Three-dimensional (3D) extra-cellular matrix coating of a microfluidic device

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Abstract

Cell migration on two-dimensional (2-D) surface is distinctively different from cell migration in three-dimensional (3-D) matrix. Although cell motility on 2-D matrix is widely studied, 3-D cell migration is just beginning to be understood. With the development of micro-fluidic systems, it is possible to study cell motility under much better controlled conditions. In this work, we present techniques for controlled coating of 3-D extracellular matrix (ECM) within microfluidic devices. This coating technique relies on the dominance of capillary forces and surface tensions in micro systems. We have successfully created uniform coating of Matrigel across microgaps of different lengths. This simple and reproducible method will have widespread biological applications such as the study of 3-D cell migration, the coating of basement membrane for cancer metastasis and angiogenesis study.

1. Introduction

Cell migration has been studied extensively in 2-D cell culture models. Although they have provided important insights into the molecular basis of cell protrusions and cell polarity, they are still not representative of the microenvironment of living tissues [1]. This makes them inappropriate for unraveling critical information like the underlying mechanisms of ECM proteolysis and different cell migration strategies in 3-D cell culture systems [2]. With the advancement of microfabrication technology,
microfluidic systems are beginning to be incorporated into a diverse field of research like analytical chemistry, tissue engineering and genetic analyses [3-5]. They are generally attractive to most biological applications due to their ability to control their physical and chemical characteristics on the micrometer and nanometer scale. Their advantage of assay miniaturization also ensures that regent consumption is and hence a reduction in cost. In this work, we combined the biological coating of Matrigel into microfluidic gaps to mimic the 3-D environment of the blood vessel. Matrigel [6], a solubilised basement membrane extracted from the Engelbreth-HolmSwarm (EHS) mouse sarcoma was used to coat the microfluidic gaps. The coating relies on the dominance of surface forces like capillary forces and surface tension in micro-systems [7]. This enables a uniform coating of the microfluidic to be achieved consistently.

2. Materials and methods

(a) Microfluidic Device

The PDMS device was fabricated by replicate molding on the master using PDMS (Sylgard Silicone elastomer 184, Dow Corning, Corp.). The master was prepared by spin coating SU8 negative photoresist (Microchem Corp.) onto a silicone wafer and crosslinked by UV for 50s. Subsequently, it was developed using SU8 developer (Microchem Corp.) and cleaned with isopropyl alcohol and nitrogen gas. The PDMS device was peeled off and bonded to a microscope glass slide to prevent leakage.

(b) Contact angle measurement

Contact angle measured were with a goniometer equipped with an optical system and a CCD camera (Dataphysics, Germany). The measurements were taken at 4°C and ambient humidity. Matrigel was used as the wetting liquid. For the measurements, 5μl of the liquid was added to the surface and the receding and advancing angle were measured. Laplace fitting was used to calculate the contact angles. Scanning electron microscopy. The specimens were cut into small pieces; sputter coated with platinum at 20mA for 80s and examined using FESEM (Joel, JSM 6300F, Japan).

3. Results and discussion

In microfluidic system, surface forces dominate like gravitational and inertial forces [7] due to the large surface area to bulk ratio. By exploiting these surface effects, they have been constantly employed for filling of microstructures [8-9] and liquid displacement [10]. In this coating technique, Matrigel was used to form a 3-D coating of the microgaps in microfluidic channels. We exploited the surface tension in holding the fluid along the walls of the microgaps and the capillary force to move the fluid across the microgaps.
Fig. 1 shows schematically our approach for coating of Matrigel into the microgaps. The coating process takes place on a cold plate to prevent the polymerization of the Matrigel. Firstly, 1:1 ratio of DMEM to Matrigel solution is prepared in a 1.0ml vial and kept at 4°C until use. 30μl of the mixture is pumped into top channel of the device to fill up the microgaps partially (Fig.1a). The device is then left alone at 4°C until the entire microgaps are filled by capillary force (Fig.1b). Theoretically, the coating of the device can be explained by an examination of the capillary pressure in the microfluidic channels. The capillary pressure $P_c$ of a liquid-air meniscus in a rectangular microchannel [11] is

$$P_c = -\gamma \left( \frac{\cos \alpha_b + \cos \alpha_t}{d} + \frac{\cos \alpha_l + \cos \alpha_r}{w} \right)$$

where $\gamma$ is the surface tension of the liquid, $\alpha_{b,t,l,r}$ are the contact angles of the liquid on the bottom, top, left and right wall respectively, and $d$ and $w$ are the depth and width of the microchannel respectively. Fig.2a shows the respective contact angles of Matrigel on the glass and PDMS. Using equation (1), the normalized values of the capillary pressure/surface tension, $P_c/\gamma$ within the microchannel and microgap showed that it is impossible for the bottom channel to be filled as its capillary pressure/surface tension is lower than that in the microgaps (Fig. 2b).
Figure 2 (a) Contact angles of Matrigel on the PDMS and glass. (b) The capillary pressure in the microgaps is lower than that in the main channel. We used this phenomenon to uniformly coat the microgaps.

Figure 3 (a) The nano-pores in a network of Matrigel fibers forms an intricate three dimensional structure; (b) specific Matrigel coating of the microgaps of different length (100 μm and 50 μm). The microgaps are filled with Matrigel which are then left to polymerize.

For instance, the capillary pressure/surface tension, \((P_c/\gamma)\) at a microgap of width, \(w\) of 15 μm, is 21604 PaNm⁻¹. It is lower than that in the main channel at -19654 PaNm⁻¹. This is consistent with our observation that the Matrigel fills up and remains contained within the microgaps. Finally, the top channel is flushed with cold DMEM before allowing the coated Matrigel to polymerize under room temperature. SEM images of the polymerized Matrigel showed that it is made up of tight inter-woven network of fibers that forms intricate 3-D nano-pore structures (Fig. 3a). By varying the length of the microgaps, we demonstrate the universality of the coating in applications of different lengths of microgaps (fig 4). Depending on the different applications, different length of coated microgaps can be used. For instance, the study of cell migration across the basement membrane will use a shorter microgap length and the invasion of cancer cell across the connective tissues of collagen will need a longer microgap length.
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