Glass-like transparent and heat-sealable films of cellulose nanoworms via ethanol triggered swelling of esterified cellulose

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Supporting information

Fig. S1 Modified cellulose in water.

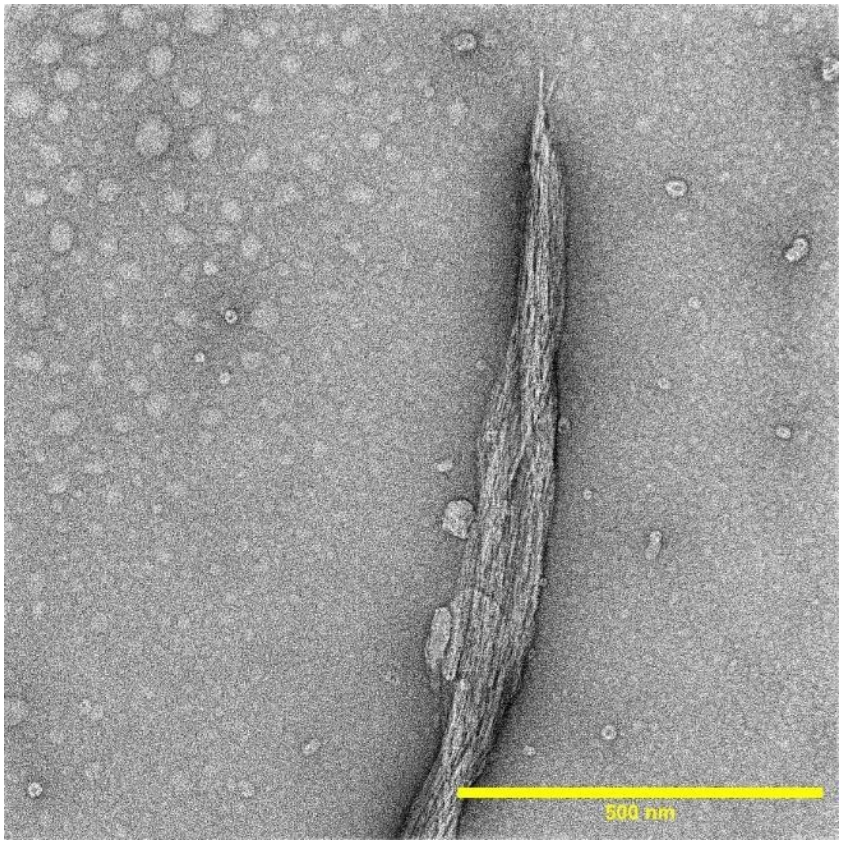

Fig. S2 TEM image of cellulose nanoworm suspension.

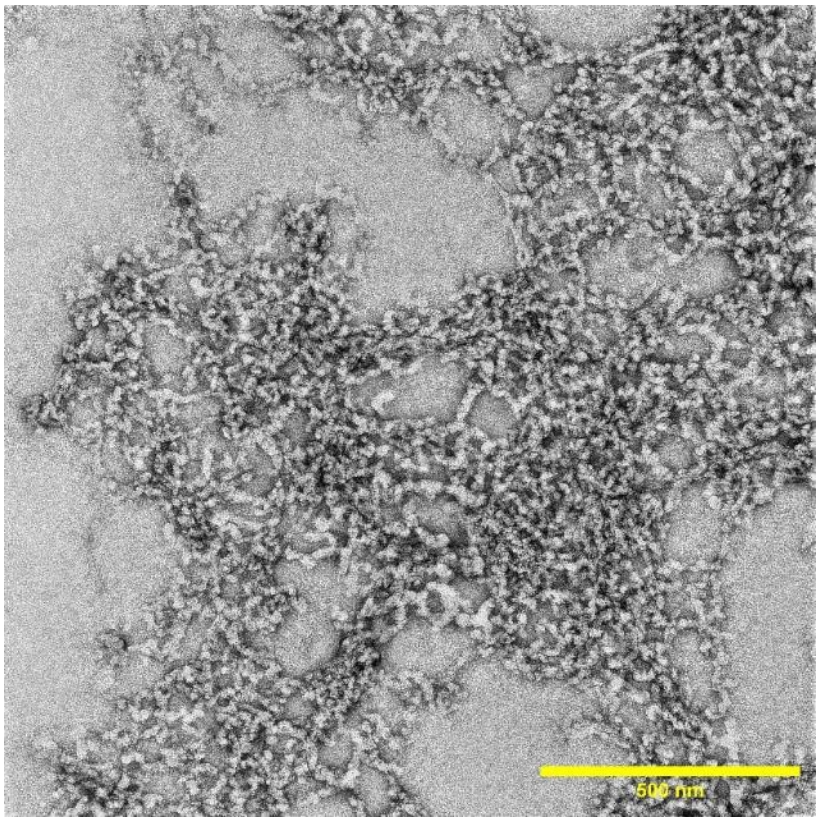

Fig. S3 TEM image of cellulose nanoworm suspension.

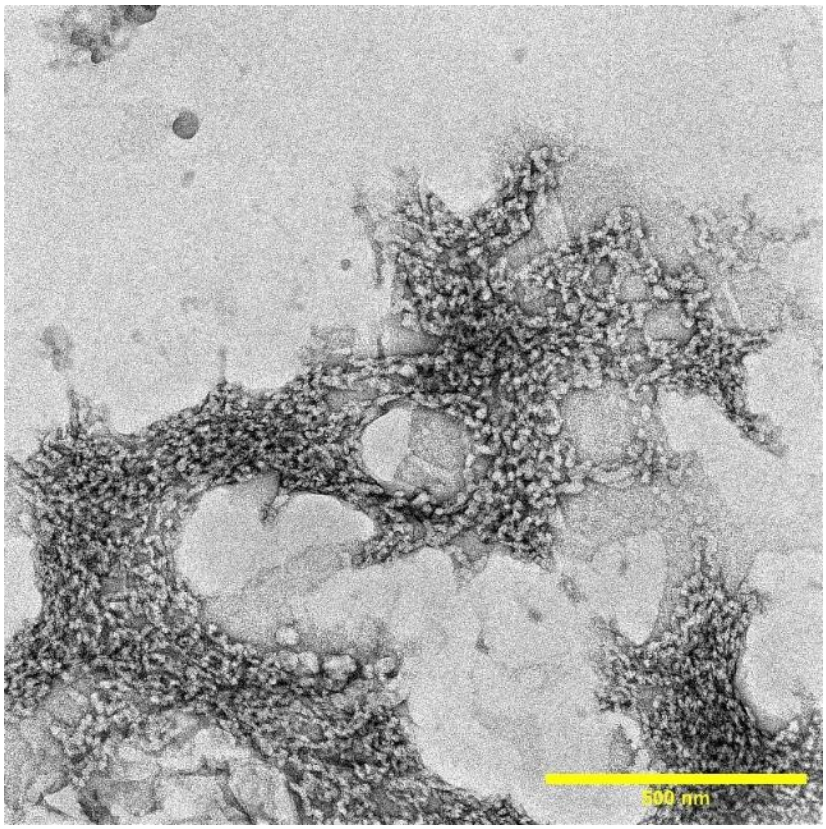

Fig. S4 TEM image of cellulose nanoworm suspension.

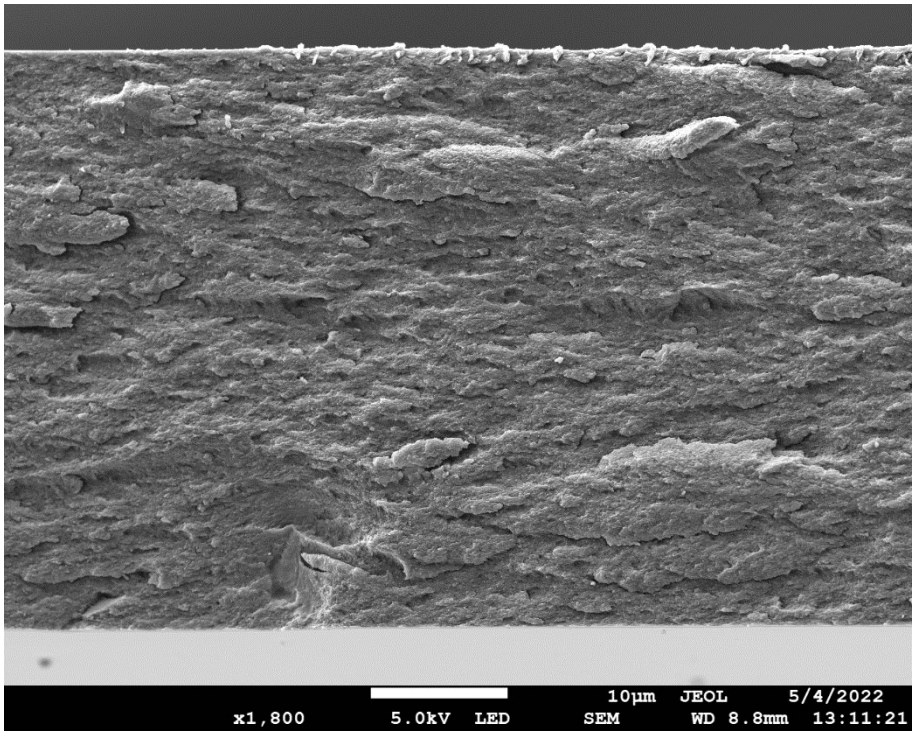

Fig. S5 FESEM cross-sectional image of nanoworm film.

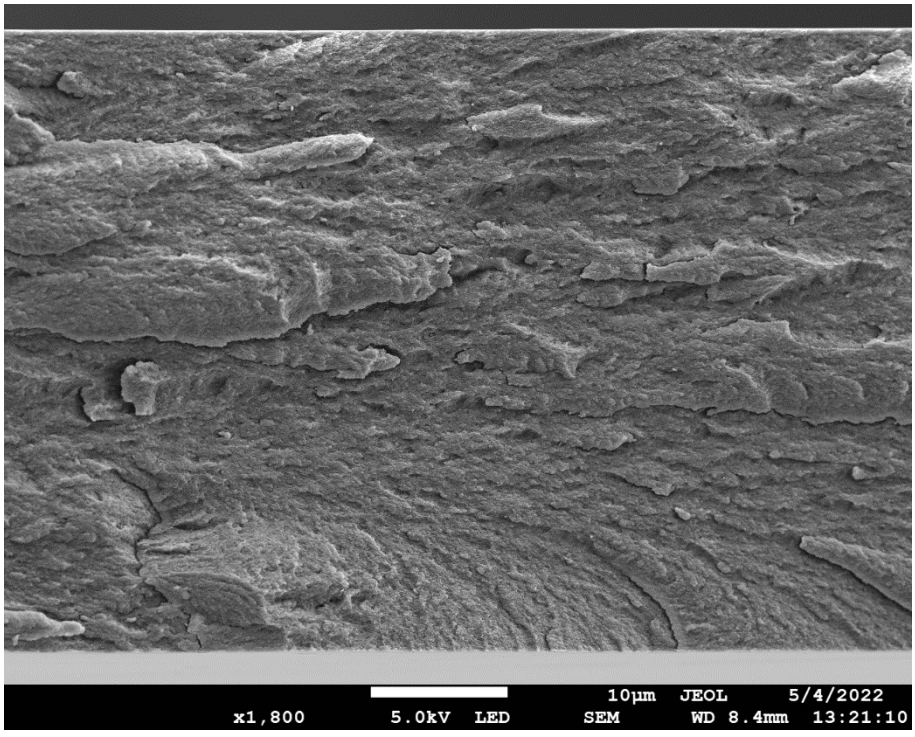

Fig. S6 FESEM cross-sectional image of nanoworm film.

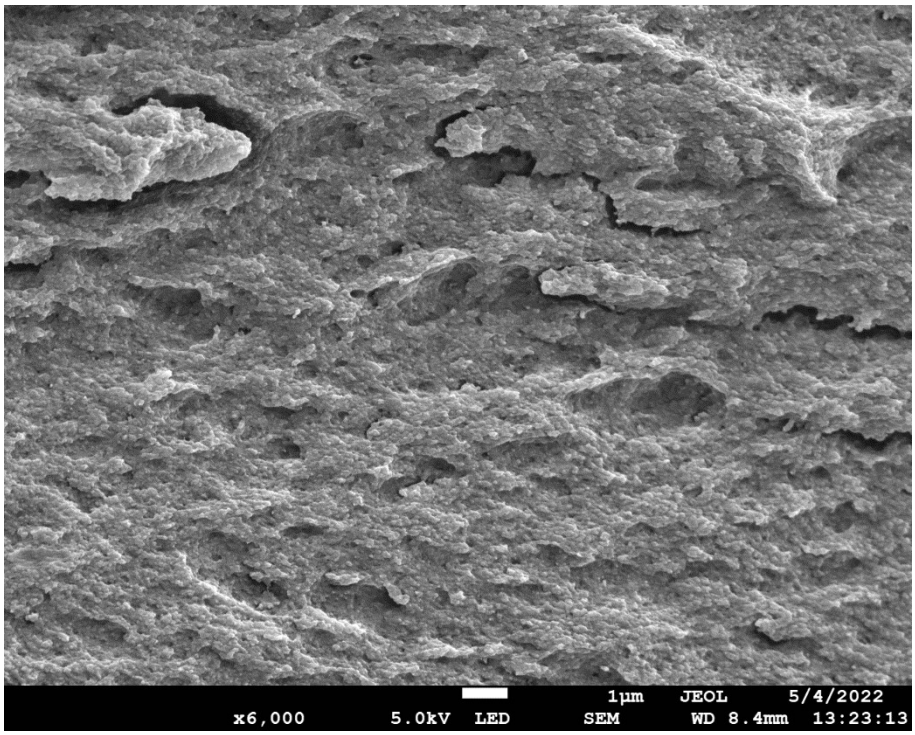

Fig. S7 FESEM cross-sectional image of nanoworm film.

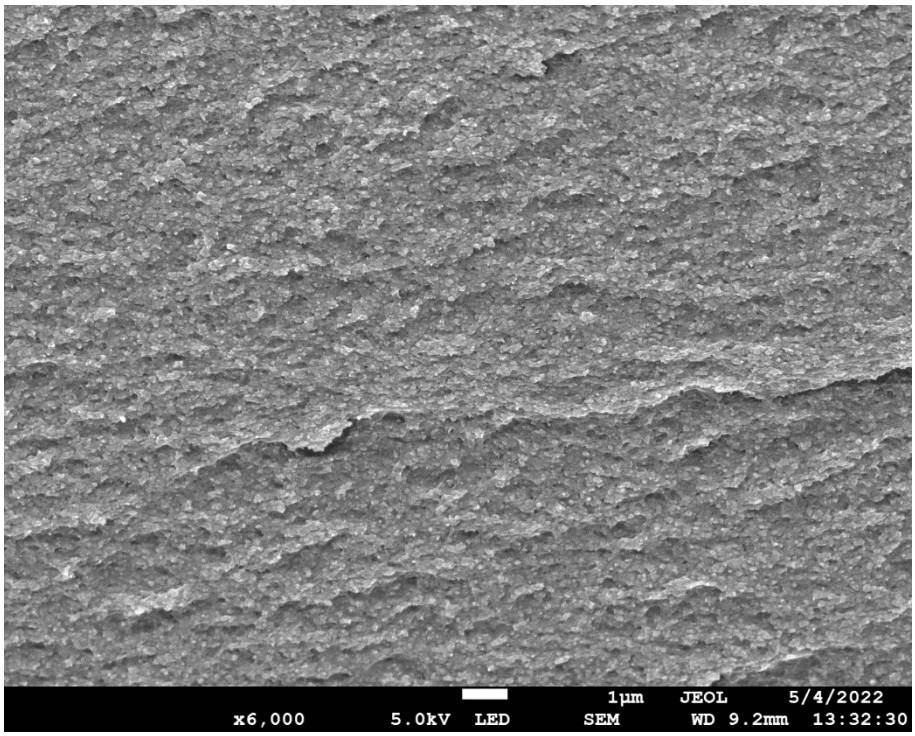

Fig. S8 FESEM cross-sectional image of nanoworm film.

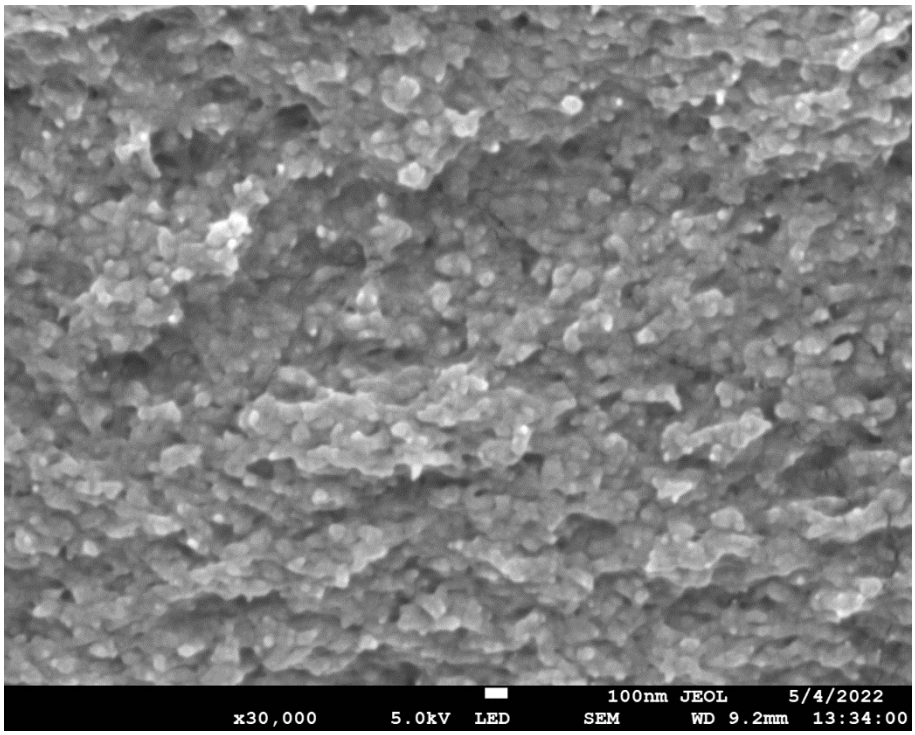

Fig. S9 FESEM cross-sectional image of nanoworm film.

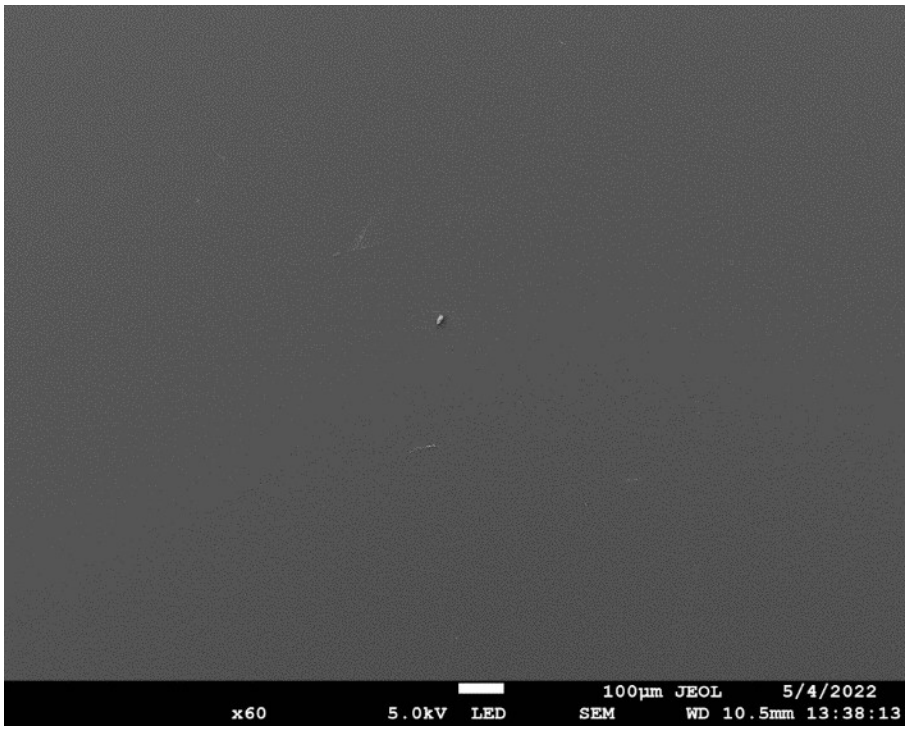

Fig. S10 FESEM surface image of nanoworm film.

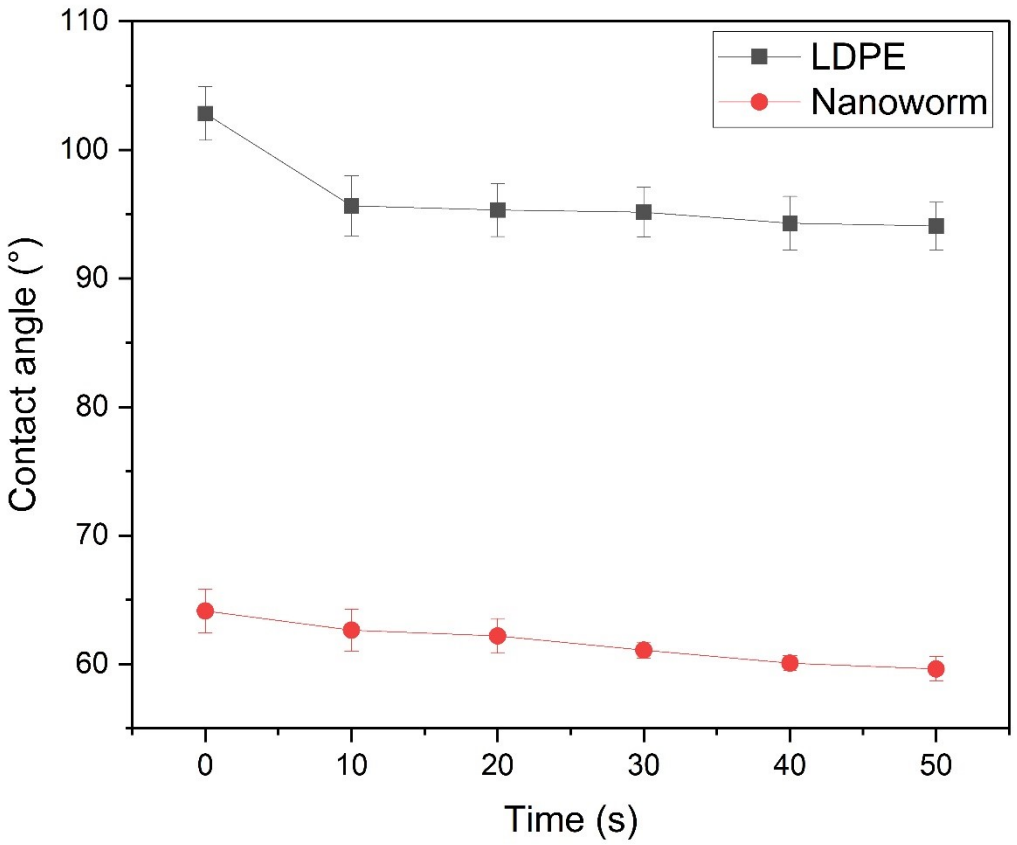

Fig. S11 Contact angle of nanoworm film and LDPE reference film as a function of time.

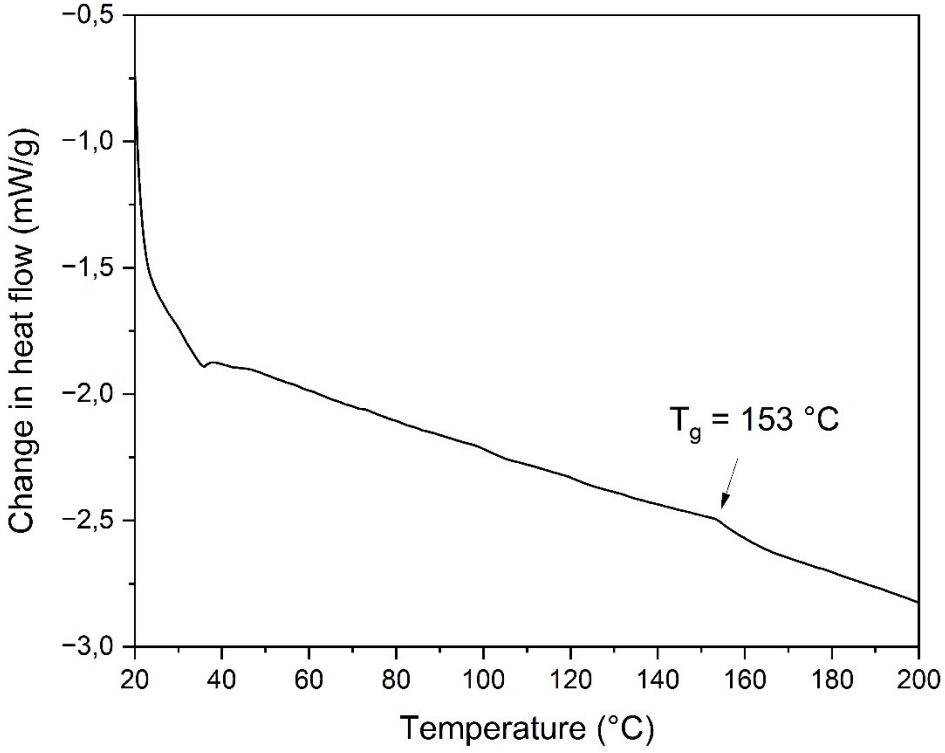

Fig. S12 DSC curve of modified cellulose.

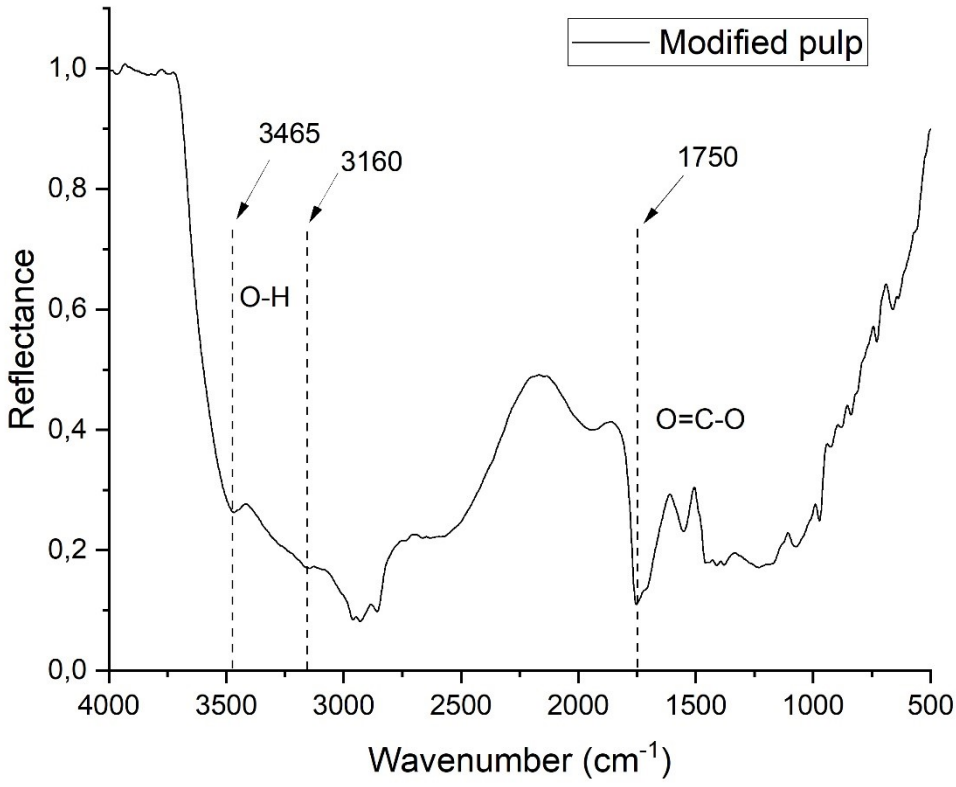

Fig. S13 DRIFT spectrum of modified cellulose.

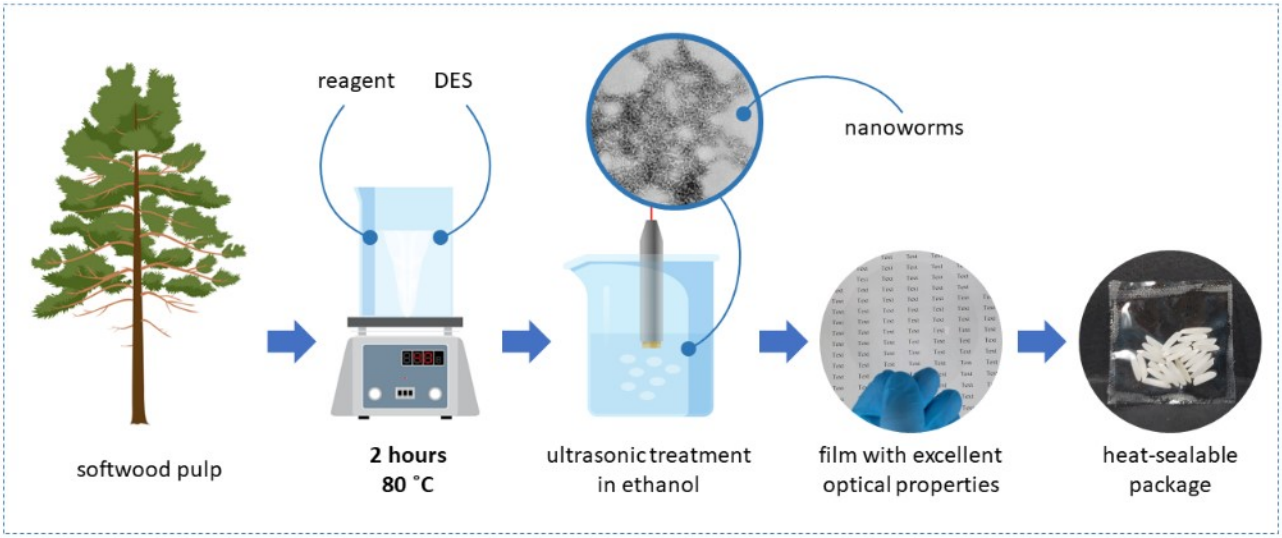

Fig. S14 Schematic diagram of production process of nanoworm film.

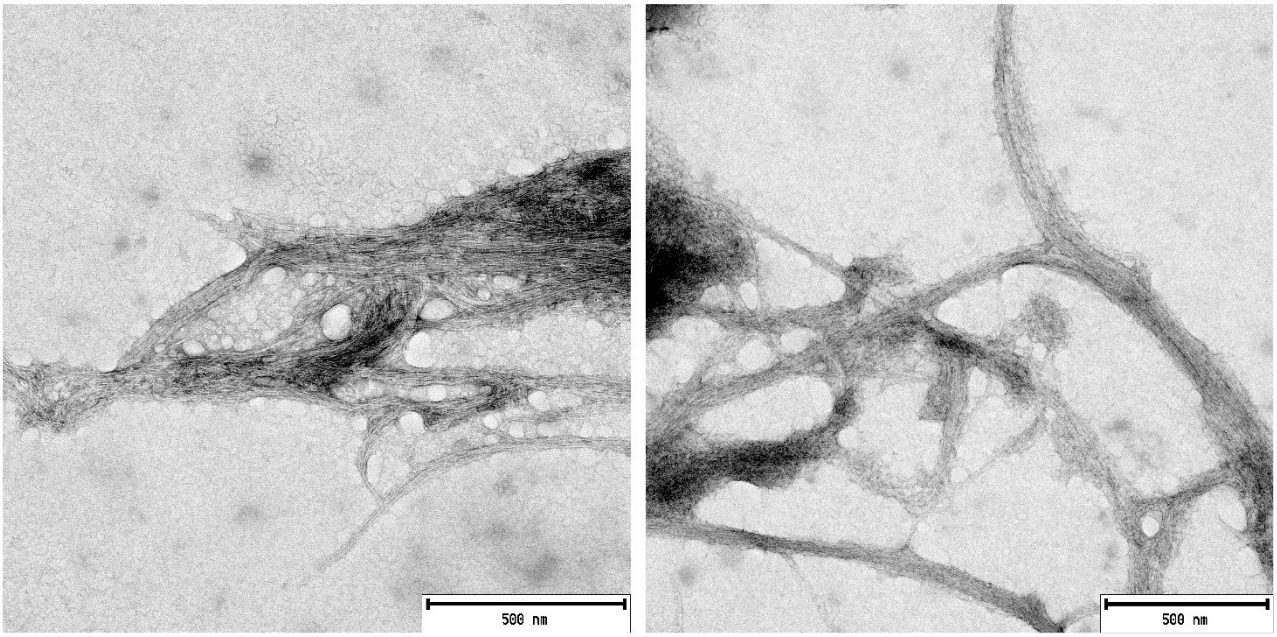

Fig. S15 TEM images of ultrasonicated cellulose pulp.

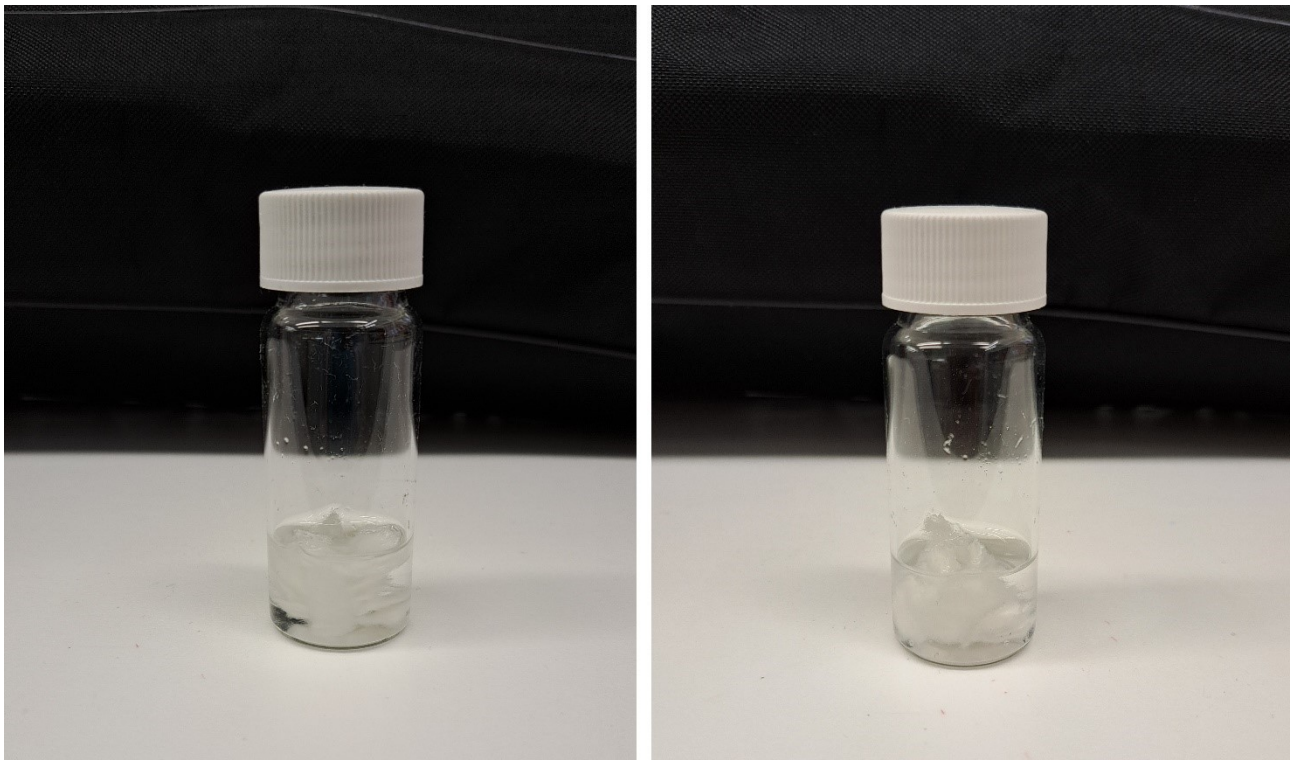

Fig. S16 Original cellulose fibers in ethanol before (left) and after (right) ultrasonication.

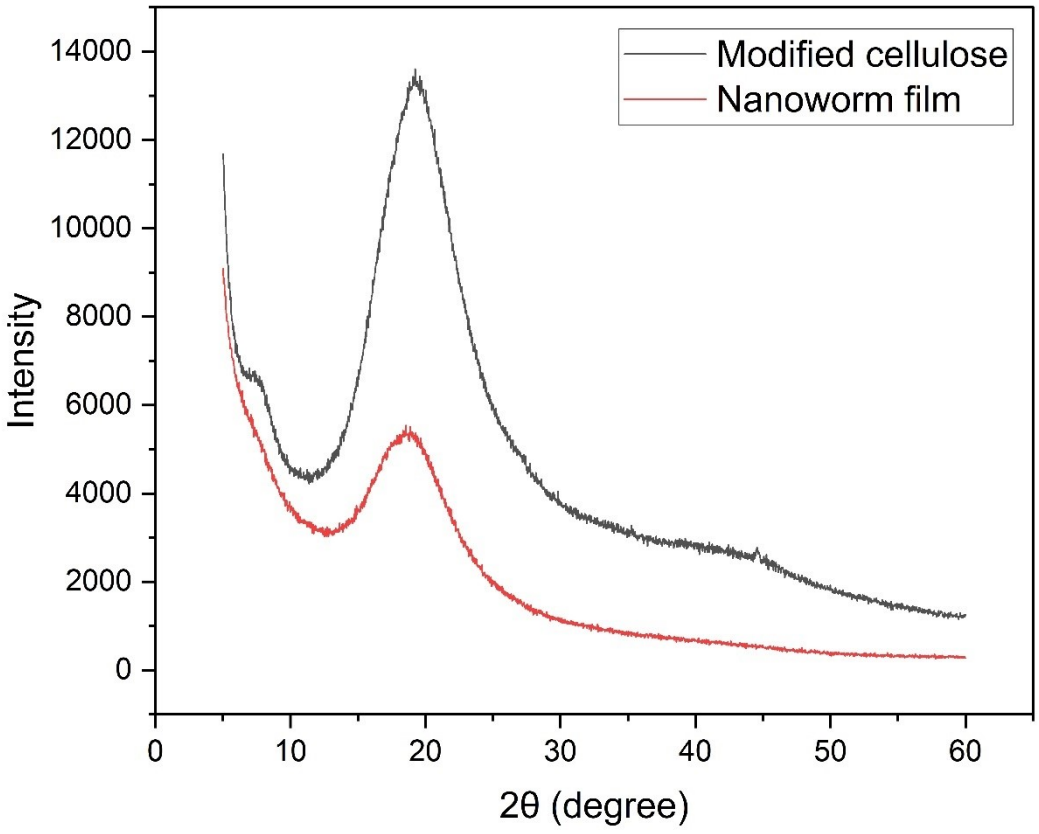

Fig. S17 XRD spectrum of modified cellulose and nanoworm film.

Analysis of antibacterial properties

A Gram positive (*Bacillus Subtilis*, *B. Subtilis*) and Gram negative (*Escherichia coli*, *E.Coli*) bacteria species were used for analyzing the antimicrobial properties of the nanoworm film. Before the experiments, glycerol stocks were streaked on LB agar to obtain 24 h cultures. Antimicrobial qualitative assessment and Minimum Inhibitory Concentration (MIC) Assay were performed using following procedure: 20 µl of bacterial suspension with OD600 of 0.6 diluted in 80 µl broth was evenly spread on plastic Petri dishes containing agar. Nanoworm films were cut to similar dimensions and were placed onto the plates to have a contact with the bacteria. The plates with the films were incubated at 37 °C for 18 h. The films were removed, and the area of bacterial growth inhibition following contact with the nanoworm films was assessed.

To establish the quantitative MIC values of the obtained nanoworm film, 150 µL of sterile broth was added in each well of sterile 96 well plates. 15 µL of microbial suspension adjusted to 106 CFU/mL was added in each well. Nanoworm films were cut to similar dimensions and were placed onto each test well. The MIC values were established after 24 h incubation at 37 °C by naked eye analysis and spectrophotometric measurement (absorbance at 600 nm). Each experiment was performed in triplicate and repeated on at least three separate occasions.

In MIC test, nanoworm film (diameter of 1.2 cm) had a small inhibition zone in *E. coli* and *B. Subtilis* (Figure R3), while filter paper (diameter of 1.2 cm) had a smaller inhibition zone only in *B. Subtilis* than nanoworm film. For absorbance measurement (Figure R4), nanoworm film had 19% of bacteria in *E. coli* and 13% bacteria in *B. Subtilis* compared to control. Both measurements indicated that nanoworm film had antibacterial properties. The nanoworm film was not a strong bacterial inhibitor, due to small inhibition zone, but nanoworm film demonstrated to exhibit strong bacterial anti-adhesive properties, since bacterial growth was significantly decreased compared to control. Antibacterial properties of nanoworm film are likely associated with imidazolium cation, which has shown antibacterial properties (DOI: 10.2174/1389557520999201209213648).

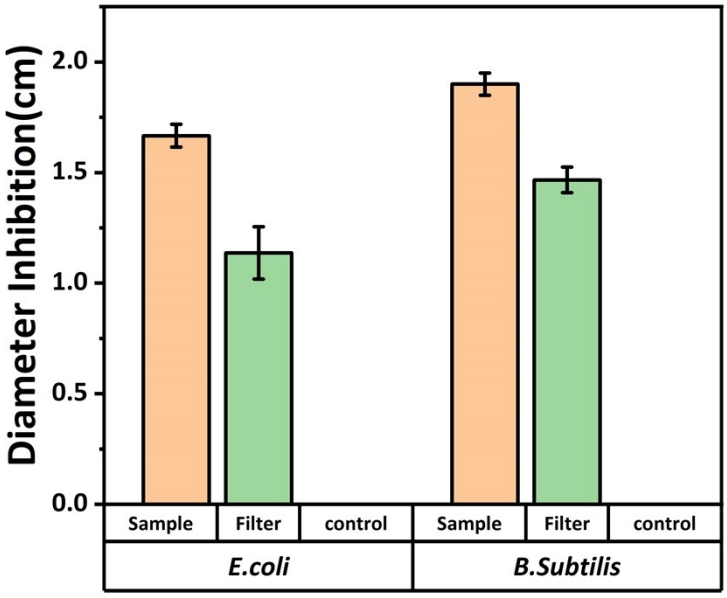

Fig. S18 Diameter area of growth inhibition in *E. coli* and *B. Subtilis* for nanoworm film (sample) and filter paper. There was not a control sample.

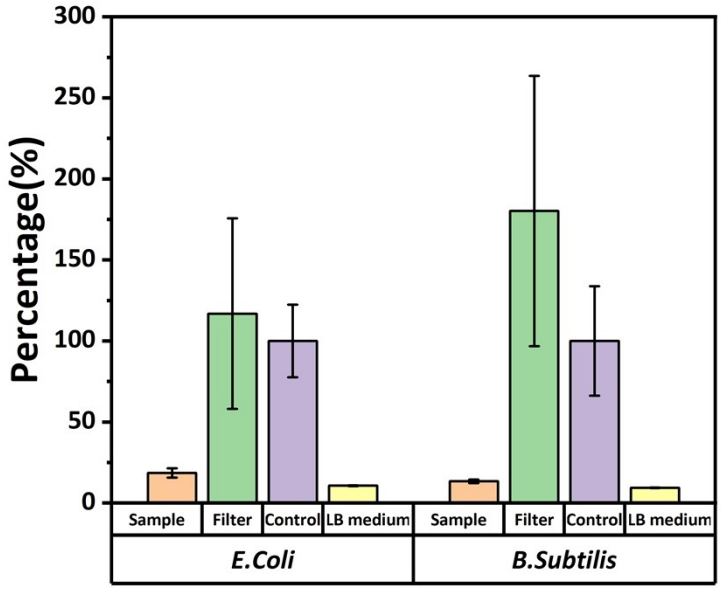

Fig. S19 Absorbance measurement of bacteria growth inhibition for nanoworm film (sample) and reference filter paper. Results are reported as a ratio of absorbance of specimen to the absorbance of control.

Biodegradability test

Biodegradability test was performed for nanoworm film, polyethylene plastics (carrier bag and plastic wrap), parafilm and a chemically nontreated CNF paper by burying the samples in soil for six weeks in a depth of 10 cm. The soil was watered once a week, and the samples were monitored periodically to assess the biodegradation degrees. After three weeks chemically nontreated CNF paper was almost completely disappeared (Figure S20) and later whole paper disappeared totally. On the contrary, none of the other films showed any signs of degradation during this time. However, the biodegradation period remained relatively brief, lasting only six weeks. This is notably shorter than the average biodegradation time of 159 days, during which bioplastics typically achieve a degradation level of 39.7% in soil (https://doi.org/10.3389/fchem.2021.671750). To attain certification as a biodegradable material in soil, the standard requires that over 90% degradation must occur within two years at a temperature of 25°C (doi: 10.1021/acs.biomac.2c00336). Therefore, a longer duration would likely yield a more significant and conclusive results. Nanoworm film was tested with two different bacteria and results (Figures S18 and S19) showed some antibacterial behavior, which may also affect biodegradability of the films. If bacteria are not able to approach and grow on the film, bacterial degradation may be prevented. However, antibacterial tests do not indicate the activity of fungi, including yeasts and molds, which are the other microorganisms responsible for biodegradation (DOI 10.1007/s40974-017-0064-9). In addition, the nanoworm film exhibited a hydrophobic nature, as illustrated in Figure 5. This hydrophobic characteristic hinders water penetration, and since water is known to play a substantial role in the biodegradation process (doi: 10.1021/acs.biomac.2c00336), additional testing is required to determine the actual biodegradability of the nanoworm film.

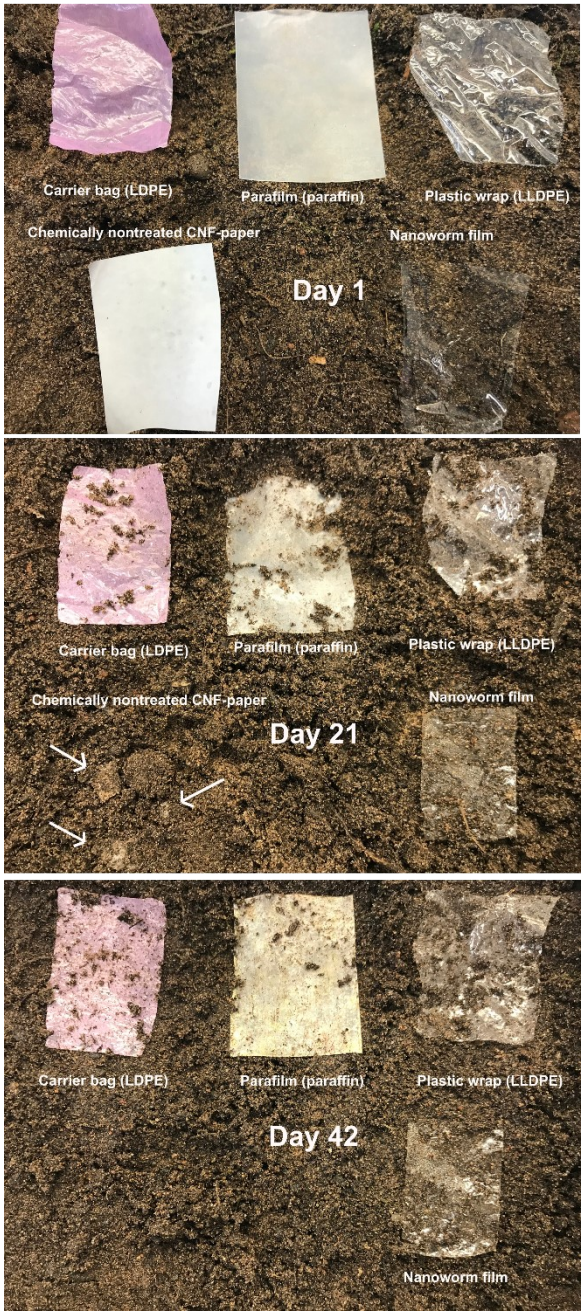

Fig. S20 Biodegradability tests of nanoworm, LDPE, paraffin, LLDPE films and chemically nontreated CNF paper on day 1 and after three and six weeks.