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An oxygen-permeable spheroid culture chip (Oxy chip) promotes osteoblastic differentiation of mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are being clinically used for bone and cartilage regeneration. However, the preparation of MSCs for implantation is still costly and time consuming, and controlling the differentiation of stem cells remains a challenge. Although much attention has been paid to three-dimensional cultures in the fields of tissue engineering and regenerative medicine, adequate oxygen supply remains a challenge for growing thicker and larger cellular constructs. To solve this problem, we have developed an oxygen-permeable spheroid culture device (Oxy chip) that enables direct oxygen supply to the cells. The aim of this study was to examine the effect of a three-dimensional culture and oxygenation to the cells on the differentiation of mouse MSC strain D1 cells. Our data demonstrated that MSCs grown in the Oxy chip differentiated into osteoblasts more quickly and efficiently than those grown in the conventional non-oxygen permeable chip and monolayer culture. DNA array and energy metabolism analyses revealed that the Oxy chip facilitated osteoblastic differentiation and aerobic glycolysis, rather than chondrogenic differentiation and anaerobic glycolysis. Together, we revealed for the first time that the oxygenation by the Oxy chip was effective on the osteoblastic differentiation and survival of three-dimensional cultured MSCs. This chip is useful for preparing differentiated cells and controlling the direction of differentiation of MSCs. Moreover, this approach may be useful for transitioning spheroid cultures as a therapy in regenerative medicine.

1. Introduction

A multicellular spheroid system is known to better reflect in vivo physiology compared to a conventional monolayer culture [1]. Some types of cells, such as hepatocytes or chondrocytes, gradually lose their cellular function and differentiation in monolayer cultures. In contrast, cells in spheroids are more functional and retain their differentiated phenotype through cell–cell interactions. Numerous reports have shown that hepatocytes [2,3], pancreatic cells [4,5], chondrocytes [6], and osteoblasts [7,8] exhibit alterations in function and cell behavior when grown in a three-dimensional (3-D) culture system.

Spheroid cultures also mimic the early avascular stage of solid tumors. Spheroids have been widely used for assessing hypoxia responses of tumor cells and screening therapeutic compounds in order to reduce experimental animal use. However, one of the biggest obstacles to the application of spheroids in regenerative medicine as a building block of 3-D tissue is the occurrence of cell death at the center of large spheroids due to a lack of oxygen [9]. In order to overcome this problem, we have developed a spheroid culture system that supplies oxygen through a highly oxygen-permeable device [10]. In our previous study, we demonstrated that the device maintains not only metabolic functions of hepatoma HepG2 cells, but also dramatically prevents hypoxia and subsequent central necrosis of relatively large hepatoma spheroids.

Oxygen tension influences the survival and differentiation of mesenchymal stem cells (MSCs). There are a number of reports on the effect of oxygen tension on osteogenic differentiation of MSCs cultured as a monolayer [11–13]. Oxygen tension has a much greater influence on 3-D cell aggregates compared to cells cultured as a monolayer due to the highly dense conditions. The oxygen concentration in spheroids depends on the balance between the amount of oxygen supply and consumption by cells. The effect of oxygen supply on the osteogenic differentiation of 3-D cultured MSCs has not been examined in any detail.

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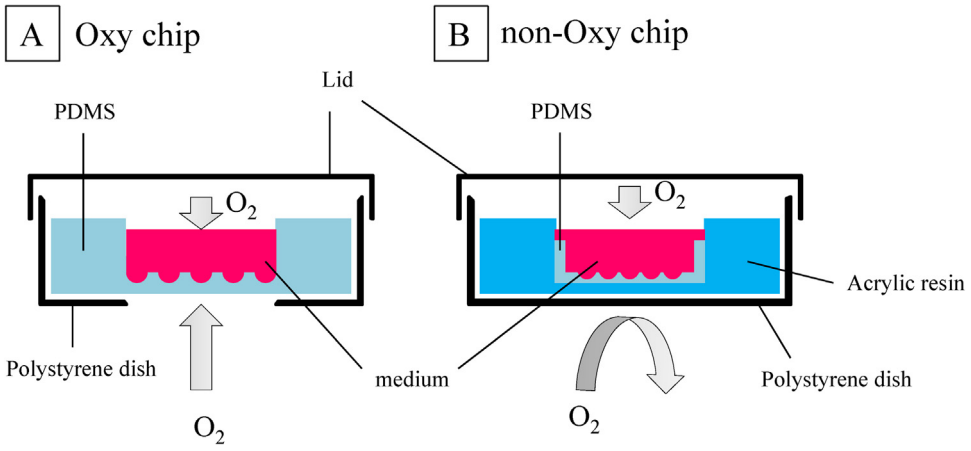

Fig. 1. An illustration of a cross-sectional view of the oxygen-permeable chip (Oxy chip) (A) and non-oxygen-permeable chip (non-Oxy chip) (B).

The present study was designed to investigate whether a 3-D culture and continuous oxygenation to MSC spheroids using an oxygen-permeable chip made with polydimethylsiloxane (PDMS) effectively promotes osteoblastic differentiation. PDMS is widely used for the fabrication of various microdevices due to its high transparency, biocompatibility, low production cost, and high diffusivity and solubility of oxygen (oxygen permeability) [14]. To demonstrate the potential application of the oxygen permeable device for bone regeneration, we compared the osteoblastic differentiation of the MSC strain D1 grown on the Oxy chip to those grown on a conventional non-oxygen permeable chip as well as a monolayer culture. In the present study, we examined the effect of the Oxy chip on differentiation of MSCs for relatively short periods of time, since faster in vitro development would be preferable for potential future therapeutic applications.

2. Experimental

2.1. Fabrication of spheroid culture chips

We prepared a spheroid culture chip as previously reported [10]. We previously reported that the spheroid culture device (prototype device) consisted of a sealed chamber and a deformable thin PDMS membrane [15]. A PDMS negative mold was replicated from the prototype culture device utilizing the thin PDMS membrane deformation by applying negative pressure [15]. A PDMS (Silpot 184, Dow Corning Toray, Co. Ltd., Tokyo, Japan) prepolymer was prepared by mixing the base and curing agent at a ratio of 10:1, respectively. The negative mold (25 × 25 × 8 mm) was treated with oxygen plasma for 3 min in a small plasma etch system (PIB-10 Ion Bombarder, Vacuum Device, Ibaraki, Japan). After plasma treatment, the mold was immersed in 4% Pluronic F-127 (Sigma-Aldrich, St. Louis, MO, USA) solution for 24 h to facilitate wetting of the surface of the mold and to prevent PDMS-to-PDMS adhesion. The PDMS negative mold was wiped with paper towels to remove excess Pluronic F-127 solution. PDMS prepolymer (base and curing agent were mixed at a ratio of 10:1) was poured into the PDMS negative mold and cured at 70 °C for 1 h. The PDMS replica was peeled off from the mold and used in the cell culture in the present study (Oxy chip, Fig. 1A). For the non-Oxy chip, the PDMS replica, which was fabricated using a very similar method as described above, was inlaid into a custom-made acrylic resin tray (Fig. 1B). The Oxy chip and non-Oxy chip were designed to be comprised of multicavities (512 wells, 1.00 mm in diameter, 1.05 mm pitch, 1.06 mm in depth) in a triangular arrangement on a 25 × 25 mm section of the cell culture area. The bottom thickness of the culture chip was adjusted to 1.5 mm.

2.2. Cell culture

Mouse bone marrow-derived mesenchymal stem cells (D1 ORL UVA [D1]) were obtained from ATCC (Rockville, MD, USA). The cells were maintained in minimum Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO) and 1% penicillin/streptomycin (PS, Invitrogen-Gibco, Carlsbad, CA) at 37 °C in a 5% carbon dioxide environment. The PDMS chips were sterilized in an oven (160 °C, 2 h). Before use, the PDMS chips were incubated with 2 ml of 4% Pluronic F-127 solution for 6 h. The polymer is adsorbed on the surface of the PDMS and prevents cell attachment [16]. The chips were then rinsed three times with DMEM to remove excess Pluronic F-127. The indicated number of D1 cells (25×10^4, 50×10^4, 100×10^4, 200×10^4, 400×10^4, and 800×10^4 cells/chip) was added to chips in 2 ml of osteogenic differentiation medium (DMEM supplemented with 10% FBS, 1% PS, 50 μg/ml ascorbate 2-phosphate, 10 mM β-glycerophosphate, and 100 nM dexamethasone). After allowing the cells to settle for 2 h, 1 ml of the medium was added to the culture chips. Cells in a monolayer culture were seeded on 6-well plates (Corning) at a cell density of 25×10^4 cells/well in 3 ml medium and used as a control group. This group is referred to as the "plate". All cells were cultured for 7 days at 37 °C, 5% CO_2, and 95% air in humidified incubators. The culture medium was changed every two days.

2.3. Spheroid diameter measurement and analysis by histochemistry

Cells were cultured on each chip and 6-well plates (n = 3). To evaluate changes in spheroid diameter, spheroids were photographed with a photomicroscope (Leica DFC300 FX, Leica Microsystems Japan, Tokyo, Japan). Spheroid diameters were analyzed using an image analysis program for Windows (Image-Pro Plus 7.0, Media Cybernetics Inc., Bethesda, MD, USA). A minimum of 30 spheroids on each chip was photographed and diameters measured. Spheroid diameter was defined as the average length of diameters measured at two-degree intervals joining two outline points and passing through the centroid.

Cells (100×10^4 cells/chip) were cultured in the Oxy chip and non-Oxy chip for 7 days, as described above. Cells were rinsed three times with PBS buffer. The spheroids collected from the culture chips were fixed in 3.7% paraformaldehyde in PBS for 2 h and embedded in paraffin. Serial sections (4 μm) were mounted onto silane-coated slides and stained with hematoxylin-eosin (HE). Pho-