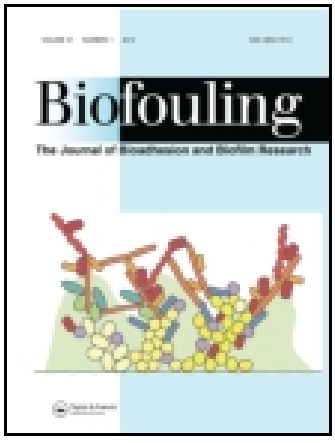

Biofouling: The Journal of Bioadhesion and Biofilm Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gbif20

Dynamic surface deformation of silicone elastomers for management of marine biofouling: laboratory and field studies using pneumatic actuation

Phanindhar Shivapooja[a], Qiming Wang[b], Lizzy M. Szott[a], Beatriz Orihuela[c], Daniel Rittschof[c], Xuanhe Zhao[bde] & Gabriel P. López[abd]

[a] Department of Biomedical Engineering, Duke University, Durham, NC, USA

[b] Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC, USA

[c] Duke University Marine Laboratory, Nicholas School of the Environment, Beaufort, NC, USA

[d] Research Triangle Material Research Science & Engineering Center, Duke University, Durham, NC, USA

[e] Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Published online: 28 Apr 2015.

To cite this article: Phanindhar Shivapooja, Qiming Wang, Lizzy M. Szott, Beatriz Orihuela, Daniel Rittschof, Xuanhe Zhao & Gabriel P. López (2015) Dynamic surface deformation of silicone elastomers for management of marine biofouling: laboratory and field studies using pneumatic actuation, Biofouling: The Journal of Bioadhesion and Biofilm Research, 31:3, 265-274, DOI: 10.1080/08927014.2015.1035651

To link to this article: http://dx.doi.org/10.1080/08927014.2015.1035651

Biofouling, 2015
Vol. 31, No. 3, 265–274, http://dx.doi.org/10.1080/08927014.2015.1035651

Taylor & Francis
Taylor & Francis Group

Dynamic surface deformation of silicone elastomers for management of marine biofouling: laboratory and field studies using pneumatic actuation

Phanindhar Shivapooja[a], Qiming Wang[b], Lizzy M. Szott[a], Beatriz Orihuela[c], Daniel Rittschof[c], Xuanhe Zhao[b,d,e] and Gabriel P. López[a,b,d]*

[a]*Department of Biomedical Engineering, Duke University, Durham, NC, USA;* [b]*Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC, USA;* [c]*Duke University Marine Laboratory, Nicholas School of the Environment, Beaufort, NC, USA;* [d]*Research Triangle Material Research Science & Engineering Center, Duke University, Durham, NC, USA;* [e]*Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*

(*Received 8 February 2015; accepted 24 March 2015*)

Many strategies have been developed to improve the fouling release (FR) performance of silicone coatings. However, biofilms inevitably build on these surfaces over time. Previous studies have shown that intentional deformation of silicone elastomers can be employed to detach biofouling species. In this study, inspired by the methods used in soft-robotic systems, controlled deformation of silicone elastomers *via* pneumatic actuation was employed to detach adherent biofilms. Using programmed surface deformation, it was possible to release > 90% of biofilm from surfaces in both laboratory and field environments. A higher substratum strain was required to remove biofilms accumulated in the field environment as compared with laboratory-grown biofilms. Further, the study indicated that substratum modulus influences the strain needed to de-bond biofilms. Surface deformation-based approaches have potential for use in the management of biofouling in a number of technological areas, including in niche applications where pneumatic actuation of surface deformation is feasible.

Keywords: pneumatic; deformation; silicones; elastomers; substrate modulus; biofilms; biofouling

Introduction

Biofouling occurs on synthetic surfaces exposed to natural aqueous environments and is a significant economic problem in the marine industry (Aftring & Taylor 1979; Characklis 1981; Callow & Callow 2002; Jain & Bhosle 2009; Mieszkin et al. 2013). Biofouling on ships' hulls results in increased hydrodynamic drag and fuel consumption, as well as an increase in maintenance and environmental compliance costs (Schultz 2007; Swain et al. 2007; Schultz et al. 2011). The performance of other kinds of marine equipment such as oceanographic sensors, seawater piping, heat exchange systems and ultrafiltration membranes is also negatively impacted by biofouling, the management of which can cost over $15 billion each year (Casanueva et al. 2003; Nebot et al. 2010; Wang et al. 2013).

Current measures to control biofouling typically involve expensive manual cleaning and the use of biocides (Sonak et al. 2009; Thomas & Brooks 2010). Increased ecological awareness and the high cost of registration of antifouling (AF) paints containing toxic ingredients (eg copper oxide and organic biocides) has led to substantial interest in the development of non-toxic coatings to reduce biofouling (Voulvoulis et al. 1999; Gittens et al. 2013). Silicone based, fouling release (FR) coatings that offer an alternative approach to biocide-containing paints are being investigated widely by various researchers. FR coatings function by minimizing the adhesion strength of attached fouling species, which can be removed (ie 'shed/released') relatively easily due to shear during cleaning procedures, such as application of water pressure or light scrubbing. The lower adhesion strength of fouling organisms to silicone surfaces has been attributed to its critical surface energy (between 20 and 30 mN m^{-1}), smoothness, and reduced opportunities for hydrogen-bonding and polar interaction at the material–liquid interface (Baire 1970; Callow & Fletcher 1994; Brady & Singer 2000; Callow & Callow 2011). Further, it was previously shown through theoretical and experimental studies that the modulus and thickness of the silicones films are also important for their efficacy and durability as FR coatings (Kendall 1971; Singer et al. 2000; Chaudhury et al. 2005). Surface active silicone compounds are also known to disrupt the curing process of adhesive glues produced by macrofouling species (eg barnacles), thereby reducing their adhesion strength (Rittschof et al. 2011; Holm 2012).

FR silicone coatings, however, have some disadvantages, and represent only a small proportion of the current total marine coatings market. They are relatively

*Corresponding author. Email: gabriel.lopez@duke.edu

less durable than other types of coatings (eg commercial acrylate self-polishing coatings) and are known to frequently foul with brown slimes (which are dominated by diatoms) that attach strongly to hydrophobic surfaces (Truby et al. 2000). The surface energy characteristics of silicone polymers are also known to be altered upon prolonged exposure to seawater, thus affecting their ability to control biofouling (Estarlich et al. 2000). Therefore, efforts are being made to further improve the performance of silicone coatings. For instance, several commercial silicone coatings incorporate silicone oils to further diminish the adhesion strength of different fouling species without significantly affecting the critical surface energy of the silicones (Stein et al. 2003; Meyer et al. 2006). In addition, the AF and FR performance of silicone coatings have been shown to be enhanced through the use of bioinspired, textured surfaces (eg Sharklet® surfaces) (Carman et al. 2006; Scardino & de Nys 2011; Halder et al. 2014; Ling et al. 2014; Zargiel & Swain 2014), incorporation of amphiphilic polymers (eg Intersleek® 900) (Martinelli et al. 2008), and tethering of AF moieties, such as quaternary ammonium salts (Majumdar et al. 2011) and zwitterionic polymers (Zhang et al. 2009; Bodkhe et al. 2015).

As an alternative and complementary method to the above examples (ie existing surface modification approaches), this work builds upon two recent reports which demonstrated that dynamic deformation of silicone elastomer surfaces can be highly effective in the release of both soft (eg bacterial biofilms) and hard (eg barnacles) foulers (Shivapooja et al. 2013; Levering et al. 2014). The approach is based on the fundamental hypothesis that biofouling on a soft, elastomeric substratum (eg polydimethylsiloxane (PDMS)) can be de-bonded if sufficient strain is applied to the substratum. Using silicone elastomers that allow manual, electric or pneumatic actuation for controlled surface deformation, it was demonstrated that a critical substratum strain is needed to de-bond bacterial biofilms from a silicone substratum. However, those studies were conducted using model, single-species bacterial biofilms grown in a laboratory environment over short time periods (eg 48 h). It is generally accepted that biofilms formed in the marine environment can be of highly complex composition and thus can exhibit robust adhesion properties (Briand 2009).

This study includes two main objectives: (1) to test the above hypothesis (ie that surface deformation can result in biofilm detachment) in the laboratory and in the field environment, and (2) to examine whether the shear modulus of the substratum affects the strain needed for biofilm detachment. Controlled, reversible surface deformation was applied through a pneumatic actuation method that has been developed for soft robotics applications (Vogel 2012). Two similar silicones (Ecoflex-10 and Ecoflex-50) that have a different shear modulus were used in this study. The field studies were conducted at the Duke University Marine Laboratory in Beaufort, NC, while the laboratory studies were conducted using *Cobetia marina* and *Escherichia coli* biofilms.

Materials and methods

Fabrication of elastomer pneumatic networks

Ecoflex® Supersoft 0010 and Ecoflex® Supersoft 0050, platinum catalyst-based silicone formulations (Smooth-On Inc., Macungie, PA, USA) (from here on, referred to in the text as Ecoflex-10 and Ecoflex-50) were used to fabricate elastomeric pneumatic networks by molding of 3D printed plastic (acrylonitrile butadiene polystyrene, ABS) templates (Shivapooja et al. 2013). The plastic template (Supplemental material Figure S1a) comprises multiple long parallel stripes and shorter perpendicular stripes, designed in such a way that the prepared elastomer pneumatic network can be subjected to controlled surface deformation. Thoroughly mixed Ecoflex silicone precursors (part A:part B = 1:1 v/v) were poured into the plastic mold and cured at room temperature for 12 h. The cured Ecoflex was removed from the mold and carefully placed with the bottom side up (ie smooth side facing upwards) on a glass slide (75 × 50 mm) that was spin-coated with a 2 mm thick layer of uncured Ecoflex. The layer was allowed to cure overnight to form a sealed pneumatic network comprising a 2 mm thick silicone layer on top of the air channels (40 mm long) that were interconnected (Figure S1b). One side of the enclosed elastomeric pneumatic network was connected to a polyurethane tube (5 mm in diameter) using a 16 gauge syringe needle. The other end of the tube was connected to a pneumatic pump, which was used to vary the pressure in the air channels. The pressure inside the air channel was measured using a pressure gauge (Cole-Parmer, USA).

Analysis of substratum shear modulus and strain

To measure the shear modulus of Ecoflex-10 and Ecoflex-50, flat rectangular films 2 mm in thickness were prepared and subjected to uniaxial tension tests (to collect stress *vs* strain data) on a micro-strain analyzer (TA Instruments, USA) at a loading rate of 1×10^{-4} s^{-1}. A thickness of 2 mm was chosen because the silicone layer above the air channels of the pneumatic network samples was also 2 mm in thickness. The stress–strain data of the Ecoflex film were fitted to the Arruda–Boyce model (Arruda & Boyce 1993) to evaluate the shear modulus. The measured shear modulus for Ecoflex-10 and Ecoflex-50 was 10.5 kPa and 50.2 kPa, respectively (Figure S2).

The strain generated on the surface of the pneumatic network was determined by measuring the contour length of the deformed surface using digital photographs taken before and after actuation. The measured surface strain for various pressures applied was in agreement with a theoretical 2D plane strain-model (see Supplemental material).

Formation of model bacterial biofilms in the laboratory

A chamber was created by fabricating Ecoflex sidewalls along the edges of the pneumatic network using 3D printed plastic supports. The bacteria *Cobetia marina* (basonym, *Halomonas marina*, ATTC 4741) and *Escherichia coli* (ATTC 15333) were chosen for laboratory studies, because their biofilms can be easily grown in commonly used media and are very commonly found in natural estuarine and seawater environments (Gerba & McLeod 1976; Shea et al. 1995). Model bacterial biofilms of *C. marina* and *E. coli* were grown on test surfaces in static media under laboratory conditions using a procedure reported previously (Shivapooja et al. 2013). In brief, *C. marina* in marine broth and *E. coli* in tryptic soy broth (TSB) were cultured in separate 50 ml conical flasks. The Ecoflex sample surface was sterilized by multiple rinses with 70% ethanol and then rinsed using sterilized deionized (DI) water. Next, 1 ml of bacterial culture was added on the sterilized Ecoflex surface along with 5 ml of sterilized artificial seawater (for *C. marina*) or TSB broth (for *E. coli*). The samples were stored in an incubator for 7 days at 26°C (for *C. marina*) or 37°C (for *E. coli*). Sterilized artificial seawater or TSB medium was added as needed to compensate for dehydration of the media over time.

Analysis of C. marina *and* E. coli *biofilm detachment*

After biofilm growth for 7 days, the test surfaces were stained with SYTO 13 (Life Technologies, Paisley, UK) using a procedure reported previously (Shivapooja et al. 2013). The stained and washed biofilm surfaces were air dried in the dark for 15 min and analyzed under a fluorescent microscope (Zeiss Axio Observer, Göttingen, Germany) using a 10× objective. At least five images at different regions across the surface of each sample were taken using the same exposure time. The percentage of biofilm detached from the surface was calculated by comparing the relative fluorescence intensities between the actuated and control (non-actuated) samples.

Field studies

Multiple replicates of Ecoflex-10 and Ecoflex-50 pneumatic network samples were prepared using the procedure mentioned above and attached to a plastic mesh that was secured to a rectangular frame of dimension 1.5 m × 1.2 m. The rectangular frame with samples was then attached to a wooden panel on the floating research dock that was previously established for biofouling studies at Duke University Marine Laboratory (May 2013, Beaufort, NC). The test samples attached to a rectangular frame were immersed perpendicular to the water surface, with the test surfaces oriented to the east (compass direction). The samples were immersed to a depth of 1 m to enable biofilm formation and growth for a period of 14 days. After this time, each of the test surfaces was individually and repeatedly actuated in place to a pre-determined pressure using a pneumatic pump (Cole Parmer, Vernon Hills, IL, USA) fitted with 5 mm flexible polyurethane tubing. One end of the tube was connected to the side inlet of the elastomer pneumatic network. By operating the pneumatic pump, the pressure in the air channels of the pneumatic network was increased, which causes a deformation of the Ecoflex surface. The deformed surface was then reverted to its initial flat state by reversing the direction of the airflow through the pneumatic pump.

Analysis of marine biofilm released using crystal violet assay

An aqueous stock solution of crystal violet (CV) (tris (4-(dimethylamino)phenyl)methylium chloride) (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 0.1% by volume was prepared and stored at room temperature. The samples removed from the field were stained using CV for 15 min and gently rinsed three times with DI water. The samples were then allowed to dry in the dark for 15 min at room temperature and at least 10 images were taken across each sample at 4X magnification using an optical microscope (Olympus SZX7, Tokyo, Japan). Control studies indicated that CV effectively stained the biofilms accumulated over the surfaces. The surface area of biofilm coverage was measured by converting the images to binary scale using ImageJ software and adjusting the threshold to differentiate precisely between areas with and without biofilm. The percentage of biofilm released due to pneumatic actuation was calculated by taking the ratio of the surface area of biofilm on actuated *vs* control samples.

Results and discussion

On-demand controlled surface deformation using pneumatic actuation

To remove adherent biofilms, this study employed pneumatic actuation for dynamic surface deformation, inspired by the recent advances in the field of hybrid soft robotic systems (Ilievski et al. 2011; Vogel 2012). Model silicones (eg based on PDMS) commonly used in

biofouling studies are also extensively used in the field of soft robotics research. A typical soft robotic system may be fabricated from easily deformable elastomers, molded using 3D printed templates, and can be powered by an external source, such as pneumatic air pumps (Kim et al. 2013). For example, Shepherd et al. (2011) reported that such 'pneumatic networks' can be used to form a simple 'starfish-like' robotic structure comprising three layers of silicones with varying moduli. Using a similar approach, pneumatic networks prepared from Ecoflex silicone elastomer were used in this study to investigate the effect of controlled surface deformation on biofilm release.

Pneumatic networks were made of Ecoflex-10 and Ecoflex-50 using the procedure detailed above. A pneumatic pump was used to increase the pressure in the air channels of the network to levels above atmospheric pressures, exerting a stress on the thin (2 mm) Ecoflex layer above the channels, causing it to stretch and generate strain along its surface (Figure 1A). The amount of deformation is proportional to the pressure in the air-channels and can be controlled. Since the air channels are well connected, the air pressure distributes uniformly inside the pneumatic network. The direction of airflow through the pneumatic pump can be reversed such that the elastomer surface can be controllably reverted to its original flat state.

The surface strain generated by pneumatic actuation was determined by measuring the contour length as described above. The strains measured for varied applied pressures (0–20 kPa) were compared with a theoretical prediction of a 2D plane-strain model (described in Figure S3). The theoretical predicted surface strain matches well with the measured experimental data for both Ecoflex-10 and Ecoflex-50, as shown in Figure 1B, and provides the relationship between applied pressure and generated strain.

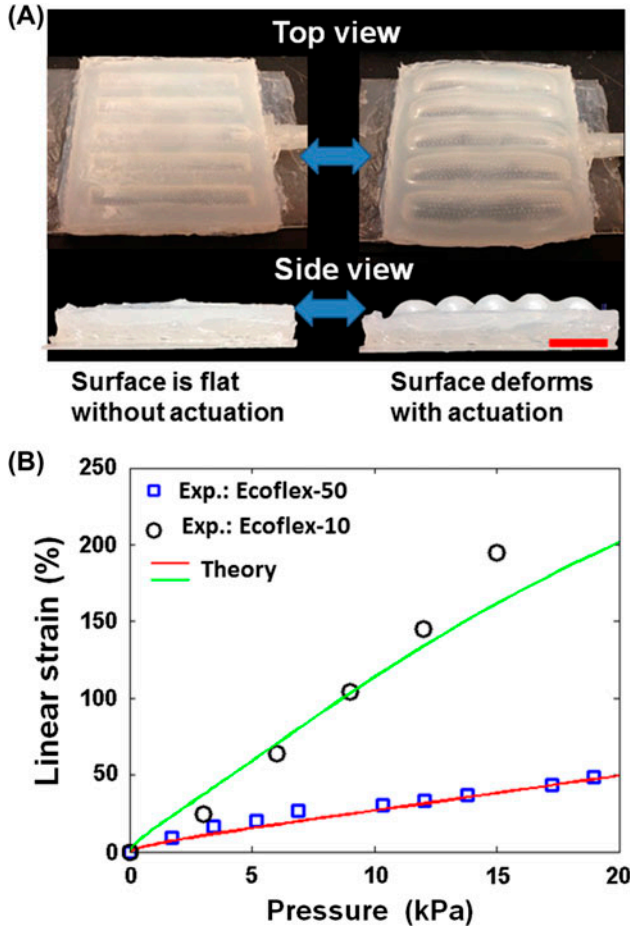

Figure 1. Optical photographs of a pneumatic network (Ecoflex-10) that shows the surface to be flat without actuation and undergoes deformation when pneumatic pressure is applied (A). The scale bar (red) represents 12 mm. Relationship of applied internal pressure by pneumatic actuation on strain generated on the surface of Ecoflex-10 and Ecoflex-50 (B); the experimental data match with the theoretical predicted strain using a 2D plane-strain model.

Detachment of model bacterial biofilms

It was recently reported that deformation of a surface caused by application of controlled dynamic stresses can be used as an effective approach to control biofouling (Shivapooja et al. 2013; Levering et al. 2014). The objective of laboratory studies was to investigate the effect of substratum strain and substratum modulus on the detachment of model bacterial biofilms. The procedure used for growing bacterial biofilms in the laboratory is detailed above.

Biofilms were allowed to grow on the surface of elastomer pneumatic networks for 7 days and then slowly rinsed using ASW (for *C. marina* biofilm) or TSB (for *E. coli* biofilm) to detach loosely adhered biofilm. The surfaces were then actuated repeatedly to a prescribed strain using pneumatic actuation. The actuation was conducted for 20 cycles at a constant strain rate (50 mm min^{-1}). The actuated and control (non-actuated) samples were gently rinsed with sterilized DI water and stained with a fluorescent dye to analyze the surface biofilm coverage. Representative optical microscope images of a fluorescently stained *C. marina* biofilm on Ecoflex-10 pneumatic before and after pneumatic actuation at a strain of 45% are presented in Figure 2A. The images clearly show that > 90% of the surface adhered biofilm (green fluorescence) present on the surface before actuation was detached after pneumatic actuation followed by a gentle rinse with DI water.

The detachment of biofilm due to deformation of a surface can be described as a de-bonding process. When a deformable silicone surface with an adherent uniform biofilm is stretched, the biofilm generates elastic energy due to its viscoelastic nature. It is hypothesized that

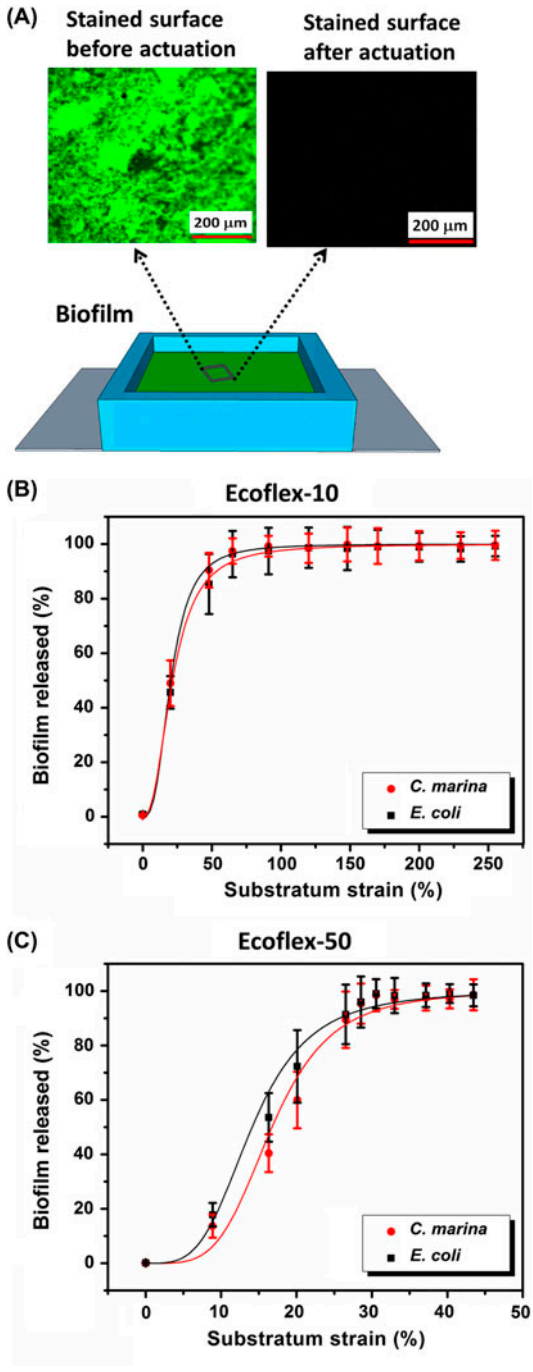

Figure 2. Detachment of model bacterial biofilms (*C. marina* and *E. coli*) from Ecoflex elastomer surfaces upon pneumatic actuation. Microscopic images of fluorescently stained *C. marina* biofilm show evidence that surface adherent biofilm (green fluorescence) present on the surface before actuation was detached significantly (by > 90%) due to pneumatic actuation followed by gentle rinsing (~ 5 ml min $^{-1}$) with DI water (A). Percentage of biofilm released from the surface of Ecoflex-10 (B) and Ecoflex-50 (C) elastomers for different amounts of applied strain *via* pneumatic actuation. Error bars represent the SD of the mean (n = 5). The curves were fitted using Equation 2 and the R^2 values of all the individual curves were ≥ 0.98.

when this generated elastic energy per unit area exceeds the adhesion energy of the biofilm to the substratum, the biofilm de-bonds from the substratum. The de-bonded biofilm can then be easily removed by applying low surface shear forces through, for example, gentle rinsing. Biofilm detachment of > 90%, such as that illustrated in Figure 2A, indicates that the applied strain (45%) was greater than or equal to that needed to de-bond the biofilm. For a given strain rate, considering the biofilm to be linearly elastic, the critical strain needed to detach the biofilm from the substratum is hypothesized to be given by Shivapooja et al. (2013):

$$\varepsilon_c = \sqrt{\frac{2\Gamma}{YH}} \tag{1}$$

where Y, H and Γ represent the biofilm Young's modulus, biofilm thickness and biofilm-polymer adhesion energy per unit area, respectively.

To validate this relationship, *C. marina* and *E. coli* biofilms were grown separately on multiple replicates of Ecoflex-10 and Ecoflex-50 pneumatic networks and subjected to varying amounts of strains. Figure 2B shows that ~ 50% of both types of biofilms (*C. marina* and *E. coli*) were released from Ecoflex-10 at low surface strain (25%), suggesting that only a small strain (< 20%) was necessary for the biofilm to de-bond from the surface. Upon applying higher strains (> 50%) > 90% of the biofilm was released from the surface. Similar biofilm detachment profiles were observed on Ecoflex-50 (Figure 2C). The experimental data were fitted using the following equation:

$$P = 1 - \left(\frac{1}{1 + \left(\frac{\varepsilon_{50}}{\varepsilon}\right)^k}\right) \tag{2}$$

where P represents the fraction of biofilm released from the surface, ε_{50} represents the strain required to detach 50% of the biofilm and k is an empirical constant. Using the fitted data, the average strain required to release 50% of the *C. marina* biofilm (ie ε_{50}) from Ecoflex-10 and Ecoflex-50 was determined to be 21 ± 0.5 and 16 ± 0.5%, respectively (the R^2 value for all the fitted curves was > 0.98). Similar trends were observed in the detachment of the *E. coli* biofilms (Figure 2B and C). More than 90% of *E. coli* biofilms were released above the critical strain and the ε_{50} values were different for Ecoflex-50 (ε_{50}=15%) and Ecoflex-10 (ε_{50}=20%) test surfaces. Figure S4b summarizes the average strains necessary to detach 20, 50 and 80% of each type of biofilm.

Ecoflex-10 and Ecoflex-50 have a similar chemical formulation (ie they are platinum-cured silicone elastomers), but different substratum moduli. This suggests that substratum modulus might be responsible for the observed difference in ε_{50} between Ecoflex-10 and Ecoflex-50 elastomer pneumatic networks. The substratum

modulus, which was measured to be different for Ecoflex-10 ($\mu = 10.5$ kPa) and Ecoflex-50 ($\mu = 50.2$ kPa), might influence each of the biofilm specific properties in Equation 1 (ie, the thickness (H), Young's modulus (Y) and surface adhesion strength (Γ) of the biofilm), which in turn affect the critical strain needed for biofilm detachment (Equation 1). The relative contributions of these variables were not investigated in the current study and further studies are needed to systematically investigate the role of surface modulus on the strain needed for biofilm detachment. It was previously reported that the modulus and thickness of PDMS coatings significantly influenced the adhesion strength of certain foulers such as the green alga *Ulva* (Chaudhury et al. 2005) and pseudobarnacles (Kim et al. 2008). In summary, the laboratory studies support the hypothesis that controlled substratum deformation (eg *via* pneumatic actuation) can effectively detach model bacterial biofilms. Also, the effect of substratum modulus on the strain required to detach biofilm (which has been not considered in the previous analysis of Equation 1 (Shivapooja et al. 2013)) is reported for the first time.

Detachment of natural biofilms in the marine environment

In contrast to most laboratory cultures, microorganisms in nature exist in mixed populations and are under constant environmental selection to adhere to surfaces and form biofilms (Briand 2009). As a result, field experimentation offers the most rigorous and realistic conditions for the testing of materials that attract, repel or release biofilms in marine applications (McLean & Simpson 2008). It should also be noted, however, that field studies generally require a longer immersion time, which can negatively impact the rate at which new coatings are developed. Hence, correlation and statistical comparison between laboratory assays and field tests is desirable to assess the performance of new biofouling management approaches (Zhang et al. 2013).

As a first step towards addressing the suitability of elastomer surface deformation for FR in relevant field environments, experiments were conducted to investigate the effect of substratum strain on detachment of surface adhered biofilms that formed naturally in a marine environment. The objective was to test the laboratory-validated hypotheses in a marine environment, ie that controlled surface deformation can be used to release biofilms and that there exists a critical strain above which biofilms de-bond from the surface easily. A secondary objective was to compare the ε_{50} of the model marine biofilm (*C. marina*) with that of biofilms formed in a marine environment. The infrastructure used and procedures followed in the field studies are detailed in the Materials and methods section.

Multiple replicates of Ecoflex-10 and Ecoflex-50 elastomer pneumatic networks were assembled on a panel and immersed in marine water at Pivers Island near Beaufort, NC (Figure 3A and B) for a period of 14 days to allow for biofilm formation and growth. Digital photographs (Figure 3C and D) of the elastomer surface before and after immersion for 14 days clearly show the accumulation of biofilm on the elastomer surfaces. To quantify the surface coverage of biofilm, a standard CV staining assay was used (Ribeiro et al. 2008). CV stains cells and other biological materials adherent on the surface, including biofilm matrix components. Control experiments (Figure 3E) showed that the CV staining protocol used here resulted in only minimal staining of silicone surfaces not subjected to field immersion. By contrast, retention of CV dye on the experimental samples provides clear evidence of biofilm accumulation on the entire surface (Figure 3F).

After biofilm accumulation for 14 days, the elastomer pneumatic networks were individually actuated while still submerged using a protocol similar to that of the laboratory studies. Actuated and control (ie, non-actuated) samples were carefully removed from the field site and analyzed in the laboratory using the CV staining assay explained above. Optical microscope images of the stained surfaces were collected, and the surface biofilm coverage was quantified using the procedure detailed above. On the Ecoflex-10 surface (Figure 4A), it was observed that only a low amount (< 15%) of adherent biofilm was detached for substratum strains up to 50%. However, when the strain was increased above 50%, a substantial increase in the amount of biofilm release was observed. These results suggest that, as for laboratory grown biofilms, a critical substratum strain is needed to detach natural biofilms formed in field environments. As the substratum strain reaches a certain critical value, adherent biofilm de-bonds from the surface and is subsequently released by the shear forces from water flow. Similarly, a sudden increase in biofilm detachment from Ecoflex-50 surfaces (Figure 4B) was observed when the applied strain was > 30%. These results demonstrate that controlled dynamic deformation of elastomeric substrata *in situ* can effectively detach (> 90%) natural biofilms formed in the marine field environment.

As with the FR experimental data on laboratory grown biofilms, Equation 2 was used to determine the strain needed to detach 50% of the biofilm grown in the marine environment (ie the ε_{50}) on each substratum. A strain of 81% was needed to detach 50% of the biofilm on Ecoflex-10, while a strain of 30% achieved the same result on Ecoflex-50. The significant difference in ε_{50} between Ecoflex-10 and Ecoflex-50 in field studies may be due to factors such as biofilm adhesion strength and thickness as mentioned above. Furthermore, the ε_{50} values for Ecoflex-10 and Ecoflex-50 obtained in the field

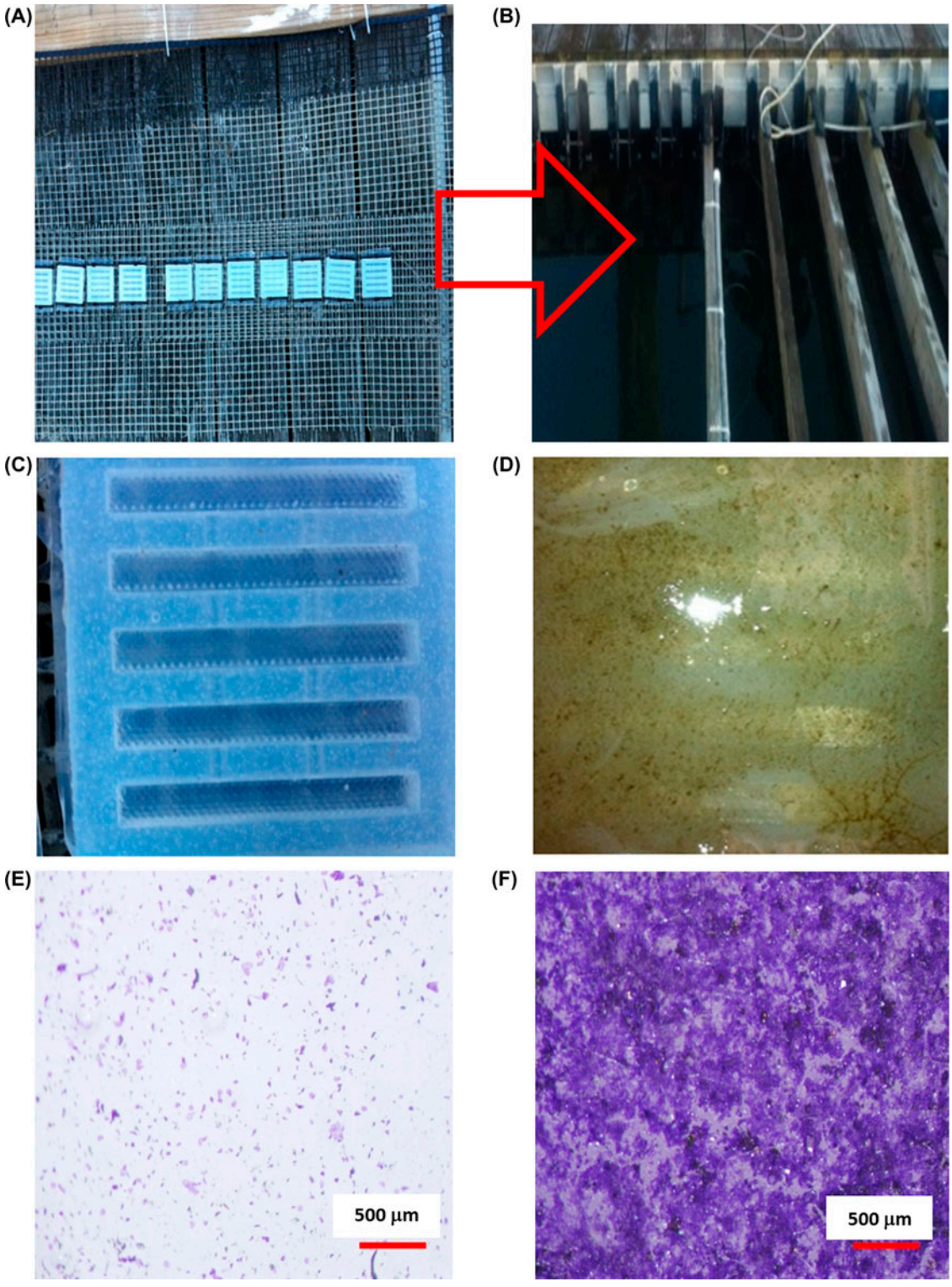

Figure 3. Field studies conducted at the research dock of Duke University Marine Laboratory (Beaufort, NC). Multiple replicates of test surfaces (elastomeric pneumatic networks) made from Ecoflex-10 and Ecoflex-50 immobilized on a mesh panel were immersed in the marine water to a depth of 1 m below water level, and subjected to biofilm formation for a period of 14 days (A–B). Representative digital photographs of the Ecoflex-10 elastomer surface before (C) and after immersion for 14 days (D) show the formation of a biofilm. Microscope images of sample surface stained using CV before (E) and after 14 days (F). Scale bars on (E) and (F) represent 500 µm.

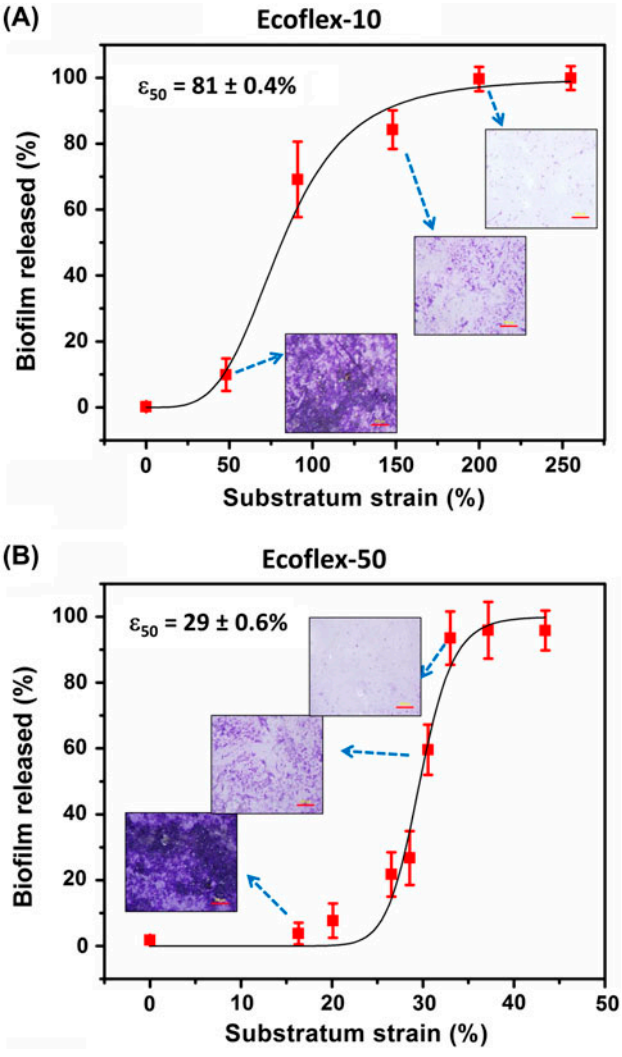

Figure 4. Effect of applied maximum substratum strain on biofilm release in a marine field environment. Multiple replicates of test surfaces (elastomer pneumatic networks) made of Ecoflex-10 (A) and Ecoflex-50 (B) were kept in the field for 14 days to allow biofilm formation. The test surfaces were then pneumatically actuated for 20 cycles to substratum strains in the range of 0–250% (for Ecoflex-10) and 0–50% (for Ecoflex-50). Error bars represent the SD of the mean (n = 5). Representative microscopy images of the amount of surface biofilm coverage after actuation are included as insets; the scale bars represent 500 μm. The data were curve fitted using Equation 2 and the R^2 values for the curves in (A) and (B) are 0.98 and 0.94, respectively.

studies were much higher than those obtained for model bacterial biofilms (*C. marina* and *E. coli*) studied in the laboratory. This difference could be due to the difference in biofilm composition between laboratory and field studies. For example, field accumulated biofilm might include diatoms, which will increase the adhesion strength of the biofilm (Holland et al. 2004; Zargiel & Swain 2014). While the experimental conditions used in the laboratory were not chosen in a deliberate attempt to mimic those in the field, the above results illustrate both the value of laboratory and field studies, and the limitations of laboratory studies. While a similar dependence of ε_{50} on substratum modulus was observed in both types of experiments, different magnitudes of ε_{50} were observed between laboratory and field experiments. The results demonstrate that the type of biofilm that forms on a surface, and the mechanical properties of the substratum, both significantly affect the substratum strain needed to release biofilm. In this present study a constant rate of substratum strain was used in order to have consistent experimental conditions between laboratory and field studies. However, it should be noted that the rate of substratum strain can also influence the detachment of biofilms. A quantitative relationship between applied strain rate, biofilm detachment, and de-bonded biofilm segment length has been reported previously (Levering et al. 2014).

It is important to emphasize that the methods to achieve FR presented here are, in principle, complementary to other established methods. The FR performance of pneumatically actuated silicones (ie the reduction in the amount of critical strain needed to de-bond biofilms) may be enhanced, for example, by modifying the silicone polymer by known approaches. For example, as mentioned earlier, addition of silicone oils (1–10% by weight) increases the FR performance of silicone coatings without significantly compromising their durability (Stein et al. 2003; Meyer et al. 2006). The non-reactive silicone oils are not covalently bound in the elastomeric network and migrate to the surface forming a weak boundary layer (at the aqueous interface) that decreases the adhesion energy of the biofilm/fouling species. Such phenomena may in turn reduce the substratum strain needed to de-bond the biofilm (Equation 1). Also, AF compounds such as triclosan and sparfloxacin (Cagni et al. 1995; Rittschof et al. 2003; Jones et al. 2006) can be impregnated in deformable silicone elastomers and subjected to controlled and triggered release *via* dynamic actuation methods to improve surface AF properties. In summary, as on-demand controlled surface deformation can be easily achieved using pneumatic methods, this soft robotics-inspired approach can have potential applications for biofouling management on certain suitable maritime equipment, such as seawater cooling pipes, oceanographic sensors and power transmitters/receivers.

Conclusions

Silicone-based FR coatings are a potential alternative to traditional biocidal coatings in biofouling management. It was recently reported that silicone substratum deformation reduces barnacle adhesion strength and allows facile removal of model biofilms. Inspired by the recent

development of silicone soft robotic devices, an approach to achieve dynamic surface deformation *via* pneumatic actuation was used in this study to investigate the effect of substratum deformation on the detachment of model microbial biofilms under laboratory conditions and naturally grown biofilms in field studies. Both laboratory and field studies showed that effective triggered detachment of biofilms occurs above a critical substratum strain. It was also found that the substratum modulus affected the strain needed to detach biofilm with higher modulus silicone substrata requiring less maximal strain for efficient detachment. Since silicone coatings are widely used in efforts to manage biofouling, it may be possible in certain applications to enhance these efforts by implementing the approach of dynamic silicone surface deformation *via* pneumatic (or other) actuation to control biofouling on maritime equipment. Though biofouling control *via* pneumatic actuation may not be practically feasible for ships' hulls, it can have potential applications for underwater autonomous environment monitoring devices such as acoustic sensors, optical sensors and gliders. Further, pneumatic actuation can also be employed in industrial water pipelines and biomedical urinary catheter devices where clogging due to biofilms is a major problem.

Conflict of interest disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Office of Naval Research [Grant N000141310828] and the NSF's Research Triangle Materials Research Science and Engineering Center [Grant DMR-1121107].

Supplemental material

The supplemental material for this paper is available online at http://dx.doi.org/10.1080/08927014.2015.1035651.

References

Aftring RP, Taylor BF. 1979. Assessment of microbial fouling in an ocean thermal-energy conversion experiment. Appl Environ Microb. 38:734–739.

Arruda EM, Boyce MC. 1993. A 3-dimensional constitutive model for the large stretch behavior of rubber elastic-materials. J Mech Phys Solids. 41:389–412.

Baire RE. 1970. Surface properties influencing biological adhesion. In: Manly RS, editor. Adhesion in biological systems. New York (NY): Academic Press; p. 15–48.

Bodkhe RB, Stafslien SJ, Daniels J, Cilz N, Muelhbert AJ, Thompson SEM, Callow ME, Callow JA, Webster DC. 2015. Zwitterionic siloxane-polyurethane fouling-release coatings. Prog Org Coat. 78:369–380.

Brady RF, Singer IL. 2000. Mechanical factors favoring release from fouling release coatings. Biofouling. 15:73–81.

Briand JF. 2009. Marine antifouling laboratory bioassays: an overview of their diversity. Biofouling. 25:297–311.

Cagni A, Chuard C, Vaudaux PE, Schrenzel J, Lew DP. 1995. Comparison of sparfloxacin, temafloxacin, and ciprofloxacin for prophylaxis and treatment of experimental foreign-body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Ch. 39:1655–1660.

Callow JA, Callow ME. 2011. Trends in the development of environmentally friendly fouling-resistant marine coatings. Nat Commun. 2:1–10.

Callow ME, Callow JE. 2002. Marine biofouling: a sticky problem. Biologist. 49:1–5.

Callow ME, Fletcher RL. 1994. The influence of low surface-energy materials on bioadhesion – a review. Int Biodeter Biodegr. 34:333–348.

Carman ML, Estes TG, Feinberg AW, Schumacher JF, Wilkerson W, Wilson LH, Callow ME, Callow JA, Brennan AB. 2006. Engineered antifouling microtopographies – correlating wettability with cell attachment. Biofouling. 22:11–21.

Casanueva JF, Sanchez J, Garcia-Morales JL, Casanueva-Robles T, Lopez JA, Portela JR, Nebot E, Sales D. 2003. Portable pilot plant for evaluating marine biofouling growth and control in heat exchangers-condensers. Water Sci Technol. 47:99–104.

Characklis WG. 1981. Fouling biofilm development – a process analysis. Biotechnol Bioeng. 23:1923–1960.

Chaudhury MK, Finlay JA, Chung JY, Callow ME, Callow JA. 2005. The influence of elastic modulus and thickness on the release of the soft-fouling green alga *Ulva linza* (syn. *Enteromorpha linza*) from poly(dimethylsiloxane) (PDMS) model networks. Biofouling. 21:41–48.

Estarlich FF, Lewey SA, Nevell TG, Thorpe AA, Tsibouklis J, Upton AC. 2000. The surface properties of some silicone and fluorosilicone coating materials immersed in seawater. Biofouling. 16:263–275.

Gerba CP, McLeod JS. 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Appl Environ Microbiol. Jul;32:114–120.

Gittens JE, Smith TJ, Suleiman R, Akid R. 2013. Current and emerging environmentally-friendly systems for fouling control in the marine environment. Biotechnol Adv. 31:1738–1753.

Halder P, Nasabi M, Jayasuriya N, Shimeta J, Deighton M, Bhattacharya S, Mitchell A, Bhuiyan MA. 2014. An assessment of the dynamic stability of microorganisms on patterned surfaces in relation to biofouling control. Biofouling. 30:695–707.

Holland R, Dugdale TM, Wetherbee R, Brennan AB, Finlay JA, Callow JA, Callow ME. 2004. Adhesion and motility of fouling diatoms on a silicone elastomer. Biofouling. 20:323–329.

Holm ER. 2012. Barnacles and biofouling. Integr Comp Biol. 52:348–355.

Ilievski F, Mazzeo AD, Shepherd RE, Chen X, Whitesides GM. 2011. Soft robotics for chemists. Angew Chem Int Ed. 50:1890–1895.

Jain A, Bhosle N. 2009. Biochemical composition of the marine conditioning film: implications for bacterial adhesion. Biofouling. 25:13–19.

Jones GL, Muller CT, O'Reilly M, Stickler DJ. 2006. Effect of triclosan on the development of bacterial biofilms by urinary tract pathogens on urinary catheters. J Antimicrob Chemother. 57:266–272.

Kendall K. 1971. The adhesion and surface energy of elastic solids. J Phys D: Appl Phys. 4:1186–1195.

Kim J, Nyren-Erickson E, Stafslien S, Daniels J, Bahr J, Chisholm BJ. 2008. Release characteristics of reattached barnacles to non-toxic silicone coatings. Biofouling. 24:313–319.

Kim S, Laschi C, Trimmer B. 2013. Soft robotics: a bioinspired evolution in robotics. Trends Biotechnol. 31:23–30.

Levering V, Wang Q, Shivapooja P, Zhao X, Lopez GP. 2014. Soft robotic concepts in catheter design: an on-demand fouling-release urinary catheter. Adv Healthc Mater. 3:1588–1596.

Ling GC, Low MH, Erken M, Longford S, Nielsen S, Poole AJ, Steinberg P, McDougald D, Kjelleberg S. 2014. Microfabricated polydimethyl siloxane (PDMS) surfaces regulate the development of marine microbial biofilm communities. Biofouling. 30(3):323–335.

Majumdar P, Crowley E, Htet M, Stafslien SJ, Daniels J, VanderWal L, Chisholm BJ. 2011. Combinatorial materials research applied to the development of new surface coatings XV: an investigation of polysiloxane anti-fouling/fouling-release coatings containing tethered quaternary ammonium salt groups. ACS Comb Sci. 13:298–309.

Martinelli E, Agostini S, Galli G, Chiellini E, Glisenti A, Pettitt ME, Callow ME, Callow JA, Graf K, Bartels FW. 2008. Nanostructured films of amphiphilic fluorinated block copolymers for fouling release application. Langmuir. 24:13138–13147.

McLean RJ, Simpson TR. 2008. Preparing for biofilm studies in the field. Curr Protoc Microbiol. Chapter 1:Unit 1B.4.1-1B.1.14.

Meyer A, Baier R, Wood CD, Stein J, Truby K, Holm E, Montemarano J, Kavanagh C, Nedved B, Smith C, et al. 2006. Contact angle anomalies indicate that surface-active eluates from silicone coatings inhibit the adhesive mechanisms of fouling organisms. Biofouling. 22:411–423.

Mieszkin S, Callow ME, Callow JA. 2013. Interactions between microbial biofilms and marine fouling algae: a mini review. Biofouling. 29:1097–1113.

Nebot E, Casanueva JF, Solera R, Pendon C, Taracido LJ, Casanueva-Robles T, Lopez-Galindo C. 2010. Marine biofouling in heat exchangers. In: Chan J, Wong S, editors. Biofouling: types, impact and anti-fouling. New York (NY): Nova Science; p. 65–104.

Ribeiro E, Stafslien SJ, Casse F, Callow JA, Callow ME, Pieper RJ, Daniels JW, Bahr JA, Webster DC. 2008. Automated image-based method for laboratory screening of coating libraries for adhesion of algae and bacterial biofilms. J Comb Chem. 10:586–594.

Rittschof D, Lai CH, Kok LM, Teo SLM. 2003. Pharmaceuticals as antifoulants: concept and principles. Biofouling. 19:207–212.

Rittschof D, Orihuela B, Harder T, Stafslien S, Chisholm B, Dickinson GH. 2011. Compounds from silicones alter enzyme activity in curing barnacle glue and model enzymes. PLoS one. 6:e16487.

Scardino AJ, de Nys R. 2011. Mini review: biomimetic models and bioinspired surfaces for fouling control. Biofouling. 27:73–86.

Schultz MP. 2007. Effects of coating roughness and biofouling on ship resistance and powering. Biofouling. 23:331–341.

Schultz MP, Bendick JA, Holm ER, Hertel WM. 2011. Economic impact of biofouling on a naval surface ship. Biofouling. 27:87–98.

Shea C, Lovelace LJ, Smithsomerville HE. 1995. *Deleya marina* as a model organism for studies of bacterial-colonization and biofilm formation. J Ind Microbiol. 15:290–296.

Shepherd RF, Ilievski F, Choi W, Morin SA, Stokes AA, Mazzeo AD, Chen X, Wang M, Whitesides GM. 2011. Multigait soft robot. Proc Natl Acad Sci USA. 108: 20400–20403.

Shivapooja P, Wang Q, Orihuela B, Rittschof D, Lopez GP, Zhao X. 2013. Bioinspired surfaces with dynamic topography for active control of biofouling. Adv Mater. 25:1430–1434.

Singer IL, Kohl JG, Patterson M. 2000. Mechanical aspects of silicone coatings for hard foulant control. Biofouling. 16:301–309.

Sonak S, Pangam P, Giriyan A, Hawaldar K. 2009. Implications of the ban on organotins for protection of global coastal and marine ecology. J Environ Manage. 90: S96–S108.

Stein J, Truby K, Wood CD, Takemori M, Vallance M, Swain G, Kavanagh C, Kovach B, Schultz M, Wiebe D, et al. 2003. Structure-property relationships of silicone biofouling-release coatings: effect of silicone network architecture on pseudobarnacle attachment strengths. Biofouling. 19:87–94.

Swain GW, Kovach B, Arthur T, Casse F, Kavanagh C. 2007. Measuring the performance of today's antifouling coatings. J Ship Prod. 23:164–170.

Thomas KV, Brooks S. 2010. The environmental fate and effects of antifouling paint biocides. Biofouling. 26:73–88.

Truby K, Wood C, Stein J, Cella J, Carpenter J, Kavanagh C, Swain G, Wiebe D, Lapota D, Meyer A, et al. 2000. Evaluation of the performance enhancement of silicone biofouling-release coatings by oil incorporation. Biofouling. 15:141–150.

Vogel V. 2012. Soft robotics: bionic jellyfish. Nat Mater. 11:841–842.

Voulvoulis N, Scrimshaw MD, Lester JN. 1999. Alternative antifouling biocides. Appl Organomet Chem. 13:135–143.

Wang DC, Qian CF, Cao SX, Liu Y, Sun JW. 2013. Effect of different biofouling characteristics on heat transfer of the heat exchanger. Adv Mater Res-Switz. 724–725: 1282–1288.

Zargiel KA, Swain GW. 2014. Static *vs* dynamic settlement and adhesion of diatoms to ship hull coatings. Biofouling. 30:115–129.

Zhang Z, Finlay JA, Wang LF, Gao Y, Callow JA, Callow ME, Jiang SY. 2009. Polysulfobetaine-grafted surfaces as environmentally benign ultralow fouling marine coatings. Langmuir. 25:13516–13521.

Zhang JW, Lin CG, Wang L, Zheng JY, Xu FL, Sun ZY. 2013. Study on the correlation of lab assay and field test for fouling-release coatings. Prog Org Coat. 76: 1430–1434.

Supplementary information

Dynamic surface deformation of silicone elastomers for management of marine biofouling: laboratory and field studies using pneumatic actuation

Phanindhar Shivapooja,[a] Qiming Wang,[b] Lizzy M. Szott,[a] Beatriz Orihuela,[c] Daniel Rittschof,[c] Xuanhe Zhao,[b] and Gabriel P. López[a, b, d]

[a] *Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA*
[b] *Department of Mechanical Engineering and Materials Science, Duke University, Durham, NC 27708, USA*
[c] *Duke University Marine Laboratory, Nicholas School of the Environment, Beaufort, NC 28516, USA*
[d] *Research Triangle Materials Research Science & Engineering Center, Duke University, Durham, NC 27708, USA*

Design and features of 3D printed plastic template

3D printed plastic (acrylonitrile butadiene polystyrene, ABS) templates (Figure S1a), consisting of vertical and horizontal strips, were used for the fabrication of elastomer pneumatic network. Each of the long (40 mm) parallel stripes in the plastic mold forms an air channel in the prepared elastomer network (Figure S1b). The air channels are separated from one another by 5 mm and interconnected by the opening formed by smaller stripes (Figure 1Sb).

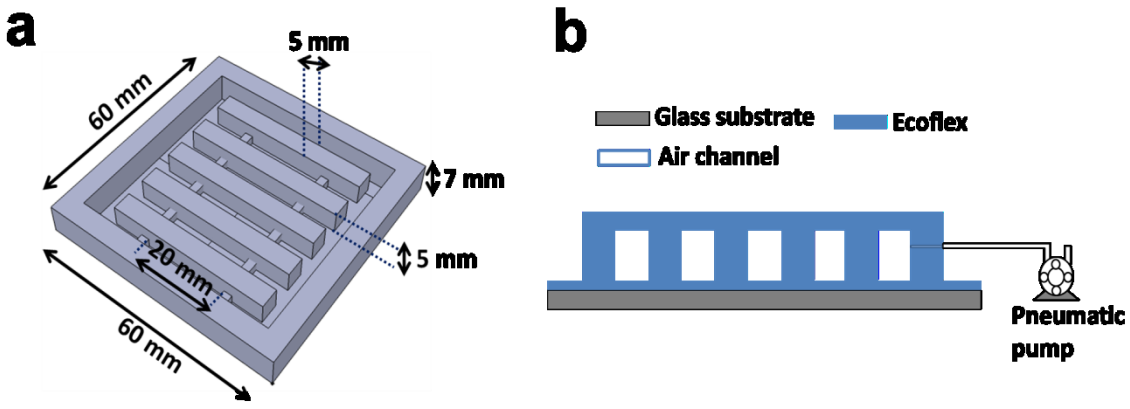

Figure S1: Fabrication and calibration of elastomer pneumatic networks. Schematic and dimensions of the plastic 3-D-printed template used for making the prototype elastomer

pneumatic networks (a), cross-section schematic of an Ecoflex elastomer pneumatic network connected to a pneumatic pump for actuation (b).

Shear moduli of Ecoflex silicone elastomer

Using the Arruda-Boyce model (Arruda and Boyce 1993), the nominal stress (s) for plane-strain uniaxial tension is given by,

$$s = \mu[(\varepsilon + 1) - (\varepsilon + 1)^{-3}]\left[1 + \frac{I_1}{5N} + \frac{11I_1}{175N^2}\right] \qquad \text{Equation (S.1)}$$

where, μ is shear modulus of the Ecoflex film, ε is the uniaxial strain, $I_1 = (\varepsilon + 1)^2 + (\varepsilon + 1)^{-2} + 1$, and N is a parameter that accounts for the stiffening effect. This equation was fitted to the experimental data obtained from uniaxial tension tests (Figure S2) using parameters μ = 10.5 kPa and N = 7.28 for Ecoflex-10, and μ = 50.2 kPa and N = 7.17 for Ecoflex-50.

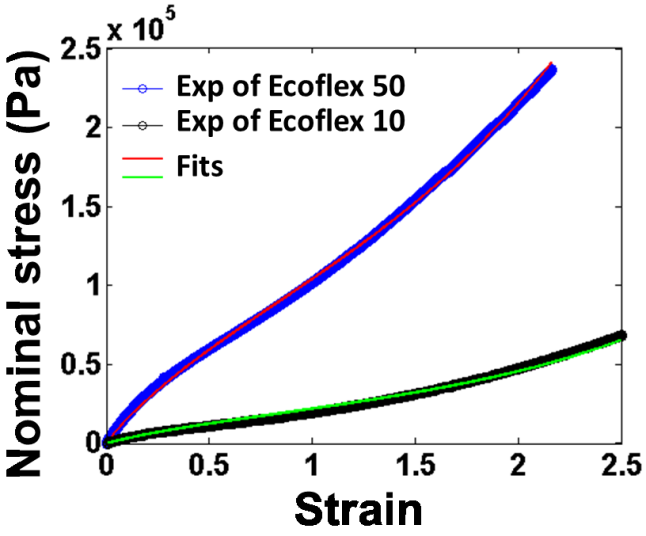

Figure S2: Effect of applied nominal stress on generated surface strain (ε).

2-D plane strain model for theoretical prediction of surface strain

A 2D plane-strain model was used by considering the deformation of a long strip of thin Ecoflex membrane on the top of a pneumatic channel. Under an applied uniform pressure P (> atmospheric pressure), the Ecoflex film on top of the pneumatic network will deform outwards as an arc with radius R (Figure S3); the force balance is given by,

$$PR = \sigma_\theta h \qquad \text{Equation (S.2)}$$

where σ_θ is the membrane stress. By denoting the initial and inflated lengths as 2L and 2l, and the initial and deformed thicknesses of the film as *H* and *h* respectively, the two principal stretches in the film are given by,

$$\lambda_\theta = \frac{l}{L} = \frac{\theta}{sin\theta},\ \lambda_r = \frac{h}{H} = \frac{1}{\lambda_\theta} \qquad \text{Equation (S.3)}$$

where 2θ is the angle of the arc (Figure 1e). The Ecoflex film obeys the Arruda-Boyce model ie,

$$\sigma_\theta = -p_0 + \mu\lambda_\theta^2\left(1 + \frac{I_1}{5N} + \frac{11I_1^2}{175N^2}\right) \qquad \text{Equation (S.4)}$$

$$\sigma_r = -p_0 + \mu\lambda_r^2\left(1 + \frac{I_1}{5N} + \frac{11I_1^2}{175N^2}\right) \qquad \text{Equation (S.5)}$$

where p_0 is the hydrostatic stress in the elastomer, μ is the shear modulus of the Ecoflex film, and $I_1 = \lambda_\theta^2 + \lambda_r^2 + 1$. Given that the radial stress $\sigma_r = 0$, the membrane stress can be expressed as

$$\sigma_\theta = \mu\left(\lambda_\theta^2 - \lambda_r^2\right)\left(1 + \frac{I_1}{5N} + \frac{11I_1^2}{175N^2}\right) \qquad \text{Equation (S.6)}$$

Based on Equations (S.2) – (S.6), the relationship between the applied pressure and applied surface linear strain is calculated using the resulting equation $\varepsilon = (\lambda_\theta - 1)$.

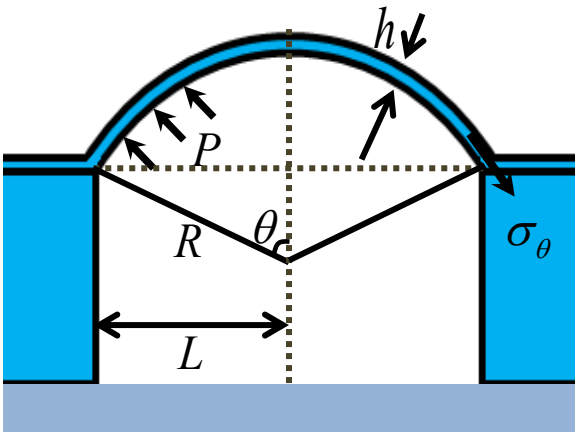

Figure S3: Schematic shows the 2D cross-section view of deformation of a thin elastomer membrane, caused by increase in air pressure (P > atmospheric pressure).

Effect of applied substrate strain on *C. marina* and *E. Coli* biofilm

The experimental data of biofilm released from Ecoflex-10 (b) and Ecoflex-50 (c) elastomer networks for different amounts of applied strain via pneumatic actuation (Figure 2) were fitted an empirical equation (Equation 2). Using the fitted data substrate strain needed to detach 20% (ε_{20}), 50% (ε_{50}) and 80% (ε_{80}) of biofilm was determined and plotted (Figure S4) for comparison.

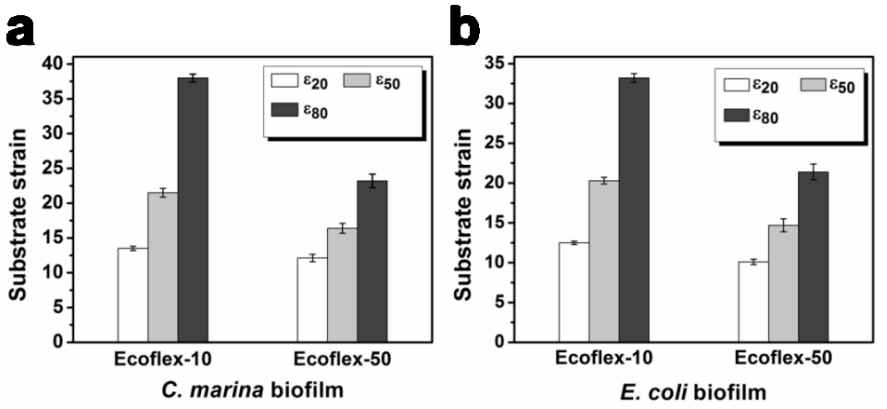

Figure S4: Effect of substrate strain on bacterial biofilm release. The percentage of substrate strain needed to detach 20% (ε_{20}), 50% (ε_{50}) and 80% (ε_{80}) of *C. marina* and *E. coli* biofilms from Ecoflex-10 and Ecoflex-50 was measured by fitting the experimental data to Equation (2).

References

Arruda EM, Boyce MC. 1993. A 3-dimensional constitutive model for the large stretch behavior of rubber elastic-materials. J Mech Phys Solids.41:389-412.