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Protocol status: Working
We use this protocol and it's working

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Automatic flow in fluid-walled dumbbells driven by Laplace pressure

In 1 collection

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ABSTRACT

This protocol describes experiments performed to quantify pressure and volume variations inside fluid-walled dumbbells when a pressure difference is generated between the two chambers. Difference in Laplace pressure automatically flows medium from the high-pressure chamber to the low pressure one. Flow stops when pressures are equilibrated.

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MATERIALS

Reagents:

- DMEM/F12 basal medium (ThermoFisher Scientific, CAT# 11320033)
- DMEM/F-12, GlutaMAX™ supplement (ThermoFisher Scientific, CAT# 10565018)
- Dulbecco's Modified EagleMedium [DMEM] (ThermoFisher Scientific, CAT# 11965092)
- FC40 (iotaSciences Ltd, CAS# 51142-49-5)
- Fetal Bovine Serum [FBS]

Note

The fluorocarbon used as overlay and jet is a processed version of the FC40 listed in the reagents table and it is called FC40star. Process to make FC40star is property of and patented by iotaSciences Ltd. Every time in the protocol we refer to FC40 it implies FC40star.

Equipment:

- CNC Router [3D traverse] (Hylewicz CNC-Technik)
- In-house Fluid Printer (Iota Sciences Ltd.)
- PHD ULTRATM Syringe pump 70-3007 (Harvard Apparatus)

Jet-Printing of Fluid-Walled Dumbbells

1 Fill a virgin uniwell plate with 5 ml of DMEM supplemented with 10% FBS. Agitate the plate to spread the 5 mL of medium to cover the whole area of the plate.

2 Remove the volume leaving a thin layer of medium wetting the plate.

3 Gently overlay FC40 (~50 mL) pouring it in one of the corners.

4 Place the plate in the 3D traverse. Adjust x, y, z positions of the traverse head to coincide with the starting point of the movement path.

5 Start the pump, setting a constant flow at 480 µL/min.

6 Wait ~10 s to allow flow to be fully developed.

7 Start traverse automatic movement.

Note

Traverse movements are controlled by scripts written in G-code.

8 At completion of the movement path, stop pump and remove plate from the traverse.

Note

The 3D traverse consists of 3 orthogonal screws that allow head movement along Cartesian axes. Traverse moving head holds a blunt needle (inner diameter = 70 um) connected to an external syringe pump through a Teflon tube. The syringe on the pump and the whole tubing/needle is prefilled with FC40.

Establishment of Pressure Difference and Volumes Variatio...

9 Place the newly printed plate into the pendant drop tensiometry machine.

10 Add FC40 to almost fill the plate entirely (~20 mL).

11 By mean of a syringe pump connected to a needle through a Teflon tube, infuse 4 µL in the right chamber and soon after 1 µL in the left chamber (infusion rate set on pump = 20 µl/min).

12 Record chambers height for 24 hours imaging every 30 minutes.