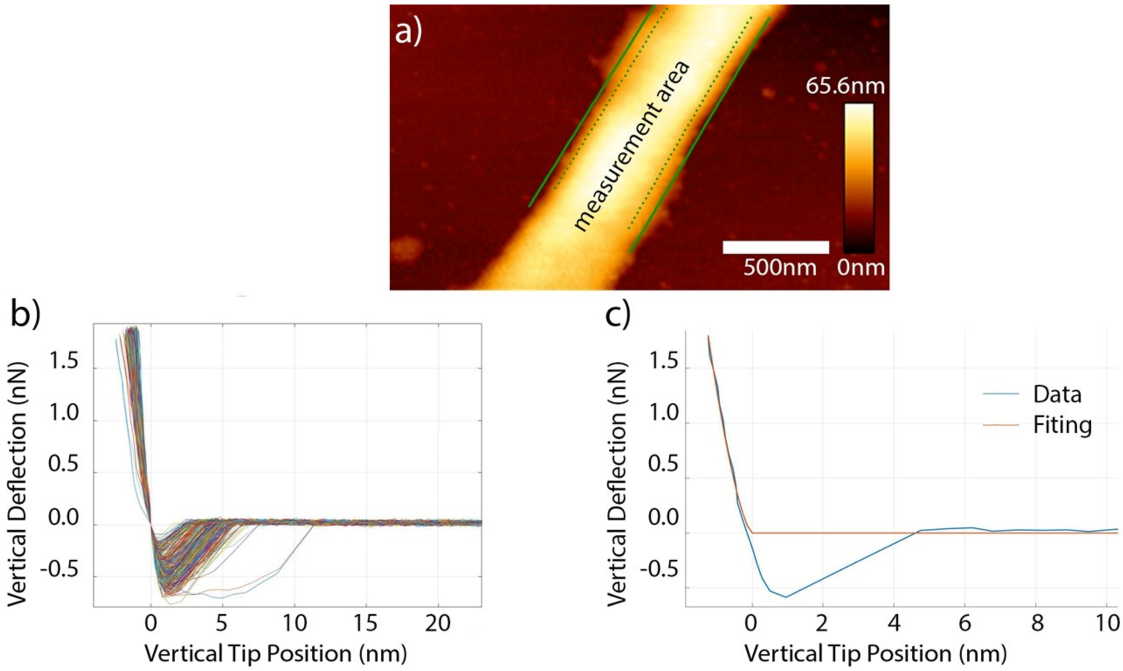
3D multiphoton lithography using biocompatible polymers with specific mechanical properties (Supplementary material)

Boris Buchroithner [ǂ], Delara Hartmann [†††], Sandra Mayr [ǂ], Yoo Jin Oh [††], Dmitry Sivun [†], Andreas Karner [ǂ], Bianca Buchegger [†,ǂ], Thomas Griesser [†††], Peter Hinterdorfer [††], Thomas A. Klar [†], and Jaroslaw Jacak [ǂ]*

*** Email: jaroslaw.jacak@fh-linz.at**



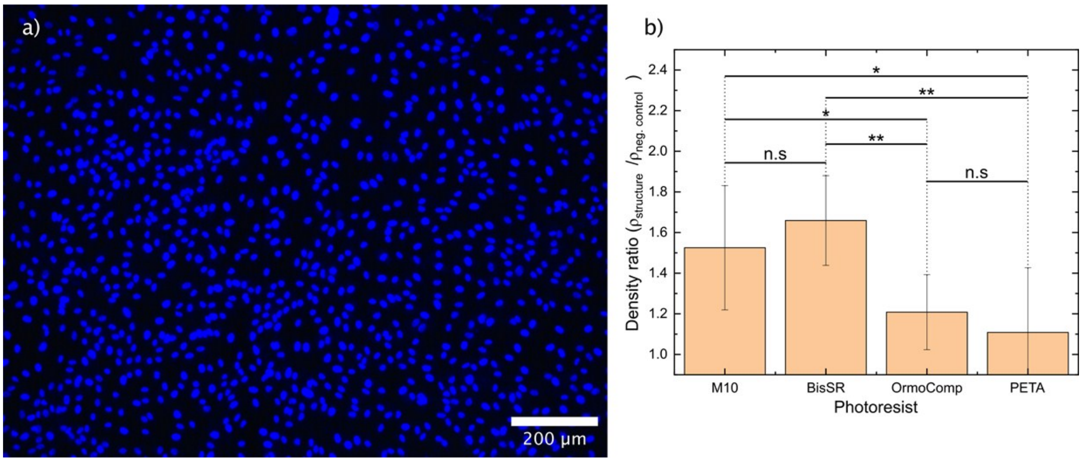
Supplementary Figure S1. a) representative polymer line measured by AFM. b) overlay of 400 force-distance curves measured on the polymer line. d) zoom of one representative force-distance curve (blue) and applied Hertz fit (orange) to retrieve the value of the Young´s modulus.

AFM analysis

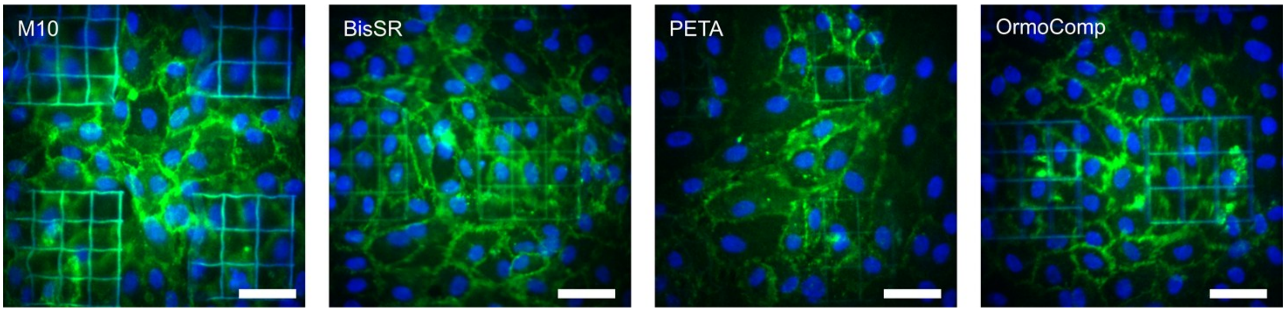
The Supplementary Figure S1 a) shows an exemplary tipped – over polymer line with a width of 517nm and a height of 66nm. The solid green lines represent the outline of the polymer line, while the dashed lines confine the measurement area. Within this region, a square area for 400 indentation measurements is chosen. The outline of the polymer line is excluded so that the tip-sample contact area stays constant. Figure S1 b) shows an overlay of all force-distance curves determined from one indentation measurement on a BisSR polymer line. A zoom of a typical AFM force-distance curve used for E-modulus analysis is shown in Fig. S1 c) (blue line). The Young´s modulus analysis has been performed using commercial JPK analysis software. The software automatically pre-processes the data and subtracts the cantilever deflection from the raw data. The data indicates that shortly before the cantilever attaches to the polymer, the AFM tip is attracted by the polymer surface (jump to contact) – indicated by a negative value of the vertical deflection force (Fig. S1 c). After the attractive indentation force is compensated, the inelastic deformation of the sample by the AFM – tip begins. For estimation of the Young´s modulus, we fitted the non-linear part of the pre-processed force-distance curves using the Hertz model (orange line). Additionally, fits determined by the Derjaguin-Muller-Toropov (DMT) model [1] were compared to the results of the Hertz model. The DMT model is an extension of the Hertz model, accounting for the jump to contact events. Both models yielded similar values.

Control measurements

Figure S2 (a) show the fluorescence microscopy image of cell nuclei (stained with DAPI) of primary endothelial cells (HUVEC) on a negative control sample (glass slide without grids). The average cell density obtained from a figure is approx. 80435 cells/cm². The density ratio (relative to negative control) is shown in figure S2 (b). Both M10 and BisSR grids show significant difference in cell density compared to the grids made of OrmoComp and PETA. Nevertheless, one can see that that density of cells in Figure S2 (a) is not homogeneous, and ranging from 70000 cells/cm² (in the upper right corner of the image) to 91000 cells/cm² (in the lower left corner of the image). The cell density on the grids in such cases can be influenced not only by the "biocompatibility" of the material, but also by the local cell density variation on the sample. In order to avoid such error, we used as a negative control, not an entirely separated sample (Fig. S2 (a)), but a bare glass in the vicinity of the grids for each sample separately.



Supplementary Figure S2. a) Fluorescence microscopy image of cell nuclei (stained with DAPI) of primary endothelial cells (HUVEC) on a negative control sample (glass slide without grids). b) Averaged (over four 2D grids) HUVEC density ratio (relative to negative control) for all four photoresists used in this work.



Supplementary Figure S3. Immunofluorescent staining of HUVEC grown on four different polymer grids. From left to right: M10, BisSR, PETA, and OrmoComp. HUVEC were stained with CD31 Ab (green), and a DAPI nuclear stain (blue). The scale bar is 50µm.

A comparison of the caspase activity of cells incubated on BisSR-polymer grids relative to cells cultivated on a plain glass substrate reveals a factor 1.68 higher caspase activity. Apoptotic cells revealed a factor 2.1 higher caspase activity compared to cells on BisSR.

[1] Derjaguin, B. V.; Muller, V. M.; Toporov, Y. P. Effect of contact deformations on the adhesion of particles. J. Colloid Interface Sci. 1975, 2, (53), 314 – 326.