## Supplementary information.

## Title:

- 1. Evaluation of the topography influence on the cellular behavior of human umbilical vein endothelial cells
- 2. Evaluation of topography high throughput assays on endothelial cell behavior
- 3. Microlens topography lowers adhesion and proliferation of human umbilical vein endothelial cells

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Supplementary Figure 1. Scanning electron microscopy (SEM) evaluation of the MARC chamber pattern replication. Micro-sized patterned substrates labeled as indicated in Table 1 and Supplementary Figure 1. White bar represents 1  $\mu$ m, 10  $\mu$ m and 50  $\mu$ m.

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**Supplementary Figure 2.** Atomic force microscopy (AFM) evaluation of MARC chamber pattern replication. Nano-sized patterned substrates labeled as indicated in Table 1 and Supplementary Figure 1. White bar represents 1  $\mu$ m and 5  $\mu$ m.



**Supplementary Figure 3.** Scanning electron microscopy evaluation of single patterned PDMS replicas.(A) Topography 11 - 1.8  $\mu$ m diameter, 2  $\mu$ m pitch, 0.7  $\mu$ m sag convex microlens, (B) Topography 22 - 1.8  $\mu$ m diameter, 2  $\mu$ m pitch, 0.7  $\mu$ m sag concave microlens, (C) Topography 8 - 500 nm pillars, 10  $\mu$ m pitch, 500 nm height, (D) Topography 6 - 2  $\mu$ m pillars, 12  $\mu$ m pitch, 2  $\mu$ m height and (E) Topography 5 - 10  $\mu$ m pillars, 10  $\mu$ m pitch, 10  $\mu$ m height. White bar represents 10  $\mu$ m and 50  $\mu$ m.



Supplementary Figure 4. Representative immunofluorescence images of collagen I adsorption on unpatterned and 41 patterned surfaces (MARC chamber). White bar represents 100  $\mu$ m. White arrows represent the gratings direction. Collagen I was stained with Alexa Fluor<sup>®</sup>488 conjugated anti-collagen I. White bar represents 100  $\mu$ m

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Bovine Collagen I coating, 2.5 µg/cm<sup>2</sup>

**Supplementary Figure 5.** Collagen I adsorption quantification on unpatterned and 41 patterned surfaces (MARC chamber). Dotted line represents unpatterned surface mean value. Data (n=30) were evaluated on outliers using Grubb's test. Statistical analysis was performed using one-way ANOVA test with Tukey's post-hoc test. Data represent mean±SD, \*P $\leq$ 0.05, \*\*P $\leq$ 0.01, \*\*\*P $\leq$ 0.001, \*\*\*P $\leq$ 0.001.



**Supplementary Figure 6.** Representative immunofluorescence images of HUVEC adhesion on Unpatterned and 41 patterned surfaces (MARC chamber) at 3000 cells/cm<sup>2</sup> seeding density with no collagen I coating. White bar represents 150  $\mu$ m. White arrows represent the gratings direction. HUVECs were stained for phalloidin and the nuclear marker – DAPI.

Non Collagen I coating, 3000 cells/cm<sup>2</sup> seeding density

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**Supplementary Figure 7.** Representative immunofluorescence images of HUVEC adhesion on unpatterned and 41 patterned surfaces at 3000 cells/cm<sup>2</sup> seeding density with bovine collagen I coating. White bar represents 150  $\mu$ m. White arrows represent the gratings direction. HUVECs were stained for phalloidin and the nuclear marker – DAPI.

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Collagen I coating, 3000 cells/cm<sup>2</sup> seeding density

150 pm

**Supplementary Figure 8.** Representative immunofluorescence images of HUVEC adhesion on unpatterned and 41 patterned surfaces at 10000 cells/cm<sup>2</sup> seeding density with no ECM coating. White bar represents 150  $\mu$ m. White arrows represent the gratings direction. HUVECs were stained for phalloidin and the nuclear marker – DAPI.

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Non Collagen I coating, 10000 cells/cm<sup>2</sup> seeding density

**Supplementary Figure 9.** Representative immunofluorescence images of HUVEC adhesion on unpatterned and 41 patterned surfaces at 10000 cells/cm<sup>2</sup> seeding density with bovine collagen I coating. White bar represents 150  $\mu$ m. White arrows represent the gratings direction. HUVECs were stained for phalloidin and the nuclear marker – DAPI.

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Collagen I coating, 10000 cells/cm<sup>2</sup> seeding density

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**Supplementary Figure 10.** Cell adhesion evaluation at 4 hour time point on unpatterned and 41 patterned surfaces (MARC chamber) with different ECM coatings and cell seeding densities. Comparison of cell density quantification on non-coated (A) and collagen I coated surfaces (B) at initial cell seeding density 3000 cells/cm<sup>2</sup> and 10000 cells/cm<sup>2</sup>. Geometry size, isotropy and anisotropy are indicated. Arrows represent increase in geometry size. Mean values of 3000 cells/cm<sup>2</sup> and 10000 cells/cm<sup>2</sup> initial cell seeding density of unpatterned surfaces are represented by dashed and dotted lines respectively. Data (n=20 for unpatterned surfaces and n=4 for patterned surfaces) were evaluated on outliers using Grubb's test. Statistical analysis was performed using one-way ANOVA test with Tukey's post-hoc test. Data represent mean±SD, \*P≤0.05, \*\*P≤0.01, \*\*\*P≤0.001, \*\*\*\*P≤0.0001.



**Supplementary Figure 11.** Cell adhesion evaluation at 24 hour time point on unpatterned and 41 patterned surfaces (MARC chamber) with different ECM coatings and cell seeding densities. Comparison of cell density quantification on non-coated (A) and collagen I coated surfaces (B) at initial cell seeding density 3000 cells/cm<sup>2</sup> and 10000 cells/cm<sup>2</sup>. Geometry size, isotropy and anisotropy are indicated. Arrows represent increase in geometry size. Mean values of 3000 cells/cm<sup>2</sup> and 10000 cells/cm<sup>2</sup> initial cell seeding density of unpatterned surfaces are represented by dashed and dotted lines respectively. Data (n=20 for unpatterned surfaces and n=4 for patterned surfaces) were evaluated on outliers using Grubb's test. Statistical analysis was performed using one-way ANOVA test with Tukey's post-hoc test. Data represent mean±SD, \*P≤0.05, \*\*P≤0.01, \*\*\*P≤0.001, \*\*\*\*P≤0.0001.



3000 cells/cm<sup>2</sup> cell seeding density 10000 cells/cm<sup>2</sup> cell seeding density



■ 3000 cells/cm<sup>2</sup> cell seeding density ■ 10000 cells/cm<sup>2</sup> cell seeding density

**Supplementary Figure 12.** Cell proliferation evaluation at 24 hour time point on unpatterned and 41 patterned surfaces (MARC chamber) with different ECM coatings and cell seeding densities. Comparison of cell proliferation on non-coated (A) and collagen I coated surfaces (B) at initial cell seeding density 3000 cells/cm<sup>2</sup> and 10000 cells/cm<sup>2</sup> (B). Geometry size, isotropy and anisotropy are indicated. Arrows represent increase in geometry size. Mean values of 3000 cells/cm<sup>2</sup> and 10000 cells/cm<sup>2</sup> initial cell seeding density of unpatterned surfaces are represented by dashed and dotted lines respectively. Data (n=20 for unpatterned surfaces and n=4 for patterned surfaces) were evaluated on outliers using Grubb's test. Statistical analysis was performed using one-way ANOVA test with Tukey's post-hoc test. Data represent mean±SD. \*P≤0.05. No significant differences were observed in B.



■ 3000 cells/cm<sup>2</sup> cell seeding density ■ 10000 cells/cm<sup>2</sup> cell seeding density