A CONTINUOUS-FLOW CELL CULTURE ARRAY WITH CHAOTIC MIXERS FOR IDENTIFICATION OF THE OPTIMUM GROWTH FACTORS COMBINATIONS FOR MOUSE EMBRYONIC STEM CELLS DIFFERENTIATION

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ABSTRACT

We designed and fabricated a continuous-flow cell culture array with chaotic mixers. By using this microfluidic device, combinations of GF's concentrations can be reliably mixed and supply to each cell culture well and the optimum combinations of GFs (VEGF & bFGF) was quickly obtained for mESC differentiation into ECs & SMCs.

KEYWORDS: Cell Array, Continuous-Flow Cell Culture, ES, Cell Differentiation

INTRODUCTION

Stem cells differentiation relies on microenvironment control for inductive signaling and initiation. In finding the optimum growth factor (GF) combinations for mouse embryonic stem cells (mESCs) differentiation into endothelial cells (ECs) and smooth muscle cells (SMCs), we developed a continuous-flow cell culture plate with integrated micro fluidics mixers. The continuous feeding of medium allows us to conduct long-term microscopic monitoring of the differentiation process of the ECs on the micro plate within a micro incubator without moving the plate conventionally needed for changing the medium and further reduces the autocrine and paracrine signaling. The micro cell culture plate with mixers to supply the cell culture array is shown in Figure 1. The need to continuously supply medium in a few 10's to 100's μl/hr to the cells etc. determines the flow velocity and the needed mixer dimensions. Since the molecular weight of these GFs are typically a few 10's~100's kDa in sizes, a conventional serpentine mixer with diffusion-limited laminar flow mixing will not mix the molecules adequately within a millimeter-size micro fluidics channel. Therefore, we design a cell culture plate consisting of an array of cell culture wells and 7-stage staggered-herringbone-ridge [1] serpentine mixers that enable GFs to mix completely within a few millimeters.

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THEORY

Microfluidic Chaotic Mixer & Microwell Arrays

Serpentine: heavy bio molecule has relative small diffusion constant and required several cm's to mix completely. However, the chaotic mixers encourage the mixing effect for laminar flows. The mixing length is determined by the logarithms of Peclet number. Thus it is considered to be more effective.

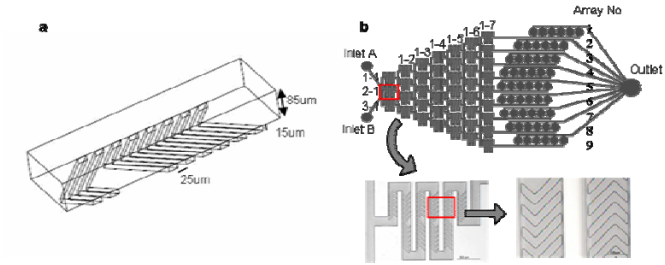

Figure 1. (a)The cell culture plate consists of 7-stage staggered-herringbone-ridge serpentine mixers that enable growth factors to mix completely within a few millimeters, and an array of cell culture wells. (b) The channel width and height of the mixers are 200 μm and 85 μm respectively. The depth and the spacing between ridges of the staggered herringbone are 15 μm, and 25 μm respectively.

EXPERIMENTAL

The negative epoxy-based photoresist SU8 2035 and SU8 3010 were used to constructed the mold of fluidic channels 85μm in height and 15μm tall ridges on silicon wafer substrate. With the SU8 mold as shown in Figure 2, the microfluidic devices were produced by PDMS molding and bonding to glasses after oxygen plasma surface treatment and a baking process.

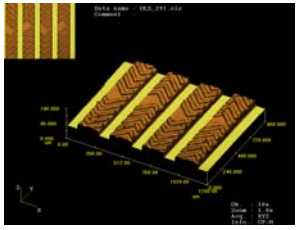

Figure 2 .The SU8 mold of the channels with herribone structures.

To verify the mixing efficiency of heavy molecules inside the chaotic serpentine mixers, a solution of 25 mg/mL blue-dextran 2000 (molecular weight 2000 kDa) and DI water were pumping into both serpentine mixers and chaotic serpentine mixers with a flow rate of 30 μL/hr from inlet B & A respectively of both devices. The gray levels of the image are measured across the channel at both entry and exit ends of mixers to assess the efficiency of mixing.

The fluidic devices were then sterilized with autoclave and immersed and decompressed in water to prevent air bubbles formation. Next, 0.1% gelatin was injected into the device and placed at 37^{o}C environment for 30 minutes for better cell

attachment. The mESC suspension was then injected into the devices and cultured standing still overnight. After the mESCs were well attached to the substrate, the continuous-flow media feeding was turn on. Since the continuous media cell culture experiments took two weeks, the incubation media with GFs were kept in 4°C inside the syringe pumps to maintain GFs' efficacies and feed into the micro fluidics devices kept in 37 °C incubator with a flow rate of 30 μL/hr. The heat transfer of the system is designed such that the media is warmed up to 37 °C when it reaches the micro fluidics devices with mESC inside.

The differentiation ability of stem cell is highly sensitive to its environment, and the current microfluidic devices provide precisely controlled microenvironment and quickly identify the optimum combination of GFs. [2] Both Vascular endothelial growth factor (VEGF) & basic fibroblast growth factor (bFGF) are used in this experiment. VEGF is requisite for vasculogenesis [3] and bFGF has less obvious effective for mESCs differentiation.[4] mESCs were cultured in an array of cell-culture wells for 7 days and 14 days, and each row of wells has a specific combination of VEGF & bFGF injected from inlet A and B as shown in Figure 4. At the end of each growth period, cells were labeled by surface markers anti CD34 & anti α-smooth muslle actin and the percentage of differentiation were counted for each GF's combination.

RESULTS AND DISCUSSION

Microfluidic Mixer Experiments: Figure 3(a) are microscope photos indicating the uniformly mixed reagents at the exit of the chaotic serpentine mixer stage in comparison to the clearly visible colored/clear boundary of reagents at the exit of the serpentine mixer at the stage 2 where the flow rate is 20 μL/hr. The intensity curves are shown in Figure 3(b) (analyzed by image-pro plus) indicating the uniform mixing of reagents of the chaotic serpentine mixer at the exit.

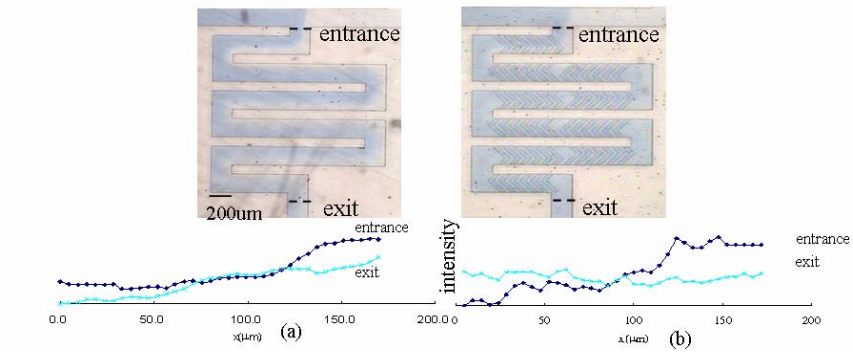

Figure 3. The heavy molecular reagent, Blue-dextran 2000(25 mg/mL), and DI water are injected to the serpentine mixer (a) and the chaotic serpentine mixers (b) to assess the mixing efficiency.

mESC Differentiation Experiments: Figure 5 (a) shows the experimental results of VEGF medium supplied from inlet A with a concentration of 10 ng/mL and

culture medium supplied from inlet B. No significant EC differentiation was observed on day 7, and significant differentiation is achieved on day 14. The differentiation ratios increase with VEGF concentration and saturated in a concentration of 5 ng/mL corresponding to those on row 5. Figure 5(b) is the experimental results of bFGF (20 ng/mL) medium supplied from inlet A and no significant influence to the mESCs differentiation into EC's. However, an optimum combination of VEGF and bFGF for EC's differentiation is found at the combination of 50%VEGF (50 ng/mL) and 50% bFGF (10 ng/mL) as shown in Figure 5(c). The developed microfluidics cell culture platform is demonstrated to be effective in quickly identifying the optimum GF combinations for stem cell differentiation.

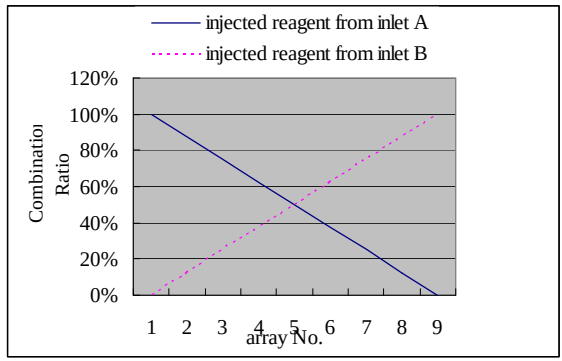

Figure 4. The cell culture array shows linear mixture compositions of those injected reagents from inlet A and inlet B.

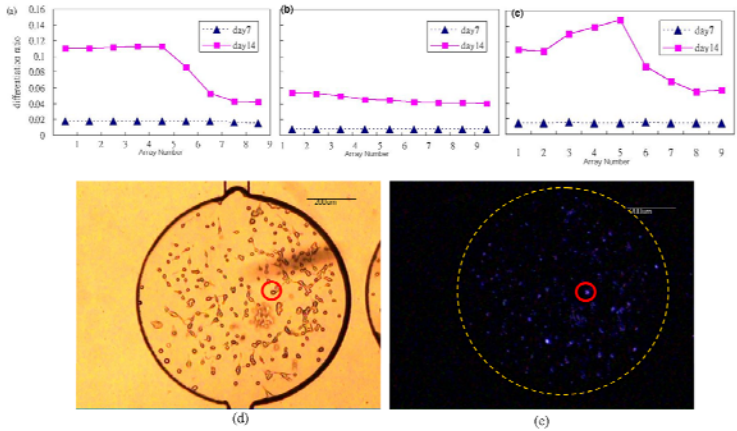

Figure 5. Differentiation ratio of mESC into EC's Medium after 7 and 14 days (a) with VEGF solution supplied from inlet A (10 ng/mL), culture medium supplied from inlet B, (b) with bFGF solution supplied from inlet A (20 ng/mL), culture medium supplied from inlet B and (c) with VEGF solution supplied from inlet A (10 ng/mL), with bFGF solution supplied from inlet B (10 ng/mL). (d) Photographs of differentiated mESCs treated by VEGF & bFGF in array 4 on day 14 (e) The fluorescence micrographs identifying the differentiated ECs. (labeled by CD34)

CONCLUSION

We have successfully designed and fabricated and demonstrated the continuous-flow cell culture array with chaotic mixers. By using the chaotic serpentine mixers, combinations of GF's concentrations can be reliably mixed and supply to each cell culture well in our continuous flow cell culture arrays. The optimum combinations of GFs (VEGF & bFGF) was quickly obtained for mESC differentiation into ECs & SMCs.

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