Localized Plasma Treatment of Poly(dimethylsiloxane) Surfaces and Its Application to Controlled Cell Cultivation

Helen M. L. Tan*, Takanori Akagi**, ***, and Takanori Ichiki ** **

*Department of Materials Engineering, School of Engineering, The University of Tokyo, 2-11-16 Yayoi, Bunkyo-ku, 113-8656, Japan
**Department of Bioengineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656, Japan
*** Center for NanoBio Integration, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656, Japan

Localized surface treatment of biocompatible polymer material, poly(dimethylsiloxane) (PDMS) using a scanning microjet (SMJ) has been studied for the control of biological cells’ attachment on it. Previously, we developed an atmospheric-pressure argon/oxygen (Ar/O₂) radical microjet system and used it for modifying PDMS surface. In this study, we examined argon/nitrogen (Ar/N₂) plasma system and compared it with the previous study using Ar/O₂ system in terms of degree of hydrophilicity, chemical state, surface morphology, and cell adhesion. The results from the Ar/N₂ system showed interesting phenomena that indicate promising potentials for application in controlling cell adhesion. Keywords: scanning microjet (SMJ), surface modification, poly(dimethylsiloxane), cell adhesion

1. Introduction

In recent years, micropatterning of cell adhesion is growing popularity in cell culture practices. This approach could allow effect of surface properties on cells function and it also allows spatial control of the cellular micro-organization [1]. This is of interest for a number of applications such as in bio-microelectromechanical systems (bioMEMs) and tissue engineering. In such fields, poly(dimethylsiloxane) polymer (PDMS) (chemical formula : -(O-(CH₃)₂-Si-O)ₙ-) is widely used as a fabrication material due to ease in micropattern fabrication at low cost, resistance to various chemicals and pH environments, and excellent transparency in the UV-vis range [2-3]. Different techniques such as chemical grafting, photo-oxidation and plasma treatment have been reported to be used for attaching different chemical groups to the surface of PDMS [4-13]. Basically, these techniques are used with the aim of achieving better wettability, adhesion or biocompatibility for cell culture practices. Plasma-assisted chemical surface functionalization is frequently used for the enhancement of biocompatibility of polymers. Oxygen, ammonia and nitrogen-based plasmas are used to chemically modify polymer surfaces such as polyethylene, polystyrene, etc by incorporating oxygen- and nitrogen-containing groups, including primary amine groups [14-24].

In previous work, we developed an atmospheric-pressure scanning radical microjet equipment using Ar/O₂ plasma [25-26]. With this system, maskless hydrophilic patterning and hence patterned cell cultivation could be achieved successfully. In the present study, we attempted localized surface modification using Ar/N₂ instead of Ar/O₂ system for controlling cells’ attachment. Effects of scanning speed on localized hydrophilic patterning on PDMS polymer surface have been first investigated. For better understanding of cell-surface interactions, contact angle measurements on the plasma-treated PDMS surface and biological effects of wettability of treated PDMS were also investigated using human
coronary artery smooth muscle cells (HCASMC). Surface morphologies and chemical states resulting from nitrogen plasma treatment were carried out using atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS), respectively. From wettability and XPS results, it is found that good hydrophilization due to surface oxidation and consequent cell adhesion could be achieved using Ar/N₂ system. The details are further discussed in this paper.

2. Experimental

2.1 Apparatus

Figure 1 shows a schematic of the apparatus used in this study. The compact plasma source is comprised of a 250-µm-thick copper antenna deposited on an alumina plate and a silica discharge tube with a 0.9 mm inner diameter. A 144-MHz VHF power was supplied to the plasma source and kept constant at 50 W [25-27]. An argon plasma jet was emitted through the pinched end of the discharge tube which has an inner diameter of 0.1 mm. Another small cylindrical tube was connected to the end of the discharge tube for the introduction of nitrogen to the downstream of the Ar plasma jet. The flow rate of Ar and N₂ used in this study were kept constant at 180 ccm and 110 ccm, respectively.

![Diagram](image)

Fig. 1. A schematic diagram of SMJ. Experimental conditions used were: N₂ flow rate: 110 ccm; Ar flow rate: 180 ccm.

2.2 Sample preparation and surface characterizations

SYLGARD 184, a commercial brand of PDMS, was obtained from Dow Corning and PDMS films were prepared with 10:1 (base:curing agent) mixture. Next, the mixture was coated on a glass substrate using a spin-coater to obtain a thin film and cured at 100°C for 2 min. Microjet was then scanned over the PDMS surface in x- or y-direction using SMJ equipment.

Subsequently, water contact angles' distributions of ~50 µm-size water droplets obtained from condensed steam of boiling water were measured across the scanned line using an in-house made contact angle measurement system with a long focal length microscope. Working parameter such as scanning speed of microjet was first investigated to find out its effects on hydrophilic patterning of PDMS surface. Then, patterned cell culture was examined using HCASMC. Moreover, surface properties such as microscopic roughness and chemical states were analyzed using AFM (Shimadzu SPM-9500) and XPS (Shimadzu-Kratos Axis Hs), respectively. In the latter analysis, a monochromated Mg Kα X-ray source was used.

2.3 Cell cultivation on nitrogen-treated PDMS films

HCASMC in CS-C complete medium kit (Cell Systems Corporation, WA, USA) were cultured on treated PDMS films and incubated for 72 hours (3 days) at 37°C in 5% CO₂ in air atmosphere. Initial area density of seeded cells was constant at 300 cells/mm² in all the experiments. Cell density on treated PDMS surfaces was counted in every 24 hours using an optical microscope.

3. Results and Discussion

3.1. Effects of scanning speed on wettability of treated PDMS

Figure 2 shows the water contact angles' distribution measured across the scanned line. From these profiles, minimal contact angle, \( \theta_{\text{min}} \) and treated line width, \( W \) are plotted in Fig. 3. \( W \) is defined here as the width at 10% of the maximum valley depth. Water contact angle for non-treated PDMS surface was measured to be approximately 112°.

From Fig. 3, it can be observed that as scanning speed increases from 0.5 to 6 mm/s, minimal contact angle, \( \theta_{\text{min}} \) increases from 59 to 80 degree while treated line-width, \( W \) decreases from 5 to 4.4 mm. This means that the surface becomes hydrophilic for longer treatment time.
3.2. Chemical states of treated PDMS

XPS analysis revealed the change in chemical states of PDMS surface with and without SMJ treatment. Although N$_2$ and Ar were used as process gases, no nitrogen peaks were detected from all the samples prepared in this study and surface oxidation was observed instead. Mechanisms of PDMS oxidation in oxygen plasmas have been studied by several researchers [28-31], and it is believed that conversion of −OSi(CH$_3$)$_2$− groups at the surface to −O$_2$Si(OH)$_4$ occurs. For further consideration of chemical state of Si on PDMS surface, Si$_{2p}$ photoelectron spectra were analyzed in detail as shown in Fig. 4. Since the Si$_{2p}$ XPS spectra are composed of 2p$_{1/2}$ and 2p$_{3/2}$ levels due to the spin-orbit interaction, for spectra analysis, they were decomposed into the Si$_{2p1/2}$ and Si$_{2p3/2}$ spectra. Si$_{2p3/2}$ spectra from PDMS films could be decomposed into three chemically shifted peaks, resulted from structures denoted as D[(CH$_3$)$_2$Si O$_2$], T[(CH$_3$)SiO$_2$], and Q[SiO$_4$] siloxy units. The Binding-energy shifts for D, T, and Q were determined by spectra fitting of a mixed Gaussian-Lorentzian product function with the equally retained FWHM for each peak, and the binding-energy values obtained were 102.3, 102.9, and 103.8 eV, respectively.

Fig. 2. Treated-line width profiles obtained from water contact angles measurements for different scanning speeds. Experimental conditions used were: N$_2$ flow rate = 110 ccm; Ar flow rate = 180 ccm.

Fig. 3. Relationship between minimal contact angle, treated line width and scanning speeds of radical jet used. Experimental conditions used were: N$_2$ flow rate = 110 ccm; Ar flow rate = 180 ccm.

Fig. 4. Si$_{2p3/2}$ spectra of the PDMS samples untreated (top) and treated at 2 mm/s (middle) or 4 mm/s (bottom). XPS spectra were obtained using Mg K$_\alpha$ X-ray source and the take-off angle of 20 deg.
As shown in the top spectra in Fig. 4, the untreated sample has a thin native oxide layer possibly formed during curing at 100 °C in the air. Number of oxygen atoms attached to the silicon atom indicates the extent of oxidation. D component spectrum is most probably originated from the bulk PDMS, while T and Q components is from oxidized layer near the surface. On the other hand, spectra from the treated samples are composed of only D and Q components. In addition, it is found that the extent of oxidation tends to be slightly higher for higher scanning speed. Although the reason is not clear at present, these data might reflect the oxidation mechanisms specific to the SMJ treatment using Ar/N₂ system.

3.3. Surface morphology of treated PDMS
Surface morphologies of treated PDMS were analyzed using AFM. Figures 5(a), (b) and (c) show the surface morphology profiles for 3 scanning speeds of 0.5, 2 and 6 mm/s, respectively. Both surface roughness values of average roughness (Rₐ) and root mean square (Rₘₙ) are plotted in Fig. 6. Rₐ and Rₘₙ values did not show much significant change from non-treated PDMS surface at any scanning speed. Rₐ and Rₘₙ of non-treated PDMS surface were measured and found to be 2.2 nm and 2.9 nm, respectively.

Surface roughness values obtained using Ar/O₂ system was reported to be much lower than those for Ar/N₂ system [26]. This discrepancy could imply that the treated PDMS surface might have undergone etching when using Ar/O₂ system, while etching did not occur when using Ar/N₂ system.

![Graph showing relationship between surface roughness (Rₐ and Rₘₙ) of treated PDMS and scanning speeds of microjet.](image)

Fig. 6. Relationship between surface roughness (Rₐ and Rₘₙ) of treated PDMS and scanning speeds of microjet.

3.4. Cell attachment analysis
Paterned cell cultures on the treated PDMS surfaces by Ar/N₂ system were performed for DIV (day in vitro) 3 and good cell proliferation results were obtained. Figure 7 shows a microphotograph of HCASMC cultivated in line on treated PDMS surfaces using scan speed of 0.5 mm/s and nitrogen flow rate of 70 ccm.

![Microphotograph of HCASMC cultivated in line on treated PDMS surfaces after DIV 3.](image)

Fig. 7. A microphotograph of HCASMC cultivated in line on treated PDMS surfaces after DIV 3. Experimental conditions used were: scan speed = 0.5 mm/s; N₂ flow rate = 70 ccm; Ar flow rate = 180 ccm.
Figure 8 illustrates the cell attachment results for various scanning speeds. It can be seen that as scanning speed increases, cell densities for DIV 1, 2 and 3 decrease. Scan speed of 0.5 mm/s contains the highest cell density of approximately 180 cells/mm² for DIV 3. Thus, lower scanning speed (i.e. longer treatment time) helps to promote better cell attachment to the treated surfaces.

![Cell Density Graph]

Fig. 8. Average cell density over total proliferated area for DIV 1, DIV 2 and DIV 3. Surface treatment conditions used were: N₂ flow rate=110 ccm; Ar flow rate= 180 ccm.

3.5. Comparison between Ar/O₂ and Ar/N₂ plasma system

As mentioned earlier, we previously reported microjet treatment using Ar/O₂ plasma system and satisfactory results for cell patterning. In this case, the PDMS surface was oxidized by oxygen radicals and resulted in hydrophilization of PDMS surface. In contrast, oxygen was not used in the current study using Ar/N₂ plasma system. However, we could still achieve good hydrophilization and consequent cells' attachment.

The XPS results showed that all surfaces of the PDMS samples were oxidized after treatment by Ar/N₂ microjet, thus causing the surfaces to become hydrophilic. Two possible explanations for causing oxidation of PDMS surface could be proposed. The Ar/N₂ system might have contaminated with trace amount of oxygen which caused oxidation and consequent hydrophilization. Another possible explanation could be due to ultraviolet light irradiation from the nitrogen plasma that can cause bond scission of the polymer surface. Thus, this process could have facilitated incorporation of the oxygen from the surrounding atmospheric air into the polymer and causes hydrophilization on the surface.

Furthermore, another interesting phenomenon which was observed using Ar/N₂ system is that there showed no significant change in surface roughness. As compared to Ar/O₂ system, surface morphology became smoother after treatment by the oxygen radical jet. Thus, etching on PDMS surface could have occurred when using Ar/O₂ system, while etching did not occur when using Ar/N₂ system.

4. Conclusion

In this work, localized surface treatment of biocompatible polymer material, poly(dimethylsiloxane) (PDMS) using a scanning microjet (SMJ) has been studied for the control of biological cells' attachment. We studied Ar/N₂ plasma system and compared it with the Ar/O₂ system in terms of degree of hydrophilicity, chemical state, surface morphology and cell adhesion. Ar/N₂ system showed as good hydrophilization results and cell adhesion results as Ar/O₂ system.

There were two interesting phenomena observed for Ar/N₂ system. Despite no use of oxygen, all the samples' surfaces were oxidized. AFM results depicted that the treated PDMS surfaces did not show significant change in surface roughness from the non-treated ones. Based on these results, likely explanations for causing oxidation of PDMS surface were discussed. Oxygen contaminants in the system and ultraviolet light irradiation were stated as the two most possible causes. Further study for better understanding of oxidation on PDMS surface using Ar/N₂ system is necessary in the near future.

Acknowledgements

This work is partially supported by the Grants-in-Aid for Scientific Research on Priority Area: Generation of micro-scale reactive plasmas and development of their new applications from the Japanese Ministry of Education, Culture, Sports, Science and Technology. The authors wish to acknowledge Ms T. Matsuoka and Mr S. Hirata for helpful contributions in XPS spectra analysis.
References