Electronic Supplementary Material (ESI) for Soft Matter

Programmed Cell Adhesion and Growth on Cell-Imprinted Polyacrylamide Hydrogels

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Materials and Equipment

Materials.
Acrylamide - Aldrich
Bisacrylamide - Promega
Ammonium persulfate (APS) - Mallinckrodt Chemicals
Tetramethylethylenediamine (TEMED) - IBI Scientific
RNase/DNase-free water - Teknova
Phosphate buffered saline (PBS) - Hyclone/Thermo Scientific
0.25% Trypsin - Hyclone/Thermo Scientific
Hydrochloric acid - Mallinckrodt Chemicals
Brilliant Blue R-250 - J.T.Baker
Ninhydrin - Sigma Aldrich
Bovine serum albumin - Sigma Aldrich
Fetal bovine serum (FBS) - PAA Laboratories
Triton X-100 - Fisher Scientific
Dulbecco's modified Eagle medium (DMEM) - Hyclone/Thermo Scientific
RPMI-1640 media - Hyclone/Thermo Scientific
Mammalian cell culture dishes - Fisher Scientific
Glutaraldehyde - Fisher Scientific
N-(3-Aminopropyl)methacrylamide hydrochloride - Polysciences, Inc
Alexa Fluor 555 phalloidin - Invitrogen
MTT reagent - Trevigen

Unless stated otherwise, all water was obtained from a Milli-Q water purification system.

Equipment. Scanning electron microscopy (SEM) images were taken on a JEOL JSM-6500 F field emission scanning electron microscope (FESEM). All microscopy images were taken on an EVOS fl fluorescence microscope from Advanced Microscopy Group (AMG). Fluorescence images of whole gels were taken on a Typhoon Trio imager.

Methods

Scanning Electron Microscopy (SEM). Hydrogels were cut into 3 mm × 3 mm pieces and incubated in 200 μL of a 25% glutaraldehyde / PBS solution for 1 hour. Hydrogels were then washed three times for 30 minutes each with PBS and twice with water for 30 minutes each. Hydrogels were then dried by sequential incubation in 500 μL of 50:50, 70:30, 80:20, 90:10, and 95:5 ethanol:water for 10 minutes each. Dehydrated gels were then washed three times with absolute ethanol for 10 minutes each and dried using the critical point drier. Gels were imaged on a JEOL JSM-6500 F field emission scanning electron microscope.

Coomassie staining. Cell-imprinted and non-cell-imprinted hydrogels were washed as described above. The hydrogels were then stained with a 50% methanol, 10% acetic acid and 0.05% Brilliant Blue R-250 solution for 1 hour at 25 °C. Gels were then washed twice with water and destained using a 50% methanol, 40% water, and 10% acetic acid solution for 1 hour. Gels were then washed with water and images were taken using a Nikon D5000 digital camera.

Ninhydrin staining. Cell-imprinted hydrogels prepared as previously described. Non-cell-imprinted amino-functionalized hydrogels were prepared with 3 mL of 32% acrylamide solution (30% N-(3-Aminopropyl)methacrylamide hydrochloride and 2% bisacrylamide (wt/vol)) and

gelation was initiated by addition of 60 μL APS and 6 μL TEMED. All hydro gels were prepared and washed as described above. Hydrogels were then incubated in 10 mL of a 1.5 g ninhydrin, 100 mL n-butanol, 3 mL acetic acid solution and heated to 100 °C for 30-40 minutes. The gels were then washed with three times with water and images were taken using a Nikon D5000 digital camera.

F-actin staining. HeLa cells were separately plated on either HeLa cell-imprinted hydrogels or a tissue culture dish in 37 °C DMEM / 10% FBS. After 12 hours the cells were washed twice with 37 °C PBS and adhered cells were allowed to grow for an additional 24 hours. The cells were first fixed with 5 mL of 4% paraformaldehyde in PBS, pH 7.4 at 37 °C for 10 minutes, then washed twice with PBS and incubated with 0.1% Triton X-100 in PBS for 5 minutes. The cells were next washed twice with PBS twice, incubated in a 1 % BSA / PBS solution for 5 minutes and then washed with PBS. Finally, cellular F-actin was stained by incubating the cells in a solution containing 50 μL of Alexa Fluor 555 phalloidin in 2 mL of PBS for 30 minutes at room temperature. Stained cells were washed twice with PBS and imaged on a EVOS fl microscope.

MTT staining. 72 hours after seeding, cells were washed 3 times with PBS. Cells were then treated with a solution containing 2 mL of DMEM / 10% FBS and 100 μL of MTT reagent. Cells were then incubated for 6 hours at 37 °C / 5% CO_2 and imaged by bright field microscopy using a Nikon D5000 inverted camera.

Figure S1. Cell viability of HeLa, HEK-293T and MRC-9 cells grown on cell-imprinted hydrogels

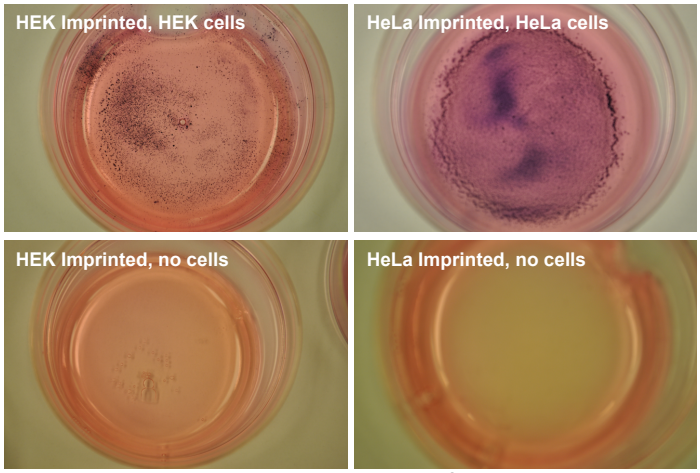

Figure S1. TACS® MTT reagent stains cells grown in cell-imprinted features, but does not stain non-imprinted gels.