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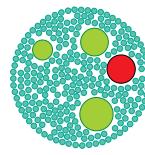
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Holding (not so) fast: surface chemistry constrains kelp bioadhesion

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ABSTRACT

Benthic macroalgae must attach firmly to the substrate to prevent being detached and washed away by water motion. The success of the bioadhesion system can be strongly influenced by surface chemistry and so this should be optimized for large-scale cultivation. This is especially important during the early stage of cultivation when the juveniles have little thigmotactic attachment, which is needed to interlock with surface rugosity. Juvenile sporophytes of *Saccharina latissima* (Phaeophyceae) were directly applied onto polymer films of varied surface composition to determine how the attachment force of the developing holdfast was influenced by surface chemistry. Eight polymer chemistries were examined: polyamide (PA), polyethylene (PE), polyester (PES), polypropylene (PP), polymethylacrylate (PMA), polyvinylalcohol (PVA), polyvinylchloride (PVC) and thermoplastic polyurethane (TPU). The PP and PE were also examined as three grades: commercial grade with additives, pure polymer, or pure polymer following a corona treatment. Additive inclusion and corona significantly reduced the water contact angle ($p < 0.0001$), indicating an increase in the surface free energy available for bioadhesion. After 6 weeks, the attachment force was greatest on PVA, PA and PVC (0.19–0.33 N), correlating strongly with the achieved biomass ($R^2 = 0.68$). Additives and corona treatment improved holdfast attachment force, particularly corona treated PE (0.28 ± 0.08 N: 0 N without corona). Generally, attachment force appeared greatest on chemistries with a contact angle of 60–75°. These results confirm that the bioadhesion of the phaeophyte holdfast is strongly influenced by the surface free energy of the substrate chosen. Through alteration of the additive composition, attachment could be improved to create bespoke cultivation substrates. Corona treatment is highlighted as a very suitable method for improving holdfast attachment force during cultivation.

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Introduction

The cultivation of macroalgae is an international industry, producing 26 million fresh weight tonnes in 2014 and estimated to be worth \$5.6 billion (FAO, 2018). Algal cultivation requires a firm attachment to be established between the holdfast of the macroalgae and the chosen cultivation substrate to prevent detachment during storms (Denny, 1988). Bioadhesion by macroalgae involves the secretion of a liquid mucilage, which connects the cell wall and the substrate. This material is then irreversibly hardened by polymerization and cross-linking (Bitton *et al.*, 2006; Tarakhovskaya, 2014). In the Phaeophyta, the secretion is proposed to be primarily composed of alginate, fucan polysaccharides and polyphenols (Vreeland *et al.*, 1993, 1998; Bitton *et al.*, 2006). This secretion initially forms a flexible gel through the interaction of alginate with calcium ions in seawater. The polyphenols are composed of phloroglucinol monomers, linked by carbon-carbon and ether bonds to form complex branched structures with a range of molecular sizes (125 Da to 650 kDa) (McInnes *et al.*, 1985; Potin & Leblanc, 2006). It has been proposed that the polyphenols form cross-links by the action of a vanadium dependent haloperoxidase, hydrogen peroxide and iodide, solidifying the bioadhesive (Bitton *et al.*, 2006).

The suitability of a surface for the bioadhesion of marine organisms has been shown to be strongly influenced by factors such as the surface free energy, surface chemistry and topography (Finlay *et al.*, 2002; Lejars *et al.*, 2012; Kerrison *et al.*, 2017, 2018b). The selection of an appropriate cultivation substrate is essential for the successful retention of macroalgae deployed at sea. A strong attachment to the substrate is especially important when the algae are at the juvenile macroscopic stage (0.5–5 mm). As they grow larger, the algae transition from a low Reynold's number environment dominated by viscous force and experience the turbulent flow of high Reynold's numbers (Vogel, 1994). In a previous study, 2 mm sporophytes of kelp were retained on numerous polymer chemistries. However once they grew to ~20 mm, increasing drag from water flow caused detachment where there was only weak bioadhesion (Vogel, 1994; Kerrison *et al.*, 2017).

With continued growth toward adulthood, further holdfast development will form thigmotactic attachment to the substrate (Candries *et al.*, 2001). This may eventually supersede the need for bioadhesion, due to mechanical interlocking into surface features. However, hatchery-reared macroalgal juveniles are

usually transplanted to the sea before this stage, and so are likely to be dependent on bioadhesion alone.

Due to the wide availability and product consistency of many synthetic polymers such as polypropylene (PP) and polyamide (PA) these are often used as a substrate for cultivation, despite few published studies having assessed their suitability (Kerrison *et al.*, 2017). Other established materials in macroalgal cultivation such as Kuralon, a form of polyvinylalcohol (PVA), have been identified by trial and error. However, without systematic analysis to compare the performance of materials, further improvements will not be made.

Recently, it was reported that solid polymer blocks show large differences in their suitability as cultivation substrates for two phaeophyte macroalgae already under cultivation within Europe: *Saccharina latissima* and *Laminaria digitata* (Kerrison *et al.*, 2017). Of the polymers examined: PA, polycarbonate, medium density polyethylene and polyvinylchloride (PVC) were highly suitable for adult attachment, but discouraged the initial settlement of meiospores. Kerrison *et al.* (2018b) revealed that this pattern was largely described by the water contact angle (θ_w), a parameter related to the surface free energy for binding (Callow & Fletcher, 1994): the kelp holdfast attached more firmly to more hydrophilic surfaces (low θ_w) allowing abundant growth. On hydrophobic surfaces with $\theta_w > 90^\circ$ holdfast attachment was weak, leading to sporophyte detachment, in agreement with similar findings in both phaeophyte and chlorophyte macroalgae (Hardy & Moss, 1979; Callow *et al.*, 1988; Finlay *et al.*, 2002; Ucar *et al.*, 2010).

Synthetic polymers have many advantages for use as macroalgal cultivation substrates. Their plastic form allows (a) the flexibility to manufacture a diverse range of structures, including novel 2D or 3D substrates that can maximize the cultivation areal productivity, compared with 1D ropes (European Commission, 2014); and (b) the inclusion of additives, or other treatments to alter the chemical structure of the polymer so as to enhance desirable characteristics, such as increased hydrophilicity (Kang & Neoh, 2009). Currently, the inclusion of complex additive mixtures such as pigments, anti-ozonants, plasticizers or stabilizers is systematic during polymer production to improve their end-use performance (Deanin, 1975; Murphy, 2001). It is anticipated that the presence/absence of such additives will modulate macroalgal attachment force by altering the surface chemistry available for bioadhesive interaction.

Surface modification of polymers such as PP allows the characteristics of the very outer layer of the material to be adjusted without affecting the main body of the material (Strobel *et al.*, 2003). Corona treatment is a commonly-used

method wherein a high frequency electrical discharge is used to alter the chemical and microtopographical structure of the polymer (Jones *et al.*, 2005; Kang & Neoh, 2009). This treatment ruptures long homogenous chains, creating low molecular weight oxidized materials (Strobel *et al.*, 2003) with new polar bonding points. This leads to increased surface free energy, lower θ_w and increased adhesion e.g. for ink, allowing label printing. Since macroalgal attachment in both brown and green macroalgae (Finlay *et al.*, 2002; Ucar *et al.*, 2010; Kerrison *et al.*, 2018b) is improved at lower θ_w , corona treatment may allow increased adhesive force.

The aim of this study was to compare the attachment force and growth form of directly applied *S. latissima* sporophytes grown on eight chemistries of flexible film which have not been tested before, including PVA and thermoplastic polyurethane (TPU). Three grades of polymer were also compared: a commercial film containing additives, a high purity film without additives and a corona treated high purity film. We hypothesized that: (1) θ_w will strongly dictate the attachment force on different films, with lower θ_w (more hydrophilic) encouraging attachment, and detachment at high θ_w (hydrophobic); (2) Additives will influence the θ_w , and so may either enhance or reduce attachment force; (3) Corona treatment will lower θ_w and therefore increase attachment force.

Materials and methods

Smooth polymer films, with different chemistries were tested: polyethylene (PE), polypropylene (PP), polyester (PES), thermoplastic polyurethane (TPU), polymethylacrylate coated polyvinylchloride (PMA) and polyvinylchloride (PVC). Additionally, high purity films (HP: without any additives) of polyamide 6 (PA; PolyOne, USA), PE (VS5580, Borealis AG, Austria) and PP (HG265FB, Borealis AG, Austria) were produced using a tape extruder (FET, UK). The conditions used are shown in Table 1. Half of the PE_{HP} and PP_{HP} films were then corona treated (HPC). This involved one side being passed five times through a 1000 W Corona Treater (Ahlbrandt Systems, Germany) at 2 m min⁻¹.

PVA film was produced by adding 1.5 g Desmodur® DN (Aliphatic polyisocyanate; Covestro AD, Germany) to 75 g PVA water-based dispersion (Bemifix PVL-P, Schmits Chemical Solutions, the Netherlands). The formulation was cast onto siliconized paper (wet thickness: 300 µm). The film was then dried for 2 min at 110°C and cured for 2 min at 155°C. Afterward, the film was separated from the siliconized paper.

There were eight polymer chemistries for experimental comparison: PA, PE, PES, PMA, PP, PVA,

Table 1. Extrusion conditions for high purity film production.

Chemistry	PE ^a	PP ^b	PA ^c
Drying conditions	n/a	n/a	Previously dried
Water content of polymer (ppm)	n/a	n/a	760
Extruder (rpm)	40	30	22
Zone 1 temperature (°C)	225	225	280
Zone 2 temperature (°C)	225	225	300
Zone 3 temperature (°C)	230	230	300
Temperature of steel disk die zone (°C)	230	230	300
Cooling roll speed (m min ⁻¹)	6	5	1.5
Heating roll 1 temperature (°C)	50	60	40
Heating roll 2 temperature (°C)	60	100	40
Fast roller (speed (m min ⁻¹)	6	5	3
Elongation ratio	1×	1×	2×
Final film thickness (μm)	80	85	270

^a Polyethylene, ^b polypropylene, ^c polyamide.

PVC and TPU. Both PE and PP had three grades: Commercial film (produced with additives), high purity with no additives (_{HP}) and corona treated high purity (_{HPC}). This gave a total of 12 film treatments ($n = 5$), which were cut to 25 × 76 mm, and secured to the surface of a glass microscope slide using 5 mm strips of Parafilm M® (Bemis, Wisconsin, USA). Glass slides were used as a control ($n = 5$). All slides were pre-cleaned: 24 h in 5% Decon90 detergent (Decon Laboratories Ltd, UK), 24 h in 10% hydrochloric acid, rinsed and soaked for 1 h in distilled water and finally dried at 35°C. Each film slide was soaked overnight in a 2% bicarbonate solution, rinsed thoroughly in distilled water, and then placed into a 300 ml borosilicate glass basin containing 150 ml of Tyndallized seawater (Kawai *et al.*, 2007) enriched with F/2 medium without silicate (F/2–Si). Glass basins were then incubated at 8.5°C for 6 h.

Sporangial tissue was collected from five *S. latissima* individuals from Seil Sound, UK (56.31724°N, -5.58309°W) and desiccated overnight at 4°C. Meiospores were released upon immersion in 8.5°C F/2–Si, in the dark for 1 h with agitation every 15 min. The suspension was then filtered at 50 μm (Kerrison *et al.*, 2016). A culture of *S. latissima* sporophytes were produced and maintained as described by Kerrison *et al.* (2018a). In brief, 500 000 meiospore l⁻¹ were bubble cultured at 8.5°C and 40–50 μmol photon m⁻² s⁻¹, 12:12 L:D in F/2–Si medium, which was 50% refreshed every 10–14 d. Germanium dioxide (0.125 ml saturated solution l⁻¹) was added during the first two weeks of cultivation at an optimized dose to prevent diatom growth whilst maintaining *S. latissima* growth (Kerrison *et al.*, 2016). After 8 weeks, sporophytes of 0.1–1 mm in size were filtered from the culture. 100 μl F/2–Si containing a 2% BinderA hydrocolloid solution (SAMS, UK) and 25 ± 2 *S. latissima* sporophytes were directly pipetted onto two locations of each prepared slide, already submerged in the medium (see Fig. 1). The BinderA solution adheres to the substrate and prevents the juvenile sporophyte from being washed away before their holdfast can adhere to the substrate

(Kerrison *et al.*, 2018a). All slides were then incubated at 8.5°C under warm fluorescent light (30–50 μmol photon m⁻² s⁻¹, 12:12 L:D).

Every 7–10 d, each slide was carefully transferred to a new basin of fresh F/2–Si and any detached sporophytes were discarded, following the cultivation protocol of Kerrison *et al.* (2016). After 8 weeks of growth, the attachment force of the five largest sporophytes, > 10 mm (where present), on each slide was measured using a digital force gauge (FK 25, Sauter, Germany): a rubber-coated clip was attached to the frond, which was pulled perpendicular to the surface until it became detached or the alga broke. The morphological characteristics of total sporophyte length, diameter of the basal holdfast and the length of all laterally spreading haptera were recorded for each sporophyte. Following this, all other sporophytes were removed from the film and measured, then patted dry with laboratory tissue (Kimtech, UK) and the fresh mass recorded to 0.01 g.

An additional set of polymer slides ($n = 3$), were cleaned and dried as described above. The static water contact angle (θ_w), was then measured using the method of Callow *et al.* (2000). On each slide, triplicate 10 μl droplets of ultra-high purity water were immediately photographed. Image J v 1.46r (National Institutes of Health, USA) with the DropSnake plugin (Stalder *et al.*, 2006) was used to determine θ_w .

Statistical analyses

All data were log transformed and tested for normality (Anderson & Darling, 1952) and homoscedasticity (Levene, 1960). Where these were satisfied, ANOVA (AN) followed by *post-hoc* Fisher's (*phF*) or 2-way ANOVA (2wAN) were used. Non-parametric data were examined using the Mann–Whitney U test (MW). Correlation between variables was explored using the Pearson's product moment correlation (PC). Excel 2016 (Microsoft Corp., USA) and Minitab® 15.1.0.0 (LEAD Technologies, Inc., USA) were used for all statistical analyses.

Results

Fresh wet mass

At the end of the experiment, the fresh wet mass of *S. latissima* attached to each polymer film was significantly different between the treatments (Table 2; AN: $p < 0.00001$, $F_{13,61} = 6.8$). Four groupings were identified: high, moderate, low and negligible biomass. The highest biomass of *S. latissima* was achieved on PVA (0.48 ± 0.13 g slide⁻¹), PE_{HPC} (0.45 ± 0.04 g slide⁻¹) and PA_{HP} (0.37 ± 0.06 g slide⁻¹). Moderate biomass was seen on PVC (0.22 ± 0.09 g slide⁻¹) and PE (0.19 ± 0.11 g slide⁻¹). Each of these films (PVA, PE_{HPC}, PA_{HP},

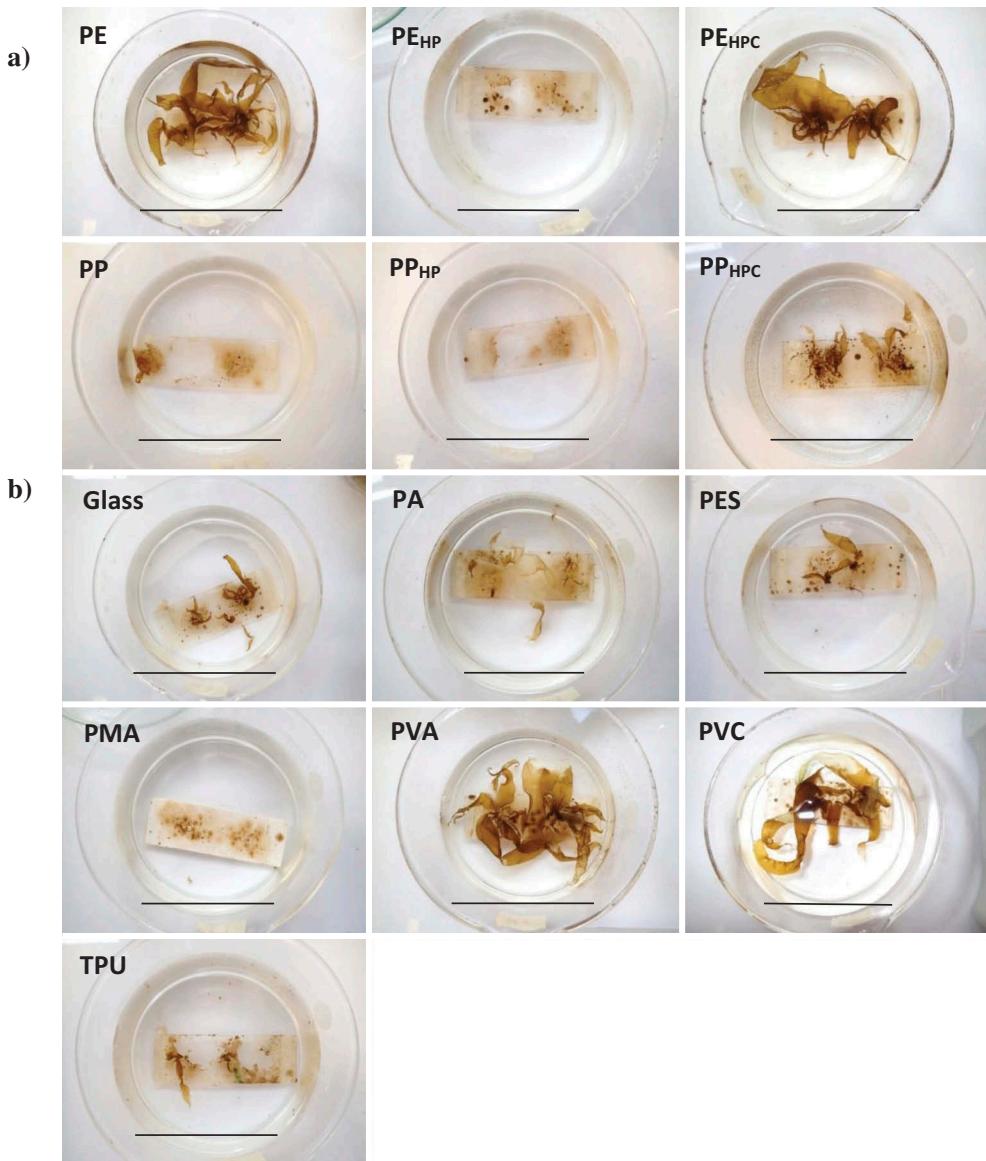


Fig. 1. Sporophyte retention and growth on *Saccharina latissima* after culture for 6 weeks on polymer slides of 13 chemistries. The median growth response is shown ($n = 5$). **(a)** The influence of polymer purity and corona treatment. HP: high purity, HPC: high purity corona treated. **(b)** Surface chemistry strongly influences sporophyte attachment and retention. PA: polyamide, PE: polyethylene, PES: polyester, PMA: polymethylacrylate, PP: polypropylene, PVA: polyvinylalcohol, PVC: polyvinylchloride, TPU: thermoplastic polyurethane. Scale bar = 75 mm.

PVC and PE) had significantly higher biomass than the glass control (0.06 ± 0.03 g slide $^{-1}$; AN: all $p < 0.05$, $F_{1,13}$). A low biomass was achieved on PP_{HPC} and PMA, which was not significantly different to the glass control ($p > 0.05$). Four films had negligible biomass (≤ 0.01 g slide $^{-1}$), less than the glass control (MW: $p < 0.05$, $n = 15$): PE_{HPC}, PP_{HPC}, TPU and PES.

Mean sporophyte length

The mean length of the sporophytes present on the films was also significantly different between the treatments, and followed a very similar pattern to the biomass (AN: $p < 0.0001$, $F_{13,61}=8.7$). PE_{HPC}, PVA, PA_{HP} and PVC had the largest mean frond lengths (42–55 mm; Table 2). These were all significant

different to the glass control ($p_{HF}: p < 0.05$). Moderately sized fronds (24–32 mm) were present on PE, PES, PP_{HPC} and glass. The smallest sized fronds, or absence of sporophytes, were seen on PE_{HP}, PP, PP_{HP} and TPU; significantly less than on the glass control ($p_{HF}: p < 0.05$).

Sporophyte attachment force

The attachment force of sporophytes was significantly different between film chemistries (AN: $p < 0.0001$, $F_{8,76} = 4.2$; Table 2). The firmest attachment was found on PVC (0.33 ± 0.17 N) and PE_{HPC} (0.28 ± 0.13 N), significantly more than the glass control ($p_{HF}: p < 0.05$; 0.12 ± 0.07 N). Moderate attachment of between 0.19–0.22 N was found on PA_{HP}, PE and PVA, which was not

significantly different from glass ($p > 0.05$). Low attachment force was measured on PP_{HPC} and PES, while no attachment force measurements were possible on five chemistries as no sporophytes > 10 mm were present (PE_{HP}, PMA, PP, PP_{HP} and TPU). On these five, the sporophytes had detached over the course of the experiment, leaving a characteristic ‘clean patch’ where the surface biofilm surrounding the sporophyte had also lifted away (Fig. 1). Since no external force was required for them to become detached, they were assigned an attachment force of 0 N.

The sporophytes used for the attachment force measurement were only those > 10 mm and so were a subset of all sporophytes present on the slides. The mean length of these sporophytes was similar (~50–60 mm, $p > 0.05$). The hapteron extent was highly variable between replicates and was not significantly different between materials ($p > 0.05$). No significant relationship was found between these physiognomy variables and the attachment force or θ_w of the film (Table 3; $p > 0.05$), indicating no morphological acclimation in response to the surface chemistry.

Correlation: sporophyte biomass and attachment force

When the final biomass at the end of the experiment was correlated against the attachment force for each film (Fig. 2), a significant relationship was evident (PC: $R^2 = 0.68$, $n = 14$). For each +0.1 N of attachment force an additional 0.114 g of *S. latissima* biomass had accumulated. A deviation from this relationship was seen in PVA, where the biomass was lower than that predicted by the attachment force. It is thought that the measurement procedure underestimated the true attachment force of PVA, as the flexible film distorted far more than the other films, applying additional torque to the sporophyte holdfast leading to detachment with lower force.

Table 2. Surface contact angle, biomass, attachment force and mean maximum length of *Saccharina latissima* sporophytes after 6 weeks of growth on 13 polymers.

Polymer	Contact angle (°)	Biomass (g slide ⁻¹)	Attachment force (N)	Length (mm)
PA _{HP}	59.1 ± 2.5	0.366 ± 0.055	0.215 ± 0.107	52.5 ± 3.5
PE	86.9 ± 0.8	0.189 ± 0.109	0.201 ± 0.125	32.7 ± 11.0
PE _{HP}	97.2 ± 4.6	0.000 ± 0.000	n.m.	2.9 ± 1.8
PE _{HPC}	63.9 ± 8.1	0.450 ± 0.042	0.278 ± 0.093	55.0 ± 6.5
PES	69.8 ± 3.1	0.010 ± 0.005	0.020 (n = 1)	30.9 ± 8.9
PMA	71.4 ± 2.4	0.027 ± 0.025	n.m.	12.2 ± 5.7
PP	84.8 ± 1.0	0.010 ± 0.006	n.m.	12.0 ± 3.1
PP _{HP}	101.5 ± 2.0	0.001 ± 0.001	n.m.	11.0 ± 3.2
PP _{HPC}	67.7 ± 7.6	0.101 ± 0.074	0.080 ± 0.057	23.7 ± 7.1
PVA	56.7 ± 1.7	0.477 ± 0.134	0.194 ± 0.074	47.4 ± 5.4
PVC	69.8 ± 2.0	0.225 ± 0.087	0.330 ± 0.164	42.4 ± 10.1
TPU	87.1 ± 1.6	0.003 ± 0.002	n.m.	16.3 ± 1.4
Glass	41.7 ± 4.2	0.061 ± 0.034	0.122 ± 0.072	22.6 ± 5.1

Shown is mean ± standard deviation. N.m.: not possible to measure as sporophytes too small.

Table 3. Physiognomy of *Saccharina latissima* sporophytes (> 10 mm) tested for attachment force after 6 weeks of growth on polymer films with different chemistries.

Surface	Sporophyte length (mm)	Hapteron extent (mm ²)	Sample number
PA _{HP} ^a	52 ± 29	4.4 ± 5.8	15
PE ^b	53 ± 25	6.3 ± 4.2	15
PE _{HPC} ^c	58 ± 21	4.6 ± 4.8	6
PES ^d	20	8.4	1
PP _{HPC} ^e	48 ± 21	5.2 ± 8.7	7
PVA ^f	53 ± 22	6.3 ± 6.6	15
PVC ^g	54 ± 27	3.2 ± 2.1	14
Glass	57 ± 34	5.7 ± 8.2	5

^apolyamide, ^bpolyethylene, ^cpolyethylene, high purity corona treated, ^dpolyester, ^epolypropylene, high purity corona treated, ^fpolyvinylalcohol, ^gpolyvinylchloride. No data were collected from polymethylacrylate, thermoplastic polyurethane or high purity polyethylene and polypropylene, as no suitable sporophytes were present. A maximum of three sporophytes were tested from each of five replicates. Shown is mean ± standard deviation.

Correlation: surface contact angle and sporophyte attachment force

The contact angle was useful to explain differences observed in the attachment force of the different surface chemistries (Fig. 3; PC: $R^2 = 0.29$). Broadly, attachment force was lowest on more hydrophobic materials at $> 85^\circ$, and maximum at $55\text{--}75^\circ$. However, this general trend had a number of outliers: PE, which had high attachment force (0.20 ± 0.13 N) despite θ_w of $87 \pm 1^\circ$, and PES and PMA, which had low attachment force despite θ_w of $70\text{--}71^\circ$.

Polymer additives influence contact angle and sporophyte attachment force

The high purity films PE_{HP} and PP_{HP}, had high θ_w ($97\text{--}102^\circ$) and no sporophytes were able to remain

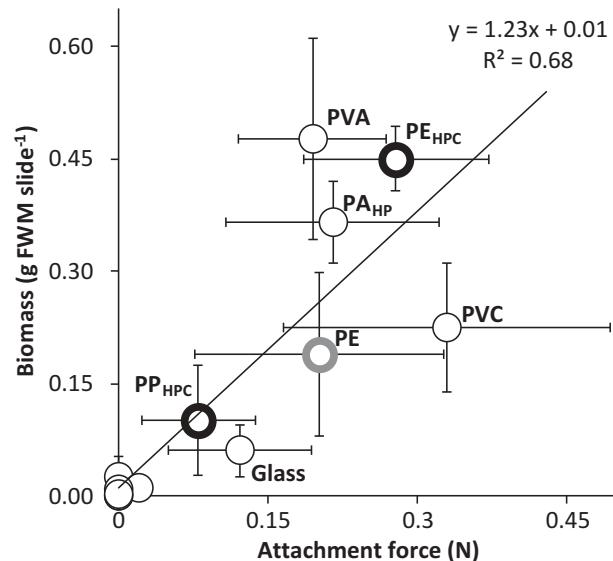


Fig. 2. *Saccharina latissima* sporophyte attachment force (N) and fresh wet mass (g) achieved on films with 13 different chemistries after 6 weeks of growth. Black ring indicates corona treatment. Light grey ring indicates an additive-containing film of PE and PP. PA: polyamide, PE: polyethylene, PP: polypropylene, PVA: polyvinylalcohol, PVC: polyvinylchloride, HP: high purity, HPC: high purity corona treated. Shown is mean ± standard deviation.

attached. Additive impurities within the PE and PP films caused a significant reduction in θ_w by 10–17° (2wAN: $p < 0.0001$, $F_{1,1,1,8} = 5.3$). In the case of PE, this also substantially increased the attachment force as just described (MW: $p < 0.0001$, $n = 21$). High purity polymer films did not necessarily lead to weak attachment: PA_{HP} has a θ_w of $59 \pm 3^\circ$ and allowed a strong attachment, despite being of high purity.

Polymer corona treatment influences contact angle and sporophyte attachment force

Corona treatment of high purity films (PE_{HPC} and PP_{HPC}) had a significant effect on θ_w , reducing it by a third or 33–38° (2wAN: $p < 0.0001$, $F_{1,1,1,8} = 5.3$). This led to a significant increase in the attachment force on both materials (2wAN: $p < 0.0001$, $F_{1,1,1,24} = 4.3$).

Discussion

The water contact angle is a large determinant of attachment force

Through experimental cultivation of the common kelp *S. latissima* on a range of smooth chemical films, we have shown that θ_w is a major determinant of bioadhesive attachment force. Specifically, hydrophobic materials with high θ_w had low or zero attachment force, in agreement with previous findings of lower adhesion on hydrophobic materials in *S.*

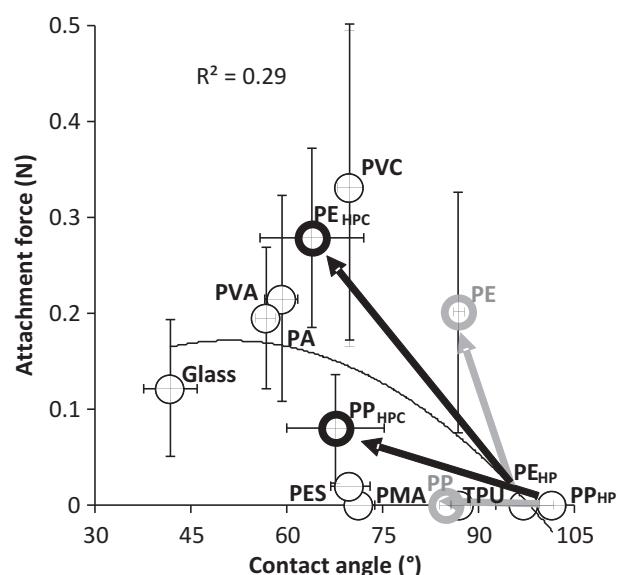


Fig. 3. The influence of contact angle (°) on the attachment force of *Saccharina latissima* sporophytes achieved after 6 weeks of growth on 13 films with different chemistries. Black arrow and ring indicates the change effected due to corona treatment. The light grey arrow and ring indicates the change effected due to additive inclusion. PA: polyamide, PE: polyethylene, PES: polyester, PP: polypropylene, PVA: polyvinylalcohol, PVC: polyvinylchloride, HP: high purity, HPC: high purity corona treated. Shown is mean ± standard deviation.

latissima – on raft panels at sea and polymer blocks – as well as findings in *Fucus* spp. and *Ulva* spp. (Hardy & Moss, 1979; Callow et al., 1988; Finlay et al., 2002; Ucar et al., 2010; Kerrison et al., 2018b). Besides aligning with these previous findings, we have further revealed that at low θ_w of $< 60^\circ$ (hydrophilic) the attachment force of *S. latissima* is also reduced, although not to the degree seen on hydrophobic surfaces. Based on the current evidence it appears that θ_w 60–75° provides the most suitable attachment surface for *S. latissima*. This range represents surfaces with a high surface free energy for chemical attachment, where there are many molecules, groups or atoms available to form bonds.

However, two of the tested films (PES and PMA) within this range gave very poor results, similar to the findings for POM-C/H and PMA in Kerrison et al. (2018b). Since these chemistries all have a high surface free energy for chemical interaction (Diversified Enterprises, 2016) and thus are generally considered to be suitable for bioadhesion (Lejars et al., 2012), surface free energy cannot be the only determinant for successful bioadhesive attachment. This supports the existence of polymer specific incompatibility (Kerrison et al., 2018b) between the surface chemistry and the cross-linked alginate-polyphenol bioadhesive system in the Phaeophyceae (Bitton et al., 2006; Salgado et al., 2009). A speculative model of this bioadhesive system, suggests that cross-linked polyphenols form micelles or globular aggregations at the surface interface (Potin & Leblanc, 2006). It is unknown why these aggregations would not adhere to the chemical structure of PES or PMA, despite a high surface free energy for chemical interaction. Future experimentation could recreate the model bioadhesive system on different polymers. A similar pattern of successful/unsuccessful attachment would be indirect evidence that the bioadhesive model is correct, while a different pattern would indicate an interface component is missing.

Controlled density allowed consistent growth

This study has also shown that the suitability of a chemical surface for the development of *S. latissima* biomass can be determined by seeding surfaces using juvenile sporophytes and then measuring the attachment force after 6 weeks. This allows faster experimentation than seeding using meiospores, where juvenile sporophytes only became visible after 5–6 weeks and attachment force was determined after 4 mo when fronds were ≤ 16 cm (Kerrison et al., 2017). A second advantage of seeding using juvenile sporophytes is that the initial density of *S. latissima* can be consistently controlled across different treatments by use of a hydrocolloid binder (Kerrison et al., 2018a). The settlement of macroalgae meiospore/zospores is

strongly influenced by the surface chemistry, leading to 15-fold differences in initial density (Kerrison *et al.*, 2017). This then influences the sporophyte development rate, with slower growth at higher density (Reed, 1990; Callow *et al.*, 2000; Kerrison *et al.*, 2018b). Consequently, by seeding a controlled density of sporophytes, we have removed the confounding influence of seeding densities from this study, allowing consistent sporophyte growth in all treatments. The similar frond lengths confirmed this across all polymers where successful bioadhesion was observed.

Fouling release coatings

On five of the chemistries examined (PE_{HP}, PMA, PP_{HP} and TPU) nearly all sporophytes had detached, taking with them the surrounding biofilm. The high θ_w in four of these chemistries indicates a low surface free energy and a relatively chemically inert surface. The holdfast bioadhesive appears to only bind weakly to these hydrophobic surfaces and so they show good fouling release performance (Lejars *et al.*, 2012), where the entire organism is lifted from the surface by water drag (Vogel, 1994) as has been shown previously in the same species (Kerrison *et al.*, 2018b). These chemistries should be avoided for kelp cultivation, although they may be useful for anti-fouling applications.

No morphological differences with chemistry

On the eight chemistries where sporophyte growth was successful, neither the sporophyte length nor the extent of the hapteron was found to be affected by the surface. It is known that bioadhesive pad formation following algal settlement, early rhizoid development in germlings and the morphology of the discoid and crustose forms can all be influenced by the surface free energy (Fletcher *et al.*, 1985; Fletcher & Callow, 1992). Nonetheless, the lack of any morphological differences between the different chemical surfaces (θ_w 42–87°) indicated that the development of the macro-scale morphology, including fronds and the holdfast, were not influenced by the surface chemistry interaction. This suggests that the juveniles have no method to sense the tenacity of their bioadhesion and so do not increase the size of their adhesion disk, produce more haptera, or limit their drag exposure (smaller fronds) when weakly attached. It is known that longitudinal tension on the frond, mimicking drag from water motion, causes kelp fronds to become thicker and more narrow, while stipes become thicker and more robust (Koehl *et al.*, 2008). Yet, no such acclimation appears possible regarding attachment tenacity. Consequently, those with weak bioadhesion will continue to grow, until they reach a few centimetres then become easily detached.

Kuralon/PVA validated as an excellent substrate

Kuralon is a synthetic twine manufactured and widely used for macroalgal cultivation in SE Asia (Werner & Dring, 2011), although the authors have found no scientific studies comparing its effectiveness to other polymers. It is composed of PVA (Kuraway, 2015, accessed 15.1.2015), which this study has shown provides an excellent substrate for the holdfast attachment of *S. latissima*. This validates its current use for members of the Phaeophyceae with the same bioadhesion system, including the widespread commercial species *Saccharina japonica* and *Undaria pinnatifida* (FAO, 2018). Both PVC and PA were also found to be highly suitable cultivation substrates, in agreement with the findings of Kerrison *et al.* (2017).

Additives can be used to reduce hydrophobicity and increase attachment force

The ubiquitous inclusion of a complex mixture of additives in plastics allows the creation of diverse variants for a range of commercial uses (Deanin, 1975). These can be used to enhance the physical properties of the native polymer, such as stability in damaging environmental conditions (e.g. UV radiation, high temperature, chemical exposure), create a new physical appearance (e.g. colour, flexibility, structural stability) or for safety features (e.g. flame retardant). Many long chain synthetic polymers lack polar groups, causing high hydrophobicity. Since most additives are expected to contain more polar groups than the native polymer, the incorporation of additives is likely to increase their hydrophilicity, regardless of the final end use intended.

The high purity PE and PP films examined had similar θ_w to those seen in previous studies (Diversified Enterprises, 2009a, 2009b). Due to their hydrophobicity, sporophytes had only very weak attachment to them. The commercial PE and PP films examined, had lower θ_w , which is surmised to be due to the ubiquitous inclusion of additives within commercially available plastics (Deanin, 1975). In the case of PE, this allowed a greatly improved sporophyte attachment force.

For certain industrial and biomedical applications, a hydrophilic surface is desired and targeted additive inclusion has been investigated as a method to achieve this (Jiang *et al.*, 2014; Kolahchi *et al.*, 2015; Turalija *et al.*, 2016). Such tests could also be used to optimize macroalgal cultivation substrates, maximizing sporophyte attachment force onto favourable materials (e.g. cheap, recyclable, low environmental impact) with other characteristics suited for deployment in the marine environment (e.g. UV resistance). This could create alternatives to the use of Kuralon, which despite being an excellent substrate for holdfast attachment, is

expensive and hard to source (authors' pers. obs.). In contrast with PE and PP, high purity PA performed well even without additives, due to a low initial θ_w .

Corona treatment increases attachment force

Corona treatment led to a more hydrophilic surface, as has been previously observed (Süzer *et al.*, 1999), and in doing so increased the suitability of the material as a macroalgal cultivation substrate. This was especially true for PE_{HP}, which changed from a hydrophobic material unsuited for bioadhesive attachment, to one of the best materials examined. Corona treatment may therefore be a cost-effective way to 'upgrade' low cost polymer materials to excellent cultivation substrates. Additionally, patterned corona treatment could potentially be used to create suitable attachment areas within a structure with fouling-release properties over the remaining surface in materials such as PE_{HP}. This could be used to regulate seeding density or reduce the need to clean fouling from connection points such as rope knots.

The selection of the substrate material for seaweed cultivation is currently based on availability, environmental resistance and cost. We have found that the suitability of polymers for the bioadhesive holdfast attachment of *S. latissima* varies widely, and that biomass accumulation on a smooth polymer is dependent on the holdfast attachment force. The water contact angle (θ_w) is a good indication of a polymer's suitability, with the best attachment usually seen at 60–75°.

The θ_w of many synthetic polymers is very high due to their non-polar repeated units. The inclusion of additives within the hydrophobic polymers, or the use of corona treatment, increases the surface's chemical complexity – the free energy for bonding – and so lowers θ_w , making a more suitable surface for holdfast bioadhesive attachment. In the case of PE, corona treatment transformed a fouling release material, highly unsuited for bioadhesion, into an excellent material for *S. latissima* attachment. Corona, or a similar treatment, may therefore be useful to create new advanced substrates for seaweed cultivation.

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Author contributions

P.D. Kerrison: Original concept, experimentation, data analysis, interpretation and commented on the manuscript; M. Stanley: Advised on experimental design and revised the manuscript draft. D. De Smet & G. Buyle: Co-developed the concept, produced polymer films, carried out corona treatment, commented on the manuscript. A.D. Hughes: Co-developed the concept, data interpretation and commented on the manuscript.

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References

- Anderson, T.W. & Darling, D.A. (1952). Asymptotic theory of certain "Goodness of Fit" criteria based on stochastic processes. *Annals of Mathematical Statistics*, **23**: 193–212.
- Bitton, R., Ben-Yehuda, M., Davidovich, M., Balazs, Y., Potin, P., Delage, L., Colin, C. & Bianco-Peled, H. (2006). Structure of algal-born phenolic polymeric adhesives. *Macromolecular Bioscience*, **6**: 737–746.
- Callow, M.E. & Fletcher, R.L. (1994). The influence of low surface energy materials on bioadhesion – a review. *International Biodeterioration and Biodegradation*, **34**: 333–348.
- Callow, M.E., Pitchers, R.A. & Santos, R. (1988). Non-biocidal anti-fouling coatings. In *Biodeterioration 7* (Houghton, D.R., Smith, R.N. & Eggins, H.O.W., editors), 43–48. Elsevier Science, London.
- Callow, M.E., Callow, J.A., Ista, L.K., Coleman, S.E., Nolasco, A.C. & López, G.P. (2000). Use of self-assembled monolayers of different wettabilities to study surface selection and primary adhesion processes of green algal (*Enteromorpha*) zoospores. *Applied and Environmental Microbiology*, **66**: 3249–3254.
- Candries, M., Altar, M. & Anderson, C.D. (2001). Foul release systems and drag. Consolidation of technical advances in the protective and marine coatings Industry. In *PCE 2001 Conference*, 273–286. Antwerp.
- Deanin, R.D. (1975). Additives in plastics. *Environmental Health Perspectives*, **11**: 35–39.
- Denny, M. (1988). *Biology and the Mechanics of the Wave-swept Environment*. Princeton University Press, Princeton, NJ.
- Diversified Enterprises (2009a). Surface energy data for PE: Polyethylene, CAS # 9002- 88-4. www.accudynetest.com/polymer_surface_data/polyethylene.pdf.
- Diversified Enterprises (2009b). Surface energy data for PP: Polypropylene, CAS #'s 9003- 08-0(atactic) and 25085-53-4 (isotactic). www.accudynetest.com/polymer_surface_data/polypropylene.pdf.
- Diversified Enterprises (2016). Surface free energy components by polar/dispersion and acid-base analyses; and Hansen solubility parameters for various polymers. [https://www.accudynetest.com/polytable_02.html](http://www.accudynetest.com/polytable_02.html).
- European Commission (2014). Innovative textiles to boost EU seaweed farming. European Commission, Brussels, BE. http://europa.eu/rapid/press-release_IP-14-962_en.htm.

- FAO (2018). Fisheries and Aquaculture Information and Statistics Services. <http://www.fao.org/figis/>.
- Finlay, J.A., Callow, M.E., Ista, L.K., Lopez, G.P. & Callow, J.A. (2002). The influence of surface wettability on the adhesion strength of settled spores of the green alga *Enteromorpha* and the diatom *Amphora*. *Integrative and Comparative Biology*, **42**: 1116–1122.
- Fletcher, R.L. & Callow, M.E. (1992). The settlement, attachment and establishment of marine algal spores. *British Phycological Journal*, **27**: 309–329.
- Fletcher, R.L., Baier, R.E. & Fornalik, M.S. (1985). The effects of surface energy on germling development of some marine macroalgae. *British Phycological Journal*, **20**: 184–185.
- Hardy, F.G. & Moss, B.L. (1979). The effects of the substratum on the morphology of the rhizoids of *Fucus* germlings. *Estuarine and Coastal Marine Science*, **9**: 577–584.
- Jiang, J.-H., Zhu, L.-P., Zhang, H.-T., Zhu, B.-K., & Xu, Y.-Y. (2014). Improved hydrodynamic permeability and antifouling properties of poly(vinylidene fluoride) membranes using polydopamine nanoparticles as additives. *Journal of Membrane Science*, **457**: 73–81.
- Jones, V., Strobel, M. & Prokosch, M.J. (2005). Development of poly(propylene) surface topography during corona treatment. *Plasma Processes and Polymers*, **2**: 547–553.
- Kang, E.T. & Neoh, K.G. (2009). Surface modification of polymers. In *Encyclopedia of Polymer Science and Technology*. 4th ed. John Wiley & Sons, New York.
- Kawai, H., Moromura, T. & Okuda, K. (2007). Isolation and purification techniques for macroalgae. In *Algal Culturing Techniques* (Andersen, R.A., editor), 133–144. Elsevier Academic Press, London.
- Kerrison, P.D., Stanley, M.S., Kelly, M., MacLeod, A., Black, K.D. & Hughes, A.D. (2016). Optimising the settlement and hatchery culture of *Saccharina latissima* (Phaeophyta) by manipulation of growth medium and substrate surface condition. *Journal of Applied Phycology*, **28**: 1181–1191.
- Kerrison, P.D., Stanley, M.S., Black, K.D. & Hughes, A.D. (2017). Assessing the suitability of twelve polymer substrates for the cultivation of macroalgae *Laminaria digitata* and *Saccharina latissima* (Laminariales). *Algal Research*, **22**: 127–134.
- Kerrison, P.D., Stanley, M.S. & Hughes, A.D. (2018a). Textile substrate seeding of *Saccharina latissima* sporophytes using a binder: an effective method for the aquaculture of kelp. *Algal Research*, **33**: 352–357.
- Kerrison, P.D., Stanley, M.S., Mitchell, E., Cunningham, L. & Hughes, A.D. (2018b). A life-stage conflict of interest in kelp: higher meiospore settlement where sporophyte attachment is weak. *Algal Research*, **35**: 309–318.
- Koehl, M.A. R., Silk, W.K., Liang, H. & Mahadevan, L. (2008). How kelp produce blade shapes suited to different flow regimes: a new wrinkle. *Integrative and Comparative Biology*, **48**: 834–851.
- Kolahchi, A.R., Ajji, A. & Carreau, P.J. (2015). Enhancing hydrophilicity of polyethylene terephthalate surface through melt blending. *Polymer Engineering and Science*, **55**: 349–358.
- Kuraway (2015). Kuralon. <http://www.sp-paper.jp/english/product/vinylon.html>. <http://www.sp-paper.jp/english/product/vinylon.html>.
- Lejars, M., Margaillan, A. & Bressy, C. (2012). Fouling release coatings: a nontoxic alternative to biocidal antifouling coatings. *Chemical Reviews*, **112**: 4347–4390.
- Levene, H. (1960). Robust tests for equality of variances. In *Contributions to Probability and Statistics* (Olkin, I., editor), 278–292. Stanford University Press, Palo Alto, USA.
- McInnes, G.A., Ragan, M.A., Smith, D.G. & Walter, J.A. (1985). The high molecular weight polyphloroglucinols of the marine brown alga *Fucus vesiculosus* L. ¹H and ¹³C nuclear magnetic resonance spectroscopy. *Canadian Journal of Chemistry*, **63**: 304–313.
- Murphy, J. (2001). *Additives for Plastics Handbook*. 2nd ed. Elsevier Science, Oxford.
- Potin, P. & Leblanc, C. (2006). Phenolic-based adhesives of marine brown algae. In *Biological Adhesives* (Smith, A.M. & Callow, J.A., editors), 105–124. Springer-Verlag, Berlin.
- Reed, D.C. (1990). The effects of variable settlement and early competition on patterns of kelp recruitment. *Ecology*, **71**: 776–787.
- Salgado, L.T., Cinelli, L.P., Viana, N.B., de Carvalho, R.T., de Souza Mourão, P.A., Teixeira, V.L., Farina, M. & Filho, G.M.A. (2009). A vanadium bromoperoxidase catalyzes the formation of high-molecular-weight complexes between brown algal phenolic substances and alginates. *Journal of Phycology*, **45**: 193–202.
- Stalder, A.F., Kulik, G., Sage, D., Barbieri, L. & Hoffmann, P. (2006). A snake-based approach to accurate determination of both contact points and contact angles. *Colloids and Surfaces A: Physicochemical and Engineering*, **286**: 92–103.
- Strobel, M., Jones, V., Lyons, C.S., Ulsh, M., Kushner, M.J., Dorai, R. & Branch, M.C. (2003). A comparison of corona-treated and flame-treated polypropylene films. *Plasmas and Polymers*, **8**: 61–95.
- Süzer, S., Argun, A., Vatansever, O. & Aral, O. (1999). XPS and water contact angle measurements on aged and corona-treated PP. *Journal of Applied Polymer Science*, **74**: 1846–1850.
- Tarakhovskaya, E.R. (2014). Mechanisms of bioadhesion of macrophytic algae. *Russian Journal of Plant Physiology*, **61**: 19–25.
- Turalija, M., Bischof, S., Budimir, A. & Gaan, S. (2016). Antimicrobial PLA films from environment friendly additives. *Composites Part B: Engineering*, **102**: 94–99.
- Ucar, I.O., Cansoy, C.E., Erbil, Y., Pettitt, M.E., Callow, M. E. & Callow, J.A. (2010). Effect of contact angle hysteresis on the removal of the sporelings of the green alga *Ulva* from the fouling-release coatings synthesized from polyolefin polymers. *Biointerphases*, **5**: 75–84.
- Vogel, S. (1994). *Life in Moving Fluids. The Physical Biology of Flow*. 2nd ed. Princeton University Press, Princeton, NJ.
- Vreeland, V., Gotkoppe, E., Espinosa, S., Quiroz, D., Laetsch, W.M. & West, J. (1993). The pattern of cell wall adhesive formation by *Fucus* zygotes. *Hydrobiologia*, **260/261**: 485–491.
- Vreeland, V., Waite, J.H. & Epstein, L. (1998). Polyphenols and oxidases in substratum adhesion by marine algae and mussels. *Journal of Phycology*, **34**: 1–8.
- Werner, A. & Dring, M.J. (2011). Cultivating *Palmaria palmata*. *Aquaculture Explained* No. 27. Bord Iascaigh Mhara (Irish Sea Fisheries Board).