

Analysis of *Giardia lamblia* Interactions with Polymer Surfaces Using a Microarray Approach

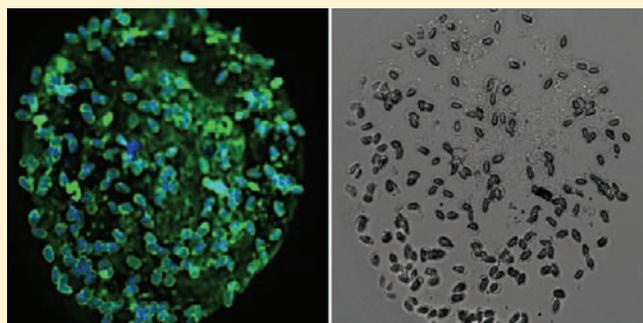
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S Supporting Information

ABSTRACT: The interaction of the waterborne protozoan parasite, *Giardia lamblia*, with polymeric materials was investigated by microarray screening of 652 polymers. Polymers were identified which either bound *G. lamblia* cysts or prevented their binding. Correlation of material properties such as wettability and surface roughness with cyst attachment revealed no influence of these factors upon *Giardia* adhesion. However, the study of polymer composition allowed the correlation of binding and generation of polymer structure function relationships; glycol and aromatic functionalities appeared to prevent adhesion, whereas secondary amine groups promoted adhesion, in agreement with previous literature. A significant reduction in attachment was observed following both cyst treatments with proteinase K and performing experiments at extremes of pH (2 and 12). It is suggested that proteinase K removes the proteins needed for specific surface interactions, whereas extremes of pH influence either protonation of the polymer or the surface charge of the cysts. The mechanism by which the protozoa attach to polymeric surfaces is proposed to be through ion–pair interactions. Improved understanding of *G. lamblia* surface interactions could assist in predicting transport and fate behavior in the environment and contribute to better design of water treatment processes, while the polymers identified in this work could find use in sensor applications and membrane filtration.



INTRODUCTION

The protozoan parasite *Giardia lamblia* (*G. lamblia*), which has a low infectious dose (1–10 cysts), contaminates water supplies across the globe and causes giardiasis.¹ Treatment of giardiasis varies depending on the patient, as does the effectiveness of different drugs, whose side effects are common.² Prevalence of *G. lamblia* is around 20–30% in the developing world,³ with up to 100% of children acquiring the infection before the age of 3.² In the developed world, where water treatment is better and more widespread prevalence is lower but outbreaks do occur. In 1985 there were particularly serious cases in both the United Kingdom⁴ and the United States.⁵ In the United States *G. lamblia* was one of the most common intestinal protozoan infections in 2001.⁶ In 2004, over 1000 cases were reported in Norway, resulting from leaking sewage and ineffective water treatment.⁷ This pathogen causes a major problem in the water industry as it is resistant to disinfection by chlorine treatment⁸ and can also pass with up to 30% efficiency through advanced membrane filters.⁹

The majority of studies investigating *G. lamblia* interactions with surfaces have focused on the postingestion trophozoite stage and its attachment through an adhesive disk.¹⁰ There has been limited investigation of the cyst stage, where the adhesive disk is internalized and fragmented.¹⁰ Cyst interaction with polymeric materials, as previously investigated in a paper by Dai

et al.,¹¹ is very important in the control of waterborne giardiasis. Since polymers are utilized in production of membrane filters for water treatment and monitoring, pathogen-specific coatings could help to improve the performance of these methods.⁹ Furthermore, the relative simplicity with which polymeric material properties can be modified provides an easy method to gain insight into structure–activity relationships with respect to parasite/material interactions. Improved knowledge of *G. lamblia* interactions could assist in the design of improved water treatment processes.

The screening of a large number of polymers can be achieved rapidly through the use of polymer microarrays.^{12–14} Such arrays have been applied to determine which materials can enrich, manipulate, or modulate a variety of cell types, including human embryonic cells,^{15,16} human skeletal progenitor cells,^{17,18} human renal tubular epithelial cells,¹² mouse bone marrow dendritic cells,¹⁹ suspension cells,²⁰ bacteria,²¹ and most recently the waterborne, protozoan pathogen, *Cryptosporidium*.²² In this paper the focus was on the study of another problematic waterborne protozoan pathogen, *G. lamblia*. This

Received: October 20, 2011

Revised: January 13, 2012

Accepted: January 13, 2012

Published: February 2, 2012

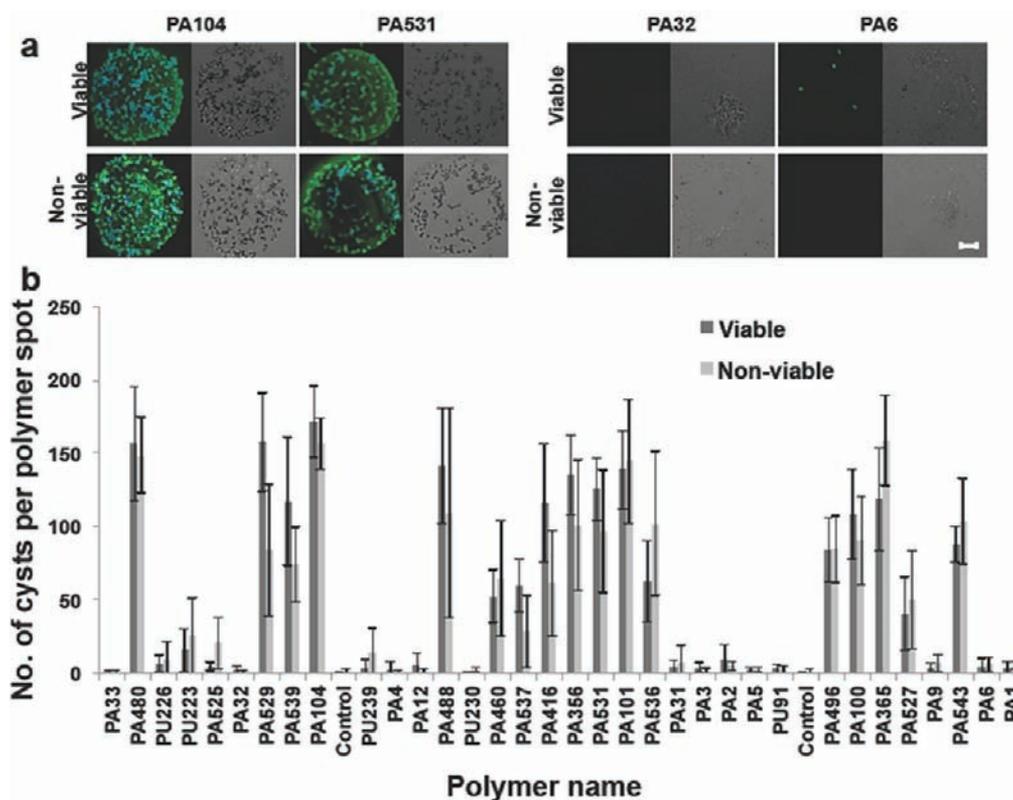


Figure 1. Microarray screening of selected polymers for *G. lamblia* cyst binding. (a) Images of the cysts stained with Giardia-a-glo (green) and DAPI (blue) bound to polymer spots. Fluorescent (left) and phase contrast (right) images of selected polymers are shown; two strong binding polymers (PA104 and PA531) and two poor binding polymers (PA6 and PA32). Scale bar = 100 μm . (b) Chart comparing the results of hit arrays with viable (dark gray) and nonviable cysts (light gray), showing strong correlation between them.

approach allowed for investigation of the mechanisms by which the protozoa attaches to polymer. Six hundred fifty two polymers were screened to determine which materials have positive or negative effects on *G. lamblia* adhesion. The effect of viability on surface interactions as well as the impact of certain polymeric properties such as hydrophobicity, surface roughness, presence of specific monomers, and relative number of ester moieties was investigated. In addition, the influence of both pH and proteinase K on *G. lamblia* interactions with surfaces was studied. Such improved understanding is likely to contribute to better design of water treatment processes for this pathogen, and the polymers identified in this paper may find applications in coatings for membrane filters or even in the development of sensing technology.

MATERIALS AND METHODS

Chemicals and Materials. All chemicals were of analytical grade and used as received without further purification. Sodium cacodylate trihydrate and all monomers used were from Sigma-Aldrich. Two and a half percent (w/v) glutaraldehyde and 1% (w/v) osmium tetroxide were from Electron Microscopy Sciences. Rectangular 4-well plates and 24-well plates were from Nunc. Silane-prep glass slides were from Sigma-Aldrich, and glass coverslips were from VWR. GeneFrames were from Thermo Scientific.

Polymer Microarray Fabrication. Polymer microarrays were fabricated as previously reported (see Supporting Information for further details).

Scanning for Cyst Interactions. *G. lamblia* cysts were obtained from Waterborne Inc., USA (catalog number P101).

The cysts were the human isolate H-3, passed through gerbils. *G. lamblia* cysts were diluted in sterilized water to a concentration of 1.66×10^5 cysts/mL. When required, heat treatment of the samples for 5 min at 70 °C was performed²³ using a Trechne Dri-Heat heating block to obtain nonviable cysts, confirmed by staining with propidium iodide (Figure S1, Supporting Information). Polymer microarrays were sterilized by exposure under UV light for 15 min, and freshly prepared 6 mL aliquots (1 million cysts per experiment) were added to the polymer microarray. The slides were incubated with cysts on a plate shaker at 20 rpm for 3 h at 25 °C. Subsequently, the slides were rinsed with sterilized water and fluorescently stained using an adapted version of the standard EPA1623 protocol.²⁴

Scale Up. Polymers were spin coated onto glass coverslips (13 mm in diameter), incubated with *G. lamblia* (0.833×10^5 cysts/mL in sterilized water) in 24-well plates, and imaged via fluorescence microscopy and scanning electron microscopy (SEM).

Fluorescent Staining of Cysts. The standard *G. lamblia* staining protocol (EPA1623) was adapted for the larger array area. Slides were rinsed and air dried; 1 mL of MeOH was added and allowed to air dry; 4 mL of a solution of DAPI (1 $\mu\text{g}/\text{mL}$) was applied for 1 min followed by a sterilized water rinse; finally, 2 mL of Giardia-a-glo, a fluorescein-labeled mouse monoclonal antibody made to a cyst wall antigenic site (at EPA1623 concentration) (Waterborne Inc., USA), was added (25 min) before rinsing in sterilized water and being left to air dry. A GeneFrame and a coverslip (1.9 \times 6.0 cm, AB-0630) were then applied to each slide, and the external surface of the slide construct was cleaned with 70% ethanol. Image capture

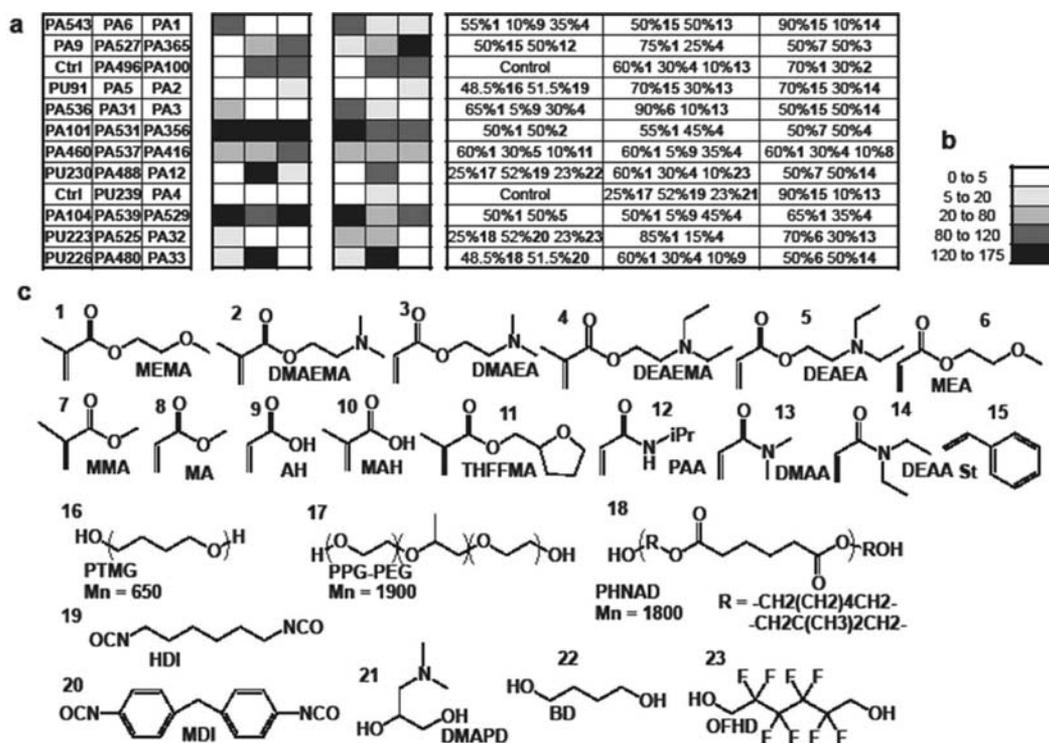


Figure 2. Analysis of microarray results and polymer structures. (a) (Left to right) Polymer identity, binding of viable and nonviable cysts, and polymer composition. (b) Bars relating color intensity to cyst binding. (c) Chemical structures of the monomers.

from the polymer microarray was performed via a Nikon 50i fluorescence microscope (20× objective) with an automated X-Y-Z stage, using the IMSTAR Pathfinder software package (IMSTAR S.A., Paris, France).

Scanning Electron Microscopy. Coated substrates, were washed (×2) with 0.1 M cacodylate buffer (pH 7.4) and then fixed with 2.5% (w/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h. Samples were postfixed with 1% (w/v) osmium tetroxide for 1 h at room temperature, dehydrated stepwise with ethanol (50%, 70%, 90%, and 100% (v/v)), critical point dried in CO₂, and gold coated by sputtering. Samples were examined with a Philips XL30CP Scanning Electron Microscope.

Wettability/Surface Roughness. Wettability was measured using the high-throughput method developed by Bradley.^{25,26} Surface roughness was quantified using atomic force microscopy (AFM). Images were taken over a 100 μm² area of the polymer surfaces using a DimensionV Nanoscope (VEECO). The scan rate ranged from 1.32 to 1.60 Hz, and the height values of the surface were obtained with a resolution of 512 × 512 pixels in the scanned region. The root mean square of the features in the hit array were calculated by averaging over three 9 μm² areas through the NanoScope analysis software (VEECO version 1.20).

For the *G. lamblia* surface roughness measurement, the sample was prepared by overnight incubation of cysts on a PA104-coated coverslip (13 mm in diameter), followed by fixing in 1% glutaraldehyde and air drying. AFM was performed with a contact cantilever in air at a scan rate of 0.788 Hz, and the roughness was determined over a scan area of 36 nm².

Proteinase K Treatment. One million cysts of *G. lamblia* were incubated at 37 °C on a plate shaker for 3 h with a solution containing 9 mg/mL proteinase k, 1 M Tris buffer, and 10 mM CaCl₂. Subsequently, the solution was centrifuged for 5

min at 12 000g. The supernatant was removed, the pellet was resuspended in 1 mL of distilled water, and this solution was centrifuged for a further 5 min. The supernatant was again removed, the pellet was resuspended in 5 mL of sterilized water, and the solution was examined using the protocol described for the 'hit' arrays.

Influence of pH. One molar hydrochloric acid and 1 M sodium hydroxide were used to prepare pH 2 and 12 aqueous solutions, respectively, and 5.2 mL of the acidified/basified solution was added to 800 μL of *G. lamblia* to give a cyst concentration of 1.66 × 10⁵ cysts/mL; these were then examined using the protocol described for the 'hit' arrays.

RESULTS

The 652 polymers examined in the initial microarray showed significant differences in their parasite binding ability (Figure S2, Supporting Information). From these results 34 polymers were selected to undergo more detailed investigation to understand how their properties influence cyst adherence. Six copies of each selected polymer were printed on a microscope slide to form 'hit arrays'. The polymers chosen were a mix of those which promoted strong adhesion (PA104, PA531, and PA480), those which prevented binding (PAs 1–6, PA32, PA33, and a group of polyurethanes), and those which showed some selection between viable and nonviable cysts (PA356, PA496, and PA527).

The results from these 'hit array' experiments (Figures 1, 2 and S3, Supporting Information) agreed with those of the initial array. Fluorescent images of the complete 'hit' arrays, with one image per polymer, can be seen in Figure S3, Supporting Information. Figure S4, Supporting Information, demonstrates the good correlation between the binding of both viable and nonviable parasites. These results confirmed PA104, PA531, and PA480 as the best binding polymers while highlighting PA6

and PA32 as polymers which prevented binding. These polymers were selected for further investigation on larger surfaces to demonstrate appropriateness for practical applications. The chosen polymers were resynthesized and spin coated onto glass surfaces, followed by exposure to cysts. Large-scale polymer performance was as expected (Figures S5–S7, Supporting Information) with PA6 and PA32 preventing cyst adhesion and PA531, PA480, and PA104 promoting strong binding, showing the robustness of the screen.

SEM imaging (Figures S5 and S6, Supporting Information) demonstrated the features expected of *G. lamblia* cysts, with their shape and sizes being consistent with results from previous studies.²⁷ They also highlighted the differences between viable and nonviable cysts, with the walls being generally rougher and thicker in the latter.²⁸

Next, the influence of wettability and surface roughness on adhesion was considered. (Figure 3, Table S1, Supporting Information). Initially it appeared as though overall high hydrophobicity or hydrophilicity (outside 50–70°) seemed to prevent cyst adhesion, as did high rms values (greater than 10 nm). This is illustrated by polymers, such as PA5 (83°; 13.67 nm) and PA32 (17°; 10 nm). Additionally, the strong binding polymers, PA480 and PA531, had wettability values within the range 60–65° and rms values of 2 nm or below. However, fitting of the results demonstrated no correlation between wettability or surface roughness on cyst adhesion.

To further understand the cysts surface interactions, viable cysts were treated with proteinase K to remove proteins from the outer layers of the cyst wall before analysis on a ‘hit’ array. The results showed that binding was severely limited for all polymers, with the number of cysts bound reduced by 70% compared to the untreated cysts (Figure 4). Changes in morphology were also observed, with cysts appearing rounded with slightly thicker outer walls.

Examining the ‘hit’ arrays at acid (pH 2) and base (pH 12) systems as opposed to the neutral system (pH 7), used in the standard arrays, made little difference to the repellent polymers while significantly reducing the adhesive capacity of the strong binding polymers. Polymers demonstrating poor binding (less than 10 cysts per spot) in the previous ‘hit’ arrays showed little change. For those polymers previously shown to support adhesion the numbers of bound cysts was significantly reduced, with the average reduction, for the binding polymers, being 94% at pH 2 and 80% at pH 12 (Figure 5).

DISCUSSION

In this work, comparison of the adhesion results with polymer properties, such as wettability, surface roughness, and polymer composition, was undertaken to elucidate which factors control cyst–polymer surface interactions. Previous literature on cellular/microbial attachment to polymer surfaces has identified structure–property relationships, noting that hydrophilic, electroneutral surfaces which contain hydrogen-bond (H-bond) acceptors but not H-bond donors resist adhesion. These factors prevent protein adhesion, which mediates cellular attachment, by binding a layer of water to the surface which acts as a steric or energetic barrier to adhesion.²⁹ It is not clear whether these factors will influence protozoan attachment, which will not proceed via protein adhesion since protozoa do not secrete proteins nor will proteins be present in the water samples used in the experiments reported here.

For both wettability and surface roughness no correlation was observed, though extremes of hydrophobicity/hydro-

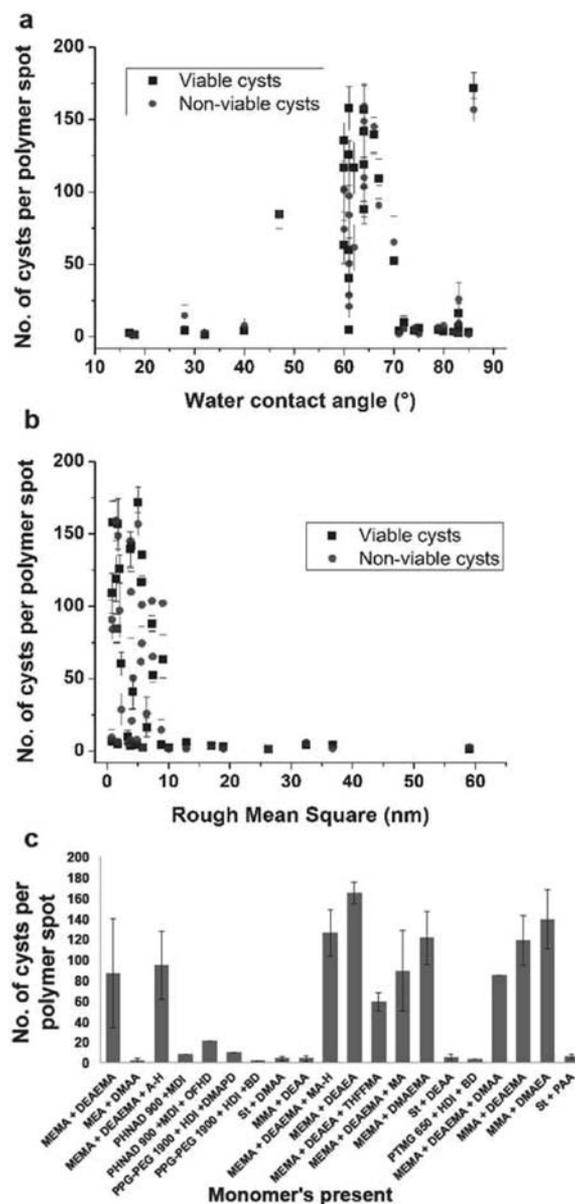


Figure 3. Correlation between *G. lamblia* cyst binding and polymer surface properties and composition. (a) Viable (square) and nonviable (circle) cyst binding versus contact angle. (b) Viable (square) and nonviable (circle) cyst binding versus surface roughness (root mean square). (c) Cyst binding in relation to monomeric composition of the polymers.

philicity and surface roughness were generally associated with poor adhesion. In terms of polymer composition, the presence of aromatic monomers (such as styrene), amide groups (in, e.g., dimethylacrylamide (DMAA) and diethylacrylamide (DEAA)) and glycol moieties (most PU monomers) appeared to prevent adhesion. In contrast, secondary amine functionalities were linked with good adhesion. Methyl ethylene methacrylate (MEMA) and methyl methacrylate (MMA) copolymerized with amine monomers, such as 2-(dimethylamino) ethyl methacrylate (DEAEMA) and 2-(dimethylamino) ethyl acrylate (DEAEA), were also identified as monomers which enhance adhesion. Certain monomers appear to be capable of participating in specific chemical reactions with the outer wall of the cysts, suggested to be ion–pair interactions between

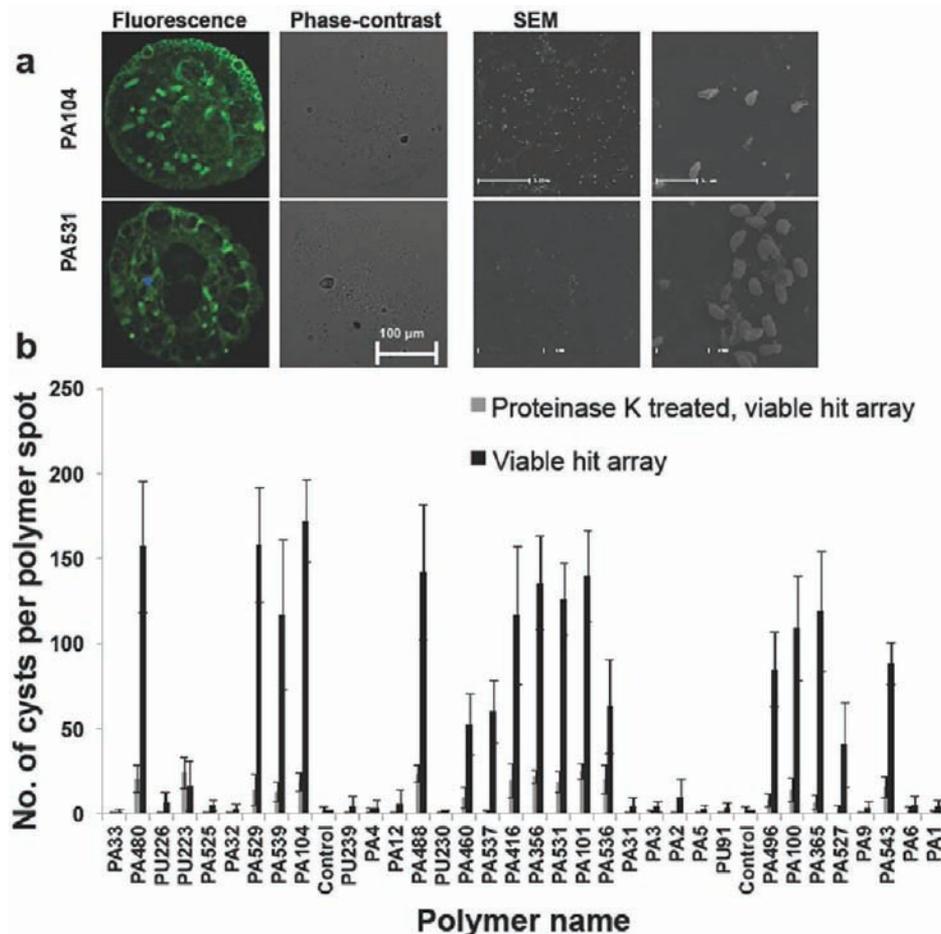


Figure 4. Proteinase K treated hit array. (a) Images of the cysts stained with Giardia-a-glo (green) and DAPI (blue) bound to polymer spots. Fluorescent (left) and phase contrast (right) images of selected polymers are shown; two strong binding polymers (PA104 and PA531) and two poor binding polymers (PA6 and PA32). (b) Chart comparing binding of viable cysts in the hit arrays before (dark gray) and after (light gray) proteinase K treatment.

protonated amines and negatively charged groups on the cyst wall. These interactions do not occur following particular cyst treatments.

G. lamblia Characteristics. The culmination of the lifecycle of *G. lamblia* in its host is the release of thick-walled cysts, which are resistant to a wide range of environmental stresses. The wall consists of a fibrillar extracellular matrix, lined by a double inner membrane and an outer filamentous wall.³⁰ This outer wall is around 300 nm thick and is the most important aspect when considering the adhesive abilities of the cysts.

The outer wall is composed of around 43% carbohydrates,³¹ 86% of which is a novel β -(1-3)-*N*-acetyl-D-galactosamine homopolymer.³² The novel galactosamine forms curled fibrils, which bind to cyst wall proteins via internal lectin domains. Binding to these proteins compresses the homopolymers into the narrow, mesh-like structure in fully formed cyst walls.³³

Most previous studies of *G. lamblia* surface interactions have focused on the postingestion trophozoite stage with the aim of understanding host susceptibility and the process of infection. Relatively little work³⁴ has considered the cyst stage, which is evidently more important in environmental analysis, for example, to understand the transport and fate of cysts in the environment, to predict and explain the performance of water

treatment technologies, and to design novel materials for membranes and filters.

Cyst Viability. Specific polymers, such as PA531 and PA104, showed high binding, regardless of the viability of the cysts. There were also a considerable number of polymers, such as PA1–6, which effectively prevented binding of both viable and nonviable cysts. Overall, the viability of the cysts had a low impact upon whether binding to the polymer surfaces was observed, as supported by an R^2 value of 0.607 (initial arrays) and an R^2 value of 0.857 ('hit' arrays) (Figure S4, Supporting Information) between viable and nonviable cyst adhesion, showing that viability is not a significant factor in the adhesion characteristics of *G. lamblia* cysts.

The absence of any viability influence on *G. lamblia* adhesion contrasts with results from a previous paper which investigated the binding of another waterborne protozoan pathogen, *Cryptosporidium parvum* (*C. parvum*).²² In this case, viable oocysts were shown to bind significantly more than nonviable oocysts. This finding was attributed to denaturation (in the heat treatment to render oocysts nonviable) of the surface glycoproteins which normally extend into solution^{11,35} and mediate specific chemical interactions with polymer materials. There are differences in both the chemical composition and the structure of *C. parvum* and *G. lamblia* and in the latter the cyst walls form a filamentous mesh-like structure.³³ Previous

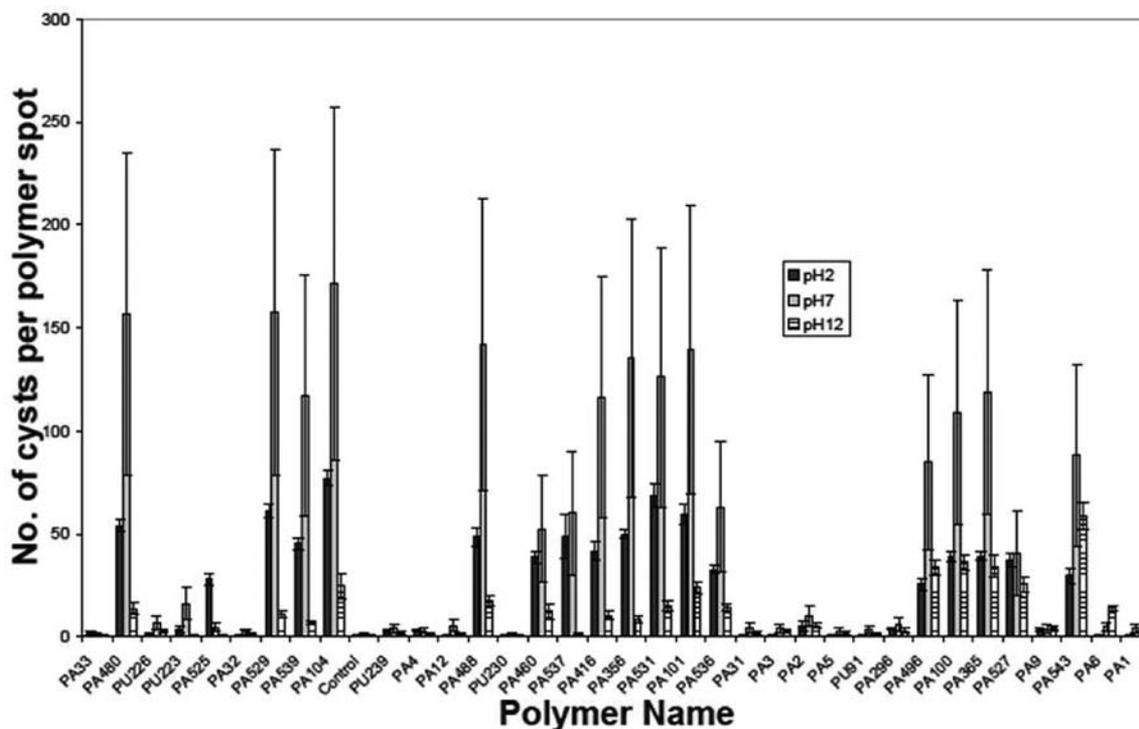


Figure 5. Effects of pH on *G. lamblia* cyst binding. Chart of viable cyst binding at pH 2 (black), 7 (light gray), and 12 (cross-hatched).

differences in adhesion characteristics for these two pathogens have been observed,¹¹ though the influence of viability was not investigated.

Hydrophobicity/Wettability. Wettability is a crucial property for analysis of polymers, with dynamic water contact angles (WCA) (this being a measurement after contact on a surface for few minutes rather than immediate, static measurements, which fail to simulate biological relevance) being the most common values used to quantify hydrophobicity. Previous work has demonstrated that hydrophobic forces dominate surface charge when considering *G. lamblia* interactions,¹¹ and although correlation between hydrophobicity and polymer binding was expected, none was observed. However, Figure 3 did show that the majority of the polymers with significant binding had a WCA between 60° and 65°. The WCAs of two of the best binding polymers, PA531 and PA480, were 61° and 64°, respectively. Values of wettability, outside of this range, generally indicated poor adhesion; for example, PA32, PA33, and PU230, which all prevented cyst adhesion, had low WCAs (17°, 18°, and 32°, respectively), PA6, PA4, and PU91, which also prevented adhesion, had high WCAs (79°, 85°, and 82°, respectively) (Table S1, Supporting Information). This is in direct contrast with the previous work by Dai et al., who observed that *G. lamblia* cysts demonstrated the strongest adhesion to hydrophobic (WCA 95°) fluorosiloxane-coated glass beads and considerable interaction with cationic polymer-coated beads (WCA 45°), whereas aminosiloxane-coated beads (WCA 70°) prevented adhesion.¹¹ Thus, from the experimental results it is concluded that the wettability/hydrophobicity of a polymer material is not a property which successfully predicts the degree of *G. lamblia* cyst interaction; other factors must play an important role.

Surface Roughness. Atomic force microscopy (AFM) was employed to investigate the influence of the surface roughness on *G. lamblia* adherence (Figure 3b). For each polymer surface,

analysis was taken over a 100 μm^2 area and the root mean square (rms) surface roughness was determined from an average of three random positions. The rms ranged from 0.01 to 59.0 nm and did not correlate with the WCA (Figure S8 and Table S1, Supporting Information).

Similar to the wettability analysis, surface roughness also demonstrated no clear correlation with cyst adhesion (Figure 3). PA6, PA32, and PU230, which were selected for preventing adhesion, had rms values of 1.71, 10.01, and 59 nm, respectively, while PA104, PA480, and PA531, the strongest binding polymers, had rms values of 5.02, 1.75, and 2.02 nm, respectively. However, it is clear from Figure 3b that high values of rms, specifically those greater than 10 nm, appear to prevent adhesion. None of the polymers with rms values of over 10 nm had more than 10 cysts bound, including polymers such as PA1 and PA3–5 and a number of polyurethanes. This suggests that while surface roughness is not well correlated with cyst adhesion, high rms values are likely to limit binding ability.

For bacterial attachment it is known that irregularities that conform to the size of the bacteria increases adhesion due to maximizing bacteria–surface contact area.^{36,37} If this hypothesis was correct for *G. lamblia*, it would imply that the surface roughness of cysts is likely to be on the order of 1–10 nm (the rms value above which no adhesion was observed for *G. lamblia*). However, AFM measurement of *G. lamblia* cysts was carried out by binding *G. lamblia* cysts on PA104-coated surface and gave a surface roughness of 53 nm, higher than all of the hit polymers except PU230 (Figure S9 and Table S1, Supporting Information).

Polymer Composition. In a previous paper investigating *C. parvum* adhesion to polymer microarrays we proposed that the interactions between oocysts and polymers were dominated by specific protein–polymer interactions, in particular ion–pair formation between carboxylate/phosphate groups on the oocyst wall and protonated amine groups in the polymers.²²

Several of the polymers in the *G. lamblia* hit array were identical or very similar to those selected for the *C. parvum* hit array both for polymers which promoted and those which prevented adhesion. This suggests that perhaps similar mechanisms control the adhesion of these two protozoan pathogens and some similarity between the composition of the oocyst and cyst outer walls. To investigate the relationship between chemical composition of the polymers and cyst adhesion, the monomeric composition was mapped against the results of the 'hit' array (Figure 2).

Comparison of Figures 1 and 2 indicated that inhibition of cyst binding was strongest in polyacrylates containing DMAA, DEAA, or styrene as well as selected polyurethanes. Monomers promoting strong binding were more variable; however, the presence of DMAEMA, DEAEEMA, DMAEA, or DEAEA was very common among the best performing polymers, such as PA104, PA480, and PA531.

Next, the nature of different functional groups present in the polymers was considered. For cellular adhesion it has been reported that glycol functionalities act in a preventative manner.^{38,39} This is normally attributed to the protein-repellent nature of these moieties; for the majority of cell types adhesion is considered to occur via initial protein adsorption, which subsequently mediates cellular adhesion. For the protozoan experiments reported here prior protein interaction with the surface is not thought to be a possible mechanism of adhesion given that the experiments are performed in water and the cysts do not secrete proteins. However, the repellent nature of glycol functionalities is still consistent with our results, since none of the polyurethanes containing monomers with glycols exhibited strong interactions with cysts. In this case, the known poor likelihood of protein interaction with glycol moieties could apply to the cyst surface proteins, thus limiting any interactions between these polymers and the cyst outer wall.

A recent paper by Yang et al. reported that aromatic functionalities were correlated with low cell adhesion, whereas amine and ester moieties were found to promote cellular adhesion.³⁸ The monomer most associated with low *G. lamblia* adhesion in the hit arrays was styrene, in agreement with the above finding that aromatic functionalities prevent adhesion. To investigate whether ester moieties correlate with adhesion for protozoan cysts, the concentration of ester moieties was plotted against the number of cysts per polymer spot (Figure S10, Supporting Information). Poor correlation was observed (R^2 values of 0.43 or less depending upon the type of fit), and the average ester concentrations are well above the values exhibited by the polymers studied by Yang et al.³⁸ In terms of amine functionalities the monomers DMAEA, DEAEA, DMAEMA, and DEAEEMA, present in the 'hit' array in polymers also containing MEMA and MMA, all contain secondary amine groups and are associated with high levels of cyst adhesion. Previous work has also shown that these monomers promote leuco binding.²⁵ For cyst adhesion, the hypothesis is that at physiological pH values the amines will be protonated and thus ion pair with the cyst wall. DMAA and DEAA contain amide groups and are present in polymers which prevent adhesion. Since amide groups will not be protonated at physiologically relevant pH this explains the lack of interaction with *G. lamblia*.

Thus, it is concluded that glycol and aromatic and amide functional groups act to prevent adhesion whereas amine groups promote adhesion. This is in agreement with previous cellular/microbial attachment literature. However, the mecha-

nism is not via prior protein attachment, as discussed above, but direct interaction of cyst wall proteins with the surface.

Proteinase K Treatment. Proteinase K has previously been employed to study the nature of surface macromolecules of *C. parvum*⁴⁰ and *Giardia*³² as well as alter the adhesion characteristics of *C. parvum*. Adhesion of *C. parvum* to quartz surfaces was improved after proteinase K treatment.³⁵ However, the 'hit' array exposed to *G. lamblia*, which had been previously treated with proteinase K, showed severely reduced binding (Figures 4 and S11, Supporting Information), although polymers which normally promoted strong adhesion, such as PA104 and PA480, still bound the highest number of cysts. Proteinase K removes any proteins stretching out from the cyst and also contributes to degradation of those involved in the mesh-like outer wall. SEM images (Figure 4) illustrate changes in cyst morphology, e.g., wall thickening, as expected; Chatterjee et al.³³ previously reported that removal of the cyst wall proteins decompresses the galactosamine fibrils, thus thickening the cyst wall. The reduction in adhesive ability suggests that the cyst wall proteins that bind the galactosamine fibrils play a crucial role in surface interactions. This supports the theory that protein-specific interactions with polymers control adhesion of cysts to these surfaces.

pH. Analysis of cyst adhesion at different pH values (pH 2 and 12) showed an overall reduction in binding (Figures 5 and S12, Supporting Information). Those polymers with weak adhesion at pH 7 did not exhibit significantly different results at extremes of pH, whereas those which had strong adhesion at pH 7 showed severe reductions in binding at both pH 2 and 12. In the previous discussion, analysis of polymer composition and proteinase K treatment on cyst adhesion both suggested that ion-pair interactions play a key role in controlling the binding of *G. lamblia* to polymer surfaces. At pH 2, below the isoelectric point for *G. lamblia*,⁴¹ the cyst wall will be mainly positively charged and therefore will not react with the protonated amines. At pH 12, while the cyst wall will be negative, the amines will be unprotonated and again no interactions will occur. Thus, performing experiments at different pH values significantly worsened the adhesive capacity of *G. lamblia* cysts to the polymers.

■ ASSOCIATED CONTENT

📄 Supporting Information

Extensive figures showing initial array results, images of the whole 'hit' array, and extra analysis graphs; table of all 'hit' array results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interests.

■ ACKNOWLEDGMENTS

We thank the University of Edinburgh, the China Scholarship Council (M.W.), and the Royal Academy of Engineering/EPSRC (H.B.) for funding. We thank Oleg Nerushev for AFM

technical support. H.B. and M.B. came up with the concept/idea. All authors cowrote this paper.

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