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RESEARCH ARTICLE

THE EFFECT OF TEMPERATURE ON DEACTIVATION FOR *E.COLI* BACTERIA

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ABSTRACT

An atmospheric cold plasma needle was developed for deactivation the Escherichia coli (*E.Coli*). The bacteria showed that the plasma jet could effectively deactivate of *E.Coli* bacteria within (5 min.) and the sterilizing efficiency depended critically on the discharge parameter of the applied voltage and distance. The plasma sterilization mechanism of *E.Coli* was attributed to the active species of OH, N_2 + and O, which were generated abundantly in the plasma jet. Our findings suggest a convenient and low-cost way for sterilization and inactivation of bacteria.

INTRODUCTION

Plasma, which is accepted as the fourth state of matter, was initially identified in 1879 by Sir C. William. In 1927 Irving Langmuir first described it as an ionized gas containing free moving charge carriers (electrons and ions). Non thermal (cold plasma), that is not in thermodynamic equilibrium, which means that the temperature of molecules, atoms and ions does not match the temperature of the electrons (Bogaests *et al.,* 2002). Due to mentioned characteristics of non-thermal plasma make it used for activating the surface of polymers, growing solar cells and etching materials . Several applications such as treatment of living cells (Shashurin *et al.,* 2008). Among the various plasma sources reported, Ar plasma jet is very effective for the sterilization of micro-organism. It was observed that Ar plasma source showed stronger emission intensity of reactive radicals and better killing effect than the He plasma source. Non equilibrium atmospheric pressure plasma needle operated with Ar gas was developed successfully (Fridman, 2008). Wounds classically are categorized as acute, that is, abrasions, scalds, burns, or post-operative incisions, or chronic, that is, long-term wounds such as diabetic ulcers, venous ulcers, arterial ulcers and pressure sores. Acute wounds can develop into a non-healing state and/or become infected, which limits their capability of successfully going through the phases of healing, and so can also become chronic in nature.

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Wound healing is a complex biological and biochemical process with a multitude of variables governing the process. However complicated the healing process might be, some things are clear: for wound healing to occur, tissue damage during treatment needs to be minimized or eliminated and wound needs to be sterilized to prevent bacterial invasion (Horsti *et al.,* 2001). Escherichia coli (*E. coli*): is a classic opportunistic pathogen found in hospitals. The World Health Organization professes that this bacterium is one of the primary pathogens of hospital acquired infection (Moreau *et al.,* 2008). *E. coli* contributing to a large percentage of nosocomial infections ranks first in the infection rate of various gram-negative nosocomial pathogens (Philippon *et al.,* 2002). In recent years, because of the multi-drug resistant mechanism of *E. coli*, infection incidents have occurred frequently, and the drug-resistance of the bacterium has gradually risen (Vincent *et al.,* 1995).

Theory

Since the kHz frequency emission of the plasma needle was influencing this measurement while using a thermocouple, the axial gas temperature was measured with simple mercury thermometer. The heat sensitive part of the thermometer was placed at different distance from the end of the needle (Backer, 2007) while the Ar gas was flowing at different flow rate through the needle, figure (1) shows plasma needle. Non-thermal atmospheric pressure plasmas are very effective in killing bacteria.

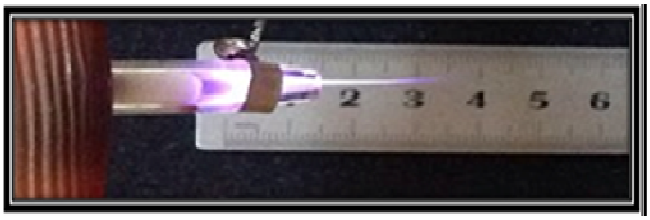

Figure 1. Plasma needle

This makes these plasmas very useful for various biological and medical applications, such as: sterilization of medical instruments, decontamination in biological warfare and air filters in hospitals (Mohammed *et al.,* 2016).

METHODS

In this study, a recently developed plasma jet device, which generates a cold plasma plume with high discharge current, is used to investigate the contribution of the charged particles to the inactivation of bacteria. Sterilization tests were performed by swabbing and culturing bacteria from human skin samples before and after cold plasma treatment, while show growth of normal skin bacteria (*E.coli*) on cultures from swabs taken on control samples cultured a large quantity of bacteria obtained from a swab from wounds were cultured on synthetic media (Mac Conkey agar) to obtain bacteria colonies, carried out investigation of effects of cold plasma on bacterial cultures to quantify the extent of sterilization and determine possible factors responsible. The prepared culture plates were treated by cold plasma, and they are then incubated for (24h) (Lederberg and Lederberg, 1982).

RESULTS AND DISCUSSION

Experimental temperature

In recent times, biological and biomedical applications of plasma are of great interest. Plasma sources are employed for bacterial inactivation and tissue sterilization, decontamination of medical instruments, surface modification of implantable biomaterials etc. The gas temperature which was determined by mercury thermometer at various distances from the tip of the plasma needle electrode, for various Ar gas flow rate**.** Optimum conditions was selected Voltage (17,22 kv), frequency (15 kHz), distance (2, 1 cm), time (10, 20, 30, 40, 50) sec, and flow rates (1, 2, 3, 4, 5) l/min, by using plasma needle for killing bacteria. There are optimal temperatures for microorganisms growth. The favorable temperature range for the growth (e.g *E. coli bacteria)* is 30^{0}C to 37^{0}C. For *E. coli* is a typical mesophilous bacterium and can be deactivated at a temperature above 43^{0}C (Jawetz *et al.,* 2007). The temperature, which was measured using a mercury thermometer at various distances. Figure (2), in applied voltage (17 Kv) and gap spacing (1cm) from end of the needle, that the temperature increased with an increase in plasma treatment time through thermocouple measuring, in time (5 min.) temperature above (45^{0}C) which bacteria may be deactivated. Same applied voltage in figure (3) but gap spacing (2cm) from end of the needle, increasing in plasma treatment time lead to increasing with temperature but above from (43^{0}C) in (5min.), this indicate role of distance, where the temperature increased with an increase in treatment time and decreased with an increase in gap spacing .

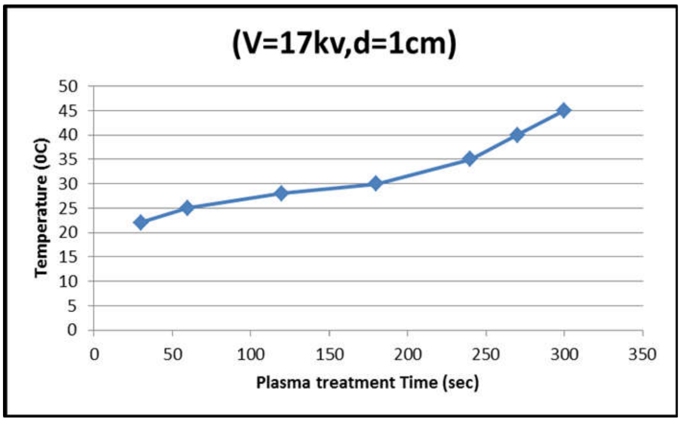

Figure 2. The temperature during the plasma treatment (under the operating conditions of a 17 kV applied voltage and distance 1cm)

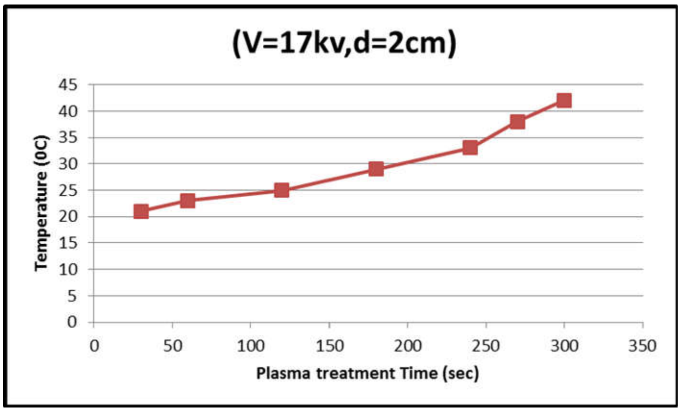

Figure 3. The temperature during the plasma treatment (under the operating conditions of a 17 kV applied voltage, distance 2cm)

Figure (4) in applied voltage (22KV) and gap spacing (1cm) from end of the needle, that the temperature increased with an increase in plasma treatment time through thermometer measuring, in time (5 min.) temperature less than (50 ^{0}C) which bacteria may be deactivated.

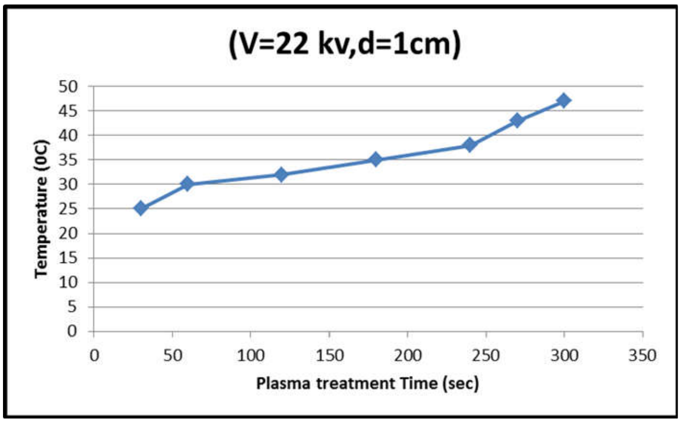

Figure 4. The temperature during the plasma treatment (under the operating conditions of a 22 kV applied voltage and distance 1 cm)

Same applied voltage but gap spacing (2cm)in figure (5), increasing in plasma treatment time led to increasing with temperature but not above from (44^0C) in (5min.), this indicate role of distance, where the temperature increased with an increase in treatment time and decreased with an increase in gap spacing. Comparing previous two figures, applied voltage and gap spacing effect on increasing temperature.

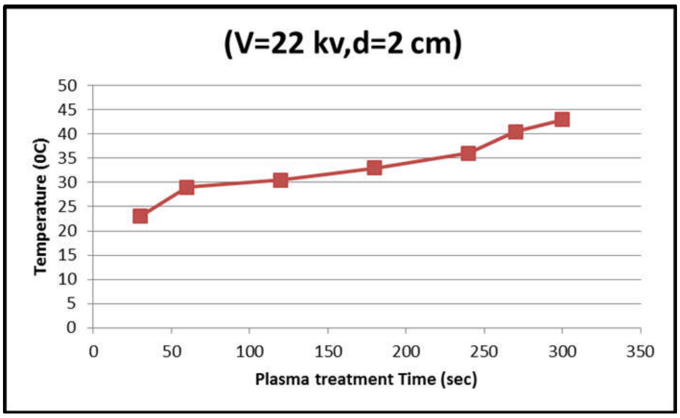

Figure 5. The temperature during the plasma treatment (under the operating conditions of a 22 kV applied voltage and distance 2cm)

Mechanism of the killing effect on microbes by plasma has been recognized. Considered the high power ultraviolet radiation in plasma played a dominant role during the bacteria inactivation (Gu Chunying and Xue Guangbo, 1999), also ozone in plasma played a major role during the process, as well as the slow combustion effect of active oxygen atoms and radicals in plasma played the dominant role during the process (Moisan *et al.,* 2001). The sterilization mechanism is proposed to be the effects of attaching, bombardment and oxidation on the bacterial cell by active species of OH, N_2 and O, respectively, which are found generated in the plasma jet, also increasing applied voltage and decreasing gap spacing temperature increased (Kuchenbecker *et al.,* 2014).

Conclusion

In summary, an atmospheric non-thermal plasma jet was well designed and applied to sterilize *E.Coli* bacteria. As many as *E.Coli* can be completely inactivated within 5min. at a 17, 22 kV applied voltage and the sterilizing efficiency improves greatly with an increase in the applied voltage. The sterilization mechanism is proposed to be the effects of attaching, bombardment and oxidation on the bacterial cell by active species of OH, N_2 and O, respectively, which are found generated in the plasma jet.

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