3D screening device for the evaluation of cell response to different electrospun microtopographies

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Supplementary information

Material and Methods

Electrospinning set up

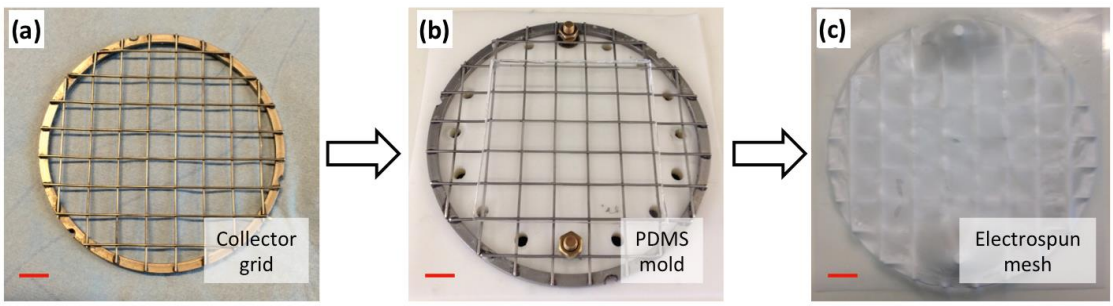

Figure S1 - Custom made collector (a) composed by a circular ring and a grid both made in aluminum. (b) A PDMS mold with 25 different geometries and size is centered on the top of the grid and used as target for the ESP jet. (c) After the ESP process, the mold is covered by the ESP mesh and the different geometries are fabricated (Scale bars: 10 mm).

Stereolithography process

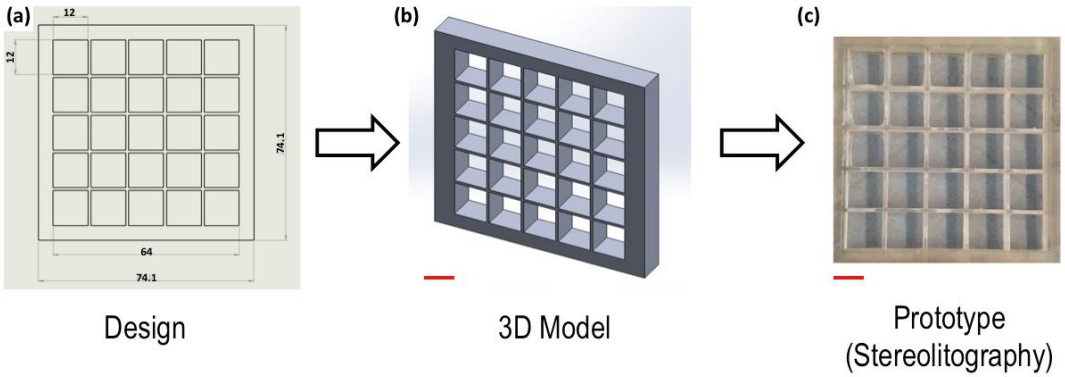

Figure S2 – Design, model and prototype of the device: the structure was designed with a CAD software (a). Subsequently, (b) a 3D model was created, the STL file was exported and (c) used in the stereolithographic system to create the prototype (Scale bars: 10 mm).

Imaging and data analysis

To show morphological changes, different features can be selected from literature [37, 38, 39]. In this study, eight non-correlated morphological parameters were selected and detailed in table S1 of supplementary information. All the features were scaled by subtracting the median and dividing by the median absolute deviation. Cell morphologies were compared within dimensions of different microtopographies. The cell morphology on random and aligned fibers without microtopographies was chosen as control.

For morphological analysis, data is also shown using Notched Box Whiskers, a graph with a box, a line in the box, and two whiskers in addition to the PhenoPlots analysis. The box shows the interquartile range (IQR) (25-75 percentile), while the line in the middle of box is the median of the data. The whiskers add 1.5 times the IQR to the 75 percentile and subtract 1.5 times the IQR from the 25 percentile. The whiskers should include 99.3% of the data, if from a normal distribution. Possible outliers were not shown on those boxplots. The Notch shows confidence interval around the median. If two notches do not overlap, there is strong evidence (95% confidence) that their medians are different [33].

Results

Imaging – artifacts removal

The results related to the removal of the artifacts are showed in figures S3 and S4. In a first outlier removal step, area ratios of cells nuclei as well as major axis ratios were determined and the objects that had ratio less than a threshold (f = 1.5) were removed.

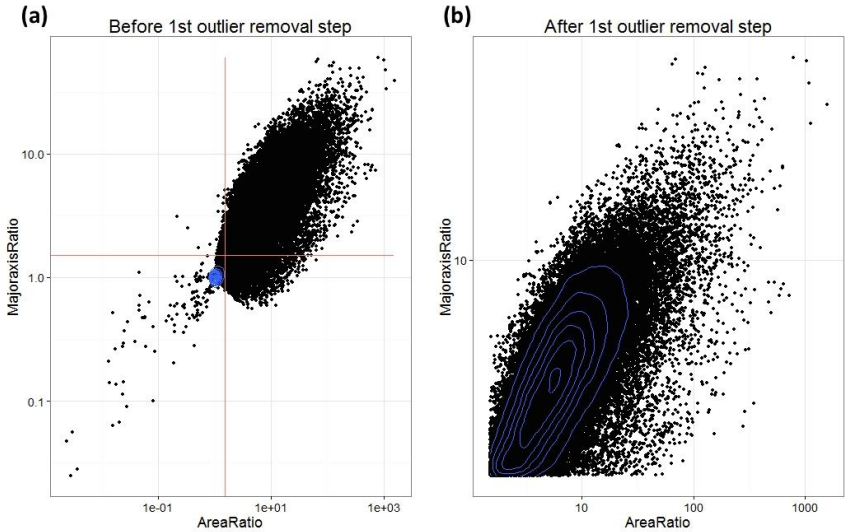

Figure S3 - First outlier detection step: Cell/nuclei major axis ratio before (a) and after (b) applying outlier removal step. Red lines represent the threshold while the blue ones represent the density of the data.

In the second outlier removal step, the distribution of each single Cell Profiler parameter for each condition was analyzed and the outliers that had a value of 1.5 interquartile ranging below or above 1st and 3rd quantile, respectively, were removed.

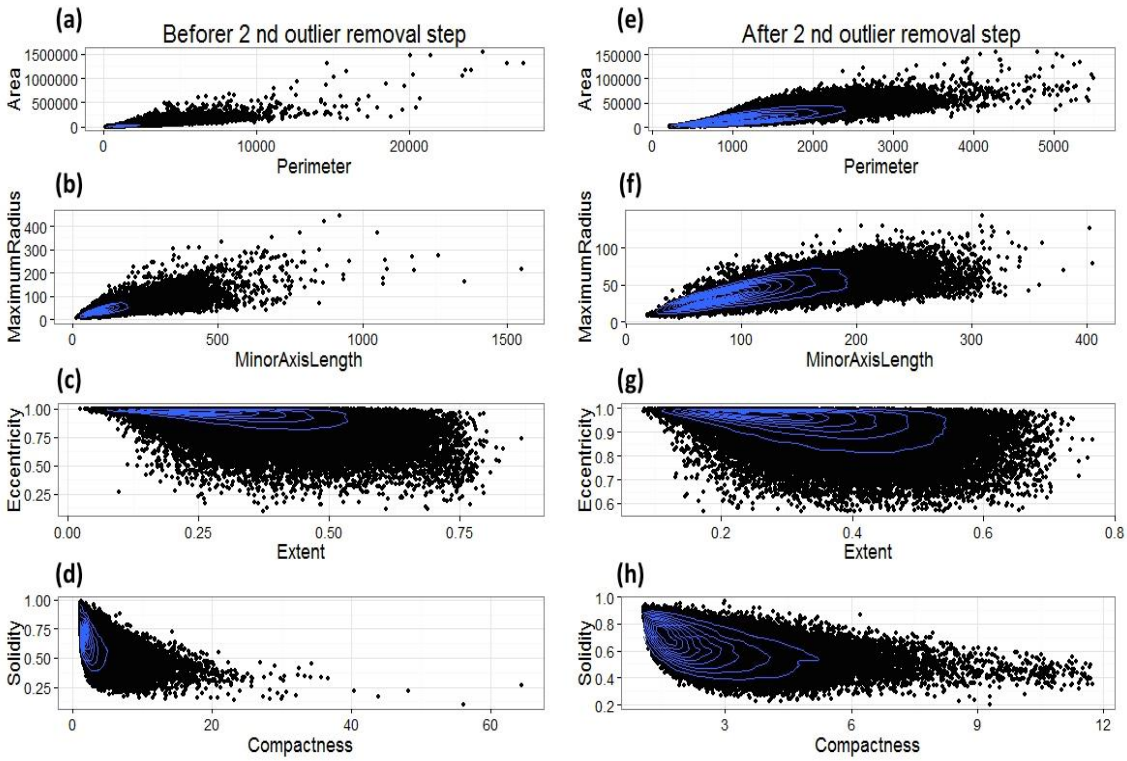

Figure S4 - Second outlier detection step: Distribution of selected morphological parameters before (a, b, c, d) and after (e, f, g, h) the step.

Morphological parameters

Parameter	Definition	Biological meaning
Solidity	The proportion of the pixels in the convex hull that are also in the object, i.e. *Object Area/Convex Hull Area*. Equals 1 for a solid object (i.e., one with no holes or has a concave boundary), or <1 for an object with holes or possessing a convex/irregular boundary.	Cells with low value of Solidity have a lot of extensions, Cells with high value of Solidity are compact and without extensions
Orientation	The angle (in degrees ranging from -90 to 90 degrees) between the x-axis and the major axis of the ellipse that has the same second-moments as the region.	Orientation of cells on a surface. Less variation in orientation means that cells are following one direction
Major Axis Length	The length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.	Max cells length
Minor Axis Length	The length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.	Max cells width
Median Radius	The median distance of any pixel in the object to the closest pixel outside of the object.	Median distance from center of the cell to all edges. It is higher for large spread cells.
Extent	The proportion of the pixels in the bounding box that are also in the region. Computed as the Area divided by the area of the bounding box	Max extent value is for a cell that has perfect rectangular shape, like thick elongated cells. Smallest value is for a cell with several extensions
Eccentricity	The eccentricity of the ellipse that has the same second-moments as the region. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length. The value is between 0 and 1. (0 and 1 are degenerate cases; an ellipse whose eccentricity is 0 is actually a circle, while an ellipse whose eccentricity is 1 is a line segment)	High the eccentricity is referred to highly elongated cells
Compactness	The variance of the radial distance of the object's pixels from the centroid divided by the area.	Large cell with irregular shape has minimum compactness value. Cells with regular shape and small area have max compactness

Table S1 – Non-correlated Morphological parameters definitions and biological meaning [32]

Comparison of cell morphology on control scaffolds

Cells on aligned and random scaffolds displayed different morphology (figure S5). Cells on aligned scaffold were more elongated then the ones on random fibers. Moreover, cells on random fibers were larger and with an irregular shape. In general, cells on random fibers had more extensions while aligned had a higher body length.

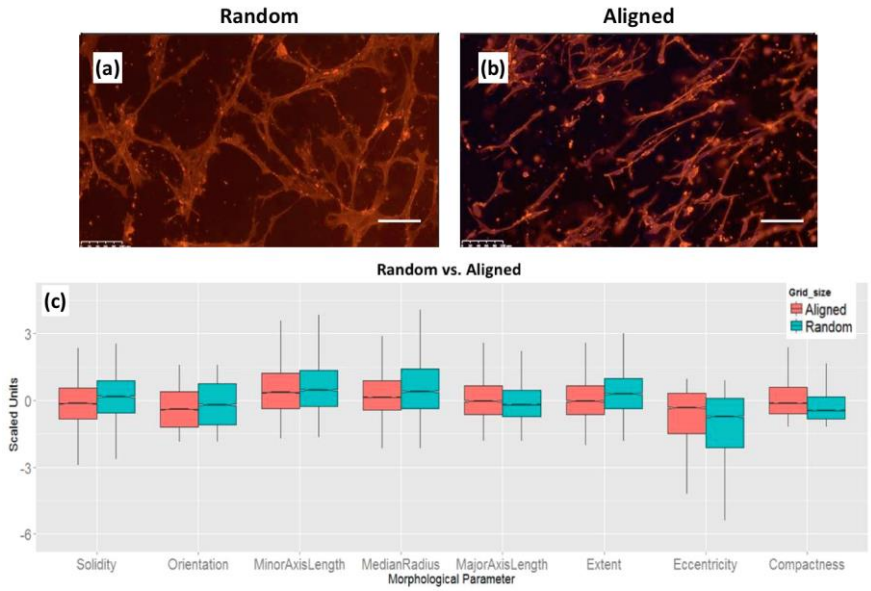

Figure S5 - Cell morphology on Control scaffolds. Representative images of cytoskeleton (a, b) and morphological parameters (c). (Scale bars: 100 μm). All data are expressed as mean ± standard deviation (SD).

Characterization of Cell morphology with connection to pattern microtopography and dimensions

As showed in figure S6, cell morphology varied on various geometries and sizes. Detailed analysis per groups is presented in the next sections.

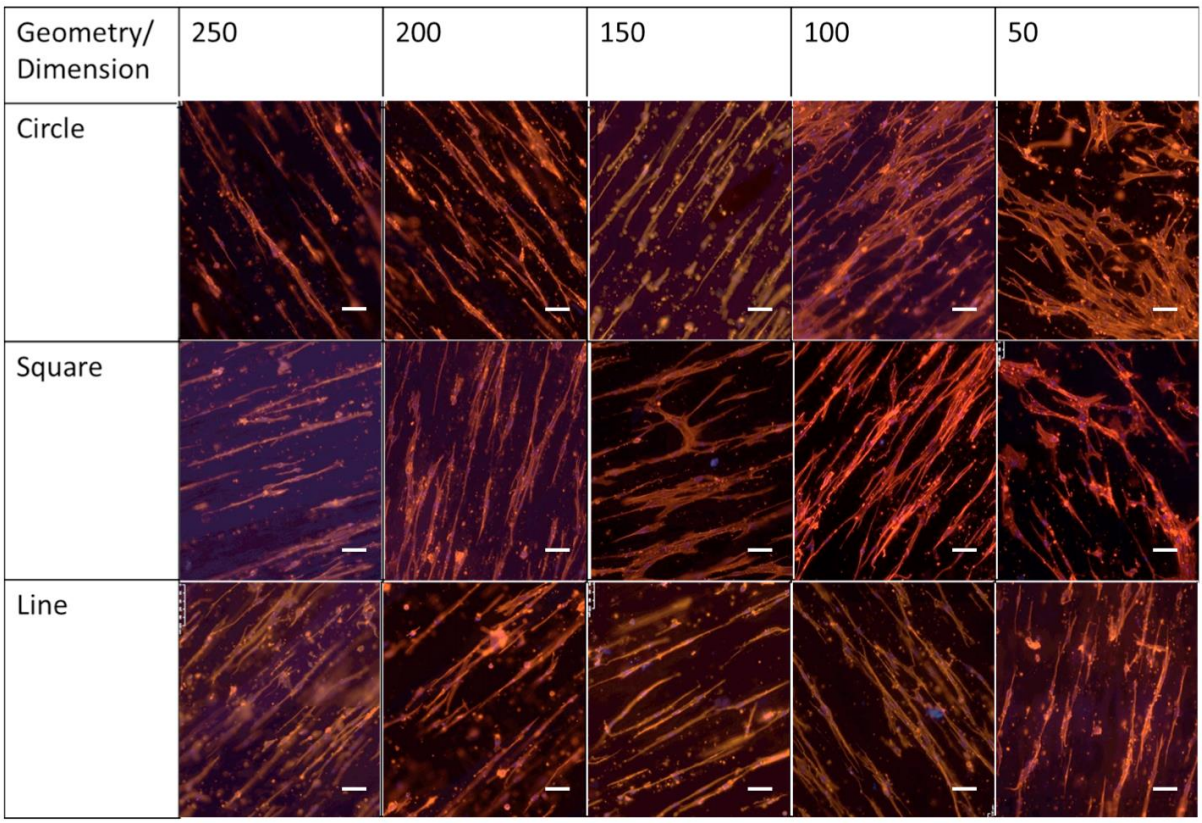

Figure S6 - Representative images of cytoskeleton: phalloidin staining on different conditions. Scale bars: 100 μm.

Cell size for different micro-topographies

Cell size

An increase of the minor axis length (cell thickness) and of the median radius were detected for circular pattern with decreasing of surface dimensionality, while no differences related to the major axis (cell length) were found (figure S7).

In contrast, cells on patterned lines had the tendency to increase the length of cells (major axis) with no clear trend for cell thickness (figure S7). In particular, on surfaces S4-S5, cell length was higher than the ones of the aligned control.

For squared patterns, cell thickness was lower than the control on surfaces S1-S4 and became similar for S5 with overlapping of notches. A similar effect was observed for cell median radius, while no differences for cell length were found, except for surfaces S1-S2. Cell length decreased with decreasing of pattern dimensionality (figure S7).

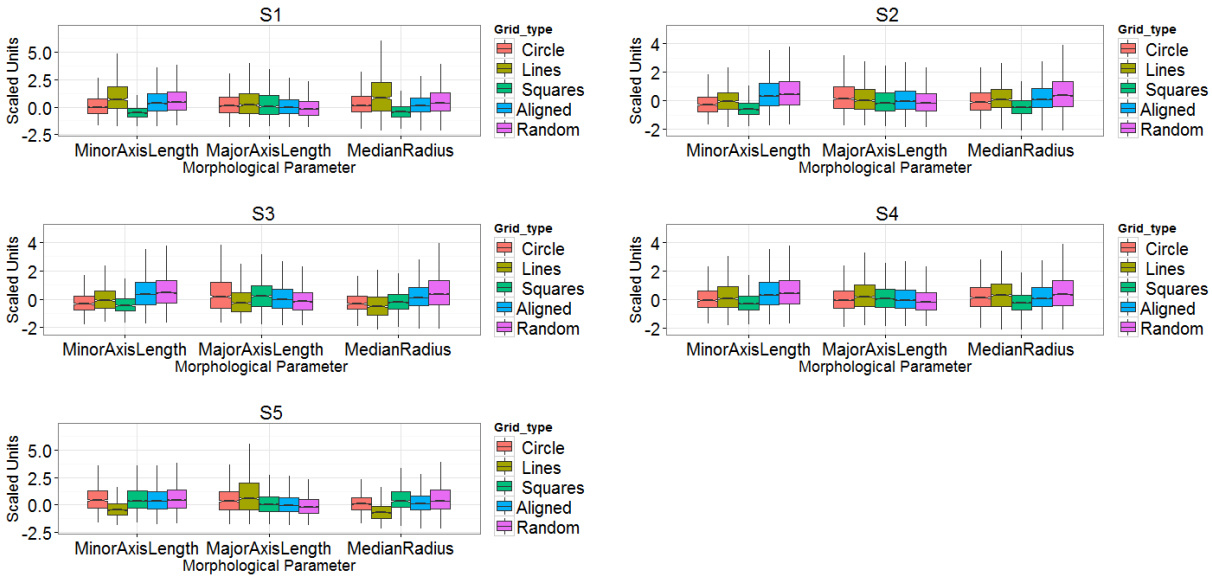

Figure S7 - Cell Size analysis for the different microtopographies grouped by surface dimensions. On S3, the minor axis for all the different patterns was lower than the controls. Median radius seems to follow a trend for minor axis.

Cell Eccentricity and compactness for different micro-topographies

Figure S8 showed cells eccentricity and compactness on different patterns. For circular pattern, cells on S2-S3 were highly eccentric; the eccentricity was similar to the one on lines S5, which was the highest. High values for compactness were detected on the same surfaces, which could mean that the cells have small number of philopodia and their body is highly compact (figure S8). The same trend can be observed for squared pattern but only for the first 4 surfaces with the highest dimension. It can be concluded that where the distance between patterns is higher than 200 µm for circles and 100 µm for squares, cells grow between patterns and form an elongated compact body.

For lines patterns, the first 4 surfaces showed a similar cell eccentricity and compactness, surprisingly lower than for circles and squares, and similar to aligned control (figure S8). However, on surface S5 there was a significant increase in both parameters. In particular, cell compactness was the highest from all geometries, which could mean that, on S5 "lines" pattern extremely compact elongated cells were present.

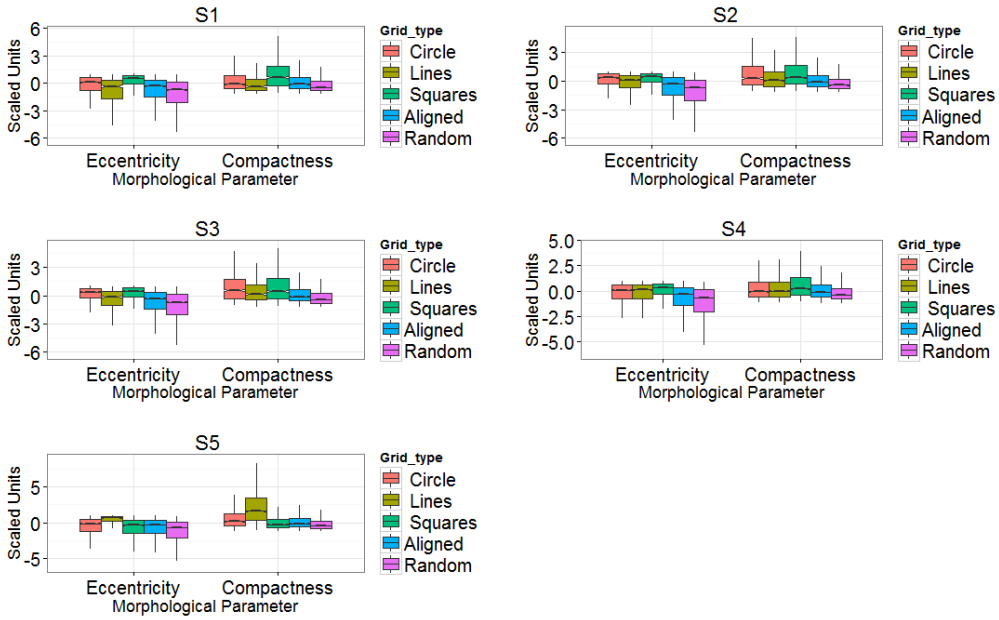

Figure S8 - Cell Eccentricity and compactness for the different microtopographies grouped by surface dimensions. The highest compactness was detected for Lines on S5. Eccentricity for lines and circles was almost equal on all surfaces, while higher values were identified on squared patterns S1, S4 and S5. On S3, high compactness and high eccentricity were observed for all the microtopographies. Therefore, S3 might represent the threshold dimension to guide cell behaviour. A similar effect was observed for S2 and S4 but less pronounced.

Cell Extent and solidity for different micro-topographies

Solidity and extent are two parameters used to evaluate cell extension. Solidity is the ratio between cell area and the area of convex hull (which is basically connections of all extreme edges of the cell), while extent is the ratio between cells area and boundary box (which makes this values very small for cells with lots of thin philopodia). Solidity for cells on squared patterns remained the same for all the different dimensions, while values for cell extent in S3 and S4 were significantly lower. This could indicate that cells on S3 and S4 had many philopodia (figure S9).

For circular pattern, the most interesting case is S5 that showed low solidity and extent similar to the other dimensions. This result indicated the presence of spread cells with irregular border. Moreover, S1 is characterized by low value of cell extent that could mean that cells had extensive philopodia (figure S9).

For lines patterns, surface S5 showed low extent and solidity and high value for compactness and eccentricity. This might indicate that cells were not perfectly aligned but had a curved shape. S3

showed low level of solidity that might indicate the presence of large cells with an irregular edge, while low level of extent for S2 indicated high number of philopodia (figure S9).

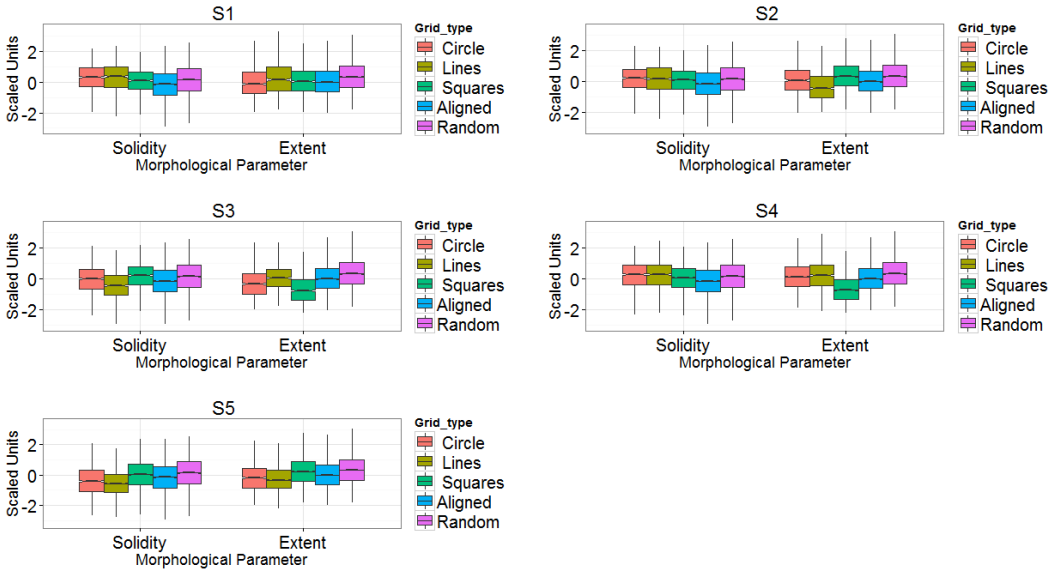

Figure S9 - Cell Extent and solidity for the different microtopographies grouped by surface dimensions. Solidity and Extent followed the same trend with the exception for S3 and S4 squared patterns where a decrease was observed. This could be due to the presence of high number of philopodia.

Principal component analysis and Clustering Surfaces based on cell morphological response

Figure S10a shows the distribution of all morphological data in the first two principal components (PCA) analysis. Using this method, cell morphology similarities related to the different surfaces and sizes can be identified. Controls (Random and Aligned) are clustered together, which indicates similar cell shapes. Cells on pattern lines and squares separately create distinct clusters. Cells on circular pattern do not occupy any distinct region in the PCA space and they are spread off all over the space. This result indicates that cells have heterogeneous morphology on circular pattern. PCA analysis for the different topographies is presented in figure S11.

As can be seen from Figure S10b, all 17 surfaces can be grouped on several clusters. Surprisingly, controls created a separate cluster with squares on S5. Lines S1 were joined in bigger cluster with second branch that includes Lines S4 and Circle S1, S2, S5. Lines S5 stood apart from all the other surfaces and created a separate cluster. This result indicates that cells on surface S5 had a unique cell morphology. The third cluster is characterized by two sub-clusters: one combines squares S1 and S2 with lines S3, while another combines squares S3, S4 with circle S3 and circle S4 with Lines S2. These results closely match the ones obtained from cell morphology analysis.

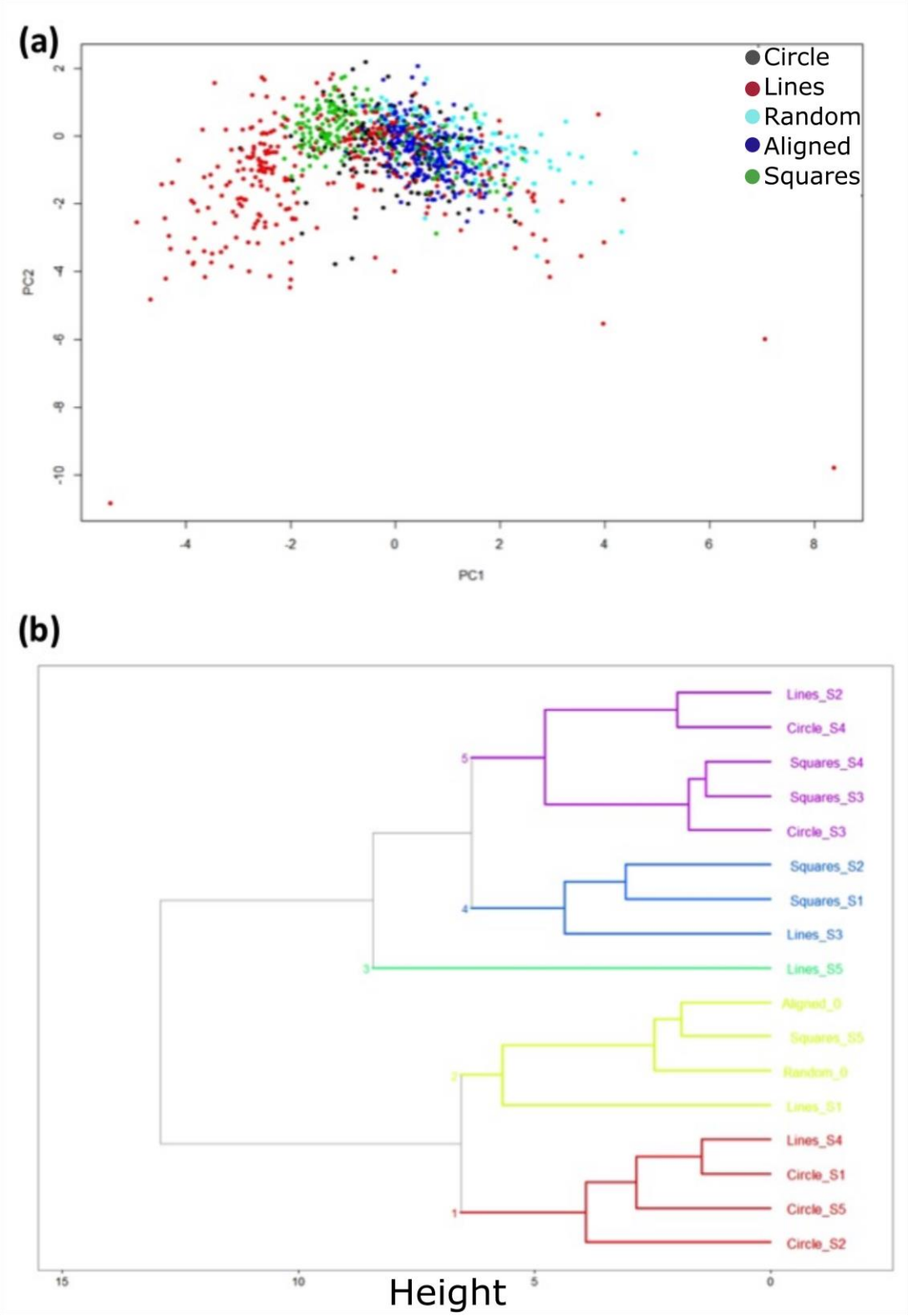

Figure S10 – (a) PCA analysis and (b) Clustering of Surfaces based on cell morphological data.

PCA analysis for different micro-topographies

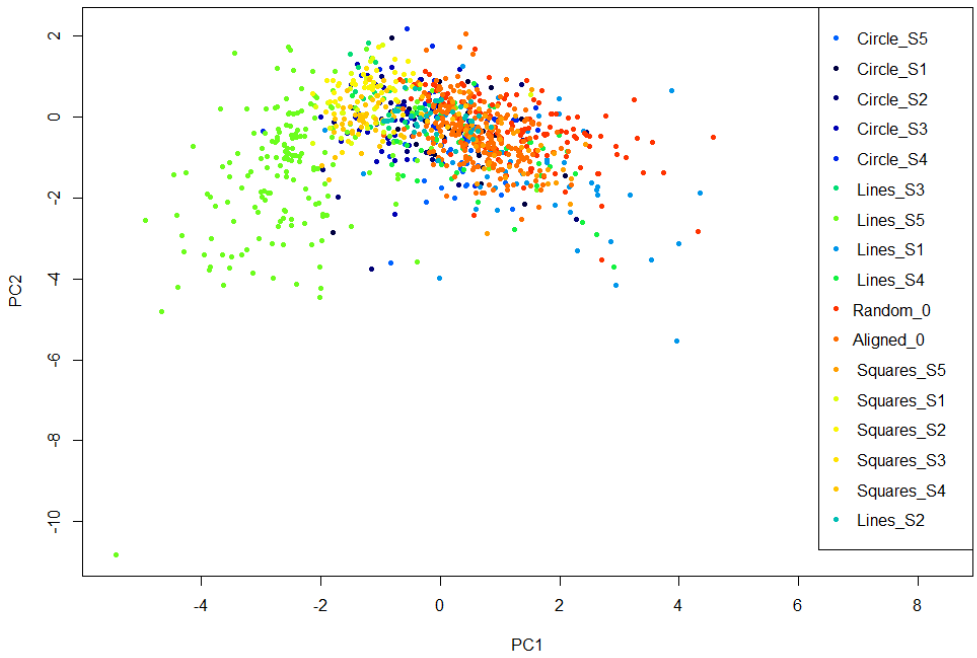

Figure S11 - PCA Analysis for different microtopographies and sizes.

DNA analysis after 7 days of culture

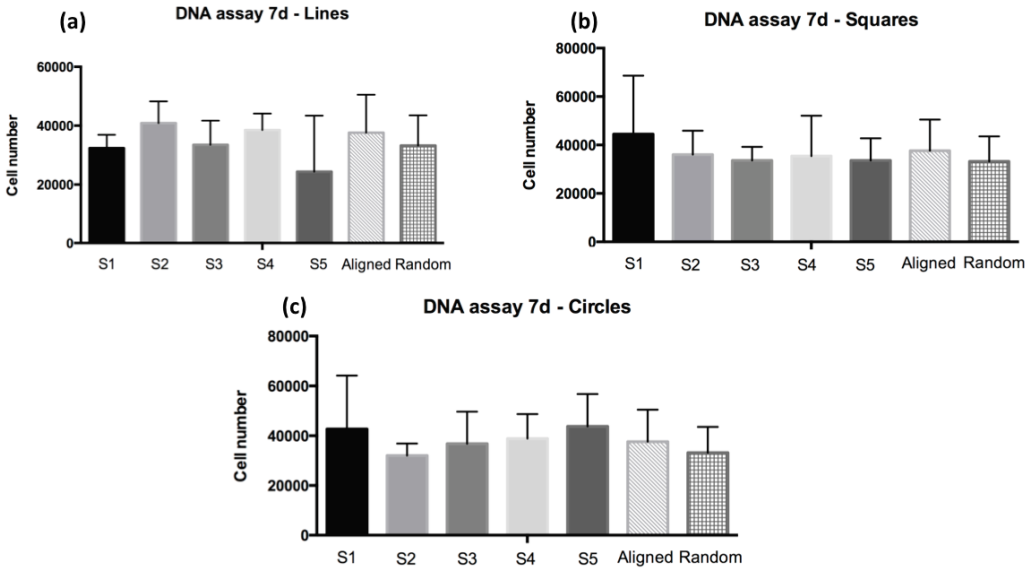

Figure S12 - Cell number after 7 days of culture. All data are expressed as mean ± standard deviation (SD). Biochemical assays were performed with triplicate biological samples. A one-way statistical analysis of variance (ANOVA) with a significant level p of 0.05 was used to determine differences between the groups. Tukey's multiple comparisons test was used to perform post hoc analysis. Statistical significance between the control group and the experimental groups are indicated with (*) which represents a p-value < 0.05, (**) which represents a p-value < 0.01, and (***) which represents a p-value < 0.001.