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Tailoring PDMS Microfluidic Channel Surfaces for Improved Cell Adhesion

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Abstract. Microfluidic on-chip platforms for cell biomechanics investigations have become very popular in the field of fundamental cell and tissue engineering. Polydimethylsiloxane (PDMS) is widely used fabrication material for such biological microfluidic applications because of its versatile nature, optical transparency, ease to replicate fine microarchitechtures and tunable mechanical properties by varying base to crosslinker ratio. However, the hydrophobic surface of native PDMS often provides unfavourable conditions for cellular attachment. Although plasma-treatment and protein physisorption methods enhances the initial cell adhesion but they are short-lived. This paper focuses on tailoring a biocompatible PDMS surface for long-term cell culture by using (3-Aminopropyl)triethoxysilane as a linking agent between PDMS and protein. Characterization of APTES+Gelatin treated PDMS surfaces has revealed changes in surface wettability, surface free energy and surface roughness as compared to pristine PDMS surface. These physico-chemical changes contribute to enhanced endothelial cell attachment and proliferation. This tailored PDMS surface can significantly prolong the cell-culture compatibility of PDMS-based microfluidic devices for mechano-biological studies and *in vitro* organ modeling.

Keywords: microfluidics, polydimethylsiloxane, surface engineering, cell adhesion, protein grafting

INTRODUCTION

Microfluidic cell culture offers numerous advantages over conventional cell-culture techniques as it provides physiologically relevant dynamic microenvironment, precise control of cell numbers in a given area and minimal reagent consumption [1,2]. The most exploited material for development of such microfluidic cell-culture platforms is Polydimethylsiloxane (PDMS), owing to its various advantages [3,4]. The salient features of PDMS include low-cost, tunable mechanical properties, optical transparency, O_2 and CO_2 gas permeability, and ease of fabrication with nano-scale precision. These advantages enables PDMS polymer to be useful for development of *ex vivo* models for fundamental studies of cell physiology, cell-cell interaction, disease models for tumor progression [5,6]. Most of these studies involve mimicking of *in vivo* microarchitectures at cellular, tissue and organ level on chip. However, the intrinsic extreme hydrophobic surface of PDMS has been identified as a poor platform for cell culture, therefore, demanding the improvement of surface biocompatibility of PDMS for long-term cell studies.

A large number of literature exists on surface modification of PDMS by physical and chemical strategies [7–11]. One such approach is plasma treatment of the PDMS surface, which renders the surface hydrophilicity by activating reactive silanol group [7]. This oxidized surface improves surface wettability but reverts back to its native hydrophobic behaviour after few hours of air exposure. While protein adsorption on hydrophobic surface enhances

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initial cell attachments but long-term culture causes cell-sheet aggregation and early detachment of cells due to uneven protein layering and protein dissociation [8,12]. Proteins often interacts with substrate surface by weak interaction forces such as electrostatic, vander Waal bondings, hydrophobic adsorption [9], which possibly causes gradual protein leaching into the cell-culture medium, results in loss of extracellular matrix (ECM) support for cellular focal adhesions. Recent studies have reported that covalent bonding of matrix protein could enhance the uniform protein layering on substrate surface in favor of stable and long term adhesion compared to conventional protein adsorption techniques [13,14].

In this regard, we employed the amino-silanization of PDMS surface using (3-Aminopropyl)triethoxysilane (APTES) for the immobilization of gelatin protein for a stable culture of endothelial cells. Gelatin is a biopolymer derived from collagen, an ECM protein, provides support for endothelial cell adhesion [15]. The endothelial cells line the entire vascular system and directly interact with blood cells. Foreseeing a need for a stable endothelial monolayer formation within microfluidic channel in order to develop a biomimetic microvascular model on-a-chip for fundamental physiological studies and disease model development, PDMS surface is chemically modified and well-characterized by various physical, chemical and biological aspects.

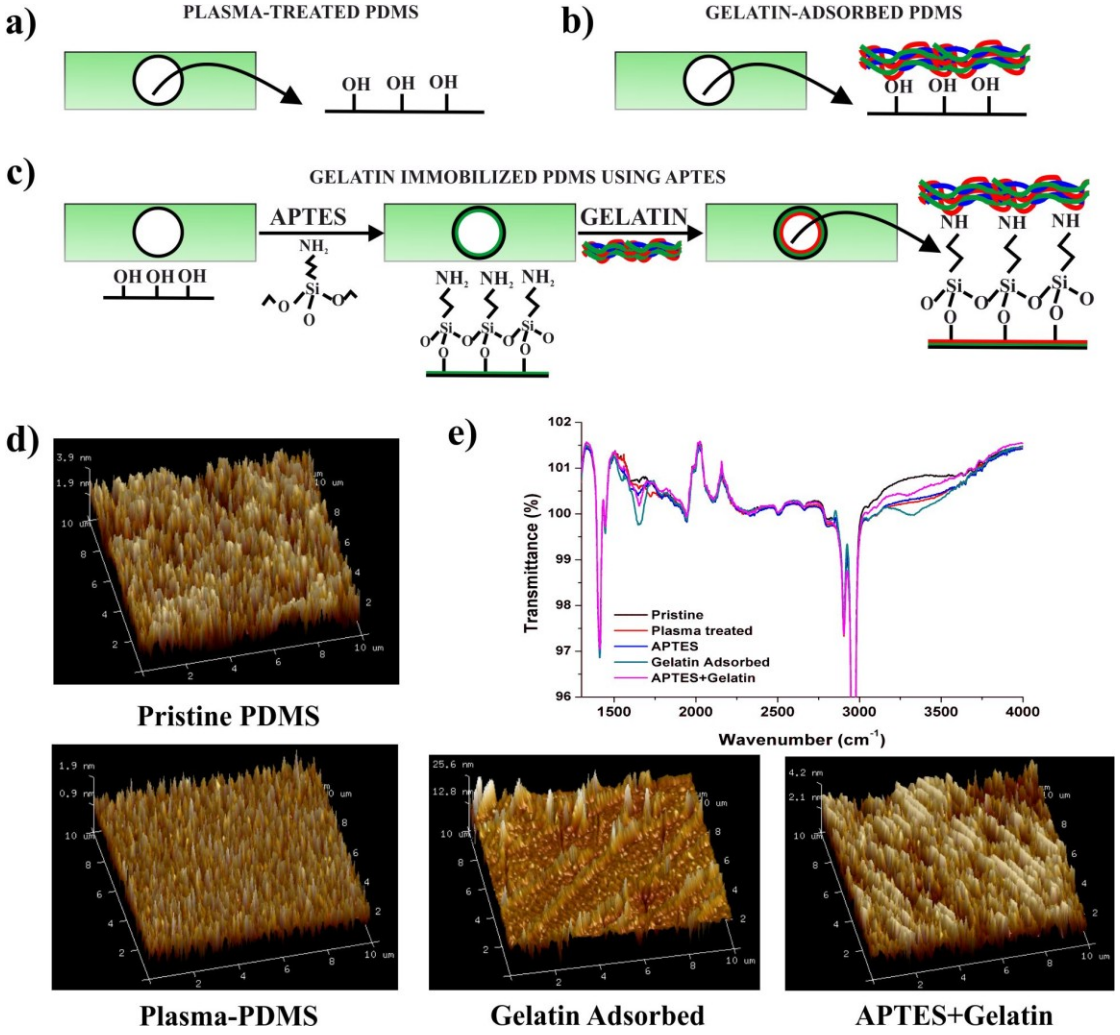

FIGURE 1. Schematic representation of surface chemistry modification: (a) Oxygen-plasma treated sample producing silanol groups (Si-OH) on the PDMS surface, (b) Physical adsorption of gelatin protein on the plasma-treated PDMS surface, and (c) Stepwise gelatin immobilization on aminosilane functionalized PDMS surface. The gelatin protein interacts with charged amine group ($-NH_2$) by ionic interactions, (d) AFM surface mapping (10 µm x 10 µm) of pristine, plasma modified, gelatin adsorbed and APTES+Gelatin surfaces, and (e) ATR-FTIR spectra of surface chemical modification

EXPERIMENTAL FRAMEWORK

PDMS base Elastomer and curing agent (SYLGARD 184, Dow Corning, USA) was mixed in a ratio of 20:1, degassed, cast to fabricate circular microfluidic channels as well as flat substrates and cured at 80ºC for 60 min on a hot plate. After cooling, microchannels and flat substrates were classified into three groups of surface modification as illustrated in Fig. 1 i.e., plasma-treated (Fig. 1(a)), gelatin adsorbed (Fig. 1(b)) and APTES-linked gelatin (APTES+Gelatin, Fig. 1(c)). All the microchannels were exposed under oxygen plasma for 3 min so that channel surface is uniformly ionized. Subsequently, the gelatin adsorbed group of microchannels were directly introduced with freshly prepared 1% gelatin solution, while APTES+Gelatin group of surfaces were treated with 10 % v/v APTES in deionized (DI) water at 50ºC for 120 min. The APTES-treated channels were washed thoroughly with DI water to remove any physically adsorbed silane molecules followed by immersion in gelatin solution. The microchannels were filled with protein solution and stored at 4ºC overnight. Finally, all the substrates were washed with DI water thrice to remove excess protein solution and prepared for further experiments.

The modified surfaces were deployed for surface chemistry analysis using various physico-chemical characterization tools such as goniometer for water contact angle (WCA) measurement, atomic force microscopy (AFM) for surface morphology and surface roughness measurement and Fourier transform infrared spectroscopy in attenuated total reflectance (FTIR-ATR) mode for understanding surface chemical changes after each step of modification. Surface free energy (SFE) was also derived from WCA experiments to understand its role in protein ligand and cell receptor interactions. Furthermore, human umbilical cord vein derived endothelial cells (HUVECs) were cultured with initial cell density of 10^5 for 5-days on modified substrates for biocompatibility analysis after UV sterilization of substrates for 60 min. Phase-contrast microscopy and Immunofluorescent microscopy techniques were employed to study cell attachment and its morphology on engineered PDMS surfaces.

RESULTS AND DISCUSSION

Physical Characterizations of Modified Surfaces

The WCA analysis of modified surfaces suggests the surface wettability changes according to stepwise chemical modification. Pristine PDMS surface possess a hydrophobic surface with a contact angle 106.57 ± 1.91º which reduces to 6.69 ± 0.68º after oxygen plasma treatment. The gelatin coating on both Plasma-treated and APTES treated substrates provide hydrophilic surface with 34.03 ± 0.58º and 33.58 ± 0.35º angles respectively. Subsequently, surface free energies (SFE) were derived from the WCA measurements as mentioned in **TABLE 1**, by using Good-Girifalco-Fowkes equation [16] as shown below:

$$\cos\theta = -1 + 2(\gamma_{sv} / \gamma_{lv})^{0.5} \qquad (1)$$

Where, 'θ' is the water contact angle, 'γ_{sv}' is interfacial free energy between solid and air, & 'γ_{lv}' is the interfacial free energy between liquid and air. For PDMS, 'γ_{sv}' is resulted from dispersive forces [16] and the value of 'γ_{lv}' for water-air interface is taken as 72.8 mJ/m^2 [17]. The calculated data suggests pristine PDMS has very low SFE value, which hinders the cellular compatibility of native surface while plasma treated surface has a high value of SFE but does not support long-term surface hydrophilicity as its property reverts back to hydrophobic surface after few hours. The protein adsorbed surface and APTES+Gelatin surface both shows wettable surface and intermediate SFEs that may support initial cell attachments [18].

TABLE 1. Water Contact Angle and Derived Surface Free Energy of Modified PDMS surfaces

PDMS Substrate	Water Contact Angle (º)	Surface Free Energy (mJ/m^2)
Pristine	106.57 ± 1.91	09.31 ± 0.82
Plasma Treated	6.69 ± 0.68	72.30 ± 0.10
Gelatin Adsorbed	34.03 ± 0.58	60.86 ± 0.38
APTES + Gelatin	33.58 ± 0.35	61.14 ± 0.22

Another factor that influences the cell attachment is surface roughness, which is obtained by AFM analysis by mapping 10 µm x 10 µm area as shown in Fig. 1(d). The surface roughness data suggests pristine PDMS surface exhibits a root-mean-square (RMS) roughness, R_q of 0.73 ± 0.02 nm, which further provides uniformly smooth surface after plasma modification having R_q, 0.27 ± 0.02 nm. The gelatin adsorbed surface has little rougher surface

with R_q, 1.95 ± 0.73 nm possibly because of uneven layering of adsorbed protein while APTES+Gelatin surface provides presence of uniform gelatin with intermediate R_q value i.e., 1.53 ± 0.55 nm.

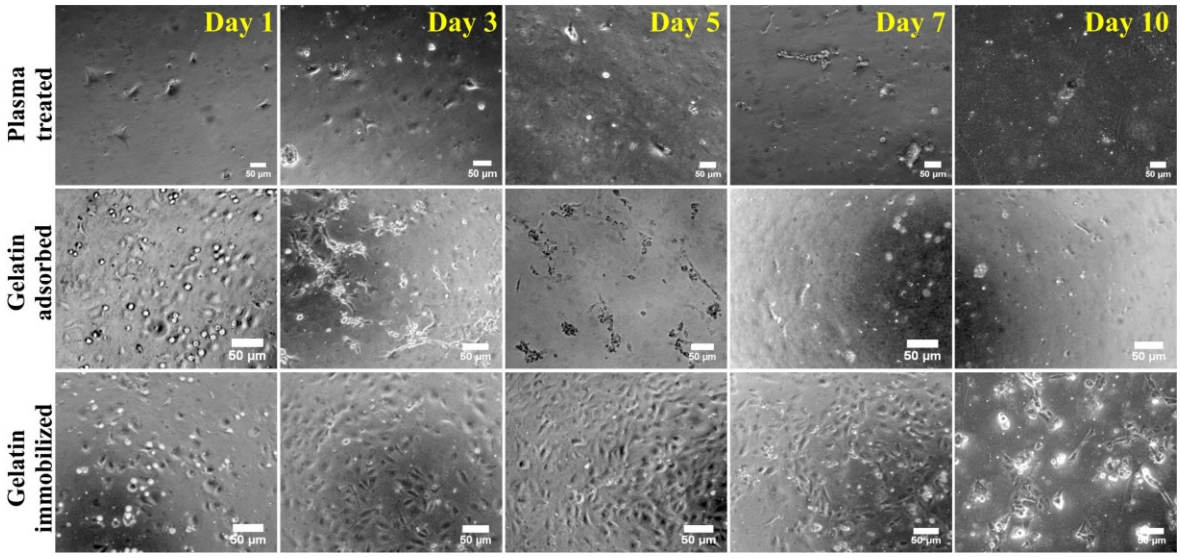

FIGURE 2. Phase contrast microscopic images of HUVECs upto 10-days of culture on each PDMS substrates

Chemical Characterization of Modified Surfaces

The FTIR-ATR was performed in the range of 500-4000 cm^{-1} at room temperature on the substrates to get the chemical composition spectra of pristine PDMS, plasma-treated PDMS and further modified PDMS surfaces in each and every step of chemical modification to compare their surface changes by using ATR-FTIR Spectrometer. The characteristic peaks of pristine PDMS, which are CH_3 rocking and Si-C stretching (789-796 cm^{-1}), Si-O-Si stretching (1020-1074 cm^{-1}) and CH_3 deformation in Si-CH_3 (1260-1259 cm^{-1}) were avoided for consideration of modified peaks only. Hence, FTIR spectra is plotted in the range of 1300- 4000 cm^{-1} as shown in Fig. 1 (e). The peak shifting in the hydroxyl and water-stretching vibrations at 3300 cm^{-1} indicate that the modified PDMS surface forms covalent bond with the -OH group of water molecules [19]. This shows Si-CH_3 groups are converted into Si-OH molecule after oxygen plasma treatment of pristine PDMS surface. The APTES conjugation with plasma-treated surface shows a small broad peak indicating vibrational modes of reactive –NH_2 functional groups at 1563 cm^{-1} [13]. The major vibrational bands of proteins are observed for amide-I (C=O stretch) and amide-II (NH bend) in the regions of 1600-1700 cm^{-1} and 1500-1580 cm^{-1} respectively. Finally, after the gelatin bond with APTES via ionic conjugation and the protein functional groups are observed around 3370 and 1635 cm^{-1} due to the association of O-H and N-H stretching in gelatin [12,14]. While in the adsorption group, only a few negligible transformations occurred due to adsorbed gelatin. Overall, FTIR-ATR results suggest chemical changes occurs after surface modification process.

Biological Characterization of Modified Surfaces

HUVECs were cultured on each group of substrates in a 24 well plate under standard cell culture condition and observed for a period of 10 days by using phase-contrast microscope as shown in Fig. 2. It is observed that the cells on APTES+Gelatin surface are more evenly spread than gelatin adsorption surface. A uniform homogeneous cell sheets are maintained on APTES-linked surfaces while the cell layer on the adsorption PDMS surface demonstrates a patchy structure possibly due to protein leaching in culture medium. Cell morphology on gelatin adsorbed surface starts aggregating from day 1 itself and a stressed condition is maintained up to 72 h, after that only fewer number of cell were observed on this surface whereas on APTES+Gelatin surface a large number of cells were present till day 10.

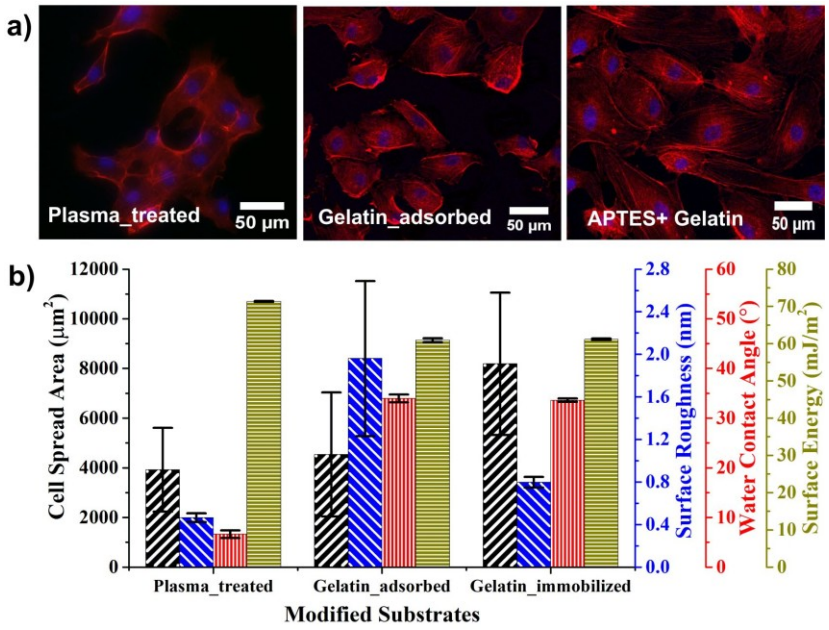

FIGURE 3. (a) Immunofluorescent images of HUVECs showing cell morphology on PDMS substrates, (b) A correlation plot showing intermediate range of surface wettability, surface roughness and surface free energy promotes cell proliferation.

Moreover, cell morphology is observed using immunofluorescence imaging by staining actin fibres of cell using Rhodamine Phalloidin (Thermo, USA) and nucleus with universal DAPI stain (HIMEDIA, India) as shown in Fig. 3(a) and correlated with other physico-chemical parameters obtained from surface chemistry analysis as represented in Fig. 3(b). It is observed that cell spread area is much higher on APTES+Gelatin surface as it provides a uniform protein layer and long-term stability to endothelial focal adhesions. Furthermore, all the physical, chemical and biological properties are summarized in **TABLE 2**.

TABLE 2. Physical, Chemical and biological characterization of tailored PDMS surfaces

PDMS Substrate	Physical properties	Chemical Properties	Biological Properties
Pristine	Hydrophobic, low SFE□, low SR□□	$(C_2H_6OSi)n$	Negligible cell attachment
Plasma Treated	Highly hydrophilic, High SFE, very low, smooth SR	Hydroxyl bond formation (Si-OH)	Supports initial cell attachment but not durable
Gelatin Adsorbed	Hydrophilic, Intermediate SFE, high SR	Gelatin adsorption on hydroxyl bonds	Supports initial cell attachment, proliferation but not durable due to protein aggregation and leaching into culture medium
APTES + Gelatin	Hydrophilic, Intermediate SFE, Intermediated SR	Silane bond formation after APTES treatment and gelatin conjugation with $-NH_2$ group via ionic bonding	Stable and prolonged cell culture

□SFE-Surface free energy; □□SR-Surface Roughness

CONCLUDING REMARKS

In order to enhance the surface properties of PDMS microchannels, protein grafting instead of adsorption on material surface is performed for better cellular adhesion and after every step of chemical modification surface chemistry is analysed by WCA, AFM and ATR-FTIR studies. The low surface wettability and high surface free energy achieved by plasma treatment may support initial cell attachment but the extent and durable cell culture is obtained by this chemical conjugation method. Among other factors such as surface roughness determines the uniformity of protein layer on the material surface. The overall results suggests that mid-range of surface hydrophilicity, roughness and surface free energy altogether promotes cell attachment and further proliferation for longer time duration to maintain endothelial monolayer in biomimetic microvessels. This study opens opportunities for development of biomimetic, simple, inexpensive and surface engineered *in vitro* platforms for disease modelling and fundamental cellular micro-environmental studies.

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