Nano-patterned bacterial cellulose hydrogel exhibits bactericidal activity against *E. coli* and *K. pneumoniae*

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Statement of Purpose: Biomedical devices are at risk of infections by microbial contamination during surgery, by hematogenous dissemination of bacteria from infections elsewhere in the body, or by the entrance of bacteria through body orifices in contact with biomaterials[1]. Once on the surface, bacteria aggregate into biofilms leading to severe complications that compromise biomaterial function and patient morbidity. Biofilms are difficult to eradicate by conventional systemic antibiotic therapy, while other strategies such as chemical modification of the material surface with biocidal compounds and delivery of nanoparticles at the infected site, can induce multidrug-resistant strains and produce a toxic accumulation of unwanted products in other tissues, respectively[2].

Contrarily to the above strategies, recent studies have demonstrated that nanoscale protrusions like those found in the cuticles of insects such as dragonfly and cicada wings, own bactericidal properties brought by the intimate interaction between bacteria and nanoscale topographies[3]. Inspired by those examples in nature, in this work, we show that argon plasma treatment of bacterial cellulose (BC) hydrogel triggers scission reactions in the polymer that result in the growth of nanopillars at its surface. *E. coli* and *K. pneumoniae* in contact with the nanopatterned BC appear flat and with significant membrane damage after one-hour incubation.

Methods: BC pellicles were air dried at room temperature and then exposed to argon plasma at low energy (1 KeV), normal incidence and high fluence. Nanoindentation, X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM) were performed to determine the stiffness, the elemental composition, and topographical changes in the BC before and after treatment. *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 13883), and *S. aureus* (ATCC 12600) were grown overnight and subsequently cultured with experimental samples for 1 hour. Attached bacteria were fixed using formalin and dehydrated in serial dilutions of ethanol. MC3T3-E1 cells were used to test the cytocompatibility of pristine and argon-treated BC using the live/dead viability assay (ThermoFisher scientific).

Results: The Young's modulus of the argon-treated BC increased from 4.86±1.05 MPa to 8.77±0.51MPa, but it was inferior to that reported for the nanopillars in cicada and dragonfly wings of 3.7 GPa and >20 MPa, respectively[4]. Elemental composition analysis of Ar-treated BC (C1S peak) obtained via XPS revealed an enrichment of the C-C/C-H bonds and depletion of oxygen with the preferential removal of C-O bonds. The C-O-C/C-OH bonds also decreased drastically, whereas the C=O/O-C-O were only slightly reduced. Because oxygen atoms are responsible for linking the glucopyranose monomers and forming the ring structure in the BC, this would cause the restructuration of the polymer chains via ring opening and chain scissoring, driving nanopillar growth in the material (**Fig. 1 (a, b)**).

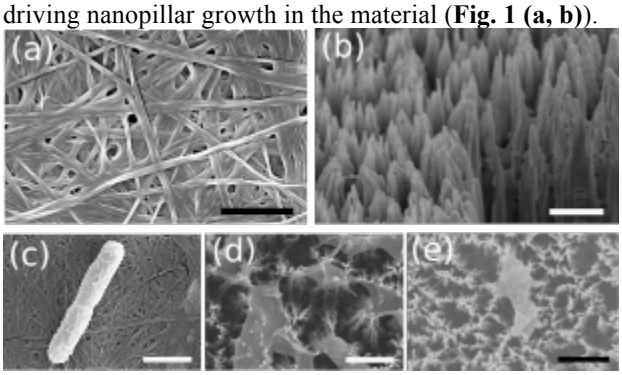

Figure 1. Argon plasma treatment induces nanopillar growth in BC hydrogel with bactericidal activity against *E. coli* and *K. pneumonieae*. (a) Fibrous profile of the pristine BC; (b) nanopillars in BC after argon plasma treatment (average height: 220 nm); (c) *E. coli* on pristine BC; (d) & (e) flat and stretched *E. coli* and *K. pneumoniae* on argon-treated BC. Scale bar: 1 um.

Both *E. coli* and *K. pneumoniae* growing on pristine BC showed a normal rod-like shape, typical of a viable bacteria (**Fig. 1 (c)**), whereas on Ar-treated BC they appeared flat, stretched, and with significant membrane damage (**Fig. 1 (d,e)**). The number of *E. coli* in Ar-treated BC (450±104.102 cells/mm^2) was lower than the one found in the pristine BC (783±270.102 cells/mm^2). Both *E. coli* and *K.pneumoniae* exhibited morphological deviations not present in the control samples, such as vertically elongation and cavitation, which has been associated with stressful environments such as the presence of antibiotics. MC3T3-E1 seeded on Ar-treated BC for 24 hours and stained for both Calcein-AM and ethidium homodimer-1 showed that the material is cytocompatible and did not produce membrane damage in those cells.

Conclusions: Argon plasma treatment induced the formation of nanopillars in BC through ring-opening and reorganization of the polymer chains. Ar-treated BC was not effective killing *S. aureus* and did not inhibit bacterial adhesion; however killed preferentially Gram-negative bacteria (*E. coli, K. pneumoniae*) upon contact via a contact killing mechanism. Further experiments will examine the role of surface charge in the killing mechanism of argon-treated BC.

References:

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