Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information for:

Cellulose nanofibrils improve dispersibility and stability of silver nanoparticles and induce production of bacterial extracellular polysaccharides

Min S. Wang,[a] Feng Jiang,[b] You-Lo Hsieh,[b] and Nitin Nitin[*a,c]

[a]Food Science and Technology Department, [b]Polymer and Fiber Science, [c]Department of Biological and Agricultural Engineering, University of California, Davis, Davis, CA 95616 USA

*Corresponding author:
Nitin Nitin
nnitin@ucdavis.edu

Dispersibility and stability of CNF-AgNP after dialysis and over time

The post-dialyzed CNF-AgNP samples remained stable after five-month of refrigerated storage at 4°C, as indicated by the minimal peak shift and height changes (grey line, Fig S1). The stability of CNF-AgNPs over extend lengths of time confirms that CNFs were excellent dispersing agents that prevent the aggregation of CNFs as well as the surface bound AgNPs.

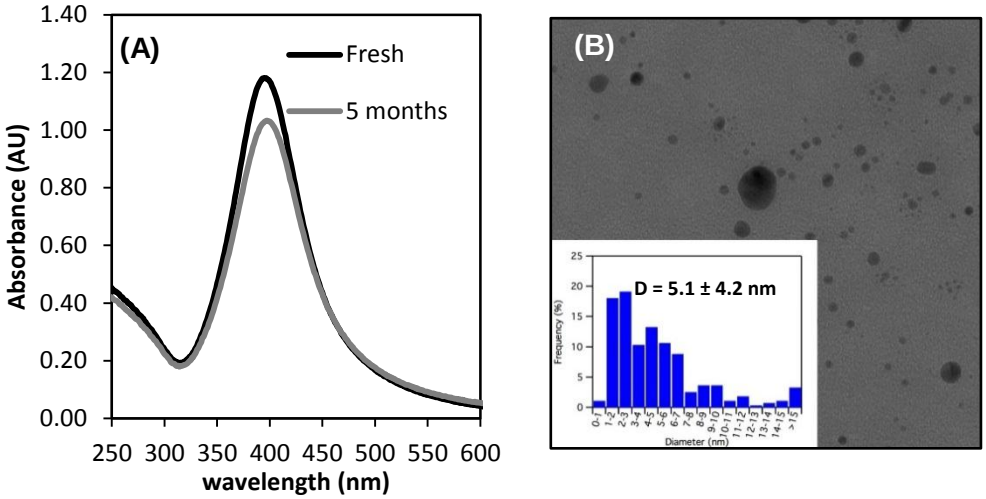

Figure S1. (A) UV-vis spectra of the post-dialyzed CNF-AgNP sample as freshly dialyzed (black line) and after 5 month storage at 4°C (grey line). (B) TEM image of the pre-dialyzed CNF-AgNPs. Inset shows the size distribution of AgNPs of the pre-dialyzed sample.

Effects of free Ag^+ ions and unbound AgNPs on bacterial growth

The importance of sample dialysis to remove free Ag^+ ions and unbound AgNPs was further illustrated using both an undialyzed and dialyzed CNF-AgNP sample (300 μM of equivalent Ag^+ concentration) and evaluated for their effects on bacterial growth. The bacterial growth kinetics using an undialyzed and dialyzed CNF-AgNPs was compared. From the bacterial growth curve, the undialyzed CNF-AgNPs caused a decrease in absorbance measurements at OD_{600nm} (Fig S2), suggesting that the undialyzed CNF-AgNP could reduce bacterial growth in the suspension and exerts antibacterial effects. On the other hand, when the dialyzed CNF-AgNPs were incubated with bacteria, only an initial suppression of growth was observed. This result indicates that the dialyzed CNF did not inhibit bacterial growth. The antibacterial effects of the undialyzed CNF-AgNP were likely due to the presence of the free Ag^+ ions and smaller unbound AgNPs, as suggested by previous studies[1, 2]

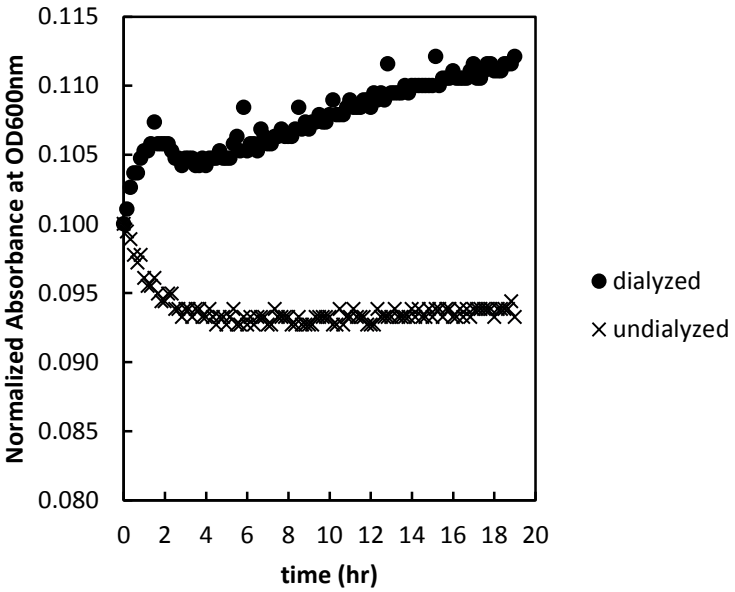

Figure S2. Bacterial growth kinetics when incubated with 300 μM of undialiyzed (×) and dialyzed (●) CNF-AgNPs samples and incubated at 37°C for 18 hr.

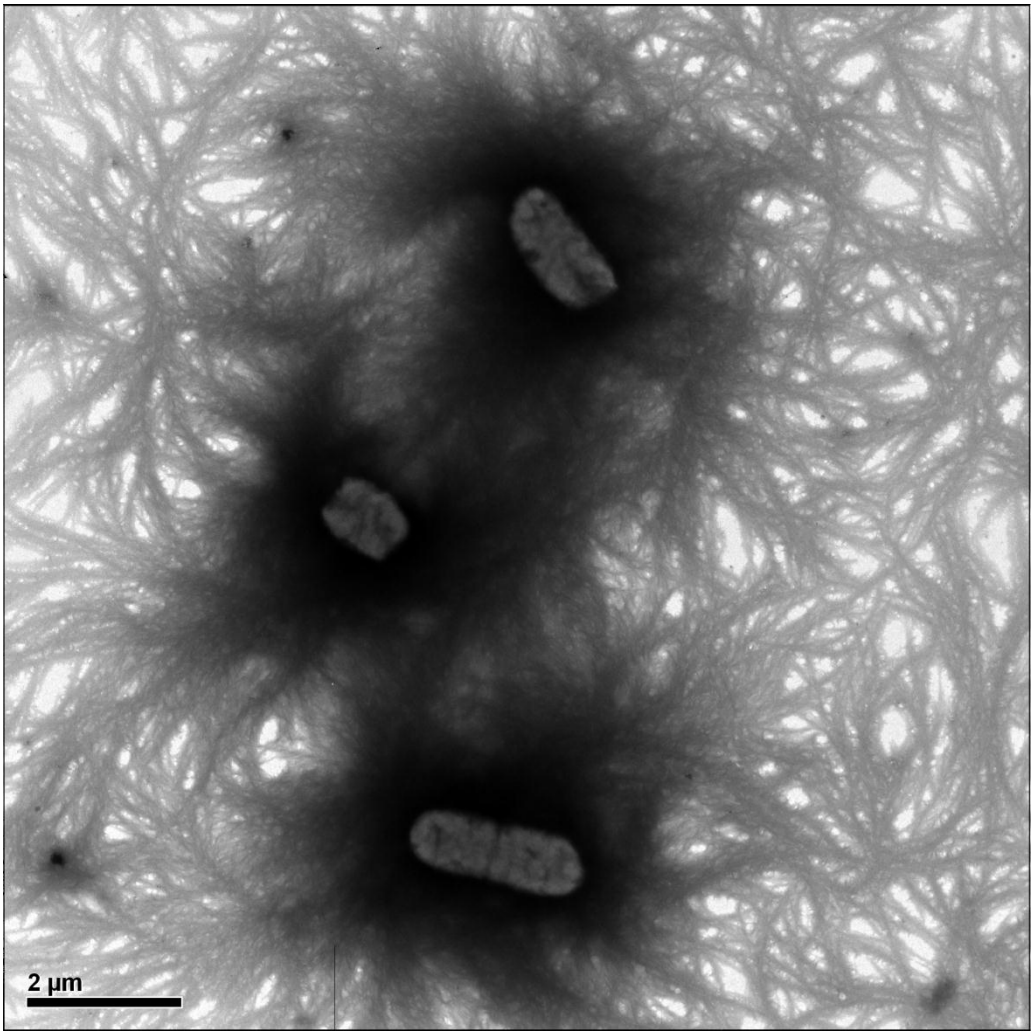

Figure S3. Clustering of multiple bacteria caused by CNF-AgNP by TEM. Bacteria (10^7 CFU/mL) was incuabted with 150 µM CNF-AgNPs and incubated at 37°C for 18 hr. The overproduction of bacterial EPS resulted in clustering between neighboring bacteria.

CNF-AgNPs caused bacterial cell death by damaging the bacterial cell wall and membrane

To further confirm that the CNF-AgNPs caused bacterial cell wall and membrane damage, an optical imaging approach using a membrane impermeable dye, propidium iodide (PI) was used. Since the PI dye can only enter cells with substantial membrane damage, a PI-labeled cell would be indicative of cell membrane damage. From the fluorescence images (Fig S4), the CNF-AgNP sample (Fig S4B) showed a large number of aggregated bacteria that are labeled with PI (red) compared to the CNF sample (Fig S4A). This suggested that the antibacterial mechanism of CNF-AgNPs was caused by the perforation of bacterial cell wall and membrane, leading to eventual cell death. Thus, the fluorescence imaging results further confirmed that the attachment of AgNPs to the bacterial cell surface as observed by TEM imaging (Fig 7H), could lead to subsequent damage to the cell wall and membrane, causing bacterial cell death (Fig S4B).

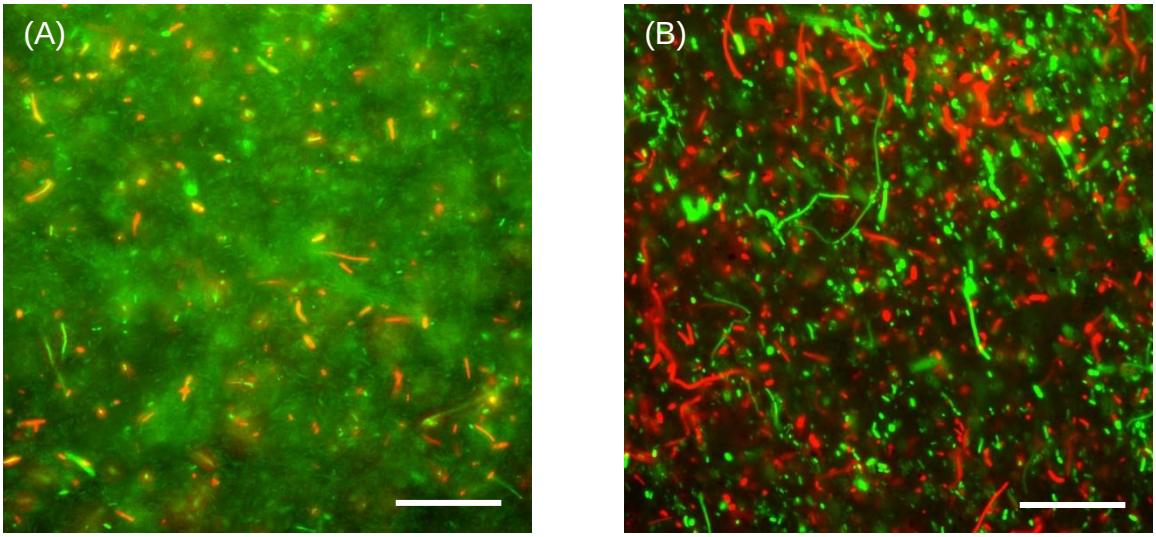

Figure S4. (A) CNF or (B) 450 μM CNF-AgNP was added to 10^7 CFU/mL bacteria in LB media in a 96-well plate and incubated at 37°C for 18 hr. Following the overnight incubation, 5 μg/mL of the propidium iodide (PI) dye was added to the bacterial suspension in the 96-well plate and incubated for 5 min to label the dead bacteria. Then, 1 μg/mL of the SYBR green dye was added to label all bacteria (dead and alive) in each sample. The fluorescence image of each sample was taken using an Olympus IX-71 fluorescence microscope with a 40× objective. The fluorescence excitation and emission for the PI and SYBR green dyes were 540 nm/605 nm and 480 nm/520 nm, respectively. Scale bar = 20 μm.

References

[1] Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., *et al.*, The bactericidal effect of silver nanoparticles. *Nanotechnology* 2005, *16*, 2346-2353.

[2] Pal, S., Tak, Y. K., Song, J. M., Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. *Appl Environ Microb* 2007, *73*, 1712-1720.