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Effect of anti-biofouling potential of multi-walled carbon nanotubes-filled polydimethylsiloxane composites on pioneer microbial colonization

Yuan Sun[a,c], Yanhe Lang[c], Qian Sun[b], Shuang Liang[b], Yongkang Liu[b], Zhizhou Zhang[a,b,c,*]

[a] School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, 150006, China
[b] School of Marine Science and Technology, Harbin Institute of Technology, Weihai, 264209, China
[c] Marine Antifouling Engineering Technology Center of Shangdong Province, Harbin Institute of Technology, Weihai, 264209 China

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ABSTRACT

In this paper, two carbon nanotube (CNT) nanofillers, namely the multi-walled carbon nanotubes (MWCNTs) and the carboxyl-modified MWCNTs (cMWCNTs), were introduced into the polydimethylsiloxane (PDMS) matrix respectively, in order to produce the PDMS composites with reinforced anti-biofouling properties. The anti-biofouling capacity of the silicone-based coatings, including the unfilled PDMS (P_0), the MWCNTs-filled PDMS (P_M) and the cMWCNTs-filled PDMS (P_C), was examined via the field assays conducted in Weihai, China. The effect of different silicone-based coatings on the dynamic variations of the pioneer microbial-community diversity was analyzed using the single-strand conformation polymorphism (SSCP) technique. The P_M and P_C surfaces have exhibited excellent anti-biofouling properties in contrast to that of the PDMS surface, with extremely low attachment of the early colonizers, such as juvenile invertebrates, seaweeds and algae sporelings. The P_M and P_C surfaces can effectively prevent biofouling for more than 12 weeks. These combined results suggest that the incorporation of MWCNTs or cMWCNTs into the PDMS matrix can dramatically reinforce its anti-biofouling properties. The SSCP analysis reveals that compared with the PDMS surfaces, the P_M and P_C surfaces have strong modulating effect on the pioneer prokaryotic and eukaryotic communities, particularly on the colonization of pioneer eukaryotic microbes. The significantly reduced pioneer eukaryotic-community diversity may contribute to the weakening of the subsequent colonization of macrofoulers.

1. Introduction

Biofouling is a ubiquitous problem for the application of materials in the marine environment [1]. The undesired accumulation of the fouling organisms adhering to the artificial structures and surfaces submerged in natural seawater have resulted in considerable economic losses and environmental problems to the shipping industry as well as other marine associated activities [2]. To date, the most effective antifouling (AF) strategy should be coating ship hulls with biocidal coatings containing tributyltin (TBT). However, considering the potential damaging effect of the TBT on the non-target marine organisms and also the aquatic environment, the application of the TBT-based coatings has been totally banned by the International Maritime Organization (IMO) in 2008 [3]. Furthermore, most TBT-free systems, like copper-based or zinc-based coatings, are also ecologically harmful, because of the bioaccumulation of heavy metals in the internal organs of the marine organisms [4,5]. Therefore, recent efforts have been dedicated to the development of eco-friendly AF materials that are more durable, stronger and safer [6,7].

Polydimethylsiloxane (PDMS) has been widely shown to function as an excellent fouling-release (FR) material in the marine environment. Considering its distinct advantages, such as non-toxicity, easy fabrication, high hydrophobicity, long-term endurance and contamination resistance [8], the PDMS has become an attractive material for anti-biofouling applications [9]. Nevertheless, the PDMS is extremely susceptible to damage because of the inherent disadvantages, such as the weak mechanical performance. Therefore, the usage of the PDMS against marine biofouling has been greatly restricted. Various biocides and nanofillers have been introduced into the PDMS matrix, in order to create the PDMS composites with improved anti-biofouling capacity [10,11]. Car-

* Corresponding author at: Faculty of Marine Science and Technology, Harbin Institute of Technology, Weihai, 264209, China.
E-mail address: zhangzzbiox@outlook.com (Z. Zhang).

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bon nanotubes (CNTs) seem to be the appropriate candidates of choice for the improvement of a wide range of polymeric materials. Beigbeder et al. have found that the AF and FR properties of the PDMS matrix to be significantly reinforced with the presence of 0.05% (w/w) multi-walled carbon nanotubes (MWCNTs) [12]. Despite many potential applications, only a few publications have focused on the preparation of the CNTs-reinforced PDMS composites for anti-biofouling applications, as compared to other polymeric matrices. In addition, the AF efficacy of most PDMS composites was only determined via the laboratory assays, rather than the field assays. Almost no detailed investigations have been performed to identify the reinforcing CNT nanoparticles in order to improve the anti-biofouling capacity of the PDMS via the long-term field studies.

Currently, the microbial biofilms that formed on the surfaces of various relatively resistant composites in natural seawater have gained extensive attention. The presence of the biofilms can either facilitate or discourage the colonization of invertebrate larvae and algae sporelings [13]. It is well believed that the degradation of the composite materials may be primarily ascribed to the colonization of the pioneer biofilm communities, like bacterial and fungi [14], because some composite constituents, such as the matrix resins, additives and plasticizers, can be readily degraded by bacterial and fungi as nutrients. Previously, most studies have primarily focused on the biofilm communities adhering to the unpainted artificial surfaces, like stainless steel, glass slides and polystyrene [15]. Only a few studies attempted to describe the biofilm communities grown on the AF coating surfaces [16–18]. Furthermore, the dynamics of the surface attachment and colonization *in situ*, particularly during the early stages of the establishment of the biofilms on the silicone-based composite surfaces, has not been investigated in depth. A better understanding of the diversity variations of pioneer microbial communities and related succession phenomena among various silicone-based coating surfaces may contribute to the future development of silicone-based AF strategy. In this study, a culture-independent molecular approach, namely the single-strand conformation polymorphism (SSCP) technique, was employed to analyze and monitor the dynamic variations of the pioneer microbial-community diversity among different silicone-based coating surfaces.

The aim of the present study was to improve the anti-biofouling capacity of the PDMS matrix by 0.1% (w/w) CNT nanoparticle incorporation. The anti-biofouling capacity of the silicone-based coatings was examined via the field studies under actual marine conditions. The wettability of the silicone-based coatings was measured by the static water contact angle. Furthermore, the effect of different silicone-based coating surfaces on the dynamic variations of the pioneer microbial-community diversity was analyzed using the SSCP technique, based on the 16SrRNA gene and the Internal Transcribed Spacer 2 (ITS-2) analysis. The possible mechanisms of the CNTs-filled PDMS composites against biofouling were also discussed.

2. Materials and methods

2.1. Description of the immersion site

The field assays were carried out at the Xiaoshi Island harbor waters (37°31′51″N; 121°58′19″E) in Weihai, China. A static, permanent wooden raft bridge allowed the immersion of numerous tested panels of the same dimension at different depths for long periods if necessary. There were rich fouling organisms at the immersion site during the field studies, such as invertebrate larvae (i.e., juvenile barnacles, oysters, mussels and ascidian), algae sporelings, seaweeds as well as sponges. All the tested panels will be faced with heavy fouling pressure in the marine environment. Furthermore, the results obtained from the field studies can be readily identified, because coating failures can be easily observed after four weeks of exposure to the natural seawater, according to the colonization of the early colonizers using visual analysis.

Table 1
Detailed information on the CNT fillers used in this study.

CNT fillers	Carboxyl content% (w/w)	Diameter (nm)	Length (μm)	SSA[a] (m^2/g)	Purity (%)
MWCNTs	–	10–20	30–100	>165	>95
cMWCNTs[b]	2.00	10–20	10–30	>200	>95

[a] SSA: Specific surface area.
[b] cMWCNTs: carboxyl-modified MWCNTs.

2.2. Test panel preparation

Ship hull steel panels, with the same dimension of 10 cm × 10 cm × 0.3 cm, were thoroughly rubbed using sandpapers, in order to obtain relatively uniform surfaces. Then all the panels were carefully washed with the sterile deionized water. Afterwards, the panels were first coated with the primer coat (i.e., the chlorinated rubber iron-red antirust paint), in order to provide a bright background for the outer layer made of a transparent silicone-based coating. The primer coat was kindly supplied by Jiamei Company (Weihai, China), which can be cured in about 72 h at room temperature.

2.3. Coating fabrication

The silicone elastomer matrix used in this study was essentially the Sylgard 184 kit (Dow Corning, USA), which was supplied as a two-part kit mainly consisting of a pre-polymer (base, part A) and a cross-linker (curing agent, part B). The CNT fillers were purchased from the *Timesnano* Company (Chengdu, China). The detailed information on the CNT fillers was presented in Table 1.

To prepare the unfilled PDMS (P_0), the PDMS pre-polymer (part A) and the curing agent (part B) were thoroughly mixed in a ratio of 10:1 (w/w), in order to obtain a cross-linked PDMS via hydrosilylation following the manufacturer's recommendations. Both the pre-polymer and the curing agent were well mixed for 15 min, and then degassed to remove air bubbles from the mixture at room temperature. For the preparation of the CNTs-reinforced PDMS composites, each CNT filler has the priority to well blend with the PDMS pre-polymer (part A) for 15 min, and then the mixture was mechanically blended with the curing agent (part B) for another 15 min, degassing at room temperature until all air bubbles were completely removed from the mixture, in order to ensure the complete mixing between the two parts. The final concentration of the CNT fillers in the PDMS matrix was 0.1% (w/w) [19]. Afterwards, these silicone mixtures were painted on one side of surfaces of the pre-treated panels respectively, using a bar-coater. After 6 h of curing at 105 °C, the silicone-based coatings with a thickness of 300 μm were obtained for the subsequent field studies. Similar preparation procedures have also been described elsewhere in our recent study [20]. The detailed information of the CNTs-filled PDMS composites was summarized as presented in Table 2.

2.4. Contact angle measurements

The static contact angle measurements were performed on the silicone-coated microscopic slides via the sessile drop technique using a JGW-360A apparatus (Chengzhou, China). The surfaces were previously cleaned with the sterile deionized water. The hydrophobic character of the silicone-based coatings was evalu-