Ablation of liver cancer cells *in vitro* by a plasma needle

Xianhui Zhang,[1,2,a] Maojin Li,[3] Rouli Zhou,[3] Kecheng Feng,[1] and Size Yang[2]
[1]*School of Science, Changchun University of Science and Technology, Changchun, Jilin 130022, People's Republic of China*
[2]*Beijing National Laboratory for Condensed Matter Physics, Institute of Physics, Chinese Academy of Science, Beijing 100080, People's Republic of China*
[3]*Health Science Center, Department of Cell Biology, Peking University, Beijing 100083, People's Republic of China*

(Received 22 March 2008; accepted 25 June 2008; published online 16 July 2008)

A plasma needle using a dielectric barrier discharge reactor at atmospheric pressure with a funnel-shaped nozzle was developed. The preliminary characteristics of the plume and applications to the ablation of cultured human hepatocellular carcinoma (HCC) BEL-7402 cell line were presented. The effect of oxygen, which was injected into argon plasma afterglow region through a steel tube, was studied. The efficiency of argon-oxygen plasma depends sensitively on the oxygen concentration in argon plasma. Large differences between spectra in atmosphere and those in Dulbecco's modified eagle medium are found. It is found that ultraviolet rays, O, OH, and Ar radicals can reach the bottom of solution and act on HCC cells and there is an optimum input power to get the most radicals. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2959735]

In recent years, the interventional therapy of cancers mainly includes intravascular interventional therapeutic[1] and percutaneous physical ablation.[2] Intravascular interventional therapeutic refers to sending medicine to the target organs or tissues through the blood vessels; then the medicine will kill the cancer cells there. Its side effect is relatively severe.[3] While percutaneous physical ablation kills the cancer cells by heating or freezing these cells by pricking the skin. This therapy takes longer than that mentioned above.[4] There is a demand in developing less side-effect and high-efficiency technologies for ablation of cancer cells.

The ablation of cancer cells by low-temperature plasma, which is partially ionized gases at atmospheric pressure, is an alternative to the conventional way. Low-temperature plasma as one of the techniques in biomedicine is gradually investigated in biology. Because of the existence of these reactive plasma species, it has proven to be an effective alternative to many existing sterilization methods and is commercially available for medical uses in 1990s.[5] Recently, it has been applied to the human body.[6]

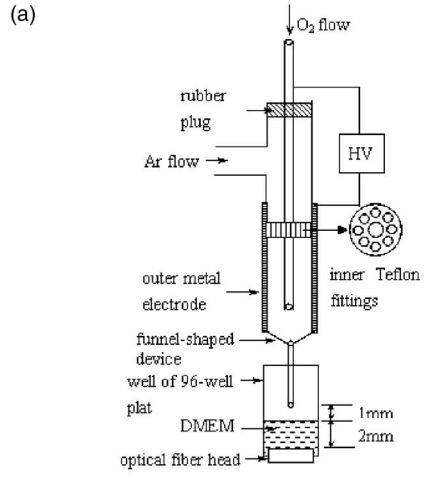
Low-temperature atmospheric plasma is a promising technology.[7] Since the atmospheric plasmas are operated in open air, the use of these plasmas can easily overcome the limitations imposed by the currently available vacuum-based plasma. Furthermore, it can generate short-lived chemical species, which can be propelled toward the surface that is to be treated. The short life of these species is desirable because they do not remain after the treatment is completed. To date, few results with regard to killing liver cancer cells by the atmospheric plasma are presented in literature. In this letter, the study of ablation of liver cancer cells by atmospheric plasma is addressed.

Through a special design, a plasma needle with a funnel-shaped nozzle by dielectric barrier discharge igniting plasma is presented, as shown in Fig. 1(a). A funnel-shaped device, which is also made of quartz, is pasted to the end of quartz tube by extremely strong cure. The funnel is 2 mm in length and its inner diameter (ID) changes from 4 to 0.5 mm; the end of funnel extends 8 mm long with 0.5 mm ID. This kind of structure provides more focusing, which helps in holding and collecting more radicals to sample. A steel tube acted as inner electrode and oxygen inlet was centered the quartz tube. A copper braid covering the quartz tube was the outer electrode. Both electrodes were connected to an ac power with a maximum peak voltage of 30 kV and a frequency from 8 to 30 kHz. A low-temperature plasma plume shown in Fig. 1(b) was generated with a gas velocity of 4.25 m/s at 32 W. As is known, it is unattractive to add gases such as O_2 directly into the gas feed because it diminishes the production of radicals due to electron attachment inside the nozzle.[8] To produce more radicals in the gas phase, Ar is fed through the side arm of the tube, and O_2 is injected into the end of quartz tube through the inner electrode to react with Ar plasma.

When Ar flow is 200 SCCM (SCCM denotes cubic centimeter per minute at STP), the addition of 5 SCCM O_2 through the inner electrode did not disturb the needle voltage and circuit current characteristics. The temperature of Dulbecco's modified eagle medium (DMEM) was measured by a platinum resistance temperature detector that was covered in DMEM 1 mm deep along the axis. The results indicated that the plasma gas temperature was not higher than 316 K when the applied power was not more than 32 W. It suggests that the temperature increase in DMEM cannot cause cell death. It should be noted that a critical power value (32 W) existed. Beyond that point, the plasma plume turned unsymmetrical and fell into a turbulent flow. In addition, the plume length began to decrease and the temperature increased a little correspondingly.

The glow is produced by electron-impact excitation of gas atoms, so that it serves as a visual indicator of the presence of energetic electrons. Figure 2 is a typical UV-visible emission spectrum (Stellarnet, EPP-2000C) of the atmospheric plasma. All emission spectra reveal the presence of excited Ar and atomic oxygen in the plasma jet, as well as

[a]Author to whom correspondence should be addressed. Electronic mail: zhang_xh16@yahoo.com.cn.



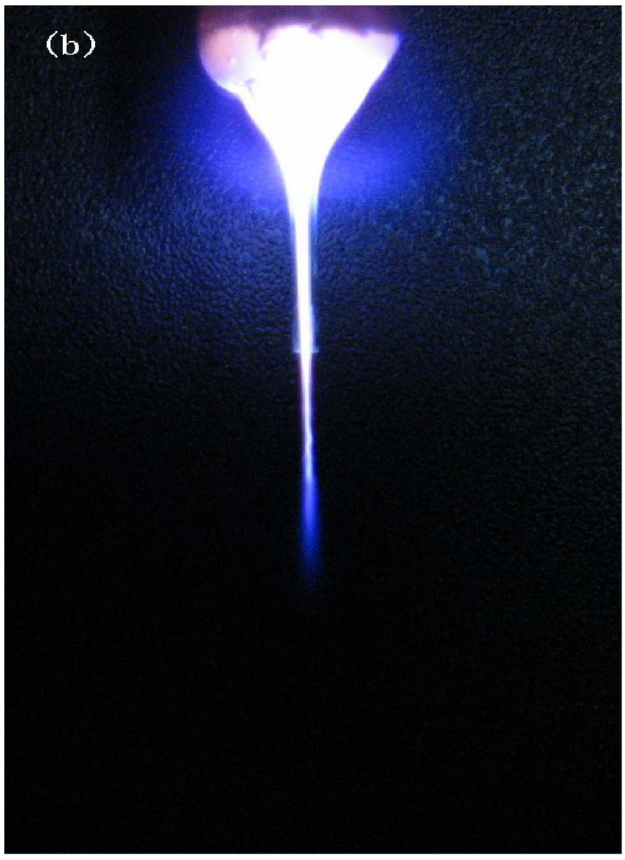


FIG. 1. (Color online) (a) Schematic diagram of the plasma needle. (b) Image of the plasma jet with 200 SCCM Ar and additional 5 SCCM O (P_j=32 W).

some excited air molecules. Although the spectrum is very rich in emission features, most of the atomic lines or molecular systems can be unambiguously identified by referring to literature.[9–14] The optical emission spectroscopy reveals the presence of OH ($2S \rightarrow 2P$), $H_\alpha(n \rightarrow 2s, 2p)$, OII, OI, and ArI species. The presence of N_2 line at 336 and 356 nm reveals the air entrainment in the reactor. N_2^+ line at 391 nm, which is formed in argon discharges, is attributed to Penning ionization ($Ar^* + N_2 \rightarrow Ar + N_2^+ + e^-$) and charge transfer ($Ar^+ + N_2 \rightarrow Ar + N_2^+$). Just because of this, it can be taken as the characteristic emission of the first negative system and has been used to detect the presence of both Ar^+ and Ar^*. The emission line at 656 nm corresponds to the H_α line, which is generated by the collision between water vapor molecules

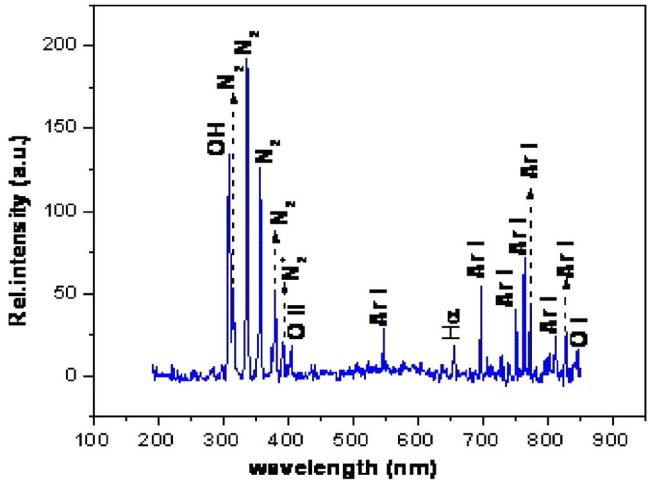


FIG. 2. (Color online) Emission spectrum of plasma with 200 SCCM Ar and additional 5 SCCM O_2 taken at 4 mm bottom the plume (P_j=32 W).

and electrons ($H_2O + e^- \rightarrow H + OH + e^-$). While the intensity of line at 309 nm is stronger than that of the emission line at 656 nm, it indicates OH radicals is also formed by the reaction of excited O with water vapor ($H_2O + O^* \rightarrow 2OH$).

In the experiment, the number (4000) of BEL-7402 cells was seeded in each well bottom of 96-well plate and cultured in DMEM 2 mm deep that contained 10% newborn calf serum. The thin and long nozzle was put in one of wells of 96-well plat. The separation between the plasma nozzle and the surface of medium was 1 mm. The treated samples were continuously cultured for 24 h at the same condition. Then the color development was measured at 405 nm using a rapid microplate reader (Bio-TEK EL311). The optical density (OD) value is directly proportional to the number of living cells in each well.

Figure 3 shows survival curves of BEL-7402 cells in Ar plasma with a different additions of O_2. The OD value of survival BEL-7402 cells of the test groups is the absorption value at 405 nm. The vertical axis is a ratio of OD value to the OD_0 value of the untreated cells, where the OD and OD_0 values are the average of triplet wells. The reference value of OD/OD_0 represents survival ratio of the treated cells compared to the control group. Increasing the acting time to 8 min, the efficacy remained almost the same. Keeping the

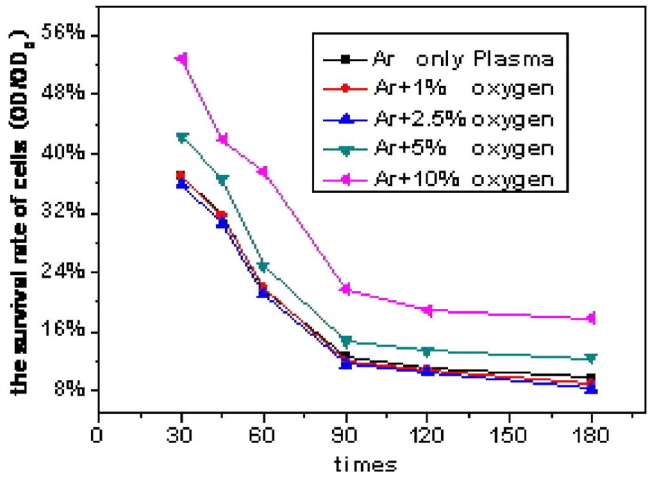


FIG. 3. (Color online) Survival curves of BEL-7402 cells in the Ar with a different O_2 additions into plasma jets at 32 W.

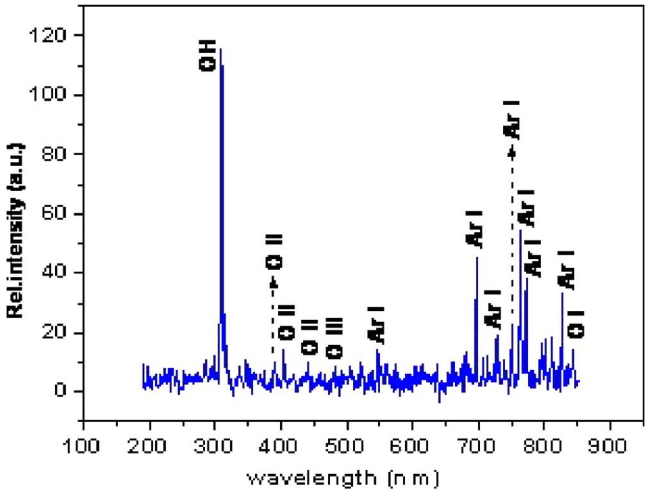


FIG. 4. (Color online) Emission spectrum of the plasma with 200 SCCM Ar and additional 5 SCCM O_2 taken at 2 mm deep in DMEM (P_j=32 W).

input power constant, the addition of O_2 was increased to 20% and there is almost no plasma at nozzle. This result suggested that O_2 diminishes the production of radicals, probably due to electron attachment inside the nozzle.

The efficacy was somewhat proportional to the input power. When power was increased to 34 W, the value of OD/OD_0 became 8.16% with a 2.5% O_2 addition. Then the value is 9.2% in only Ar plasma. At this time, the corresponding temperature of Ar plasma was the same with argon-oxygen (316 K), while the plasma got unstable. The experimental data show that efficacy of ablation cancer cells by argon-oxygen plasma is better than that by only argon plasma.

To find the critical radicals for the ablation of hepatocellular carcinoma HCC cells, an optical fiber head was buried in DMEM 2 mm deep to measure the spectrum. The separation between the plasma nozzle and surface of medium is 1 mm. Figure 4 shows the spectrum in DMEM along the axis. There are bigger differences between spectra in atmosphere and that in DMEM; all the N lines and H_α line (656 nm) lost, while the OH line (309 nm) and the O lines (403 and 844 nm) have no remarkable change. The most notable differences are some O lines (390, 481, and 442 nm) appeared in the spectrum of DMEM. It demonstrates that the solution of DMEM absorbed the N radical, but cannot absorb OH and O radicals. Perhaps the new lines of O was produced by N_2^*, electrons, and O_2 in solution ($N_2^*+O_2 \rightarrow N_2+O+O$) (Ref. 15) and ($e^-+O_2 \rightarrow O+O+e^-$).[16] Since BEL-7402 cells were seeded in the bottom of wells of 96-well plate, N species cannot reach the bottom of solution and are removed to kill the cancer cells. Although ultraviolet rays in 309 nm can arrive at the bottom of solution, the intensity of UV irradiance is about 19 mJ/cm^2 in DMEM 2 mm deep. It is harmless cell.[17] Ar, OH, and O radicals can also arrive at the bottom of solution and act on cells, which should be concerned with cell death. In aerobic metabolism, O radical and OH radical entered DMEM and diffused around. Moreover, part O radical indirectly transformed into OH radical.[18] Both can cause cells death.[19,20] Although arriving at the bottom of DMEM, it was difficult for Ar radical to diffuse as O and OH radicals. It indicated that O and OH radicals are the critical radicals to cell death.

To increase the content of OH and O radicals, increasing power is a useful method. However, the length of plasma plume shortened rapidly when the power is more than 34 W. It means that the intensity of needle plasma becomes weaker. This leads to ultraviolet rays and radicals in solution less correspondingly. Another way is the addition of O_2 through the inner electrode. It is proved by spectrum that O and OH emission profiles showed a maximum when the proportion of O_2 was 2.5%. The result was consistent with the experiment. When the flow of Ar is higher than 200 SCCM the cells in DMEM will be blown off. The results show that 34 W power and 200 SCCM Ar with 2.5% O_2 addition are the best. The separation has little effect on efficiency of cancer cell death because the argon atom is heavier than air molecules that penetrate deep into the air blanket creating a path for oxygen radical species to surface of solution.[21]

In summary, a plasma needle with a funnel-shaped nozzle was designed and fabricated. The efficiency in ablation of cultured liver cancer cells *in vitro* showed that oxygen addition to the argon plasma could improve the efficiency. By comparing the spectra, it is found that Ar, OH, and O radicals all can arrive at the bottom of solution, while the critical radicals to cell death are just O and OH radicals. An effective way to get more OH and O radicals is to increase the input power and the addition of O_2.

[1]H. Ohishi, H. Uchida, Y. Kurosaki, H. Yoshimura, S. Ohue, M. Katsuragi, N. Matsuo, and Y. Hosogi, J. Air Waste Manage. Assoc. **6**, 104 (1983).
[2]N. Honda, Q. Guo, H. Uchida, H. Ohishi, and Y. Hiasa, Radiology **190**, 53 (1994).
[3]T. Vogl, K. Eichler, S. Zangos, C. Herzog, R. Hammerstingl, J. Balzer, and A. Gholami, J. Cancer Res. Clin. Oncol. **133**, 177 (2007).
[4]D. Choi, H. K. Lim, H. Rhim, Y. S. Kim, B. C. Yoo, S. W. Paik, J. W. Joh, and C. K. Park, Ann. Surg. Oncol. **14**, 2319 (2007).
[5]P. T. Jacobs, and S. M. Lin, in *Disinfection, Sterilization and Preservation*, 5th ed., edited by S. S. Block (Lippincott, Willams and Wilkins, Philadelphia, 2001), pp. 747–763.
[6]T. C. Montie, K. Kelly-Wintenberg, and J. R. Roth, IEEE Trans. Plasma Sci. **28**, 41 (2000).
[7]M. Laroussi and X. Lu, Appl. Phys. Lett. **87**, 113902 (2005).
[8]J. Goree, B. Liu, D. Drake, and E. Stoffels, IEEE Trans. Plasma Sci. **34**, 1317 (2006).
[9]V. L'eveill'e and S. Coulombe, Plasma Sources Sci. Technol. **14**, 467 (2005).
[10]F. J. Andrade, W. C. Wetzel, G. C. Y. Chan, M. R. Webb, G. Gamez, S. J. Ray, and G. M. Hieftje, J. Anal. At. Spectrom. **21**, 1175 (2006).
[11]N. K. Bibinov1, A. A. Fateev, and K. Wiesemann, J. Phys. D: Appl. Phys. **34**, 1819 (2001).
[12]N. Masoud, K. Martus, and K. Becker, J. Phys. D: Appl. Phys. **38**, 1674 (2005).
[13]S. Wang, V. Schulz-von der Gathen, and H. F. Döbele, Appl. Phys. Lett. **83**, 3272 (2003).
[14]T. Shimoo, K. Okamura, and Y. Morisada, J. Mater. Sci. **37**, 1793 (2002).
[15]G. V. Naidis, J. Phys. D: Appl. Phys. **30**, 1214 (1997).
[16]B. Eliasson, M. Hirth, and U. Kogelschatz, J. Phys. D: Appl. Phys. **20**, 1421 (1987).
[17]E. A. Sosnin, E. Stoffels, M. V. Erofeev, Ingrid E. Kieft, and S. E. Kunts, IEEE Trans. Plasma Sci. **32**, 1544 (2004).
[18]B. A. Freeman and J. D. Crapo, Lab. Invest. **47**, 421 (1982).
[19]D. Dreher and A. F. Junod, Eur. J. Cancer **32A**, 30 (1996).
[20]B. Halliwell and O. I. Aruoma, FEBS Lett. **281**, 9 (1991).
[21]H. S. Uhm, J. P. Lim, and S. Z. Li, Appl. Phys. Lett. **90**, 261501 (2007).