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Seeding V.2

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Bio-X-Space

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This protocol describes the procedures to seed the *Bacullis subtilis* inside the microfluidic chip to create the biofilms.

Materials

- Pressure pump.
- Needles
- Microfluidics chip (see design in this link https://cad.onshape.com/documents/d01540193290530cad6c1bea/w/108b7d3735006ad86309beb7/e/58c9223ab6c2555e1ce67f02).
- Shaker.
- 50 ml falcon tubes.
- Centrifuge.

MSgg biofilm-forming medium:
-5 mM potassium phosphate buffer pH 7.0 (0.0536 M K2HPO4+ 0.0464M KH2PO4).
-100 mM MOPS buffer ;pH 7.0, adjusted using NaOH (10X: 0.2M MOPS free acid+ 0.05M Sodium Acetate+ 0.01M Na2EDTA).
-2 mM MgCl2
-700 µM CaCl2
-50 µM MnCl2
-100 µM FeCl3
-1 µM ZnCl2
-2 µM thiamine HCl
-0.5% (v/v) glycerol
-1X (30 mM) of glutamate.

LB medium:
-1% Bacto tryptoney.
-0.5% Bacto yeast extract.
-1% NaCl.
-1 mM NaOH.

Media were solidified through the addition of Bacto agar (Difco) to 1.5%, and the plates were allowed to dry at 25°C for 16 h before use.

Before start

Make sure to clean all the workspace with alcohol and bleach.

Day before experiment

1 Cellls from stock were streaked onto LB agar plate and incubated at 37 ºC overnight.

Day of the experiment

2 A single colony was picked from the plate and inoculated into 3 ml of LB broth in a 50 ml conical tube, and then incubated at 37 uC in a shaker.

3 After 2.5 h of incubation, the cell culture was centrifuged at a relative centrifugal force of 2,100 for 1 min.

4 The cell pellet was re-suspended in MSgg and then immediately loaded into microfluidics.

5 After the loading, cells in the microfluidic chamber were incubated at 37 ºC for 90 min, and then the temperature was kept at 30 ºC for the rest of the experiment.