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Rapid Sterilization of Cell Phones Using a Novel Portable Non-Thermal Plasma Device

Kerry A. Morrison,[1] Ope Asanbe,[1] Xue Dong,[1] Andrew L. Weinstein,[1] Yoshiko Toyoda,[1] Diana Guevara,[1] Eleonora Kirkels,[1] Wilmina Landford,[1] Czeslaw Golkowski,[2] Mark Golkowski,[3] & Jason A. Spector,[1,4,*]

[1]Laboratory for Bioregenerative Medicine and Surgery, Division of Plastic Surgery, Weill Cornell Medical College, New York, NY; [2]Sterifre Inc., Cornell University, Ithaca, NY; [3]Department of Electrical Engineering, University of Colorado Denver, Denver, CO; [4]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY

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*Address all correspondence to: Jason A. Spector, M.D., F.A.C.S., Professor of Surgery (Plastic Surgery) and Otolaryngology; Weill Cornell Medical College Adjunct Professor, Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University; Director, Laboratory for Bioregenerative Medicine & Surgery, Weill Cornell Medical College, Payson 709-A, 525 E. 68th St., New York, NY 10065; Tel.: 212-746-4532, Fax: 212-746-8952; E-mail: jas2037@med.cornell.edu

ABSTRACT: Non-thermal plasma has become an increasingly useful technology across a wide variety of disciplines. Currently a number of plasma-based technologies, which deliver reactive oxygen and nitrogen species (RONS), are being investigated for therapeutic sterilization in the biomedical field. We report on a novel, non-thermal plasma/free radical system developed by SteriFre Inc., the Sterifre Countertop Sterilizer (SCS), which uses a remote source to deliver a sustained mixture of highly active RONS in a closed loop system. Our technology significantly reduces the footprint of traditional non-thermal plasma generators, and it can be packaged into a desktop unit. Herein, we demonstrate that SCS reliably and rapidly sterilizes one of the more ubiquitous and sensitive personal electronic devices, the cellular phone, which is well known to harbor microorganisms, including pathogenic species, such as *Staphylococcus aureus*. Cell phones treated with SCS for 10 minutes had 100% reduction in bacterial growth and sterilization with no detrimental or residual effects on phone performance or appearance. Effective against a wide variety of microorganisms, SCS is an innovative, low cost, and portable technology that could potentially revolutionize the current practice of device sterilization in both industrial and private settings, including hospitals and other health-care environments.

KEY WORDS: plasma medicine; sterilization methods; biomedical engineering; hospital-acquired infections

I. INTRODUCTION

Despite best practices, the number of hospital-acquired infections has continued to rise considerably over the past two decades, with a total of 1.7 million hospital-acquired infections documented, resulting in approximately 99,000 deaths in the United States in 2002 alone.[1] Nosocomial outbreaks of colonization and infection with multidrug-resistant strains, including *Acinetobacter baumannii-calcoaceticus* complex (ABC)

and methicillin-resistant *Staphylococcus aureus* (MRSA), have been extensively reported.[2–12] Environmental contamination has been implicated as the underlying etiology of many nosocomial infections, and the challenge posed by an environmental reservoir is greatly enhanced in hospitals.[13,14] Movement of health-care personnel, their personal devices, and medical equipment between patients' rooms within hospitals makes thorough cleaning a more onerous task; and the risk of recontamination due to the frequent influx of many health-care workers, patients, and the equipment used in their care is a formidable challenge.

Moreover, multidrug-resistant infections often limit treatment options by physicians and surgeons.[6] Pathogenic bacteria are often transferred between patients via intermediary inanimate sources, such as personal cell phones and tablets, which are frequently used by health-care personnel, and not easily sterilized because of the delicate nature of their components and electronic materials. In a series of 114 health care–associated outbreaks in 39 states investigated onsite by the Centers for Disease Control and Prevention (CDC) personnel over a 10-year period, outbreaks related to invasive medical procedures, devices, and surgeries predominated. Twenty (17%) were linked to contaminated products, and 21 (18%) of the infectious disease outbreaks were associated with multidrug-resistant bacteria, including vancomycin-resistant *S. aureus* and vancomycin-resistant *Staphylococcus epidermidis*.[15] Thus, there is a dire need for rapid and effective methods to eradicate resident bacteria from these ubiquitous personal devices. Furthermore, such a means of sterilization would simultaneously lessen the financial impact of health care-associated infections, which have led to increased inpatient length of stay, morbidity, and mortality.[16]

Although multiple agents, such as ethylene oxide, glutaraldehyde, formalin gas, chlorine dioxide gas, and vaporized hydrogen peroxide, can be used for sterilization, the vast majority of these treatments are toxic, require multiple hours to ensure bacterial reduction, and necessitate highly specialized, expensive equipment and trained personnel to operate. Thus, these current sterilization processes are utterly impractical for routine disinfection of fomites carried into the hospital or office/point of care setting.[17,18]

Alternatively, non-thermal plasma offers a unique, rapid, and safe approach to the sterilization of sensitive inanimate objects, such as electronic devices.[19] Specifically, non-thermal plasmas produce the same highly active species that thermal plasmas do, but do not require high bulk temperatures (ion and heavy particle temperatures) or energies to be sustained. Notably, only electrons have high temperatures up to 10,000K, and the heavier species (neutral species and ions) are at ambient temperature. Furthermore, non-thermal plasmas do not require a large population of electrons to produce the highly active and germicidal reactive oxygen and nitrogen species (RONS).[20]

As the impetus for a clinically translatable application of non-thermal plasma sterilization treatments in health-care and hospital settings has never been stronger, we investigated a recently developed novel, non-thermal plasma/free radical system that uses a remote source to deliver a sustained, highly active, and efficacious concentration of RONS. It is a closed loop system that is placed up to 3 meters from the treatment site,

and it does not require specialized hardware, rigorous personnel training, or health-care expertise. Further, our technology significantly reduces the footprint of traditional non-thermal plasma generators and can be packaged into a desktop unit (Figure 1).

Herein, we demonstrate that the SteriFre Countertop Sterilizer (SCS) reliably and rapidly sterilizes one of the more ubiquitous and sensitive electronic components found in the hospital setting—the cell phone, a device well known to harbor microorganisms,[21-24] including pathogenic ones, such as *S. aureus*. Furthermore, sterilization is complete within minutes, without damaging the device or leaving any undesirable residue.

II. MATERIALS AND METHODS

A. SteriFre, Inc. Non-Thermal Plasma Technology

The plasma device used in this work was developed and constructed by Sterifre, Inc. (Ithaca, NY) and is described in detail by Golkowski et al.[25] In summary, the device creates a hydrogen peroxide–enhanced room temperature effluent from atmospheric air that is passed through a dielectric barrier discharge. The system consists of a cold plasma generator, flow distributor, evaporator, circulating blower, and effluent collector arranged in a closed loop flow system (Figure 2). A subsequent investigation of this technology by Plimpton et al.[26] illustrated that the system effectively generates H_2O_2, O_3, N_2O, NO_2, and OH^-, which collectively lyse cell membranes and deactivate bacteria. Precision measurements of active plasma components created

FIG. 1: The SteriFre Countertop Sterilizer, highlighting its desktop unit size

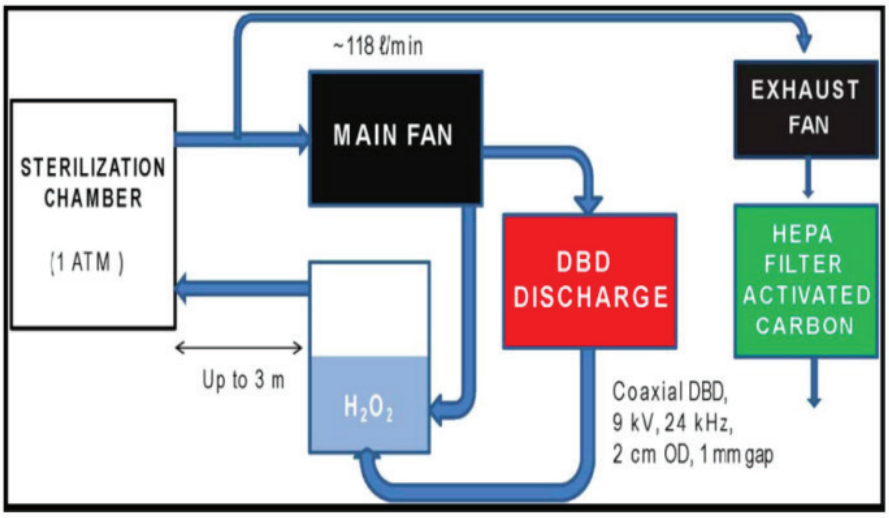

FIG. 2: Schematic depiction of closed-loop reactive oxygen and nitrogen species (RONS) generation within the SCS device

by the device are shown in Figure 3. Any exhaust from the system is filtered through a free radical destroyer, which mitigates the release of any free radical species to the surrounding environment.

B. Non-Thermal Plasma Treatment of Cell Phones

To test the efficacy of sterilization of cell phones using the SCS, we collected cell phones from volunteer personnel at the Laboratory for Bioregenerative Medicine and Surgery and analyzed them for adherent bacterial colony forming units (CFU) before and after SCS treatment. All cell phones were included in the study regardless of design type or electronic display, including smartphones and touchscreen phones; and all available marketed brands, ranging from Apple™ to BlackBerry™ to Samsung™. All cell phones were aseptically swabbed and samples were streaked onto trypticase soy agar (TSA) plates using sterile technique before treatment. Chocolate, blood, MacConkey, and Columbia Nalidixic Acid (CNA) agars (Hardy Diagnostics, Santa Monica, CA) were used in 24-well plates. Phones were similarly swabbed following non-thermal plasma treatment for either 5 or 10 minutes. Following 24 hours of agar incubation in a standard 37°C, 5% CO_2 incubator, number of CFU grown from cell phone swabs before and after treatment on each of the respective TSA plates were analyzed (Figure 4). Microbial colonization of cell phones before and after treatment was evaluated by counting the number of viable adherent CFU and subsequent identification of bacterial species present.

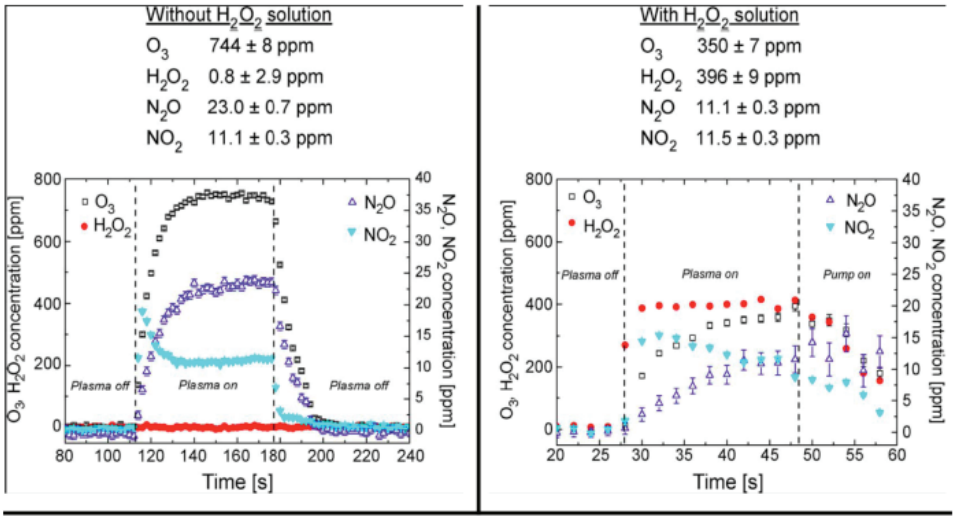

FIG. 3: Time dependence of chemical species concentrations measured with direct frequency comb spectroscopy as the device (DBD, dielectric barrier discharge and fans) is turned on and off (measured at JILA, Joint Institute of Laboratory Astrophysics; NIST, National Institute of Standards and Technology and the University of Colorado Boulder).[26]

C. Microbiology Speciation

For microbiology speciation, samples were collected with swabs from cell phones, immediately before and after treatment using sterile technique. A 24-well primary media tray was inoculated with each sample and allowed to incubate for 24 hours at 37°C. After incubation, the trays were observed for growth. Any colonies found were subcultured to blood agar plates and chocolate plates because these two plates have nonselective media properties. After 24 hours of subculture incubation at 37°C, Gram stains were performed on each mature colony. Gram stain results included gram-positive cocci and gram-positive rods. There was no gram-negative bacterial growth; therefore, no MacConkey plates were needed for subculture, and CNA was not needed for its selective gram-positive properties because all bacteria were gram-positive. For further identification, the MicroScan WalkAway *plus* System (Dade Behring Inc., West Sacramento, CA) was used with the positive combo panel type 33.

D. Statistical Analysis

An a priori power analysis for sample size estimation based on pilot study results of 10-minute data to determine effect size (Cohen's $d = 0.87$) was performed using PASS 13 (NCSS, LLC., Kaysville, UT); by convention, Cohen's $d = 0.2$, $d = 0.5$, and $d > 0.8$ correspond to small, medium, and large effect sizes, respectively. This analysis revealed

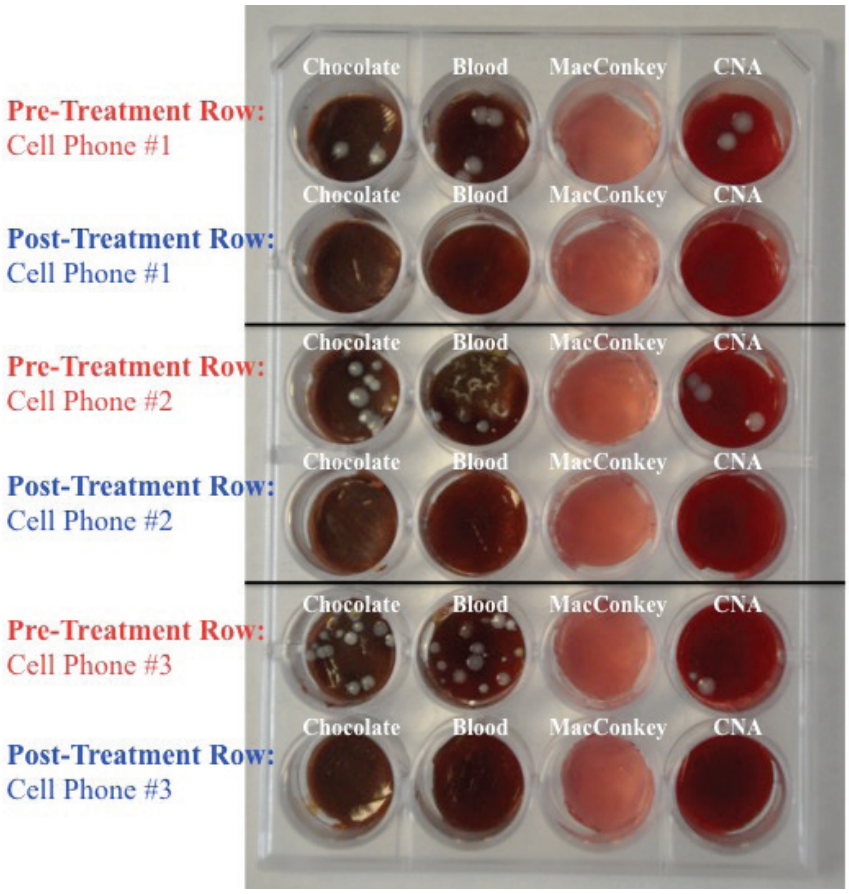

FIG. 4: Representative example of 24-well plate demonstrating bacterial CFU growth from cell phones. Rows 1, 3, and 5 demonstrate growth pre-treatment from three different phones; rows 2, 4, and 6 demonstrate absence of growth post 10-minute SCS treatment of the same phones, respectively.

that a minimum sample size of 12 was needed to achieve a power level of at least 0.800 for a two-sided Wilcoxon signed-rank test assuming a logistic actual distribution consistent with graphical assessment. Based on the actual 10-minute data (Cohen's $d = 0.94$), a post hoc power analysis revealed a final power level of 0.997.

The data were assessed for normality using both graphical techniques and the Shapiro-Wilk test for normality and found to be non-normal. Thus, Wilcoxon signed-rank tests were performed to determine whether treatment of cell phones with a non-thermal plasma system for 5 minutes and 10 minutes leads to a significant CFU reduction. Given that data for the pre-treatment and post-treatment groups for the 5-minute time interval were collected independently from those for the 10-minute time interval, an application of the Bonferroni correction was not indicated.

Data were reported as mean ± standard deviation (SD) and median and interquartile range (IQR). For all tests, a p value less than 0.05 was considered statistically significant. Analyses were performed using NCSS 10 Statistical Software (NCSS, LLC., Kaysville, UT).

III. RESULTS

A total of 51 cell phones were studied with SCS non-thermal plasma treatments. Each cell phone was treated in the SCS with non-thermal plasma for either 5 minutes or 10 minutes, respectively, while powered down. Twenty-six cell phones (51%) were treated for 5 minutes, and 25 cell phones (49%) were treated for 10 minutes; each cell phone in each group was treated only once.

Following treatment of cell phones with the non-thermal plasma system for 5 minutes and 24 hours of agar incubation in a standard 37°C, 5% CO_2 incubator, the number of CFU pretreatment (median 15, IQR 6.5-34.25; mean 59.2, SD 186.7) was significantly reduced compared to post-treatment (median 0, IQR 0-1; mean 0.6, SD 1.6), corresponding to $p < 0.001$, d = 0.31. The treatment of cell phones with non-thermal plasma for 5 minutes produced a 93% CFU reduction with only 7% residual CFUs in the cohort of 26 cell phones (Figure 5).

Following treatment of cell phones with non-thermal plasma system for 10 minutes and 24 hours of agar incubation in a standard 37°C, 5% CO_2 incubator, there was 100%

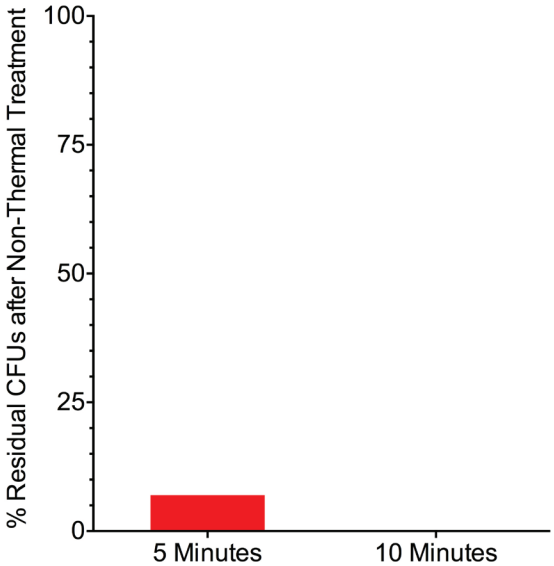

FIG. 5: Percent residual CFU after SCS treatment for 5 and 10 minutes. The treatment of cell phones with non-thermal plasma system for 5 minutes (n = 26) produces a 93.0% CFU reduction, and treatment for 10 minutes (n = 25) produces a 100% CFU reduction.

CFU reduction with no CFU on all TSA plates for all cell phones (Figure 5). The number of CFU pre-treatment (median 13, IQR 6.5–33.5; mean 24.6, SD 26.3) were significantly reduced compared to post-treatment (median 0, IQR 0-0; mean 0, SD 0), corresponding to $p < 0.001$, d = 0.94. After a 10-minute non-thermal plasma treatment with the SCS system, all 25 of the cell phones in this group were 100% sterilized (Figures 6A and B).

Speciation of the colonies determined the identities of the cell phone resident bacteria to be *S. aureus*, *S. epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*, *Staphylococcus capitis-urea*, *Kocuria kristinae*, *Bacillus* spp., and *Micrococcus* spp. Of the colonies isolated from cell phones, the most common pathogens in decreasing order of prevalence were *S. aureus*, *S. epidermidis*, and *S. hominis*. The majority of cell phones had *S. aureus* and *S. epidermidis*, and fewer phones had *S. hominis*.

IV. DISCUSSION

Currently, there is no effective technology that can expeditiously and safely sterilize colonized and/or contaminated personal electronic devices without subjecting them to either harmful heat or corrosive chemicals that would destroy the devices along with their resident bacteria. As presented herein, we have developed a novel non-thermal plasma dielectric barrier discharge (DBD) system for decontamination, sterilization, and medical applications. As the data demonstrate, a brief room temperature treatment of only 10 minutes resulted in complete sterilization of all cell phones tested. Further, all devices functioned normally after treatment, without any evidence of damage or change in appearance.

Although non-thermal plasma has innumerable potential applications to clinical medicine as recently reviewed by Isbary et al.,[27] in the realm of acute and chronic wounds and pruritic diseases, we believe that there is a crucial intermediary clinical application that is being overlooked—sterilization of mobile phones and other inanimate objects, such as personal pagers and stethoscopes, used ubiquitously by health-care workers while caring for their patients. Cell phones have inevitably become an invaluable asset in the health-care setting because they provide a quick and convenient means of team communication, access to laboratory and imaging results, and facilitation of the management of life-threatening emergencies.[28,29] In fact, census data reveal that nearly every health-care professional has a cell phone, which further emphasizes its vital role in patient care.[30]

Yet, the indispensable mobile phone simultaneously serves as a transmission vector of bacteria and nosocomial infection between health-care providers and patients.[21,22,31] As reviewed by Brady et al.,[32] in the hospital setting, an alarming 96.2% of cell phones had evidence of bacterial contamination, and 13.3% of the phones contained bacteria known to cause nosocomial infections.[33] Likewise, Ulger et al.[34] reported that 94.4% of cell phones used by health-care workers had evidence of bacterial contamination with nosocomial pathogens. In our sample, 100% of cell phones were contaminated with bacteria, so bacterial colonization is ubiquitous. In our study, the most common bacteria contaminating cell phones were *S. aureus* and *S. epidermidis*, which are, as expected,

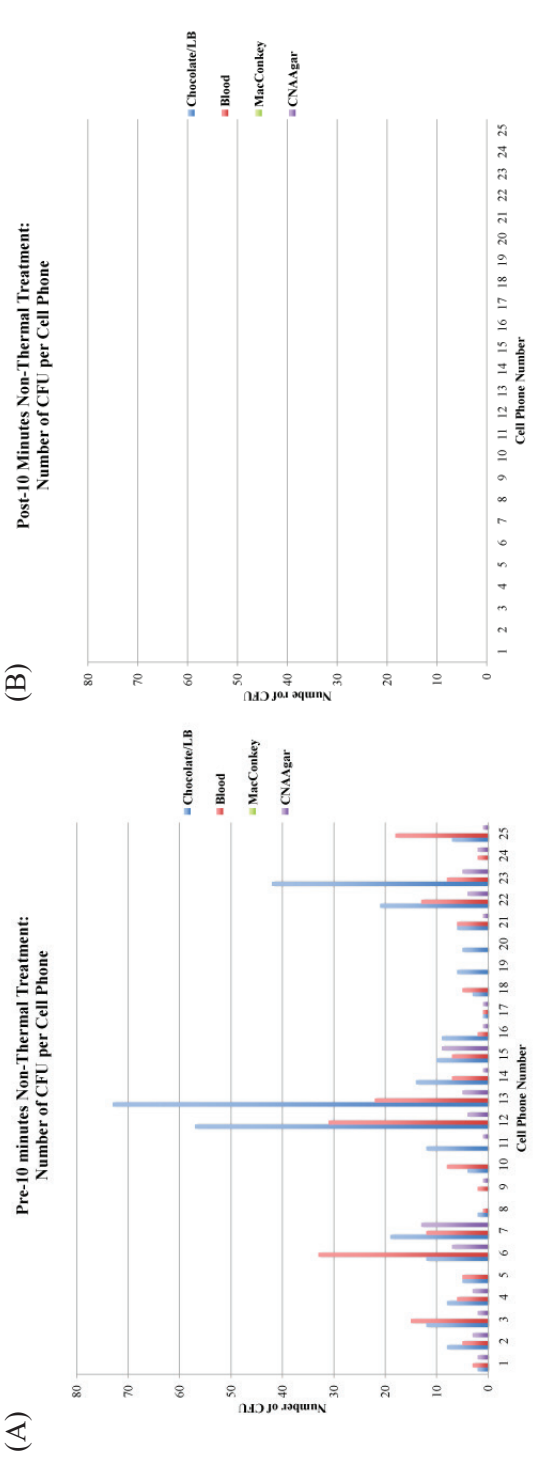

FIG. 6: Number of CFU grown per cell phone on respective agars before (Figure 6A) and after 10-minute (Figure 6B) non-thermal plasma treatment. Note that a 10-minute treatment with the SCS system results in 100% bacterial sterilization of all phones tested.

representative of the most common bacterial flora of normal human skin.[35-37] Furthermore, the bacteria identified from our sample of devices are consistent with Bhalla et al.,[38] who reported that *S. aureus* was one of the top two most commonly acquired nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. Additionally, there was no gram-negative bacterial growth from any of the cell phones, which is not unexpected, given that gram-negative bacteria are not typically a component of skin flora of the human hand[35] and by extension handheld cell phones.

Unlike traditional heat and chemical sterilization processes, SCS technology uses a free radical gaseous method to kill microorganisms, including bacteria, vegetative cells, spores, and viruses, which have been deposited or attached to delicate surfaces.[25] Gaseous free radical disinfection uses several gaseous oxidizing species, such as hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), ozone (O_3), and excited molecular oxygen (O_2); these agents strip an electron from the microorganisms, resulting in their death.[39,40]

The SteriFre Countertop Sterilizer device has several distinguishing features when compared to other existing non-thermal plasma devices. First, the electrical discharge (plasma) does not directly contact the sample to be disinfected. To elaborate, the setup does not involve any pressurized gases, and a hydrogen peroxide additive is used to enhance the bactericidal efficacy. Secondly, the system employs a closed-loop flow with a sterilization chamber, and this closed-loop flow system allows for maximal buildup of concentration of free radicals and RONS. The closed loop must remain continuously intact for 10 minutes to provide adequate non-thermal plasma treatment for sterilization.

For safety purposes, the system operates at slightly lower pressure than ambient pressure to prevent uncontrolled leakage of RONS. Specifically, two identical 100-W fans provide circulation (main fan) and exhaust (exhaust fan). The role of the exhaust fan is to keep the closed-loop system in a state of under pressure preventing any escape of free radicals or pathogens, except through the exhaust exit, which contains an activated carbon RONS deactivation bed and a HEPA filter.[25] Therefore, the working gas is ambient air that is supplied to the system through the HEPA filter while the exhaust and purging is performed using an activated carbon RONS deactivation bed as well as the HEPA filter. Hence, use of the SCS system does not require a chemical hood or a biosafety cabinet, despite the relatively high concentrations of RONS produced.[25,26] Furthermore, flow from the main fan is fractionally split, with 33.3% allocated to the dielectric barrier discharge (DBD), and 66.6% bypassing the DBD and directly connecting to the hydrogen peroxide bubbler. The DBD component consists of two independent units, each with two concentric cylindrical electrodes, which are 5 cm in length, respectively. Each DBD unit is driven by a voltage waveform with an amplitude of 9 kV and a frequency of 24 kHz. After the DBD, the flow streams connect and proceed to the hydrogen peroxide bubbler, which contains a 50% H_2O_2 solution. In contrast to other non-thermal plasma systems, the sterilization chamber can be up to 3 meters from the bubbler exit and still provide efficacious and efficient sterilization at this distance, while operating at a flow rate of 90 L/min through the SCS system at a temperature of 37°C. Detailed optical spectroscopy measurements demonstrate that the device is able

to produce a copious stream of free radicals, including ozone (O_3), hydrogen peroxide (H_2O_2), nitrous oxide (N_2O), and nitrogen dioxide (NO_2).[25] Electron paramagnetic spin resonance spectroscopy showed the presence of hydroxyl radicals (OH^-) generated in secondary chemical reactions.[26] Importantly, for clinically translatable application, the SCS device does not require the proximity of high voltage electrodes or a water supply. Any exhaust from the system is filtered as described earlier, and thus there is no release of active free radical species into the environment (Figure 2).

The SCS technology may be used immediately to perform sterilization in hospital wards; since our technology significantly reduces the footprint of traditional non-thermal plasma generators it can be packaged into a desktop unit. Our SCS system is unique in that it employs an indirect plasma source by definition, but its innovative features make it more aptly a hybrid plasma source. To enumerate, the hydrogen peroxide additive robustly enhances the bactericidal properties of the free radical effluent, and the concentration of hydrogen peroxide additives in our model is undoubtedly a vital variable in inactivation efficacy, and it means that the active species are different from those in other DBD configurations.[25] Given that the physical distance between the discharge point, featuring a stream of plasma-induced free radicals, and the treatment surface can be up to 3 meters, our technology is primed for clinical application (e.g., decontamination of wounds, sterilization of biofilms). As SCS demonstrates, complete sterilization of sensitive electronic devices after a brief 10-minute treatment is revolutionary compared to other non-thermal plasma devices, which are not nearly as inexpensive, facile to use, or compact. As the delicate components contained within cell phones are also present within numerous similar electronics (e.g., tablets, medical monitors, laptops, pagers), which have likewise proven to be contaminated,[41,42] we believe the results obtained in this study, namely safe and efficacious sterilization, may be extrapolated to those devices as well. However, to ensure the highest level of confidence when treating any individual device, further testing will ultimately need to be performed for each one.

V. CONCLUSION

Effective against a wide variety of microorganisms, SCS non-thermal plasma technology is a novel, low-cost, and portable technology that has the potential to revolutionize the current practice of device sterilization in both industrial and private settings. Treatment of cell phones for 10 minutes in SCS completely eradicated resident bacteria from all phones without any detrimental effect on the devices. Hence, SCS technology has the ability to transform the hospital environment, providing rapid, efficient, and total elimination of bacteria on cell phones, personal pagers, and other ubiquitous devices, such as stethoscopes, for use on hospital wards with a convenient desktop model.

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