

# Adaptation of a simple microfluidic platform for high-dimensional quantitative morphological analysis of human mesenchymal stromal cells on polystyrene-based substrates

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**Keywords:** microfluidics, MSC, mesenchymal stromal cells, high dimensional morphological analysis, substrate modification

## Supplemental Tables and Table Legends:

**Supplemental Table 1:** Single cell morphological shape features as measured by the CellProfiler software. Formal definitions of each feature were obtained from the CellProfiler manual (<http://cellprofiler.org/manuals.shtml>) under the “MeasureObjectSizeShape” section.

Cell Shape Features	Definition
<i>Area</i>	“The actual number of pixels in the region.”
<i>Compactness</i>	“The variance of the radial distance of the object’s pixels from the centroid divided by the area.”
<i>Eccentricity</i>	“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.”
<i>Extent</i>	“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.”
<i>FormFactor</i>	“Calculated as $4 \cdot \pi \cdot \text{Area} / \text{Perimeter}^2$ . Equals 1 for a perfectly circular object.”
<i>MajorAxisLength</i>	“The length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.”
<i>MaxFeretDiameter; MinFeretDiameter</i>	“The Feret diameter is the distance between two parallel lines tangent on either side of the object. The minimum and maximum Feret diameters are the smallest and largest possible diameters, rotating the calipers along all possible angles.”
<i>MaximumRadius; MedianRadius; MeanRadius</i>	“The maximum, median, and mean distance of any pixel in the object to the closest pixel outside of the object, respectively.”
<i>MinorAxisLength</i>	“The length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.”
<i>Perimeter</i>	“The total number of pixels around the boundary of each region in the image.”
<i>Solidity</i>	“The proportion of the pixels in the convex hull that are also in the object, i.e. $\text{ObjectArea} / \text{ConvexHullArea}$ . 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.”

**Supplemental Table 2:** CellProfiler algorithm (pipeline) setup to quantify high dimensional morphological features of MSCs.

<b>Module</b>	<b>Description</b>
Load Images	Images loaded for each color channel: Cells (green), Nuclei (blue)
ApplyThreshold	<p>Sets pixel intensities below or above a certain threshold to zero</p> <p><u>Parameters</u></p> <p>Set pixels below or above the threshold to zero: below threshold</p> <p>Subtract the threshold value from the remaining pixel intensities: no</p> <p>Threshold strategy: Adaptive</p> <p>Thresholding method: RobustBackground</p> <p>Select the smoothing method for thresholding: no smoothing</p> <p>Threshold correction factor: 1.3</p> <p>Lower and upper bounds on threshold: 0.0 – 1.0</p>
IdentifyPrimaryObjects	<p>Individual nuclei analyzed as primary objects.</p> <p><u>Parameters</u></p> <p>Discard objects touching border of image: yes</p> <p>Thresholding Strategy: Adaptive</p> <p>Thresholding Method: Background</p> <p>Select the smoothing method for thresholding: no smoothing</p> <p>Threshold Correction Factor: 1.3</p> <p>Method to distinguish clumped objects: none</p> <p>Retain outlines of the identified objects: yes</p> <p>Fill holes in identified objects: yes</p>
IdentifySecondaryObjects	<p>Cells identified as secondary objects associated with nuclei</p> <p><u>Parameters</u></p> <p>Method to identify secondary objects: Watershed – Image</p> <p>Thresholding Strategy: Adaptive</p> <p>Thresholding Method: Background</p> <p>Select the smoothing method for thresholding: no smoothing</p> <p>Threshold Correction Factor: 1.0</p> <p>Fill holes in identified objects: yes</p> <p>Discard secondary objects that touch the edge of the image: yes</p> <p>Retain outlines of the identified secondary objects: yes</p>
MeasureObjectSizeShape	All features from Supplementary Table 1 measured here for each cell/nucleus
ExportToSpreadsheet	Measurements exported to .csv file
GrayToColor	Creating composite green/blue image for thresholding evaluation
OverlayOutlines	Overlaying cell and nucleus outlines onto composite image to visually evaluate quality of thresholding process
DisplayDataOnImage	Unique numbering of individual cells to allow for identification of poorly thresholded cells (or debris) that could then be removed from analysis in the exported spreadsheet
SaveImages	Save outlined composite images

**Supplemental Table 3:** Results from the statistical effects test (JMP12) of the main effects and interaction analysis of the MSC morphological (a) area, (b) eccentricity, (c) form factor, and (d) solidity for the full factorial bgPS microchannel experimental design. Nparm refers to the number of parameters associated with the effect (one less than the number of levels). The F Ratio is the ratio of the effect mean square divided by the error mean square. Italics indicate statistical significance ( $p < 0.05$ ). (e) For each morphological feature, the data are presented as mean  $\pm$  the standard error. Morphological features not connected by the same letter are significantly different ( $p < 0.05$ ).

<b>a. Factors (Area)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>2.03E+08</i>	<i>76.3954</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	3	<i>2.63E+09</i>	<i>330.163</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	3	<i>1.6E+09</i>	<i>200.561</i>	<i>&lt;.0001</i>

<b>b. Factors (Eccentricity)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>1.86902</i>	<i>92.5772</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	3	<i>2.3053</i>	<i>38.0624</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	3	<i>1.078373</i>	<i>17.8048</i>	<i>&lt;.0001</i>

<b>c. Factors (Form factor)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.928072</i>	<i>88.4744</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	3	<i>0.703558</i>	<i>22.3571</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	3	<i>1.297238</i>	<i>41.2225</i>	<i>&lt;.0001</i>

<b>d. Factors (Solidity)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>2.268471</i>	<i>143.4908</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	3	<i>1.468931</i>	<i>30.9721</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	3	<i>0.930657</i>	<i>19.6227</i>	<i>&lt;.0001</i>

<b>e. Group</b>	<b>Area</b>	<b>Eccentricity</b>	<b>FormFactor</b>	<b>Solidity</b>
10000 cells per cm <sup>2</sup> ,0h	7079.1 $\pm$ 135.4 <sup>B</sup>	0.807 $\pm$ 0.012 <sup>A,B</sup>	0.189 $\pm$ 0.009 <sup>D,E</sup>	0.659 $\pm$ 0.010 <sup>B,C</sup>
10000 cells per cm <sup>2</sup> ,2h	7909.3 $\pm$ 141.9 <sup>A</sup>	0.807 $\pm$ 0.012 <sup>A,B</sup>	0.219 $\pm$ 0.009 <sup>C,D</sup>	0.688 $\pm$ 0.011 <sup>B</sup>
10000 cells per cm <sup>2</sup> ,4h	3482.4 $\pm$ 145.9 <sup>F</sup>	0.748 $\pm$ 0.013 <sup>C</sup>	0.304 $\pm$ 0.009 <sup>A</sup>	0.766 $\pm$ 0.011 <sup>A</sup>
10000 cells per cm <sup>2</sup> ,16h	3061.8 $\pm$ 133.2 <sup>F</sup>	0.665 $\pm$ 0.012 <sup>D</sup>	0.263 $\pm$ 0.008 <sup>B</sup>	0.764 $\pm$ 0.010 <sup>A</sup>
50000 cells per cm <sup>2</sup> ,0h	5245.3 $\pm$ 70.6 <sup>C</sup>	0.821 $\pm$ 0.006 <sup>A,B</sup>	0.217 $\pm$ 0.004 <sup>C,D</sup>	0.647 $\pm$ 0.005 <sup>C</sup>
50000 cells per cm <sup>2</sup> ,2h	4739.1 $\pm$ 69.3 <sup>D</sup>	0.837 $\pm$ 0.006 <sup>A</sup>	0.181 $\pm$ 0.004 <sup>E</sup>	0.637 $\pm$ 0.005 <sup>C</sup>
50000 cells per cm <sup>2</sup> ,4h	4537.2 $\pm$ 61.7 <sup>D,E</sup>	0.826 $\pm$ 0.005 <sup>A</sup>	0.179 $\pm$ 0.004 <sup>E</sup>	0.628 $\pm$ 0.005 <sup>C</sup>
50000 cells per cm <sup>2</sup> ,16h	4322.1 $\pm$ 59.6 <sup>E</sup>	0.801 $\pm$ 0.005 <sup>B</sup>	0.217 $\pm$ 0.004 <sup>C</sup>	0.682 $\pm$ 0.005 <sup>B</sup>

**Supplemental Table 4:** Results from the statistical effects test (JMP12) of the main effects and interaction analysis of the MSC morphological (a) area, (b) eccentricity, (c) form factor, and (d) solidity for the full factorial tctPS microchannel experimental design. Nparm refers to the number of parameters associated with the effect (one less than the number of levels). The F Ratio is the ratio of the effect mean square divided by the error mean square. Italics indicate statistical significance ( $p < 0.05$ ).

<b>a. Factors (Area)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>4.84E+08</i>	<i>77.9503</i>	<i>&lt;.0001</i>
<i>Serum Concentration</i>	2	<i>1.05E+08</i>	<i>8.4408</i>	<i>0.0002</i>
<i>Cell Seeding Density*Serum Concentration</i>	2	<i>4.03E+08</i>	<i>32.4676</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	2	<i>7.93E+09</i>	<i>638.1282</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>7.54E+08</i>	<i>60.7136</i>	<i>&lt;.0001</i>
<i>Serum Concentration*UV Treatment</i>	4	<i>4.53E+08</i>	<i>18.254</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*Serum Concentration*UV Treatment</i>	4	<i>4.25E+08</i>	<i>17.1004</i>	<i>&lt;.0001</i>
<b>b. Factors (Eccentricity)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.695245</i>	<i>45.0172</i>	<i>&lt;.0001</i>
<i>Serum Concentration</i>	2	<i>0.010806</i>	<i>0.3498</i>	<i>0.7049</i>
<i>Cell Seeding Density*Serum Concentration</i>	2	<i>0.065683</i>	<i>2.1265</i>	<i>0.1197</i>
<i>UV Treatment</i>	2	<i>10.44226</i>	<i>338.0687</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>0.359182</i>	<i>11.6285</i>	<i>&lt;.0001</i>
<i>Serum Concentration*UV Treatment</i>	4	<i>0.662666</i>	<i>10.7269</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*Serum Concentration*UV Treatment</i>	4	<i>0.182513</i>	<i>2.9544</i>	<i>0.0191</i>
<b>c. Factors (Form factor)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.003328</i>	<i>1.0042</i>	<i>0.3165</i>
<i>Serum Concentration</i>	2	<i>0.098489</i>	<i>14.8596</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*Serum Concentration</i>	2	<i>0.029199</i>	<i>4.4054</i>	<i>0.0124</i>
<i>UV Treatment</i>	2	<i>1.850197</i>	<i>279.1499</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>0.01717</i>	<i>2.5905</i>	<i>0.0754</i>
<i>Serum Concentration*UV Treatment</i>	4	<i>0.509015</i>	<i>38.399</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*Serum Concentration*UV Treatment</i>	4	<i>0.213755</i>	<i>16.1252</i>	<i>&lt;.0001</i>
<b>d. Factors (Solidity)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.322368</i>	<i>23.9874</i>	<i>&lt;.0001</i>
<i>Serum Concentration</i>	2	<i>0.577354</i>	<i>21.4805</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*Serum Concentration</i>	2	<i>0.028056</i>	<i>1.0438</i>	<i>0.3524</i>
<i>UV Treatment</i>	2	<i>5.879849</i>	<i>218.7602</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>0.291469</i>	<i>10.8441</i>	<i>&lt;.0001</i>
<i>Serum Concentration*UV Treatment</i>	4	<i>0.582125</i>	<i>10.829</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*Serum Concentration*UV Treatment</i>	4	<i>0.292725</i>	<i>5.4454</i>	<i>0.0002</i>

**Supplemental Table 5:** Results from the statistical effects test (JMP12) of the main effects and interaction analysis of the MSC morphological (a) area, (b) eccentricity, (c) form factor, and (d) solidity for the full factorial tctPS open-well experimental design. Nparm refers to the number of parameters associated with the effect (one less than the number of levels). The F Ratio is the ratio of the effect mean square divided by the error mean square. Italics indicate statistical significance ( $p < 0.05$ ). (e) For each morphological feature, the data are presented as mean  $\pm$  the standard error. Morphological features not connected by the same letter are significantly different ( $p < 0.05$ ).

<b>a. Factors (Area)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>1.74E+09</i>	<i>426.5897</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	2	<i>2.27E+09</i>	<i>277.387</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>8.47E+08</i>	<i>103.5872</i>	<i>&lt;.0001</i>

<b>b. Factors (Eccentricity)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.108813</i>	<i>9.3634</i>	<i>0.0022</i>
<i>UV Treatment</i>	2	<i>0.067792</i>	<i>2.9168</i>	<i>0.0542</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>0.01327</i>	<i>0.5709</i>	<i>0.565</i>

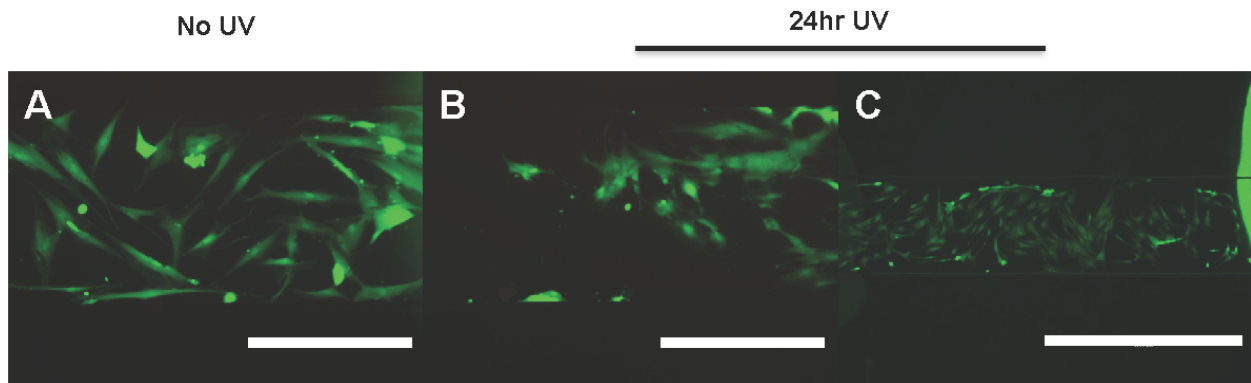
<b>c. Factors (Form factor)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.003931</i>	<i>1.6168</i>	<i>0.2036</i>
<i>UV Treatment</i>	2	<i>0.1767</i>	<i>36.3338</i>	<i>&lt;.0001</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>0.099834</i>	<i>20.5283</i>	<i>&lt;.0001</i>

<b>d. Factors (Solidity)</b>	<b>Nparm</b>	<b>Sum of Squares</b>	<b>F Ratio</b>	<b>P Value</b>
<i>Cell Seeding Density</i>	1	<i>0.951251</i>	<i>78.9087</i>	<i>&lt;.0001</i>
<i>UV Treatment</i>	2	<i>0.063458</i>	<i>2.632</i>	<i>0.072</i>
<i>Cell Seeding Density*UV Treatment</i>	2	<i>0.203468</i>	<i>8.4391</i>	<i>0.0002</i>

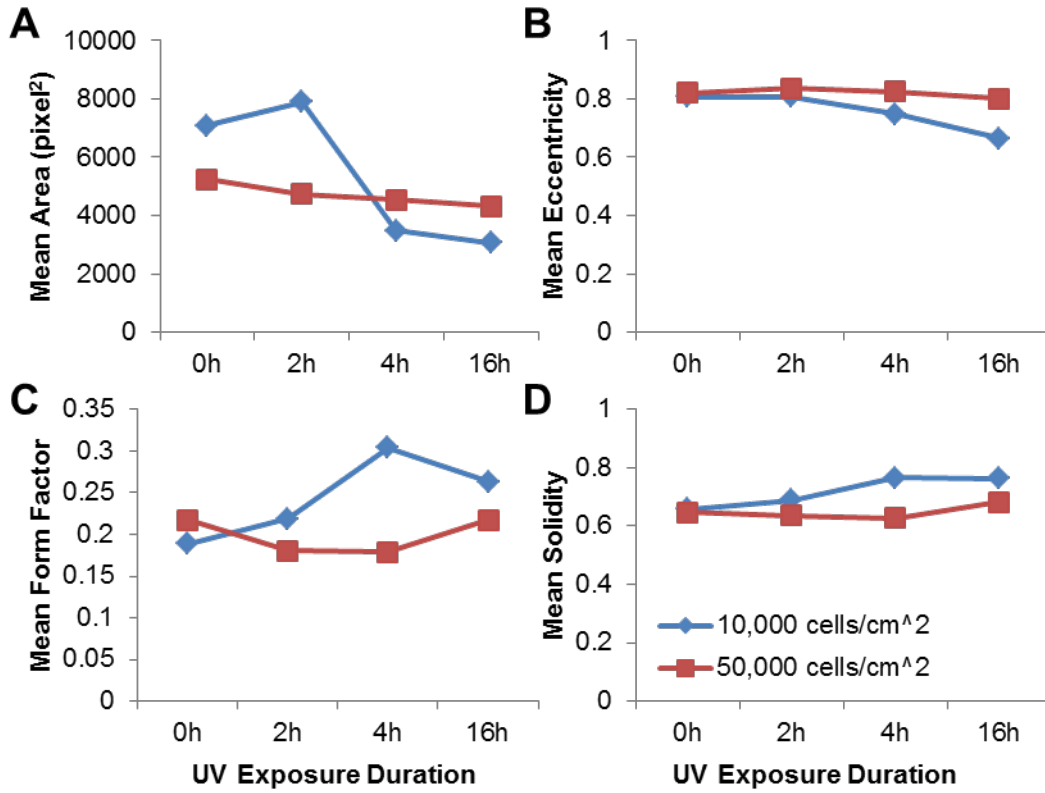
<b>e. Group</b>	<b>Area</b>	<b>Eccentricity</b>	<b>FormFactor</b>	<b>Solidity</b>
5000 cells per cm <sup>2</sup> ,0h	6980.0 $\pm$ 69.5 <sup>A</sup>	0.904 $\pm$ 0.004 <sup>A</sup>	0.099 $\pm$ 0.002 <sup>B,C</sup>	0.597 $\pm$ 0.004 <sup>B,C</sup>
5000 cells per cm <sup>2</sup> ,4h	7064.2 $\pm$ 59.5 <sup>A</sup>	0.903 $\pm$ 0.003 <sup>A</sup>	0.102 $\pm$ 0.001 <sup>B</sup>	0.599 $\pm$ 0.003 <sup>B,C</sup>
5000 cells per cm <sup>2</sup> ,16h	6498.5 $\pm$ 64.6 <sup>B</sup>	0.900 $\pm$ 0.003 <sup>A,B</sup>	0.102 $\pm$ 0.002 <sup>B</sup>	0.586 $\pm$ 0.004 <sup>C</sup>
10000 cells per cm <sup>2</sup> ,0h	6947.6 $\pm$ 45.0 <sup>A</sup>	0.898 $\pm$ 0.002 <sup>A,B</sup>	0.095 $\pm$ 0.001 <sup>C</sup>	0.608 $\pm$ 0.002 <sup>B</sup>
10000 cells per cm <sup>2</sup> ,4h	5980.2 $\pm$ 39.9 <sup>C</sup>	0.898 $\pm$ 0.002 <sup>A</sup>	0.098 $\pm$ 0.001 <sup>B,C</sup>	0.618 $\pm$ 0.002 <sup>A</sup>
10000 cells per cm <sup>2</sup> ,16h	4869.5 $\pm$ 38.9 <sup>D</sup>	0.889 $\pm$ 0.002 <sup>B</sup>	0.113 $\pm$ 0.001 <sup>A</sup>	0.621 $\pm$ 0.002 <sup>A</sup>

## Supplemental Figures and Figure Legends:

**Supplemental Figure 1:** Compared to (A) control PS substrates that were not UV exposed, MSCs are viable 24h after being seeded at 50000 cells/cm<sup>2</sup> on (B) PS substrates that were exposed to UV for 24h prior to cell seeding as indicated by LIVE (green)/DEAD (red) staining. A lower magnification of MSCs seeded on PS substrates subjected to 24h of UV exposure before cell seeding is shown in (C). Scale bar is 400 microns for A and B, and 1 mm for C.

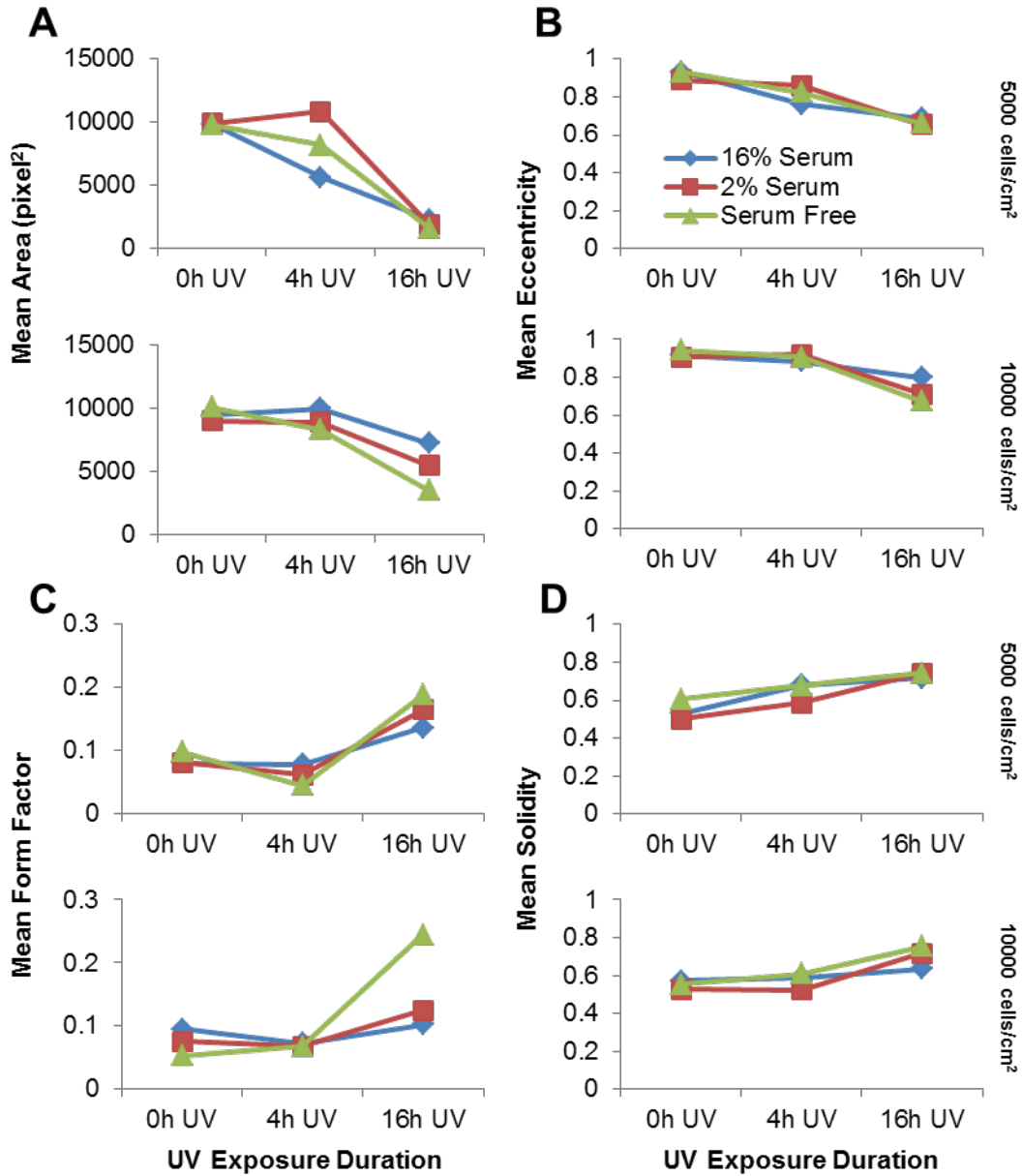


**Supplemental Figure 2:** Interaction plots illustrating cross effects between cell seeding density and substrate UV exposure duration on the MSC morphological features of (A) area, (B) eccentricity, (C) form factor, and (D) solidity are shown for bgPS microchannels. Blue diamonds and red squares indicate MSCs seeded at 10000 and 50000 cells/cm<sup>2</sup>, respectively.





**Supplemental Figure 3:** Interaction plots illustrating cross effects between cell seeding density, substrate UV exposure duration, and serum concentration on the MSC morphological features of (A) area, (B) eccentricity, (C) form factor, and (D) solidity are shown for tctPS microchannels. Blue diamonds green crosses, and red circles represent serum free, 2%, and 16% serum conditions, respectively.



**Supplemental Figure 4:** Interaction plot illustrating cross effects between cell seeding density and substrate UV exposure duration on the MSC morphological area feature are shown for macroscale tctPS well plates. Red squares and blue diamonds indicate MSCs seeded at 5000 and 10000 cells/cm<sup>2</sup>, respectively.

