ACANTHAMOEBA TROPHOZOITE AND CYST ADHERENCE TO FOUR TYPES OF SOFT CONTACT LENS AND REMOVAL BY CLEANING AGENTS

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SUMMARY

Trophozoite and cyst adherence of two *Acanthamoeba* keratitis strains (PHL/530 and PHL/978) to four types of unworn soft contact lens and their removal by cleaning agents were studied. Greater adherence of the trophozoites compared with the cysts was recorded for both strains. Trophozoites of PHL/530 adhered in greater numbers to type I lenses (61.4% poly[2-hydroxyethyl methacrylate–38.6% water), with no differences between type II (30% lidofilcon A–70% water), III (55% bufilcon A–45% water) and IV lenses (42% etafilcon A–58% water). Adherence of PHL/978 trophozoites to type II lenses was decreased compared with their adherence to the other lenses. Cysts of both strains showed greater adherence to type I and III lenses. Interstrain differences in trophozoite adherence occurred, with PHL/530 showing greater adherence to type I and II lenses. Recommended cleaning procedures using three commercial solutions were effective in removing *Acanthamoeba* from the lenses. This study indicates the possible role of adherence to contact lenses in the acquisition of *Acanthamoeba* keratitis, but shows that correct use of commercial cleaning agents may be important in the prevention of infection.

Acanthamoeba is a genus of small free-living amoeba found in virtually all soil and aquatic environments.[1] The organism is characterised by a feeding and replicating trophozoite which, under adverse conditions, can form a dormant cyst stage resistant to extremes of temperature, desiccation and disinfection.[1] *Acanthamoeba* are pathogenic to man, causing a rare but invariably fatal encephalitis in the immunocompromised host and, more frequently, a potentially blinding infection of the cornea in previously healthy persons termed *Acanthamoeba* keratitis.[2,3] Since the disease was first recognised in 1973, several hundred cases of *Acanthamoeba* keratitis have been reported, mainly from the United States.[4] Contact lens wearers are most at risk from *Acanthamoeba* keratitis, and account for approximately 80% of reported cases.[3,4] Poor lens hygiene practices, notably the use of tap-water-prepared saline rinsing solutions and defaulting on standard lens disinfection procedures, are recognised risk factors in acquiring the disease.[3–5]

Acanthamoeba have been observed adhering to the surface of contact lenses recovered from keratitis patients and have also been isolated from contact lens storage containers of both symptomatic and asymptomatic patients.[5–7] It therefore seems likely that adherence of *Acanthamoeba* to the contact lens is a potential vehicle by which the organism is inoculated on to the surface of the cornea in the acquisition of keratitis. Although the activity of contact lens disinfecting methods against *Acanthamoeba* has been extensively investigated,[8–13] few studies have addressed the efficacy of cleaning procedures in removing the organism adhering to lens surfaces.[14,15] As this may have an important role in the prevention of *Acanthamoeba* keratitis, the adherence of trophozoites and cysts to four types of unworn soft contact lenses and the efficacy of three commercial cleaning agents in removing the organism were investigated. The demonstration that *Acanthamoeba* trophozoites and cysts adhered to all lens types but could be removed effectively by routine cleaning procedures has prompted this report.

MATERIALS AND METHODS

Test Organisms

Two *Acanthamoeba* strains (PHL/530 and PHL/978), isolated from keratitis cases in the United Kingdom, were used in this study. The strains were isolated by culture of corneal scrapings on non-nutrient agar plates seeded with a living suspension of *Escherichia coli*[1,5] and the trophozoites adapted to axenic (bacteria-free) growth in a serum–casein–glucose–yeast extract medium[16,17] modified by the inclusion of 0.1% filter sterilised Panmede liver digest (Paines & Byrne, Greenford, UK). Cultures were maintained in tissue culture flasks at 32°C. Mature cysts

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were produced from the trophozoites using the constant pH encystment medium of Neff *et al.*[18] in stationary tissue culture flasks incubated in air at 32°C. Strain PHL/978 was isolated and adapted to axenic growth shortly before the start of this study, whilst PHL/530 had been maintained in axenic culture since 1990.

Lens Types and Cleaning Agents

Four types of soft contact lens, as defined by the USA Food and Drug Administration (FDA), were studied: type I (Soflens U3: 61.4% poly[2-hydroxyethyl methacrylate–38.6% water); type II (Bausch & Lomb 70: 30% lidofilcon A–70% water); type III (Softmate B: 55% bufilcon A–45% water) and type IV (Bausch & Lomb 58: 42% etafilcon A–58% water). Lenses were stored in sterile normal saline (0.9% w/v NaCl) at room temperature until used.

Three types of contact lens cleaning agents were studied for their efficacy in removing adhering *Acanthamoeba* from the lenses: Bausch & Lomb ReNu Multi-Purpose Solution (poloxamine, 0.00005% polyaminopropyl biguanide, 0.1% EDTA), Alcon Opti-Clean II Daily Cleaner (Tween 21, polymeric cleaning beads, 0.1% EDTA, 0.001% polyquarternium-1) and Ciba MiraFlow Daily Cleaner (20% isopropyl alcohol, poloxamer 407, amphoteric 10).

Adherence to Lenses

Immediately prior to testing, trophozoites or cysts were washed three times with normal saline by centrifugation at 1000*g* for 10 minutes at room temperature and adjusted to a final concentration of 1×10^6/ml with cell counts performed in a Modified Fuchs Rosenthal haemocytometer. Contact lenses were placed into individual wells of a 12-place microtitre plate (Falcon, Becton Dickenson, Oxford, UK) and 1 ml of the calibrated trophozoite or cyst suspension added to give an inoculum of one million organisms per lens. Plates were left undisturbed at room temperature for 4 hours before the lenses were removed and gently immersed in two separate 10 ml volumes of normal saline at room temperature. The lenses were then placed into microcentrifuge tubes containing 1 ml of ice cold normal saline, incubated on ice for 15 minutes and the tubes vortexed for 10 seconds. Previous studies had shown this treatment to be effective in removing all adhering trophozoites or cysts from the lens surfaces. Cell counts of detached amoebae were then performed using a Modified Fuchs Rosenthal haemocytometer. Each lens type was tested in triplicate against the trophozoites and cysts of the two *Acanthamoeba* strains and repeated on one other occasion.

The results of the trophozoite and cyst adherence studies were compared using a one-way analysis of variance (ANOVA). Then a two-tailed *t*-test was used in analysis and the level of significance taken as 5% ($p<0.05$).

Efficacy of Cleaning

Acanthamoeba were allowed to adhere to the four lens types and rinsed gently in normal saline as described above. The lenses were then mechanically cleaned according to the manufacturer's instructions and rinsed in a stream of sterile saline or ReNu Multi-Purpose Solution for 5 seconds (approximately 15 ml of solution) and examined by light microscopy for the presence of remaining amoebae. In control experiments, lenses were rinsed only in a stream of saline or ReNu Multi-Purpose Solution. Each lens type was tested in triplicate against the trophozoites and cysts of the two *Acanthamoeba* strains.

RESULTS

Adherence to Lenses

Direct microscopic examination of the lenses before and after immersion in normal saline showed that *Acanthamoeba* trophozoites and cysts adhered to all four types of soft contact lens. Although not quantified, this also revealed that some detachment of trophozoites and cysts from the lenses occurred during the two gentle saline rinses. However, sufficient amoebae remained to enable haemocytometer estimation of *Acanthamoeba* adherence to be made. Trophozoite and cyst adherence of the *Acanthamoeba* strains to the four types of soft contact lens is shown in Figs. 1 and 2 respectively. Table I gives the mean adherence, with standard deviations, for the replicate experiments.

For both strains, greater adherence of trophozoites than cysts to all the lens types was recorded ($p<0.01$). For strain PHL/530, greatest adherence of trophozoites occurred to the type I lenses ($p<0.001$), with no significant difference in the degree of adherence to the type II, III and IV lenses ($p\geq0.2$). For strain PHL/978, no difference in trophozoite adherence to type I, III or IV lenses was found ($p\geq0.1$), although the decreased adherence to type II lenses was significant ($p\leq0.002$). For the cysts of strain PHL/530, significantly greater adherence to type I and III lenses was found ($p<0.05$). No difference in cyst adherence to type II or IV lenses was found ($p = 0.5$). For strain PHL/978, cyst adherence to type I and III lenses was also

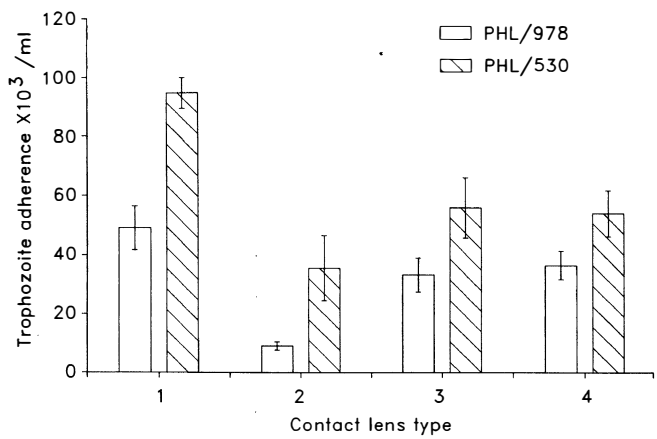

Fig. 1. *Adherence of* Acanthamoeba *trophozoites to four types of unworn soft contact lens. 1, FDA type I (Soflens U3: 61.4% poly[2-hydroxyethyl methacrylate–38.6% water); 2, FDA type II (Bausch & Lomb 70: 30% lidofilcon A–70% water); 3, FDA type III (Softmate B: 55% bufilcon A–45% water); 4, FDA type IV (Bausch & Lomb 58: 42% etafilcon A–58% water).*

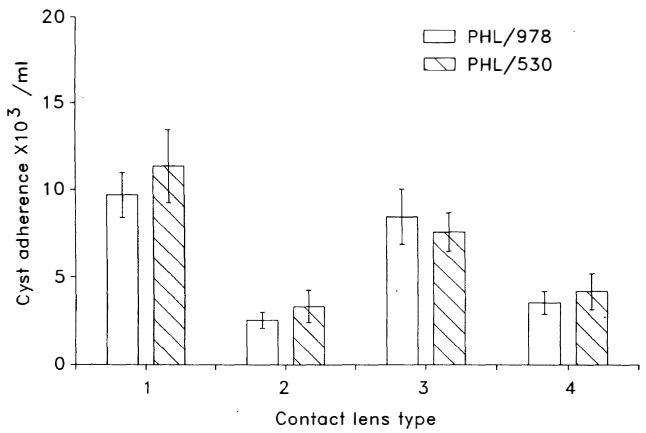

Fig. 2. *Adherence of* Acanthamobea *cysts to four types of unworn soft contact lens. 1, FDA type I (Soflens U3: 61.4% poly-[2-hydroxyethyl methacrylate–38.6% water); 2, FDA type II (Bausch & Lomb 70: 30% lidofilcon A–70% water); 3, FDA type III (Softmate B: 55% bufilcon A–45% water); 4, FDA type IV (Bausch & Lomb 58: 42% etafilcon A–58% water).*

significantly higher compared with the others ($p<0.05$). Overall differences in the degree of trophozoite adherence between the two *Acanthamoeba* strains were found, with PHL/530 showing greater adherence to type I and type II lenses compared with PHL/978 ($p<0.05$). No difference in cyst adherence between the two strains was noted ($p>0.4$).

Efficacy of Cleaning

Mechanical cleaning of the lenses according to the manufacturer's instructions with either ReNu Multi-Purpose Solution, Opti-Clean II Daily Cleaner or MiraFlow Daily Cleaner, followed by rinsing with saline or ReNu Multi-Purpose Solution was found to be effective at removing all trophozoites and cysts adhering to the four contact lens types. Microscopic examination of the lenses immediately after cleaning but prior to rinsing showed some *Acanthamoeba* floating in liquid retained on the surface of the lenses. Rinsing of the lenses with saline or ReNu Multi-Purpose Solution alone was not effective in removing all adhering *Acanthamoeba*, as a small number of trophozoites of strain PHL/530 were observed still remaining on type I and type III lenses on one or more occasion.

DISCUSSION

Although previous studies have demonstrated the ability of *Acanthamoeba* to adhere to the surface of soft contact lenses, the work has been confined to only one or two lens types.[14,15,19] Here, these observations have been extended to show that *Acanthamoeba* trophozoites and cysts can adhere to all types of soft contact lens. Differences in attachment between the two test strains was also noted, with significantly greater adherence of PHL/530 trophozoites to type I lenses (Soflens U3: 61.4% poly[2-hydroxyethyl methacrylate–38.6% water) and significantly lower adherence of PHL/978 to type II lenses (Bausch & Lomb 70: 30% lidofilcon A–70% water). The cyst forms of both strains adhered less well to the lenses but both showed greater attachment to the type I followed by type III lenses (Softmate B: 55% bufilcon A–45% water). The reason for such variations in lens adherence is unclear but may be due to the different chemical composition or water content of the four lens types. Attachment of *Acanthamoeba* trophozoites to the surface of glass has been reported to occur through the presence of long slender pseudopods, termed filopodia, on the underside of the amoeba.[20] Similar processes may therefore characterise the adherence of trophozoites to contact lenses. Filopodia are not present in the cyst forms and attachment would seem to be due to the presence of adhesive substances on the outer cyst wall.

The reasons for the greater adherence of PHL/530 trophozoites to type I and II lenses compared with those of PHL/978 is unclear. Strain PHL/530 had been in continuous axenic culture for more than 1 year whilst PHL/978 was isolated and adapted to axenic growth shortly before the commencement of this study. It was also observed that strain PHL/530 adhered more avidly to the surface of tissue culture flasks during axenic culture compared with PHL/978. As it has been reported that prolonged axenic culture results in the attenuation of virulence in pathogenic *Acanthamoeba*, it would appear that the degree of adherence to soft contact lenses may not necessarily be a function of pathogenicity.[21] It should be noted that the lenses used in this study were unworn and that that may result in a decreased level of *Acanthamoeba* attachment. In the bacterial corneal pathogen *Pseudomonas aeruginosa* it has been shown that proteinaceous material increases the degree of adherence to unworn contact lenses.[22] *Acanthamoeba* cysts have also been observed adhering to a hard gas-permeable contact lens from a patient with keratitis,[5] but in studies with unworn lenses of this type no cyst attachment was found to occur.[15] As worn lenses contain proteinaceous deposits, this may increase the affinity of *Acanthamoeba* adherence.

Whilst the activity of contact lens disinfecting methods against pathogenic *Acanthamoeba* has been extensively investigated,[8–13] only limited studies have determined the efficacy of cleaning agents in removing the organism

Table I. Mean values with standard deviation for *Acanthamoeba* trophozoite and cyst adherence to four soft contact lens types

Strain	Form	No. of lenses	Mean adherence $\times 10^3$/ml ± SD			
			Type I	Type II	Type III	Type IV
PHL/530	Trophozoites	6	94.5 ± 12.9	35.4 ± 26.8	55.8 ± 25.1	53.8 ± 19.0
PHL/978	Trophozoites	6	49.0 ± 18.1	9.0 ± 3.3	33.2 ± 14.1	36.4 ± 11.8
PHL/530	Cysts	6	11.3 ± 5.1	3.3 ± 2.2	7.6 ± 2.7	4.2 ± 2.5
PHL/978	Cysts	6	9.7 ± 3.15	2.55 ± 1.1	8.45 ± 3.8	3.5 ± 1.6

adhering to lenses.[14,15] In this study it was found that cleaning of lenses according to the manufacturer's instructions with either ReNu Multi-Purpose Solution, Opti-Clean II or Mira Flow, followed by rinsing with saline or ReNu Multi-Purpose Solution, was effective at removing both trophozoites and cysts from four soft lens types. Penley *et al.*[14] investigated the efficacy of 10 cleaning agents (including those used here) in removing *Acanthamoeba* from soft contact lenses. In contrast to the findings of this study, only MiraFlow was found to be effective, as judged by the absence of *Acanthamoeba* growth on culture of the lenses after cleaning. However, it is unclear whether the workers rinsed the lenses after cleaning, as this was found to be necessary in this study for the removal of detached *Acanthamoeba* from the residual cleaning solution.

Acanthamoeba keratitis is a rare but potentially blinding infection associated with contact lens wear.[3,4] *Acanthamoeba* have been seen adhering to contact lenses recovered from keratitis patients[5,7] and were shown here to attach readily to unworn soft contact lenses. It therefore seems likely that adherence to contact lenses is a potential vehicle by which the organism is inoculated on to the cornea. Clinicians and eye care professionals should be aware of the risk to contact lens wearers from *Acanthamoeba* keratitis and instill the need for strict compliance with recommended lens hygiene procedures. This should include the regular cleaning and replacement of contact lens storage containers and use of sterile commercial cleaning, disinfection and rinsing solutions.

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Key words: *Acanthamoeba,* Adherence, Cleaning, Contact Lenses.

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