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Efficacy of low-temperature plasma-activated gas disinfection against biofilm on contaminated GI endoscope channels CME

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Background and Aims: It has been increasingly recognized that the safety of GI endoscopes needs to be improved by addressing the small margin of safety of high-level disinfectants (HLDs) and the failure of HLDs to clear multidrug-resistant organisms and biofilms. There is also an unmet need for effective low-temperature sterilization techniques that have a clear pathway for U.S. Food and Drug Administration clearance. Here, we report the results of our investigation of a novel argon plasma-activated gas (PAG) for disinfection and potentially sterilization of biofilm-contaminated endoscopic channels.

Methods: Test polytetrafluoroethylene channel segments were contaminated with 4-, 24- and 48-hour luminal biofilms of methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa,* or *Escherichia coli* and were treated by PAG flowing for up to 9 minutes. After PAG treatment, inactivation and dispersal of luminal bacterial biofilms and their regrowth in 48 hours were evaluated. Reactive species induced by PAG were measured with colorimetric probes and electron spin resonance spectrometry. Surface morphology and elemental composition of PAG-treated channel material were analyzed with scanning electron microscopy.

Results: PAG treatment for 9 minutes led to more than 8 log reduction of viable cells and dispersal of 24- and 48-hour luminal biofilms of all 3 bacteria and to suppression of their regrowth, whereas it resulted in little morphologic abnormalities in channel material. Ozone concentration of PAG fell to below .01 ppm within 30 seconds of switching off the plasma. PAG-treated deionized water was acidified with numerous types of reactive species, each with a concentration some 3 orders of magnitude or more below its bacterial inhibition concentration.

Conclusions: PAG is capable of effectively and rapidly disinfecting luminal bacterial biofilms and offers an alternative to the step of HLDs and/or ethylene oxide in the endoscope reprocessing procedure with safety to personnel and environment. (Gastrointest Endosc 2019;89:105-14.)

Recent years have witnessed increasing episodes of infection transmission by contaminated GI endoscopes both in the United States and Europe,[1-5] thus highlighting the need to improve the safety of GI endoscopes.[1,6-8] Key reasons attributed to the reprocessing failure include the structural complexity of existing GI endoscopes, the low margin of safety of current high-level disinfectants (HLDs), and the failure of HLDs to clear multidrug-

Abbreviations: CAP, cold atmospheric plasma; CV, crystal violet; DIW, deionized water; ETO, ethylene oxide; HLD, high-level disinfectant; MRSA, methicillin-resistant Staphylococcus aureus*; PAG, plasma-activated gas; PBS, phosphate-buffered saline; PTFE, polytetrafluoroethylene; RNS, reactive nitrogen species; ROS, reactive oxygen species.*

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resistant organisms and biofilms.[7-11] Most outbreak sites have now added an ethylene oxide (ETO) step after HLD treatment after each procedure, because ETO has eradicated persistent culture positivity[7,8] and may reach surfaces of complex endoscopes that HLD fails to access.[7] However, ETO is not an adequate long-term solution because of its cost, long aeration time (>16 hours), toxicity to personnel, and becasue it has not been cleared by the U.S. Food and Drug Administration for sterilizing GI endoscopes.[7,12,13] Ultimately, the assurance of a sufficient margin of safety for patients will require redesign of endoscopes and the adoption of new low-temperature sterilization techniques.[7,12]

Low-temperature gas plasmas generated at atmospheric pressure are capable of rapid inactivation, often within a few minutes, of a wide spectrum of microorganisms including drug-resistant bacteria, bacterial spores, microbial biofilms, and fungi.[14,15] Recently, gas plasmas have been shown to eradicate bacterial persisters through synergy with antibiotics.[16] Such plasmas are commonly known as cold atmospheric plasmas (CAPs), and their antimicrobial properties are associated with their reactive oxygen species (ROS) and reactive nitrogen species (RNS),[14,15] some of which, singlet oxygen ($^1O_2$) and hydroxyl radicals ($^{\bullet}OH$) for instance, cannot be enzymatically detoxified by bacteria.[17] CAPs are considered to be a low-temperature sterilization technique.[15,17,18]

We report here a novel plasma-activated gas (PAG) technique for low-temperature disinfection and potentially sterilization of endoscopes. Used as a remote mode of CAP, PAG is formed inside an enclosed channel from the effluent of an upstream cold atmospheric argon plasma plume. Confirmation of PAG efficacy for disinfecting channels of the greatest length of GI endoscopes would remove the cumbersome need of sustaining gas plasma within a long endoscopic channel. The main objective of this study was to establish the efficacy of PAG for eradicating biofilm-contaminated channels and its safety to channel materials. A secondary objective was to provide a basis to inform further investigations involving a greater array of test microorganisms and clinical endoscopes. It is worth noting that cold atmospheric argon plasma configured in GI endoscopes has been FDA-approved for coagulation,[19] and this offers a clear pathway toward 510(k) clearance for PAG.

METHODS

Bacteria and cultures

To represent bacteria associated with endoscope contamination, methicillin-resistant *Staphylococcus aureus* (MRSA) MW2 BAA-1707, *Pseudomonas aeruginosa* PA01 BAA-47085, and *Escherichia coli* K12 14948 were sourced from ATCC (Manassas, Va). *E coli* and MRSA were grown on Luria-Bertani agar, and *P aeruginosa* was grown on brain heart infusion agar (both from Difco Laboratories, Detroit, Mich). A single colony from the agar plate was inoculated in 10 mL of broth and incubated overnight at 37°C while shaking at 160 rpm, harvested in the mid-logarithmic phase by centrifugation (500 ×*g*, 5 minutes), and then washed twice with 1× phosphate-buffered saline (PBS). The inoculum concentration was adjusted to about 1.0 to 2.0 × 10^7 CFU/mL by broth dilution.

Test channels and their contamination

New 1.93-mm inner diameter polytetrafluoroethylene (PTFE) channels (Clear Air, Palatine, Ill) were used to simulate endoscopic channels with channel sizes of 2.0 to 4.8 mm.[20] The PTFE channels were either 1200 mm or 2200 mm long, representing an average length and the longest length of all GI endoscopes, respectively.[20] Before use, the PTFE channels were sterilized. Similar PTFE channels were recently used to model endoscopic biofilms.[21]

To contaminate a PTFE channel, a channel segment of 70 mm in length was added with 180 μL of the bacteria culture using a sterile syringe. Each contaminated channel segment was placed in a sterile petri dish and incubated without shaking for 4, 24, or 48 hours at 37°C. Nonadhesive bacteria were removed by pumping approximately 135 mL sterile water through the contaminated channel segment with a peristatic pump at a rate of 66 mL/min for 2 minutes.

PAG and PAG treatment

Details of the atmospheric argon plasma device is reported elsewhere.[22] Briefly, it produced a plasma plume confined inside a quartz tube that was tight fit into a sterile PTFE channel of either 1200 mm or 2200 mm in length (Fig. 1A). The argon plasma plume was sustained with an applied voltage of 10 kV and an electrical power of 15.9 W at 23 kHz and with a flow of argon (99.9%) at 4 L/min. The distal point of the plasma plume was about 200 mm into the PTFE channel. To test the decontamination efficacy of PAG, a 7-cm contaminated PTFE channel segment (again with an inner diameter of 1.93 mm) was used as the test channel and connected to the distal end of the long and sterile PTFE channel (Fig. 1A). The flowing PAG was used to treat the test segment before being collected in a bleach container. Because reactive species in PAG are most abundant near the plasma, efficacy of PAG to sterilize a test channel downstream of the 2200-mm channel suggests its sterilization efficacy for the entirety of an endoscopic channel of up to 2200 mm.

Evaluation of eradication and regrowth

After PAG treatment, the test channel segment was disconnected from its upstream PTFE channel. The exterior of the test segment was swabbed with 70% alcohol to ensure that bacteria recovery was from the interior of the segment only. Each 70-mm-long test segment was then cut into five 14-mm-long segments. The latter were submerged in 2 mL of .1 M glycine buffer (pH = 7.0) and then vortexed for 1 minute, sonicated at 40 kHz in a

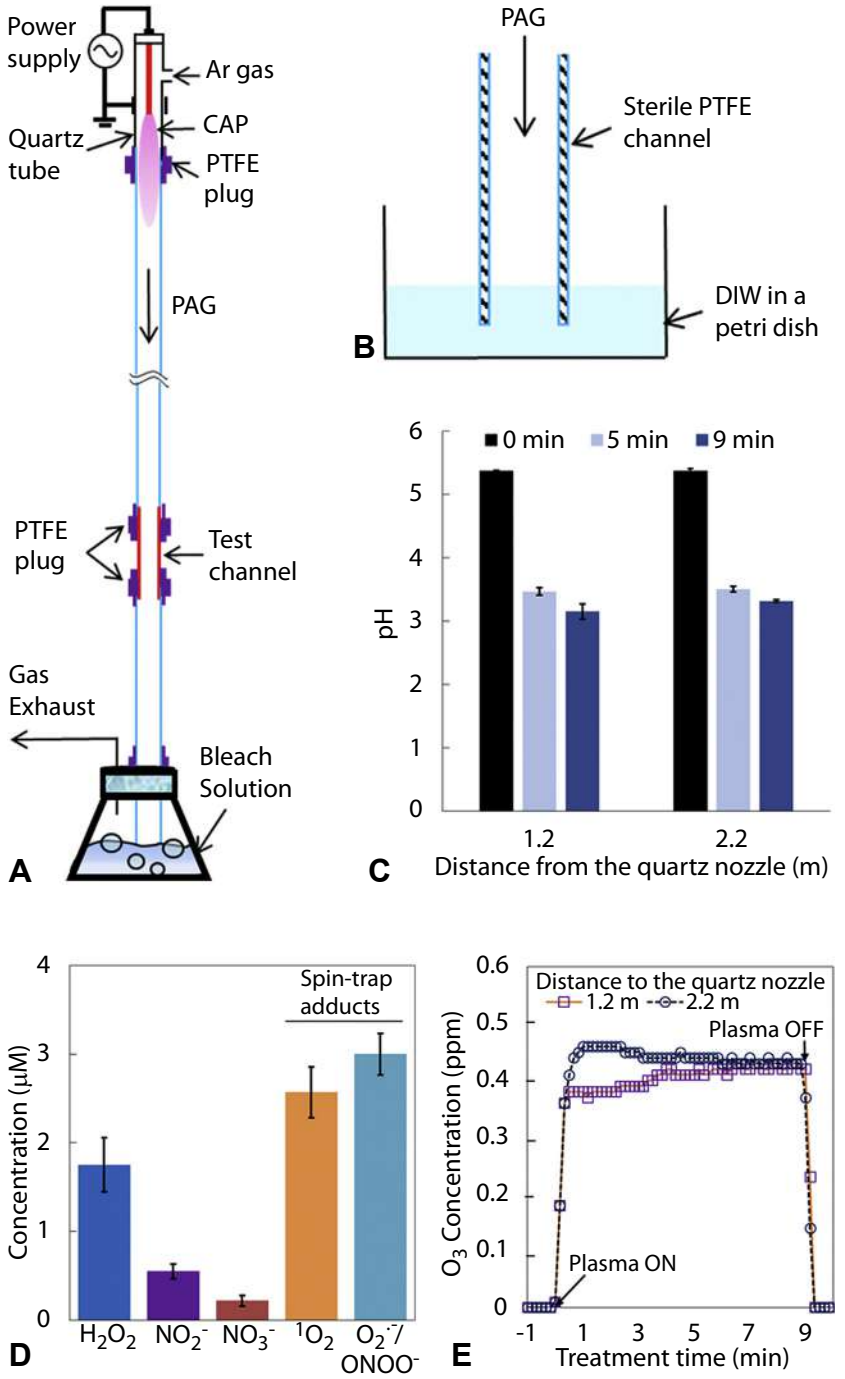

Figure 1. PAG, its treatment of contaminated channels, and its liquid- and gas-phase species. **A,** Schematic of the CAP device, its production of PAG in a long PTFE channel, and PAG treatment of a test channel segment. **B,** PAG treatment of DIW at the distal end of the PTFE channel. **C,** pH of the DIW. **D,** Aqueous reactive species in DIW at the end of a 2200-mm PTFE channel. **E,** Ozone concentration inside the long PTFE channel. n = 3. *PAG*, Plasma-activated gas; *CAP*, cold atmospheric plasma; *PTFE*, polytetrafluoroethylene; *DIW*, deionized water.

room temperature water bath for 1 minute, and vortexed for an additional minute. The recovered contents from each 14-mm segment were serially diluted and enumerated using a plate counter with a detection limit of 10 CFU/mL. The quantification was evaluated per length of PTFE segment cut, similar to that in the literature.[21]

To evaluate microbial regrowth, the 14-mm-long test channel segments from each eradication treatment were placed in a sterile 15-mL Falcon conical tube (Thomas Scientific, Swedesboro, NJ) filled with 2 mL of appropriate broth and incubated overnight with shaking at 37°C. After incubation, the segments were vortexed for 1 minute and

sonicated for 1 minute. A 200-μL aliquot of the sonicated broth was used for serial dilution and plating. Plates were incubated overnight at 37°C before colony enumeration. Untreated and gas-treated channels were used as controls.

Quantification of biofilms

Before and after PAG treatment, adhesive materials on the inner wall of a test channel segment were stained with .1% crystal violet (CV; BD, Franklin Lakes, NJ) for 15 minutes at room temperature. Unbound dyes were washed away with 1× PBS, and the samples were then left in a biosafety cabinet to air dry. The air-dried test segments were cut into five 14-mm segments and submerged in 2 mL of 95% molecular grade ethanol for 30 minutes to dissolve the CV. Dissolved CV in the ethanol was then quantitated by means of optical absorption at 590 nm.[23]

To see directly adhesive materials on a PAG-treated surface, a supplementary test was performed where glass chamber slides (Thermo Fisher Scientific, Fair Lawn, NJ) were inoculated with 200 μL of microbial cultures at 1.0 to 2.0 × 10^7 CFU/mL and then incubated for 24 hours to form biofilm on the surface of the slides. To remove planktonic cells, the culture medium was aspirated off and washed 3 times with 1 mL of 1× PBS. After gentle washing, the biofilm-contaminated glass slides were placed at the distal end of a 1200-mm PTFE channel for 1-minute PAG treatment. Before and after the treatment, various wells of the inoculated slides were stained with CV for imaging and quantification of any adherent material.[23]

Measurement of reactive species, pH, and oxidation-reduction potential

For use after the manual cleaning step, PAG is likely to treat wet endoscopes, and as such its gaseous species form aqueous reactive species at the site of microbes on the endoscopes.[24] To mimic this, the distal 10-mm section of a sterile 2200-mm PTFE channel was immersed in 2 mL of sterile deionized water (DIW) in a 15-mL Falcon conical tube (Thomas Scientific) for measurement of aqueous reactive species, pH, and oxidation-reduction potential after a 5-minute PAG treatment (Fig. 1B). Long-lived ROS were measured with a microplate reader (FLUOStar; BMG Labtech, Cary, NC) using the Amplex Red assay kit (Thermo Fisher Scientific, Waltham, Mass) for hydrogen peroxide (H_2O_2) and the Griess Reagent assay (Cayman Chemical Co, Ann Arbor, Mich) for nitrite (NO_2^-) and nitrate (NO_3^-). Short-lived reactive species were measured with an electron spin resonance spectrometer (EMX+, Bruker, Germany) and spin traps.[25] Specifically, 5,5-dimethyl-1-pyrroline N-oxide was used for hydroxyl radicals ($^{\bullet}OH$) and diethyldithiocarbamate and N-methyl-D-glucamine dithiocarbamate for nitric oxide ($NO^{\bullet}$), all from Dojindo Laboratories (Kumamoto, Japan), and 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine hydrochloride (Enzo Biochem, Farmingdale, NY) was used for both superoxide ($O_2^{\bullet-}$) and peroxynitrite ($ONOO^-$). pH and oxidation-reduction potential of the sterile DIW were measured with a pH meter (Accumet AB 200; Thermo Fisher scientific, Ashville, NC).

In the gas phase, the time-resolved ozone concentration was measured with an ozone analyzer (model 106-M; 2B Technologies, Boulder, Colo) with the detection limit of 0.01 ppm. The optical emission spectrum of the CAP and its effluent were collected using an UV/VIS spectrometer with a wavelength range of 350 to 1100 nm (RED Tide USB 650; Ocean Optics, Largo, Fla).

Surface characterization

Surface topology of the inner wall of test PTFE channel segments was examined by scanning electron microscopy with a JSM-6060LV scanning electron microscope (JEOL, Tokyo, Japan) equipped with energy dispersive x-ray analysis facilities. To show the maximum impact of the plasma plume, a new 70-mm PTFE channel segment was connected to the nozzle of the plasma-housing quartz tube for up to 9 minutes. After the PAG treatment, the test segment was cut open for access to their inner surface. They were then gold-coated and examined with scanning electron microscopy at a working voltage of 15 kV. The untreated segments were used as the control.

Statistical analysis

All test conditions were tested in at least 3 independent experiments. Data are presented as mean ± standard deviaiotn, and statistical analysis was performed using the Student t test to determine significance between data points and to establish statistical significance. $P < .05$ was considered significant.

RESULTS

Measurement of acidity, temperature, and reactive species

After treatment of PAG contained in an immersed PTFE channel (Fig. 1B) for more than 30 seconds, the DIW became acidic with a pH of 3.2 to 3.5 after 5 to 9 minutes of PAG treatment (Fig. 1C). For 9 minutes of PAG treatment, the oxidation-reduction potential was found to increase from 99.0 mV (untreated) to about 215 mV for the DIW sample at the end of a 1200-mm or 2200-mm long PTFE channel (data not shown). After 9-minute PAG treatment, the temperature of the exterior of the PTFE channel rose to 61, 43, 28, and 25°C at 5, 20, 1200, and 2200 mm from the proximal end of the channel (in contact with the quartz tube), respectively, and then fell to room temperature of 25°C in 3 minutes after switching off the plasma (data not shown).

As shown in Figure 1D, aqueous reactive species in the DIW were of many types but at low concentrations. For a 5-minute PAG treatment and at 2200-mm downstream of the quartz tube nozzle, long-lived aqueous H_2O_2, NO_2^-,

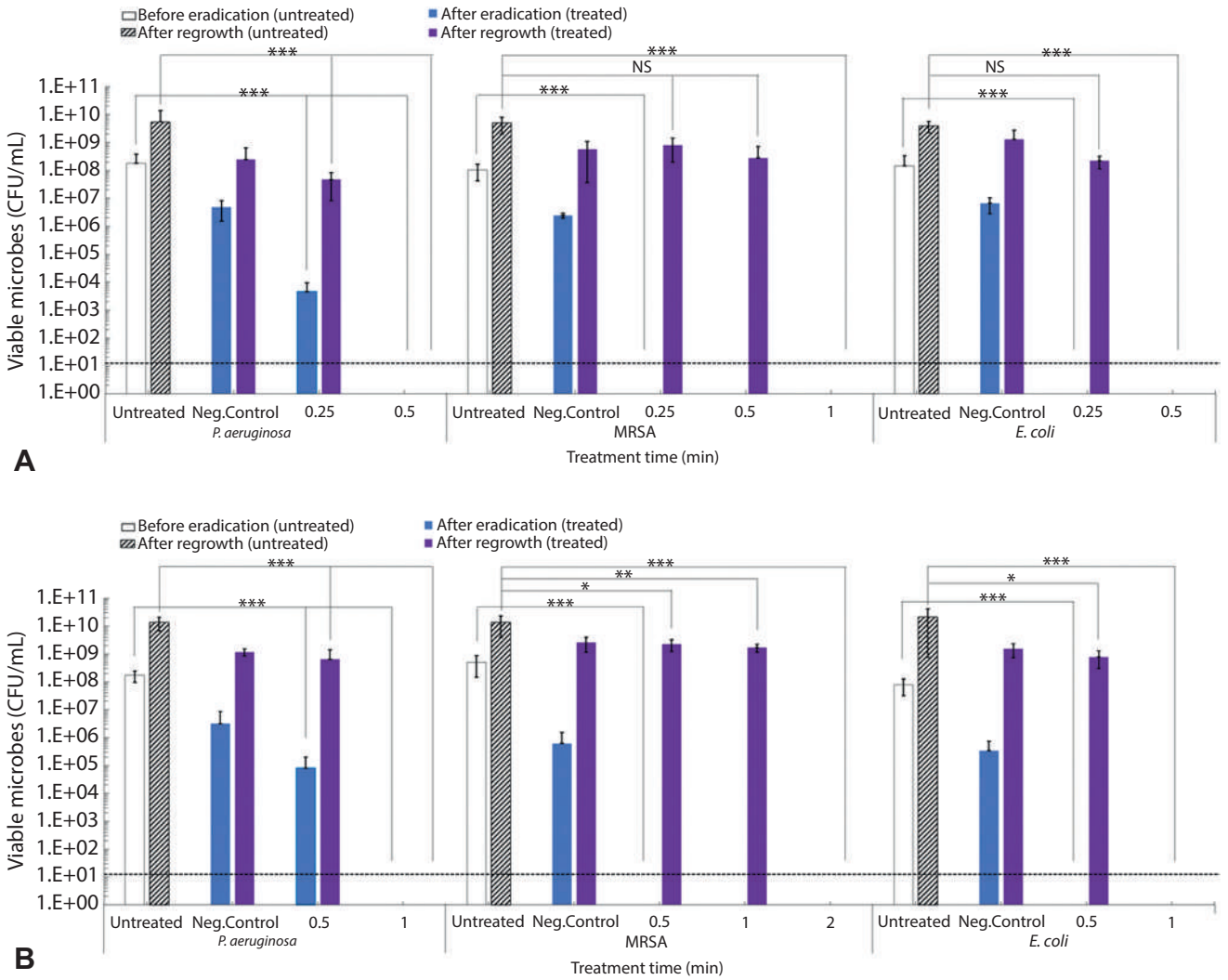

Figure 2. Eradication and regrowth by plasma-activated gas of luminal biofilms on test channel segments placed at the distal end of a 1200-mm sterile channel. Biofilms of *Pseudomonas aeruginosa*, MRSA, and *Escherichia coli* were formed in (**A**) 4 hours and (**B**) 24 hours. n = 3. ***$P < .001$, **$P < .01$, *$P < .05$. *NS*, Not significant; *MRSA*, methicillin-resistant *Staphylococcus aureus*.

and NO_3^- reached their peak concentrations of 1.74, .54, and .21 μM, respectively. These were 1 to 2 orders of magnitude lower than those found in liquid under direct treatment of a CAP plume.[26-28] Similarly, spin-trap adducts of short-lived ROS and RNS were found to be at a low level with the peak concentration of spin-trap adducts of $^1O_2$ at 2.57 μM and that of spin-trap adducts of $O_2^{\bullet-}$ and $ONOO^-$ at 2.99 μM (Fig. 1D). Concentrations of these spin-trap adducts are known to be at least 2 to 4 orders of magnitude above those of their corresponding short-lived ROS.[25] $^{\bullet}OH$ and $NO^{\bullet}$ were found to be below the electron spin resonance detection limit; however, they were found present when the DIW sample was placed near the quartz tube nozzle (data not shown).

In the gas phase, the ozone concentration inside the long PTFE channel was found to rise rapidly after switching on the argon plasma and reached a plateau within 1 minute to .38 ppm and .47 ppm at 1200 mm and 2200 mm from the proximal end of the PTFE channel (Fig. 1E). For the 1200-mm case, the ozone concentration rose again during 2 to 3.5 minutes to a new plateau of .43 ppm. When the plasma was switched off, the ozone concentration reduced rapidly in 30 seconds to below the detection limit of .01 ppm. Optical emission spectrum detected at 200-mm downstream of the quartz tube nozzle showed the presence of excited argon atoms (at 764 nm), nitrogen molecules (357 nm and 384 nm), and oxygen atoms (777 nm) (data not shown). No optical emission was observed at locations between 1200 mm and 2200 mm from the proximal end of the long PTFE channel (data not shown).

PAG decontamination of luminal bacteria

Test channel segments infected with biofilms of *P aeruginosa*, MRSA, and *E coli* were connected to the distal point of a 1200-mm sterile PTFE channel for PAG treatment. Figure 2 suggests a progressive increase in

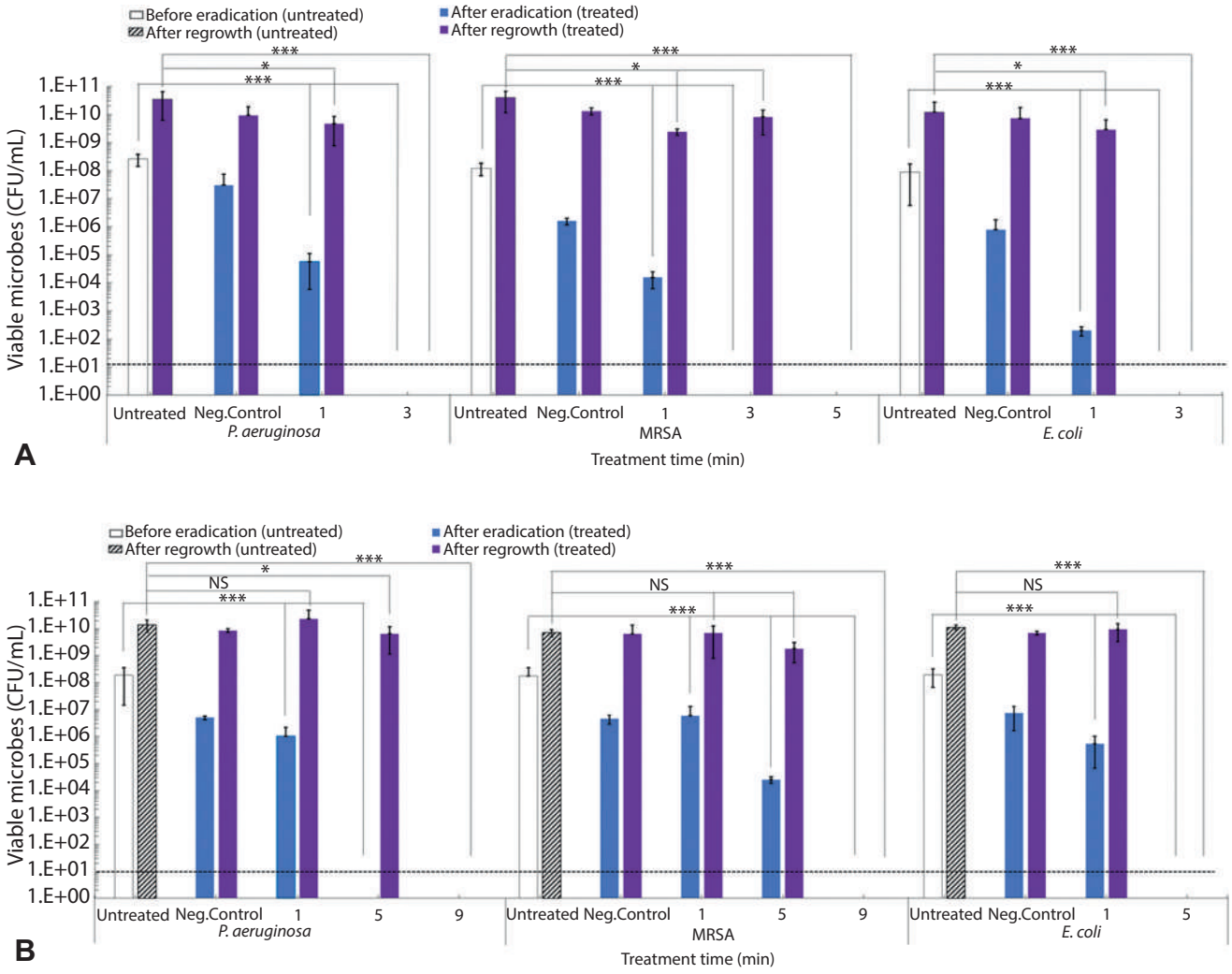

Figure 3. Eradication and regrowth by plasma-activated gas of luminal biofilms on test channel segments placed at the distal end of a 2200-mm sterile channel. Biofilms of *Pseudomonas aeruginosa*, MRSA, and *E coli* were formed in (**A**) 4 hours and (**B**) 24 hours. n = 3. ***$P < .001$, **$P < .01$, *$P < .05$. *NS*, Not significant; *MRSA*, methicillin-resistant *Staphylococcus aureus*.

eradication efficacy with increasing PAG treatment time. The 4- and 24-hour luminal biofilms of all 3 bacteria were eradicated with ≥8 log reduction of viable cells without regrowth with 1-minute and 2-minute PAG treatment, respectively. For luminal biofilms formed in 48 hours, a 3-minute PAG treatment was found to achieve both complete eradication and regrowth suppression for all 3 bacteria (data not shown).

With contaminated test channels connected to the distal point of a 2200-mm sterile PTFE channel, it was necessary to extend the PAG to 3, 4, and 5 minutes for complete eradication without regrowth of 4-hour biofilms of *P aeruginosa*, *E coli*, and MRSA, respectively (Fig. 3A). For luminal biofilms formed for 24 hours, complete eradication without regrowth was achieved with the PAG treatment extended to 5, 7, and 9 minutes for *E coli*, *P aeruginosa*, and MRSA (Fig. 3B), respectively. For all cases in Figures 2 and 3, PAG treatment achieved more than 8 log reduction for all 3 bacteria and the negative control of argon gas flow without the plasma attained about .5 to 1.5 log reduction.

PAG dispersal of luminal bacterial biofilm

To evaluate whether PAG treatment can disperse luminal biofilms formed in 24 hours, all adhesive materials on the inner wall of test channel segments were stained with CV, and the CV staining was dissolved in PBS for quantification by optical absorption at 590 nm. For test channel segments connected to the distal end of a 1200-mm sterile PTFE channel, optical absorption of dissolved CV was the most with *P aeruginosa* (~48% of the untreated) and minimal with *E coli* (5.5%) and MRSA (2.0%) after a .5-minute PAG treatment (Fig. 4A). After the PAG treatment was extended to 1 minute, optical absorption of dissolved CV was found to reduce to below 1%. To supplement the above with direct visualization of surface-

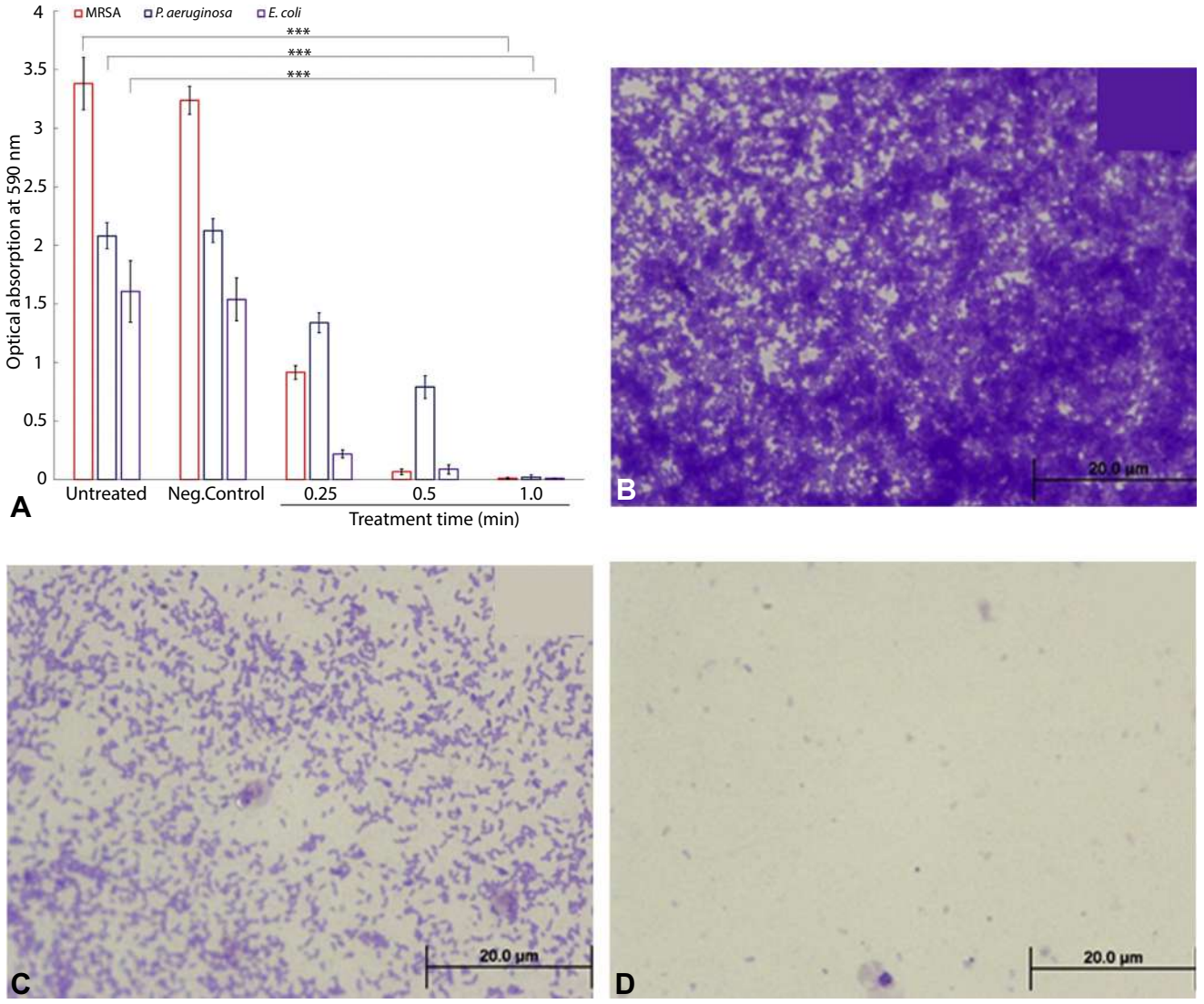

Figure 4. Plasma-activated gas (PAG) dispersal of 24-hours luminal *Pseudomonas aeruginosa* biofilm in test channel segments placed at the distal end of a 1200-mm sterile polytetrafluoroethylene channel. **A,** Optical absorption at 590 nm of crystal violet dyes dissolved in 2 mL ethanol from the contaminated segments (n = 3, ***$P < .001$). Images of crystal violet–stained 24-hour biofilms on glass chamber slides for (**B**) untreated sample, (**C**) samples PAG-treated for .5 minutes, and (**D**) samples PAG-treated for 1 minute.

borne biofilms after the PAG treatment, *P aeruginosa* was used to form 24-hour biofilms on a glass chamber slide, and the biofilm was stained with CV. Figure 4 shows significant biofilm dispersal after a 1-minute PAG treatment as compared with the very dense soil of untreated *P aeruginosa* biofilm. Similar biofilm dispersal was confirmed for test channel segments connected to the distal end of a 2200-mm sterile PTFE channel (data not shown).

Effects of PAG on channel surface morphology

To study the maximum PAG impact on surface morphology of endoscopic channels, test channel segments were connected directly to the nozzle of the quartz tube and treated by PAG for 9 minutes. Figure 5 shows little sign of fissures, cracks, or other morphologic abnormalities on the inner surface of the PAG-treated channel segments. Indeed, little difference was observed in scanning electron microscopy images of untreated and treated samples. In addition, elemental compositions revealed by energy dispersive x-ray analysis were found to be very similar between untreated and treated test channels (data now shown).

DISCUSSION

In this study, we demonstrated the efficacy of PAG for eradication, regrowth suppression, and dispersal of luminal biofilms, aged up to 48 hours, of *P aeruginosa*, MRSA, and *E coli* on test PTFE channel segments located at the distal end of a sterile PTFE channel of either 1200 mm or 2200 mm in length (Figs. 2-4), thus establishing for the first

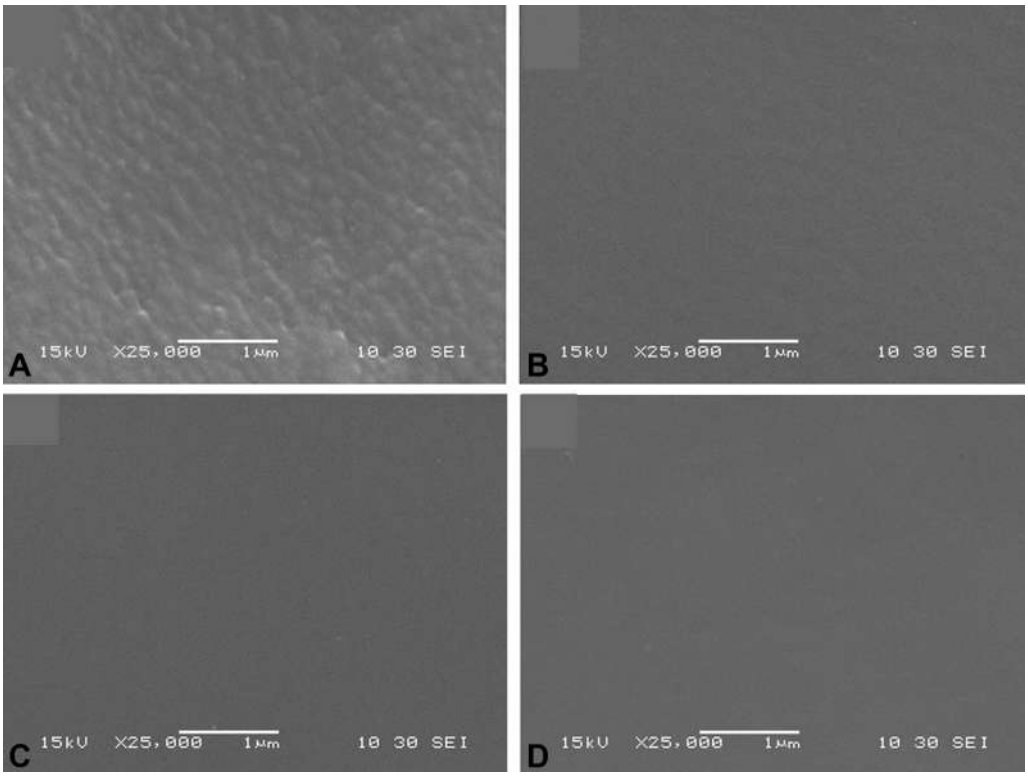

Figure 5. Scanning electron micrographs of the inner wall of test channel segments (**A**) as received from the manufacturer and those of test segments directly connected to the nozzle of the quartz tube for plasma-activated gas treatment of (**B**) 2 minutes, (**C**) 5 minutes, and (**D**) 9 minutes. Orig. mag. ×25,000.

time its effectiveness to disinfect and potentially sterilize endoscopic channels of up to 2200 mm, the longest length of current GI endoscopes.[20] Gas plasma is regarded as a low-temperature sterilization technique,[18,29] and previous reports have confirmed that both CAP and its effluent are capable of eradicating *Bacillus subtilis* spores in minutes.[30,31] Given these, PAG appears to offer better assurance of sterility than HLD, which is ineffective against drug-resistant bacteria (eg, MRSA), bacteria embedded in biofilm, and bacterial spores.[10,32] PAG enables a novel route to address the small safety margin of HLD and the desire to move toward low-temperature sterilization.[12] Within the current endoscope reprocessing procedure,[32] PAG may be introduced to replace the HLD step after the precleaning, manual cleaning, and rinsing steps, and as a result the subsequent rinsing and drying steps may become redundant, thus availing an opportunity to shorten the reprocessing procedure. As an indicator of its practicality, the PAG-producing system used in this study was housed in a small box measuring 10 inches × 15 inches × 8 inches and with a material cost of less than $2000. It can be readily integrated into the current endoscope reprocessing procedure.

It is of interest to compare PAG with ETO because an ETO step is sometimes added after the HLD step.[7] Although ETO offers the assurance of sterilization, it is costly, toxic to personnel, and very long in its aeration time (>16 hours).[7,12] Despite its long history, ETO has not been cleared by the FDA for sterilizing GI endoscopes and is not considered as an adequate long-term solution.[7,12,13] PAG does not require aeration because of the short half-lives of its reactive species, and therefore its capital cost is drastically lower than that of an ETO sterilization facility. Its running cost is largely associated with the use of argon, a common medical gas. Together, PAG offers a fast, effective, environmentally safe, and economical solution to contaminated endoscopes. No evidence of change was observed between untreated and PAG-treated test channels at the proximal area of the PTFE channel in terms of surface morphology and elemental composition (Fig. 5).

Similar to CAP,[14,15] PAG achieves rapid eradication of bacterial biofilms through synergy of its diverse ROS and RNS significantly enhanced by PAG-induced acidity (Fig. 1C and E). Reactive species produced by PAG were all below their individual minimum inhibition concentration when acting alone. For example, the minimum inhibition concentration of H_2O_2 is 2 to 10 mM against *E coli,*[33] and ozone exposure for 6 minutes at 300 ppm is needed for only 4 log reduction of viable *S aureus,*[34] both some 3 orders of magnitude above H_2O_2 and O_3 concentrations induced by PAG (Fig. 1D and E). This suggests that PAG elicits its lethality not through high doses but through the diversity of its ROS and RNS in attacking different cellular targets at low

concentrations.[15] Antimicrobial properties of PAG are further enhanced by the acidity with pH < 3.5 (Fig. 1C), a phenomenon previously reported[35,36] and partly associated with a significant increase of oxidation-reduction potential to about 215 mV that is known to damage cell membrane of bacteria and oxidize their cellular glutathione.[37,38]

Short-lived species in Figure 1D (ie, $^1O_2$, $O_2^{\bullet-}$, $ONOO^-$) tend to recombine to form harmless molecules (eg, H_2O and O_2) within microseconds,[28] and the concentration of ozone produced by PAG fell within less than 30 seconds to below the detection limit of .01 ppm (Fig. 1E) and below the safe threshold of .06 ppm of the U.S. Environmental Protection Agency for the ozone national ambient air quality.[39] The 1.7-μM peak concentration of H_2O_2 (Fig. 1D) is about 2 orders of magnitude below its minimum toxic level to mammalian cells.[40] Together with material impact data (Fig. 5), these suggest that PAG is safe to both personnel and environment. As a result, PAG unlikely requires aeration or rinsing.

This study tested PAG against 3 representatives of bacterial biofilms, and its activity in fungal biofilms and mixed biofilms remains unknown. We used PTFE channel segments with dimensions and bulk materials identical to those used clinically but not segments cut from endoscope channels. In addition, the work did not test PAG for endoscope accessories that are hard to access. These are limitations of the study.

In conclusion, PAG offers a viable alternative to HLD and ETO for disinfection and potentially sterilization of GI endoscopes. It can be integrated into the current endoscope reprocessing procedure and readily deployed in endoscopy departments with low capital and consumable advantages. Its main advantages over HLD are rapid eradication of drug-resistant bacteria, bacterial biofilms, and bacterial spores, whereas its advantages over ETO include safety to personal and environment, cost-effectiveness, and potentially clear pathway for 510(k) clearance.

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