Supporting Information

Construction of chevaux-de-frise from cellulose nanocrystals to enable mechano-bactericidal activity on recycled waste cotton films

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Materials and methods

Materials

Waste cotton fabrics were collected from a second-hand shop in Montreal, Canada. Sulfuric acid, sodium hydroxide, and live/dead cell double staining kit were purchased from Sigma-Aldrich (MO, USA). ROS-Glo™ H_2O_2 assay kit was purchased from Promega (WI, USA). Glutaraldehyde was purchased from Fisher Scientific (ON, Canada). Bacteria strains used in this study, *E. coli* K12 ATCC 25404 and *L. monocytogenes* LM 1870, were respectively provided by the American Type Culture Collection (VA, USA) and Health Canada (ON, Canada).

Fabrication of CNC coated regenerated cellulose films

The fabrics were hydrolyzed by H_2SO_4 aqueous solution, and after dialyzing in distilled water for 48 hours, CNC suspension with a concentration of 0.1% was obtained. The fabrics were also dissolved in H_2SO_4 aqueous solution that was pre-cooled to -20 ℃, and then regenerated in NaOH solution. The RC films were washed with distilled water. Subsequently, a desired amount of CNC suspension was gradually poured onto a RC film under vacuum filtration. The obtained CNC/RC films were air dried at room temperature, and coded as RC, 3%CNC/RC, 5%CNC/RC, and 10%CNC/RC corresponding to a CNC content (based on the dry weight of RC film) of 0, 3, 5, and 10 wt%, respectively.

Structure and mechanical properties

The structures of CNCs, RC films, and CNC/RC films were studied by Nicolet 6700 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA) equipped with an ATR accessory.

The spectra were recorded as the average of 64 scans with the resolution of 4 cm^{-1} from 400-4000 cm^{-1} at 25 ℃, using the empty accessory as blank. XRD patterns were recorded by a Bruker D8 Discovery diffractometer (Bruker, Billerica, MA, USA), operating at 40 kV and 44 mA with the speed of 2°/min. The measurements were performed from 4° to 45°. SEM images were taken by a field-emission SEM (LEO ZEISS 1530 SEM, Germany) at 1 kV under 5000× magnification. Bacterial suspension (100 μL, ~10^4 CFU/mL) was dropped on CNC/RC films (1 cm × 1 cm) and allowed to incubate for 3 min in a Petri dish. Then, 2% (w/v) glutaraldehyde in PBS was added on the films to fix bacteria. After 20 min, the films were gently washed with Milli-Q water and freeze-dried for SEM observation. AFM images were acquired by directly scanning the surfaces in a tapping mode of silicon cantilevers with a force constant of 37 N m^{-1}, a frequency range of 100-500 kHz, and a nominal tip radius of 6 nm. The tensile strength, Young's modulus, and elongation at break of RC and CNC/RC films were tested by an Instron 5967 universal testing machine (Instron Corp., MA, USA) at the speed of 5 mm/min with the initial grip separation distance of 20 mm. Five strips, with the dimension of 6 cm×1 cm (length × width), were cut from each film. The thickness of each strip was detected by a digital micrometer (Fisherbrand, Fisher Scientific, Canada) with the precision of 1 μm.

Bactericidal activity

RC and CNC/RC films were cut into squares (1 cm × 1 cm) and laid in a Petri dish with no specific sterilization treatment. *E. coli* and *L. monocytogenes* strains were stored at -80°C. To reanimate strains, *E. coli* and *L. monocytogenes* were streaked onto Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) agar, respectively and then incubated at 37°C for 24 hours. A well isolated colony was used to inoculate 20 mL of Tryptic Soy Broth (TSB) with *E. coli* or 20 mL of BHI broth with *L. monocytogenes*. Cultures were incubated in a shaker incubator at 37°C for 18 hours. A 1:2000 dilution of the overnight culture was made into fresh broth, and incubated for 2.5 hours at 37°C (~10^8 CFU/mL). The cells were washed 3 consecutive times with phosphate-buffered saline (PBS) and diluted to a concentration of 10^4 CFU/mL in PBS. Bacterial suspensions (200 μL, 10^4 CFU/mL) were placed on the films and incubated at either room temperature (RT) or 4°C for 3 min, 1 hour, or 2 hours. For the free CNC suspensions with different concentrations, 200 μL bacterial suspensions were added in 5 mL of CNC solutions (3, 5, and 10 wt%) and incubated. After incubation, the film was washed with 1.8 mL of PBS to recover any attached cells and to obtain a dilution of 1:10, which was further diluted to 10^{-5}. CFUs were enumerated by spread plating of 100 μL of each dilution on TSA for *E. coli* and BHI agar for *L. monocytogenes* suspensions. The plates were incubated at 37 ℃ for 24 h. A logarithmic reduction was calculated and expressed as Log_{10} CFU following plate count. As a

contrast, CNC suspensions (5 mL) were directly mixed with 2 mL of bacterial suspensions. The inoculated tubes were positioned onto a tube mixer and rotated at low speed for either 3 minutes, 1 hour, or 2 hours. After the contact period, 100μL of the mixture of bacterial suspensions and CNC suspensions were recovered and diluted to 10^{-5}, and plated on the respective agar and incubated at 37°C for 24 hours.

Cell viability assay and reactive oxygen species production

Viability of bacterial cells after contacting with RC and CNC/RC films was evaluated using live/dead cell double staining kit (Sigma-Aldrich). The recovered bacterial suspensions were added onto a glass slide and observed using the multiphoton confocal Nikon A1R MP microscope (NY, USA) with an excitation wavelength of 490 nm. Production of reactive oxygen species (ROS) by bacteria after contacting with RC and CNC/RC films was measured using the luminescence kit ROS-Glo™ H_2O_2 Assay (Promega). The recovered bacterial suspensions were added into a white clear-bottom 96-well plate with 20 μL of H_2O_2 substrate solution and incubated for 6 hours. Other reagents were added as per the instruction. Luminescence was measured using BioTek Synergy HTX Multi-Mode Reader (VT, USA).

Statistical analysis

The experimental results were presented as the mean of three replicates ± SD. Statistical evaluation was carried out by ANOVA followed by multiple comparison tests using Duncan's multiple-range test with significant differences within samples at $p < 0.05$. All analyses were performed through SPSS statistical software (IBM, New York, NY, USA).