

Investigation of Cells Migration Effects in Microfluidic Chips

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Abstract

Microfluidic chips offer the unique opportunity to establish novelty *in vitro* cells models where the *in vivo* cells microenvironment could be precisely reconstituted. Although they have significances in the fields of cell biology, the real applications are extremely limited by ambient materials for cells culture, i.e., cells morphology and migration are greatly influenced by substrate material. In this study, we investigated the cells migration effects in four kinds of microfluidic chips with the same geometry. They are PDMS mold structures bonded on culture dish, glass slide, and PDMS substrates, respectively, and another PMMA mold structure bonded on PMMA substrate. For convenient description, we denoted these four chips as PDMS-DISH, PDMS-GLASS, PDMS-PDMS, and PMMA-PMMA, respectively. We compared and summarized the relationship of cells migration effects on different substrate. The cells are initially introduced into the culture area. The experiment results indicate that cells spreading time, spreading area and cells migration on these chips has obvious diversities. To further investigate the cells migration in these chips, a new model is prepared using trypsin/EDTA solution and cell culture medium. It shows a good repeatability. Most of cells could be formed a good morphology and monolayer growth in these microfluidic chips. The cells migrated furthest is in the PDMS-DISH chip after monitored 24 hours. The migration rates are 20.30 $\mu\text{m}/\text{h}$, 18.63 $\mu\text{m}/\text{h}$, 15.00 $\mu\text{m}/\text{h}$, and 10.75 $\mu\text{m}/\text{h}$ in PDMS-DISH, PDMS-GLASS, PDMS-PDMS, and PMMA-PMMA, respectively. This study turns open up opportunities for new biochips in prospective applications of wound healing and anti-scarring expected in drug screening and the related fields.

Keywords: Microfluidic chip; Cell migration; Cell spreading; Laminar flow

Introduction

The cells dynamic behaviors in culture has been the subject of research over several decades. Cells culture and cells migration *in vitro* is the basis of modern biology. Cells culture has made substantial contribution to understanding of many phenomena, such as intracellular enzyme activities, and cells interactions [1,2]. Cells migration is defined as the movement of individual cell, cell sheet and cluster from one location to another [3-6]. It is critical to a variety of different pathologic and physiologic processes across many areas of biology including wound healing, cancer metastasis, inflammation, cells growth and differentiation [7-9]. The conventional *in vitro* cells culture are used culture dishes or microliter plates and the typical cells migration assays are used Boyden chamber and physical scraping [10,11]. Although these methods are widely used, there are several limitations in this conventional methods [12,13]. For example, a traditional cells culture *in vitro*, there is neither cellular structure nor extracellular matrix for the cells to attach to, neither interaction nor communication with other cells. Cells lines and those in organs are different a lot in behavior, which growth rate, morphology, and intracellular metabolic activity will change correspondingly [14]. Furthermore, the membrane of Boyden chamber is not transparent. Therefore, it is difficult to perform the microscopy. In addition, the method is limited to tracing migration of individual cell. The cells are at the wound edges that can be damaged by physical scraping, which are important to cells migration assays [12,13]. In order to overcome these drawbacks, a solid and reliable approach for cells migration assay is necessary.

Recently, microfluidic systems have significant implications for cell biology and cell-based assay. They can provide precise cells pattern and controllable reagents distribution in a reproducible platform, which is not easily achieved by standard culture dish. In addition, they enable conventional assays to be performed using an automated and high-throughput approach [15]. In the past few years, microfluidic chips have been applied for use in cell culture and wound healing

assay [1,16-19]. Unlike conventional *in vitro* cell culture methods, microfluidics can provide small and complex structures mimicking *in vivo* environment. Recent research has shown that microfluidic cell culture systems convey more reliable results due to their ability to grow cells as biological systems, and they could outperform those from conventional cell cultures and assay systems [20]. A microfluidic wound healing assay was reported in the literature [21-23]. In an attempt to reconstruct a wound edge, a confluent cell sheet inside a microchannel was partially digested using multiple laminar flows with and without trypsin/EDTA. By using these operations, the interface resembled the actual wound edge was fabricated by physical scraping, without damaging to the cells on the leading edge [12].

In the study, we used the classical Y-shape microfluidic chip to investigate the cells normal physiological functions, such as migration, growth, reproduction and wound healing. We found that activities of cells are related to chemical composition and physical properties of base material of culture. In the process of cells growth, cells spreading is the first step in the interaction of cells and extracellular matrix (extracellular matrix, ECM). Cells adhesion and spreading on substrates are not only associated with the chemical properties of the substrate, but also related to surface physical properties of substrates, such as stiffness, hydrophobicity and geometric structure. We investigated the cells migration effects in four types of microfluidic chips. For this purpose, Human lung fibroblasts cell (HLF) was utilized, they were

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inoculated into the culture district. Laminar flow in the microchannel is used to injure cell layer to form a wound. Two of these flows contained protease trypsin, whereas one of them contained culture medium. Cells that were exposed to the trypsin-containing flow detached from the surface, whereas cells that were exposed to normal medium remained attached. Cell spreading, monolayer growth and cell migration in the microchannel were monitored.

Preparation and Fabrication of Microfluidic Chips

Four classical Y-shape microfluidic chips with the same feature sizes were used in this study. They are PDMS mold structures bonded on culture dish, glass slide, and PDMS substrates, respectively, and PMMA layer bonded on PMMA substrate. For convenient description in this study, they are denoted as PDMS-DISH, PDMS-GLASS, PDMS-PDMS and PMMA-PMMA, respectively. The feature sizes of four chips are the main channel with $900\ \mu\text{m} \times 100\ \mu\text{m} \times 5\ \text{mm}$ (width \times height \times length), three inlet channels with $300\ \mu\text{m} \times 100\ \mu\text{m} \times 5\ \text{mm}$ (width \times height \times length), respectively. The fabrication process of PDMS mold structures were prepared by a silicon mold with micrometer-sized SU-8 exposed to chlorotrimethylsilane vapor for 3 minutes and then released after carrying out the baking process. Secondly, PDMS prepolymer and curing agent (8:1 w/w, RTV615A, RTV615B) were mixed at an 8:1 ratio and then poured onto the mold. Thirdly, the samples were degassed under vacuum for 30 minutes and then baked for 45 minutes at 85°C . Fourthly, the cured PDMS layers with the desired structures were peeled and punched using a metal pin at the terminals of the inlet and outlet channels as shown in Figure 1. Finally, the PDMS layers were bonded on a culture dish, glass slide, and PDMS substrates, respectively. The fourth microfluidic chip is used PMMA material. This chip consists of two pieces of PMMA bonded by an adhesive film (model: ArcareMH-90445). Three parallel streams were observed by means of a fluorescence microscope equipped with a CCD digital camera (model: SS-300, TUSUN Co. Ltd.). Cells culture and migration were investigated by a phase contrast microscope.

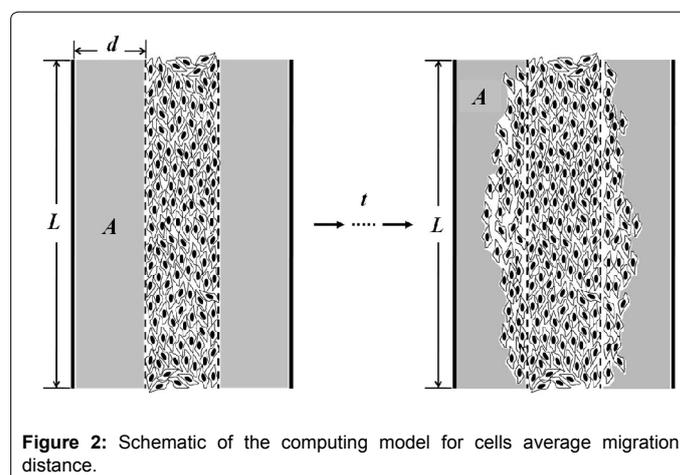
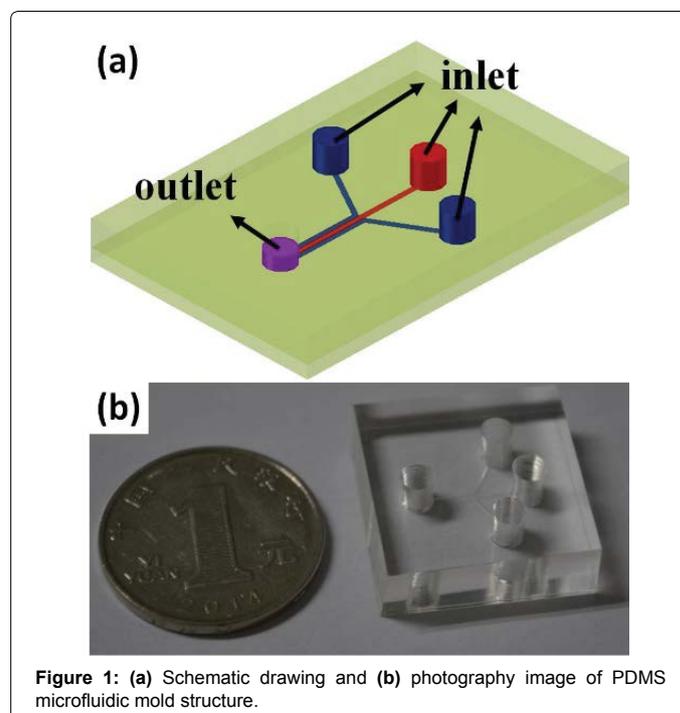
HLF cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin at 37°C and 5% CO_2 . When the cells reached confluence, they were dissociated from culture dishes with trypsin/EDTA. The cell suspension was then centrifuged at a rotational speed of 1,000 rpm for 5 min, after which it was re-suspended in DMEM containing FBS. The microfluidic channels were pretreated with DMEM containing FBS before cell seeding. The cell suspension (1×10^7 cell/mL) were carefully pipetted into four chips with different substrate material using a 3 μL micropipette. Due to pressure difference from micropipettes, cells flowed from the inlets to the outlets. To facilitate cell adhesion on the microchannel of chip, the microfluidic chip was kept stationary for 10 min at 37°C . Next, fresh medium using a 2 μL micropipette was then introduced. After cell adhesion on the inlet, the medium was replaced per every 6 hour. The growth and proliferation of cells was monitored at 0, 4, 8, 12, 16, 20, and 24 hours using an inverted fluorescence microscope.

Cells were ready for migration experiments when 80%-90% confluence was established in four Y-shape microfluidic chips. To prepare a wound model of monolayer cells, each of three inlets of the microfluidic channel was washed with PBS without divalent cations and trypsin/EDTA solution was introduced from two inlets, while DMEM containing 10% FBS was introduced from the middle simultaneously. The outlet was connected to its own 5 mL syringe in a syringe pump using polyethylene tubing, which were pulled through the microfluidic channel at a total rate of 20 $\mu\text{L}/\text{min}$ for 15 min. After this, the pump was

stopped, and the channels were flushed with DMEM containing 10% FBS, and stored in the incubator.

Experimental Results and Discussion

The schematic of the computing model for the cells average migration distance in four microfluidic chips is shown in Figure 2. The cells average migration distance can be expressed by $D=d-A/L$, where d is the distance between baseline and the edge of microchannel, L is the length of microchannel, and A is the no cell zone. Cells propagation on chip generally includes the following steps (1) The adhesion between cell and substrate initializes. (2) Cells protruded around. (3) New adhesion sites form between pseudopodia tips and substrate. (4) Cell spreading driving force and resistance achieve a balance, and cells no longer spread out forward. The initial contact of cell and the substrate is mainly a passive process, and only depend on physical properties of cell and the substrate, such as the viscoelasticity of cell, cell membrane tension and substrate stiffness.



During the migration assay, the cells were monitored and photographed at 0, 4, 8, 12, 16, 20, and 24 hours under a phase contrast microscope with a 80 times multiplied objective. The optical images of fixed positions in the wounds were taken with a CCD digital camera that was mounted on the microscope. Each experiment was repeated at least three times. Subsequently, the wound area in each image was determined by outlining the wound and measuring the area using Image-Pro Plus 6.0 software. From the wound area, the average wound width could be obtained by dividing the area by the length of the analyzed region. Figure 3 shows cell spreading and monolayer growth in four individual microfluidic chips at different culture time. Cell image analysis indicated that most of cells could be formed a good morphology and monolayer growth status by culture time. Therefore, these four substrate materials have biological safety and are suitable for cell growth. However, cell spreading time and spreading area on four microfluidic chips with different substrate material is distinct. Cell spreading speed in the increasing order is PDMS-DISH, PDMS-GLASS, PMMA-PMMA and PDMS-PDMS as shown in Figure 4a.

In order to observe material effect on cell growth and obtain monolayer for cell migration, bare material and cell seeding are very important. At the beginning of the test, a syringe was used to force air bubbles out of the microfluidic chip. When cells were introduced into a channel without surface modification, the phenomenon of cell aggregation occur for intercellular adhesion. Therefore, before the seeding of the cells in the microchannel for culture, the microchannels were pretreated with DMEM containing FBS. It is 3 μL of cells were introduced into each inlet at a density of 1×10^7 cell/mL. A micropipette was put at the outlet to aspirate the waste and fluid making them flowing out quickly. With the aid of pressure difference, cells flowed from the inlets to the outlets with a fast speed. When the liquid flow was stopped in the microchannel and the cells were uniformly distributed on the substrate surface, aspirating was stopped and the chip was put into the incubator to further incubate. Dynamic

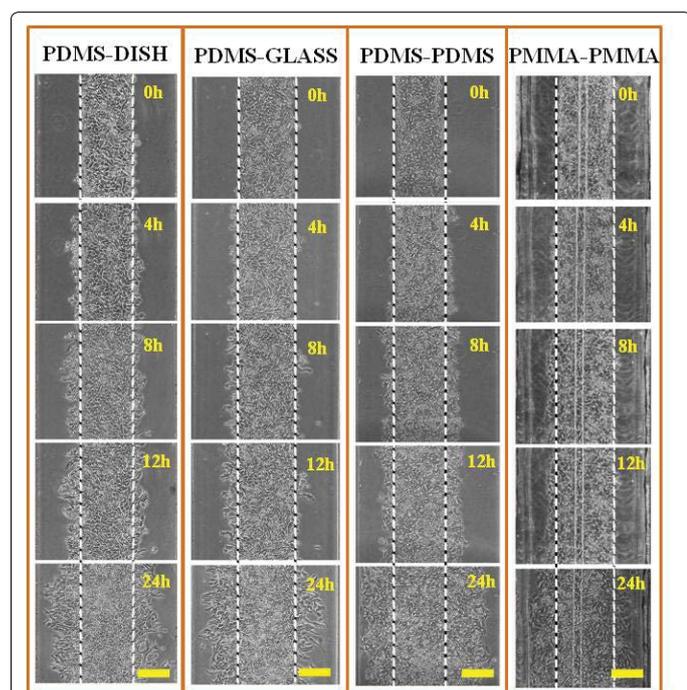


Figure 3: Optical images of human lung fibroblasts (HLF) cells migration in the four chips taken at 0, 4, 8, 12, and 24 hours, respectively. The white dashed lines are the baselines for the migration assay (Scale bar is 200 μm).

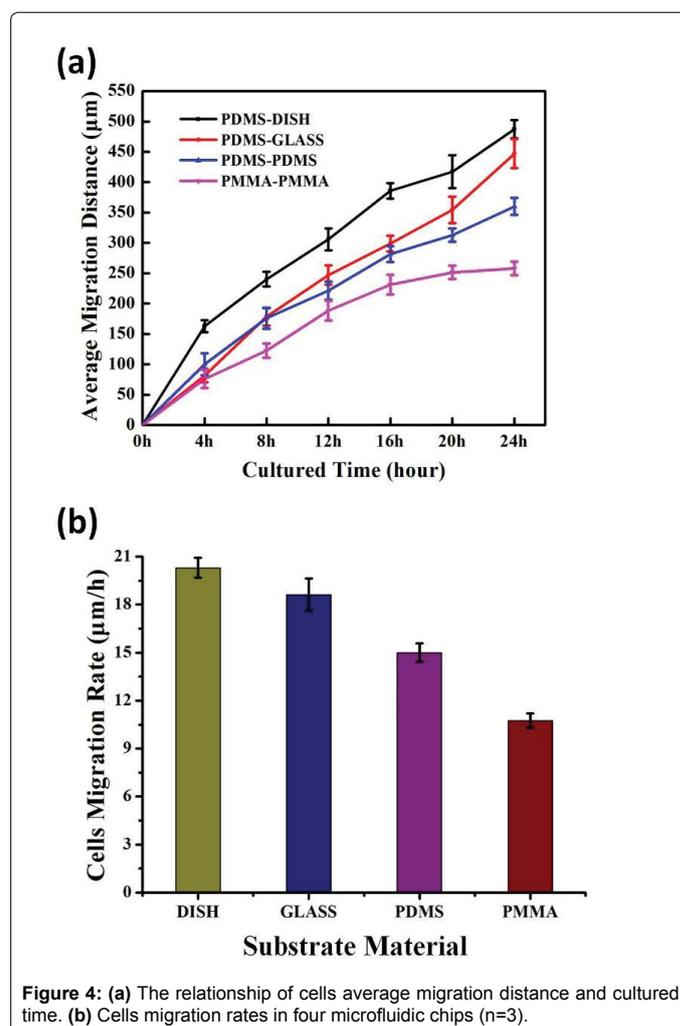


Figure 4: (a) The relationship of cells average migration distance and cultured time. (b) Cells migration rates in four microfluidic chips ($n=3$).

culture was offered by supplying fresh culture medium every 6 hour to provide enough nutrition for cells to grow and reduce cell injury. Cell morphology and migration movement is influenced to a great degree by substrate mechanical properties, such as permeability or hardness. The wound had been introduced in the monolayer. The migration rate was quantified by calculating migration distance of images at fixed position at fixed intervals. To quantify cell migration in this experiment, the initial wound edge was defined as the baseline. The cell migration distances were calculated from the baseline and the cell area move. The number of cells on the both sides of the baseline was also included. The photo images of cell migration at different times were taken at 0, 4, 8, 12, 16, 20, and 24 hours analyzed using Image-Pro Plus 6.0 software. Figure 4a indicates that four materials have distinct effects on cell migration. After cultured for 24 hours, cells migrated furthest in the PDMS-DISH chip. The migration rates are 20.30 $\mu\text{m}/\text{h}$, 18.63 $\mu\text{m}/\text{h}$, 15.00 $\mu\text{m}/\text{h}$, and 10.75 $\mu\text{m}/\text{h}$ in PDMS-DISH, PDMS-GLASS, PDMS-PDMS, and PMMA-PMMA, respectively, as summarized in Figure 4b. These differences may be caused by different materials with different hydrophilicity and hardness stiffness. The culture dish material is made of high quality crystal polystyrene, covalent binding of hydrogel layer on surface, also has good hardness and hydrophilicity. Glass material has better hardness and hydrophilicity than PDMS and PMMA materials. These results provide supportive evidence that cell migration distance associates with substrate material properties, such as hardness and hydrophilicity of culture material.

Conclusion

In this study, we investigated cells migration effects in four types of microfluidic chips. In cell biology, the real applications are extremely limited by ambient materials for cells culture, i.e., cells morphology and migration are greatly influenced by substrate material. A new wound model is prepared using trypsin/EDTA solution and culture medium, showing good repeatability. Most of cells could be formed a good morphology and monolayer growth in these microfluidic chips. For cells migration, cells spreading time and spreading area on these chips with different substrate material are distinct. The furthest cell migration rate is 20.30 $\mu\text{m}/\text{h}$ in the PDMS-DISH chip. This assay paves a way to deepen our understanding of biological behavior in cells culture and aid in developing novel material for biochip to achieve different aims. For example, the migration of cancer cells would help to understand cancer invasion, mechanism of metastasis and anti-cancer drugs treatment and so on.

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