Biofilm induction using mother machine microfluidic devices

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Abstract

According to eh IUPAC definition a biofilm is an *"aggregate of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface."* [1] Five stages have been described to occur in a biofilm formation which are Initial attachment, irreversible attachment, maturation 1, maturation 2, and finally dispersion. [2] [3] The most common example of biofilm is the dental plaque which is often related to gum disease and tooth decay. Formation of Biofilm is also common in hospitals and are one of the most relevant causes of nosocomial bacterial infections. [4] [5] [6]

Contents

1 Chip preparation

1.1 Materials

Reagents:

1. PDMS
2. Curing Agent
3. Isopropyl alcohol
4. KOH 1M
5. Sterile Water

Equipment:

1. Plasma Cleaner
2. Hot plate
3. Drying Oven
4. Degasser
5. Sonicator
6. Cover glass (0.13 - 0.17 mm; Fisherbrand , cat. no. 12-542B)
7. Single Edge Razor Blades (Americanline, 66-0089)
8. Aluminum foil
9. Cotton Swab
10. Puncher (0.75 mm; WPI , cat. no. 504529)
11. Scotch magic tape (Amazon.com)

Reagent setup:

1. Prepare 10:1 PDMS:Curing Agent mix.

1.2 Procedure

1.2.1 Baking the PDMS chips • TIMING ~ 5 hours

1. Make a circular Aluminum foil bed for the control master.
2. Insert the silicon wafer in its bed.
3. Pour the 5:1 PDMS mix over the master.
4. Using a Cotton swab gently push the master towards the bottom releasing any excess air trapped under it.
5. Place the bed in a Degasser chamber until the PDMS is bubble free (approx. 30 minutes). ◆ TROUBLESHOOTING
6. Remove the aluminum foil bed from the degasser chamber and place it in a covered plastic petri dish (with a slight opening)
7. ▲ CRITICAL STEP let the air flow back into the chamber slowly to prevent dust particles from sticking to the PDMS mix.
8. Place the petri dish in a drying oven for 4 hours.

1.2.2 Cutting out the chips • TIMING ~ 5 hours

1. Using a new single edge razor cut out the chips according to the pattern.
2. ▲ CRITICAL STEP make the cut by the marked edge of the chip. If you can't, cut it 1-1.5 mm outside of the marked lines.
3. Remove the PDMS from the master wafer.
4. Using a 0.75 mm puncher make holes across the PDMS layer.
5. ▲ CRITICAL STEP Remove any residual PDMS that remain in the punched holes.
6. Sonicate the chips in isopropyl alcohol at 42°C for 40 minutes.
7. Blow dry any residual liquid from the PDMS.
8. ▲ CRITICAL STEP You want to minimize dust exposure as much as possible in this stage.

9. Place the PDMS piece in a covered petri dish.

10. Place the petri dish in a drying oven at 65°C for 4 hours.

1.2.3 Preparing the cover glass • TIMING ∼ 1 hour

1. Sonicate the Cover glass in KOH 1M at 42° C for 30 minutes.

2. ⚠ CAUTION Necessary precautions should be taken when handling highly basic substances.

3. Dispose of the KOH safely. Rinse the cover glass in deionized filtered water 6 times.

4. Sonicate the cover glass in deionized filtered water at 42° C for 10 minutes.

5. Place the cover glass in a drying oven at 65° C for 15 minutes.

1.3 Plasma Bonding • TIMING ∼ 24 hours

1. Start the plasma cleaner with both the PDMS pieces and the cover glasses at 200 mTorr, 100 W for 11 s.

2. Place the cover slips over the PDMS pieces.

3. ▲ CRITICAL STEP It is likely that the instant the cover glass touches the PDMS the bonding would be too strong to reverse, so this step must be done with special care.

4. Place the chips with the cover glass facing downwards on a hotplate at 150°C for 5 minutes.

5. Place the chips on a covered petri dish.

6. Place the petri dish on a drying oven at 65°C overnight.

1.4 TROUBLESHOOTING

Process	Step	Problem	Possible Reason	Possible Solution
Making the PDMS chips	5	Bubbles won't disappear	This happens sometimes as the vacuum in the degasser isn't enough to break the superficial tension of the PDMS surface	Gently blow (particle-free) filtered air directly over the trapped bubble.

Table 1: Troubleshooting table.

1.4.1 • TIMING

Process	Timing
Making the PDMS chips	∼ 5 hours
Cutting out the chips	∼ 5 hours
Preparing the cover glass	∼ 1 hour
Plasma Bonding	∼ 24 hours

Table 2: Timing table.

2 Mother machine Setup

To use the mother machine Chips in an experiment requires a certain amount of preparation. which will be described in this section.

2.1 Chip loading

2.1.1 Getting the cells ready

1. Grow single colonies overnight in 5 mL LB
2. Dilute the overnight culture 1:1000

2.1.2 Inserting the cells in the channels

1. Attach a 200 μL gel-loading tip to the end of the mother machine feeding channel.
2. Using a micro-pipette Take 20 μL from the diluted ON culture using a different gel-loading tip.
3. Attach both micropipette and gel loading tip to the other end of the mother machine feeding channel.
4. Press on to the micropipette until the fluid is level in both gel loading tips.
5. ▲ CRITICAL STEP Do not pierce or exert any extra pressure to the chip
6. For the purpose of procedure wait for 5 seconds.
7. Remove both tips from the microfluidics device.
8. Centrifuge the chip in the direction of the growth channels at 5000 g for 1 minute.

2.1.3 Cleaning the channel

1. Attach a pump to the feeding channel
2. Push media through the channel at 20 $\frac{mL}{h}$ for about 10 seconds
3. Only cells within the growing channels should be left inside the chip.

2.1.4 Attaching the chip to the microscope

1. Take the microfluidics chip and tape it upwards from the bottom to the stage mount using labeling tape.
2. Secure the mount to the stage.
3. Treat the glass side of the chip as any cover slip while taking microscopy images.

2.2 Inducing Biofilm formation

1. Start a flow of nutrients through the feeding channel at a rate of $1\frac{mL}{h}$
2. Within a few hours Biofilm formation should be visible in the outlet of the growing channels.

3 Experiment

The chip was prepared as described in Section 1 and setup as in Section 2 by duplicate and a 14 hour timelapse of the growing biofilm was acquired.

Results for the first biofilm induction can be seen in Figure 1.

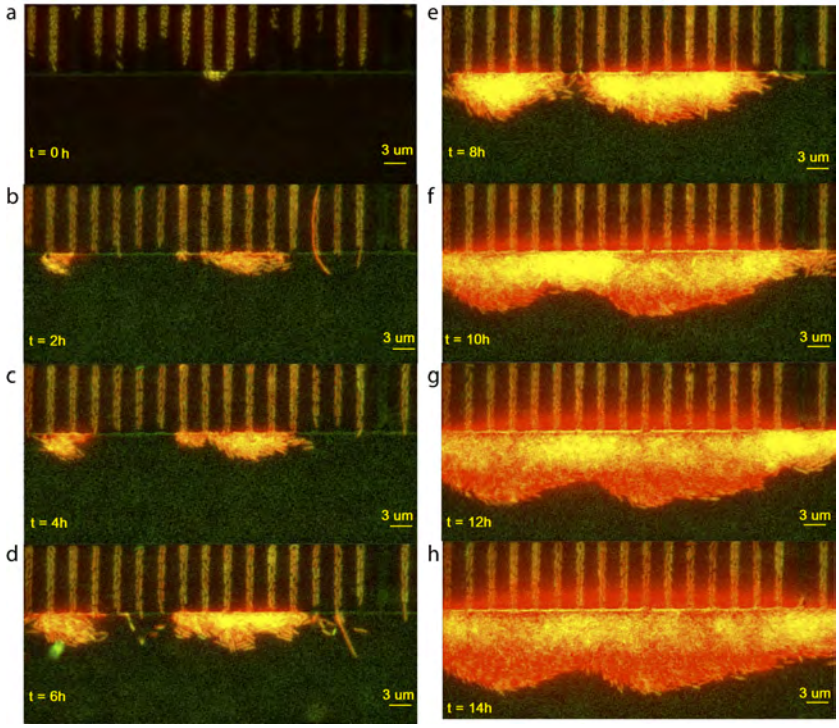

Figure 1: Biofilm growth as seen in the microscope

Results for the second biofilm induction can be seen in Figure 2

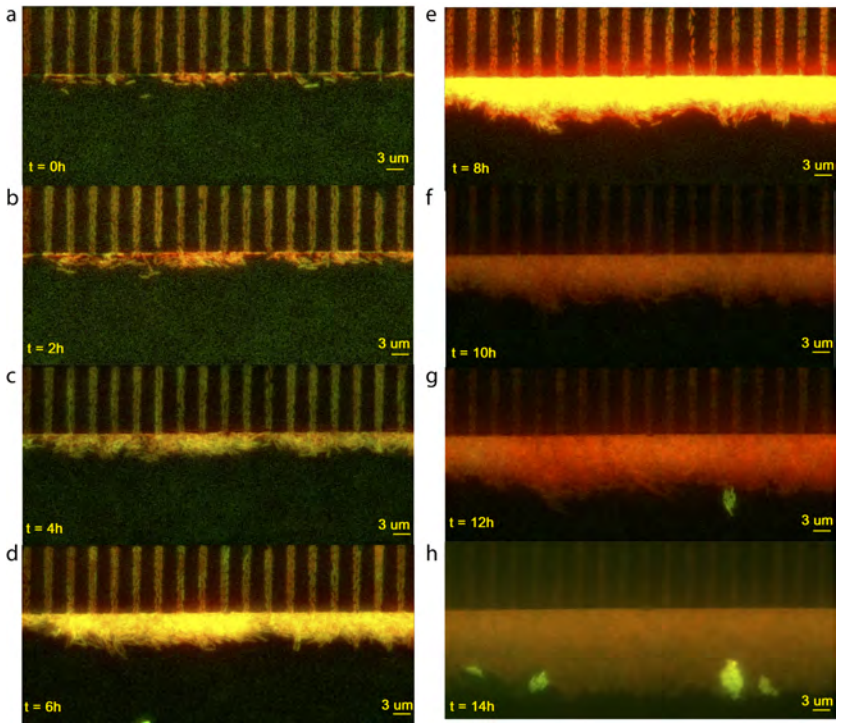

Figure 2: Biofilm growth as seen in the microscope

4 limitations

Biofilm produced is confined to the microfluidics chamber and therefore recovery for further testing becomes a difficult matter.

It seems that the biofilm forms preferentially as a two dimensional layer, instead of how its commonly known to manifest. However traditional microscopes are not able to capture the three dimensional essence of the biofilm structure and the need for more sophisticated microscopic imaging techniques becomes relevant.

5 Conclusions

We have found a consistent reproducible protocol for inducing biofilm in *Escherichia coli*. Although not reported in this document biofilm induction was achieved using the same procedure more than twice.

This procedure can be used for studying growth patterns in biofilm formation, gene expression of cells within the extracellular matrix. Also to test the biofilm's resistance to a variety of different stressors (antibiotics, flow rate, to name a few).

References

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