

Tailored Emulsion-templated Porous Polymer Scaffolds for iPSC-derived Human Neural

Precursor Cell Culture - SUPPLEMENTARY INFORMATION

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Contents: formulations and reaction conditions used to prepare polyHIPE materials; pore diameter and porosity data; Ellman's assay results; 2D culture of hNPCs; 3D culture isotype controls showing scaffold autofluorescence in the blue channel.

Table S1. HIPE formulations and reaction conditions for the synthesis of polyHIPE materials^a

	TMPTA_80%	HDDA_80%	PEGDA_80%	PEGDA_85%
Aqueous Phase Volume (% v/v)	80	80	80	85
Organic Phase Fraction (% v/v)	20	20	20	15
Molar ratio (thiol:acrylate)	1:1	2:3	2:3	2:3
Concentration of trithiol in organic solvent (M)	1.67	1.67	0.79	0.79
Stirrer Speed (rpm)	350	470	350	350
Additional	5	5	120	120

Stirring Time (min)				

^a Surfactant fraction in organic phase was 3% w/w and photoinitiator fraction in organic phase was 5% w/w

Pore diameter distributions

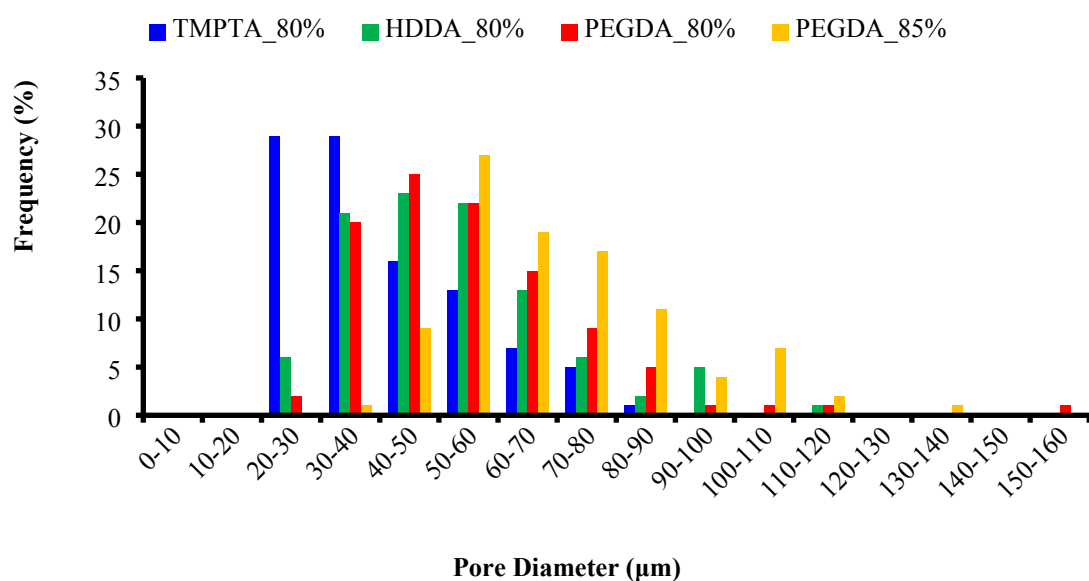


Figure S1. Pore diameter distributions of four polyHIPE materials determined using Image J imaging software (N=100).

Table S2. Average pore diameter and porosity measurements determined by helium pycnometry^a

	Average Pore Diameter (µm) ^b	Porosity (%)
TMPTA_80%	30.3 ± 14.9	77 ± 0.77
HDDA_80%	44.2 ± 23.1	78 ± 0.78
PEGDA_80%	45.3 ± 19.4	81 ± 0.82
PEGDA_85%	63.2 ± 31.5	86 ± 2.0

^a mean \pm standard deviation; ^b determined by analysis of SEM images.

Ellman's Assay Procedure

The polyHIPE samples were crushed under liquid nitrogen into a fine powder. 10-15 mg of powder was immersed in 1 mL of tetrahydrofuran and was then incubated at room temperature for 10 minutes on a shaker. Thereafter, 1 mL of Ellman's reagent solution (5 mmol prepared in methanol) and 5 μ L of ethyldiisopropylamine were then added to each flask and were incubated at room temperature in the dark, shaking for additional 10 minutes. Samples were then diluted to 5 mL with methanol and filtered. Samples were then diluted to a concentration between 5 μ M and 5 mM and added to a 96 well plate in triplicate for each dilution. The absorbance of the test samples was then recorded with a spectrophotometer against an absolute methanol blank at 412 nm.

Table S3. Average residual thiol chemical groups present on polyHIPE materials after photopolymerisation as determined by Ellman's Assay^a

Average Residual Thiol Groups (μ mol/gram)	
TMPTA_80%	27.6 \pm 2.82
HDDA_80%	39.2 \pm 5.02
PEGDA 80%	53.6 \pm 3.44
PEGDA 85%	62.7 \pm 5.84

^a mean \pm standard deviation

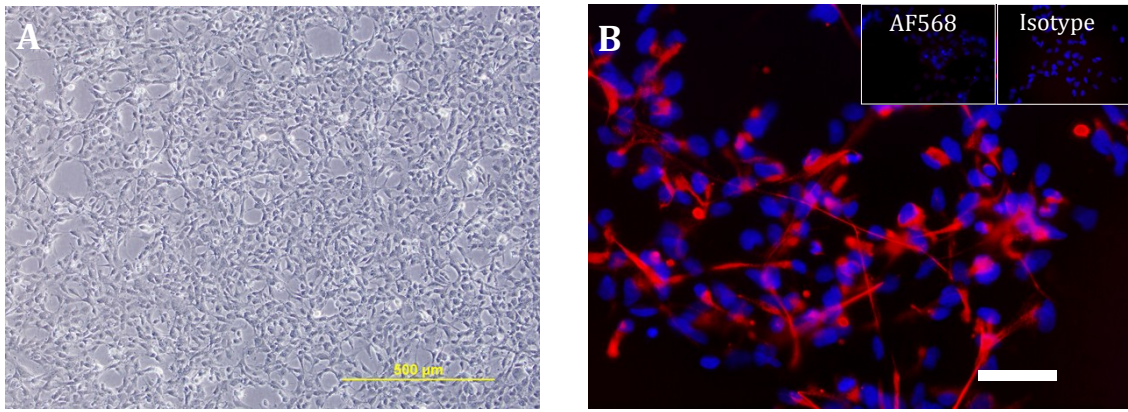


Figure S2. Human HDF51i509 NPCs cultured for 2 days (passage 14) on 2D laminin-coated TCPS, bright field phase contrast image (scale bar 500 μm) (A). Human HDF51i509 NPCs cultured for 4 days (passage 16) on 2D laminin-coated glass slide, showing the detection of vimentin (Alex Fluor AF568, red) following PFA fixation and immunostaining (3CB2 anti-vimentin, 4.4 $\mu\text{g}/\text{ml}$) and DAPI counter staining (blue), compared with goat anti-mouse IgM AF568 and Mouse IgM isotype controls, (Scale bar = 50 μm) (B).

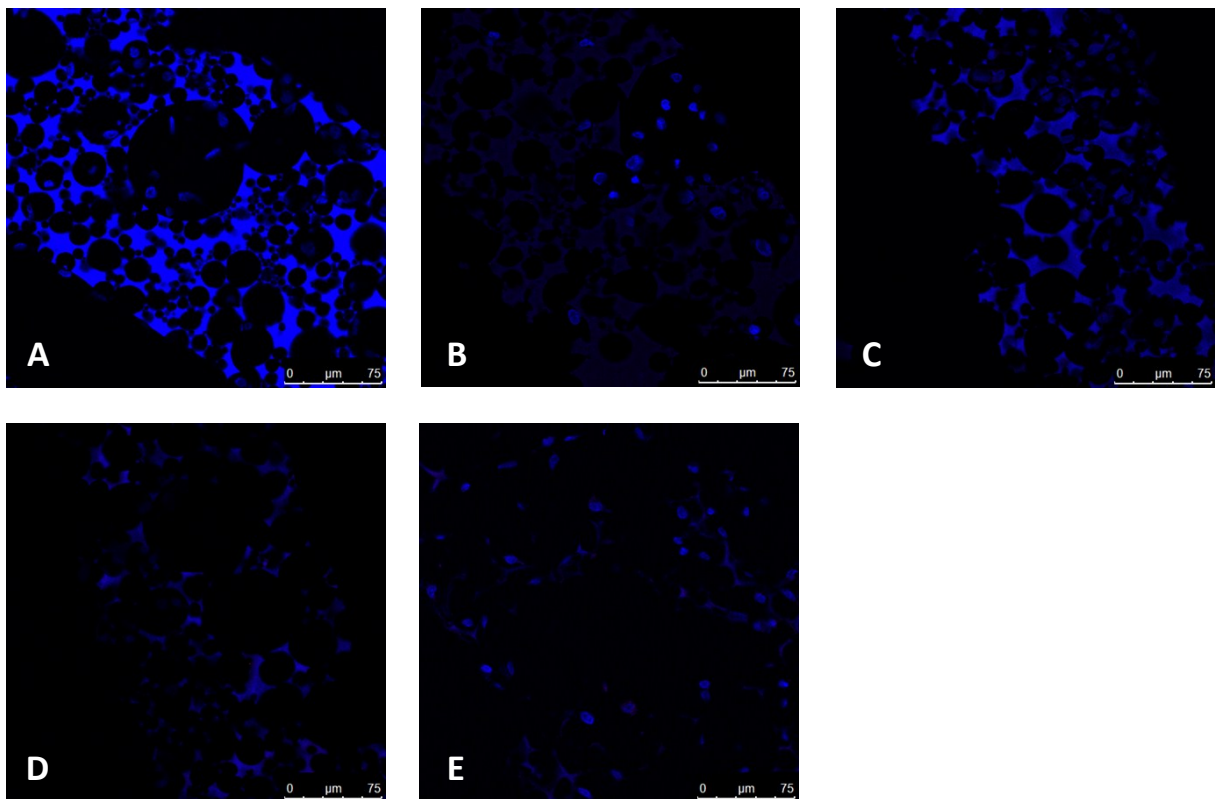


Figure S3. HDF51i509 hNPCs (passage 18) seeded at 1×10^6 cells/scaffold and cultured for 3 days on laminin-coated scaffolds, PFA-fixed. Cells

stained with negative control Mouse IgM isotype and detected with Alexa Flour 568 (red). Cell nuclei are counterstained with DAPI (blue). TMPTA (A), HDDA (B), PEGDA_80% (C), PEGDA_85% (D) and Alvetex control (E). Scaffolds autofluoresce in the blue channel.