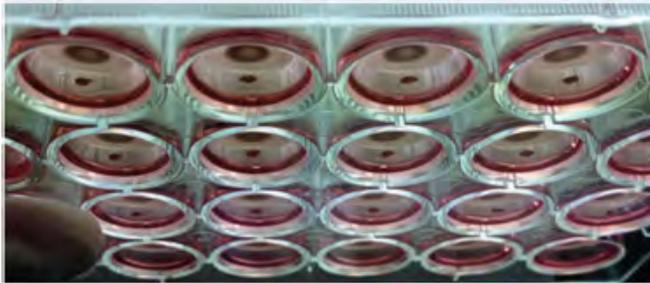


# Three-dimensional cell culturing by magnetic levitation

William L Haisler<sup>1,4</sup>, David M Timm<sup>2,4</sup>, Jacob A Gage<sup>3</sup>, Hubert Tseng<sup>3</sup>, T C Killian<sup>2</sup> & Glauco R Souza<sup>3</sup>

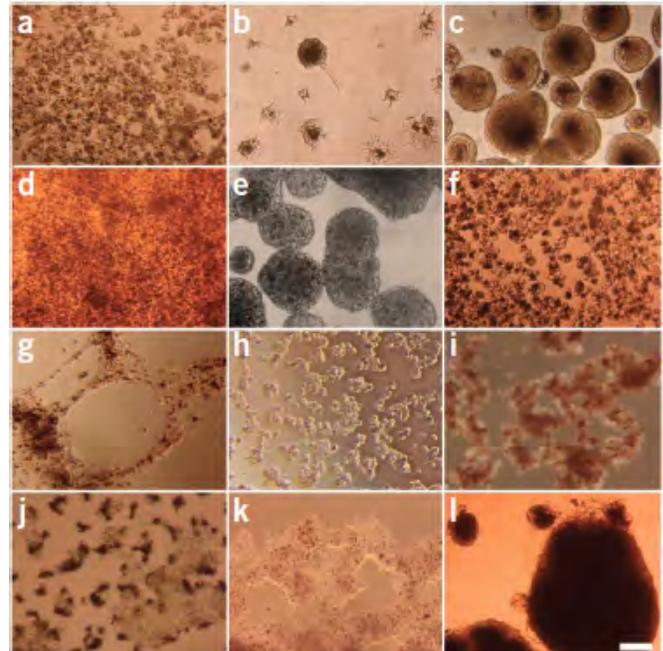
<sup>1</sup>Department of Bioengineering, Rice University, Houston, Texas, USA. <sup>2</sup>Department of Physics, Rice University, Houston, Texas, USA. <sup>3</sup>Nano3D Biosciences, Houston, Texas, USA. <sup>4</sup>These authors contributed equally to this work. Correspondence should be addressed to G.R.S. ([gsouza@n3dbio.com](mailto:gsouza@n3dbio.com)).

**Abstract:** Recently, biomedical research has moved toward cell culture in three dimensions to better recapitulate native cellular environments. This protocol describes one method for 3D culture, the magnetic levitation method (MLM), in which cells bind with a magnetic nanoparticle assembly overnight to render them magnetic. When resuspended in medium, an external magnetic field levitates and concentrates cells at the air-liquid interface, where they aggregate to form larger 3D cultures. The resulting cultures are dense, can synthesize extracellular matrix (ECM) and can be analyzed similarly to the other culture systems using techniques such as immunohistochemical analysis (IHC), western blotting and other biochemical assays. This protocol details the MLM and other associated techniques (cell culture, imaging and IHC) adapted for the MLM. The MLM requires 45 min of working time over 2 d to create 3D cultures that can be cultured in the long term (>7 d).



**Magnetically levitated 3D cultures.** 3D cultures as seen from the bottom of a 24-well plate

**Different cell types magnetically levitated.** Micrographs of 3D magnetically levitated cultures of: (a) human embryonic kidney cells (HEK293); (b) human tracheal smooth muscle cells; (c) human pulmonary fibroblasts; (d) human glioblastoma; (e) rat hepatoma (H-4-II-E); (f) human mammary epithelial cells (MDA-231); (g) human umbilical vein endothelial cells (HUVEC); (h) human mammary epithelial cells (MCF-10A); (i) human prostate epithelial (LNCaP); (j) human hepatocytes (HepG2); (k) human adenocarcinoma alveolar epithelial cells (A549); (l) murine embryonic fibroblasts (3T3). Note the variety in morphology and density between cell types. Scale bar = 100  $\mu$ m.



- **Magnetic levitation can be easily used to rapidly generate 3D cultures of any cell type with relevant ECM**
- **3D magnetically levitated cultures can be experimented on using traditional techniques, such as IHC, Western blotting or other biochemical assays**



**Scan the QR code for the PDF version!**

Nano3D Biosciences • 7000 Fannin St. • Ste. 2140  
Houston, TX 77030 USA • [www.n3dbio.com](http://www.n3dbio.com) • [info@n3dbio.com](mailto:info@n3dbio.com)  
Tel: +1 713 790 1833