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Bactericidal efficacies of nebulized non-thermal atmospheric plasma-treated liquids

Utku Kursat Ercan, Fatma Ibis

Department of Biomedical Engineering, Izmir Katip Celebi University, Izmir, Turkey

ABSTRACT

Objectives. Antimicrobial activities of non-thermal atmospheric plasma-treated liquids on various bacterial and fungal strains in their planktonic and biofilm forms have been widely reported. Since most of the plasma-treated liquids are water based, they might be washed off certain surfaces and they cannot be applied for the infections of respiratory tract. In the present study we have tested antimicrobial activities of plasma-treated N-acetyl cysteine solution (NAC), phosphate buffered saline (PBS) solution and deionized water when nebulized over planktonic forms of *E. coli* and *S. aureus*. ***Methods.*** Antimicrobial activities of nebulized plasma-treated liquids were evaluated with zone of inhibition test and colony counting assay. Moreover pH of NAC, PBS solution and deionized water were measured before, after plasma treatment and during nebulization since low pH is well known consequence observed in plasma-treated liquids. ***Results.*** Our results have revealed that pH of plasma-treated NAC, PBS solution and DIW decreases after plasma treatment consistent with previous reports and does not change during nebulization. Moreover, antimicrobial activity assessment indicates that nebulized plasma-treated NAC shows the strongest antimicrobial activity, which leads complete inactivation of bacteria for 10^3 to 10^6 CFU/ml initial bacterial load and 5-log reduction for 10^7 CFU/ml initial bacterial load on both *E. coli* and *S. aureus*. ***Conclusions.*** Plasma-treated liquids could retain their antimicrobial activity during nebulization and nebulization could be considered as a future alternative method for delivery of plasma-treated liquids for respiratory tract infections.

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Keywords: Non-thermal plasma, plasma medicine, nebulization, antimicrobial, biomedical engineering

Introduction

Even though the word "plasma" defines the liquid component of blood in medical terminology, Irving Langmuir used the same word in order to define physical plasma since the complex structure of physical plasma reminded him the blood plasma [1]. Basically, plasma is defined as the ionized gas and the fourth state of the matter next to solid, liquid and gas. When a gas is subjected to an electric field and when the voltage reaches the breakdown voltage of the gas, an electric discharge will form which then leads formation of ionized gas (or plasma) via removal of electrons from gas atoms and/or molecules. Plasma is

Address for correspondence:
Utku Kursat Ercan, PhD., Izmir Katip Celebi University, Department of Biomedical Engineering, Cigli; 35620 Izmir, Turkey
E-mail: utkuercan@gmail.com; utkuk.ercan@ikc.edu.tr
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the most abundant state of the matter and comprises more than 99% of the universe. Sun, lightning, aurora borealis are examples of natural plasmas [2]. Plasmas are classified as thermal (hot or equilibrium) and non-thermal (cold or non-equilibrium) plasmas according to gas and electron temperature. In all plasmas generated under electric field electron temperature reaches thousands of Kelvin. In thermal plasmas, the gas temperature is in equilibrium with electron temperature and also reaches thousands of Kelvins. In non-thermal plasmas, cooling of heavy particles (uncharged molecules and ions) is more effective that the energy flux from electrons and gas remains in room temperature [2]. Therefore non-thermal plasmas can be used for the treatment of temperature sensitive materials including cells and living tissue [3]. During generation of plasma, formation of ultraviolet (UV) light, free electrons, electric field, reactive oxygen species (ROS) such as hydroxyl radical, superoxide, atomic oxygen, hydrogen peroxide, singlet oxygen and reactive nitrogen species (RNS) such as nitric oxide, nitrite, nitrate, peroxynitrite make plasma a reactive medium [4].

Plasma can be utilized in various modalities. In direct plasma treatment modality, the material that is being treated serves as a counter electrode and comes in direct contact with plasma discharge. In indirect plasma treatment, plasma is generated remotely and plasma products are carried to the material that is being treated via gas flow. Therefore in indirect plasma treatment effects of UV light, electric field and free electrons can be excluded [5]. Moreover, Ercan *et al.* [6] have defined fluid mediated plasma treatment in which a particular fluid is first treated with plasma and then the treated fluid is exposed to material that is being treated.

The use of non-thermal plasmas on cells and living tissues created a new multidisciplinary, emerging area called as plasma medicine that investigates therapeutic effects of physical non-thermal plasmas [7]. Non-thermal atmospheric plasma technology found itself vastly growing various application areas in biomedicine such as disinfection, cancer therapy, wound healing, blood clotting, tooth whitening, biomaterial modification [8-13]. Broad range antimicrobial activity of non-thermal plasmas on planktonic and biofilm forms of bacteria - including multi drug resistant (MDR) strains -, fungi, viruses and even prions have been reported [6, 14-17]. In addition to antimicrobial activity of non-thermal plasmas, also various materials such as liquids, gels may acquire antimicrobial activity when treated with plasma [6, 18-20]. Poor *et al.* [18] have shown that calcium alginate gel wound dressings gains strong antimicrobial activity over several bacterial and fungal strains upon non-thermal plasma treatment. Furthermore, different groups have shown strong antimicrobial activity acquisition of fluids such as water, phosphate buffered saline (PBS) solution, saline solution, N-acetyl cysteine (NAC) solution against biofilm and planktonic forms of bacteria and fungus when treated with non-thermal plasma [6, 21-23]. Antimicrobial activity of plasma-treated liquids was attributed to presence of ROS and RNS in plasma-treated liquids that are generated during plasma treatment [24, 25]. Besides, plasma-treated liquids can maintain acquired microbial activity up to several years [6]. Therefore, plasma-treated liquids are considered as novel antimicrobial solutions that may be conveyed to clinical practice. Despite broad range and strong antimicrobial activities of plasma-treated liquids, their use for eradication of certain infections such as respiratory tract infections is not applicable since presence of liquid in respiratory tract leads suffocation. Thus, a delivery method of plasma-treated liquids should be established and investigated for possible future applications. Nebulization is a drug delivery method that is used for administration of medications in liquid phase towards respiratory tract. Nebulization is a process in which a liquid is converted to form of mist that can be inhaled directly and therefore leads to medications to reach higher concentration in respiratory tract [26].

Present proof of concept study aims to investigate whether plasma treated liquids could still exert their antimicrobial activity when nebulized for possible future applications such as eradication of respiratory tract infections. In the present study, we have chosen three liquids - deionized water (DIW), PBS solution and NAC solution - that are previously reported to gain antimicrobial activity following plasma treatment [6]. Those liquids were nebulized over gram-negative and gram-positive model organisms (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) after treatment with non-thermal dielectric barrier discharge (DBD) air plasma to evaluate antimicrobial activity.

Methods

Bacterial Cultures

E. coli (ATCC 25922) and *S. aureus* (ATCC

25923) strains were generously donated by Ege University School of Medicine, Department of Microbiology.

Frozen stocks of microorganisms were thawed and cultures were grown on trypticase soy agar (TSA) plates. Single isolated colony of each organism was collected from TSA plate using a 10 µl loop and transferred into 10 ml of trypticase soy broth (TSB) medium and incubated in shaker incubator for overnight at 120 rpm and 37°C. After overnight incubation, suspended cultures of each strain was diluted appropriately in 1X sterile PBS solution in order to achieve desired number of bacteria by measuring absorption of each diluted culture using a spectrophotometer.

Plasma Treatment of Liquids

In the present study, DIW, PBS solution and 5 mM of NAC solution were treated separately with non-thermal atmospheric air DBD plasma in order to evaluate antimicrobial activities after nebulization on planktonic forms of *E. coli* and *S. aureus*. DIW was collected from a water purification system. 100 mM stock solution of NAC solution was prepared appropriately by weighing and dissolving NAC powder in PBS solution, and stored at -20°C until used. 5 mM of working solution of NAC solution was prepared by diluting NAC stock solution in 1X PBS solution.

An alternating current (AC) microsecond power supply was operated at 31 kV of voltage, 1.5 kHz of frequency with 10 µs of pulse duration, which yields 0.29 W/cm^2 power distribution. A custom-made glass fluid holder was used to maintain liquids during plasma treatment, which provides 1mm long liquid column. 1 ml of each liquid was treated with non-thermal atmospheric air DBD plasma for 3 minutes separately by fixing the discharge gap as 2 mm (Figures 1a and 1b).

pH Measurements of Plasma-Treated Liquids

Each liquid was transferred into microcentrifuge tubes after 3-minute plasma treatment. An ultrasensitive pH probe that is connected to a pH meter was immersed in plasma-treated liquids for pH measurement. Moreover, during nebulization, pH probe was fixed about 1cm away from outlet of nebulizer to allow proper contact of mist with pH meter, and pH of nebulized liquids were measured during the course of nebulization. pH measurement were repeated at least 9 times for each liquid.

Nebulization of Plasma-Treated Liquids

After 3-minute plasma treatment, total 2 ml of each plasma-treated liquid was transferred into reservoir of the nebulizer. 2 ml of each plasma-treated liquid was nebulized over bacteria for ZOI and inactivation tests (Figure 1c). Required time for the nebulization 2 ml of each liquid was determined as approximately 7 minutes.

Zone of Inhibition (ZOI) Experiments:

1 ml of 10^7 CFU/ml *E. coli* and *S. aureus* cultures were transferred over TSA plates and spread using disposable spreader. Then, plates were kept in biological safety cabin in order to let excess liquid to be evaporated. Then, 1 ml of each liquid was separately nebulized over *E. coli* and *S. aureus*. After nebulization, plates were incubated in stationary incubator for 24 hours at 37°C. After incubation, plates were visually examined to determine zone of inhibition. Plates without nebulization, with nebulization of untreated liquids and with nebulization of 3% H_2O_2 solution were used as internal, positive and negative controls respectively. Examined plates were incubated further 48 hours in stationary incubator

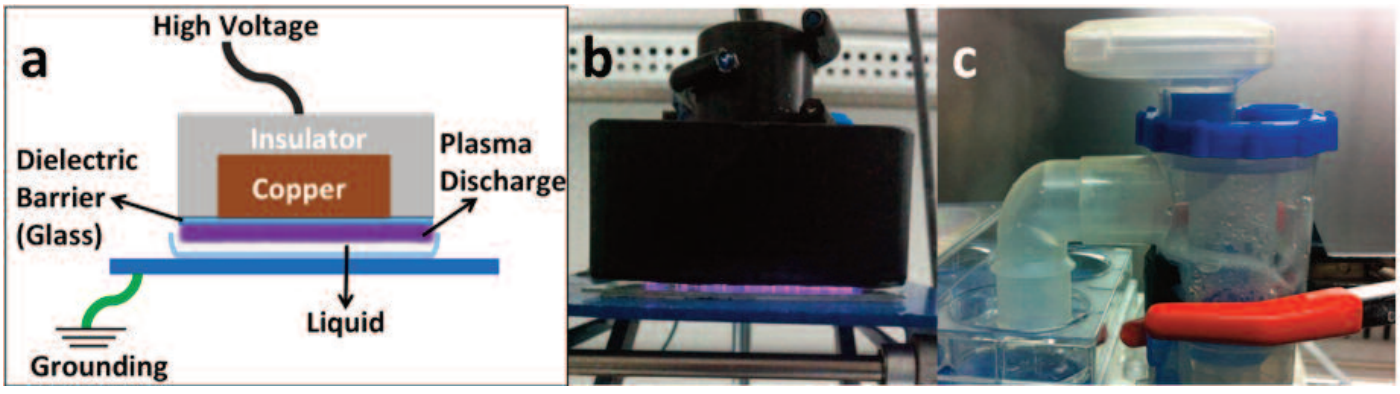

Figure 1. Plasma treatment set up and nebulization of plasma-treated liquids. Schematic view (a), and image of plasma treatment procedure of liquids (b). Plasma discharge (in violet color) is clearly visible in between DBD electrode and liquid surface (b). Plasma-treated liquids were nebulized over planktonic forms of bacteria that are kept in wells of 6-well plate (c).

at 37°C to rule out any possible dormancy of microorganisms. Each experiment was carried out thrice in triplicate.

Bactericidal Effect of Nebulized Plasma-Treated Liquids

Bactericidal activity of nebulized plasma-treated liquids were tested on 10^3, 10^4, 10^5, 10^6 and 10^7 colony forming unit /ml (CFU/ml) of initial bacterial numbers of *E. coli* and *S. aureus*. After overnight incubation of *E. coli* and *S. aureus* cultures, concentration of each bacterial culture was set to 10^7 CFU/ml using spectrophotometer. Afterwards, cultures of *E. coli* and *S. aureus* were further diluted to obtain desired initial concentration (10^3, 10^4, 10^5, and 10^6 CFU/ml) using sterile 1X PBS solution.

100 µl of each initial concentration of *E. coli* and *S. aureus* cultures were transferred into wells of 6-well plate and held in biological safety cabin to allow evaporation of excess liquid. Afterwards, 1 ml of each plasma treated liquid was nebulized over various initial concentrations of *E. coli* and *S. aureus*. Following completion of nebulization, samples were held for 15 minutes to allow complete interaction of bacteria and nebulized plasma treated liquids. Then, samples were homogenized using 1 ml of sterile 1X PBS and serially diluted and plated on TSA plates to carry out colony counting assay. Plated samples were transferred into a stationary incubator and incubated for 24 hours at 37°C. After 24 hours of incubation, surviving colonies on plates were counted to determine bactericidal activity of plasma treated liquids. Plates were incubated further 48 hours in stationary incubator at 37°C to exclude possible dormancy. Nebulization of untreated liquids and nebulization of 3% H_2O_2 were used as positive and negative controls respectively. Each experiment was carried out thrice in triplicate.

Statistical Analysis

Each experiment was performed thrice in triplicate unless otherwise stated. Statistical analysis was performed using Prism software v4.03 for Windows. Student's t-test was used for pair comparisons and one-way analysis of variance was used for multiple comparisons. A p value of <0.05 was considered as statistically significant.

Results

pH Measurements of Plasma-Treated Liquids

As presented in Figure 2, pH of NAC, PBS solutions and DIW were measured as 6.7, 7.1 and 6.1 respectively. All liquids have had acidic pH following non-thermal atmospheric plasma treatment. After plasma treatment, pH of NAC, PBS solutions and DIW were dropped to 2.5, 2.8 and 2.2 respectively. The pH drop of each liquid after plasma treatment was statistically significant. Furthermore, acidic pH of all tested liquids was not significantly changed during nebulization and measured as 2.6, 2.8, and 2.3 for NAC, PBS solutions and DIW respectively (Figure 2).

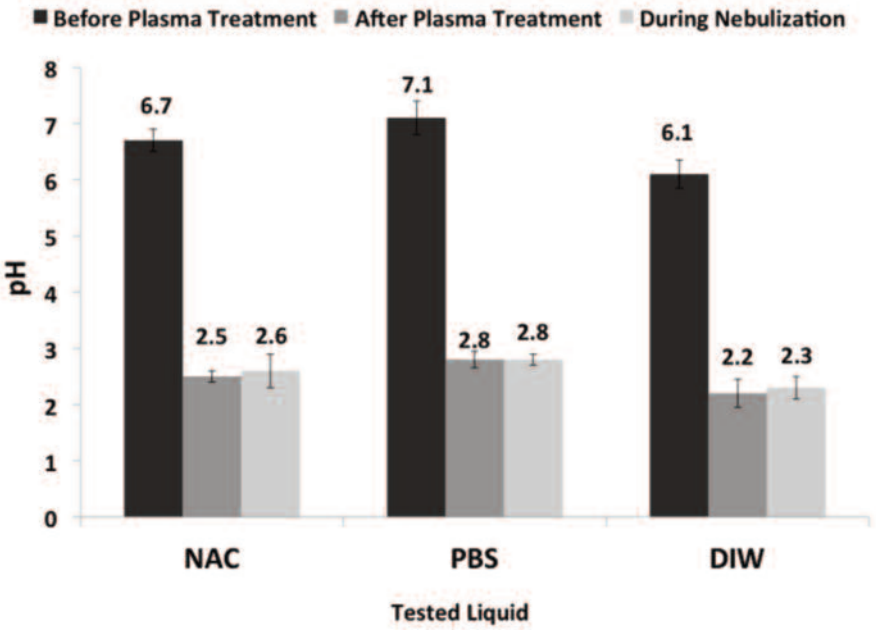

Figure 2. pH of tested liquids before, after and during nebulization. pH of all liquids were close to neutral before plasma treatment. Following plasma treatment pH of all liquids dropped to acidic pH and has not significantly changed during nebulization.).

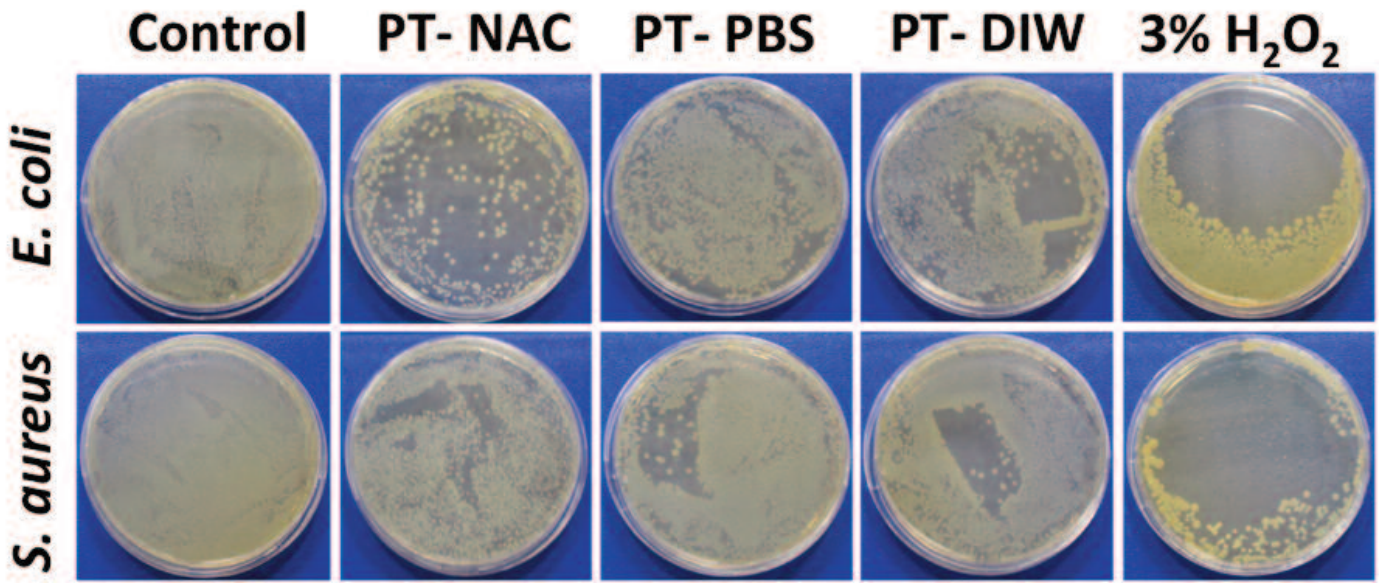

Figure 3. Zone of Inhibition Test of nebulized plasma-treated liquids. Nebulization of plasma-treated NAC solution led the strongest antimicrobial activity for both E. coli and S. aureus. More diluted and separated colonies were grown after nebulization of plasma-treated NAC solution, which corresponds nebulized plasma-treated NAC solution shows its antimicrobial activity over a larger area while nebulized plasma-treated PBS solution and DIW showed inhibition zone on the area where they came in contact first. 3% H2O2 was used as negative control.

Zone of Inhibition Experiments

Antibacterial activities of nebulized NAC, PBS solutions and DIW following non-thermal atmospheric plasma treatment were qualitatively determined on *E. coli* and *S. aureus* and represented as shown in Figure 3. In positive control groups (in which no plasma-treated liquids were nebulized) bacterial lawn growth on TSA plates were observed. 3% H_2O_2 was nebulized over *E. coli* and *S. aureus* on TSA plates as negative control and a clear bacterial inhibition zone was observed on TSA plates for both *E. coli* and *S. aureus* where nebulized hydrogen peroxide solution came in contact with bacteria. For the tested plasma-treated liquids, the most significant inhibition zone was obtained after nebulization of plasma-treated NAC solution over *E. coli* and this effect was dispersed all over the plate in which single colonies with spaces between them were observed. However similar effect following nebulization of plasma-treated NAC solution over *S. aureus* was less pronounced and even though, presence of less frequent bacterial colonies also, inhibition zone was observed on the plate in which nebulized NAC solution first came in to contact with bacteria. After nebulization of plasma-treated DIW, inhibition zones were clearly observable on both *E. coli* and *S. aureus* which also were limited in areas where nebulized plasma-treated DIW first contacted to bacteria. Moreover, nebulization of plasma-treated PBS solution was resulted as a similar inhibition zone on *S. aureus* that was limited to contact area. The inhibition zone over *E. coli* was less pronounced and remained limited on the edges of the TSA plate along with little dilution of bacterial lawn following nebulization of plasma-treated PBS. Furthermore, untreated NAC, PBS solutions and DIW were also nebulized over bacteria to ensure that the flow of mists of liquids generated during nebulization were not responsible for removal of bacteria and for generation of inhibition zone. Following nebulization of untreated NAC, PBS solutions and DIW no inhibition zone was observable on TSA plates for both *E. coli* and *S. aureus* (data not shown).

Bactericidal Effect of Nebulized Plasma-Treated Liquids

Colony counting assay was performed for determination of bactericidal efficacy of nebulized plasma-treated liquids at different initial concentrations of *E. coli* and *S. aureus*. As depicted in the Figures 4a and 4b, for 10^3, 10^4, 10^5 and 10^6 CFU/ml initial bacteria number, after nebulization of plasma-treated NAC, PBS solutions and DIW on both *E. coli* and *S. aureus* complete inactivation was achieved. However, after nebulization of all plasma-treated liquids over 10^7 CFU/ml initial bacterial number of *E. coli* and *S. aureus*, complete inactivation was not observed. After nebulization of plasma-treated liquids over 10^7 CFU/ml initial bacterial number of *E. coli*, NAC solution, PBS solution and DIW led >5-log,

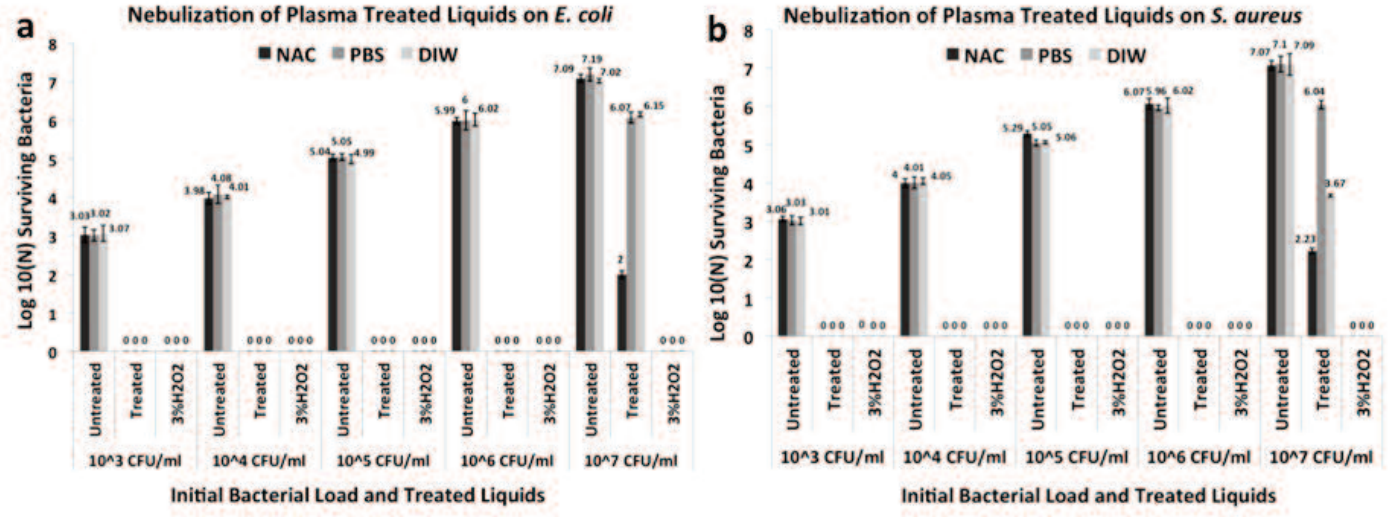

Figure 4. Bactericidal effects of nebulized plasma treated liquids were quantified with colony counting assay on E. coli (a) and S. aureus (b). All tested liquids have led complete inactivation of *E. coli* (a) and S. aureus (b) for the initial bacterial load of 10^3, 10^4, 10^5 and 10^6 CFU/ml. However, when initial bacterial load was increased to 10^7 CFU/ml nebulized plasma-treated liquid have shown the strongest antimicrobial activity with around 5-log reduction over E. coli (a) and S. aureus (b). Nebulized plasma-treated PBS solution and water led around 1-log reduction on *E. coli* (a) and around 1-log and 3-log reduction on *S. aureus* (b) respectively when the bioburden was 10^7 CFU/ml.

around 1-log and <1-log inactivation respectively. Moreover, after nebulization of plasma-treated liquids over 10^7 CFU/ml initial bacterial number of S aureus, NAC solution, PBS solution and DIW led about 5-log, 1-log and 3.5-log inactivation respectively. Bactericidal effects of all nebulized plasma-treated liquids for each various initial bacterial load of *S. aureus* and *E. coli* were found to be statistically significant. 3% H_2O_2 was used as negative control and for all initial bacterial numbers of both *E. coli* and *S. aureus*, it led complete inactivation.

Discussion

As discussed in the previous reports in detail, not only non-thermal atmospheric plasma by itself, but also liquids modified by it exerts strong antimicrobial activity over wide range of gram positive and negative bacteria - including antibiotic resistant strains - and fungus in their both planktonic and biofilm forms [6, 14, 22]. Strong antimicrobial activities of non-thermal plasma and non-thermal plasma-activated solutions have drawn attention for their possible future clinical use including for control and prevention of hospital acquired infections [27]. However, utilization of non-thermal plasma-treated liquids in respiratory tract is not applicable since presence of liquids in respiratory tract may cause asphyxia. In the present study, possible use of non-thermal atmospheric plasma-treated liquids was investigated through nebulization.

During plasma treatment of various liquids, different plasma generated ROS and RNS may react and/or diffuse into liquid, which then make plasma-treated liquids strong antimicrobial solutions. Presence of various ROS such as hydrogen peroxide, superoxide, hydroxyl radical and various RNS such as nitrate, nitrite, nitric oxide, peroxynitrite, in plasma-treated liquids were reported [4, 25, 28]. Even though plasma-treated liquids are complex mediums containing ROS and RNS and synergetic effects of those species were pronounced, researchers attempted to determine the most dominant species in plasma-treated liquids for antimicrobial activity [24, 25, 29, 30]. Dominant species for antimicrobial activity of plasma-treated liquids depends on the type of plasma and the treated liquid [25, 31]. Ercan *et al.* [25] have shown that peroxynitrite as the dominant species for bactericidal efficacy in NAC solution when treated with an atmospheric pressure non-thermal air DBD plasma [25]. Wu *et al.* [31] have detected various ROS in He/O_2 microjet plasma treated water and indicated singlet oxygen ($^1O_2$) as contributing the most of the inactivation.

Besides formation of ROS and RNS in plasma-treated liquids, also acidification of liquids after non-thermal atmospheric plasma treatment is one of the most common finding reported in various studies [32]. Decreased pH in plasma-treated liquids was mainly attributed to formation of nitric acid, nitrous acid, hydrogen cation and superoxide anion during plasma treatment [23]. Ercan *et al.* [6] have reported

pH drop in PBS, NAC solutions and DIW from 6.48, 6.24 and 6.80 to 2.58, 2.35 and 2.00 respectively after 3-minutes non-thermal atmospheric DBD plasma treatment. Also in the present study, we have shown statistically significant pH drop for all three liquids, which are consistent with the literature. However, low pH was not attributed to antimicrobial activity. In a previous study Machala *et al.* [29] have correlated reduced pH of plasma treated water with antimicrobial activity. In that study, low pH was found to be leading rapid oxidation of nitrites - formed during plasma treatment - to nitrates, which then associated as dominant species for antimicrobial activity. However, when buffers were used to prevent drastic pH drop, a weaker antimicrobial activity was observed instead of complete loss [29].

Moreover, in another study, antimicrobial activity of plasma treated NaCl solution was tested. Following surface DBD plasma treatment, pH of NaCl solution was decreased to acidic range along with formation of other chemical species. Treatment of NaCl solution led formation of nitrite, nitrate and hydrogen peroxide along with reduction of pH to around 3 [23].

As reported previously, acidification of nitrate and nitrite leads strong antimicrobial activity on various bacterial strains [33-35]. Formation of nitrates and nitrites and their diffusion to liquids during plasma treatment could enlighten the role of low pH for antibacterial effects of plasma treated liquids. In previous studies, effect of pH was investigated to clarify its contribution to antimicrobial activity. pH of various acid solution were set to pH values determined after plasma treatment and then exposed to bacteria. However, significant antibacterial effect of low pH was not observed In summary, as it was mentioned previously, low pH could play a supportive role instead of being main reason for antimicrobial activity arising from plasma treated liquids since low pH is not sufficient for microbial inactivation by itself [23, 25, 36].

In the present study antimicrobial activities of nebulized plasma-treated NAC, PBS solutions and DIW were evaluated on *E. coli* and *S. aureus*. As depicted in the Figure 2, inhibition zones on which nebulized plasma-treated liquids came in contact were presented. Zone of inhibition test was conducted to evaluate if plasma-treated liquids whether loose or could conserve antimicrobial activity during nebulization. Moreover, even though zone of inhibition test is not a quantitative method, depending on the obtained area of inhibition zone the antimicrobial power of plasma-treated liquids could be compared qualitatively [37]. As shown in Figure 2 when plasma treated NAC solution was nebulized over *E. coli*, the obtained inhibition zone was more generalized and bacterial growth turned out as separated single colonies as oppose to inhibition zones and lawn like bacterial growth obtained from nebulization of plasma-treated PBS and DIW. Moreover, nebulized plasma-treated PBS solution led inhibition on a smaller are at the edges of the plate, compared to nebulized plasma treated NAC solution and DIW. Inhibition zone results on *S. aureus* were similar to results obtained from *E. coli*. Effect of nebulized plasma-treated NAC solution over *S. aureus* was not prominent as observed over *E. coli* however this effect was spread over the plate leading separated single colony growth on the plate. Similarly effects of nebulized plasma-treated PBS and DIW on *S. aureus* mostly remained on the area where the nebulized mist first contacted on the plate.

As represented on Figures 4a and 4b bactericidal effects of plasma treated NAC, PBS solutions and DIW both on *E. coli* and *S. aureus* were initial bacterial load dependent. All nebulized plasma-treated liquids led complete statistically significant inactivation on tested bacterial strains for the initial bacterial load from 10^3 to 10^6 CFU/ml. However when initial bacterial load was increased to 10^7 CFU/ml, even though all liquids led statistically significant inactivation, the strongest bactericidal effect was exerted by nebulized plasma-treated NAC solution. The nebulized plasma-treated NAC solution led about 5-log reduction both on *E. coli* and *S. aureus*. Results of zone of inhibition test and colony counting assay were consistent and both showed nebulized plasma-treated NAC solution as the strongest antimicrobial solution among all plasma-treated liquids. Also, our findings were consistent with literature in which plasma-treated NAC solution was presented as a stronger antimicrobial solution compared to plasma-treated PBS solution and DIW [6]. Previously, chemical modifications in the NAC solution during plasma treatment, was reported and stronger antimicrobial activity mainly attributed to formation of RNS and especially peroxynitrite [25].

In consequence of nebulization size of mist particles were reduced to the order of micrometers and whole liquid had a bigger surface area to react with ambient air, which then would lead loss of antimicrobial effect. However, our results indicates that, plasma-treated liquids could retain their antimicrobial effect when

they were nebulized.

Best of our knowledge, this study is the first report, which shows antimicrobial activity of nebulized plasma-treated liquids and presents nebulization as a delivery method for plasma-treated liquids for their possible future use on respiratory tract infections. Moreover, sterilization of endoscopes is a common challenge due to presence of lumen and associated with infection outbreaks [38]. Thus, nebulization of plasma-treated liquids could be considered as an alternative method for sterilization of endoscopes.

Conclusions

In the present study we have tested the antimicrobial activities of plasma-treated NAC, PBS solutions and DIW when nebulized over *E. coli* and *S. aureus* gram positive and negative model organisms respectively. Zone of inhibition test and colony counting assay consistently showed that nebulized plasma-treated NAC solution exerted the strongest effect over two model organisms. Taken together, nebulization of plasma-treated liquids could lead novel applications of plasma treated liquids for the respiratory tract infections and sterilization of endoscopes. Experiments regarding the efficacy of nebulized plasma-treated liquids on biofilm forms of bacteria and fungus and cytotoxic effects of nebulized plasma-treated liquids are underway.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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References

[1] Langmuir I. Oscillations in ionized gases. Proc Natl Acad Sci USA 1928;14:627-37.
[2] Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. Plasma Process Polym 2008;5:503-33.
[3] Haertel B, von Woedtke T, Weltmann KD, Lindequist U. Non-thermal atmospheric-pressure plasma possible application in wound healing. Biomol Ther (Seoul) 2014;22:477-90.
[4] Graves DB. The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. J Phys D Appl Phys 2012;45.
[5] Fridman G, Brooks AD, Balasubramanian M, Fridman A, Gutsol A, Vasilets VN, et al. Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria. Plasma Process Polym 2007;4:370-5.
[6] Ercan UK, Wang H, Ji H, Fridman G, Brooks AD, Joshi SG. Nonequilibrium plasma-activated antimicrobial solutions are broad-spectrum and retain their efficacies for extended period of time. Plasma Process Polym 2013;10:544-55.
[7] Kong MG, Kroesen G, Morfill G, Nosenko T, Shimizu T, van Dijk J, et al. Plasma medicine: an introductory review. New J Phys 2009;11:115012.
[8] Laroussi M, Mendis DA, Rosenberg M. Plasma interaction with microbes. New J Phys. 2003;5:41.
[9] Tanaka H, Nakamura K, Mizuno M, Ishikawa K, Takeda K, Kajiyama H, et al. Non-thermal atmospheric pressure plasma activates lactate in Ringer's solution for anti-tumor effects. Sci Rep 2016;6:36282.
[10] Fathollah S, Mirpour S, Mansouri P, Dehpour AR, Ghoranneviss M, Rahimi N, et al. Investigation on the effects of the atmospheric pressure plasma on wound healing in diabetic rats. Sci Rep 2016;6:19144.
[11] Fridman G, Peddinghaus M, Balasubramanian M, Ayan H, Fridman A, Gutsol A, et al. Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air. Plasma Chem Plasma Process 2006;26:425-42.
[12] Nam SH, Lee HW, Cho SH, Lee JK, Jeon YC, Kim GC. High-efficiency tooth bleaching using non-thermal atmospheric pressure plasma with low concentration of hydrogen peroxide. J Appl Oral Sci 2013;21:265-70.
[13] Dolci LS, Quiroga SD, Gherardi M, Laurita R, Liguori A, Sanibondi P, et al. Carboxyl surface functionalization of poly (L-lactic acid) electrospun nanofibers through atmospheric non-thermal plasma affects Fibroblast morphology. Plasma Process Polym 2014;11:203-13.
[14] 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.
[15] Panngom K, Lee SH, Park DH, Sim GB, Kim YH, Uhm HS, et al. Non-thermal plasma treatment siminishes fungal viability and up-regulates resistance genes in a plant host. Plos One 2014;9:e99300.
[16] Alekseev O, Donovan K, Limonnik V, Azizkhan-Clifford J. Nonthermal dielectric barrier discharge (DBD) plasma suppresses Herpes Simplex Virus type 1 (HSV-1) replication in corneal epithelium. Transl Vis Sci Technol 2014;3:2.
[17] Julak J, Janouskova O, Scholtz V, Holada K. Inactivation of prions using electrical DC discharges at atmospheric pressure and ambient temperature. Plasma Process Polym 2011;8:316-23.
[18] Poor AE, Ercan UK, Yost A, Brooks AD, Joshi SG. Control of multi-drug-resistant pathogens with non-thermal-plasma-treated alginate wound dressing. Surg Infect 2014;15:233-43.
[19] Yorsaeng S, Pornsunthorntawee O, Rujiravanit R. Preparation and characterization of chitosan-coated DBD plasma-treated natural rubber latex medical surgical gloves with antibacterial activities. Plasma Chem Plasma Process

2012;32:1275-92.
[20] Monetta T, Scala A, Malmo C, Bellucci F. Antibacterial activity of cold plasma-treated titanium alloy. 2011;1:205-14.
[21] Yost AD, Joshi SG. Atmospheric nonthermal plasma-treated PBS inactivates Escherichia coli by oxidative DNA damage. Plos One 2015;10:e0139903.
[22] Ma R, Wang G, Tian Y, Wang K, Zhang J, Fang J. Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. J Hazard Mater 2015;300:643-51.
[23] Oehmigen K, Winter J, Hahnel M, Wilke C, Brandenburg R, Weltmann KD, et al. Estimation of possible mechanisms of Escherichia coli inactivation by plasma-treated sodium chloride solution. Plasma Process Polym 2011;8:904-13.
[24] Joshi SG, Cooper M, Yost A, Paff M, Ercan UK, Fridman G, et al. Nonthermal dielectric-barrier discharge plasma-induced inactivation involves oxidative DNA damage and membrane lipid peroxidation in Escherichia coli. Antimicrob Agents Chemother 2011;55:1053-62.
[25] Ercan UK, Smith J, Ji HF, Brooks AD, Joshi SG. Chemical changes in nonthermal plasma-treated N-Acetylcysteine (NAC) solution and their contribution to bacterial inactivation. Sci Rep 2016;6:20365.
[26] OCallaghan C, Barry PW. The science of nebulised drug delivery. Thorax 1997;52:S31-S44.
[27] Cahill OJ, Claro T, O'Connor N, Cafolla AA, Stevens NT, Daniels S, et al. Cold air plasma to decontaminate inanimate surfaces of the hospital environment. Appl Environ Microbiol 2014;80:2004-10.
[28] Lukes P, Dolezalova E, Sisrova I, Clupek M. Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H2O2 and HNO2. Plasma Sources Sci Technol 2014;23:015019.
[29] Machala Z, Tarabova B, Hensel K, Spetlikova E, Sikurova L, Lukes P. Formation of ROS and RNS in water electro-sprayed through transient spark discharge in air and their bactericidal effects. Plasma Process Polym 2013;10:649-59.
[30] Brisset JL, Hnatiuc E. Peroxynitrite: a re-examination of the chemical properties of non-thermal discharges burning in air over aqueous solutions. Plasma Chem Plasma Process. 2012;32:655-74.
[31] Wu HY, Sun P, Feng HQ, Zhou H, Wang R, Liang Y, et al. Reactive oxygen species in a non-thermal plasma microjet and water system: generation, conversion, and contributions to bacteria inactivation-An analysis by electron spin resonance spectroscopy. Plasma Process Polym 2012;9:417-24.
[32] Chen CW, Lee HM, Chang MB. Influence of pH on inactivation of aquatic microorganism with a gas-liquid pulsed electrical discharge. J Electrostat 2009;67:703-8.
[33] Xia DS, Liu Y, Zhang CM, Yang SA, Wang SL. Antimicrobial effect of acidified nitrate and nitrite on six common oral pathogens in vitro. Chin Med J (Engl) 2006;119:1904-9.
[34] Dykhuizen RS, Frazer R, Duncan C, Smith CC, Golden M, Benjamin M, et al. Antimicrobial effect of acidified nitrite on gut pathogens: importance of dietary nitrate in host defense. Antimicrob Agents Chemother 1996;40:1422-5.
[35] Cunningham R, Mustoe E, Spiller L, Lewis S, Benjamin M. Acidified nitrite: a host defence against colonization with C. difficile spores? J Hosp Infect 2014;86:155-7.
[36] Oehmigen K, Hahnel M, Brandenburg R, Wilke C, Weltmann KD, von Woedtker. The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. Plasma Process Polym 2010;7:250-7.
[37] Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis 2009;49:1749-55.
[38] Humphries RM, McDonnell G. Superbugs on duodenoscopes: the challenge of cleaning and disinfection of reusable devices. J Clin Microbiol 2015;53:3118-25.