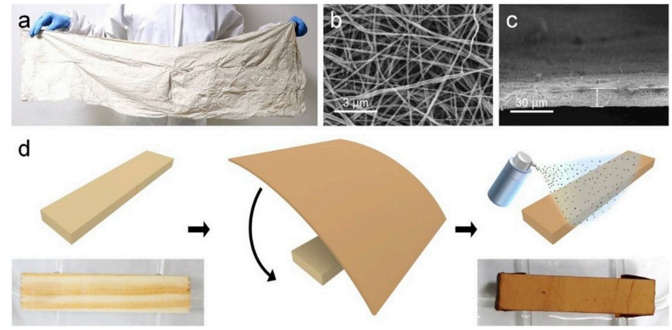

Fig. 2. (a) Photograph of the fabricated large-area lignin-based NF mat comprising four pieces. (b) Top-view and (c) cross-sectional SEM images of the NF mat. (d) Schematic of the fungal infection test process. Insets show photos of uncovered and mat-covered sapwood specimens.

2.2. *Fungal species and culture conditions*

The sapstain fungi, *Grosmannia koreana* KUC2078 and *Ophiostoma floccosum* KUC2014, were obtained from the Korea University Culture collection (KUC). These fungi were pre-cultivated on malt extract agar (MEA, Difco) at 25 °C for 14 days in the dark for sporulation. The spore suspension was prepared according to the ASTM D4445 Standard test method. Each spore suspension was uniformly inoculated onto the surface of all the sterilized pine sapwood (*Pinus densiflora*) specimens, i.e., uncovered and mat-covered sapwood specimens. Note that the mat-covered specimens were prepared by wrapping the specimens with the fabricated lignin-based NF mat (see Fig. 2d). Sterilization and preparation of the specimens were conducted according to the ASTM D4445 Standard Reference. The cross-section and length of each specimen were 5 × 10 mm^2 and 50 mm, respectively, and the moisture content was 40%. All the samples were incubated for two weeks and five replicate experiments were performed to verify the experimental reliability of the data. Eight specimens were used for each experiment, i.e., four uncovered and four mat-covered specimens. To maintain humidity in the Petri dishes for sufficient growth of the fungi during the tests, a filter paper soaked with 5 ml of distilled water was placed at the bottom of the Petri dish. A sterilized U-shaped glass rod was also placed above the filter paper as a spacer to prevent direct contact of the samples with the water-soaked filter paper.

2.3. *Characterization*

The lignin-based NF mats and the fungi grown on the surface of sapwood and mats were characterized by field-emission scanning electron microscopy (FE-SEM, S-5000, Hitachi) and optical microscopy (SZ61, Olympus). Note that the mats detached from the samples for the scanning electron microscopy (SEM) analysis were dried for one day and then sputtered with a thin platinum metallization layer to obtain clear images without undesirable charging. The average diameter of the NFs was estimated by measuring the diameter of two hundred NFs from

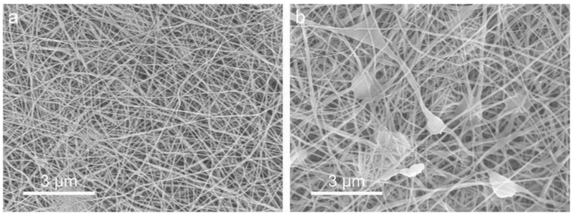

Fig. 3. SEM images of (a) the bare NF mat and (b) the fully-dried NF mat after water imbibition.

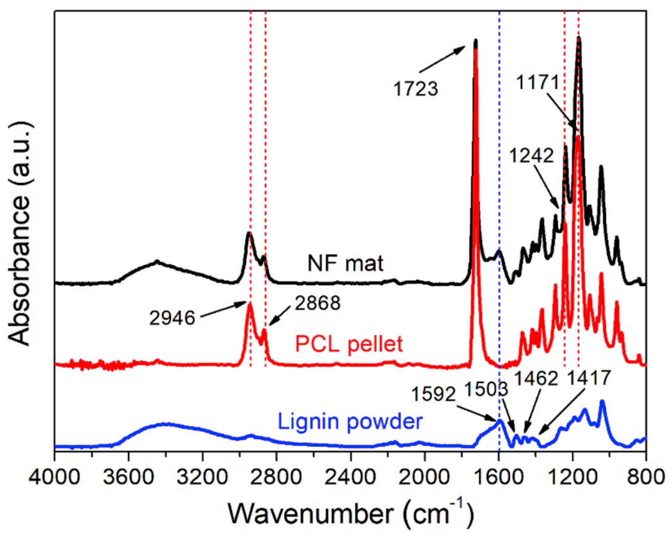

Fig. 4. FTIR spectra of lignin powder, PCL pellet, and NF mat.

the SEM images. The average pore size of the NF mat was determined by analysis of 30 pores from five SEM images. The presence of lignin and PCL in the NF mat was confirmed by Fourier transform infrared (FTIR) spectroscopy (Horiba LabRam Aramis IR2, Japan). Tensile and blister tests were conducted by using a universal testing machine (Model 5942, Instron, USA) equipped with a 100 N load cell. The size of the NF mats was 15 mm × 60 mm and the strain rate was set to 10 mm/min. For the blister test, a shaft of 1 mm in diameter was used to delaminate the NF mat from the surface of sapwood. The shaft was driven by Instron 5942, with the corresponding shaft speed being 5 mm/min. The NF mat was video-recorded during the blister test to measure the radius of the appearing blister. Note that the detailed procedure of the blister test of highly stretchable materials and the corresponding fundamental theory were developed and discussed in our previous studies [22,23].

3. Results and discussion

Figs. 2b and 2c show the SEM images of the lignin-based nanofiber (NF) mat. The average diameter of the NFs was 109 ± 19 nm with an average pore size of 0.23 μm^2, and the thickness of the mat was about 12.3 μm. Even though the NFs dissolved slightly after prolonged contact with water because of the presence of water-soluble lignin, as shown in Fig. 3, the fibrous structure was still maintained due to the presence of water-insoluble polycaprolactone (PCL).

To better characterize the presence of lignin and PCL in the NF mat, FTIR analysis of the lignin powder, PCL pellet, and the NF mat was performed, as shown in Fig. 4. The broad peaks between 3700 and 3000 cm^{-1} in the spectrum of the NF mat (see black line in Fig. 4) are attributed to the stretching vibrations of the lignin CH and OH groups [24,25]. Among the characteristic bands of lignin at 1592, 1503, 1462, and 1417 cm^{-1} (see blue line in Fig. 4), which are related to the aromatic rings and CH bonds, a band was observed at 1592 cm^{-1} only in the profile of the NF mat because the other peaks were covered by the PCL bands. The bands of the PCL pellet at 2946 and 2868 cm^{-1} (see red line in Fig. 4), which respectively correspond to the asymmetric and symmetric CH_2 stretchings, were clearly observed in the spectrum of the NF mat [26]. Bands were also observed at 1723 cm^{-1} for the carbonyl stretching and at 1242 and 1171 cm^{-1} for the asymmetric and symmetric COC stretchings, respectively, in the profile of the NF mat.

To investigate the protective effect of the NF mat on the sapwood specimens, the fungal growth on the specimens was optically studied, as shown in Fig. 5. *Grosmannia koreana* KUC2078 and *Ophiostoma floccosum* KUC2014 were used for the study (cf. Sec. 2.2). Note that *O. floccosum* is one of the most common sapstain fungi found in coniferous logs and lumber [27–29], whereas *G. koreana* is found in East Asia [30–32].

Figs. 5a–5f show the uncovered specimens after incubation for two weeks. The entire surface of the sapwood specimens was covered with spores and hyphae of both *G. koreana* and *O. floccosum* during the

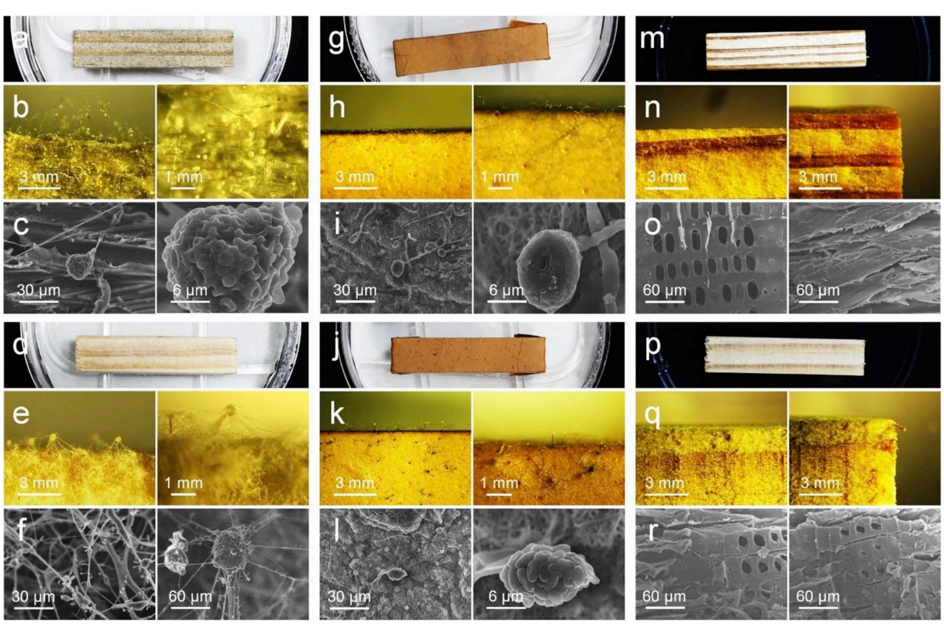

Fig. 5. Images of (a–f) uncovered and (g–l) mat-covered samples exposed to (a–c, g–i) *G. koreana* and (d–f, j–l) *O. floccosum* and incubated for two weeks: (a, d, g, j) photographs, (b, e, h, k) optical microscope images, and (c, f, i, l) SEM images. Images of (m–r) the mat-covered samples exposed to (m–o) *G. koreana* and (p–r) *O. floccosum* after removing the mat: (m, p) photographs, (n, q) optical microscope images, and (o, r) SEM images.

incubation period via fungal infection, resulting in discoloration of the specimens (Figs. 5a and 5d). The spores were entangled in a complicated manner and the dark-colored hyphae of *G. koreana* could be clearly observed (Figs. 5b and 5c). The spores and bright-colored hyphae of *O. floccosum* were also observed (Figs. 5e and 5f). The spores and hyphae of *O. floccosum* grew densely compared to those of *G. koreana*. However, the discoloration was more pronounced when *G. koreana* was used than when *O. floccosum* was used (Figs. 5a and 5d).

Figs. 5g–5j show the results for the mat-covered specimens after two weeks of incubation. A considerable number of spores and hyphae (though less than those observed in the case of the uncovered specimens) could be clearly observed on the specimens covered with both *G. koreana* and *O. floccosum*. Broad spherical spores of *G. koreana* and *O. floccosum* with dimensions of a few micrometers, along with fiber-shaped hyphae, were observed (Figs. 5i and 5l). The spore growth was retarded in the case of the mat-covered specimens (cf. "swollen" surfaces of the NF mats in Figs. 5h and 5k with those that appear plane in Figs. 5n and 5q) because the densely deposited NF mat inhibited hyphae penetration as well as spreading of the spores into the covered mats.

For detailed evaluation of whether the mat-covered samples were protected against fungal infection or not, the mats on the covered samples were carefully detached and the inner sapwood specimens were analyzed for *G. koreana* and *O. floccosum*. Unlike the uncovered specimens, the sapwood enclosed in the mat cover was perfectly protected against fungal infection (Figs. 5m–5r) during exposure to the fungi for two weeks. Optical microscopy and SEM analysis of the mat-covered specimens revealed that no spores or hyphae of *G. koreana* and *O. floccosum* were detectable on the surfaces of the wood specimens covered by the protective mats, thus confirming that these samples were completely protected against fungal infection (Figs. 5n, 5o, 5q, and 5r).

In addition to the protective effect of the NF mat on the wood samples demonstrated in the single-sample experiments described above, a test aiming at field applications, especially relevant for international trade, is also required. A study related to the wood trade reported that the number of stained woods infected by sapstain fungi increased at the final destination, indicating an obvious quarantine implication in international trade [2]. Fig. 6 shows the fungal growth in the stacked samples during incubation for two weeks, where the conditions mimic the process of wood transportation. Only the sapwood specimen located in the middle (marked with red in Fig. 6a) was inoculated with *O. floccosum*, whereas the other eight samples were not inoculated. Among them, four samples were not covered with the NF mats (indicated by yellow in Fig. 6a); the other samples were covered with such mats (indicated by blue in Fig. 6a). After two weeks of incubation, the surfaces of both the uncovered and mat-covered samples were found to be fungal-infected by the spread of *O. floccosum* from the inoculated sample located in the middle (Fig. 6b). It should be emphasized that the fungal growth was extremely severe at the surfaces facing the other samples (Figs. 6d, 6e, and 6f). These surfaces (which were not exposed to the environment) had a high moisture content ($>$ 60%), which resulted in accelerated fungal infection [33]. In spite of the significant fungal infection on the sample surface, the inner sapwood specimens were completely preserved (Fig. 7), as observed in the single-sample test (cf. Figs. 5p–5q).

The mechanical durability of the NF mat upon exposure to moisture should be explored because wood may be exposed to humid conditions during transportation, and fresh-cut wood also has a high moisture content in itself (cf. Section 2.2). Even though neither the fibrous nor porous structures underwent serious deterioration (see Fig. 3), the water-imbibed NF mats as well as the fully-dried NF mats (after water imbibition) showed considerable changes in the mechanical properties, as shown in Fig. 8. The averaged values of the Young's modulus (E), stress at failure (σ_{max}), and strain at failure (ε_f, see the purple-rectangles on the blue solid line in Fig. 8a) are listed in Table 1. Notably, for the water-imbibed and fully-dried NF mats, it was commonly observed that

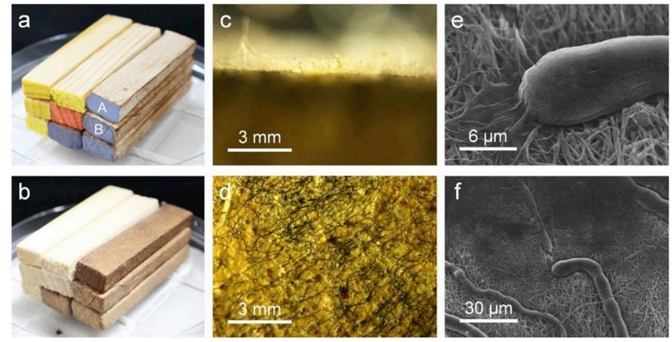

Fig. 6. Images of stacked samples: (a) at the beginning and (b) after incubation for two weeks. Only the specimen located in the middle was inoculated with *O. floccosum*. Optical microscope images: (c) top-surface of sample marked "A" in panel (a), and (d) top-surface of sample marked "B", which faced the bottom-surface of sample "A". (e, f) SEM images of the surface corresponding to panel (d).

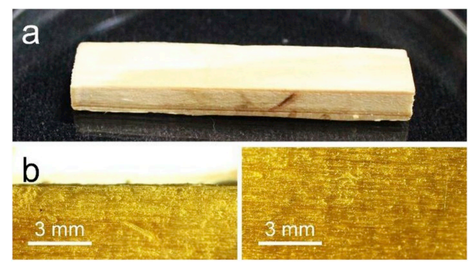

Fig. 7. (a) Photograph and (b) optical microscope images of sample marked "B" in panel (a) in Fig. 6 after removing the NF mat.

some parts of the mats remained unbroken until the perfect failure point (or strain at perfect failure, ε_{pf}, see purple circles on blue solid line in Fig. 8a), where failure seemingly occurred primarily at the junctions of the NFs and subsequently where there was dissolved lignin, resulting in a gradual decrease in the mechanical strength, even after failure.

The E and σ_{max} values decreased significantly by 0.1 MPa and 0.5 MPa, respectively, as water was imbibed into the NF mats, whereas the corresponding ε_f values increased from 59% to 71% (Fig. 8 and Table 1). Specifically, the relatively stiff NF mat became soft due to the presence of water-soluble lignin, which can be regarded as a positive effect when considering the periodic swelling/shrinking of wood depending on the moisture content [34,35]. On the other hand, although the E value of the fully-dried NF mat did not change significantly compared to that of the bare NF mat, the σ_{max} and ε_f values decreased remarkably (Fig. 8 and Table 1). Considering this mechanical degradation (cf. Fig. 8) as well as the fungally polluted surface of the NF mats after use (cf. Fig. 6), reuse of the NF mats does not appear feasible. However, it should be emphasized that the thermoplastic features of PCL and lignin with accompanying sterilization can facilitate refabrication of the NF mats after use, where studies on imparting thermoplasticity to lignin have been actively explored in recent years [36]. Thus, the developed mats are still expected to provide environmentally friendly and cost-effective alternatives for wood protection.

On the other hand, the adhesion energy T between the surface of sapwood and the lignin-based NF mat was measured to evaluate the delamination propensity of the NF mat at the interface. This was done was done using the blister test (cf. Sec. 2.3). Note that the blister test was conducted shortly after the sapwood specimen was wrapped by the NF mat (cf. Fig. 2d), which means that the NF mat was partially wetted due to the high moisture content of wood (cf. Sec. 2.2). The adhesion energy T of the NF mat to the sapwood can be expressed as [22,23]:

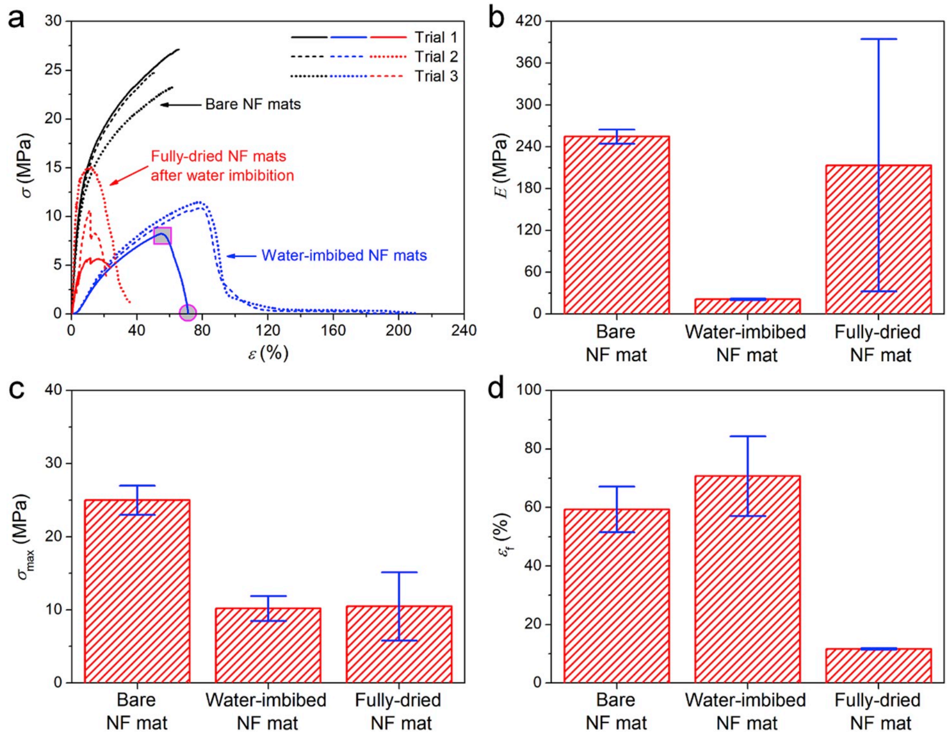

Fig. 8. (a) Stress-strain curve of the bare NF (black lines), the water-imbibed NF (blue lines), and the fully-dried NF mats after imbibition (red lines). Note that purple-rectangle and -circle marks correspond to points for ε_f and ε_{pf}, respectively. Corresponding results for the average (b) Young's modulus, (c) σ_{max}, and, (d) ε_f.

Table 1
Mechanical properties of bare NF, water-imbibed NF, and fully-dried NF mats after water imbibition.

	Bare NF mat	Water-imbibed NF mat	Fully-dried NF mat after water imbibition
E (MPa)	254.7 ± 10.2	20.9 ± 0.9	213.5 ± 181.0
σ_{max} (MPa)	25.0 ± 2.0	10.2 ± 1.7	10.5 ± 4.7
ε_f (%)	59.3 ± 7.8	70.7 ± 14.6	11.6 ± 0.2
ε_{pf} (%)		161.2 ± 77.8	27.1 ± 7.7

$$T = \frac{3}{8}\left(\frac{1}{\pi^4 Eh}\right)^{1/3}\left(\frac{P}{a}\right)^{4/3} \tag{1}$$

where E is the Young's modulus of the NF mat, which corresponds to the value of E of water-imbibed NF mat in Table 1 and the pushing force P by the shaft, which corresponds to the resistance force of the NF mat against delamination at the moment of blister formation (cf. Sec. 2.3). In Eq. (1), h and a are the thickness of the NF mat (12.3 μm) and the radius of the blister (Fig. 9a), respectively. The adhesion energy measured using the blister test and based Eq. (1) was 0.17 J m^{-2}. Note that the adhesion energy at the interface can vary according to the moisture content and periodic swelling/shrinking of wood.

4. Conclusions

The industrially feasible electrospinning technique equipped with a drum collector was used to fabricate large-area, eco-friendly lignin-based nanofiber (NF) mats for potential wood protection in the global wood trade. The presence of lignin and polycaprolactone (PCL) in the NF mat was confirmed by Fourier transform infrared analysis. To confirm the protective effect of the mats on wood samples, fungal infection tests were conducted with the sapstain fungi, *Grosmannia koreana* and *Ophiostoma floccosum*, mimicking a realistic wood-shipping situation. Although the uncovered pine sapwood specimens were fungally infected by the adjacent polluted specimen, the mat-covered pine sapwood specimens were perfectly protected against fungal infection during the incubation period of two weeks. In addition, the water-

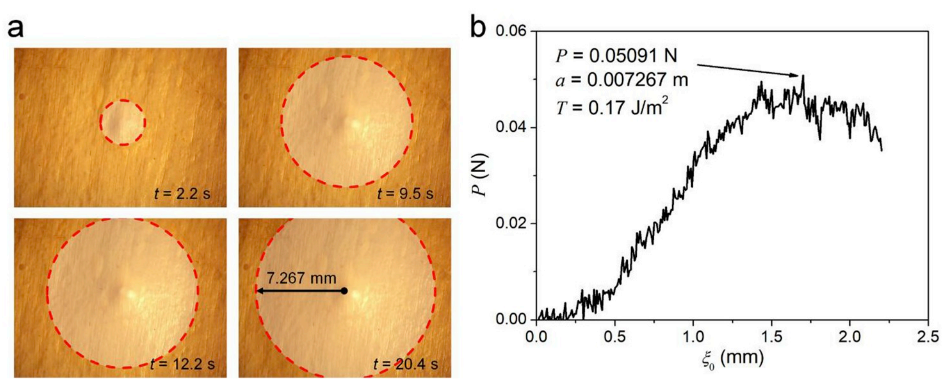

Fig. 9. (a) Images of the blistering NF mat during the blister test as a function of time t, with the radius of the blister shown. (b) The corresponding pushing force P as a function of the extension ξ_0 which corresponds to the blister elevation at the center.

imbibed NF mats showed reliable mechanical durability against the periodic swelling/shrinkage of wood exposed to different amounts of moisture. The new protective coating approach holds great promise for the prevention of fungal infections in the global wood trade.

Acknowledgement

This research was supported by the Technology Development Program to Solve Climate Changes of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016M1A2A2936760), NRF-2017R1A2B4005639, and Advanced Research Center Program (NRF-2013R1A5A1073861).

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