## **Supporting Information**

## Nanoarchitectonics of a Microsphere-Based Scaffold for Modeling Neurodevelopment and Neurological Disease

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Figure S1. FTIR analysis of the microsphere chemical structure. (A) FTIR spectra of PVA, gelatin, PLGA, microspheres, microspheres coated with HA for 12 h, and microspheres coated with HA for 24 h. The spectra for microspheres coated with HA for 12 h exhibit -OH stretching vibrations (3000 - 3650 cm<sup>-1</sup>), -CH, -CH2, -CH3 stretching vibrations (2850 - 3000 cm<sup>-1</sup>), carbonyl C=O stretching vibrations (1700 - 1750 cm<sup>-1</sup>), a O-hydroxyl band due to intramolecular hydrogen bonding (1663 cm<sup>-1</sup>), -CH deformation vibrations (1300 - 1500 cm<sup>-1</sup>), C-O-C stretching vibrations (1300 - 1200 cm<sup>-1</sup>) and C-O stretching vibrations (1045 cm<sup>-1</sup>). A band shift from 1040 cm<sup>-1</sup> to 1045 cm<sup>-1</sup> occurred due to interaction of HA with C-O. A band at 1624 cm<sup>-1</sup> due to the H—O—H bending vibration of interlayer water in HA was also observed. Microspheres coated with HA for 24 h exhibit -OH stretching vibrations (3000 - 3650 cm<sup>-1</sup>), -CH, -CH2, -CH3 stretching vibrations (2850 - 3000 cm<sup>-1</sup>), carbonyl C=O stretching vibrations (1700 – 1780 cm<sup>-1</sup>), intramolecular hydrogen bonding of O-hydroxyl band (1660 cm<sup>-1</sup>), -CH deformation vibrations (1300 - 1500 cm<sup>-1</sup>), C-O-C stretching vibrations (1300 - 1200 cm<sup>-1</sup>) and C-O stretching vibrations (1048 cm<sup>-1</sup>)<sup>93</sup>. (B, C) Comparison of spectra between uncoated, 12 h HA coated, and 24 h HA coated microspheres. A band shift from 1040 cm<sup>-1</sup> to 1048 cm<sup>-1</sup> due to interaction of HA with C-O was identified. We also observed bands at 1624 cm<sup>-1</sup> in 12 h coated microspheres and 1632 cm<sup>-1</sup> in 24 h coated microspheres resulting from H—O—H bending vibration of interlayer water in HA.



Figure S2. Neural stem cell derivatives exhibit broad distribution within the entirety of the threedimensional microsphere scaffold. (A) d56 microsphere cultures (~200  $\mu$ m in diameter) were paraffin embedded, sectioned, and analyzed by H&E immunohistochemical stain for nuclear identification. (B-D) Representative H&E staining of nuclei at multiple cross-section depths within microsphere cultures. Nuclei were easily identified in cross-sections taken approximately 60  $\mu$ m (B), 110  $\mu$ m (C), and 165  $\mu$ m (D) from the surface of the scaffold. Inset in (D) demonstrates high cellularity within the internal microsphere structure.