Non-Cell-Adhesive Substrates for Printing of Arrayed Biomaterials

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S.1 Instrumentation and Materials

FTIR spectroscopy was performed using a Bruker Alpha-E FTIR spectrometer equipped with a universal ATR sampling accessory. Water contact angle measurements were performed with a Kr uss DSA100 drop shape analyzer using a circle fitting algorithm for contact angle determination. Atomic force microscopy (AFM) images were aquired with a Veeco Multimode 8 Atomic Force Microscope using a Bruker NCHV-A probe and images were analyzed with NanoScope analysis software. Printing was performed with a Nano eNabler™ nanoarrayer from Bioforce Nanosciences. Microscopy was performed using an EVOS® FL cell imaging system from Life Technologies.

Glass slides (75x25x1 mm) were purchased from VWR and the surface treated with UV and ozone with a device from Bioforce Nanosciences for 5 min prior to use. Monomethoxy-poly(ethylene glycol) (PEG; 550 and 2000 Da) and poly(ethylene glycol) methacrylate (PEGMA) were purchased from Aldrich and was purified by azeotropic distillation with toluene. All other materials were purchased from Aldrich and used as received.

S.2 Experimental Protocols

S.2.1 Synthesis of non-cell-adhesive substrates

PEG (1.00 g, 1.82 mmol), PEGMA (0.10 g, 0.18 mmol), and triethoxy(3-isocyanatopropyl)silane (0.49 g, 2.0 mmol) were dissolved in anhydrous dichloromethane (DCM, 10 mL) with a catalytic amount of dibutyltin dilaurate (TDL; ∼3 drops) and stirred overnight at room temperature.[1] This solution was then diluted with anhydrous toluene to 100 mL and poured into a glass slide holder containing surface-treated glass slides. The slides were submerged in the silane reaction medium overnight at room temperature, then rinsed with anhydrous ethanol and acetone, followed by drying and storage in a desiccator.

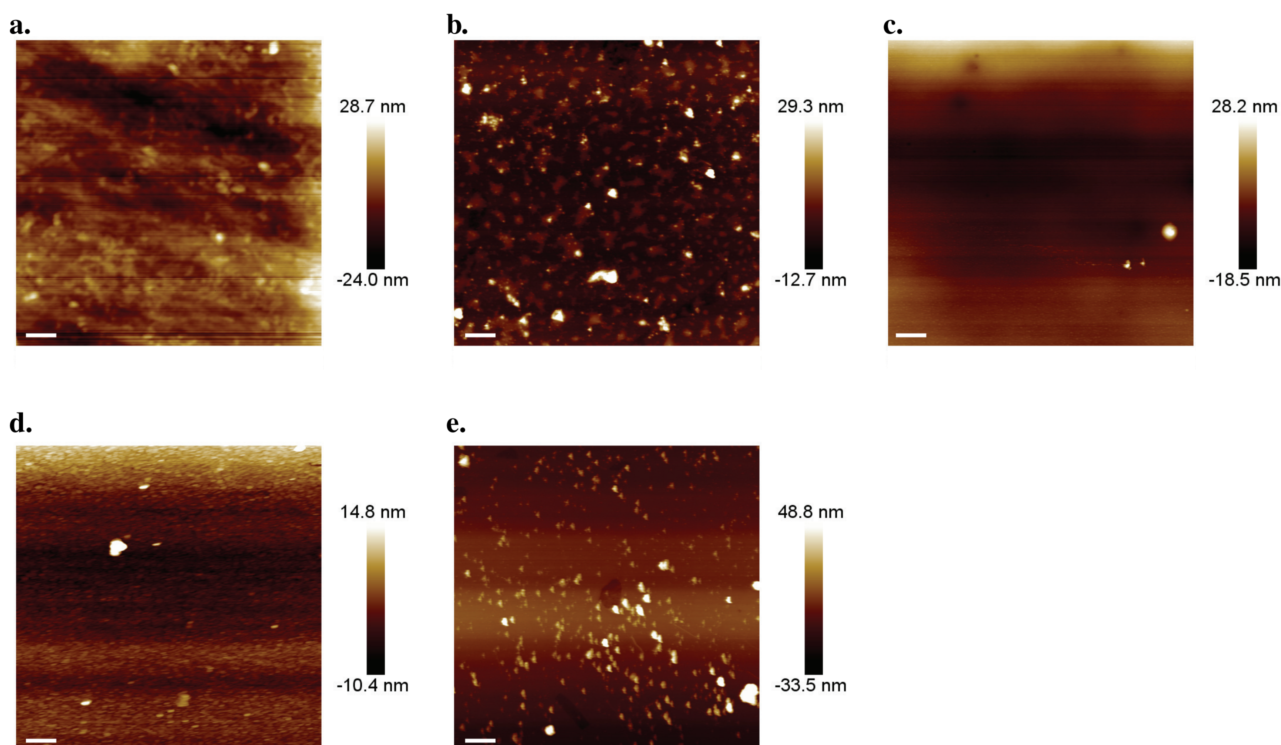

Figure S1: Atomic force microscopy (AFM) images of **a.** TCPS, **b.** PEG_{500}/PEGMA, **c.** PHEMA, **d.** glass, and **e.** PEG_{2k}/PEGMA substrates (scale bars = 1 μm).

S.2.2 Cell culture

To assess the ability of cells to adhere to treated glass slides we cultured several cell types, including NIH 3T3 Fibroblasts, HeLa, and human mesenchymal stem cells (MSCs) from bone marrow stroma, for 24 hours on the slides. NIH 3T3 fibroblasts were cultured in DMEM (+ Glutamate / - Pyruvate; Invitrogen), 10% FBS (Atlanta Biologicals, Flowery Branch, GA), 1% penicillin/streptomycin (Invitrogen) at 37°C in 5% CO_2. MSCs were obtained from the Center for the Preparation and Distribution of Adult Stem Cells that supplies standardized preparations of MSCs under the auspices of an NIH/NCRR grant (P40 RR 17447-06) (medicine.tamhsc.edu/irm/msc-distribution). Both MSCs and HeLa cells were cultured in complete culture medium that consisted of alpha minimal essential medium (α-MEM) (Invitrogen, Carlsbad, CA), 16.5% FBS (Atlanta Biologicals, Flowery Branch, GA), 1% penicillin/streptomycin (Invitrogen), and 2 mM glutamine (Invitrogen).

S.2.3 Cell adhesion assays

For adhesion experiments, substrates were UV treated for 5 min prior to cell culture. For all cell types, cells were seeded at a density of 5,000 cells/cm^2 onto the slides. After 24 h the substrates were removed from the culture media and rinsed gently with Hank's Balanced Salt Solution (HBSS; + Ca^{2+} / + Mg^{2+}; Invitrogen). For visualization, adhered cells were fixed in methanol-free paraformaldehyde (3.4%) for 30 min, washed three times with HBSS, and stained with Hoechst nuclear stain. In order to count cells adhered to the substrates, 10 fluorescence microscopy images (4x magnification) were taken at random across the substrates and the stained cellular nuclei were counted with ImageJ software.

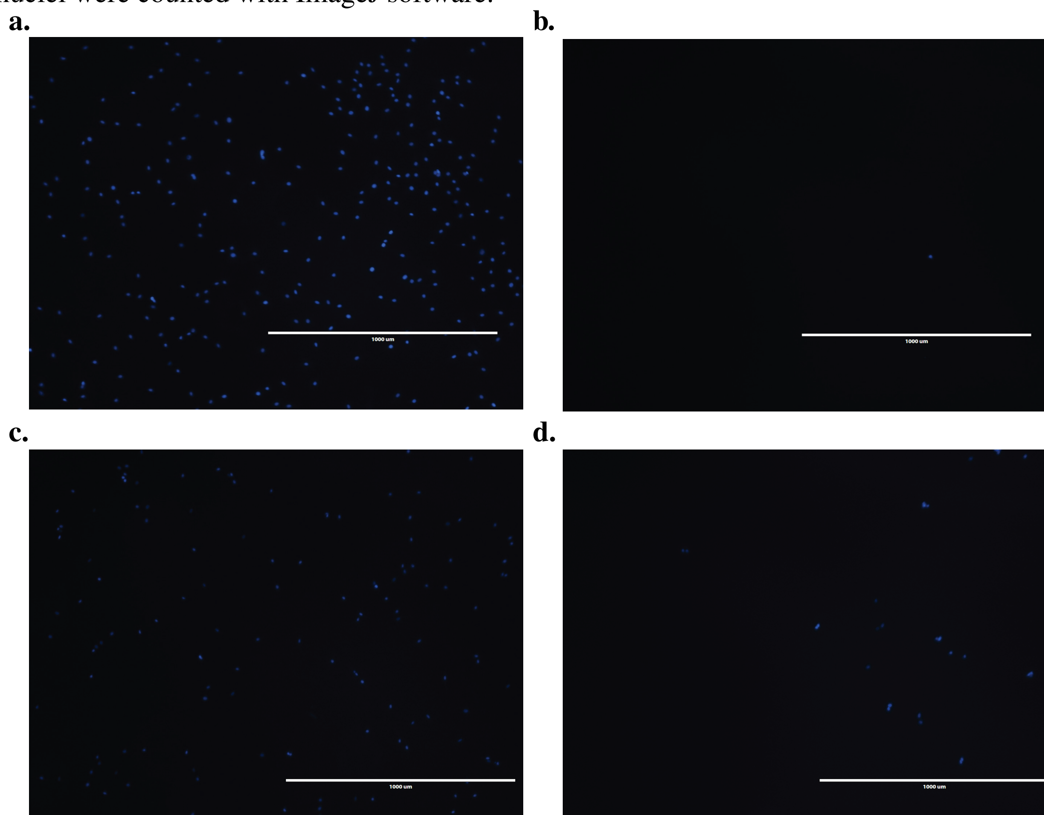

Figure S2: DAPI-stained NIH 3T3 Fibroblast cells grown on substrates for 24h before fixing in paraformaldehyde. **a.** TCPS. **b.** PEG_{500}/PEGMA. **c.** PEG_{2000}/PEGMA. **d.** PHEMA. Scale bars = 1000 μm.

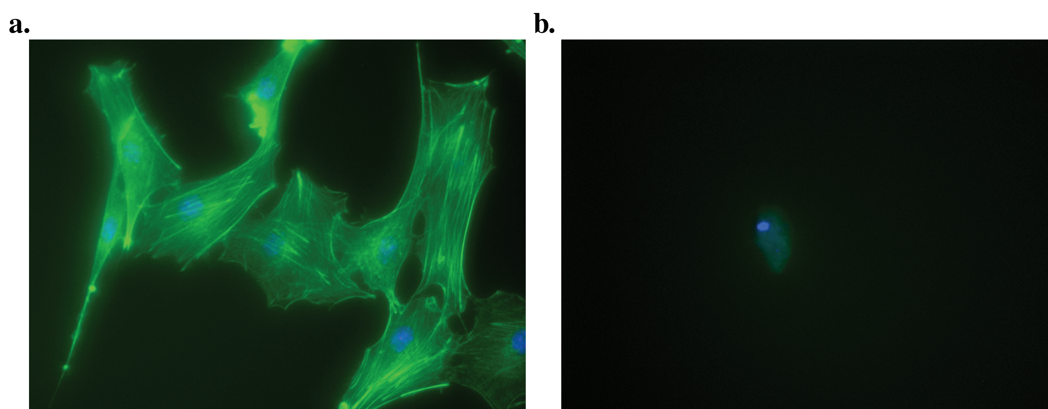

Figure S3: Fixed NIH 3T3 Fibroblast cells grown on **a.** TCPS. **b.** PEG_{500}/PEGMA substrates and stained with DAPI (blue; nuclei) and Phalloidin (green; actin). The actin staining clearly demonstrates broad spreading of the cells on TCPS, a good substrate for cell adhesion, while negligible spreading is observed on PEG_{500}/PEGMA substrates, highlighting the poor adhesion of the very few cells found attached to these substrates.

S.2.4 Printing of biomaterials

Printing of biomaterial dots onto treated slides was performed with a nanoarrayer from Bioforce Nanosciences. Synthetic biomaterials were prepared from a monomer solution was composed of trimethylolpropane triacrylate (22.5%), poly(ethylene glycol) methyl ether acrylate (52.5%) and dimethylformamide (25%), measured by weight and containing 2,2-dimethoxy-2-phenylacetophenone (DMPA). Initially, the cantilever (Bioforce Nanosciences) is treated with UV/O_3 for 30 min and the cantilever reservoir is filled with 0.5 μL of monomer solution. Then, grids (10 x 10) of synthetic polymer dots (d = 8 - 60 μm) were printed in triplicate onto treated slides. Following printing the dots were irradiated with UV light (λ=365 nm) for 5 min to cure and were washed with ethanol to remove any solvent and unreacted monomer from the materials. Additionally, lines ($w \sim 40$ μm) of both synthetic and natural biomaterials were printed. Synthetic materials were treated as described above and poly(*d*-lysine) (info) was printed in borate buffer (pH=10), which promoted covalent attachment by Michael reaction with the methacrylated surfaces of the PEG_{500}/PEGMA-coated substrates. Following printing, the poly(*d*-lysine) were allowed to react with the slide surface overnight before washing twice with HBSS.

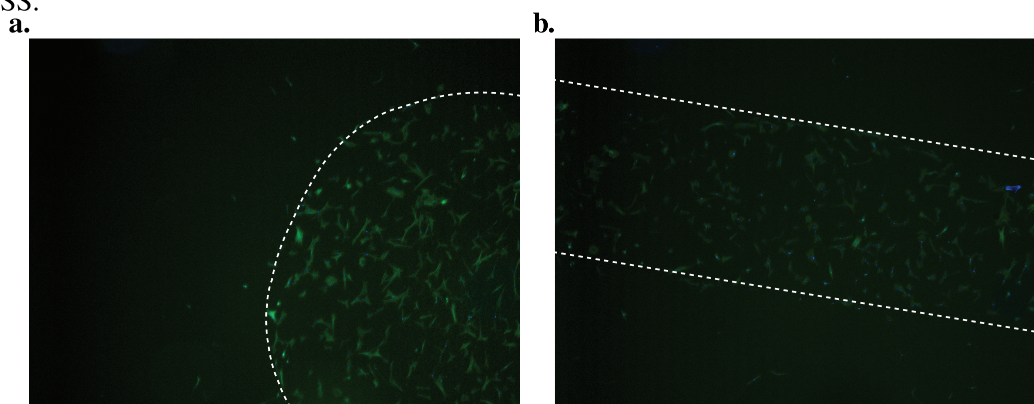

Figure S4: Mesenchymal stem cells (MSCs) grown on poly(*d*-lysine) features printed onto PEG_{500}-coated substrates. Poly(*d*-lysine) was printed in a borate buffer solution (pH=10) to promote conjugation of the amine-functionality on the polymer to the methacrylate functionality of the substrates *via* Michael-addition.

S.2.5 Printed-feature stability assays

Slides printed with arrayed biomaterials were submerged in solvent, either ethanol or PBS (pH=7.4), and agitated on an orbital shaker at either 20°C (EtOH) or 37°C (PBS). At each time-point the slides were observed with a microscope and the proportion of dots remaining on the substrates was quantified.

References

[1] Biedermann, F.; Appel, E. A.; del Barrio, J.; Gruendling, T.; Barner-Kowollik, C.; Scherman, O. A. *Macromolecules* **2011**, *44*, 4828–4835.