Magnetic levitation-based adipose tissue engineering using horizontal magnet deployment

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***Abstract*— Magnetic levitation is a promising technique for tissue engineering with contact- and label-free approach. Levitation-based biofabrication systems emerge as a simple, rapid and versatile alternative to traditional tissue culture systems, since biofabrication specs can easily be tailored via magnet shape and configuration. This study aims at possible magnetic levitation systems for culture of adipose tissue cells. In this study, we performed two different magnet configurations, vertical and horizontal deployment, in an effort to be utilized in adipose tissue engineering.**

Keywords—adipose tissue, magnetic levitation, adipocytes, stem cells

I. INTRODUCTION

Diamagnetic levitation, which is a technique working with the principle of removing of diamagnetic objects to lowest magnetic flux density produced by magnets [1], has been used for various biological applications such as density-based analysis, cell manipulation and 3D biofabrication [2-7]. One of the first applications of magnetic levitation system is detection of the cell density which is an indicator of cell state due to density changes occurred during some cellular processes such as differentiation, disease state, cell cycle and cell death [8]. With high resolution of the system, small differences in cell densities were successfully detected between healthy and sickled red blood cells [9, 10], resistant and nonresistant bacteria and yeast strains [8], and most recently mesenchymal stem cells that remain dormant, commit to osteogenesis or commit to adipogenesis [11]. Later on, the system was considered as an emerging technology for tissue engineering studies with its advantage of manner of bioprinting through label-free and contact-free approach. It was shown that it was possible to form various structures such as spheroid, line and star-shaped with different magnet configurations and shapes [12, 13]. In recent studies, magnetic levitation system was used on 3D culture of various cell types such as cancer cells, stem cells and fibroblasts having similar density in cell groups [3, 14-17].

Adipocytes are the main components of adipose tissue. Since they include lipid droplets in the cytoplasm, adipocytes have lower density compared to other cell types and they exhibit heterogeneity in terms of density related to the lipid content. In our previous study, it was demonstrated that adipocytes can be detected by density-based magnetic levitation system [11]. In this study, it is aimed at adipose tissue biofabrication by using the magnetic levitation principle enabling self-assembly of the cells in lower magnetic field. In light of this, the study was conducted by two configurations of the system for 3D cellular culture of adipocytes and mesenchymal stem cells.

II. METHODS

A. Design of Magnetic Levitation Platform

Magnetic levitation device was designed with two different configurations. In the vertical magnetic levitation system, two vertical N52-grade neodymium magnets (NdFeB, Supermagnete) were positioned with the same poles facing each other and a capillary channel (Vitrocom) was inserted between the magnets to culture cells in a paramagnetic medium (Gadavist®, Bayer). Two mirrors were placed at 45˚ for visualization of the levitated and cultured cells. Secondly, horizontal magnetic levitation system was consisted of two

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magnets positioned with the like poles facing each other on the horizontal axis. Unlike the vertical device, the platform enables to image cellular structures without any reflective mirrors. The parts of both magnetic levitation platforms were assembled by holders printed by 3D printing system (Formlabs Form 2).

B. Cell Culture

D1 ORL UVA (mesenchymal stem cells) and 7F2 (mouse osteoblast) cells were used in this study (Fig. 1). D1 ORL UVA cells were cultured in Dulbecco's Modified Eagle's medium (DMEM high glucose, Gibco) containing 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (P/S, Gibco). 7F2 cells were cultured in alpha Modified Essential Medium (αMEM, Biological Industries) supplemented with 10% FBS and 1% P/S. The media were refreshed every 2-3 days. For adipogenic differentiation of 7F2 cells, the cells were seeded with concentration of 2500 cells/mL in 6-well plates. After two days, the growth medium was replaced with adipogenic induction medium containing 50 µM indomethacin, 10 nM dexamethasone and 5 µg/mL insulin [18]. The cells were cultured for 7 days and the adipogenic induction medium was replaced every 2-3 days. Also, control group (growth) cultured in non-induction medium was maintained in the same conditions for same culture time. All cells were incubated in a humidified 5% CO2-air incubator at 37°C. The cells were imaged at 10× under an inverted microscope (Olympus IX-83).

C. Biofabrication in magnetic levitation platform

Firstly, adipogenic-differentiated cells were trypsinized and centrifuged at 125 × g for 5 min. Then, pellet was resuspended with the concentration of 1×10^5 cells/mL in culture medium. After that, cell suspensions with Gd^{3+} concentration of 50 mM were prepared and loaded into microcapillaries as 50 µl of samples in vertical magnetic levitation device. After the cells were cultured in the levitation device for 24 hours in incubator with humidified 5% CO_2-air at 37°C, 3D cellular structures were imaged under inverted microscope at 4× magnification. Secondly, D1 ORL UVA and 7F2 cells were detached from culture plates and loaded into horizontal magnetic levitation system with the same protocol as explained above. For culture in horizontal device, it was used 25 mM, 50 mM and 100 mM Gadavist (Gd^{3+}). After culture for 24 hours, 3D structures were visualized under microscope at 4× and 10× magnifications.

III. Results and Discussion

In brief, magnetic levitation principle was used to conduct 3D cellular structures of adipose tissue with label-free system.

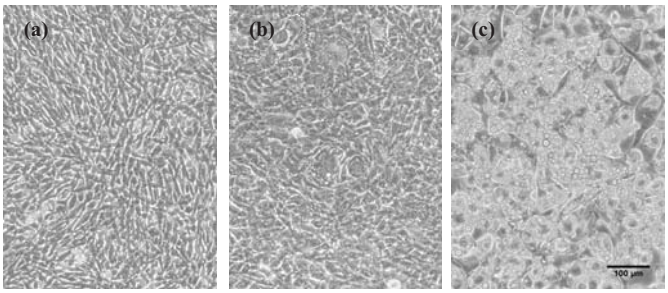

Fig. 1. 2D culture images of (a) D1 ORL UVA, (b) 7F2 and (c) adipogenic-differentiated 7F2 cells. Scale bar: 100 µm.

In this context, mesenchymal stem cells (D1 ORL UVA) and adipogenic-differentiated cells (7F2) were used as models for adipose tissue cells (Fig. 1). Both of them were separately cultured with the same cell concentration of 1×10^5 cells/mL in two different magnetic levitation systems containing gadolinium-based paramagnetic medium, abbreviated to Gd^{3+}.

In our previous study, it has been shown the capability of forming 3D structures of D1 ORL UVA cells in vertical magnetic levitation system [3]. Here, we aimed to create 3D adipogenic structures with vertical magnetic levitation system (Fig. 2) by using adipogenic-differentiated 7F2 cells having lower buoyant density compared to the most of cells types (Fig. 3). When the cells were levitated with 50 mM Gd^{3+} concentration, which is known that is proper for cell culture in vertical levitation platform [3], it was seen that single adipocytes were located at different levitation levels because of heterogeneous density of the cells (Fig. 3a). Concomitantly, the cells formed 3D adipocyte clusters with a loose structure after culture for 24 hr. (Fig. 3b).

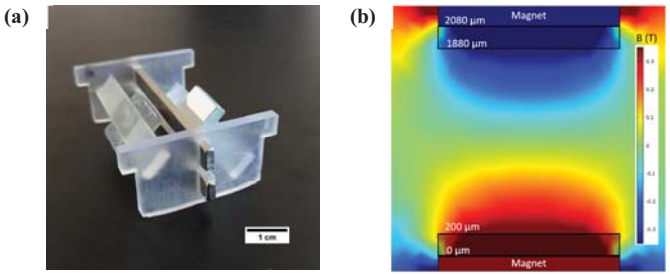

Fig. 2. (a) Vertical magnetic levitation platform. (b) Cross-sectional representation of the magnetic induction between vertical magnets. Scale bar: 1cm. Adapted from [7].

Alternatively, we hypothesized that levitation using horizontal magnets would minimize the differences in cell positions that is based on single-cell density (Fig. 4a). COMSOL simulation of magnetic field for cross-section of the horizontal device was performed to determine magnetic field gradient and density profile between the magnets (Fig. 4b-c). By simulation, it was confirmed that in the same density range, cell distribution was narrower. Namely, adipogenic-differentiated cells with heterogeneous density are expected to be located closer to each other compared with vertical levitation system [8].

Firstly, using the horizontal levitation system, D1 ORL UVA cells were cultured with different Gd^{3+} concentrations to

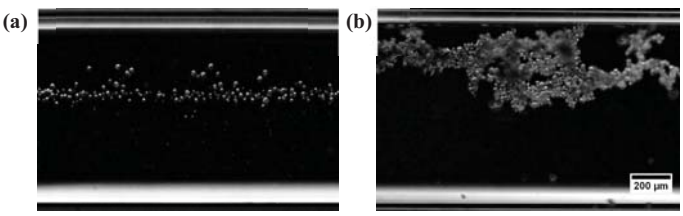

Fig. 3. Adipogenic-differentiated 7F2 cells in vertical magnetic levitation device. (a) Single cell levitation of the cells in 50 mM Gd concentration. (b) Levitation culture of the cells in magnetic levitation system for 24 hr. Scale bar: 200 µm.

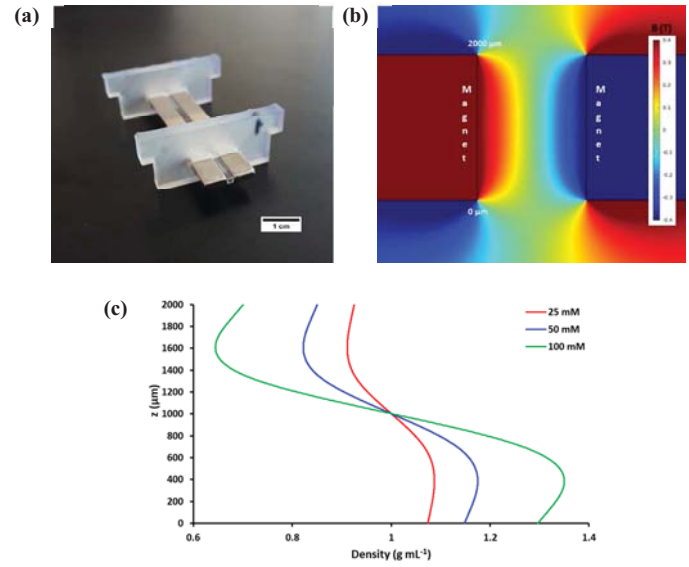

Fig.4. (a) Horizontal magnetic levitation platform. (b) Cross-sectional representation of the magnetic induction between horizontal magnets. (c) Simulation results of levitation heights of cells with different densities for simulated paramagnetic medium concentrations. Scale bar: 1 cm.

determine appropriate concentrations for culture of cells (Fig. 5). Consistent with the simulation results indicating that the cells are located at lower levitation heights when using low Gd^{3+} concentrations, it was seen that 25 mM Gd^{3+} were not strong enough for levitation culture of the cells and it caused attachment to the capillary surface during levitation culture. However, with 50 mM and 100 mM Gd^{3+}, the cells were successfully cultured and they formed 3D bar-like structures. Secondly, the platform

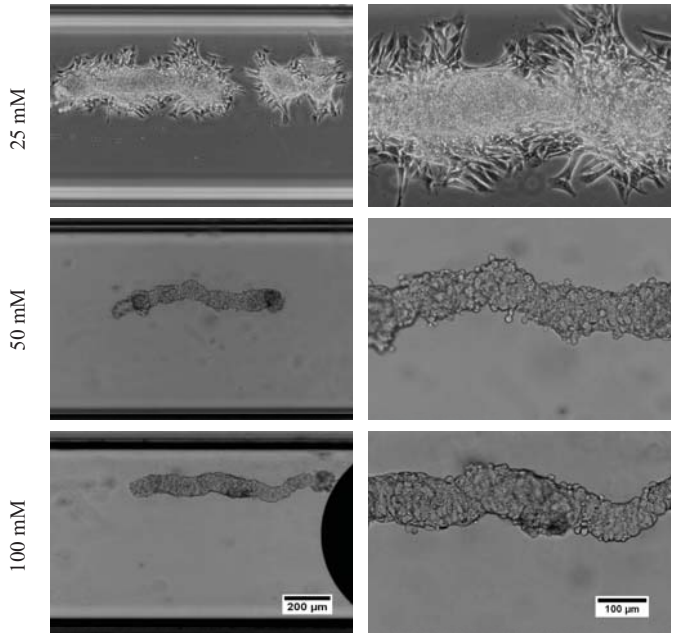

Fig. 5. Micrographs of levitation-cultured D1 ORL UVA cells in different Gd^{3+} concentrations and magnifications. Scale bar: 200 µm (left) and 100 µm (right).

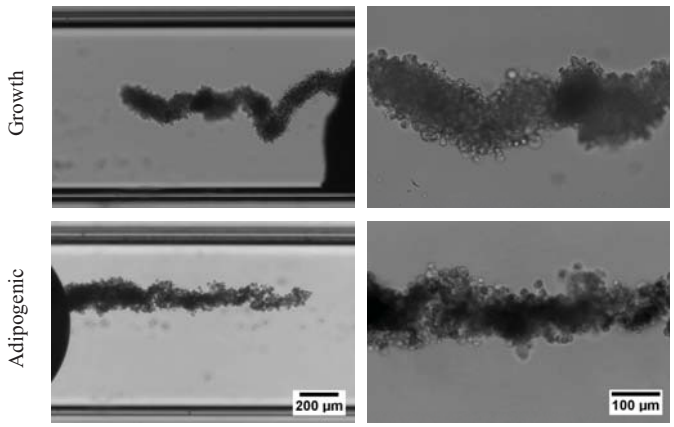

Fig. 6. Micrographs of levitation-cultured adipogenic-differentiated and undifferentiated 7F2 cells with 50 mM Gd^{3+}. Scale bar: 200 µm (left) and 100 µm (right).

was used for adipogenic cell culture (Fig. 6). For that, 50 mM Gd^{3+} was chosen. When adipogenic differentiated and undifferentiated 7F2 cells were cultured in horizontal levitation device, the formed 3D structures were similar in terms of compactness. Besides, 3D adipogenic clusters were not loose as in vertical device. Thereby, it was shown that, second levitation system can enable to create more uniform adipogenic structures. These forms can also be seen at high magnification (Fig. 6, right). In this design, under favour of magnet configuration, imaging is easier and it is possible to visualize the cellular structures with higher magnifications than 10×.

IV. Conclusion

In this study, we used two different configurations of magnetic levitation system to biofabricate adipose tissue cells with a label-free and contactless approach. Both of systems were successful in homotypic culture of adipose tissue cells. Because of mechanically sensitive nature of adipocytes, conventional nozzle-based tissue engineering techniques are not appropriate for adipose tissue biofabrication. Therefore, this proposed technique can be a preferable alternative to other techniques. Also, it offers the opportunity to simulate complex nature of adipose tissue which consists of various cells types. With the development of the system, it may be possible to produce structures that will be used for further molecular studies for adipose tissue-related diseases or soft tissue transplantation.

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