Non-thermal atmospheric pressure plasma sources for biomedical applications

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Abstract: It was presented that various types of non-thermal atmospheric pressure plasma sources operated with low frequency(~kHz) or microwave frequency feeding argon or helium gas, or in the ambient air could be used for the treatment of cancer cells, sterilization, tooth whitening, and blood coagulation. The antibody-conjugated GNPs enhanced to kill cancer cells exposed to plasmas. The plasmas treatment made tooth bleaching three times effective..

Keywords: non-thermal plasma, cancer treatment, sterilization, tooth-bleaching, coagulation.

1. Introduction

Atmospheric pressure plasmas (APPs) have been recognized as new tools in the biomedicine. APPs has proved their effectiveness to be used in bio-medical applications such as treatment of living cells, sterilization, blood coagulation, wound healing, and air purification [1]. APPs are inexpensive thanks to no need for vacuum system, robust to operate for a long time, and safe to be touched by living tissue such as a human body directly. The most attractive one in these features of APPs is their low temperature property which leads to minimize thermal damage of living cells and heat-sensitive polymers. Many devices for APPs were operated by DC, low frequency(~kHz), radio-frequency, microwave frequency and pulsed power sources [2]. Because APP generated by each power source has unique property, it is important to select the proper power source for desired biomedical application.

Air, argon (Ar) and helium (He) plasma sources driven by AC or microwave power which dose not need additional matching network were presented for bio-medical applications such as the treatment of cancer cells, sterilization, tooth whitening, and blood coagulation in this study.

The non-thermal APPs have shown their effectiveness in killing cancer cells [3,4]. We used drug delivery nanotechnology by means of conjugated-gold nanoparticles to achieve the selectivity between normal and cancer cell. For tooth bleaching which is a popular esthetic service in dentistry, hydrogen peroxide (H_2O_2) has been a widely used bleaching material [5]. Although the exact mechanism of bleaching action by H_2O_2 has not been completely understood, hydroxyl radicals (·OH), which H_2O_2 breaks down into by means of external high-intensity light source such as laser, is a crucial agent for tooth bleaching [5]. Because plasma consists of energetic charged particles, several reactive radicals, U.V., and strong electric field, the non-thermal APPs can break down H_2O_2 effectively. Among the various plasma sources reported, Ar plasma jet is very effective for the sterilization of microorganism [6-8]. It was observed that Ar plasma source showed stronger emission intensity of reactive radicals and better killing effect than He plasma source. The microwave-excited plasma jet needs very low power (a few watts) to sustain due to the very efficient power coupling to plasma. Furthermore, a number of low-cost power modules and solid-state components for the design of a suitable and portable power source make it possible to construct a compact low-cost plasma generator at microwave frequencies. Thus compact microwave excited microplasmas operating at low power may meet the need for the portability in areas such as skin regeneration, blood coagulation and wound healing.

2. Results and discussion

Non-thermal air plasmas were generated between two electrodes covered with a dielectric material as shown in **Fig. 1a** and **1b**. One electrode was connected to sinusoidal (22 kHz) high voltage (5 kV) source and the other electrode was grounded. This setup operates in an ambient air without any gas tanks and a vacuum system. Although the high voltage used to operate the device, the dielectric material covering the electrodes limits the current across the plasma itself. It makes to be safe to touch the plasma during operation as shown in **Fig. 1b**.

This non-thermal air plasma has shown its effectiveness in killing cancer cells. The target was G361 human melanoma skin cancer cells and was placed 2mm from the plasma source and exposed to 40s plasma treatment. The point type air plasma source in **Fig. 1a** makes direct and indirect contact regions. The apoptosis induced by air plasma was observed in plasma direct treatment region. In **Fig. 2a**, the region B shows round shaped death cells. The apoptosis phenomena were proved even by DNA fragmentation of cell in **Fig. 2b**.

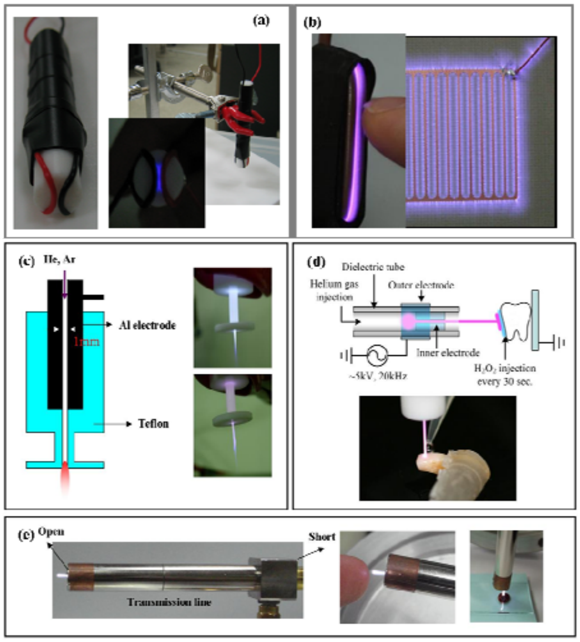

Fig.1 (a) Point plasma source, (b) line-type plasma source, (c) Long plasma jet and (d) multi-plasma jet driven by AC, (e) micro-plasma induced by microwave

Antibody conjugated 30 nm gold nanoparticles which enhance the therapeutic effects of the plasma were used for the selectivity between normal and cancer cells [3,4]. Cells were treated with anti-FAK antibody-conjugated gold nanoparticles (FAK-GNPs) and then kept in 4% paraformaldehyde for 10 min [3]. After 40 sec treatment, the death rate of cancer cells was increased about 74% compared to only plasma treatment without any conjugation [3]. This air plasma coupled with FAK-GNPs resulted in a 5× increase in cell death over the case with the plasma alone.

The atmospheric pressure plasma jet (APPJ) for the

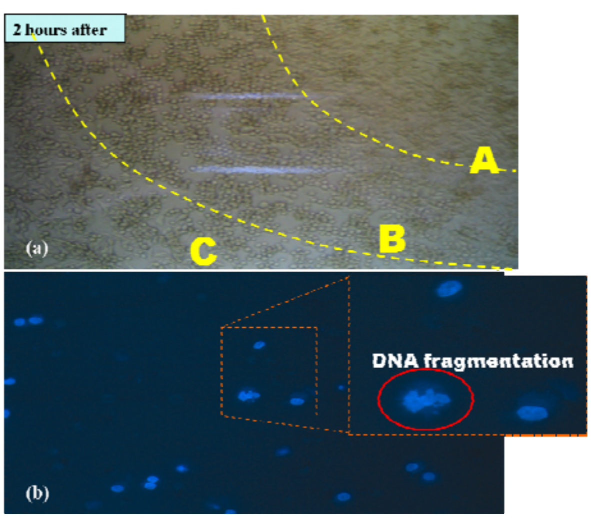

Fig.2 (a) Apoptosis induced by air plasma, (b) DNA fragmentation as a proof of apoptosis

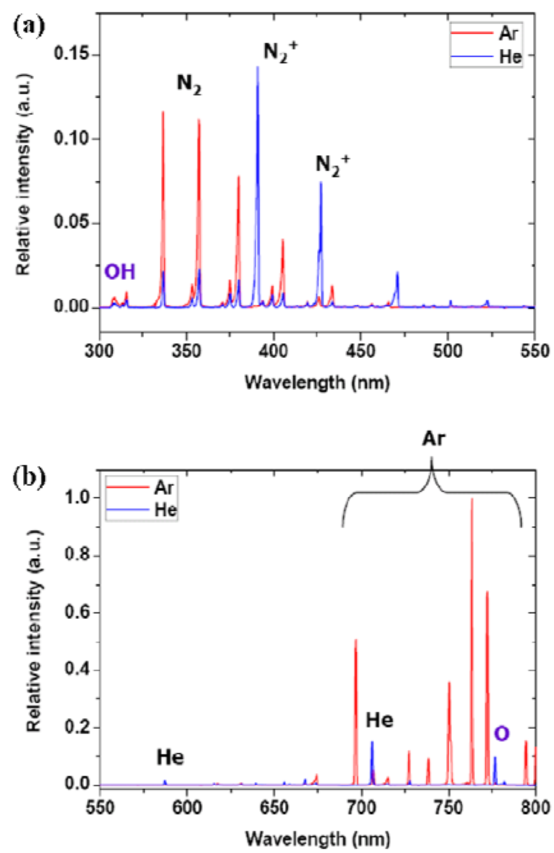

Fig.3 (a) Emission spectrum comparison of reactive species (OH, N_2, N_2^+) intensity, (b) of reactive oxygen line in Ar and He plasma

sterilization consists of aluminum electrode with 1mm hole and dielectric tube. A sinusoidal (20 kHz) high voltage source (7kV) was applied to the aluminum electrode in **Fig. 1c** and argon and helium gas with a flow rate of 4 slm were used as working gases. The temperature of Ar and He plasma was around room temperature (300K) [5]. It was observed through the measurement of the optical emission spectrum the important reactive species (OH, O) as well as N_2, N_2+, He+, Ar+ in **Fig. 3a**. The emission intensity of reactive oxygen atom in Ar plasma was much larger compared to the He plasma in **Fig. 3b**. It is believed that reactive atomic oxygen is one of essential factors of sterilization of microorganism such as bacteria and E-coli. In our experiments (**Fig. 4a** and **4b)**, Ar plasma treatment was more effective for sterilization than He plasma treatment. It took several tens of seconds to kill the 90% bacteria and the survival curve also showed Ar plasma jet killed bacteria more faster than He plasma jet as shown in **Fig. 4c**.

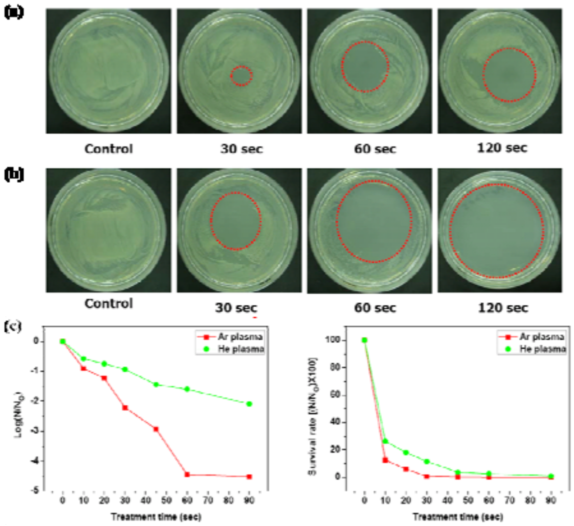

Fig.4 Bacteria killing effect due to the treatment time by (a) He plasma source and (b) Ar plasma source, and (c) E. coli survival curve comparison between He and Ar plasma sources.

Another non-thermal APPJ was developed for enhancement and acceleration of tooth whitening effect with H_2O_2 [5]. This APPJ used He gas as a feeding gas and was driven by low frequency and consisted of a tube structure dielectric material (Teflon, $\varepsilon_r = 2.6$) and inner and outer electrodes made of aluminum (**Fig. 1d**). Extracted human canine teeth were used in these *in vitro* experiments. The bleaching results are analyzed by comparing the overall color changes in the tooth surface using photos taken before and after treatments [5]. After the plasma treatment with H_2O_2 (28%, 20 µl every 30 s) for 10min, a 220% improvement of the tooth whitening as compared with H_2O_2 alone treatment for the same duration was observed in **Fig. 5**. The enhancement was attributed to considerable OH radical generation. The amounts of ·OH generated from H_2O_2 before and after the plasma treatment were measured using electron spin resonance (ESR) spin-trapping method [9-11]. A clear ·OH signal was detected after treating the tooth for 1 min. This signal was 1.9 times greater when using plasma with H_2O_2 than when using H_2O_2 alone [5]. The teeth surface temperature increased from room temperature (~25°C) and stabilized near 38°C after 1.5 min operation [5].

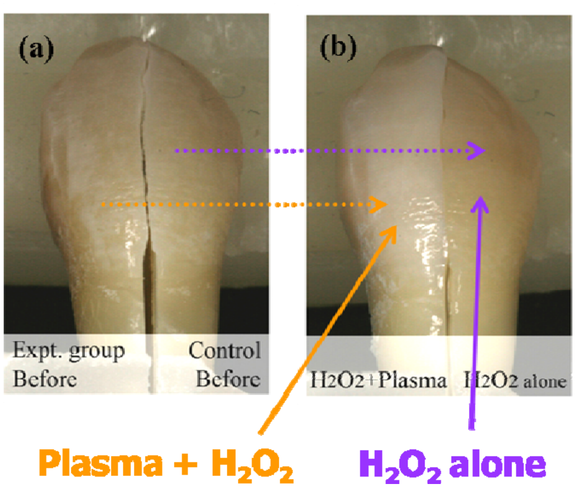

Fig.5 (a) Both side; the photographs of canine tooth used before treatments, (b) left side; after plasma treatment using H_2O_2 (28 %, 20 µl every 30 s) for 10 min and right side; after using H_2O_2 alone for the same duration.

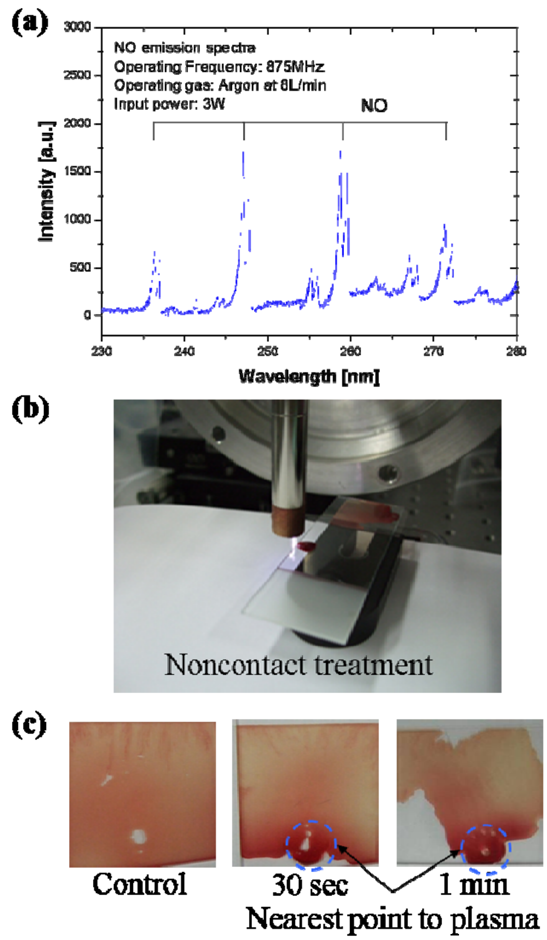

Fig.6 (a) NO emission of the microwave-excited APPJ at 8 slm argon flow and 3 W input power, (b) side-view (noncontact) of *in vitro* blood coagulation, (c) *In vitro* blood coagulation using APPJ at 3.5 W input power and 3 slm argon flow rate: (left) control; (center) after noncontact treatment for 30 s; (right) after noncontact treatment for 60s (10 µl anti-coagulated blood was used)

The micro-plasma source based on a coaxial transmission line resonator (CTLR) has been designed and applied to the blood coagulation. The CTLR plasma source consists of a coaxial transmission line where one end point is electrically short and the other end point is open as shown in **Fig. 1e**. The CTLR was capable of self-igniting below 2.5 W input power with argon gas at an atmospheric pressure. A small metal tip is used to enhance the electric field at the open end of the device. The gap between the metal tip and the inner conductor is 200 μm. The length of the APPJ could be varied from 5 to 10 mm depending on the gas flow rate and input power. The rotational temperature of OH molecules in the discharge is around 400K, and the Ar plasma could be touched without any thermal damages [12]. Nitrogen Oxide (NO) molecule can promote blood coagulation and wound healing [1]. **Fig. 5a** showed that emission bands corresponding to NO were observed in the emission spectra of the APPJ from the CTLR. The NO formation results from the high gas temperature gradient of the APPJ between the ignition point and the afterglow [1]. The NO generated by the microwave-excited discharge suggests that this device could be useful for biomedical applications, particularly blood coagulation and wound healing. After 15~20 seconds of treatment for mouse blood, the blood was changed into the clot in part and coagulated [12]. Indirect coagulation was also observed in **Fig. 5c** after treatment for 30 and 60 seconds, when the APPJ was held 3 mm away from the blood sample. Even though the APPJ did not contact the blood directly, coagulation occurred. It is noted that the non-contact coagulation could result from the NO radicals of the APPJ.

3. Conclusion

We have presented various types of non-thermal APPs operated in low frequency (20kHz) and microwave frequency (900MHz and 2.45GHz) power feeding argon, helium, and even in the ambient air and have shown their effectiveness to several biomedicine applications such as treatment of cancer cells, sterilization, tooth whitening, and blood coagulation. Due to their low temperature property, they can be used for the treatment of living tissues. The antibody-conjugated GNPs enhanced to kill cancer cells exposed to plasmas. The plasma treatment to bacteria can achieve efficient sterilization and Ar plasmas were more efficient to kill bacteria than He plasmas. APP jet also showed to be effective to make a tooth white with H_2O_2, resulting from that plasmas can promote to generate OH radicals dissociating H_2O_2. APP jet generated by microwave can be self-ignited with low power below 2.5 W and produce abundant NO to promote to coagulate blood. Through understanding characteristics of the various types of plasma sources presented in this study, it is expected to achieve the optimization for each bio-medical application.

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