Antifouling Activity of Hydroxyl Functional Groups in PVA Thin Films Against the Settlement of Sessile Organisms in Laboratory and Field Conditions

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Sessile organisms cause serious fouling problems on artificial submerged surfaces all around the world. Tributyltin (TBT)-based antifouling paints have been widely used and show high antifouling performance against sessile organisms. However, TBT was banned from use in ship paints globally in 2008 by IMO because of its considerable endocrine disrupting effects in marine organisms. This has created a demand for ecofriendly antifouling materials. Recently, the antifouling properties of specific chemical and physical surface properties have attracted attention for the development of green antifouling technologies. Therefore, we focused on the relationship between surface chemical composition and antifouling activity against the settlement of sessile organisms. In this study, we prepared chemically crosslinked PVA thin films and investigated the antifouling activities against sessile organisms in both laboratory and field conditions. Our results highlight the inhibitory activity of hydroxyl functional groups in PVA against larval settlements with no toxicity detected in laboratory tests. Furthermore, the chemically crosslinked PVA coatings were demonstrated to display easy release activity against settled barnacles in the sea.

Keywords: Antifouling, Biofouling, Barnacles, Polyvinyl alcohol, Sessile organisms, Settlement

1. Introduction

Sessile organisms easily adhere to ship hulls and cooling water channels of power plants, thus causing serious fouling problems such as increased water resistance and higher fuel consumption in ships, and reduced cooling water intake rate due to pipe clogging [1,2]. In the past, tributyltin (TBT) compounds were widely used in antifouling paints; however, the use of TBT paint has been banned globally on ship hulls because TBT causes severe shellfish deformities and sex changes (imposex) [3]. The development of alternative environmentally friendly antifouling technologies is therefore crucial. Mainstream antifouling paints on the market belong to the dissolution and self-polishing antifouling categories of products [4-7]. Recently, progress has been made in the screening of marine natural products for antifouling compounds, and in the
development of synthetic biodegradable antifouling compounds [8-10]. Besides these approaches, environmentally friendly antifouling materials (e.g., silicone elastomers and hydrogels) have been developed. Silicone elastomers show high release activity against settled barnacles due to their specific properties (low surface energy, low elasticity, and high smoothness) [11-13]. Hydrogels prevent the larval settlement of barnacles because of their low elasticity and high swelling properties [14-17]. Furthermore, biomimetic antifouling materials, inspired by the specific surface texture or chemical composition of marine livings (e.g., shark and crab) have been recently reported [18-20].

Thus, the biological effect of chemical and physical properties of green antifouling technologies have been under study, and it is known that many factors affect barnacle settlement [21-24]. However, the crucial factors for preventing barnacle settlement are not completely understood. In particular, few studies have focused on the antifouling effects of surface chemical composition against barnacles. Previously, K. Ohkawa et al. reported on the relationship between the settlement of barnacles and mussels and the surface free energy of glass surfaces with different functional groups [25]. In addition, Rasmussen et al. reported that polyvinyl alcohol (PVA) (Fig. 1 (a)) hydrogels with abundant surface hydroxyl group (-OH) show high antifouling effects against barnacle cypris larvae [14].

In this study, we focused on the antifouling activity of OH groups against marine sessile organisms. We prepared chemically crosslinked PVA thin films and conducted settlement tests in laboratory and field conditions. In the laboratory test, we evaluated the antifouling activity of the surfaces with OH groups against barnacle cypris attachment. We also evaluated the antifouling activity against sessile organisms and the durability of the PVA thin films in the ocean for about 4 months.

2. Experimental
2.1. Preparation of PVA thin film-coated glass substrates for laboratory assay
The soda-lime glass substrates (0.7 mm thick, 40 mm \( \times \) 40 mm) were cleaned by UV-ozone treatment for 30 min using an ozone cleaner (UV/Ozone ProCleaner™ Plus, BioForce Nanosciences, Inc.). The glass substrates were washed with ethanol and ultra-pure water, and then dried at room temperature.

![Fig. 1. The chemical structures of (a) polyvinyl alcohol (PVA) and (b) the crosslinking agent (Orgatix® TC-315).](image)

PVA (Wako 1st Grade, degree of polymerization = 1,500 ~ 1,800, saponification value = 78 ~ 82 mol%), and the crosslinking agent (Orgatix® TC-315, a titanium lactate product) (Fig. 1 (b)) were purchased from FUJIFILM Wako Pure Chemical Corporation and Matsumoto Fine Chemical Co. Ltd., respectively.

5 wt% PVA solution was prepared by dissolving PVA in ultra-pure water at room temperature. The solution was subsequently mixed with the crosslinking agent. The concentration of crosslinker in PVA solution was varied: 10, 20, 30, 40, and 50 wt% (molar ratios of 0.19, 0.38, 0.56, 0.75, and 0.94, respectively, when the saponification value of PVA is estimated as ca. 80%). Each PVA solution was dripped onto the glass substrate and spin-coated using a spin coater (1H-D7, Mikasa Co., Ltd.). The samples thus prepared were dried in a vacuum oven (~0.1 Pa, 100 °C, 24 h). After drying, a thin film of chemically crosslinked PVA on the glass substrate was obtained.

Static contact angles of air bubbles and droplets (ultra-pure water or diiodomethane (FUJIFILM Wako Pure Chemical Corporation)) were measured on each PVA substrate using the contact angle meter (Drop Master and the analysis software FAMAS, Kyowa Interface Science Co., Ltd.). The sessile drop method was used; the droplet volume was 2 µL.

To analyze the surface free energy, the Kaelble–Uy's theory (1) was adopted because its formulation considers the surface free energy \( \gamma \) as consisting of two components (the dispersion component \( \gamma^d \) and the polar component \( \gamma^p \)) [26]. The surface free energy of each substrate was calculated using equations (1) and (2) and the measured values of the contact angle \( \theta \):

\[
\gamma = \gamma^d + \gamma^p \quad (1)
\]

\[
\gamma_L \left(1 + \cos \theta \right) = 2 \sqrt{\gamma_S^d \gamma_L^d} + 2 \sqrt{\gamma_S^p \gamma_L^p} \quad (2)
\]

herein, \( \gamma_S \) and \( \gamma_L \) are the surface free energies of the solid and the liquid, respectively, \( \gamma_S^d \) and \( \gamma_L^d \) are the...
are the dispersion components of the solid and the liquid, respectively, \( \gamma_L^D \) and \( \gamma_L^P \) are the polar components of the solid and the liquid, respectively. The values of \( \gamma_L, \gamma_L^D, \) and \( \gamma_L^P \) for ultra-pure water (20 °C) are 72.8, 21.8, and 51.0 mJ/m², respectively. The values of \( \gamma_L, \gamma_L^D, \) and \( \gamma_L^P \) for diiodomethane (20 °C) are 50.8, 48.5, 2.3 mJ/m², respectively.

2.2. Preparation of barnacle cypris larvae for laboratory assay

The cypris larva (settlement stage larva) of the barnacle *Amphibalanus amphitrite* was used for settlement tests in the laboratory. The adult barnacles were collected in Shizuoka, Shizuoka Prefecture (34°59’32” N 138°30’40” E). The cypris larvae obtained from adults were cultured according to standard procedures [27]. Autoclaved seawater (120 °C, 20 min) was used in all laboratory assays. The cypris larvae were kept at 5 °C ~ 10 °C in dark conditions in autoclaved seawater for two days before the settlement tests were carried out.

2.3. Settlement tests in the laboratory

Settlement tests were carried out in wells with an inside diameter of 15 mm, a chemically crosslinked PVA bottom, and silicone walls (flexiPERM disc, Sarstedt K.K.). Settlement tests were conducted according to standard procedures [28]. For this, 500 µL of autoclaved seawater containing 15 cypris larvae was poured into each well. The wells loaded with cypris larvae were cultured in an incubator (LPH-120SP; NK System) and held at a temperature of 25 °C with a photoperiod of 8 h of light under a cool white fluorescent lamp, followed by 16 h of darkness for 5 days. Half of the amount of seawater in each well was changed each day during the test period. Polystyrene (PS) wells (VSC24, AS-ONE, 15 mm in diameter) containing 30 cypris larvae were used as negative controls. Cypris larvae that had metamorphosed into juvenile barnacles were counted as “settlement,” as schematically illustrated in Fig. 2. The settled barnacles and non-settled cypris larvae were observed by using a stereomicroscope (SZX-16, Olympus) 5 days after loading. The number of settled, non-settled, and dead cypris was averaged over 6–9 wells. Statistical analysis was performed by Steel–Dwass multiple comparison test. The results were defined as statistically significant when \( p < 0.05 \), and statistically highly significant when \( p < 0.01 \).

2.4. Preparation of PVA thin film-coated aluminum substrates for the field test

Aluminum (Al) plates (1.0 mm thick, 100 mm × 100 mm, Japanese Industrial Standards JIS-A1050P) were cleaned under the same conditions as the PVA coated glass substrates for laboratory assays. Previously it was reported that nanoscale protrusions on the Al surface, which modify its surface wettability, can be formed by immersion in boiling water [29]. To enhance the hydrophilicity of the Al substrate and the adhesiveness between PVA films and Al substrates, the Al substrates were boiled for 25 min in ultra-pure water. After boiling, the formation of the nanoscale protrusion structure on the Al surface was confirmed (Fig. 3). We applied the same PVA coating treatment adopted for the laboratory assays to the Al substrate modified...
through hot water immersion, and obtained thin chemically crosslinked PVA film coated Al substrates. Al substrates just after UV-ozone treatment were used as a negative control.

2.5. Field experimental system

The field test was conducted at the private berth of Hokkaido soda group in Tomakomai port, Hokkaido, Japan (42°39'05"N 141°40'40"E). The design water depth of the berth is 10 m. The current velocity near the experimental site was usually under 10 cm/s, and almost never over 15 cm/s [30]. Experimental periods were between 10 May 2018 and 20 September 2018.

The field test system consisted of the test pieces (two PVA-coated Al plates, and two bare Al plates), a stainless frame, two stud plate fasteners per test piece, and three stainless chains. Photographs of the stainless frame, which can take several test pieces (SUS304, Kowa K.K.), used for the field test are shown in Fig. 4. Each set of test pieces, which were fixed on the upper site and the lower site of the stainless frame using stud plates, was placed vertically at the depth of 5 m beneath the sea surface with three stainless chains from a quay. The actual exposure size of the test piece was ca. 100 mm × 80 mm excluding the surface area covered with stud plate fasteners. The samples were temporarily removed from the sea, and photographs of the surfaces were taken in air, after exposure for 34, 63, 102 and 133 days. Data were collected from photographs of two specimens for each sample. The observation area on the specimen was defined as the 60 mm × 40 mm area near the center of the surface, to omit organisms that had settled on the stainless frame or fasteners. Living barnacle, dead barnacle, basis of the barnacle, or other sessile organisms were classified as settlements. We subsequently analyzed the surface coverage of sessile organisms using an image analysis software (Image J Ver. 1.42q, NIH). Furthermore, the histogram of barnacle coverage was analyzed by another image analysis software (Image-Pro Premier Ver. 9.0, Media Cybernetics).

| Sample code | PVA 10 | PVA 20 | PVA 30 | PVA 40 | PVA 50 | PS |
|-------------|--------|--------|--------|--------|--------|----|
| Concentration of crosslinker in PVA (wt %) | 10 | 20 | 30 | 40 | 50 | - |
| Theoretical degree of crosslinking (%) | 75 | 150 | 225 | 300 | 375 | - |
| Number of cypris larvae per well | 15 | 15 | 15 | 15 | 15 | 30 |
| Number of replicants | 9 | 6 | 9 | 6 | 9 | 9 |
| Number of settlements on bottom | 0.4 ± 0.7 | 0.8 ± 1.1 | 2.6 ± 1.8 | 2.2 ± 0.7 | 3.6 ± 2.0 | 13.3 ± 3.7 |
| Number of settlements on wall | 12.0 ± 2.9 | 12.7 ± 1.8 | 10.6 ± 1.8 | 11.0 ± 1.4 | 10.0 ± 2.2 | 14.1 ± 2.1 |
| Number of non-settled cypris larvae | 2.3 ± 3.0 | 0.5 ± 0.5 | 0.7 ± 0.9 | 0.7 ± 0.7 | 0.7 ± 1.2 | 0.8 ± 0.8 |
| Number of dead cypris larvae | 0.2 ± 0.4 | 0.8 ± 0.9 | 0.8 ± 0.9 | 0.8 ± 1.1 | 0.6 ± 0.7 | 1.2 ± 1.3 |
3. Results and discussion
3.1. Laboratory assay

Table 1 summarizes the composition of the samples and the results of the settlement tests. Figure 5 (a) shows the relationship between contact angles on the substrates and crosslinker concentrations. The value of the contact angles of air bubbles decreased from 130.3° to 122.6° with increasing crosslinker concentration, while the values of ultra-pure water and diiodomethane increased from 71.5° to 99.0° and from 40.9° to 59.0°, respectively. Figure 5 (b) shows the relationship between the surface free energies of the substrates and the crosslinker concentrations. The surface free energies of the substrates decreased from 41.9 mJ/m² to 29.2 mJ/m² with increasing crosslinker concentration. These results indicate that the density of OH groups on the PVA surface is decreased by crosslinking reactions; thus, the hydrophilicity of the surface decreases with an increase in concentration of the crosslinker. When the crosslinker concentration is 20 wt%, the theoretical degree of crosslinking is 150%. However, the results of the measurements of contact angles indicate that full crosslinking was not achieved on PVA surfaces up to a crosslinker concentration of 40 wt%.

Figure 6 (a) shows the percentage of cypris settlement on the bottom surfaces covered with PVA films and bare PS control. It was found that 45% of the cypris larvae settled on the PS bottom surface, in contrast only 3 to 24% the cypris larvae settled on PVA surfaces.

![Figure 5](image1.png)

![Figure 6](image2.png)
Figure 6 (b) shows the percentage of cypris settlement on the silicone wall surface in PVA wells and PS wall surface of the PS control well. The result of settlement on walls indicates that most of the cypris larvae settled on the silicone wall surfaces instead of the PVA bottom surfaces. Silicone elastomers have also been applied as antifouling materials against sessile organisms, however this result indicates that the antifouling activity of PVA films is relatively higher compared to silicone elastomers.

The percentage of non-settled cypris is shown in Fig. 6 (c). Most of cypris larvae settled in the PVA wells with a crosslinker concentration of 20 to 50 wt% and in the PS control well, however relatively high non-settled cypris percentage (16%) was found in the PVA well with a crosslinker concentration of 10 wt%. From the results of the PS control well, the settlement direction (bottom or wall) was not found to be important for cypris settlement selectivity (Fig. 6 (a), (b)). It was found that only a few of the cypris larvae were dead on all PVA substrates, and the value was similar to that of the PS controls. This result indicates that none of the PVA films were toxic to the barnacle cypris (Fig. 6 (d)).

As shown in Fig. 6 (a), all the PVA films showed an antifouling effect in contrast to the PS control. Furthermore, our results reveal that the cypris settlement tended to increase with increasing crosslinker concentration in PVA. In PVA with low crosslinker concentrations (10 and 20 wt%), only a small percentage of the settlements was found; however, in PVA with high crosslinker concentrations (30, 40, and 50 wt%), about 20% cypris were settled. Thus, the cypris settlement behavior underwent a dramatic change around the crosslinker concentration of 30%. The settlement activity followed the trend of change in the surface free energy values of the substrates. So, the results indicate that residual OH groups on PVA surfaces affected the settlement behavior of barnacle cypris larvae.

3.2. Field test

Figure 7 shows the top view photographs of the PVA-coated and bare Al substrates after exposure for 34, 63, 102, and 133 days. After 34 days, considerable barnacle fouling was found on bare Al plates, in contrast there was limited barnacle fouling on the PVA-coated plates. The species of settled barnacles that could be identified was *Balanus crenatus*. After 63 days, the settled barnacles on bare Al surfaces grew up and covered a large part of the substrate, and many small settled barnacles were found on the PVA-coated plates. After 102 days, ascidiacea, corophiidae, and diatoms were found to add to the fouling of barnacles on bare Al surfaces, and slight fouling of sessile organisms was found on the PVA surfaces. After 133 days, the bare Al surfaces were completely fouled by sessile organisms. On the PVA-coated plates, a large number of corophiidae settlements was found, thus it appears that the antifouling activity of PVA was lost after about 4 months of exposure.

Figure 8 shows the percentage area of surface coverage of living barnacles, barnacle shell remains and other sessile organisms, which were found on the surfaces. After 34 days, 13 to 25% of the bare Al surface area was covered with settled barnacles, however the coverage on PVA surfaces was almost 0% (< 1%). After 63 days, the bare Al plate was almost completely covered with barnacles (42 to 91%). On PVA surfaces, 32 to 40% of the area was covered with living barnacles and shell remains of barnacles. After 102 days, the coverage of barnacles adhered to the bare Al surface was 28 to 65%, and 10 to 25% coverage of other sessile organisms was found. In contrast, distinct foul release effect against barnacles was observed on the PVA coatings, the coverage of barnacles and other
barnacles that could be identified was on the PVA-coated plates. The species of settled plates, in contrast there was limited barnacle fouling considerable barnacle fouling was found on bare Al substrates. 

Field test indicate that residual OH groups on PVA surfaces PVA-coated and bare Al substrates after exposure free energy values of the substrates. So, the results activity followed the trend of change in the surface behavior underwent a dramatic change around the concentrations (30, 40, and 50 wt%), about 20% low crosslinker concentrations (10 and 20 wt%), PVA films were toxic to the barnacle cypris (Fig. 6 (c)). Most of cypris larvae settled in the PVA wells with a crosslinker concentration of 20 to 50 wt% and in the PS control well, however relatively low non-settled cypris percentage (16%) was found. Furthermore, our results reveal that the cypris settlement tended to increase with increasing concentrations (30, 40, and 50 wt%) of PVA and slight fouling of sessile organisms was found on the PVA coating. However, almost completely covered with barnacles (42 to 91%). On PVA surfaces 32 to 40% of the area was covered with living barnacles and shell remains of other sessile organisms (46 to 55%), however the shell remains of barnacles were very few (0 to 2%).

Figure 8 shows the top view photographs of the settlement on wall surfaces indicates that most of the settlement on the silicone wall surface in PVA wells shows high antifouling activity against cypris larval settlements. However, the antifouling activity of PVA was found to be important for cypris settlement. Furthermore, we can assume that PVA coatings showed high antifouling activity against cypris larval settlements. However, the anti-larval-settlement activity became weak due to the accumulation of dusts (e.g., minerals, proteins, polymers, etc.) in the sea water. In addition, the PVA surface gradually underwent hydrolysis reactions, and settled barnacles were detached from the surfaces by the PVA dissolution. After 4 months of exposure, the PVA coatings were peeled away and the base Al substrate exposed, and at that point, many adhesions of corophiidae occurred on the substrates.

4. Conclusion
In conclusion, all chemically crosslinked PVA coatings used in the laboratory tests inhibited the settlement of barnacle cypris larvae without toxicity. The antifouling performance of PVA increased with decreasing crosslinker concentrations in the film. From the results of the field test, the PVA coatings showed excellent antifouling performance against barnacles for at least 1 month in the sea. Furthermore, we can assume that PVA coatings show easy-release activity against settled barnacles, because there are few large-size barnacles and shell remains on PVA surfaces. Thus, the antifouling mechanisms of chemically crosslinked PVA thin films consist of the inhibitory activity of OH functional groups against larval settlements and the easy release activity against settled barnacles by surface hydrolysis.

In future work, we aim to achieve long-term (at least 1 year) protection by improving the coating techniques, and to investigate more details of the
antifouling activity of chemically crosslinked PVA coatings in the sea. The authors believe that chemically crosslinked PVA shows potential as an excellent candidate for ecofriendly antifouling coatings.

Acknowledgments
The authors gratefully acknowledge AGC Inc. Kowa K.K. and Hokkaido Soda K.K. for supporting them to perform the field test, and Dr. K. Tanaka of Tokai University for providing them with the barnacle *Amphibalanus amphitrite*. This research was financially supported by JSPS Grant-in-Aid for Scientific Research(B) (No. 18H01645), Grant-in-Aid for Scientific Research(C) (No. 18K05812), and The Sumitomo Foundation, Grant for Basic Science Research Projects (170078). We would like to thank Editage (www.editage.com) for English language editing.

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