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Microfluidic Model to Evaluate Astrocyte Activation in Penumbral Region following Ischemic Stroke

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Supplementary Materials: Figure 1A shows astrocytes stained with an anti-GFAP antibody, which demonstrates their astrocytic origin. Figure 2A shows the hypoxia analysis in the PDMS and the polystyrene-based microdevices. PDMS oxygen permeability led to no oxygen gradient formation across the microchamber, whereas in the polystyrene-based microdevices we observed the formation of an oxygen gradient after 24 hours in culture. Figure A3 shows that the number of astrocytes in the 3D hydrogel did not increase over a period of 3 days in culture. Figure A4 illustrates the variety of morphologies exhibited by astrocytes recovered form the microdevice.

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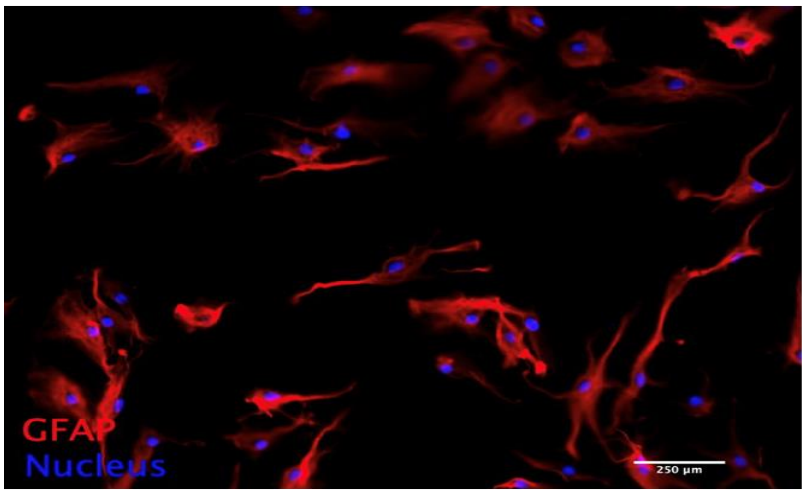

Figure S1. Primary rat cortical astrocytes cultured on Poly-L-lysine coated coverslips were stained with anti-GFAP to confirm astrocyte origin. The GFAP stain is shown in red and DAPI stained nu-clei are shown in blue.

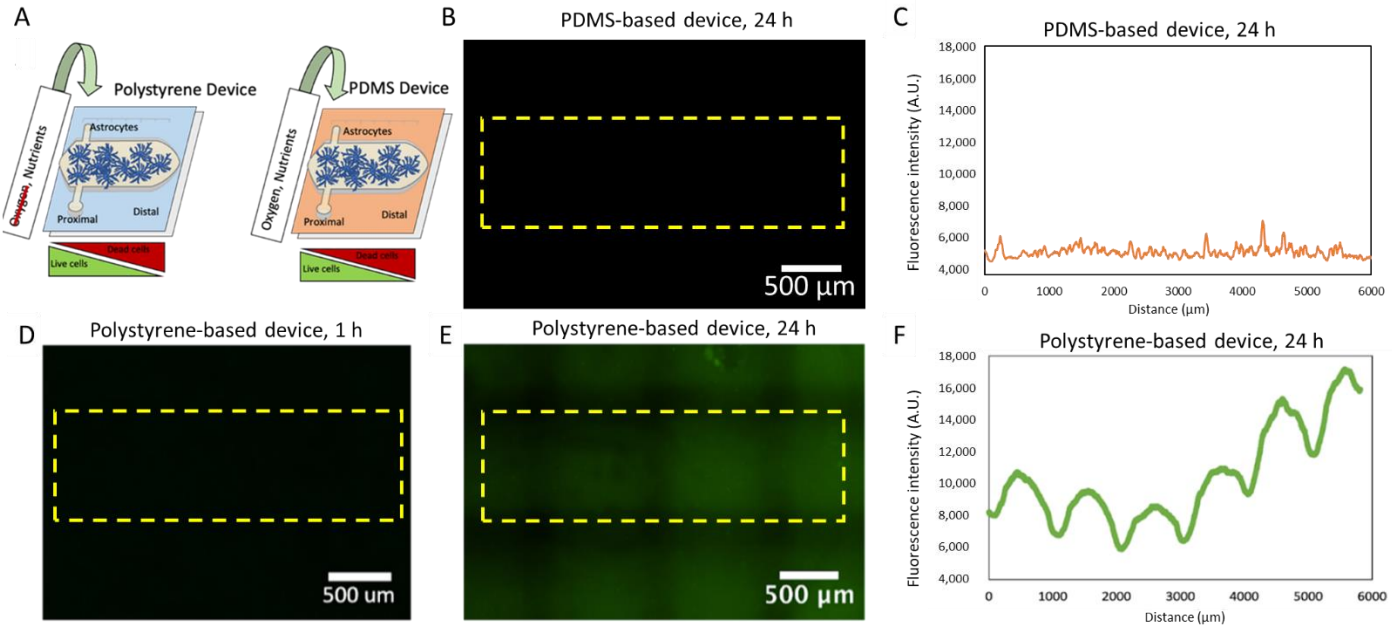

Figure S2. (**A**) Schematic of the polystyrene and PDMS-based microdevices. (**B**,**C**) Hypoxia-sensing die within the PDMS-based microdevice imaged after 24 h in culture. Graph shows the hypoxia-induced fluorescence across the delimited region (yellow rectangle) in (**B**,**D**–**F**) Hypoxia-sensing die in the Polystyrene-based microdevice imaged at 1 and 24 h in culture. Graph shows the hypoxia-induced fluorescence across the delimited region (yellow rectangle) in E.

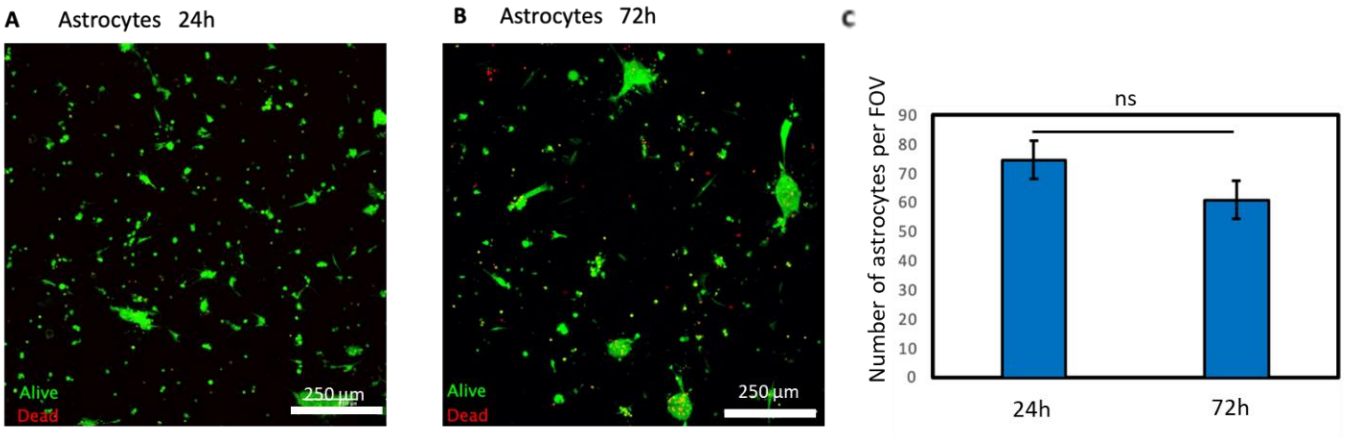

Figure S3. Astrocytes proliferation in the microdevice. (**A**,**B**) Confocal microscopy images show viabile and dead astrocytes in green and red respectivly after 24 and 72 h in culture. (**C**) Graph shows the number of astrocytes in the microdevice after 24 and 72 h in culture.

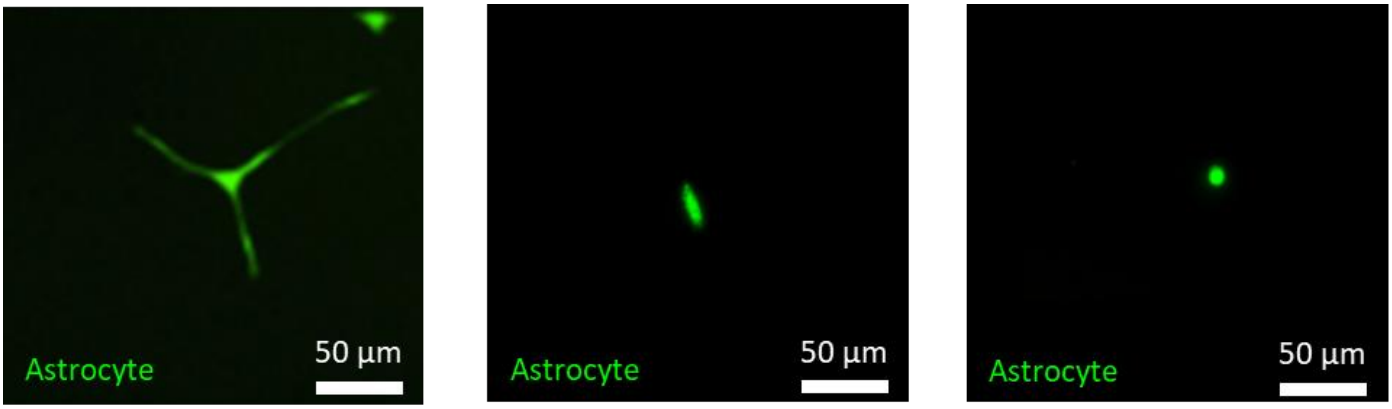

Figure S4. Astrocyte morphologies. Astrocytes were cultured in the microdevice for 3 days and then retrieved from the hydrogel and cultured in regular flasks for 5 days. Recovered astrocytes exhibited a broad range of cell morphologies.

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