

1st International & 28th National Conference on Nuclear Science & Technology 2022 (ICNST22)



Destruction of Staphylococcus aureus Bacteria by Atmospheric Cold Plasma Mazandarani A^{1*}, Goudarzi Sh²

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Abstract

The rapid increasing in bacterial resistance to known antibiotics is one of the major challenges in medicine and a lot of researches have been done to find alternatives for antibiotics. Among the methods that have been studied so far, cold plasma technology has achieved good results, and it seems that this technology will find high applications in future. Staphylococcus aureus is a human pathogen that is present in a high percentage of the world's population and is one of the most important and abundant causes of nosocomial infections. In this paper the results of the experiments that have been done using a cold jet plasma device in Nuclear Science and Technology Research Institute of IRAN have been presented and analyzed. In these experiments, the effect of cold plasma on this bacteria has been investigated for different treated times. The results have shown that this method can significantly kill these bacterias.

Keywords: Cold Plasma, Staphylococcus aureus bacteria, Antibiotics

Introduction

Staphylococcus aureus is one of the most widespread pathogenic bacteria. This bacteria is a gram-positive cocci and coagulase positive of the Staphylococcus family and an opportunistic infection agent in humans. Staphylococcus aureus is present as a human pathogen in 30% of the population and is one of the important causes of severe and healthy diseases. The clinical manifestations of Staphylococcus aureus are highly variable. Infections associated with this bacterium include bacteremia, sepsis, pneumonia, osteomyelitis, and skin infections in human. This bacterium is found on the mucous membranes and skin of mammals, various foods and the environment and causes pneumonia after viral infections, urinary tract infections, inflammation of the veins and superficial skin lesions, and is sometimes accompanied by shock [1,2].

One of the most important toxins produced by this bacteria are enterotoxins. More than 95% of these enterotoxins cause food poisoning in humans [3,4].

In recent decades, plasma technology has found a lot of applications in various fields, Cold Atmospheric Plasma technology has been found Significant progress in the biological sciences. The results of researches have shown that plasma can have a positive effect on the destruction of bacteria and microbes as an effective method. In this paper, the effects of cold plasma on Staphylococcus aureus was investigated. This research has been done in the Atomic Energy Organization of Iran's Nuclear Science and Technology Research Institute.

Experimental Method

In this experiments an atomospheric cold plasma jet device have been used. This device has 2 parts. Power

supply and plasma generator jet nozzle. Electrons, ions, hydroxyl radicals, oxygen atoms, ozone and hydrogen peroxide are the major products at the nozzle output.

A voltage transformer applies a voltage of 2.6 kV and 60 kHz frequncy to nozzle. The nozzle consists of a cylindrical rod of aluminum as the outer electrode, and a small-diameter rod as the inner electrode. A cylindrical quartz dielectric is also located between the inner and outer electrodes. The air flow to the nozzle is puffed with a flow rate of 3 liters per minute. The length of the plasma jet formed in the nozzle under these conditions is equal to 4 mm.

Bacterial samples used in the present study were prepared from the microbial bank in Baqiyatallah Medical Sciences and kept in LB Agar culture medium. After 24 hours and the growth of colonies of this microorganism inoculate some colonies from each plate in sterile PBS buffer and add to the cuvette tubes and using a spectrophotometer. The optical density was measured in a wavelength 600 nm and the bacterial counting cell number was calculated. The plate was then incubated at 37 ° C for 24 hours after plasma treatment. After this time, the established colonies were counted.

Plasma treatment of this bacteria at different times have been studied [5]. In our experiments, the treatment times were 5, 7 and 10 minutes. It should be noted that a control plate (without plasma treatment) was used to compare the effect.

After determining the number of Staphylococcus aureus bacteria inoculated in PBS, a certain amount of bacteria was transferred to the designated medium from the plate surface containing LB Agar culture medium. The bacteria were then allowed to sit in the environment for



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2 minutes and the bacterial medium was treated with atmospheric cold plasma.

Results and discussion

In these experiments, Staphylococcus aureus were cultured on 4 plates of LB Agar medium each at 1.5×10^3 CFU / ml. One group was selected as the control group and the other three groups were treated with cold atmospheric plasma for 5, 7 and 10 minutes. Then, The plates were then incubated at 37 ° C for 24 hours.

Figures

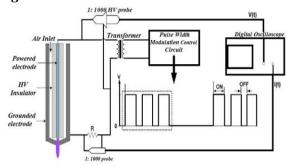


Figure 1. The schematic of Plasma jet



Figure 2. Overview of all groups treated with air plasma and Staphylococcus aureus control group

Tables

Counting of Staphylococcus aureus colonies under the influence of cold atmospheric plasma to evaluate the power of the device.

Table 1. The Countong cell number of Staphylococcus aureus after treatment by plasma in different times.

Control: 1.5×10^3 CFU/ML

5 min air plasma: 0.09×10^3 CFU/ml

7 min air plasma: 0.05×10^3 CFU/ml

10 min air plasma: 0

As can be seen Increasing the duration of treatment significantly reduces bacterial colonies.

Conclusions

Atmospheric cold plasmas technology would be hopeful for therapeutic applications in the coming years. They can be applied to living tissue without heat damage. Plasma utilization produces special s such as reactive oxygen species and nitrogen species (RONS) that lead to antibacterial effects.

Some of these studies showed differences in the susceptibility of MRSA (Methicillin-resistant Staphylococcus aureus) and MSSA (methicillin-susceptible Staphylococcus aureus) strains to plasma therapy for planktonic or suspended form after biofilm culture. In these studies, it was found that using 2 minutes of atmospheric plasma reduces bacterial survival by 6 to 10%. [6] .

The results of our experiments showed that in vitro the device was able to kill most of the bacteria in the culture medium within 5, 7 and 10 minutes. The antibacterial mechanism of atmospheric cold plasma is due to ROS and RNS free radicals that were generated by plasma in the bacterial environment , free radicals damages bacterial DNA, which results in complete destruction and extinction of bacteria.

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