Supporting Information for

Disinfection and Electrostatic Recovery of N95 Respirators by Corona Discharge for Safe Reuse

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This PDF file includes:

Text S1 to S12

Table S1 and S2

Figures S1 to S12

SI References

S1. Acronym definitions

CD: Corona Discharge

R: Log Reduction

V: Discharge Voltage

I: Discharge Current

d: Electrode-Sample Distance

t: Treatment time

n: Cycle time

s: Storage time between each cycle

S2. Preparation of cell cultures

Fresh *E. coli* cell cultures were prepared using 100 mL of lysogeny broth (LB) and incubated overnight at 37 °C for each experiment. The cell pellets were collected at 2,500 rpm for 10 min and washed one time with 1 mL of phosphate buffer (PBS, pH = 7.4) to obtain an initial concentration of 10^{10} CFU/mL. For *Pichia pastoris* SMD1163 (a species of methylotrophic yeast), fresh cell cultures were prepared using 5 mL of Yeast Extract–Peptone–Dextrose (YPD) broth with 2% glucose and incubated overnight at 30 °C at 300 rpm to achieve an initial concentration of 10^8 CFU/mL). *Geobacillus stearothermophilus* (which produces heat resistant spores) cells were prepared using 100 mL of nutrient broth (Difco™ nutrient broth for cultivation of non-fastidious microorganisms, 3 g of beef extract and 5 g of peptone per liter) and incubated overnight at 50 °C at 120 rpm (reaching an initial concentration of 10^4 CFU/mL).

S3. Electric field simulation

COMSOL simulation was performed to calculate the electric potential distribution in the CD controlled by different parameters. For this purpose, a one dimensional model was setup using a point to plane configuration.[S1] The electron and ions continuity and momentum equation is solved using the drift-diffusion equation coupled with Poisson's equation.[S2] The electrostatic potential V_s is computed with Equation (2).

$$-\nabla \cdot \varepsilon_0 \varepsilon_r \nabla V_s = \rho \quad (2)$$

where, ρ is the space charge density, ε_0 is the permittivity in free space, and ε_r is the relative permittivity.

S4. Expression and Purification of sfGFP

sfGPF was expressed using arabinose inducible plasmid pBad-sfGFP (AddGene). The sfGFP gene was codon-optimized for *E. coli* with a C-terminal 6-His affinity tag. DH10B *E. coli* cells transformed with pBad-sfGFP were used to inoculate 500 mL of LB medium containing 100 μg/mL Amp. After the *E. coli* in the LB medium culture grown to OD600=0.1 with shaking at 37 °C, 0.1% Arabinose was added to induce the sfGFP expression. After 40 h of shaking at 37 °C, cells from 500 mL of culture were collected by centrifugation.

The protein was purified using TALON Metal Affinity Resin (Takara Bio USA, Inc.). The cell pellet was resuspended and broken by an Ultrasonic Cell Disruptor in Lysis buffer (50 mM PBS, 300 mM sodium chloride, pH 7.4). The supernatant was applied to 1 mL talon resin and bound for 30 min. The bound resin was washed with > 50 volumes of Lysis buffer. Protein was eluted from the bound resin with 5 mL volumes of Elution buffer (50 mM PBS, 300 mM sodium chloride, 300 mM imidazole, pH 7.4) and concentrated to 1 mL. See SDS page for electrophoresis details.

S5. SDS-PAGE

Preparation of the separation gel. Mix in the following order: 30% acrylamide, 4 mL; 4xTRIS, pH 8.8, 2.5 mL; H_2O, 4.4 mL; 10% SDS, 75 µL; 10% APS, 12.5 µL; TEMED, 7.5 µL. Pour gel, leaving ~2 cm below the bottom of the comb for the stacking gel. Make sure to remove bubbles by adding isopropanol. Layer the top of the gel with isopropanol. In ~30 min, the gel is completely polymerized. Remove the isopropanol and wash out the remaining traces of isopropanol with distilled water.

Preparation of the stacking gel. Mix in the following order: 30% acrylamide, 0.65 mL; 4xTRIS/SDS, pH 6.8, 1.1 mL; H_2O, 3.5 mL; 10% SDS, 25 µL; 10% APS, 25 µL; TEMED, 5 µL. Pour stacking gel on top of the separation gel and add combs. Incubate samples with loading buffer for 20 min at room temperature. Load samples and molecular mass protein markers into wells for separation by electrophoresis after the gel is polymerized. Run at 120 V for 70 min.

How to stain and visualize it, write a couple of sentences here.

S6. Fluorescence Measurement

The treated sfGPF samples were transferred to the wells of a fluorescence compatible 96 well plate. The total volume in each well was 150 µL. sfGFP fluorescence readings (Excitation 483 nm, Emission 535 nm) were taken by Bio Synergy 2 Multi-Detection Microplate Reader. Each well was repeated in triplicate with Elution buffer being used as a blank.

S7. DNA Gel Electrophoresis

1% agarose (Bio-Rad only) was made using 1X TAE buffer. Move gel into a chamber and samples were loaded into the wells as follows: GeneRuler ladder (5 µL), plasmid with different treatment

time (5 µL). The chamber was filled with 1X TAE buffer and ran at 100 V for 40 minutes. How to visualize it? Write a couple of sentences here.

S8. Filtration efficiency test

The filter tester used in testing was a TSI 8130 Automated Filter Tester that is capable of efficiency measurements of up to 99.999%. It produced a particle size distribution with a count median diameter of 0.075 ± 0.020 microns (µm) and a geometric standard deviation not exceeding 1.86 µm. The mass median diameter was approximately 0.26 µm, which is generally accepted as the most penetrating aerosol size. All test method acceptance criteria were met. Testing was performed in compliance with US FDA good manufacturing practice (GMP) regulations 21 CFR Parts 210, 211 and 820.

S9. Effect of cycle time (*n*) and storage time between cycles (*s*)

To enhance the disinfection effect further, the samples were exposed to multiple treatment cycles with a storage time between each cycle to allow *E. coli* to completely react to the applied CD.[S3,S4] With treatment time of $t = 7.5$ mins and storage time of $s = 20$ min for $n = 2$ cycles, the log reduction increased to $R = 2.52 \pm 1.14$ from 1.21 ± 0.33 achieved with $t = 15$ mins of continuous treatment as shown in Figure S1a. Reducing s from 20 min to 5 min did not seem to have any effect (Figure S3). Thus, it can be learned that adding storage time, $s \geq 5$min, between each cycle of treatment can significantly enhance the disinfection. This observation could be attributed to the fact that reactive species can remain active even during the storage time, allowing the cell membranes to be completely disrupted before the next cycle.[S4,S5,S6]

S10. Disinfection efficacy against yeast and spores

To test the broad-spectrum disinfection efficacy of CD, we also conducted disinfection experiments on *Pichia pastoris* (a species of methylotrophic yeast) and *Geobacillus* (which produces heat resistant spores), both of which possess thicker cell walls than *E. coli.*, meaning better tolerance against membrane rupture and DNA leakage. For yeast, the effect of treatment time was investigated with the discharge parameters set to V = 25 kV, I = 0.05 mA and d = 3.5 cm and varying t from 2.5 min to 30 mins. As shown in Figure S4a, the disinfection efficacy against yeast slightly increased from R = 0.34 ± 0.06 for t = 7.5 min to 0.50 ± 0.07 for t = 30 min. It matches with the previous result that after 15 min, no significant disinfection effect improvement can be achieved by simply increasing the treatment time. Instead, when the samples were treated with n = 2 cycles, the log reduction was increased to 1.04 ± 0.10, as indicated in Figure S4b. Further, when the samples were treated in a closed environment, the efficacy was slightly increased to R = 0.96 ± 0.10 for n = 1 cycle (Figure S4c). As discussed in the previous result, the samples in closed environment were consistently exposed to ozone (up to 8.8 ppm after 20 mins), which could lead to more oxidative damage to cell membranes compared to an open environment. Spores are generally more challenging to be deactivated compared to vegetative forms.[S7,S8,S9]. When 100 μL of freshly prepared *Geobacillus* cells were spread on a nutrient agar plate and exposed to CD, a log reduction of 2.52 was observed after two cycles of treatment as shown in Figure S4d. Higher log reduction can be achieved if yeast and spores are treated using a tungsten wire electrode in a closed environment. The disinfection results on yeast and spores indicate that CD can provide broad-spectrum disinfection for different types of microorganisms, including traditionally challenging ones such as spores. The mechanisms leading to different effectiveness of CD on various types of microorganisms will be further studied in the future.

S11. SEM images of treated *E. coli* compared with control

To explain the mechanism of CD inactivating bacteria, we firstly observed the influence of CD on global morphology of *E. coli* by high-resolution SEM images. Figure S5a-d shows homogenous populations in both control condition and CD treated samples. Although a little cell destruction and pore formation in the cell wall were observed (Figure S5b-d) compared to control (Figure S5a), the global results suggested no significant damage of the morphology after eradication by CD, indicating the sterilization mechanism of CD was not through physical damage but others.

S12. Comparison of traditional disinfection methods vs. corona discharge

Table S2 compares various disinfection methods based on their safety, cost, process time, disinfection efficacy and recharge effect. Although traditional methods have very good disinfection efficacy (up to 6 log reduction), they use toxic chemicals or gasses to achieve them. Prolonged human exposure to such environments can lead to harmful effects including respiratory ailments and cancer. Disinfection methods such has dry heat and HPV rely on thermal excitation to achieve the specified log reduction. This can cause structural damages over time leading to a significant drop in filtration efficacy. Indirect treatments such as HPV and ozone rely on prolonged exposure to the dispersed gas for effective disinfection. This requires a tightly controlled environment and long process time making it expensive to use. None of them can electrostatically recharge N95 masks. However, CD based disinfection method is a relatively safe non thermal process with quick process time and low cost. It can reach up to 6 log reduction and simultaneously recharge N95 masks for up to 15 times of safe reuse.

Table S1. Filtration efficiency results received from Nelson Labs

Test Article	Corrected[a] Airflow Resistance (mm H_2O)	Particle Penetration (%)	Filtration Efficiency (%)
J0531	6.4	5.88	94.42
J0532	6.9	5.14	94.86
J0533	6.7	5.88	94.12

[a] The final airflow resistance value for each test article was determined by subtracting out the background resistance from the system

Table S2. Comparison of traditional disinfection methods vs. corona discharge for N95 mask reuse

Disinfection Method	Safety	Non-Thermal	Process Time	Cost	Log Reduction	Electrostatic Recharge	Reuse Time
Chemical[10,11]	Low	Yes	7 days	Low	1 to 6	No	0
UV[10,11]	Low	Yes	1 hour	Nominal	1 to 5	No	3 to 5
HPV[10,11]	Low	No	4 hours	High	3 to 6	No	3
Heat[10,11]	Low	No	1 hours	Nominal	2 to 6	No	3
Ozone[11]	Low	Yes	2 hours	High	2 to 6	No	3 to 5
CD	High	Yes	5 to 10 min	Low	3 to 6	Yes	15

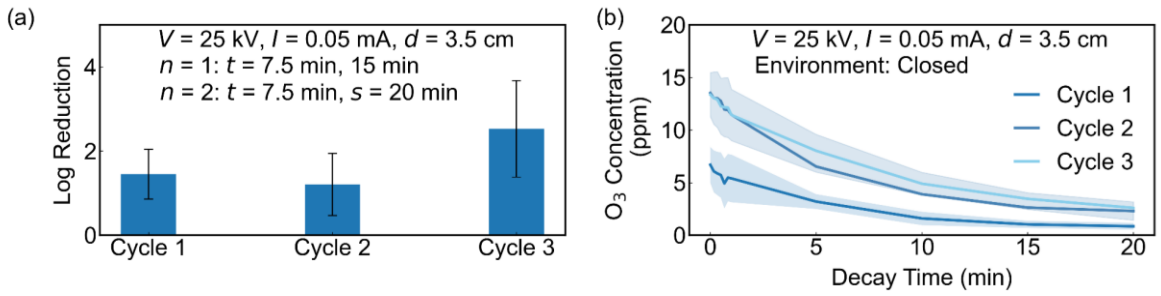

Figure S1. (a) Log reduction achieved with introducing 2 treatment cycles compared with continuous treatment (b) Ozone decay inside a closed environment measured after each cycle for 3 treatment cycles using tungsten wire. All results were obtained with discharge parameters set to V = 25 kV, I = 0.05 mA and d = 3.5 cm.

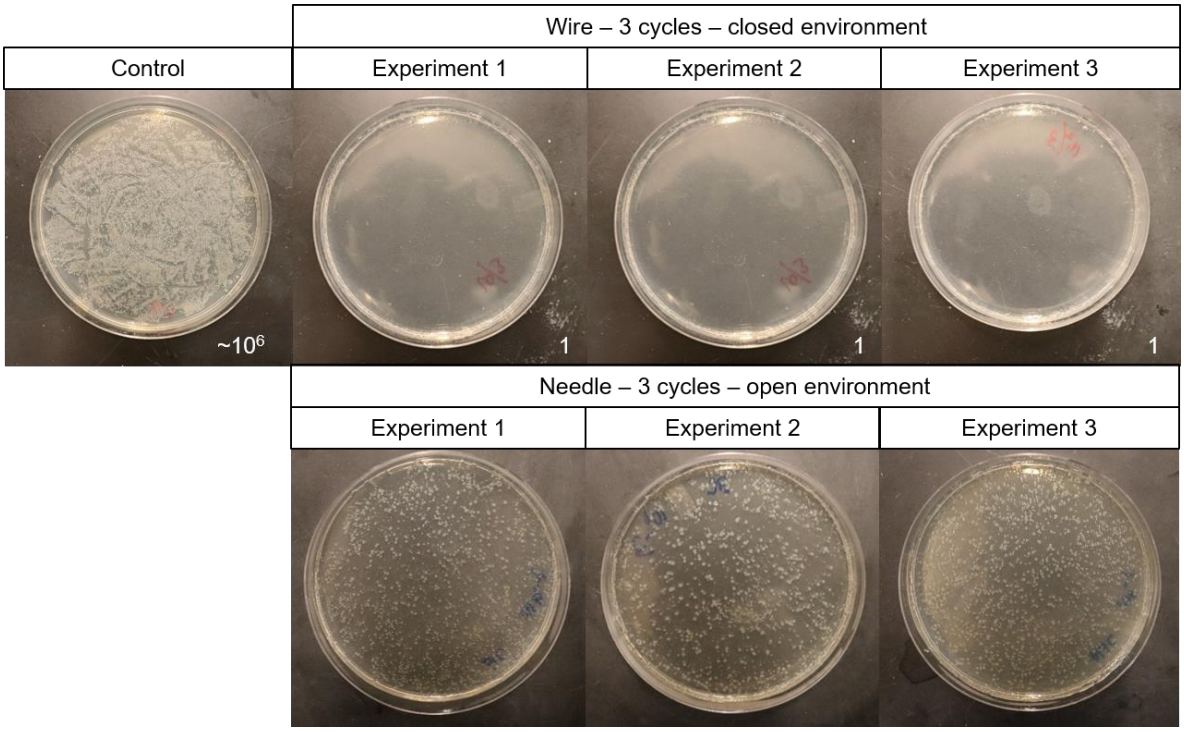

Figure S2. *E. coli* colonies survived after treated with tungsten wire in closed environment compared to tungsten needle in open environment when exposed to 3 cycles of CD wire (n = 3, t = 7.5 min, I = 0.05 mA, V = 25 kV, d = 3.5 cm).

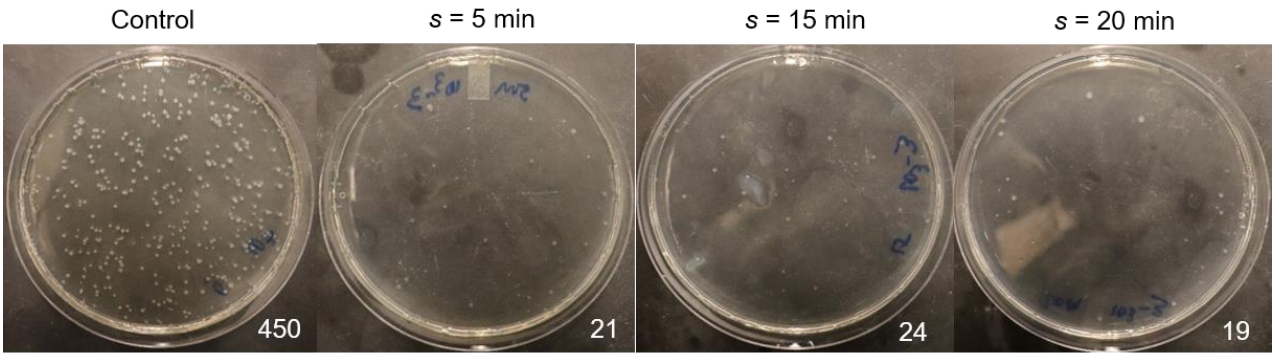

Figure S3. *E.coli* colonies before and after CD treatment with varying storage times from s = 5 min to 20 min at 2 cycles (n = 2, t = 7.5 min, s = 20 min, I = 0.05 mA, V = 25 kV, d = 3.5 cm).

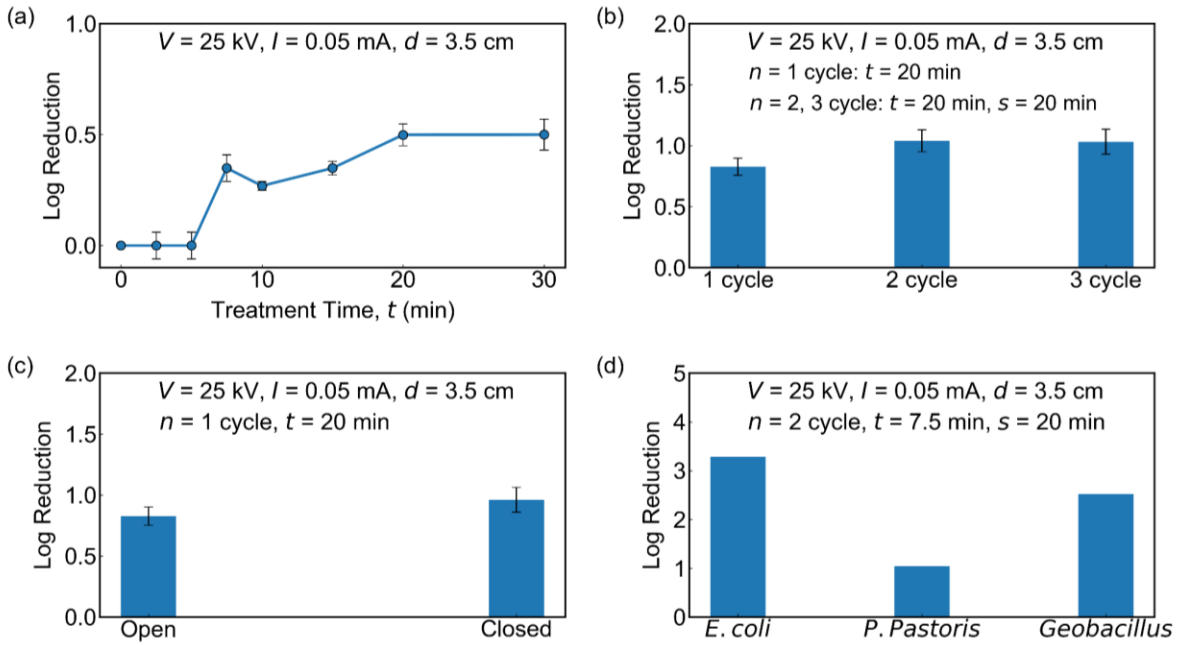

Figure S4. Log reduction achieved (a) with increase in treatment time (b) with introducing treatment cycles (c) in open and closed environment. (d) Comparison of Log reductions achieved for *E. coli*, yeast, and spores. All results were obtained with discharge parameters set to $V = 25$

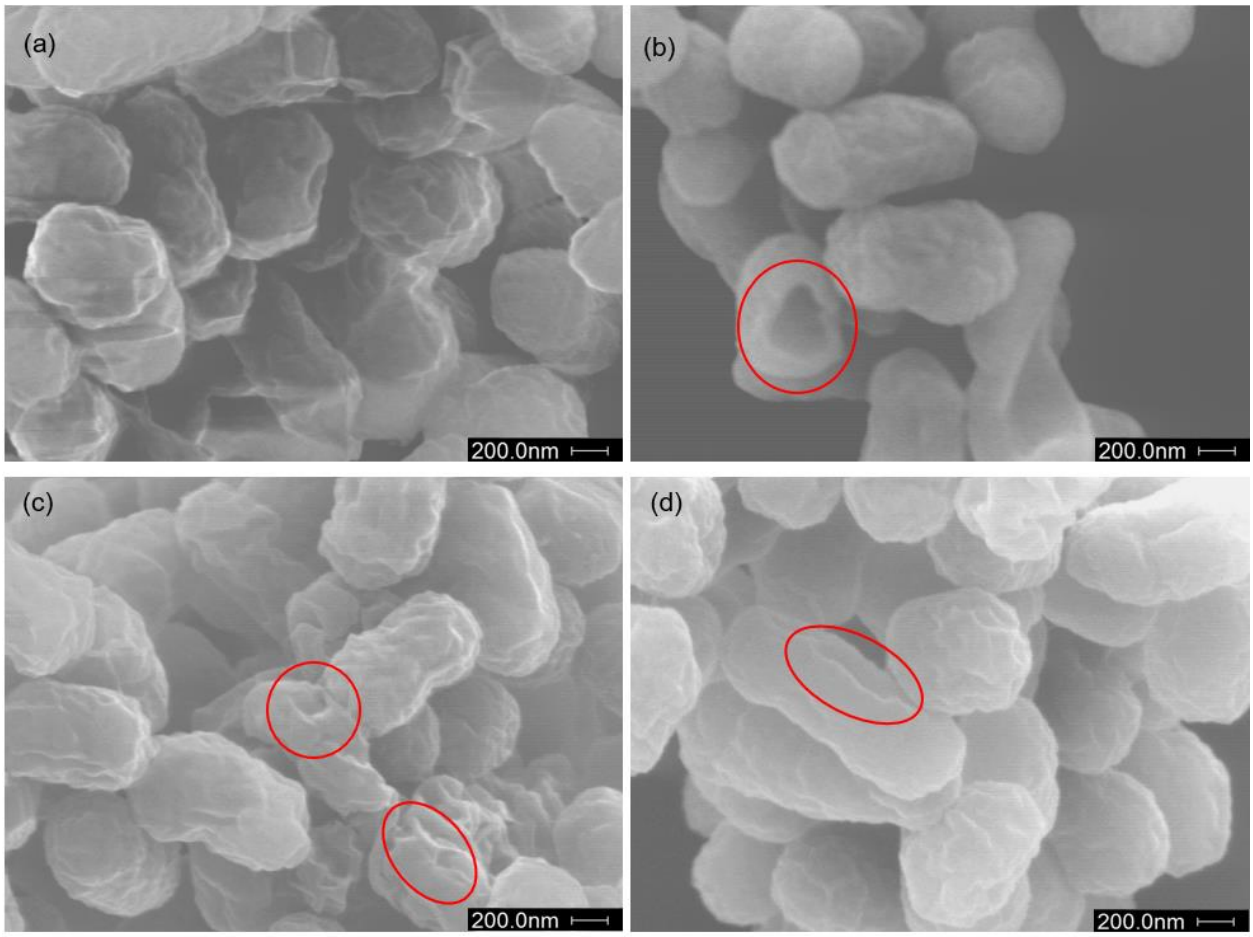

Figure S5. SEM images of (a) untreated *E. coli* (b-d) *E. coli* after 7.5 mins of CD treatment showing membrane rupture.

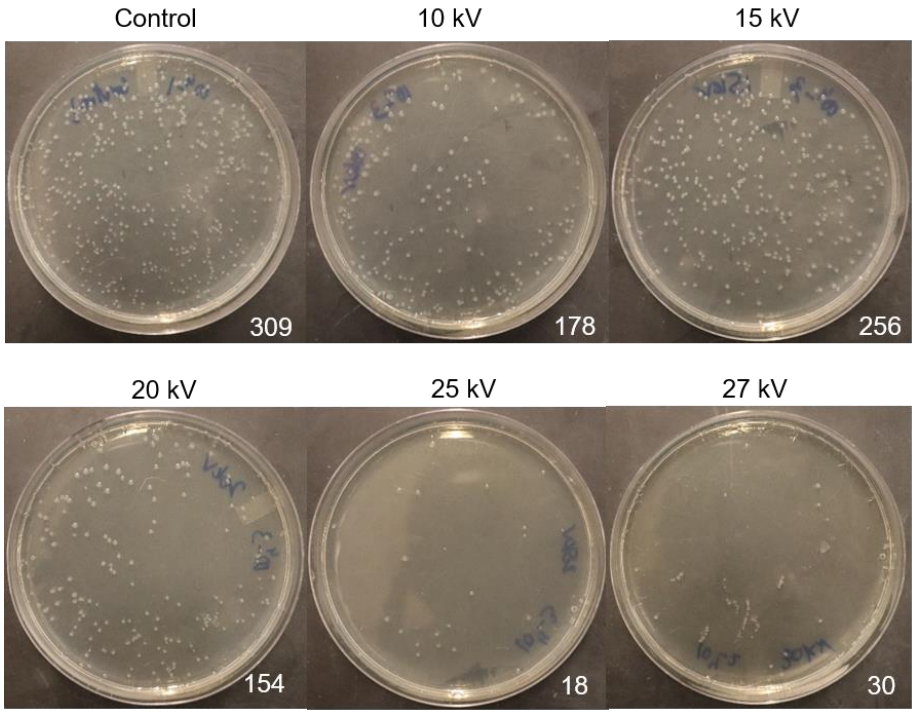

Figure S6. *E.coli* colonies before and after CD treatment for voltage study (I = 0.05 mA, d = 3.5 cm, t = 7.5 min, V = 10 kV to 27 kV).

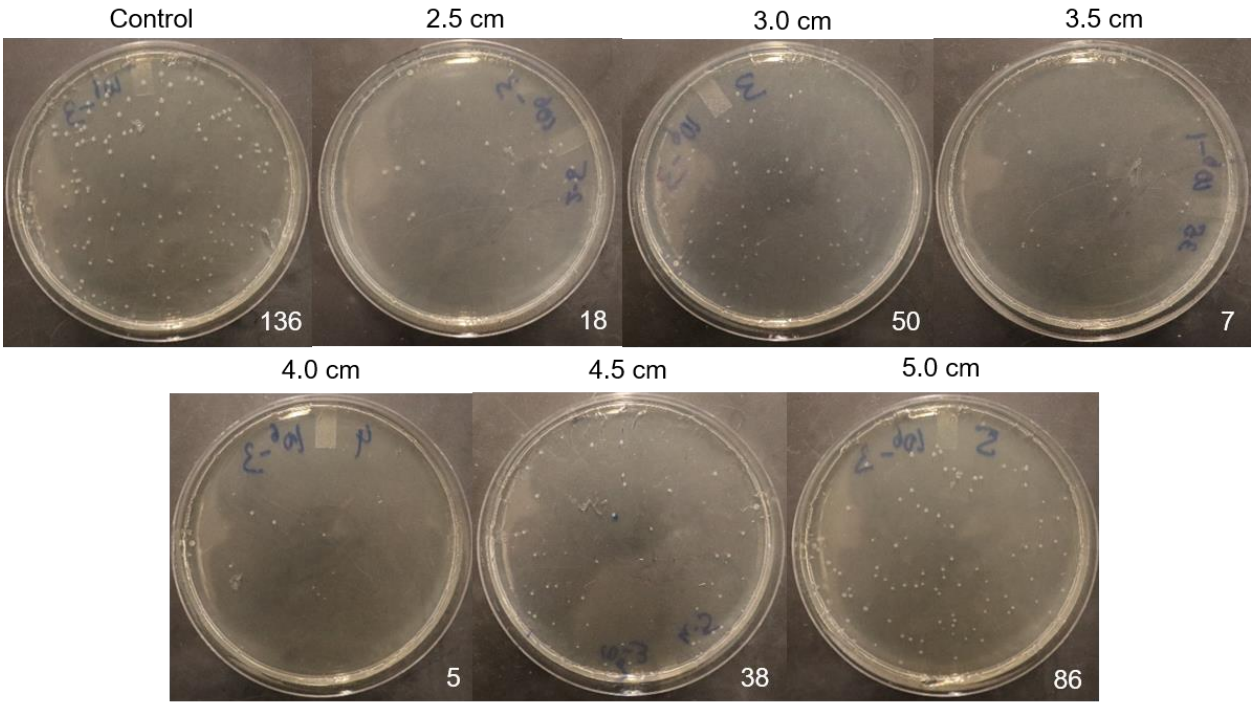

Figure S7. *E.coli* colonies before and after CD treatment for distance study (I = 0.05 mA, V = 25 kV, t = 7.5 min, d = 2.5 cm to 5 cm).

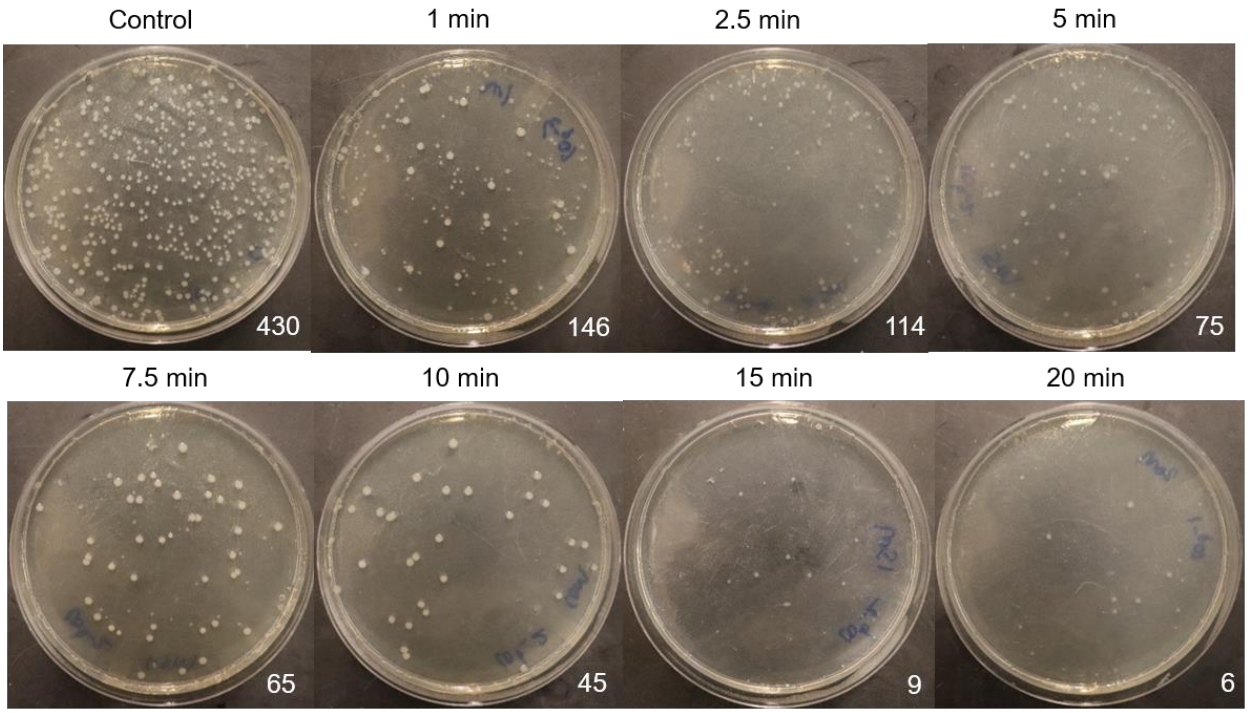

Figure S8. *E.coli* colonies before and after CD treatment for distance study (I = 0.05 mA, V = 25 kV, d = 3.5 cm, t = 1 min to 20 min).

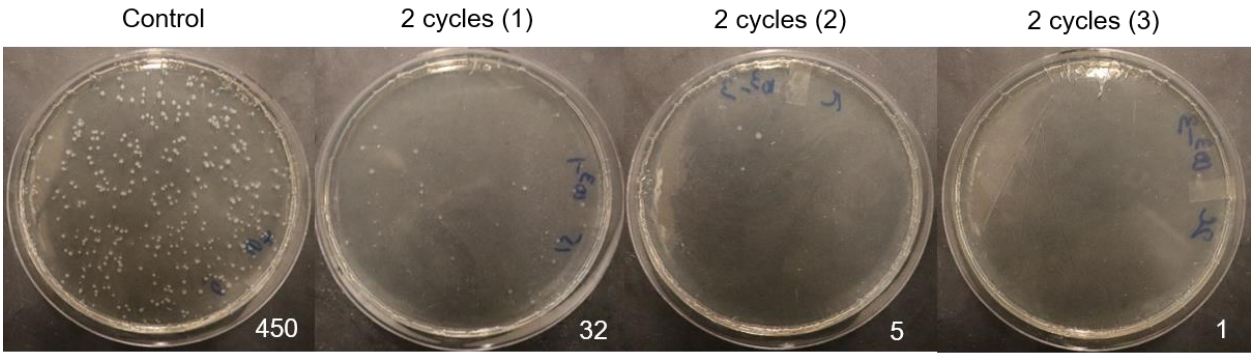

Figure S9. *E.coli* colonies before and after CD treatment with 2 cycles (n = 2, t = 7.5 min, s = 20 min, I = 0.05 mA, V = 25 kV, d = 3.5 cm).

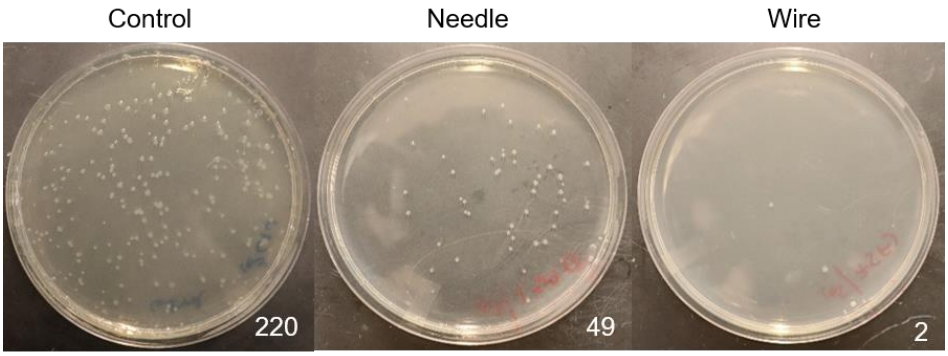

Figure S10. *E.coli* colonies before and after CD treatment with tungsten needle and 5 cm long tungsten wire (n = 1, t = 7.5 min, I = 0.05 mA, V = 25 kV, d = 3.5 cm).

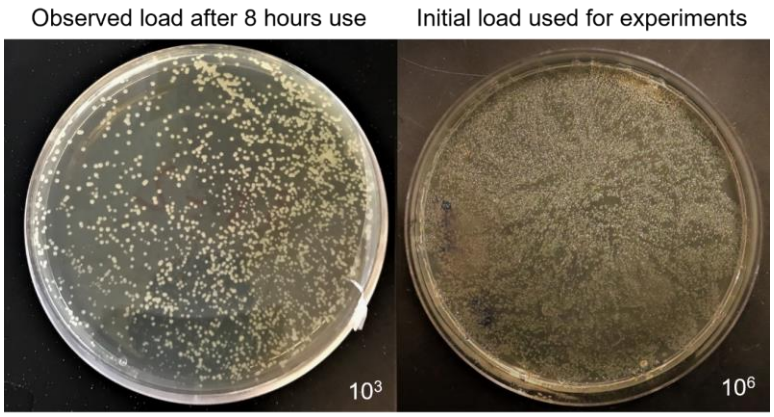

Figure S11. (a) Bacterial load of 10^3 CFUs observed on a 5 x 5 cm sample of N95 mask after 8 hours of usage in lab environment (b) Initial bacterial load of 10^6 CFUs applied on each sample for all reported experiments.

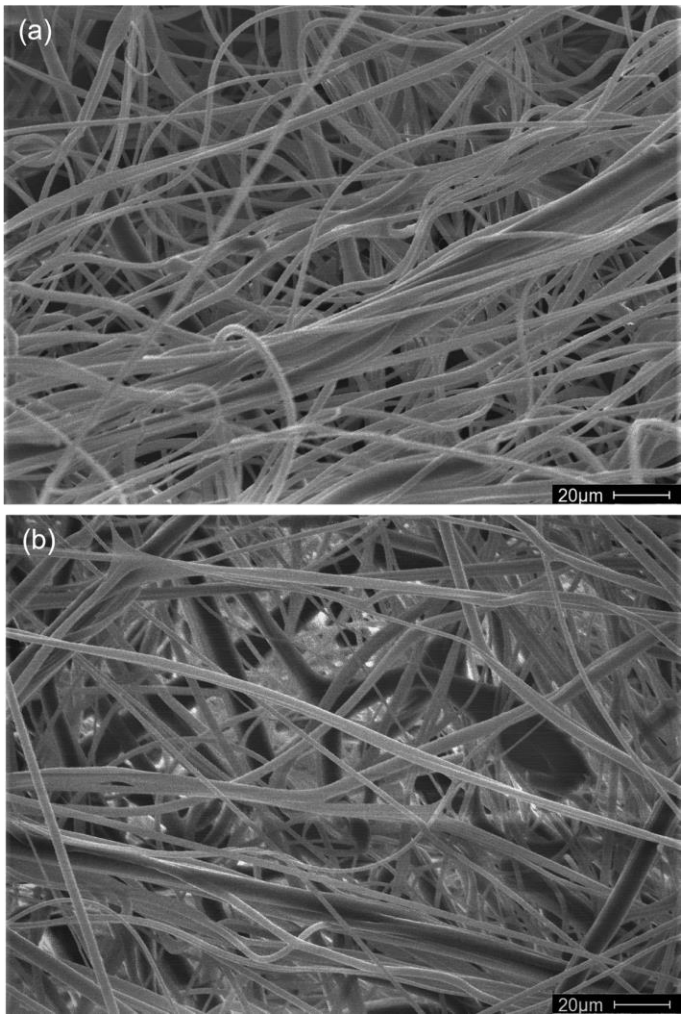

Figure S12. SEM images of the filter layer (a) untreated new N95 respirator (b) after 10 cycles of CD treatment showing no physical damage to fibers.

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