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<td>Author(s)</td>
<td>Wang, Yilei; El-Deen, Ahmed G.; Li, Peng; Oh, Bernice Hui Lin; Guo, Zanru; Khin, Mya Mya; Vikhe, Yogesh Shankar; Wang, Jing; Hu, Rebecca G.; Boom, Remko M.; Kline, Kimberly A.; Becker, David Laurence; Duan, Hongwei; Chan-Park, Mary B.</td>
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High Performance Capacitive Deionization Disinfection (CDID) of Water with Graphene Oxide-\textit{Graft}-Quaternized Chitosan Nanohybrid Electrode Coating

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Figure S1. Synthesis schematic of quaternized chitosan (dimethyldecylammonium chitosan).
Figure S2. GPC spectrum of synthesized quaternized chitosan (QC, specifically dimethyldecylammonium-chitosan).
Figure S3. Size distribution of (a) GO and (b) GO-QC (1:5). At least 100 nanoflakes were measured for each sample to obtain the average size and distribution.
Figure S4. XPS spectroscopy. (I) Wide scans of (i) GO and (ii) GO-QC. (II) High resolution C1s spectra of (i) GO, (ii) QC, (iii) GO-QC. (III) High resolution O1s spectra of (i) GO, (ii) QC, (iii) GO-QC. (IV) High resolution N1s spectra of (i) QC and (ii) GO-QC.
Figure S5. The time dependence of antimicrobial activity (killing curve) was investigated by varying the incubation time of *E. coli* with GO, QC or GO-QC (1:5) dispersions (100 µg mL⁻¹) from 0.5 h to 24 h. The % kill of *E. coli* by GO, QC and GO-QC increased monotonically with incubation time. QC and GO-QC (1:5) produced high % kill quickly and reached nearly 100% after 4 h. The % kill of GO increased more gradually and plateaued beyond 12 h in the range of 70% kill.
Antimicrobial Activity In the Presence of NaCl, KCl, MgCl$_2$ and CaCl$_2$

The combination of QC with GO in a nanohybrid enhances the microbicidal potency synergistically. GO-QC (1:5) and QC also retain their antimicrobial activities, unlike GO, in the presence of physiologically important salts, including NaCl and KCl, CaCl$_2$ and MgCl$_2$ (Supporting information Figure S6a – S6d). The GO-QC nanohybrid is salt-insensitive, like pristine QC, because the microbial killing action does not depend on secondary structures of the QC molecule but on its cationic charge.

The antimicrobial activity of GO declines with increasing KCl concentration between 0 mM and 150 mM (Figure S6b). The antimicrobial activities of GO-QC and QC are unaffected by KCl concentration up to 150 mM. Biological concentrations of divalent ions such as Mg$^{2+}$ and Ca$^{2+}$ are much lower than those of monovalent ions; the effect of these divalent ions was tested over the concentration range 0 to 5 mM. The antimicrobial activity was not affected by adding Mg$^{2+}$ or Ca$^{2+}$ up to 5 mM, while the % kill of GO for *E. coli* decrease slightly along with the increasing divalent ion concentration (Figure S6c and S6d).
Figure S6. Antimicrobial activity (1 h) of GO, QC and GO-QC (1:5) (100 µg mL⁻¹) vs concentration of (a) NaCl (b) KCl, (c) CaCl₂ and (d) MgCl₂.
**Figure S7.** UV-vis absorption of water exiting out of the GO-QC/AC CDID cell after 10 min and 1 h. Reference used is GO (concentration is 0.15 mg L$^{-1}$).
Figure S8a. FESEM observations of *E. coli* cells, (i) immediately (0 min) after contact with GO-QC (1:5). (ii) After 1 h contact with GO-QC (1:5), the morphology of *E. coli* cells was changed. (iii) Dead cells separated from the GO-QC nanohybrids by centrifugation, major damages to bacteria cell walls are indicated with arrows.
**Figure S8b.** Microscope images of GO-QC flake conjugated with Texas-red dye under (i) darkfield imaging and (ii) fluorescence imaging.
Detailed Antimicrobial mechanism study of (I) QC and GO individually and (II) GO-QC nanohybrid

(I) QC and GO individually

We hypothesize that free QC in solution and grafted QC in GO-QC both penetrate the cell surface but with different modes. Free QC molecules in solution form are absorbed into the bacterial cell wall and electrostatically bind with the anionic cell envelope so that they cannot be removed by centrifugation (Figure S9.1).

Experimental Procedure

Optical Microscopy for visualization of microbes attachment

10 µL of suspensions of GO, QC, GO-QC and different microbes were pipetted onto a piece of glass slide, then protected with a coverslip before viewing under a confocal microscope (Olympus BX51, Germany) and images were taken using the software, analySIS (Olympus, Germany). To assess the viability of the attached bacteria, the bacteria-GO-QC suspension was first incubated with BacLight bacterial viability kit (L13152, Invitrogen) for 30 min at room temperature before being placed onto glass slide and viewed under a fluorescence microscope.
Figure S9.1 Optical micrographs of mixtures of (i) *S. aureus* and QC and (ii) *S. aureus* and GO; arrows point to bacteria in suspension.
(II) **GO-QC nanohybrid**

**Experimental Procedure**

*Contact angle measurements*

A suspension of microbes was drop casted onto glass slides and a drop of either water or 10 mg mL$^{-1}$ QC solution was deposited on top of the slides. The contact angles were then measured using a contact angle analysis system (FTA200, First Ten Angstroms Inc.)

**Discussion**

*Figure S9.II.a* shows bacteria clustering together and sticking onto the planes of the GO-QC nanohybrids rather than internalizing them. *Figure S9.II.b(ii)* further shows that these bacteria sticking to GO-QC nanohybrids are dead. These images corroborate our hypothesis that GO-QC nanohybrids are not internalized by microbes due to their relatively larger size, although the tethered QC likely penetrate partially into the microbe envelope. A FESEM image of the mixture of GO-QC with bacteria (Supporting information *Figure S8.a(ii)*) shows that the bacteria surfaces are fuzzy, suggesting that they are covered with GO-QC nanohybrid.

We qualitatively distinguished the attachment forces between GO-QC (1:5) and different microbes by adapting the recovery process with low and variable centrifugal force and measuring the relative fractions of microbes that detached from pre-challenged nanohybrids. The amounts of microbes detached were determined by
pelleting the free microbes by centrifugation, and then re-suspending and measuring the optical density. For all the microbes, a consistent trend is seen in which the fraction of microbes pelleted, *i.e.* detached from GO-QC as indicated by the optical density, increases with the relative centrifugal force applied (Supporting information Figure S9.III.a). The Gram negative bacterium (*E. coli*) has the highest fraction of microbes dislodged, followed by Gram positive bacteria *S. aureus* and finally the fungus *C. albicans* which has the lowest fraction dislodged. This trend mirrors the MBC values (Table 1): the Gram negative bacteria which are the most poorly killed by the GO-QC nanohybrid (*i.e.* they have the highest MBCs) have the smallest attachment force to the nanohybrids, while the fungi with the lowest MBC values have the highest attachment force. It appears that high attachment force correlates to high killing efficacy and low MBC values.

Zeta potentials of the various microbes were also measured (Figure S9.III.b) and it appears that the Gram positive *S. aureus* is more negatively charged than the Gram negative *E. coli*. Comparing the Gram positive and Gram negative bacteria, Figures S9.III.a and S9.III.b indicate a correlation between the magnitude of the microbe negative charge and the strength of the bacterium/GO-QC adhesion. It appears that more negative bacterial charge and higher attachment force correlate with lower MBCs for *S. aureus* (Gram positive) compared to *E. coli* (Gram negative) (Table 1). Increased electrostatic attraction between the anionic bacterial envelope and cationic GO-QC may explain why GO-QC is more effective against *S. aureus*
than it is against *E. coli*.

We further hypothesize that compositional compatibility of our QC polymer with bacterial cell wall will also contribute to microbes membrane disruption. Bacteria have cell walls that are rich in polysaccharides; the peptidoglycan cell wall layer is made from a polysaccharide of poly(muramic acid-co-glucosamine). Fungi, on the other hand, have a cell wall rich in chitin, which is very similar to the chitosan backbone of our QC. We qualitatively characterized the compatibility of QC with microbe cell wall by measuring the wettability by water and QC solution of glass slides coated with microbes. The results in Table S1 show that QC solutions have lower contact angles than the water medium, indicating that they wet the microbes better than water. Also, the lowest contact angle of QC solution (6.5 ± 1.5°) was achieved with *C. albicans* film, indicating that the QC tethered to the GO-QC nanohybrid probably has the highest compatibility with the *C. albicans* cell wall. *C. albicans* is also the most vulnerable of the microbes to GO-QC (Table 1, see sample 3c). Although *C. albicans* is much less anionic than the bacterial species (Figure S9.III.b), the MBC value against it is the lowest, suggesting that cell wall compatibility is a major contributor to the adhesion of *C. albicans* cells to GO-QC (Figure S9.III.a) and to the high killing efficacy of GO-QC against this microbe. We hypothesize that increased compatibility of QC with microbe cell wall enhances the adsorption of the grafted cationic QC on the surface of the microbe cell wall which in turn increases charge density near the cytoplasmic membrane to enhance disruption.

A unique feature of our GO-QC is the employment of polysaccharide as the
compatibilizing component, unlike other contact-active nanoparticles that employ hydrophobic polymers to enhance the interaction with membrane lipids. Others have found that hydrophobicity of the cationic polymer enhances its ability to penetrate the cell membrane to avoid the aqueous environment but this entails significant toxicity to mammalian cells as well as to microbial pathogens.² Our polymer is tuned to be compositionally similar to microbe cell wall so as to improve microbe selectivity and reduce toxicity to mammalian cells.

Further, the QC appears to “blunt” the GO edge in the nanohybrid so that GO-QC is less hemolytic than GO. We also tested a series of three different GO-QC sizes which have different edge lengths per unit area of the nanohybrid; we found that all the different sizes (from small to medium and to large sizes) have low hemolysis corroborating that mechanical cutting due to the particle edges is not prominent in the GO-QC action (see Supporting information Figures S10.I-IV and Table S2).
Table S1. Compatibility study of the various microbes surfaces with QC by contact angle analysis at 25 °C. (No. of samples per datum =10).

<table>
<thead>
<tr>
<th>Surface</th>
<th>Medium</th>
<th>Contact angle (°)</th>
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<tbody>
<tr>
<td></td>
<td>Water</td>
<td>QC solution</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>15.5±4.0</td>
<td>10.5±0.6</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>12.8±0.8</td>
<td>9.7±1.1</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>12.7±0.9</td>
<td>6.5±1.5</td>
</tr>
<tr>
<td>Glass slide</td>
<td>25.5±1.7</td>
<td>26.8±1.3</td>
</tr>
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</table>
Figure S9.II Optical and fluorescence micrographs of *S. aureus* attachment to 2 different pieces of GO-QC. (a) A larger piece of GO-QC (chosen for better visualization) is shown with the attached bacteria. (b) Another piece of GO-QC: (i) optical microscopy image and (ii) fluorescence microscopy image. Bacteria staining with BacLight bacterial viability kit indicates that the attached bacteria on GO-QC were dead (indicated by red color).
Figure S9.III (a) Fraction of microbes detached from pre-challenged GO-QC (1:5) determined by measuring the optical density of detached microbes after centrifuging at different Relative Centrifugation Forces (RCF). The absorbance is normalized to the initial concentration of microbes added to GO-QC. (b) Zeta potentials of the various microbes.
Antimicrobial activity of different nanotemplates: GO-QC (Small), GO-QC (Medium), GO-QC (Large) and Polystyrene (PS)-QC

To investigate if the GO-QC nanohybrid kills bacteria by mechanical cutting due to the edge or charge concentration, we prepared different sizes of GO-QC. The small GO-QC nanohybrid has greater edge length per unit area but also more polymer molecules conjugated per unit area as the functional groups on the GO for polymer attachment are concentrated at the edge defects.

Small, medium and large GO-QC nanohybrids were prepared by varying the GO sonication duration prior to functionalization with QC (Supporting information Figure S10.I and Table S2). The average lateral dimensions of GO-QC (Small), GO-QC (Medium) and GO-QC (Large) are approximately 100 nm, 500 nm and 1 µm respectively (see Supporting information Figure S10.I). At sub-MBC, the three sizes have different rates of killing with GO-QC (Small) having the highest rate (Supporting information Figure S10.II and Table S2); for example, at 25% MBC, the % kill for GO-QC (Small), GO-QC (Medium) and GO-QC (Large) are 99.7%, 97.0%, and 48.6% respectively (Supporting information Figure S10.II.a). At MBC, GO-QC (Small) also produces the most extensive damage over the bacterial surface, as shown by AFM analysis of challenged bacterial cells (Supporting information Figure S10.II.B). From only these sub-MBC data, we cannot differentiate between the contributions due to the charge concentration of QC versus mechanical cutting by GO edges on bacterial killing efficacy; with the small GO-QC, either the higher QC concentration leading to increased electrostatic killing or the higher edge length leading to increased mechanical cutting could increase the killing efficacy for these smaller sizes.
The “mechanical cutting” versus “charge concentration” interpretations can be tested by consideration of nanohybrid size effect on hemolysis. The hemolysis values for all three sizes were found to be the same, 10,000 µg mL\(^{-1}\) (Table S2). From the fact that GO is rather hemolytic but GO-QC is significantly less hemolytic (Table 1), we infer that hemolysis is mainly due to the sharp GO edges and not the QC tethered at the edges. Though there is more edge length per unit area in GO-QC (Small), it is also not more hemolytic than GO-QC (Large), which indicates that mechanical edge cutting effects are not a significant contributor to the killing action of these nanohybrids. We infer that QC makes the GO-QC relatively less-hemolytic compared to GO because of edge blunting and makes the antibacterial activity greater because of higher charge density per unit area. Zeta potential measurements (Table S2) corroborate this interpretation, with potential increasing as the GO-QC nanohybrid size decreases, strongly so for the smallest sized nanohybrid.

We also conjugated QC to polystyrene (PS) spheres with diameter of 500 nm. The PS-QC spheres are more hemolytic since QC is tethered over the entire surface and not primarily at the edges, and so the areal density of QC is high. QC coated spheres are highly hemolytic in spite of having no sharp edges. This shows that high areal density of cationic charge, above that present in GO-QC, can be harmful to Red Blood Cells. The high areal density of QC on PS-QC also leads to the low normalized MBC value for PS-QC (for normalization, we multiplied the measured MBC value of PS-QC by the approximate weight fraction of QC measured by TGA which is about 14%, see Supporting information Figure S10.III).

The MBC values of the different sizes of GO-QC were found to be similar (at 31.3 µg mL\(^{-1}\), Supporting information Table S2) but the smaller nanohybrids are more effective at killing bacteria at sub-MBC. Large GO-QC nanohybrids are less
numerous and fewer large GO-QC nanohybrids will attach per bacterium. Conversely, the smaller sizes permit higher counts of GO-QC (Small) nanohybrids to adhere onto the typical bacterium leading to more complete killing (Supporting information Figure 10.IV). Statistically, for the small GO-QC nanohybrids which are smaller in size than the average bacterium size, at concentrations lower than the MBC, a smaller fraction of the bacterial cells will have fewer than the critical number of GO-QC nanohybrids needed for killing, as compared to GO-QC (Large). This explains the sharp rise but non-100% kill at slightly lower than the MBC values for GO-QC (Small). For the large GO-QC nanohybrids, there is not such a steep increase in the killing rate with increase in concentration as the probability of a bacterium not contacting with GO-QC (Large) nanohybrids is higher at a given concentration. The behavior of the % kill for GO-QC (Medium) is intermediate between that of (Small) and (Large).
Figure S10.1. (a) AFM images of GO in small/medium/large sizes, the respective height curve and lateral dimension histograms of GO in small/medium/large size. The scale bar = 1 µm. (b) Average size value of GO in small/medium/large size. (30 pieces of GO were measured to calculate the average size and standard deviation for each sample.)
Figure S10.II (a) % kill vs. concentration of GO-QC for *S. aureus* for the three different sizes of GO-QC and PS-QC. (b) AFM of the different particles, GO-QC (Small), GO-QC (Medium), GO-QC (Large) and PS-QC, as well as their effect on bacteria morphology. (Scale bar represents 1 µm and the totally lysed bacteria are outlined in black.)
Figure S10.III TGA of (i) polystyrene spheres and (ii) PS-QC.
Figure S10.IV. Probability of GO-QC (Small) and GO-QC (Large) in bacteria contact at concentrations lower than the minimum bactericidal concentrations.
Table S2. Minimum bactericidal concentrations (MBC), % kill, vs. concentration, hemolytic activities and ζ-potentials for the three different sizes of GO-QC and PS-QC

<table>
<thead>
<tr>
<th>Materials</th>
<th>MBC (µg mL⁻¹)</th>
<th>% kill</th>
<th>HC₅₀ (µg mL⁻¹)</th>
<th>ζ potential (mV)</th>
</tr>
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<tr>
<td>GO-QC (Small)</td>
<td>31.3</td>
<td>99.7</td>
<td>99.7</td>
<td>99.4</td>
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<tr>
<td>GO-QC (Medium)</td>
<td>31.3</td>
<td>99.7</td>
<td>97.0</td>
<td>71.5</td>
</tr>
<tr>
<td>GO-QC (Large)</td>
<td>31.3</td>
<td>69.2</td>
<td>48.6</td>
<td>27.8</td>
</tr>
<tr>
<td>PS-QC</td>
<td>8.1</td>
<td>99.9</td>
<td>99.8</td>
<td>98.9</td>
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Table S3. Minimum bactericidal concentrations (MBC), hemolytic activities and $\zeta$-potentials for the PS-QC with three different PS:QC ratio

<table>
<thead>
<tr>
<th>Materials</th>
<th>MBC (µg mL$^{-1}$)</th>
<th>HC$_{50}$ (µg mL$^{-1}$)</th>
<th>$\zeta$ potential (mV)</th>
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<tbody>
<tr>
<td>PS-QC (1:1)</td>
<td>312.5</td>
<td>&gt;10000</td>
<td>9.8 ± 1.7</td>
</tr>
<tr>
<td>PS-QC (1:5)</td>
<td>78</td>
<td>5000</td>
<td>23.3 ± 0.49</td>
</tr>
<tr>
<td>PS-QC (1:10)</td>
<td>8.1</td>
<td>650</td>
<td>48.1 ± 0.7</td>
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Figure S11. $^1$H NMR of nCS in D$_2$O.
Reference:
