Electronic Supplementary Material (ESI) for Lab on a Chip.

An integrated microfluidic chip for studying the effects of neurotransmitters on neurospheroids

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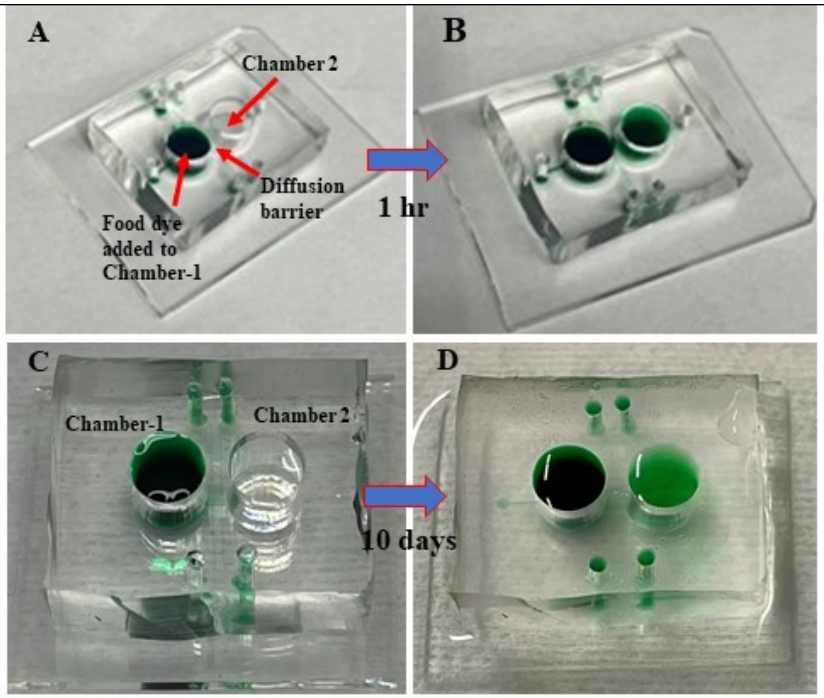

Figure S1. Photos showing the blue food dye diffuses from Chamber-1 to Chamber-2 through different barriers (a-b) for Chip1 and (c-d) for Chip2.

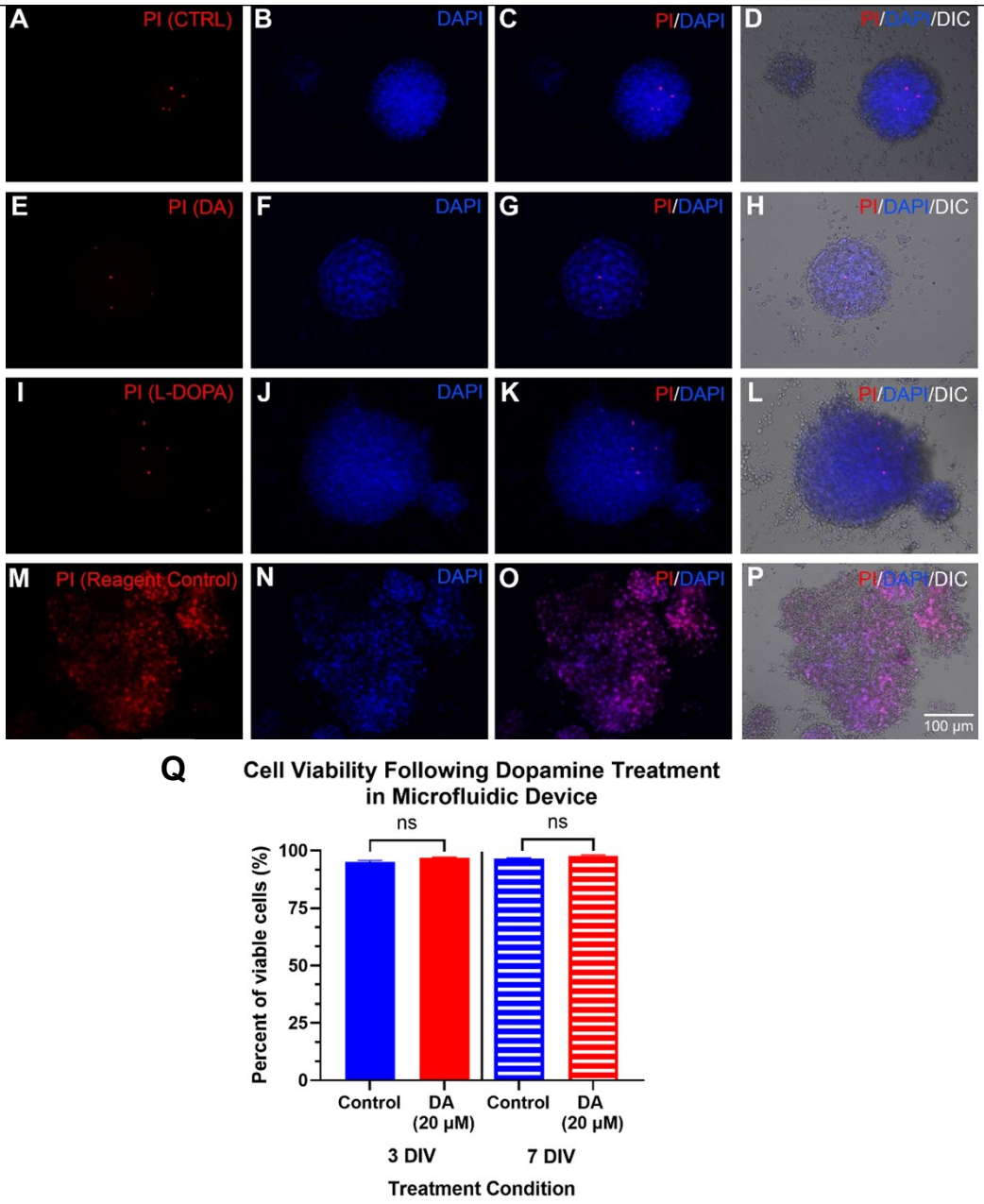

Figure S2. Viability of AHPC Neurospheroids under DA Treatment in Microfluidic Devices. A-P: Fluorescence images of AHPC neurospheroids at 7 DIV following dopamine (DA) treatment in microfluidic devices. Propidium iodide staining of dead cells (PI, red; A, C, D, E, G, H, I, K, L, M, O, and P) and cell nuclei counterstained with DAPI (blue; B, C, D, F, G, H, J, K, L, N, O, and P). Cells intentionally killed with 70% ethanol acted as a positive reagent control for PI staining (M-P). Scale bar = 100 µm. Q: Quantitative analysis of AHPC cell viability following drug treatment within microfluidic devices at 3 and 7 DIV. No significant difference was detected between any of the culture conditions at 3 or 7 DIV, though the DA treated spheres had a slight decrease in the percentage of PI-labeled cells compared to the control group. Bars represent the mean percentage of PI-labeled cells, and the error bars represent the standard error of the mean. N=3 independent experiments, 30-41 images were quantified for each treatment

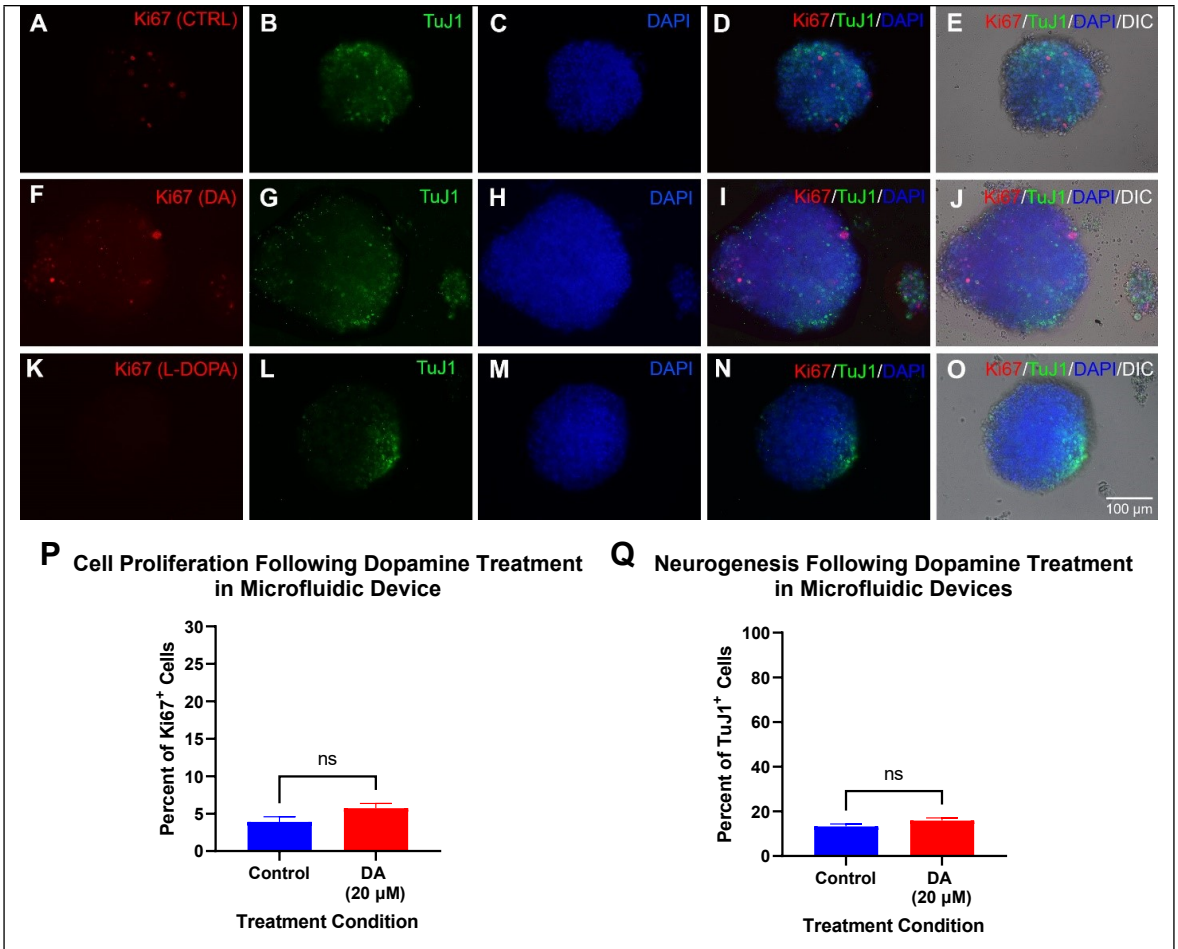

Figure S3. Proliferation and Differentiation of AHPC Neurospheroids under DA Treatment in Microfluidic Devices. A-P: Fluorescence images of AHPC neurospheroids at 7 DIV following dopamine treatment in microfluidic devices. Cells were immunolabeled with cell proliferation marker (Ki67, red; A, D, E, F, I, J, K, N, and O), immature neuron marker (TuJ1, green; B, D, E, G, I, J, L, N, and O), and cell nuclei marker (DAPI, blue; C, D, E, H, I, J, M, N, and O). Scale bar = 100 µm. P-Q: Quantitative analysis of AHPC cell proliferation and differentiation following drug treatment within microfluidic devices at 7 DIV. P: There was a small, yet non-significant increase in the percent of Ki67-labled cells following DA treatment compared to the control. Q: A slight increase in neurogenesis was observed following DA treatment compared to the control as shown by the slight increase in the percent of TuJ1-labeled cells; however, the DA treated group was not significantly different from the control group. Bars represent the mean percentage of PI-labeled cells, and the error bars represent the standard error of the mean. N=3 independent experiments, 24-29 images were quantified for each treatment condition.

Table S1. **Estimated sizes of Green Food Dye (Fast Green FCF), Dopamine and Serotonin**

Chemical Name	Formula	Molar Mass	Density	Molecule volume
Fast Green FCF	$C_{37}H_{34}N_2Na_2O_{10}S_3$	808.84 g·mol^{-1}	0.35 g/cm^3	**372×10^{-23} cm^3**
Dopamine	$C_8H_{11}NO_2$	189.64 g·mol^{-1}	1.2 g/cm^3	**26×10^{-23} cm^3**
Serotonin	$C_{10}H_{12}N_2O$	212.68 g·mol^{-1}	1.3 g/cm^3	**27×10^{-23} cm^3**

Molar volume is $Vm = M/\rho$

Volume of molecule is $Vmolecule = Vm/NA$

M is the molar mass and ρ is the density

$V_{molecule}$ is the volume of the molecule,

N_A is Avogadro constant (6.02214076×10^{23} mol^{-1}).