Differential responses of human liver cancer and normal cells to atmospheric pressure plasma

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(Received 8 March 2011; accepted 22 May 2011; published online 10 August 2011)

When treated by atmospheric pressure plasma, human liver cancer cells (SK-HEP-1) and normal cells (THLE-2) exhibited distinctive cellular responses, especially in relation to their adhesion behavior. We discovered the critical threshold voltage of 950 V, biased at the electrode of the micro-plasma jet source, above which SK-HEP-1 started to detach from the substrate while THLE-2 remained intact. Our mechanical and biochemical analyses confirmed the presence of intrinsic differences in the adhesion properties between the cancer and the normal liver cells, which provide a clue to the differential detachment characteristics of cancer and normal cells to the atmospheric pressure plasma. © 2011 American Institute of Physics. [doi:10.1063/1.3622631]

Atmospheric pressure plasma (APP) has been suggested as a biomedical tool for its simplicity in application and its capability to generate abundant chemically reactive species.1–3 While sterilization of medical devices and removal/prevention of biofilm have been widely reported as the representative applications of atmospheric pressure plasma,1–4 recent attempts have been made on the direct application of plasma to wounded skin for enhancement of cellular healing process5,6 and to dental cavities for sterilizing the infected tissues.7 Recently, in vitro studies on a few mesenchymal and epithelial cell types have indicated that APP induces cellular necrosis, apoptosis, and detachment, suggesting its potential use in cancer cell removal.8–11 However, induction of such cellular changes would be meaningful in cancer therapy practices if and only if removal was selective to cancer cells leaving the normal cells unaffected.9 This requires a parallel comparison of cancer and normal cells from the same tissue under the controlled plasma condition. Therefore, in the present study, the responses of cancer and normal cells to the plasma treatment are compared to discover the behavioral difference between two cell types in their detachment characteristics from the substrate. We hypothesized that either physical or bio-chemical stresses were responsible for the plasma-induced changes in cells and performed sets of experiments to elucidate the mechanism behind the distinct response between cancer and normal cells [Figs. 1(a)–1(c)]. For instance, physical shear stress imposed by the gas flow, as shown in Fig. 1(b), could possibly deliver a shearing force to the adherent cells to peel them off from the substrate. In addition, bio-chemical stress induced by reactive oxygen species (ROS) can inflict damage on intracellular proteins as well as surface proteins such as those involved in focal adhesions (FAs), leading to the detachment of the cells [Fig. 1(c)].

For the plasma applicator, we used a single pin electrode type micro jet plasma, whose detailed description is reported elsewhere12 and the additional information is provided in the supplementary material.13

To study the effects of plasma on cellular viability, we stained cells using live/dead assay14 following a brief exposure of cells to APP (950–1200 V applied voltage, 50 kHz driving frequency, 2 min treatment time with 2 slpm Helium gas) and noticed the presence of differential responses between two cell types. As shown in Figs. 2(a)–2(d), cells begin to detach from the surface leaving a void above a critical voltage. At higher voltages, over 1000 V, three distinctive regions are observed; the dead cell region at the center, the live cell region at the periphery and the void region along the interface between the live and dead zones [Figs. 2(c) and 2(d)]. The dead zone consists of necrotized cells, and the void zone is the blank area of no cells, both of which collectively refer to the plasma effective zone (PEZ) [Figs. 2(b)–2(d)].

When the plasma above a critical voltage impinged on the monolayer of the cells covered by phosphate buffered saline solution, we observed cells starting to detach from the substrate and forming a sheared area, as shown in Fig. 1(b). The detached cells are then released from the substrate, as shown in Fig. 1(c), after a few minutes. The mechanism of the plasma detachment is followed in the supplementary material.13

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FIG. 1. (Color online) The description of (a) an adherent cell to ECM coated substrate through surface receptor protein integrins, (b) a cell detachment forced by physical stress, and (c) bio-chemical stress.
substrate along the periphery of the plasma edge while cells at the center region became necrotized.\textsuperscript{14} At the lowest applied voltage of 950 V, the liver metastatic cancer SK-HEP-1 cells started to detach from the substrate as shown in Fig. 2(b). Unlike the case for SK-HEP-1, the normal liver THLE-2 cells remained intact at the same applied voltage of 950 V [Fig. 2(a)]. At the highest applied voltage of 1200 V, SK-HEP-1 exhibited a much enlarged plasma effective zone including both the dead and void zones as shown in Fig. 2(d). To quantify the distinctive response of these two different cell types, we plotted the fraction of live cells as depicted in Fig. 2(e). SK-HEP-1 cells, having a larger PEZ and a smaller fraction of live cells, seem more susceptible to the plasma than THLE-2 at all applied voltages. Also, the quantitative results in Fig. 2(f) clearly show that the SK-HEP-1 void zone is distinctively larger than that of the THLE-2 cells by more than 1.5 times, which led us to question the possible existence of fundamental differences in the adhesion strength and ability to respond to external disturbance among different cell types.\textsuperscript{11,14}

In our results with metastatic cancerous SK-HEP-1 and normal THLE-2 cells, the important question was whether the differential detachment characteristics arise from the intrinsic distinction between cancer and normal cell types in the liver epithelium. Based on our observations, we hypothesized that SK-HEP-1 cells would have weaker adhesion strength than THLE-2 cells resulting in easier detachment, which would also be consistent with the characteristics of highly motile metastatic cells compared to that of sessile type normal cells. In this scenario, when certain plasma properties, either physical or biochemical, trigger cells to detach from the substrate, cancer cells would detach more readily due to their weaker nature in adhesion. To investigate which of the physical or biochemical stress would have a dominant effect on cellular detachment, we performed a couple of experiments. Although our previous study on THLE-2 cells has shown that the gas flow alone does not inflict intracellular changes,\textsuperscript{14} there still remains a possibility that the drag force of the liquid medium induced by the impinging gas flow can impose physical shear stress on the adherent cells. To test the possibility of functional dominance of physical shear stress in the plasma induced detachment of cells, we designed a set of experiments using commercial micro-channels (µ-Slide V0.4 ibidi GmbH) and investigated whether SK-HEP-1 and THLE-2 responded differentially to shear stress.\textsuperscript{13}

The results shown in Fig. 3(a) indicate no significant difference in the adhesion strength against physical shear stress between SK-HEP-1 and THLE-2 in all tested magnitudes from 10 to 40 dyn/cm$^2$. The critical shear stress at which only 50% of the cells remain attached reflects the physical adhesion strength of each cell. In our case, the critical shear stresses for SK-HEP-1 and THLE-2 were measured to be 25.62 dyn/cm$^2$ and 25.87 dyn/cm$^2$. Since our shear experiment confirms that the difference in cellular detachment characteristics induced by physical shear stress is insignificant between the two cell types, we conclude that the observed differential responses in SK-HEP-1 and THLE-2 upon plasma treatment are unlikely due to physical shearing imposed by the gas flow.

The next candidate for cell detachment is the biochemical stresses from various plasma species. Since the chemical species in the plasma have been shown to play critical roles to induce changes in intracellular cytoskeletal proteins as well as surface adhesion proteins,\textsuperscript{8,11,14} we designed a set of experiments to test the susceptibility of cells to biochemical stresses causing them to detach from the surface. Because the chemical species originated from the plasma are likely to alter membrane proteins causing cellular detachment in such a short term treatment of 5 min, the detachment experiment due to biochemical stress was limited to the digestion of integrins serving as the linkage between the extracellular matrix (ECM) on the substrate and the cells. To realize the appropriate experimental conditions, we treated the cells with trypsin-ethylenediaminetetraacetic acid (EDTA), which is the most well known clipper of biotic anchors including integrins. As shown in Figs. 3(b) and S1(c), when cells were...
As shown in Fig. 4(c), we find that there exists almost twice repeatedly counted as mature FA and their lengths are measured. Insets in (a) and (b) show the z axis-sectioned confocal microscopy images (scale bar = 20 μm). (c) Number of FA of THLE-2 and SK-HEP-1 obtained by paxillin dots from the confocal images. (d) Distribution of focal adhesion dots of THLE-2 and SK-HEP-1.

FIG. 4. (Color online) Immunofluorescence image of paxillin dots (green) and actin stress fibers (red) of (a) THLE-2 and (b) SK-HEP-1 (scale bar = 20 μm). Consistent to these results, the immunofluorescence images indicate stronger and more pronounced paxillin dots in the FA. Interestingly, the distribution of the lengths of the number of mature FA in a single THLE-2 cell than those in a single SK-HEP-1 cell averaged over 30 cells in 10 different images. Interestingly, the distribution of the lengths of focal dots is similar in both cell types except that only THLE-2 cells feature very large FA whose length is longer than 7 μm [Fig. 4(d)], possibly indicating more mature and stronger focal dots is similar in both cell types except that only THLE-2 cells feature very large FA whose length is longer than 7 μm [Fig. 4(d)], possibly indicating more mature and stronger FA. To test whether these phenotypic differences in FA correlate with their activity, western blot assay was performed for focal adhesion kinase (FAK) and z5 integrin protein [Figs. S4(a) and S4(b)]. As shown in Fig. S4(a), more FAK proteins exist in THLE-2 than in SK-HEP-1. Moreover, the quantitative value of z5 integrin protein, which is a part of FA, consistently reveals that THLE-2 has stronger coupling to the ECM by showing more z5 integrins on THLE-2 [Fig. S4(b)]. These results from liver cells are consistent with our aforementioned hypothesis, where the presence of intrinsic difference in adhesion properties between cancer and normal cells leads to differential detachment behavior upon application of the atmospheric pressure plasma, and the detachment is achieved by biochemical disruption of anchorage proteins rather than physical tearing off from the substrate.

To support our hypothesis, the similar experiments were performed on another set of cancer and normal cells from the mammary gland epithelium (MDA-MB-231 vs. MCF10A) [Figs. S5–S7]. These two cells also exhibited consistent detachment characteristics where the void zone of cancer cells formed after plasma treatment was larger than that of normal cells [Fig. S5(b)].

In summary, we found that the cancer cells (SK-HEP-1) detached more readily compared to normal cells (THLE-2) upon a short treatment by atmospheric plasma. Based on the results from the biophysical and biochemical assays, SK-HEP-1 was found to feature weaker adhesion strength than THLE-2, exhibiting different responses against plasma treatment, which seems to inflict biochemical stress to disrupt the integrin anchorage of cells at the cell-ECM interface. This difference in cellular adhesion property between two cell types attributes to the intrinsic physiological difference of focal adhesions between the two cancer and normal cells in hepatocytes.

The authors thank Wonjong Song, Sunghyun Kim, Unghyun Ko Sukhyun Song, Se Youn Moon, and Sunhee Kim for their initial contribution in experiments. This work was in part supported by KAIST.