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High-throughput generation of spheroids using magnetic nanoparticles for three-dimensional cell culture

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ABSTRACT

Various attempts have been made to develop three-dimensional (3-D) cell culture methods because 3-D cells mimic the structures and functional properties of real tissue compared with those of monolayer cultures. Here, we report on a highly simple and efficient 3-D spheroid generation method based on a magnetic pin-array system to concentrate magnetic nanoparticle-incorporated cells in a focal direction. This system was comprised only of external magnets and magnetically induced iron pins to generate a concentrated magnetic field for attracting cells in a focused direction. 3-D spheroid generation was achieved simply by adding magnetic nanoparticle-incorporated cells into a well and covering the plate with a magnetic lid. Cell clustering occurred rapidly within 5 min and created more compact cells with time through the focused magnetic force. This system ensured not only reproducible and size-controlled generation of spheroids but also versatile types of spheroids such as random mixed, core–shell, and fused spheroids, providing a very useful tool for various biological applications.

1. Introduction

Cells form close cell–cell contacts in tissues or organs and construct 3-dimensional (3-D) structures that contact with the extracellular matrix (ECM) and a variety of cell–cell junctions that play an important role in regulating cell proliferation, migration, and differentiation [1–4]. Initial stable cell–cell interactions affecting overall cell survival and cellular structure result in different cellular activities and functions. Thus, 3-D cells have attracted increasing attention as they resemble and simulate various functions of real tissue [5,6].

Therefore, 3-D cells are important as a cell culture model and have been used for various applications such as stem cell research [7,8], tumor studies [1,3,4], drug screening [9–11], and tissue engineering [8,12]. For these reasons, several techniques have been developed to form 3-D cells based on traditional methods such as the spinner flask [4,13] or hanging-drop method [4,14–16] to microfabrication or patterning techniques [17–19], which were developed recently. However, such systems have their limitations to achieve uniform and high-throughput generation of 3-D cells and to broaden their applications [4,20]. In particular, generating uniform and size-controlled multicellular spheroids is critical to use spheroids as a tumor model for drug screening or to provide appropriate cues for stem cells to differentiate into a specific cell lineage [10,11].

Recently, the magnetic cell levitation method has been introduced based on magnetic particles [21] or combined with magnetic particles, viruses and hydrogels [22]. In these methods, cells incorporated with magnetic particles were lifted up by magnetic force, triggered cell–cell contact, and concentrated using these methods. These methods simply create cell aggregates in suspension by applying a magnetic force, yet they still have limitations in that they require significant time for cells to come in close contact with each other, and precise size control is impossible. In this system, magnetic force lifts the magnetized cells and brings them together in the medium, but the magnetic force gradient is insufficient to concentrate cells at a specific point.

To overcome these hurdles, we developed a method to generate uniformly sized 3-D cell clusters by applying magnetic nanoparticles and a magnetic pin system without any other exogenous

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materials. We created a focused magnetic field using magnetically induced iron pins to produce 3-D spheroids. We hypothesized that combining an iron pin and magnet would generate a strong and concentrated magnetic field at a specific point to aggregate cells and this physical force could reinforce cell–cell contact in a 3-D cell aggregate for enhanced cell functions. To explore the capability of this method, various environmental conditions for aggregating cells such as cell number and concentration of magnetic particles have been examined.

2. Materials and methods

2.1. Manufacture of magnetic spheroid generation system

The culture plate lid was manufactured with the magnet and iron pins to generate spheroids using magnetism. The dozens of cylinder-shaped NdFeB magnets (N35, radius: 10 mm; height: 10 mm), whose magnetization was parallel to the symmetry axis, were used for manufacturing magnetic lids and arrayed in an alternate magnetized direction to make them have a strong magnetic force. The magnets were placed to the top side of the plate lid (96-well plate), and each iron pin was attached to the inner side of the plate lid under each magnet. Each magnet was regularly apart from other magnets. The overall height of iron pin was about 8.4 mm and fixed to the plate by the magnet.

2.2. Preparation of magnetic nanoparticles

The magnetic nanoparticles (Fe_3O_4) used in this study were ferromagnetic nanoparticles with a size of 40–50 nm. These nanoparticles were isolated from magnetic bacteria, *Magnetospirillum* sp. AMB-1, which was cultured in magnetic spirillum growth medium for 5 days at 27 °C under anaerobic conditions. The detailed procedure for preparing the magnetic nanoparticles and their characteristics was previously described [23,24]. Isolated and purified nanoparticles were finally dispersed in PBS and their concentration was determined using ICP-AES (ICPS-7500, Shimadzu, Japan). Nanoparticles were concentrated to 1 mg ml^{-1} and fully dispersed using a bath sonicator (JAC 1505, Korea) for 15 min just before experiments.

2.3. Cell preparation

Bone marrow-derived human MSCs (BM-hMSCs) were purchased from Lonza (Switzerland) and were used to generate spheroids. Cells were grown in 25 cm^2 tissue culture flasks in nonhematopoietic expansion medium (Miltenyi Biotec GmbH, Germany) at 37 °C in a humidified CO_2 incubator. The cells were maintained until reaching approximately 90% confluence, detached with trypsin-EDTA (Sigma, USA) and re-seeded for passage [25].

2.4. Spheroid generation

The hMSCs were incubated with magnetic nanoparticles for 16–24 h in a dose-dependent manner (10–40 μg ml^{-1}). Magnetically induced cells were detached from the plate with trypsin-EDTA and suspended in the medium to generate spheroids. The cell suspension (130–135 μl) was added to wells of 96-well plates at various cell concentrations (250–2000 cells/well). And then the plates were covered with the magnetic lid that was prepared as described above. Cells were focused toward the magnetic pinpoint and spheroids were generated underneath the medium surface by magnetic force. The cells were stained with two different fluorescent dyes (PKH67 green fluorescent dye, PKH26 red fluorescent dye, Sigma) according to the manufacturer's manual just before spheroid generation to clearly observe cells. Aggregated cells were incubated in a humidified CO_2 incubator and observed using a fluorescence microscope (IX71, Olympus, Japan) mounted with CCD camera (12.8 MP, DP72, Olympus, Japan).

2.5. Formation of versatile type spheroids

Three types of spheroids were performed to validate the magnetic generation techniques to produce versatile types of spheroids. Prior to experiments, magnetic nanoparticle-incorporated cells were divided into two groups, and each group of cells was stained with two different fluorescent dyes such as PKH67 and PKH26,

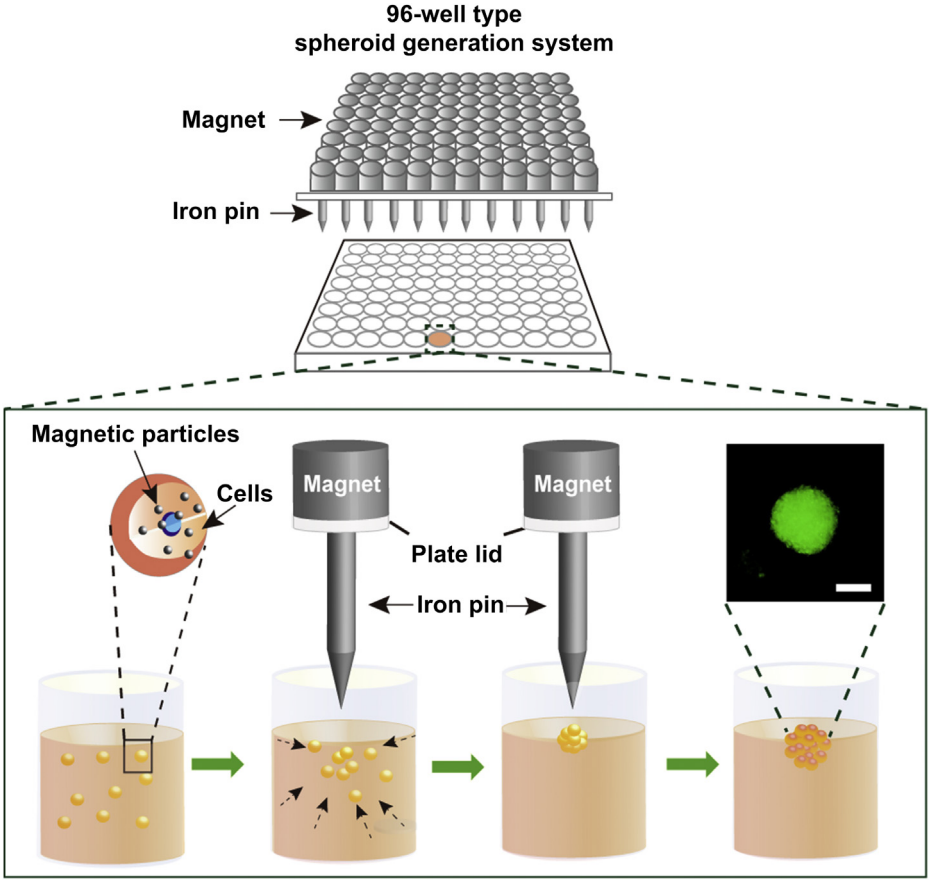

Fig. 1. A schematic showing the spheroid generation platform using magnetic nanoparticles and iron pins. Magnetic nanoparticle-incorporated cells were concentrated to a specific point underneath the medium surface using a focused magnetic force that was induced by iron pins and magnets. Scale bars represent 200 μm.