Electronic Supplementary Information for

Vascular cell responses to silicone surfaces grafted with heparin-like polymers: surface chemical composition vs topographic patterning

Wei Sun, Sheng Jin, Aiyang Zhang, Jialei Huang, Yuepeng Li, Xiaoli Liu,* Hong Chen*

State and Local Joint Engineering Laboratory for Novel Functional Polymeric Materials, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, 199 Ren-Ai Road, Suzhou 215123, P. R. China.

Corresponding Author

*Tel: +86-512-65880827; Fax: +86-512-65880583;

E-mail: liuxiaoli@suda.edu.cn (X. Liu); chenh@suda.edu.cn (H. Chen).

Materials

Sylgard 184 was obtained from Dow Corning (Midland, MI, USA). D-(+)-glucosamine hydrochloride was from Tokyo Chemical Industry Company. Sodium 4-vinyl-benzenesulfonate (SS), 10-undecen-1-ol (99%), paraformaldehyde, dimanganesedecacarbonyl ($Mn_2(CO)_{10}$, 98%), Triton X-100, Actin-Tracker Green (Phalloidin-FITC), 4',6-diamidino-2-phenylindole (DAPI) and human serum albumin (HSA) were from Sigma-Aldrich Chemical Company. Methacryloyl chloride (stabilized with hydroquinone monomethyl ether) was from Aladdin Reagent Inc. $Na^{125}I$ was from Chengdu Gaotong Isotope Co., Ltd (China). Human umbilical vein endothelial cells (HUVECs), human umbilical vein smooth muscle cells (HUVSMCs), endothelial cell medium (ECM) and smooth muscle cell medium (SMCM) were from ScienCell (USA). All the other solvents were purchased from the Sinopharm Chemical Reagent Co. Ltd. (China), and purified before use according to standard methods. Silicon template was obtained from Suzhou Institute of Nano-Tech and Nano-Bionics (SINANO), Chinese Academy of Sciences. Human VEGF enzyme-linked immunosorbent assay (ELISA) kit was from Boster (China, catalog #EK0539).

Undec-10-enyl-2-bromo-2-methylpropanoate and 2-(methacrylamido)glucopyranose (MAG) were synthesized according to previously reported methods.[1, 2] Undec-10-enyl-2-bromo-2-methylpropanoate: ^1H NMR (400 MHz, CD_3OD), δ(ppm): 5.79-5.87 (m, 1H, =C***H***), 4.89-5.02 (d, 2H, =C***H***$_2$), 4.19 (t, 2H, -C***H***$_2$O), 2.06 (m, 2H, =CHC***H***$_2$), 1.93 (s, 6H, -C***H***$_3$), 1.70 (m, 2H, -C***H***$_2$CH$_2$O), 1.41 (m, 2H, -C***H***$_2$CH$_2$CH$_2$O), 1.35 (m, 10H,-

C***H***$_2$C***H***$_2$C***H***$_2$C***H***$_2$C***H***$_2$-). MAG: ^{1}H NMR(D_2O, 400 MHz), δ(ppm): 5.69 (s, 1H, =C***H***H), 5.46 (s,1H, =CH***H***), 5.21 (d, 0.53H, anomeric α-C***H***), 4.70-4.74 (d, 0.53H, anomeric β-C***H***), 3.40-4.00 (m, 6H, sugar moiety 6×C***H***), 1.93 (s, 3H, -C***H***$_3$)

In vitro stability test

The samples were immersed in PBS at 37 °C for 24 h and washed three times by deionized water. The dried samples were then characterized by water contact angle and FT-IR.

Protein adsorption from PBS

To measure protein adsorption on the surfaces, the radiolabeled protein was first mixed with unlabeled protein at an approximate concentration of 5% of the endogenous protein level. The surfaces were immersed in PBS (pH = 7.4) overnight prior to the protein adsorption experiments and then immersed in PBS containing the radiolabeled protein (BSA) at 25 °C for 3 h. Samples were rinsed three times (10 min each time) with PBS, wicked onto filter paper and transferred to clean tubes for radioactivity determination using a Wallac 2480 Wizard 3″ Automatic Gamma Counter (PerkinElmer Life Sciences, Shelton, CT).

Vascular cell culture

HUVECs were seeded on sample surfaces at a cell density of 25 000/cm^2 and cultured in ECM at 37 °C under 5% CO_2 for 4 h or 48 h. After incubation, the samples were washed

three times with PBS and then treated with 4% paraformaldehyde for at 25 °C for 10 min to fix the adherent cells. The samples were treated with 0.1% Triton X-100 for 5 min, washed three times with PBS, and then incubated with 3% BSA in PBS for 40 min, Phalloidin-FITC for 40 min and DAPI for 10 min in the dark. The stained cells were observed using a fluorescence microscope (Olympus IX71 Carl Zeiss, Germany). Three replicate experiments were performed. The density of HUVECs adherent to the surfaces was calculated from at least 10 images for each sample using Image-Pro Plus software.

HUVSMC culture experiments were conducted in the same way. In these experiments, SMCM was used as the culture medium. And HUVSMCs were seeded on sample surfaces at a cell density of 12 000/cm^2.

The images of HUVECs and HUVSMCs cultured on surfaces after fixation by 4% paraformaldehyde for 10 min and dehydration by graded ethanol solutions (30−100%) were taken by scanning electron microscope (SEM, S-4700, Hitachi, Japan).

Adsorption of vascular endothelial growth factor

The adsorption of vascular endothelial growth factor (VEGF165, Boster Bioengineering) was measured using an enzyme-linked immunosorbent assay (ELISA) kit. First, samples were equilibrated overnight in PBS at 4 °C. They were then incubated in VEGF solution (1.5 ng/mL) at 4 °C for 2 h, washed three times with PBS, and the washes combined. The VEGF concentration in each remaining solution and wash was measured according to the ELISA kit manufacturer's instructions. The quantity of VEGF adsorbed

on the surfaces was calculated from the difference between the initial and remaining amounts of VEGF in the solutions. The experiment was carried out in three replicates.

Statistical analysis

All experiments were performed independently at least in duplicate and quantified with at least three parallel samples per condition in each experiment. The results are expressed as the mean ± standard error of each sample. Comparison of data between PDMS-Br and PDMS surfaces grafted with heparin-like polymers was carried out by one-way ANOVA (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

Table S1 Atomic concentrations of flat/patterned PDMS-Br, PDMS-pSS, PDMS-pSG and PDMS-pMAG surfaces measured by XPS. The symbol "-" indicates non-detectable level.

Surface		elemental composition (%)						
		Si	C	O	Br	N	S	N/S
Flat	PDMS	28.40	44.00	27.60	-	-	-	-
	PDMS-Br	25.54	45.16	29.20	0.11	-	-	-
	PDMS-pSS	17.66	52.53	27.52	-	-	2.30	-
	PDMS-pSG	18.18	50.66	28.49	0.24	1.43	1.00	1.43
	PDMS-pMAG	16.62	51.40	29.65	0.20	2.14	-	-
Pattern	PDMS-Br	23.22	46.02	30.63	0.14	-	-	-
	PDMS-pSS	20.71	50.94	27.46	0.18	-	0.71	-
	PDMS-pSG	21.53	48.28	28.38	0.34	0.88	0.59	1.49
	PDMS-pMAG	23.74	46.81	28.10	0.29	1.07	-	-

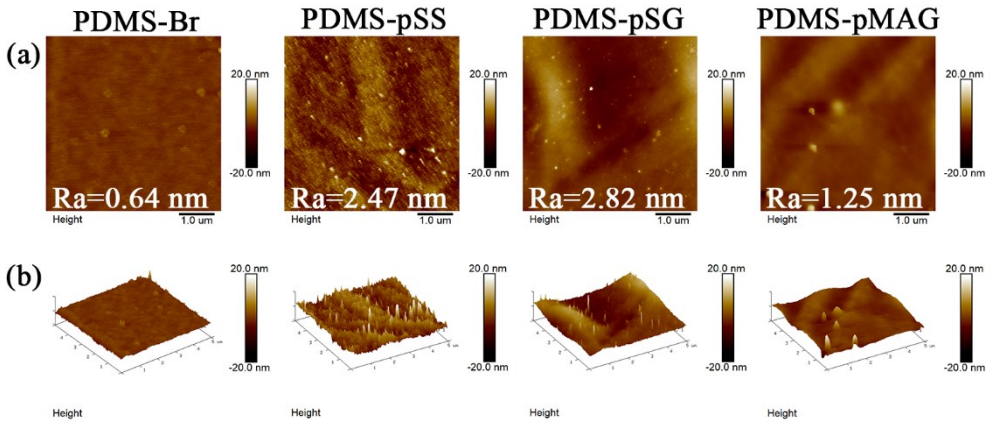

Figure S1 AFM height images (a) and 3D images (b) of flat PDMS-Br, PDMS-pSS, PDMS-pSG and PDMS-pMAG surfaces.

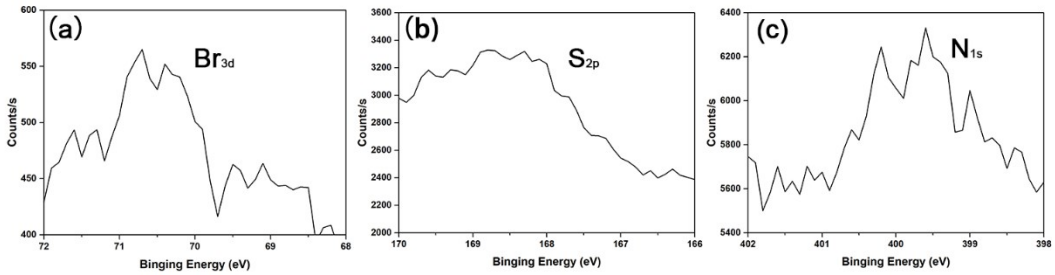

Figure S2 XPS high-resolution spectra of patterned PDMS-Br (a), PDMS-pSS (b), and PDMS-pMAG (c) surfaces.

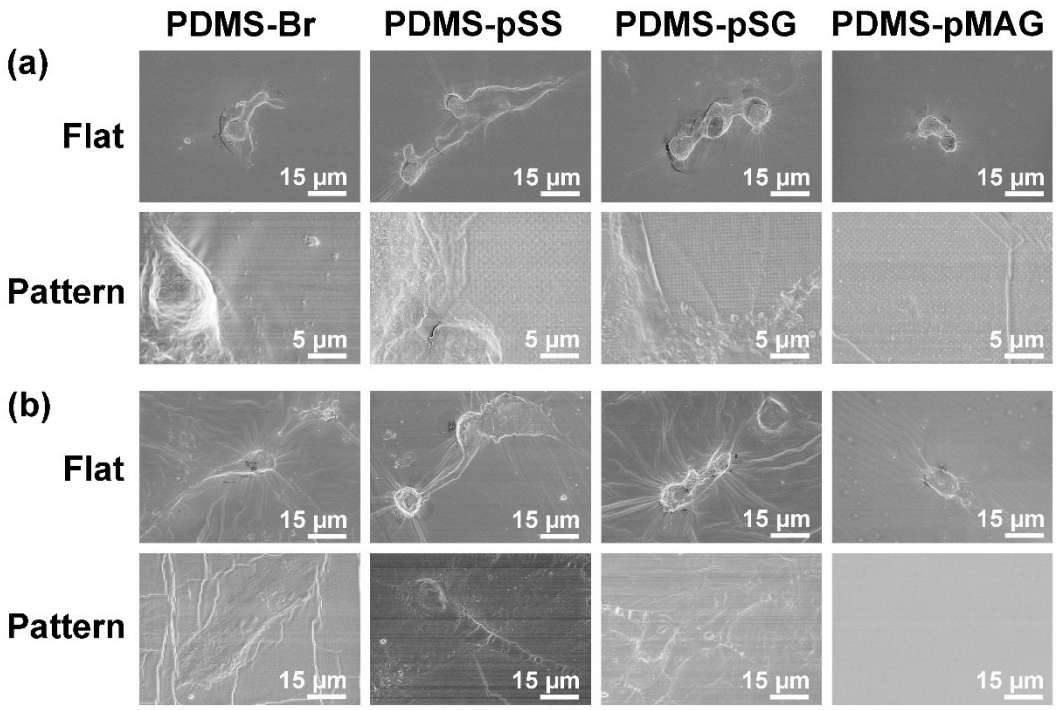

Figure S3 SEM images of HUVECs on the flat and patterned PDMS-Br, PDMS-pSS, PDMS-pSG and PDMS-pMAG surfaces after 4 h culture (a) and 48 h culture (b).

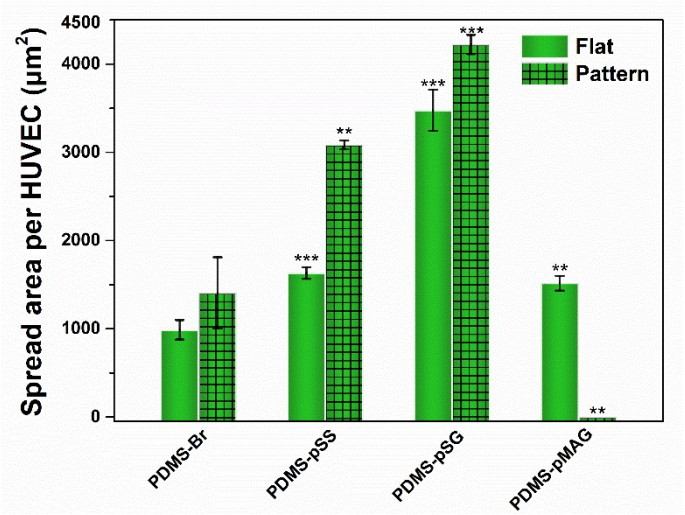

Figure S4 The average area of HUVECs spreading on the flat and patterned PDMS-Br, PDMS-pSS, PDMS-pSG and PDMS-pMAG surfaces after 48 h culture (mean ± SD, n = 6). Comparison of data between flat and corresponding patterned surfaces was carried out by one-way ANOVA (**$p < 0.01$, and ***$p < 0.001$).

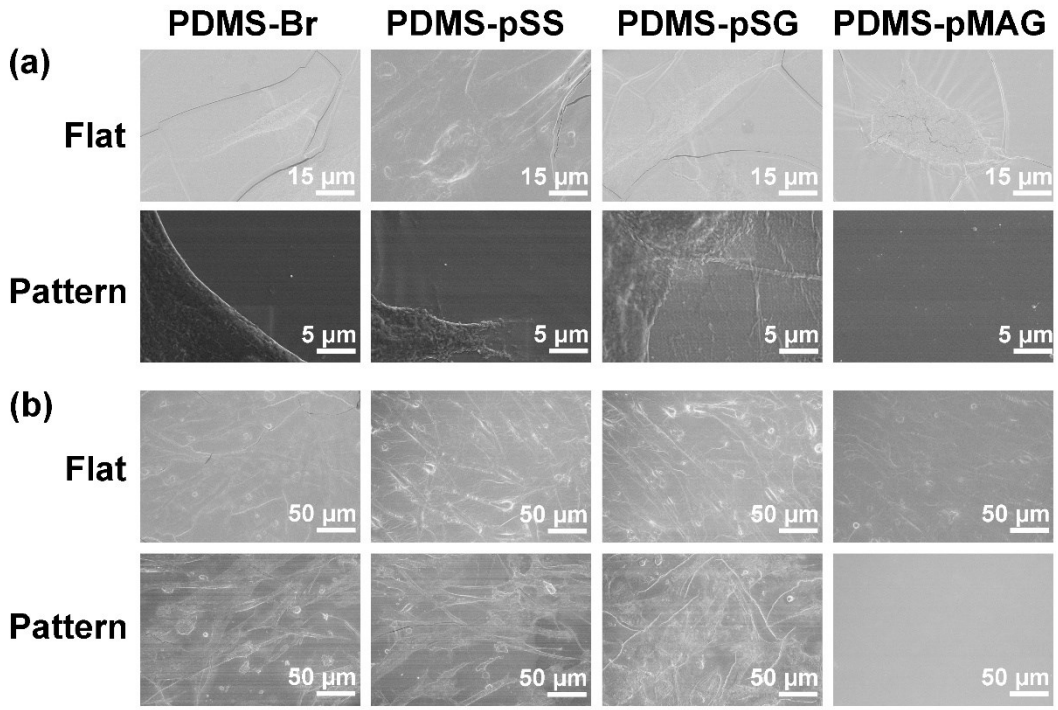

Figure S5 SEM images of HUVSMCs on the flat and patterned PDMS-Br, PDMS-pSS, PDMS-pSG and PDMS-pMAG surfaces after 4 h culture (a) and 48 h culture (b).

References

1. J. Huang, H. Murata, R. R. Koepsel, A. J. Russell and K. Matyjaszewski, *Biomacromolecules*, 2007, **8**, 1396-1399.
2. S. R. S. Ting, E. H. Min, P. B. Zetterlund and M. H. Stenzel, *Macromolecules*, 2010, **43**, 5211-5221.