Supporting Information

Polyelectrolyte Multilayers of Poly (L-lysine) and Hyaluronic Acid on Nanostructured Surfaces Affect Stem Cell Response

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The Supporting Information contains six figures, one table and additional methodological information on nine pages.

EXPERIMENTAL SECTION

Cell adhesion

Adherent hADSC were fixed with 4% paraformaldehyde (Sigma-Aldrich) solution at room temperature for 15 min and washed three times with PBS. After permeabilization using 0.1% (v/v) Triton X-100 (Sigma-Aldrich) for 10 min, the non-specific binding sites were blocked with a 1% bovine serum albumin solution (BSA, Roth) in PBS at room temperature for 1 h. Actin cytoskeleton and the nucleus were stained with BODIPY®-phalloidin (Invitrogen, Germany) and TO-PRO3® (Invitrogen), respectively. Focal adhesion complexes were visualized with a primary mouse antibody raised against vinculin (Sigma-Aldrich) and a CY2-conjugated secondary anti-mouse antibody (Dianova, Germany). Simultaneously, cells in another set of samples were stained for the small GTPase Ras homolog family member A (RhoA) using a primary mouse antibody (Santa Cruz Biotechnology, Germany), which was visualized with a secondary, FITC-conjugated goat anti-mouse antibody. Further, the cells were stained for the focal adhesion kinase (FAK) using a primary rabbit antibody (Santa Cruz Biotechnology, Germany), which was visualized with a secondary, CY3-conjugated goat anti-rabbit antibody (Dianova). Each of the staining steps was stopped after 30 min by rinsing the samples twice with PBS. Finally, all samples were dipped into ultrapure water, mounted to object holders, and examined with CLSM. At least five images per samples (10X, 20X objectives) were used to determine cell count and morphology using Zeiss efficient navigation (ZEN 2012) and ImageJ (version 1.49m) software.

Cell differentiation

Human adipose-derived stem cells (hADSC) were seeded at density of 25,000 cells/sample in control media (α-MEM supplemented with 10% FBS and 1% Pen/Strep) for seven days and incubated in specific induction media for additional 21 days (**Table S1**).

Table S1 - Composition of different induction media used in the differentiation study

	Adipogenic medium	**Osteogenic medium**	**Chondrogenic medium**
Pen/Strep	1%	1%	1%
FBS	1%	1%	1%
ITS+1	1%	-	1%
Dexamethasone	0.5 µM	10 nM	20 nM
TGF-β3	-	-	10 ng mL^{-1}
Ascorbic acid	-	50 µg mL^{-1}	5 µg mL^{-1}
β-Glycerophosphate	-	20 mM	-
BMP-2	-	500 ng mL^{-1}	-
IBMX	0.5 mM	-	-
Indomethacine	50 µM	-	-
All components were dissolved in α-MEM.			

Thereafter, cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) solution at room temperature for 15 min and washed three times with PBS. After permeabilization using 0.1% (v/v) Triton X-100 (Sigma-Aldrich) for 10 min, the non-specific binding sites were blocked with a 1% bovine serum albumin solution (BSA, Roth) in PBS at room temperature for 1 h. For visualization of adipogenic markers, the cells were incubated with primary monoclonal antibodies raised against perilipin (rabbit) and glucose transporter 4 (Glut4, mouse) and conjugated secondary anti-mouse (FITC) or anti-rabbit (CY3) antibodies. For visualization of chondrogenic markers, the cells were incubated with primary monoclonal antibodies raised against collagen II (rabbit) and aggrecan (mouse) and conjugated secondary anti-mouse (FITC) or anti-rabbit (CY3) antibodies. For visualization of osteogenic markers, the cells were incubated with primary monoclonal antibodies raised against collagen I (mouse) and osteocalcin (rabbit) and conjugated secondary anti-mouse (FITC) or anti-rabbit (CY3) antibodies.

RESULTS

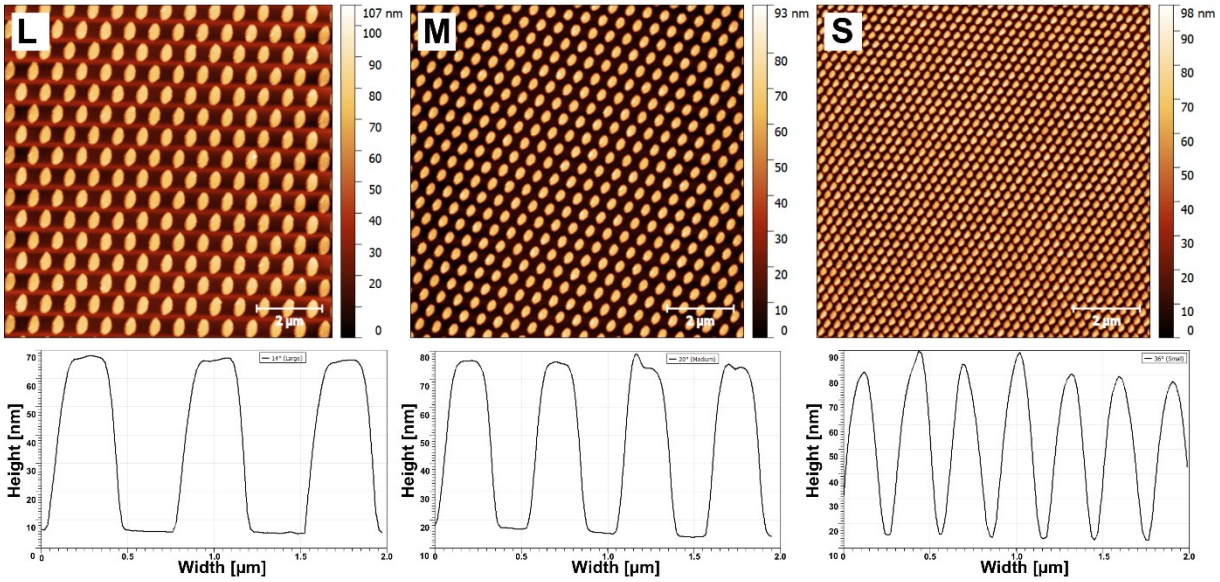

Figure S1. AFM analysis of hexagonally arranged, unmodified nanostructures of different feature dimensions obtained using three-beam laser interference lithography (LIL) at different angles of incidence (large (L) = 14°, medium (M) = 20°, and small (S) = 36°). The height profiles show the progression of structures in lateral dimension. [Scale: 2 µm]

Figure S2.

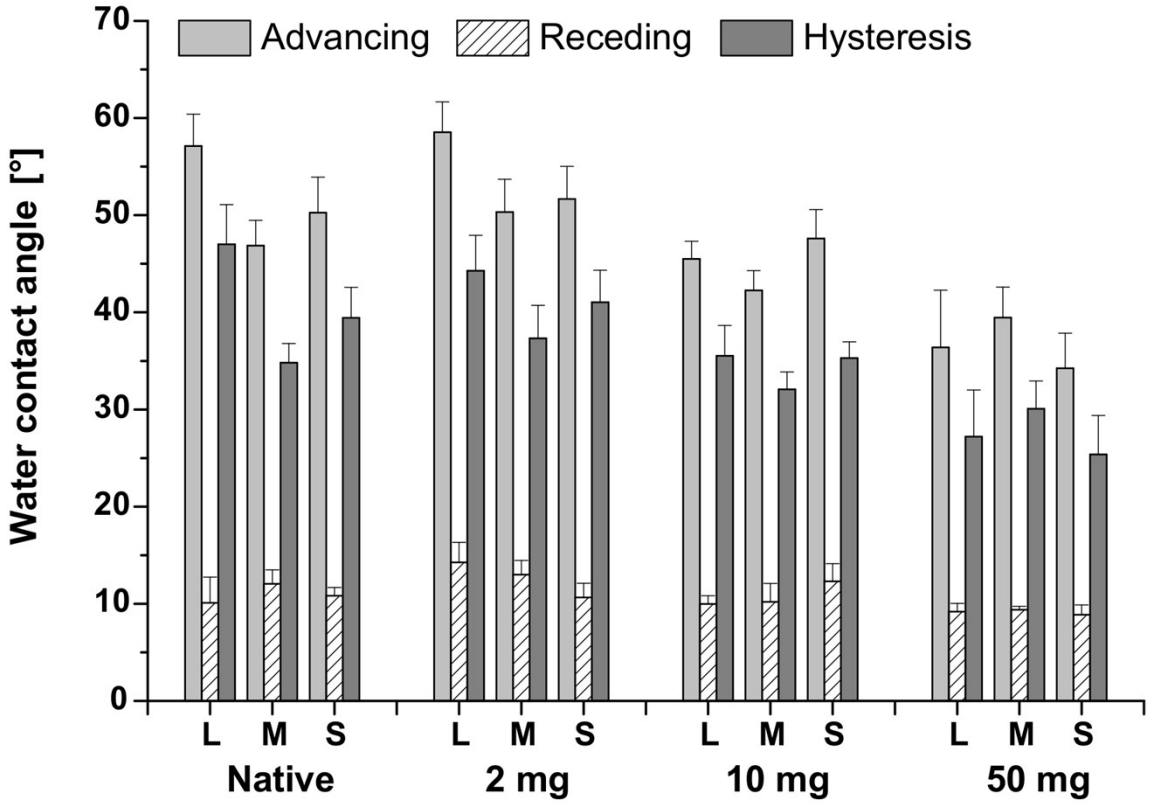

Figure S2. Results of dynamic WCA measurements on PEM-modified nanostructures. The different nanostructures have been modified with polyethylene glycol (PEG) and, subsequently, with mercaptoundecanoic acid (MUDA) and a multilayer system containing 11 bilayers of (PLL/HA) sandwiched by a primary PEI and terminal HA layer (PEI-$[HA\text{-}PLL]_{11}$-HA). Further, the multilayer system was cross-linked with different EDC concentrations (2, 10, or 50 mg mL^{-1}) or used native. Advancing (light gray) and receding (hatched) angles were measured and the hysteresis (dark gray) was calculated thereafter. The letters L, M, and S denote the feature dimensions large, medium, and small.

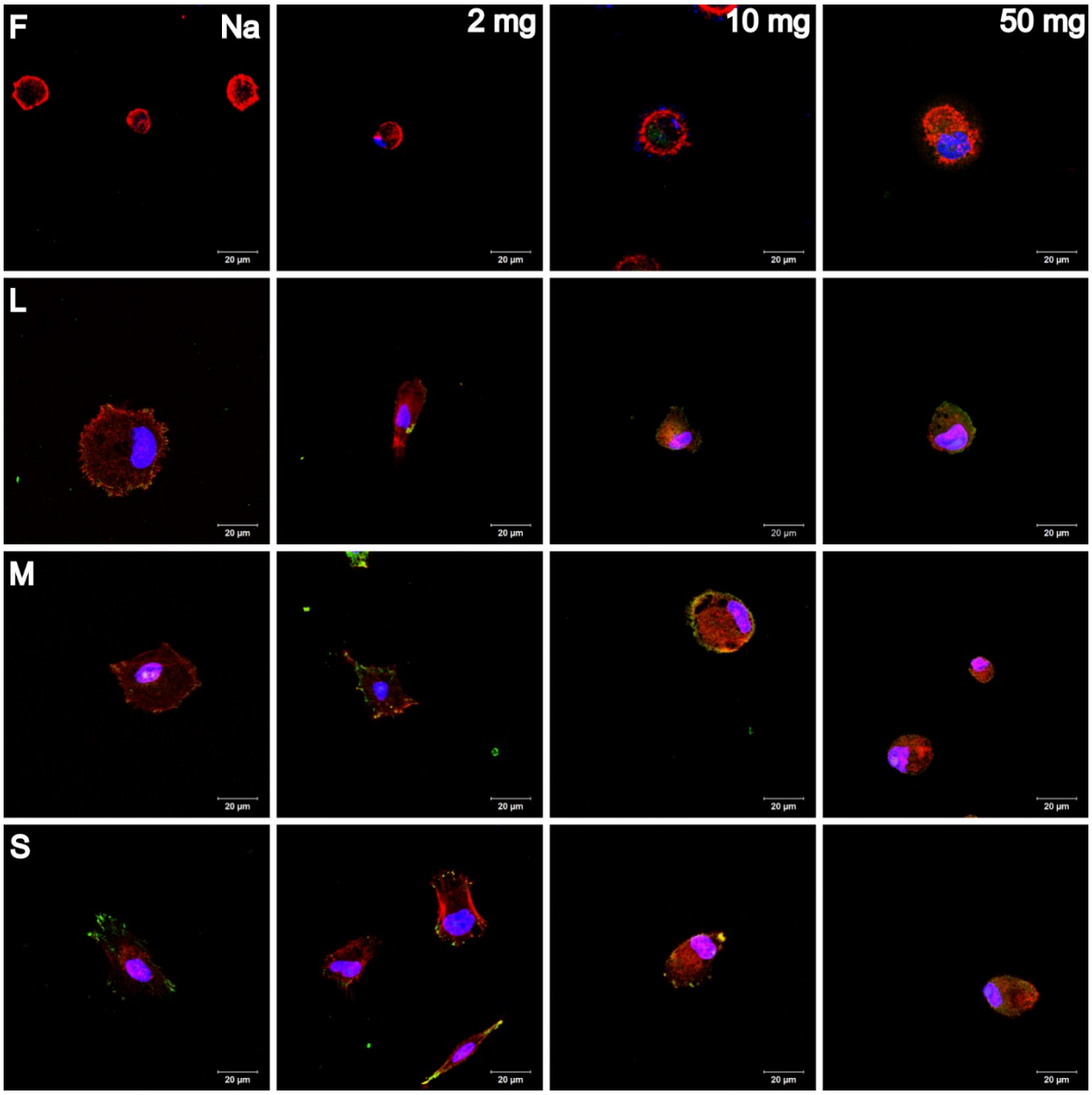

Figure S3. CLSM images of adherent hADSC cultured on flat (F) PEM or on S-PEM. The multilayers were cross-linked with different EDC concentrations (2, 10, or 50 mg mL^{-1}) or used native (Na). Afterwards, the cells were cultivated for 4 h and stained for the nucleus (blue), actin cytoskeleton (red), and vinculin in focal adhesions (green). The letters L, M, and S denote the feature dimensions large, medium, and small. [Scale: 20 µm]

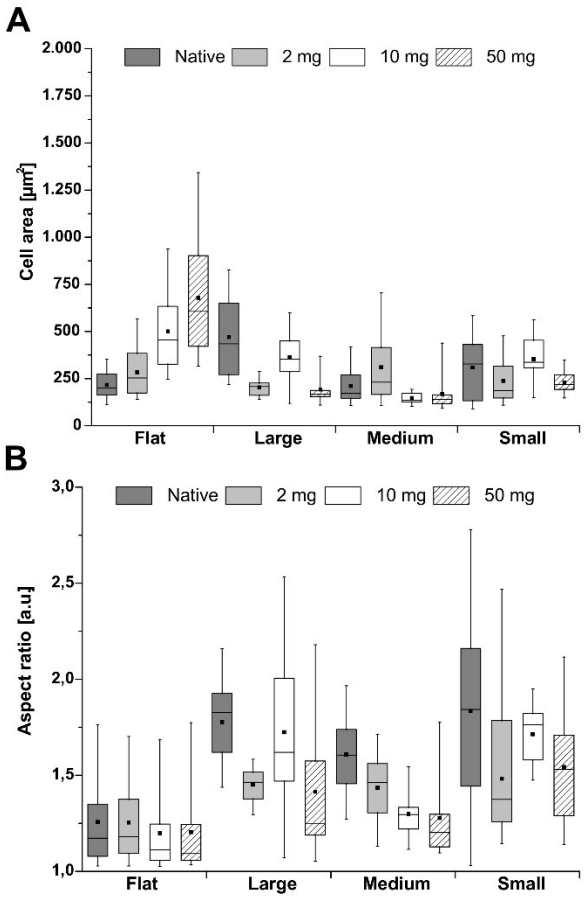

Figure S4. Quantification of cell area (A) and aspect ratio (B) of hADSC cultured on F-PEM or S-PEM (Large – 14°, Medium – 20° and Small – 36°). The multilayers were cross-linked with different EDC concentrations (2, 10, or 50 mg mL^{-1}) or used native. The cells were cultured for 4 h and image analysis was performed using ImageJ software with investigation of at least 10 images per condition. The box indicates the 25^{th} and 75^{th} percentile, the median (dash) and mean value (black square), respectively, whereas the 95-5% confidence interval is represented by the whiskers.

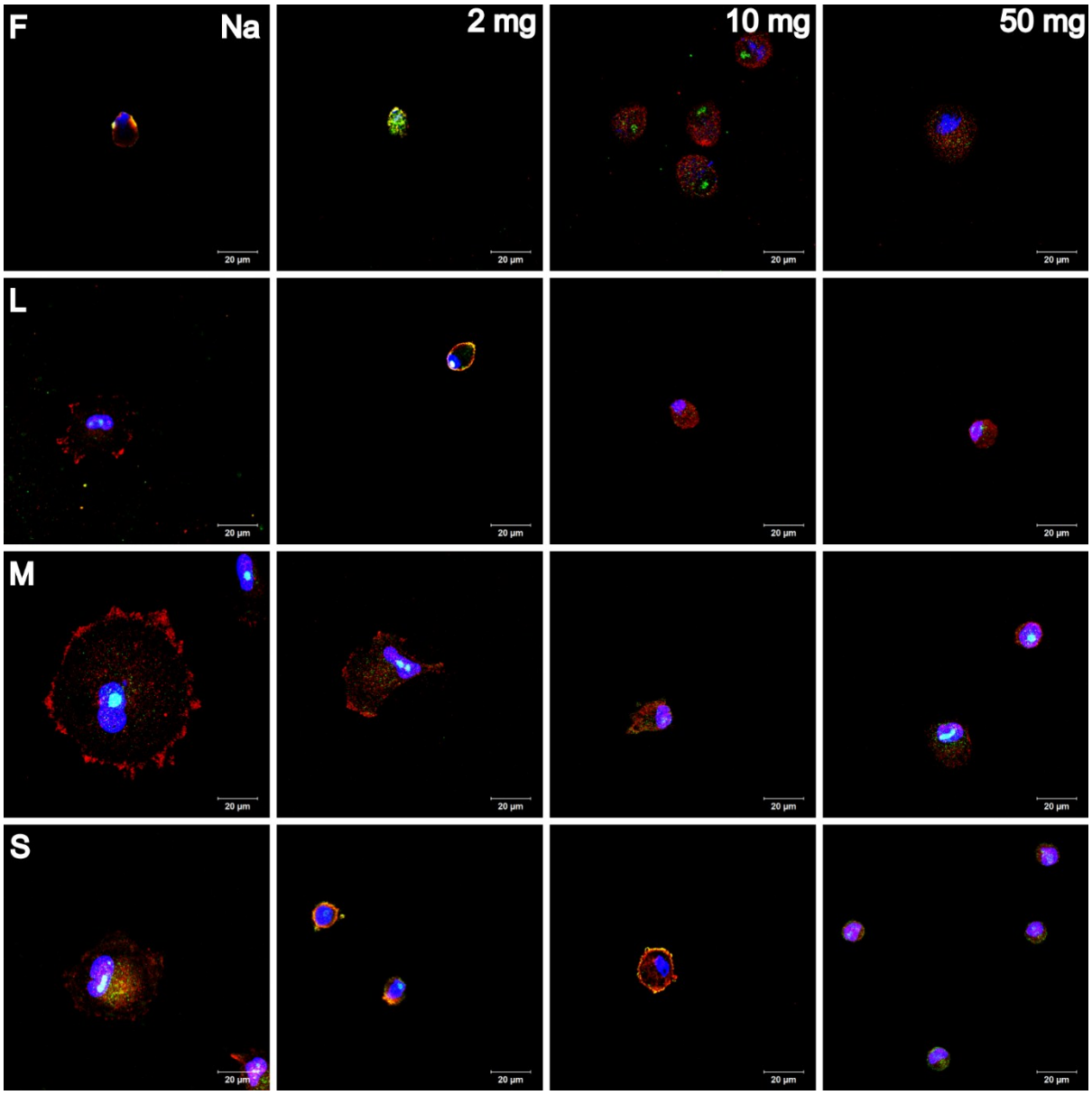

Figure S5. CLSM images of adherent hADSC cultured on flat (F) multilayers or on S-PEM. The multilayers were cross-linked with different EDC concentrations (2, 10, or 50 mg mL^{-1}) or used native (Na). Afterwards, the cells were cultivated for 4 h and the cells were stained for the nucleus (blue), focal adhesion kinase (FAK, red), and the small GTPase RhoA (green). The letters L, M, and S denote the feature dimensions large, medium, and small. [Scale: 20 µm]

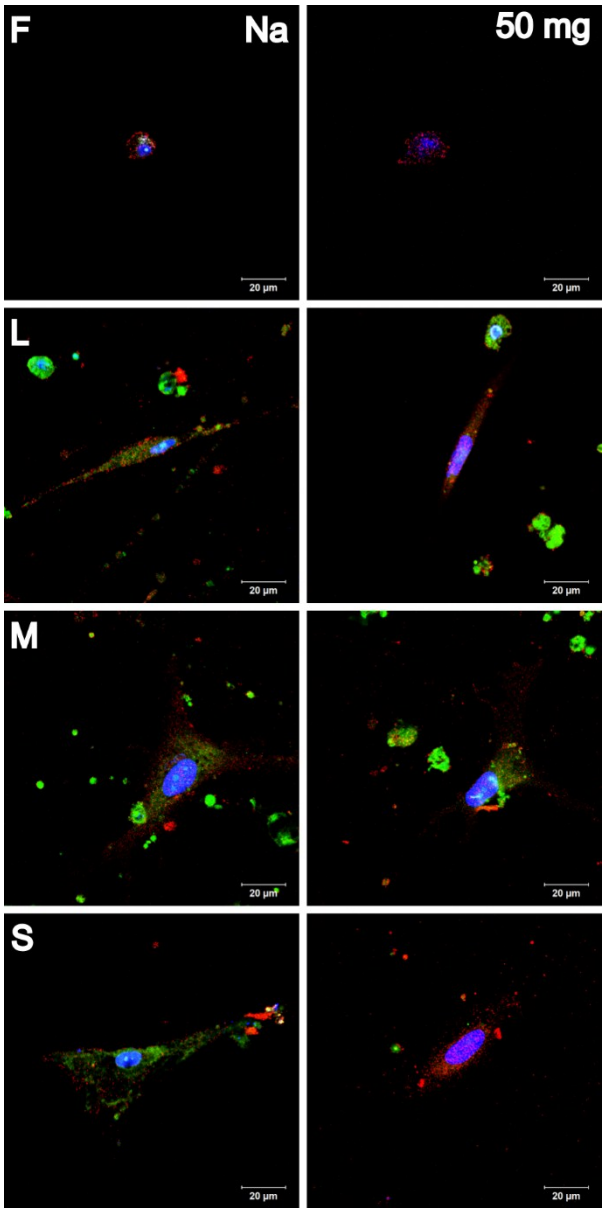

Figure S6. Visualization of adipogenic differentiation of hADSC. Nanostructured substrates were modified with (PLL/HA) multilayers and these layers were cross-linked with 50 mg mL^{-1} EDC or used native (Na). After 21 days of induction, hADSC were stained for specific differentiation markers. Here, collagen I (Col I, green) and osteocalcin (red) were stained together with the nucleus (blue). The letters L, M, and S denote the feature dimensions large, medium, and small. [Scale: 20 µm]