Supplementary material

Matrix Deformation and Mechanotransduction as Markers of Breast Cancer Cell Phenotype Alteration at Matrix Interfaces

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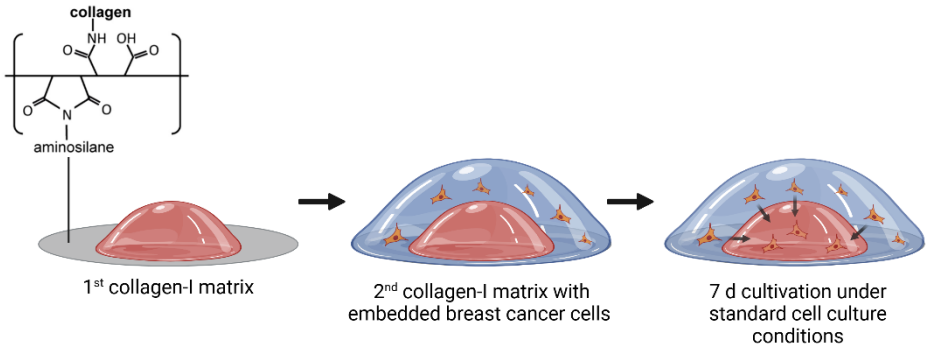
Topological characteristics of collagen I matrices

Supplementary Table S1: *Topological parameters of the reconstituted collagen I matrices and matrix interfaces. Pore diameter and fibril diameter are shown as the mean value with standard deviation. The compact letter display method was used to indicate statistical groupings ($p > 0.05$). The analysis was carried out in triplicate, with at least three matrices analyzed per condition.*

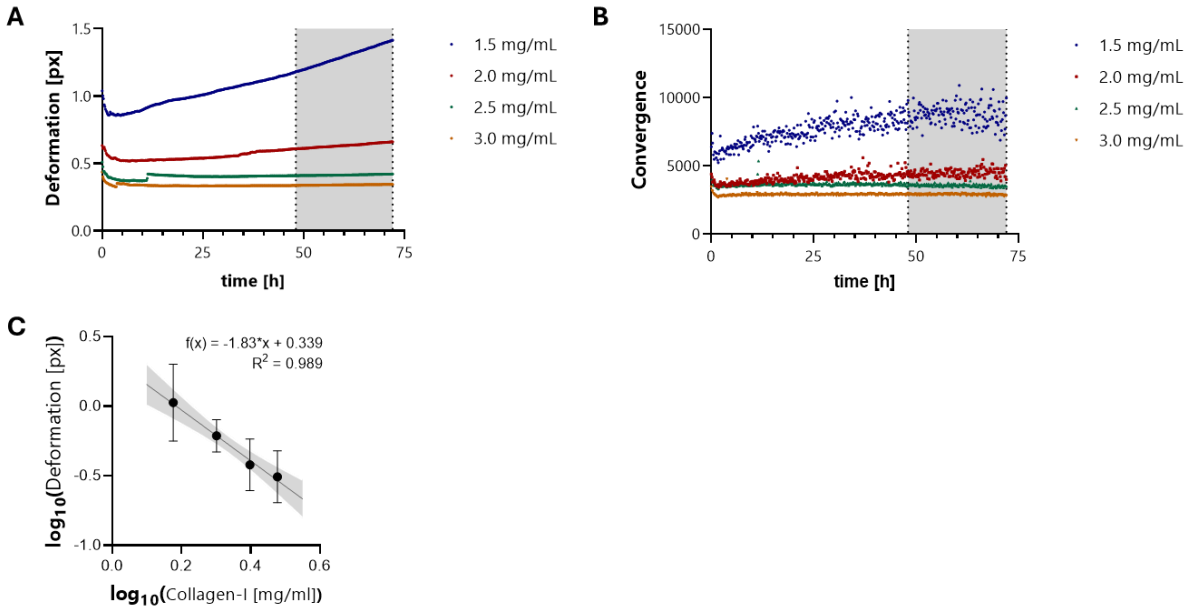
	Pore diameter [µm]	**Fibril diameter [µm]**
1.5 mg/mL	5.10 ± 0.75 ^{ef}	0.94 ± 0.13 ^{ab}
2.0 mg/mL	4.46 ± 0.27 ^{ab}	0.92 ± 0.06 ^{a}
2.5 mg/mL	4.29 ± 0.15 ^{ac}	0.93 ± 0.06 ^{ab}
3.0 mg/mL	3.65 ± 0.54 ^{cd}	1.01 ± 0.09 ^{b}
d → o (nt)	4.14 ± 0.22 ^{ac}	0.92 ± 0.09 ^{a}
d → **o** (t)	4.85 ± 0.34 ^{be}	0.93 ± 0.07 ^{ab}
o → d (nt)	5.37 ± 0.58 ^{f}	0.92 ± 0.07 ^{a}
o → **d** (t)	3.60 ± 0.35 ^{d}	0.92 ± 0.07 ^{ab}

Preparation of collagen I matrix interfaces



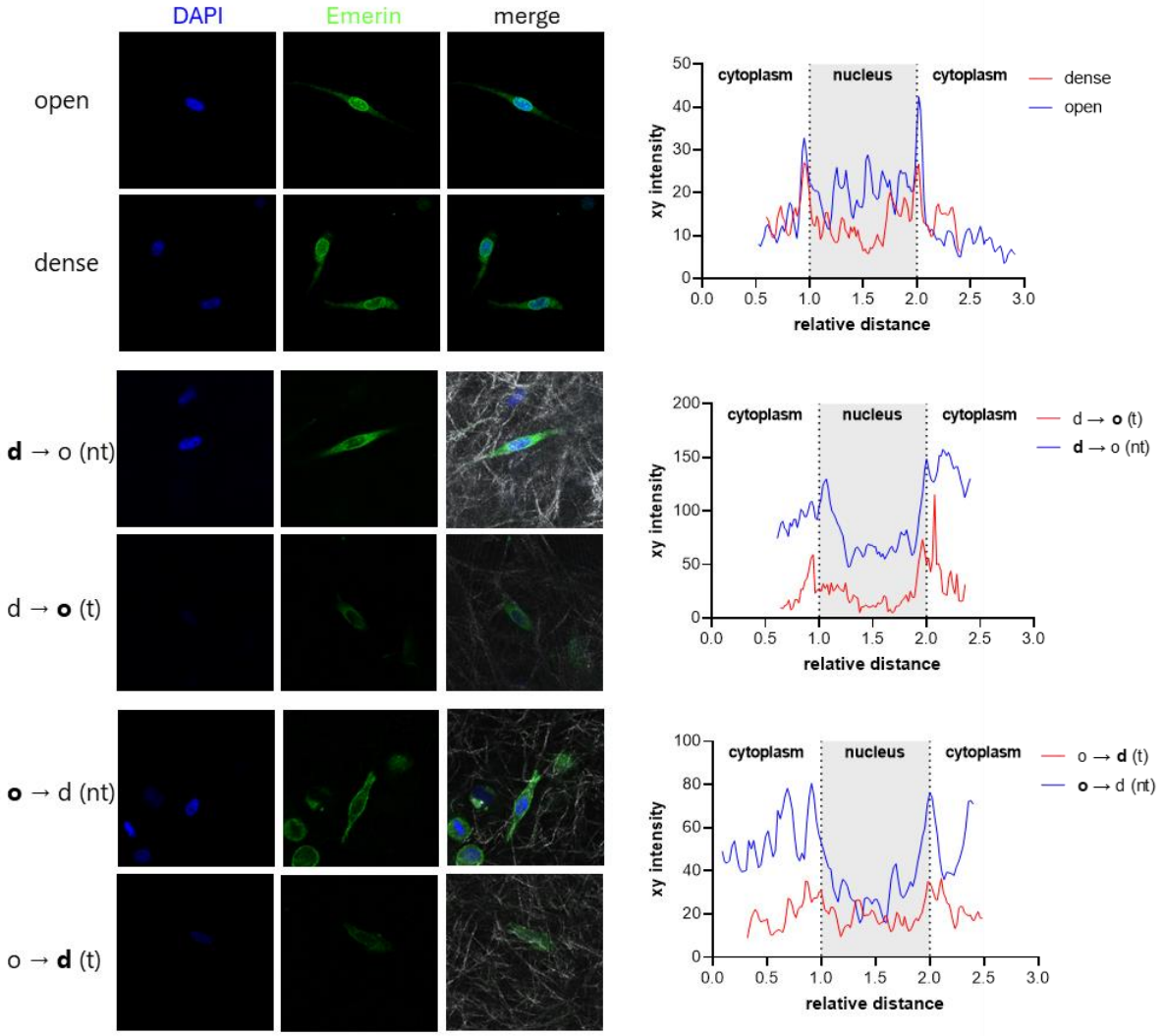
Supplementary Figure S1*: Schematic representation of the reconstitution of collagen I interface matrices on PSMA-coated coverslips. A collagen I solution is applied as a droplet to the PSMA-coated coverslip and polymerized at 37 °C in a wet chamber. A second collagen I solution with a contrasting concentration is then mixed with an MDA-MB-231 breast cancer cell suspension, overlaid, and polymerized under the same conditions. This results in a clearly defined interface between two compartments with contrasting topological and / or mechanical properties.*

Matrix deformation analysis of MDA-MB-231 cells embedded in homogeneous matrices

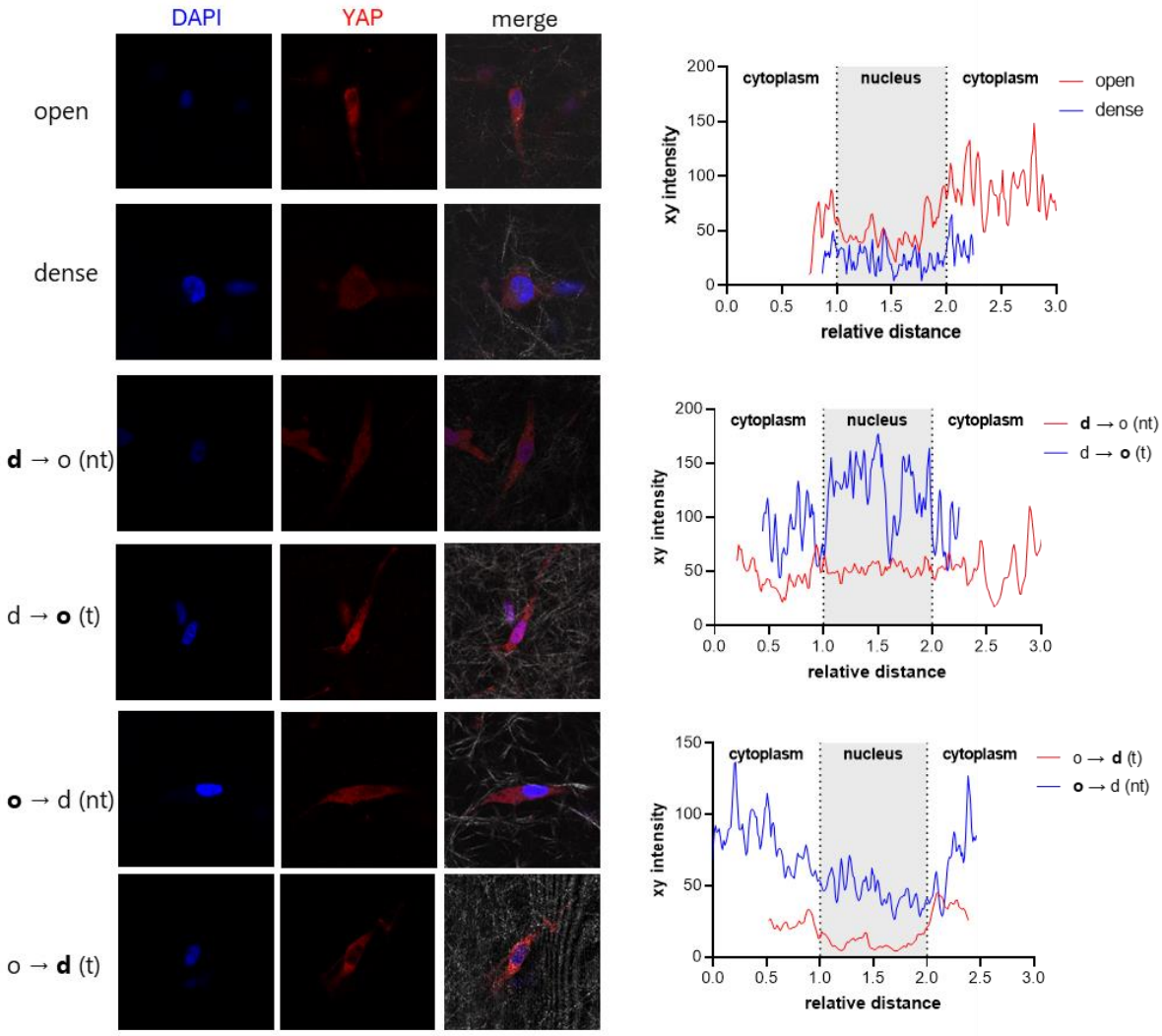


***Supplementary Figure S2**: Quantification of matrix deformation and convergence of the displacement vector field from the PIV-based analysis of live imaged MDA-MB-231 breast cancer cells in collagen I matrices with different matrix densities. Time-resolved **A|** matrix deformation and **B|** convergence over a period of three days. **C|** Correlation of deformation and collagen I concentration c shown using a double logarithmic plot and linear regression, indicating a scaling of deformation with $c^{-1.8}$. The analysis was carried out in triplicate, with at least three matrices analyzed per condition.*

Immunostaining of nuclear mechanotransduction proteins emerin and YAP



***Supplementary Figure S3**: Immunofluorescence images of emerin in matrices with different densities as well as before and after transmigration across defined collagen I matrix interfaces. Left: Confocal images of emerin (green) in MDA-MB-231 breast cancer cells cultivated in collagen I matrices with varying density and matrix interfaces after 7 d of cultivation. DAPI staining is shown in blue, collagen matrices were imaged in reflection mode, shown in grey. Right: Fluorescence intensity plot along a cross section of the nucleus of the cell on the left. The xy distance was normalized according to the position of the nucleus assessed by the DAPI channel. DAPI staining of the nucleus (blue). Image size: 100 µm x 100 µm. d – dense, o – open, d→o – dense to open, o→d – open to dense, nt – not transmigrated, t – transmigrated.*



***Supplementary Figure S4**: Immunofluorescence images of YAP in matrices with different densities as well as before and after transmigration across defined collagen I matrix interfaces. Left: Confocal images of YAP (red) in MDA-MB-231 breast cancer cells cultivated in collagen I matrices with varying density and matrix interfaces after 7 d of cultivation. DAPI staining is shown in blue, collagen matrices were imaged in reflection mode, shown in grey. Right: Fluorescence intensity plot along a cross section of the nucleus of the cell on the left. The xy distance was normalized according to the position of the nucleus assessed by the DAPI channel. DAPI staining of the nucleus (blue). Image size: 100 µm x 100 µm. d – dense, o – open, d→o – dense to open, o→d – open to dense, nt – not transmigrated, t – transmigrated.*