Standard Operating Procedure **SOP202**

Title: Elveflow microfluidic system for organ on the chip assays

Location: H25 in CBE

1. PURPOSE

The intent of this SOP is to describe the method of how to use Elveflow OB1 MK4 pressure-driven flow control technology for microfluidics.

2. SCOPE

This SOP applies to CBE personnel using the Elveflow OB1 MK4 microfluidic system located in CBE laboratory H25 for culturing cells on the chip.

3. RESPONSIBILITES

CBE Laboratory Users

Shall
1) Work in accordance with the instructions for the operation and maintenance detailed in this SOP, Risk Assessment and the operator manual.
2) Carry out the routine inspection, cleaning, and maintenance of the equipment, as required.
3) Record any adverse events and alarms that indicate non-conformance or malfunction on the Maintenance Record and notify the Laboratory Manager/Responsible Person

Responsible Person (RP)/Laboratory Manager (LM)

Shall
1) Ensure that authorized laboratory personnel are given suitable information, instruction, training and supervision in the correct use and maintenance of the equipment.
2) Investigate any reported problems, adverse event, alarms, or non-conformities associated with equipment usage.
3) Organise the maintenance, repair, or servicing of the equipment by trained and authorized contract/service personnel.

4. EQUIPMENT AND MATERIALS

a) Equipment & devices

1) Elveflow OB1/MK4 pressure controller and accessories
2) Hyundai 8 Litre Air Compressor

3) Biological Safety Cabinet
4) BD 6220 CO2 incubator
5) Bench-top Centrifuge
6) Water bath (37°C)
7) Countess automatic counting system
8) Microscope, inverted

b) Consumables

1) Appropriate PPE (labcoat, gloves, overshoes and eye protection (where appropriate))
2) Serological Pipettes: 1ml, 2ml, 5ml, 10ml, 25ml)
3) 50ml Centrifuge Tubes
4) Corning® BioCoat™ Collagen I 75cm² Rectangular Canted Neck Cell Culture Flask with Vented Cap (356485)
5) microfluidic chip/ μ-Slide I Luer Collagen IV (Ibidi, C-80176)
6) 2ml Cryo S tubes
7) Eppendorf tubes (1ml, 5ml)
8) Syringe BD Plastipak 20ml
9) Sterile filter (0.2μm)
10) Pipette tips (20μl, 200μl, 1250μl)
11) Endothelial Cell Growth Medium 2, Basal Medium 2 (Promocell, C-22211)
12) Growth Medium Kit 2 SupplementPack (Promocell, C-39211)
13) Dulbecco's Phosphate Buffered Saline (DPBS), w/o Ca and w/o Mg
14) Trypan blue stain (0.4%)
15) TrypLE Select (1X) no phenol red (Gibco)
16) Human brain microvascular endothelial cells (Creative Bioarray, CSC-C1503)

5. PROCEDURE

5.1 Set-up of the Elveflow microfluidic system (see Fig.1):

*All the equipment must be placed on a stable surface (e.g., workbench, floor) and kept in a dry environment (except the microchip with cells- placed inside the incubator or the sterile workbench).
*OB1 Pressure controller should be higher or at least the same level as the other elements (never placed below the level of the Flow Sensor or the reservoirs).
*The flow sensor should be laid flat on the setup bench

Hyundai 8 Litre Air Compressor set-up:

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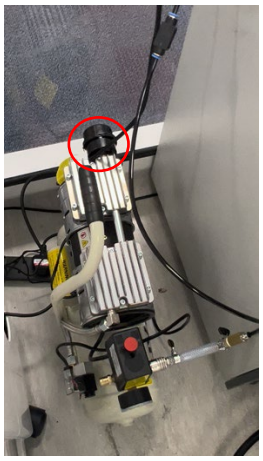
1) The Hyundai 8 Litre Air Compressor is factory set to deliver up to 8bar. It provides compressed air to an OB1 pressure regulator with two 0-2 bar channels. The pressure source offers a steady pressure of 2000mbar, making it the perfect companion for 2000mbar OB1 pressure regulators.
2) Connect air intake cap with compressor that allows to set the output pressure as low as 1.5 mbar.



4) Connect Air drier/Particle Filter between OB1 and air compressor using 6mm cables.

4) Connect the power supply. Once the valve is open, the compressor is running and connected to the OB1. When the tank is filled with compressed air, the pump stops. You can then pull the cap upwards to be able to turn it and adjust the output value. Then secure it again by putting it back in the low position.

OB1 Mark 4 Pressure controller installation (see Figure 1-3 for system set-up):

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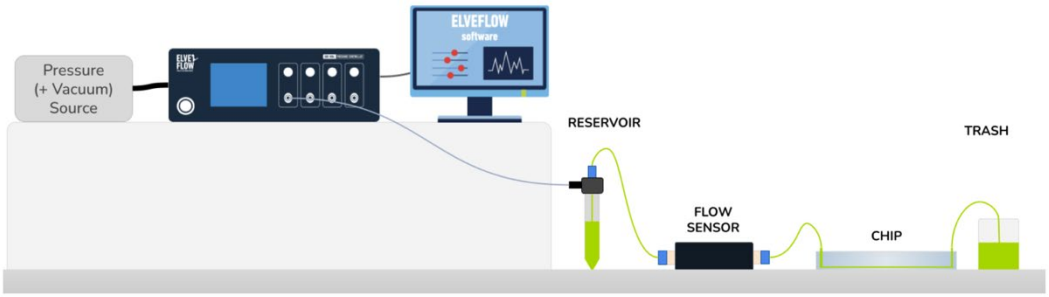


Fig 1. Overview of the simplified Elveflow pressure-driven flow control system setup.

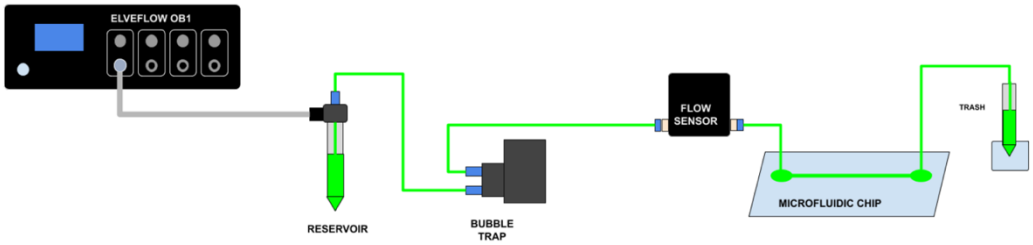


Fig. 2. Example setup using bubble-trap to remove possible in-line bubbles injected into microfluidic chip

Solenoid valves 1-4

MUX valve controller

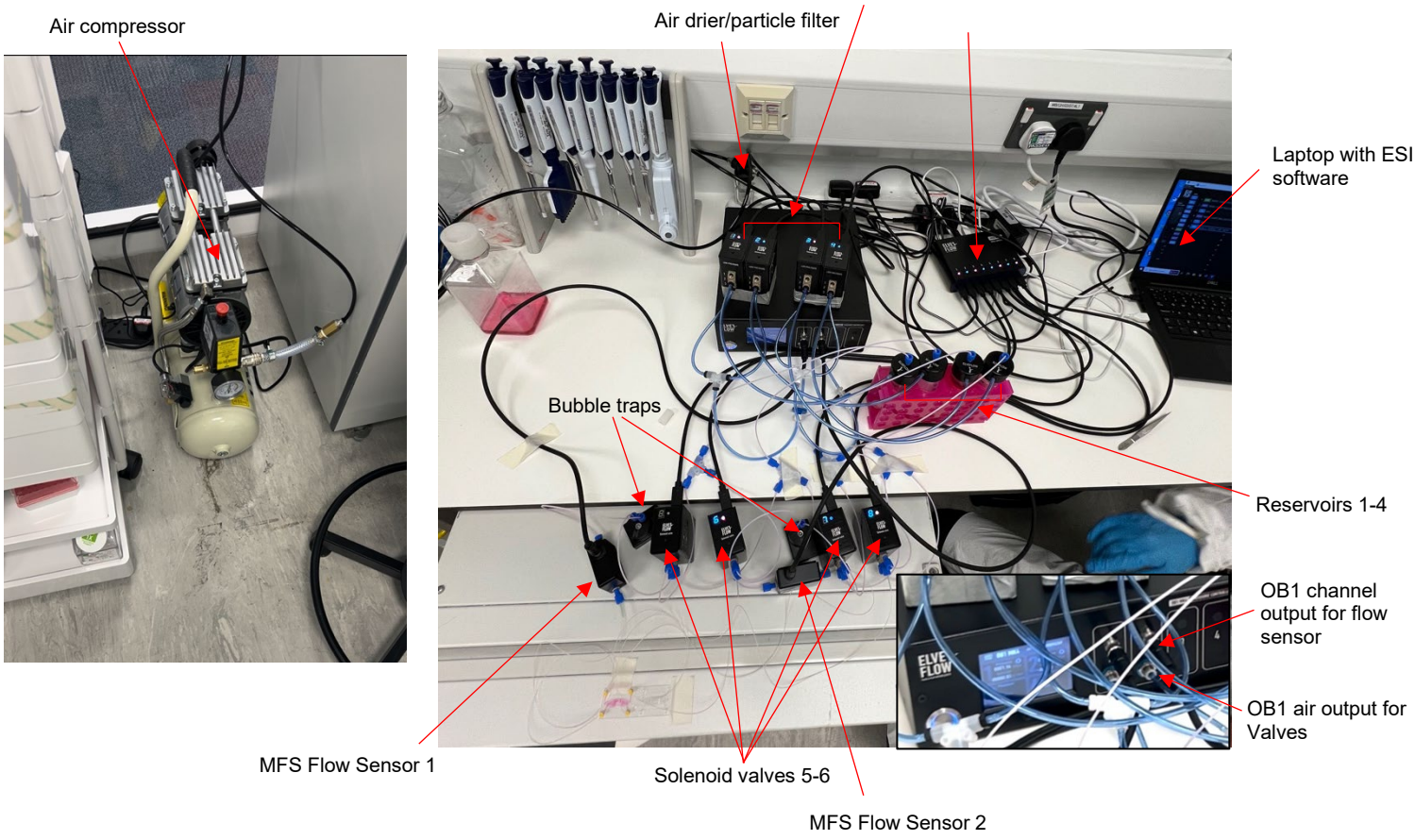
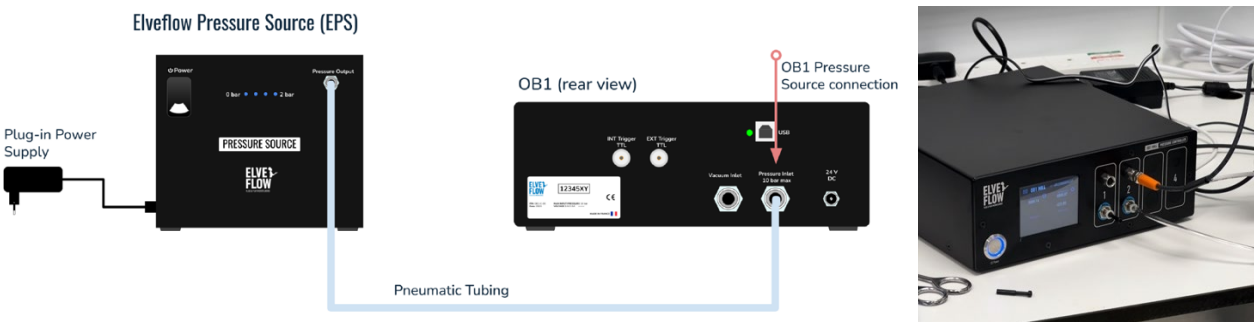
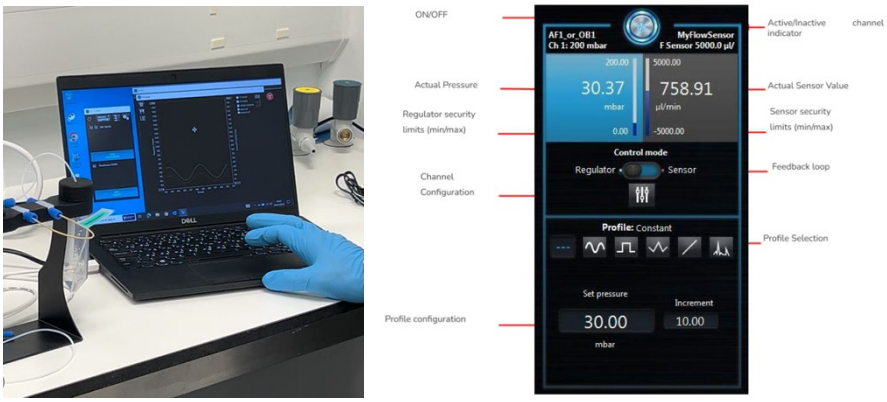


Fig 3. Final Elveflow OB1 setup in the lab.

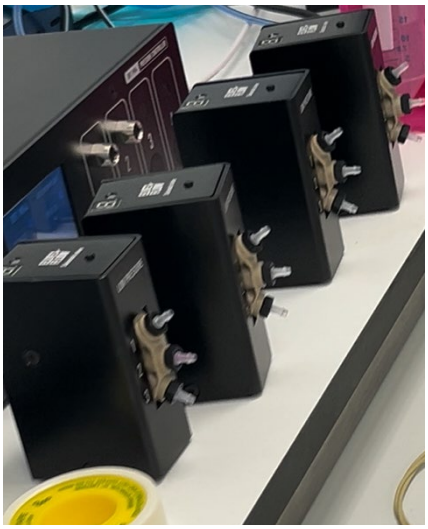
1) Place the OB1 Mark 4 Pressure controller on the working bench and connect the power supply.



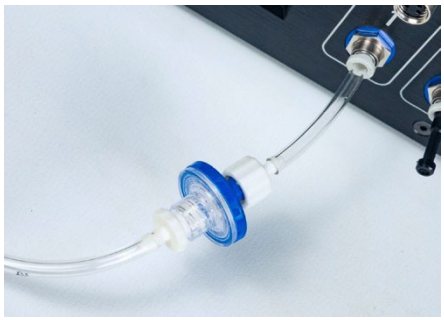
2) Place the computer with installed Elveflow Smart Interface (ESI) software next to the OB1 controller, connect via the USB cable and connect the power supply.



4) Prepare 8 low-pressure valves connected with MUX valve controller using 6mm cables.

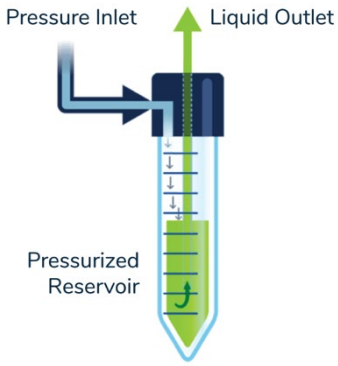


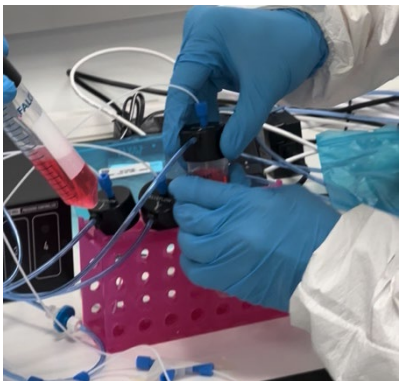
5) Install anti-backflow filters between the OB1 air outlets (2 channels) and valves using 4mm tubing.



6) Insert T-junction (splitter) between the tubes from OB1 outlets to connect Valves 1-4 with the OB1 (Channel 1 for Valve 1&2, channel 2 for Valve 3&4).

7) Prepare the Reservoirs (4x 50ml Falcon tube) filled with culture medium (place in the rack (and water bath)).

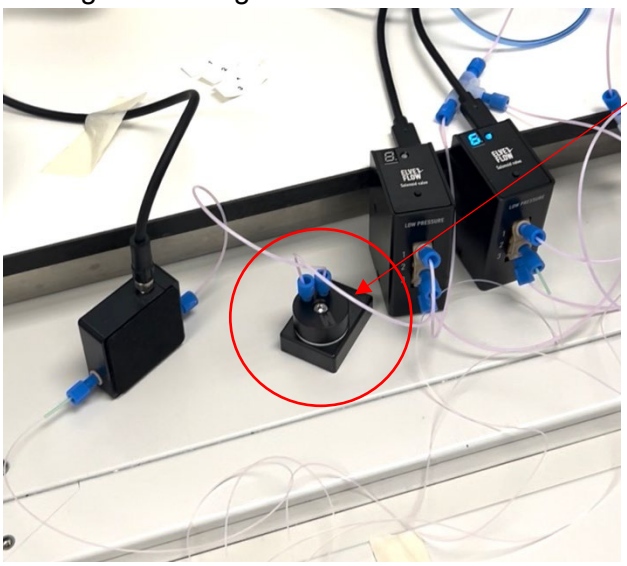




8) Connect Valves 1-4 with reservoirs using 4mm tubing.

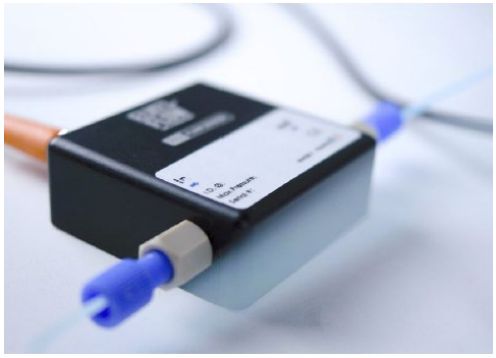
9) Insert T-junction to each tube coming from reservoirs to divide tubes into valves: Reservoir 1 to Valve 5 and Valve 6; Reservoir 2 to Valve 6 and 5; Reservoir 3 to Valve 7 and 8; Reservoir 4 to Valve 8 and 7.

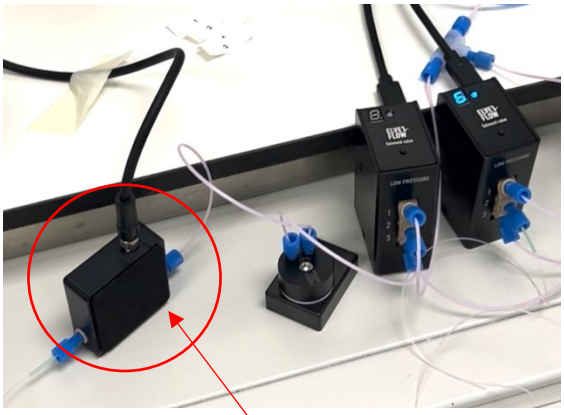
10) Place Bubble trap between Valve 5 and MFS flow sensor 1 and between Valve 7 and MFS flow sensor 2 using 1/16 tubing.



Bubble trap

11) Insert 1/16 tubing from flow sensors and Valves 6 and 8 that are to be connected to the microfluidic chip once the cells are seeded and tubes filled with medium





Flow sensor connected with Bubble trap, chip and OB1 (feedback loop)

5.2 Cell preparation

Trypsinization of endothelial cells

1) Remove flask with 85-95% confluent endothelial cells from the incubator
2) Remove media with pasture pipette connected to the vacuum pump
3) Wash the culture one time with prewarmed DPBS by rinsing it carefully to the edge of the flask
4) Close the lid and swirl flask carefully 2-3x, aspirate DPBS with pasture pipette
5) Add 20ml TrypLE select (1x) in the flask and place into the incubator for 3-5min. Monitor trypsinization by microscopy
6) Add 25ml prewarmed medium to cell/TrypLE select solution to block trypsin reaction. Transfer cell suspension to a 50ml falcon tube
7) Centrifuge with 190Xg, Acc 9, for 5 minutes at room temp
8) Remove supernatant and dissolve pellet in prewarmed medium

Cell counting using Invitrogen countless cell counter

1) Start the machine
2) Mix 10µl of Trypan blue stain with 10µl cell suspension
3) Pipette 10µl cell/trypan mix into countless cell counter chamber slide and place it with the loaded pocket in front into the counter
4) Parameters (adjust in settings):
Sensitivity: 5
Min size (µm): 5

Max size (μm): 60
Circularity :80
5) Push the zoom button and adjust the machine so that the white cells on the display are sharp
6) Push the sample button and record the calculated cell number per ml as well as cell viability
7) After counting, discard the chamber, shut down the machine

5.3 Coating the chips

BBB chips are coated as per manufacturer's instructions

Reagents:
Acetic acid (e.g., 695092, Sigma)
Human Collagen Type IV (e.g., CC076, Sigma)
Distilled water
DPBS (-Mg^2, -Ca^2)

Collagen needs to be dissolved in 0.02M (1.2mg/ml) acetic acid (MW: 60,05 g/mol; density: 1.049 mg/ml).

Standard volumes of acetic acid solution:

Final volume (ml)	Volume of glacial acetic acid (μl)	Volume of distilled water (ml)
10	11.4	10
15	17.2	15
20	22.9	20
30	34.3	30
50	57.2	50

1) Pipette desired amount of water into a 50ml Falcon tube
2) Add right amount of glacial acetic acid in the water (see table).
3) Mix by pipetting up and down/invert the tube 5x.

4) Concentration of the collagen should be 50μg/ml.
5) C1V1=C2V2, C1- 0.05mg/ml concentration of collagen
V1- desired amount of collagen solution
C2- concentration of supplied collagen type IV solution
V2- amount of supplied collagen diluted
6) Example: To prepare 3ml coating solution when the supplied concentration is 4.42mg/ml, dilute 33,9μl of collagen solution in 3ml of 0.02M acetic acid.
6) Pipette right amount of 0.02M acetic acid into 50ml Falcon tube
7) Add calculated amount of collagen to the 0.02M acetic acid solution (coating solution)
8) Mix well by pipetting up and down/ inverting 5x

9) Filter with 0.2μm filter

10) The inner surface of the microchannels in the chip are coated with collagen coating solution and incubated overnight at 37°C (Volume of collagen solution for coating the chips is 5μg/cm^2)
Before seeding the cells, the surface must be washed 2x with PBS to remove residuals of acetic acid:
Remove coating solution
Add PBS, slew 2-3x
Incubate 15min
When ready to seed cells, remove PBS and proceed to seeding. If not, keep PBS on the surface to prevent trying.

5.4 Setting up an experiment with cells

1) Pre-warm all required materials, such as microfluidic chips/μ-slides, medium and tubing inside the incubator at 37°C and 5% CO_2.
2) Prepare the cell suspension: to achieve a final cell number of 1x10^5 cells/cm^2, prepare a suspension of 1.6x10^6 cells/ml. This will be a total of 2.5x10^5 cells/μ-slide.
3) Fill 150μl of the cell suspension into the channel by putting the pipet tip directly onto the channel's inlet.
4) Incubate the chips/slides for 1-2h at 37°C and 5% CO_2. After this time, cells should have formed a confluent layer crucial for enabling the cells to resist the shear stress.
5) Add some surplus cell-free medium (60μl) to the slides (avoid pointing pipet tip directly towards the channel inlet) and place the chip/slide back to the incubator while preparing the system.

Initializing microfluidic system:

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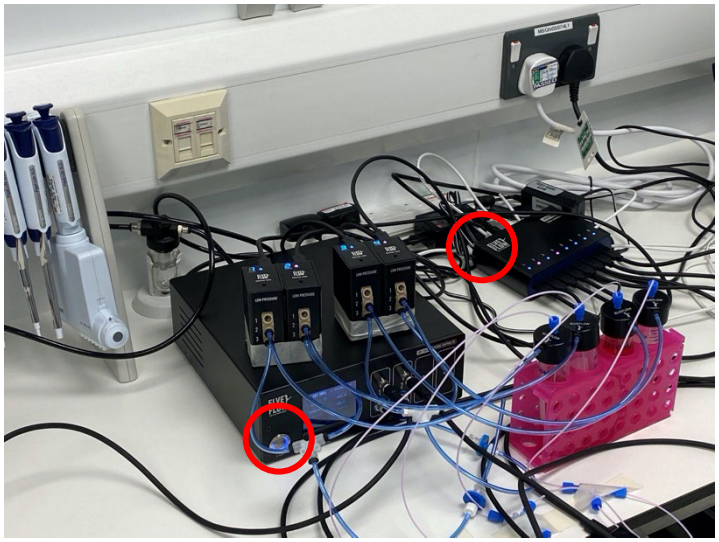
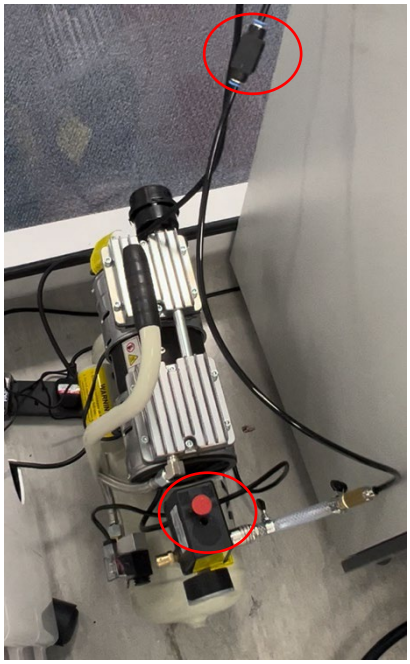
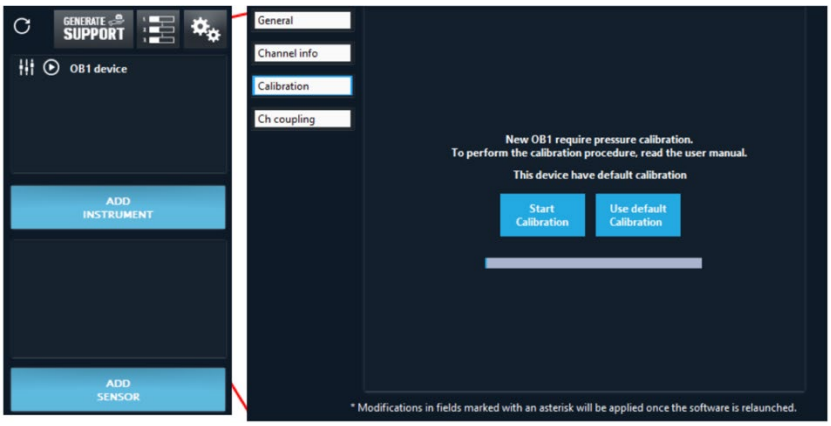
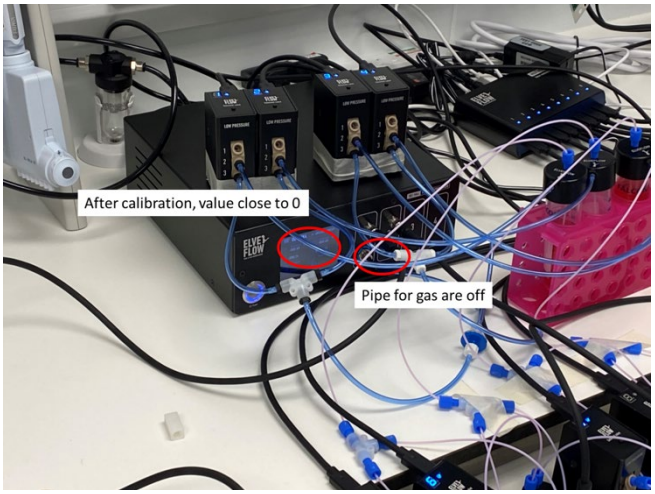
1) Turn on the OB1 pressure controller, valve controller, air compressor and the ESI software.



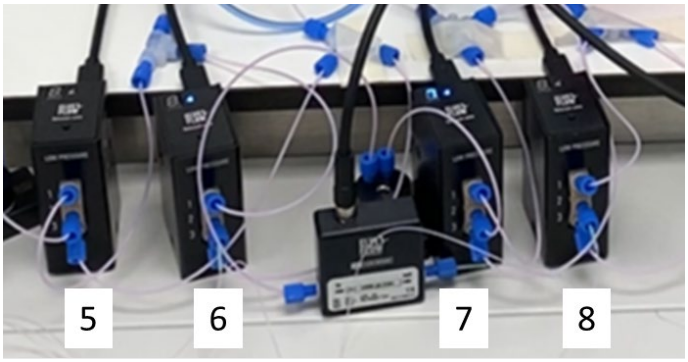


2) Calibrate the system.

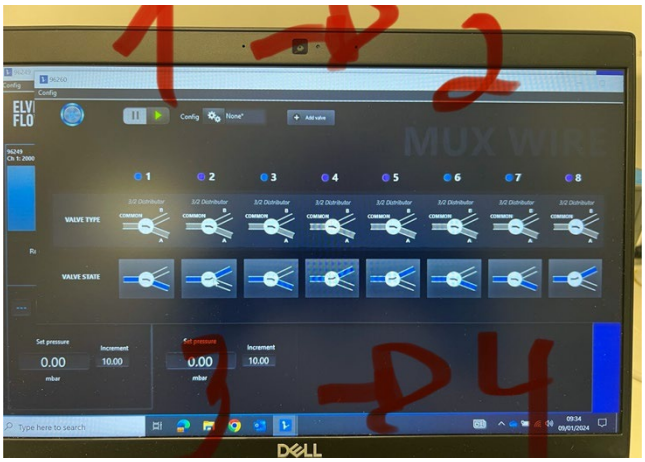


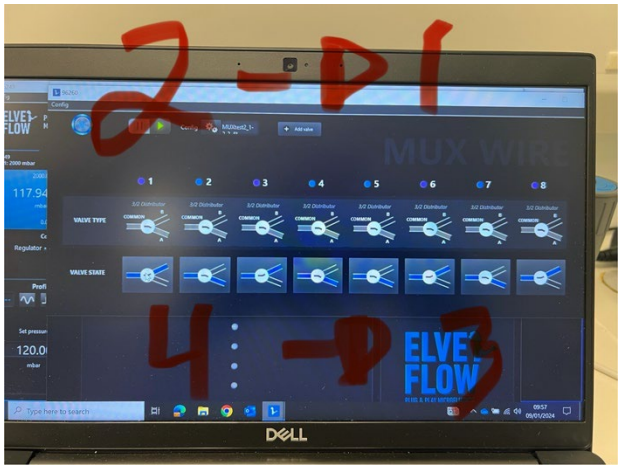


3) After calibration, 1-4 valves are opened (air from way 2 to way 3, blue light). When valves are deactivated, the light is purple (air from way 2 to way 1). When valves 2 and 4 are deactivated (switched to purple), one loop is created. To initialize the flow cycle, valves 5 and 7 is switched to purple (1 to 2 and 2 to 3) and valves 6 and 8 to blue. That means flow is directed from reservoir 1 to 2 and 3 to 4. To transfer the loop, all the switches are turned to opposite and flow is moving from 2 to 1 and 4 to 3. Changing the loops (i.e., reservoirs) ensures the tubes will not run out of liquid.



3) Create 2 MUX valve configurations (flow from reservoirs 1-2 and 3-4, and flow from reservoirs 2-1 and 4-3). SAVE





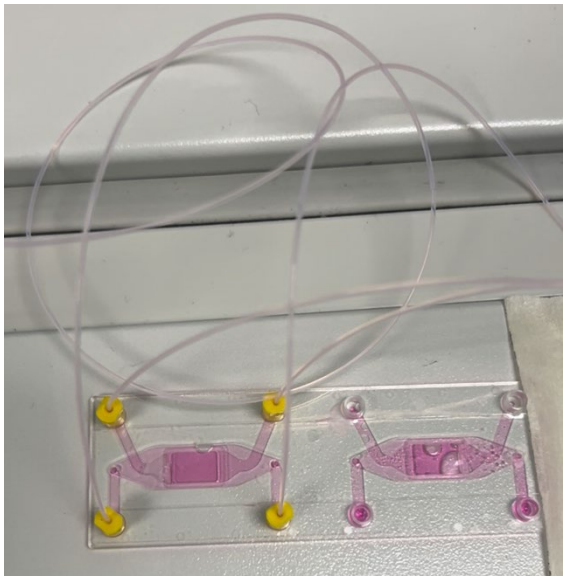
4) Create OB1 configuration by modifying pressure levels to obtain certain flow rate (e.g.,to achieve 100ul/min fllow, set 1st loop to 130mbar and 2nd loop to 110mbar). SAVE

*when initializing the system, adjust pressure (Regulator mode)
*when system is filled with liquid and stable, adjust sensors (P and I) to reach balance in both loops.

5) Create another OB1 configuration to end the experiment (0 flow rate).
6) Create sequence to run experiment: 1) load the WUX valve configuration; 2) load the OB1 configuration; 3) set time; 4) set loop (GO button); 5) load OB1 END configuration. SAVE

7) To ensure medium flow in the tubes before introducing the system to the cells, run the system by placing the end of all tubes (x4) into “trash” reservoir instead of the chip. Run the sequence. Check if both loops are balanced. Modify pressure/sensor levels if necessary.
8) Once the medium is flowing in every tube, stop the run and insert yellow screws to the end of each tube.
9) Pull tube a bit lower from the bench to create medium drop at the end of it (**to avoid bubbles in the chip!**) and connect to the chip. Do it with all 4 tubes.

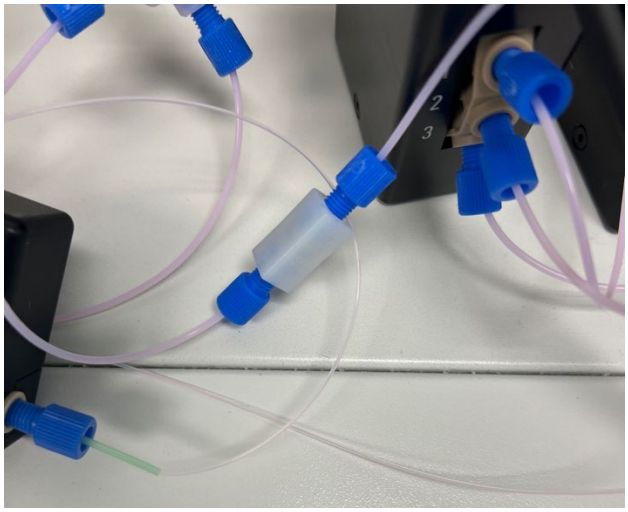
10) Start the same sequence again. Check if flow rates match in each loop. If not- check the potential leak, connection tightness, medium levels, or presence of a potential bubble.



Cleaning the microfluidic system:

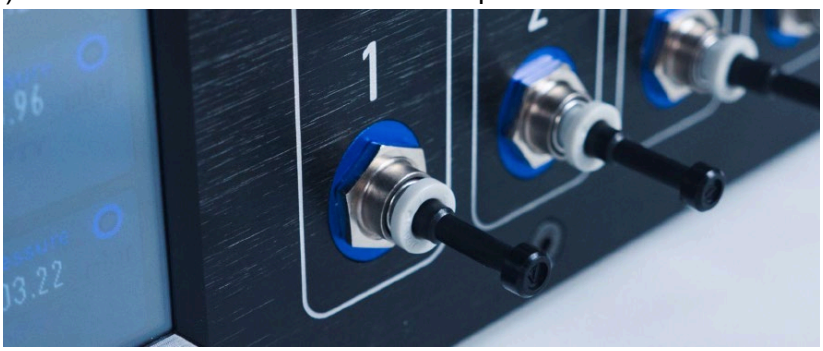
1) Once the experiment is complete (and cells collected), replace the medium in reservoirs with ethanol or 70% IMS. Avoid ethanol to get into contact with the membrane in the bubble trap. Replace if this happened or what the membrane in sterile water and dry.

2) Remove the bubble traps and replace it with the union (to connect valves 5 and 7 with flow sensor).



2) Initialize the same sequence, observe the color change in the tubing (from red to transparent). Switch the flow direction in valves 5-8 once the medium has been replaced in 1st loop (negative flow in the software). Cleaning takes 5-10min. Increase the pressure (e.g., up to 1bar) if faster flow is needed.
3) To dry the tubes: lift the tubes in reservoirs 1 and 3 high enough to avoid contact with liquid. Push the air through the air until see bubbles in 2 and 4. Switch the flow direction in 5-8 if needed. Dry up to 10min.

To shut down the system:
1) Remove air tubes from OB1 and replace with short black endings.



2) Turn off OB1.
3) Turn off MUX valve.
4) Close the ESI software.
5) Turn of the switch in pressure controller and push the red button down.

Keeping the system sterile:
1) Tubing can be replaced in regular intervals if contamination is observed.
2) Tubes can be washed with water, PBS or bleach, just not the bubble trap.
3) The rubber sealing in the lids of the 50ml falcon tubes can be cleaned and kept in 70% ethanol solution in a closed container.
4) Add appropriate amounts of antibiotics during the experiments.
5) Air coming into the system is not as clean as air under the hood. Pay close attention to changes in media color that could indicate a contamination.

Maintenance/inspection of the air compressor:
1) Do not tamper with or attempt to adjust the pressure switch or safety valve. Before moving or carrying out maintenance on the compressor, make sure that the ignition switch is OFF and the air tank pressure has been vented and the compressor allowed to cool down for a period of time.

Draining the air tank:
1) You should train the tank at the end of each day

2) Place a suitable container capable of holding water underneath the compressor.
3) With compressed air in the tank, slowly turn the drain know to the open position. The water in the tank will drain out.
4) Once the water has drained, turn the drain knob to the closed position.
5) Draining the tank reduces the risk of corrosion inside the tank.

Air filter:
1) The air filter is designed to reduce noise and help prevent particulates in the air from entering and damaging the air compressor.
2) After being used for a period of time, the air filter will become clogged. This will reduce the air intake capabilities of the compressor, reducing performance. Therefore, the air filter must be cleaned and replaced regularly.
3) Open the cover on the air filter and remove the filter element.
4) Inspect the filter element and if damaged or worn, replace immediately.
5) Blow the dirt from the air filter from the inside out. You can use a low-pressure airline to do this.
6) Reinstall the air filter.

Leak testing:
1) A small leak in any hose or connection will reduce the air compressors performance.
2) To test for leaks, spray a small amount of soapy water on the area suspected of leaking. If soap bubbles appear, replace a broken part.

Cleaning:
1) Clean the compressor with a soft brush or moist cloth
2) Do not use a pressure washer or hose pipe as water can penetrate the motor and cause dilute that will not be covered by the warranty.
3) Do not use solvent-based cleaning products, these could damage parts.

Storage:
1) Turn off the power and wrap the power cord around the compressor.
2) Pull the relief valve and release all the pressure form the air tank.
3) Clean the air compressor to remove dirt and dust.
4) Cover the air compressor with a cover to protect the unit from dust and moisture.
5) Do not stack or store other items on top of or around the air compressor.

6. DOCUMENTATION

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The following records are outputs of this SOP:

1)FSOP202.1 **:Checklist to complete every time before using Hyundai 8 Litre Air Compressor**

2) FSOP202.2 : **Maintenance checklist of Hyundai 8 Litre Air Compressor to complete**

- **monthly**

These records will be filed in …… [Insert where filed, stored, or otherwise archived for future review or retrieval]. [Insert any record retention requirements]

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Title: Elveflow microfluidic system for organ on the chip assays

Location: H25 in CBE

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
		[Insert specific changes from previous SOP] < e.g. changes in accountabilities, process steps, deviation actions, or records>.	

Standard Operating Procedure **SOP202**

Title: Elveflow microfluidic system for organ on the chip assays

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Appendix

FSOP202.1 :Checklist to complete every time before using Hyundai 8 Litre Air Compressor

Task	Drain the water from the tank (see SOP for details)	Check that the pressure is not above max level	Make sure compressor is positioned evenly and feet secured	Visual check (scratches/dents on the vessel)	Listen for potential leaks
Date/Name					

Standard Operating Procedure **SOP202**

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FSOP202.2 Maintenance checklist of Hyundai 8 Litre Air Compressor to complete monthly (or whenever error is detected)

*Before moving or carrying out maintenance on the compressor, make sure that the ignition switch is **OFF** and the air tank pressure has been vented and the compressor allowed to cool down for a period of time.

Task	Check if air filter needs cleaning/replacing (see SOP for details)	If leak is suspected, test (see SOP for details) and replace broken part	Clean the compressor (see SOP for details)	If compressor is stored, check if it is covered properly and no other items are placed on top of it
Date/Name				

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