Enhanced Attachment of *Acanthamoeba* to Extended-wear Silicone Hydrogel Contact Lenses

A New Risk Factor for Infection?

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Purpose: To establish if silicone hydrogel (S-H) contact lenses could be a risk factor for *Acanthamoeba* infection by facilitating the attachment of trophozoites to their surface and transfer to the cornea and to determine the effect *Acanthamoeba* culture technique, patient wear, and *Pseudomonas* biofilm coating have on attachment to the S-H lens.

Design: Experimental material study.

Participants and Controls: Attachment to a S-H lens was compared with that of a conventional hydrogel control lens. Sixteen replicates were carried out for both lens types under each test condition.

Methods: Unworn S-H (PureVision; Bausch & Lomb, Kingston-Upon-Thames UK) and conventional hydrogel (Acuvue; Vistakon, Johnson & Johnson, Jacksonville, FL USA) lens quarters were incubated for 90 minutes in suspensions of liquid or plate-cultured *Acanthamoeba castellanii* trophozoites. Unworn, worn, and *Pseudomonas* biofilm coated S-H and hydrogel quarters were incubated for 90 minutes with plate-cultured trophozoites.

Main Outcome Measures: Trophozoites attached to one surface of each lens quarter were counted by direct light microscopy. Logarithmic transformation of data allowed the use of a parametric analysis of variance.

Results: Lens polymer had a significant effect on attachment ($P < 0.001$), with higher numbers of trophozoites attaching to the S-H lens. Culture technique also had a significant effect on attachment ($P = 0.013$), with higher numbers of liquid-cultured organisms attaching to both lens types. A significant increase in attachment was demonstrated with worn and *Pseudomonas* biofilm-coated hydrogel lenses ($P < 0.001$); however, this difference was not seen with the S-H lens.

Conclusions: Acanthamoebal attachment to the S-H lenses was significantly greater than to the conventional hydrogel. Liquid-cultured trophozoites demonstrated a higher affinity for the lenses tested. Wear and bacterial biofilm coating had no effect on attachment to S-H lenses. The increased attachment found with the S-H lens may be an inherent characteristic of the polymer or a side effect of the surface treatment procedure to which the lenses are exposed. It is possible that S-H lenses are at greater risk of promoting *Acanthamoeba* infection if exposed to the organism because of the enhanced attachment characteristic of this new material. *Ophthalmology 2003;110:765–771 © 2003 by the American Academy of Ophthalmology.*

As an opportunistic pathogen of humans, free-living protozoa of the genus *Acanthamoeba* can cause a painful sight-threatening disease of the cornea: *Acanthamoeba* keratitis. The first documented cases of ocular infection resulting from *Acanthamoeba* appeared in the early 1970s,[1,2] but it was not until the mid 1980s that a link between *Acanthamoeba* keratitis and the use of contact lenses was established.[3] Ineffective lens disinfection systems,[4] home-made saline,[5] tap water,[6,7] and contaminated lens storage cases[8–10] have been cited as important risk factors for the

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disease. A direct chain of causation of *Acanthamoeba* keratitis has been identified using DNA matching of isolates of *Acanthamoeba griffini* from the corneal scrapping of an infected individual, their lens storage case, and their bathroom water supply.[11] In such an incident, the storage case becomes contaminated by rinsing with tap water containing *Acanthamoeba*, the organisms in turn attach to the lens, which acts as a mechanical vector transmitting the amoebae onto the corneal surface, where invasion and subsequent infection can occur. The initial incidence of *Acanthamoeba* keratitis among contact lens wearers was thought to be low.[5] However, recent work has suggested a much higher incidence[12–14]; however, it is possible that this may decrease as a results of the widespread use of multipurpose storage and disinfecting solutions.[4]

The attachment of *Acanthamoeba* to contact lenses is influenced by various parameters.[15–22] First, the material from which the lens is manufactured has an effect on attachment. Previously, when investigating the attachment of *Acanthamoeba* trophozoites to soft contact lenses from all four of the Food and Drug Administration lens groups, our research group has consistently shown the greatest affinity for ionic, high water-content materials such as etafilcon A (58% H_2O).[18,20,21] This concurs with work by Lema et al,[23] who found higher attachment to etafilcon A as compared with polymacon (38.1% H_2O) lenses. Kilvington and Larkin[16] and Gorlin et al[19] also demonstrated *Acanthamoeba* attaching more readily to high water-content polymers than low water-content materials. However, Kilvington[24] found higher attachment to polymacon than lidofilcon (70% H_2O), bufilcon A (45% H_2O), and etafilcon A when testing a different strain of *Acanthamoeba* than that of previous work.[16] Dissimilarity in results has also been found when investigating attachment to rigid lens materials (silicone acrylate and fluorosilicone acrylate). Our group only found amoebal attachment in the absence of a postincubation washing step.[18] This was in contrast to work carried out by Kelly et al[25] and Sharma et al,[26] who found higher attachment to rigid contact lenses as compared with soft lenses. To date, no studies have been published on the attachment of *Acanthamoeba* to the new extended-wear S-H lens, PureVision (Bausch & Lomb; Kingston-Upon-Thames). The current study therefore was undertaken to establish the level of acanthamoebal attachment to this new lens polymer (balafilcon A, 36% H_20) and to compare this with attachment to a conventional hydrogel.

It has been suggested that differing methodologies, that is, using different strains of *Acanthamoeba*, differing levels of inocula, differing rinse time, and so forth, are responsible for the inconsistencies in the level of attachment found by different research groups.[19] It is yet to be established if the method used to culture the organism has any effect on attachment. At present no standard culturing technique exists; researchers generally cultivate *Acanthamoeba* in either a liquid culture media, that is, peptone-yeast extract-glucose,[19] or solid plated media, that is, nonnutrient agar (NNA).[20] In the current study, the effect of culture technique (liquid vs. plate) on the attachment of *Acanthamoeba* to S-H and conventional hydrogel lenses was assessed.

Acanthamoebal attachment to conventional hydrogel contact lenses is affected by the presence of a bacterial biofilm on the lens surface. Studies by Gorlin et al[27] and Simmons et al[21] demonstrated that the presence of a *Pseudomonas* biofilm on lenses increased levels of attachment. Therefore, the effect that *Pseudomonas* biofilm coating may have on attachment to the S-H lens was investigated.

Previous work has shown that wear of most hydrogel contact lenses increases the attachment of *Acanthamoeba*,[20] probably as a result of surface contamination with tear film deposits. The final factor investigated in the current study was the effect wear had on the level of attachment to S-H lenses.

Materials and Methods

Acanthamoeba Culture

An axenic culture of *Acanthamoeba castellanii* (1501/1A) was obtained from the Culture Collection of Algae and Protozoa (Cumbria, UK). The organism was maintained axenically in 75-cm^2 tissue culture flasks containing 30 ml of proteose peptone glucose broth[28] and was adapted for growth on NNA plates seeded with heat-killed (70° C for 40 minutes) *Klebsiella aerogenes* (WPRL CN345).

To produce liquid-cultured trophozoites, organisms were subcultured from established tissue culture flasks into fresh flasks containing 30 ml proteose peptone glucose broth and incubated at 30° C for 2 days. A sterile cell scraper was used to gently remove the trophozoites adhered to the base of the tissue culture flasks. The proteose peptone glucose broth containing the trophozoites was centrifuged at 3000 rpm for 10 minutes at room temperature, the supernatant removed, and the *Acanthamoeba* pellet resuspended in 10 ml Page's amoebal saline (PAS).[28] Centrifugation and resuspension was repeated a further two times.

To produce plate grown trophozoites, organisms were subcultured from established plate cultures onto fresh NNA plates seeded with heat-killed *K. aerogenes* and incubated at 30° C for 2 days. The plates were flooded with PAS and a sterile spreader used to gently dislodge the amoebae from the surface of the plates. The PAS containing the trophozoites was centrifuged and resuspended as described for the liquid cultured trophozoites.

Page's amoebal saline cultures of liquid and plate-cultured trophozoites were enumerated with a Neubauer hemocytometer and adjusted to 10^5 trophozoites/ml by dilution or centrifugation. Cultures were dispensed in 1-ml volumes into sterile glass bijou bottles for lens incubation.

Pseudomonas Culture

A culture of *Pseudomonas aeruginosa* (NCIMB12469), obtained from the National Collections of Industrial and Marine Bacteria (Aberdeen, Scotland), was maintained on nutrient agar (Oxoid, Basingstoke UK) slopes at 4° C. When required, the organism was subcultured onto fresh nutrient agar plates and incubated for 18 to 24 hours at 37° C, then suspended in PAS to give a concentration of 10^7 colony forming units (cfu)/ml by comparison with a McFarland standard and subsequent culture of serial dilutions. Previous work has shown 10^7 cfu/ml to be the ideal inoculum level to produce adequate biofilm on the lenses.[21] The PAS culture was dispensed in 1-ml volumes into sterile glass bijou bottles for lens incubation.

Table 1. Mean, Median, and Standard Deviation of Trophozoites Attached per Centimeter Squared of Lens under All Test Conditions (n = 16)

Test Condition	Lens Type	Mean	Median	Standard Deviation
1. Plate-cultured trophozoites attached	S-H	1298	641	1450.2
to unworn lenses	hydrogel	84	32	101.6
Liquid-cultured trophozoites	S-H	4124	2707	5378.6
attached to unworn lenses	hydrogel	225	159	225.6
2. All remaining test conditions used plate-cultured trophozoites				
a. Attachment to unworn lenses	S-H	1562	617	1818.2
(control condition)	hydrogel	84	73	59.8
b. Attachment to bacterial biofilm-	S-H	2394	1412	2469.9
coated lenses	hydrogel	220	118	256.6
c. Attachment to worn lenses	S-H	2613	1723	2375.4
	hydrogel	337	153	411.0

S-H = Silicone hydrogel.

Contact Lenses

The S-H lens used in the present study was PureVision (Bausch & Lomb, UK), a cast-molded lens composed of balafilcon A (36% H_20). For these experiments, its performance was compared with that of a conventional hydrogel lens, Acuvue (Vistakon, UK), a cast-molded lens composed of etafilcon A (58% H_20). The etafilcon A lens was chosen as a comparison standard in this, and in previous studies by our group, because this lens has consistently shown the highest affinity for acanthamoebal attachment.[18,20,21] All lenses were quartered before use; this prevented overlap and folding of the lenses during preparation for microscopic observation.

Procedure

Test Condition 1. Test condition 1 examined the effect of lens polymer and *Acanthamoeba* culture method on trophozoite attachment. The S-H and conventional hydrogel lens quarters were incubated individually on an orbital shaker (80 rpm) at 25° C for 90 minutes in 1 ml of a suspension containing approximately 10^5 liquid- or plate-cultured trophozoites per milliliter.

Test Condition 2. Test condition 2 examined factors affecting acanthamoebal attachment. Three test conditions were investigated, all using plate-cultured trophozoites.

a. Attachment to unworn S-H and conventional hydrogel lenses (control condition). Lens quarters were incubated individually on an orbital shaker (80 rpm) at 25° C for 90 minutes in 1 ml of a suspension containing approximately 10^5 trophozoites per milliliter.
b. Attachment to S-H and conventional hydrogel lenses coated with a *Pseudomonas* biofilm. Lens quarters were incubated individually on an orbital shaker at 25° C for 12 to 18 hours in 1 ml of a suspension containing approximately 10^7 cfu/ml *P. aeruginosa*. Quarters were then rinsed in PAS for 5 minutes on an orbital shaker (80 rpm) before incubation in trophozoite suspension as described in test condition 2a.
c. Attachment to worn S-H and conventional hydrogel lenses. Lenses were worn on a daily basis for 7 days, using the ReNu MultiPlus (Bausch & Lomb) lens care system for cleaning and storage. Before wear on the eighth day, lenses were collected for testing. Quartered lenses were rinsed in PAS for 5 minutes on an orbital shaker (80 rpm) to remove any ReNu MultiPlus, before incubation in trophozoite suspension as described for test condition 2a.

After incubation in the various trophozoite suspensions, all lens quarters were rinsed in PAS for 1 minute on an orbital shaker (80 rpm) and were mounted on microscope slides under a coverslip, and the trophozoites attached to one surface of each quarter were enumerated by direct light microscopy. Quarters were also measured to enable counts to be expressed as trophozoites attached per centimeter squared of lens. Sixteen replicates were carried out for each lens type under each test condition. To increase the rigor of the statistical analysis, test condition 2a replicated the plate-cultured trophozoite test condition for test condition 1.

Biofilm Enumeration

Enumeration of the biofilm formed during test condition 2b was achieved by serial dilution and culturing as previously described.[21] Enumeration was carried out on eight S-H and conventional hydrogel lens quarters.

Statistical Analysis

Attachment of *Acanthamoeba* to hydrogel contact lenses has been shown to have an inherently high variability both within and between experimental runs.[18,20–22] Amelioration of this effect was achieved by comparison of trophozoite attachment to unworn conventional hydrogel lenses (exposed to plate-cultured trophozoites, control condition) among experimental runs. Data were adjusted by reference to these control values. With the inherent skewness in the variables under consideration, natural logarithmic transformations were necessary before parametric statistical analyses were performed. One- and two-factor balanced analysis of variance were used to analyze the transformed data, with Tukey's pairwise comparison for follow-up testing where appropriate.

Results

The mean, median, and standard deviation of trophozoites attached per centimeter squared of lens under all test conditions are shown in Table 1. Figure 1 shows typical sections of S-H and conventional hydrogel lenses with liquid- or plate-cultured trophozoites attached. Figures 2 and 3 show the mean number of trophozoites attached per centimeter squared of lens under test conditions 1 and 2(a–c), respectively. Figure 4 shows the extent of biofilm formation on the S-H and conventional hydrogel lenses used for test condition 2b.

1. Effect of lens polymer and *Acanthamoeba* culture method on trophozoite attachment (Table 1, Figs 1 and 2). Lens polymer

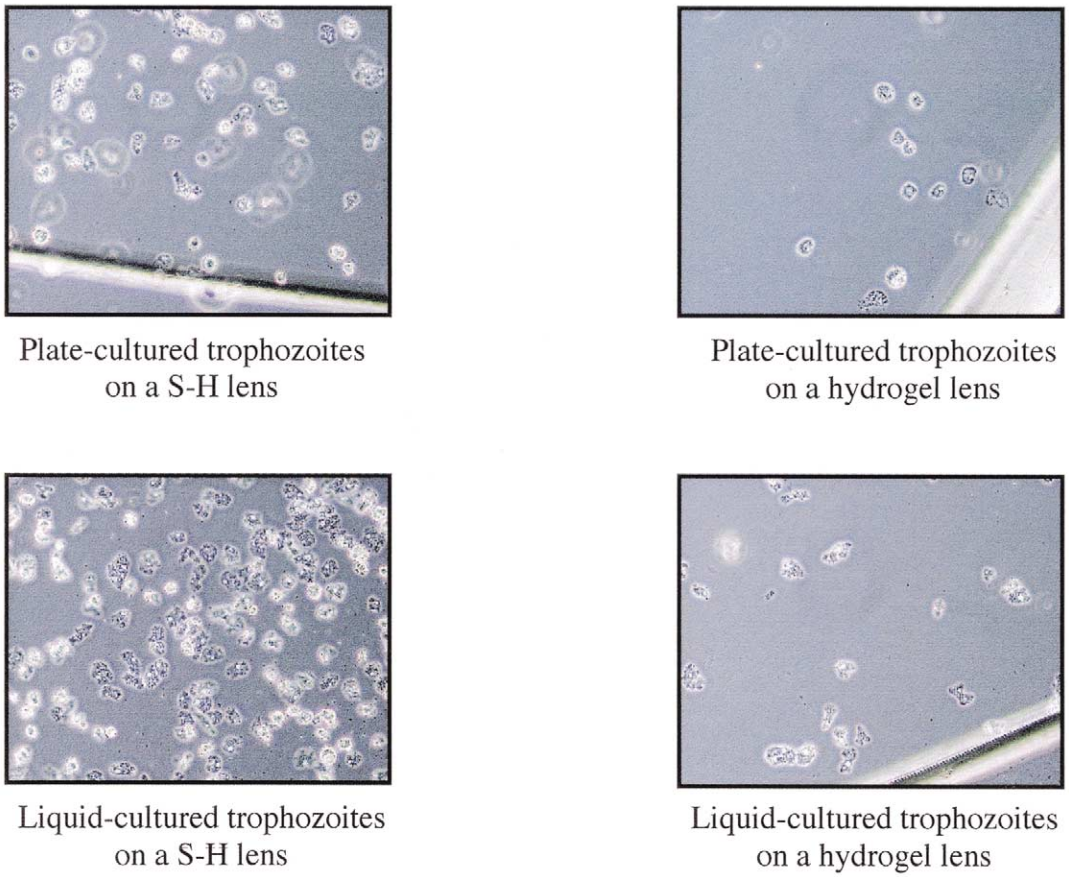

Figure 1. Typical sections of silicone hydrogel (S-H) and conventional hydrogel contact lenses with plate- or liquid-cultured trophozoites attached (original magnification, ×100).

had a significant effect on acanthamoebal attachment ($P < 0.001$). As can be seen from Figures 1 and 2 the number of trophozoites attached to the S-H polymer was much higher than that of the conventional hydrogel control lens. Culture method was also found to have a significant effect on the level of acanthamoebal attachment to both polymers ($P = 0.013$), with higher numbers of liquid-cultured trophozoites enumerated from both lens types. No interaction between lens type and culture method was established ($P = 0.826$).

2. Factors affecting acanthamoebal attachment (Table 1, Figs 3 and 4).
   a. Attachment to unworn S-H and conventional hydrogel lenses (control condition). As with test condition 1, higher levels of attachment were found with the S-H lenses.
   b. Attachment to S-H and conventional hydrogel lenses coated with a *Pseudomonas* biofilm. As can be seen by Table 1, the large standard deviation in attachment to unworn and biofilm coated S-H lenses meant that although a difference between the means was detected, it was not found to be significant. However, attachment to biofilm coated conventional hydrogel lenses was, on average, significantly different from that of the unworn conventional hydrogels ($P < 0.001$), with higher numbers of trophozoites detected on the surface of the biofilm-coated hydrogels. Enumeration of the bacterial biofilm gave mean bacterial counts (n = 8) of 3.8×10^5 and 3.3×10^5 cfu/lens quarter for the S-H and the conventional hydrogel lenses, respectively; no significant difference was established.
   c. Attachment to worn S-H and conventional hydrogel lenses. As with test condition 2b, the large standard deviation found in attachment to the unworn and worn S-H lenses meant that the difference found between the means was not significant. Significance was detected between the worn and unworn conventional hydrogels ($P < 0.001$), with higher average attachment to the worn hydrogels.

Discussion

Our group has consistently shown the conventional hydrogel used in the current study, Acuvue, to have the highest predisposition for acanthamoebal attachment of any contact lens.[18,20,21] However, the present study has demonstrated a much higher level of attachment to the S-H (PureVision) lens. Incorporating silicone into a hydrogel polymer gives the advantage of high oxygen transmissibility, but the disadvantage of decreased wettability and increased lipid interaction.[29] To overcome these disadvantages, the lenses are exposed to a plasma oxidation surface treatment that produces "glassy silicate islands" on the surface of the lens. Between these silicate islands are areas of hydrophobic balafilcon material. The wettability of the glassy silicate areas "bridges" over the balafilcon regions because of their relatively small size.[29] The high level of attachment found

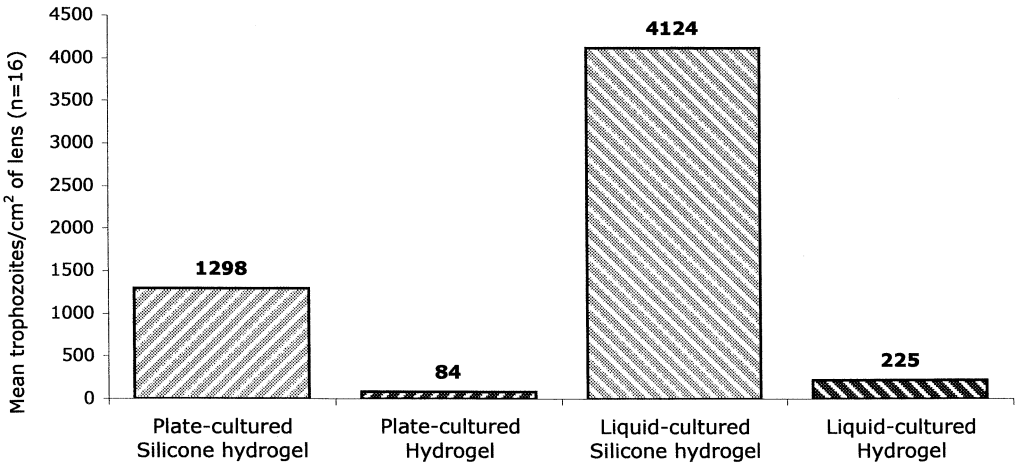

Figure 2. Test condition 1: mean number of plate- or liquid-cultured trophozoites attached to silicone hydrogel and conventional hydrogel contact lenses after 90 minutes of exposure to solutions containing approximately 10^5 trophozoites per milliliter (n = 16).

with the S-H lenses may be the result of an inherent property of the polymer itself or could be related to the surface treatment procedure and the areas of hydrophobic material left unoxidized after treatment. Further work is planned to clarify the reason for this higher level of attachment.

Crawford et al [Invest Ophthalmol Vis Sci 41(4): 802, 2000] demonstrated that culture technique affects the formation, physical appearance, and viability of *Acanthamoeba* cysts. However, no literature could be found citing the effect that culture technique has on trophozoites and their ability to attach to lens surfaces. The current study has shown that higher numbers of trophozoites cultured in liquid media attached to S-H and conventional hydrogel lenses as compared with trophozoites cultured on solid plated media, seeded with heat-killed *Klebsiella*. When observed during growth, the liquid-cultured trophozoites appeared inactive, with little movement or winking, that is, contraction of the water expulsion vacuole. The plate-cultured trophozoites, however, demonstrated a high level of activity (winking was frequent and large numbers of trails could be seen in the film of *Klebsiella* covering the surface of the NNA plate). It is possible that liquid-cultured trophozoites adapt to a more sedentary lifestyle, attaching to a surface and absorbing the soluble nutrients. The main priority for these organisms may be surface attachment. In contrast, the plate-cultured trophozoite are highly active, seeking out bacteria to ingest and hence do not attach to the lenses as readily.

As with previous studies,[20] patient wear of the conventional hydrogel lens produced a significant increase in acanthamoebal attachment. Such an increase was not seen with the S-H lens, possibly because attachment was at such a high level for the unworn lenses that any increase with wear is minimal possibly the result of a lack of available attachment sites.

Gorlin et al[27] noted that fewer trophozoites attached to a *Pseudomonas* biofilm coated lens were removed by rinsing, as compared with those attached to a clean hydrogel lens, suggesting that a stronger attachment forms between the *Acanthamoeba* and the biofilm coated lens than the clean lens. *Acanthamoeba* have been shown to possess binding sites for bacterial flagella,[30] and it would seem likely that the amoebae attach more readily and form a stronger bond with the polar flagella on the surface of *Pseudomonas* than to a clean hydrogel lens. In accordance with the results of Gorlin et al[27] and Simmons et al,[21] an increase in attachment was seen with the *Pseudomonas* biofilm-coated conventional hydrogel lenses; however, no such increase in attachment was detected with the biofilm-coated S-H lenses. It is possible that, as with the worn S-H lenses, attachment to the unworn lens is at such a high level, as a result of an innate characteristic of the material, that any increase resulting from biofilm coating and the presence of bacterial flagella is minimal.

Enumeration of the *Pseudomonas* biofilm formed on both lens types failed to establish any significant difference. This concurs with a clinical study by Keay et al,[31] who compared the number of bacteria adhering to S-H lenses (lotrafilcon A) worn on an extended basis of 30 nights with conventional hydrogel lenses (etafilcon A) worn for six nights continuously. They found similar levels of bacteria attached to both lens types, regardless of the difference in the length of extended wear. These results indicate that the increased propensity for acanthamoebal attachment found with the S-H lens does not apply to all microorganisms.

Holden[32] reported 14 cases of microbial keratitis associated with extended wear of S-H lenses in approximately 221,500 patient years. This gives an incidence for S-H lens-associated microbial keratitis of 1 in 15,800 patient years, which is approximately 30 times lower than for previous studies with extended wear of conventional hydrogel lenses,[14,33,34] suggesting a vast improvement in patient safety with this new lens polymer. Although *Acanthamoeba* has proved to have a high affinity for S-H lenses, none of the microbial keratitis cases reported so far have only been associated with the organism. However, the lenses have been on the market for a short period, and it may take several more years before enough patient wear years have been recorded for corneal infections with this organism to be identified.

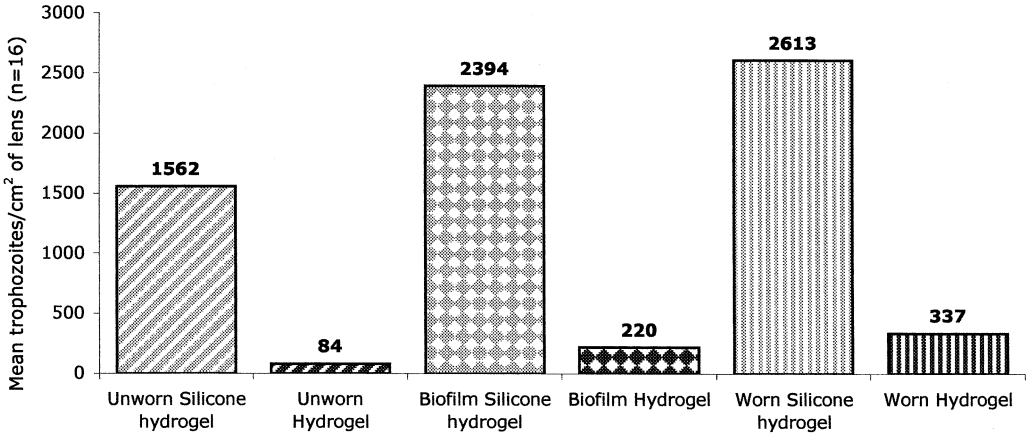

Figure 3. Test condition 2 (a–c): mean number of plate-cultured trophozoites attached to silicone hydrogel and conventional hydrogel contact lenses after 90 minutes of exposure to a solution containing approximately 10^5 trophozoites per milliliter (n = 16).

The incidence of *Acanthamoeba Keratitis* (AK) with rigid silicone acrylate lenses is extremely low.[34] This is not unexpected because our research group only found acanthamoebal attachment to rigid lenses in the absence of a postincubation rinse step.[18] This was in contrast to work carried out by Kelly et al[25] and Sharma et al,[26] who found significant attachment to silicone acrylate lenses. If this were the case, then low infection rates would most likely be the result of the improved oxygen transfer to the cornea associated with rigid lenses as compared with standard soft hydrogel lenses. The S-H lenses may demonstrate the same effect. The increased oxygen transmission of S-H lenses reduces corneal hypoxia, leading to reduced bacterial binding to corneal epithelium cells,[35] improving the safety of extended wear with this lens. It is possible that the ultra oxygen-permeable S-H lenses may also reduce acanthamoebal binding to corneal epithelium cells, hence lowering infection rates.

Wear regimen may also play a role in the reduced infection rates seen with this lens. The S-H lenses are designed, and have been approved, for 30 days of continuous wear before disposal. Under this regimen, it is unlikely that the lenses would be exposed to *Acanthamoeba* unless worn when swimming, showering, and so forth. However, some lens practitioners prescribe S-H lenses on a 6-night and 7-day extended wear basis, with a cleaning and disinfection stage on the seventh night. This regimen requires the lenses to be stored in a case and the use of lens care solutions, both of which have been cited as risk factors in the development of *Acanthamoeba* keratitis.[4,7–10] Ideally, the lenses should only be prescribed for 30 days of continuous wear, thus avoiding the need for a lens storage case and lens care solutions, reducing the risk of exposure to *Acanthamoeba*. If a 6-night and 7-day regimen must be prescribed, wearers should use disposable storage cases and should disinfect their extended wear S-H lenses with a multipurpose solution.[4,7,10] Present disinfecting solutions do not specifically prevent attachment of trophozoites to the lens, but may be able to do so in future.[22] Tap water washing of lenses or the storage case must be avoided at all times. Clinicians should be aware that the S-H lens is very "sticky" for *Acanthamoeba* trophozoites and of the theoretical possibility of devastating infection associated with it. It is therefore vital that patients are informed of the danger of wearing these lenses when exposed to sources of the organisms, that is, swimming, showers, hot tubs, and so forth. It is also important that clinicians are familiar with the early clinical signs of the disease and of its effective treatment with 0.02% chlorhexidine and 0.1% Brolene (propamidine isethionate).[36]

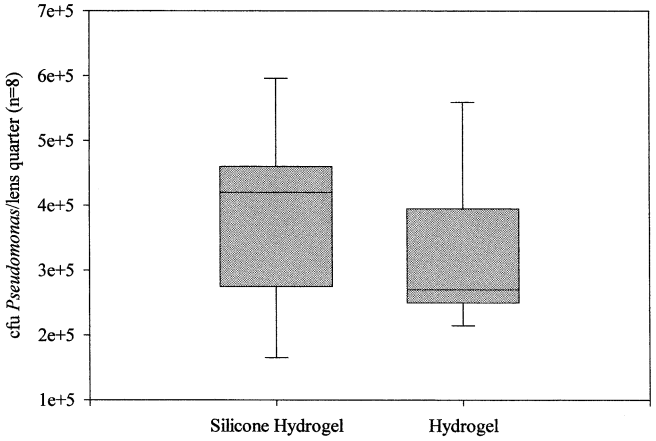

Figure 4. Extent of *Pseudomonas* biofilm formation on silicone hydrogel and conventional hydrogel contact lenses used for test condition 2b (n = 8; cfu, colony forming units).

Conclusions

Acanthamoeba have been shown to have a higher affinity for new extended-wear S-H lenses as compared with conventional hydrogel lenses. Liquid-cultured trophozoites attached to the lenses tested more readily than plate-cultured organisms. This may explain some of the inconsistencies found in attachment studies carried out by different research groups using differing culture techniques. Factors previously known to increase acanthamoebal attachment to conventional hydrogels, such as patient wear and presence of a bac-

terial biofilm, did not produce any significant increase in attachment with the new lens polymer. Such an increase may have been minimal because of the high level of attachment seen with unworn S-H lenses. The increased level of attachment seen with the S-H lenses may be an inherent characteristic of the material or an effect of surface treatment.

The new S-H lens is highly 'sticky' for attachment of *Acanthamoeba* trophozoites and must be considered a risk factor for *Acanthamoeba* infection. All clinicians should be aware of this problem and ideally should prescribe these lenses on a 30-day continuous wear regimen. If the lenses have to be removed for disinfection, then the clinician should advise the correct hygiene procedures to prevent contamination with *Acanthamoeba*.

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