Supplementary Material

Combined Effect of Shear Stress and Laser-Patterned Topography on Schwann cell Outgrowth: Synergistic or Antagonistic?

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A. Parametric Studies

Figure 1S depicts the top-view SEM images of PET coverslips that were ablated by the femtosecond laser at a constant fluence of 11.9 J/cm^2 and scan velocity of 7 mm/s. Using a x_{step} of 50 μm, a x_{step} and y_{step} of 50 μm, and a x_{step} of 200 μm, the geometries of microgrooves, chess, and net were fabricated, respectively. The spacing and the width, w, of the microgrooves were equal to 28.76 ± 0.50 μm and 28.68 ± 0.47 μm, respectively. Moreover, the spacing of the chess was 24.52 ± 1.62 μm and the diameter of each chess piece was 21.91 ± 1.12 μm. Finally, the spacing of the net was equal to 168.12 ± 1.38 μm.

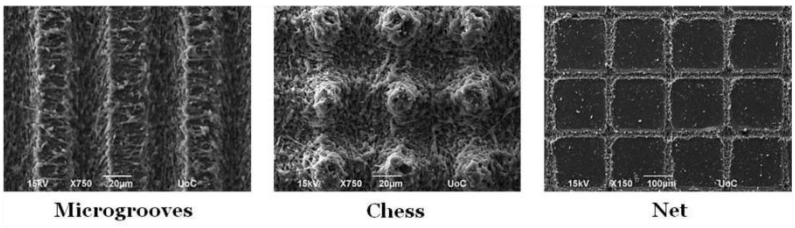

Figure 1S: *Scanning electron microscopy (SEM) images of laser-microstructured substrates with different geometries (microgrooves, chess, net).*

When we used the topography of microgrooves, Schwann (SW10) cells appeared to be oriented along the direction of microgrooves (Figure 2S) while they showed a random orientation on the other geometries, chess (Figure 3S) and net (Figure 4S).

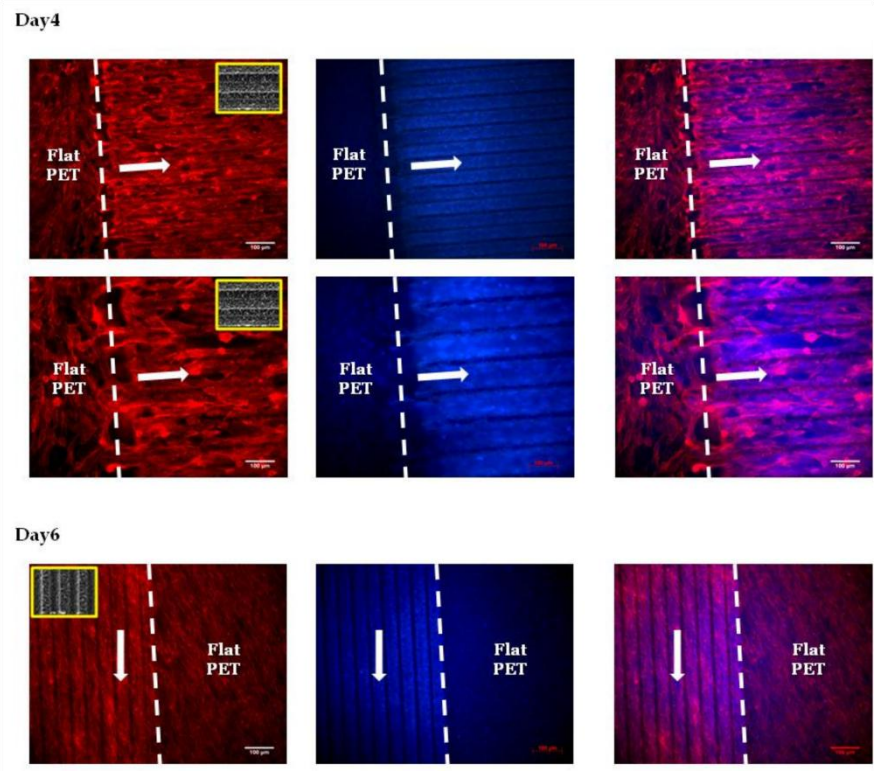

Figure 2S: *Fluorescent images of Schwann cells cultured on the PET substrates (PET-MG and PET-Flat) for 4 and 6 days. The cytoskeleton of the cells is visualized with red color (Alexa Fluor® 568 Phalloidin), while the nuclei are indicated with blue color (DAPI). The white arrows represent the directionality of Schwann cytoskeleton, which is according to the direction of the microgrooves. The inset SEM images, indicated by the yellow box, show the geometry of microgrooves. This figure has been reprinted from Babaliari et al., 2018* [1] *under a Creative Commons Attribution License.*

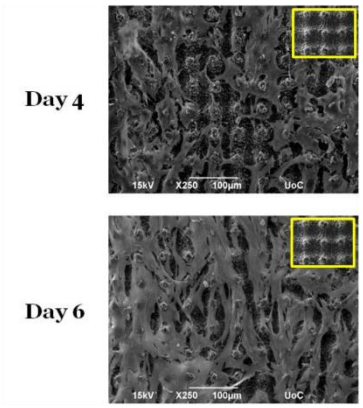

Figure 3S: *Scanning electron microscopy (SEM) images of Schwann cells cultured on the PET substrates (PET-Chess) for 4, 6 days. The inset SEM images, indicated by the yellow box, show the geometry of chess.*

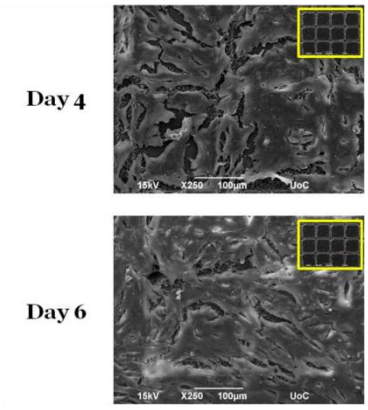

Figure 4S: *Scanning electron microscopy (SEM) images of Schwann cells cultured on the PET substrates (PET-Net) for 4, 6 days. The inset SEM images, indicated by the yellow box, show the geometry of net.*

Thus, we decided to focus on the geometry of microgrooves. Moreover, we determined that it would be more effective to quantify the cell length after a 3-day culture period.

Regarding the dynamic experiments, before using SW10 cells, we used NIH 3T3 cells as a well-studied and characterized experimental model for the study of cellular functions (adhesion, proliferation, and orientation) to optimize the parameters of the experiment. Specifically, Figure 5S illustrates the NIH 3T3 cells (50000, 25000 cells) cultured on the PET coverslip under dynamic conditions (15, 30 μL/min). We noticed that the cells adhered strongly and proliferated well on the PET coverslip, but no orientation was observed. Thus, we decreased the number of cells, 15000 cells, to examine if the lack of orientation was due to the cell number (Figure 6S). However, by decreasing the cell number and applying the same flow rate (30 μL/min), cells proliferated well on the PET coverslip but similarly appeared to have a random orientation (Figure 6S). So, we increased the flow rate to examine if the cells will be oriented along the direction of the flow. However, by applying 120 and 60 μL/min, cells detached from the PET coverslip (Figure 6S).

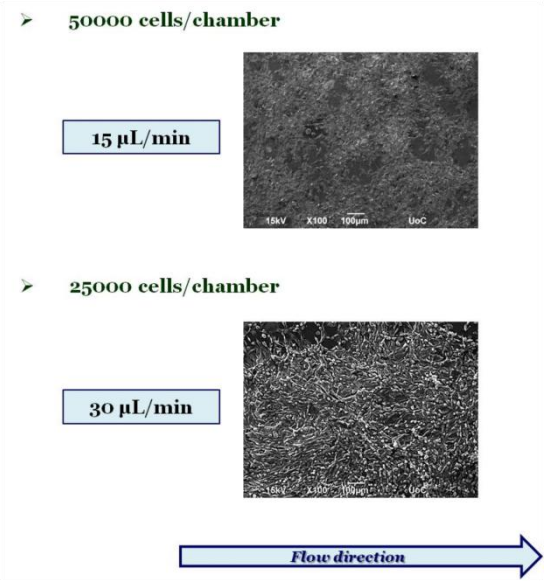

Figure 5S: *Scanning electron microscopy (SEM) images of NIH 3T3 cells (50000, 25000) cultured on the PET coverslip under dynamic conditions applying 15 and 30 µL/min on the third day.*

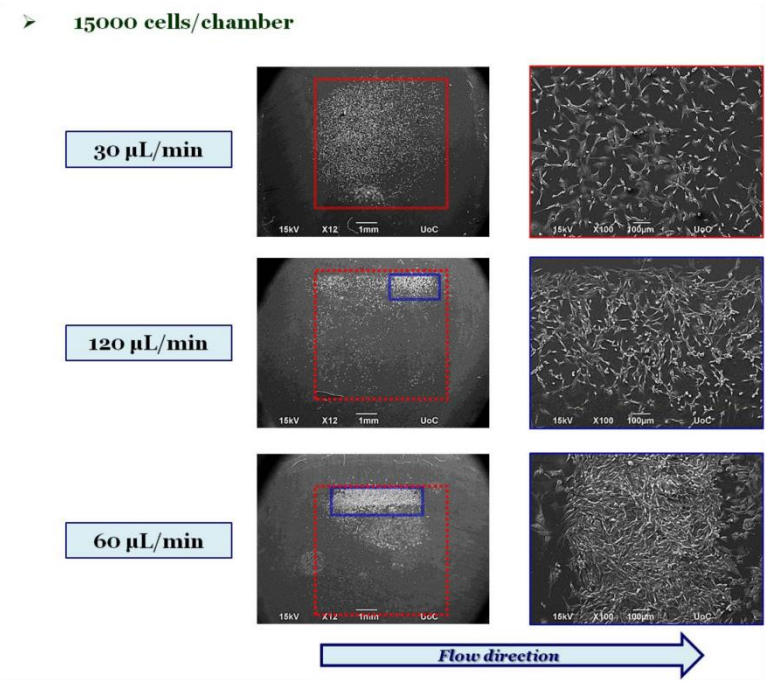

Figure 6S: *Scanning electron microscopy (SEM) images of NIH 3T3 cells (15000) cultured on the PET coverslip under dynamic conditions applying 30, 120, and 60 µL/min on the third day.*

Thus, after optimizing some basic parameters of the dynamic experiments using NIH 3T3 cells, such as cell number (25000 cells) and flow rate (starting point 30 µL/min), we performed the experiments using SW10 cells. By applying a flow rate of 30 µL/min, we noticed that the SW10 cells seem to be oriented across the direction of the microgrooves and parallel to flow while a random orientation observed on the flat PET (Figure 7S). So, we

decided to increase the flow rate (50 μL/min) to examine if the cells will be oriented on the flat PET (results presented in the paper).

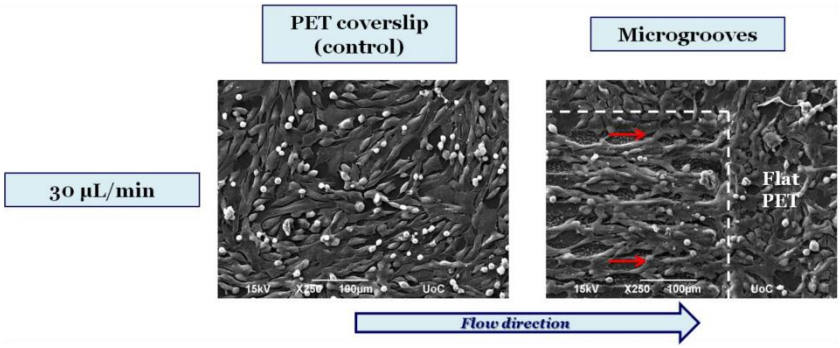

Figure 7S: *Scanning electron microscopy (SEM) images of Schwann cells (25000) cultured on the PET coverslip and the microgrooves under dynamic conditions applying 30 μL/min on the third day.*

To conclude, following the abovementioned parametric studies (different substrates, cells, cell densities, days of culture, flow rates) we came up with the results presented in the paper.

B. SW10 Cells' Orientation under Static and Dynamic Culture Conditions

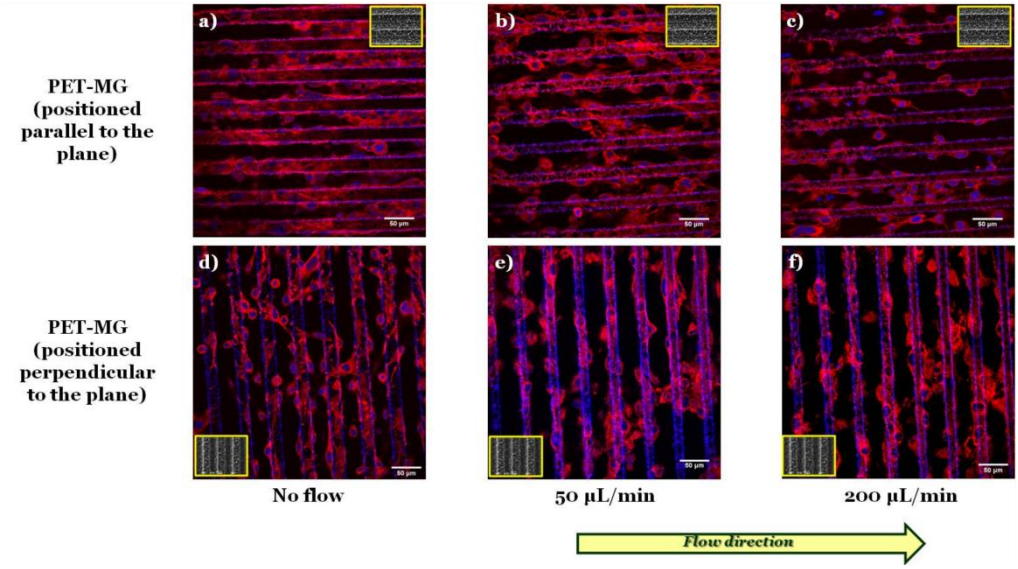

Figure 8S: *Confocal images of SW10 cells cultured on top of the MG of the PET-MG substrates under static (a, d) or dynamic conditions, applying 50 (b, e) and 200 (c, f) μL/min, on the third day of culture. The cytoskeleton of the cells is visualized with red color (Alexa Fluor® 680 Phalloidin) while the nuclei with blue color (DAPI). The direction of the flow was parallel (b, c) or perpendicular (e, f) to the microgrooves. The inset SEM images, framed by a yellow box, depict the geometry of microgrooves.*

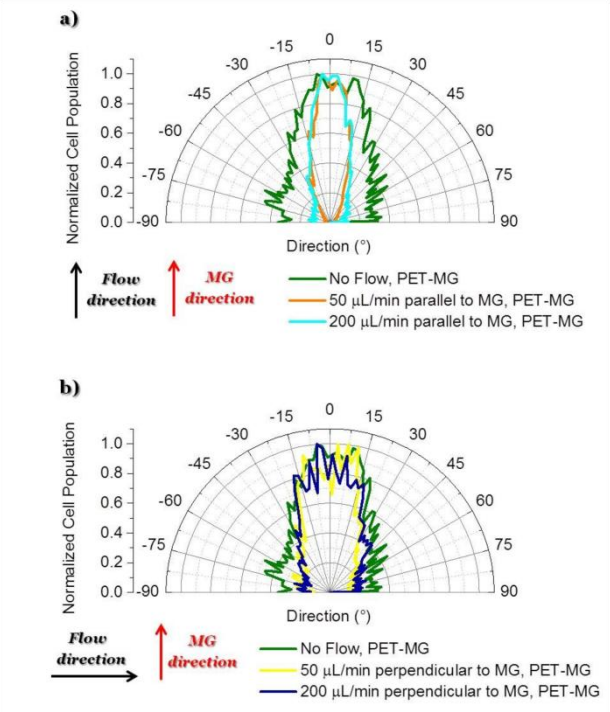

Figure 9S: *Directional polar plots of cells' cytoskeleton on top of the MG of the PET-MG substrates. The directional polar plots were generated using the Fiji ImageJ plug-in "Directionality"* [2]*. In this way, the data of the amount of cells, presented in the input image, in each direction was extracted and plotted as a polar plot. To compare the different cases, the cell population per angle was normalized with the maximum value in each case and expressed as normalized cell population. Specifically: a) No flow, PET-MG (green line) - 50 μL/min parallel to MG, PET-MG (orange line) - 200 μL/min parallel to MG, PET-MG (turquoise line), b) No flow, PET-MG (green line) - 50 μL/min perpendicular to MG, PET-MG (yellow line) - 200 μL/min perpendicular to MG, PET-MG (dark blue line). The black and red arrows represent the direction of the flow and the microgrooves, respectively. The statistical analysis of the data was performed using post hoc Tukey HSD test. A p-value < 0.05 was considered significant. In particular: a) No flow, PET-MG is significantly different from 50 μL/min parallel to MG, PET-MG and 200 μL/min parallel to MG, PET-MG (* p<0.05). No significant difference was observed between 50 μL/min parallel to MG, PET-MG and 200 μL/min parallel to MG, PET-MG; b) No significant difference was observed between No flow, PET-MG, 50 μL/min perpendicular to MG, PET-MG and 200 μL/min perpendicular to MG, PET-MG.*

References

1 E. Babaliari, P. Kavatzikidou, D. Angelaki, L. Chaniotaki, A. Manousaki, A. Siakouli-Galanopoulou, A. Ranella and E. Stratakis, *Int. J. Mol. Sci.*, 2018, **19**, 2053.

2 J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat. Methods*, 2012, **9**, 676–82.