

Acanthamoeba Adherence to Contact Lenses and Removal by Cleaning Agents

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Summary

The quantitative adherence of a keratitis isolate of *Acanthamoeba polyphaga* to low- and high-water content non-ionic soft contact lenses and one type of rigid gas-permeable lens was investigated. Adherence of trophozoite and cyst forms of the organism was observed *in vitro*, and adherent amoebae counted by a plaque assay method following detachment. Trophozoites adhered to all lens types with adherence being statistically significantly greater to high water content soft lenses. Cyst attachment occurred only to the soft lenses but not to gas-permeable lenses, and was significantly higher for the high water content lenses. Attachment of cysts was significantly lower than that of trophozoites to each lens tested. Recommended cleaning procedures using two commercial solutions removed all adherent trophozoites and cysts from lenses. These studies demonstrate

- (i) that lenses may act as a vector in *Acanthamoeba* keratitis, particularly for high-water content lenses and trophozoite amoebae,
- (ii) that lens cleaning agents may prevent keratitis by removing adherent *Acanthamoeba*.

Acanthamoeba is a genus of free-living amoebae found in most soil and aquatic environments.¹ Keratitis caused by *Acanthamoeba* is significantly linked with wear of contact lenses; especially soft lenses.² *Acanthamoeba* have been isolated from the lens storage cases of many patients in whom *Acanthamoeba* keratitis has been diagnosed.³ *Acanthamoeba* contaminating a storage case might be transmitted to the cornea by adhering to the lens. Increasing incidence of *Acanthamoeba* keratitis, especially in wearers of soft lenses, prompted us to study adherence of this amoeba to lenses.

It has been reported that amoebae may adhere to soft contact lenses.⁴ We used a quantitative method to study soft lenses of dif-

fering water content and a rigid gas-permeable lens in order to ascertain whether preferential adherence might explain higher incidence of infection in wearers of certain lens types. New lenses were studied, using trophozoite and cyst forms of a clinical isolate of *A. polyphaga*. In addition we tested the efficacy of lens cleaning solutions in removing the adherent amoebae. Demonstration and quantification of *Acanthamoeba* trophozoite and cyst adherence to lens surfaces, and their removal by recommended cleaning agents has prompted this report.

Materials and Methods

Test Organism

A strain of *A. polyphaga* (Shinh) which had

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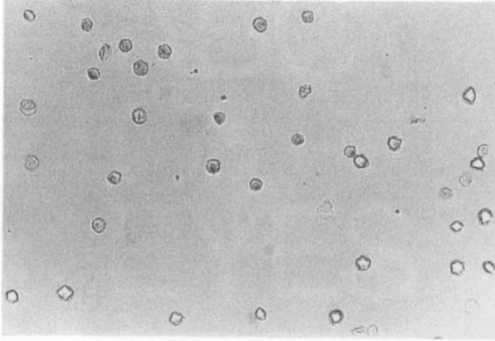


Fig. 1. *Acanthamoeba* cysts adherent to a lidofilcon lens, following immersion in cyst suspension for four hours and prior to detachment by addition of ice-cold saline and agitation ($\times 200$).

been isolated from a keratitis patient was used in this study (kindly given by Dr. David Warhurst, London School of Hygiene and Tropical Medicine). The strain was adapted to axenic culture in a serum-casein-glucose-yeast extract medium,⁵ modified here by the inclusion of 0.1% filter-sterilized Panmede liver digest (Paines and Byrne Ltd, Greenford, England). Trophozoites were maintained in tissue culture flasks at 32°C. Mature cysts were produced from these cultures using the constant pH encystment medium of Neff⁶ in stationary tissue culture flasks incubated in air at 32°C.

Lenses

Three types of contact lenses were studied:

- (a) soft extended-wear lidofilcon A (70% water, 30% non-ionic lidofilcon A [poly (n-vinyl pyrrolidone + methylmethacrylate) with allyl methacrylate and ethylene dimethacrylate]),
- (b) soft daily-wear polymacon (38.6% water, 61.4% non-ionic polymacon [2-hydroxyethyl methacrylate cross-linked with ethylene glycol dimethacrylate])
- (c) rigid gas-permeable sulphylcon A (fluorosilicon-acrylate).

Lenses were stored in sterile vials in normal saline at room temperature until used.

Adherence to lens materials

Immediately prior to testing, the trophozoites or cysts were washed three times with normal saline. Cell counts were performed using a haemocytometer and a concentration of

1×10^5 /ml prepared. Three lenses of each type were placed into individual wells of a 24 well tissue culture plate (Nunc Tissue Culture Products, Gibco BRL Ltd., Paisley, United Kingdom) and 1 ml of the calibrated trophozoite or cyst suspension added. Trophozoite attachment was allowed to occur for two hours and cyst attachment for four hours, at room temperature. The culture plate was stationary during this time and the process was monitored using light microscopy.

Lenses were then removed and rinsed twice in separate 10 ml volumes of normal saline. Amoebae remaining adherent (Figs. 1 and 2) were then detached by adding the lenses to 10 ml of ice cold normal saline and placing in ice for 15 minutes. At five minute intervals, the lenses were agitated on a vortex mixer for ten seconds. This treatment caused complete detachment of both trophozoites and cysts, verified by microscopic observation of the lens surface. Serial ten fold dilutions of the saline solution were made in Page's amoebal saline (PAS)¹ and 1 ml volumes plated out in triplicate on to the surface of nutrient agar plates (2.5% agar, 0.1% glucose and 0.1% yeast extract in PAS). Before use, the plates had been seeded with live *Escherichia coli* using a sterile swab moistened in a turbid suspension of the bacterium in PAS. Plates inoculated with the test dilutions were allowed to dry at room temperature before incubation in a humidified incubator at 35°C for up to seven days. Under these conditions, *E. coli* divided to form a confluent lawn on the plate with zones of clearing, termed plaques, resulting from growth of individual trophozoites, or hatched cysts.

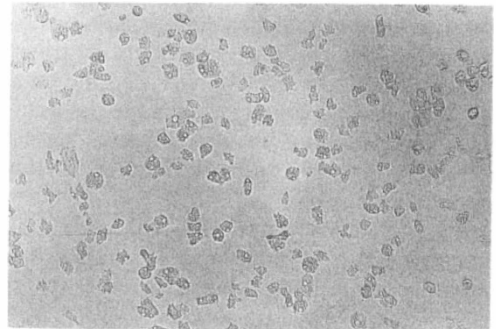


Fig. 2. Trophozoites adherent to a lidofilcon lens following immersion in trophozoite suspension for two hours ($\times 100$).

Table I *Acanthamoeba polyphaga* cyst and trophozoite adherence to contact lenses.

Amoeba	Lens material	water content (%)	PFU/ml at dilution:				
			Undiluted	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
cysts	lidofilcon A	70	9.3±2.0	1.2±0.6	0	-	-
	polymacon	38.6	6.1±2.0	0.3±0.1	0	-	-
	sulphylcon A	-	0	0	0	-	-
trophs	lidofilcon A	70	-	-	>15	>15	5.0±1.7
	polymacon	38.6	-	-	>15	7.9±2.4	0.4±0.1
	sulphylcon A	-	-	-	>15	7.0±1.6	0.3±0.1

Values given are mean ± standard deviation.

Significantly more cysts adhered to lidofilcon than polymacon ($t = 3.25$; 2-tailed t -test; $p = 0.005$).

More trophozoites adhered to lidofilcon than polymacon ($t = 6.94$; $p < 0.001$). The difference between adherence of trophozoites to polymacon and gas-permeable (sulphylcon A) was not significant ($t = 0.87$; $p = 0.4$).

The degree of attachment of amoebae to a lens surface was expressed as the number of plaque-forming units (PFU)/ml. A one-way analysis of variance (ANOVA)⁷ was carried out simultaneously on the results of adherence to all three lenses to assess overall differences. Then a two-tailed t -test was used in analysis and the level of significance taken as 5% ($p < 0.05$).

Efficacy of Cleaning

The efficacy of two contact lens cleaning solutions in removing *Acanthamoeba* adherent to lenses was investigated. Trophozoites or cysts were attached to the lens surfaces as described above. Cyst adherence to gas-permeable lenses was found only to occur when $c.10^3$ cysts were air dried on to the lens surface. The lenses were then cleaned according to the solution manufacturer's instructions using

- (i) 10% sodium tridecylether sulphate ("Cleaner for rigid gas-permeable lenses", Bausch and Lomb, Middlesex, England) for the gas-permeable lenses
- (ii) EDTA and sorbic acid ("Daily Cleaner for soft contact lenses", Bausch and Lomb) for the lidofilcon and polymacon lenses.

Following one rinse in 10 ml of normal saline, the lenses were examined for the presence of remaining amoebae using light microscopy, with phase contrast and magnification $\times 100$.

Results

Adherence to lens materials

Counts of adherent trophozoites and cysts are shown in Table I. Three counts were obtained

from each lens tested, and three lenses of each type were studied. From the nine counts, a mean and standard deviation was calculated for each lens type.

One-way ANOVA showed a significant overall difference in adherence to all lenses for trophozoites ($p < 0.001$) and cysts ($p < 0.001$). A 2-tailed t -test indicated significantly greater adherence, after two hours, of trophozoites to lidofilcon than to polymacon lenses ($t = 6.94$; $df = 16$; $p < 0.001$). No significant difference was observed between adherence to polymacon and gas-permeable lenses ($t = 0.87$; $df = 16$; $p = 0.4$).

Significantly greater adherence, after four hours, of cysts to lidofilcon than to polymacon lenses was observed ($t = 3.25$; $df = 16$; $p = 0.005$). No cysts were observed to adhere to gas-permeable lenses.

Efficacy of Cleaning

No trophozoites or cysts were observed to remain attached to the surface of the lenses following cleaning and rinsing using the solution manufacturer's instructions.

Discussion

We found adherence of substantial numbers of *Acanthamoeba* trophozoites to each lens type. *Acanthamoeba* trophozoite adherence is significantly higher to high water-content lidofilcon lenses than low water-content polymacon or gas-permeable lenses. We found that cyst adherence to lidofilcon was also substantially higher than to polymacon lenses, and that no cysts remained adherent to gas-permeable lenses. The difference in water

content (70% and 38.6%) may alone account for the differential soft lens adherence, both lenses being composed of non-ionic polymers. However lens surface characteristics may also be a factor: lidofilcon A lenses are lathe-cut and polymacon lenses spun cast. It is interesting to note that studying adherence of *Pseudomonas aeruginosa* to a number of soft lenses of varying hydration, Miller and Ahearn⁸ found no strict correlation between adherence and percent hydration but found that slightly more bacteria adhered to lidofilcon than polymacon lenses. Other workers have found decreased bacterial adherence with increased water content.⁹⁻¹¹

It has been shown that adherence of bacteria is facilitated by mucin¹² or protein¹¹ coating of lenses and that bacteria preferentially adhere to organic debris and lens scratches.¹³ These factors may also influence *Acanthamoeba* adherence, and it is likely that study of new lenses underestimates the adherence which occurs *in vivo*. Studies of bacterial adherence to contact lenses have included electron microscopy of sectioned soft lens fragments.^{8,14,15} The difficulty in cutting hard lenses prompted us to compare adherence of both lens types by quantitative plaque assay, a technique ideally suited to study of *Acanthamoeba*.

Acanthamoeba keratitis has been increasingly recognised in recent years and contact lens wear is the predominant risk factor. Epidemiological study of 208 cases in the United States indicated that 160 (85%) wore contact lenses, of which 89 (56%) wore daily-wear soft, 31 (19%) wore extended-wear soft, and 14 (9%) wore hard or gas-permeable lenses.² These data may reflect the popularity of, and numbers wearing different lens types. The data may also reflect the role of the soft lens material in disease pathogenesis. In any event, it is probable that daily-wear lens users inoculate *Acanthamoeba* from contaminated lens storage cases to the cornea each day: these patients therefore have a higher probability of developing keratitis than those using extended-wear lenses, irrespective of surface adherence characteristics.

Contamination of lens solutions and storage cases is a major risk factor in *Acanthamoeba* keratitis.³ Contact lens storage cases

provide an environment which can readily be contaminated by *Acanthamoeba*.¹⁶ This is one reason why so many *Acanthamoeba* keratitis patients are lens wearers. Our results demonstrate another reason: *Acanthamoeba* trophozoites and cysts can adhere to lenses, which are a suitable vehicle for transfer of contaminating amoebae to the cornea. It is possible that a further factor is lens-induced epithelial trauma providing an entry route for the *Acanthamoeba* to the stroma, but the necessity of an epithelial break in induction of keratitis remains uncertain.

Whether *in vivo* a loose or a tight adhesion to the lens allows greater numbers of amoebae to be inoculated on the cornea is uncertain. The effects of lid movement or the tear film on adherence here demonstrated *in vitro* remain uncertain. Tightly adherent *Acanthamoeba* will not be washed away by lid movement or the tear film, but may not transfer to the cornea and cause keratitis.

Stern and Zam¹⁷ have demonstrated that effective enzymatic cleaning agents remove mucin coating of lenses such that the adherence of *Ps. aeruginosa* is inhibited. The complete removal of *Acanthamoeba* from lenses by use of lens cleaning agents in this study indicates that these compounds are effective for this purpose also. Clinical impressions suggest that many lens wearers default on regular use of disinfectant and cleaning agents. This report demonstrates a role for cleaning agents in prevention of *Acanthamoeba* keratitis.

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