Development of a cold atmospheric pressure microplasma jet for freeform cell printing

Halim Ayan,[1,a)] Eda D. Yildirim,[2] Daphne D. Pappas,[3] and Wei Sun[4,5,b)]
[1]*Department of Engineering and Physics, Murray State University, Murray, Kentucky 42071, USA*
[2]*Bioengineering Department, University of Toledo, Toledo, Ohio 43606, USA*
[3]*United States Army Research Laboratory, Aberdeen Proving Ground, Maryland 21005, USA*
[4]*Department of Mechanical Engineering, Drexel University, Philadelphia, Pennsylvania 19104, USA*
[5]*Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China*

(Received 8 July 2011; accepted 23 August 2011; published online 12 September 2011)

An atmospheric pressure non-thermal microplasma jet (Ø 50 μm) was developed for localized functionalization of various substrates, including polymers, to allow maskless freeform cell printing. The applied microplasma jet power ranged from 0.1 to 0.2 W without causing any damage to the polyethylene substrate. The surface characterization results demonstrate that the microplasma treatment locally changes the surface roughness and the concentration of oxygen-containing functional groups on the polyethylene surface. The biological characterization confirms that the osteoblast cells attach and survive on the plasma activated line while untreated surfaces show almost no attachment and viability. © *2011 American Institute of Physics*. [doi:10.1063/1.3638062]

The ability to align cells and proteins and to guide their functions by providing engineered and designed environments has been of strong interest for a wide range of diagnostic, therapeutic application, and fundamental studies.[1] In living tissue environments, cells are surrounded by topographical and biochemical cues which assist them to attach, align, and guide their cell-cell and cell-substrate interactions.[2] However, most biopolymers are often lacking in adequate surface structural or biochemical cues themselves without additional surface functionalization.

Today, a wide variety of techniques have been developed to enhance the surface functionality with patterns to align cells. Examples include conventional photolithography,[3,4] soft lithography,[5] microcontact printing,[6] direct writing,[7] and laser ablation.[8] These enabling surface treatment techniques can provide additional structural, chemical, and/or biological cues that regulate cell morphology as well as the subsequent cellular function.[9,10] However, in these techniques, the surface functionalization has been done by promoting cell adhesive or resistant biomolecules to the surface through patterned masks or with master stamps and requires preparatory steps. The masking and master stamping techniques further necessitate clean room instrumentation, long processing time, complex chemistry, and use of solvents that may denature or degrade the deposited bioorganic layers.[11–13] Above all, the requirement of mask and master stamps restricts the flexibility in patterning process while increasing the operating costs.[14]

Due to the aforementioned limitations, plasma-based surface treatment has recently gained considerable interest.[15] In general, microplasma discharge technology (miniaturization of the plasma down to characteristic micro feature size) has found application in display panels,[16] material processing,[17] and analytical instruments.[18] Also, microplasmas have been of interest in biology and biomedicine, particularly for cancer therapy,[19] disinfection of liquids,[20] decontamination of living human tissue,[21,22] and surgery.[23] Though versatile and efficient, most enabling plasma technologies have to use "hot" plasma discharges and operate at low pressure (below atmospheric pressure) which makes it difficult for modifying heat sensitive biopolymers and biological substrates. Besides, the conventional plasma needle discharges are in the order of millimeters in size[22,23] and is inadequate to produce the micro-scale surface patterning needed for cell and biomolecule printing.

In this paper, the authors introduce an approach for cell and biologics printing, namely, freeform surface patterning and cell printing. A helium-oxygen microplasma jet system operating at atmospheric pressure was developed to functionalize the surface of polyethylene in a freeform pattern. Microplasma is capable of creating tens-of-micron size patterns and printing cells on biopolymers without using any masks, master stamps, or any prior chemical treatments as would be required in the lithography and soft-lithography techniques. The microplasma nozzle system is driven by a computer controlled 3-D motion system. An in-house developed computer program was utilized to fully manipulate motion along each of the 3 axes.[24]

The plasma system was based on the concept of dielectric barrier discharges (DBD) combining the advantages of non-thermal and atmospheric pressure plasma processing.[25,26] Fig. 1 shows the schematic view of the plasma system and its components. The microplasma jet system contained a high voltage copper electrode (Ø 1 mm) which was inserted coaxially in a dielectric (borosilicate glass) tube with an outer diameter of 6 mm and a wall thickness of 0.8 mm. The ground electrode was wrapped around the dielectric tube. Once the high voltage is applied to the centric electrode, breakdown is initiated in the annular space between the high voltage electrode and the ground electrode. Plasma is allowed to propagate through the flow of the operating gas (or gas mixture) from the plasma generation point onto the biopolymer surface through a nozzle tip with a 50 μm diameter. Fig. 2 shows the nozzle tip with no plasma (a) and with plasma (b), respectively.

[a)]Electronic mail: hayan@murraystate.edu.
[b)]Electronic mail: sunwei@drexel.edu.

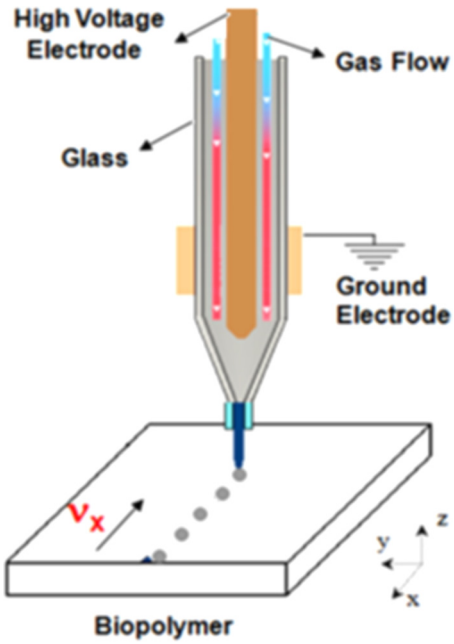

FIG. 1. (Color online) Microlasma jet system and its components (drawing is not to scale).

The microplasma jet was powered by a pulsed power supply that can generate up to 35-kV peak voltage with 100 Hz-1 kHz repetition rate and 1.5 μs full-width at half-maximum (FWHM) of the voltage pulse. The microplasma system was typically operated in the range of 0.1-0.2 W. The gas mixture consisted of 200 ml/min He and 0-25 ml/min O_2. Oxygen is required to produce active oxygen species for the functionalization of the polymer surface.[27,28] However, oxygen is a highly electronegative gas and causes excessive attachment and eventually quenching of the discharge if it is not accompanied by another gas that can sustain a stable non-thermal plasma, such as helium.[29,30] In Fig. 3 microplasma images with various He-O_2 mixtures are presented. The plasma exhibits its highest intensity when 100% He is used (Fig. 3(a)) and gets dimmer and more restricted as the O_2 levels increase (Fig. 3(b)). Eventually, it cannot be carried out of the nozzle because of the quenching effect of elevated O_2 content (Fig. 3(c)).

To obtain the simple pattern on the polyethylene surface, the microplasma jet system was moved with a speed of

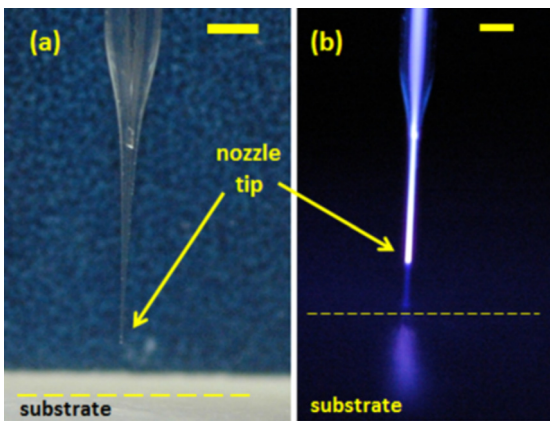

FIG. 2. (Color online) The microplasma nozzle system (a) power-off, (b) microplasma in contact with the substrate (scale bars: 500 μm).

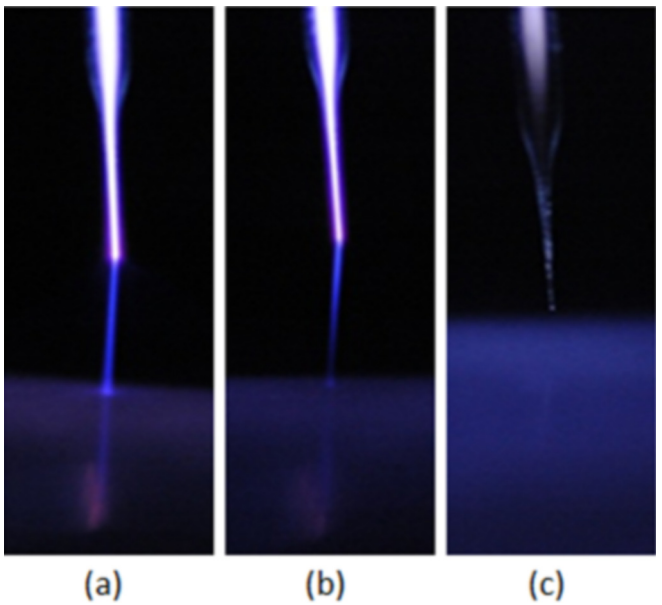

FIG. 3. (Color online) The microplasma with various He-O_2 content. (a) 100% He, (b) 97.5% He + 2.5% O_2, and (c) 87.5% He + 12.5% O_2. Images were taken in a dark room with 190 Hz pulse repetition rate and 0.62 s exposure time corresponding to superposition of about 120 voltage pulses.

2 mm/s on a straight line over the polyethylene surface while the plasma was ignited with 200 ml/min He and 2.6 ml/min O_2. The distance of the nozzle tip from the polymer surface was 2 mm. The effects of microplasma jet treatment on the polyethylene were characterized by several methods. The scanning electron microscopy (SEM) image of a patterned polyethylene sample is given in Fig. 4. The elongated lines seen on the virgin polyethylene surface are due to the manufacturing process as the material is biaxially stretched (Goodfellow, 75 μm thick). However, after the plasma treatment the surface topography of the patterned area looks very different than the as-received polyethylene surface. It is clear that the surface topography and roughness were changed along the plasma treated line while the rest of the substrate remained unaffected. A well-defined ~10 μm thick line was patterned on the polyethylene surface without using any mask or master stamps.

The surface chemical composition changes on the microplasma patterned surface, expressed through the oxygen and carbon concentration on the plasma functionalized surface, were measured by x-ray photoelectron spectroscopy (XPS). A 100 W monochromatic A1 Ka (1486.7 eV) beam irradiated a 12 μm × 12 μm sampling area with a take-off angle of 90°. Elemental high resolution scans for C1s and O1s were taken at the pass energy of 20 eV. The XPS

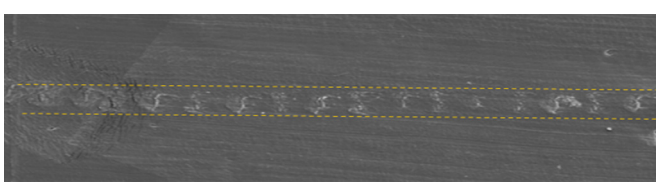

FIG. 4. (Color online) SEM image of freeform maskless patterned polyethylene surface. Distance between dashed lines is approximately 10 μm. (Gas flow rate: 202.6 ml/min, gas composition: 98.8% He + 1.2% O_2, nozzle distance: 2 mm, nozzle speed: 2 mm/s).

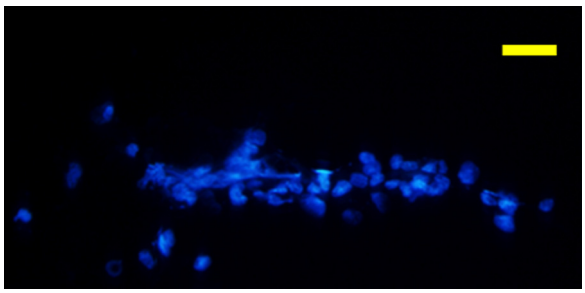

FIG. 5. (Color online) Labeled osteoblast cells attached on patterned area (scale bar: 50 μm).

data shows that the fraction of carbon-containing groups decreased with the plasma exposure while the concentration of oxygen containing functional groups increased. The atomic concentration of oxygen increased from 5% for the virgin polymer to 18% for the plasma treated material along the center of the plasma footprint. The highest concentration of oxygen (or lowest amount of carbon) was observed on the center location of the patterned line and started to decrease by the lateral distance (up to ~2 mm).

Following the patterning process, mouse osteoblast cells (7F2 – #CRL-12557, ATCC, VA) were deposited on the polyethylene surface to assess the effect of microplasma treatment on cell organization. Cell suspensions, with 10^6 cells/ml concentration, were pipetted immediately after the microplasma treatment, and samples were placed into an incubator (37 °C, 5% CO_2) for 2 h. After incubation, the substrates were vigorously rinsed with phosphate-buffered saline (PBS) to remove the unattached cells. The remaining cells were labeled with bisbenzimide (Hoechst 33258) to make the nuclei of the attached cells visible.

Fig. 5 shows the fluorescence images of cell nuclei labeled with bisbenzimide that are attached on the patterned area after the PBS wash. Images show that the attachment and survival of the osteoblast cells takes place mostly along the plasma treated line while untreated surfaces showed almost no attachment and viability. This demonstrates that the cells which adhered on the surface showed significantly high degree of adhesion strength. Although the observed widths of the morphologically modified surface (Fig. 4) and the chemically activated areas were found to be 10 μm and ~2 mm, respectively, the size of the patterned cell is about that of microplasma (50 μm). This suggests that the reason for the enhanced cell adhesion is due to the combinatorial effect of increased surface roughness and modified surface chemistry along the pattern. Slight dispersion of the cells (Fig. 5, left-hand side) can be attributed to the presence of micro-roughness and traces of oxygen residue on the virgin polyethylene.

In conclusion, a He-O_2 microplasma jet was developed and operated at atmospheric pressure. Surface functionalization/modification (physical and chemical) was achieved on polyethylene. The biological test showed that surface functionalization resulted in the attachment and survival of the osteoblast cells, specifically along the plasma treated pattern. This simple and effective single-step process enables cell and/or biomolecule printing for future biofabrication and tissue engineering applications.

This study was funded in part by the National Science Foundation.

[1]R. Singhvi, A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. C. Wang, G. M. Whitesides, and D. E. Ingber, Science **264**, 696 (1994).
[2]K Anselme, Biomaterials **21**, 667 (2000).
[3]R. Michel, J. W. Lussi, G. Csucs, I. Reviakine, G. Danuser, B. Ketterer, J. A. Hubbell, M. Textor, and N. D. Spencer, Langmuir **18**, 3281 (2002).
[4]F. L. Yap and Y. Zhang, Biomaterials **28**, 2328 (2007).
[5]G. M. Whitesides, E. Ostuni, S. Takayama, X. Y. Jiang, and D. E. Ingber, Annu. Rev. Biomed. Eng. **3**, 335 (2001).
[6]A. Offenhausser, S. Bocker-Meffert, T. Decker, R. Helpenstein, P. Gasteier, J. Groll, M. Moller, A. Reska, S. Schafer, P. Schulte, and A. Vogt-Eisele, Soft Matter **3**, 290 (2007).
[7]D. J. Odde and M. J. Renn, Trends Biotechnol. **17**, 385 (1999).
[8]P. Li, U. Bakowsky, F. Y. Yu, C. Loehbach, F. Muecklich, and C. M. Lehr. IEEE Trans. Nanobiosci. **2**, 138 (2003).
[9]G. J. Bakeine, J. Ban, G. Grenci, A. Pozzato, S. Dal Zilio, M. Prasciolu, L. Businaro, M. Tormen, and M. E. Ruaro, Microelectron. Eng. **86**, 1435 (2009).
[10]L. Altomare and S. Fare, J. Appl. Biomater. Biomech. **6**, 132 (2008).
[11]H. Hwang, G. Kang, J. H. Yeon, Y. Nam, and J. K. Park, Lab Chip **9**, 167 (2009).
[12]D. Falconnet, A. Koenig, T. Assi, and M. Textor, Adv. Funct. Mater. **14**, 749 (2004).
[13]A. Khademhosseini, G. Eng, J. Yeh, P. A. Kucharczyk, R. Langer, G. Vunjak-Novakovic, and M. Radisic, Biomed. Microdevices **9**, 149 (2007).
[14]K. B. Lee, Y. Kim, and I. S. Choi, Bull. Korean Chem. Soc. **24**, 161 (2003).
[15]J. P. Frimat, H. Menne, A. Michels, S. Kittel, R. Kettler, S. Borgmann, J. Franzke, and J. West, Anal. Bioanal. Chem. **395**, 601 (2009).
[16]S.-J. Park, C. J. Wagner, C. M. Herring, and J. G. Eden, Appl. Phys. Lett. **77**, 199 (2000).
[17]D. Staack, B. Farouk, A. Gutsol, and A. Fridman, Plasma Sources Sci. Technol. **14**, 700 (2005).
[18]M. Miclea, K. Kunze, J. Franzke, and K. Niemax, Spectrochim. Acta, Part B **57**, 1585 (2002).
[19]J. Y. Kim, S.-O. Kim, Y. Wei, and J. Li, Appl. Phys. Lett. **96**, 203701 (2010).
[20]Y. Sakiyama, T. Tomai, M. Miyano, and D. B. Graves, Appl. Phys. Lett. **94**, 161501 (2009).
[21]M. Laroussi and X. Lu, Appl. Phys. Lett. **87**, 113902 (2005).
[22]Y. C. Honga and H. S. Uhm, Appl. Phys. Lett. **89**, 221504 (2006).
[23]E. Stoffels, A. J. Flikweert, W. W. Stoffels, and G. M. W. Kroesen, Plasma Sources Sci. Technol. **11**, 383 (2002).
[24]E. Yildirim, X. Yin, K. Nair, and W. Sun, J. Biomed. Mater. Res., Part B: Appl. Biomater. **87B**, 406 (2008).
[25]H. Ayan, D. Staack, G. Fridman, A. Gutsol, Y. Mukhin, A. Starikovskii, A. Fridman, and G. Friedman, J. Phys. D: Appl. Phys. **42**, 125202 (2009).
[26]H. Ayan, G. Fridman, A. Gutsol, V. N. Vasilets, A. Fridman, and G. Friedman, IEEE Trans. Plasma Sci. **36**, 504 (2008).
[27]E. D. Yildirim, H. Ayan, V. N. Vasilets, A. Fridman, S. Guceri, G. Friedman, and W. Sun, Plasma Processes Polym. **5**, 397 (2008).
[28]E. Yildirim, R. Besunder, D. Pappas, F. Allen, S. Güçeri, and W. Sun, Biofabrication **2**, 1 (2010).
[29]S. J. Kim, T. H. Chung, S. H. Bae, and S. H. Leem, Appl. Phys. Lett. **94**, 141502 (2009).
[30]D. Staack, B. Farouk, A. Gutsol, and A. Fridman, Plasma Sources Sci. Technol. **17**, 025013 (2008).