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## Supporting Information

# Controlling differentiation of neural stem cells using extracellular matrix protein patterns**

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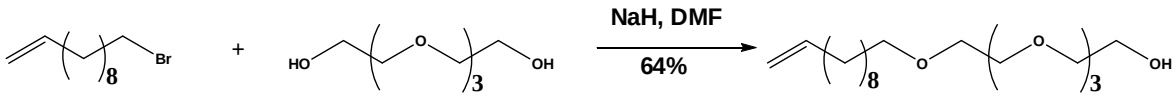
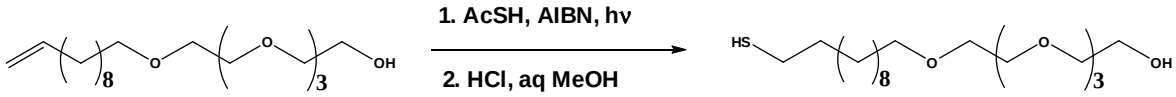
# Table of Contents (Supporting Information)

**Synthesis and Characterization of passivation molecule $EG_4$-$(CH_2)_{11}$-SH**:

Procedure: The procedure was adopted and modified from References [1,2].



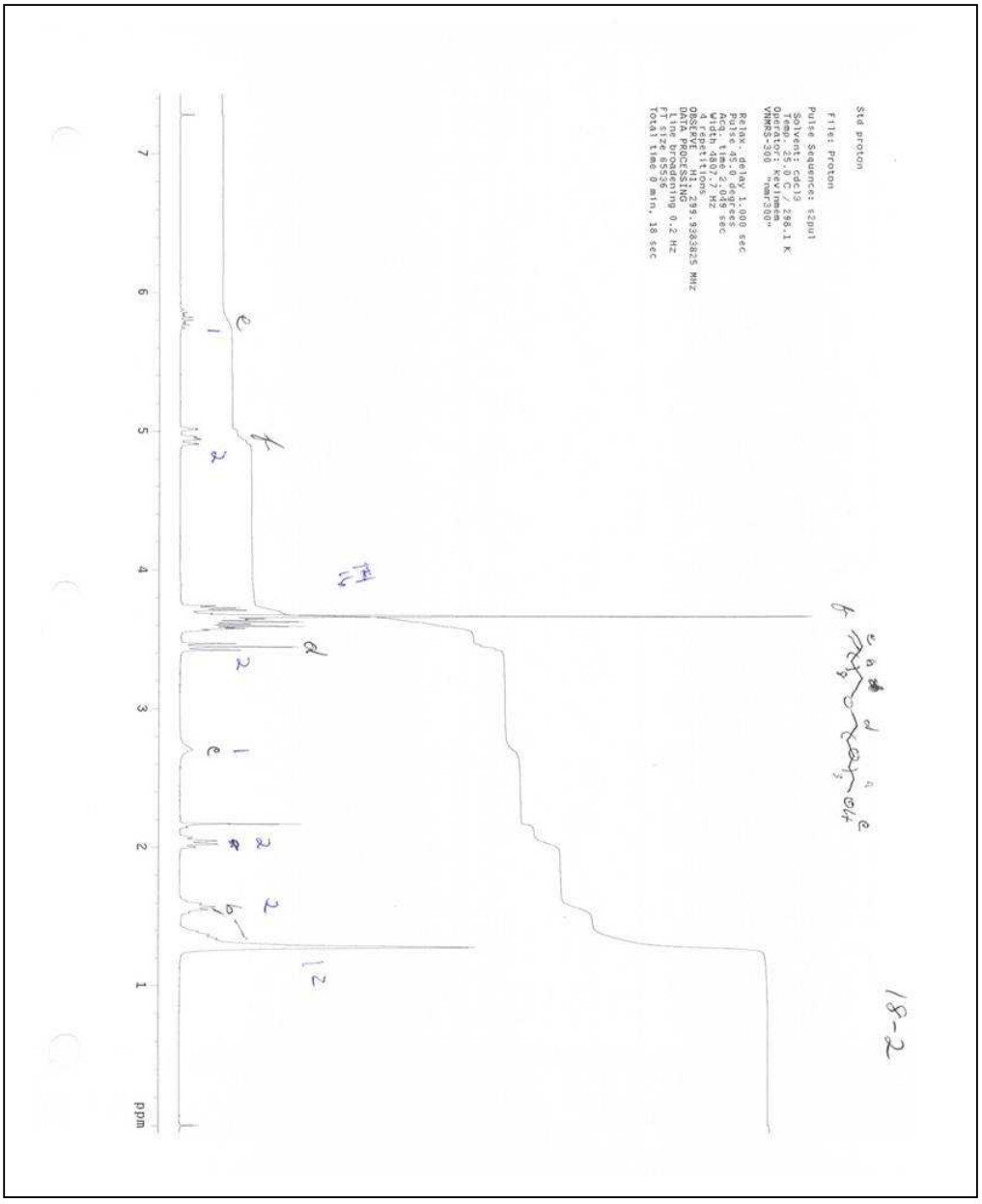

Tetraethyleneglycol (29.7 g, 153 mmoles) was dissolved in 75 ml of dry dimethylformamide under nitrogen. This solution was cooled to 0° C and NaH (1.22 g of 60% in mineral oil, 30.6 mmoles) was added in portions. After stirring at room temperature for one hour, 7.5 g of undecenyl bromide (30.6 mmoles of 95% purity) was added and the reaction was stirred at room temperature overnight. The reaction was then diluted with 75 ml of water and extracted with 4 x 50 ml of hexane. The combined extracts were then washed with 2 x 20 ml of water, 20 ml of saturated brine, dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. The crude product thus obtained was chromatographed on silica gel eluting with 2:1, 1:2 hexane/ethyl acetate and then with 100% ethyl acetate. The product was a light-yellow oil weighing 7.1 g (64%). The proton NMR was consistent with the desired product (see S-4).




The alkene obtained previously (7.1 g, 20.5 mmoles) and thiolacetic acid (6.24 g, 82.0 mmoles) were dissolved in 75 ml of THF and this solution was deoxygenated with nitrogen. After the addition of 50 mg of AIBN, it was irradiated with 254 nm UV light overnight. The solvent was then removed *in vacuo* and the residue was evaporated with 3 x 50 ml of toluene to remove thiolacetic acid. The crude product so obtained was chromatographed on silica gel eluting with 2:1, 1:1, 1:2 hexane/ethyl acetate and finally with 100% ethyl acetate. The intermediate thioacetate product was a colorless liquid weighing 7.2 g. The proton NMR was consistent with the desired product (see S-5).

The intermediate obtained above (3.0 g, 7.10 mmoles) was dissolved in a mixture of 1.5 ml of conc. hydrochloric acid and 30 ml of 95% EtOH which had been deoxygenated with nitrogen and this solution was refluxed under nitrogen overnight. It was then cooled to room temperature and the solvent removed *in vacuo*. The residue was partitioned between 30

ml of saturated $NaHCO_3$ solution and 30 ml of ethyl acetate. The phases were separated and the aqueous phase was extracted with 2 x 30 ml of ethyl acetate. The combined extracts were dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The product was a yellow liquid weighing 2.6 g (96%). The proton NMR was consistent with the desired product (see S-3). MS: $M^+$ 380.9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Solvent: Cdcl3
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Pulse 45.0 degree
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Width 4801.7 Hz
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Line broadening 0.2 Hz
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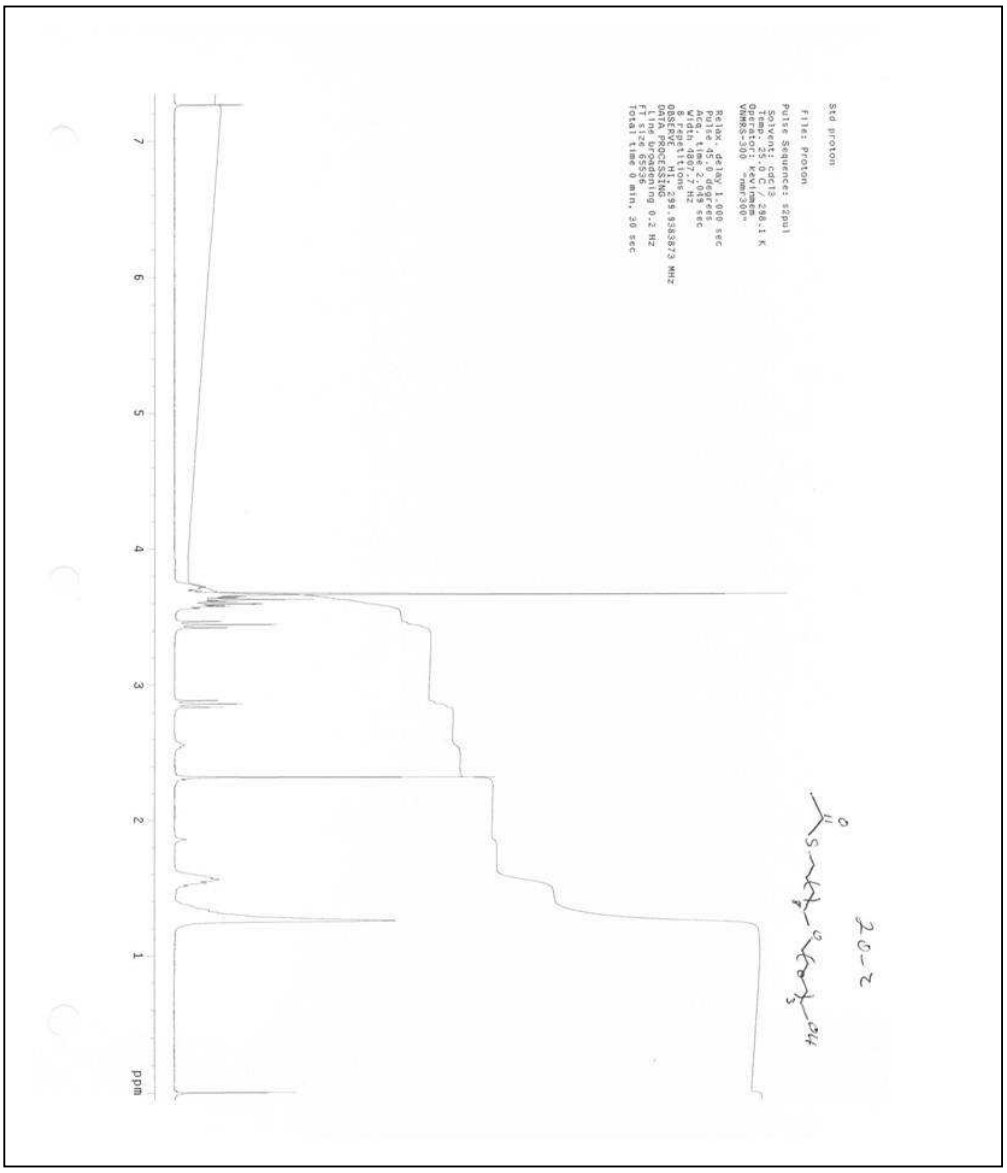
ppm
18-2




**References for the Synthesis and Characterization:**

[1] Langmuir, 2005, 21 (23), 10311-10315

[2] Langmuir, 2007, 23(22), 11164-11167

**Generating ECM protein patterns:**

Polycrystalline Au films were prepared by thermally depositing 5 ~6nm thick Ti layer followed by 10~20nm Au deposition (1.5 cm x 1.5 cm) on cover glass substrates (Fisher No. 1) under a high vacuum condition (base pressure ~$5 \times 10^{-6}$ torr). For micro contact printing, the molded PDMS stamps was prepared by conventional photolithography and 5mM 1-octadecanethiol (ODT) was used as ink molecule. The various patterns were designed using AutoCAD so as to incorporate more multiple geometries having varying dimensions on each stamp. Pattern dimensions and spacings for each of the geometries (stripes, squares, and grids) ranged from 10 µm to 250 µm. After patterning the ODT SAMs on the thin gold films, the background was passivated using $EG_4$-$(CH_2)_{11}$-SH, a protein resistant thiol. The ECM proteins (from Sigma) such as laminin (10 µg/ml), collagen (50 µg/ml), and fibronectin (50 µg/ml) and their combinations were adsorbed on the ODT SAMs by incubating the protein solution of the SAMs for 3 h at room temperature. The protein micropatterns were then rinsed with sterile phosphate buffer saline pH 7.4 (PBS) multiple times and 2 ml suspensions of NSCs were seeded with density of $7.5x10^4$/ml (in basal medium) in a 6-well plate. The samples were incubated for 30 min. at 37°C and each culture well containing the samples was washed gently with the NSC basal medium to remove the NSCs weakly attached on the substrate. It was observed that laminin provided the most optimum microenvironment for the adhesion and growth of the NSCs, hence all the experiments were carried out with laminin as the ECM protein. The laminin micropatterns were confirmed using anti-laminin.

**Rat neural stem cell (NSC) culture and differentiation:**

Rat neural stem cell line (Millipore) were purchased and routinely expanded according to the manufacture's protocol. The NSCs were maintained in laminin (Sigma, 20 µg/ml) coated culture dishes precoated with poly-L-lysine (10 µg/ml) in DMEM/F-12 media (Invitrogen) supplemented with B-27 (Gibco) and containing L-Glutamine (2 mM, Sigma), and antibiotics penicillin and streptomycin (Invitrogen) in the presence of basic fibroblast growth factor (bFGF-2, 20 ng/ml, Millipore). All the cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$. For consistency, the experiments were carried out on the cells between passages 2 and 5. Neural differentiation was initiated by changing the medium to basal medium (without bFGF-2) on the laminin micropatterns. The cells were allowed to differentiate for 6 days with the basal medium in each being exchanged every other day.

**Immunocytochemistry:**

To investigate the extent of neuronal differentiation, at Day 6, the basal medium was removed and the cells fixed for 15 minutes in Formalin solution (Sigma) followed by two PBS washes. Cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes and non- specific binding was blocked with 5% normal goat serum (NGS, Invitrogen) in PBS for 1 hour at room temperature. To study the extent of neuronal differentiation the primary mouse antibody against TuJ1 (1:500, Covance) and primary mouse antibody against Synapsin (1:500, Santa Cruz Biotechnology) was used and for glial differentiation the primary rabbit antibody against GFAP (1:500, Dako) was used. The fixed samples were incubated overnight at 4°C in solutions of primary antibodies in PBS containing 10% NGS. After washing three times with PBS, the samples were incubated for 1 h at room temperature in solution of anti-mouse secondary antibody labelled with cy3 and anti-rabbit secondary antibody labelled with cy2 (1:400, Jackson ImmunoResearch), Hoechst (1:500, Invitrogen) in PBS containing 10% NGS to observe neuronal and glial differentiation. After washing the samples thrice with PBS the substrates were mounted on glass slides using ProLong Gold antifade (Invitrogen) to minimize quenching by gold. To confirm that the NSCs on the micropatterns were undifferentiated at Day 2, the cells were similarly fixed and immunostained with primary and secondary antibodies. The primary rabbit polyclonal antibody against neural stem cell marker, nestin (1:400, Santa Cruz Biotechnology, Inc) was used. The secondary anti-rabbit antibody used was labelled with cy5 (1:400, Jackson ImmunoResearch). Phalloidin (1:75, Invitrogen) labelled with Alexa-546 was added to the secondary antibody solution to observe the actin cytoskeleton and alignment of the NSCs along the patterns. The mounted samples were imaged using Ziess. ImageJ (NIH) was used for comparative analysis of fluorescence signals of TuJ1 on the various pattern geometries of different dimensions, and on control samples (only laminin, no patterns).

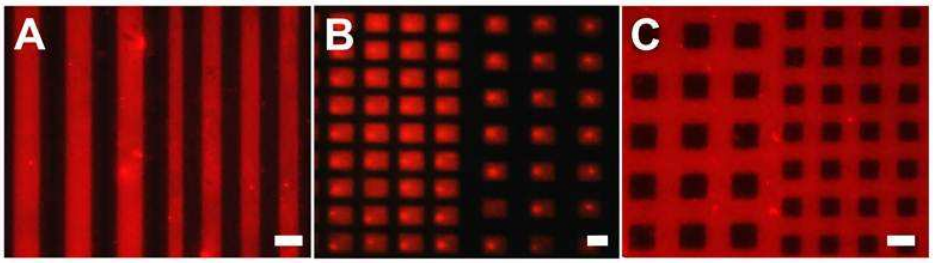



**Figure S1. Immunostaining with anti-laminin IgG**

The laminin patterns generated were confirmed using anti-laminin IgG (Sigma). Consistent results were obtained and reproduced for the three different geometries (A) Stripes, (B) Sqaures, and (C) Grids having varying dimensions. Scale bars: 20 µm

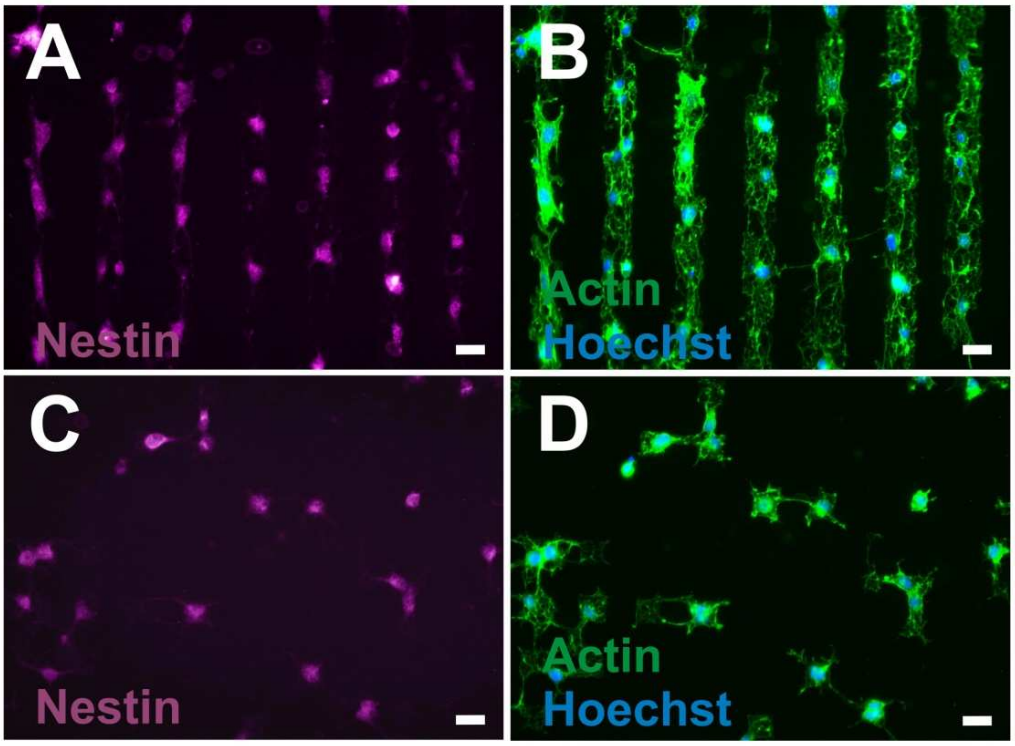



**Figure S2. Actin and nestin staining for NSCs on squares and stripes of laminin**
NSC alignment and differentiation on combinatorial ECM protein arrays. NSCs on stripes (A) and squares (C) of laminin express the neural stem cell marker, nestin (purple) on Day 2 after seeding, thus confirming that the NSCs are undifferentiated. NSCs stained for actin (green) on stripes (B) and squares (D) of laminin on Day 2 after seeding. Scale bars: 20 µm

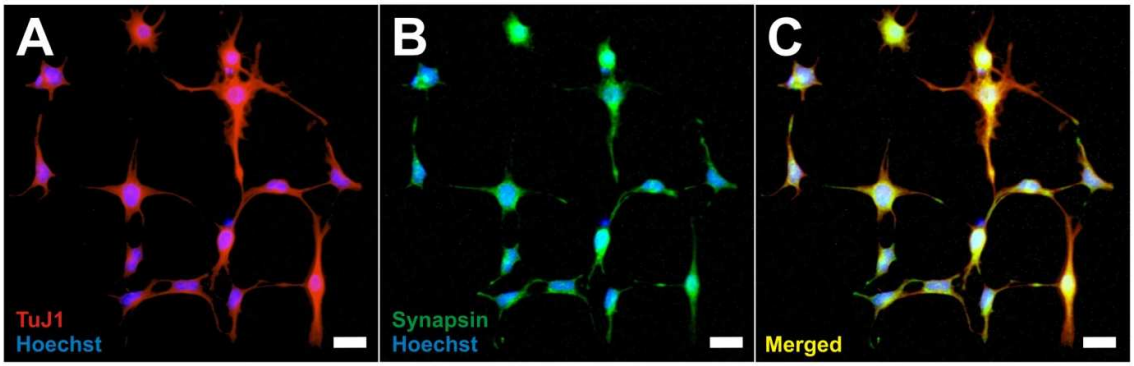



**Figure S3. Colocalization of TuJ1 and synapsin within cells on grid patterns**
(A) NSCs differentiated into neurons, on grid patterns, expressing the neuronal marker TuJ1 (red). (B) The differentiated NSCs also express another neuronal marker, synapsin (pseudocolored green). (C) Merged image showing the overlap of the two neuronal markers TuJ1 and synapsin. The overlapping regions within the neurons are yellow in color. Scale bar: 20 µm

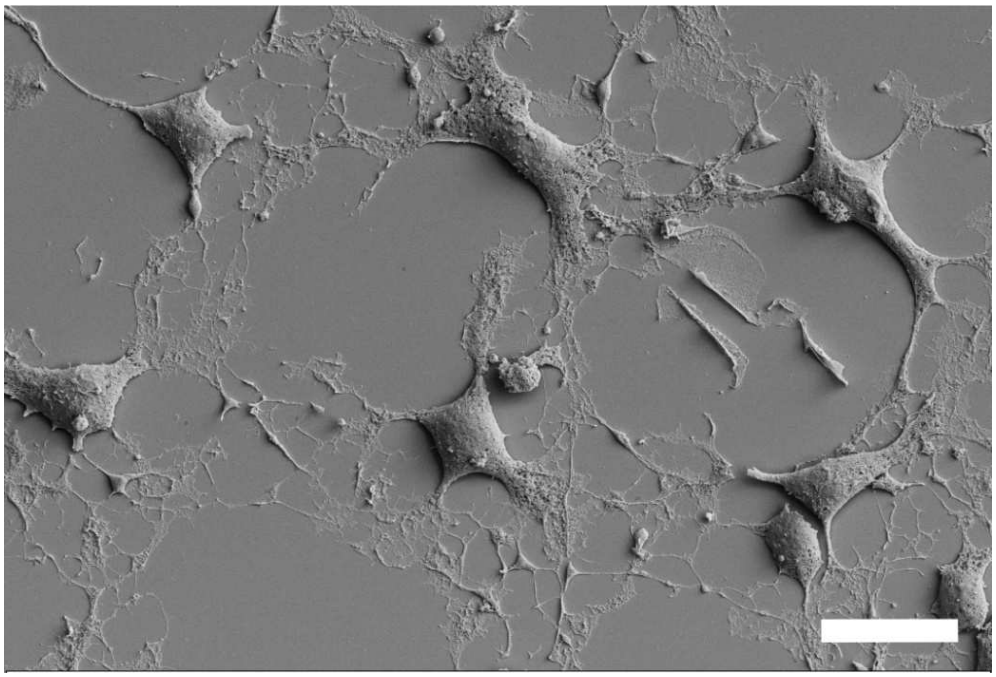


**Figure S4. FESEM image of NSCs on grids of laminin**
SEM image of NSCs on Day 2 after seeding, showing the early alignment and extension of processes on grid patterns of laminin. Scale bar: 20 µm