DIFFERENTIATION OF MULTIPOTENT DFAT CELLS INTO SMOOTH MUSCLE-LIKE CELLS IN 3D TUBULAR MICROENVIRONMENT FOR TISSUE REGENERATION APPLICATIONS

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ABSTRACT

This paper describes the differentiation of dedifferentiated fat (DFAT) cells cultured within alginate shell microfibers into smooth muscle-like cells. A microfluidic co-axial device was developed and used to fabricate DFAT cell fibers. DFAT cells embedded in extracellular matrix (ECM) composed of fibrin and collagen inside the alginate shell of microfibers were cultured for one week in control and differentiation induction media. Using such technique, we successfully cultured DFAT cells as 3D fibers and demonstrated differentiation of DFAT cells into smooth muscle-like cells. These findings suggest the use of DFAT 3D cell fibers as a promising and efficient approach for tissue regeneration.

KEYWORDS

3D culture, alginate, DFAT, smooth muscle cells, tissue engineering, regenerative medicine

INTRODUCTION

Recently the establishment of DFAT cells has brought promising light to the field of regenerative medicine. DFAT cells are originally derived from adipose tissue and generated through ceiling culture, which leads to dedifferentiation of the cells. DFAT cells with multilineage differentiation potential (adipogenic, osteogenic, chondrogenic, muscle) could be expanded with high purity without gene manipulations, which provides greater efficacy and safety in clinical applications [1,2]. When DFAT cells cultured in 2D were induced in differentiation medium, cells have been shown to differentiate into smooth muscle α-actin (ASMA, a smooth muscle lineage specific marker) positive cells, but did not express markers of mature smooth muscle cells (SMCs) [2]. Alternative culture condition must be defined to induce differentiation into more mature and functional SMCs, and physiological 3D culture microenvironment is anticipated to provide more efficient differentiation. Nevertheless, it has been traditionally challenging to culture DFAT cells in 3D and induce differentiation into SMCs. Our preliminary data suggests that 3D spheroid culture of DFAT cells is not optimal for differentiation into SMCs due to the lack of elongated cellular morphology when cells are aggregated as spheroids. Therefore, to induce efficient differentiation of DFAT cells into SMCs, it is essential to preserve cells in their stretched morphology while culturing them in a biomimetic 3D microenvironment.

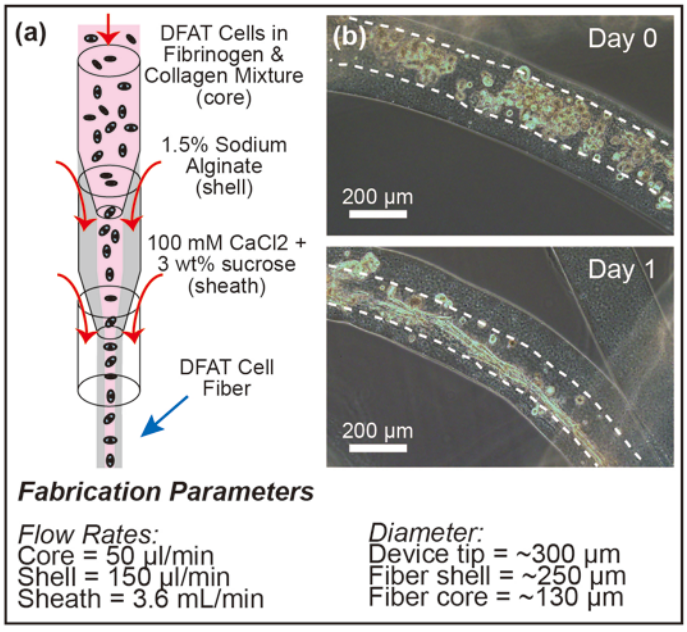

Figure 1. (a) Schematic of the microfluidic device for DFAT cell fiber fabrication. DFAT cells were encapsulated within alginate shell fibers through the double coaxial laminar flow achieved in the device with the parameters listed. (b) Actual images of the microfiber immediately after seeding and 1 day after initial fabrication.

EXPERIMENTAL

Microfluidic co-axial device [3] (Figure 1a) was used to encapsulate DFAT cells inside alginate microfibers (Figure 1b). The device was fabricated from two pulled glass capillary tubes assembled in tandem and joined by three-way connectors. DFAT cells suspended in a 1:1 mixture of fibrinogen (33.3 mg/ml) and 0.4% collagen at 1 x 10^7 cells/ml was introduced as the core stream at a flow rate of 50 μl/min using syringe pump. 1.5 wt% sodium alginate solution and 100mM $CaCl_2$ with 3 wt% sucrose solution were similarly introduced as the shell and sheath solutions at 150 μl/min and 3.6 ml/min. Using such condition, DFAT cells (isolated and derived from rabbit adipose tissue) in fibrinogen-collagen matrix were successfully encapsulated within alginate shell fibers. Immediately following fabrication, the fibers were incubated with thrombin (4.2 units/ml) for 15 min at 37°C to convert fibrinogen into fibrin. The fabricated DFAT core-shell fibers were then cultured in normal DFAT growth media (DMEM + 10% heat-inactivated fetal bovine serum (HI FBS) + 1% Penicillin-Streptomycin (P/S)) supplemented with 20 μg/ml of aprotinin (fibrinolysis inhibitor) overnight. On the following day when DFAT cells formed into cell fiber constructs, differentiation of DFAT cells into SMCs was induced by replacing the media with differentiation media (DMEM + 5% HI FBS + 1% P/S + 5 ng/ml TGFβ). DFAT cell fibers were cultured in differentiation media for a total of 6 days, with media exchanges performed on differentiation day 2 and 4. Similarly a control condition was carried out using normal DFAT growth media. On the last day of experiment (differentiation day 6), the DFAT cell fibers were fixed in 4% paraformaldehyde and subsequently stained with Hoechst 33342 and anti-ASMA antibodies.

RESULTS AND DISCUSSION

We first characterized the behavior of DFAT cells cultured under various conditions, and found a moderately low cell density in a mixture of fibrin and collagen as the optimal cell-extracellular matrix environment for DFAT cells to form into fiber constructs and be kept as such forms for a 1-week period. Following encapsulation of DFAT cells into alginate shell fibers, DFAT cells still exist as rounded single cells within the core ECM (Figure 1b). DFAT cells subsequently self-arranged into long fiber shapes within a few hours of seeding, and formed into solid cell fibers by day 1 (Figure 1b). Figure 2 further shows the images of DFAT 3D cell fibers on Day 1 and Day 7 of culture. Under both control and differentiation conditions, DFAT cells formed into cell fibers by day 1 (Figure 2a, c), spontaneously coiled into spring-like shapes by day 3 (data not shown) due to the cells' strong contractile force, and stayed in such form throughout the rest of the culture (Figure 2b, d).

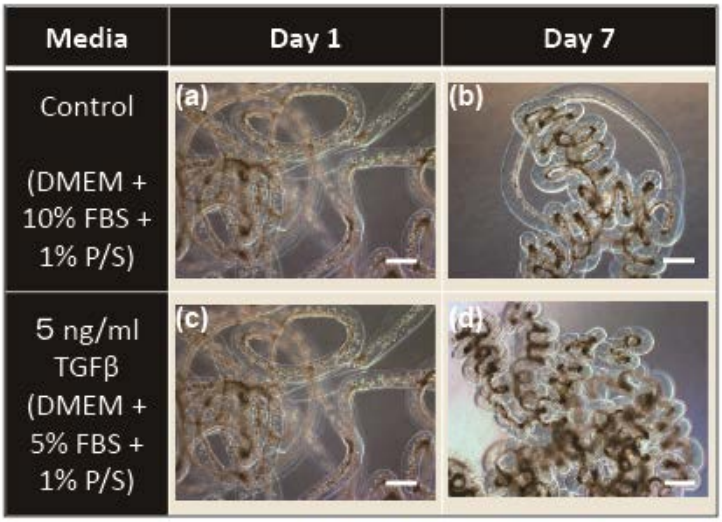

Figure 2. Images of DFAT-fibrin-collagen cell fiber inside alginate shell on Day 1 (Differentiation Day 0) and Day 7 (Differentiation Day 6) under control (a-b) and differentiation induction (c-d) conditions. Scale bar = 500 μm.

On the last day of culture (day 7), DFAT cell fibers were evaluated for the presence ASMA. The staining results (Figure 3) showed that only a small portion of DFAT cells in the control 3D cell fiber expressed ASMA; whereas almost all cells in the fiber induced for SMC differentiation were found to be positive for ASMA. In addition to such high rate of DFAT cell differentiation into ASMA positive cells, the orientation of the ASMA expressed in the differentiated cell fiber was very well-aligned in the longitudinal direction. Differentiation of DFAT cells into smooth muscle-like cells with such alignment of the ASMA has not been achieved in DFAT cells differentiated in conventional 2D dish cultures. This suggests that 3D cell fiber construct provides a better microenvironment for more efficient DFAT cell differentiation into SMC compared to conventional 2D dish cultures. Thus far, we have successfully induced efficient differentiation of DFAT cells into ASMA positive cells in our 3D cell fiber culture system over a 1-week culture period. However, ASMA is only an early marker of smooth muscle differentiation [4]. Currently long-term culture (> 1 week) of DFAT cell fibers induced for smooth muscle differentiation is underway in our group, in which the expression of mid- to late-phase smooth muscle markers such as calponin, smoothelin, and myosin heavy chain (MHC) is being investigated for differentiation into more mature and functional SMCs. Since

cell therapies based on SMC differentiation may provide treatment for various diseases concerning SMC dysfunction, efficient differentiation of a reliable source of precursor cells into mature and functional SMCs is especially critical. 3D DFAT cell fibers are anticipated to bring positive impacts to the regenerative medicine field as they could be easily handled and transplanted to injured organ tissues for reconstitution of tissue function.

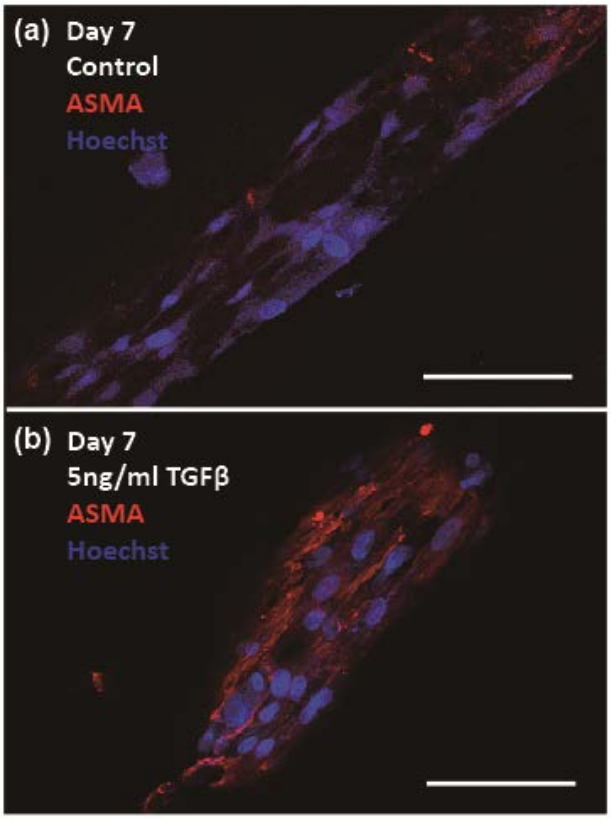

Figure 3. Confocal section images of control (a) and differentiated (b) DFAT fiber samples stained with Hoechst 33342 (blue) and anti-ASMA antibodies (red). Scale bar = 100 μm.

CONCLUSION

Here, we successfully developed a microfluidic device to encapsulate DFAT cells into 3D alginate shell fibers. We subsequently demonstrated that such 3D fiber construct provides an excellent environment for efficient differentiation of DFAT cells into smooth muscle-like cells. Using such 3D cell fiber culture system, we showed that DFAT cells expressed ASMA in well-aligned pattern that has not been recapitulated in 2D dish cultures. 3D DFAT cell fibers are expected to be useful in tissue engineering and regeneration of functional smooth muscle cells.

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