Marine inspired textured materials for reduction of biofouling on surfaces

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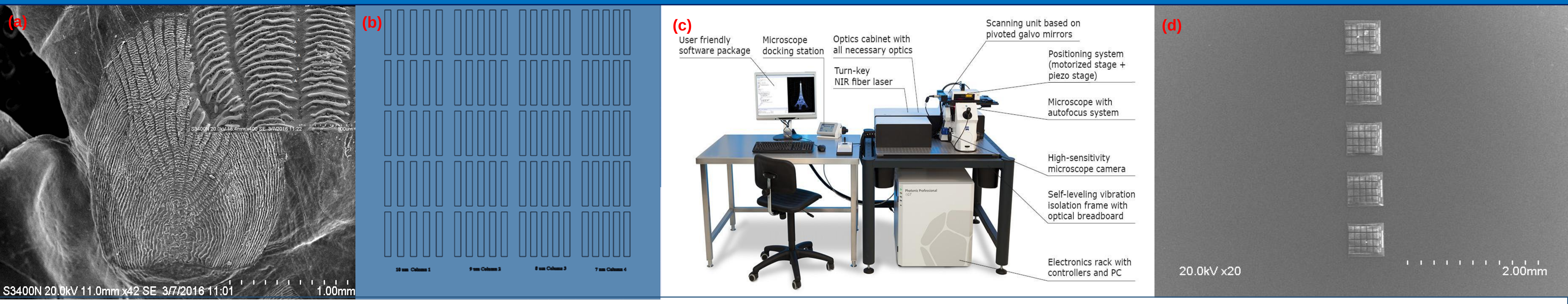
Introduction

Biofouling on deployed in-situ sensors without regular removal or cleaning can disrupt sensor data collected. The current replacement antifouling (AF) materials under development are largely unsuited to sensor technologies as they have been developed with large scale applications in mind, such as those required by the shipping industry. Therefore, a strategy for the development of novel, sustainable, antifouling materials for sensor applications is required. Bio-inspiration refers to adapting strategies already developed in the natural world to problems encountered in modern science and technology. Engineered surfaces capable of controlling cellular behaviour under natural conditions are challenging to design due to the diversity of attaching cell types in environments such as marine waters, where many variations in cell shape, size and adhesion strategy exist. Nevertheless, understanding interactions between a cell and a potential substrate for adhesion, including topographically driven settlement cues, offers a route to designing surfaces capable of controlling cell settlement. Biomimetic design of artificial surfaces, based upon microscale features from natural surfaces, can be utilized as model surfaces to understand cell-surface interactions. In this study it was hypothesized that an AF effect could be induced through the replication of a synthetic surface. *Scophthalmus rhombus* (Brill) is a small flatfish occurring in marine waters of the Mediterranean as well as in Norway and Iceland. It inhabits sandy and muddy coastal waters from 5 to 80 metres. Its skin changes colour depending on the environment but is generally brownish with light and dark freckles and a creamy underside. *S. rhombus* is oval in shape and its flesh is white[1], [2]. In this study, the micro topography of the brill scale is characterized for the first time which may serve as a trend for the design of a marine inspired biomimetic surface texture. Natural dermal scales of *S. rhombus* are artificially replicated using 3-D printing and mould casting technologies. The replication methods are then tested for initial colonization of fouling species using 3 h immersion testing using diatom species, CCAP 1052/1B, *Phaeodactylum tricornutum.* The aim of this study was to discover the potential of using textured surfaces inspired by nature in particular marine organisms to combat fouling. This work identifies simple textures that can reduce fouling in its early stages which can contribute to antifouling coatings on sensors for monitoring in the marine environment.

Methodology



Legend: (a) Single scale SEM micrograph from *S. rhombus* showing characteristic growth rings. (b) Design of synthetic surface based on *S. rhombus* characterization fabricated using SolidWorks 2017. (c) Nanoscribe two-photon 3-D printer instrument (image source: https://www.nanoscribe.de/en/). (d) SEM micrograph showing three-dimensional printed (array = 5) design printed using Nanoscribe two-photon 3-D printer.

1. Replication of S. rhombus topography using 3-D printing
Based on previous characterization studies of *S. rhombus*, a 3-D design was constructed using the modelling software, SolidWorks. The design was fabricated using the Nanoscribe 2-photon 3D printer. The Nanoscribe uses a direct laser writing process whereby polymer structures are formed by deflecting a laser beam into a photosensitive material.

2. Replication of S. rhombus topography using mould casting technologies
This design was fabricated as a negative template of the originally designed 3-D printed part. The texture was then vapour treated in a vacuum chamber with the solution of 30 mL of hexane mixed with 4.7 µl of Octadecyltrichlorosilane (OTS) for 2 h and then dried in an oven at 100 °C for 30 min. Mould casting was carried out by casting a liquid PDMS (10:1, Polydimethylsiloxane) against the surface of the 3-D printed part. The PDMS layer was then peeled off resulting in a positive structure of the original template.

Results

Rapid testing was performed using a culture suspension of *Phaeodactylum tricornutum* (CCAP 1052/1B, SAMS Limited). Textures were immersed in the cell suspension for 3 h followed by fixation in 2.5 % glutaraldehyde. Colonization of textures was observed by observation with scanning electron microscopy at 20 kV. *P. tricornutum* was chosen as the model diatom species as they are easy to grow, their size is relevant to the textures being produced and they are a complex diatom species existing in three different morphotypes.

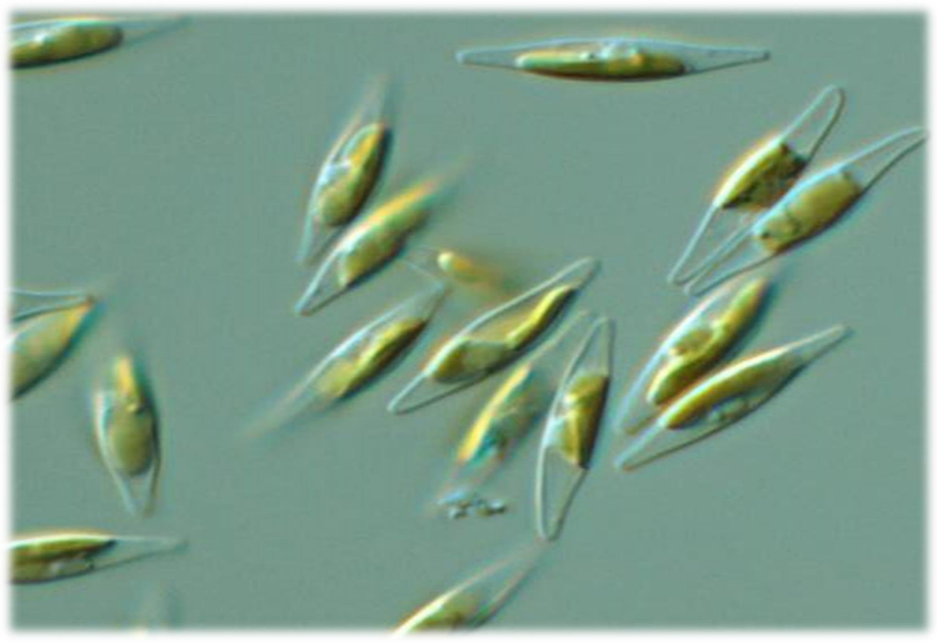


Figure 1. Diatom species, *P. tricornutum* under light microscopy (image source: https://algaeresearchsupply.com).

(a) Effect of surface topography in surfaces produced using a 3-D printing process

The texture reproduced using a 3-D printing process displayed a WCA of 65.59° making it hydrophilic . Cell attachment to the 3-D printed texture was minimal indicating that surface texture did not play a role in cell attachment but rather the hydrophilicity of the surface causing decreased cell attachment. This study highlights the need for further study of hydrophilic biomimetic surfaces.

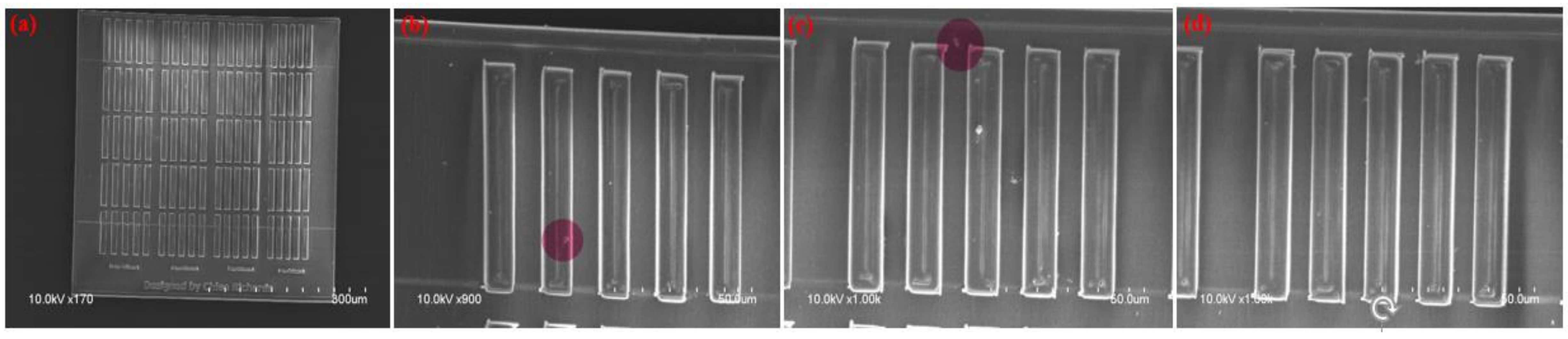


Figure 1. SEM observations of diatom species, *Phaeodactylum tricornutum*, settlement on surfaces inspired by *S. rhombus* and using a 3-D printing process. (a) Overview of surface texture reproduced using 3-D printing after cell testing. (b) Texture with 10 µm spacing between features. (c) Texture with 9 µm spacing between features. (d) Texture with 8 µm spacing between features. Cell attachment is highlighted in red in each micrograph for convenience.

(***b) Effect of surface topography in surfaces produced using mould casting technologies***

Two main morphotypes are observed; oval and triradiate. The oval form of this diatom species can be seen adhered to or between raised surface features in spacing of approx. 10 µm to 8 µm. On the other hand, the triradiate form of the species is observed with two of the mucilage pads adhering to the end of raised surface features while the another pad stabilizes its attachment by adhering to the smooth PDMS surface[3]. This settlement pattern is most likely caused by the ability of diatoms to locate and re-orientate their position on a surface to find one optimum for habitation[3]. In this case, *Phaeodactylum tricornutum*, may appear in both oval and triradiate morphologies depending on where they are positioned when firstly exposed to a substratum. Cells exposed to areas with raised surface features (75 µm x 10 µm) will most likely be occupied by cells of the form, 'triradiate' for habitat stabilization, colony formation, sessile adhesion and motility[3]. Further work is required however this work provides a promising approach to designing textured surfaces inspired by nature that can potentially reduce fouling in it's early stages.

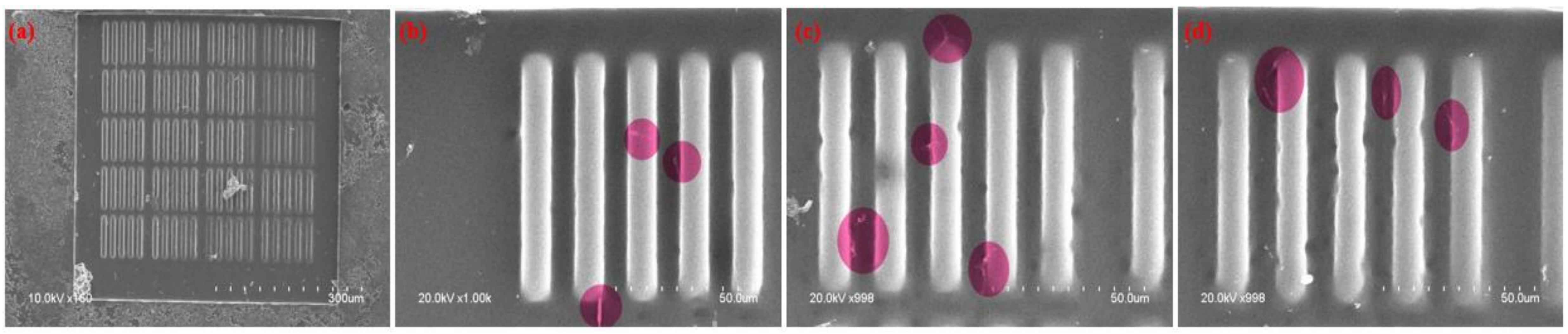


Figure 2. (a) Overview of texture created in PDMS from a mould casting process. (b) Micro-texture with spacing of 10 µm between features. (c) Micro-texture with spacing of 9 µm between features. (d) Micro-texture with spacing of 8 µm between features. Cell attachment is highlighted in red for convenience

References

[1] P. Taylor, V. Caputo, G. Candi, S. Colella, and E. Arneri, "Reproductive biology of turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) (*Teleostei, Pleuronectiformes*) in the Adriatic Sea Reproductive biology of turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) (*Teleostei* , *Pleuronectiformes*)," March 2014, pp. 37–41.
[2] W. Sweetser, *The Connoisseur's Guide to Fish & Seafood*. 2009.
[3] P. J. Molino and R. Wetherbee, "The biology of biofouling diatoms and their role in the development of microbial slimes.," *Biofouling*, vol. 24, no. 5, pp. 365–379, 2008.

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