Supplementary Information

Low-Cost Rapid Prototyping of Flexible Microfluidic Devices using a Desktop Digital Craft Cutter

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In this supplementary information, the protocol for overnight C3A cell culture is provided.

**Overnight C3A cell culture**

Cryopreserved C3A cells, a derivative of HepG2/C3A human hepatoblastoma cell line (CRL-10741™, American Type Culture Collection (ATCC), Manassas, VA, USA) were first thawed and cultured in Eagle’s Minimum Essential Medium (EMEM) (ATCC® No.: 30-2003, ATCC, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Catalog No.: 16000-077, Invitrogen Corporation, Carlsbad, CA, USA) and 1% Penicillin-Streptomycin (Catalog No.: 15140-163, Invitrogen Corporation, Carlsbad, CA, USA). Then, the inside and outside of the microchannel cell culture devices were thoroughly washed with 70% ethanol, rinsed with deionized water and blown dry with air before cell seeding. Next, C3A cells were seeded inside the microchannel cell culture devices using Minimum Essential Medium (MEM) (Catalog No.: 41090-036, Invitrogen Corporation, Carlsbad, CA, USA) and incubated overnight in a CO₂ HEPA incubator (Model: 3130, Forma Scientific, Inc., Marietta, OH, USA) at 37 °C, 95% humidity and 5% CO₂. After overnight incubation, the LIVE/DEAD® Viability/Cytotoxicity Assay Kit for mammalian cells (Molecular Probes, Inc., Eugene, OR, USA) was used to determine viability (live and dead) of the C3A cells inside the microchannel cell culture devices. Finally, fluorescent live and dead staining images were collected using a Zeiss Axiovert 200 inverted fluorescence microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA).