PDMS Well Chip

iGEM Pasteur Paris team - Institut Pasteur

Axelos Etienne Dejoux Alice Dumont Claire Ehret Antoine
Fyrillas Andreas Ghaneme Aymen Guerassimoff Léa
Jaoui Samuel Picaud-Lucet Emma Porte Sarah
Redheuil Ellyn Richard Charlotte Starck Thomas
Thomas Florian Trang Kelly Madelénat Manon
Paillarès Éléa Naccache Jonathan Sachet Gabriela

2018

Introduction

The well chip was designed and assembled by our team. It was used to test the biocompatibility of our membranes, as well as the culture of bacteria in the presence of current. Here we show how the molds were made, how the chip itself was assembled, how well's conductivity was measured and how biofilm culture was performed on it.

I. Molds

Molds were made of aluminium according to the following plans (Figure 1). Part 1 Mold's center cylinder part is detachable from the bottom to make the demolding ot PDMS easier.

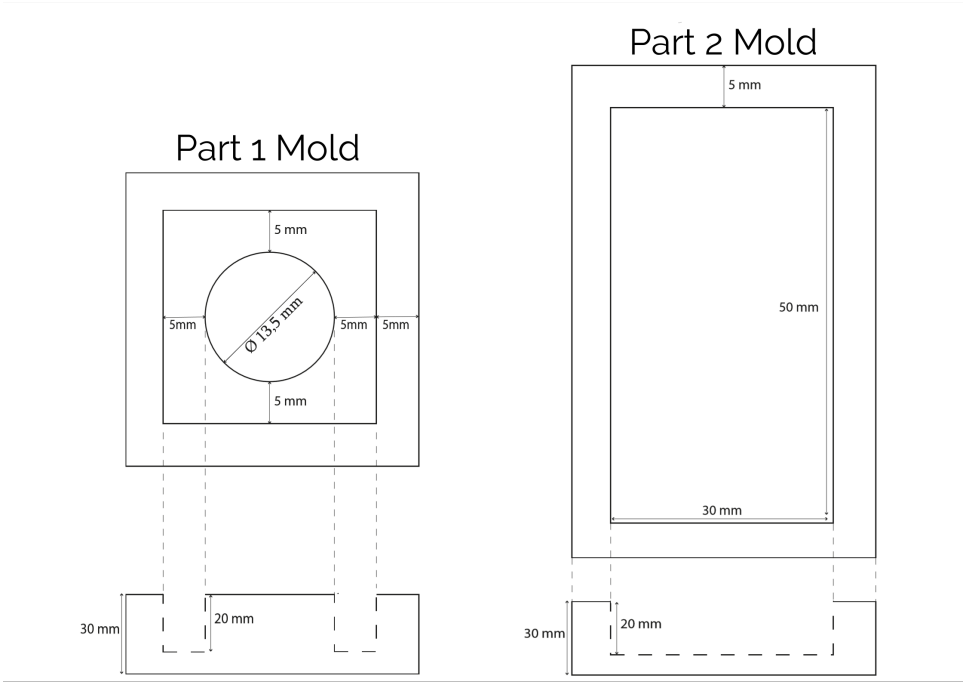

Figure 1 – *Mold plans*

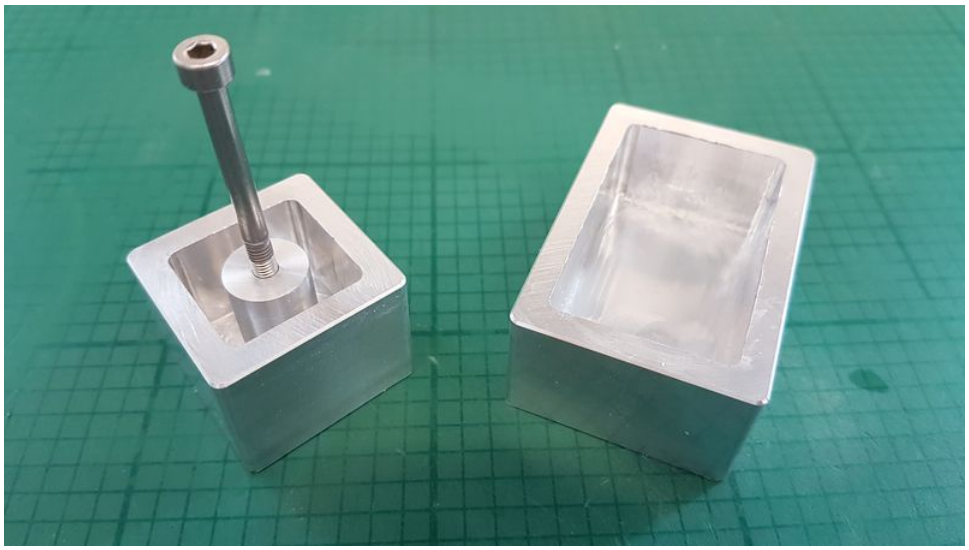

Figure 2 – *Molds*

II. Chip fabrication protocol

II.1. Materials

- Molds
- Syringe (Terumo syringe without needle, 10 mL)
- Platinum 24 mm x 2 mm strip (mechanically flattened 24mm long 0.7mm diameter platinum wire)
- Polycarbonate gold-coated membrane filters, 0.4 micron, 13mm diameter (Sterlitech) or polymerized membrane (see Microfluidics : Membranes, **link to protocol**)

Refer to sections 1 and 2 of Microfluidics : General Protocols (**Link**) for further needed materials.

II.2. Procedure

Step 1 : Prepare 20 g of PDMS monomer using section 1 of Microfluidics : General Protocols (**Link**). Replace step 5 by : Fill the syringe with PDMS. Fill part 1 mold until it's full and part 2 mold until the PDMS layer is more or less 1 cm thick. Keep the PDMS that is left.
Step 2 : Demold the chip following section 2 of Microfluidics : General Protocols (**Link**). Ignore step 2.

Step 3 : Put membrane and platinum strip on PDMS part 1. Refer to figure 2 for their position.

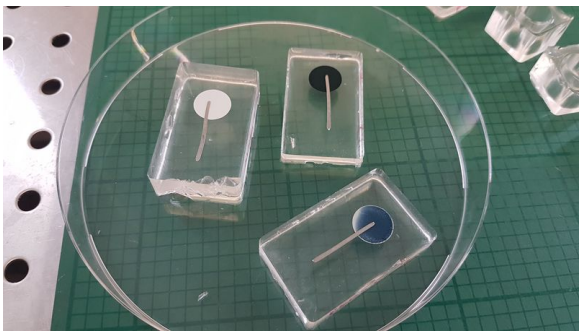

Figure 3 – *Multiple PDMS parts 1 with membrane and platinum strip*

Step 4 : Refer to section 3 of Microfluidics : General Protocols (**Link**) to bond PDMS part 2 to the PDMS part prepared in the previous step. It should look like figure 4.

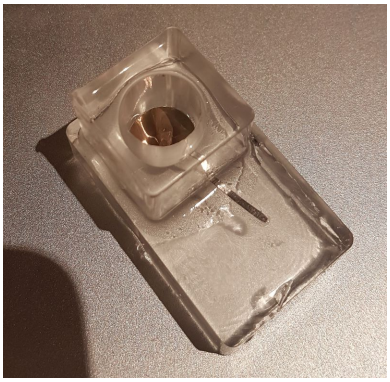

Figure 4 – *PDMS well chip*

Step 5 : Apply a small layer of PDMS with the syringe. Refer to figure 5 . This way, the well is watertight.

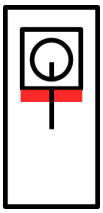

Figure 5 – *Apply PDMS on the red zone*

Step 6 : Put the chip in the stove for 3 hours.

III. Well conductivity measurement

III.1. Materials

- Oscilloscope (Tektronix TDS 2002C)
- Function generator (GW Instek SFG-2010)
- Solderless breadboard assembly (Twin Industries TW-E41-102B)
- 4 Electric wires with banana connectors
- 1 coaxial cable (RG58C/U BNC Plug Coaxial Cable Assemblies - 50 Ω Transmission Line with BNC Male Connectors)
- Male BNC to 2 female banana connectors converter (TNP BNC Male Plug to 2x 4mm Dual Banana Female Jack Socket Binding Post RF Coax Coaxial Splitter Connector Adapter Adaptor)
- BNC Splitter (BNC Male Connector to BNC Double Female (T-Shape) Adaptor)
- 1 kΩ resistor

III.2. Procedure

Reproduce the following electric circuit (figure 6 and 7).

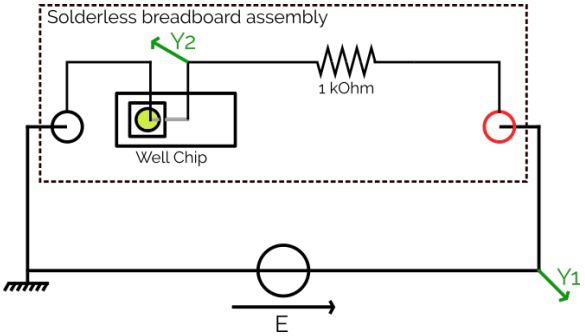

Figure 6 – *Electric circuit for well conductivity measurement, Y1 and Y2 being the oscilloscope inputs*

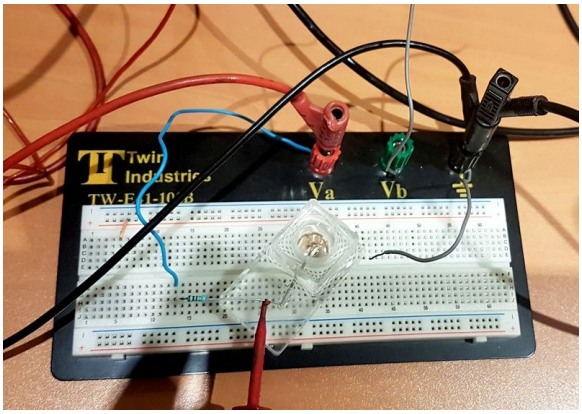

Figure 7 – *PDMS well chip on breadboard assembly*

IV. Biofilm assay

IV.1. Materials

- BL21 liquid culture, see Molecular Biology : DNA Assembly and Microbiology (**Link**)
- PDMS well chip
- Crystal violet (Thermofisher, 0.1 % in water)
- Distilled water
- Acetone
- Ethanol 96%
- P1000, P200, P20 (Gilson) + tips
- Gloves (Kimtech PFE)
- Biochrom WPA CO8000 Cell density meter
- glass jar of bleach
- plastic jar
- Falcon tube 15 mL

IV.2. Procedure

According to Dr Jean-Marc Ghigo.

IV.2.1 Biofilm formation

Step 1 : Pour 600 μL of liquid culture in the well.
Step 2 : Incubate well at 37 degrees Celsius for 24 hours.

IV.2.2 Well wash

Step 1 : Discard the supernatant in microbiological waste bin. Do not pipet.
Step 2 : Immerse well in the plastic jar with distilled water (let the water softly enter the wells).

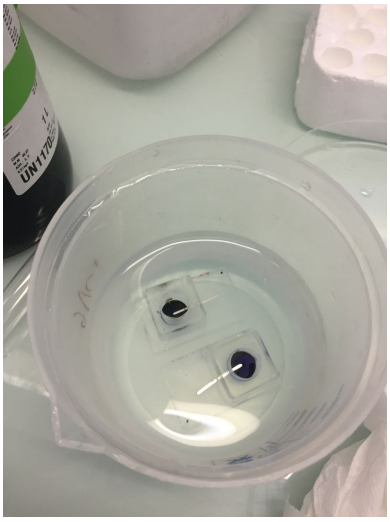

Figure 8 – *Immersed well*

Step 3 : Take the well out of the water and discard water sharply over the waste container.
Step 4 : Repeat this operation twice.
Step 5 : Bang on blotter paper to eliminate residual water.

IV.2.3 Crystal violet staining

Step 1 : Add 125 μL Crystal violet in the emptied well.
Step 2 : Wait 15 minutes for staining.

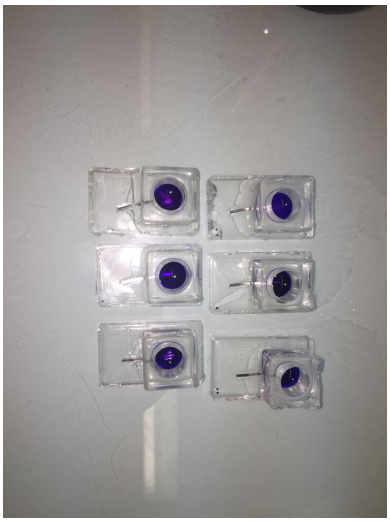

Figure 9 – *Wells with crystal violet*

Step 3 : Wash 3 times with distilled water as described before.
Step 4 : Bang on blotter paper to eliminate residual water.
Step 5 : Suspend colored biofilm by adding 150 μL ethanol/acetone solution (80 :20).
Step 6 : Transfer 50 μL of the solution in a falcon tube and add 1.5 mL of ethanol/acetone solution (80 :20).

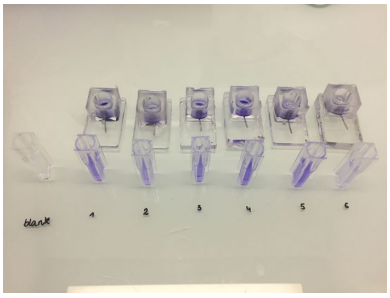

Figure 10 – *Solution ready for optical density measure*

Step 7 : Read optical density of 1 mL of the falcon tube's solution at 600 nm.