GENERATION OF DYNAMIC MICROENVIRONMENT IN A HYDROGEL-BASED MICROFLUIDIC DEVICE FOR CELL CULTURE STUDY

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ABSTRACT

Many cellular characteristics such as cell attachment, migration, morphology and proliferation are affected by porosity and pore size of bioactive gradients. Therefore, engineering a gradient that mimics the porosity relevant features of different living tissues can provide a better understanding for some of these cellular characteristics. In this work, a simple method is proposed to generate an injectable hydrogel gradient that allows the pore structure to be altered post injection resulted in a continuous porosity scaffold gradient under. This gradient was employed to study the cell metastasis phenomenon through monitoring the chemotactic response of human fibrosarcoma (HT1080) cells, an invasive cancer cell model, in a cell culture housed in a polydimethylsiloxane (PDMS) microfluidic chip. It was found that the migration of cells through this gradient is sensitive to the mean pore size where cells migrated toward the largest pores. This gradient provides the advantage of giving users the flexibility to observe cell migration in a user-controlled dynamic microenvironment.

KEYWORDS

Hydrogel, microfluidic device. Porosity gradient, chemotaxis

INTRODUCTION

Biochemical, biomechanical and biophysical stimuli from microenvironment play crucial roles in biological processes such as morphogenesis, differentiation and carcinogenesis [1,2,3]. Understanding the mechanisms involved in cell-substratum interaction is essential for creation of therapeutic systems. Various biomaterials have been developed to mimic the in vivo extracellular matrix (ECM) structure and porous architecture for cell behavior study [4,5]. However, most of the existing systems can only provide static porosity gradient. This study demonstrates the preparation of dynamic porosity gradient in a hydrogel-based microfluidic device to mimic the dynamic ECM 3D topology. The device was utilized to study cell migration.

EXPERIMENT

To prepare the microfluidic device, a master mold was constructed using a 3D printer. The desired microstructure was designed using AutoCAD design software. PDMS was casted onto the master mold using soft lithography technique, the desired microstructures were imprinted onto the PDMS layer, which consist of a main reservoir, inlet and outlet reservoirs, microchannels that connect the reservoirs, and microchannels that allow injection of solution into each reservoir (Fig. 1).

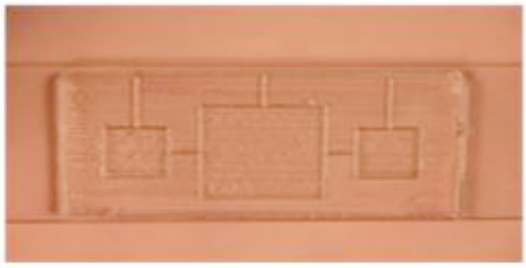

Fig. Top view of PDMS chip comprises of inlet reservoir, outlet reservoir, one main chamber and microchannels

The PDMS layer was thermal cured and subsequently treated with oxygen plasma in preparation for bonding onto a glass slide. Hydrogel was employed to mimic the 3D extracellular matrix (ECM). Hydrogel precursor solution was prepared and sterilized. The hydrogel precursor solution mixed with component responsible for pore formation was injected into the main reservoir and allowed to gel. To generate a 3D porosity gradient, a biocompatible pore etching solution was injected into the inlet reservoir, blank solution was injected into the outlet reservoir to generate a pore etching concentration gradient along the hydrogel by diffusion; a porosity gradient was etched simultaneously. For visualization, a model FITC-BSA biochemical gradient was generated and examined using laser scanning confocal microscopy (LSCM). To visualize the generation of porosity gradients, fluorescence labeled hydrogel was examined using LSCM. The pore size and porosity at different regions along the hydrogel were measured. Hydrogel precursor solution and HT1080 fibrosarcoma cells were injected into the main reservoir and allowed to gel, the cells were immobilized evenly. To generate a dynamic microenvironment, porosity gradient was generated in situ; a chemoattractant concentration gradient was then generated in situ in similar manner. Cell migration in response to the changing microenvironment was monitored at different time points. Cell viability was assessed using Calcein AM assay.

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The concentration gradient generated in the microfluidic chip can be observed from the diffusion of the fluorescent material (FITC-BSA) as shown in Figure 2. The average fluorescence intensity measurement across these regions shows a reduction in the fluorescence intensity through the scaffold in the main chamber from left to right.

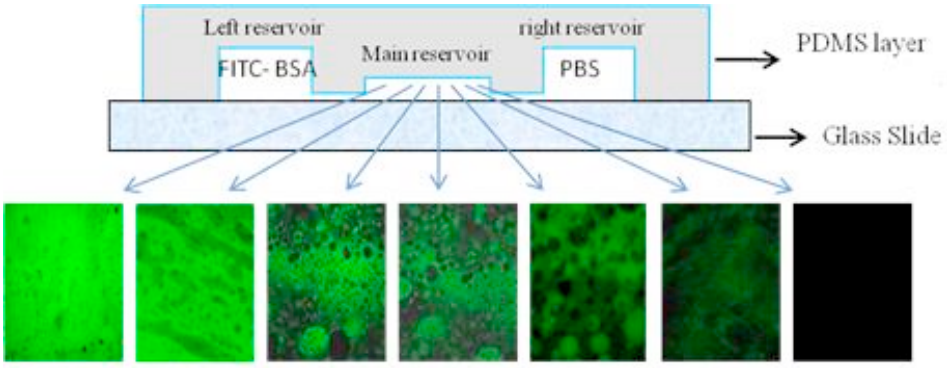

Figure 2. FITC-BSA concentration gradient generated across the hydrogel filled reservoir

Hydrogel precursor was loaded in liquid form into the main chamber and formed a gel *in situ*, the hydrogel was fluorescence labelled for visualization. To form a porosity gradient within the hydrogel matrix, pore etching reagent was added to the left reservoir while blank buffer solution was added to the right reservoir. As shown in Fig. 3 the porosity in the generated gradient gradually increased from the left to the right of the main chamber after 3 days of degradation step, the hydrogel gradient is comprised of three different regions: the right region with the smallest pore size (average 20μm), the middle region with the average of 27 μm and the left region with the largest pore size (average of 33 μm). Table 1 shows that the pore sizes in the region of the main chamber close to the left reservoir were on average larger than in the middle or right regions, reflecting the gradual increase in porosity from the left to the right regions.

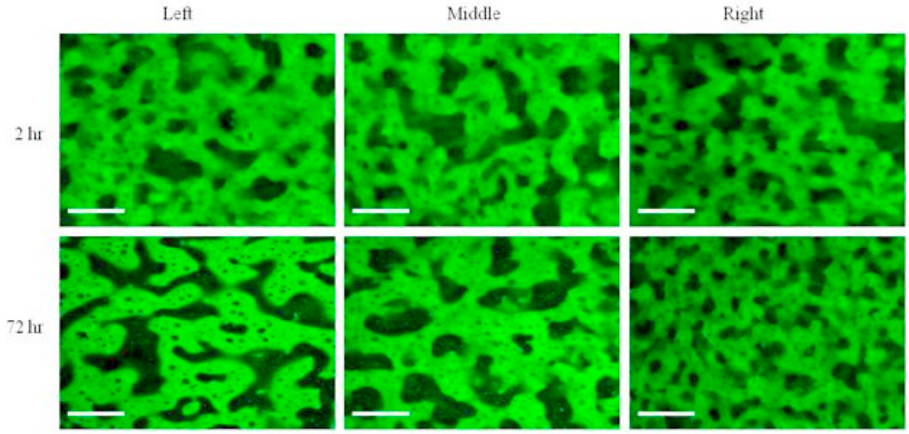

Figure 3. LSCM showing fluorescence labeled hydrogel in main reservoir before and after porosity gradient generation.

	Left region (μm)	Middle region (μm)	Right region (μm)
Before pore etching	22±8.9	23±6.5	19±6.4
After pore etching	33±2.2	27±4.1	20±5.5

Table 1. Average pore size in the left, middle and right regions of hydrogel-based device before and after porosity gradient generation.

Cell migration assay revealed that HT1080 cells were distributed uniformly at the beginning; but cells migrated to highly porous region after 3 days (Fig. 4). The pore size appears to have a considerable effect on the cell migration within the scaffold. The confocal images acquired shows that cell stained by Calcein AM remain viable (appear in green), indicating that this device is biocompatiable to HT1080 cells.

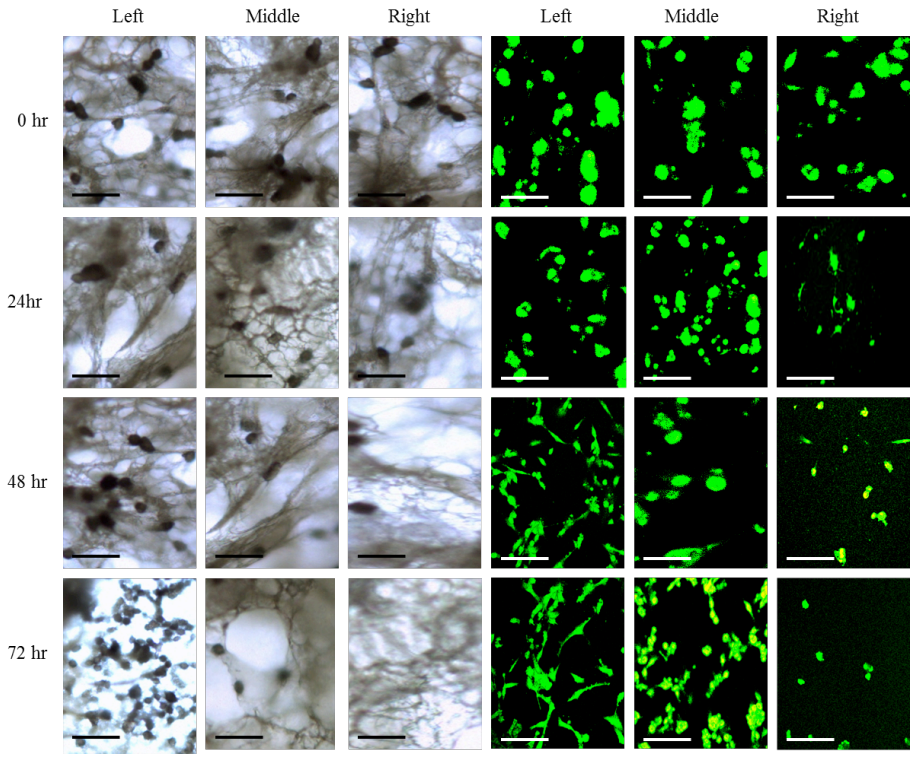

Table 5. Images of cell migration in porosity gradient obtained using SEM and laser scanning confocal microscope. Live cell (green) are stained with Calcein AM.

The microfluidic platform presented here, which incorporates the use of perfusable hydrogels with complementary photolithographic fabrication methodologies, demonstrates the potential for the development of a robust tool set to construct more complex, micro-scale, biomimetic, *in vitro* tissue analogues.

CONCLUSION

Biochemical In conclusion, this study reports the successful generation of dynamic microenvironment in situ using a hydrogel-based microfluidic device. Cell migration in response to the changing topology and biochemical gradient can be easily examined in situ using this device.

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