Supplementary Information for

Photolithographic microfabrication of hydrogel clefts for cell invasion studies

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Supplemental movies

Supplemental movie S1: Characterization of hydrogel swelling depending on z-position from substrate (3mM PEG-NB, cross-link ratio 0.6, D=30 µm)

Final strip width of the hydrogel-hydrogel clefts depending on the distance z from channel bottom with a PEG-NB monomer concentration of 3mM, a cross-linker ratio of 0.6 and an initial block-to-block distance of 30 µm. Fluorescently labelled Dextran (MW: 10kDa) is used for visualization.

Supplemental movie S2: Characterization of hydrogel swelling depending on z-position from substrate (3mM PEG-NB, cross-link ratio 0.6, D=50 µm)

Final strip width of the hydrogel-hydrogel clefts depending on the distance z from channel bottom with a PEG-NB monomer concentration of 3mM, a cross-linker ratio of 0.6 and an initial block-to-block distance of 50 µm. Fluorescently labelled Dextran (MW: 10kDa) is used for visualization.

Supplemental movie S3: HT-1080 cells deform the hydrogel while migrating through.

LifeAct GFP labelled HT-1080 cells transmigrating in the hydrogel-hydrogel cleft casted with a PEG-NB monomer concentration of 2mM, a cross-linker ratio of 0.6 and an initial block-to-block distance of 30 µm. Fluorescently labelled nanobeads are embedded in the hydrogel showing the hydrogel deformation while cells are migrating through the cleft.

Supplemental movie S4: HT-1080 cells invading into a hydrogel-hydrogel cleft (2mM PEG-NB, cross-link ratio 0.6, D=30 µm)

HT-1080 cells invading into a hydrogel-hydrogel cleft casted with a PEG-NB monomer concentration of 2mM, a cross-linker ratio of 0.6 and an initial block-to-block distance of 30 µm.

Supplemental movie S5: HT-1080 cells invading into a hydrogel-hydrogel cleft (2mM PEG-NB, cross-link ratio 0.65, D=40 µm)

HT-1080 cells invading into a hydrogel-hydrogel cleft casted with a PEG-NB monomer concentration of 2mM, a cross-linker ratio of 0.65 and an initial block-to-block distance of 40 µm.

Supplemental movie S6: HT-1080 cells invading into a hydrogel-hydrogel cleft (2mM PEG-NB, cross-link ratio 0.7, D=50 µm)

HT-1080 cells invading into a hydrogel-hydrogel cleft casted with a PEG-NB monomer concentration of 2mM, a cross-linker ratio of 0.7 and an initial block-to-block distance of 50 µm.

Supplemental movie S7: MDA-MB-231 cells invading into a hydrogel-hydrogel cleft (2mM PEG-NB, cross-link ratio 0.65, D=50 µm)

MDA-MB-231 cells invading into a hydrogel-hydrogel cleft casted with a PEG-NB monomer concentration of 2mM, a cross-linker ratio of 0.65 and an initial block-to-block distance of 50 µm.

Supplemental movie S8: MDA-MB-231 cells invading into a hydrogel-hydrogel cleft (2mM PEG-NB, cross-link ratio 0.7, D=50 µm)

MDA-MB-231 cells invading into a hydrogel-hydrogel cleft casted with a PEG-NB monomer concentration of 2mM, a cross-linker ratio of 0.7 and an initial block-to-block distance of 50 µm.

Supplemental figures

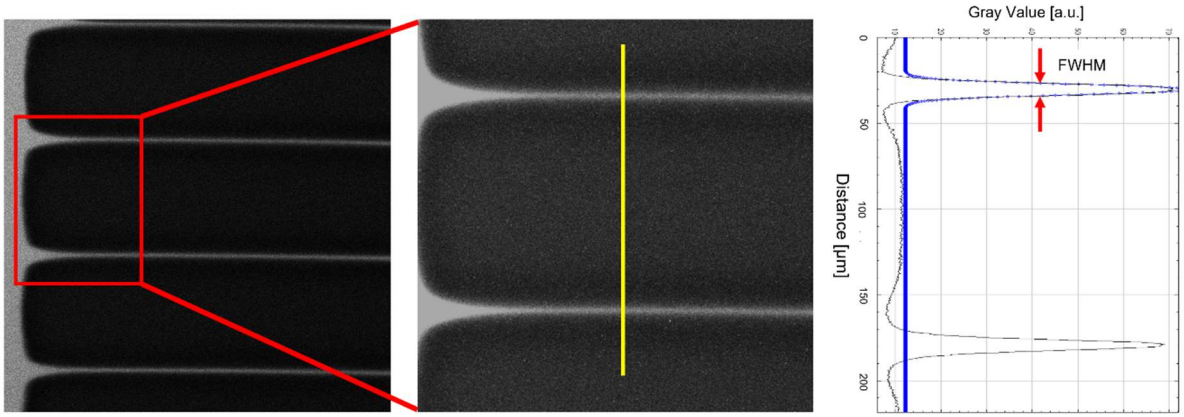

Figure S1: Measurement and definition of the hydrogel-hydrogel gap width. The gap between the hydrogel strips is visualized via the use of GFP-labelled Dextran (MW 10kDa) which is not able to diffuse into the polymerized hydrogel due to its molecular size. The intensity profile perpendicular to the hydrogel clefts (yellow line) is measured and fitted with a Gaussian fit (blue line) afterwards. The gap width is defined as the FWHM of these Gaussian fits.

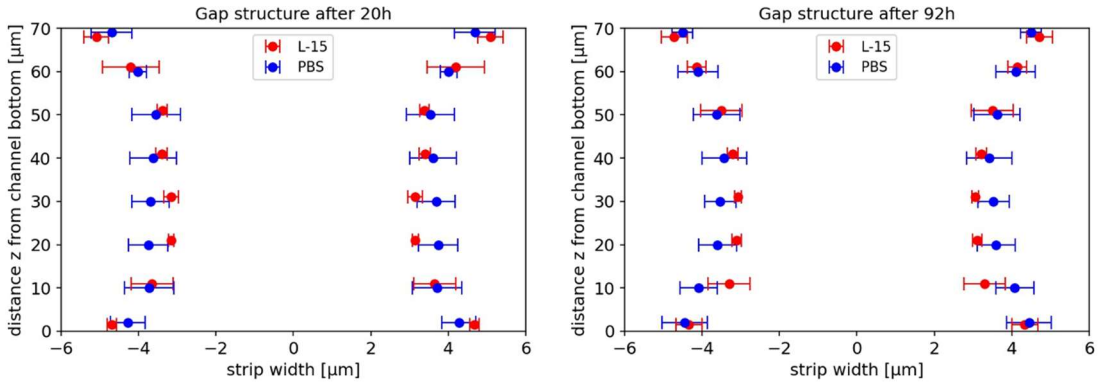

Figure S2: Reproducibility and long-term stability of the hydrogel clefts. 'Sponge clamps' were casted with the same conditions and imaged 20h and 92h after the fabrication (used GFP-labelled Dextran (MW 10kDa) to visualize the gap (n = 5 'sponge clamps' for PBS and L-15, respectively. Results show mean with standard deviation). No significant changes in the gap structure are noticeable over time and in terms of the batch-to-batch variability. The influence of medium components on the swelling behavior was characterized by analyzing hydrogel profiles swollen in PBS or cell culture medium (L-15 + 10% FBS). No significant influences of the used medium on the swelling characteristics was detected.