

Biomimicking Patterns

Technical Field

5 The present invention generally relates to bio-inspired patterns, products comprising the pattern and a method for fabricating the pattern for preventing biofouling on surfaces in aquatic environments.

Background

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Biofouling is found in almost all circumstances where water-based liquids are in contact with other materials. It is one of the most challenging issues affecting the marine industry as it compromises durability and functionality of infrastructure. As a result, additional costs are generated in relation to energy consumption and infrastructure maintenance. For example, biofouling affects the shipping industries significantly, since high levels of fouling on a ship's hull significantly increases drag, reducing the overall hydrodynamic performance of the vessel and increases fuel consumption. Other areas affected by biofouling include, but are not limited to, maintenance of mariculture, membrane systems in membrane bioreactors or reverse osmosis membranes and cooling water cycles of large industrial equipment and power stations.

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To overcome the problem of biofouling, traditional anti-fouling methods have included the use of biocides, bi-dispersants and use of antifouling coatings. However, the use of such antifouling methods can damage the aquatic environment. For example, the use of paints containing biocides has become significantly restricted due to their damaging effects on the environment. In this method, the biocide is incorporated into an

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antifouling surface coating, typically through physical adsorption or through chemical modification of the surface. Because the biocide, which can be toxic to both microorganisms and larger aquatic organisms, can leach out of the coating, it can lead to significant damage of aquatic organisms, the ecosystem and the environment. For example, the tributyltin (TBT) moiety which is a commonly used biocide in antifouling coatings is known to cause, amongst others, defective shell growth in certain shell fish. As such, the international maritime community has banned coatings containing TBT. Similarly, coatings containing biocides are gradually being phased out, requiring the need for an alternative antifouling method.

Several non-toxic alternatives have been considered in recent years, including chemical coatings such as fluoropolymers and silicon coatings that impart low friction and low surface energies. These coatings may also create a smooth surface which can prevent attachment of larger microorganisms. These coatings are ecologically inert but have problems in mechanical strength and long term stability.

There is therefore a need to provide a product and/or method to prevent biofouling which may at least partially ameliorate one or more of the disadvantages described above.

Summary

In a first aspect, there is provided a substrate for aquatic environments, the substrate having a patterned surface comprising a plurality of protruding structures extending from the surface of the substrate, said structures being comprised of a first structure type and a second structure type that has different dimensions to the first structure type, the structures

being selectively arranged on the substrate to at least partially decrease biofouling when exposed to an aquatic environment compared to a substrate without said patterned surface, wherein the first structure type has a base diameter in the range of 0.05 μm to 50 μm and an aspect ratio in the range of 1 to 100 and the second structure type has a base diameter in the range of 50 μm to 350 μm and an aspect ratio in the range of 0.05 to 4.

Advantageously, the substrate may comprise a patterned surface comprising a bio-mimetic pattern. The bio-mimetic pattern may be inspired by naturally occurring patterns found on the surfaces of various decapods. Further advantageously, the patterned surface on the substrate may be inspired by decapods that naturally exhibit low levels or absence of biofouling in heavy fouling conditions. Advantageously, these decapods may not exhibit burrowing behaviour which may result in decreased fouling of their surface. Therefore, the low levels or absence of biofouling may be inherent to the properties of their outer surface. Advantageously, the patterned surface may comprise a bio-mimetic pattern inspired by the microtopography on the surface, in particular the carapace, of decapods such as *Myomenippe hardwickii*, *Gaillardiiellus orientalis* or *Acanthodromia margarita*. Advantageously, these decapods may show low levels or absence of biofouling despite their occurrence in heavy fouling environments and their lack of burrowing behaviour.

Advantageously, the patterned surface may comprise protruding structures of various shapes and sizes that may prevent the settlement of aquatic organisms. That is, the substrate comprising the patterned surface may possess antifouling properties. Further advantageously, the substrate may comprise protruding structures of various shapes and sizes

that may reduce the strength of adhesion of aquatic organisms and/or biofilms on surfaces. That is, substrate may have fouling-release properties.

Advantageously, the substrate may comprise protruding structures of various shapes and sizes arranged such that it prevents biofouling. Advantageously, the interplay between the first structure type and second structure type may facilitate the biofouling properties of the pattern. Further advantageously, the first structure type and the second structure type may have different dimensions to each other to prevent fouling by different types of organisms.

Advantageously, the substrate may have protruding structures which may correspond to the size of organisms that cause biofouling, therefore resulting in the prevention of biofouling. That is, the substrate may advantageously have a patterned surface that is comprised of smaller protruding structures to prevent biofouling by small organisms and larger protruding structures to prevent biofouling by larger organisms. The dimensions and spacing between the features may affect the fouling potential of a given pattern. Advantageously, the size, shape, orientation, relative distance, frequency, population density or any mixture thereof, of the protruding structures may impart the anti-fouling and fouling-release properties to a surface.

Further advantageously, the substrate may be coated with a polymeric coating. Advantageously, the polymeric coating may enhance the anti-fouling or fouling-release properties of the substrate. More advantageously, the physical modification of the substrate in combination with chemical modification of the substrate may facilitate enhanced the anti-fouling or fouling-release properties of the substrate.

In a second aspect, there is provided a use of a substrate for aquatic environments, the substrate having a patterned surface comprising a plurality of protruding structures

extending from the surface of the substrate, said structures being comprised of a first structure type and a second structure type that has different dimensions to the first structure type, the structures being selectively arranged on the substrate to at least partially decrease biofouling when exposed to an aquatic environment compared to a substrate without said patterned surface, wherein the first structure type has a base diameter in the range of 0.05 μm to 50 μm and an aspect ratio in the range of 1 to 100 and the second structure type has a base diameter in the range of 50 μm to 350 μm and an aspect ratio in the range of 0.05 to 4.

Advantageously, the substrate may prevent biofouling on surfaces regardless of how it is formed. Advantageously, the patterned surface may be formed directly on the surface of the substrate by imprinting the structures thereon or by applying liquid medium comprising particles capable of self-arrangement on a surface. That is, the particles may spontaneously form 3-dimensional structures based on a 2-dimensional design. Advantageously, the patterned surface may prevent antifouling regardless of the material of the substrate. Advantageously, the substrate may be any material that may be used in an aquatic environment.

Further advantageously, patterned substrate may facilitate the fabrication of a mechanically stable protective surface (antifouling and/or foul-release) that is durable and is not susceptible to corrosion in an aquatic environment. Advantageously, the patterned substrate comprising the patterned surface may be permanent, allowing the preventative effect of biofouling to last longer. More advantageously, the substrate may have minimum effect on the performance of underwater equipment and aquatic vessels.

Further advantageously, the patterned substrate may facilitate the fabrication of a protective surface (antifouling/foul-release) that is non-toxic and does not adversely affect the organisms that are present in the aquatic

environment in which it is used. In particular, the patterned substrate may circumvent the use of biocides such as tributyltin (TBT) that are toxic to aquatic organisms.

5 In a third aspect, there is provided a method for fabricating a substrate comprising a patterned surface to at least partially decrease biofouling in aquatic environments, the method comprising the steps of: (a) providing protruding structures, the protruding structures comprising of a first structure type and a second structure type that has different
10 dimensions to the first structure type on the substrate; and (b) selectively arranging the protruding structures on the substrate, wherein the first structure type has a base diameter in the range of 0.05 μm to 50 μm and an aspect ratio in the range of 1 to 100 and the second structure type has a base diameter in the
15 range of 50 μm to 350 μm and an aspect ratio in the range of 0.05 to 4.

Definitions

20 The following words and terms used herein shall have the meaning indicated:

The term "aquatic environment" refers to any environment in a body of water including fresh water, salt water, buffer, or any mixture thereof. The body of water may be of natural or
25 artificial composition.

The term "bio-mimicry" refers to the design and production of materials, structures, and systems that are modelled on biological entities and processes. The terms "bio-mimicking" and "bio-mimetic" should be construed accordingly.

30 The term "biofouling" refers to the accumulation of microorganisms, plants, algae, animals or any mixture thereof,

on wetted surfaces and adsorption of substances such as proteins and polymers that are excreted by these organisms on the wetted surfaces.

The term "base diameter", with reference to structures of a
5 circular or spherical shape, refers to the diameter or

equivalent diameter of a structure at the site where it protrudes from.

The term "aspect ratio" refers to the height:width ratio of a structure.

5 The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

10 Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

15 As used herein, the terms "about" and "approximately", in the context of concentrations of components of the formulations, or where applicable, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value,
20 even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and
25 brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of
30 a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to

4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

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Disclosure of Optional Embodiments

Illustrative, non-limiting embodiments of a pattern in accordance with the first aspect will now be disclosed.

10 Decapods have been extensively studied in relation to underwater robotics (mechanism of movement), sound generation (mechanical energy and acoustics) and, more recently, as biomimetic models for understanding biomineralisation. However, there has been little work done on the surface microstructure of
15 decapods. The surface armory in a number of crustaceans may have an unprecedented complexity, comprising of complex configurations with functions yet to be determined. The decapod's surface feature may be multifunctional. The decapod's morphology may be related to function. The decapod's morphology
20 may be used to elucidate which features are directly associated with prevention of biofouling, and how the combination of surface structures facilitate the prevention of biofouling in environmentally relevant conditions.

 Low fouling observed on decapod shells may be attributed to
25 burrowing, grooming and molting behaviours as well as excretion of bioactive substances. However, some decapods are known to have relatively low fouling on their surfaces despite the lack of these behaviours.

 Decapods may excrete substances that naturally render the
30 decapod shells to have anti- or low fouling properties.

Decapod shells may comprise a patterned surface which may naturally have anti- or low-fouling properties. The patterned surface of the decapod shells may be artificially mimicked to achieve an anti- or low-fouling surface.

5 Decapod shells that may have naturally anti- or low-fouling properties may be selected to provide an artificial bio-mimicking surface that has anti- or low-fouling properties. The decapods from which the artificial surface may be mimicked from to provide an anti- or low-fouling artificial biomimetic surface,
10 may fulfil at least one of the following criteria:

- 1) occurrence in shallow water tropical habitats with heavy fouling conditions;
- 2) generally low level or absence of fouling on their surface, particularly their carapace; and
- 15 3) lack of burrowing behaviour or occurring in a habitat where burrowing is unlikely.

Structures found on the surfaces of the selected decapods may be significantly different in shape and sizes.

The surface of the decapod may exhibit hierarchical
20 structures with shape and size ranges that may prevent adherence of both larger organisms such as barnacles as well as smaller organisms such as algae. The surface microtopography which comprises protruding structures as well as a series of folds and creases may be associated with improved foul-release properties
25 and reduced organic matter retention.

A substrate for aquatic environments may have a patterned surface comprising a plurality of protruding structures extending from the surface of the substrate, said structures may be comprised of a first structure type and a second structure
30 type that may have different dimensions to the first structure type, the structures may be selectively arranged on the

substrate to at least partially decrease biofouling when exposed to an aquatic environment compared to a substrate without said patterned surface.

5 The substrate may further comprise N different structure types that have different dimensions to the first structure type, second structure type or each other. N may be any integer. N may be any integer in the range of 1 to 100. N may be any integer in the range of 1 to 10. N may be 1.

10 The structures on the substrate may be arranged on the surface in an interspersed manner. The first structure type, the second structure type, the third structure type, the Nth structure type or any mixture thereof, may be arranged on the surface in an interspersed manner.

15 The structures of the first structure type may be adjacent to structures of the second structure type. The structures of the first structure type may be adjacent to structures of the third structure type. The structures of the first structure type may be adjacent to structures of the Nth structure type. The structures of the second structure type may be adjacent to the structures of the third structure type. The structures of the second structure type may be adjacent to the structures of the Nth structure type. The structures of the third structure type may be adjacent to the structures of the Nth structure type. The structures of the first structure type, the second structure type, the third structure type, the Nth structure type or any combination thereof may be adjacent to each other.

25 Each structure type may be independently arranged in a generally hexagonal array or generally square array. The different structures types may be independently arranged in a general array shape selected from the group consisting of hexagonal array, square array and combinations thereof. The

hexagonal array may be the 2-dimensional lattice pattern, also known as the equilateral triangular lattice pattern, whereby three nearby points may form an equilateral triangle. The hexagonal array may have horizontal rows with triangles pointing up and down, or have vertical rows, with triangles pointing left and right. The horizontal rows and the vertical rows may differ by an angle of 30° . The hexagonal array with horizontal rows may be a special case of a centered rectangular (i.e. rhombic) grid, with rectangles which may be $\sqrt{3}$ times as high as wide. The hexagonal lattice with vertical rows may have the rectangles that are $\sqrt{3}$ times as wide as high. Hexagonal array patterns may have a symmetry category of wallpaper group p6m.

The square array may be the 2-dimensional lattice pattern, whereby four nearby points may form a square. The square lattice may be up-right square lattices, or diagonal square lattices. The up-right and diagonal lattices may differ by an angle of 45° . An upright square lattice may be viewed as a diagonal square lattice with a mesh size that is $\sqrt{2}$ times as large, with the centers of the squares added. Hexagonal lattice patterns may have a symmetry category of wallpaper group p4m.

The structures on the substrate may be at least partially overlaid with each other. The first structure type, the second structure type, the third structure type, the N^{th} structure type or any mixture thereof, may be at least partially overlaid with each other. The first structure type may or may not be at least partially overlaid over the second structure type. The first structure type may or may not be at least partially overlaid over the third structure type. The second structure type may or may not be at least partially overlaid over the third structure type.

The first structure type may or may not be at least partially overlaid with the N^{th} structure type. The second structure type may or may not be at least partially overlaid with the N^{th} structure type. The third structure type may or may not be at least partially overlaid with the N^{th} structure type. The first structure type, the second structure type, and the third structure type may be at least partially overlaid with each other. The first structure type, the second structure type and the N^{th} structure type may all be at least partially overlaid with each other. The first structure type, the second structure type, the third structure type and the N^{th} structure type may all be at least partially overlaid with each other.

Within the same pattern comprising a first structure type, second structure type, third structure type, N^{th} structure type or any mixture thereof, a part of the first structure type may be at least partially overlaid with the second structure type while a part of the first structure type may not be overlaid with the second structure type. Within the same pattern comprising a first structure type, second structure type, third structure type, N^{th} structure type or any mixture thereof, a part of the first structure type may be at least partially overlaid with the third structure type while a part of the first structure type may not be overlaid with the third structure type. Within the same pattern comprising a first structure type, second structure type, third structure type, N^{th} structure type or any mixture thereof, a part of the second structure type may be at least partially overlaid with the third structure type while a part of the second structure type may not be overlaid with the third structure type.

Each structure shape may be independently selected from the group consisting of a cylinder, cone, prism, pyramid and hemisphere.

5 The cylinder may be a solid formed by the points at a fixed distance from a given line segment that is the axis of the cylinder, and two planes (base faces) perpendicular to this axis. All cross-sections parallel to the base faces may be the same. The cross-section may be a circle, ellipse, parabola or hyperbola. The cylinder may be a right circle cylinder, elliptic
10 cylinder, parabolic cylinder or hyperbolic cylinder.

The cone may be a solid formed by a base in a plane and by a surface (lateral surface) formed by the locus of all straight line segments joining the apex to the perimeter of the base. The base may be a circle or ellipse. The cone may be a right
15 circular cone or oblique circular cone.

The prism may be a solid formed by an n-sided polygonal base, a translated copy (not in the same plane as the first) and n other faces joining the corresponding sides of the two bases. The prism may be a right prism in which the joining edges and
20 faces are perpendicular to the base faces. The prism may be an oblique prism where the joining edges and the faces are not perpendicular to the base faces. All cross-sections parallel to the base faces may be the same. The cross-section may be a polygon. The polygon may be any n-gon. The polygon may be a
25 triangle, quadrilateral, pentagon, hexagon, heptagon, octagon, nonagon, decagon, hendecagon, dodecagon, pentadecagon, icosagon, hectagon, chiliagon, myriagon or megagon.

The polygon may be any regular n-gon that is equiangular and equilateral. The polygon may be an equilateral triangle,
30 square, regular pentagon, regular hexagon, regular heptagon, regular octagon, regular nonagon, regular decagon, regular

hendecagon, regular dodecagon, regular pentadecagon, regular icosagon, regular hectagon, regular chiliagon, regular myriagon or regular megagon.

5 The hemisphere may be half of a sphere. The sphere is a solid formed by a set of points that are all the same distance from a given point in three-dimensional space.

The structure may be a hemiellipsoid.

10 The first structure type may be selected from the group consisting of a cylinder and cone. The second structure type may be selected from the group consisting of a cylinder, cone and hemisphere. The third structure type may be selected from the group consisting of a cylinder, cone and hemisphere.

15 Each structure type may independently be in a cluster arranged in a generally hexagonal array or generally square array.

The cluster may comprise 1 to 10, 1 to 3, 1 to 5, 1 to 7, 3 to 5, 3 to 7, 3 to 10, 5 to 7, 5 to 10 or 7 to 10 structures of a single structure type selected from the group consisting of a first structure type, second structure type, third structure type or N^{th} structure type. The cluster may comprise 1, 5 to 7, 3 to 7 or 4 to 10 structures of a single structure type.

25 Each cluster may be arranged in a polygonal or circular shape. The polygon may or may not be a regular polygon. The circle may or may not be a regular circle. The polygon may be any n-gon. The polygon may be a triangle, quadrilateral, pentagon, hexagon, heptagon, octagon, nonagon, decagon, hendecagon, dodecagon, pentadecagon, hexadecagon, icosagon, hectagon, chiliagon, myriagon or megagon. The polygon may be a trapezoid or an octagon. The first structure type may have at least one of the following features: (a) a base diameter in the range of about 0.05 μm to about 150 μm ; (b) an aspect ratio in

the range of about 0.5 to about 150; or (c) the distance between each structure of the first structure type is in the range of about 0.1 μm to about 500 μm .

5 The base diameter of the first structure type may be in the range of about 0.05 to about 0.1, about 0.05 to about 0.2, about 0.05 to about 0.5, about 0.05 to about 1.0, about 0.05 to about 1.2, about 0.05 to about 2.5, about 0.1 μm to about 2.5 μm , about 0.1 μm to about 0.2 μm , about 0.1 μm to about 0.5 μm , about 0.1 μm to about 1.2 μm , about 0.2 μm to about 0.5 μm ,
10 about 0.2 μm to about 1.2 μm , about 0.2 μm to about 2.5 μm , about 0.5 μm to about 1.2 μm , about 0.5 μm to about 2.5 μm , about 1.2 μm to about 2.5 μm , about 0.5 μm to about 8 μm , about 0.5 μm to about 1 μm , about 0.5 μm to about 2 μm , about 0.5 μm to about 4 μm , about 0.5 μm to about 6 μm , about 1 μm to about 2
15 μm , about 1 μm to about 4 μm , about 1 μm to about 6 μm , about 1 μm to about 8 μm , about 5 μm to about 150 μm , about 5 μm to about 10 μm , about 5 μm to about 20 μm , about 5 μm to about 50 μm , about 5 μm to about 70 μm , about 5 μm to about 100 μm , about 10 μm to about 20 μm , about 10 μm to about 50 μm , about 10 μm to
20 about 70 μm , about 10 μm to about 100 μm , about 10 μm to about 150 μm , about 20 μm to about 50 μm , about 20 μm to about 70 μm , about 20 μm to about 100 μm , about 20 μm to about 150 μm , about 50 μm to about 70 μm , about 50 μm to about 100 μm , about 50 μm to about 150 μm , about 70 μm to about 100 μm , about 70 μm to
25 about 150 μm or about 100 μm to about 150 μm .

The base diameter of the first structure type may be in the range of about 0.1 μm to about 2.5 μm , 0.2 μm to about 1.2 μm , about 0.5 μm to about 8 μm , about 1 μm to about 6 μm , about 10 μm to about 100 μm , about 20 μm to about 70 μm about 5 μm to
30 about 150 μm or about 10 μm to about 100 μm .

The aspect ratio of the first structure type may be in the range of about 0.5 to about 1, about 0.5 to about 5, about 0.5 to about 10, about 0.5 to about 12, about 0.5 to about 15, about 0.5 to about 20, 0.5 to about 30, about 0.5 to about 50, about 0.5 to about 100, about 0.5 to about 150, about 1 to about 5, about 1 to about 10, about 1 to about 12, about 1 to about 15, about 1 to about 20, about 1 to about 30, about 1 to about 50, about 1 to about 100, about 1 to about 150, about 5 to about 10, about 5 to about 12, about 5 to about 15, about 5 to about 20, about 5 to about 30, about 5 to about 50, about 5 to about 100, about 5 to about 150, about 10 to about 12, about 10 to about 15, about 10 to about 20, about 10 to about 30, about 10 to about 50, about 10 to about 100, about 10 to about 150, about 12 to about 15, about 12 to about 20, about 12 to about 30, about 12 to about 50, about 12 to about 100, about 12 to about 150, about 15 to about 20, about 15 to about 30, about 20 to about 30, about 30 to about 50, about 30 to about 100 or about 30 to about 150.

The aspect ratio of the first structure type may be in the range of about 0.5 to about 30, about 1 to 15, about 1 to about 20, about 2 to about 12, about 0.5 to about 150 or about 1 to about 100.

The distance between the structures of the first structure type is in the range of about 0.5 μm to about 2 μm , about 0.5 μm to about 0.8 μm , about 0.5 μm to about 1 μm , about 0.5 μm to about 1.6 μm , about 0.8 μm to about 1 μm , about 0.8 μm to about 1.6 μm , about 0.8 μm to about 2 μm , about 1 μm to about 1.6 μm , about 1 μm to about 2 μm , about 1.6 μm to about 2 μm , about 1 μm to about 8 μm , about 1 μm to about 1.5 μm , about 1 μm to about 2 μm , about 1 μm to about 4 μm , about 1 μm to about 6.5 μm , about 1.5 μm to about 2 μm , about 1.5 μm to about 4 μm , about 1.5 μm to about 6.5 μm , about 1.5 μm to about 8 μm , about 2 μm to about

4 μm , about 2 μm to about 6.5 μm , about 2 μm to about 8 μm ,
about 4 μm to about 6.5 μm , about 4 μm to about 8 μm , about 6.5
 μm to about 8 μm , about 5 μm to about 250 μm , about 5 μm to
about 10 μm , about 5 μm to about 50 μm , about 5 μm to about 100
5 μm , about 5 μm to about 150 μm , about 5 μm to about 200 μm ,
about 10 μm to about 50 μm , about 10 μm to about 100 μm , about
10 μm to about 150 μm , about 10 μm to about 200 μm , about 10 μm
to about 250 μm , about 50 μm to about 100 μm , about 50 μm to
about 150 μm , about 50 μm to about 200 μm , about 50 μm to about
10 250 μm , about 100 μm to about 150 μm , about 100 μm to about 200
 μm , about 100 μm to about 250 μm , about 150 μm to about 200 μm ,
about 150 μm to about 250 μm , about 200 μm to about 250 μm ,
about 0.1 μm to about 500 μm , about 0.1 μm to about 0.5 μm ,
about 0.1 μm to about 1 μm , about 0.1 μm to about 10 μm , about
15 0.1 μm to about 100 μm , about 1 μm to about 10 μm , about 1 μm to
about 100 μm , about 1 μm to about 500 μm , about 10 μm to about
100 μm , about 10 μm to about 500 μm or about 100 μm to about 500
 μm .

The distance between the structures of the first structure
20 type is in the range of about 0.5 μm to about 2 μm , about 0.8 μm
to about 1.6 μm , about 1 μm to about 8 μm , about 1.5 μm to about
6.5 μm , about 5 μm to about 150 μm , about 10 μm to about 100 μm ,
about 5 μm to about 250 μm or about 10 μm to about 200 μm .

The cluster of the first structure type may comprise 2 to
25 12 structures of the first structure type. The cluster of the
first structure type may comprise 3 to 7 or 5 to 7 structures of
the first structure type.

The cluster may be in a polygonal shape. The cluster of the
first structure type may be in a trapezoidal shape.

30 The distance between each cluster of the first structure
type may be in the range of about 0.5 μm to about 1500 μm , about

0.5 μm to about 1 μm , about 0.5 μm to about 5 μm , about 0.5 μm to about 10 μm , about 0.5 μm to about 50 μm , about 0.5 μm to about 100 μm , about 0.5 μm to about 500 μm , about 0.5 μm to about 1000 μm , about 1 μm to about 5 μm , about 1 μm to about 10 μm , about 1 μm to about 50 μm , about 1 μm to about 100 μm , about 1 μm to about 500 μm , about 1 μm to about 1000 μm , about 1 μm to about 1500, about 5 μm to about 10 μm , about 5 μm to about 50 μm , about 5 μm to about 100 μm , about 5 μm to about 500 μm , about 5 μm to about 1000 μm , about 5 μm to about 1500 μm , about 10 μm to about 50 μm , about 10 μm to about 100 μm , about 10 μm to about 500 μm , about 10 μm to about 1000 μm , about 10 μm to about 1500 μm , about 50 μm to about 100 μm , about 50 μm to about 500 μm , about 50 μm to about 1000 μm , about 50 μm to about 1500 μm , about 100 μm to about 500 μm , about 100 μm to about 1000 μm , about 100 μm to about 1500, about 500 μm to about 1000 μm , about 500 μm to about 1500 μm or about 1000 μm to about 1500 μm .

The distance between each cluster of the first structure type may be in the range of about 0.8 μm to about 10 μm or about 200 μm to about 100 μm .

The second structure type may have at least one of the following features: (a) a base diameter in the range of about 5 μm to about 350 μm ; (b) an aspect ratio in the range of about 0.05 to about 150; or (c) the distance between each structure of the second structure type is in the range of about 0.5 μm to about 1500 μm .

The base diameter of the second structure type may be in the range of about 5 μm to about 350 μm , about 5 μm to about 10 μm , about 5 μm to about 50 μm , about 5 μm to about 100 μm , about 5 μm to about 150 μm , about 5 μm to about 250 μm , about 5 μm to about 250 μm , about 5 μm to about 300 μm , about 10 μm to about 50 μm , about 10 μm to about 100 μm , about 10 μm to about 150 μm ,

about 10 μm to about 250 μm , about 10 μm to about 250 μm , about
10 μm to about 300 μm , about 10 μm to about 350 μm , about 50 μm
to about 100 μm , about 50 μm to about 150 μm , about 50 μm to
about 250 μm , about 50 μm to about 250 μm , about 50 μm to about
5 300 μm , about 50 μm to about 350 μm , about 100 μm to about 150
 μm , about 100 μm to about 250 μm , about 100 μm to about 250 μm ,
about 100 μm to about 300 μm , about 100 μm to about 350 μm ,
about 150 μm to about 250 μm , about 150 μm to about 250 μm ,
about 150 μm to about 300 μm , about 150 μm to about 350 μm ,
10 about 250 μm to about 300 μm , about 250 μm to about 350 μm or
about 300 μm to about 350 μm .

The base diameter of the second structure type may be in
the range of about 30 μm to about 300 μm , 50 μm to about 250 μm ,
about 5 μm to about 150 μm , about 10 μm to about 100 μm , about
15 100 μm to about 350 μm or about 150 μm to about 300 μm .

The aspect ratio of the second structure type may be in the
range of about 0.05 to about 0.1, about 0.05 to about 0.5, about
0.05 to about 1, about 0.05 to about 2, about 0.05 to about 3,
about 0.05 to about 4, about 0.05 to about 5, about 0.1 to about
20 0.5, about 0.1 to about 1, about 0.1 to about 2, about 0.1 to
about 3, about 0.1 to about 4, about 0.1 to about 5, about 0.5
to about 1, about 0.5 to about 2, about 0.5 to about 3, about
0.5 to about 4, about 0.5 to about 5, about 1 to about 2, about
1 to about 3, about 1 to about 4, about 1 to about 5, about 2 to
25 about 3, about 2 to about 4, about 2 to about 5, about 3 to
about 4, about 3 to about 5, about 4 to 5 or about 5 to 10,
about 5 to 25, about 5 to 50, about 5 to 100, about 10 to 25,
about 10 to 50, about 10 to 100, about 25 to 50, about 25 to 100
or about 50 to 100.

30 The aspect ratio of the second structure type may be in the
range of about 0.05 to 5, about 0.1 to about 3, about 0.1 to

about 4, about 0.2 to about 3, about 5 to 150 or about 10 to about 100.

The distance between each structure of the second structure type may be in the range of about 0.5 μm to about 1500 μm , about
5 0.5 μm to about 1 μm , about 0.5 μm to about 2 μm , about 0.5 μm to about 5 μm , about 0.5 μm to about 10 μm , about 0.5 μm to about 20 μm , about 0.5 μm to about 30 μm , about 30 μm to about 50 μm , about 30 μm to about 100 μm , about 30 μm to about 300 μm , about 30 μm to about 500 μm , about 30 μm to about 600 μm , about
10 30 μm to about 700 μm , 50 μm to about 100 μm , about 50 μm to about 300 μm , about 50 μm to about 500 μm , about 50 μm to about 600 μm , about 50 μm to about 700 μm , about 100 μm to about 300 μm , about 100 μm to about 500 μm , about 100 μm to about 600 μm , about 100 μm to about 700 μm , about 300 μm to about 500 μm ,
15 about 300 μm to about 600 μm , about 300 μm to about 700 μm , about 500 μm to about 600 μm , about 500 μm to about 700 μm , about 600 μm to about 700 μm , about 500 μm to about 750 μm , about 500 μm to about 1000 μm , about 500 μm to about 1250 μm , about 500 μm to about 1500 μm , about 750 μm to about 1000 μm ,
20 about 750 μm to about 1250 μm , about 750 μm to about 1500 μm , about 1000 μm to about 1250 μm , about 1000 μm to about 1500 μm or about 1250 μm to about 1500 μm .

The distance between each structure of the second structure type may be in the range of about 30 μm to about 600 μm , about
25 50 μm to about 500 μm , about 50 μm to about 700 μm or about 100 μm to about 600 μm .

The cluster of the second structure type may comprise 4 to 12 structures of the first structure type. The cluster of the second structure type may comprise 4 to 10 structures of the
30 second structure type.

The cluster may be in a polygonal shape. The cluster of the second structure type may be in the shape of half an equiangular polygon. The cluster of the second structure may be in the shape of half an equiangular hexadecagon. The cluster of the second structure type may be in an octagonal shape.

The distance between each cluster of the second structure type may be in the range of about 0.5 μm to about 1500 μm

The N different structure types may have at least one of the following features: (a) a base diameter in the range of about 10 μm to 750 μm ; or (b) an aspect ratio in the range of about 0.05 to 10.

The base diameter of the N^{th} type of structure may be in the range of about 0.1 μm to about 800 μm . The aspect ratio of the N^{th} type of structure may be in the range of about 0.05 to about 250. The distance between each structure of the N^{th} type of structure may be in the range of about 0.5 μm to about 1500 μm .

Each cluster of the N^{th} type of structure may comprise 2 to 12 structures of the third structure type. The cluster may be in a polygonal shape. The distance between each cluster of the N^{th} type of structure may be in the range of about 0.5 μm to about 1500 μm .

The third structure type may have at least one of the following features: (a) a base diameter in the range of about 10 μm to about 750 μm ; or (b) an aspect ratio in the range of about 0.05 to about 10.

The base diameter of the third structure type may be in the range of about 30 μm to about 600 μm , about 30 μm to about 50 μm , about 30 μm to about 100 μm , about 30 μm to about 200 μm , about 30 μm to about 250 μm , about 30 μm to about 300 μm , about 30 μm to about 500 μm , about 50 μm to about 100 μm , about 50 μm to about 200 μm , about 50 μm to about 250 μm , about 50 μm to about

300 μm , about 50 μm to about 500 μm , about 50 μm to about 600 μm ,
100 μm to about 200 μm , about 100 μm to about 250 μm , about 100
 μm to about 300 μm , about 100 μm to about 500 μm , about 100 μm
to about 600 μm , about 200 μm to about 250 μm , about 200 μm to
5 about 300 μm , about 200 μm to about 500 μm , about 200 μm to
about 600 μm , about 250 μm to about 300 μm , about 250 μm to
about 500 μm , about 250 μm to about 600 μm , about 300 μm to
about 500 μm , about 300 μm to about 600 μm or about 500 μm to
about 600 μm .

10 The base diameter of the third structure type may be in the
range of about 30 μm to about 300 μm , about 50 μm to about 250
 μm , about 200 μm to about 600 μm or 300 μm to about 500 μm .

The aspect ratio of the third structure type may be in the
range of about 0.05 to about 0.1, about 0.05 to about 0.2, about
15 0.05 to about 0.5, about 0.05 to about 1, about 0.05 to about 2,
about 0.05 to about 3, 0.1 to about 0.2, about 0.1 to about 0.5,
about 0.1 to about 1, about 0.1 to about 2, about 0.1 to about 3,
0.2 to about 0.5, about 0.2 to about 1, about 0.2 to about 2,
about 0.2 to about 3, about 0.5 to about 1, about 0.5 to about 2,
20 about 0.5 to about 3, about 1 to about 2, about 1 to about 3 or
about 2 to 3.

The aspect ratio of the third structure type may be in the
range of about 0.05 to about 5, about 0.1 to about 2 or about
0.1 to about 3.

25 The distance between each structure of the third structure
type may be in the range of about 100 μm to about 150 μm , about
100 μm , to about 200 μm , about 100 μm , to about 300 μm , about
100 μm to about 500 μm , about 100 μm to about 600 μm , about 100
 μm to about 900 μm , about 100 μm to about 1000 μm , about 150 μm ,
30 to about 200 μm , about 150 μm , to about 300 μm , about 150 μm to
about 500 μm , about 150 μm to about 600 μm , about 150 μm to

about 900 μm , about 150 μm to about 1000 μm , about 200 μm , to about 300 μm , about 200 μm to about 500 μm , about 200 μm to about 600 μm , about 200 μm to about 900 μm , about 200 μm to about 1000 μm , about 300 μm to about 500 μm , about 300 μm to about 600 μm , about 300 μm to about 900 μm , about 300 μm to about 1000 μm , about 500 μm to about 600 μm , about 500 μm to about 900 μm , about 500 μm to about 1000 μm , about 600 μm to about 900 μm , about 600 μm to about 1000 μm or about 900 to about 1000 μm .

10 The distance between each structure of the third structure type may be in the range of about 100 μm to about 600 μm , about 150 μm to about 500 μm , about 200 μm to about 1000 μm or about 300 μm to about 900 μm .

15 The cluster of the third structure type may comprise 2 to 12 structures of the third structure type. The cluster may be in a polygonal shape. The distance between each cluster of the third structure type may be in the range of about 0.5 μm to about 1500 μm .

20 At least one of: (a) the distance between each cluster of the first structure type; (b) the distance between each cluster of the second structure type; or (c) the distance between each cluster of the third structure type may be in the range of 0.5 μm to 1500 μm .

25 The distances between the features may determine whether a given organism may be able to penetrate the space between the structures on the surface of the substrate. The structures may be present on the surface at a sufficient density to at least partially prevent biofouling.

30 The distance between each structure of the first structure type and each structure of the second structure type may be in the range of about 1 μm to about 500 μm , about 1 μm to about 5

10 μm to about 500 μm , about 50 μm to about 100 μm , about 50 μm to about 500 μm or about 100 μm to about 500 μm .

The distance between each cluster of the first structure type and each cluster of the third structure type may be in the
5 range of about 1 μm to about 500 μm , about 1 μm to about 5 μm , about 1 μm to about 10 μm , about 1 μm to about 50 μm , about 1 μm to about 100 μm , about 5 μm to about 10 μm , about 5 μm to about 50 μm , about 5 μm to about 100 μm , about 5 μm to about 500 μm , about 10 μm to about 50 μm , about 10 μm to about 100 μm , about
10 10 μm to about 500 μm , about 50 μm to about 100 μm , about 50 μm to about 500 μm or about 100 μm to about 500 μm .

The distance between each cluster of the second structure type and each cluster of the third structure type may be in the range of about 1 μm to about 500 μm , about 1 μm to about 5 μm ,
15 about 1 μm to about 10 μm , about 1 μm to about 50 μm , about 1 μm to about 100 μm , about 5 μm to about 10 μm , about 5 μm to about 50 μm , about 5 μm to about 100 μm , about 5 μm to about 500 μm , about 10 μm to about 50 μm , about 10 μm to about 100 μm , about 10 μm to about 500 μm , about 50 μm to about 100 μm , about 50 μm
20 to about 500 μm or about 100 μm to about 500 μm .

The distance between each structure of the first structure type and each cluster of the second structure type may be in the range of about 1 μm to about 500 μm , about 1 μm to about 5 μm , about 1 μm to about 10 μm , about 1 μm to about 50 μm , about 1 μm
25 to about 100 μm , about 5 μm to about 10 μm , about 5 μm to about 50 μm , about 5 μm to about 100 μm , about 5 μm to about 500 μm , about 10 μm to about 50 μm , about 10 μm to about 100 μm , about 10 μm to about 500 μm , about 50 μm to about 100 μm , about 50 μm to about 500 μm or about 100 μm to about 500 μm .

30 The distance between each structure of the first structure type and each cluster of the third structure type may be in the

about 10 μm to about 50 μm , about 10 μm to about 100 μm , about 10 μm to about 500 μm , about 50 μm to about 100 μm , about 50 μm to about 500 μm or about 100 μm to about 500 μm .

5 The distance between each structure of the third structure type and each cluster of the second structure type may be in the range of about 5 μm to about 150 μm , about 5 to about 10 μm , about 5 μm to about 20 μm , about 5 μm to about 50 μm , about 5 μm to about 100 μm , about 10 μm to about 20 μm , about 10 μm to about 50 μm , about 10 μm to about 100 μm , about 10 μm to about 150 μm , about 20 μm to about 50 μm , about 20 μm to about 100 μm , about 20 μm to about 150 μm , about 50 μm to about 100 μm , about 50 μm to about 150 μm or about 100 μm to about 150 μm .

The pattern may comprise an overlap of: (i) a first hexagonal or square pattern comprising structures of a first structure type or clusters of a first structure type, wherein;
15 (a) each structure of the first structure type has a base diameter in the range of about 0.1 to about 150 μm and an aspect ratio in the range of about 0.5 to about 150, wherein the distance between each structure of the first structure type is in the range of about 0.5 μm to about 250 μm ; and (b) each cluster of the first structure type comprises about 1 to about 10 structures of the first structure type, arranged in a first polygonal shape, wherein the distance between each cluster of the first structure type is in the range of about 0.5 μm to about 1500 μm ;
25 (ii) a second hexagonal or square pattern comprising structures of a second structure type or clusters of a second structure type, wherein; (a) each structure of the second structure type has a base diameter in the range of about 5 μm to about 350 μm and an aspect ratio in the range of about 0.05 to about 150, wherein the distance between each structure of the second structure type is in the range of about 0.5 μm to
30

about 250 μm ; and (b) each cluster of the second structure type comprises about 1 to about 10 structures of the second structure type arranged in a second polygonal shape, wherein the distance between each cluster of the second structure type is in the range of about 5 μm to about 1500 μm ; and (iii) the distance between the clusters of the first structure type and the clusters of the second structure type is in the range of about 1 μm to about 100 μm .

The pattern may further comprise an overlap of: (iv) a third hexagonal or square pattern comprising structures of a third structure type or clusters of a third structure type, wherein; (a) each structure of the third structure type has a base diameter in the range of about 25 μm to about 750 μm and an aspect ratio in the range of about 0.05 to about 5, wherein the distance between the structures of the third structure type is in the range of about 100 μm to about 1000 μm ; and (b) each cluster of the first structure type comprises about 1 to about 10 structures of the first structure type, wherein the distance between each cluster of the third structure type is in the range of about 0.5 μm to about 1500 μm ; and (v) the distance between the clusters of the second structure type and the clusters of the third structure type is in the range of about 1 μm to about 500 μm ; and (vi) the distance between the clusters of the first structure type and the clusters of the third structure type is in the range of about 1 μm to about 500 μm .

The substrate may mimic the patterned surface on carapaces of *Myomenippe hardwickii*.

The substrate may have a patterned surface comprising the first structure type having a cylindrical or conical shape that has a base diameter in the range of about 0.5 μm to about 8 μm and an aspect ratio in the range of about 0.5 to about 30,

wherein the distance between the structures of the first structure type is in the range of about 0.5 μm to about 8 μm ; and the second structure type having a cylindrical or hemisphere shape that has a base diameter in the range of about 30 μm to about 300 μm and an aspect ratio in the range of about 0.05 to about 5, wherein the distance between the structures of the second structure type is in the range of about 30 to about 600 μm . The distance between the structures of the first structure type and the structures of the second structure type may be in the range of about 0.5 μm to about 8 μm . The substrate may have a patterned surface comprising the first structure type of cylindrical or conical shape that has a base diameter in the range of about 0.1 μm to about 2.5 μm and an aspect ratio in the range of about 0.5 to about 30, wherein the distance between the structures of the first structure type is in the range of about 0.1 μm to about 2.5 μm ; and each cluster of the first structure type comprises about 5 or about 7 structures of the first structure type arranged in a trapezoidal shape and the distance between each cluster of the first structure type is in the range of about 0.1 μm to about 10 μm ; and the second structure type of cylindrical or half sphere shape that has a base diameter in the range of about 30 μm to about 300 μm and an aspect ratio in the range of about 0.05 to about 5, wherein the distance between the structures of the second structure type is in the range of about 30 to about 600 μm . The distance between the clusters of the first structure type and the structures of the second structure type may be in the range of about 0.1 to about 10 μm .

The substrate may mimic the patterned surface on carapace of *Gaillardielliellus orientalis*.

The substrate may have a patterned surface comprising the first structure type of cylindrical or conical shape that has a

base diameter in the range of about 5 μm to about 150 μm and an aspect ratio in the range of about 0.5 to about 150, wherein the distance between the structures of the first structure type is in the range of about 5 μm to about 150 μm ; and each cluster of the first structure type comprises about 3 to about 7 structures of the first structure type, wherein the distance between each cluster of the first structure type is in the range of about 100 μm to about 1500 μm ; the second structure type of cylindrical or conical shape that has a base diameter in the range of about 5 μm to about 150 μm and an aspect ratio in the range of about 5 to about 150, wherein the distance between each structure of the second structure type is in the range of about 150 μm to about 1500 μm ; and each cluster of the second structure type comprises about 4 to about 10 structures of the second structure type arranged in a polygonal shape, wherein the distance between each cluster of the second structure type is in the range of 5 μm to 1500 μm ; the third structure type of cylindrical or hemisphere shape that has a base diameter in the range of about 30 μm to about 300 μm and an aspect ratio in the range of about 0.05 to about 5, wherein the distance between the structures of the third structure type is in the range of about 100 μm to about 600 μm ; the distance between the structures of the first structure type and the structures of the second structure type is in the range of about 5 μm to about 1500 μm ; the distance between the structures of the first structure type and the structures of the third structure type is in the range of about 5 μm to about 1500 μm ; and the distance between the clusters of the second structure type and the third structure type is in the range of about 5 μm to about 150 μm .

The substrate may mimic the patterned surface on the carapace of *Acanthodroma margarita*.

The substrate may have a patterned surface comprising the first structure type of conical structures having polygonal or round cross-sections that has a base diameter in the range of about 10 μm to about 100 μm and an aspect ratio in the range of about 1 to about 15, wherein the distance between the structures of the first structure type is in the range of about 5 μm to about 250 μm ; the second structure type of conical structures having polygonal or round cross-sections that has a base diameter in the range of about 100 μm to about 350 μm and an aspect ratio in the range of about 0.1 to about 4, wherein the distance between the structures of the second structure type is in the range of about 50 μm to about 700 μm ; the third structure type of conical structures having polygonal or round cross-sections that has a base diameter in the range of about 250 μm to about 600 μm and an aspect ratio in the range of about 0.05 to about 5, wherein the distance between the structures of the third structure type is in the range of about 200 μm to about 1000 μm ; wherein the distance between the structures of the first structure type is in the range of 5 μm to about 1500 μm , the distance between the structures of the second structure type is in the range of about 5 μm to about 1500 μm ; and the distance between the structures of the third structure type is in the range of about 5 μm to about 1500 μm .

The substrate may be any solid or semi-solid medium that may be used in an aquatic environment. The substrate may be a free-standing solid, a film, a coating or gel. The substrate may be a liquid medium that comprises particles capable of self-arrangement on a surface. The substrate may be a hydrogel. The substrate may be a polymer, copolymer or polymer composite. The polymer may be any polymer that is used in the marine industry or in the biomedical industry. The polymer may be selected from

the group consisting of polydimethylsiloxane, polyurethane, polymethylmethacrylate, polystyrene, polyvinylchloride, rubber and any mixture thereof. The polymer may be selected from the group consisting of polydimethylsiloxane, polyurethane, and any
5 mixture thereof. The substrate may be non-toxic to organisms. The substrate may be a polydimethylsiloxane film.

The copolymer may be derived from more than one species of monomer. The polymer composite may be a composite between a polymer and a non-polymeric substance. The polymer composite may
10 be polymer clay, polymer metal, polymer nanocomposite and any combination thereof.

The substrate may further comprise a polymeric surface coating. The polymeric surface coating may be any coating that may enhance the anti-fouling or fouling-release properties of
15 the substrate. The polymeric surface coating may be a polymer brush, layer-by-layer deposited film, molecular monolayer or any combination thereof.

A use of a substrate for aquatic environment, may comprise the use of the substrate having a patterned surface comprising a
20 plurality of protruding structures extending from the surface of the substrate, the structures being comprised of a first structure type and a second structure type that has different dimensions to the first structure type, the structures being selectively arranged on the substrate, to at least partially
25 decrease biofouling when exposed to an aquatic environment compared to a substrate without said patterned surface.

The use may be non-toxic to organisms.

The use may be in free-standing surfaces, films or coatings which can be used in on- or off-shore stationary equipment such
30 as underwater sensors, pipe systems, cables, harbour structures, oil platforms, oil rigs, bridges, water desalination plants,

water purification plants, and the like, as well as mobile objects such as ships, submarines, floating bridges, pontoons and the like, to prevent biofouling and or to form foul-release coatings.

5 The use may at least partially decrease antifouling of a substrate.

10 A method for fabricating a substrate comprising a patterned surface to at least partially decrease biofouling in aquatic environments, may comprise the steps of: (a) providing protruding structures, the protruding structures comprising of a first structure type and a second structure type that has different dimensions to the first structure type on the substrate; and (b) selectively arranging the protruding structures on the substrate.

15 The method may further comprise the step of providing N different structure types that have different dimensions to the first structure type, the second structure type or each other, after the first providing step.

20 The method may be selected from the group consisting of moulding, imprinting, coating and any mixture thereof.

 The method may be imprinting on a surface. The imprinting may be performed by fabricating a mould and applying pressure against the surface using the mould to form a surface comprising the patterned surface. The imprinting may be hot embossing.

25 The method may be coating on a surface. The coating may be performed by application of a liquid medium comprising particles capable of self-arrangement on a surface. The coating may be paint. Self-arrangement of molecules or particles may allow spontaneous formation of 3-dimensional structures based on a 2-
30 dimensional design. A substrate may be covered with a monolayer of a first reagent. The substrate may be subsequently covered

with a mask and exposed to UV radiation in such way that after the removal of the mask there will be areas with and without the first reagent on the substrate. Thus prepared substrate may be subsequently exposed to the suspension of particles in a second
5 reagent having affinity only to the first reagent. Upon contact, particles of the second reagent may assemble onto the first reagent. The pattern of the first reagent may also be printed on the substrate. Partial magnetisation of the substrate and subsequent exposure to metal particles may also facilitate self-
10 assembly of particles.

The method may be moulding.

The method may comprise the step of forming the structures using a moulding method.

The moulding may be performed by fabricating a mould and
15 casting a substrate comprising the patterned surface.

The substrate may be any solid or semi-solid medium that may be used in an aquatic environment. The substrate may be a free-standing solid, a film, a coating or gel. The substrate may be a liquid medium that comprises particles capable of self-
20 arrangement on a surface. The substrate may be a hydrogel. The substrate may be a polymer, copolymer or polymer composite. The polymer may be any polymer that is used in the marine industry or in the biomedical industry. The polymer may be selected from the group consisting of polydimethylsiloxane, polyurethane,
25 polymethylmethacrylate, polystyrene, polyvinylchloride, rubber and any mixture thereof. The polymer may be selected from the group consisting of polydimethylsiloxane, polyurethane, and any mixture thereof. The substrate may be non-toxic to organisms. The substrate may be a polydimethylsiloxane film.

30 The copolymer may be derived from more than one species of monomer. The polymer composite may be a composite between a

polymer and a non-polymeric substance. The polymer composite may be polymer clay, polymer metal, polymer nanocomposite and any combination thereof.

The providing step may further comprise the steps of: (i) providing an image of a crustacean surface; (ii) determining the dimensions of the structures on the crustacean surface from the image of (i); (iii) constructing histograms of the dimensions of structures on the crustacean surface determined in (ii); (iv) extracting the most common dimensions of structures on the crustacean surface from the histograms of (iii); (v) approximating a pattern based on the dimensions extracted in (iv); and (vi) fabricating the protruding structure in the pattern of (v).

The crustacean may be any crustacean that may comprise a patterned surface which may naturally have anti- or low-fouling properties. The crustacean may be a decapod. The crustacean may occur in shallow water tropical habitats with heavy fouling conditions. The crustacean may have generally low level or absence of fouling on their surface, particularly their carapace. The crustacean may lack burrowing behaviour or occur in a habitat where burrowing is unlikely.

The image may be any image that has sufficient resolution of the surface structures. The image may be a macroscopic or microscopic image. The image may be a microscopic image. The image may be a scanning electron microscopy image.

The determining step may be physical measuring.

The constructing step may be plotting the dimensions of structures on the crustacean surface. The constructing step may comprise plotting the dimensions with dimension on the x-axis and count or frequency on the y-axis.

The extracting step may comprise analysing what measurement value is most common for each parameter of dimension. The extracting step may comprise analysing what dimension of the structures is the most common. The extracting step may comprise
5 using a Gaussian curve approximation to analyze what dimensions are most common.

The approximating step may be done manually or with a computer program.

A method for fabricating a substrate comprising a patterned
10 surface for aquatic environments to at least partially decrease biofouling may comprise the steps of: (a) providing a photoresist coated silicon wafer; (b) exposing the photo-resist coated silicon wafer through a first photolithography mask comprising holes having a diameter in the range of 0.1 to 200
15 μm ; (c) etching the silicon wafer; (d) coating the silicon wafer of (c) with photoresist; (e) exposing the silicon wafer of (d) through a second photolithography mask comprising holes having a diameter in the range of 5 to 400 μm ; (f) etching the silicon wafer of (e); (g) coating the silicon wafer of (f) with a
20 polymer; and (h) separating the polymer from the silicon wafer.

The method may further comprise the steps of; (i) coating the etched silicon wafer of (f) with photoresist; (j) exposing the silicon wafer of (i) through a third lithography mask comprising holes having a diameter in the range of 30 to 600 μm ;
25 and (k) etching the silicon wafer of (j); between etching step (f) and coating step (g).

The photo resist may be a light-sensitive material. The photo resist may be any light-sensitive material used in photolithography. The photoresist may be Hoechst AZ4620 or
30 OiR674-05.

The silicon wafer may have a diameter in the range of about 80 mm to about 120 mm, about 90 mm to about 110 mm or about 99.5 to about 100.5 mm. The silicon wafer may have an orientation of <100>. The silicon wafer may be a P-type or N-type wafer. The silicon wafer may be doped with Boron. The silicon wafer may have a thickness of 450 μm to about 600 μm or about 500 μm to about 550 μm . The silicon wafer may have a resistivity of about 1 Ω to about 100 Ω .

The first photo lithography mask may have holes with diameters in the range of about 0.1 μm to about 200 μm . The first photo lithography mask may have holes with diameters in the range of about 0.5 μm to about 1.5 μm , about 0.75 μm to about 1.25 μm , about 1 to 5 μm , 2 to 4 μm or 2.5 to 3.5 μm . The distance between the holes of the first lithography mask may be in the range of about 5 μm to 10 μm , about 6 μm to about 9 μm or about 7 μm to about 8 μm .

The second photo lithography mask may have holes with diameters in the range of about 50 μm to about 150 μm , about 70 μm to about 130 μm or about 95 μm to about 105 μm . The distance between the holes of the second lithography mask may be in the range of about 200 μm to about 300 μm or about 225 μm to about 275 μm .

The polymer of steps (g) and (h) may be selected from the group consisting of polydimethylsiloxane, polyurethane, polymethylmethacrylate, polystyrene, polyvinylchloride, rubber and any mixture thereof. The polymer may be selected from the group consisting of polydimethylsiloxane, polyurethane, and any mixture thereof.

The exposing steps (b), (e) or (j) may be performed at an energy density suitable for etching a hole with the appropriate dimension. The exposing steps (b), (e) or (j) may be performed

at an energy density in the range of about 20 mJ/cm³ to about 500 mJ/cm³, about 20 mJ/cm³ to about 50 mJ/cm³, about 20 mJ/cm³ to about 100 mJ/cm³, about 20 mJ/cm³ to about 150 mJ/cm³, about 20 mJ/cm³ to about 200 mJ/cm³, about 20 mJ/cm³ to about 300 mJ/cm³,
5 about 20 mJ/cm³ to about 400 mJ/cm³, about 50 mJ/cm³ to about 100 mJ/cm³, about 50 mJ/cm³ to about 150 mJ/cm³, about 50 mJ/cm³ to about 200 mJ/cm³, about 50 mJ/cm³ to about 300 mJ/cm³, about 50 mJ/cm³ to about 400 mJ/cm³, about 50 mJ/cm³ to about 500 mJ/cm³, about 100 mJ/cm³ to about 150 mJ/cm³, about 100 mJ/cm³ to about
10 200 mJ/cm³, about 100 mJ/cm³ to about 300 mJ/cm³, about 100 mJ/cm³ to about 400 mJ/cm³, about 100 mJ/cm³ to about 500 mJ/cm³, about 150 mJ/cm³ to about 200 mJ/cm³, about 150 mJ/cm³ to about 300 mJ/cm³, about 150 mJ/cm³ to about 400 mJ/cm³, about 150 mJ/cm³ to about 500 mJ/cm³,. about 200 mJ/cm³ to about 300 mJ/cm³, about
15 200 mJ/cm³ to about 400 mJ/cm³, about 200 mJ/cm³ to about 500 mJ/cm³, about 300 mJ/cm³ to about 400 mJ/cm³, about 300 mJ/cm³ to about 500 mJ/cm³ or about 400 mJ/cm³ to about 500 mJ/cm³.

The etching steps (c), (f) or (k) may be performed for a duration in the range of about 2 minutes to about 30 minutes,
20 about 2 minutes to about 5 minutes, about 2 minutes to about 10 minutes, about 2 minutes to about 20 minutes, about 5 minutes to about 10 minutes, about 5 minutes to about 20 minutes, about 5 minutes to about 30 minutes, about 10 minutes to about 20 minutes or about 20 minutes to about 30 minutes.

25 The method may further comprise coating the substrate with a surface polymeric coating. A use of the polymer film, wherein the use may be in an aquatic environment. The aquatic environment may be fresh water, salt water and any mixture thereof. The polymer film may prevent biofouling. The use may be
30 non-toxic to organisms.

Brief Description of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed
5 embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1 comprises Fig. 1(a) to 1(f) and is a set of histograms constructed based on measurements of the dimension of
10 the structures on the surface of *Myomenippe hardwickii*. Fig. 1(a) is a histogram of the distance between tubercles, Fig. 1(b) is a histogram of the diameter of tubercles, Fig. 1(c) is a histogram of the distance between clusters, Fig. 1(d) is a histogram of the number of spines within clusters, Fig, 1(e) is
15 a histogram of the diameter of spines and Fig. 1(f) is a histogram of the length of spines. Gaussian curve approximation was applied for each histogram to determine the most common dimension.

Fig. 2 is a set of Scanning Electron Microscope (SEM)
20 images of the carapace surface of *Momenippe hardwickii*.

Fig. 3(a) is a schematic showing the G1 pattern. Images are not to scale.

Fig. 3(b) is a set of Scanning Electron Microscope (SEM) images of G1 patterned PDMS pieces.

25 Fig. 4(a) is a schematic showing the G2 pattern. Images are not to scale.

Fig. 4(b) is a set of Scanning Electron Microscope (SEM) images of G2 patterned PDMS pieces.

Fig. 5 is a set of Scanning Electron Microscope (SEM)
30 images of the carapace surface of *Gaillardielliellus orientalis*.

Fig. 6 is a schematic showing the G3 pattern.

Fig. 7 is a set of Scanning Electron Microscope (SEM) images of the carapace surface of *Acanthodromia margarita*.

Fig. 8 is a schematic showing the G4 pattern.

Fig. 9 is a graph showing the settling of fouling species on smooth and G1 patterned PDMS pieces. "Other Hard" stands for other hard foulers and "Other Soft" stands for other soft foulers.

Fig. 10 is a table showing photographic images of smooth and G1 patterned PDMS pieces subjected to fouling in a marine environment. "Control" refers to smooth PDMS pieces.

Examples

Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Example 1: Generation of the patterns

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To generate the patterns, the structures on the decapod carapaces were examined using images taken with a Scanning Electron Microscope (SEM). Analysis of the dimensions of the structures on the carapaces was performed. The dimensions of the structures were then correlated with the settlement of various aquatic organisms. Subsequently, fabrication limitations for the structures were considered and any structures that were not feasible to manufacture were removed.

More specifically, *Myomenippe hardwickii* specimens were collected from fouling communities located in Tuas, on the west coast of Singapore. A fragment from the dorsal carapace was

removed for examination using scanning electron microscopy (SEM). The fragments were critically dried at temperatures up to 40 °C in a Balzers CPD 030, then were platinum-coated in a JEOL JFC-1600 sputter coater, and finally examined with a JEOL JSM 6510LV SEM microscope. Samples from 19 specimens in total were examined.

The carapace surface of *M. hardwickii* displays low-aspect-ratio calcareous structures (tubercles) in two size classes, as well as very few hairs. To the naked eye, the carapace appears clean and smooth, with setae only found in bundles at the edges of the carapace and legs. Examination with scanning electron microscopy (SEM) showed that the carapace surface is covered by almost flat circular tubercles (5 µm high and 25 - 170 µm in diameter). Between the tubercles, clusters (4 - 8) of small spines (0.6-1.2 µm in diameter, 3-13 µm long) are present.

It is known that 2-µm-size features may deter the settlement of algal spores, whereas features on the order of 20 µm may be effective against settlement of barnacle larvae, and microtopographies of similar size (2 µm width x 4 - 16 µm length) may reduce and delay the formation of biofilms of *Staphylococcus aureus* without the need for prophylactic antibacterial agents. Since the *M. hardwickii* model exhibited hierarchical features across both of these size classes, it was speculated that the high aspect-to-ratio features on carapaces may have potential antifouling properties, while the purpose of larger low-aspect-ratio features may be to reduce mechanical damage to the small spines.

Analysis was performed on data obtained from two-dimensional (2D) SEM images of the surfaces of at least five different specimens of *M. hardwickii* by measuring the dimension of the features and the distance between these features. At

least 100 measured values for the distance between tubercles, diameter of tubercles, distance between clusters, number of spines within clusters, diameter of spines and length of spines were obtained and histograms were constructed for each parameter showing the distribution of measurements (Fig. 1). Histograms of the dimensions provide a robust analysis for extracting the most common features of each dimension. These histograms were used to determine what dimension is the most common for each parameter of the structures on the surface by applying a Gaussian curve approximation. For example, the most common distance between tubercles, as shown in Fig. 1(a), is in the range of 100 μm to 150 μm , the most common diameter of tubercles as shown in Fig. 1(b) is in the range of 100 μm to 110 μm , the most common distance between the clusters as shown in Fig. 1(c) is in the range of 4 μm to 5 μm , the most common number of spines within a cluster as shown in Fig. 1(d) is 5, the most common diameter of spines as shown in Fig. 1(e) is in the range of 0.8 μm and 0.9 μm and the most common length of spines as shown in Fig. 1(f) is in the range of 6 μm to 7 μm . The histograms were also used to approximate the arrangement of the structures/clusters to be a hexagonal array. The resolution, that is the minimum separation between the structures, and the minimum diameter of each structure for which the lithography and etching equipment was capable of producing, was also taken into account when designing the patterns.

Example 2: Detailed descriptions of the patterned PDMS surface

G1 Pattern

The G1 pattern is a bio-mimetic pattern mimicking the patterned surface on carapaces of *Myomenippe hardwickii*.

Myomenippe hardwickii may be found living in rocky shores in shallow water, hiding under rocks, crevices and boulders. This species may also be very abundant in sub-tidal fouling communities. The shell may have calcerous features (tubercles) in two size classes, both having low aspect ratios. The shell may also have very few hairs. The carapace may appear clean and smooth. Seta may be found in bundles at the edges of the carapace and legs. The carapace surface may be covered by almost flat (approximately 5 μm) and oval shape tubercles (30 to 120 μm in diameter x 20 to 90 μm log). Between the tubercles, small groups of about 3 to 5 small spines (1 to 4 μm in diameter, 8 to 12 μm long) may be present (Fig. 2).

Fig. 3(a) is a schematic showing the G1 pattern. G1 consists of two overlapping hexagonal patterns: the first pattern consists of cylindrical to conical structures of a diameter in the range of 1 μm to 6 μm and an aspect ratio (height:width) in the range of 1 to 20. The distance between the features (A) is in the range of 1.5 μm to 6.5 μm . The second pattern consists of cylindrical or hemisphere structures of a diameter in the range of 50 μm to 250 μm and an aspect ratio in the range of 0.1 to 3. The distance between the features (B) is in the range of 50 μm to 500 μm . Fig. 3(b) shows a set of Scanning Electron Microscope (SEM) images of G1 patterned PDMS pieces.

G2 Pattern

The G2 pattern is a bio-mimetic pattern mimicking the patterned surface on carapaces of *Myomenippe hardwickii*. Fig. 4(a) is a schematic showing the G2 pattern. G2 consists of two overlapping hexagonal patterns: the first pattern consists of clusters of cylindrical to conical structures of a diameter in

the range of 0.2 μm to 1.2 μm and an aspect ratio (height:width) in the range of 1 to 20, arranged in clusters of five or seven. The clusters are of trapezoid shape and are arranged in a checkered pattern or in a line. The distance of the features within a cluster is in the range of 0.8 μm to 1.6 μm and the distance between the clusters (A) is in the range of 0.8 μm to 10 μm . The second pattern consists of cylindrical or hemisphere structures of a diameter in the range of 50 μm to 250 μm and an aspect ratio in the range of 0.1 to 3. The distance between the features (B) is in the range of 50 μm to 500 μm . Fig. 4(b) shows a set of Scanning Electron Microscope (SEM) images of G2 patterned PDMS pieces.

G3 Pattern

The G3 pattern is a bio-mimetic pattern mimicking the patterned surface on carapace of *Gaillardielliellus orientalis*. *Gaillardielliellus orientalis* may be found in rocky shores. It may possess large tubercles (approximately 2 mm) with deep and clean grooves on the surface, as well as bundles of thick hair comprising 4 to 8 strands and having lengths of 300 μm to 1200 μm . These bundles of hair may often be associated with small (approximately 200 μm), evenly distributed tubercles. The average distance between the tubercles may vary from 400 μm to 700 μm . The seta may exhibit a distinct surface, where the lower region may be smooth whereas the upper region may have hundreds of setula having two morphologies; (i) round, thin and long (>15 μm) or (ii) flat, wide and short (<2 μm) (Fig. 5).

Fig. 6 is a schematic showing the G3 pattern. G3 consists of three overlapping hexagonal patterns. The first pattern consists of clusters of cylindrical or conical hair-like structures of a diameter (C) in the range of 10 μm to 100 μm and

an aspect ratio (height:width) in the range of 1 to 100, arranged in clusters of three to seven. The distance between the features within each cluster (D) is in the range of 10 μm to 100 μm and the distance between the clusters (E) is in the range of 200 μm to 1000 μm . The second pattern consists of cylindrical or conical hair-like structures of a diameter in the range of 10 μm to 100 μm and an aspect ratio in the range of 1 to 100, arranged in clusters of four to ten. The clusters are of half-equiangular polygonal shape with equal distribution of shapes. The third pattern consists of cylindrical or hemisphere structures of a diameter (B) in the range of 50 μm and 250 μm and an aspect ratio in the range of 0.1 to 4. The distance between the centres of the features (A) is in the range of 150 μm and 500 μm . The distance between the clusters of the second pattern and features of the third pattern is in the range of 10 μm to 100 μm .

G4 Pattern

The G3 pattern is a bio-mimetic pattern mimicking the patterned surface on carapace of *Acanthodroma margarita*. *Acanthodromia margarita* may be found in deep water, with possibly muddy substrates, where fouling may be lower. The shell may be covered by conical spines occasionally accompanied by one or two protruding hairs. The abdominal area may be covered by numerous tubercles and protuberances of the same size. The orientation of the spines may be perpendicular to the carapace. The typical spine may have a tip with several holes and ridges, and this feature may also be visible at the tip of the spine buds. Larger spines, at their surface, may have small (1 to 2 μm) protuberances aligned in groups of 2 to 5 and perpendicular to the spine axis. Some undulations may also be visible following the same general orientation. The spine base may be a

widened structure having a ring like shape with the seta placed on that structure. The spine sizes may vary, but there may be 4 prominent size classes of $53 \pm 13 \text{ }\mu\text{m}$, $153 \pm 30 \text{ }\mu\text{m}$, $427 \pm 34 \text{ }\mu\text{m}$ and $835 \pm 171 \text{ }\mu\text{m}$. Typically, spines in the two smallest size classes (spinules) may fill the space between the spines of the two larger size classes. The average distance between the larger spines may be $708 \pm 182 \text{ }\mu\text{m}$, while the average distance between the smaller spines (spinules) may be $226 \pm 43 \text{ }\mu\text{m}$. *A. margarita* is unique in having a small aspect ratio. That is, structures on the surface of *A. margarita* may generally be much broader at the base (Fig. 7). The tubercles may be multifunctional.

Fig. 8 is a schematic showing the G4 pattern. G4 consists of three overlapping hexagonal patterns of conical features having polygonal or round cross-sections. The first pattern consists of structures of a diameter in the range of $20 \text{ }\mu\text{m}$ to $70 \text{ }\mu\text{m}$ and an aspect ratio (height:width) in the range of 2 to 12. The distance between the features is in the range of $10 \text{ }\mu\text{m}$ to $200 \text{ }\mu\text{m}$. The second pattern consists of structures of a diameter in the range of $150 \text{ }\mu\text{m}$ to $300 \text{ }\mu\text{m}$ and an aspect ratio in the range of 0.2 to 3. The distance between the features is in the range of $100 \text{ }\mu\text{m}$ to $600 \text{ }\mu\text{m}$. The third pattern consists of structures of diameter between $300 \text{ }\mu\text{m}$ to $500 \text{ }\mu\text{m}$ and an aspect ratio in the range of 0.1 and 2. The distance between the features is in the range of $300 \text{ }\mu\text{m}$ to $900 \text{ }\mu\text{m}$.

Example 3: Fabrication of silicon moulds

Microtextured surfaces biomimicking *M. hardwickii* were fabricated using two mask designs. Each mask design consists of $20 \text{ mm} \times 20 \text{ mm}$ hexagonal patterns of circular holes, and a smooth control area.

Mask A1, used for generating first generation patterns (G1), consists of 3 μm diameter holes with a distance of 7.5 μm between the centre of the holes.

Mask A2, used for generating second generation patterns (G2), consists of clusters of 1 μm diameter holes with a distance of 2 μm between the centre of the holes.

Mask B, used for both G1 and G2, consists of 100 μm diameter holes with a distance of 250 μm between the centre of the holes.

Masks A1 and A2 were used to fabricate silicon moulds with etched cones/cylinders with a depth of 20 μm ($\varnothing$ 100 μm) and 10 μm ($\varnothing$ 6 μm and $\varnothing$ 1 μm) for G1 and G2, respectively. The moulds were used to fabricate PDMS pattern replicas, as described below in Example 4.

15

A silicon wafer with the following properties:

Prime silicon wafer 4"

Diameter: 100 $\pm$ 0.5 mm

Orientation: <100>

Type/Dopant: P/Boron

Thickness: 525 $\pm$ 25 μm

Resistivity: 1-100 Ω/cm

Flat: semi-standard

Polish: single side polished

obtained from MOS group Pte Ltd was vapour-coated with hexamethyldisilazane (HMDS, Fluka Analytical 52619-250 mL).

For the G1 pattern, the silicon wafer was spin-coated with photo-resist AZ4620 (AZ Electronic Materials, USA) and soft-baked at 100 $^{\circ}\text{C}$ for 6.5 minutes. The photo-resist was exposed at 30 mJ/cm^2 through the photolithography mask A1 (Mask and Bond Aligner, SUSS Microtec) and developed in an AZ Developer 400k

(AZ Electronic Materials, USA) diluted with deionised water (1:5) for 30 seconds, rinsed with deionised water and blown dry with N₂ gas. The wafer was then subjected to Reactive Ion Etching (RIE), with a total etching time of 20 minutes to form 10 µm deep holes of 6 µm diameter.

For the G2 pattern, the silicon wafer was spin-coated with photo-resist OiR674-05 (FujiFilm) and soft-baked at 100 °C for 1 minute. The photo-resist was exposed at 140 mJ/cm² through the photolithography mask A2 (Canon FPA-2000il Fine Pattern Aligner) and developed in an AZ Developer 400k (AZ Electronic Materials, USA) diluted with deionised water (1:5) for 30 seconds, rinsed with deionised water and blown dry with N₂ gas. The wafer was then subjected to Deep Reacting Ion Etching (DRIE), with a total etching time of 4 minutes during the Bosch process for holes of 1 µm diameter.

As dry etching of Si-wafer with gases is an isotropic process, i.e. the etching progresses with the same rate in every direction, the side walls of the opening must be protected during the process in order to achieve a cylindrical profile of the etched opening (anisotropic etching). RIE and DRIE offer different methods to do so with different advantages.

RIE relies on a carefully established ratio of reactive and inert gases, whereas DRIE relies on a polymer coating process in-between the etching steps. Thus, it is slightly more challenging to control RIE with respect to obtaining the vertical walls of the etched opening, especially if these openings are of a diameter below 3 µm. The etch time is also longer in RIE than in the DRIE process which is important considering the stability of the photoresist.

The used Bosch process (DRIE) allows for fast etching of high aspect ratio structures with flat bottoms and nearly

vertical side walls in short period of time. Thus it was used for structures of 1 μm in diameter.

RIE was used for structures of 6 and 100 μm as it resulted in sufficiently good cross-sectional profiles. Moreover, RIE was more advantageous for forming hemispherical structures of base diameter of about 100 μm , as such profile cannot be easily formed using the DRIE (Bosch) process.

For both G1 and G2 pattern wafers, the residual photo-resist was removed by rinsing with acetone (Honeywell Specialist Chemicals Seelze GmbH, 10189037). Subsequently, the wafer was cleaned with hot piranha solution (1 part 30% H_2O_2 (Electronics grade, MGC Pure Chemicals Singapore Pte. Ltd) and 2 parts 95–97% H_2SO_4) for 20 minutes, rinsed with deionized water and dried. The wafer was then vapour-coated with HMDS and photo-resist AZ4620.

Following this, the second pattern of holes of Mask B ($\varnothing$ 100 μm) was aligned with the already etched pattern using a SUSS Mask and Bond Aligner. The second pattern was subjected to RIE using an etching time of 20 minutes. Residual photo-resist was removed by rinsing with acetone. The resultant moulds were cleaned with hot piranha solution for 20 minutes then vapour-coated with 1H,1H,2H,2H-perfluorododecyltrichlorosilane (FDTS, Alfa Aesar, L16584).

Vapour-coating with FDTS was performed in the following manner: a syringe filled with approximately 0.1 mL of FDTS, placed in a 30 mL glass container was placed in the desiccator containing the moulds. The FDTS solution was vaporised by lowering the pressure in the desiccator and left to react with the moulds overnight. During the reaction, silane and water vapour reacts with the surface, forming silanols with fluoroalkyl chains, rendering the surface hydrophobic and of low energy.

The quality of the moulds and the depth of the etched features were inspected with a Scanning Electron Microscope (JEOL SEM JSM5600) and Surface Profiler.

5 **Example 4: Fabrication of patterned polymer surfaces**

Polydimethylsiloxan (PDMS)

Patterns were replicated in polydimethylsiloxane (PDMS) using standard PDMS casting procedures. The quality of the PDMS
10 microstructured samples was controlled with an optical microscope (Olympus BX51 microscope equipped with Olympus TH4-200 light source and Olympus DP70 digital camera).

Polydimethylsiloxane (PDMS, SYLGARD 184 Silicone Elastomer Kit, Best Chemical Co(s) Pte Ltd) base was mixed with the cross-
15 linker Sylgard 184 in a 10:1 volume ratio. The mixture was poured over the FDTS-coated silicon mould of Example 3 and placed in a disposable aluminium dish with the microstructured surface facing upwards). Once the mould was covered with PDMS, the dishes were placed in a desiccator to degas under low
20 pressure for 30 to 40 minutes. The degassed samples were cured in an oven at 60 °C overnight, under atmospheric pressure. After curing, the PDMS microstructured samples were separated from the moulds, cut into 2 x 2 cm pieces and stored for further tests.

25 Polymethylmethacrylate (PMMA)

Patterned PMMA samples were fabricated from polymer pellets using compression moulding (manual hot press, LabTech Engineering Company Ltd). During this process, the polymer pellets were placed onto the FDTS-coated silicon mould of
30 Example 3, placed within a metal frame which determined the sample dimensions and acted as a spacer in between two parallel

plates. Subsequently, the prepared stack was placed in the hot press and heated up to the polymer melting temperature. Once the polymer was softened, the sample was degassed by a means of fifteen quick pressurization-de-pressurization cycles, followed by a stack pressurization to 250 and 500 Psi, at the polymer melting temperature, for 15 minutes. Following this, the samples were demoulded by cooling the samples down to 150°C and demoulding while hot. This was done due to the material shrinkage upon cooling which may lead to the damage of the silicone mould.

Polyurethane (PU)

Patterns were replicated in polyurethane (PU) using two methods. The first method was the same as the method used for PMMA described above, except during the demoulding step, the PU samples were gradually cooled down under pressure to room temperature before being separated from the mould.

Alternatively, patterns were replicated in PU by pouring a UV-curable solution of PU over the FDTS-coated silicon mould of Example 3 and subsequently degassing the samples by a means of pressurization and depressurization cycles. After removal of gas bubbles, the samples were exposed to UV radiation and the cured samples were separated from the mould.

Example 5: Laboratory testing of the patterned PDMS surface

Barnacle settlement test

Amphibalanus Amphitrite barnacle larvae were spawned from adults collected from Kranji mangrove, Singapore. The nauplius larvae were fed with an algal mixture 1:1 v/v of *Tetraselmis*

suecica and *Chaetoceros muelleri* (CSIRO Microalgae Research Centre, Australia) at a density of approximately $5 \times 10^5 \text{ mL}^{-1}$ and reared at 27 °C in 27 parts per trillion (ppt), 0.2 µm filtered sea water. Nauplii metamorphosed into cyprids in 5 days and cyprids were aged for 2 days at 4 to 6 °C prior to use in settlement assays.

A choice experiment was set up using 2 day old cyprids suspended in a 50 µm Nitex mesh basket in approximately 800 mL of 27 ppt, 0.22 µm filtered sea water. A total of 3200 cyprids were added to give a final concentration of 4 cyprid/mL.

5 pieces each of Smooth PDMS (control) and G1 patterned PDMS were rinsed with distilled water prior to use, randomly immersed vertically in the cyprid culture and incubated in the dark at 27 °C for 24 hours. After 24 hours, the PDMS pieces were removed and the number of settled cyprids on each piece of the PDMS was counted. The $\log_{10}$ data analysis of variance (ANOVA) indicated that the surface structure (patterned or smooth) has a significant effect on the settlement of barnacle cyprids. Significantly higher settlement occurred on the patterned PDMS surface compared to the smooth PDMS ($p = 0.017$).

Amphora adhesion assay

Amphora coffeaeformis (UTEX number B2080) was maintained in F2 medium in tissue culture flasks at 24 °C under a 12 hour light:12 hour dark regime for a week prior to use. Algae were gently removed from culture flasks with a cell scraper and clumps were broken up by continuous pipetting and filtering through a 35 µm Nitex mesh. Cell count was determined with a haemocytometer and a suspension of 10,000 cells/mL was made up in 30 ppt, 0.22 µm filtered seawater. Smooth PDMS (control) and

G1 patterned PDMS pieces and glass cover slips were randomly placed into wells in a 6-well plate. To each well, 5 mL of algal cell suspension was added.

The dish was incubated for 24 hours in a 12 hour light:12 hour dark regime at 24 °C. At the end of the incubation period, all PDMS pieces and slides were dip-rinsed in 30 ppt, 0.22 µm filtered sea water to remove unattached cells. The rinse step was repeated thrice. The PDMS pieces or slides were then dripped dry and the number of cells in ten random fields of view at a 20 x magnification (0.916 mm²) were counted under a fluorescence microscope. The result of the Amphora adhesion assay showed that the surface microtopography has a highly significant effect (p<0.001) on cell adhesion.

Example 6: Field testing of the patterned PDMS surface

The samples were tested in at the Republic of Singapore Yacht Club (TMSI testing platform). Smooth PDMS (control) and G1 patterned PDMS pieces were fastened in a per-cut polyethylene (PE) slide box holder to allow seawater flow. The samples were equally spaced out in the box, and the box was immersed in seater. Four replicate samples each of the smooth and patterned polymers were threaded with a nylon thread and suspended in the slide-box holder frame at 0.5 m sea depth.

This was covered with a netting to reduce grazing by marine animals. The samples were photographed and inspected every week. The number of settling organisms was counted on each sample, disregarding 10% of the area around the edges of each sample during counting

The test was carried out for approximately 7 weeks. After 8 weeks, the surfaces became heavily covered with sediments,

making the identification of settled organisms difficult. Thus, the test was terminated after 8 weeks and only results from the first 7 weeks are shown.

As shown in Fig. 9 and Fig. 10, the field test clearly demonstrates that the surface micropattern on the PDMS pieces has a significant effect on the adhesion of fouling species. Generally less fouling was observed on the patterned PMDS pieces compared to the smooth PMDS (control) pieces. It should be noted that as seen in Fig. 9, there was an initial build-up of a biofilm followed by a decrease in the attached biomass. This suggests that the patterned surface has fouling-release properties.

The first design tested under laboratory conditions suppresses adsorption of algae and enhances adsorption of barnacles, as demonstrated in Example 5. This is in agreement with previous studies that suggest the size and dimensions of the surface structures that suppress the adsorption of fouling organisms correspond to the size and dimensions of the fouling organisms themselves. However, the G1 pattern was shown to reduce overall fouling under field conditions.

Applications

The disclosed pattern and the pattern formed on surfaces may have applications in fabrication of free-standing surfaces, films or coatings which can be used in on- or off-shore stationary equipment such as underwater sensors, pipe systems, cables, harbour structures, oil platforms, oil rigs, bridges, water desalination plants, water desalination and purification plants, and the like, as well as mobile objects such as ships,

submarines, floating bridges, pontoons and the like, to prevent biofouling and or to form foul-release coatings.

The disclosed pattern and the pattern formed on surfaces may have applications in prevention of biofouling in medicine,
5 such as the interior of catheters, tubing for dialysis, and the like.

The disclosed pattern and the pattern formed on surfaces may have applications in prevention of biofouling in the food industry, cosmetics industry, pharmaceutical industry and the
10 like in manufacturing plants at facilities such as reactors, coolers, piping systems and the like.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure
15 without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A substrate for aquatic environments, the substrate having a patterned surface comprising a plurality of protruding structures extending from the surface of the substrate, said structures being comprised of a first structure type and a second structure type that has different dimensions to the first structure type, the structures being selectively arranged on the substrate to at least partially decrease biofouling when exposed to an aquatic environment compared to a substrate without said patterned surface, wherein the first structure type has a base diameter in the range of 0.05 μm to 50 μm and an aspect ratio in the range of 1 to 100 and the second structure type has a base diameter in the range of 50 μm to 350 μm and an aspect ratio in the range of 0.05 to 4.

2. The substrate according to claim 1, further comprising N different structure types that have different dimensions to the first structure type, the second structure type or each other, wherein N is any integer.

3. The substrate according to claim 2, wherein N is 1.

4. The substrate according to any one of the preceding claims, wherein the structures are arranged on the surface in an interspersed manner.

5. The substrate according to claim 4, wherein the structures of the first structure type are adjacent to structures of the second structure type.

6. The substrate according to any one of the preceding claims, wherein the different structure types are independently arranged in a general array shape selected from the group consisting of hexagonal array, square array and combinations thereof.

7. The substrate according to any one of the preceding claims, wherein the different structure types are at least partially overlaid with each other.

5 8. The substrate according to any one of the preceding claims, wherein the different structure types are independently arranged in grouped clusters on said substrate.

10 9. The substrate according to claim 8, wherein the grouped clusters are independently arranged in a general array shape selected from the group consisting of hexagonal array, square array and combinations thereof.

15 10. The substrate according to any of the preceding claims, wherein each structure type has a shape that is independently selected from the group consisting of a cylinder, cone, prism, pyramid and hemisphere.

20 11. The substrate according to any one of the preceding claims, wherein
the distance between each structure of the first structure type is in the range of 0.1 μm to 500 μm .

25 12. The substrate according to any one of claims 1 to 10, wherein
the distance between each structure of the second structure type is in the range of 0.5 μm to 1500 μm .

30 13. The substrate according to any one of claims 2 to 10, wherein the N different structure types have at least one of the following features:

- (a) a base diameter in the range of 10 μm to 750 μm ; or
- (b) an aspect ratio in the range of 0.05 to 10.

14. The substrate according to any of the preceding claims,
wherein at least one of:

5 (a) the distance between each cluster of the first structure
type;

(b) the distance between each cluster of the second structure
type; or

(c) the distance between each cluster of the third structure
type;

10 is in the range of 0.5 μm to 1500 μm .

15 15. The substrate according to any of the preceding claims,
wherein the substrate is a polymer or copolymer, optionally selected
from the group consisting of polydimethylsiloxane, polyurethane,
polymethylmethacrylate and any mixture thereof.

16. The substrate according to any of the preceding claims,
further comprising a polymeric surface coating.

20 17. A use of a substrate for aquatic environments, the
substrate having a patterned surface comprising a plurality of
protruding structures extending from the surface of the substrate,
said structures being comprised of a first structure

type and a second structure type that has different dimensions to the first structure type, the structures being selectively arranged on the substrate to at least partially decrease biofouling when exposed to an aquatic environment compared to a substrate without said patterned surface, wherein the first structure type has a base diameter in the range of 0.05 μm to 50 μm and an aspect ratio in the range of 1 to 100 and the second structure type has a base diameter in the range of 50 μm to 350 μm and an aspect ratio in the range of 0.05 to 4.

10

18. A method for fabricating a substrate comprising a patterned surface to at least partially decrease biofouling in aquatic environments, the method comprising the steps of:

(a) providing protruding structures, the protruding structures comprising of a first structure type and a second structure type that has different dimensions to the first structure type on the substrate, wherein the first structure type has a base diameter in the range of 0.05 μm to 50 μm and an aspect ratio in the range of 1 to 100 and the second structure type has a base diameter in the range of 50 μm to 350 μm and an aspect ratio in the range of 0.05 to 4; and

(b) selectively arranging the protruding structures on the substrate.

19. The method according to claim 18, wherein the providing step further comprises the steps of:

- (i) providing an image of a crustacean surface;
- (ii) determining the dimensions of the structures on the crustacean surface from the image of (i);
- (iii) constructing histograms of the dimensions of the
5 structures on the crustacean surface determined in (ii);
- (iv) extracting the most common dimensions of the structures on the crustacean surface from the histograms of (iii);
- (v) approximating a pattern based on the dimensions
10 extracted in (iv); and
- (vi) fabricating the protruding structures in the pattern of (v).

20. The method according to claim 18 or 19, further comprising the step of providing N different structure types that have different dimensions to the first structure type, the
5 second structure type or each other, wherein in N is an integer, after the providing step (a).

21. The method according to any one of claims 18 to 20, wherein the method is selected from the group consisting of
10 moulding, imprinting, coating and any mixture thereof.

22. The method according to claim 21, wherein the method comprises the step of forming the structures using a moulding method.
15

23. The method according to claim 22, wherein the substrate is a polymer or copolymer selected from the group consisting of polydimethylsiloxane, polyurethane, polymethylmethacrylate and any mixture thereof.
20

24. The method according to claim 23, further comprising coating the substrate with a surface polymeric coating.