Barnacle Settlement Behaviors on Microstructured Surfaces with Different Geometric Parameters

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Marine sessile organisms easily adhere to submerged surfaces (e.g., rocks, metals and plastics), and cause serious economic problem. Previously, tributyltin (TBT) has been used widely as antifoulant to inhibit the fouling by sessile organisms. However, TBT was banned to use globally due to its high endocrine disruption effects against marine organisms. Recently, antifouling activities of microstructured surfaces against marine sessile organisms have attracted attention. In this study, we prepared honeycomb patterned microstructured surfaces with different geometric parameters, and investigated the relationship between barnacle settlement and geometric parameters of microstructured surfaces. The results found the number of settled barnacles increased with the increasing of roughness factor of the microstructured surfaces with shallow pits. However, the number of settled barnacles was few without dependence of roughness factor of the microstructured surfaces with deep pits.

Keywords: Antifouling, Biofouling, Barnacles, Surface topography, Sessile organisms, Settlement

1. Introduction

Marine sessile organisms (barnacles, sea squirts, seaweeds, etc.) cause serious fouling problems on artificial submerged surfaces (ship’s full, fishnet, intake channel, etc). To inhibit the fouling by sessile organisms, several antifouling paint agents have been developed. Previously, tributyltin (TBT) has been used widely as antifoulant, and it shows high antifouling activity against sessile organisms. However, TBT based antifouling paint was banned globally due to its high endocrine disruption effects [1]. So, development of alternative environmentally benign antifouling technologies is necessary.

Soft materials [2,3] and artificial microstructures [4] are good candidates for non-biocidal antifouling materials. Recently, biomimetic antifouling technologies inspired by surfaces of marine organisms have attracted attention. M. E. Callow et al. reported the settlement of green algal zoospores on microstructured surfaces with valleys.

Fig. 1. The geometric parameters of honeycomb patterned microstructured surfaces (pore size, rim width, and depth).
or pillars, and they discussed the topographic preferences of zoospores [5]. K. K. Chung et al. reported the antifouling effect of the sharklet AF inspired by shark skin, and it inhibits the bacterial adhesion and growth [6]. A. M. Brzozowska et al. reported the synergistic antifouling activities of the bioinspired microstructured surface pattern and chemical surface modifications [7].

In this study, we report the antifouling effects of the geometric parameters of microstructured surfaces. To investigate the antifouling properties of the surface topography, we prepared the honeycomb patterned surfaces with different three characteristic geometric parameters (pore size, rim width, and depth) (Fig. 1), and investigate the settlement behaviors of barnacle cypris larvae on microstructured surfaces.

2. Experimental

2.1. Preparation of honeycomb patterned microstructured surfaces

Polydimethylsiloxane (PDMS, Sylgard184™, Dow Corning Toray Co., Ltd.) was used as the settlement test plates with honeycomb patterned microstructured surfaces. The precursor of PDMS resin and the crosslinker of PDMS (catalyst of Sylgard184™) were used as purchased.

At first, a photomask which have 9 square sections with different honeycomb pattern (pore sizes; 5, 15, 30 µm, rim sizes; 2, 6, 12 µm) was purchased from Toyo Precision Parts Mfg. Co., Ltd. The size of each section is 20 mm × 20 mm. And then, silicon molds used for the replication of PDMS microstructured surfaces were fabricated on 4 inch diameter silicon wafers using the photomask in photolithographic processes. In this case, two types of mold with different depth sizes (5, 15 µm) were fabricated using same photomask on each silicon wafer (a series of 5 or 15 µm depth with 5, 15, and 30 µm diameter pillars and 2, 6, and 12 µm rim width). PDMS pre-polymer was casted on the Si negative molds of honeycomb patterned microstructures and polymerized in the oven (70 °C, 7 h). And then, PDMS test plates peel off from molds (Fig. 2). Thus, we prepared 18 types of honeycomb patterned microstructured surfaces with different geometric parameters used for settlement test. The surface structures of the honeycomb patterned microstructured surfaces were observed by using a field emission-scanning electron microscope (FE-SEM; S-5200, Hitachi, Japan).

2.2. Preparation of barnacle cypris larvae

In this study, the cypris larva (settlement stage larva) of the barnacle Amphibalanus Amphitrite was used for settlement test. The barnacle cypris larvae were cultured according to standard procedures [8].
Autoclaved seawater (120 ºC, 20 min) was used in all experiments. Cypris larvae were kept at 6 ºC ~ 10 ºC in the dark condition in autoclaved seawater for two days before the settlement tests were carried out.

2.3. Preparation of settlement test system

Settlement tests were carried out in the wells with an inside diameter of 15 mm, and they consist of PDMS microstructured bottom and agarose gel wall (Fig. 3a). In previous studies reported that agarose gel inhibits barnacle settlement [2,9]. In this case, to inhibit barnacle settlement on the wall surfaces, we prepared the wall with agarose gel. Agarose (Agarose XP) was purchased from Wako pure Chemicals (Osaka, Japan). To apply the agarose gel wall, a 2 wt% agarose solution was prepared by dissolving agarose powder in autoclaved seawater and the solution was stirred for 20 min at 90 ºC. The hot agarose solution was poured in the well molds consist of PDMS microstructured bottom and acrylic cylindrical shape mold of 15 mm in diameter. The well molds were then kept at 4 ºC over 6 hours to allow the gel to set. After gelation, the cylindrical molds were removed, and then PDMS microstructured bottoms with agarose gel walls were immersed in a large volume of autoclaved seawater for two days to leach out residuals from the agarose gel.

2.4. Settlement test

Settlement tests were conducted according to standard procedures [10]. 0.5 mL of autoclaved seawater containing 15 cypris larvae was poured into each well. The cypris larvae loaded wells were cultured in an incubator (LPH-120SP; NK System, Japan) and held at a temperature of 25 ºC with a photoperiod of 8 h light under a cool white fluorescent lamp and 16 h dark for 3 days. Cypris larvae that had metamorphosed into juvenile barnacles were counted as ‘settlement’ (Fig. 3b). The settled barnacles were observed by using a stereomicroscope (SZX-16; Olympus, Japan) at 3 days after loading. The number of settled barnacles on three wells with same bottom was counted for each microstructured surface.

3. Results and discussion

Figure 4 shows the SEM images of the 18 types of honeycomb patterned microstructured surfaces with different geometric parameters. Large figures show top view of the surfaces, and upper right small figures show side view of them.
Figure 5 shows the result of settlement tests on 18 types of honeycomb patterned microstructured surfaces with different geometric parameters and the flat PDMS surface. It was found from the result that there is no relationship between barnacle settlement and pore sizes of honeycomb patterned microstructured surfaces in the range of 3.0 ~ 28.9 µm diameter. On the other hand, the barnacle settlement tended to decrease with increasing of the rim width and depth of microstructured surfaces.

The relationship between the total number of settled barnacles and (a) rim width, and (b) depth of honeycomb patterned microstructured surfaces is shown in Figure 6. The definition of roughness factor ($r$) is given in Figure 7.
The relationship between the total number of settled barnacles and rim width, and depth of honeycomb patterned microstructured surfaces are shown in Fig. 6(a) and Fig. 6(b), respectively. The number of barnacle settlement decreased with an increase in the rim width of microstructured surfaces. As shown in Fig. 6(b), the number of barnacle settlement decreased with an increase in the depth of microstructured surfaces. From these results, antifouling properties of honeycomb patterned microstructured surfaces might depend on the rim width and the depth of the honeycomb pattern pits.

To make a quantitative comparison of the roughness of honeycomb patterned microstructured surfaces, the roughness factor \( r \) is defined as the ratio of true area of the surface to the projected area (Fig. 7). Figure 8 shows the relationship between the total number of settled barnacles and the roughness factor \( r \) of honeycomb patterned microstructured surfaces. The number of settled barnacles increased with increasing roughness factor \( r \) on the surfaces with 5.2 µm of depth. On the other hand, surfaces with 16.1 µm of depth, few settlements were found without dependence of roughness factor, and the number of settled barnacles was lower compared to the flat PDMS surface \( (r = 1) \) in most cases. From these results, the depth might be the dominant geometric parameter for antifouling activities of honeycomb patterned microstructured surfaces.

In pre-settlement stage, cypris larvae explore on substrates with their two sensory organs, and determine the suitable place for their settlement. When cypris larva settles on the microstructured surface with large roughness factor, it obtains large surface area and establish strong adhesive cement layer. So, cypris larvae might prefer to settle on the surfaces with large roughness factor in the case of the surfaces with 5.2 µm of depth. During the exploring behavior, cypris larvae release temporary adhesive cement onto the substrates [11,12]. On the surfaces with deep pits (like the surfaces with 16.1 µm of depth), the adhesiveness of two sensory organs with temporary cement might be not good because temporary cement hard to fill the deep pits. So, their settlements might be inhibited on the surfaces with 16.1 µm of depth as a result.

4. Conclusion

In conclusion, we succeeded to fabricate the honeycomb patterned microstructured surfaces with different geometric parameters. In the results of settlement test, the barnacle settlement decreased with the increasing of the sizes of rim width and depth of the honeycomb pattern pits. From the analysis of roughness factor \( r \), barnacle settlement depended on the roughness of the microstructured surfaces with shallow pits. On the other hand, the number of barnacle settlement was few without dependence of roughness factor of the microstructured surfaces with deep pits. In future work, we will observe the 3D bottom surface structure of settled barnacles on several microstructured surfaces using 3D laser measuring microscope, and investigate the mechanical properties of cement layer on the microstructured surfaces with different roughness factor.

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