

A Pilot Study of Atmospheric Nonthermal Plasma Jet Application on *Staphylococcus aureus* and *Staphylococcus epidermidis*

Wenhui Du,¹ Chunjun Yang,^{1,*} Cheng Cheng,² Jie Shen,² Jilu Shen,³
Shumei Zhang,¹ Shi Chen,⁴ Longdan Liu,¹ Jing Gao,¹ Shengxiu Liu,¹
& Shenghai Huang⁵

¹Institute of Dermatology and Department of Dermatology, First Affiliated Hospital, Anhui Medical University, Hefei, People's Republic of China; ²Institute of Plasma Physics, Chinese Academy of Sciences, Hefei, People's Republic of China; ³Clinical Laboratory, First Affiliated Hospital, Anhui Medical University, Hefei, People's Republic of China; ⁴Department of Medicine, Anhui Medical University, Hefei, People's Republic of China; ⁵Department of Microbiology, Anhui Medical University, Hefei, People's Republic of China

*Address correspondence to: Dr. Chunjun Yang, Institute of Dermatology and Department of Dermatology, First Affiliated Hospital, Anhui Medical University, Hefei, China, 230032; Tel: +86 551 65161002; Fax: +86 551 65161016; E-mail: yangchunjun9@163.com

ABSTRACT: *Background:* Infectious skin diseases caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* continue to increase worldwide even with management by current infection control methods. Novel methods for disinfecting these drug-resistant strains would be useful. We experimentally tested the effectiveness of nonthermal atmospheric pressure plasma jets at killing *S. aureus* and *S. epidermidis* in *in vitro* settings. *Methods:* The strains of *S. aureus* and *S. epidermidis* were propagated in tryptic soy agar plates after isolation and cultivation, and all of the plates were then exposed to nonthermal atmospheric pressure plasma jets for varying lengths of time (10, 20, 40, 60, 180, and 300 seconds). The colony forming units were quantified after 48-hour incubation at 37°C. *Results:* Exposure of *S. aureus* and *S. epidermidis* to nonthermal atmospheric pressure plasma jets at different lengths of time resulted in all strains killed on tryptic soy agar plates within 3 minutes. *Conclusions:* The nonthermal atmospheric pressure plasma jet is a promising instrument in controlling skin infection disease, which warrants further study in dermatology.

KEY WORDS: *Staphylococcus aureus*, *Staphylococcus epidermidis*, plasma

I. INTRODUCTION

Staphylococcus aureus and *Staphylococcus epidermidis* cause various skin and mucous membrane infections in children and adults, and these strains are also the most common pathogens of hospital-acquired infections worldwide.^{1,2} *S. aureus* skin infections are divided into superficial and deep pyoderma infections based on clinical morphological and treatment aspects.³ Superficial infections with *S. aureus* include Impetigo contagiosa, superficial folliculitis, perifolliculitis, furuncle, and furunculosis. Deep infections with *S. aureus* include cutaneous abscess, phlegmon, and local soft-tissue infections. *S. epidermidis*, a type of coagulate-negative staphylococcus and a facultative pathogen, is a commensal bacterium of the human skin. *S. epidermidis* can trigger a human body

infection if there is a local or generalized immune deficiency. In addition, *S. epidermidis* can cause synthetic implant- or catheter-related infections as well as infections in immunocompromised patients.⁴

Methicillin-resistant *S. aureus* (MRSA), first reported in 1960, is frequently detected in hospitals worldwide. All sorts of infections caused by MRSA are increasing problems throughout the world in many fields, such as dermatology, ophthalmology, and rhinolaryngology.⁴ The increased antibiotic resistance of nosocomial isolates of *S. aureus* and *S. epidermidis* aggravates these problems and poses a great challenge for the management of hospital-acquired infections.⁵

Plasma medicine offers a new form of therapy by combining potent physical effects such as ultraviolet and infrared reactive species and particle radiation, which has been used to successfully treat many different illnesses, especially in the field of dermatology.⁶ Nonthermal atmospheric pressure plasma, such as atmospheric uniform glow discharge plasma, dielectric barrier discharge, surface discharge, and atmospheric pressure plasma jets (APPJs), and atmospheric pressure plasma plumes (APPPs), have received significant attention because of their low operating temperatures, high efficiency, and cost-effective operation. We previously demonstrated that an atmospheric plasma jet with an argon and oxygen mixture gas was highly effective in the sterilization of *Bacillus subtilis* spores.⁷ Based on these findings and the results of other groups,⁸ this pilot study investigated the ability of plasma to treat *S. aureus* and *S. epidermidis* in *in vitro* settings.

II. MATERIALS AND METHODS

A. Bacterial Strains and Growth Conditions

S. aureus and *S. epidermidis* were isolated from skin lesions in one patient with severe pemphigus vulgaris and in another patient with severe drug eruption, respectively, at Anhui Medical University Hospital's clinical laboratory. The *S. aureus* and *S. epidermidis* strains were identified by API and VITEK 2 automatic systems (BioMérieux Inc, Durham, NC). All strains were stored with trypticase soy broth (TSB) at -20°C and were used as overnight cultures in TSB for primary inoculations. Colonies of *S. aureus* and *S. epidermidis* were cultivated at 37°C on TBS broth (to late-log phase) and agar plates. After overnight incubation at 37°C , the colony forming units (CFUs) were quantified.

B. Plasma Discharge Apparatus and Parameters

A schematic of the APPJ device used in this pilot study is shown in Fig.1A. In brief, one copper rod (2 mm diameter, 100 mm length) was embedded in a quartz tube, and one side of the rod was sealed as a single electrode. The rod was tightly wrapped in a Teflon shell. A quartz tube (20 mm length, 4 mm inner diameter, 6 mm outer diameter) was connected to the shell as a nozzle. The electrode was connected with a sinusoidal AC high-voltage supply (36 kHz, 20 kV) injected into the APPJ device as the working

gas. A typical photograph of the plasma jet is shown in Fig. 1B. All bacteria samples were exposed to the atmospheric pressure plasma discharge in an argon gas medium with a distance of 1 cm away from the nozzle. The discharge reached the surface of the specimen through diffusion aided by a rotary fan that generated air currents across the top of the plates.

C. Plasma Bactericidal Activity

S. aureus and *S. epidermidis* strains were prepared by adding 100 μl of bacterial suspension (the original CFUs of *S. aureus* and *S. epidermis* strains were 5.1×10^6 and 2.8×10^6 , respectively, after inoculation overnight at 37°C) to tryptic soy agar plates in the 6×4 wells. All of the plates were then exposed to the APPJ device for varying lengths of time (10, 20, 40, 60, 180, and 300 seconds). The final step was to count the CFUs after 48-hour incubation at 37°C .

III. RESULTS

Trypticase soy plates in the 6×4 wells were inoculated with an equal number of organisms in the A group (*S. epidermidis*) and B group (*S. aureus*) (two lines per group), respectively. The relationship diagram between the effectiveness of plasma and exposure

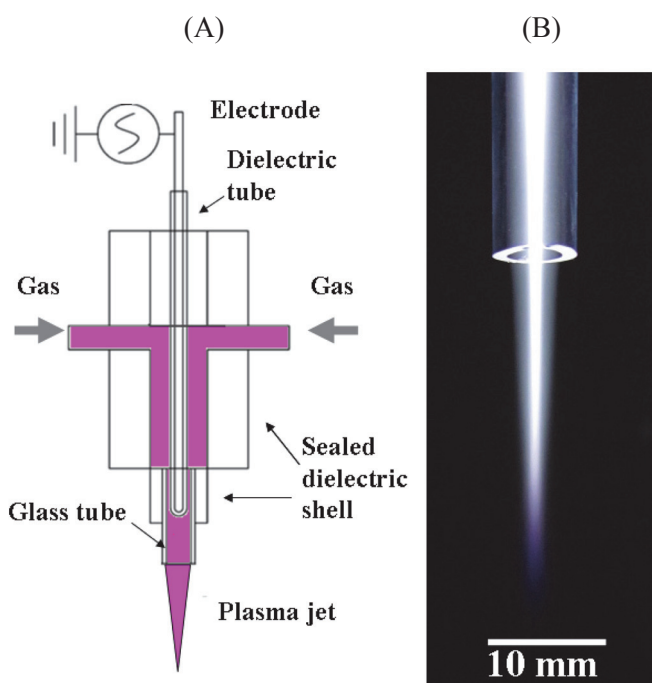


FIG. 1: (A) Schematic of the APPJ device. (B) Photograph of the APPJ device.

time is depicted in Fig. 2. With the increase of irradiation time of the plasma, the delivery area was enlarged and almost all of the *S. aureus* and *S. epidermidis* strains were killed within 3 minutes. We also counted the surviving organisms after different plasma exposure times for the *S. aureus* and *S. epidermidis* strains (corresponding line average value), and the results are shown in Fig. 3.

IV. DISCUSSION

This pilot study aimed to evaluate the ability of nonthermal atmospheric plasmas to manage *S. aureus* and *S. epidermidis* *in vitro*. As an initial test, inocula of commonly used laboratory strains of *S. aureus* and *S. epidermidis* were propagated in agar plates and exposed to plasma for 10, 20, 40, 60, 180, and 300 seconds followed by incubation at 37°C for 48 hours. The strains in this study were isolated from the clinic laboratory in our hospital and were resistant to common antibiotics (unpublished data). Both *S. aureus* and *S. epidermidis* were highly sensitive to the effects of plasma and were killed completely within 3 minutes (Fig. 2).

There are many pilot studies on nonthermal atmospheric plasma applications on pathogenic microorganisms, and recent progress in understanding nonthermal atmospheric plasmas has led to clinical applications.⁹ Studies investigating the interaction of plasma with microorganisms or living cells have shown applications on inactivation of

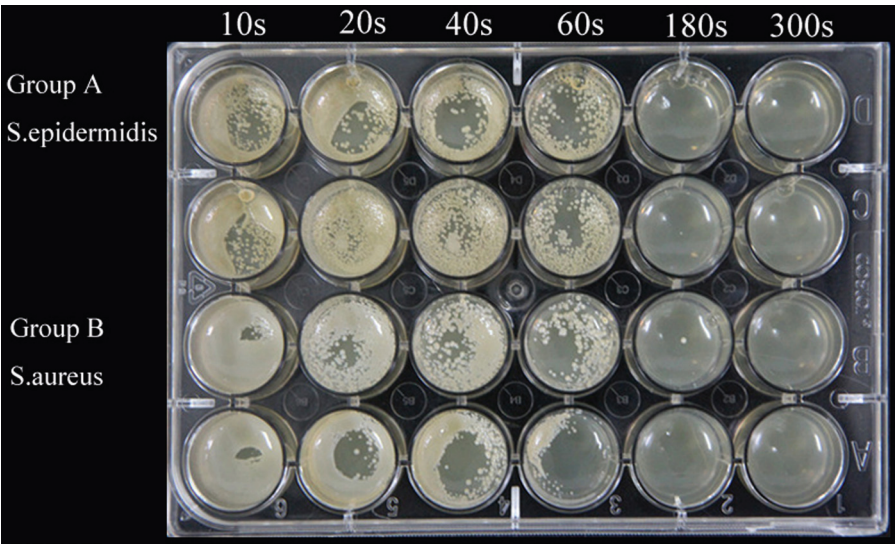


FIG. 2: Relationship diagram between the effectiveness of the APPJ device and exposure time. Along with increased exposure time to the plasma, the delivery area was enlarged and almost all of the *S. aureus* and *S. epidermidis* strains were killed within 3 minutes.

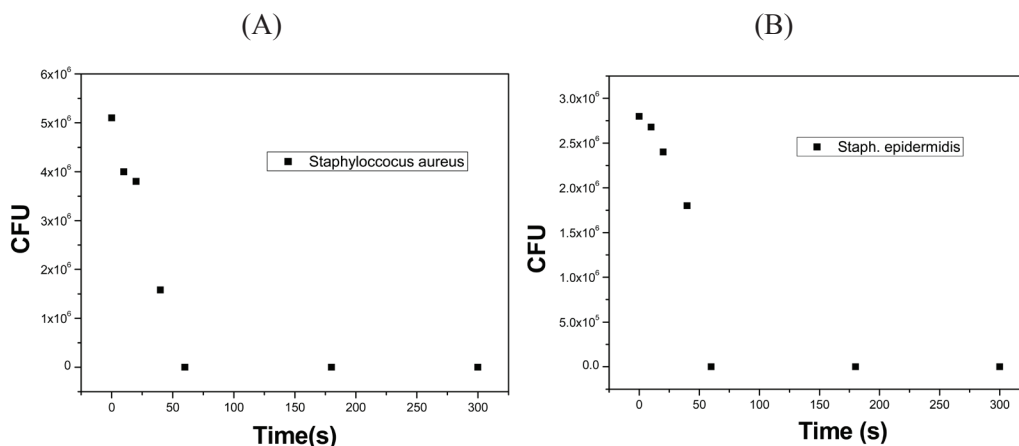


FIG. 3: Survival curves of *Staphylococcus* treated with the APPJ device at different plasma exposure times. (A) *S. aureus*. (B) *S. epidermidis*.

pathogens,¹⁰ wound healing,¹¹ fungal infections,¹² tissue sterilization,^{13,14} and even ablation of cultured cancer cells.^{15,16} The effects of nonthermal atmospheric plasmas on reactive species (e.g., oxygen/hydroxyl radicals and nitric oxide) generated in the plasma or in the tissues brought into contact with the plasma are numerous. We previously demonstrated that charged particles and ozone play a major role in the plasma application on the *B. subtilis* spore, contributions from heat and UV radiation can be neglected, and the change of the shapes of the spores may be due to reactive oxygen radicals.⁷

Burts et al. reported that exposing *S. aureus* to atmospheric nonthermal plasma discharge at different concentrations and for varying lengths of time resulted in up to a 4- to 5-log (10) kill on tryptic soy agar plates within 10 minutes and was not toxic to epithelial cells.¹⁷ We demonstrated that *S. aureus* and *S. epidermidis* were killed by atmospheric plasma discharge in our setting for varying lengths of time resulting in up to 5-log (10), respectively, on tryptic soy agar plates within 3 minutes (Fig. 3). There may be several reasons for the different killing times of the same order strains between our findings and those of Burts et al. First, we used a different work gas because we believed that an argon/oxygen plasma jet may be more effective. Second, the set parameters of the nonthermal atmospheric plasma were different. Third, *S. aureus* may have different resistance ability in different locations; however, this difference cannot negate the fact that the nonthermal atmospheric plasma jet may be a highly effective device in killing *S. aureus* and *S. epidermidis*, which are usually resistant to common antibiotics.

There are some shortcomings of the device that must be addressed in the future, including its complexity, effective spray area (limited per times), nonportability, and so forth. Although the non-thermal atmospheric plasma jet device has strong bacteria-destroying abilities, there is a long way to apply it in clinical.¹⁸ Several authors have documented the mechanisms of action of plasma on bacteria,^{13,19,20} but further studies regarding the effect of plasma on normal tissues or cells, as well as the target bacterium, are needed.

REFERENCES

1. Klein EY, Sun L, Smith DL, Laxminarayan R. The changing epidemiology of methicillin-resistant *Staphylococcus aureus* in the United States: a national observational study. *Am J Epidemiol*. 2013;177:666–74.
2. Du X, Zhu Y, Song Y, Li T, Luo T, Sun G, Yang C, Cao C, Lu Y, Li M. Molecular analysis of *Staphylococcus epidermidis* strains isolated from community and hospital environments in china. *PLoS One*. 2013;8:e62742.
3. Schöfer H, Bruns R, Effendy I, Hartmann M, Jappe U, Plettenberg A, Reimann H, Seifert H, Shah P, Sunderkötter C, Weberschock T, Wichelhaus TA, Nast A; German Society of Dermatology (DDG)/Professional Association of German Dermatologists (BVDD); Infectious Diseases Society of Germany (DGI); German Society for Hygiene and Microbiology (DGHM); German Society for Pediatric Infectious Diseases (DGPI); Paul Ehrlich Society for Chemotherapy (PEG). Diagnosis and treatment of *Staphylococcus aureus* infections of the skin and mucous membranes. *J Dtsch Dermatol Ges*. 2011;9:953–67.
4. Tavares A, Miragaia M, Rolo J, Coelho C, de Lencastre H; CA-MRSA/MSSA Working Group. High prevalence of hospital-associated methicillin-resistant *Staphylococcus aureus* in the community in Portugal: evidence for the blurring of community-hospital boundaries. *Eur J Clin Microbiol Infect Dis*. 2013;32:1269–83.
5. Gould IM, David MZ, Esposito S, Garau J, Lina G, Mazzei T, Peters G. New insights into methicillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. *Int J Antimicrob Agents*. 2012;39:96–104.
6. Heinlin J, Morfill G, Landthaler M, Stolz W, Isbary G, Zimmermann JL, Shimizu T, Karrer S. Plasma medicine: possible applications in dermatology. *J Dtsch Dermatol Ges*. 2010;8:968–76.
7. Shen J, Cheng C, Fang S, Xie H, Lan Y, Ni G, Meng Y, Luo J, Wang X. Sterilization of *Bacillus subtilis* spores using an atmospheric plasma jet with argon and oxygen mixture gas. *Appl Phys Express* 2012;5:036201.
8. Joshi SG, Paff M, Friedman G, Fridman G, Fridman A, Brooks AD. Control of methicillin-resistant *Staphylococcus aureus* in planktonic form and biofilms: a biocidal efficacy study of nonthermal dielectric-barrier discharge plasma. *Am J Infect Control*. 2010;38:293–301.
9. Idlibi AN, Al-Marrawi F, Hannig M, Lehmann A, Rueppell A, Schindler A, Jentsch H, Rupf S. Destruction of oral biofilms formed in situ on machined titanium (Ti) surfaces by cold atmospheric plasma. *Biofouling*. 2013;29:369–79.
10. Heller LC, Edelblute CM, Mattson AM, Hao X, Kolb JF. Inactivation of bacterial opportunistic skin pathogens by nonthermal DC-operated afterglow atmospheric plasma. *Lett Appl Microbiol*. 2012;54:126–32.
11. Isbary G, Heinlin J, Shimizu T, Zimmermann JL, Morfill G, Schmidt HU, Monetti R, Steffes B, Bunk W, Li Y, Klämpfl T, Karrer S, Landthaler M, Stolz W. Successful and safe use of 2 min cold atmospheric argon plasma in chronic wounds: results of a randomized controlled trial. *Br J Dermatol*. 2012;167:404–10.
12. Maisch T, Shimizu T, Isbary G, Heinlin J, Karrer S, Klämpfl TG, Li YF, Morfill G, Zimmermann JL. Contact-free inactivation of *Candida albicans* biofilms by cold atmospheric air plasma. *Appl Environ Microbiol*. 2012;78:4242–7.
13. Fricke K, Koban I, Tresp H, Jablonowski L, Schröder K, Kramer A, Weltmann KD, von Woedtk T, Kocher T. Atmospheric pressure plasma: a high-performance tool for the efficient removal of biofilms. *PLoS One*. 2012;7:e42539.

14. Brun P, Brun P, Vono M, Venier P, Tarricone E, Deligianni V, Martines E, Zuin M, Spagnolo S, Cavazzana R, Cardin R, Castagliuolo I, Valerio AL, Leonardi A. Disinfection of ocular cells and tissues by atmospheric-pressure cold plasma. *PLoS One*. 2012;7:e33245.
15. Ishaq M, Evans MM, Ostrikov KK. Effect of atmospheric gas plasmas on cancer cell signaling. *Int J Cancer*. 2014;134:1517–28.
16. Volotskova O, Hawley TS, Stepp MA, Keidar M. Targeting the cancer cell cycle by cold atmospheric plasma. *Sci Rep*. 2012;2:636.
17. Burts ML, Alexeff I, Meek ET, McCullers JA. Use of atmospheric non-thermal plasma as a disinfectant for objects contaminated with methicillin-resistant *Staphylococcus aureus*. *Am J Infect Control*. 2009;37:729–33.
18. Isbary G, Shimizu T, Li YF, Stolz W, Thomas HM, Morfill GE, Zimmermann JL. Cold atmospheric plasma devices for medical issues. *Expert Rev Med Devices*. 2013;10:367–77.
19. Kvam E, Davis B, Mondello F, Garner AL. Nonthermal atmospheric plasma rapidly disinfects multidrug-resistant microbes by inducing cell surface damage. *Antimicrob Agents Chemother*. 2012;56:2028–36.
20. Ahn HJ, Kim KI, Kim G, Moon E, Yang SS, Lee JS. Atmospheric-pressure plasma jet induces apoptosis involving mitochondria via generation of free radicals. *PLoS One*. 2011;6:e28154

