DUAL DIELECTROPHORETIC ASSEMBLY OF CO-CULTURES FOR THE STUDY OF CELL MIGRATION INDUCED BY CELL-CELL INTERACTIONS

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ABSTRACT

We present the differences in cell migration patterns of endothelial cells when they interact with cancer cells through a porous membrane barrier (1.2 µm pore size). Cells were dielectrophoretically trapped on opposite sides of an 11 µm thick polyester membrane assembled between two microfluidic channels perpendicular to each other. Endothelial cells at the crossover area, where they interacted with the HepG2 cancer cells, had larger displacements than cells away from that region. However, when endothelial cells were in monocultures, they showed no significant difference in their migration (i.e., directionality) behavior.

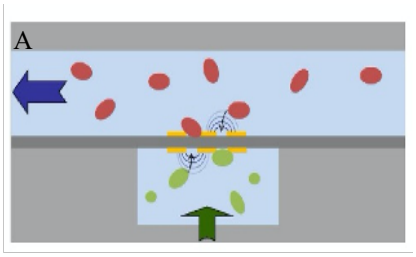
KEYWORDS: Cell migration, Endothelial cells, Cancer, Dielectrophoresis, Polyester (PET) membranes

INTRODUCTION

The interactions between cancer and endothelial cells are poorly understood and are of utmost importance during cancer metastasis. Specifically, steps such as cell intravasation and extravasation require exquisite communication and coordination between the cells involved in these processes [1]. Cell-cell communication is achieved via paracrine signaling and physical interactions, and represents a critical aspect of cell migration [2]. Thus, decoupling the intravasation rate-limiting stages by physically separating cells while permitting communication through a membrane will help provide new information about the mechanisms involved in cancer metastasis. Our method uses microfabricated electrodes on porous membranes (≈ 1.2 µm pore size) to dielectrophoretically (DEP) trap cells on opposite sides of the membrane, thus allowing them to interact in a controlled way by preventing the complete migration of cancer cells to the area where endothelial cells are located.

EXPERIMENTAL

Microfluidic devices were fabricated using poly(dimethylsiloxane) (PDMS), and assembled by bonding of oxidized PDMS surfaces to each side of a polyethylene terephthalate (PET) porous membrane. Fabrication of gold electrodes, ≈ 50 nm thick, was done by standard photolithographic processes. A hybrid cell adhesive material (hCAM) which consist of fibronectin and polyallylamine hydrochloride (PAH) was deposited layer-by-layer and incubated in the channels for a period of 90 min and 20 min, respectively [3]. This hCAM helped to hold cells in place after dielectrophoretic trapping. HUVEC (human umbilical veins endothelial cells) and HepG2 cells were harvested from tissue culture flasks. For dielectrophoretic trapping, cells were re-suspended in a sucrose solution with osmolality similar to the cell culture media used for each of these cells. Cells were DEP trapped using sine waves between 10 MHz and 12 MHz at < 2 V peak to peak (Fig. 1A) for a period of up to 10 min. Cell media was promptly replaced after dielectrophoretic trapping. Fluorescent cell trackers (PKH26 and PKH67 red- and green-fluorescent dyes, respectively) were used to monitor cell migration on both areas (Fig. 1B).



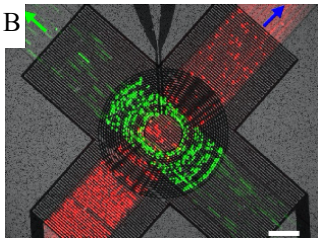


Figure 1: A. Scheme of the microfluidic device used for dielectrophoretic trapping of cells to gold electrodes on both sides of the membrane (dark gray). B. Micrograph of HepG2 cells (red) and HUVECs (green) dielectrophoretically trapped at the crossover area. Arrows show the direction of the flow. Scale bar: 250 µm.

RESULTS AND DISCUSSION

The cell-cell interactions between HUVEC and HepG2 cells were apparent starting 24 h after DEP trapping. Fig. 2A shows a mono-culture of HUVECs, 24 h after DEP trapping, covering mainly the crossover area. On the other hand, HUVECs gathered away from the crossover section (Fig. 2B, yellow ovals) when in co-culture with

HepG2 cells (green) 24 h after DEP trapping. The notion of migration guided by cell-cell interactions is supported by the observation of invadopodia-like formations (white arrowheads) by HepG2 cells after establishing a co-culture with HUVECs (Fig. 2C). Net cell migration distance on HUVECs was measured both inside and outside the crossover region (Fig. 3A). The ratio of net displacement (Δr) to the distance traveled (Δs) for the HUVECs at the crossover was about double the Δr/Δs observed outside that region (Fig. 3B). These results provide a view that could help illuminate the influence of cell-cell interactions between cancer and endothelial cells, thus providing an approach that could be used to gain knowledge of cancer metastasis.

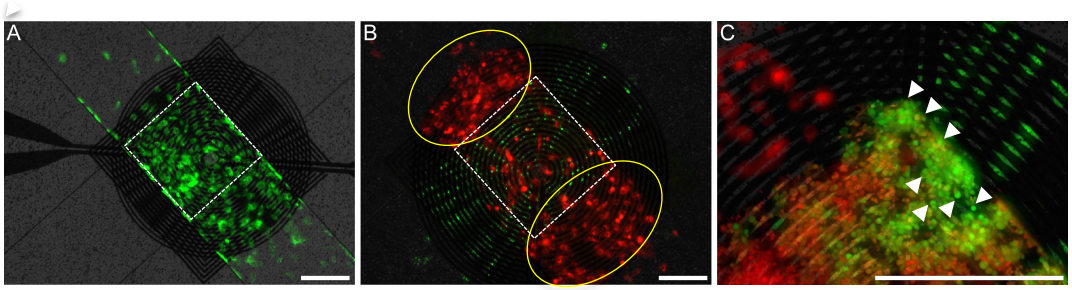


Figure 2. A. Mono culture of HUVECs (green) 24 h after trapping showing cell coverage within the crossover area. B. Co-culture of HUVEC (red) and HepG2 (green) 24 h after simultaneously trapping shows each cell type on opposite sides of the membrane. HUVECs are located mainly on opposite ends of the crossover area (yellow ovals). C. Micrograph of a close look at HepG2 cells showing projections in the form of invadopodia-like formations after 113 h in co-culture with HUVECs. Scale bars: A - 250 µm*, B and C - 200* µm*.*

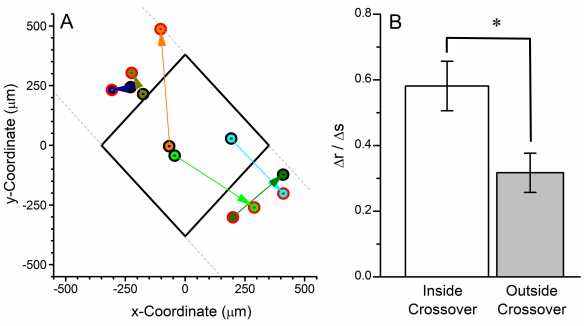


Figure 3. A. Comparison of HUVEC migration (in vector representation) in co-culture over a period of 113 h. The black square represents the crossover region. Each inner circle color represents a different cell. The black outer circle represents the starting point of cell migration, and the outer orange circle represents the ending point of the cell migration process. (The measured position uncertainty is comparable to the symbol size.) B. Migration calculated by the ratio of the magnitude of displacement (Δr) (i.e., the straight-line distance between the starting and ending points) to the distance traveled (Δs) (i.e., the total distance of the complete trajectory of the cells). Differences between cell migration behavior inside versus outside the crossover area are statistically significant at the 95 % confidence level. The error bars denote standard deviations from at least 10 cells.

CONCLUSION

Our method offers a unique approach to study cell-cell interactions while they are under optimal cell culture conditions (*i.e.*, cell media, cell adhesive material). Cell-cell interactions were controlled by using membranes with 1.2 µm pore sizes, thus preventing cancer cells from completely invading the side where endothelial cells were cultured. Optical monitoring of cancer-endothelial cell interactions demonstrated a difference in cell migration based on their environment (*i.e.*, HepG2-HUVECs interactions and HUVECs with no HepG2 interactions). This approach provides a tool to study cell-cell interactions and processes related to intra- and extravasion of cancer cells.

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