Supplementary Information

A microfiber scaffold-based 3D in vitro human neuronal culture model of Alzheimer's disease

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<table>
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<th>Target Gene</th>
<th>Primer sequence (5'-3')</th>
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| **Ki67**    | **F** ACGCCTGGTTACTATCAAAAGG  
|             | **R** CAGACCCATTTACTTGTGTTGGA  |
| **TuJ1**    | **F** CACCCAGCAGATGTTCGATG  
|             | **R** CTTCACGTTGTTGGGATCC  |
| **NeuN**    | **F** GCAGGCTACACGTCTCAAATCC  
|             | **R** ATCGTCCCATTCAGCTTCTCCC  |
| **PSEN1**   | **F** ACAGGTGCTATAAGGTCATCCA  
|             | **R** CAGATCAGGAGTGCAACAGTAAT  |
| **ADAM17**  | **F** GGCAAATGTGAGAAAC  
|             | **R** TGGACAAGAATGCTGAAAGGA  |
| **Actin**   | **F** GCGCAAGTGGTTTTGTCA  
|             | **R** AGATGTGGACAGCAAGCAG  |
Figure S1. Characterization of 3D PLGA microfiber scaffolds. (A) High-resolution XPS spectra of C1s region of PLGA microfiber surface (a) without and (b) with atmospheric air plasma treatment (300 s); (B) Ultimate compressive strengths ($\sigma$) of 3D PLGA microfibrous scaffolds obtained for mechanical testing under dry and wet conditions; Data is expressed as mean ± SD (n = 5), **$p$ < 0.01.
Figure S2. Encapsulation and characterization of iPSC-derived NPC inside 3D PLGA microfiber scaffolds. Confocal fluorescent microscopy images indicating (i-ii) differentiation of iPSC-derived NPCs (8529 cell line) inside 3D scaffold as assessed via staining for TuJ1 (green) and Nestin (red) markers on D13; (iii) cross-section of 3D microfiber scaffold after sectioning (dotted line indicates top surface of 3D scaffold); (iv) cell infiltration and distribution of D13 differentiated NPC inside 3D scaffold without orbital shaking as assessed via staining for Ki67 (green) and DAPI (blue) markers.
**Figure S3. Comparison of glial differentiation between 2D and 3D cultures.** (A) Confocal fluorescent microscopy images indicating glial differentiation in 2D and 3D cultures stained for GFAP (red) marker on D13 and D19 respectively; Nuclei were counterstained with DAPI (blue); (B) Quantification of immunostaining results showing percentage positive staining of glial differentiation markers normalized to DAPI for D13 and D19; Data is expressed as mean ± SD (n = 3), **p < 0.01, ***p < 0.001; Scale bar: 50 µm.
Figure S4. Confocal fluorescent microscopy images indicating pluripotency of iPSCs. Fibroblast-derived iPSCs were immunostained and positive for various iPSC markers such as SOX2, OCT4, Nanog, SSEA4, and TRA-1-60; Nuclei were counterstained with DAPI (blue).
Figure S5. 3D culture elevates expression of specific genes linked to the APP pathway. qPCR analysis of APP-linked pathway genes in D19 FAD-iPSC derived neurons; PSEN1 expression in (A) 2D culture and (B) 3D culture; ADAM17 expression in (C) 2D culture and (D) 3D culture; Data is expressed as mean ± SD (n = 3), *p < 0.05, **p < 0.01, ***p < 0.001.