

Radio-Frequency Plasma-Induced Biocompatibility of Polyimide Substrates

Magdaleno R. Vasquez, Jr.,^{a,*} Eloise I. Prieto,^b & Motoi Wada^c

^aDepartment of Mining, Metallurgical, and Materials Engineering, College of Engineering, University of the Philippines, Diliman, Quezon City 1101, Philippines; ^bNational Institute of Molecular Biology and Biotechnology, College of Science, University of the Philippines, Diliman, Quezon City 1101, Philippines; ^cGraduate School of Science and Engineering, Doshisha University, Kyotanabe, Kyoto 610-0321, Japan

*Address all correspondence to: Magdaleno R. Vasquez, Jr., DMMME Bldg., Velasquez St. cor. C.P. Garcia Ave., UP Diliman, Quezon City 1101, Philippines; Tel.: +63 2 981 8500, x3132, E-mail: mrvasquez2@up.edu.ph

ABSTRACT: Surfaces of polyimide (PI) sheets were modified using 13.56-MHz radio-frequency discharges to enhance their affinity with fibroblast cells. Physico-chemical analysis of pristine and plasma-treated PI sheets showed different responses against argon (Ar), oxygen (O₂), and nitrogen (N₂) as the plasma process gases. Overall, hydrophilicity of treated PI surfaces was enhanced, and its surface free energy increased from 53 dyn/cm to at least 73 dyn/cm. Surface roughness values also increased from 1.3 to 40.8 nm, as demonstrated by atomic force microscopy analyses. Infrared spectral analyses showed a decrease of imide functional group peak intensities on plasma exposure, corresponding to chemical surface structural changes. In addition, plasma-treated surfaces significantly increased cell adhesion and proliferation compared to pristine samples. N₂-containing plasma exhibited the greatest increase among the test gases, due to the possible inclusion of N₂-based functional groups that enhance biochemical affinity of fibroblast cells.

KEYWORDS: polyimide, plasma treatment, biocompatibility, biofunctionalization, cell adhesion, cell proliferation

I. INTRODUCTION

Polyimides (PIs) are characterized by their superior flexibility, high thermal stability, high chemical resistance, and low dielectric constant.^{1,2} Thus, PIs are useful for industrial applications such as microelectronics^{3,4} that include wire insulation, liners, and substrates. In addition to their excellent physical properties and ability to be sterilized, PIs have been determined to elicit no cytotoxic response and low hemolysis,⁵ making them ideal for biomedical applications. Recent work includes the use of PIs as biosensor encapsulants and substrates for neural implants,⁶ regenerative-medicine applications,⁷ cell-culture applications,⁸ and other implantable electronic sensor and stimulation systems.⁹ PIs, although demonstrated to be biocompatible, still lack cell-interactive properties that would make them appropriate for tissue-repair applications.⁷ PI substrates therefore need to be surface-modified to improve cell-adhesion and -proliferation properties. Among the surface treatment techniques, plasma-based treatment has been used to improve biomolecular affinity of polymeric substrates^{10–12} and adhesion of different materials.¹³ This dry process is quick, does not affect the bulk properties of the substrates, and does not pro-

duce toxic by-products. Plasma discharge provides a mixture of neutrals, ions, electrons, radicals, metastables, and ultraviolet radiation, making it a versatile process for tailoring properties of polymeric materials that are located a few nanometers from the surface. By varying gas composition and operating parameters such as incident power, exposure time, and gas-flow rate, precise surface functionalization and activation can be realized.

The present work explores the biochemical response of pristine and plasma-treated PI substrates. Degreased substrates were subjected to a 13.56-MHz radio-frequency (RF) plasma discharge at subatmospheric pressures using different process gases. After plasma treatment, physical and chemical changes of the substrates were compared to untreated samples. Cell-adhesion and -proliferation properties were also examined using a human fibroblast cell line.

II. EXPERIMENTAL DETAILS

A. Surface Preparation and Characterization

Commercially available PI (Kapton, The Nilaco Corp.; Tokyo, Japan), with a thickness of 0.05 mm, was cut into 1-cm squares. The substrates, degreased through subsequent sonication in acetone, ethanol, and water baths for 10 min each, were blow-dried using nitrogen gas. Immediately after degreasing, the samples were transported in ambient conditions, mounted on a flat glass plate, and placed inside a plasma reactor (Fig. 1), a glass bell jar with a diameter of 165 mm and height of 170 mm. The chamber was terminated by a flange with an RF-powered electrode that also acted as a sample mount. The distance between electrode and gas outlet was ~55 mm. Details of the plasma system can be found in Cagomoc and Vasquez.¹⁴ The system was evacuated by a rotary pump to ~8 Pa before the working gas was introduced through a needle valve. We used argon (Ar),

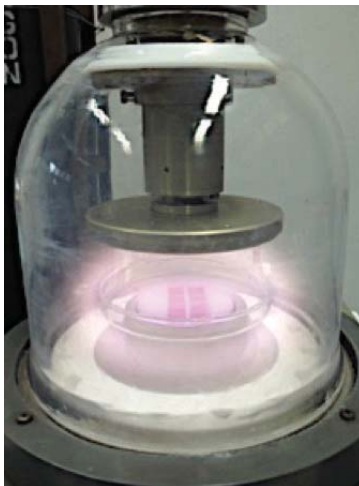


FIG. 1: Image of PI sheets undergoing plasma treatment

nitrogen (N₂), and oxygen (O₂) gases. Operating pressure was maintained at 100 Pa for all experiments, and exposure time was set to 30 min. Plasma was excited by capacitively coupled 13.56-MHz RF power, with a manual matching circuit that minimized the reflected power. Incident RF power was set to 50 W for all runs. After plasma treatment, the samples were wrapped in aluminum foil for sterilization, which was conducted in a convection oven (Heratherm, Thermo Fisher Scientific; Waltham, MA) for 3 hr at 180°C. After sterilization, samples were cooled and prepared for biocompatibility experiments.

We used atomic force microscopy (AFM) for topographic analyses of untreated and plasma-treated PI substrates using XE-Bio AFM (Park Systems; Suwon, Korea) under an intermittent mode. Silicon- and aluminum-coated cantilevers (PPP-NCHR, 10 M; Park Systems Inc.; Suwon, Korea) were used that had a typical spring constant of 42 N/m⁻¹. Surface free energy (SFE) was determined from the Owens–Wendt equation¹⁵ through contact-angle (θ) measurements of water (polar) and diiodomethane (nonpolar) test liquids as follows:

$$\frac{\gamma_l(1+\cos\theta)}{2} = \left[\sqrt{\gamma_l^d \gamma_s^d} + \sqrt{\gamma_l^p \gamma_s^p} \right] \quad (1)$$

where γ_l corresponds to surface tension of the liquid; γ_l^d and γ_l^p to dispersive and polar components of liquid surface tension, respectively; and γ_s^d and γ_s^p to dispersive and polar components of solid surface energy. A Fourier-transform infrared (FTIR) spectrophotometer (Shimadzu IR-Prestige 21; Kyoto, Japan) was used to investigate chemical characteristics of the untreated and plasma-treated PI surfaces.

B. Cell Adhesion and Proliferation

1. Cell Culture and Seeding of Substrates

We used human gingival fibroblasts (HGF-1) from the American Type Culture Collection (Rockville, MD). These cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Millipore Sigma; St. Louis, MO) that was supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific; Waltham, MA) and 0.5 % penicillin–streptomycin (Thermo Fisher Scientific; Waltham, MA) at 37°C in a 5% CO₂ humidified incubator. Cells were seeded onto PI substrates inside a 24-well cell-culture plate at 15,000 cells per well. Cell-attachment and proliferation assays were then performed on days 1 and 3 after seeding.

2. Cell-Attachment Assays

Cell adhesion on plasma-treated PI substrates was assessed using the LIVE/DEAD Viability/Cytotoxicity Assay Kit (Molecular Probes/Invitrogen; Carlsbad, CA). Substrates were stained with 2 μ M calcein acetoxymethyl and 4 μ M ethidium homodimer (EthD-1)

solution, and the percentage of cell coverage was analyzed by fluorescence micrographs using ImageJ software.¹⁶ Polystyrene tissue-culture (TC) dishes served as a positive control, and pristine substrates served as a negative control.

3. Cell-Proliferation Assays

Cell proliferation on plasma-treated PI substrates was evaluated using PrestoBlue (Molecular Probes/Invitrogen; Carlsbad, CA), a reagent that is reduced by metabolically active cells. The substrates seeded with HGF-1 were incubated with PrestoBlue, after which solution fluorescence was measured with Varioskan Flash (Thermo Fisher Scientific; Waltham, MA) at an excitation and emission wavelength of 560 and 590 nm, respectively. Higher fluorescence values corresponded to greater total metabolic activity. Polystyrene TC dishes served as positive control and pristine substrates as negative control. Obtained fluorescence values were normalized with cells grown on TC dishes.

III. RESULTS AND DISCUSSION

A. Physico-Chemical Analyses of Surfaces

One of the major surface parameters that determine cell–material interaction is surface hydrophilicity. It has been demonstrated that cell-adhesion proteins show better binding to hydrophilic surfaces than to hydrophobic surfaces, leading to more effective cell attachment on hydrophilic materials.¹⁷ We found that hydrophilicity of PI surfaces significantly improved after plasma treatment, with Ar plasma having a noteworthy increase in hydrophilicity [Fig. 2(a)]. Compared to the 60° contact angle of the pristine PI surface, treated samples had considerably lower mean contact angles of 10°, 11°, and 31° for Ar, O₂, and N₂ plasma treatment, respectively. Correspondingly, SFE also increased for all treated substrates [Fig. 2(b)]. Because surface hydrophilicity is influenced by modifications in surface morphology and chemistry, analyses of PI substrates were conducted via AFM and FTIR spectroscopy to determine the effect of plasma treatment on these surface parameters.

Figure 3(a)–(d) show representative 2 × 2-μm² AFM images of pristine and plasma-treated substrates and their corresponding root-mean-square (RMS) roughness. It is evident from the images that plasma treatment of PI substrates resulted in variations in surface morphology. This was confirmed with increased surface roughness, from 1.3 nm for pristine samples to 9.8, 14.6, and 40.8 nm for Ar, O₂, and N₂ plasma-treated samples, respectively [Fig. 3(e)]. These physical changes may be attributed to plasma energetic particles impinging on the PI substrate, thereby etching its surface. Reactive gases in the discharge such as O₂ and N₂ also contributed to the increase in roughness through attachment of functional groups on the surface.

FTIR spectral analyses of pristine and treated samples are shown in Fig. 4 (FTIR spectrum images). Figure 4(a) shows typical IR absorption spectra of pristine and plasma-modified PI surfaces. Imide functional groups are apparent at 1780 cm⁻¹ (C=O

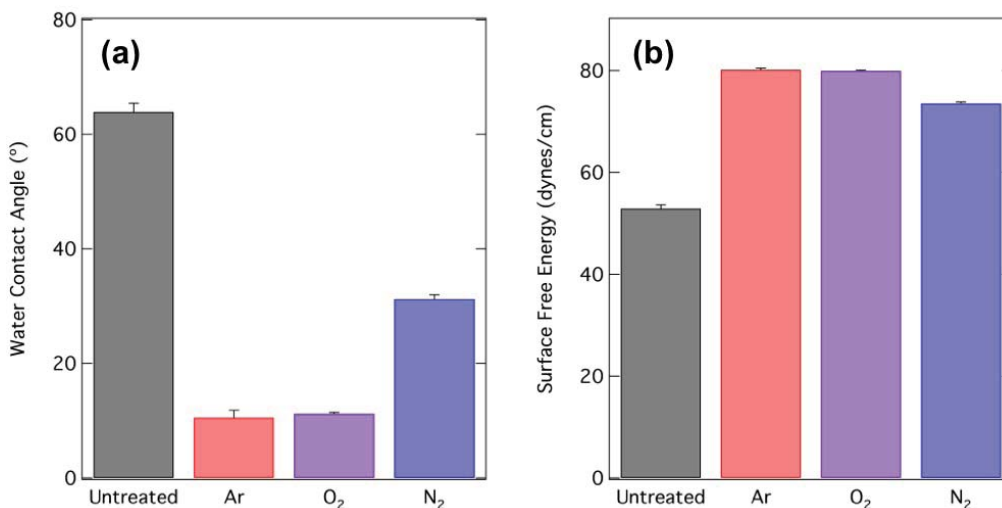


FIG. 2: (a) Water contact angles and (b) SFEs of pristine and plasma-treated substrates

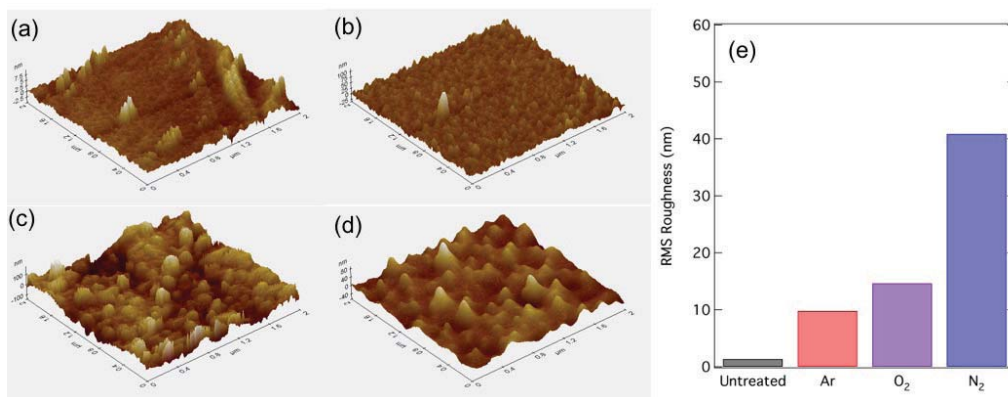


FIG. 3: Morphologies of pristine (a) and plasma-treated samples of (b) Ar, (c) N₂, and (d) O₂. (e) Comparison of RMS roughness

asymmetrical stretch), 1715 cm^{-1} (C=O symmetrical stretch), 1375 cm^{-1} (C-N stretch), and 720 cm^{-1} (C=O bending). We used these peaks that were relevant to the chemical structure of PIs to evaluate changes in IR peaks of plasma-treated surfaces. After reviewing the difference in FTIR spectra of plasma-treated samples [Fig. 4(b)], we observed a decrease in intensity of the imide functional groups. This decrease indicates changes in surface chemical properties such as the reduction of imide functional groups, although the degree of chemical change is challenging to determine from the spectra. This difficulty in detecting chemical changes on PI surfaces may be due to the very thin layers of chemical modifications formed on the substrate after plasma treatment. In addition, the number of detected signals using FTIR spectroscopy was less than the actual amount

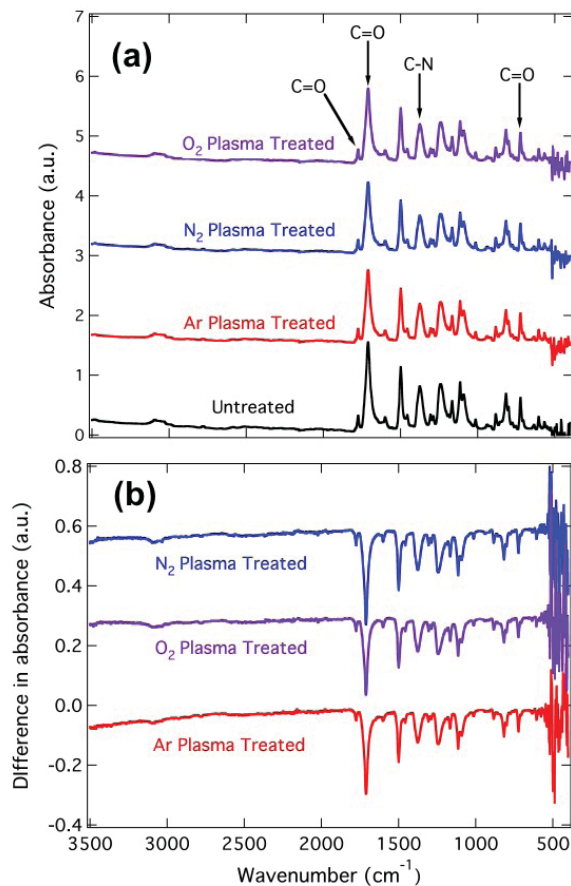


FIG. 4: (a) Typical FTIR spectra of pristine and plasma-modified PI samples; (b) difference in FTIR absorbance of plasma-treated samples

(detection of chemical changes may be improved with X-ray photoelectron spectroscopy). Although FTIR spectral analyses were unable to identify additional functional groups on the treated surfaces, attachment of functional groups on the PI surfaces cannot be discounted, because reactive species are present in the N_2 and O_2 plasma.

B. Biocompatibility Assays

Effects of PI surface plasma treatment on cell behavior were investigated for 3 d using fibroblast cells. Figure 5 shows cells grown on PI surfaces, and analyses of cell morphology revealed the absence of any significant morphological changes between cells grown on pristine and plasma-treated PI. Moreover, their morphologies were similar to cells grown on TC dishes, demonstrating that plasma treatment of PI is not cytotoxic to fibroblast cells. Quantification of cell adhesion in Fig. 5 demonstrated better cell cover-

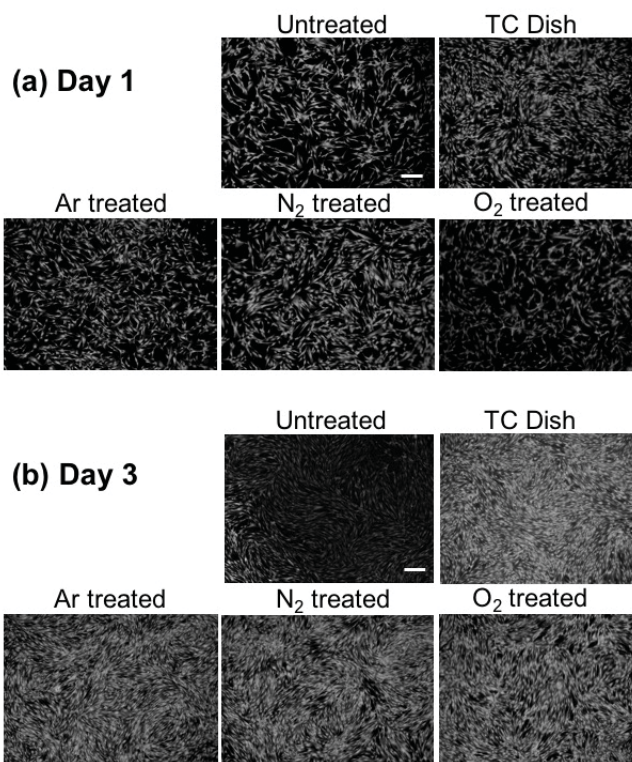


FIG. 5: Representative cell-adhesion profiles of fibroblast cells on pristine and plasma-treated PI surfaces after 1 and 3 d in culture. Cells were stained with calcein and visualized with fluorescence microscopy. TC, Tissue culture. (Bar, 200 μm .)

age for plasma-treated PI substrates than for pristine samples. Initial attachment significantly improved with N_2 plasma treatment, as revealed by increased cell coverage at day 1 [Fig. 6(a)]. Cell coverage increased for all plasma-treated substrates compared to cells grown on TC dishes, indicating that plasma modification of the PI surface improved cell attachment [Fig. 6(b)].

Figure 7 depicts cell viability on PI substrates relative to untreated controls after 1 and 3 d of incubation. Although the amounts of viable cells on plasma-treated substrates were initially comparable to those of untreated PIs with only the TC dish showing high viability, significant increase in viability between the treated and pristine samples was apparent at day 3, indicating that plasma treatment of PI positively influences cell proliferation.

The enhanced initial attachment of fibroblast cells on N_2 plasma-treated PI may be due to its hydrophilic nature brought about by its high RMS roughness and the presence of polar groups on the PI surface. N_2 -containing plasma may include the attachment of amine-based functional groups. Amine functionalities provide positive charges that encourage cell adhesion under physiological conditions.¹⁸ PI substrates treated with Ar plasma would have surface modifications associated mainly with the etching process as

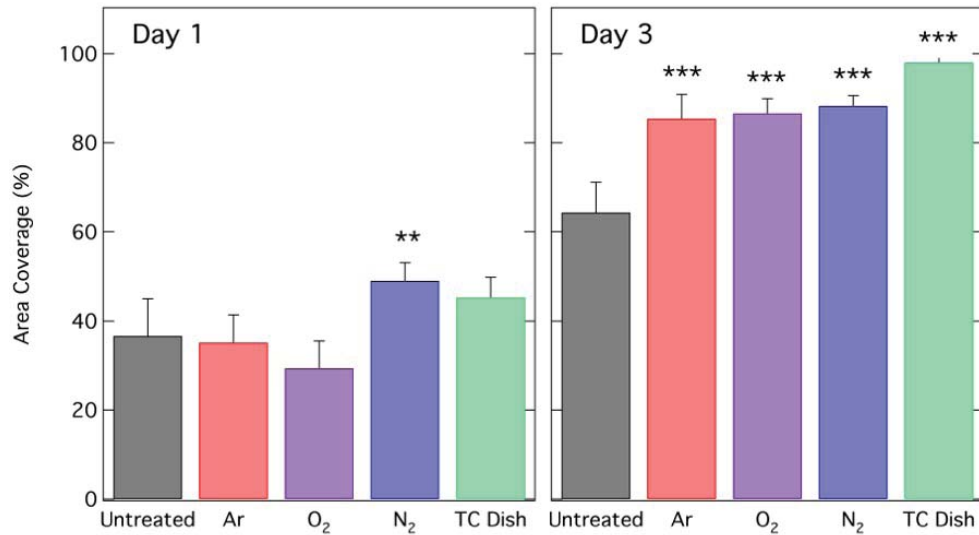


FIG. 6: Cell adhesion on plasma-treated PI substrates measured as percentage of area coverage after 1 and 3 d of culture. Cells were stained with calcein and the percentage of area coverage was determined using ImageJ. Values are reported as mean \pm standard deviation (SD). TC, Tissue culture. ** $p \leq 0.01$ and *** $p \leq 0.001$ statistical significance, with one-way analysis of variance (ANOVA) and Dunnett's post-test.

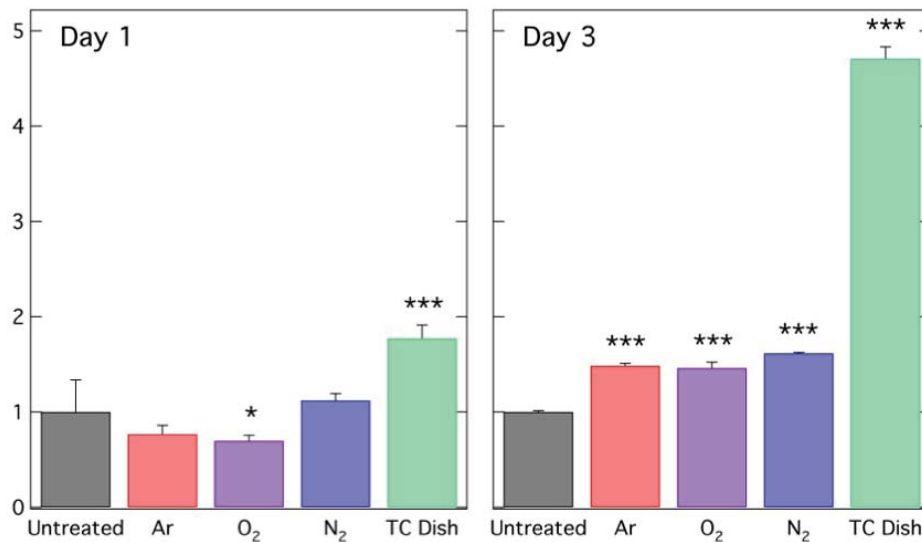


FIG. 7: Cell proliferation on plasma-treated PI substrates normalized with untreated PI after 1 and 3 d of culture. Cell proliferation was measured using the PrestoBlue cell-viability assay. Values are reported as mean \pm SD. TC, Tissue culture. * $p \leq 0.05$ and *** $p \leq 0.001$ statistical significance, with one-way analysis of variance (ANOVA) and Dunnett's post-test.

a result of the inert nature of the Ar gas, whereas O₂-treated surfaces will attach hydroxyl groups on the surface. In general, the improved biocompatibility properties of plasma-treated PI substrates at day 3 may also be attributed to the increase in hydrophilicity of the PI surfaces through morphological modifications and changes in SFE brought about by plasma treatment.^{19,20}

IV. CONCLUSIONS

Improving directed cell responses to biofunctionalized interfaces requires the ability to tune surface properties for desired cell adhesion and subsequent proliferation. Plasma-treated PI surfaces exhibited improvements in terms of cell attachment as well as proliferation in comparison to untreated surfaces. Among the three gas species used, N₂ plasma-treated surfaces showed strong affinity to biomolecules, especially during the first day of cell seeding, compared to that of untreated samples. This may be attributed to the possible nitrogen-containing functional groups on the surface that further enhance cell adhesion and proliferation. Surface functionalization using N₂ plasma must be conducted to further clarify the mechanisms of action between the cell and polymer substrate interactions.

ACKNOWLEDGMENTS

M.R.V. is grateful for the support of the Balik-PhD research grant OVPAA-BPhD-2014-01. E.I.P. acknowledges support of the Balik-PhD research grant OVPAA-BPhD-2015-03.

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