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Rapid prototyped biomimetic antifouling surfaces for marine applications

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Abstract

Fouling-release coatings typically rely on a mechanical shear force (usually generated by movement in the marine environment) to remove bio-matter. This is problematic in situations where the surface of interest will remain in a static state (e.g. marine sensors). Here we investigate the antifouling properties of textured surfaces of regular patterns in poly(methyl methacrylate) (PMMA) in a static marine environment. Nine PMMA samples were prepared and the effect of hole-size and spacing on marine diatom adhesion was studied. Self-replenishing silicone oil was tested in each textured surface, and impact of oil transfer on diatom settlement was also investigated.

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Keywords: biomimetic; PMMA; textured surface; diatoms; antifouling; fouling-release; rapid prototyping

Introduction

Bio-inspiration refers to adapting strategies already developed in the natural world to problems encountered in modern science and technology. Often the solution to a particular problem or a means of improving an existing solution can be seen if the natural solutions to a similar problem are studied in detail. As an example of this, synthetic materials and devices with advanced structures and functions can often be traced back to being based on biological concepts and design features [1]. Even a cursory review of the literature shows that the terms bio-inspired and biomimetics are very much in vogue in recent published research papers. A search of the Web of Science database using the terms, "bio- inspired or bioinspired or biomimetic" reveals that there are over two thousand papers published from the period 2000- 2009, but just over 100 from for the period 1950-2000. This trend may be due to the fact that as the need for environmentally benign research becomes more important, the scientific community is increasingly looking at how nature has solved a particular problem before going on to create analogous solutions if possible. Recent work has focused on the ability of textured surfaces present on crustaceans and bivalves to prevent fouling. Specifically the work of Bers and Wahl, 2004 [2] has shown that microtopography present on a range of marine organisms such as *Cancer pagurus* and *Mytilus edulis* are likely to be in part responsible for the lack of intense fouling on their shells and carapaces. The authors prepared high-resolution resin replicas from natural surfaces and tested them in tandem with the natural surfaces to ensure that the topographies were responsible and not some as yet unidentified chemical defence. Their results showed that, as would be intuitively expected, different organisms showed different responses based on the scale of the microtopography encountered, in particular the texture of *C. pagurus* was repellent to barnacles in the early stages of immersion while replicas of the brittle star, *Ophiura texturata*, showed resistance to micro-fouler settlement. This finding and subsequent further work led to the authors proposing a theory of how microtopography can influence the rate of fouling, which they have called attachment point theory [3]. In this theory they propose that the size of the micro-texture present and its relation to the size of the settling organism is important in selection of attachment sites. Laser processing has been shown to be capable of cutting and texturing material surface to high levels of dimensional accuracy [4, 5].

Inspiration for this investigation has been taken from this previous laser processing work and studies of the surface micro-structures of *Cancer pagurus* [6, 7]. The materials are tested against a marine diatom to determine if their settlement is impacted by the size and spacing of micrometer scale pattern surfaces.

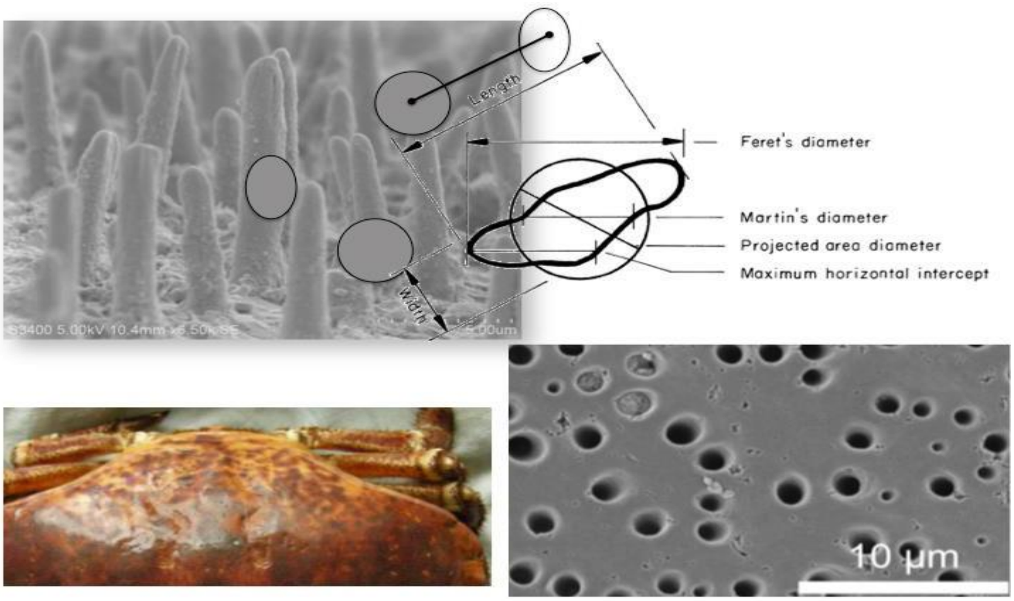


Fig. 1. Illustration shows the surface of Cancer pagurus and associated surface features (Above = SEM micrographs of typical microtrichia from C. pagurus) and below right (imprint of surface features of the epicuticle) and their distribution. Above right shows the consideration of distribution and spacing of features [6].

1. Experimental

1.1. Material design and development

Nine surface designs with three different hole sizes and spacing were created using Solidworks 2014 (x64 edition), see Fig. 2 A. Designs were exported as two-dimensional .dxf files and input into the Epilog® laser processing software (Laser Dashboard™). Clear poly(methyl methacrylate) (PMMA) sheets of 1.5 mm (RS components, Ireland) were subsequently laser cut using a 40W CO_2 laser (Epilog Zing™ 24. Input hole diameters of 200, 300 and 400 µm resulted in laser cut hole sizes of 550, 600 and 700 µm respectively, hole spacing were 1, 1.5, and 2 mm. The cut PMMA samples were sonicated in water to remove debris and air dried at room temperature for 2 h. After fabrication the laser textured slides were characterised using a Keyence VX-2000 digital microscope to measure actual hole size and spacing (Fig. 2 B).

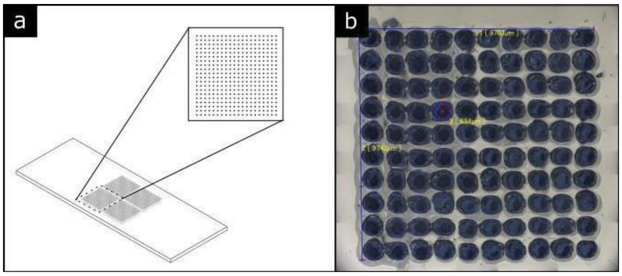
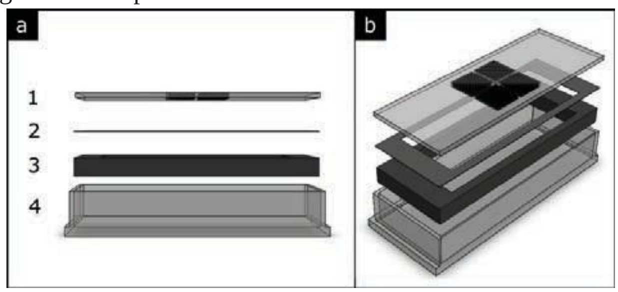


Fig. 2. a) CAD drawing of PMMA slide with four 1 cm^2 sections of laser patterned holes, and b) microscope picture of laser cut 1 cm^2 section.

Static contact angles for the prepared PMMA slides were measured. Nunc 4-well slide processing dishes (Thermo Fisher Scientific, Ireland, catalog number 267061) were used to house the PMMA slides on top of a silicone oil reservoir for testing. Closed-cell spongy neoprene spacers of 6 mm thickness (Abbey Seals International, Ireland) were cut into rectangular sections to act as a reservoir and gasket for the silicone oil. A 200 µm thick general purpose rubber (TWI Textile Machinery Fabrics Co., Ireland) was laser cut with a 1064nm Nd:YAG laser to act as the gasket surface between the PMMA slide and the neoprene well. The gasket was glued to the neoprene using Loctite® waterproof superglue. The experimental set up is shown in Fig. 3. Stainless steel cylindrical weights (Miko metals Ireland, www.miko.ie) of 100 g each were placed either side of the textured area to create a hydrostatic pressure of



4kPa, and force the oil to partially extrude through the surface.

Fig. 3. a) 2D exploded view of experimental set up with parts from top down (1) Laser textured PMMA slide (2) Rubber Gasket (3) Closed-cell neoprene spacer (4) Nunc slide holder and b) 3D exploded view.

1.2. Diatom preparation

Cell compatibility testing was performed using a culture of the marine diatom *Amphora coffeaformis*. The cells were originally sourced from CCAP Scottish Marine Institute (algal strain number 1001/2). A 3 mL volume of CCAP culture was subcultured into 50 mL of Guillard's medium for diatoms (f/2+Si). The cells were stored for two weeks under Gro-Lux® lamps with a 12 h light/dark cycle at a temperature of 20 ± 5 °C. The diatom culture was then passed through a 20 µm nylon mesh using vacuum assisted filtration to ensure the suspension was primarily planktonic single cells.

1.3. Materials testing

The experimental reservoir encompassing the neoprene and gasket (Fig. 3.) was filled with silicone oil with a viscosity of 100 mPa.s (Sigma Aldrich, Ireland) and placed into the Nunc 4 well dish. A 6 mL aliquot of the diatom filtrate was added to each well to submerge the set up. The stainless steel weights were applied to force the silicone oil to partially extrude through the textured holes in the PMMA surface. A blank PMMA slide was used as a control surface to account for variation between tests. The culture was left for two hours to allow the diatoms to adhere. Non-adhered plankton organisms were rinsed away by submerging the each surface in 200 mL of mili-Q purified water 3 times. Epifluorescence microscopy was used to enumerate adhered cells using a Leica DM2500 Light Microscope with UV filter (I3 – excitation 450 - 490nm, dichroic filter: 510 nm, suppression filter LP515 nm) under 10x objective. The chlorophyll a and b present in viable diatom cell's chloroplast fluoresce red light under this parameter and illuminate the cells for imaging. Images were recorded using a Leica DC300F microscope mounted camera with Leica Image Manager image processing software. ImageJ image processing software was used to count the number of adhered cells per image (1.1 mm by 1.5 mm) (Rasband, W.S., Image J, U. S. National Institutes of Health, Bethesda,Maryland, USA).

2. Results

2.1. Testing of cell settlement

The materials in this work were designed based on the surface features of *C. pagurus* however, these test materialshad regular patterns unlike the random patterns studied in this organism and previously reported. It was found that compared with control, the textured materials illustrated greatly reduced adhesion of *Amphora coffeaformis* under thetest conditions. Fig. 4. illustrates the epifluorescence images representing a control (red fluorescence due to organism) and a textured surface. In all cases of texturing there is reduced adhesion of the test organism.

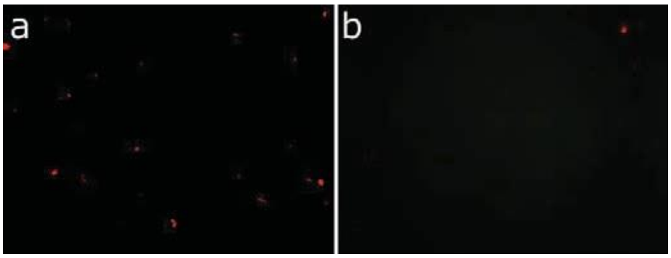


Fig. 4. Illustration of epifluorescence measurements of diatom settlement on (a) control and (b) textured material (hole diameter = 0.4 mm, spacing = 1 mm).

2.2. Effect of hole sizing and spacing

In this work three hole sizes and three spacings were studied (in total nine variations). Three replicates of each texture were measured. Results of cell count versus, hole size and spacing is shown in Fig. 5. It was found that, the greatest cell count occurred in the material with a spacing between features of 1500 µm and a hole size of approximately 620 ± 20 µm. The largest reduction in adhesion was observed with the material comprising 1000 ± 10 µm spacing and 720 ± 25 µm hole size. The greater the hole size and smaller the spacing between holes led to the lowest number of cells measured (<100 cells measured). In the case of all textured materials, the measured contact angle was between 68 and 78o. There was no significant effect of hole size or spacing on the measured contact anglehowever, these features showed significant effect on the degree of settlement, indicating that features (holes) and nearest neighbour, is an important factor in terms of biofouling organism attachment and settlement. Further work todetermine optimal transfer of oil through the holes, and oil viscosity will be continued.

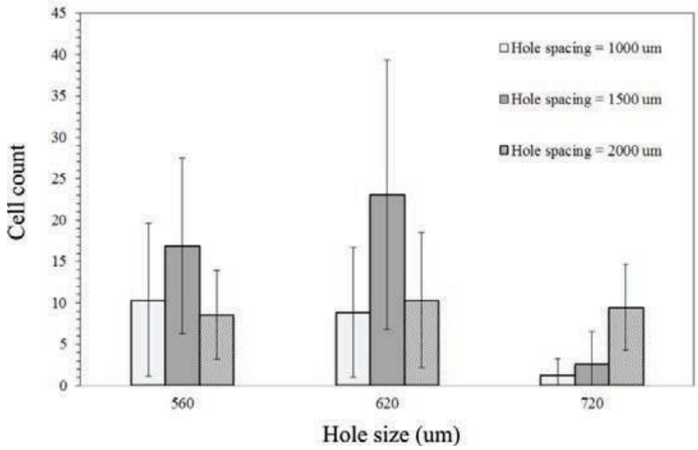


Fig. 5. Effect of hole size and spacing on cell count with standard error (number of repetitions, n = 15). Hole size of 714 μm and hole spacing of 1000 μm was found to inhibit cell adhesion significantly compared with other test samples. A cell count of 499 was observed for a blank PMMA control slide.

3. Conclusions

The inspiration for conducting this investigation has been taken from previous studies of the surface microstructures from the marine organism C. pagurus. Nine different textured surfaces were prepared and materials were tested against a marine diatom to determine if settlement was impacted by size and spacing of regularly patterned holes and associated texture on PMMA. This initial study found that compared with a control, there is a clear advantage in using such regular patterning as found by a reduction in diatom viability on these surfaces. Greater spacing between features resulted in increased numbers of settled diatoms. The largest average contact angle was found for the hole size of 720 μm and hole spacing of 1000 μm. As the most hydrophobic surface, this surface was also found to have the lowest cell count. Further investigation will examine silicone oils with different viscosities in order to optimise transfer through the holes and prevent cell viability.

Acknowledgements

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