Biofabrication of Cellular Structures Using Weightlessness as a Biotechnological Tool

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***Abstract*— Gravity is an important biomechanical signal effecting the morphology and function of organisms. Reduction of gravitational forces, as experienced during spaceflight, cause alterations in the biological systems. Magnetic levitation technique is one of the most recent ground-based technology to mimic weightlessness environment. In addition to providing a platform to investigate biological effects of the weightlessness, this platform presents a novel opportunity to biofabricate 3-dimensional (3D) structures in a scaffold- and nozzle- free fashion. In this study, various controllable self-assembled 3D living structures were fabricated via magnetic levitation technique. This strategy may offer an easy and cost-effective opportunity for a wide range of space biotechnology researches.**

***Keywords*— Magnetic levitation, simulated weightlessness, stem cells, space biotechnology, gravitational biology.**

I. Introduction

Gravitational force is a physical stimulus that play role in regulation of biological structure and functions [1, 2]. Reduction or cessation of gravity, such as experienced during space flights causes critical alterations in living systems [3, 4]. Although space experiments provide good opportunities to understand the biological effects of the weightlessness environment, practical and cost-effective ground-based techniques are required to accelerate the accumulation of knowledge in space biology. Magnetic levitation technique [5, 6] is one of the most recent ground based technology to mimic weightlessness [7] with eliminating external shear forces created on cells by other *in vitro* ground-based techniques such as rotating wall vessel and random positioning machine [8, 9]. In principle, cells, most of which are diamagnetic objects, are pushed away from the high magnetic field region to the low magnetic field region in the paramagnetic medium, whereby a magnetic force equal to gravitational force is generated on the cell in a label-free manner and the actual weightlessness condition is simulated. Magnetic levitation technique was firstly applied to living cells for cell separation and single-cell density measurement [10-13]. The technique has a serious potential to provide an important opportunity to study the effects of the weightlessness environment with its use for cell culture.

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II. Methods

A. Magnetic Levitation Platform

Magnetic levitation platform consists of two high grade (N52) neodymium magnet (l: 50 mm, w: 2 mm, h: 5 mm, Supermagnete) positioned at 1,5 mm distance with same poles facing each other, a capillary channel (1 mm × 1 mm cross-section, l: 50-mm, Vitrocom) between two magnets and side mirrors (12,7 × 12,7 × 3,2 mm, Thorlabs) at 45° for real-time observation under microscope (Fig. 1). The components of the magnetic levitation platform are assembled using holders printed by 3D printer (Formlabs Form 2).

Cells suspended in a gadolinium (Gd, Gadavist)-containing paramagnetic medium are migrated from a region of high magnetic field strength to low magnetic field strength. This movement, that is caused by the magnetic susceptibility difference between cells and the surrounding medium (x_{cell}-x_{medium}), ends when magnetic force (F_{mag}, Eq. 1) is equal to gravitational force (F_g, Eq. 2). In the magnetic field gradient created between the magnets, the point where the F_{mag} and F_g forces are equal is the equilibrium position of the cells for levitation, at which point the cells are in the weightlessness environment.

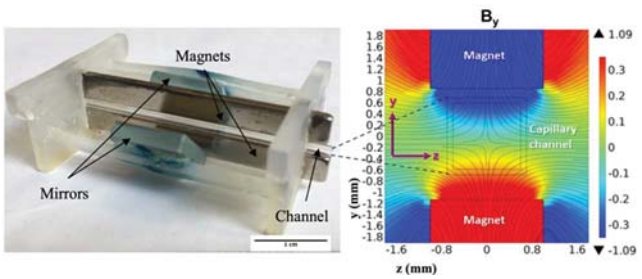

Fig. 1 Photograph of magnetic levitation platform and magnetic induction (y component, B_y) between two opposing magnets via Finite Element Methodology. Streamlines show total magnetic induction (B_y+B_z). Scale bar: 1 cm.

$$\overrightarrow{F_{mag}} = \frac{V.\,(x_{cell} - x_{medium})}{\mu_0}(\vec{B}.\nabla)\vec{B} \quad (1)$$

$$\overrightarrow{F_g} = V\Delta\rho g \quad (2)$$

Here, V is the volume of cell, μ_o is the magnetic permeability of free space (1.2566×10^{-6} kg·m·A^{-2}·s^{-2}), B is the magnetic flux density, $\Delta\rho$ is the density difference between cell and the surrounding medium and g is the vector of gravity.

B. Cell culture

D1 ORL UVA [14], D1 ORL UVA^{eGFP} (bone marrow stem cell line) and MDA-MB-231^{dsRed} (breast cancer cell line) cells were cultured in DMEM (Gibco), 7F2 (mouse osteoblasts) cells were cultured in alpha modified essential medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All cells were grown in a humidified 37 °C incubator with 5% CO_2. The growth medium was refreshed every 2-3 days and the cells were passaged every 4-6 days. Adipogenesis of 7F2 cells was induced by the induction medium containing 10 nM dexamethasone, 50 mM indomethacin, and 5×10^{-3} mg/mL insulin for 6 days.

C. Assembly and Culture of 3D cellular structures under weightlessness

Cells were harvested by trypsinization and suspended at final concentrations of 10^5 cells/mL (5,000 cells per capillary), 10^6 cells/mL (50,000 cells per capillary) and 10^7 cells/mL (500,000 cells per capillary) in 100 mM Gd-containing culture medium. Approximately 50 µL cell suspension were loaded into the capillary channel and the channel was sealed. The cells were cultured under weightlessness condition by magnetic levitation for 48 h at 37 °C and 5% CO_2 in a humidified atmosphere and visualized under the inverted microscope (Olympus IX-83).

In order to test different cell seeding strategies in magnetic levitation culture, either two cell type were mixed together and the 3D structure formed after 24 hours of levitation was visualized, or the first cell type was levitated for 24 hours to allow formation of 3D structure and the second cell type was added to the culture, after formation of the biphasic 3D structure, cells were visualized.

D. Live/Dead Assay

The viability of D1 ORL UVA cells exposed to 100 mM Gd for 5 days in 2D culture and 3D magnetic levitation cultures (5000 cells/capillary channel) were assessed by live/dead assay (calcein-AM/propidium iodide, Sigma Aldrich). After a 15-min staining, cells were visualized under the fluorescence microscope (Olympus IX-83).

III. Results and Discussion

In order to assess cellular assembly of living cells under weightlessness environment with different cell seeding densities, D1 ORL UVA cells were seeded at densities of 5000, 50000 and 500000 cells/capillary and levitated in 100 mM Gd-containing medium for 48 hours (Fig. 2). In all groups, cells were assembled under weightlessness condition, after 24 hours formed a stable structure and at the 48th hour of the culture, the 3D shape and levitation of the structures were maintained. The increase in the number of cells cause an increase in length and thickness of the cellular structures. The average length of the resulting structures after a 24-hour levitation was approximately 0.3, 2.8 and 15 mm, for cultures with cell seeding density of 5000, 50000 and 500000 cells/capillary, respectively. The average thickness of these constructs was 0.10, 0.17 and 0.28 mm, respectively. Observed size increase occurred in length direction rather than in thickness, due to the tendency of the cells to stay in the low magnetic field region. This result shows that shape of the structure can be controlled by culturing cells within the different magnetic field gradients.

Self-assembly of different cell types during weightlessness was firstly investigated using D1 ORL UVA^{eGFP} cells, that are able to form tightly packed aggregates and MDA-MB-231^{dsRed} cells that form only loose aggregates. Biofabrication of co-culture assemblies under weightlessness was performed using different cell loading strategies; by (1) simultaneously loading of both of cells, (2) seeding MDA-MB-231^{dsRed} cells onto D1 ORL UVA^{eGFP} self-assembled cluster and (3) seeding D1 ORL UVA^{eGFP} cells onto MDA-MB-231^{dsRed} self-assembled clusters (Fig. 3). Simultaneous loading of cells resulted in clusters with random positioning of cells. Second loading strategy showed that loose MDA-MB-231^{dsRed} cell layer started to cover the tightly packaged D1 ORL UVA^{eGFP} clusters. Third loading strategy resulted in integration of D1 ORL UVA^{eGFP} cells into gaps within loose MDA-MB-231^{dsRed} clusters.

Following this experiment, adipogenic differentiated 7F2 cells and quiescent D1 ORL UVA stem cells, which are

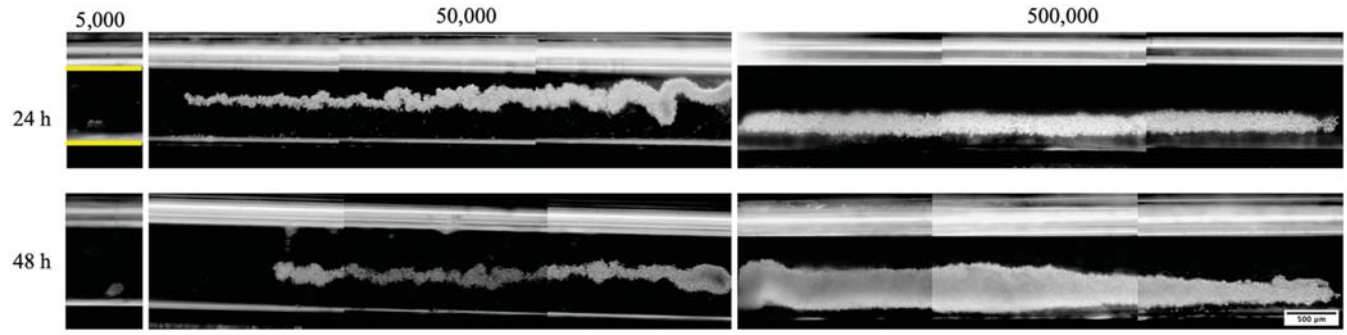

Fig. 2 3D cellular organization of D1 ORL UVA cells under weightlessness environment after 24 and 48 h of magnetic levitational assembly (100 mM Gd) with different cell seeding densities (total 5000, 50000 and 500000 cells per capillary). Yellow lines in the first picture represent upper and lower levels of the inside of the capillary. Scale bar: 500 µm.

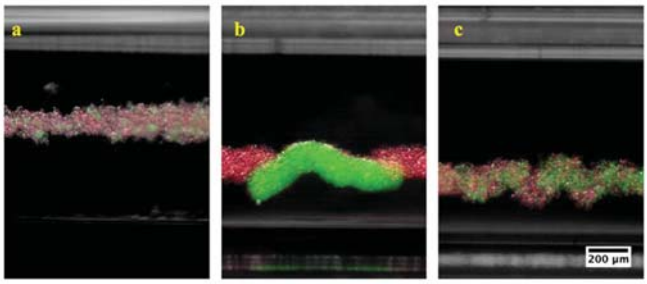

Fig. 3 Self-assembled D1 ORL UVA^{eGFP} and MDA-MB-231^{dsRed} coculture clusters formed with weightlessness condition by magnetic levitation (100 mM Gd, 50,000 total cells/capillary) and different cell loading strategies; (a) simultaneously loading of MDA-MB-231^{dsRed} and D1 ORL UVA^{eGFP} cells, (b) MDA-MB-231^{dsRed} cells onto D1 ORL UVA^{eGFP} clusters formed with magnetic levitation and (c) D1 ORL UVA^{eGFP} cells onto MDA-MB-231^{dsRed} clusters formed with magnetic levitation. Scale bar: 200 µm.

known have distinct single-cell density [10], were co-cultured with magnetic levitation. In this experiment, adipogenic cells, which were expected to be positioned higher level due to their lipid accumulation and hence low cell density, were taken into magnetic levitation culture and after 24 hours, D1 ORL UVA stem cells were added to the culture (Fig. 4). After 3 hours, stem cells formed a layer under the adipogenic cell cluster. This layered co-culture model supports that the weightlessness environment may allow for the biofabrication of different complex living models and for *in situ* examination the effect of the weightlessness environment on these models. In addition, to evaluate the effect of this method on the viability of the cells, both cells were exposed to the paramagnetic medium in 2D culture and with magnetic levitation for 5 days (Fig. 5). For both of the cultures, it was observed that most of the cells were alive. This suggests that both the chemical and physical environment in this method can be used to biofabricate living 3D models without causing any adverse effect on cellular viability.

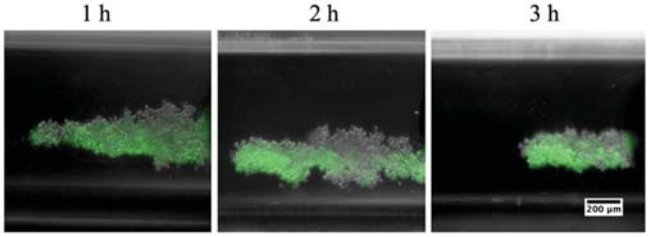

Fig. 4 Assembly of D1 ORL UVA^{eGFP} cells onto adipogenic 7F2 cells cultured for 24 hours with magnetic levitation (100 mM Gd, 5,000 total cells/capillary). Scale bar: 200 µm.

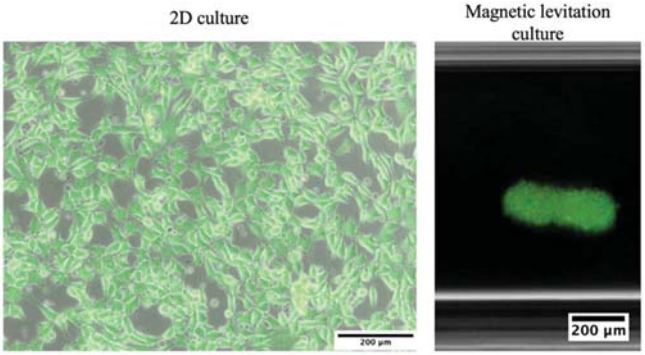

Fig. 5 Live-dead analysis of D1 ORL UVA cells cultured with 100 mM Gd-containing medium in 2D culture or magnetic levitation culture for 5 days. Scale bar: 200 µm.

IV. Conclusions

Reduction in gravitational forces on cells, as in spaceflights, cause a set of morphological and physiological stresses. Weightlessness condition lead to serious biological changes in all living organisms, including humans, such as bone loss. Due to infrequency and cost constraints of the spaceflight experiments, ground-based technologies are gaining importance. Simulated weightlessness via magnetic levitation is a promising technique for space biology researches.

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