VASCULAR MODELS: WHY CHOOSE A PRESSSURE-BASED FLOW CONTROLLER OVER A PULSATILE PERISTALTIC PUMP?

INTRODUCTION

Microfluidic cell culture has significant advantages over macroscopic culture in flasks, petri dishes and well-plates. This technology offers new possibilities to accurately reproduce the cellular environment and enables the analysis of biochemical processes that were not accessible before. Cost reduction due to volume reduction is also a major benefit of microfluidics as many reagents for bioassays or cell culture studies can be costly.

One of the primary contributions of microfluidics to cell biology is the capability to perfuse cells. The first experiments that showed unprecedented results compared to static conditions were conducted on vascular cells. Endothelial cells grown under biomimetic shear stress expressed physiological phenotype, proliferated, aligned and met living tissue permeability.

In order to mimic in vivo conditions, the type of perfusion system used is critical. Peristaltic pumps are widely used. Although relatively inexpensive, they deliver a highly pulsatile flow that oscillates around the set flow rate value. These erratic oscillations are not representative of any physiologic condition in the body and can damage cells. Conversely, pressure-based system can deliver either constant flow or on-demand realistic pulsatile flow patterns simulating aortic flow. In addition, both flow rate and pressure values are accessible for monitoring the parameters of an experiment.

Regarding cost reduction, in addition to volume reduction, cell culture medium can be recirculated. This function can be implemented in pressure-based system circuit by coupling an automatic valve to the flow controller.

Table 1: Advantages and disadvantages of pressure-based flow controllers and peristaltic pumps

	Fluigent pressure-based flow controllers	Peristaltic pump
Flow stability	High (<1% variation)	Low (>20% flow variation)
Response time	High	Medium
Pressure control	Yes	No
Price	Medium - High	Low
Implementation	Requires pressure source	Easy

To demonstrate the importance of flow stability in vascular models, endothelial cells seeded in microfluidic chip were perfused either using peristaltic pump or pressure-based flow controllers.

MATERIALS AND METHODS

Materials

Microfluidic flow controller

The Flow EZ is the most advanced flow controller for pressure-based fluid control. Two Flow EZ with 1 bar are used in the system presented here.

Flow sensor

The Flow Unit is a flow sensor that allows real time flow rate measurement. One Flow Unit M is used here to monitor and control the flow rate.

L-SWITCH

The L-Switch™ is a bidirectional 6-port / 2 position valve for injection or switching different fluids. Its configuration make it ideal for fluid recirculation experiments.

SWITCH-EZ

The LineUp™ SWITCH EZ is a module allowing one to control Fluigent's microfluidic valves. The module has 6 ports and can be combined with other LineUp™ products to have a complete and compact system for benchtop use.

Microfluidic chip:

BeOnChip - BE-Flow**:** is an easy-to-use device dedicated to cell culture under flow. It allows the performance of long term 2D or 3D culture in two independent channels.

Fluid recirculation for cell perfusion

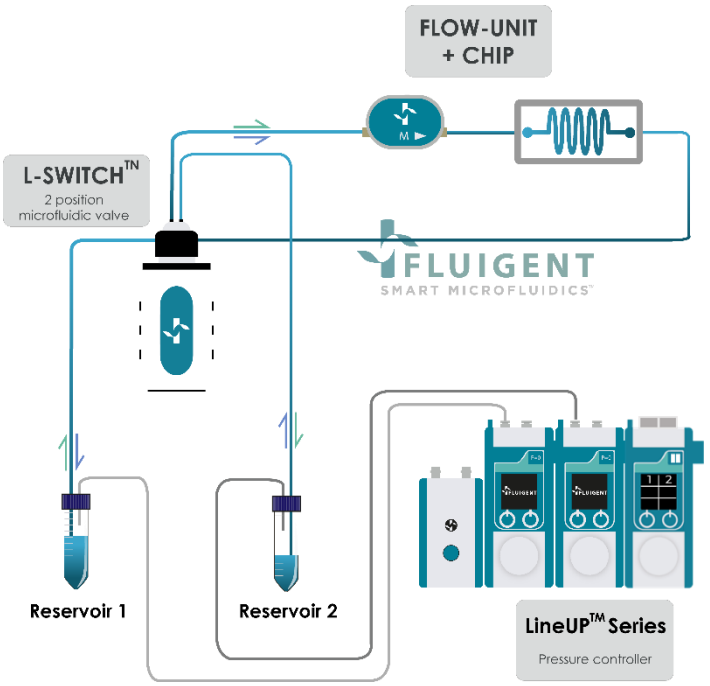

Figure 1: Working principle of the recirculation system using pressure-based flow control. Two Flow EZ are connected to two reservoirs. Tubing pass through the L-SWITCH to permit recirculation, a flow unit, and the microfluidic device. Fluigent software are used for protocol automation

Figure 1 shows the working principle of the recirculation system using pressure-based flow controllers.
Two Flow EZ are connected to two 15 ml reservoirs. Tubing pass through the L-SWITCH (allowing media recirculation), a flow unit, and the microfluidic device. The software used to control the system is OxyGEN. In the system using the peristaltic pump, the inlet and outlet tubing are both placed in a reservoir containing media which continuously flows within the microfluidic device. In both systems, the flow rate was monitored with a Flow Unit to evaluate fluctuations.

Protocol for microfluidic cell culture

To compare both flow systems, Be-flow microfluidic devices (BeOnChip) were seed with endothelial cells (HBEC-5i: ATCC® CRL-3245™). HBEC-5i cells grow using DMEM-F12 culture media supplemented with 10% FBS, 40 µg/mL endothelial growth supplement, and 1% antibiotics.

Steps

1) Coat the BE-Flow channels with a solution of 0,1 mg/mL collagen type 1 at 37°C
2) Seed cells in a suspension until monolayer is formed (48 h).
3) Connect the BE-Flow to the microfluidic system following the instructions of https://www.youtube.com/watch?v=uDObVW0MqsA&feature=emb_logo&ab_channel=BEONCHIPSL.
4) Set the flowrate at 50 µL/min and leave it working in this condition for 24h inside and incubator at 37◦, 5% of CO_2.

Brightfield and fluorescence microscopy was used to visualize cells before and after flow (Nikon Ti-E microscope). Nuclei were stained with Hoestch blue fluorescent dye. Image analysis was performed using FIJI software.

RESULTS & DISCUSSION

1. Cell seeding (and fluid actuation)

After coating the microfluidic device with collagen, HBEC-i cells are seeded within it. Figure 2 shows cell adhesion to the chips before perfusing with the different pumps. Cells reach a~80% confluency in both chips. Cells are well spread, with polygonal or trigonal shape, suggesting that they are healthy. It is noteworthy that there is no significant difference in term of confluence nor shape between the two cultures. Before flowing liquid to the microfluidic devices, the two cell cultures are similar in term of viability and confluency.

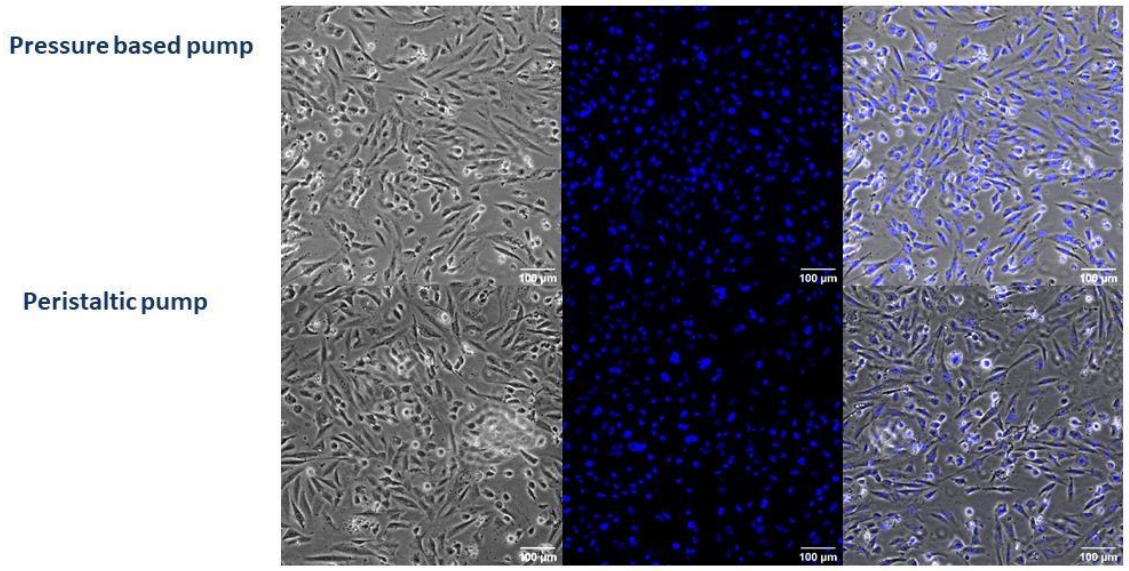

Figure 2: Contrast and fluorescence microscopy images of HBEC-5i cells seeded in BE-Flow microfluidic devices before flow. Nuclei are stained in blue with Hoestch dye. Upper row: Pressure pump at t = 0 h. Lower row: Peristaltic pump at t = 0 h. Scale bar is 100 µm.

2. Fluid Perfusion

Once cells are well adhered, media recirculation is performed by connecting Fluigent Flow EZ (pressure based flow controllers) or peristaltic pump to each microfluidic device with a set flow rate of 50 µL/min for t = 24h. Figure 3 shows the flow rate as a function of time using the peristaltic pump (in orange) and the Flow EZ (in blue). Using the peristaltic pump the flow rate highly fluctuates, with flow minimum and maximum respectively of about 30 µL/min and 65 µL/min. This corresponds to more than 40% flow variation compared to the desired value of 50 µL/min. It is a critical parameter, as strong and unbalanced pulses of over 40% of the ordered flow rate can induce cell detachment, cell death (loss of plasma membrane integrity), and can also impact cell function (proliferation, gene expressions). When using the Flow EZ, we observe a highly stable flow rate with flow maximum and minimum of 50 µL/min and about 49 µL/min (figure 3), corresponding to less than 2% flow variation. Flow rate is highly stable using the Flow EZ, exposing cells to a smooth constant shear stress comparable to the one experienced in vivo.

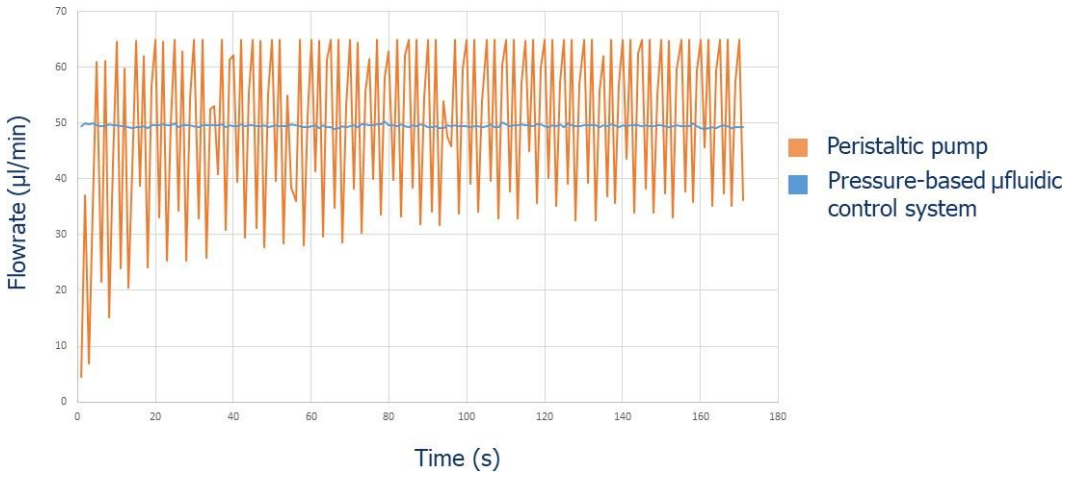

Figure 3: Flow rate as a function of time using peristaltic pump and pressure-based flow controller (Fluigent Flow EZ). Flow rate was set at 50 µL/min on both devices

3. Cell viability after 24h

After performing media recirculation for 24h, cell cultures are observed under a microscope. Figure 4 shows micrographs of HBEC-i cells after 24h in the microfluidic devices perfused with the Flow EZ and the peristaltic pump. In the cell culture perfused with the peristaltic pump, we observe a decrease in cell density when compared to t=0. In fact, we observe cells at ~ 50% confluency, while cells were at ~ 80% confluency at the start of the experiment. This decrease of about 30% of cell density suggests that the large flow rate fluctuations led to cell detachment. In addition, cells are roundly shaped, as opposed to the well spread cells with trigonal shape observed before perfusion. This suggests that, even though not detached from the device, cells show less adhesion, are less viable, and cell function might have been impacted by the poor flow conditions provided by the peristaltic pump. In the cell culture perfused with the Flow EZ, we observe a similar cell confluency of ~80% when compared to t=0. In addition, cells have similar spreading, with trigonal shape. These results confirm that cells perfused with the Flow EZ remained healthy and viable because of ideal flow conditions.

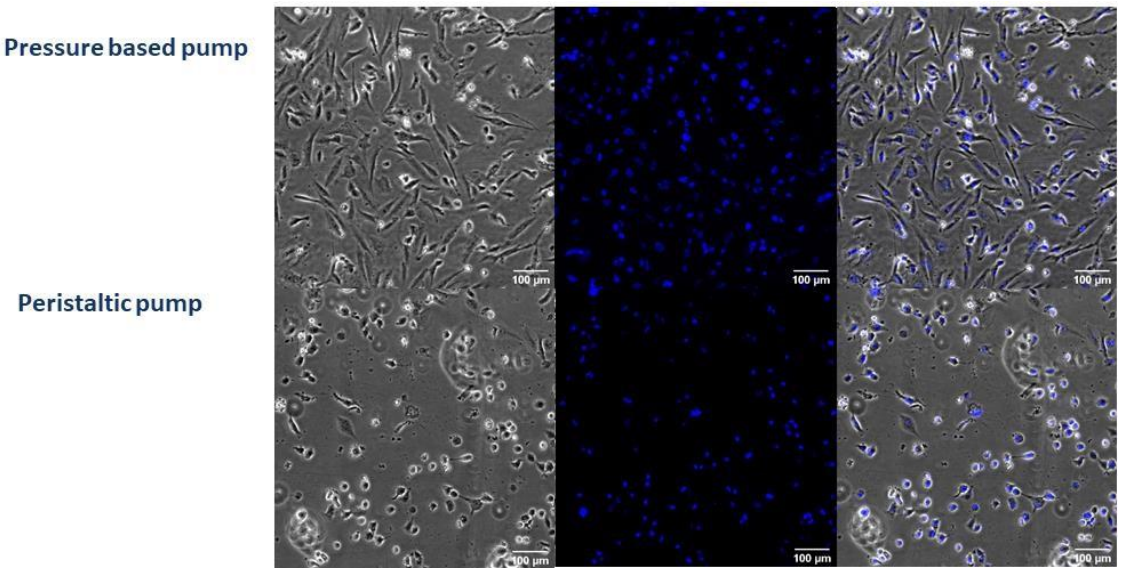

Figure 4: Contrast and fluorescence microscopy images of HBEC-5i cells seeded in BE-Flow microfluidic devices after t=24h of flow at 50 µL/min. Nuclei are stained in blue with Hoestch dye. Upper row: Pressure pump. Lower row: Peristaltic pump. Scale bar is 100 µm.

Table 2 summarizes the results obtained. It demonstrates that pressure-based flow controllers are less aggressive to cells compared to peristaltic pumps under identical experimental conditions.

Table 2: Summary of results obtained using the two flow controllers

	Fluigent Flow EZ (pressure-based flow controller)		Peristaltic pump	
	t=0	t=24h	t=0	t=24h
Cell confluency (density)	~80%	~80%	~80%	~50%
Cell Morphology	trigonal	trigonal	trigonal	round shape

CONCLUSION

When switching from conventional cell culture in flasks to microfluidics, attention is focused on the chip. We have demonstrated that the perfusion instrument is as important as the chip. Choosing the right instrument to reproduce the flow conditions cells experience in vivo is of major importance as it will impact cell survival, spreading, phenotype and extend their genetic expression.

In the above application, the results are striking. After only 1 day of perfusion, vascular cells grown under erratic pulsatile flow are dying. On the opposite, when submitted to laminar constant shear stress, similar to living conditions, cells survive and are nicely spread in the chip. The main results of this application note can be summarized as follows:

- The flow rate varied considerably using the peristaltic pump, with 40% flow variation.
- Using the Flow EZ, a stable flow rate was observed, with less than 2% flow variation.
- Perfusing endothelial cells for 1 day with a pulsatile flow induced a 30% cell detachment and strongly impacted cell spreading and phenotype as they appear roundish and unhealthy.
- A stable constant flow rate for a day promoted endothelial cell survival and the maintenance of a physiological phenotype.