Colloids and Surfaces B: Biointerfaces 115 (2014) 118–124

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Effect of ultrasound on cyprid footprint and juvenile barnacle adhesion on a fouling release material

Shifeng Guo[a,b,*], Boo Cheong Khoo[a,d], Serena Lay Ming Teo[c], Shaoping Zhong[e], Chwee Teck Lim[a,e,f], Heow Pueh Lee[a,d]

[a] Department of Mechanical Engineering, National University of Singapore, Singapore 117576, Singapore
[b] Institute of Materials Research and Engineering (IMRE), A*STAR, 3 Research Link, Singapore 117602, Singapore
[c] Tropical Marine Science Institute, National University of Singapore, Singapore 119223, Singapore
[d] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Jiang Su 215123, People's Republic of China
[e] Department of Bioengineering, National University of Singapore, Singapore 117576, Singapore
[f] Mechanobiology Institute, National University of Singapore, Singapore 117411, Singapore

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 4 November 2013
Accepted 10 November 2013
Available online 23 November 2013

Keywords:
Ultrasound
AFM
Barnacle cyprid footprint
Settlement
Adhesion strength
Silicone substrates

ABSTRACT

In our earlier studies, we have demonstrated that low and high intensity ultrasound can prevent barnacle cyprid settlement. In this study, we found that ultrasound treatment reduced the adhesion of newly metamorphosed barnacles up to 2 days' old. This was observed in the reduction of adhesion strength of the newly settled barnacles from ultrasound treated cyprids on silicone substrate compared to the adhesion strength of barnacles metamorphosed from cyprids not exposed to ultrasound. Atomic force microscopy (AFM) was used to analyze the effect of ultrasound on barnacle cyprid footprints (FPs), which are protein adhesives secreted when the larvae explore surfaces. The ultrasound treated cyprids were found to secrete less FPs, which appeared to spread a larger area than those generated by untreated cyprids. The evidence from this study suggests that ultrasound treatment results in a reduced cyprid settlement and footprint secretion, and may affect the subsequent recruitment of barnacles onto fouling release surfaces by reducing the ability of early settlement stage of barnacles (up to 2 days' old) from firmly adhering to the substrates. Ultrasound therefore can be used in combination with fouling release coatings to offer a more efficient antifouling strategy.

1. Introduction

Barnacles are a major problem in marine fouling due to their size, hard shell, and gregarious nature [1]. In earlier studies, it has been demonstrated that both low and high intensity ultrasound can prevent barnacle cyprid settlement [2–5]. The probable mechanism for the prevention of cyprid settlement using high intensity ultrasound was attributed to ultrasonic cavitation [3], and it was also shown that cavitation bubbles can be used to remove early stages of barnacle settlement [6]. In this paper, we further examine the feasibility of using ultrasound to control the barnacle fouling on fouling release surfaces.

It has been reported that attachment strength plays a significant role in the recruitment of marine organisms onto a surfaces, and stronger forces are required to remove organisms on surfaces with higher settlement [7,8]. For example, the settlement of *Mytilus galloprovincialis* was found to be positively correlated to adhesion strength [7]. Also, barnacle cyprids preferred to settle on the substrates where the possibility of subsequent removal is least likely to occur [8]. Fouling release coatings (FRCs) are now widely used as antifouling for ship hulls [9,10]. Hydrophobic FRCs was reported to be beneficial to vessels operating above 10 knots and resulted in less fuel consumption in comparison to biocide-containing self-polishing coatings [11]. One of the main features of the FRCs is that the interfacial bond between the organism and coating is weak. As a result, the attached organisms are more easily removed by the hydrodynamic forces created from a vessel's movement through the water, or by simple mechanical cleaning [9,12].

The cyprids and juvenile barnacles have been previously proposed as appropriate experimental models for the evaluation of antifouling performance [12]. The lifecycle of the barnacle, *Amphibalanus amphitrite* (=*Balanus amphitrite*), includes planktotrophic nauplius stages, a non-feeding cypris larval stage, and a sessile adult stage. The pre-settlement cypris stage actively explores surface and metamorphoses into the juvenile barnacle once a suitable site is found. Surface exploration is conducted using the antennules, in a form of bi-pedal 'walking', which appears to be

* Corresponding author at: Institute of Materials Research and Engineering (IMRE), A*STAR, 3 Research Link, Singapore 117602, Singapore. Tel.: +65 65148745.
E-mail address: gsf830@hotmail.com (S. Guo).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.11.020

affected by the surface texture, material properties, chemical clues, and presence of adult or cyprid nonspecific proteins [9,13]. During exploration, temporary proteins secretion is deposited as footprint (FP) through the antennular attachment discs and it has been implicated to act as settlement cues for other exploring cyprids [1,9]. The FP has been described as a temporary adhesive, as it enables reversible attachment to surfaces [14,15]. A settlement-inducing protein complex (SIPC), which also functions as a settlement cue, has been found in FPs [14,15]. It has also been reported that surfaces which are apt to the adsorption of FPs would lead to higher settlement [16]. Ultrasound treated cyprids have been shown to have an altered exploration behavior [2]. Since cyprids secrete temporary adhesive proteins during surface exploration, it is conceivable that the altered walking behavior may also be accompanied with change in the secreted FPs.

Based on the preliminary experiments, juvenile barnacles metamorphosed from ultrasound treated cyprids were more easily detached. It is surmised that the adhesion strength may be reduced. Therefore, the effect of ultrasound on juvenile barnacle adhesion strength was evaluated using a nano-tensile tester. Also, since FPs play a significant role on cyprid settlement, the effect of ultrasound on the FPs was explored using AFM, with the objective of gaining a better understanding of the mechanism behind the ultrasound induced inhibitory effects.

2. Materials and methods

2.1. Barnacle culture

Larvae of the barnacle *Amphibalanus amphitrite* were reared at 26 °C, fed with an algal mixture of 1:1 (v/v) of *Tetraselmis suecica* and *Chaetoceros muelleri* at a density of approximately 5×10^5 cells/ml [17]. Barnacle larvae metamorphosed into cyprids within 5–7 days. The cyprids were stored at 4 °C and used for experiments after 3 days. Juvenile barnacles metamorphosed from ultrasound treated and untreated cyprids were reared and fed daily with algal mixture of 1:1 (v/v) of *Tetraselmis suecica* and *Chaetoceros muelleri* at a density of approximately 5×10^5 cells/ml.

2.2. Ultrasonic experimental setup

The ultrasonic experimental setup is same as that described in Guo et al. [3]. As low frequency of 23 kHz generated most significant effect on barnacle cyprid settlement and exploration behavior [2], in the present study, only this frequency was chosen, with the pressure set at 20 kPa. The cyprids were then subjected to ultrasound exposure for 5 min.

2.3. Surfaces choice for the barnacle adhesion and cyprid FPs study

There are two surfaces used to study the effect of ultrasound on juvenile barnacle adhesion strength and cyprid footprints, respectively.

The fouling release surfaces (silicone substrates) were used to evaluate the effect of ultrasound on barnacle adhesion strength. The surfaces were chosen because the interaction between juvenile barnacle cement and the surface is low. Therefore, the barnacles were easier to be fully detached, and the forces measured can be regarded as the adhesive forces. The fouling release surfaces used to evaluate barnacle adhesion strength were reported widely [9,12,18].

To study the effect of ultrasound on FPs, the NH_2 terminated glass cover slips were used as the surfaces were easier for the FPs detection using AFM and shown more concentrated and distinct FP patterns [19,20].

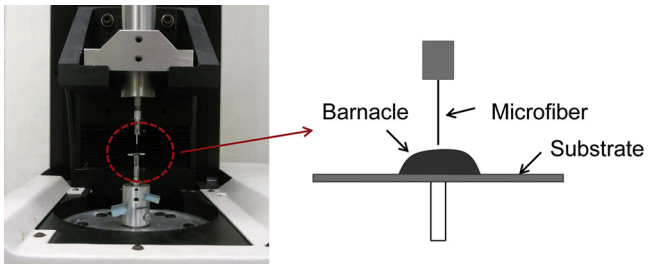

Fig. 1. The schematic of nano-tensile tester on juvenile barnacle detachment forces measurement.

To prepare the NH_2 terminated surfaces, the glass microscopy cover slips were firstly immersed in 5% Decon 90 solution and cleaned using ultrasonic clean-tank for 20 min. The use of Decon 90 solution to effectively clean surfaces for increasing surface density of silanol groups and/or prior to silanization has been well documented in the previous literature [21,22]. The slips were then rinsed thoroughly with ultrapure water and dried with nitrogen gas. The amino (NH_2—) terminated surfaces were obtained by immersing the cleaned slips in 5% 3-aminopropyl triethoxysilane (APTES) solution and were put in the shaker (GFP MBH, Germany) for 30 min. After that the surfaces were rinsed with ultrapure water thoroughly and dried using nitrogen gas.

Static water contact angles (CA) of NH_2 terminated surfaces and silicone substrates were measured at 25 °C, using the sessile drop method with a 2 μl water droplet, in a telescopic goniometer (model 100-00-(230), Rame-Hart, Inc., Mountain Lake, NJ, USA). To measure the contact angle of coated cover slips, eight samples were replicated and the averaged contact angle was $55° \pm 2.4°$ and the value was approximately similar with reported results [19,20]. The contact angle of silicone substrates was 94°.

2.4. Cyprid settlement on silicone substrate

Cyprid settlement was performed with a 'no choice' droplet assay, as described in Refs. [8,23]. The experiments were conducted on the medical grade silicone substrates (Bioplexus, USA). The material was cleaned following the provided instructions before usage.

It was reported that the gregarious behavior of cyprids did not interfere with settlement rate at cyprid densities of 5–200 per 5 ml [24]. In our experiments, after ultrasound exposure, 500 μl volume of filtered seawater (FSW), containing approximately 15–20 cyprids, was deposited on each silicone substrate (2 cm × 2 cm), forming a droplet on the substrate. The substrates were placed in Petri dishes and sealed with parafilm to prevent water evaporation. The assay was incubated at 26 °C for 48 h, on a 15 h light and 9 h dark cycle. The cyprids were then examined under a stereo microscope (Nikon SMZ 1500, Japan), and the number of cyprids which had settled and metamorphosed into barnacles was enumerated. To evaluate the ultrasonic effect, eight replicate silicone substrates were prepared for both ultrasound treated and control cyprids.

2.5. Juvenile barnacle adhesion strength measurement

Measurement of barnacle adhesive force was conducted using a calibrated Nano-tensile tester (Nano Bionix System, MTS, USA), and the schematic of the experimental setup is shown in Fig. 1. The silicone substrate (2 cm × 2 cm) with barnacles settled was clamped in the lower grip. A micro steel fiber with diameter of 80 μm and length of 10 mm was clamped in the upper grip. The fiber was controlled and lowered until it was slightly above the top of barnacle. Then the tip of the fiber was wetted with a small drop of superglue (Selleys Pty Ltd, Australia) and lowered to touch the top of