Plasma Medicine, 2(4): 179–189 (2012)

The Sterilization Technology Research on the Non-thermal Atmospheric Plasma

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ABSTRACT: In modern medicine, with the overuse of antibiotics, bacterial resistance has increased, and the efficacy of traditional sterilization methods has gradually weakened. Therefore, new sterilization methods have attracted attention. Recently, a non-thermal atmospheric plasma has been developed, and its interaction with living objects has been studied.[1] Plasma is capable of bacterial sterilization, operates at room temperature, and is generated at one atmosphere of pressure. The plasma has a low temperature, and it will not cause thermal damage to normal tissue. Furthermore, plasma has a large number of charged particles and active species. These particles interact with bacteria, resulting in the death of the bacteria. Consequently, non-thermal atmospheric plasma systems are uniquely suited for medical sterilization applications, especially in the treatment of oral diseases and the sterilization and disinfection of the medical apparatuses and instruments. Non-thermal atmospheric plasma is expected to become a perfect alternative to traditional sterilization methods.

KEY WORDS: Plasma; atmospheric pressure, non-thermal, security, sterilization, bacteria, cell

I. INTRODUCTION

Sterilization and disinfection are important parts of medical procedures and are therefore directly related to human health. Traditional sterilization methods have been widely used in clinical medical treatments. However, modern medical and health services are facing severe challenges due to the variation in bacteria and viruses and their increasing drug resistance.[2,3] During practical sterilization, existing methods usually do not work effectively and do not best meet the needs of medical applications. For example, in the treatment of oral diseases, as in the preparation of cavities prior to filling[4] and root canal treatments,[5] the efficacy of current sterilization procedures could be improved upon. Furthermore, the sterilization of heat-sensitive, reusable tools requires alternative methods to autoclaving.[6] In contrast to currently used methods, non-thermal atmospheric plasma contains free electrons and ions, various active species, and energetic UV photons and radicals. This new sterilization method has gained increasing attention and has recently been the focus of intensive research. It combines strong germicidal ability with quick sterilization

1947-5764/12/$35.00

speed, excellent safety, and simple operation. It leaves no poisonous residues, and it may be used in a wide range of applications.

II. STERILIZATION SETUP

A. Overall Setup Scheme

In this study, we aimed to test a non-thermal atmospheric plasma device used for small areas of sterilization, as in the treatment of oral disease. From the technical point of view, two critical elements must be taken into account: temperature and pressure. Low temperature assures that the plasma does not cause thermal damage to the human body during the practical application. Low pressure means that the non-thermal plasma can be generated at atmospheric pressure. In addition, the plasma generation device is open in the atmosphere, with helium gas blowing, which decreases the cost of plasma generation, guaranteeing the device's economic availability and convenience. See Figure 1.

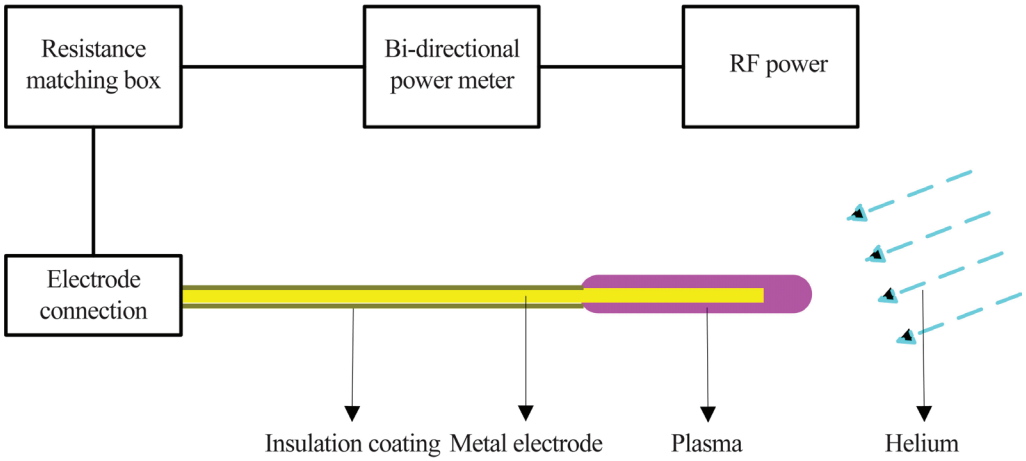


FIG. 1: Overall scheme of the setup.

B. Discharge Diagram

The overall scheme of the non-thermal atmospheric plasma sterilization system is similar to that of the plasma needle.[7] The discharge electrode is covered by a coating (0.1~0.3 mm thick), except at both ends. This design ensures a very good insulation effect. At the same time, the length of the coating can be adjusted to the length of the exposed part. Thereby, the generation location and the area of plasma can be controlled according to the application. In addition, the electrode structure is simple, has a very small diameter, and is flexible. These features facilitate the use of this system in specialized applications. See Figure 2.

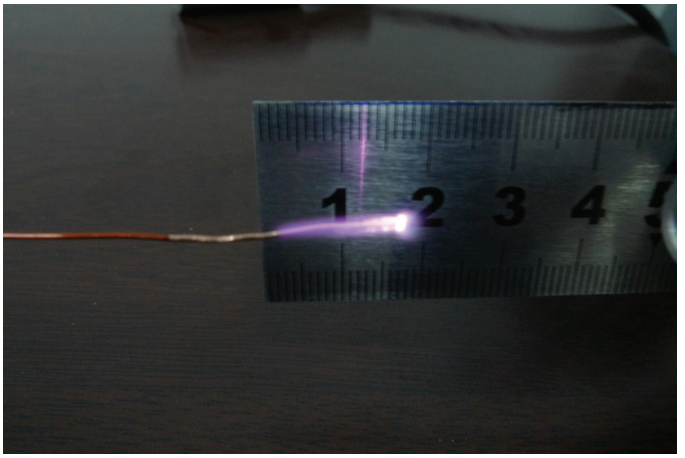


FIG. 2: Cold plasma discharge device.

III. PHYSICAL PROPERTIES OF NON-THERMAL ATMOSPHERIC PLASMA

A. Voltage Analysis of Non-thermal Atmospheric Plasma

To establish whether the plasma generated by the sterilization setup can be safely applied, voltage measurements were performed. Using the RIGOL DS5000 digital storage oscilloscope to measure the plasma voltage, we adjusted the radiofrequency (RF) power to 6W, 10W, and 15W, respectively. The corresponding voltages were approximately 60V, 70V, and 100V. The electrical resistance of the human body ranges from 1000 to 2000 ohms,[8] and the maximum currents corresponding to our RFs were 30 mA, 35 mA, and 50 mA, which are relatively small. In addition, the high frequency of 13.56 MHz RF power greatly reduces the possibility of damage to the human body and fully guarantees the safety of the procedure. During the practical application for sterilization, safety can be further strengthened by decreasing the RF power and by ensuring the driest possible environmental conditions.

B. Temperature Analysis

1. Temperature Measurement

To establish whether the plasma generated by the setup can be safely applied, temperature measurements were performed. PT-100 (platinum)-resistance temperature detectors (RTDs) were used to measure temperature. RTDs contain a sensing element that changes the resistance with temperature.[9] The system for measuring the temperature contains the thermal resistor PT-100. An SBWZ-PT100 temperature transmitter and a PCI2002 data acquisition card were used. The environmental temperature was approximately 22°C, and the effective power was 10W.

An agar-agar layer (2 mm thick) was used to simulate human tissue, and the temperature of the agar-agar layer. A thermal resistor PT-100 was placed beneath the agar-agar layer. The plasma generator was placed over the agar-agar layer where the PT-100 was located, and the agar-agar layer was exposed to the RF-plasma with an effective power of 10W. The temperature was measured before and during plasma exposures.

As exposure time increased, the temperature of the PT-100 increased somewhat at first, but that increase stabilized after approximately 30 seconds, with a maximum temperature of approximately 30°C. Obviously, this temperature is rather low, and it is well within the bearable range of the human body and will not cause tissue damage.

2. Factors Affecting Plasma Temperature

The relationships among plasma temperature and power, helium flow, and the distance between the electrode and the treated surface were analyzed experimentally. The experimental results are beyond the scope of this paper, but briefly, they showed that plasma temperature increases with the increase of applied RF power and decreases with the increase of helium flow and the distance between the electrode and the treated surface.

C. Spectral Analysis of Non-thermal Atmospheric Plasma

1. Elements of Non-thermal Plasma

The spectrum of the light emitted by the plasma was measured using a spectrograph. From the spectrum ,See Figure 3, we concluded that the main species generated by the plasma are OH•, N_2, N_2^+, He, and O• particles. The He is, of course, the working gas, and the nitrogen is introduced from the atmosphere of the open system. The most important species for sterilization are the free radicals OH• and O.

2. Factors Affecting Plasma Composition

The sterilization character of plasma is determined by its constituent particles. To achieve a plasma with good sterilization capability, the constituent particles and their concentration must be effectively controlled to optimize the sterilization and to reduce the presence of particles that have no effect on sterilization.

Experiments regarding OH•, O•, and N_2^+ intensities and power and helium flow were conducted. Our results show that the OH•, O•, and N_2^+ intensities increased with the increase of power but decreased with the increase of helium flow. Charged particles, therefore, likely play a significant role in the rupture of the outer membranes of bacterial cells.[10]

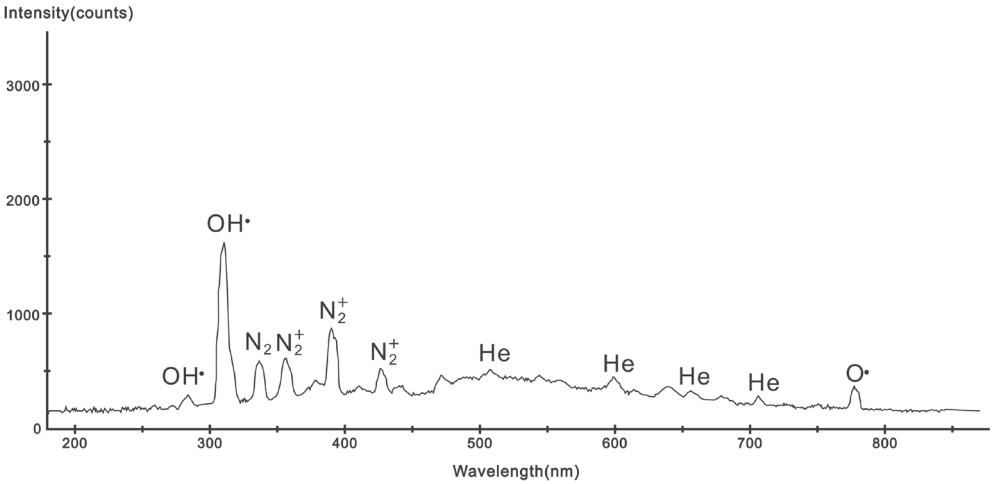


FIG. 3: Spectrum of non-thermal atmospheric plasma

IV. ATMOSPHERIC NON-THERMAL PLASMA EXPERIMENTS

A. Evaluating Sterilization Using *Escherichia coli*

1. E. coli Experiments

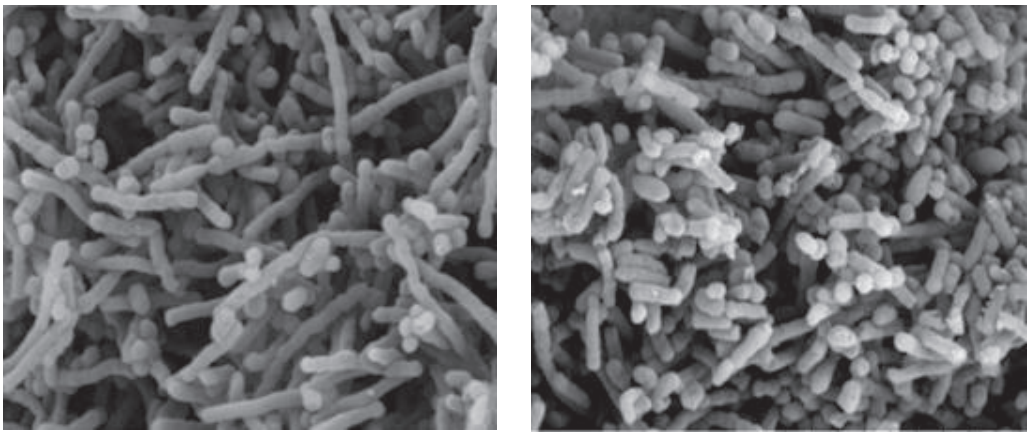
Oral bacteria are known to be much more vulnerable to sterilization than *E. coli*,[10] which normally resides in the lower intestinal tract. Therefore, *E. coli* was used to test the sterilization capability of the plasma system. Sterilization effects on *E. coli* treated with non-thermal atmospheric plasma were observed using a scanning electron microscope and a transmission electron microscope.

In Figure 4(a), untreated *E. coli* are long and rod-shaped. In Figure 4(b) on the other hand, many small fragments of *E. coli* remain after plasma treatment. *E. coli* cells were broken into fragments by the plasma treatment; the structures of the *E. coli* cells were seriously damaged, resulting in death.

This effect becomes more clear in Figure 5(a) and (b). In Figure 5(a), untreated *E. coli* are long rod-shaped and the structures are complete; the black objects are organelles. In Figure 5(b), the *E. coli* treated with plasma are white; even though they are long and rod-shaped, the internal structure of the cell has been almost completely destroyed. The inner structures of the *E. coli* were seriously damaged by treatment with plasma, and the plasma treatment ultimately killed the *E. coli*.

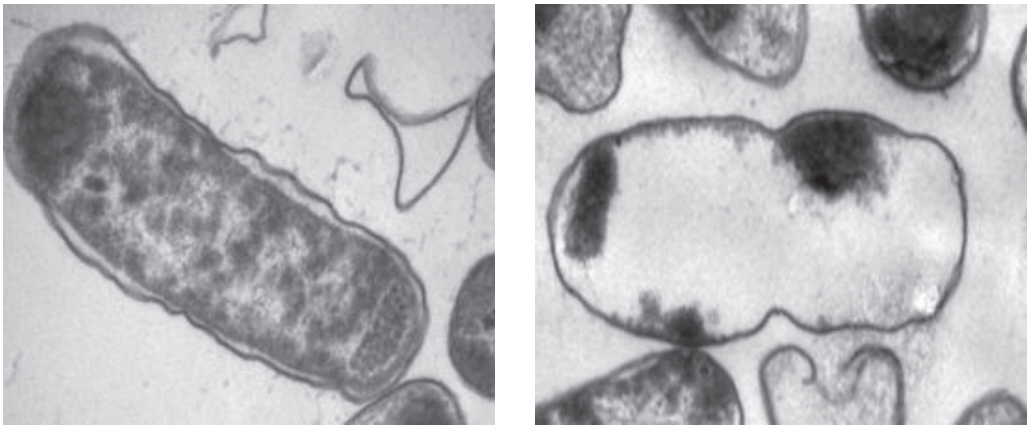
2. Sterilization Efficiency

To determine the sterilization efficiency of the plasma generated by this device, we calculated the decimal reduction time (D-value) of the bacteria; the D value is a parameter



(a) (b)

FIG. 4: Sterilization effects observed by scanning electron microscope.



(a) (b)

FIG. 5: Sterilization effects observed by transmission electron microscope.

commonly used to describe decontamination efficiency. It is defined as the time needed to kill 90% of cells, which is one decade on a log plot.[11] Using the plasma generated by this device, we treated five *E. coli* samples containing the same number of bacteria for 10 s, 20 s, 30 s, 60 s, and 90 s, respectively. The samples were transferred to culture dishes and cultured at 37°C for 48 hours. We then recorded the number of bacterial agglutinin. According to these numbers, the D values were obtained.

The resulting D values were on the order of 10 s, so the number of *E. coli* decreased to 10^4 ORG/ml (organisms/ml) within approximately 20 s, a decrease of three orders of magnitude. Comparing our results to previous research on the sterilization of *E. coli*, we have concluded that the sterilization efficiency of plasma is

quite good and meets the need of killing bacteria quickly and effectively in clinical medical applications.

V. NON-THERMAL ATMOSPHERIC PLASMA EXPERIMENTS

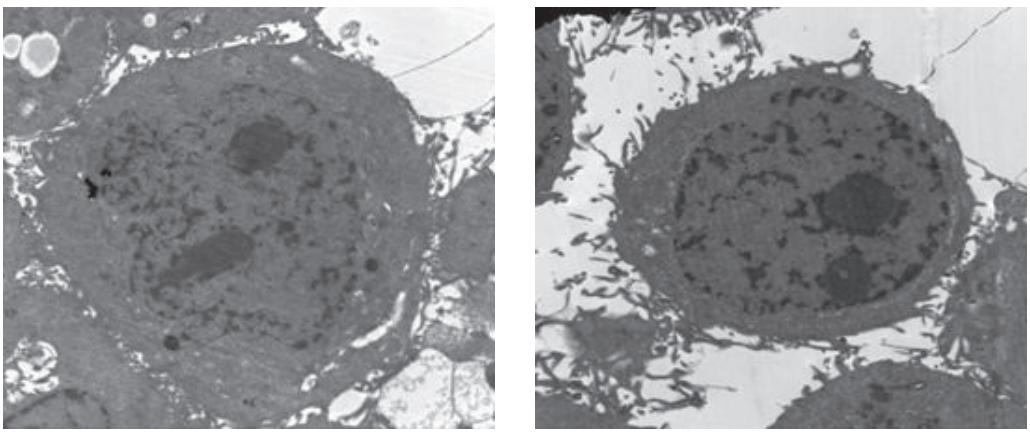
Non-thermal atmospheric plasma sterilization devices are designed for clinical medical applications and to interface with the human body. Therefore, in addition to the sterilization experiments, cell experiments on human tissue were also performed to test the safety of the plasma sterilization system. Plasma interactions with eukaryotic cells are much more complex than interactions with bacteria. These cells are more resistant, and they can initiate many interesting responses in self-defense. Therefore, some fundamental studies are required to identify the reactions of living cells to plasma treatment.[12] Although some experiments have been performed and reported by researchers,[13,14] we used human tongue cancer cells to perform our experiments.

A. Human Tongue Cancer Cells

Experiments using human tongue cancer cells were conducted using a power of 10W, a helium flow of 1 L min^{-1}, and a distance between the electrode and the treated surface of 2 mm. After centrifuging and affixing the cells, they were subjected to plasma treatment. The cells were then observed using a transmission electron microscope.

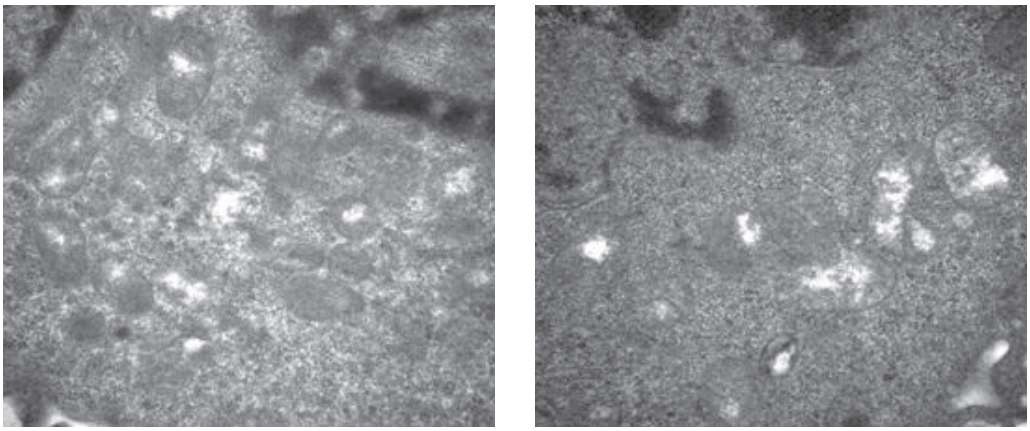
Figure 6(a) shows the appearance of untreated cells. The cells are round in shape, the cell membrane is intact, and the cell structure is complete. Figure 6(b) shows the cells after the plasma treatment, and the cells are virtually identical.

Figure 7(a) shows the inner structure of untreated human tongue cancer cells. The organelles, visible as dark grains, are complete, there is no cellular swelling, and the cell is not damaged. Figure 7(b) shows that the inner structure of the human cells subjected to the plasma treatment were not affected at all.



(a) (b)

FIG. 6: Appearance of human tongue cancer cells.

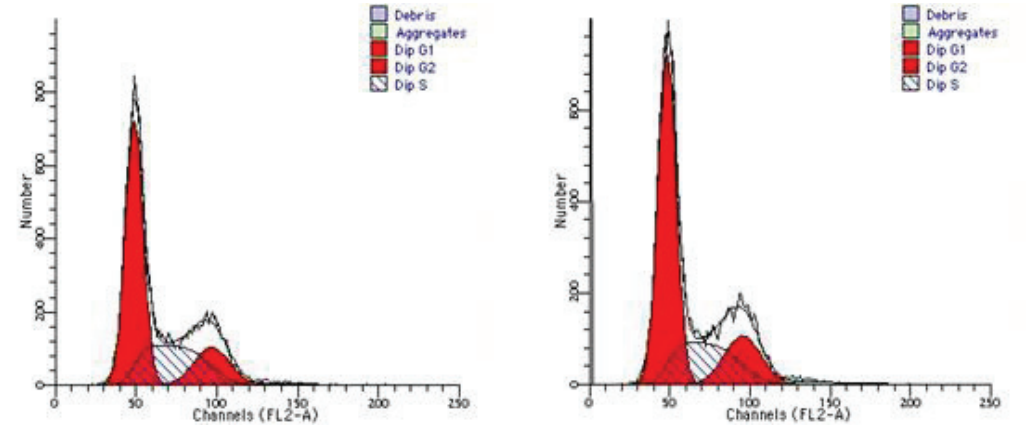


(a) human tongue cancer cells not treated (b) human tongue cancer cells treated

FIG. 7: Inner structure of human tongue cancer cells.

1. Cell Cycle Experiment on Human Tongue Cancer Cells

The cells subjected to the plasma treatment were centrifuged and collected, then flow cytometry was used to study the cell cycle.[15,16] Figure 8(a) shows the cell cycle curve of the untreated human tongue cancer cells; Figure 8(b) shows the curve of treated cells. Almost no variation occurred between Figure 8(a) and (b) and the S growth period. It is clear that the non-thermal plasma had no effect on the regular growth of these human cells and did not affect the regular growth cycle.



(a) human tongue cancer cells not treated (b) human tongue cancer cells treated

FIG. 8: Cell cycle curves of human tongue cancer cells.

2. *Apoptosis Experiment on Human Tongue Cancer Cells*

When cell apoptosis occurs (programmed cell death), the DNA is fragmented, a condition called echelonment.[17,18] See Figure 9.

We extracted the DNA of cells treated using the plasma and dispersed them using electrophoresis. Figure 9 shows the result of the apoptosis experiment. Figure 10(a) shows the DNA electrophoresis result of the untreated cells. Figure 10(b) shows the DNA electrophoresis figure of the cells treated by plasma. No observable change occurred as a result of the plasma treatment. The DNA is still in one strip; it was not fractured. Therefore, cell apoptosis did not occur as a result of treatment with non-thermal atmospheric plasma.

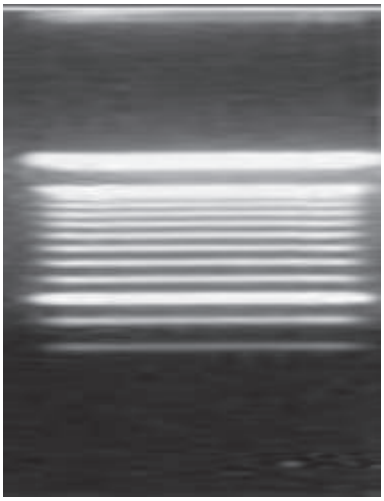
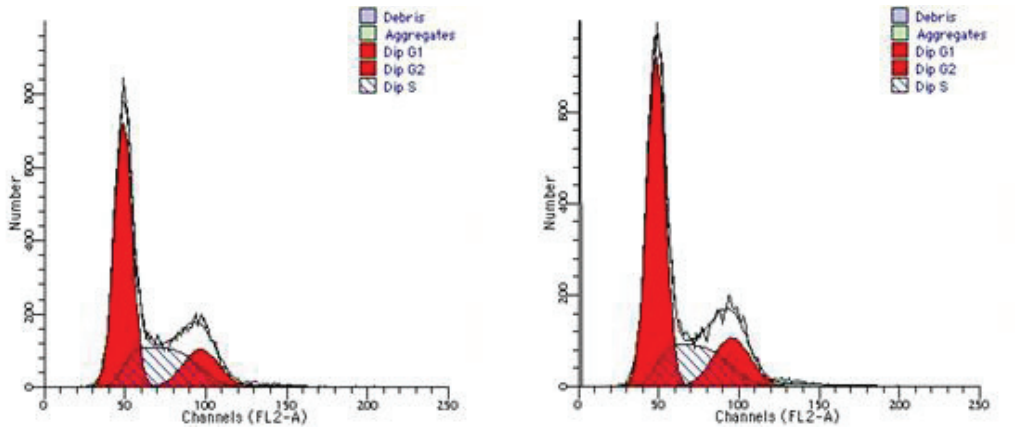


FIG. 9: Photo of DNA fragmented.



(a) human tongue cancer cells not treated (b) human tongue cancer cells treated

FIG. 10: DNA electrophoresis figure.

VI. CONCLUSION

Fundamental research on the use of a non-thermal atmospheric plasma for sterilization is presented in this paper. A radio-frequency plasma sterilization device was designed to be used for small areas of sterilization, as in the treatment of oral disease. Our biomedical experiments illustrate the safety and effectiveness of the plasma system for sterilization, that is, to kill bacteria on human tissue. All of our results show that human cells (in this case tongue cancer cells) were not damaged by non-thermal atmospheric plasma treatment; therefore, non-thermal atmospheric plasma is completely safe for sterilization uses.

REFERENCES

1. Stoffels E. "Biomedical applications of electric gas discharges." High Temper Mater Process. 6(2002)191–202.
2. English BK, Gaur AH. The use and abuse of antibiotics and the development of antibiotic resistance[J]. Adv Exp Med Biology. 2010;659,DOI 10.1007/978-1-4419-0981-7_6.
3. Bin Zhou, Jianjun Wu , Tianxiang Hu, Saying Du, Shiping Liao. Discussion on overuse of antibiotics. [J] Tibet Sci Technol.2003(5):45–6.
4. Yip HK, Samaranayake LP. Caries removal techniques and instrumentation: a review. Clinical Oral Invest. 1998;2:148–54.
5. Lu X, Cao Y, Yang P, et al. An RC plasma device for sterilization of root canal of teeth[J]. IEEE Trans. Plasma Sci, 2009, 37(5): 668-673.
6. Laroussi M. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. IEEE Trans Plasma Sci. 2002;30(4):1409–15.
7. Stoffels E, Flikweert AJ, Stoffels WW, Kroesen GMW. Plasma needle: a non-destructive atmospheric plasma source for fine surface treatment of (bio)materials. Plasma Sources Sci Technol. 2002;4:383–8.
8. Li Z. The overview of safety power use in lab. Exp Teaching Instrument. 2009;2.
9. Sladek REJ, Stoffels E, Walraven R, Tielbeek PJA, Koolhoven RA. Investigation of possibilities of plasma treatment for dental caries. Part 2. IEEE Trans Plasma Sci. 32(4):1540–3.
10. Mendis DA, Rosenberg M, Azam F. A note on the possible electrostatic disruption of bacteria. IEEE Trans Plasma Sci. 2000;28:1304–6.
11. Monna V, Nguyen C, Kahil M, Ricard A, Sixou M. Sterilization of dental bacteria in a flowing N2-O2 postdischarge reactor. IEEE Trans Plasma Sci. 2002;30:1437–9.
12. Sladek REJ, Stoffels E, Walraven R, Tielbeek PJA, Koolhoven RA. Plasma treatment of dental cavities: a feasibility study. IEEE Trans Plasma Sci. 2004;32(4): 1540–3.
13. Kieft IE, Darios D, Roks AJM, Stoffels E. Plasma treatment of mammalian vascular cells: A quantitative description. IEEE Trans. Plasma Sci. 2005;33(2):771–5.
14. Stoffels E, Kieft IE, Sladek REJ, Slaaf DW, van der Laan EP, Jimenez-Moreno P, Steinbuch M. Towards Plasma Surgery: Plasma Treatment of Living Cells. AIP Conf. Proc. 2004;740(309).
15. Zhang Y. Medical technology application of flow cytometer. Chinese J Lab Med. 1997;20.
16. Geng H, Wang L, Wang Q. Biomedical sciences application of flow cytometer. J Biology. 2005;22(4).

17. Fang XY, Sheng W. The concept source of cell apoptosis and the research progress[J]. Sci Life. 2003;23(2):122–4.
18. Xiaohong Han, Shihe Shao, Yanjun Xue. The 4HPR effects of cervical cancer cell apoptosis. [J] Acad J ShanDong Univ. 2008;46(12):1120–3.