Supporting Information

Hydrophilic Cell-derived Extracellular Matrix as A Niche to Promote Adhesion and Differentiation of Neural Progenitor Cells

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Materials and Methods

Culture and identification of mouse embryonic fibroblasts. Mouse embryonic fibroblasts (MEFs) were harvested as described previously with slight modification.[1] Briefly, uteri were isolated from 13.5-day-old C57BL/6 mice and were washed with PBS. The head, arms, legs and visceral tissues were removed from isolated embryos. The remaining bodies were washed in fresh PBS, minced using a pair of scissors, and transferred into 0.25% trypsin with EDTA solution and incubated at 37 °C for 30 min. After trypsinization, DMEM containing 10% FBS was added and pipetted up and down a few times to help with tissue dissociation. Cells were collected by centrifugation (1000 rpm for 5 min at 4 °C) and resuspended in fresh medium. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 with DMEM supplemented with 10% FBS and 100 U/mL penicillin/streptomycin. The MEFs were characterized using immunofluorescence staining against vimentin, which is a protein marker for fibroblasts (Figure S1).

Neural progenitor cell culture and differentiation. NPCs were derived from the forebrains of 13.5-day-old C57BL/6 mice. The forebrain of each embryo was dissected and dissociated into single cells by triturating the tissue with a pipette. Cells were seeded on a 25 cm^2 culture flask at a density of 2×10^5 cells/mL in mouse NeuroCult NSC Proliferation Medium supplemented with epidermal growth factor and basic fibroblast growth factor for neurosphere culture (Figure S2). Cells were incubated at 37 °C in a humidified incubator with 5% CO_2.

For differentiation, in the first 24 h, the medium was supplemented with 1% B-

27 supplement, 100 ng/mL sonic hedgehog, 100 ng/mL fibroblast growth factor-8, and 10 ng/mL bone morphogenetic protein-9 (BMP-9). For the next 48 h, the medium was supplemented with 10 ng/mL BMP-9 and 1% B-27 supplement, after which the cells were never again exposed to BMP-9. From the 4th day to the 19th day, the medium was supplemented with 1% B-27, 2 mM glutamic acid, and 2.66 μM AraC.[2] All neural cells used in this study were between passages 2 to 5.

2. Tables

Table S1. Primers used for real-time polymerase chain reaction analysis.

Gene	Primer	Sequence
Ki67	forward (5’-3’)	CCTGCCTGTTTGGAAGGAGT
	reverse (5’-3’)	ATTGCCTCTTGCTCTTTGACT
Vinculin	forward (5’-3’)	GGAATCCTTTCTGGCACATCTG
	reverse (5’-3’)	ACCTCTGCCACTGTAAGATATTCCA
MAP-2	forward (5’-3’)	AGACCTTCCTCCATCCTCCC
	reverse (5’-3’)	ATTTGTACATTTCCGCCCCCA
p75	forward (5’-3’)	ACAACACCCAGCACCCAGGA
	reverse (5’-3’)	CACAACCACAGCAGCCAAGA
ChAT	forward (5’-3’)	TGTCATCGTGGCCTGCTGCAA
	reverse (5’-3’)	CTCGCTCCTCCCGTCTGACGT
VAChT	forward (5’-3’)	TTGATCGCATGAGCTACGAC
	reverse (5’-3’)	CCACTAGGCTTCCAAAGCTG
β-actin	forward (5’-3’)	TACAGCTTCACCACCACAGC
	reverse (5’-3’)	AAGGAAGGCTGGAAAAGAGC

Table S2. List of the 10 most abundant proteins. (Decreasing from 1 to 10 according to counts of peptides)

No.	Protein	Function
1	Fibrillin-1	Fibrillin is a glycoprotein, which is essential for the formation of elastic fibers found in connective tissue. Fibrillin is secreted into the extracellular matrix by fibroblasts and becomes incorporated into the insoluble microfibrils, which appear to provide a scaffold for deposition of elastin.
2	Plectin	Plectin is a giant protein found in nearly all mammalian cells which acts as a link between the three main components of the cytoskeleton: actin microfilaments, microtubules and intermediate filaments.
3	Collagen alpha-3(VI) chain	Collagen alpha-3(VI) chain is an alpha chain of type VI collagen that aids in microfibril formation.
4	COL6A3 protein	Collagen alpha-3(VI) chain is a protein that is encoded by the COL6A3 gene in humans.
5	Collagen alpha-1(XII) chain	Type XII collagen is a homotrimer found in association with type I collagen, an association that is thought to modify the interactions between collagen I fibrils and the surrounding matrix.
6	Myosin-9	Cellular myosin that appears to play a role in cytokinesis, cell shape, and specialized functions such as secretion and capping.
7	HSPG2	Perlecan (HSPG2) is a HSPG highly expressed in the basal lamina of the developing neuroepithelium. The growth factor binding ability of HSPG2, including to FGFs and SHH, is thought to be the primary mechanism by which HSPG2 regulates NSPC behaviors.
8	Fibronectin	Fibronectin has numerous functions that ensure the normal functioning of vertebrate organisms.It is involved in cell adhesion, growth, migration, and differentiation. Cellular fibronectin is assembled into the extracellular matrix, an insoluble network that separates and supports the organs and tissues of an organism.
9	Filamin-A	Filamin A is a widely expressed protein that regulates reorganization of the actin cytoskeleton by interacting with integrins, transmembrane receptor complexes, and second messengers. Filamin-A-dependent regulation of cytoskeletal dynamics is thought to direct neural progenitor migration and proliferation.
10	TNC variant protein	TN-C is classified as an adhesion-modulating protein, because it has been found to inhibit cellular adhesion to fibronectin. Consistent with the dynamic interplay of factors within the NPCs niche, the TNC-mediated alterations in growth factor responsiveness may be due, in part, to secondary alteration heparan sulfate proteoglycan

3. Figures

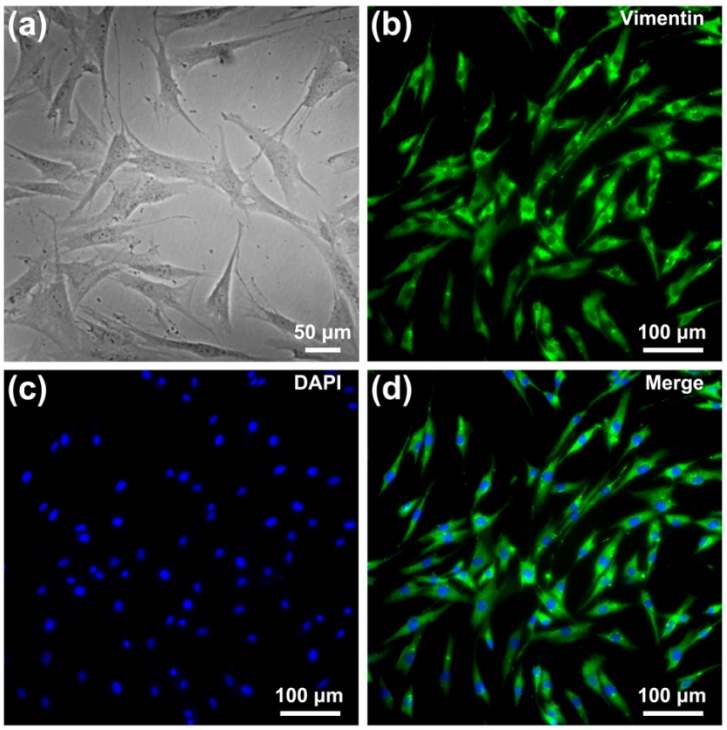

Figure S1. Characterization of mouse embryonic fibroblasts. (a) Morphology of MEFs under phase-contrast microscopy. Immunofluorescence staining image of (b) Vimentin (green), (c) DAPI (blue) and their merge (d) in MEFs.

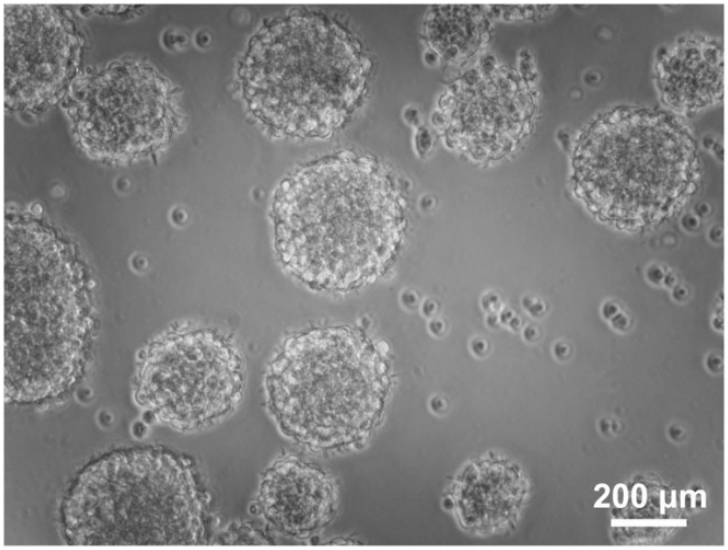

Figure S2. Morphology of NPCs (P2) under phase-contrast microscopy.

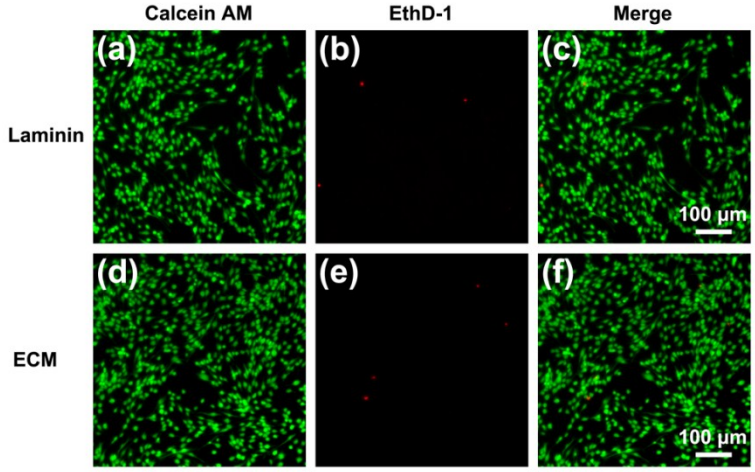

Figure S3. Cell viability of NPCs cultured on MEF-derived ECM and laminin after 7 d, as determined by a live/dead assay, in which live cells are stained green and dead cells are stained red.

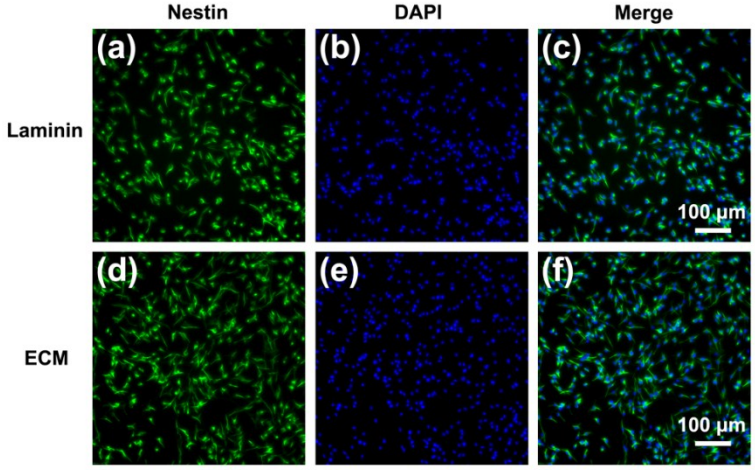

Figure S4. Immunofluorescence images of NPCs cultured on MEF-derived ECM and laminin for 7 d, immunostaining markers were nestin for NPCs (green) and DAPI for nuclei (blue).

References:

1. K Takahashi and S Yamanaka, *Cell*, 2006, **126**, 663-676.
2. C. J. Bissonnette, L. Lyass, B. J. Bhattacharyya, A. Belmadani, R. J. Miller and J. A. Kessler, *Stem Cells*, 2011, **29**, 802-811.