

## Supplementary Information for

### Investigation of cotton functionalized with ZnO nanorods and its interaction with *E. coli*

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**Chemicals, Materials and other Supplies:** Bleached desized cotton fabric (#400) was purchased from TestFabrics (West Pittston, PA). The following chemicals were purchased from Sigma-Aldrich: zinc acetate dihydrate ( $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ , ACS reagent,  $\geq 98\%$ ), triethylamine (99.5%), isopropyl alcohol (Anhydrous, 99.5%), sodium hydroxide (NaOH, ACS reagent,  $\geq 97.0\%$ , pellets), citric acid (ACS reagent,  $\geq 99.5\%$ ), Triton X-100 (laboratory grade nonionic surfactant), zinc nitrate hexahydrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , reagent grade, 98%), and hexamethylenetetramine (ACS reagent,  $\geq 99.0\%$ ). All supplies for *E. coli* cell culture (strain BL21DE3) were purchased from Edvotek (EDVO-Kit #223).

**Scouring of the cotton fabric:** Before the ZnO nanostructures were grown, the cotton fabric was cut into square swatches (2 cm x 2 cm) and scoured to remove wax, grease, and other finishing chemicals. The scouring solution was prepared by first dissolving 5.0 g of NaOH in 20 mL of deionized water followed by the addition of 1.5 g of Triton X-100 and 0.75 g of citric acid. The resulting solution was then diluted to 500 mL with deionized water. In the scouring process, four cotton swatches were then placed in a 500 mL round bottom flask containing 200 mL of the scouring solution. The mixture was then stirred at 100 °C for 1 hour. The scoured swatches were removed from the solution, rinsed thoroughly with DI water, and dried in air.

**Preparation of ZnO seed solution:** The ZnO seed solution (0.100 M in Zn) was prepared as follows. First, 1.10 g of zinc acetate dihydrate (5.0 mmol) was dissolved in 50.0 mL of isopropyl alcohol. The resulting solution was then stirred vigorously at 85 °C for 15 min. After this time, 701  $\mu\text{L}$  of triethylamine (5.0 mmol) was added dropwise to the stirred solution. The resulting solution, now clear, was stirred at 85 °C for an additional 10 min. After this time, the solution was cooled to room temperature and incubated without stirring for 3 hours. The pH of the seed solution was 7.01 (pH meter). The average particle size of the ZnO nanocrystal seed solution was  $31.5 \pm 10.0$  nm, as measured by a Zetatrack (Microtrac) particle size analyzer. We have used this seed solution up to two weeks after its preparation with no discernable change in the final ZnO nanostructures.

**Preparation of ZnO growth solution:** Equimolar aqueous solutions of zinc nitrate hexahydrate and hexamethylenetetramine were used to grow ZnO nanorods on cotton swatches. First, a 0.100 M solution of hexamethylenetetramine was prepared by dissolving 7.71 g (0.055 mmol) in 550 mL DI water. Once dissolved, 16.4 g of zinc nitrate hexahydrate (0.055 mmol) was added and the resulting solution was stirred for 24 hours at room temperature. The final pH of this ZnO growth solution was 6.11 (pH meter).

**Growth of ZnO nanostructures on cotton swatches:** The scoured cotton swatches were first dip-coated with the seed solution for 5 min and then rinsed with ethanol. The dip-coated swatches were then suspended from a wire rack (Figure S1), cured at 120 °C for 1 hour in an oven, and then further dried in air for 12 hours at room temperature. To ensure uniform deposition of ZnO nanostructures on cotton surfaces in subsequent steps, the swatches were then immobilized on a glass cover slip using an epoxy glue (LOCTITE stik'n seal ultra, Flextec Technology). The immobilized swatches were then suspended vertically in  $\sim 70$  mL of the growth solution (Figure S2) and incubated at 95 °C for 8 hours in an oven. The container was removed from the oven, cooled to room temperature, and then further incubated at room temperature for 10-12 hours. Finally, the swatches were removed from the growth solution, thoroughly rinsed with DI water, and allowed to air-dry at room temperature.

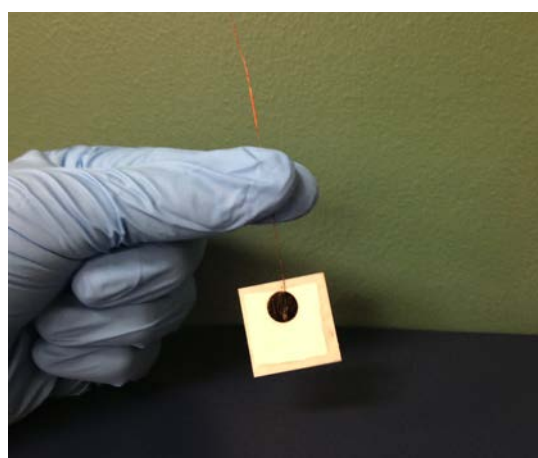


**Figure S1:** Cotton swatches after dip-coating in ZnO seed solution. The drying rack was mounted directly in an oven and cured at 120 °C for 1 hour.

A)



B)



C)



D)



**Figure S2:** A) Cotton swatch glued to cover slip (front view). A copper wire is taped to the back of the cover slip. The jar contains the ZnO growth solution and the lid has a small hole for suspending the swatch by the wire. B) Cotton swatch glued to cover slip with copper wire taped to back of cover slip (tape: PELCO Tabs, 9 mm OD, Ted Pella Inc.) (rear view). C) Cotton fabric (supported on cover slip) suspended in ZnO growth solution. D) Top view of swatch suspended in ZnO growth solution. The copper wire was secured to the lid with label tape (Fisher Scientific) before incubating at 95 °C in an oven.

**Characterization Methods:** The morphology of the ZnO nanostructures grown on cotton swatches was investigated using a JEOL JSM 6060 LV scanning electron microscope (SEM). The samples were coated with 5-10 nm Au layer before the SEM imaging. Elemental analyses of the samples were performed on the SEM system equipped with EDAX TEAM system operating at an accelerating voltage of 20 kV. Crystal structures were analyzed using a Shimadzu XRD-6100 X-ray Diffractometer with Cu K $\alpha$  radiation, employing a scanning rate of 0.02° s<sup>-1</sup> within the range of 2 $\theta$ =10°-70° operating at 40 kV and 33 mA (1320 watts). Thermogravimetric analysis (TGA) was performed using a Mettler Toledo 851 with a TSO 801RO robotic arm. The samples were heated from 40 °C to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere at a flow rate of 40 mL/min. Static water contact angles (SWCA) were measured using an AST Products VCA Goniometer which uses a computer-controlled syringe to dispense a 5  $\mu$ L DI water droplet onto the substrates while calculating the contact angles at room temperature.

### Scanning electron microscopy (SEM) analysis

The surface morphology of the cotton and ZnO-modified cotton was investigated using a field emission scanning electron microscope (FESEM). Figure S3A and S3B show the smooth surface of unmodified and ZnO seed-deposited cotton, respectively. Note that the ZnO nanocrystal seed coating is not visible under the FESEM conditions. The FESEM images in Figure S3C-F reveal the ZnO nanostructures prepared after incubation in the ZnO growth solution. The single-crystalline hexagonal ZnO nanorods are radially aligned on the cotton surface with high uniformity and dense coverage. It can be clearly observed from the cross-sectional images (Figure S3E-F) that the nanorods grow perpendicular to the cotton surface. The cluster morphology shown in Figure S3E-F also suggests that multiple nanorods grow from a single aggregate of the ZnO nanocrystals deposited onto cotton surface.

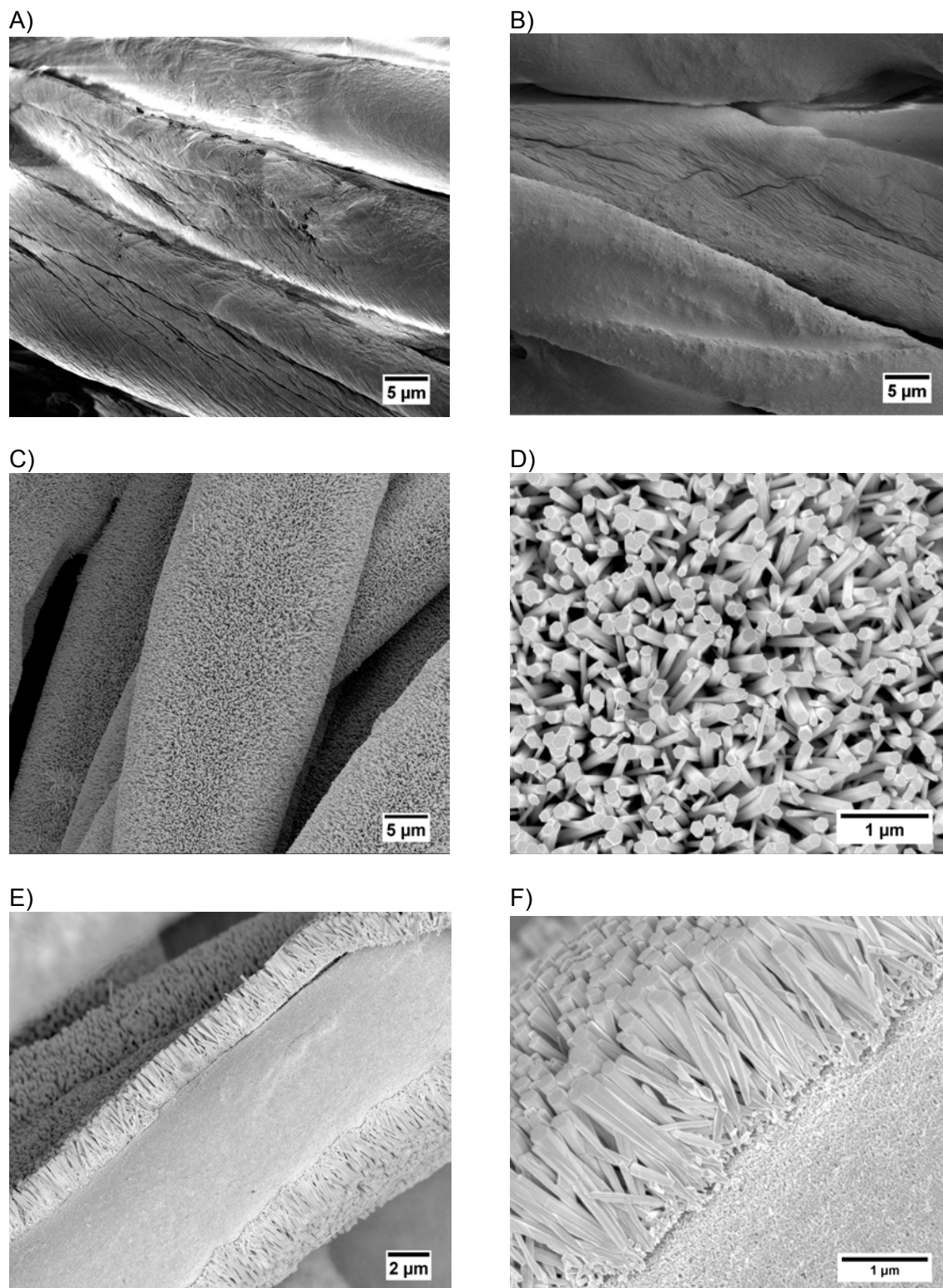
The dimensions of the nanorods were calculated using imaging processing software ImageJ with the scale bar obtained during SEM acquisition as a reference. At least 10 nanorods from three independent ZnO modified swatches were used in this analysis. The results are tabulated below.

Average length of ZnO nanorod: 1.98  $\mu$ m

Standard deviation for length of ZnO nanorod: 0.07  $\mu$ m

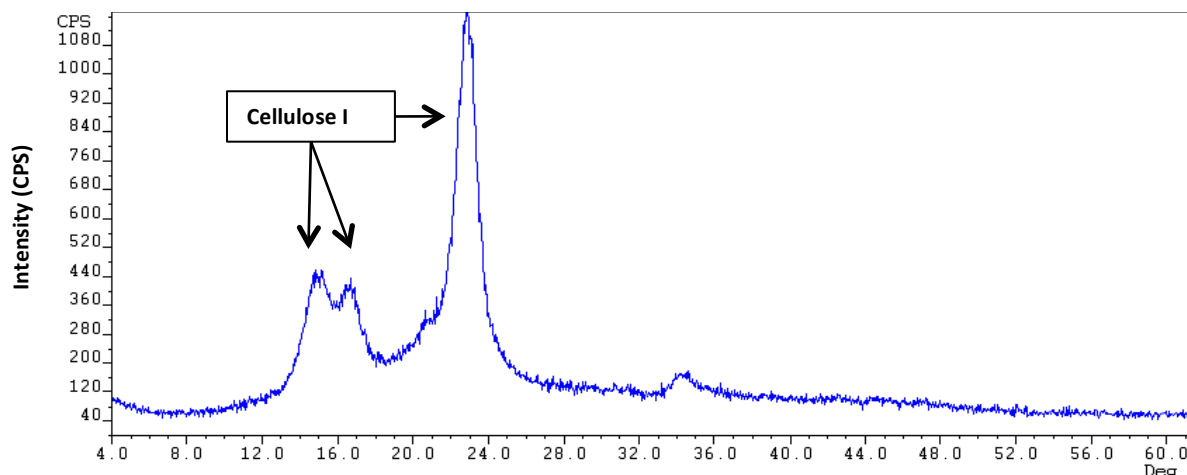
Average diameter of ZnO nanorod: 172 nm

Standard deviation for diameter of ZnO nanorod: 18 nm



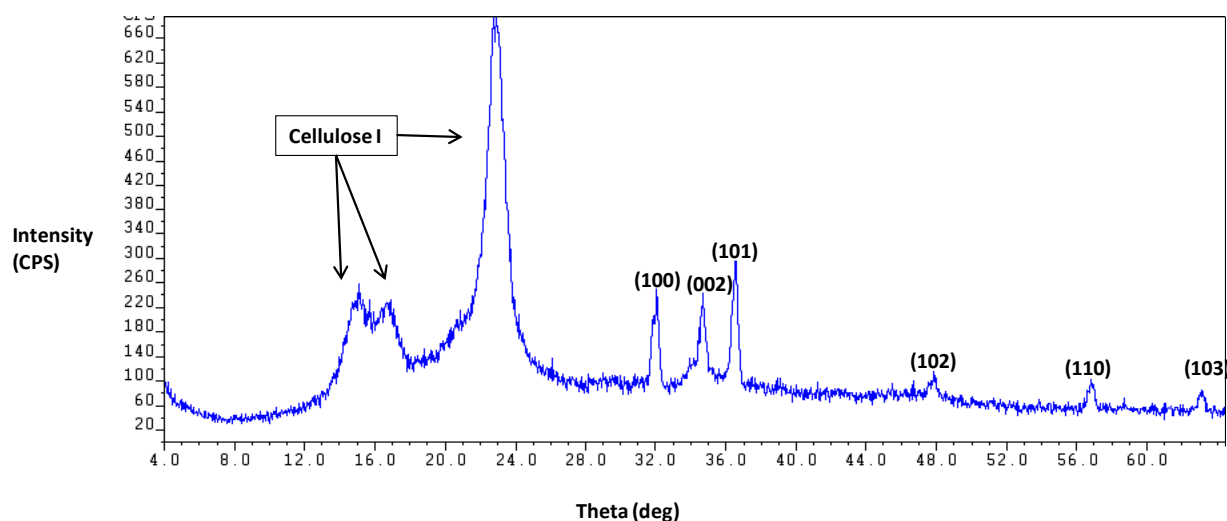
**Figure S3:** SEM images of (A) unmodified cotton, (B) ZnO-seed deposited cotton, (C) and (D) the ZnO nanorods hydrothermally grown on cotton at different magnifications, (E) and (F) cross-sectional images of ZnO nanorods hydrothermally grown on cotton at different magnifications.

XRD pattern of unmodified cotton sample is shown in Figure S4. The peaks at  $2\theta$  values of  $15.2^\circ$ ,  $16.7^\circ$ , and  $23.1^\circ$  corresponding to (101), (101), (002) planes, respectively, are the diffraction peaks of cotton fiber (cellulose I structure) (JCPDS. No. 03-0226).<sup>1</sup>



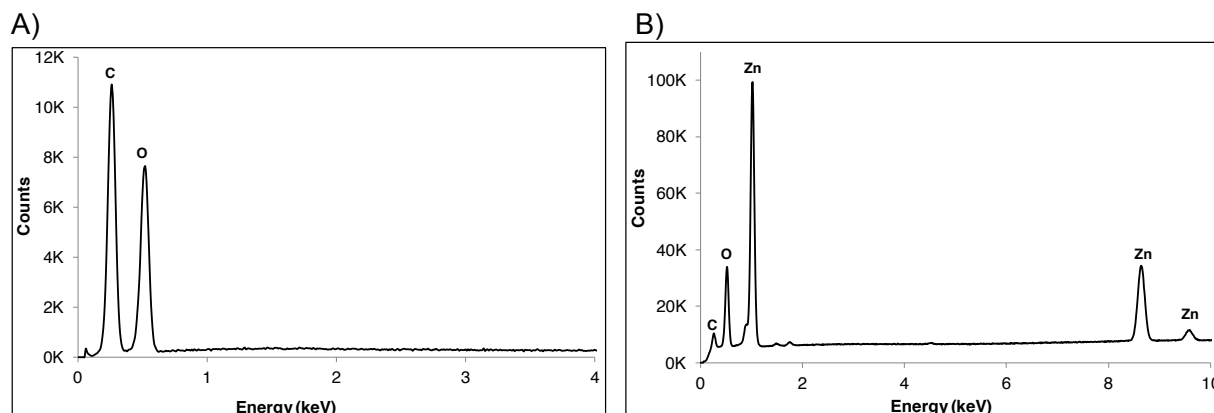
**Figure S4:** XRD pattern of unmodified cotton

Figure S5 presents the XRD pattern of ZnO nanorods grown on cotton swatch. Six reflection peaks appeared at  $2\theta$  values of  $31.9^\circ$  (100),  $34.6^\circ$  (002),  $36.5^\circ$  (101),  $47.7^\circ$  (102),  $56.8^\circ$  (110),  $63.1^\circ$  (103), and all the diffraction peaks could be indexed as the hexagonal wurtzite structure of ZnO, which were consistent with the values in the standard card (JCPDS 36-1451). No diffraction peaks from any other impurity phases are found, confirming that the only single-phase hexagonal wurtzite ZnO is present. The enhanced (002) diffraction peak implies that the hexagonal ZnO nanorods are grown along the *c*-axis orientation that is the theoretical and most stable crystal form.



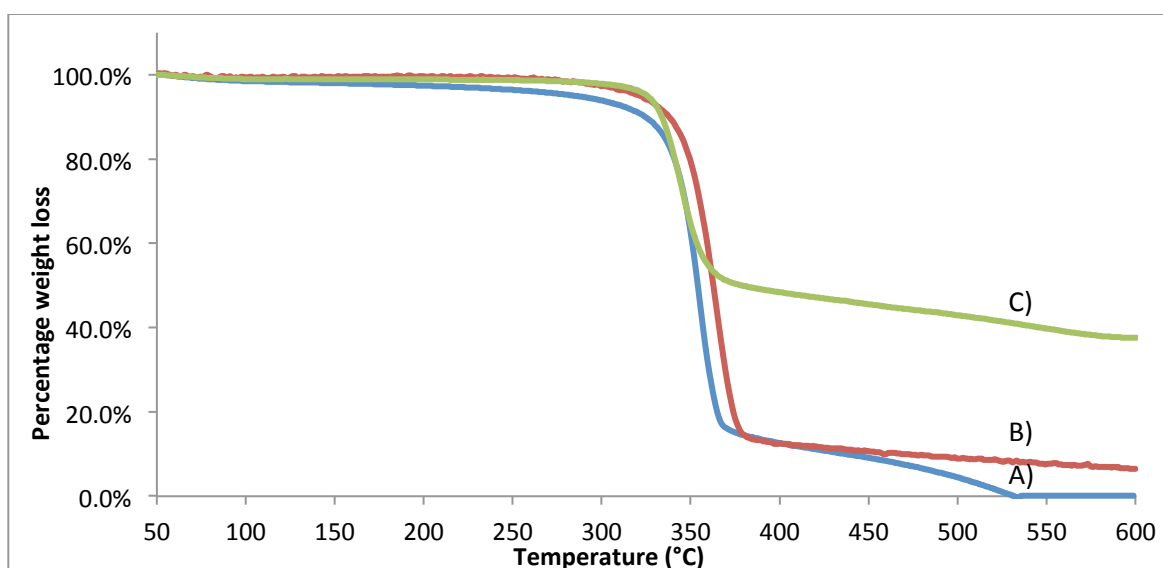
**Figure S5:** Indexed XRD patterns of highly oriented ZnO nanorods hydrothermally grown on cotton

The chemical composition of the ZnO nanorods was verified by energy dispersive X-ray (EDX) spectroscopy. Figure S6 shows the EDX analyses of unmodified cotton and cotton swatches modified with ZnO nanorods. The scoured cotton swatches do not reveal any contaminants and the presence of only Zn, C, and O atoms are clearly indicated on the treated samples.



**Figure S6:** EDX analyses of (A) unmodified cotton, (B) the ZnO nanorods hydrothermally grown on cotton

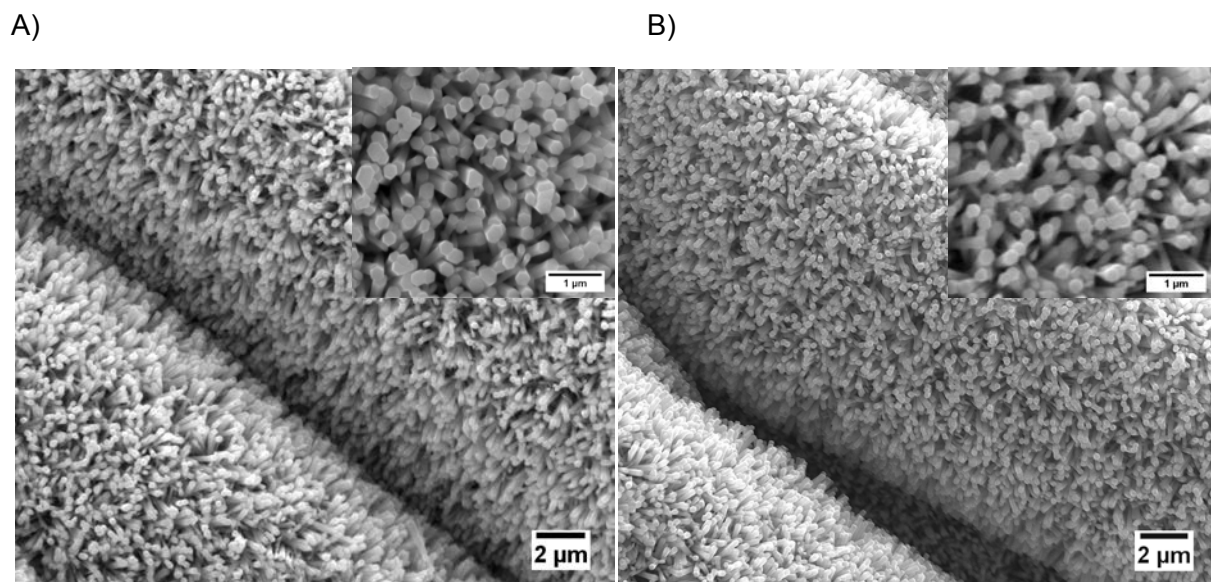
The ZnO content of the functionalized cotton swatches was further assessed by thermogravimetric analysis (TGA). Figure S7 shows the thermograms of an unmodified cotton, a cotton swatch treated with ZnO nanocrystal seeds, and a cotton swatch functionalized with ZnO nanorods. The sample weight, which is normalized against the final weight, was observed to drop sharply between 300-350 °C. Unmodified cotton starts to thermally decompose around 250 °C, and the total decomposition is completed around 500 °C. The cotton modified with ZnO nanorods, in contrast, displays a higher initial thermal decomposition at 325 °C. The amount of ZnO (wt.%) was calculated by the weight loss of the samples at the end of the heating cycle. For the cotton modified with the ZnO nanocrystal seed solution only: 7 ( $\pm$  1) wt% ZnO. For the cotton modified with with ZnO nanorods: 37.9 ( $\pm$ 0.5) wt % ZnO.



**Figure S7:** Thermograms of (A) unmodified cotton, (B) seed-deposited cotton, and (C) ZnO nanorods hydrothermally grown on cotton.

### **Durability test of the ZnO nanorods on cotton swatches:**

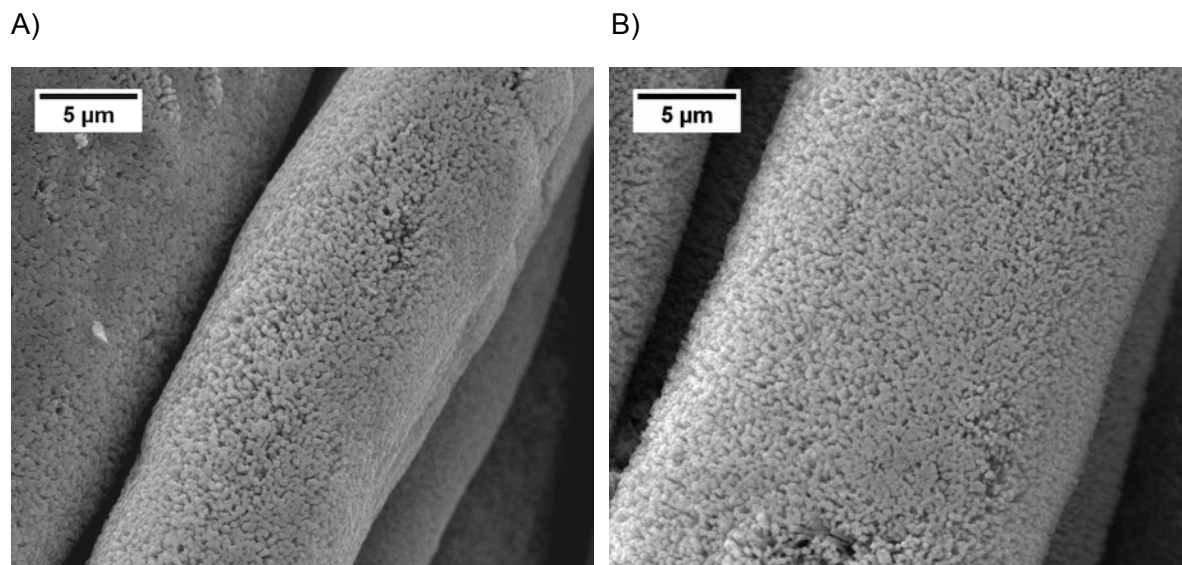
**Wash at room temperature:** The durability of the ZnO nanorods grown on cotton surface was tested by vigorously stirring swatches in a 250 mL crystallization dish containing deionized water at room temperature for one hour. After this time, the samples were removed and allowed to air-dry. SEM images before and after the process show no visible changes in the morphology and surface coverage of the ZnO nanorods, which indicate that ZnO nanorods on cotton surfaces withstand prolong exposure to water under agitation. SEM images are shown below (Figure S8).



**Figure S8:** SEM images of cotton swatches functionalized with ZnO nanorods (A) before, (B) after agitation in DI water for an hour at room temperature.

**Wash at 90 °C with detergent:** A second durability test was undertaken to simulate real washing conditions. Accordingly, 50 mL of tap water containing 1 wt. % of Arm and Hammer laundry Oxi Clean™ detergent was heated to 90 °C in a round bottom flask. Cotton swatches modified with ZnO nanorods was then added to the solution and stirred rapidly at 90 °C for 1 hour. After this time, the swatches were removed and washed thoroughly with running tap water to remove detergent. Thermogravimetric analysis were conducted using three samples taken from different spots of the same swatch. The average loading of the ZnO on cotton swatches before and after washing were found as  $21.7 \pm 1.5$  wt% and  $22.3 \pm 1.5$  wt%, respectively. The results of thermogravimetric analysis indicate that there is no statistical difference in ZnO content before and after detergent washing and the ZnO nanorods were durable under the detergent washing conditions. Comparison of SEM images before and after washing (Figure S9) was also consistent with little to no change in the ZnO content and morphology. These experiments demonstrate the durability of the cotton modified with ZnO nanorods.

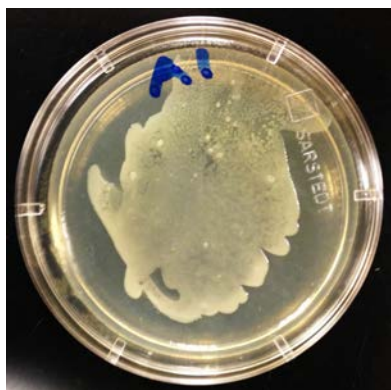




**Figure S9:** SEM images of cotton swatches functionalized with ZnO nanorods (A) before, (B) after washing for 1 hour at 90 °C in tap water containing 1 wt% commercial laundry detergent. No significant change in the ZnO crystals is apparent from these images. Moreover, the TGA analysis for the two samples shown above indicated no significant change in ZnO content before and after washing.

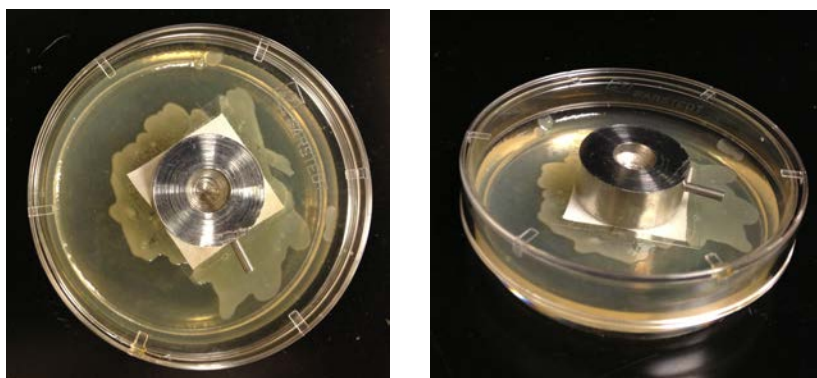
**Wettability Tests:** Surface-wetting properties were evaluated on Dynamic Contact Angle Goniometer (AST products Inc.) which uses a computer controlled syringe to dispense a 5  $\mu\text{L}$  DI water droplet on substrates and calculate contact angles at room temperature. Both untreated and treated cotton fabric samples show the static water contact angle (SWCA) of 0°, indicating that both surfaces are hydrophilic. However, it should be noted that when the 5  $\mu\text{L}$  DI water droplet is added to the untreated cotton, it is immediately absorbed. When the 5  $\mu\text{L}$  DI water droplet is added to the cotton modified with ZnO nanorods, the water is absorbed over the course of several seconds.

***E. coli* culture:** Source plates were prepared using EDVOTEK ReadyPour™ Luria Broth agar (sterile, no antibiotics) and stored at 4 °C. Starter cultures were prepared by placing one *E. coli* BactoBead™ (*E. coli* strain BL21DE3, EDVOTEK 223/AP08) on a source plate, and then allowing to dissolve (3-5 min, room temperature). After the *E. coli* BactoBead™ was dissolved, primary and secondary steaks were made using a sterile inoculating loop and the source plate was incubated for 48 hours at room temperature. After this time, 3-5 colonies were transferred to 1.3 mL of sterile Luria Recovery Broth (EDVOTEK, AP08) and further incubated for 1 hour at 37 °C. After this time, the culture was transferred in 100  $\mu\text{L}$  aliquots to each agar plate used in subsequent experiments. The liquid culture was spread uniformly across each plate with an inoculating loop and then incubated for 48 hours at room temperature or until a uniform coverage of *E. coli* was observed. A typical plate of *E. coli* used in this series of experiments is shown in Figure S10:

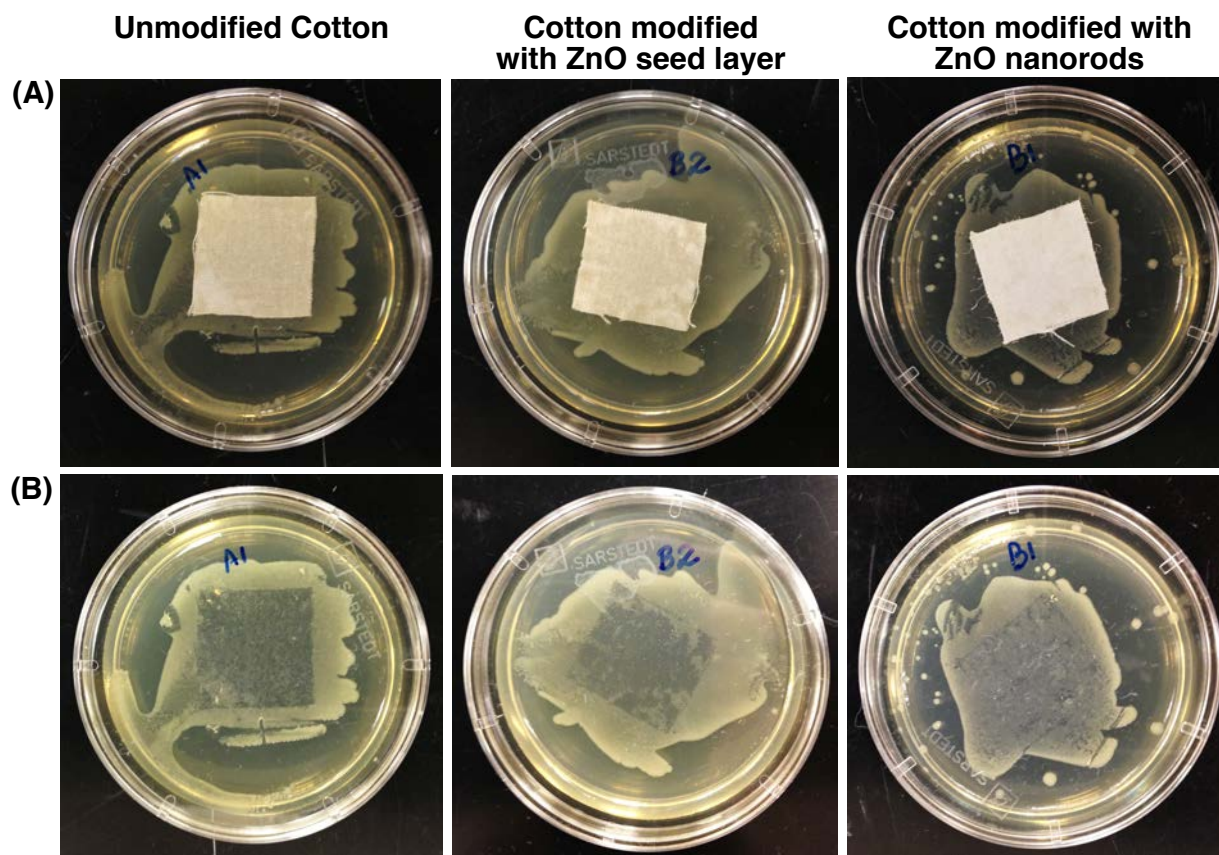


**Figure S10:** *E. coli* BL21DE3 grown on agar.

***E. coli* adhesion experiments with cotton samples:** Three types of cotton swatches were used to study the interaction with *E. coli* biofilms: unmodified cotton, cotton modified with the ZnO seed solution, and the cotton modified with the ZnO nanorods. The cotton swatches prepared as described above were carefully removed from the cover slips with forceps. As a control measure, unmodified and ZnO seed coated cotton swatches were also glued to a cover slip and removed to ensure the same handling as the cotton modified with ZnO nanorods. Using sterile forceps, the cotton swatches were gently placed directly on an *E. coli* biofilm (each sample was prepared in triplicate). Care was taken so that the side of the swatch that was glued to the cover slip was facing up and *not* in contact with the *E. coli*. The cotton was flattened by carefully placing a cover slip on top of the fabric, followed by a 17.5 g weight. The force of the weight was applied for 1 minute, after which time the weight and cover slip were removed (Figure S11). The cotton samples, now in direct contact with the biofilm, were incubated at room temperature for 22 hours. After this time, the appearance was noted and recorded using a digital camera. The cotton swatches were then removed carefully using sterile forceps. Again, the appearance was noted and the results recorded with a digital camera. Typical results are shown in Figure S12.

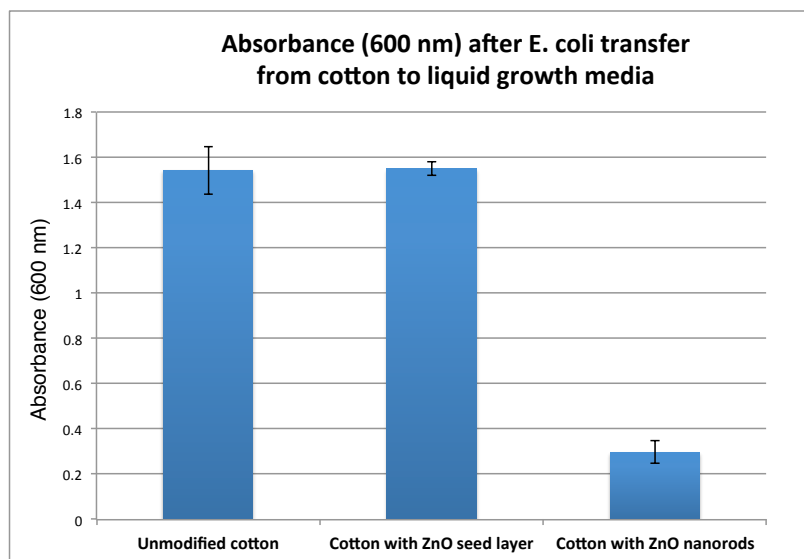


**Figure S11:** Cotton swatches were placed on *E. coli* film and pressed with a cover slip and weight for 1 minute before further incubation.



**Figure S12:** (A) Cotton swatches after 22 hours of direct contact with *E. coli*. Note that the unmodified cotton and the cotton with the ZnO seed layer appear to have absorbed moisture. The cotton modified with ZnO nanorods appears dry. (B) Agar plates after removal of cotton swatch. More *E. coli* appear to be transferred to the unmodified cotton and the cotton modified with the ZnO seed layer than to the cotton modified with ZnO nanorods.

**Quantification of *E. coli* transfer to cotton:** After the cotton swatches were removed from the *E. coli* sample, they were immediately transferred to a 15 mL centrifuge tube containing 1 mL of sterile Luria Recovery Broth (EDVOTEK, AP08). The suspension was then agitated by vortex at a maximum rate (~3000 rpm) for 1 minute to shed cells from the cotton swatches to the liquid media. An 800  $\mu$ L aliquot of the media containing the shed cells was transferred to a cuvette and the absorbance was measured at 600 nm to determine relative concentration of cells. (Absorbance data was obtained using a dual beam UV-1800 Shimadzu UV spectrophotometer interfaced with UVProbe 2.34 software). Absorbance values were corrected by subtracting the absorbance obtained from the same procedure for cotton swatches that were not exposed to the *E. coli* film (a small, but non-zero, background absorbance was observed for unmodified cotton, cotton modified with ZnO seed solution, and the cotton modified with ZnO nanorods). The *E. coli* transfer and absorbance measurements were carried out for three separate samples of unmodified cotton, cotton modified with ZnO seed solution, and the cotton modified with ZnO nanorods (9 total samples). The results are summarized in Figure S13.



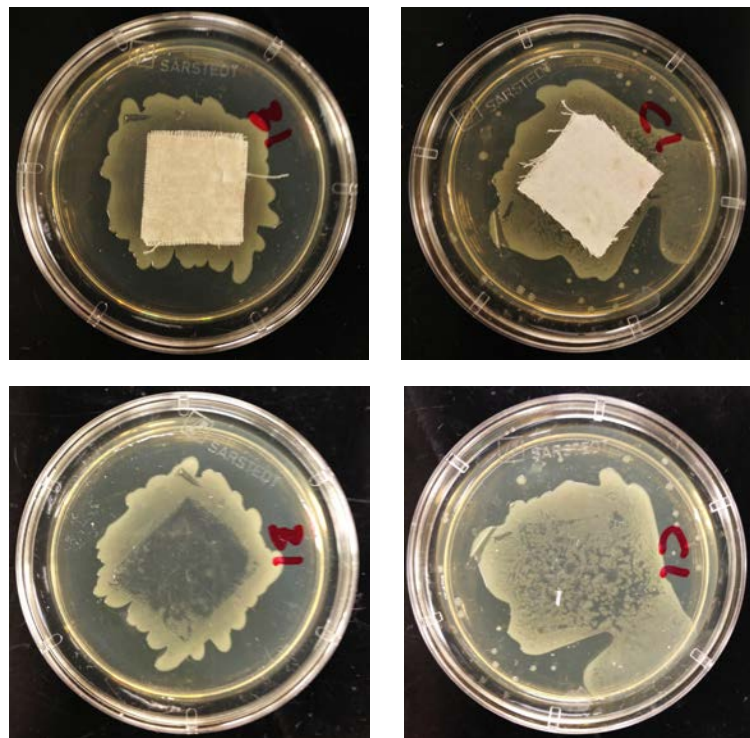
**Figure S13:** Absorbance (600 nm) after *E. coli* cells were shed from cotton swatches. Error bars indicate  $\pm 1$  standard deviation from the mean absorbance. The *E. coli* transfer, as inferred by absorbance, is significantly lower than the *E. coli* transferred by the unmodified cotton ( $P < 0.0001$ , two-tailed t-test) and the cotton modified with the ZnO seed layer ( $P < 0.0001$ , two-tailed t-test).

***E. coli* adhesion experiments in darkroom:** Since ZnO is photoactive, it was necessary to determine if ambient light has any effect on the *E. coli* adhesion, the experiment described above was repeated in a dark room. Only unmodified cotton and cotton with the ZnO nanorods were examined since no difference in adhesion was observed between cotton and cotton with the ZnO seed layer. The samples were prepared in the same manner as described above (triplicate for both cotton and cotton with ZnO nanorods), with the exception that after placing the swatch on the *E. coli* plate, the samples were incubated in a dark room for 22 hours. After this time, the plates appeared the same as the experiment conducted in ambient light: the unmodified cotton appeared wet and transferred more bacteria from the plate when removed. The cotton modified with ZnO nanorods appeared dry and did not remove as many bacteria. The cells were shed from the swatches as previously described and the relative *E. coli* transfer was inferred by absorbance at 600 nm. The results are summarized in Figures S14 and S15.

Representative plates for adhesion experiments in darkroom:

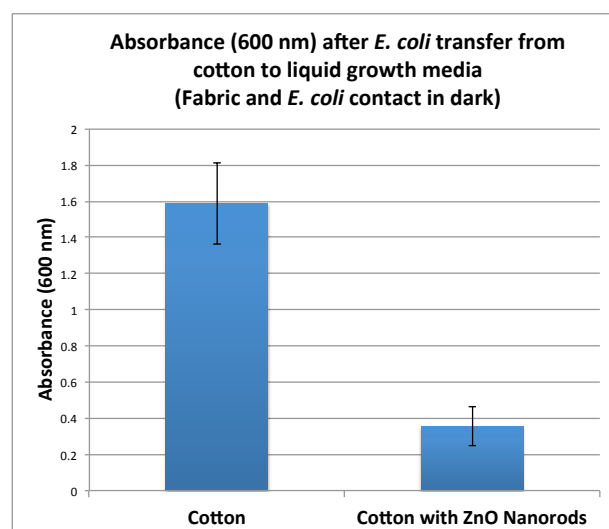
Unmodified cotton

Cotton modified with ZnO Nanorods



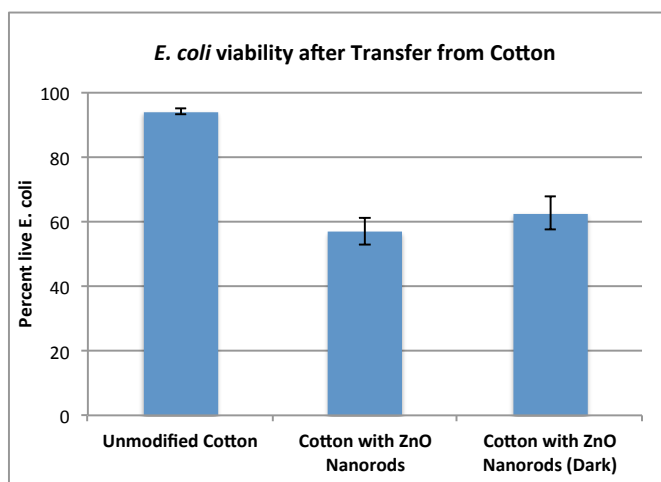
**Figure S14:** Unmodified cotton (left panels) and cotton modified with ZnO nanorods (right panels) were incubated on *E. coli* plates in a darkroom for 22 hours. Like the experiments conducted in light, more moisture permeates the unmodified cotton and more *E. coli* are transferred to the unmodified cotton.

Absorbance data after shedding *E. coli* from cotton swatches:



**Figure S15:** Absorbance (600 nm) after *E. coli* cells were shed from cotton swatches after incubation in dark room. Error bars indicate  $\pm 1$  standard deviation from the mean absorbance. The *E. coli* transfer, as inferred by absorbance, is significantly lower than the *E. coli* transferred by the unmodified cotton ( $P = 0.001$ , two-tailed t-test) Note that these results are comparable to the same experiment in ambient light (Figure S13).

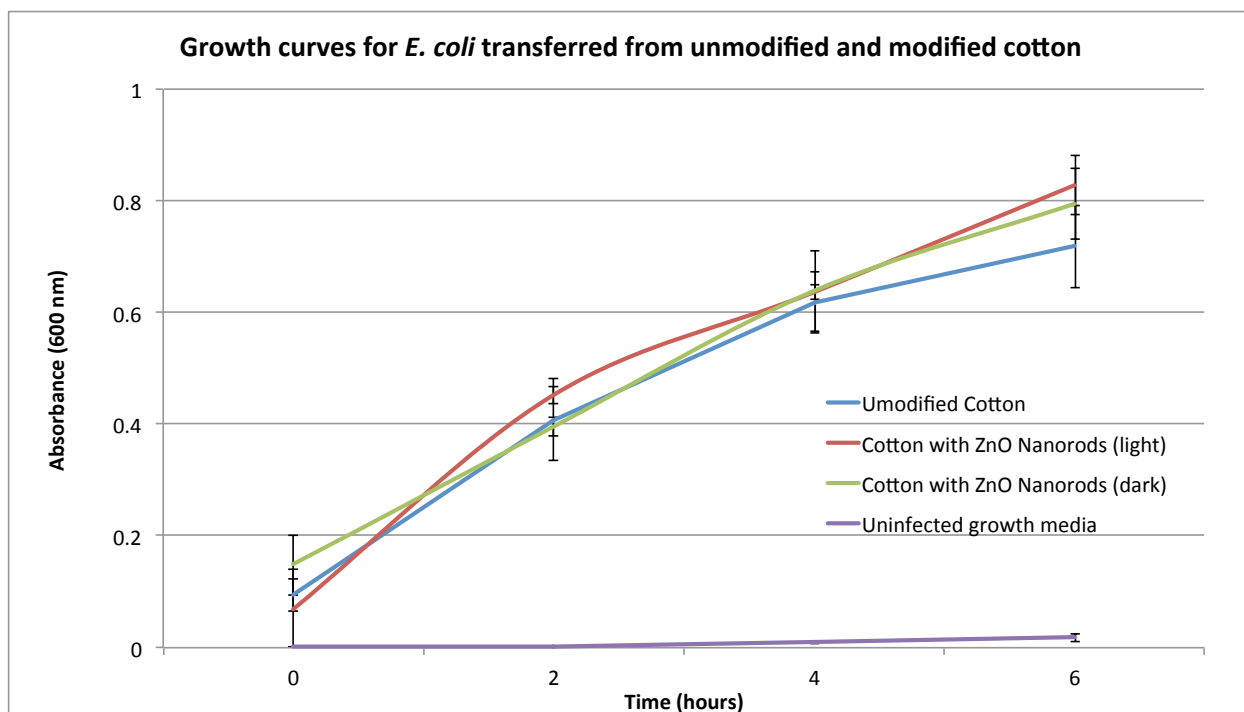
***E. coli* viability test after contact with unmodified and ZnO modified cotton swatches:** The percent of live and dead *E. coli* was assessed using the Molecular Probes LIVE/DEAD<sup>®</sup> BacLight<sup>™</sup> Bacterial Viability Kit (Invitrogen L7012). Samples of unmodified cotton and cotton modified with ZnO nanorods were used in this study. Parallel experiments were carried out in both ambient light and in a dark room to determine what effect, if any, light plays in the viability of *E. coli* that have prolonged contact with cotton modified with ZnO nanorods. Briefly, agar plates were prepared and inoculated as described on page S9. Unmodified cotton swatches and cotton swatches modified with ZnO nanorods were placed in contact with the *E. coli* as described on page S9-S10. Each sample was prepared in triplicate and incubated for 22 hours at room temperature. Three of the cotton samples modified with ZnO were incubated in a dark room; three separate samples ZnO modified cotton were incubated in ambient light. After this incubation, the swatches were removed with sterile forceps and the *E. coli* were shed into 2 mL of 0.85% NaCl (vortex at ~3000 rpm, 1 min). The concentration of all *E. coli* samples was adjusted to uniformity ( $OD_{670} = 0.06$ ) by dilution with a sterile filtered solution of 0.85% NaCl before further analysis. A 100  $\mu$ L aliquot of each sample was added to an individual well of a 96-well microplate suitable for fluorescence analysis. (A SAFIRE TECAN Multifunctional microplate reader was used for this fluorescence analysis). Each sample was then mixed with a 100  $\mu$ L of mixture of SYTO 9 dye (1  $\mu$ M) and propidium iodide (60  $\mu$ M), per the manufacturers instructions. (*E. coli* with intact cell membranes stain green with SYTO 9 dye; *E. coli* with damaged cell membranes—dead cells—stain red with propidium iodide. The ratio of green (530 nm) to red fluorescence (630 nm) can be used to calculate the ratio of live to dead cells). A calibration curve was constructed according to the manufacturer's instructions using known concentrations of live and dead *E. coli*. The ratio of fluorescence at 530 nm to 630 nm for the experimental samples was then obtained and the percent live cells was calculated using the calibration curve. The results are summarized Figure S16. *E. coli* transferred from unmodified cotton displayed a high *E. coli* viability ( $94 \pm 1\%$  live cells) while cotton modified with ZnO nanorods had fewer live cells:  $57 \pm 4\%$  for the sample incubated in ambient light and  $63\% \pm 5\%$  live cells when incubated in the dark. While this antibacterial activity is modest, both ZnO samples (light and dark) had a significantly lower live cell count than the sample from unmodified cotton (For ZnO sample in light,  $P = 0.0002$ ; for the ZnO sample in the dark  $P = 0.0002$  in a two-tailed t-test). There was no statistically significant difference between the ZnO samples incubated in the light and dark ( $P = 0.18$ , two-tailed t-test).



**Figure S16:** Percent of live *E. coli* cells after transfer from cotton and cotton modified with ZnO nanorods. Error bars indicate  $\pm 1$  standard deviation from the mean live cell count. Cotton modified with ZnO nanorods resulted in a modest, but statistically significant ( $P = 0.0002$ , two-tailed t-test), reduction in live cell count. There was no difference in live cell count observed for ZnO samples in ambient fluorescent light and samples incubated in a dark room ( $P = 0.18$ , two-tailed t-test).

### Growth curves of *E. coli* shed from unmodified cotton and cotton modified with ZnO nanorods:

While the live cell count was lower for *E. coli* transferred from cotton modified with ZnO nanorods (page S14), it should be noted that the live cells are indeed viable and display logarithmic growth regardless of what type of cotton they were transferred from. To show this, three conditions were used in this experiment and repeated in triplicate: unmodified cotton with incubation in ambient light, cotton modified with ZnO nanorods with incubation in ambient light, and cotton modified with ZnO nanorods with incubation in a dark room. Uninfected growth media was used as a control in this experiment. After incubation of the cotton swatches on the *E. coli* plates for 22 hours, cells were shed from the cotton swatches as described above into liquid LB media. Next, a suspension of cells from each cotton sample was diluted to approximately the same concentration in sterile growth media ( $OD_{600} = 0.09 (\pm 0.03)$ ) and allowed to grow at room temperature. Three blank samples of Luria Recovery Broth were also incubated in the same fashion as a control. Cell growth was monitored by absorbance at 600 nm over the course of 6 hours. All samples showed similar logarithmic growth curves (Figure S17). This experiment was carried out to show that the viable cells transferred from all cotton samples are capable of exponential growth. We therefore issue a cautionary note: while the ZnO imparts modest bactericidal properties to the cotton and results in the transfer of significantly fewer cells (live and dead), the ZnO cannot entirely prevent infection by contact transfer.



**Figure S17:** Growth curves for *E. coli* transferred from cotton and cotton modified with ZnO nanorods.

**References:**

1. Lin, H., Yao, L. R., Chen, Y. Y. & Wang, H. Structure and properties of silk fibroin modified cotton. *Fibers and Polymers* **9**, 113–120 (2008).