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Effects of atmospheric nonthermal plasma on invasion of colorectal cancer cells

Chul-Ho Kim,[1,2] Seyeoul Kwon,[3] Jae Hoon Bahn,[1] Keunho Lee,[4] Seung Ik Jun,[4] Philip D. Rack,[3,a)] and Seung Joon Baek[1,a)]
[1]*Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37996, USA*
[2]*Department of Otolaryngology, School of Medicine, Ajou University, Suwon 443-721, Republic of Korea*
[3]*Department of Materials Science and Engineering, University of Tennessee, Knoxville, Tennessee 37996, USA*
[4]*PSM America Inc., Colorado Springs, Colorado 80910, USA*

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The effect that the gas content and plasma power of atmospheric, nonthermal plasma has on the invasion activity in colorectal cancer cells has been studied. Helium and helium plus oxygen plasmas were induced through a nozzle and operated with an ac power of less than 10 kV which exhibited a length of 2.5 cm and a diameter of 3–4 mm in ambient air. Treatment of cancer cells with the plasma jet resulted in a decrease in cell migration/invasion with higher plasma intensity and the addition of oxygen to the He flow gas. © *2010 American Institute of Physics*. [doi:10.1063/1.3449575]

Tumor cell migration and invasion are critical steps in tumor progression and metastasis.[1] Half of all cancer deaths are either directly or indirectly due to local invasion, with or without involvement of regional lymph nodes.[2] Thus, controlling tumor invasion and metastasis is a crucial goal for treatment of various cancers.

Nonthermal atmospheric plasma treatments are gradually being investigated for biomedical applications and recent progress in understanding nonthermal, atmospheric plasmas has led to clinical applications.[3,4] Studies investigating the interaction of a plasma with living cells have shown inactivation of pathogens,[3,5,6] wound healing,[7] blood coagulation,[8] tissue sterilization,[8,9] and the ablation of cultured cancer cells.[10] The effects of nonthermal, atmospheric plasmas are due to active species, mainly oxygen/hydroxyl radicals and nitric oxide, generated in the plasma or in the tissue brought into contact with the plasma.[11] In fibroblast cells, plasmas affect migration. At a mild level of plasma exposures, the migration of fibroblasts is decreased, whereas at a medium level exposure, cells are detached from the extracellular matrix.[9] In this study, we investigate whether plasma treatment in colorectal cancer cells results in decreasing cell migration and invasion, and if so, whether the effects of the plasma on cell migration and invasion depend on the plasma intensity (i.e., plasma voltage) and oxygen concentration.

The technical specifications of the nonthermal atmospheric pressure plasma system, "torch with spray type," are presented schematically in Fig. 1. We have designed and manufactured a spray type plasma system with a designed arc-free and antistatic plate to provide uniform plasma for biological research applications. The plasma source is equipped with a pair of electrodes (high voltage and ground electrodes) that is isolated from direct contact with the plasma by a ceramic barrier, as shown in Fig. 1(a). The specifications of the power supply with this system are 2 kV minimum, 13 kV maximum, and mean frequency 20–30 kHz; these specifications can vary with the type and amount of gas used. In this study, helium (He) and oxygen (O_2) gases were used. Due to its unique inertness, high thermal conductivity and other unique physical properties, He allows the most stable low temperature, atmospheric plasma to be formed.[9] The visible plasma had a length of approximately 2.5 cm that varied with gas flow and voltage [Fig. 1(b)].

The emission spectra of several different nonthermal atmospheric pressure plasma plumes were measured by optical emission spectroscopy (Ocean Optics, S2000) in which the distance between nozzle and spectrometer was fixed at 10 mm; this was the same distance for the gas irradiated on the cell surface. Figure 2 shows the emission spectrum with different gases in the nonthermal, atmospheric pressure plasma, which compares the spectra for He and He+O_2 gases. All peaks were referenced from the Atomic Spectra Database of National Institute of Standards and Technology (http://

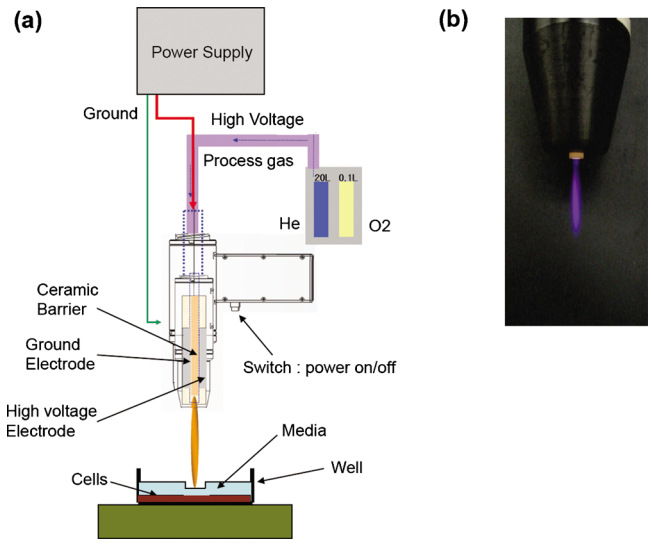

FIG. 1. (Color online) (a) Schematic diagram of the plasma torch. (b) Image of the plasma jet with helium and oxygen.

[a)]Authors to whom correspondence should be addressed. Electronic addresses: prack@utk.edu and sbaek2@utk.edu. Tel.: (865) 974-5344 and (865) 974-8216. FAX: (865) 974-4115 and (865) 974-5616.

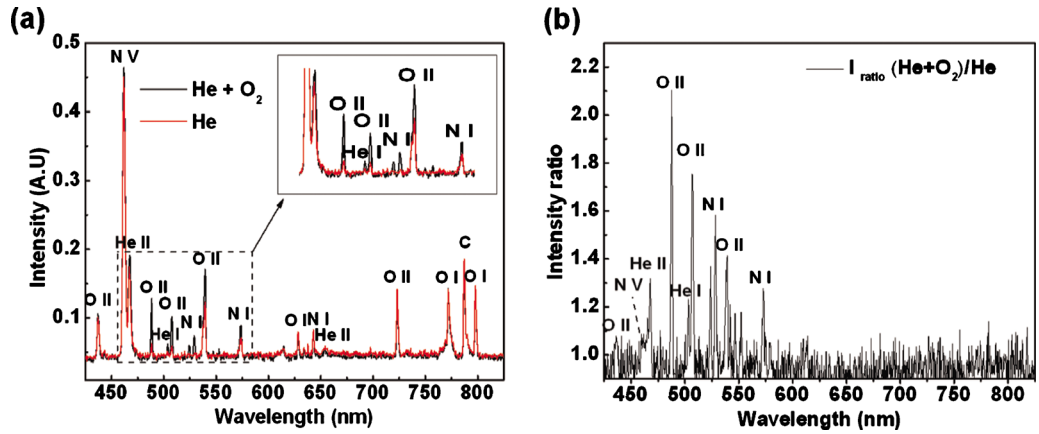

FIG. 2. (Color online) Plasma emission spectra with different gases, (a) comparison of He vs $He+O_2$ gases and (b) plot of peak ratio vs wavelength $[I_{ratio},(He+O_2)/He]$ at 5 kV.

physics.nist.gov/asd3). The plotted spectra were normalized in order to compare the relative intensity of the various peaks and to find any unique peaks for the different gas combinations. All emission peaks have O I, O II, He I, He II, N I, C I, and N V, which correspond to spectral lines of neutral (I), singly ionized (II), or quadruply ionized (V) species over the measured wavelength range; the peaks include signatures from air molecules, i.e., nitrogen, oxygen, and even carbon. In particular, different intensities appear in the range of 475 to 575 nm, as shown in the inset of Fig. 2(a). To better illustrate the spectral differences, the plasma emission spectra ratio $[(He+O_2)/He]$ is shown in Fig. 2(b). There were three particularly large peak changes with O II ($O_2 \rightarrow O_2^+ + e^-$ or $O \rightarrow O^+ + e^-$) at 488.5, 507.8, and 539.4 nm wavelengths, showing a 110% increase at 488.5 nm for the $He+O_2$ as compared with only He gas. On the other hand, between 625 and 800 nm, the oxygen peaks have the same ratio as do the nitrogen and carbon peaks, indicating that most of the gas peaks between 625 and 800 nm are associated with contributions from ambient air.

According to Moisan *et al.*,[12] there are two basic mechanisms involved in the plasma inactivation of microorganisms: (1) UV irradiation and (2) reactive oxygen atoms or radicals in plasma. We attribute the cell inactivation rate to reactive oxygen content since there were no measurable UV emission peaks in the range of 300 to 400 nm (spectra not shown). Therefore, plasmas with $He+O_2$ is more effective in cell inactivation and sterilization than He gas only, because of the higher reactive oxygen density as shown in Fig. 2(b); O II is believed to be the dominant contributor to cell inactivation and sterilization.

Figure 3 shows the result of plasma ($He+O_2$) emission peaks with different voltages, namely, 5 and 10 kV with a fixed distance between the plasma nozzle and the cells. All the spectral line intensities were significantly increased (up to 2–3×). These results are also consistent with cell sterilization behavior (data not shown), which was enhanced with increasing voltage. We attribute the effect to the fact that by applying higher voltage to the electrodes causes the O_2 dissociation rate to increase, due to the increased electron energy distribution in the plasma, thereby generating more reactive oxygen atoms or radicals.[13]

To investigate the effect of the plasma treatment on cell migration and invasion in colorectal cancer in the presence

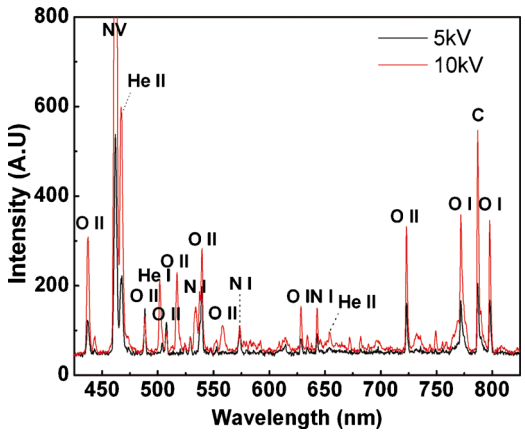

FIG. 3. (Color online) Comparison of atmospheric pressure plasma emission spectrum of $He+O_2$ gases with different voltages at 5 and 10 kV.

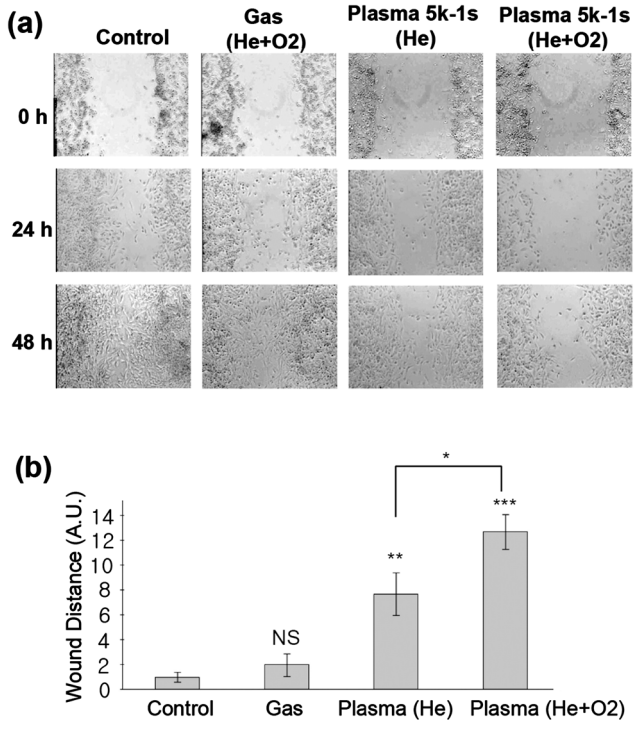

FIG. 4. Cell migration of colorectal cancer cells. (a) Photographs of the SW480 cells at 24 and 48 h after treatment. 100× magnification. (b) Quantification of cell migration assay from three independent experiments. $^{*}p<0.05$. $^{**}p<0.01$, and $^{***}p<0.001$ by student *t* test compared to control. NS, no significant; AU, arbitrary unit.

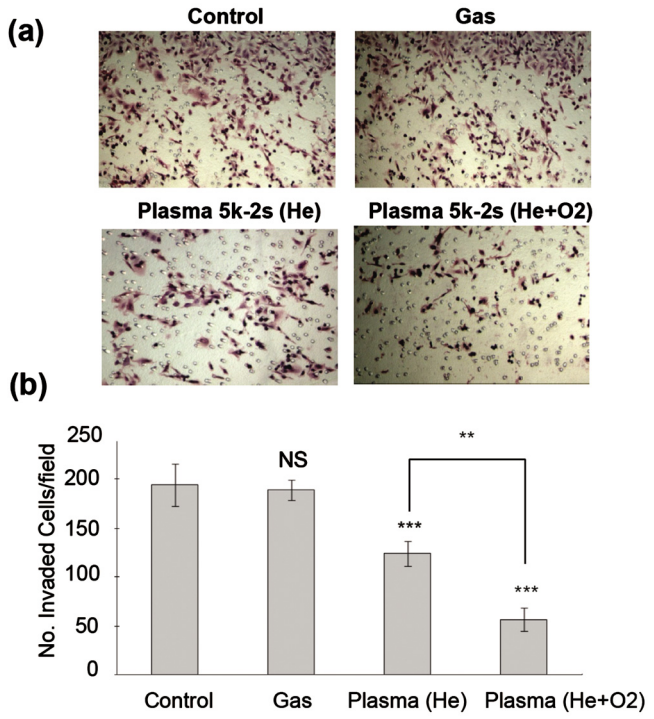

FIG. 5. (Color online) Invasion assay for SW480. (a) Photographs of the SW480 cell at 48 h after treatment. 100× magnification. (b) Quantification of invasion assay from three independent experiments. $^{**}p<0.01$, $^{***}p<0.001$ by student t test compared to control. NS, no significant.

or absence of oxygen, and whether the plasma optical emission spectra for He and $He+O_2$ correlate with the results of cell behavior, we performed migration and invasion assays. For the cell migration assay, SW480 cells were plated in culture plates at a density of approximately $1\times$ x 10^5/well and grown to confluency. The monolayer was scratched with a sterile pipette tip, and cells were exposed to gas only, a 5 kV He, and 5 kV $He+O_2$ plasma, respectively, for 1 s. The migration ratio was documented by photography at 0, 24, and 48 h. As shown in Figs. 4(a) and 4(b), the plasma significantly inhibited the migration and proliferation of SW480 cells ($p=0.0085$). However, inhibition of cell migration was improved in the $He+O_2$ plasma, compared to He-only plasma.

Tumor invasion requires degradation of basement membranes, proteolysis of extracellular matrix, pseudopodial extension, and cell migration. To make an *in vitro* environment for invasion assays, we used Transwell chambers (Costar) and matrigel. SW480 cells were seeded on filters (pore size 8 μm) coated with matrigel in the upper compartment of the Transwell chamber. After 48 h of incubation, cells plugged in 8 μm pores or cells attached to the undersurface of membrane were counted, and the cells attached in the lower section were stained with hematoxylin and eosin, and counted by optical microscopy. Figures 5(a) and 5(b) demonstrate that the plasma treatments significantly inhibited the number of cells that invaded, compared to untreated and gas only controls, and this invasiveness was more significantly inhibited in $He+O_2$ plasma, compared to control or gas only treatment ($p=0.0015$).

Gas minimally affects the migration and invasion of SW480 but not significantly. Since He, hydroxyl, and O_2 radicals induce cell death in cell culture systems,[10] it is likely that these radicals contribute to plasma-inhibited migration and invasion in colorectal cancer cells.

While stationary adult cells use the adhesion molecule–extracellular matrix interactions for anchorage, cancer cells appear to use these interactions for traction to migrate.[14] During cancer development, the increased expression of adhesion molecules (e.g., integrins, cadherins, and focal adhesion kinase) enables cancer cells to convert from a stationary to a migratory and invasive phenotype.[15] Therefore, inhibiting the expressions of adhesion molecules may be a viable strategy in cancer treatment. The observed decrease in migration and invasion for plasma treated cells reported here are attributed to the interactions of oxygen radicals generated by the plasma with the cell membrane; the reactive oxygen species can oxidize and thus break the cell adhesion molecules.[3,9,16]

In summary, nonthermal, atmospheric plasmas significantly inhibited cell migration and invasion in SW480 colorectal cancer cells. Oxygen addition to the helium plasma appears to improve efficiency in inhibition of migration and invasion of SW480 cells. *In vitro* results are consistent with the spectral data, showing that increasing the input power and adding oxygen to the gas produce effective reactive oxygen radicals which are critical to cell function.

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