Supporting Information

Magnetically Guided Self-assembly and Coding of Three-dimensional Living Architectures

Alessandro Tocchio[1], Naside Gozde Durmus[2,3], Kaushik Sridhar[1], Vigneshwaran Mani[1], Bukre Coskun[4], Rami El Assal[1], Utkan Demirci[1]*

[1]Bio-Acoustic MEMS in Medicine (BAMM) Laboratory, Canary Center at Stanford for Cancer Early Detection, Department of Radiology, Stanford School of Medicine, Palo Alto, California 94304, USA;

[2]Department of Biochemistry, School of Medicine, Stanford University, Stanford, CA 94304;

[3]Stanford Genome Technology Center, Stanford University, Stanford, CA 94304;

[4]Department of Molecular and Cellular Biology, University of California, Davis, CA 95616;

*Corresponding Author's E-mail: utkan@stanford.edu;

Experimental Section

Aggregation time and size study. Magnetic levitation (LEV) and hanging drop (CTR) methods were compared. To this aim, NIH 3T3 cells were assembled with both methods, then extracted and imaged at different time points (2, 4, 8, 24, 48 and 72 hours). 1500 cells, 10 µl of total volume and 50mM Gd^{3+} were used for 2, 4 and 8 hours experiments. 1500 cells, 30 µl of total volume and 50mM Gd^{3+} were used for 24, 48 and 72 hours experiments.

Effects of Gd^{3+} concentration on cell viability and metabolism in 2D. NIH 3T3 cells were seeded in 48-well plate (25000 cells/well) and exposed to different concentrations of Gd^{3+} (0, 25, 50, 75, 100mM) over 3 days. Viability was assessed every 24 hours with live/dead assay (calcein/ethidium homodimer-1). In a similar experiment, NIH 3T3 cells were exposed to different concentration of Gd^{3+} (0, 25, 50, 75, 100mM) for 3 days and their metabolism was measured every 24 hours with PrestoBlue assay (Thermo Fisher Scientific). In the recovery experiment, NIH 3T3 cells were exposed to different concentrations of Gd^{3+} (0, 25, 50, 75, 100mM) for 48 hours. Cells were then recovered, counted, seeded and cultured again for 3 days in the absence of Gd^{3+}. Cell metabolism was measured every 24 hours with PrestoBlue assay (Thermo Fisher Scientific). Changes in the fluorescence of the samples was measured by using TECAN multi plate reader with the excitation/emission wavelengths set at 560/590 nm.

Gd^{3+} concentration and levitation time effect on cell viability in 3D. In one experiment, NIH 3T3 cells were exposed for different times (24, 48 and 72 hours) to different concentration of Gd^{3+} (0 and 50 mM) during magnetic levitation (LEV) and hanging drop (CTR) assembly. In a second experiment, cells were exposed for 48 hours to different concentration of Gd^{3+} (0, 25, 50 and 100mM) during LEV and CTR assembly. Cell viability was assessed with live/dead assay (calcein/ethidium homodimer-1). Individual greyscale images were analyzed using ImageJ Fiji software. The area of the spheroid was selected in the red (dead) and green (live) channels and analyzed for mean gray value. A cell-free area was selected in the red and green channels to be subtracted from mean gray values of the spheroid areas. Percent viability was calculated using this formula: (Mean spheroid green – mean background green) / [(Mean spheroid green – mean background green) + (mean spheroid red - mean background red)]*100.

Method	Advantages	Disadvantages
Non-adherent surfaces	- Simple to perform. - Fast formation. - Low size variability.	- Relatively expensive. - Time-consuming. - Labor-intensive. - Surface effect. - Limited control on cell organization.
Hanging Drop	- Relatively inexpensive. - Simple to perform. - Fast formation .	- Difficult to scale-up. - Instability of the liquid-air interface. - Variation in size/cell number. - Time-consuming. - Labor-intensive. - Difficult imaging and long-term culture. - Limited control on cell organization.
Spinner Flasks	- Simple to perform. - Ease to scale-up. - Long-term culture.	- Specialized equipment is required. - Variation in size/cell number. - Difficult imaging. - High shear stress. - Minimal control on cell organization.
NASA Rotary System	- Simple to perform. - Ease to scale-up. - Provide long-term culture.	- Specialized equipment is required. - Expensive. - Difficult imaging. - Variation in size/cell number. - Minimal control on cell organization.
Magnetic particle Assembly	- Fast formation. - Reconfigurable method. - Control on cell organization.	- Required magnets and magnetic particles. - Relatively expensive. - Concerns about nanoparticle toxicity
Microwell	- Simple to perform. - Ease to scale-up. - Low size variability. - Long-term culture.	- Relatively expensive. - Surface effect. - Minimal control on cell organization.

Table 1. Comparison of cell assembly techniques.

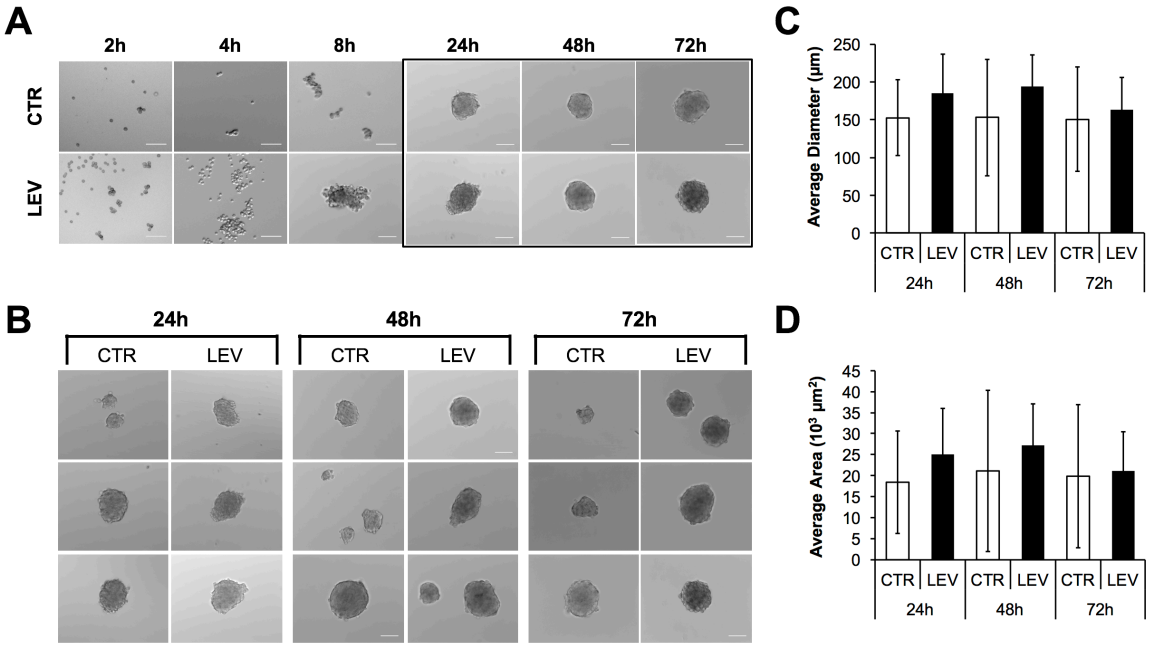

Figure 1. Aggregation time and size study. Magnetic levitation (LEV) and hanging drop (CTR) methods were compared. Brightfield images are presented (**A**). Additional representative images of 24, 48 and 72 hours time points are presented (**B**). Scale bar, 100 µm. Average diameter (**C**) and area (**D**) of assembled cells with CTR compared with LEV (1500 cells, 50mM Gd^{3+}). The data are presented as a mean ± standard deviation (SD) (n>6).

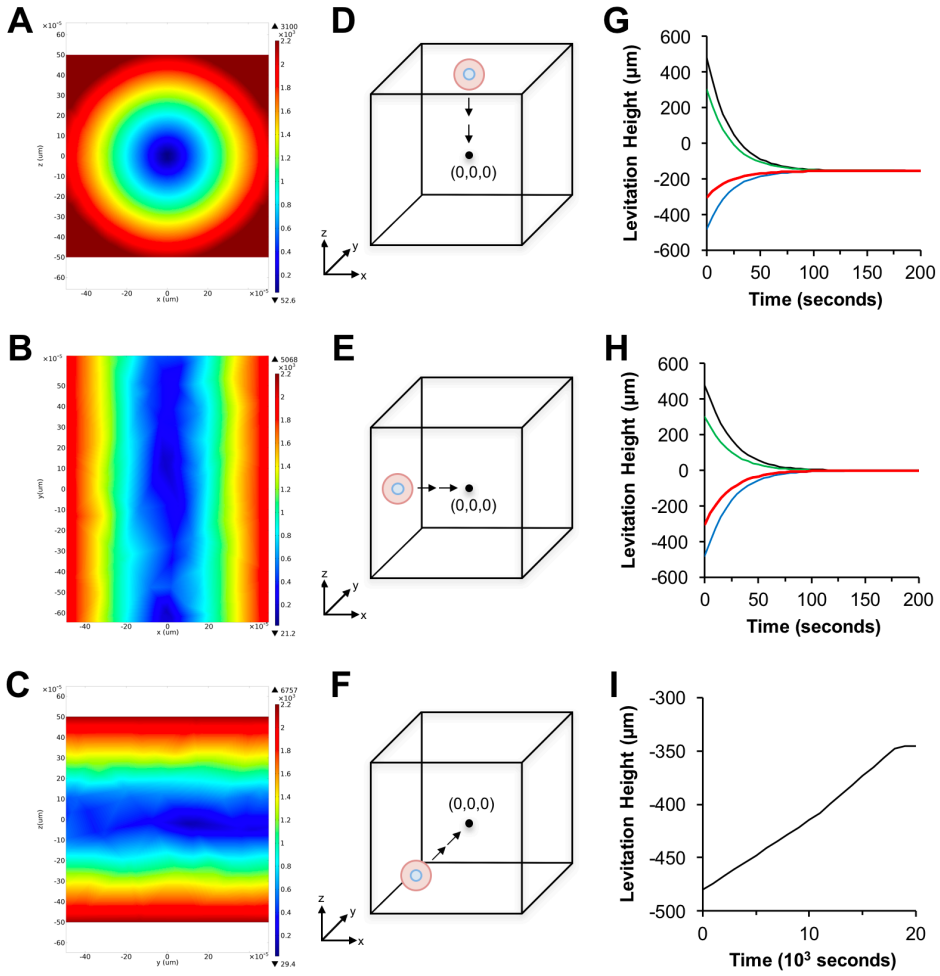

Figure 2. 3-D simulation model of the magnetic field distribution, presented using colors. x-z section at y=0 (**A**), x-y section at z=0 (**B**) and y-z section at x=0 (**C**) are presented. The lowest value point of the magnetic field is at the centroid of the channel and the magnet field is symmetric with the channel center at x-z (**A**) x-y (**B**), and y-z (**C**) sections. Schematic of the unit cube around the capillary center (0.5x0.5x0.5mm) and cell movement for different spatial orientation and release points in z (**D**), x (**E**) and y (**F**). Simulation of the time required for equilibrium for a cell-like bead (20µm diameter, density 1.06 g/cc, 50mM Gd^{3+}) for different release points and spatial orientations in z (**G**), x (**H**) and y (**I**). The simulation was performed using Comsol Multiphysics™.

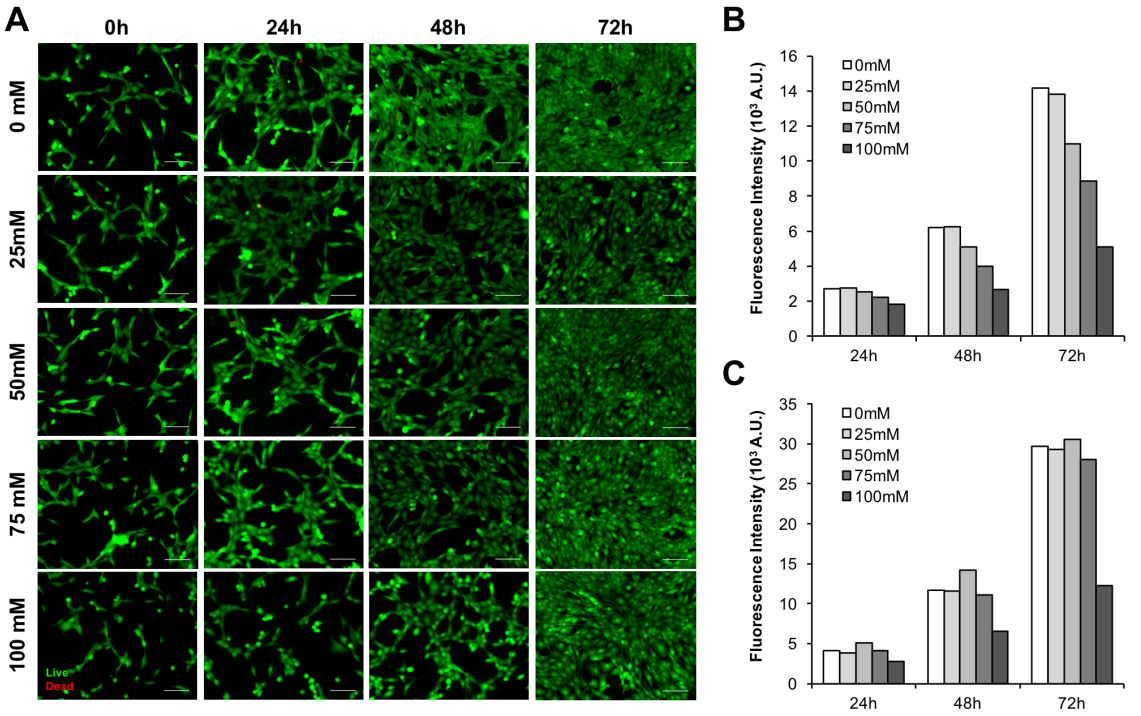

Figure 3. Effects of Gd^{3+} concentration on cell viability and metabolism. NIH-3T3 cell viability was evaluated at different Gd^{3+} concentrations (0, 25, 50, 75, 100mM) and different time points (0, 24, 48, 72 hours) with live/dead assay (green: live cell, red: dead cell). Fluorescent images are presented (**A**). Scale bar, 100 µm. Cell metabolism measured at different Gd^{3+} concentrations (0, 25, 50, 75, 100mM) and at different time points (24, 48, 72 hours) (n=1) (**B**). Metabolism of recovered cells previously exposed to Gd^{3+} (48 hours and 0, 25, 50, 75, 100mM) was evaluated after re-seeding and culturing in the absence of Gd^{3+} for 3 days (n=1) (**C**).

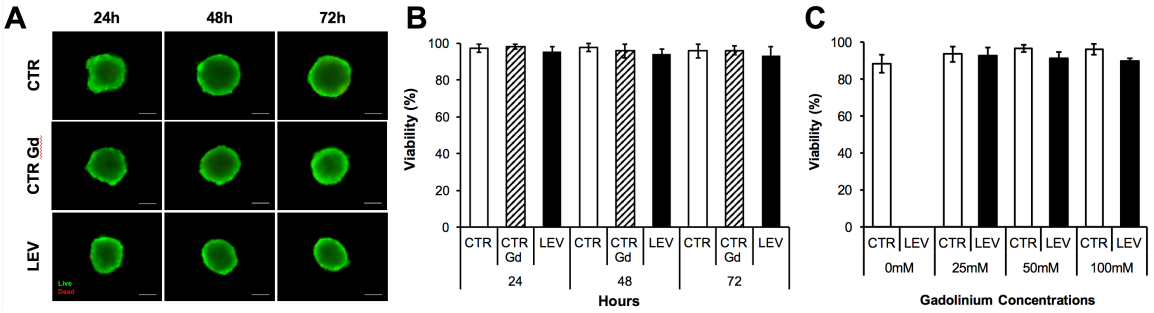

Figure 4. Effects of levitation time and Gd^{3+} concentration on cell viability. NIH 3T3 cell viability was evaluated during magnetic levitation (LEV) and hanging drop (CTR) assembly at different Gd^{3+} concentrations (0 and 50 mM) and at different time points (24, 48 and 72 hours). Live/dead fluorescent images are presented (green: live cell, red: dead cell) (**A**). Scale bar, 100 µm. Viability was quantified as described in the experimental section. Percent viability is presented. (**B**). NIH 3T3 cell viability was evaluated during magnetic levitation (LEV) and hanging drop (CTR) assembly (48 hours) at different Gd^{3+} concentrations (0, 25, 50 and 100mM mM). Percent viability is presented (**C**). The data are presented as a mean ± standard deviation (SD) (n>6).