

Paper-based plasma sanitizers

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Edited by John A. Rogers, University of Illinois, Urbana, IL, and approved March 28, 2017 (received for review December 23, 2016)

This work describes disposable plasma generators made from metallized paper. The fabricated plasma generators with layered and patterned sheets of paper provide a simple and flexible format for dielectric barrier discharge to create atmospheric plasma without an applied vacuum. The porosity of paper allows gas to permeate its bulk volume and fuel plasma, while plasma-induced forced convection cools the substrate. When electrically driven with oscillating peak-to-peak potentials of ± 1 to ± 10 kV, the paper-based devices produced both volume and surface plasmas capable of killing microbes. The plasma sanitizers deactivated greater than 99% of Saccharomyces cerevisiae and greater than 99.9% of Escherichia coli cells with 30 s of noncontact treatment. Characterization of plasma generated from the sanitizers revealed a detectable level of UV-C (1.9 nW·cm⁻²·nm⁻¹), modest surface temperature (60 °C with 60 s of activation), and a high level of ozone (13 ppm with 60 s of activation). These results deliver insights into the mechanisms and suitability of paper-based substrates for active antimicrobial sanitization with scalable, flexible sheets. In addition, this work shows how paper-based generators are conformable to curved surfaces, appropriate for kirigami-like "stretchy" structures, compatible with user interfaces, and suitable for sanitization of microbes aerosolized onto a surface. In general, these disposable plasma generators represent progress toward biodegradable devices based on flexible renewable materials, which may impact the future design of protective garments, skin-like sensors for robots or prosthetics, and user interfaces in contaminated environments.

paper-based electronics | plasma | touch sensors | kirigami | sanitization

S tate-of-the-art, plasma generators are capable of manipulating surface chemistries in manufacturing processes (1, 2), sterilizing medical devices (3, 4), providing thrust for space vehicles (5, 6), manipulating lift-to-drag ratios on airfoils (7, 8), manipulating heat transfer (9), healing wounds (10), and killing microbes in atmospheric environments (11, 12). These plasmabased generators typically use rigid components, which cannot bend or conform to irregularly shaped objects. The lack of flexibility limits their potential use as protective skins for prosthetics, wearable garments, robotics, or hard-to-access areas where microbes might collect. In addition, chamber-free ionizers with atmospheric plasma typically have small active areas (\sim 50 cm²) not designed for scalable antimicrobial protection over large surfaces. Scalable and flexible atmospheric plasma generators might be suitable in varied urban and rural environments to reduce healthcareassociated infections.

Previous studies have suggested that plasma generators have the potential to be fast, efficient, and safe devices for healing wounds (10, 13), modifying structural surfaces (14), and assisting in tissue engineering (15). In general, plasma-based sanitization/disinfection/sterilization takes advantage of three synergistic mechanisms: (*i*) interacting free radicals, (*ii*) radiative effects from UV light, and (*iii*) volatilization of microorganisms (16). Sanitization reduces the number of disease-causing microbes, disinfection provides further reduction, and sterilization is the most stringent form of decontamination with demonstrated deactivation of spores. Plasma-based treatments have the potential to provide comparable sterilization to conventional methods that use heat (17), chemicals (17), or radiation (18–21). Plasma treatments have deactivated a

range of microbes, such as *Geobacillus stearothermophilus* (22), *Staphylococcus aureus* (MRSA) (23), and adenoviruses (24) on varied substrates [e.g., food (25)].

Cellulose-based paper has tunable porosity to allow gases to permeate its bulk volume, and it is capable of handling temperatures up to 250 °C (26). These properties make paper a fitting material for atmospheric plasma generators, as its permeability allows the flow of gas through the substrate to provide fuel for the plasma and to cool the paper with plasma-induced forced convection. Stemming from advances in paper-based microfluidics (27–29), paper-based electronics and photonics (papertronics) are demonstrating advances in energy, sensing, actuation, communication, and biodiagnostic applications (30–34). There is a gradual transition from conventional, nonresponsive paper products (e.g., printing, food packaging, labels, and decorations) to active, communicative, and "smart" devices (35–38).

In this work, we report the design, fabrication, and experimental characterization of a simple and disposable plasma generator that is mechanically flexible and capable of antimicrobial sanitization. Beyond characterizing the effectiveness of the sanitizers, this work also demonstrates garment-like protection, multifunctional capacitive touchpads, and a kirigami-based "stretchy" device. Potential applications of kirigami-based, stretchable plasma generators might include comfortable protective garments. Ultimately, the engineering and science demonstrated in this work is a step toward large-scale, skin-like sensors that provide active antimicrobial protection.

Experimental Design

Fabrication of Plasma-Generating Devices. For each paper-based plasma generator, we prepared two 150-µm sheets of metallized

Significance

Conventional plasma-based treatments deactivate microbes in confined, nonatmospheric chambers. During the last few decades, new concepts have led to portable, chamberless generators with rigid configurations of electrodes. This work explores unique designs and methods of antimicrobial sanitization with flexible plasma generators consisting of laminated assemblies of patterned, metallized paper. Under oscillating potentials (\pm 1 to \pm 10 kV from 100 Hz to 8 kHz), the paperbased devices produced plasma that deactivated 99% of *Saccharomyces cerevisiae* and *Escherichia coli* cells with 30 s of treatment. Future uses of this type of plasma generator might include the sanitization of protective garments, origami- or kirigami-like devices, and human-machine user interfaces in healthcare and/or contaminated environments.

This article is a FNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10. 1073/pnas.1621203114/-/DCSupplemental.

Author contributions: J.X., Q.C., S.R., J.F.W., and A.D.M. designed research; J.X., Q.C., P.S., J.F.W., and A.D.M. performed research; J.X., Q.C., S.R., J.F.W., and A.D.M. analyzed data; and J.X., Q.C., S.R., J.F.W., and A.D.M. wrote the paper.

Conflict of interest statement: US Provisional Patent was filed (62/291,082) for "Low-Cost, Flexible, Paper-Based Plasma Sterilizer" on February 4, 2016. US Patent Application was filed (15/425,474) for "Flexible Plasma Applicators Based on Fibrous Layers" on February 6, 2017. This article is a PNAS Direct Submission.

paper (A-550; AR Metallizing) patterned with a laser engraver (VLS 2.3; Universal Laser Systems). Fig. 1 depicts the laminated structure of the devices and the metallized paper. In this study, we engraved repeated hexagons (honeycombs) on the devices. Such a pattern uses a minimal amount of total perimeter to cover a surface (39) and permits generation of atmospheric plasma along the edges of the hexagonal grid. By tuning the size/spacing of the honeycombs, it is possible to ensure the plasma has coverage over the entirety of a patterned surface.

As illustrated in Fig. 1*A*, we bonded the nonconductive sides of two sheets of laser-engraved metallized paper with a 30-µm-thick adhesive layer (Ad-Tech 5645; Adhesive Technology). To connect the patterned conductive regions to an external power supply, we bonded wires to the metallized paper with water- and silver-based inks (Conductive Compounds Company) in similar fashion to that described in ref. 35.

Generation of Plasma. The paper-based plasma generators in this work rely on the working principle of dielectric barrier discharge (DBD). The first demonstration of DBD-based plasma was in 1857 by Siemens (14), but the first report of DBD for deactivation of microbes was not until the mid-1990s (40). The plasma originates from the discharge between two electrodes separated by a dielectric medium, which, in our work, is a porous matrix of cellulose fibers. To produce plasma, we first produced sinusoidal signals with frequencies ranging from 1 to 8 kHz and peak-to-peak voltages V_{p-p} ranging from ±1 to ±5 V using a function generator (4011A; BK Precision). Then, we amplified this signal using a high-voltage amplifier (model 10/10; TREK) with a gain of 1,000 to output a high oscillating potential V_{p-p} ranging from ±1 to ±5 kV. The generation of plasma was also frequency dependent, which suggests the existence of an optimal frequency at a given electric potential to generate uniform coverage of plasma (Movie S1). The

power consumptions for typical paper-based plasma generators demonstrated in this work were less than 20 W (\sim 18 W for RMS 2.2 kV at \sim 8 mA) going into the device.

DBD can produce two types of plasma (i.e., volume plasma and surface plasma) depending on the configuration of the device. Although both configurations consist of one or more dielectric insulators sandwiched by two electrodes, volume plasma usually has a gap of air between the two electrodes, whereas surface plasma does not. The plasma presented in this work was a combination of both volume plasma and surface plasma because of the porosity of the metallized paper. Fig. 1*A* illustrates the typical geometry and locations of the volume and surface plasmas.

The paper-based plasma generators produced glowing plasma and perceptible ozone. Fig. 1*C* shows a flexible, functioning plasma generator in the form of a logotype with an internally etched, honeycomb pattern. This sample was under the excitation of a peak-to-peak voltage of ± 3 kV at 1.7 kHz. Fig. 1*D* shows another functioning rectangular plasma generator conforming to a cylindrical substrate (diameter of ~100 mm) while generating glowing plasma activated with a peak-to-peak voltage V_{p-p} of ± 2 kV at 1 kHz. Characterization of the plasma (*Supporting Information*) also showed a detectable level of UV-C (1.9 nW·cm⁻²·nm⁻¹; Fig. S1), modest surface temperature (60 °C with 60 s of activation; Fig. S2*B*), and a high level of ozone (13 ppm with 60 s of activation; Figs. S2*A* and S3 and Table S1).

Noncontact Experiments. To characterize the efficacy of paperbased plasma sanitizers, we performed experimental studies with two setups (i.e., a noncontact experiment and a direct-contact experiment for both *Saccharomyces cerevisiae* and *Escherichia coli*). In noncontact experiments, we prepared circular plasma sanitizers with metallized paper and measured the ozone levels (*Supporting Information*). Each sanitizer had a diameter of 90 mm,



Fig. 1. Atmospheric plasma generators made from metallized paper. (*A*) A hexagonal unit of a paper-based plasma generator. (*B*) The laminated structure of metallized paper used in this work. (*C*) A paper-based plasma generator with and without applied potential. (*D*) A flexible plasma generator made from etched metallized paper in straight and bent states. (*F*) Volume plasma goes through the porous matrix of cellulose-based fibers, while surface plasma is above/below the metallized substrate. (*F*) A flat, circular plasma generator without patterned honeycombs shows surface plasma. (*G*) A circular plasma generator with the same design as *F* has one-half of the top layer curling upward to demonstrate the production of both volume and surface plasmas. The plasma glowed with the application of a V_{p-p} of ± 3 kV at 1.7 kHz for *C*, a V_{p-p} of ± 2 kV at 1 kHz for *D*, and a V_{p-p} of ± 2.5 kV at 2 kHz for *F* and *G*.

matching the inner diameter of the lid of a Petri dish. By attaching the sanitizer to the inner surface of the lid, we avoided touching the sanitizer during experiments, which would have led to unintentional contamination. When closed, the surface of the paperbased sanitizer was 10 mm away from the surface of the yeast extract-peptone-dextrose (YEPD) and Luria-Bertani (LB) solid media, on which the *S. cerevisiae* or *E. coli* suspension was inoculated aseptically in the Petri dish. Fig. 2*A* and *B* illustrates the setup of the noncontact experiments.

We inoculated 100 μ L of *S. cerevisiae* and *E. coli* cell suspensions on YEPD and LB solid media, respectively, and then covered the Petri dish with the plasma generator attached to the lid. The lead of the circular sanitizer ran through the gap between the lid and the Petri dish to an AC input with a frequency of 2 kHz and a peak–peak voltage of ± 3.15 kV. There were six groups of experiments with varied times of exposure, each consisting of five repetitive samples for both *S. cerevisiae* and *E. coli*.

Direct-Contact Experiments. Our noncontact experiments demonstrated the ability to deactivate bacteria on agar a set distance away from the sanitizers. Nonetheless, it is desirable to deactivate microbes that have landed on a substrate itself. Keypads, shared user interfaces, clothing, and garments exemplify direct-contact applications involving surface contamination with microbes.

A human sneeze is one method of spreading infectious microbes. To simulate a sneeze—an abrupt expulsion of secretions, saliva, and microorganisms from the respiratory tract—and evaluate the efficacy of paper-based plasma generators in decontaminating themselves, we developed an experimental setup (Fig. 3*A*) with a pneumatic dispensing system (Performus II; Nordson EFD). Using a gauge pressure of 11 psi and a dispensing time of 50 µs, an intranasal drug delivery device (MAD Nasal; LMA) aerosolized a liquid suspension of *S. cerevisiae* or *E.coli* onto our paper-based plasma generators. The fine mist of generated droplets had diameters ranging from ~30 to 100 µm. Having a diameter less than 100 µm, these droplets are similar in size to 95% of droplets in a sneeze (41).

Results and Discussion

Noncontact Experiments. The plasma generators applied treatments for 0 (control), 5, 10, 20, 30, and 60 s. Immediately after timed treatment, we incubated each sample at 30 °C for 48 h. Fig. 2D shows the resulting quantities of colonies of *S. cerevisiae* after incubation. After 10 s of active treatment, the mean number of colonies decreased to 16.14, or an inactivation rate of 91.85%. After 20 and 30 s of treatment, the inactivation rate for yeast became 97.89% and 99.34%, respectively. Fig. 2*E* shows the efficacy of using plasma to kill *E. coli*. With only 10 s of treatment, the resulting inactivation rate was as high as 99.93%. Treatments longer than 10 s resulted in an average of less than one remaining colony, representing efficiencies greater than 99.9%. Fig. 2*C* shows the comparison between the control group and the 10-s group of *E. coli*. These results for both *S. cerevisiae* and *E. coli* indicate efficiencies as high as 99% with plasma treatment for only 30 s.

One of the common measures in microbiology is the decimal reduction time, or D value. As shown in Eq. 1, it is the time t required to inactivate 90% of the cells of a given microorganism in a medium at a specified temperature:

$$D \text{ value} = \frac{t}{\log N_0 - \log N_t},$$
 [1]

where N_0 is the initial population and N_t is the population at the end of the test. Based on the experimental results, the *D* values for both *S. cerevisiae* and *E. coli* were less than 10 s.

Direct-Contact Experiments. After dispensing suspensions of *S. cerevisiae* or *E. coli* directly onto the honeycomb-patterned side of a circular paper-based generator, we activated plasma for 0 (control),



Fig. 2. Experimental setup for verifying the efficiency of noncontact sanitization using the designed paper-based plasma generators. (*A*) Exploded view of the setup: a circular paper-based plasma generator with hexagonal patterns fits the lid of a Petri dish. (*B*) The assembly of the setup when conducting experiments. (*C*) Images of experiments with *E. coli* showing the comparison of a control group and a sample after 10 s of treatment. (*D*) Plotted number of colonies versus sanitization time for *S. cerevisiae* cells. (*E*) Plotted number of colonies versus sanitization time for *E. coli*.

5, 10, 20, 30, and 60 s. Then, we transferred the cells attached to the surface of the plasma generator onto a solid media of YEPD or LB. During transfer, we manually applied pressure to the back of the generator for 10 s, and then discarded the generator after use. The inoculated media stayed in the oven at a temperature of 30 °C for 48 h. We also adopted a blank control (BC) group, which contained the same type of paper-based plasma generator but was not activated. Fig. 3B shows quantitative results from sanitizing *S. cerevisiae* in the direct-contact experiments. With 60 s of plasma treatment, there were no observable cells on the YEPD media.



Fig. 3. Experimental results showing the efficiency of paper-based plasma sanitizers exposed to aerosolized liquid suspensions of *S. cerevisiae* and *E. coli.* (*A*) Setup used in direct-contact experiments. (*B*) A histogram showing the number of colonies formed by *S. cerevisiae* after being incubated for 48 h. (C) A histogram showing the number of colonies formed by *E. coli* after being incubated for 48 h. BC, blank control.

Fig. 3C shows the quantitative results from sanitizing E. *coli* in the direct-contact experiments. With only 10 s of treatment, there were no observable colonies on the LB media.

Contamination and Self-Sanitization of Metallized Paper. Metallized paper, generally used for labeling and printed media, is not an

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inherently sterile product. Thus, the metallized paper likely possessed contaminants on its surfaces and within its porous structure. When examining the experimental results, we observed nonspecific contaminants/colonies (i.e., microbes not associated with E. coli or S. cerevisiae). By examining the DNA sequence of the unspecified colonies, we identified them as Bacillus, a type of bacteria that can be difficult to irradiate with chemical solvents, such as isopropyl alcohol. In our experiments, there were two control groups: one with inoculation and one without inoculation. In both control groups, we found some of the media had Bacillus contamination. However, none of the samples appeared to be contaminated after 30 s of active plasma. Thus, experimental results suggest that, by generating volume plasma, the metallized paper also treated itself and removed Bacillus from its fibrous interior. This result is particularly notable because Bacillus species are generally difficult to kill due to the production of resistant endospores.

Paper-Based Disposable Garments. After exposure to hazardous microbes, self-sanitizing garments might decontaminate themselves before being transported offsite and reduce the risk of harmful release of contaminated materials during shipping. In addition, low-cost plasma generators may reduce the rate of morbidity and mortality resulting from nosocomial infections (i.e., diseases or infections acquired in a hospital or healthcare facility) (42). After the outbreak of the highly contagious Ebola virus, there was an increased interest in developing viable, cost-effective techniques for disinfection and enhanced personal protection equipment (43–45).

We have experimentally characterized the effectiveness of paper-based plasma generators for active antimicrobial sanitization. To demonstrate the potential use of the devices in garmentlike systems, we prepared a rectangular, paper-based band with one-half of the surface area covered with a hexagonal, conductive layer. There was no conductive layer on the other half as we removed it with laser ablation. This design produced plasma on only one-half of the surface area and reserved the other half as a control group with Kapton tape attached to its surface. Fig. 4 A and D shows the configuration. In the experiments, we first wrapped the paper-based band around a researcher's wrist, and then carefully sprayed an aerosolized suspension of E. coli on the surface of the band to ensure approximately equal distribution of the *E. coli*. The concentration of the suspension was $\sim 3 \times 10^8$ cells/mL. Then, we removed the band from the wrist and connected the electrodes. Fig. 4 B-D depicts these steps. After activating the atmospheric plasma, we removed the electrodes and transferred the cells to the surface of a preprepared LB medium. Finally, we incubated the medium at 37 °C for 48 h. Fig. 4E shows the results. Movie S2 shows the experiment in motion.

Scalability. Within the limits on the size of acquired metallized paper and working area of the laser engraver, we created paperbased, plasma generators with dimensions up to 400 mm × 276 mm, which is ~20 times larger than the logotype-based, plasma generator shown in Fig. 1*C*. For the device shown in Fig. 5*A*, the frequency of excitation (100 Hz at a voltage V_{p-p} of ±3kV) to generate plasma was much lower than that used with the smaller devices previously described. As the electrical resistance scales with the size of the device, especially considering the 10-nm-thick layer of aluminum in the metallized paper (35), this observation agrees with previously reported methods of decreasing the frequency of applied voltage to generate plasma through resistive barrier discharge (46).

Self-Sanitizing Capacitive Touchpad. Based on previously developed capacitive touch sensors, we etched traces to fabricate a keypad (35). By integrating atmospheric plasma generators with this keypad, these devices can potentially sanitize themselves after being touched. Fig. 5B shows a sequential operation, including touching the button with two fingers to activate corresponding LEDs, and activating the plasma to sanitize the buttons with a

20 M



Surface covered by Kapton tape

Fig. 4. A paper-based plasma generator as a potential protective garment. (A) A rectangular (166 mm \times 100 mm) device is in the activated state. The left-hand side has coverage with plasma under the excitation of an AC source with a peak-peak voltage of ± 2.3 kV and a frequency of 1.7 kHz. The right-hand side had a layer of Kapton tape, which prevented the generation of plasma as it cut off the supply of air. *B* and *C* show the procedure for spraying the atomized *E. coli* suspension to the paper-based garment. (*D*) An image of the unfolded device attached to the a high-voltage connector. (*E*) The growth of *E. coli* after being transferred to LB agar and incubation for 48 h at 37 °C. From a qualitative perspective, the number of colonies is inversely proportional to the duration of plasma treatment.

frequency of 500 Hz and a V_{p-p} of ± 2.5 kV. It is worth mentioning that the conductive traces on the touchpad were at least 2.5 mm away from each other. Narrower gaps resulted in discharges and nonuniform ablation of the conductive layer of aluminum during activation. Movie S3 shows this demonstration in motion.

Kirigami-Like Plasma Generators. Kirigami uses the cutting of paper to create decorative or functional objects. As a proof-of-concept example, we created a kirigami-based device with an initial geometry of a 2D square as shown in Fig. 5C. When stretched, it opened into a 3D structure. The excitation frequency of this device was 500 Hz while the peak-to-peak voltage was ± 2.5 kV. Kirigami-based devices might be useful for building conformable electronics that require stretching or bending about more than one axis.

Conclusions

In this article, we have introduced a concept for antimicrobial sanitization with flexible and disposable plasma generators made

of paper. The design is simple, with back-to-back bonding of two sheets of metallized paper. The porosity of paper allows gas to permeate its bulk volume and fuel plasma, while forced convection cools the substrate. These mechanisms suggest fibrous, paper-like substrates may be appropriate in the design and fabrication of flexible devices to produce both volume and surface plasmas capable of killing microbes. With oscillating peak-to-peak potentials ranging from ± 1 to ± 10 kV, the paper-based plasma generators deactivated greater than 99% of S. cerevisiae and greater than 99.9% of E. coli cells with 30 s of treatment on neighboring substrates with an offset distance of 10 mm. We have also demonstrated how paper-based generators are conformable to curved surfaces, suitable for sanitization of aerosolized microbes onto a substrate, appropriate for kirigami-like stretchy structures, and compatible with user interfaces. In the future, paper-based plasma generators may be appropriate as antimicrobial protectors for skin-like sensors, self-sterilizing garments, devices for sterilizing laboratory or biomedical equipment, smart bandages for wound healing, or sacrificial components in manufacturing processes that apply patterned surface treatments.



Fig. 5. Scalable and multifunctional plasma sanitizers made from metallized paper. (A) A 400-mm \times 276-mm rectangular, paper-based plasma generator with a surface area ~20 times larger than the R-shaped plasma generator shown in Fig. 1. The streak of green light in the upper right was from an external source. (B) A paper-based, capacitive touchpad served as an input device and plasma generator. (C) A kirigami-like plasma generator transformed from a planar to a 3D geometry.

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Materials and Methods

Strains of Microbes. Saccharomyces cerevisiae strain AH109 (Clontech Laboratories) and Escherichia coli strain TOP10 (Invitrogen) served as samples of fungus and bacteria in our experiments. S. cerevisiae strain AH109 is a yeast strain usually used for two-hybrid screening in biological research. E. coli TOP10 is an ideal bacterial strain for high-efficiency cloning and plasmid propagation.

Preparation of Media and Microbes. We cultured *S. cerevisiae* strain AH109 and *E. coli* strain TOP10 with YEPD medium and LB medium, respectively. The YEPD broth contained 1% [mass/volume (m/vol)] yeast extract (Difco; Becton Dickinson), 2% (m/vol) peptone (Sigma-Aldrich), 2% (m/vol) dextrose (VWR International), and the rest was distilled water. The YEPD solid medium consisted of 0.3% (m/vol) yeast extract, 1% (m/vol) peptone, 1% (m/vol) dextrose, 2% (m/vol) agar (Difco; Becton Dickinson), with the rest being distilled water. We prepared LB medium with the dehydrated culture media of LB (powder form) (Difco; Becton Dickinson) and proper hydration with distilled water. The prepared LB medium contained 2.5% (m/vol) LB powder, and the rest was distilled water. The LB solid medium consisted of 2.5% (m/vol) LB powder, 1.5% (m/vol) agar, with the rest being distilled water. Autoclavation of all media lasted for 20 min at 121 °C. In the solid media of both YEPD and LB, it contained 25 mL of media in each Petri dish.

We cultured *S. cerevisiae* and *E. coli* in YEPD broth and LB broth, respectively, at 150 rpm in an orbital incubator shaker (model 3527; Lab-Line Instrumentations). The culturing lasted for 24 h at room temperature (25 °C). We collected the microbes by centrifuging (Clinical 100; VWR International) the cultures at 4,000 rpm (~1,520 × g) for 5 min. Both *S. cerevisiae* and *E. coli* cells were in suspension with sterilized distilled water. To determine the

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concentration of *S. cerevisiae* and *E. coli* in the suspension, we used a spectrophotometer (Genesys 10s UV-VIS; Thermo Scientific) to measure the value of OD₆₀₀, which indicated the optical density of samples measured at a wavelength of 600 nm. The measured OD₆₀₀ of *S. cerevisiae* and *E. coli* were 1.037 and 0.867, indicating concentrations of ~ 6.22×10^7 and 6.94×10^8 cells per mL, respectively. Finally, the concentrations were diluted to 2.07×10^3 and 2.50×10^4 cells per mL, respectively.

Image Acquisition. Unless otherwise specified, this work used a Nikon D7100 camera with an 18- to 105-mm lens to acquire images. For some still images of the plasma, we used extended exposure. The only digital modification of images was in contrast and brightness.

ACKNOWLEDGMENTS. We thank Wilson Rodriguez and Kris Mohan from Rutgers Environmental and Occupational Health Sciences Institute for providing equipment for ozone measurements. We also thank the anonymous reviewers for their helpful suggestions and comments. Joe Formosa from AR Metallizing, Ltd. (A Nissha Company), provided the metallized paper. Maxim Lazoutchekov helped with initial experimental measurements of the dielectric strength of the metallized paper. We acknowledge support from National Science Foundation Award 1610933 and from Rutgers University through the School of Engineering, the Department of Mechanical and Aerospace Engineering, the University Research Council, and an A. Walter Tyson Assistant Professorship Award. We acknowledge support from the John E. and Christina C. Craighead Foundation, US Department of Agriculture–National Institute of Food and Agriculture Multistate Project W3147, and the New Jersey Agricultural Experiment Station. S.R.'s work was partially supported by Air Force Office of Scientific Research Grant FA9550-15-1-0424.

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