Nie, Y.; Deng, Z.; Wang, W.; Bhuvanesh, T.; Ma, N.; Lendlein, A.:
Polydopamine-mediated Surface Modification Promotes the Adhesion and Proliferation of Human Induced Pluripotent Stem Cells.
In: MRS Advances . Vol. 5 (2020) 12 - 13, 591 - 599.
First published online by Cambridge University Press: 04.11.2019

DOI: 10.1557/adv.2019.405
https://dx.doi.org/10.1557/adv.2019.405

Polydopamine-mediated Surface Modification Promotes the Adhesion and Proliferation of Human Induced Pluripotent Stem Cells

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ABSTRACT

With their abilities of self-renewal and pluripotency to differentiate into all three germ layers, human induced pluripotent stem cells (hiPSCs) are promising cell sources for cell-based drug and implant testing. However, the large-scale expansion and maintenance of hiPSCs require following strict protocols. Advanced cell culture systems for high-quality hiPSCs are highly demanded to meet the application requirements. In this study, we probe the possibility of modifying the polymeric substrates for maintaining the self-renewal and pluripotency of hiPSCs. Here, polydopamine (PDA) was employed to immobilize the Laminin 521 (LN521) onto the surface of polyethylene terephthalate (PET). An aqueous solution of dopamine with concentrations ranging from 0 to 2.0 mg/mL was applied on PET surfaces. These PDA-modified surfaces were further functionalized with LN521. Surface wettability was evaluated by measuring the water contact angle (WCA) and surface properties of the modified substrate were analyzed using an atomic force microscope (AFM). Initial hiPSC attachment (1h after seeding) and cell proliferation were evaluated by counting the total cell number. The maintenance of pluripotency was evaluated at designed time points. WCA of the PDA-LN521 surfaces gradually decreased from 62.1°±6.3° to 8.1°±2.9°. The maximum peak-to-valley height roughness (R_t) of those surfaces determined by AFM increased in a dopamine-concentration-dependent manner, ranging from 43.9±1.6 nm to 126.7±7.6 nm. Young's modulus of those surfaces was substantially increased from 0.98±0.36 GPa to 4.81±2.41 GPa. There was a significant enhancement (13.0±7.2% and 24.2±8.1%) of hiPSC adhesion on PDA-LN521 (dopamine concentration at 0.125 and 0.25 mg/mL). When increasing the

dopamine concentration to 0.5 and 1.0 mg/mL, there was no further increase in hiPSC adhesion on PDA-LN521 surfaces. Moreover, hiPSC proliferation was remarkably enhanced on PDA-LN521 surface (dopamine solution at concentration from 0.125 to 1.0 mg/mL). Pluripotency of hiPSC was not affected by PDA treatment. In conclusion, PDA-mediated surface modification is an effective approach for the robust expansion and maintenance of hiPSCs on polymer substrates.

INTRODUCTION

Human induced pluripotent stem cells (hiPSCs) are a promising cell source for disease modeling, drug discovery, and cell therapy [1, 2]. Research on hiPSC is progressing from pre-clinical investigations to the clinical applications [3, 4]. However, several important issues remain to be addressed. For example, the cultivation of high-quality hiPSCs with high self-renewal capacity and pluripotency as well as low variations in differentiation efficiency still faces challenges. Great progress has been made in developing chemically defined, xeno-free media for hiPSC cultivation in order to solve these problems [5].

Moreover, the culture of hiPSCs requires the support from extracellular matrix (ECM) proteins that provide cell adhesion sites and act as reservoirs for various signaling factors to enhance the viability and preserve pluripotency of hiPSCs [6]. Importantly, increasing evidence has demonstrated the importance of using biologically relevant and defined matrices [7]. Among several adhesion molecules, such as fibronectin, vitronectin, laminin 521 (LN 521) that contains α1, β5, and γ1 laminin chains has been identified as a unique protein permitting the long-term self-renewal of single-cell cultured hiPSCs under completely defined and xeno-free culture conditions [8]. However, the routine protein coating procedure through physical adsorption usually lead to the inhomogeneous distribution of those proteins, which would compromise their function in supporting hiPSC maintenance [9].

Polydopamine (PDA), typically used to join two materials including a wide range of inorganic and organic materials [10, 11], was employed to immobilize LN 521 onto polyethylene terephthalate (PET) surfaces to solve the problem mentioned above. An aqueous solution of dopamine with concentrations ranging from 0 to 2.0 mg/mL was applied on PET surfaces to form PDA layers. These PDA-modified surfaces were further functionalized with LN 521 to provide binding ligands for hiPSCs. Surface properties and the effect of PDA-LN521 modification on hiPSC adhesion, proliferation, as well as the maintenance of pluripotency were explored. Data presented here should be able to improve our understanding of the effects of an engineered surface on hiPSC culture.

METHODS

Preparation of substrates

Dopamine (3, 4-dihydroxyphenethylamine, Sigma-Aldrich, Germany) solution with concentrations ranging from 0 to 2.0 mg/mL was prepared using a Tris-HCl buffer solution (50 mM, pH 8.5) as a solvent. PET substrates (ThermoFisher Scientific, Germany) were immersed in the freshly prepared dopamine solution in an open vessel. After reacting for 1 h, the substrates were washed with distilled water.

Characterization of PDA-coated substrates

Water contact angle (WCA) measurements were performed using the sessile drop method. Briefly, 2 μL deionized water was placed on each surface and image was taken immediately. The contact angle was analyzed using ImageJ software (National Institutes of Health). The surface topography and Young's modulus of different surfaces were analyzed on an atomic force microscope (AFM, Nanowizard4, JPK, Germany) using a cantilever OMCL-AC200TS-R3 (OLYMPUS, Japan) at room temperature. The amount of LN 521 adsorbed on the substrate surface was quantified through immunostaining (anti-laminin gamma 1 antibody, ab80580, Abcam, USA).

Hipsc Culture

BIHi001-A hiPSC cell line [12] was used for study cell adhesion, proliferation, and pluripotent maintenance on those substrates. Detailed information of this cell line is available in the hPSCreg database (http://hpscreg.eu/cell-line/ BIHi001-A). For cell maintenance, hiPSCs were cultured on 5 μg/mL LN 521 (BioLamina, Sweden) coated tissue culture plate (TCP, Corning, Germany) using Essential 8 medium (ThermoFisher Scientific, Germany). For studying the influence of the engineered surfaces on hiPSC behaviors, PET substrates with or without PDA modification as well as TCP (used as a positive control) were functionalized with 5 μg/mL LN521 overnight at 4 °C before cell seeding.

Quantification of hipsc number

The number of hiPSCs was evaluated based on the DNA content using the FluoReporter™ Blue Fluorometric dsDNA Quantitation Kit (ThermoFisher Scientific, Germany). The standard curve that represents the relationship between the fluorescent intensity and the cell number was obtained according to the manufacture's instruction. To evaluate hiPSC adhesion, samples were harvested 1 h after seeding; to measure hiPSC proliferation, samples were harvested on Day 1, 3, and 7. The cell number was interpolated from the standard curve.

Characterization of hipscs

hiPSCs were cultured on each substrate and harvested on Day 3. Real-time PCR reactions were performed by using the resulting cDNA from each sample and designed primers (OCT4: 5'-ACATCAAAGCTCTGCAGAAAGAACT-3' and 5'-CTGAATACCTTCCCAAATAGAACCC-3'; SOX2: 5'-TGCGAGCGCTGCACAT-3' and 5'-GCAGCGTGTACTTATCCTTCTTCA-3'; SSEA4: 5'-TGGACGGGCACAACTTCATC-3' and 5'-GGGCAGGTTCTTGGCACTCT-3'; β-ACTIN: 5'-GCTCGTCGTCGACAACGGCTC-3' and 5'-CAAACATGATCTGGGTCATCTTCTT-3'). Real-time PCR reactions were amplified for 40 cycles on a StepOnePlus real-time PCR System (Applied Biosystems, ThermoFisher Scientific, Germany) in triplicate and normalized to β-ACTIN in the same run. Immunofluorescent staining was performed on Day 7 using 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, Germany) and antibodies, including Nanog and Sox2 (ThermoFisher Scientific, Germany).

Statistical analysis

All data presented were from at least three independent experiments and data are expressed as the mean ± standard deviation (SD). Statistical analysis was carried out using independent samples t-test to compare the difference between corresponding groups. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Surface modification

Properties of PDA-LN521 coated surfaces were characterized by measuring WCA, maximum peak-to-valley height roughness (R_t), and Young's modulus (Table 1). With increasing concentration of dopamine, PDA-LN521 functionalized surfaces showed reduced WCA from 62.1 ± 6.3 ° to 8.1 ± 2.9 °, demonstrating the higher concentration of dopamine solution used correlate with higher hydrophilicity [13, 14]. AFM measurements were carried out at room temperature. It showed that the R_t value of PDA-LN521 modified surfaces increased in a dopamine-concentration-dependent manner, ranging from 43.9±1.6 nm to 126.7±7.6 nm. Similarly, Young's modulus of PDA-LN521 modified surfaces was substantially increased from 0.98±0.36 gpa to 4.81±2.41 gpa. Together, these resulted showed the presence of PDA-LN521 layer could provide an extracellular environment with a mechanically controllable and tunable surface for c culture.

Table 1. WCA, R_t, and Young's modulus of PDA-LN 521 modified surfaces at room temperature

Dopamine concentration [mg/mL]	WCA [°]	R_t [nm]	Yong's modulus [GPa]
0	62.1±6.3	43.9±1.6	0.98±0.36
0.125	56.9±4.8	54.7±2.5	1.05±0.24
0.25	47.8±2.5	68.8±3.2	1.14±0.41
0.5	27.9±3.1	84.0±2.0	1.71±0.61
1.0	18.9±2.6	100.4±2.5	2.92±0.86
2.0	8.1±2.9	126.6±7.6	4.81±2.41

Quantification of LN 521 adsorption to PDA engineered surfaces

As LN 521 is able to sustain the self-renewal and pluripotency of cs [7, 15], it is important to quantify the amount of LN 521 presented on the substrate surface. PDA layer induced a higher amount of LN 521 adsorption (Figure 1), which may subsequently promote cs attachment and proliferation.

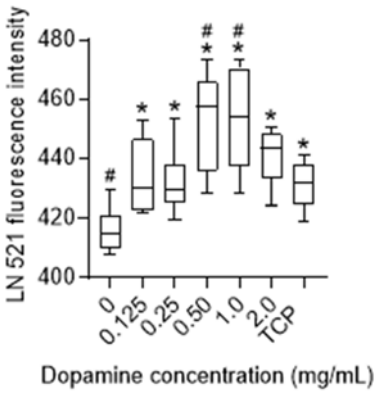

Figure 1. Quantification of LN 521. Amount of LN 521 presented on substrate surfaces. (n=6, PET and TCP surfaces modified only with LN 521, shown as 0 and TCP respectively, * $p < 0.05$ versus 0, # $p < 0.05$ TCP).

Cell adhesion and proliferation on PDA-LN 521 modified surfaces

C adhesion on PDA-LN521 was observed 1 h after cell seeding (Figure 2A). A 13.0±7.2% increase and a 24.2±8.1% increase were observed on the surfaces modified with 0.125 and 0.25 mg/mL dopamine solution. There was no further increase in C adhesion on pre-treated surfaces using dopamine solution with higher concentration. This may be closely related to WCA of the surfaces, as previous studies demonstrated that surfaces with moderate hydrophilicity (WCA, around 40-60°) promoted cell adhesion [16, 17].

The proliferation of hiPSCs was evaluated on Day 1, 3, and 7 after seeding (Figure 2B). hiPSCs showed no decrease of their proliferation on PDA-LN 521 surfaces (from 0 to 1.0 mg/mL) on Day 1. The enhanced proliferation rate of hiPSCs was observed within a concentration range of 0.125 to 0.5 mg/mL on Day 3 and Day 7 as compared to PET and TCP only treated with LN 521. While elevated content of PDA (2.0 mg/mL) could hamper hiPSC proliferation, consisting with the result that high PDA dosage inhibits cell proliferation [18].

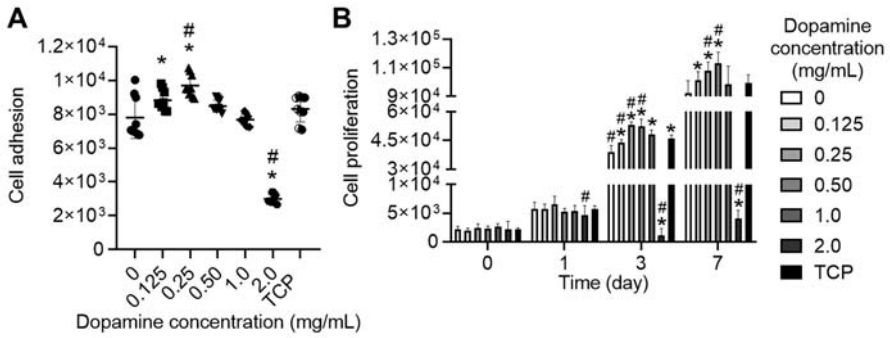

Figure 2. hiPSC adhesion and proliferation. (A) the number of hiPSCs adhered to the surfaces 1 h after cell seeding (n=9, PET and TCP surfaces modified only with LN 521, shown as *0* and *TCP* respectively, * $p < 0.05$ versus *0*, # $p < 0.05$ *TCP*); (B) the number of hiPSCs were quantified at different time points after seeding. (n=8, PET and TCP surfaces modified only with LN 521, shown as *0* and *TCP* respectively, * $p < 0.05$ versus *0*, # $p < 0.05$ *TCP*).

Maintenance of hipsc pluripotency

Continued growth in biotech-sector in the field of drug testing and cell therapy creates an increasing demand to expand and maintain undifferentiated hiPSCs [3]. Previous studies report that LN 521 is able to support renewal and maintaining hiPSC pluripotency [15]. Here, the expression of OCT4, SOX2, and SSEA4 of hiPSCs on PDA-LN 521 surfaces was examined on Day 3 (Figure 3A), and stable expression of key pluripotency markers, including Nanog and Sox2, was detected on Day 7 as shown by immunofluorescence (Figure 3B). In conclusion, the long-term maintenance of hiPSCs was not influenced by various PDA content, although these might induce variations on temporal gene expression.

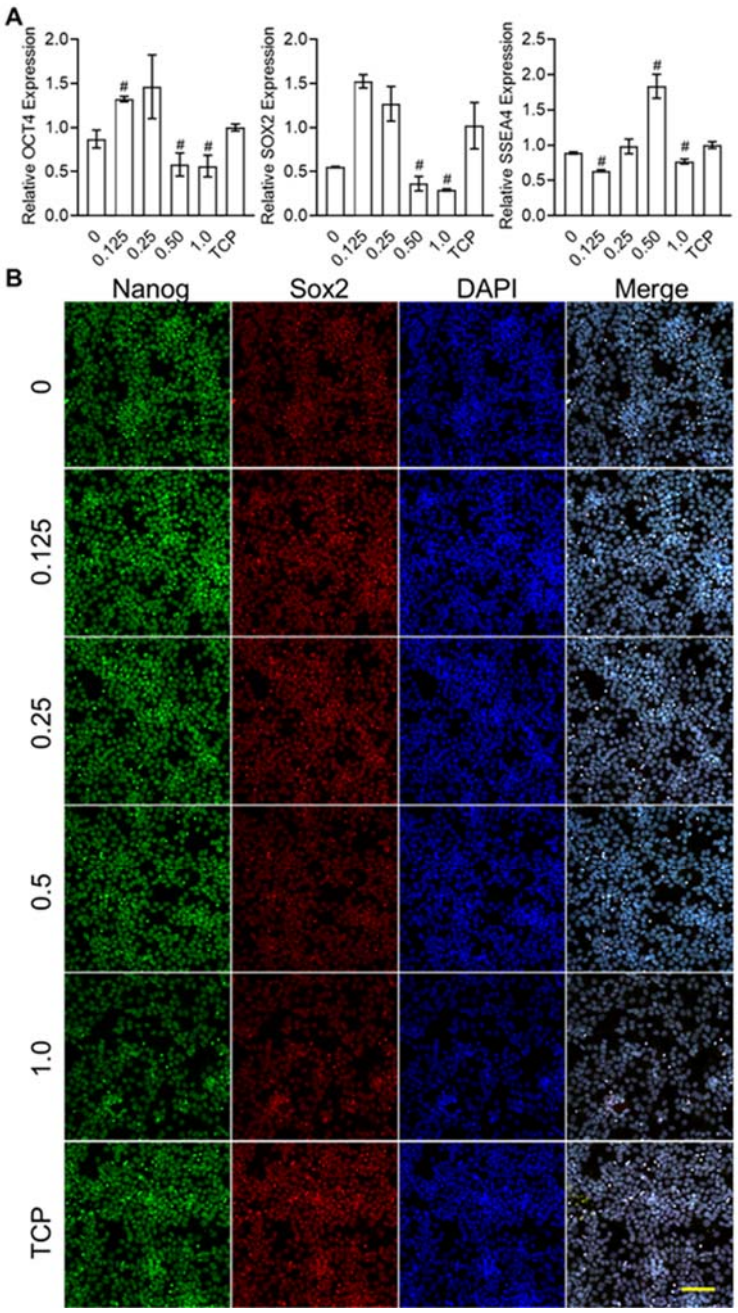

Figure 3. Relative pluripotent gene expression level. (A) Relative gene expression of hiPSCs on Day 3. (n=3, PET and TCP surfaces modified only with LN 521, shown as *0* and *TCP* respectively, # $p < 0.05$ versus *TCP*). (B) Immunofluorescent staining of hiPSCs on Day 7 (scale bar = 50 µm).

CONCLUSION

In our study, the influence of PDA-LN521 modified PET on hiPSCs behaviors was investigated. Surface properties of substrates could be modified in a controllable manner by adjusting the concentration of dopamine. A result of particular importance is that the use of 0.25 mg/mL dopamine solution could not only favor the adhesion and proliferation of hiPSCs but also preserve hiPSC pluripotency. Conclusively, PDA modification of bulk material surfaces showed tremendous potential for the cultivation of hiPSCs, as it was able to promote the *in vitro* expansion of hiPSCs and preserve their pluripotency.

ACKNOWLEDGEMENTS

We thank the Berlin Institute of Health (BIH) Stem Cell Core Facility for kindly providing us with the used hiPSC line BIHi001-A. This work was financially supported by the Helmholtz-Association of German Research Centers (through program-oriented funding, Helmholtz-Portfolio Topic "Technology and Medicine", Helmholtz Virtual Institute "Multifunctional Biomaterials for Medicine (VH-VI-423), as well as through Helmholtz Graduate School of Macromolecular Bioscience (MacroBio), grant no. VH-GS-503) and the Federal Ministry of Education and Research, Germany through the Program Health Research, grant no. 13GW0098. Yan Nie is grateful for support from the China Scholarship Council (No. 201706205049).

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