

Microfluidic Control of Adipose-Derived Stem Cell Growth and Positioning

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ABSTRACT

Microfluidic devices provide powerful new tools for quantitative stem cell research owing to its advantages in cellular microenvironmental control and experiment throughput over conventional methods. Particularly, microfluidics is playing a growing role in studying stem cell growth, differentiation and trafficking mediated by chemical factors such as different growth and differentiation factors and physical parameters such as direct current electric fields (dcEF). In the present study, we focused on adipose-derived stem cells (ADSCs) and we quantitatively characterized ADSCs growth and positioning mediated by epidermal growth factor (EGF) and dcEF using microfluidic devices. Our results revealed that ADSCs preferentially grow toward the EGF gradient. In addition, we showed that ADSCs tend to orient perpendicularly to the dcEF. These microfluidics-based results demonstrated the interesting roles of EGF and dcEF in controlling ADSCs growth and trafficking.

Keywords: adipose-derived stem cells, microfluidic device, EGF, cell migration, electric field.

INTRODUCTION

Recent development of microfluidic devices provides important experimental platforms for quantitative cell biology research owing to the unique capability of microfluidic devices for precise control of cellular microenvironments mimicking the tissue situations as well as the potential for significantly improving experiment throughput[3]. Notably, increasing number of studies using microfluidic devices has focused on stem cell biology to better understand the complex biological mechanisms for regulating stem cell growth, differentiation and migration[4-6]. Of particular relevance to this study, microfluidic gradient generators have been used to study neural stem cell growth and differentiation under different

conditions[4]. In addition, microfluidic devices have been used to investigate the migration and positioning of different cell types in response to applied DC electric fields (dcEF)[7]. Recently, growing studies have shown that different stem cell types are also capable of undergoing dcEF directed migration[8] although microfluidic devices were not yet used in these studies. These previous studies demonstrated the potential and advantages of microfluidic devices for quantitatively studying stem cell responses to chemical and electrical stimulations.

Among the many stem cell types, adipose-derived stem cells (ADSCs) is a type of mesenchymal stem cells (MSCs) and can differentiate into different cell lineages[9, 10], including adipocytes, osteoblasts, chondrocytes, and neurons. Because adipose tissue is abundant in most of the population and contains significantly high number of ADSCs than that of MSCs in bone marrow, it serves as a unique source of stem cells for regenerative biomedical applications[10-17]. Therefore, it is important to investigate the factors and mechanisms for regulating ADSCs growth, differentiation and trafficking *in vitro*. Epidermal growth factor (EGF) has been routinely used for promoting ADSCs growth *in vitro*. However, the role of EGF in guiding the spatial growth of ADSCs is not clearly defined. In addition, a previous study showed that murine adipose-derived stromal cells adjust their orientation and migration in an applied dcEF and such electrical controls of cell migration and positioning may apply to other types of ADSCs as well.

Thus, we in the present study focused on rat ADSCs to study its growth and positioning mediated by EGF and dcEF using microfluidic devices.

MATERIALS AND METHODS

Cell Preparation

ADSCs were isolated from the subcutaneous adipose tissues of the inguinal and interscapular regions of the

inbred Lewis rats following the established protocol. Fibroblasts, macrophages, lymphocytes, and hemopoietic stem cells were removed by immuno-depletion techniques. ADSCs were transfected with a GFP vector to generate cells stably expressing GFP using lentiviral transduction methods. GFP-ADSCs were routinely cultured and passaged in ADSCs culture medium (DMEM with 10% FBS, 1% MEM/NEAA and 20ng/mL EGF) before experiments.

Microfluidic Device Preparation

The previously developed microfluidic chemotaxis and electrotaxis devices[1, 2] were designed in Freehand 9.0 (Macromedia) and the design was printed to a transparency mask by a high resolution printer with at least 2400 dpi resolution. The device masters were fabricated in The Nano Systems Fabrication Laboratory (NSFL) at the University of Manitoba. Briefly, the design was patterned on a silicon wafer by contact photolithography with SU-8 photoresist (MicroChem, MA), yielding $\sim 100\ \mu\text{m}$ thickness [1]. The PDMS replicas were then fabricated by molding PDMS (Dow Corning, MI) against the master. For the device used for the EGF study[2], two 1 mm diameter holes for the 2 fluidic inlets and one 4 mm diameter hole for the fluidic outlet of a $350\ \mu\text{m}$ (W) x 1cm (L) main channel were punched out respectively in the PDMS device using sharpened needles. For the device used for the dcEF study[1], 2 wells (4 mm diameter holes) at the 2 ends of the main channel for inserting electrodes were punched out of PDMS. The surface of the PDMS replica and a clean glass coverslide were treated with air plasma for 1 min using a plasma cleaner (PDC-32G, Harrick, NY) and brought together to form an irreversible seal. This assembly produced the required systems of microfluidic channel. A new microfluidic device was used for each experiment.

Experimental Setup

The microfluidic channel was coated with fibronectin (BD Biosciences, MA) for 1 hour at room temperature and blocked with 0.4% BSA for another hour before the experiment. ADSCs were then loaded into the microfluidic device and allowed to settle in the main channel for a few hours in a 37°C incubator with 5% CO_2 injection. After the cells were seeded, culture medium with or without 20ng/mL EGF were infused into the device by a syringe pump (KD Scientific, MA) with two 1mL KD syringes through PE-20 tubing (Becton Dickinson, MD) connecting the pump and the two fluidic inlets of the device. This created an EGF gradient across the main channel width at the total flow rate of $0.4\ \mu\text{L}/\text{min}$. The device was kept in the incubator and cells inside the channel were imaged using a Nikon Ti-U microscope at different time points at multiple positions along the channel. For the dcEF experiments, after cell seeding in the device, $\sim 150\ \mu\text{L}$ of culture medium was added to each well with inserted platinum electrodes (SPPL-010, Omega Engineering, Inc), which were connected to a DC power supply (Central Scientific, NY) to

complete the circuit. The device was then placed on the Ti-U microscope stage and cells in the channel were monitored by time-lapse microscopy for 2 hours using the NIS Elements software. Throughout the time-lapse experiments, the microscope stage was maintained at 37°C with 5% CO_2 using a stage incubation system (In-Vivo Scientific).

Data Analysis

For the EGF experiment data, cell numbers were counted for the left half (low EGF) and the right half (high EGF) of the channel from the images and were compared between day 1 and day 2 upon applying the EGF gradient. The cell count was averaged from images acquired at different positions along the channel from multiple experiments and presented as the average value \pm the standard error of the mean (SEM). For the dcEF experiment data, cell orientation was analyzed in ImageJ (v.1.34s) by drawing a line along the cell polarity and the angles of the line with respect to the dcEF was measured to indicate the relative cell orientation. The cell orientation was averaged for all cells from the time-lapse images and presented as the average value \pm SEM.

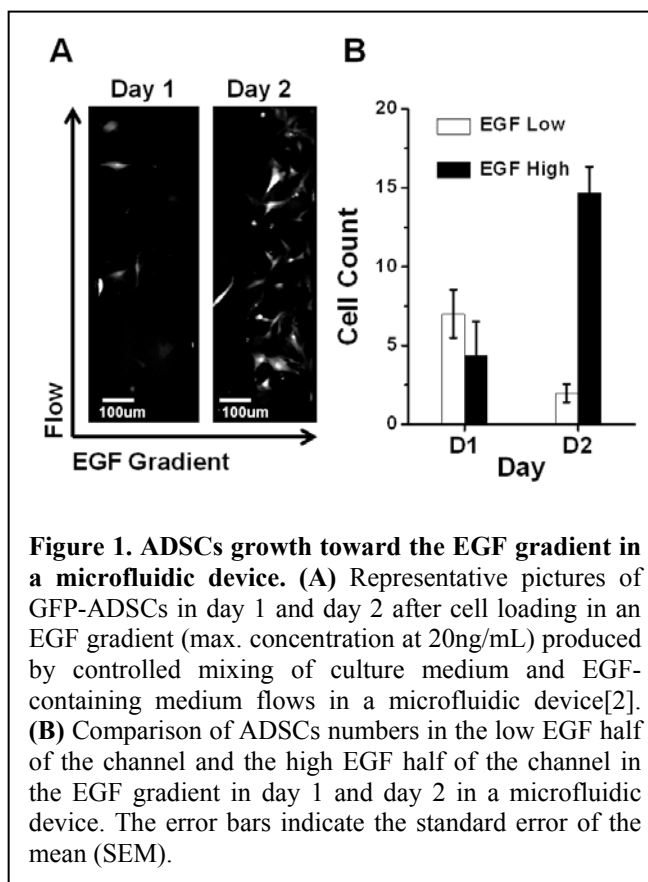


Figure 1. ADSCs growth toward the EGF gradient in a microfluidic device. (A) Representative pictures of GFP-ADSCs in day 1 and day 2 after cell loading in an EGF gradient (max. concentration at 20ng/mL) produced by controlled mixing of culture medium and EGF-containing medium flows in a microfluidic device[2]. (B) Comparison of ADSCs numbers in the low EGF half of the channel and the high EGF half of the channel in the EGF gradient in day 1 and day 2 in a microfluidic device. The error bars indicate the standard error of the mean (SEM).

RESULTS AND DISCUSSION

ADSCs Growth and Positioning in EGF Gradients

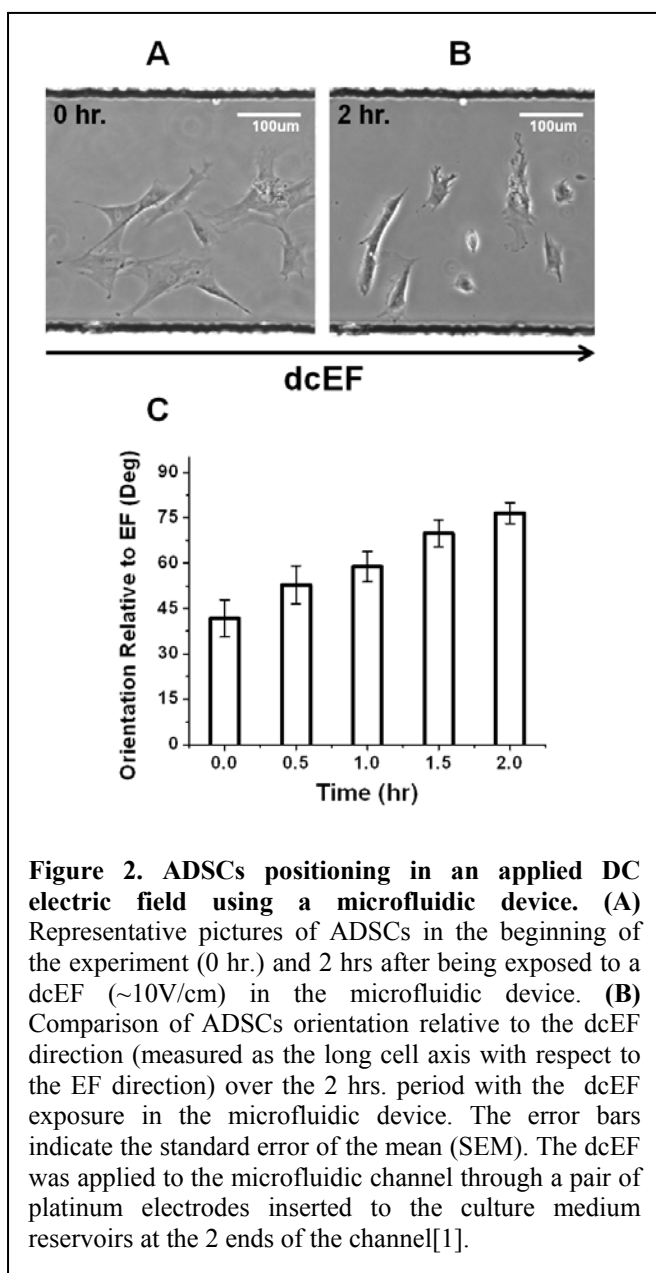


Figure 2. ADSCs positioning in an applied DC electric field using a microfluidic device. (A) Representative pictures of ADSCs in the beginning of the experiment (0 hr.) and 2 hrs after being exposed to a dcEF (~10V/cm) in the microfluidic device. (B) Comparison of ADSCs orientation relative to the dcEF direction (measured as the long cell axis with respect to the EF direction) over the 2 hrs. period with the dcEF exposure in the microfluidic device. The error bars indicate the standard error of the mean (SEM). The dcEF was applied to the microfluidic channel through a pair of platinum electrodes inserted to the culture medium reservoirs at the 2 ends of the channel[1].

Using a previously developed “Y” shape microfluidic gradient-generating device[2], we analyzed the growth and positioning of ADSCs in a defined stable EGF gradient over an extended period of time. Comparing cells in day 1 and day 2 upon applying the EGF gradient, our results showed that ADSCs underwent significant proliferation in the microfluidic device. In addition, it was clearly shown that ADSCs grew more in the high EGF region of the gradient after 2 days even if there were more cells initially seeded in the low EGF region of the gradient (**Fig. 1**).

Such preferential growth of ADSCs toward the EGF gradient was not clear using conventional culture flasks or microfluidic devices without an EGF gradient. Thus, our studies using microfluidic devices revealed the previously unclear importance of EGF gradient for regulating ADSCs

growth and positioning. Further studies will clarify if this effect simply resulted from the EGF stimulated ADSCs proliferation or from a combined proliferation and chemotaxis mechanism.

ADSCs Orientation in dcEF

As a potential new guiding mechanism, dcEF mediated cell orientation and migration (i.e. electrotaxis) has drawn growing attention to the life science community in recent years. Various stem cell types including mouse ADSCs were demonstrated to be capable of undergoing electrotactic orientation and migration. Using the simple PDMS electrotaxis device we previously developed[1], we performed live cell imaging experiments to monitor the migration and orientation of ADSCs in an applied dcEF. Our results showed that although no significant migration of ADSCs was observed over a 2 hrs period, ADSCs tend to orient perpendicular to the dcEF direction (**Fig. 2**). This observation is consistent with previously reported results of murine adipose-derived stromal cells using a dish-based electrotaxis chamber[6], and suggest the unique electric field mediated ADSCs orientation and positioning *in vitro*. Longer time-lapse experiments upon future studies may further verify the electrotactic migration of rat ADSCs.

CONCLUSION

In conclusion, our studies using microfluidic devices revealed the previously unclear importance of EGF gradient for regulating ADSCs growth and positioning, and the unique electric field mediated ADSCs orientation and positioning *in vitro* with implications for stem cell growth and trafficking in tissues that can be further investigated.

ACKNOWLEDGEMENTS

This study was supported by grants from the Manitoba Medical Service Foundation (MMSF), the Natural Sciences and Engineering Research Council of Canada (NSERC), Manitoba Health Research Council (MHRC), the Canada Foundation for Innovation (CFI) and the University of Manitoba, and the Rh award to F.L.. We thank The Nano Systems Fabrication Laboratory (NSFL) at the University of Manitoba for research support. We also thank the Faculty of Science at the University of Manitoba for fellowships to N.W. and H.K., and MHRC for a fellowship to S.N..

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