Cell-Cell Communication: Cell Proliferation and Migration in Microfluidic Flow Cells

Scratch wound assays are commonly performed to assess cellular migration and cell proliferation in response to potentially therapeutic compounds in development. Further, wound healing assays are used to drift down to the molecular phenomena related to wound healing, including cell migration, matrix and healing response to bacterial cell-wall infections, and tissue remodeling. Most wound healing assays are performed using an approach by which the wound is established by scratching away a section of a monolayer of adhered cells, creating a gap by a scratch of a specified depth. This physically damages the monolayer as desired, but also damages the cells, causing a partial population of the monolayer to die or become non-viable. This complicates the interpretation of data and analysis and it is desired to control experimental conditions. In this study we generated wounds in microfluidic channels using parallel flow streams, which eliminate cell damage at the wound edge. The microfluidic channels in the BioFlux plate are identical within ±5%, conferring the ability to generate wounds by fluid flow that are highly regular in shape and similar in size.

Cytosolic leakage or debris were not a factor in migration, proliferation or the interpretation of the data. The other drawback of this method is that wound size and shape is highly variable contributing to difficulty in data analysis and inability to directly compare experimental conditions. In this study we generated wounds in the microfluidic channel using parallel flow streams, which eliminate cell damage at the wound edge. The microfluidic channels in the BioFlux plate are identical within ±5%, conferring the ability to generate wounds by flow streams that are highly regular in size and similar in shape.

Cytosolic leakage at the leading edge of migration was observed at 5.5 hours after wounding. The highest concentration of EGF led to the furthest migration under starvation conditions at 47 microns, predictably both the media alone control and the lower concentration of EGF led to migration as well. The CytoD treated cells (treated as early as 2 hours post-wounding and the monolayer began to contract. The anomaly in the migration distance for the 5 ug/mL concentration at 5.5 hours post-wounding is an example of mounted cells moved in the channel and was externally measured as an increase in the distance to the wall.

To study cell proliferation leading to wound closure, all treatments were delivered in complete media containing fetal cell serum which is permissive for growth. The cells in the media alone control were highly packed together structured up to 50% and closed the wound without spreading out by 20 hours and the gap was completely closed. EGF treatment caused cells to migrate away from the wall and spread out before closing the wound by protruding towards the wall. Cells exposed to the lowest concentration however came close to complete wound closure within 24 hours.

Summary

We adapted the commercially-available BioFlux system to perform a novel wound healing assay using parallel flow streams. A uniform and fully confluent monolayer is formed in one channel of the microfluidic device. A wound was generated in the parallel flow streams by closing the channel at one end while keeping the other open to create a single stream. To visualize the migration rate of the monolayer, the cells are stained with calcein, a non-fluorescent dye that fluoresces when exposed to microwaves. To assess cell proliferation, the cells are stained with calcein, a non-fluorescent dye that fluoresces when exposed to microwaves. To assess cell proliferation, the cells are stained with calcein, a non-fluorescent dye that fluoresces when exposed to microwaves. To assess cell proliferation, the cells are stained with calcein, a non-fluorescent dye that fluoresces when exposed to microwaves. To assess cell proliferation, the cells are stained with calcein, a non-fluorescent dye that fluoresces when exposed to microwaves.

We also showed that cell proliferation can be assessed in the same manner.

Well Plate Microfluidics

A Well plate microfluidic is based on an SBS plate with up to 24 shear controlled microfluidic channels, a.k.a. micro-sized flow cells, coupled to the walls (top right). All reagents and cells are loaded in the wells to be introduced into the microfluidic channels (above).

The device is self-contained and flow is controlled using pneumatic pumping. Data are collected using an inverted microscope and digital camera.

Control of parallel flow inter-device using the 24-well BioFlux plate.

Applied pressure (PSI) was constant on inlet A (black) and varied on inlet B (green). Area covered by calcein fluorescent dye was measured using morphometry analysis in Montage. Error bars were measured standard deviation for a total 14 channels.

When flowed under laminar flow dynamic conditions, fluids were shown to remain separated in non-mixing streams. A change in pressure (green trace) at the end of the channel. Error bars were ±2 standard deviation of the mean (black trace).

In panel B, laminar flow conditions were demonstrated by flowing a fluorescent dye from one inlet (starved) and a non-fluorescent dye from another inlet (feeding). No mixing was applied. No pressure of the inlet with dye or the inlet with buffer resulted in a channel with no fluorescent dye or buffer. Equilibrated applied to both inlet resulted in equal mixed channels of fluorescent dye and non-fluorescent buffer (green trace).

Create a wound monolayer using parallel flow. Cell proliferation and migration after wounding. Measurements were made at t=0, 0.5, 5, and 24 hours post-wounding.

(a) Cells migration was measured as the distance from the monolayer edge to the leading edge of the wound at 3 time points for each channel. Distance migration was calculated by subtracting the leading edge distance from the starting position of the leading edge. Distance measurement includes the area of the wound, which indicates contraction of the area or loss of cells from the monolayer.

(b) Cell proliferation was measured as the total area covered by cells in motion. The change in area is assessed in micrometers for each of the 0.5, 5, and 24 hour points using a non-fluent micrometer. The measurement includes the area of the wound, which indicates contraction of the area or loss of cells from the monolayer.