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APPLICATION NOTE

BIOSYNTHESIS ENHANCEMENT OF TROPODITHIETIC ACID (TDA) ANTIBACTERIAL COMPOUND THROUGH BIOFILM FORMATION BY MARINE BACTERIA *PHAEOBACTER INHIBENS* ON MICRO-STRUCTURED POLYMER SURFACES

by

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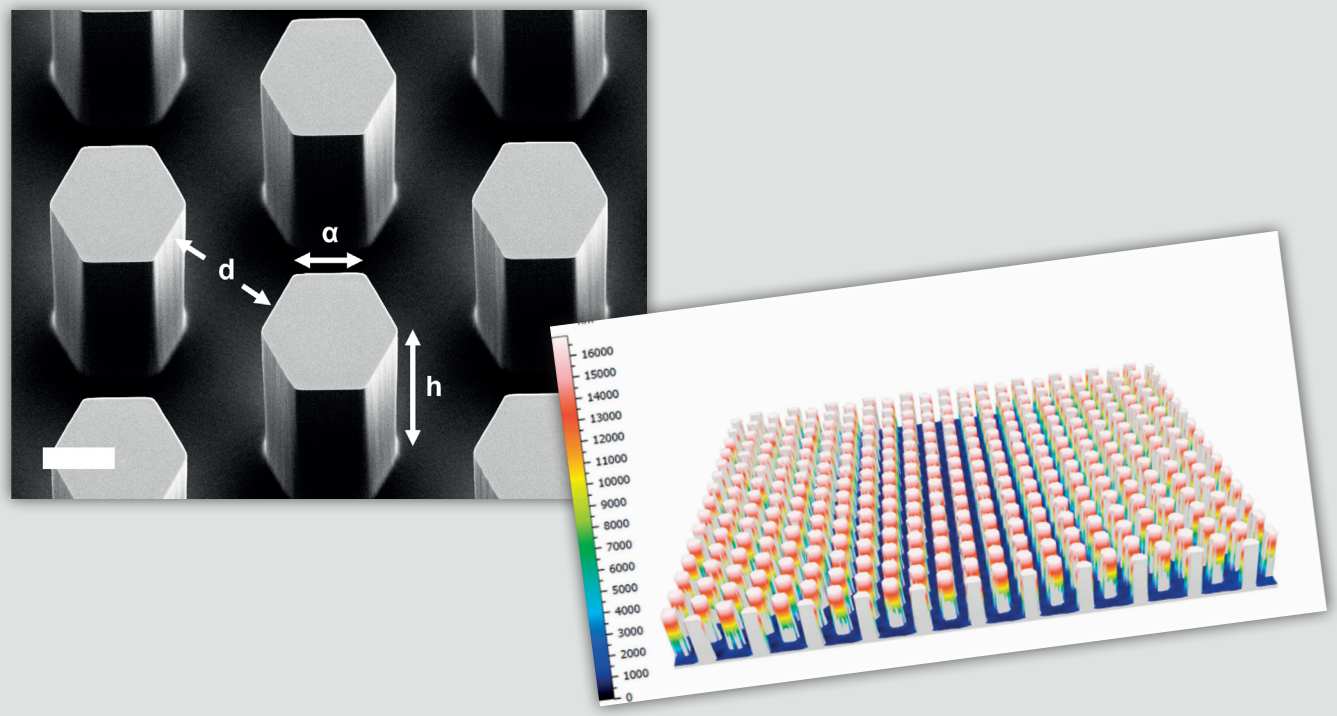

This application note describes the fabrication processes of the Micro-Structured Polymer Surfaces used in the paper "Biosynthesis enhancement of tropodithietic acid (TDA) antibacterial compound through biofilm formation by marine bacteria Phaeobacter inhibens on micro-structured polymer surfaces" published by RSC Advances, DOI: 10.1039/D3RA05407A, Modifications: Figures modified is licensed under CC BY 4.0, accessible here:
https://doi.org/10.1039/D3RA05407A

And PhD thesis "Fabrication of surfaces for the promotion of bacterial biofilm" by Droumpali, Ariadni, accessible here:
https://orbit.dtu.dk/en/publications/45f25228-5812-46a7-a5de-7421f7fa1693

EXPERIMENTAL DETAILS

FLOW CELL AND PATTERN DESIGN

In this study, we use microfluidic flow channels that enable culture of *Phaeobacter inhibens* biofilms. Here, the biofilms were grown on weakly hydrophobic hard polymer surfaces comprising two different micro-fabricated honeycomb structures and a planar reference surface composed of the same material. As shown in **Figure 2**, the flow channels are 40 mm long, 4 mm wide, and 1 mm deep. The flow cells comprised polymer substrates with surfaces having small 3x3 mm micro-structured fields comprising planar reference fields, hexagonal pillar- and pit- array fields. Both surfaces have a hexagon side length ***a***= 5 µm, and depths ***h***≈ 12 µm, while the trench widths ***d*** for the pillar array is 10 µm, the corresponding wall widths for the pit array surface is only 5 µm.

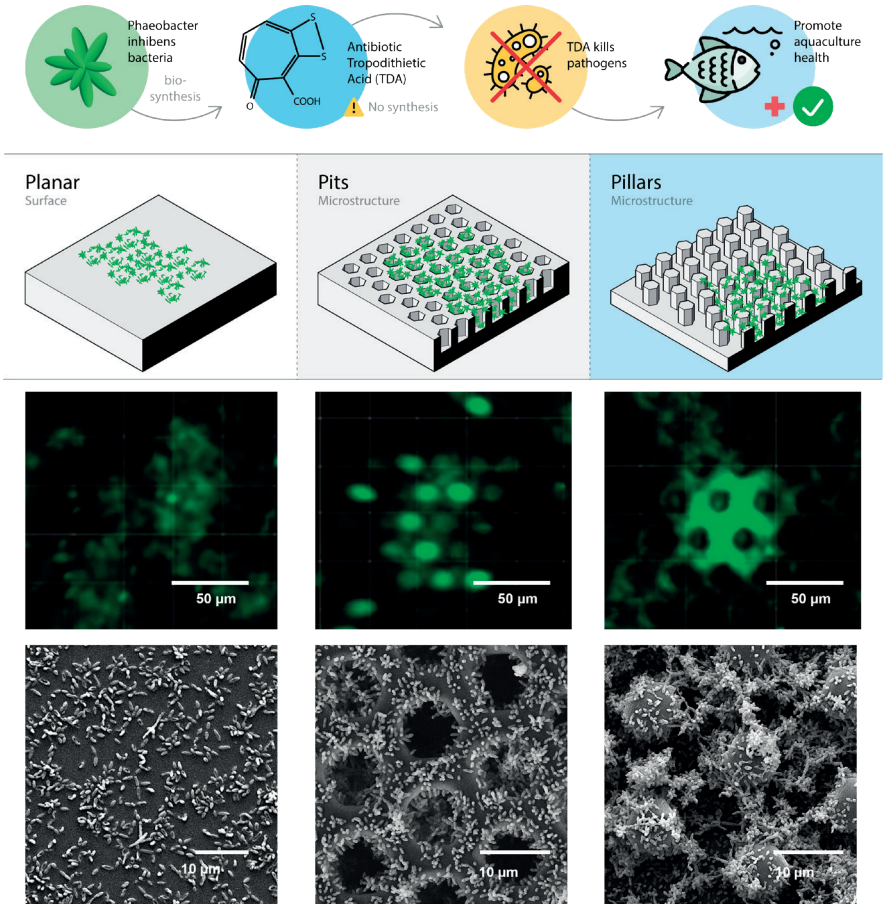

Figure 1. Illustration demonstrating the motivation, 3D structures, and the bacterial growth on the micro-patterned surfaces. "Biosynthesis enhancement of tropodithietic acid (TDA) antibacterial compound through biofilm formation by marine bacteria *Phaeobacter inhibens* on micro-structured polymer surfaces", Modifications: Figures modified is licensed under CC BY 4.0.

FABRICATION PROCESS

The so-called DEEMO process (dry etching, electroforming, and moulding) was employed to micro-fabricate the substrates in a transparent and low auto-fluorescence polymer. (see **Figure 3**).

UV lithography. Silicon surfaces comprising arrays of hexagonal micro-pillars and pits with the same hexagon side lengths and depths, but different pitches were originated on 100 mm diameter n-type <100> single-sided polished silicon wafers with a thickness of 525 ± 20 µm. A layer of 1.5 µm photoresist (AZ5214E) was applied using a spin coater.

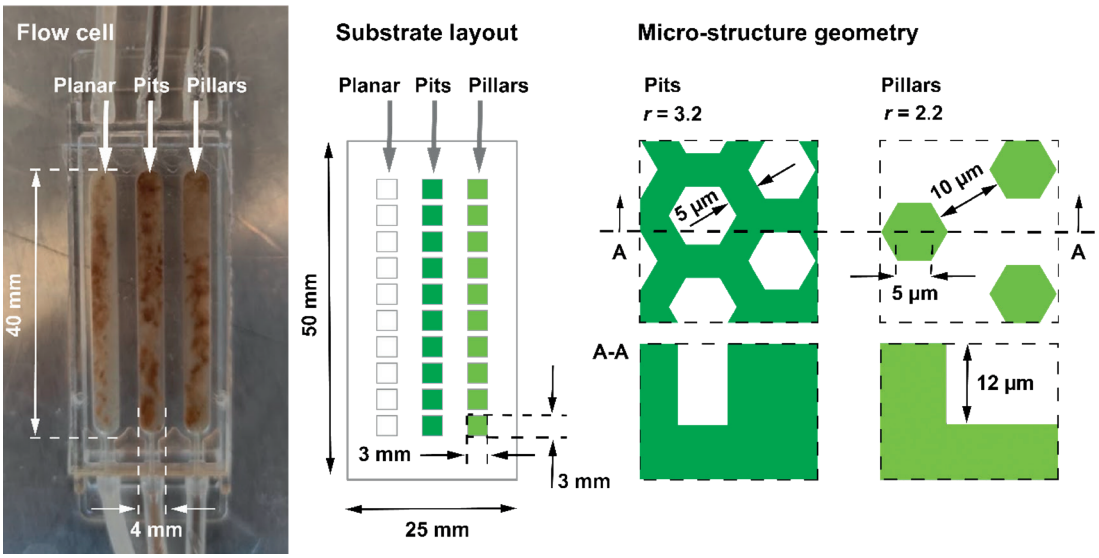

Figure 2. Flow cell system and structure design. "Biosynthesis enhancement of tropodithietic acid (TDA) antibacterial compound through biofilm formation by marine bacteria Phaeobacter inhibens on micro-structured polymer surfaces", Modifications: Figures modified is licensed under CC BY 4.0.

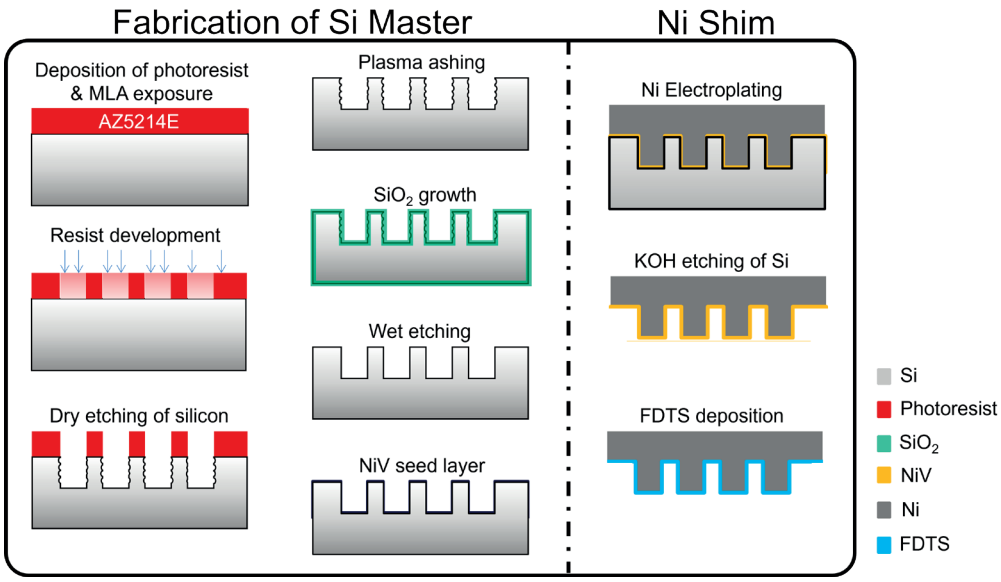

Figure 3. Process flow. "Fabrication of surfaces for the promotion of bacterial biofilm" by Droumpali, Ariadni.

The patterns were designed using CleWin5 software. The design was transferred to the photoresist using an MLA 150 maskless aligner by means of a dose of 65 mJ/cm^2, using a laser emitting at 375 nm. The development of samples was done for 60 s in a tetramethylammonium hydroxide (TMAH)-based solution (AZ 726 MIF) to develop the pattern.

Si etching and smoothing. Approximately 12 µm of Si was etched by deep reactive ion etching (DRIE). An inner coil and an outer coil with 13.56 MHz RF generators produced the plasma, where the maximum power is 5 kW. A deposit-removal-etch-multiple-times (DREM) process, 1kW power with 380 cycles was used with a descum/ashing step before etching to remove resist residues. An oxygen plasma ashing process removed the remaining photoresist. In **Figure 4**, the SEM image on the top shows the pit and pillar structure after dry etching. After DREM process, the scallops are replication step. Therefore, the Si oxide growth and removal processes were introduced for smoothening the side wall.

For smoothing the scallops, a layer ~530 nm of SiO_2 in a furnace with O_2 gas at 1100 °C for 70 min was grown by wet oxidation. Subsequently, an annealing process of 20 min in N_2 gas was carried out. Before starting the furnace, a standard RCA cleaning process was used to clean the samples from any residual traces. After the SiO_2 growth, the silicon master was dipped in a buffered hydro-fluoric acid (BHF) bath with wetting agent, featuring a SiO_2 etch rate of 75-80 nm/min, for 5-7 min. As shown in Figure 4, the side wall is much smoother for easy demoulding.

Fabrication of nickel shim. A 100 nm seed layer of NiV alloy was deposited onto the Si master using sputter deposition. Subsequently, a 340-350 µm thick layer of Ni was electroformed. Deposition started at a low current of 100 mA and remained at a max current of 1.5 A, the process lasted for 12.5 hours. After electroforming, the remaining Si wafer was etched away using an aqueous KOH solution. The etching was performed at 80 °C. The produced Si stamp and Nickel shim are shown in Figure 5.

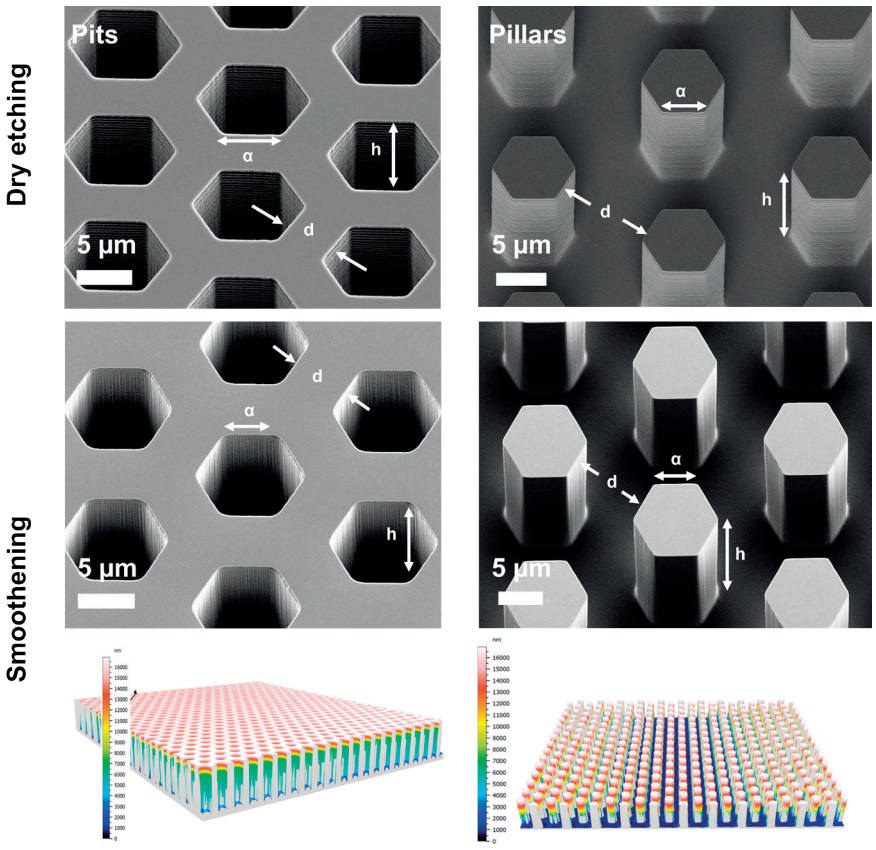

Figure 4. The SEM images of Si stamp before and after smoothening. "Fabrication of surfaces for the promotion of bacterial biofilm" by Droumpali, Ariadni

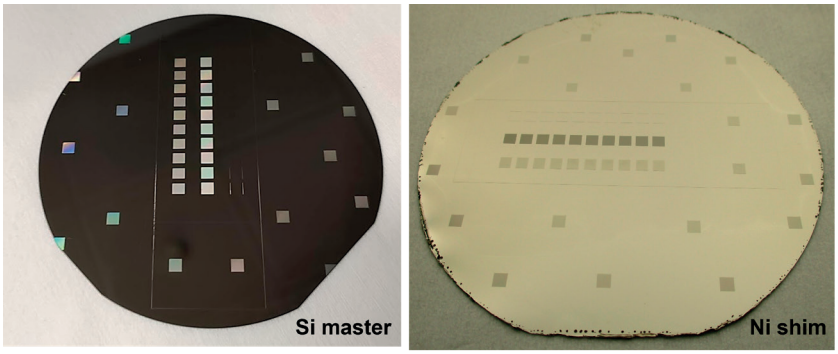

Figure 5. Fabricated Si master and Nickel shim. "Fabrication of surfaces for the promotion of bacterial biofilm" by Droumpali, Ariadni.

For better anti-stiction properties, the produced Ni mould was coated by vapour deposition with a self-assembled monolayer from the precursor per-fluorodecyltrichlorosilane (FDTS).

Fabrication of polymer surfaces. Finally, the polymer substrates were produced by injection moulding on a standard microscopy slide of size 75 mm × 25 × 1 mm^3. The microstructures were obtained by using the micro-structured negative relief Ni shim as a mould. Before moulding, the Ni shim was cut into a 80 mm diameter plate by a green (532 nm) laser micromachining tool, comprising a flat edge to allow alignment against two alignment pins mounted on the fixed part of the Injection moulding tool. The nickel shim was mounted in an industrial injection moulding machine with a back plate of 1 mm thickness, and aligned with the pins. The injection moulding was done using a vario-thermal process, whereby the heated polymer was filled and packed on the inverse relief polarity Ni shim, to ensure proper filling of the microstructures and subsequently allowed to cool down and solidify before demoulding. The polymer used for injection moulding was a cyclic-olefin-copolymer grade TOPAS 5013-L10. The microstructures were well replicated as shown in **Figure 6**.

To clean the nickel shim, after use, the remaining polymer was dissolved in a toluene bath (100% w/v) at room temperature overnight. Before reusing the shim in the machine, it was again coated with an FDTS anti-stiction layer.

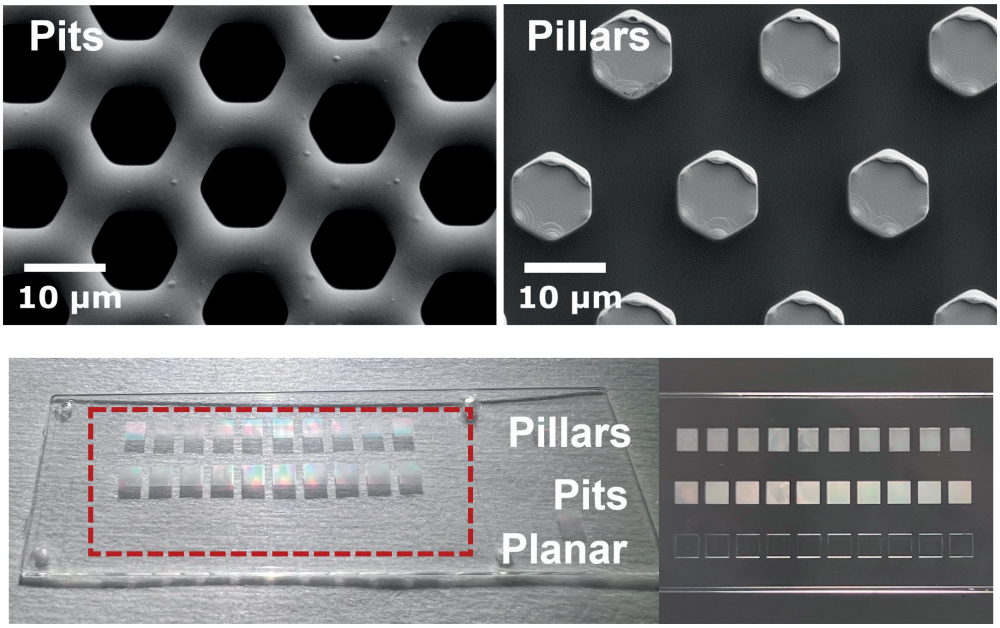

Figure 6. Fabricated polymer chip. "Fabrication of surfaces for the promotion of bacterial biofilm" by Droumpali, Ariadni.

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HIMT - DTU AN 10 V1.1.1 | 03/2024