

# **Direct rapid prototyping of PDMS from a photomask film for micropatterning of biomolecules and cells**

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## Cell culture method

**Human hepatocellular carcinoma cells and fibroblasts.** The human hepatocellular carcinoma cell line (HepG2/C3A, ATCC CRL-10741) and human skin fibroblast (CCD-986sk, ATCC CRL-1947) were selected for cell patterning with PLL-FITC on glass. The cell layers were briefly rinsed with PBS with a pH of 7.4 (Gibco, Grand Island, NY). Then, the trypsin-EDTA solution (0.25% trypsin and 1 mM EDTA·4Na; Gibco) was used to detach the cells at 78%-80% confluent cell layer and the Dulbecco's modified Eagle's medium (DMEM; Gibco) with 10% fetal bovine serum (FBS, Gibco) was supplemented to the dispersed cell layer. The cell cultures were maintained at 37 °C under 5% CO<sub>2</sub> in a humidified water-jacketed incubator. The appropriate aliquots of the cell suspension was refreshed and diluted to suitable concentrations of  $1 \times 10^6$  cells/ml. Before cell spreading on culture dish, the PLL-FITC patterned glass was sterilized with 70% ethanol and cell culture media was changed after 1 h for cell attachment.

**Hippocampal neurons.** Primary hippocampus dissected from E-18 Sprague-Dawley rat embryos was incubated with trypsin 2.5% for 15 min in a 37 °C water bath. After rinsing residual trypsin with Hank's Buffered Salt Solution (HBSS), hippocampus was triturated using fire-polished Pasteur pipette in Neurobasal medium (Invitrogen, CA) supplemented with B27 (Invitrogen), 2 mM L-glutamine (Invitrogen), 12.5 μM glutamate (Sigma-Aldrich) and penicillin-streptomycin (Invitrogen). Dissociated neurons were centrifuged at 1000 rpm for 3 min. Supernatant was removed and pellet was triturated again by Pasteur pipette with 1 ml of fresh medium. Then neurons were seeded on the sterilized substrate at the density of 100 cells/mm<sup>2</sup>. Plated neurons were kept in a humidified incubator with 5% CO<sub>2</sub> and 37 °C for 3 days in vitro.