Supporting Information

Self-assembled monolayers of enantiomerically functionalized periodic mesoporous organosilicas and the effect of surface chirality on cell adhesion behavior

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Materials: *D*- and *L*-mannose pentaacetate, (purum, ≥97.0%), hexadecyltrimethylammonium bromide (CTAB, 98%), 1,2-bis(trimethoxysilyl)ethane (BTME, 96%), 3-aminopropyltrimethoxysilane (APTES, 99%), 3-chloropropyltrimethoxysilane (CP-TMS, 97%), paraformaldehyde (PFA), *N*-hydroxy-succinimide (NHS), and trypsin, were purchased from Sigma-Aldrich. Ethanol (absolute for analysis), ammonia solution (32%, pure) and hydrochloric acid (32%, for analysis), were purchased from Merck (stored over molecular sieves, puriss., H_2O_0.005%). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid (EDC) was obtained from ABCR. Hoechst 33342, Phalloidin Alexa Fluor® 546 and Phalloidin Alexa Fluor® 546 488 were purchased by Invitrogen. Toluene was purchased from Merck (stored over molecular sieves, puriss, H_2O 0.005%). Glass plates (1.8 cm x 1.8 cm) used for PMO monolayers were obtained from Servoprax GmbH. The cell medium (supplemented with 200 µg/mL Penicillin/Streptomycin, 200 µg/mL Gentamycin) and 10% (v/v) fetal bovine serum (FBS) were obtained from Biochrom, Germany. The enzymes (Collagenase/Dispase) for isolating porcine brain capillary endothelial cells (PBCECs) were purchased from Roche (Germany). PBCECs were isolated and cultured according to Franke *et al*. (H. Franke, H. J. Galla, C. T. Beuckmann, *Brain Res. Brain Res. Protoc.* 2000, **5**, 248). ANOVA test was used as statistical analysis to show the significance of the quantitative number of adhered cells in different functional groups. **ANOVA test**: This test is a single-step multiple comparison procedure and statistical test generally used in conjunction with an ANOVA to find which means are significantly different from one another. The significance level is usually denoted by the Greek symbol α (lowercase alpha). Popular levels of significance are 5% (0.05 = *), 1% (0.01 = **) and 0.1% (0.001 =

***). If tests of significance gives a p-value lower than the α-level, the null hypothesis is rejected. Such results are informally referred to as 'statistically significant'. The lower the significance level, the stronger the evidence required. Choosing level of significance is an arbitrary task, but for many applications, a level of 5% is chosen, for no better reason than that it is conventional.

Synthesis of mannose amido-acids [D(L)-MAN]: D- and *L*-MAN were prepared according to literature (K. El-Boubbou, C. Gruden, X. Huang, *J. Am. Chem. Soc.* 2007, **129**, 13392). Comparison of ^{1}H NMR spectra of *D*- and *L*-MAN with literature confirms their structure.

^{1}H NMR of *D*-MAN, (CD_3OD, 300 MHz): δ_H 1.76-1.84 (m, 2H), 2.49 (t, J = 6.3 Hz, 2H), 2.62 (t, J = 6.4 Hz, 2H,), 3.25-3.30 (m, 2H), 3.45-3.88 (m, 8H), 4.75(d, J = 1.6 Hz, 1H).

HRMS *m*/*z* calcd. for *D*-MAN: $C_{13}H_{23}NNaO_9$ $[M + Na]^+$: 360.1271, found: 360.1265.

^{1}H NMR of *L*-MAN, (CD_3OD, 300 MHz): δ_H 1.76-1.84 (m, 2H), 2.49 (t, J = 6.2 Hz, 2H), 2.62 (t, J = 6.4 Hz, 2H,), 3.25-3.30 (m, 2H), 3.45-3.83 (m, 8H), 4.75(d, J = 1.6 Hz, 1H).

HRMS *m*/*z* calcd. for *L-MAN*: $C_{13}H_{23}NNaO_9$ $[M + Na]^+$: 360.1271, found: 360.1265.

Synthesis of PMO-NH$_2$ was done according to modified literature procedure (W. Guo, J. Wang, S. J. Lee, F. Dong, S. S. Park, C. S. Ha, *Chem. Eur. J.* 2010, **16**, 8641)*:* 484.5 mg of CTAB was dissolved in 88 mL of H_2O, 33 mL of ethanol, and a 28 wt% ammonia (0.075 g) solution. The reaction mixture was stirred at room temperature for 1 h before the addition of BTME (1.27 g) and APTES (0.26 g). The above reaction mixture was continuously stirred for an additional 72 h at room temperature. The CTAB mesoporous template was removed by stirring the sample in ethanol (50 mL) with a 36 wt% aqueous solution of HCl (1.5 g) at 50 ^{o}C for 6 h. The resulting solid was recovered by centrifugation, washed with ethanol and acetone several times, and dried at 60 ^{o}C under vacuum.

DXP loading of PMO-NH$_2$: PMO-NH_2 (100 mg) was suspended in 10 mL toluene and mixed with DXP (8 mg). This reaction mixture was refluxed at 120 ^{o}C for 2 days. The final

product was obtained by centrifugation, washed with toluene x 2, ethanol x 2, and dried at room temperature.

Hoechst 33342 loading of PMO-NH_2: PMO-NH_2 (10 mg) was suspended in 1 mL water and mixed with Hoechst 33342 (0.1 mg). This reaction mixture was stirred for 1 day at room temperature. The final product ^{Hoechst}PMO-NH_2 was obtained by centrifugation, washed with water x 2 and dried at room temperature.

Functionalization of ^{DXP}PMO-NH_2 with D(L)-MAN: A solution of 10 mM *D(L)*-MAN, 30 mM EDC, and 60 mM NHS in 1 mL DMSO was added dropwise to a suspension of the ^{DXP}PMO-NH_2 (20 mg) in 1 mL DMSO. The reaction mixture was stirred for 16 h at room temperature. Subsequently the suspension was centrifuged 10 min at 4400 rpm and the isolated solid was washed with DMSO x 2 and ethanol x 2. Finally the solid was dried at room temperature.

Functionalization of ^{Hoechst}PMO-NH_2 with D-MAN: A solution of 10 mM *D*-MAN, 30 mM EDC, and 60 mM NHS in 1 mL DMSO was added dropwise to a suspension of the ^{Hoechst}PMO-NH_2 (10 mg) in 1 mL DMSO. The reaction mixture was stirred for 16 h at room temperature. Subsequently the suspension was centrifuged 10 min at 4400 rpm and the isolated solid was washed with DMSO x 2 and ethanol x 2. Finally the solid was dried at room temperature.

General procedure of the preparation of SAMs of functionalized PMOs: Glass plates were cleaned with "piranha solution" (H_2SO_4/H_2O_2, 3/1) by refluxing at 100°C for 1 hour, the plates were washed with large amounts of bidistilled water following with ethanol and finally dried at 80°C. The clean substrates were then set into a Teflon support to avoid any surface overlap and the system was placed in a 100 ml round bottom flask containing toluene (20 ml). CP-TMS was added (0.8 ml) and the reaction flask was heated at 115ºC for 3 hours. Subsequently, plates were washed with toluene dried with nitrogen and placed one glass plate in 1 mg/ml suspension of PMOs in toluene. Catalytic amount of triethylamine (10 µl) was

added to the reaction mixture and the final suspension was sonicated for 30 minutes; the plates were taken out, washed with toluene and dried in air.

Cell adhesion experiments on glass slide and on SAMs of ^{DXP}PMO-D-MAN, ^{DXP}PMO-L-MAN, and ^{DXP}PMO-NH$_2$: The cells were carefully thawed and resuspended in their specific medium (dulbecco's modified eagle's medium for HeLa and C-6-glioma, earle's medium M199 for endothelial cells, McCoy's 5A for macrophages) for cell experiments in serum free medium The cell medium was supplemented with 200 µg/mL Penicillin/Streptomycin, 200 µg/mL Gentamycin and with 10% (v/v) fetal bovine serum (FBS) for cell experiments in serum containing medium. HeLa, C-6-Glioma (cell line rat astrocyte glioma cancer cells), primary macrophage and primary porcine brain capillary endothelial cells (PBCEC) were seeded on glass slide and on SAMs of functionalized PMOs and incubated for 24 hours at 37°C and 5 % CO_2. After the incubation period the adhered cells on glass slide and on PMO monolayers was washed twice with PBS^{++} (supplemented with 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$) to remove non-adhered cells, fixed with 4% PFA solution and stained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI).

Counting of adhered cells on glass slide and on SAMs of ^{DXP}PMO-D-MAN, ^{DXP}PMO-L-MAN, and ^{DXP}PMO-NH$_2$: The cells were carefully thawed and resuspended in their specific medium (dulbecco's modified eagle's medium for HeLa and C-6-glioma, earle's medium M199 for endothelial cells, McCoy's 5A for macrophages). The cell medium was supplemented with 200 µg/mL Penicillin/Streptomycin, 200 µg/mL Gentamycin and with 10% (v/v) fetal bovine serum (FBS). HeLa, C-6-Glioma (cell line rat astrocyte glioma cancer cells), primary macrophage and primary porcine brain capillary endothelial cells (PBCEC) were seeded on glass slide and on SAMs of functionalized PMOs and incubated for 24 hours at 37°C and 5 % CO_2. After the incubation period the adhered cells on glass slide and on PMO monolayers was washed twice with PBS^{++} (supplemented with 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$) to remove non-adhered cells. Subsequently, glass slide and monolayers were treated

with trypsin for 10 min incubation time and cells were counted immediately with an automatic cell counter

Cell adhesion experiments on ^{Hoechst}PMO-D-MAN: HeLa cells were seeded on SAM of ^{Hoechst}PMO-*D*-MAN and incubated for 24 hours at 37°C and 5 % CO_2. After the incubation period the adhered cells on PMO monolayer was washed twice with PBS^{++} to remove non-adhered cells and fixed with 4% PFA solution.

Separation of cells on SAM of PMO-D-MAN: HeLa or C-6-Glioma cells were incubated with Hoechst 33342 (0.1 mg) and endothelial cells were incubated with Phallodine (30 µl) in their specific cell culture medium (2ml) for 24 h at 37°C and 5 % CO_2 in cell culture plate. Hoechst 33342 and Phallodine stained cells were washed twice with PBS^{++} to remove excess fluorescence dye molecules. Cells were treated with trypsin for 10 min incubation time and centrifuged. A 1:1 mixture of blue emitting dye stained HeLa or C-6-Glioma cells and red emitting dye stained endothelial cells were prepared and seeded on SAMs of PMO-*D*-MAN in cell culture plate and incubated for 24 hours at 37°C and 5 % CO_2. After the incubation period SAMs of PMO-*D*-MAN and cell culture plate were separated and the adhered cells on both surfaces were washed twice with PBS^{++} (supplemented with 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$) to remove non-adhered cells and fixed with 4% PFA solution.

Characterization: The morphology of the PMO-NH_2 and SAM of PMOs were investigated using a Zeiss 1540 EsB dual beam focused ion beam/field emission SEM with a working distance of 9 mm and an electronic high tension (EHT) of 3 kV. ATR-IR spectra were carried out with Golden Gate ATR Accessory (resolution: 4 cm^{-1}, number of scans: 16). Zeta potential measurements and DSL were done at pH 7.2 in PBS buffer with Malvern Zetasizer Nano Series. Cells were counted with TC10™ automatic cell counter from Bio-Rad.

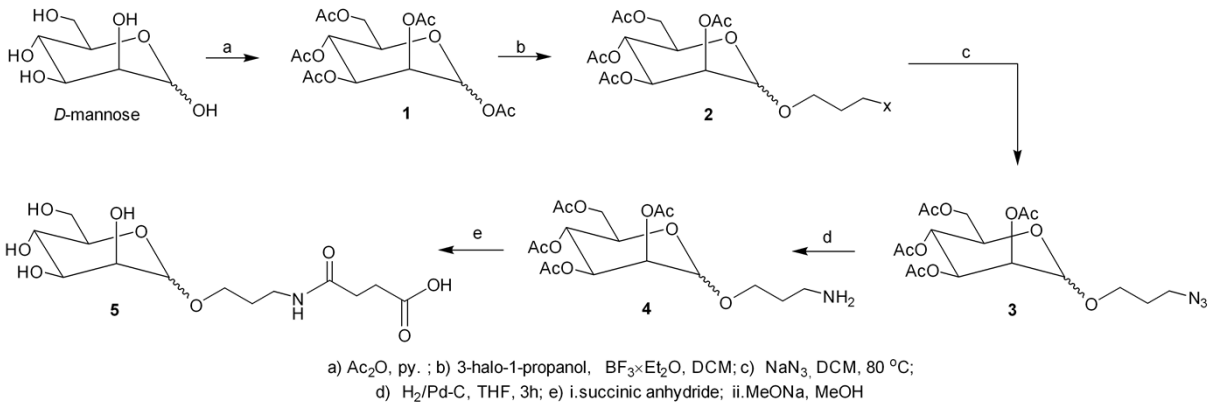

Fig. S1. Synthesis of mannose amido-acids [*D(L)*-MAN].

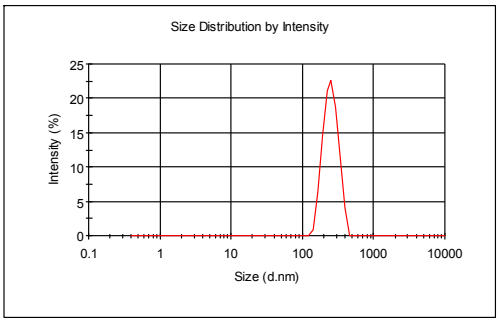

Fig. S2. Size distribution histogram of ^{DXP}PMO-NH_2 [Z-AVg (d.nm) = 243].

The curvature of ^{DXP}PMO-NH_2 is calculated according to literature: K. P. Browne, B. A. Grzybowski, Langmuir, 2010, 27, 1246:

κ=1/radius
κ=1/121,5 nm
κ=0,0082 nm^{-1}

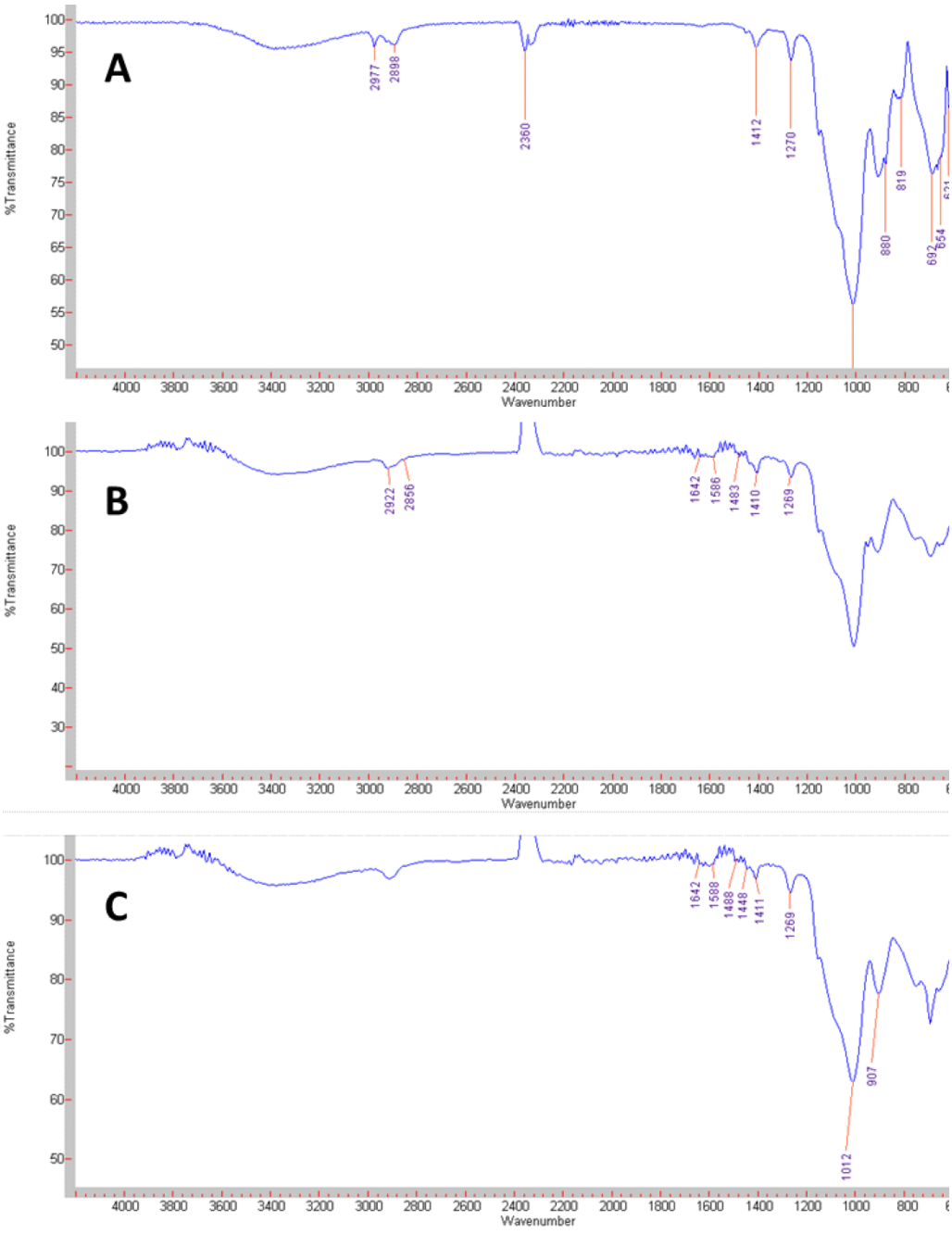

Fig. S3. ATR-IR spectra of **A**.^{DXP}PMO-NH_2, **B**. ^{DXP}PMO-*D*-MAN, **C**. ^{DXP}PMO-*L*-MAN.

Table S1. TG analysis: weight composition of the PMO derivatives

Sample	Silica	Ethane+AP[a]	DXP	Func. group
PMO-NH_2	85.20 %	14.80 %		
^{DXP}PMO-NH_2	81.00 %	14.00 %	5.00 %	
^{DXP}PMO-*L*-MAN	72.50 %	12.40 %	4.30 %	10.80 %
^{DXP}PMO-*D*-MAN	72.55 %	12.30 %	4.35 %	10.80 %

[a.] Ethane and AP (amino propyl) are originated from the organic backbone and the organic functional groups of the respective PMO particles.

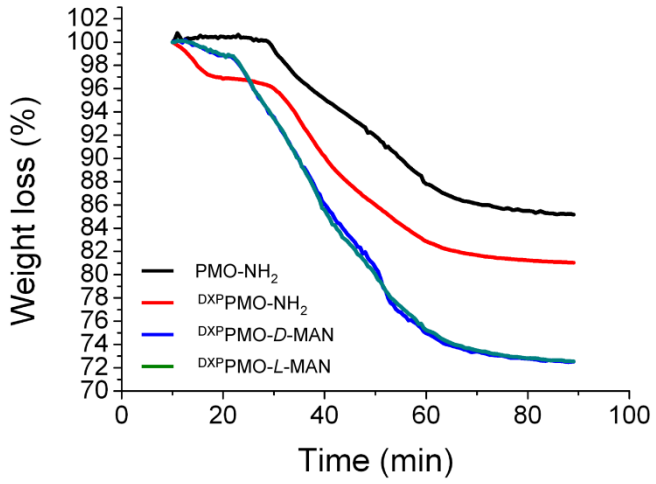

Fig. S4. TG analysis of PMO derivatives.

Table S2. Quantitative numbers (× 10^4) of alive adhered endothelial / macrophage / HeLa / C-6-Glioma cells after 24 h incubation time (37 °C) on glass slide, SAMs of ^{DXP}PMO-NH_2, ^{DXP}PMO-*L*-MAN, and ^{DXP}PMO-*D*-MAN in serum containing media (cell viability was given in parentheses).

	Glass slide	^{DXP}PMO-NH_2	^{DXP}PMO-*L*-MAN	^{DXP}PMO-*D*-MAN
Endothelial cells	n.o.	n.o.	n.o.	n.o.
Macrophage	n.o.	0.5 ± 0.1 (95%)	0.6 ± 0.1 (91%)	2.3 ± 0.2 (90%)
HeLa cells	1.0 ± 0.2 (98%)	1.8 ± 0.1 (95%)	2.5 ± 0.1 (86 %)	5.6 ± 0.2 (85 %)
C-6-Glioma cells	1.4 ± 0.5 (98%)	10.5 ± 0.4 (96%)	16.0 ± 1.0 (87%)	30.0 ± 1.0 (91%)

n.o. : not observed

Table S3. Quantitative numbers (× 10^4) of adhered endothelial / macrophage / HeLa / C-6-Glioma cells after 24 h incubation time (37 °C) on glass slide, SAMs of ^{DXP}PMO-NH_2, ^{DXP}PMO-*L*-MAN, and ^{DXP}PMO-*D*-MAN in serum free media.

	Glass slide	^{DXP}PMO-NH_2	^{DXP}PMO-*L*-MAN	^{DXP}PMO-*D*-MAN
Endothelial cells	n.o.	n.o.	n.o.	n.o.
Macrophage	n.o.	n.o.	n.o.	n.o.
HeLa cells	0.7 ± 0.2	1.0 ± 0.1	1.7 ± 0.3	2.0 ± 0.1
C-6-Glioma cells	0.7 ± 0.1	1.4 ± 0.1	6.1 ± 0.4	6.6 ± 0.3

n.o. : not observed