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OBSERVATION OF TRACTION FORCES DURING GALVANOTAXIS

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Introduction

Cell migration plays a critical role in many biological functions within multicellular organisms such as wound healing, immune response, and embryogenesis. A cell's inability to migrate can cause severe complications such as inflammatory or autoimmune diseases, defective wound healing or cancer metastasis [1].

External cues such as chemical gradients or mechanical stimulus can activate cell migration and guide a cell in one direction. However, in galvanotaxis, cell migration is directed by electric fields. Previous studies have shown these fields can polarize cells and examined the actin filament in the cell's cytoskeleton [2]. However, a cell's ability to sense the direction of the field and the underlying mechanisms involved in migration and orientation remain unknown.

A key factor in cell migration is the traction force transmitted from the cell to the substrate it moves along by means of focal adhesions. Currently, most galvanotaxis studies have not examined these forces. Here, we apply an electric field to cells *in vitro* and measure their traction forces using a micropost array, implemented in previous studies [3]. We hypothesize that the traction forces will increase with the application of an electric field.

Materials and Methods

Human pulmonary artery endothelial cells (HPAECs) were cultured in a media comprised of CO_2 Independent Media with 10% fetal bovine serum, 1% penicillin, 0.1% heparin, and 0.5% endothelial cell growth supplement at 5% CO_2.

Polydimethlysiloxane (PDMS) microposts were made on No. 2 cover slips and stamped with fibronectin. The microposts had 6 µm spacing and a diameter of 2.3 µm. HPAECs were seeded onto the posts and allowed to spread for four hours. HPAECs were incubated for 48 hours in tissue culture dishes before being seeded. The micropost array was placed in a 35 mm dish and two PDMS blocks were used to hold the array in place as well as creating a channel (Fig 1).

Platinum electrodes were placed into CO_2 independent media at the end of the channel, approximately 3 cm apart. A direct current electric field of approximately 4 V/cm was applied. The set up was placed in a live chamber that maintained 37°C and 5% CO_2 throughout the entire experiment.

Cell migration with and without the electric field was observed with time-lapse videos. Without the electric field, images were taken every five minutes for 1.5 hours. With the application of an electric field, images were taken every minute for an hour before toxicity in the chamber blurred the image.

Results

Cell migration was observed on the microposts. Without the electric field, the cell had random orientation and protrusions were observed (Fig 2). With an electric field, the cell changed its orientation and began to migrate towards the cathode (Fig 3), which was seen in bovine aortic endothelial cells previously [2]. The deflection of the microposts was apparent as the cell protruded forward. The results are promising for further analysis and long term studies.

Summary

Although galvanotaxis on microposts was observed, there were many limitations of the presented method and thus, changes will be implemented in future experiments. Because direct contact of electrodes in media with serum can create toxic products, future experiment will use a pair of agar salt bridges, a feature of many studies [2,4]. The agar salt bridge will help control ion and pH gradients in the electrotactic chamber. Image analysis in MATLAB

will measure the deflection of the post and quantify the traction force generated.

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References

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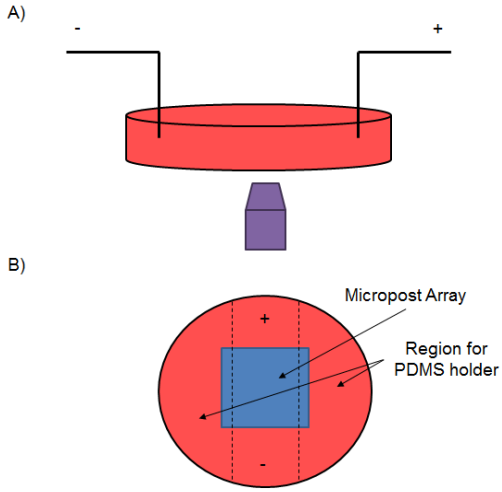

Figure 1: Experimental Design of the electrotactic chamber. (A) Side view with microscope lens centered in between the electric field lines. (B) Top view of the chamber.

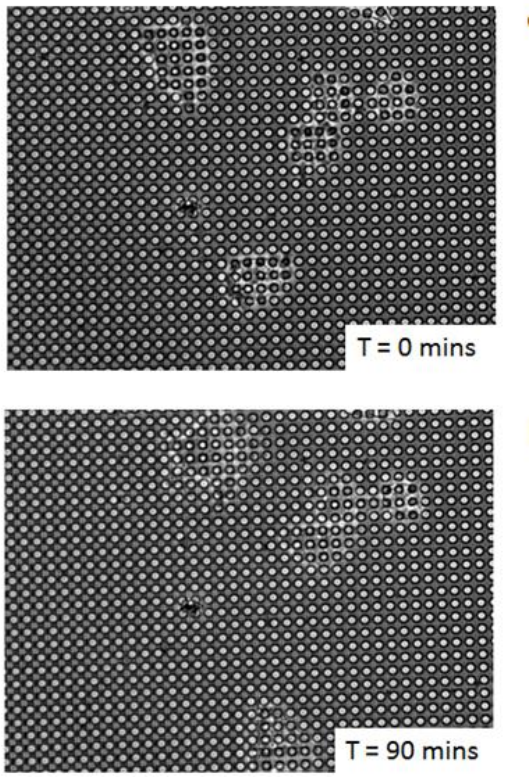

Figure 2: Cell migration on microposts without electric field at 0 and 90 minutes respectively.

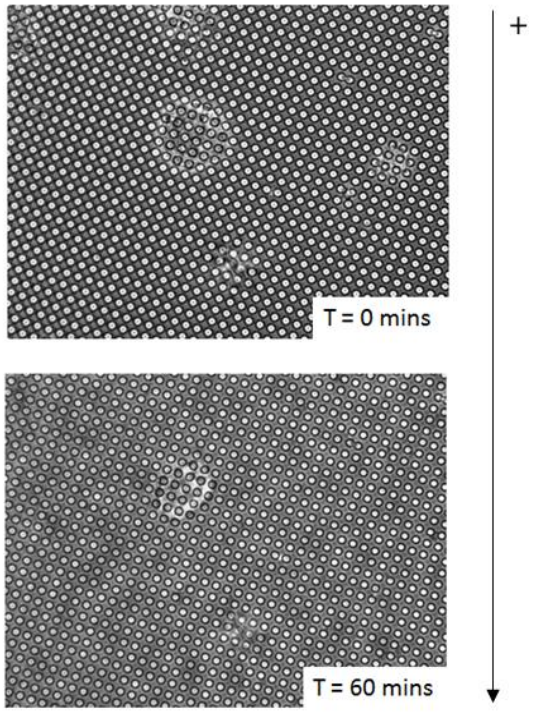

Figure 3: Cell migration on a micropost array with a 4 V/cm electric field at 0 and 60 minutes respectively.