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## Wound healing assay in a low-cost microfluidic platform

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**Abstract.** Cell migration is a very important stage in different pathological processes such as wound healing, vascularisation, immune response, tissue regeneration and cancer. Traditional wound healing assays are done by scratching a monolayer on standard culture surfaces. However this method can lead to data misinterpretation and poor comparability in results from different experiments. The microfluidic wound healing assay is a mature alternative to the traditional method, but it is expensive and bulky due to the need of syringe pumps and can be difficult to implement by the end user. Here we show an alternative low-cost microfluidic platform based on the MainSTREAM platform. We also demonstrate the feasibility of a microperistaltic pump to carry out these kind of assays. A laminar flow of PBS-Trypsin-PBS confined in a microfluidic channel with a previously cultured monolayer of IIB-Mel LES melanoma cells was used to generate the wound. Cell migration rate could be quantified successfully.

### 1. Introduction

Cell migration is the movement of cells from one location to another and it can involve single cells or groups of them and it is regulated by different phenomena such as physicochemical gradients [1], cell-cell contacts [2] and substrate topology [3] among others. Cell migration is an essential step in different pathologic and physiological processes such as wound healing, cancer, inflammation, cell growth and differentiation [4]. There are several in-vitro cell migration assays [5]. The most common are the wound healing assay and the Boyden chamber assay. The first is performed by introducing a wound by scratching the cell monolayer with a pipette tip, a blade or similar. The migration rate can be quantified by monitoring cell movement towards the artificially generated wound. The Boyden chamber assay is performed by seeding cells on top of a permeable membrane that separates two culture chambers. Cell migration can be quantified by observing the number of cells that migrate through the membrane.

Although the traditional wound-healing assay is simple, economical and can result in very high throughput assays [6], the inherent nature of the scratching can damage the cells on the monolayer which can cause leaking of the intracellular contents towards the wound complicating the interpretation of data [7]. Also the artificially generated wound size in this method can vary up to 33% [8]. With these drawbacks in mind, data analysis and direct comparison of experimental conditions can



be misleading. Furthermore, in the traditional assay, cell culture media and reagents volumes are not insignificant.

Some of the aforementioned shortcomings have been already addressed utilizing microfluidics [9] and it has been demonstrated that this new assay is a mature alternative for the conventional wound-healing assay [10]. In this assay the cells are cultured inside a microfluidic channel and the wound is generated by parallel flows of trypsin and buffer/media that mix only by diffusion (due to the laminar flow condition). The trypsin flow detaches the exposed cells, while the cells exposed to the buffer remain attached. Migration rate of the cells towards the generated wound can be quantified like the standard assay. Another advantage of microfluidic systems is that physicochemical gradients and shear stress can be easily generated [10, 11]. Furthermore, it offers a much better control over the cell microenvironment being possible, for example, to reduce dilution of secreted cell signaling molecules in the culture media (due to the small volume of the microchannels) [12].

Although such devices offer an alternative solution to the traditional wound healing assay, they are quite difficult to implement by the end user, i.e. biologists, and have a high cost associated with support equipment, i.e. syringe pumps can cost more than 4.000 US dollars each and two of them are needed at least. Moreover, they might be difficult to place near or within detection instruments, incubators and so on limiting system portability. Furthermore, long tubing is required for interfacing the microfluidic chips to macro world, resulting in large dead volumes and poor liquid control due to pressure buildups [13].

Here, we propose a low-cost microfluidic system for the implementation of the wound healing assay based on the MainSTREAM platform developed by Sabourin et. al. [13]. This platform incorporates all components required for the flow of sample to waste through the Lab On a Chip system (i.e., pumps, valves, microfluidic reaction chips, reagent vials, waste containers, component interconnections, and computer controlled actuation). We demonstrate for the first time the use and feasibility of a low-cost microperistaltic pump for the generation of the multiple parallel laminar flows needed for the wound generation. IIB-Mel LES melanoma cells with well-characterized migratory phenotype [14] were used for this study.

## 2. Materials and Methods

### 2.1. Microfluidic chip and microfluidic platform

Microfluidic channels were milled in polymethyl methacrylate (Clarex, Japan) layers by a CNC milling machine (Minuth, Argentina). For best optical results, the cell culture channels were all-through milled in a 0.5 mm thick PMMA layer and other two 1.0 mm thick layers were used for sealing and connection purposes according to the MainSTREAM platform standards [13]. The PMMA layers were then bonded via a UV-assisted bonding process [15]. Bonding faces of the PMMA layers were exposed to a 250W mercury UV light for 2 minutes, aligned in a CNC milled aluminum frame and subsequently bonded in a bonding press (Shimeq, Argentina) at 80°C and 2MPa for 15 minutes. The bonding press was then fan cooled until the plates' temperature reached 40°C in order to avoid deformations and cracking of the chips.

PDMS (Sylgard 184; Dow Corning, USA) used for elastomeric components was mixed in a 10:1 mass ratio of elastomer to curing agent and placed under vacuum to remove air bubbles. PDMS components were cured overnight at 65°C.

The PMMA base plate, pumps, connectors, vial holders, tubing interfaces were also milled with a CNC machine, designs are described elsewhere [13].

Inlets and outlets of the chips were connected to 10 ml glass vials (Sigma Aldrich, USA) via 1.6mm OD and 0.2mm ID PTFE tubing (Bola, Germany). Vials were held in position by custom made PMMA vial holders attached to the base plate. In order to avoid bubbles the inlet and the outlet vials were pressurized with 0.5 bar filtered air.

LEGO Mindstorms NXT 2.0 servomotors and control unit were used to drive the pumps.

A photograph of the complete system can be seen in Figure 1.



**Figure 1.** The complete microfluidic system for the wound-healing assay is shown on the left. On the right, it is shown that the complete system can fit easily within an incubator, only a power cable and gas connection for pressurization are needed. Note the small footprint and portability.

### 2.2. Assembly, sterilization and preparation of the chip and microfluidic platform

The complete system was assembled in a laminar hood under sterile conditions. Glass vials, screw caps and PTFE tubing were previously autoclaved. When the system was fully assembled, sterilization of pumps, connectors and the chip was done by flushing a 0.5 M NaOH solution at 5  $\mu$ l/min for 30 min. This was followed by 30 min washing with sterile H<sub>2</sub>O to remove all the remaining NaOH and finally coating the chip by flushing it with complete medium (10% FBS (Natocor, Argentina) in DMEM (Invitrogen, USA)) at 5  $\mu$ l/min for 1 hour at 37°C.

### 2.3. Cell loading and culture

Subconfluent IIB-Mel LES cells were detached with 0,05% trypsin-EDTA, counted in a haemocytometer and resuspended at 2x10<sup>6</sup> per ml in complete medium.

Cell loading was done from the outlet connector chip (previously removing the outlet tubing) of the system and with the pump in reversal mode, i.e. sucking. LEGO motors power was set to 90% in order to have a good cell seeding distribution [16].

Cells were left to attach with no flow for 2 hours. Then, the cells were flushed with a 50 nl/min flow for 5 hours and finally were left in culture with a 500 nl/min flow overnight or until the monolayer was formed.

### 2.4. Laminar flow confirmation and microfluidic wound assay

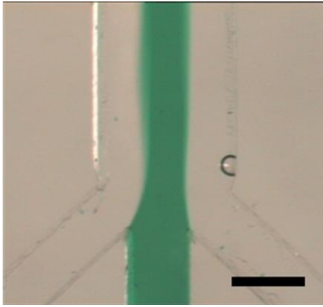
In order to confirm the laminar flow inside the cell culture channel, the outer vials were loaded with miliQ water and the center vial with green food dye. Pumps were started and observation of the laminar flow was carried out with a stereoscopic microscope coupled with a CCD camera.

For the wound assay, the middle vial was replaced with 0,05% trypsin-EDTA (Invitrogen, USA) and the outer vials with PBS or media with no serum in order to avoid deactivation of the trypsin. In this way, only the center portion of the cells are to be removed. The pumps were set to 50  $\mu$ l/min at 37°C for 20 min. Confirmation of the wound was observed in a contrast phase inverted microscope. Then, the cells were left at 500 nl/min flow with fresh media and pictures were taken at 1 hour, 2 hours, 3 hours, 5 hours and 18 hours.

### 3. Results

#### 3.1. Visualization of laminar flow

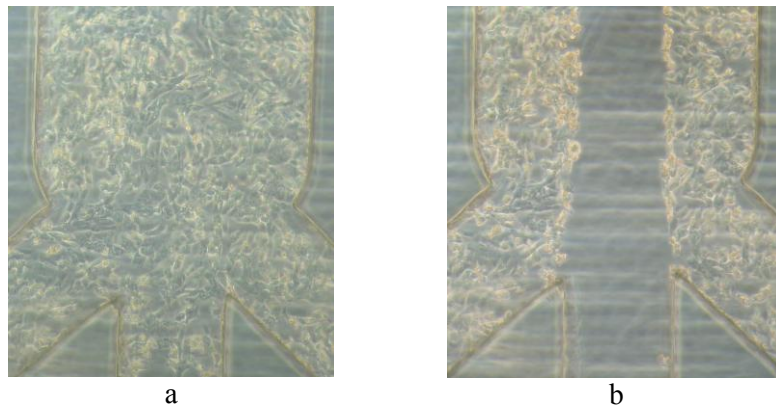
We were able to effectively obtain a clearly defined laminar flow in the centre of the culture channel. A photo of the assay can be observed in Figure 2. In this experiment, the Reynolds number was 2.12 ( $D=500\ \mu\text{m}$ ,  $\nu=1.10\text{e-}6\ \text{m}^2/\text{s}$ ,  $V=0.00424\ \text{m/s}$ ), confirming that the laminar flow was stably formed.



**Figure 2.** Laminar flow confirmation inside the microfluidic channel. Scale bar is 500  $\mu\text{m}$ .

#### 3.2. Visualization of the wound

As expected, cell detachment was observed in the centre of the channel confirming also the laminar flow inside the microfluidic channel. Photographs of the cells cultured inside the channel before and after trypsin flushing can be seen in Figure 3. We show the beginning/merging point of the channel in order to be able to compare with the laminar flow seen on Figure 2.

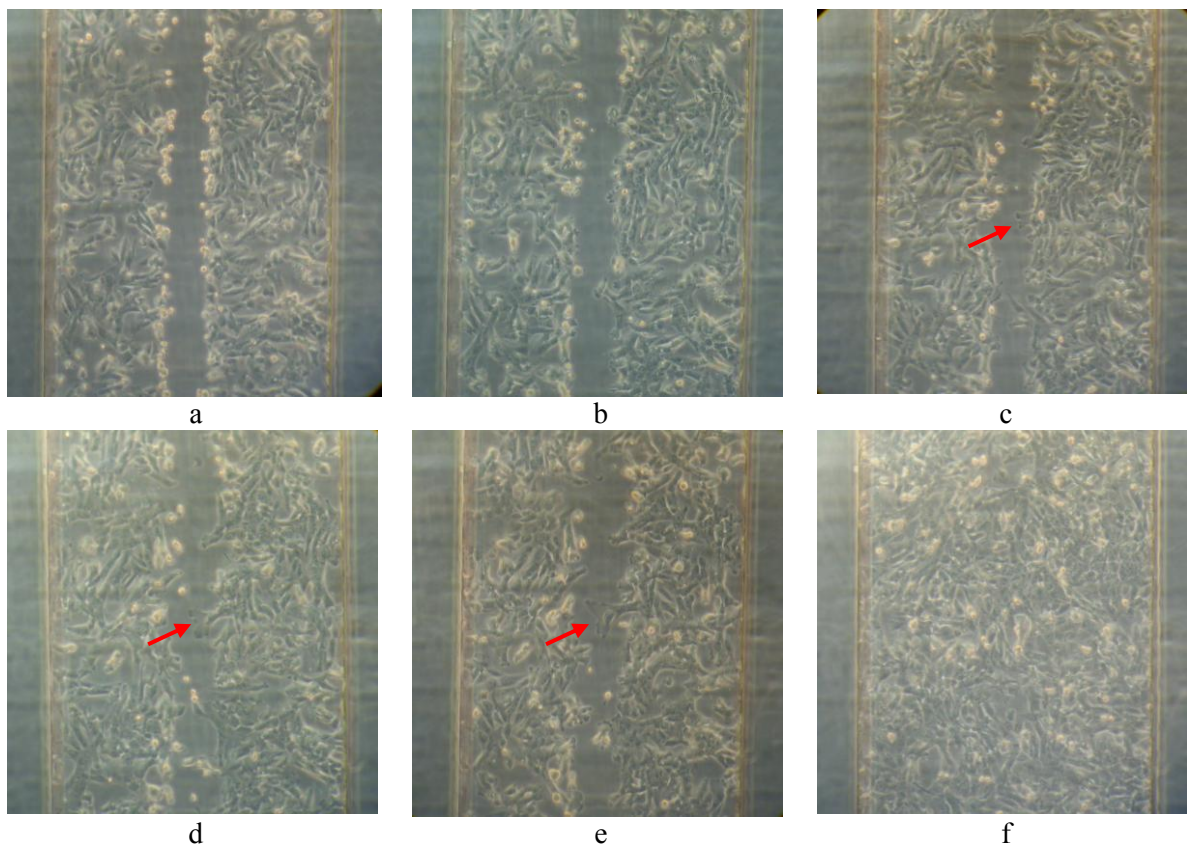


**Figure 3.** Cell monolayer inside the microfluidic channel after 24 hours of culture a) before and b) after the trypsin flushing.

#### 3.3. Cell migration after the wound assay

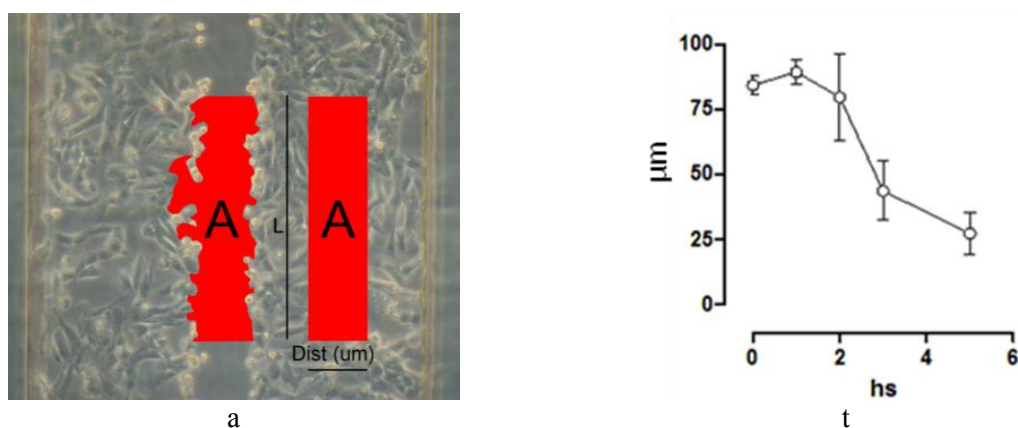
Photos of the cell migration towards the wound at different times can be observed in Figure 4. Pictures were taken at the center region (related to the length) of the microfluidic channel instead of the beginning/merging point as showed in Figure 3. This was done in order to ensure that the flow was fully developed. Note the invasion and pseudopodia extension of some of the cells in the centre of the photos pointed out by the red arrow.





**Figure 4.** Cell migration observation. a) Just after the trypsin flush, b) 1 hour after the wound, c) 2 hours after the wound, d) 3 hours after the wound, e) 5 hours after the wound and f) 18 hours after the wound. The red arrow shows migration and pseudopodia expansion of a particular cell at different times.

Quantification of the migration process can be seen in the graph of Figure 5. The migration distance was calculated as the short side of the rectangle with 500  $\mu\text{m}$  of length without cell in the wound. Such area was measured in the segmented image using ImageJ software.



**Figure 5.** Quantification of the cells' migration towards the wound. a) Representation of migratory distance calculus. Wounds areas were measured in segmented images and  $l$  (Dist.) were calculated as  $l=A*L^{-1}$ . t The average of three areas of the wound was calculated. Values represent the mean  $\pm$ SD in every time measured.

#### 4. Discussion

We have demonstrated that it is possible to carry out microfluidic wound healing assays in a low-cost and easy to use microfluidic platform. A complete microfluidic system (including the control units and motors) can cost down to 20 times less than the systems based on syringe pumps. We have also demonstrated the feasibility of the microperistaltic pump for performing this kind of assays. Moreover, multiple migration assays can be carried out at the same time and in the same chip because of the pump's 8-channels design. Just for numbers, 4 simultaneous migration assays can be carried out with one pump, eight with two pumps and 16 with four pumps. It is very easy and straightforward to put these 4 pumps in a single platform because of the innate modular design of the MainSTREAM platform. Thus, considering the cost per migration assay, using the four pumps at a time, we could roughly estimate the initial cost per assay in our platform as 35 USD.

#### 5. Conclusion

A low cost and easy to use microfluidic system with a microperistaltic pump was developed and tested. Wound edges of confluent cell monolayers for cell migration assay were obtained using a combination of laminar flows with media/PBS and trypsin in such a way that the central portion of the channel is in contact with the trypsin flow. This central channel can be coated with different matrix proteins as well as with proteins and factors or combinations to be tested. In addition blood flow can be simulated for endothelial cell assays and dynamic adhesion assays with tumor cells on endothelial monolayer. Although the preferred method is the Boyden chamber, it has the disadvantage that it cannot be monitored in real time, and gradients through pores could be disrupted by cell migration itself.

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