Influence of Cold Plasma Atmospheric Jet on Surface Integrin Expression of Living Cells

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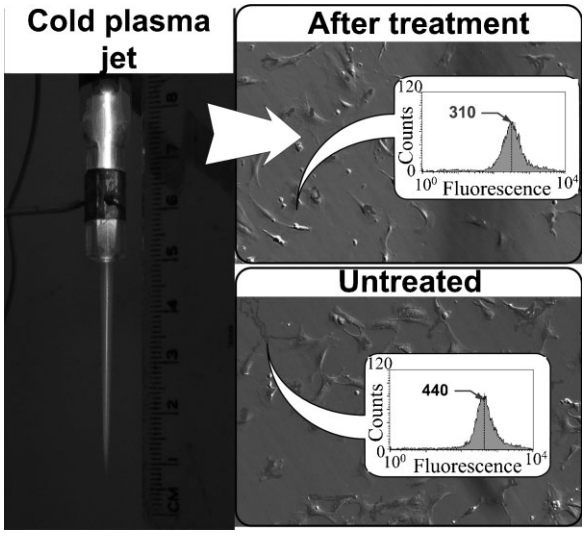
The effects induced in cells due to treatment with cold atmospheric plasma jet are studied. Cell migration rate is measured by means of time-lapse microscopy. In order to characterize cell surface integrin expression, the fluorescent response of cells after surface integrins are stained with specific antibodies is measured by flow cytometry. We show that treatment of cells with plasma jet affects the cells on sub-cellular level, namely decreases expression of cell surface integrins (β_1 and α_v integrins were tested). This change in integrin expression might be the original cause for the effects observed on cellular level, such as reduced cell migration rate and cell detachment observed experimentally.



Introduction

Atmospheric plasmas are being intensively applied in biomedicine at present time.[1] Stoffels et al.[2,3] demonstrated the non-traumatic nature of plasma needle driven by radio-frequency discharge and observed the detachment of cells from the extracellular matrix. It was concluded that plasma can interact with organic materials without causing thermal/electric damage to the surface. Other effects of atmospheric plasma on living tissue includes the eradication of yeast grown on agar,[4] ablation of cultured liver cancer cells,[5] blood coagulation and tissue sterilization[1] and decrease of migration rate of fibroblast cells.[6]

Plenty of possible applications of jets in biomedicine have stimulated an interest in studying their physical properties and involved mechanisms. Time-resolved visual observations of the jets by means of intensified CCD cameras indicated a discontinuous nature of the jet. It was shown that the jet consists of sequence of "bullet-like" structures shooting from the discharge channel at each breakdown event and propagating with velocities from $\approx 10^6$ up to $8 \times 10^7\ \mathrm{cm \cdot s^{-1}}$.[7–10] Another series of studies looked at the radiation from jets by means of optical emission spectroscopy.[11–13] Ionized and excited states of oxygen, nitrogen, helium, and hydroxyl radicals were detected, and the temperature of heavy species was measured and found to be close to room temperature. Recently, scattering of the microwave radiation on the jet was studied.[10] It was found that afterglow plasma column

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Plasma Process. Polym. **2010**, *7*, 294–300

DOI: 10.1002/ppap.200900086

remains on the path of the streamer passing. The plasma column had longer lifetimes, than streamer, and thus may be responsible for the effects induced in cells.

Thus, various effects of atmospheric plasma on living tissue have been demonstrated recently.[1–6] However all the studies cited consider the responses of whole cells or group of cells (cellular level effects) to plasma jet treatment such as detachment from the extracellular matrix, slowing down the migration rate, while its influence on the components of individual cell (sub-cellular level effects) and mechanism of plasma affect were not studied. So, the elementary cell components being affected by the jets are unknown. The understanding of plasma jet effect on a level of elementary cell components (on sub-cellular level) is required to predict and/or control the cell responses. In this study we evaluated the influence of plasma jet on integrin expression at the cell surface using fibroblast cells (α_v and β_1) by means of the flow cytometry technique. Integrins are the receptors responsible for the cell adhesion to extracellular matrix and they define cellular shape and mobility.[14] In order to explore the elementary cell components affected by plasma jet treated and to shed light on the mechanism of jet–tissue interaction at an "elementary level", the primary objective of this paper was to determine whether plasma treatment altered the integrin expression at the cell surface.

Experimental Part

New insights on fundamentals about interaction of a plasma jet with a living tissue could be gained by investigation of its interaction with primary cultured cells. The primary fibroblast cells and PAM cells, a mouse epithelial cell line (PAM cells were the generous gift of Dr. Luowei Li, Laboratory of Cancer Biology and Genetics, NCI, NIH) were used in this study.[15] Fibroblasts are a type of cells that synthesizes and maintains the extracellular matrix of many animal tissues and PAM cells are skin cancer cells. Cell cultures were grown on the bottom of the six-well plates (35 mm in diameter) using methodology described in ref.[16] Cells were treated by plasma gun from the distance of 2 cm from the outlet syringe hole as shown in Figure 1a. Plasma gun used high voltage resonant transformer and operated at voltages up to 10 kV and frequencies 25–30 kHz. More details about the plasma gun can be found in ref.[6,10] Typical photo of the plasma jet is presented in Figure 1b. Three groups of cells were examined: treated with plasma jet, treated with He only, and untreated cells.

Cell Tracking

Immediately, following the plasma jet treatment cells were tracked in several locations of the well by repetitive taking images of those locations (the fibroblast cells grown in standard media for 3 days were used). Imaging was performed on an Olympus IX81 research microscope equipped with a Proscan motorized stage and a

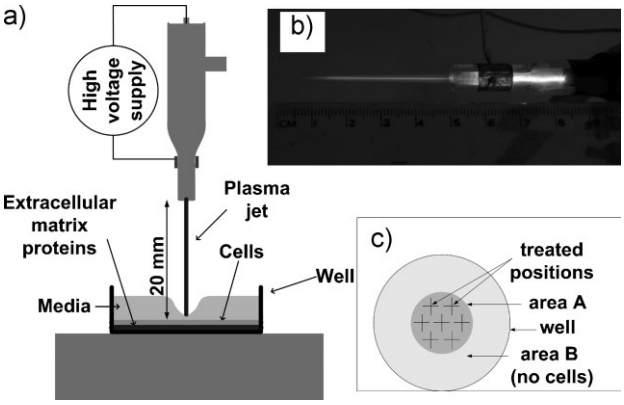

Figure 1. a) Schematic view of the plasma gun, b) typical photograph of plasma jet at $U = 3.5$ kV and $\upsilon_{fl} = 15\ \mathrm{L \cdot min^{-1}}$, and c) well containing cells only in area A. Cells in area A were treated in seven positions, at points labeled by crosses. Diameter of plasma jet at point of contact with media was 2–3 mm.

temperature- and CO_2-controlled chamber. Cells were maintained in normal cell culture media containing serum at 37 °C and 7% CO_2. Using relief-contrast optics, 10 × images were taken of each well every 10 min (for 16 h 40 min) until 100 images were captured. Images were then transferred to a workstation equipped with Metamorph image analysis software, where velocities were calculated using the track cell module (20 cells per location were tracked). In the cell tracking experiments Helium flow rate was $17\ \mathrm{L \cdot min^{-1}}$, discharge voltage was $U = 5$ kV (±200 V), and the treatment duration was 5 min (±5 s). The amount of protecting media was kept the same during whole treatment by periodical adding of fresh media (to compensate desiccation). Note, that media was flushed away from the treatment center so that the media thickness far away from the treated location was about 3 mm and in the center of treated zone – less than 1 mm (Figure 1a). This depleted area had diameter of about 4–5 mm.

Flow Cytometry

Flow cytometry studies were conducted immediately after the plasma treatment. Fibroblast cells grown in standard media for 4 d were used (4-day cells were used for flow cytometry instead of 3-day cell used for cell tracking in order to obtain denser cell culture and reach number of cells sufficient for flow cytometry analysis).[16] Cells were scratched from the area B of the well using a pipette tip, but were left undisturbed in area A (≈15 mm in diameter) as shown in Figure 1c. Treatment with plasma jets consisted in consecutive exposition of seven adjacent positions shown by crosses in Figure 1c. Each position was exposed to the plasma jet for 5 min and thickness of protecting media was kept about 2 mm during whole treatment by periodical (once in every 100 s) addition of fresh media. The 2 mm thickness of protecting media refers to the undisturbed media level far away from the treated location and was thinner at the very point of treatment (less than 1 mm) as shown in Figure 1a. The characteristic diameter of this depleted area was about 4–5 mm. Cell scratching from part of well was used

in order to achieve trade-off between two goals, namely make sure that all cells in the well were treated (to prevent the contribution of untreated cells located outside from the area A on flow cytometry results) and keep the overall treatment time in reasonable limits. Plasma source was operated in flow cytometry studies at more moderate conditions (namely He flow of 15 $L \cdot min^{-1}$ and discharge voltage −3.5 kV), than that in cell tracking experiments in order to decrease the risk of "frozen" cell appearance (see Results below).

The procedure of sample preparation for the flow cytometry was as follows (refers to a single well of the six-well plate). The well was washed twice in PBS in order to remove fibroblast cell culture media and cells were then trypsinized using 2 mL of 0.25% Trypsin-EDTA for 10 min in a 37 °C-7% CO_2 incubator. As a result of trypsinization, the cells were detached from the well and were distributed in the solution. Thereafter, 2 mL of fibroblast media containing 10% serum was added in order to inactive the trypsin. Cells were then counted using a Beckman Coulter counter and aliquoted into three tubes in volumes corresponding to 2×10^5 cells per tube. Then cells were centrifuged at 1 000 rpm for 5 min, solution removed, and staining antibody was added to 1st and 2nd tubes: 1 μL of a phycoerythrin PE-conjugated antibody diluted in 100 μL of blocking buffer (PBS supplemented with 3% BSA). Cells from the 1st tube were stained with either β_1 or α_v integrin antibody conjugated with PE; cells from the 2nd tube – with a non-specific PE conjugated control antibody; and cells from the 3rd tube were supplemented with 100 μL of blocking buffer alone (Biolegend, http://www.biolegend.com, #102207 and 104105 were used for β_1 and α_v integrin antibodies, respectively). All three tubes were then incubated for 30 min at 4 °C. Cells from the 1st, 2nd, and 3rd tubes will be indicated as Stained, Control, and Unstained cells, respectively. After staining, cells were washed in blocking buffer to remove unbound antibody and then resuspended in 1 mL of PBS. A FACSCalibur (BD Biosciences) flow cytometer with an air-cooled argon laser and CELLQuest Software was used. The flow cytometer measured light scattering properties and fluorescence intensity of cells excited by 488 nm laser. It should be noted that fluorescence patterns were unique for each cell culture plating session. Variations in average fluorescence intensity of cells stained with antibody plated on different days often reached up to 30%, due to changes in culture, media etc. At the same time, such variations within same plating session were small – up to 5%–7% (will be explained in more details below). Therefore, all parameters and comparisons using the absolute values of fluorescence intensities were based on cells from the same plating session.

Flow cytometry procedure described in previous paragraph represents a standard procedure used in studies of integrin expression at the cell surface. [17–20] We use the term integrin expression at the cell surface to refer to the number of integrin heterodimers that are at the cell surface and to differentiate the data we obtain using flow cytometry from data obtained using other methods that measure total integrin steady state protein levels. Cell surface integrins and the ability of integrins to bind antibodies have been shown to be insensitive to trypsin-EDTA treatment.[18] In addition, immediately after trypsination, test tubes containing harvested cells were kept at 4 °C in order to prevent movement of integrins present at the cell membrane into intracellular vesicles during the flow cytometry studies and backwards from the cell inside to its membrane. This standard approach is used to ensure that integrin expression at the cell surface does not change during the analysis and results obtained reflect that seen immediately following treatment.[21]

Results and Discussion

Cell Detachment and Migration

Typical photographs of fibroblast cells and PAM cancer cells after treatment with plasma gun are presented in Figure 2 (He flow-17 $L \cdot min^{-1}$, $U = 5$ kV). The results presented in this paragraph were obtained for the cells covered with only 1.5 mm of protecting media (this small amount of media was used to obtain complete flushing of the media from the point of treatment and provide direct contact of jet with cell culture). The effect observed on PAM cancer cell was similar to that observed recently on fibroblasts,[6] namely three zones with different cell morphologies were observed. First, corresponded to the highest intensity of treatment (media was completely displaced away from this zone due to flow from the syringe and the cells were not covered by the media) and located in vicinity of treated center was filled with cells which did not migrate or proliferate over 16.6 h of observation under the microscope (were called "frozen" cells in our previous work[6]). Subsequent use of the trypan blue assay indicated that the cells in this zone were dead.[22] The cells in this central zone which did not move stained positive with trypan blue indicating that they had been killed by the treatment. A second zone, located further from the treatment center (about 2 mm from the center), corresponded to mild treatment intensity and was filled with live, actively migrating cells. Third region, located on the interface between 1st and 2nd regions had no cells. Cells were detached from this region forming voids shown in Figure 2 (located at about 2 mm from the jet center for experimental conditions specified in caption to the Figure 2) and were released into the media. The size of the voids was much larger with fibroblast cells than that with PAM cells indicating that PAM cell culture was more firm with respect to plasma jet treatment. Those detached cells were collected

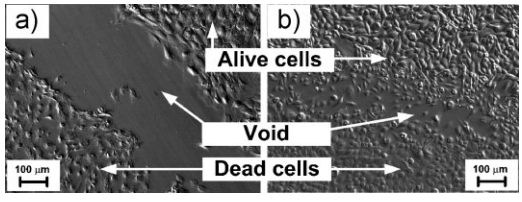

Figure 2. Photographs of a) fibroblast cells and b) PAM cancer cells immediately after treatment with plasma gun $U = 5$ kV, He flow – 17 $L \cdot min^{-1}$, 1.5 mm of protecting media. Photographs were taken at the jet periphery – about 2 mm from the jet center.

DOI: 10.1002/ppap.200900086

and re-plated into new well. None of the released cells attached and grew leading to conclusion that detached cells were not viable.[22]

We performed simulation of helium plasma jet interaction with background air. Our analysis was limited to a single axial cut through the beam and azimuthal symmetry was assumed. These simplifications reduced the problem to one dimension. We used the reaction rates identical to the ones used by Sakiyama and Graves.[23] The simulated distributions of electric field and potential are shown in Figure 3 (position $r = 0$ indicates the jet center, 1D model in radial direction). One can see that electric field has a peak shifted about 0.7 mm from the jet axis. It is interesting to note that cell detachment area was shifted from the jet center as well (see voids in Figure 2).

Results presented below in this section (migration rate and flow cytometry) were obtained with larger amount of protecting media, so that media was never completely flushed away from the treated location and plasma jet was always separated from the cells by some amount of protecting media. Details on the amounts of media in migration rate and flow cytometry studies have been described above in Methodology. In experiments below, no voids or "frozen" cells were found and the cells remained migrating.

Cell tracking experiments were carried out using a large amount of media (3 mm instead of 1.5 mm). In this case cells were covered by the media during whole time of treatment. The typical tracks obtained after 16.6 h of migration for cells treated with plasma (jet was directed to the center of well and 20 cells in each of four positions located in 1–2 mm from the well center were tracked, 80 cells total) and untreated cells (20 cells in each of four positions located in 1–2 mm from well center and two locations in 1 cm from the well center were tracked, 120 cells total) are presented in Figure 4a and b, respectively. It was found that treated cell tracks are significantly shorter than that of untreated cells and correspond to about a two-fold decrease of cell migration rate.[6]

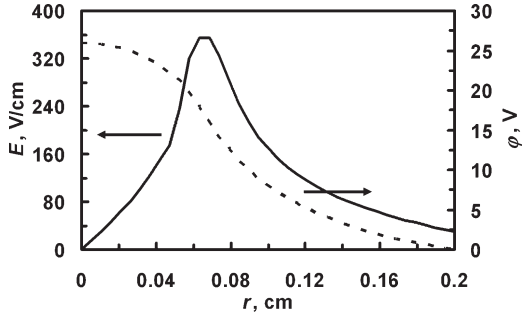

Figure 3. Radial distributions of potential and electric field in and in the vicinity of the plasma jet.

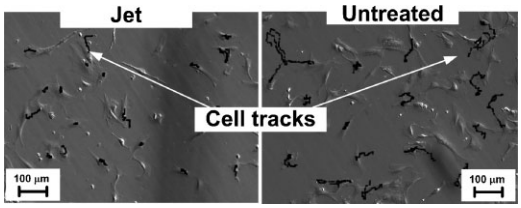

Figure 4. Cells tracks after 16.6 h of migration: a) cells treated with plasma jet – 5 kV, 3 mm of protecting media and b) untreated cells.

Integrin Expression

The scattering pattern of cytometer laser beam is presented in Figure 5. Columns indicate different types of treatment: cells treated with plasma, cells treated with He flow only, and untreated cells; while rows present different types of staining: Stained, Controls, and Unstained. This dot plot correlates forward scatter (small angle light scatter) and side scatter (right angle light scatter), indicators of cellular size and complexity (shape, optical properties). It was observed that the black contour chosen as shown in Figure 5 includes >94% of total number of cells for all types of treatment and staining. This means that cell scattering patterns were similar irrespective of type of treatment and staining, indicating that structural cell properties were not affected by treatment and/or staining. This bounded area contains typical undamaged cells and those cells were taken into account for the subsequent fluorescent signal analysis.

Typical flow cytometry histograms of untreated cells undergo different types of staining: a) stained with β_1 antibody, b) controls, and c) unstained are presented in left column of Figure 6. Plots in Figure 6 show the fluorescence response of cells upon irradiation with cytometer laser. The distributions shown are obtained by accumulation of fluorescence signals from 10^4 cells; the x-axis shows fluorescence intensities in relative units, and the y-axis represents the number of events with corresponded intensity. It was observed for all experiments that average fluorescence intensities ($I_{\beta 1}$) of unstained cells and controls (Figure 6b and c) were about 4–7. This value indicates cellular autofluorescence and gives level of background. Cells whose surface integrins were stained with the β_1-integrins-PE-conjugated antibody expressed about two orders of magnitude higher fluorescence response – $I_{\beta 1} = 440$ (Figure 6a). This increase of fluorescence intensity is caused by fluorescence of antibodies binding to the β_1-integrins on the cell surface.

The right column of Figure 6 presents flow cytometry histograms of cells treated with plasma jet (Figure 6d–f indicate cells stained with β_1-integrins-PE-conjugated antibody, controls and unstained cells, respectively). Controls and unstained cells demonstrate the level typical

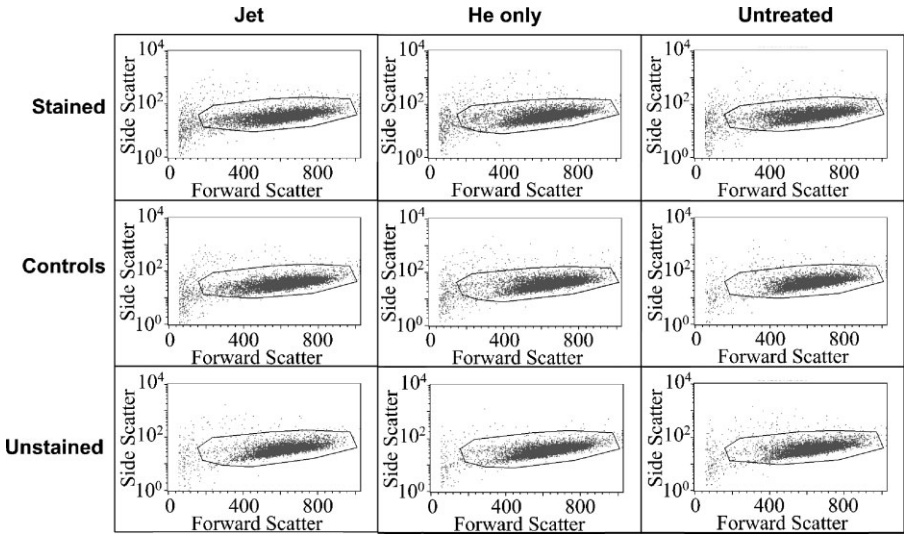

Figure 5. Light scattering properties of cells, forward scatter vs. side scatter. Area limited by black contour indicates typical undamaged cells (these cells were taken into account below). Columns – different cell treatment (with plasma jet, with He flow only, untreated); rows – different types of staining.

for autofluorescence. $I_{\beta 1} = 5.5$. Similar as above Stained cells demonstrate much higher $I_{\beta 1} = 310$ (Figure 6a). However the mean fluorescence intensity is significantly less (about 30%) than that for untreated cells (440).

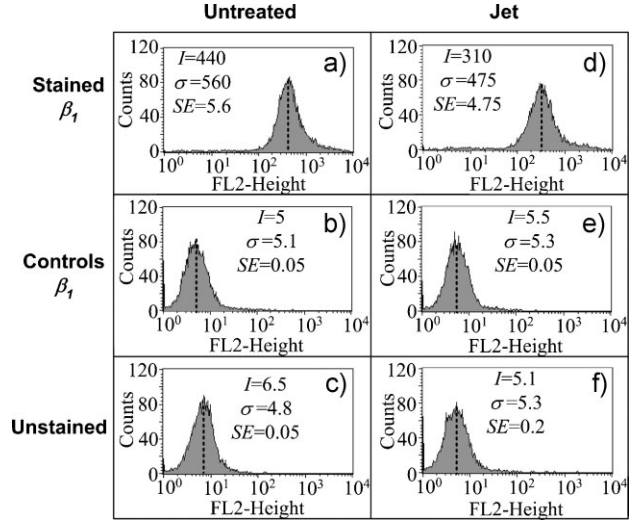

Figure 6. Flow cytometry histograms indicating the distribution of fluorescence intensities of 10^4 cells ($\beta 1$ integrin staining). Rows – types of staining (stained, controls and unstained); columns – types of treatment (untreated and treated with plasma jet). Low level of fluorescence for unstained cells and controls gives the cell autofluorescence level. Much higher fluorescence of stained cells is caused by antibodies bound to cell surface integrins. β_1 integrin expression for stained treated cells was about 22% lower than that for untreated. The locations of distribution peaks are indicated by dotted lines.

Flow cytometry histograms for α_v-integrins are shown in Figure 7: left column – untreated cells (stained (a), controls (b), unstained(c)), right column – treated cells (stained (d), controls (e), unstained (f)). Similar to the previous case controls and unstained cells demonstrated a low level of fluorescence $I_{\alpha v} = 5$–6, while stained cells expressed much higher $I_{\alpha v}$ (49 and 56 for treated and untreated cells, respectively).

The histograms summarizing change of average fluorescence responses after cell treatment, and their deviations are presented in Figure 8. Let us consider in more details the statistical set used to confirm the trends presented in Figure 8. We analyzed cells (cultured in different plating sessions and on different days) from 5 wells treated with plasma and from 18 untreated wells. Cells from each well were run on the flow cytometer typically three times. The total number of flow cytometery runs for treated with plasma cells was 13 and untreated cells was 43. Each flow cytometer run used the statistical sample of 10^4 cells ensuring that standard errors are small. For the data presented in Figures 6 and 7 the means (I) and standard deviations (σ) of treated stained cells were $I_{\beta 1} = 310$ and

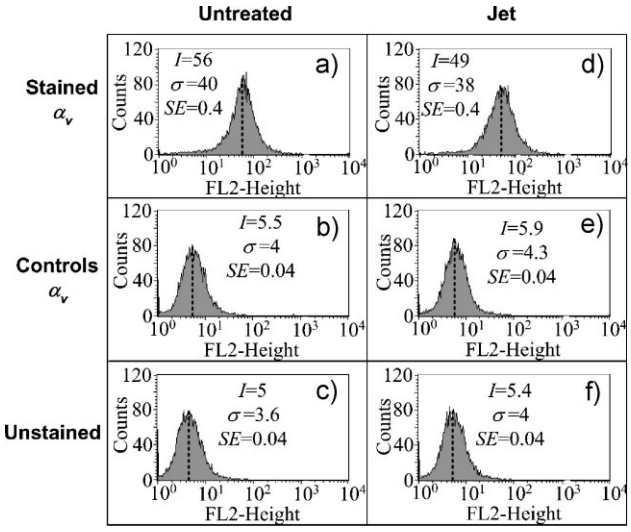

Figure 7. Flow cytometry histograms for different types of cell treatment (untreated and treated with plasma jet) and staining (stained with α_v integrin antibody, controls α_v, unstained). α_v integrin expression for stained treated cells is about 10% lower than that for untreated. The locations of distribution peaks are indicated by dotted lines.

DOI: 10.1002/ppap.200900086

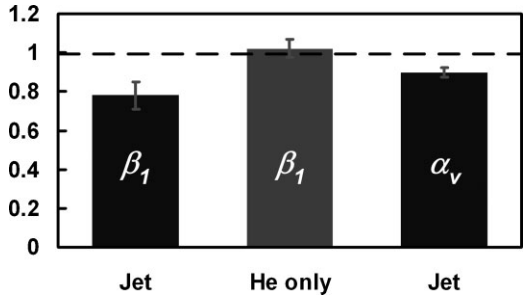

Figure 8. Average fluorescence of treated with plasma cells (stained with β_1 and α_v -integrins-PE-conjugated antibodies) and treated with He only cells (stained with β_1-integrins antibody) normalized to fluorescence of untreated cells stained with corresponded antibody.

$\sigma_{\beta 1} = 475$ for β_1-integrins, and $I_{\alpha v} = 49$ and $\sigma_{\alpha v} = 38$ for α_v-integrins, respectively. Using that statistical sample of $N = 10^4$ cells was used, the standard errors of means ($SE = \sigma/\sqrt{N}$) were 4.75 and 0.4 for β_1 and α_v-integrins, respectively, or in percents from the corresponding means were 1.5 and 1% for β_1 and α_v-integrins, respectively (Figures 6 and 7). The error bars presented in Figure 8 indicate the whole range of experimental points observed in flow cytometry studies (no experimental points were observed outside the bars). Thus, the fluorescence intensity of cells treated with plasma in percents from fluorescence intensity for untreated cells was $78\% \pm 8\%$ for β_1 integrins and $90\% \pm 2.5\%$ for α_v-integrins. The fluorescence intensity from cells treated with He in percents from that for untreated cells was $102\% \pm 4.6\%$.

It should be noted that in this work we addressed the influence of plasma jets integrin expression at the cell surface and cell mobility, and we have not addressed the causes of these changes. These causes may include enhancement of chemical reactions in cells due to activation of cell signaling induced by species presented in plasma jet (ions, radicals, etc.). Establishing correlations between plasma jet effects on cellular/sub-cellular levels and the accompanying changes in cell signaling can help us to better understand the underlying reasons and mechanisms of the observed effects, and together with exploring the influence of treatment intensity on effects induced in cells may be the subject of future work.

To summarize, we observed that treatment of cells with plasma jet resulted in a decrease of cell fluorescence responses: 22% and 10% for staining of β_1 and α_v-integrins, respectively. This clearly indicates that plasma treatment reduces expression of β_1 and α_v surface integrins. Thus, it may be suggested that one of the elementary cell components being affected by plasma treatment is cell surface integrins. Since cell surface integrins are responsible for cell adhesion and mediate cell migration,[14] changes in integrin expression may be responsible for the effects observed experimentally including the decrease in migration rate and cell detachment after plasma jet treatment (note, the dependence of migration rate on cell-substrate adhesiveness has non-monotonic bell-shaped type, with maximal migration rate corresponded to the intermediate level of cell adhesiveness to substrate).[24–26]

Acknowledgements: This research was supported by the *Institute of Bioengineering of George Washington University*. The authors would like to thank *M. Taghioskoui* for preparing the fibroblast cell cultures used in these studies. This work was also supported in part by *Princeton Plasma Physics Laboratory University Support program* (sponsored by DOE). We thank *Dr. Yevgeny Raitses* for useful discussions. The efforts of *M. A. Stepp, S. Pal-Ghosh and R. A. Jurjus* were supported by *NIH RO1-EY08512* to *M. A. Stepp*.

Received: June 1, 2009; Revised: November 1, 2009; Accepted: November 24, 2009; DOI: 10.1002/ppap.200900086

Keywords: cold plasma; cell culture; flow cytometry; integrin expression; radiation

[1] G. Fridman, G. Friedman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, A. Fridman, *Plasma Processes Polym.* **2008**, *5*, 503.
[2] E. Stoffels, I. E. Kieft, R. E. J. Sladek, L. J. M. van den Bedem, E. P. van der Laan, M. Steinbuch, *Plasma Sources Sci. Technol.* **2006**, *15*, S169.
[3] E. Stoffels, A. J. Flikweert, W. W. Stoffels, G. M. W. Kroesen, *Plasma Source Sci. Technol.* **2002**, *11*, 383.
[4] J. F. Kolb, A. A. H. Mohamed, R. O. Price, R. J. Swanson, A. Bowman, R. L. Chiavarini, M. Stacey, K. H. Schoenbach, *Appl. Phys. Lett.* **2008**, *92*, 241501.
[5] X. Zhang, M. Li, R. Zhou, K. Feng, S. Yang, *Appl. Phys. Lett.* **2008**, *92*, 021502.
[6] A. Shashurin, M. Keidar, S. Bronnikov, R. A. Jurjus, M. A. Stepp, *Appl. Phys. Lett.* **2008**, *93*, 181501.
[7] X. Lu, M. Laroussi, *J. Appl. Phys.* **2006**, *100*, 063302.
[8] N. Mericam-Bourdet, M. Laroussi, A. Begum, E. Karakas, *J. Phys. D: Appl. Phys.* **2009**, *42*, 055207.
[9] B. L. Sands, B. N. Ganguly, K. Tachibana, *Appl. Phys. Lett.* **2008**, *92*, 151503.
[10] A. Shashurin, M. N. Shneider, A. Dogariu, R. B. Miles, M. Keidar, *Appl. Phys. Lett.* **2009**, *94*, 231504.
[11] M. Laroussi, X. Lu, *Appl. Phys. Lett.* **2005**, *87*, 113902.
[12] X. Lu, Z. Jiang, Q. Xiong, Z. Tang, X. Hu, Y. Pan, *Appl. Phys. Lett.* **2008**, *92*, 081502.
[13] X. Lu, Z. Jiang, Q. Xiong, Z. Tang, Y. Pan, *Appl. Phys. Lett.* **2008**, *92*, 151504.
[14] R. O. Hynes, *Cell* **1987**, *48*, 549.
[15] D. R. Roop, P. Hawley-Nelson, P. C. K. Cheng, S. H. Yuspa, *J. Invest. Dermatol.* **1983**, *81*, 144s.
[16] R. A. Jurjus, Y. Liu, S. Pal-Ghosh, G. Tadvalkar, M. A. Stepp, *Wound Rep. Regul.* **2008**, *16*, 649.
[17] A. Buda, D. Qualtrough, M. A. Jepson, D. Martines, C. Paraskeva, M. Pignatelli, *Gut* **2003**, *52*, 729.

[18] *Integrin Protocols (Methods in Molecular Biology)*, A. Howlett, Eds., Humana Press Inc., Totowa, NJ 1999.
[19] M. A. Stepp, S. Spurr-Michaud, I. K. Gipson, *Invest. Ophthalmol. Vis. Sci.* **1993**, *34*, 1829.
[20] M. M. Sczekan, R. L. Juliano, *Eur. J. Biochem.* **1990**, *189*, 83.
[21] M. A. Stepp, Y. Liu, S. Pal-Ghosh, R. A. Jurjus, G. Tadvalkar, A. Sekaran, K. Losicco, L. Jiang, M. Larsen, L. Li, S. H. Yuspa, *J. Cell Sci.* **2007**, *120*, 2851.
[22] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, 4th edition, Garland Science, New York 2002.
[23] Y. Sakiyama, D. Graves, *J. Appl. Phys.* **2007**, *101*, 073306.
[24] S. P. Palecek, J. C. Loftus, M. H. Ginsberg, D. A. Lauffenburger, A. F. Horwitz, *Nature* **1997**, *385*, 537.
[25] L. Y. Koo, D. J. Irvine, A. M. Mayes, D. A. Lauffenburger, L. G. Griffith, *J. Cell Sci.* **2002**, *115*, 1423.
[26] E. Puklin-Faucher, M. P. Sheetz, *J. Cell Sci.* **2009**, *122*, 179.

DOI: 10.1002/ppap.200900086