THE PATHOGENESIS AND EPIDEMIOLOGY OF CONTACT LENS RELATED DISEASE IN COSMETIC CONTACT LENS WEARERS

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DECLARATION

Subject to the discretionary powers of the University Librarian, copies of parts of this thesis may be made for the purpose of personal study only.

ABSTRACT

A prospective case control study was performed to evaluate the relative risk (RR) and population attributable risk percentage (PAR%) for all exposures of microbial keratitis and for a range of contact lens (CL) related disorders. The study population comprised new casualty attenders at Moorfields Eye Hospital presenting between 22nd April 1988 and 21st April 1989.

CL wear for the correction of low refractive errors was found to be the major cause of new keratitis cases in this population. Compared to eyes with no predisposing condition, the RR of keratitis in CL wearers was found to be 80.2x (95% confidence limits were 38.5-166.9x) greater. The RR for trauma and previous ocular surface disorders were estimated at 13.9x (6.0-32.2x) and 7.4x (2.2-25.3x) higher respectively. The PAR% for CL wear was found to be 62%. Increased RR for CL wear compared with other exposures were maintained for all severities of keratitis and persisted despite controlling for age, sex and socioeconomic class.

Hydrogel CL's were found to account for 80% of all cases of lens related keratitis (n=60). The RR for EWSCL was found to be 20.8-36.8x higher than for a gas permeable lens (GPCL). The RR for keratitis in DWSCL was found to be 3.6- 4.1x higher.

1611 lens wearers were identified from 29,242 new casualty attenders. Lens related disorders were classified according to their probable pathogenesis. EWSCL were found to have the greatest overall risk for any complication occurring at 2.7x (1.73-4.16x) higher than for GPCL. The overall risk for DWSCL was found to be 1.3x (1.0-1.72x) higher than for GPCL. EWSCL showed the greatest risk for metabolic disorders and sterile infiltrates at 2.1-3.7x and 2.4-4.7x that of GPCL. DWSCL were found to have the greatest RR for toxic and hypersensitivity disorders at 5.8-5.9x that of GPCL.

Possible relevant factors in the pathogenesis of lens related keratitis were investigated. Bacterial adherence to unworn hydrogel lenses was demonstrated using a lens homogenisation and colony counting technique. Using this technique, significant numbers of viable organisms, adherent to worn hydrogel CL from wearers with CL related keratitis, were recovered. Bacteria enclosed in a polysaccharide-rich film were demonstrated on the back surface of an EWSCL using scanning and transmission electron microscopy.

KEY TO ABBREVIATIONS

BOZR	Back optic zone radius
cas	Keratitis cases
CL	Contact lenses
CLRRE	Contact lens related red eye
con	Controls
CPD	Critical point drying
DWSCL	Daily wear soft contact lens
EWSCL	Extended wear soft contact lens
GPC	Giant papillary conjunctivitis
GPCL	Gas permeable contact lens
HEMA	Poly-Hydroxyethylmethacrylate
HMDS	Hexamethyldisilazane
Misc	Miscellaneous
ml	Millilitres
OSD	Ocular surface disorders
PAR%	Population attributable risk percentage
PBS	Phosphate buffered saline
PMMACL	Polymethylmethacrylate contact lens
RCL	Rigid contact lens
r.p.m.	Revolutions per minute
RR	Relative risk
SCL	Soft contact lens
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
P. aeruginosa	*Pseudomonas aeruginosa*
P. fluorescens	*Pseudomonas fluorescens*
A. castellanii	*Acanthamoeba castellanii*
E. coli	*Escherichia coli*

CHAPTER 1. INTRODUCTION

1.1 SCOPE OF THE THESIS

Numerous complications associated with contact lens wear have been documented for cosmetic lens users. These include both short and long term disorders, the effects of which are likely to be dose related. Most disorders cause some degree of morbidity. Microbial keratitis is the most severe complication of lens wear, which can commonly cause a loss in vision. Most attention has been previously focussed on microbial keratitis due to it's potential for visual loss. However, less severe but more common complications of lens wear are still associated with significant morbidity, when factors such as hospital casualty or outpatient attendances, unscheduled practitioner visits and time off needed from work are considered. This thesis describes a case control study of lens related disorders in a group of wearers using lenses for the correction of low refractive errors. The objective was to evaluate the relative risks of different types of lenses and to estimate the population attributable risk percentages in different conditions. The study design also enabled other lens related risk factors, such as lens hygiene and lens age, to be determined.

Relative risks and population attributable risk percentages for all exposures resulting in microbial keratitis were estimated. This enabled the impact of contact lens wear on new keratitis cases in this casualty population to be evaluated. Previous studies have implied that 30% of new keratitis cases can be attributable to contact lens wear.

The second part of the study examined factors relevant to the development of microbial keratitis in lens wearers. This is an important area since keratitis has the potential to cause visual loss and the pathogenesis of this disorder is unclear. The epidemiology of *Pseudomonas aeruginosa*, which is the organism most frequently associated with lens related infections, was investigated in a group of lens wearers

with culture positive ulcers. Environmental samples were taken from the homes of these wearers and from a group of asymptomatic control wearers.

There has been increased reporting of *Acanthamoeba* keratitis amongst lens wearers, a study was therefore performed to evaluate the epidemiology of *Acanthamoeba*. Environmental samples from the domestic water supply, domestic drains and dust were analsyed.

Bacterial adherence to hydrogel lenses has been suggested as a possible initial stage in the pathogenesis of lens related infections. Bacterial adherence in vitro was evaluated using a lens homogenisation and bacterial culture technique. These techniques were evolved to examine bacterial adherence with respect to time, and to compare adherence to ionic and non-ionic lens materials. Similar techniques were subsequently used to evaluate material collected from wearers with lens related keratitis.

Lenses and lens cases from wearers with lens related keratitis, were evaluated by quantitative bacteriology, scanning and transmission electron microscopy, in order to assess possible formation of a bacterial biofilm on these surfaces. Bacterial adherence to lenses or lens cases, with subsequent colonisation and formation of a protective bacterial biofilm, has been implicated as a possible step in the development of keratitis. The lens may act as a potential delivery system for a large inoculum of organisms to the cornea. Formation of such a bacterial biofilm has important implications for the safety of lens hygiene regimes.

1.2 COMPLICATIONS OF CONTACT LENS WEAR

During the past decade there has been a large increase in the numbers of contact lens wearers. Lenses are currently worn by approximately 3% of the population in the UK[1] (data from MORI survey for the Association of Optical Practitioners, 1988). Market statistics[2] have shown that between 1982 and 1988 the number of lenses dispensed has increased threefold and there have been changes in the

trends of lens use (Figure 1.1).

Coincident with the rise in numbers of lens wearers, has been increased reporting of complications, particularly microbial keratitis, associated with lens wear for the correction of low refractive errors. A literature search during the period of 1986 to 1989, performed using Medline, Excerpta Medica and Contact Lens Update, confirms (Table 1.1) this increased reporting of microbial keratitis in cosmetic lens wearers.

TABLE 1.1

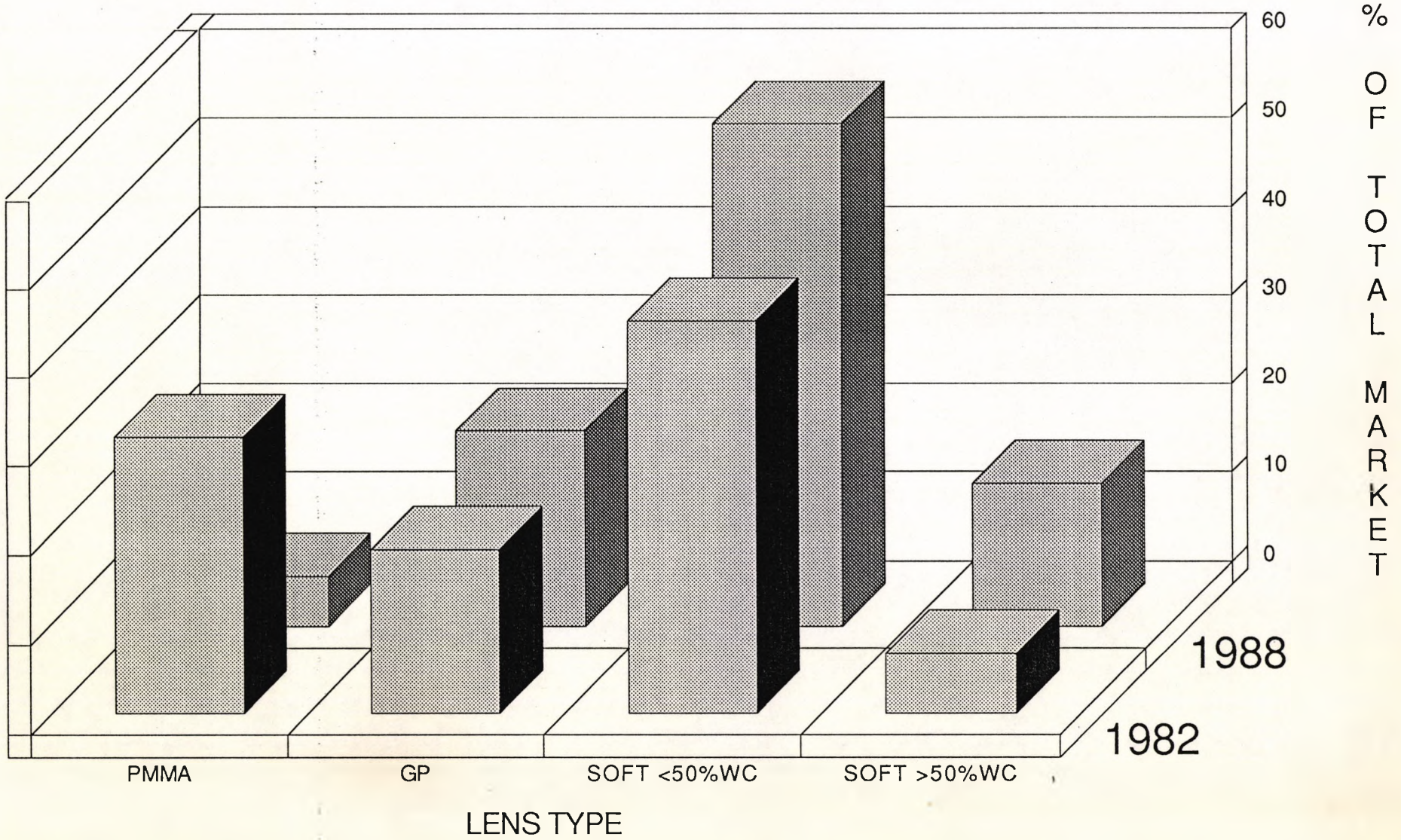
1986	5 Reports	111 Cases
1987	11 Reports	145 Cases
1988	11 Reports	121 Cases
1989	15 Reports	479 Cases

Descriptive studies have also implied that the risk of complications differs with different lens types[3-8]. Information has rarely been collected on the lens wearing population at large, hence quantifying the risks has not been possible, and comparisons between studies cannot be made.

Cohort studies of lens wearers have been largely innappropriate for the estimation of incidence of rare complications of lens wear[9-13], and bias has existed where wearers have been carefully selected and monitored[14-16]. Many of these studies of cosmetic lens users have been biased towards infections and severe complications in lens wearers, rather than other, less severe complications of lens wear. Although these other more common complications are rarely sight threatening, they involve significant morbidity in lens users since large numbers of wearers are affected.

Retrospective studies have shown a high rate of red eyes and corneal staining amongst extended wear soft lens (EWSCL) users[14,16], at 24-27% and 11-17% respectively. However, a lower overall rate of complications estimated at 13.1% in

FIGURE 1.1 UK MARKET ANALYSIS BY LENS TYPE FOR 1982 AND 1988



successful EWSCL users, has been shown[11]. A comparative study of complications associated with cosmetic soft contact lens (SCL) use, estimated that the overall rate of complications in EWSCL users was 50% compared with 32% in daily wear soft lens (DWSCL) users[12]. A large retrospective Japanese study on 66,218 patients[5], demonstrated a significantly lower complication rate amongst rigid lens (RCL) wearers compared with SCL wearers. Complications amongst RCL wearers were less severe and required a shorter healing time, compared with SCL related complications. This study made no distinction between DWSCL and EWSCL. Similarly, a lower rate of complications was found amongst RCL wearers (8%) compared with DWSCL wearers (41%) and EWSCL wearers (43%) in a Swedish study of 224 wearers[7].

Such retrospective studies and case reports have identified a need for prospective epidemiological studies to establish the risks associated with lens wear. The influence of other risk factors such as lens hygiene, lens age and wearing schedule also needs to be evaluated.

Few prospective epidemiolgical studies on wearers using lenses for the correction of low refractive errors have been performed. Prospective case studies carried out in the USA[17] on 137 lens wearers, and in Singapore[18] on 147 lens wearers have both confirmed a higher incidence of severe complications with hydrogel lenses, particularly EWSCL. Rigid lenses were found to have a higher incidence of corneal abrasions.

A pilot case control study was performed at Moorfields Eye Hospital[19], to determine the relative risks for different lens types for a range of lens related disease. Over a 3 month period, 393 wearers were identified. EWSCL users were found to have the highest relative risk for any complication occurring, at 2.2x that of gas permeable lens (GPCL) users. This pilot study demonstrated that a larger prospective study carried out at this centre would be feasible, and that sufficient

numbers of lens wearers would attend to allow relative risks to be estimated for more rare complications in addition to common disorders. A case control study design would allow high risk lenses to be identified for specific lens related complications, and would identify additional risk factors for each complication.

This information is relevant to practitioners involved in the fitting of lenses, and in the management of lens related disease. Quantification of the varying risks between lens types is important in suggesting strategies to avoid complications, and is expected to increase understanding of the pathogenesis of specific diseases occurring in association with different types of lens.

1.2.1 Microbial Keratitis in Contact Lens Wear.

Microbial keratitis represents the most severe complication of contact lens wear, due to its potential to cause visual loss by corneal scarring and perforation. Prior to the widespread use of contact lenses, microbial keratitis was rare in normal healthy eyes. Predisposing factors include:

1. Pre-existing ocular surface disorders, such as bullous keratopathy, dry eyes or herpetic eye disease[20,21].

2. Corneal trauma or surgery[22].

3. Contact lenses worn for the correction of aphakia[23-25], or for bandage or therapeutic indications[26- 29].

Contact lens wear as an alternative to spectacles in healthy eyes, has become one of the major predisposing factors for microbial keratitis. The proportion of ulcers attributed to contact lens wear has risen to 30% in major centres[30-33].

Descriptive studies have also implied that the risk of microbial keratitis differs for different lens types. There has been considerable anecdotal and circumstantial evidence from hospital based studies, to suggest that the risk of keratitis is higher with EWSCL[6,30,31,32,34-47]. A review of accumulated case reports[48], has indicated that cosmetic lens wearers are at risk and particularly those wearing

EWSCL.

Cohort studies have been largely inappropriate for the estimation of the incidence of microbial keratitis in cosmetic lens wearers. Since the disease is rare, such studies require a large and often unmanageable cohort of wearers. A study of lens wearers in Japan[5] reported on 66,218 lens wearers, of these 7 infections occurred in 42,721 eyes wearing hydrogel lenses, compared with 4 infections in 61,562 eyes wearing polymethylmethacrylate (PMMA) lenses.

One group for which this type of study has been carried out sucessfully is aphakic lens wearers. The incidence of microbial keratitis amongst aphakic EWSCL users has been estimated at between 1-3% per year[49,50,24]

Prospective epidemiological studies carried out in the USA have confirmed evidence from descriptive studies, that the incidence of microbial keratitis associated with contact lens wear, has increased significantly over the past 5 years. The incidence of microbial keratitis in EWSCL users in the USA has been estimated at between 1 in 300 and 1 in 450 per year[51]. This annualised incidence is based on data collected over a four month period, from June to September inclusive[52]. However, a seasonal variation in the number of lens related ulcers has been documented[53], with a higher proportion of ulcers presenting during the summer months.

As the penetrance of lens wear into the population increases, this low level of risk still represents significant numbers of healthy eyes exposed to corneal infections. Pattern of lens wear has been identified as a major risk factor in microbial keratitis. Overnight wear poses a 10-15x higher risk of infection compared with strict daily wear, despite reduced lens handling[54]. Descriptive studies have suggested that DWSCL use poses a higher risk compared with RCL use[48], but prospective studies carried out to date have not been able to validate this.

Most epidemiological data have been derived from studies in the USA and these results are not necessarily applicable in the UK. Recent studies have identified a need for prospective epidemiological studies of microbial keratitis and other complications, in wearers using lenses for the correction of low refractive errors in the UK. Rigid lens wearers account for a larger proportion of the market in the UK, compared with the USA, such that it would be possible to quantify more accurately the risks associated with rigid lens wear.

1.2.2 The Spectrum of Microbial Keratitis.

1.2.2 (i) Bacterial Keratitis.

The spectrum of bacteria associated with lens related disease differs significantly from that associated with non-lens related disease. *Pseudomonas aeruginosa (P. aeruginosa)*, a Gram negative rod, is frequently associated with soft contact lens related infections[41,45,6], although other Gram negative rods such as *Serratia species*[35,55], *Proteus species*[31] and other *Pseudomonas species*[37], have been reported. Of the Gram positive organisms, *Staphylococcus species* are the most prevalent[30,35,45].

In non-lens related keratitis, Gram positive species such as *Staphylococcus species* and *Streptococcus species* predominate, followed by *P. aeruginosa* and other Gram negative organisms[22,56]. In several hospital series[32,46,47], *P. aeruginosa* has been isolated as the causative organism in 70% of all culture positive cases of lens related infections.

P. aeruginosa infections have been associated with all types of lenses, although the majority have been associated with hydrogel, particularly with EWSCL[6,30,39-45,57]. There have been several recent reports of *P. aeruginosa* keratitis associated with disposable EWSCL[58-61] and one report associated with rigid GP extended wear[62].

Table 1.2 shows a summary of reports of cosmetic lens associated *P. aeruginosa*

TABLE 1.2

Summary of Reports of Cosmetic Lens Associated Pseudomonas aeruginosa Keratitis.

Author	Year	EWSCL	DWSCL	PMMA/GPCL
Golden et al[34]	1971	-	-	1
Krachmer et al[36]	1978	-	2	-
Wilson et al[37]	1981	-	4	-
Sjostrand et al[38]	1981	1 (Bilateral ulcers)		-
Adams et al[39]	1983	3	-	-
Hassman et al[40]	1983	1	-	-
Galentine et al[30]	1984	3	5	1
Weissman et al[41]	1984	6	-	-
Patrinely et al[42]	1985	4	-	-
Donnenfield et al[43]	1986	11	5	-
Mondino et al[44]	1986	7	2	-
Ormerod et al[45]	1986	5	5	1
Spindel et al[6]	1986	1	-	-
Schivitz[57]	1987	1 (Bilateral ulcers)		-
Dunn et al[58]	1989	1 (Disposable EWS)		-
Kent et al[59]	1989	2 (Disposable EWS)		-
Killingsworth et al[60]	1989	1 (Disposable EWS)		-
Sawusch et al[61]	1989	1 (Disposable EWS)		-
Ehrlich et al[62]	1989	1 (GP Extended Wear)		-

keratitis.

1.2.2 (ii) Fungal Keratitis.

A review of fungal keratitis in lens wearers[63], has shown that fungal infection in cosmetic lens wearers is rare, and is more likely to be due to filamentous fungi than yeasts. Fungi have been isolated from lens storage cases in asymptomatic wearers using rigid lenses[64] (3%) and hydrogel lenses[65,64] (8-14%). Fungal contamination of hydrogel lenses has been reported in several studies, and the incidence of lens contamination has been estimated at 2-5%[66]. The adherence of *Candida albicans* to hydrogel lenses in vitro has been shown to be enhanced by tear components[67], which may be a factor in the pathogenesis of lens related infections.

1.2.2 (iii) *Acanthamoeba* Keratitis.

Acanthamoeba keratitis is a rare but potentially blinding corneal infection, previously associated with corneal trauma. Increasing numbers of case reports from the USA and Europe have been published recently and the majority of cases have been in association with contact lens wear[68,69]. Two hundred and five cases were reported in the USA between 1973 and 1988[68], 85% (160/189) of cases for which full information was obtained, wore contact lenses. *Acanthamoeba* keratitis has been associated with all types of lens wear[70-72], although it has been most commonly reported in SCL wearers using home made saline[70,73]. Three recent cases have been reported associated with disposable EWSCL wear[74,75].

Acanthamoeba is a ubiquitous, free living, dimorphic organism, isolated from a variety of environmental sources[76]. These include well water, fresh water, chlorinated water, tap water, hot tub water[77], air[78] and soil. *Acanthamoeba* species have been isolated from domestic water supplies in Strasbourg[79]. The genus *Acanthamoeba* has been divided into 3 major morphological groups which have been confirmed serologically[80]. Group II species including *Acanthamoeba (A.) castel-*

lanii, *A. polyphaga* and *A. rhysodes*, have been implicated in contact lens related keratitis.

The source of the organism in contact lens wearers remains unclear, although a study of lens case contamination in asymptomatic wearers has identified Group II *Acanthamoeba* species in 7% of lens storage cases[81]. The presence of bacteria and fungi found in association with *Acanthamoeba*, suggests that these organisms may be important in the survival and growth of amoebae[81,82].

Acanthamoeba, particularly in their cystic form, have been shown to be resistant to several contact lens disinfection systems. Two recent studies[83,84] have shown that trophozoites and cysts from several corneal isolates of *A. polyphaga* and *A. castellanii* were able to survive the recommended disinfection times for a range of cold chemical systems. Studies by Ludwig et al. in 1986[85], and Sylvaney et al. in 1988[86], showed that the efficacy of disinfection systems was species dependent. Heat disinfection was effective against trophozoites and cysts from all species[85]. The efficacy of disinfection systems was investigated using both new and worn hydrogel lenses contaminated with *Acanthamoeba*[87]. Thermal disinfection was found to be effective in eradicating *Acanthamoebae*, but quaternary ammonium disinfection and hydrogen peroxide disinfection were found to be ineffective.

Another factor possibly associated with the pathogenesis of lens related *Acanthamoeba* keratitis, is the adherence of organsims to the surface of contact lenses. Cysts and trophozoites of *A. castellanii* and *A. polyphaga* have been shown to adhere to the surface of new hydrogel lenses[88,89] and to worn lenses[87].

1.2.3 The Pathogenesis of Bacterial Invasion of the Cornea.

Bacterial infection of the cornea requires an inoculum of organisms which must adhere to the ocular surface; colonisation and subsequent invasion of the site may then occur[90]. Possible sources of organisms in contact lens wearers will be discussed in Section 1.2.5. Animal models have implied that corneal epithelial trauma

is an important prerequisite for bacterial adherence [91-93]. *P. aeruginosa* has been shown to adhere preferentially to damaged corneal epithelial cells in mouse [92] and rabbit models[94]. Stern et al. in 1985[95] used electron microscopy to monitor the early events in the infection process in a rabbit model. Interaction occured between the bacterial cell membrane and the damaged or exposed basal epithelial cell membrane, resulting in irreversible adherence.

Bacterial adherence to mucosal surfaces is mediated by interaction between bacterial adhesins (specific bacterial sites for adherence) and mucosal cell receptors (complementary host site for adherence). Bacterial adhesins have been demonstrated to be pili for certain species of *Pseudomonas*. Pili are flexible polar filaments comprised of a single protein; pilin[96]. In vitro adherence of *P. aeruginosa* to human buccal epithelial cells[97,96], has been shown to be mediated by pili. Similarly, in vitro adherence of *P. fluorescens* to human corneal epithelial cells was shown to correlate with bacterial pili[98]. Ramphal et al. in 1984[99] showed that bacterial adhesins differ for mucoid and non-mucoid strains of *P. aeruginosa*. Pili were found to mediate adherence for non-mucoid strains to injured mouse tracheal epithelial cells, but that the mucoid exopolysaccharide may be the adhesin for mucoid strains of the organism [100].

Mucosal cell receptors for Gram negative organisms are likely to be composed of either single, or complex carbohydrates in the cell membrane [101]. The tracheal epithelial cell receptor (host site for adherence) for *P. aeruginosa*, is thought to be a sialic acid moiety in the mouse model [102]. Similarly, a sialic acid moiety has been proposed as the corneal epithelial receptor in an immature mouse model [103]. The epithelial cell receptor in the traumatised adult mouse cornea has been shown to be N- Acetylmannosamine [104]. Mannose has been shown to mediate the adherence of *Escherichia coli (E. coli)* to human mucosal epithelial cells [105]. Carbohydrate moieties are present on all epithelial cell membranes, but *P. aeruginosa* will only adhere to damaged cells. It may be that trauma exposes these specific recep-

tor sites.

Fibronectin is a cell surface glycoprotein produced by fibroblasts, which plays a role in wound healing. This may inhibit bacterial adherence to human cells. Fibronectin is known to modulate bacterial adherence to epithelial cells in the respiratory tract. Loss of fibronectin, as a result of increased levels of salivary proteases, has been shown to cause increased bacterial adherence to buccal epithelial cells in debilitated patients[97]. Similarly, normal buccal cells treated with trypsin showed reduced surface fibronectin and increased bacterial adherence[106]. It has been postulated that contact lens wear may reduce corneal cell surface fibronectin and facilitate bacterial adherence[107]. However, the presence of fibronectin has not yet been demonstrated on intact superficial corneal cells or in tears.

Once adhered, bacteria colonise the epithelial surface and appear to be engulfed by epithelial cells and reach the corneal stroma. Stromal inflammation and destruction is caused by enzymes secreted by the bacterial cell membranes. *P. aeruginosa* produces rapid stromal necrosis by the release of extracellular enzymes, such as Exotoxin A[108,109], other proteases, haemolysins and toxins[110,111]. Enzymes or toxins are thought to cause damage to the stromal keratocytes and to degrade the collagen and proteoglycan ground substance[112].

1.2.4 Clinical Features of Microbial Keratitis.

Clinically, microbial keratitis is characterised by a greyish-white sub-epithelial and deeper stromal infiltrates (collections of leucocytes, migrating to the site of the lesion) and usually an overlying epithelial defect. In the case of sterile corneal infiltrates, or early infective keratitis, the epithelium may be intact[113]. Sterile infiltrates may be of toxic, allergic or microbial origin. These are usually small (<2mm), peripheral lesions, not associated with severe pain or anterior chamber reaction[114]. Larger, central lesions associated with pain, anterior chamber activity and hypopyon are more likely to be infective. However, there is likely to be con-

siderable overlap between these conditions. Most hospital based series have shown a culture positive rate of 60% for suspected microbial keratitis[30,31,21] with 40% of ulcers clinically appearing to be microbial and being treated as such, but from which no organism can be recovered. A culture negative result is strongly associated with previous antibiotic therapy[41,21], or may represent an early stage in the disease process.

The epithelium and stroma may be oedematous, with folds in Descemets layer often appearing to radiate from the ulcer site. Fibrinous endothelial plaques may form, and conjunctival hyperaemia with limbal vessel engorgement occurs. Often there is considerable anterior chamber activity with a hypopyon present. A mucopurulent discharge is seen, and in Gram negative keratitis, particularly that caused by *P. aeruginosa*, mucous may be adherent to the epithelial defect, with a surrounding circular infiltrate[56]. Left untreated, extracellular enzymes destroy the stromal ground substance causing the cornea to melt. Perforation can occur rapidly; *P. aeruginosa* can penetrate a cornea in less than 24 hours.

P. aeruginosa ulcers may also be associated with a ring shaped stromal infiltrate. This is thought to be caused by a type of immune response to bacterial toxins[115,116]

Figure 1.2 and 1.3 show a *P. aeruginosa* ulcer in an EWSCL user.

1.2.5 Factors Predisposing to Keratitis in Contact Lens Users.

The non-lens wearing eye is resistant to bacterial invasion due to a combination of the blinking action and washing effects of the tears, an intact epithelial layer with tight cell junctions, the antibacterial effects of tear lysozyme and the phagocytic functions of the conjunctival mucosal and polymorphonuclear cells.

Contact lenses may increase the susceptibility of the cornea to infection in several ways:

Figure 1.2 and Figure 1.3

These demonstrate presumed microbial keratitis in an EWSCL wearer. No organisms were isolated from the ulcer, the lens storage case or any of the lens care materials. Lenses were worn on a weekly basis, consisting of 5 consecutive days of lens wear and 2 lens free days. The ulcer developed within 1 day of lens insertion and the patient, a 20 year old myope, was admitted for 1 week and was treated with intensive topical gentamicin. Lens hygiene was reportedly carried out according to practitioner instructions. Surfactant cleaning and chemical disinfection were performed weekly using fresh solutions, which were less than 28 days old. Enzyme cleaning was performed fortnightly, and the Hema lenses were 8 months old.

The photographs both with and without fluorescein show the extent of the excavated but resolving central epithelial defect with a dense stromal infiltrate and associated oedema. Two months later, with residual central scarring, the visual acuity was measured at L. 6/12 with spectacle correction. The clinical picture in this case with the large central lesion resolving to dense scarring, deep excavation and anterior chamber activity is strongly indicative of a classic microbial lesion, despite no organisms being recovered.

Figure 1.2 Resolving lesion shown with fluorescein staining.

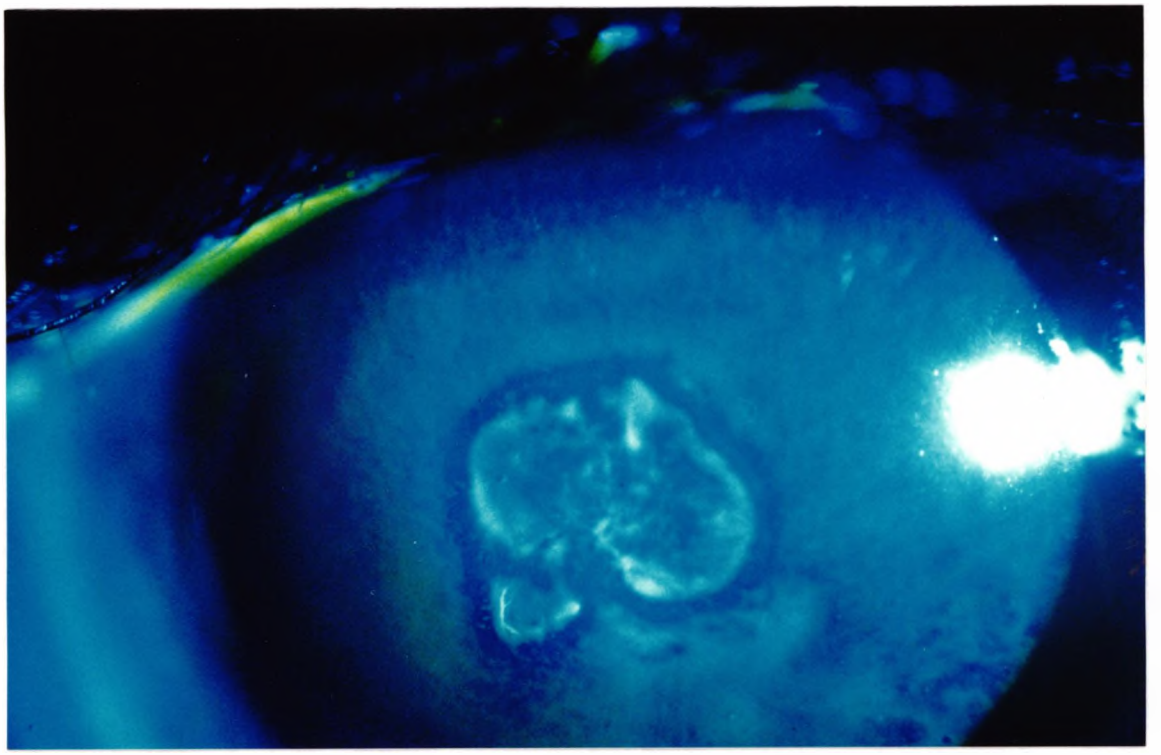
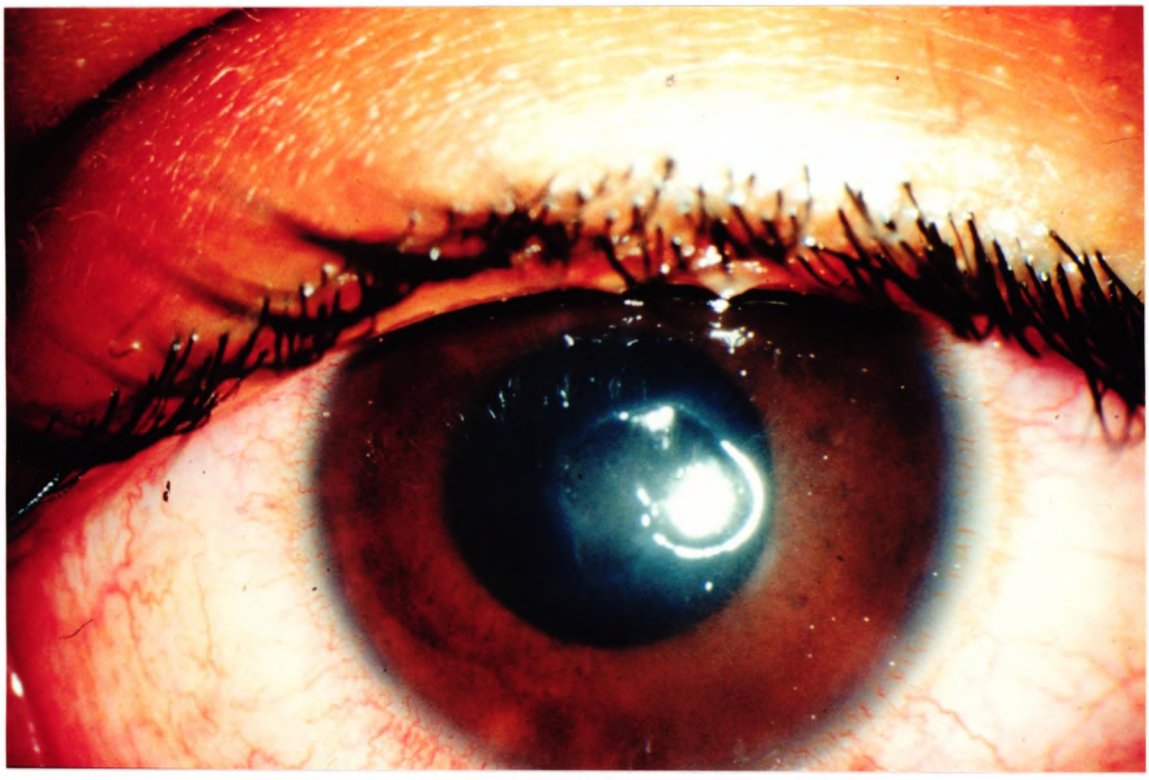


Figure 1.3 Resolving lesion shown without fluorescein staining.



1. Epithelial Trauma.

Physical trauma may arise through lens insertion or removal, poor lens fitting, poor lens edge or surface, from foreign bodies trapped under the lens or from deposits on the lens. Epithelial stress during contact lens wear is common with all types of lenses and may manifest itself as punctate staining. Lamer in 1983[14], estimated that 17.5% of EWS users had punctate staining.

Ultrastructural alterations have been demonstrated in both animal models and in human lens wearers. Specular microscopy has demonstrated slowed epithelial cell turnover under hydrogel extended wear lenses in humans[117]. Studies on a rabbit model of hydrogel lens wear have shown reduced basal cell mitosis[118] and increased surface cell desquamation with a loss in surface microvilli[119]. Such minor trauma to the cornea during lens wear may prevent the epithelium from fulfilling it's normal barrier function to microorganisms.

2. Hypoxia.

Contact lens wear induces relative corneal hypoxia compared with the non-lens wearing state, which will be compounded by the closed eye situation in overnight lens wear. This hypoxic state can result in a fragile epithelium, which may be predisposed towards tiny epithelial defects. Hypoxia is thought to suppress aerobic epithelial metabolism and hinder epithelial growth, causing thinning[120,121]. Compromised epithelial attachment has been readily demonstrated in the cat. The epithelium becomes readily detatched from the basement membrane in eyes exposed to extended wear of lenses, compared with the normal eye[122].

Epithelial microcysts are seen in 85-100% of EWS lens users[120,123-125] and are thought to arise due to reduced aerobic metabolic activity[120]. Their presence, particularly in large numbers, implies impaired epithelial metabolism.

It has been suggested that these alterations due to hypoxia, result in a more fragile, traumatised epithelium with reduced barrier function, which is more susceptible to infection.

3. Disturbance of Normal Tear Flow

Contact lenses disrupt normal tear flow dynamics and normal lid/tear resurfacing mechanisms. This may affect the clearance of adherent bacteria from the corneal surface. Contact lens wear may result in altered blink rates, partial blinking, tear stasis and reduced tear exchange beneath lenses. A build up of trapped cellular and metabolic debris, may lead to an inflammatory response[126,127]. Changes in levels of tear proteins, especially lysozyme, with lens wear[128] may affect the anti-microbial efficacy of the tears.

4. Toxic Epithelial Disturbances

Contaminated contact lenses may absorb bacterial enzymes and toxins[129], which may chemically injure the cornea and render it more susceptible to infection. Similarly preservatives may become concentrated within the lens itself or by adsorption onto its surface[130]. This may cause epithelial damage either by a hypersensitivity[131] or toxic response.

5. Other Factors

Contact lens wear causes a temperature increase at the corneal surface due to the insulating effect of the contact lens[132,133]. Bacterial propagation may be enhanced due to this raised temperature between the lens and cornea. However, ef fects of lens related temperature and pH changes on bacterial invasion have not been investigated.

Osmotic oedema, due to either hypotonicity of tears in the closed eye situation or during adaptation to lens wear, will cause the epithelium to become more fragile as the cells become less tightly packed.

Extended wear of lenses may allow a greater opportunity for colonisation of the lens surface by micro-organisms, during the interval between disinfection cycles. A preliminary study, performed on a limited number of lenses worn under closed eye conditions, has demonstrated a higher bacterial count compared with lenses worn under daily wear conditions[134].

1.2.6 Sources of Bacteria.

Contact lens wear alone is thought not to modify the spectrum of organisms which can be recovered from the normal non-lens wearing conjunctiva[135-138]. Common conjunctival bacteria rarely cause infection in normal eyes. Gram negative pathogens are rarely isolated from the conjunctiva of asymptomatic wearers[139], hence other sources of organisms are likely. Bacteria may be spread from other regions in the body, such as the upper respiratory tract[56], the skin or faecal contamination.

There is undoubtedly a link between contaminated lens care material (lenses, lens storage cases and lens solutions) and corneal ulcers. Home prepared saline for heat disinfection, found to be contaminated with *P. aeruginosa*, has been implicated in the development of lens related keratitis[35,56]. Mayo et al. in 1986[140] and 1987[141], have confirmed using biotyping techniques, that identical organisms have been isolated from home prepared saline and corneal ulcers. However, significant bacterial contamination of lens care materials, particularly lens cases, occurs in approximately 50% of asymptomatic wearers[64,65,81,138,142,143] It does appear however, that *P. aeruginosa* is rarely found in lens care material from asymptomatic lens users. Lens care systems have reportedly been contaminated despite proper use of hygiene systems[64]. Lens care material contamination cannot explain the greater risk of microbial keratitis with EWSCL, since extended wear would involve less exposure to this source of organisms. Half of EWSCL users with microbial keratitis are found to have sterile lens care materials[144,33]. Clearly, other sources of bacteria are important in the pathogenesis of lens related keratitis.

P. aeruginosa is a ubiquitous Gram negative organism with the ability to survive in water and other moist environmental areas, including soil and vegetation[145]. It is able to metabolise a wide variety of organic compounds[145]. The external environment may cause contamination either via the lens case, other lens care materi-

als, eye drops or make-up[146]. Most lens case contamination is thought to arise from the fingers when inserting and removing the contact lens from the storage case. There is a well established link between ulcers in EWSCL users and recent lens manipulation[39,30]. Other routes for environmental contamination arise from the fingers when inserting the lenses[35,39,30], by rubbing the eyes or from airborne contaminants.

1.3 THE ROLE OF BACTERIAL ADHERENCE TO LENSES IN LENS RELATED KERATITIS

Bacterial adherence to a surface is dependent on both bacterial components and surface characteristics. Several forces interact to bring about permanent or temporary adhesion. These include Van der Waals forces, electrostatic forces of repulsion, covalent and hydrogen bonds, dipole forces and hydrophobic interactions. The effects of these different factors have been reviewed by Marshall in 1985[147].

Animal models and in-vitro studies have confirmed that viable bacteria can actively adhere to new and worn, rigid and hydrogel lenses. Subsequent to this initial adherence, bacteria are able to colonise the contact lens surface, with the production of a polysaccharide-containing glycocalyx. Glycocalyx-enclosed microcolonies form a biofilm, which may also contain material derived from host and exogenous origins. This biofilm encloses the bacteria, providing a favourable microenvironment for bacterial propagation and protection from host defences[148]. Part of its function may be in the continued adherence of bacteria to the substratum, and the biofilm itself may allow subsequent corneal inoculation with bacteria in the event of an epithelial defect. Clusters of bacteria may slough off the bulk of the biofilm layer on the posterior surface of the lens onto the cornea, which may predispose the cornea to bacterial infection.

Scanning electron microscopy has demonstrated that bacteria can adhere to the surfaces of both worn and unworn hydrogel lenses and adherence to new lenses is

thought to be an active, time dependent process[149] with killed or damaged bacteria adhering less[150]. Worn lenses become coated soon after insertion with a complex mixture of organic and inorganic materials derived both from host and exogenous sources[151]. Several workers have concluded that tear components particularly mucin, may facilitate bacterial adherence and subsequent colonisation of lens surfaces[152,153]. Formation of this coating is likely to be time dependent and extended wear of lenses is likely to develop a thicker coating, which may enhance bacterial adherence. The effect of enzyme cleaning on bacterial attachment to mucin coated lenses in vitro, has been investigated using electron microscopy[154]. Enzyme use was found to reduce bacterial adherence to these coated lenses, although this was not investigated for worn lenses.

In vitro studies using electron microscopy[153] and quantitative scintillation counting of organisms[155], have investigated bacterial adherence to new hydrogel lenses coated with various tear components. It was concluded that mucin may facilitate the adherence of *P. aeruginosa* to lenses. Using similar radiolabelling techniques, worn lenses, particularly those with visible surface deposits, have shown significantly greater numbers of adherent organisms than new lenses[156,157]. Bacterial adherence demonstrated using this technique was found to be reduced when lenses were bathed in a sialic acid solution[157]. This was felt to support previous evidence that sialic acid, which is a major sugar component in mucin[158], is a receptor for *P. aeruginosa*. The presence of sialic acid on worn EWSCL has been demonstrated using specific lectins to identify sugar components contained in glycoproteins such as mucin[159]. This is likely to be relevant in adherence to worn lenses. However, *P. aeruginosa* will also bind to unworn lenses without sialic acid, hence other binding sites on the plastic surface must exist. These in vitro studies have used bovine sub-maxillary gland mucin, which is similar to, but not identical to human ocular mucin. In addition, these studies have not examined the combined effects of mucin with other tear components.

Lens coatings formed in vivo were found to behave less predictably in another study; both enhanced and reduced adherence were seen[155] when adherence of bacteria to worn lenses was compared with new lenses in different individuals. A quantitative colony counting technique for bacteria adherent to lenses in vitro has shown no preferential bacterial adherence to deposits or surface defects on previously worn lenses compared with new lenses[160]. This method assessed the bacterial viability of adherent organisms (i.e. those not removed on rinsing), which allows differentiation from dead organisms or bacterial residues. This is important, considering that viable organisms are more likely to be significant in the subsequent development of infection.

Studies examining adherence to worn lenses have been criticised, since the amount and composition of the formed deposits is unknown and uncontrolled. However, these studies have reflected the situation during wear of lenses.

Bacterial adherence is also likely to be material dependent. Bacteria have been shown to adhere in greater numbers to non-ionic hydrogel materials compared with ionic materials, irrespective of the nominal water content of the material[161]. Greater adherence has been shown to unworn rigid lenses, compared with unworn hydrogel lenses[160]. For worn lenses, the in vitro bacterial adherence has been shown to be greater with hydrogel compared with rigid materials, although there appears to be wide variation amongst individuals[162].

There is considerable controversy as to whether greater numbers of bacteria adhere to worn lenses compared with unworn lenses. Certainly, the application of pure solutions of mucin and other human tear components in vitro appear to increase bacterial adherence to lenses. However, adherence to worn lenses seems to be less predictable. Comparison between different studies is difficult, since different techniques have been used to quantify adherent bacteria. These include:

a. Electron microscopy, which poses problems with the processing of lenses. Bacteria may separate from the lens during the dehydration process, artefacts may

be introduced, bacteria may adhere selectively to different areas of the lenses and the technique cannot differentiate between viable adherent bacteria and dead cells or bacterial residues.

b. Scintillation counting of radiolabelled bacteria seems to produce variable results. It is a difficult technique and also cannot differentiate between viable and non-viable bacteria.

c. A pour plate agar counting method[160] is effective for small numbers of bacteria. It relies on colony counting and is less accurate for larger numbers of bacteria. This agar sandwich technique does not provide adequate conditions for aerobic bacteria.

Differences between studies may also arise from different strains of bacteria used. Strains of bacteria vary in tissue adherence characteristics[99] and pathogenicity[163,164]. Variation in adherence properties between different species and strains of organisms to contact lens materials may account for the different spectrum of causative bacteria in lens related infections.

A recent study investigated the adherence of different strains of *P. aeruginosa* to new hydrogel lenses[165]. These results showed that strains isolated from corneas (from lens and non-lens related keratitis cases), adhered in greater numbers, compared with strains isolated from other body sites.

Different types of hydrogel materials used in different studies may have different adherence properties. Adherence is dependent on material, ionic charge, water content, chemical composition and monomer contamination. Finally, in-vitro findings may not accurately represent the situation in-vivo, due to the presence of a biofilm derived from host, bacterial and exogenous components.

1.4 THE RELATIONSHIP BETWEEN BACTERIAL ADHERENCE AND BIOFILM FORMATION

Bacteria in most natural ecosystems are surrounded by a polysaccharide-containing matrix of fibres (glycocalyx), outside the cell wall[166]. The glycocalyx mediates the formation of microcolonies which constitutes the predominant mode of growth. Microcolonies can adhere either to inert substrata or living cells by the interaction of the glycocalyces, or pili, with the surface components[167]. Colonisation of a surface, whereby adherent bacteria persist on a surface, is likely to be facilitated by glycocalyx enclosed microcolonies. The bulk of organisms within a microcolony will remain adherent, but swarmer cells are released from the body of the film to colonise adjacent sites.

This feature of bacteria existing in microcolonies, appears to be an important step in the development of certain infections[168,169]. Colonisation of biomaterials[170] such as heart valves, catheters and artificial joints has been implicated in biomaterial mediated infections. The formation of *P. aeruginosa* microcolonies has been suggested as a factor in the resistance of this organism to specific antibodies and antibiotics[171]

Bacterial adherence to, and subsequent colonisation of lenses has been described as; 'irreversible sorption - a time dependent process facilitated by the synthesis of an extracellular polymeric material that bridges the bacterial and substratum surface'[161]. Several studies have described an amorphous material produced by bacteria visible on lenses[149,152,172]. Bacterial adherence and subsequent colonisation of the surface involves the formation of a biofilm. This is likely to be derived from both organism and host factors. Bacteria are embedded within this polysaccharide-containing film which seems to facilitate adherence to the substratum and provides a micro-environment in which organisms are able to survive. Cytochemical staining techniques[172] have demonstrated that the film is of bacterial origin, although the 'in-use' biofilm is likely to be a complex mixture of host

and bacteria derived material. A bacteria-containing biofilm has been demonstrated in humans, having developed on an extended wear soft contact lens from a patient with *P. aeruginosa* keratitis. This has been found to correlate with in vitro models[173].

Bacteria enclosed within a biofilm produced on hydrogel lenses in-vitro have been shown to be significantly more resistant to antimicrobials compared with a similar number of bacteria in solution[174]. Currently, the antimicrobial efficacy of contact lens disinfection regimes in the UK is assessed using free bacteria in solution. It appears that the bacterial biofilm on worn contact lenses or on contact lens storage cases would provide a much greater challenge to these solutions. This may be a more appropriate method for assessing the efficacy of disinfectants.

Several in vivo animal models of lens related keratitis, have shown that bacteria inoculated onto a lens wearing eye establish a bacterial biofilm. This biofilm increases in thickness with time, and it's formation appears to be necessary for sucessful colonisation of the contact lens surface.

Using a rabbit model, it has been shown that bacteria inoculated into a non-traumatised, non-lens wearing rabbit eye, were cleared from the ocular surface within 4 hours[175]. A contact lens in-situ did not appear to enhance bacterial adherence to the cornea. However, the numbers of bacteria recoverable from the lenses throughout a one week wearing period, increased progressively. The presence of a polysaccharide-rich film was demonstrated on all lenses, but was thicker in the presence of bacteria, which were enveloped in the material. Similar findings were reported using a monkey model of lens wear[176]. In all eyes, the corneal surfaces showed changes after lens wear, but no epithelial breaks were reported. Lid suturing in a rabbit lens wearing model[177], to simulate the closed eye situation in extended wear, was found to be associated with the development of keratitis. Eyes with a tarsorrhaphy and an equivalent direct bacterial inoculum

did not develop keratitis[178]. Bacterial keratitis was only found to arise in one study[177], if worn rabbit lenses were incubated with bacteria and then reinserted. New lenses, incubated and inserted, did not result in keratitis. This feature was not reported in a more recent study[178], where both new and worn contaminated lenses caused keratitis.

Animal studies have reported biofilm on contact lenses but not on animal corneas. No cases of keratitis resulted from colonised lenses unless the cornea was put under gross hypoxic stress. This would suggest that a large inoculum of organisms on the contact lens does not necessarily cause corneal infection, unless an epithelial breach occurs, providing a favourable environment for bacterial invasion. Further support for this hypothesis has been demonstrated in a rabbit model, comparing the effects of mucin coated lenses and trauma on the development of keratitis[179]. Coated lenses and trauma resulted in a higher proportion of ulcers compared with non-coated lenses and trauma, and seemed to be associated with more severe ulcers. No infections occurred in non-traumatised eyes, (either with coated or uncoated lenses), even if a higher bacterial inoculum was used. A similar rabbit study[180], demonstrated that hydrogel lenses contaminated with bacteria, only caused infections if the epithelium was injured. Fewer infections in the rabbit arose where contaminated lens wear was combined with an epithelial abrasion, compared with an epithelial abrasion plus a direct bacterial inoculum.

The pathogenesis of microbial keratitis in contact lens wear is poorly understood, although it does appear that contact lens wear predisposes normal eyes to this disease. Bacterial adherence to, and subsequent colonisation of contact lenses and lens storage cases, may be an important initial stage in the pathogenesis of this disease.

CHAPTER 2. METHODS

2.1 CASE CONTROL STUDY

2.1.1 Summary

The study aimed to determine reliable estimates for the risks of microbial keratitis and for other lens related disease in cosmetic contact lens wearers, by performing a case control study. A case control study design allows two estimates of risk to be made; population attributable risk percentage and relative risk.

The population attributable risk, which is the percentage of the disease eliminated by control of the risk factor, was determined for all exposures of microbial keratitis. These included contact lens wear, trauma and previous ocular surface disorders.

The relative risk, which is a measure of how many times more likely a condition is to be associated with one exposure than another, was determined for microbial keratitis. The relative risks for a range of lens related disease, for the different lens types, were estimated. The importance of predisposing factors in the pathogenesis of lens related disease was evaluated. Lens hygiene, lens age, wearing patterns, time since last aftercare visit, patients age, gender and socioeconomic grouping were evaluated for all contact lens wearers.

The prevalence of microbial contamination of lens care materials was evaluated for patients with microbial keratitis, sterile infiltrates and a selected group of control wearers.

Investigation of the epidemiology of *Pseudomonas aeruginosa* infections in contact lens wearers was carried out. Personal carriage of *P. aeruginosa* was investigated, and domestic sites were sampled for likely sources of this organism. In parallel with this study, environmental samples were cultured for *Acanthamoebae*, to investigate possible sources of this organism in contact lens wearers.

2.1.2 Data Collection and Management.

Data collection was from new attenders of the Accident and Emergency Department at Moorfields Eye Hospital over a twelve month period from 21st April 1988 to 20th April 1989.

2.1.2 (i) Identification of Patient Groups.

Casualty attenders were divided into five groups (A-E) for analysis, according to diagnosis and whether or not contact lenses were worn. Data sheets and questionnaires are shown in Appendix 1.

Attenders with Keratitis: Group A and B.

These groups included those patients with a clinical diagnosis of presumed microbial keratitis, where a diagnostic corneal scrape was taken for culture. Cases were initially identified from routine bacteriology report cards completed by the casualty officers.

Group A patients were those attending with lens related keratitis, and were either interviewed and examined as in-patients or were seen as outpatients (Data Sheet A). Previous studies carried out in this department had estimated 50 such cases to attend during the study period.

Group B patients were those presenting with non-lens related keratitis, often associated with trauma or pre- existing ocular surface disorders. Information on these patients was abstracted from the hospital notes (Data Sheet B). Patients were asked to complete Questionnaire 2, to provide data on the socioeconomic classification of the head of household. From previous studies, 40 patients were estimated in this category.

Contact Lens Wearers: Groups C and D

All new casualty attenders wearing contact lenses for the correction of low refrac-

tive errors were identified by the nursing staff. Hospital notes for these patients were identified by a coloured sticker for later examination. All contact lens wearers were asked to complete Questionnaire 1, regarding their lens type, wearing schedules and hygiene. Information was also collected to allow the socioeconomic classification of the patients' head of household to be determined. The protocol excluded wearers using lenses for aphakia or therapeutic indications.

Further subdivisions were made according to whether wearers attended as a result of lens related disease (Group D). Those lens wearers attending without a lens related disorder (Group C), such as chalazia, adenovirus conjunctivitis, vitreous floaters etc, were entered onto Data sheet C. Diagnoses of lens related disease were made by the attending casualty surgeons, using a diagnostic classification employed in a previous pilot study[14]. Table 2.1 summarises this classification. Disorders were classified by pathogenesis to aid statistical analysis. Bacterial conjunctivitis was excluded from the analysis since the relationship between lens wear and conjunctivitis is poorly understood.

Diagnoses were broadly divided into:

1. Toxic and Hypersensitivity disorders, including thiomersal keratopathy, giant papillary conjunctivitis, enzyme keratopathy and toxic keratopathy.

2. Metabolic disorders, including overwear, tight lens syndrome and hypoxic epitheliopathy.

3. Microbial keratitis

4. 'Sterile' keratitis, including non-progressive peripheral infiltrates which were presumed to be non- infective.

5. Abrasions

6. Tear resurfacing disorders, including three and nine o'clock staining and inferior closure stain.

7. Miscellaneous, including old scarring, poor lens tolerance and discomfort.

A control group comprising all contact lens wearers not having microbial keratitis

TABLE 2.1

DIAGNOSES OF LENS RELATED DISORDERS

CLASSIFICATION	DISEASE	SYMPTOMS	CORNEAL SIGNS	CONJUNCTIVAL SIGNS
METABOLIC				
Epithelial	Acute epithelial necrosis (Overwear syndrome)	Often blurred vision before onset due to corneal oedema. Delayed pain & epiphora from necrosis. Resolves in hours (or days if severe)	Central punctate epithelial erosions may coalesce into an ulcer. Involved area is larger in SCL users. Stromal oedema in severe cases.	Ciliary injection
	Tight lens syndrome	As overwear, starting in morning after overnight anoxia. Vision usually affected.	As above but stromal oedema and an epithelial defect common.	Ciliary injection and limbal indentation from tight lens.
	Microcystic epitheliopathy	Recurrent brief episodes of pain & epiphora.	Mini erosions during symptomatic episodes. Clear or opaque epithelial cysts and punctate keratitis.	None
	Epithelial oedema (Sattler's veil)	Blurred vision after some hours of wear. May recover on lens removal/progress to acute epithelial necrosis.	Dull corneal reflex from central epithelial oedema.	None
Stromal	Stromal Oedema (Striate epitheliopathy)	Blurring of vision in some cases only.	Deep stromal folds from corneal oedema occurring in severe acute epithelial necrosis.	None except when associated with acute epithelial necrosis.
	Neovascularisation - superficial and deep	None unless lipid keratopathy results from deep vessels, when vision is lost.	Superficial/deep stromal vessels. Lipid keratopathy associated with deep vessels	None
Endothelial	Endothelial polymegethism	None	Polymegethism	None
MICROBIAL INFECTIONS				
Keratitis	Microbial keratitis	Rapid onset & progression of pain, redness & discharge.	Epithelial ulcer with underlying white stromal infiltrate. Pseudomonas common & associated with fulminating course, adherent mucous & gross corneal oedema.	Ciliary injection
Conjunctivitis	Microbial conjunctivitis	Mild discomfort & muco-purulent discharge.	Normal in bacterial infections. Punctate keratitis & infiltrates in viral.	Hyperaemia & papillae in bacterial, follicles in viral
TOXIC DISORDERS				
	Enzyme keratopathy	Severe pain arising after inserting a lens soaked in proteolytic enzyme.	Widespread punctate stain	Ciliary injection
ALLERGIC DISORDERS				
	Thiomersal keratopathy	Chronic irritation & redness soon after inserting lenses each day. Vision affected in severe cases.	Superior limbal injection & neovascularisation. Opacity, punctate keratitis & microcysts affecting superior quadrant in classic cases. Variable signs in atypical cases.	Intense hyperaemia with lens in. Little except follicular changes when lens out.
	Sterile keratitis	Discomfort, redness & discharge.	Appearances similar to marginal keratitis. Peripheral infiltrates +/- ulceration.	Hyperaemia

	Giant or lens related papillary conjunctivitis	Increased discharge & greasing of lenses. Itching on lens removal, in early stages, later severe irritation. Resolves within days of lens disuse.	None	Upper tarsal hyperaemia, mucous & fine papillary response. 'Giant' (Compound) papillae in advanced disease.
TRAUMA				
	Corneal abrasion	Sudden onset of pain & epiphora. Resolves in hours.	Linear or sharply circumscribed epithelial defect.	Hyperaemia
	Anterior stromal opacity	Asymptomatic. Rarely loss of vision.	Central superficial stromal opacity.	None
LENS SPOILATION				
	Contact lens related red eye	Chronic redness, discomfort & loss in tolerance. Vision may be blurred.	Punctate stain common.	Hyperaemia. Papillae & follicles common.
CORNEAL DISTORTION				
	Corneal warpage	Uncorrectable spectacle blur but clear vision in lenses.	Irregular keratometry & photokeratoscopy.	None
TEAR RESURFACING DISORDERS				
	3 and 9 o'clock stain (Dellen in severe cases)	Interpalpebral redness. Rarely discomfort.	Punctate keratopathy in 3 & 9 positions +/- vascularised superficial stromal scars.	Interpalpebral hyperaemia
	Inferior closure stain	Inferior redness & discomfort.	Inferior/interpalpebral punctate stain	Inferior limbal hyperaemia
	Dimple veil	None or blurred vision.	Fluorescein pooling in epithelial depressions.	None

(Groups C and D) was used to calculate relative risks for the different lens types associated with microbial keratitis.

A previous pilot study[19] estimated 750-2800 wearers attending during a twelve month period. This gave an expected relative risk for microbial keratitis of 10x for extended wear soft lens users compared with gas permeable lens users.

A further control group, comprising all lens users without lens related disease (Group C) was also used to provide further estimates for the relative risks for different lens types associated with microbial keratitis. This was expected to give higher estimates of relative risk compared with the first control group, since extended wear soft lens users are likely to be overrepresented in other groups of complications. This group also provided an estimate of the proportions of different types of lenses in use in the population.

A selected group of controls (Group C1) were identified from Group C, as those patients attending immediately subsequent to a patient with lens related keratitis. These patients underwent home interviews and served as controls for the environmental survey, investigation of the epidemiology of *P. aeruginosa* keratitis and microbiological investigation.

Non-Lens Wearing Casualty Controls: Group E.

Group E comprised 1 in 100 of all new casualty attenders who were identified prospectively by the casualty clerical staff. These patients were selected to provide a representative sample of the hospital casualty population, serving as a control group for the estimation of the population attributable risk percentage for risk factors in microbial keratitis. New casualty attenders have been estimated at 25,000 to 30,000 patients per year. Exposures in this group, such as contact lens wear, trauma and previous ocular surface disorders, were compared with the exposures for all new cases of keratitis.

Patients were asked to complete Questionnaire 2, regarding the socioeconomic

grouping of the head of household. Information on patients' age, gender and diagnosis was abstracted from the case notes.

2.1.2 (ii) Data Collection and Questionnaires.

The data abstracted from casenotes, questionnaires and interviews were transferred to the data sheets shown. This information was added into a database for statistical analysis.

Where possible, patients were identified prospectively and issued with questionnaires on their first visit. Patients missed at registration, or those presenting at night and not registering, were either identified at a subsequent visit or contacted by telephone and letter. Similarly, those patients who had difficulty with questionnaires were interviewed by telephone or contacted by letter.

All lens wearers were asked to complete Questionnaire 1. This provided information to allow the identification of additional risk factors. These included; lens type, lens age, duration of wear, current wearing schedule, frequency of cleaning, disinfection and enzyme use, type of cleaning and disinfection system, type of saline used, time since the lenses were last checked and socioeconomic grouping for the patient and head of household.

Practitioners were contacted where lenses were worn overnight, to investigate the type of lenses dispensed. This information was used to evaluate the proportions of wearers misusing daily wear lenses for wearers both with and without keratitis.

A hygiene score was compiled for each lens wearer, based on the frequency of lens cleaning, lens disinfection and enzyme use. Scores ranged from 0 (poor lens hygiene) to 18 (good lens hygiene) and were calculated by adding frequency of lens cleaning per week (maximum 7) with frequency of disinfection (maximum 7) with frequency of use of enzyme tablets per month (maximum 4). Scores for extended wear lens users were based on the level of lens hygiene which occurred each time

the lenses were removed. This allowed extended wear lenses to be compared with other lens types.

2.1.2 (iii) Socioeconomic Classification.

Socioeconomic coding was carried out using the information derived from the questionnaires. Codings were based on the employment, status and industry of the head of the patients household and patient themselves where possible, according to the 1981 Census[181]. Scores ranged from 1.1 to 18.3.

2.1.2 (iv) Statistical Analysis.

Relative risks for each group of complications, including microbial keratitis, were estimated by calculating the odds ratio from contingency tables. Cases were compared with two sets of controls. Firstly, all lens users without the specific complication, but who may or may not have had a lens related disorder. Secondly, lens users who had a complication unrelated to lens wear. Gas permeable lenses were chosen to be the referent with a relative risk of 1.0, since the previous pilot study has shown this type of lens to have the lowest overall risk to wearers. Relative risks for other lens types were compared to gas permeable lenses. The significance of the trend of increasing risk was estimated using a Mantel-Haenzel Chi-squared test of trend[182] and confidence limits using Miettinen's test based approximate confidence limits[183].

The proportion attributable risk percentage for associations of microbial keratitis, was calculated from the relative risks for the different exposures and the proportion of the population exposed in the control group. Relative risks for different exposures for microbial keratitis, were compared to a risk of 1 for keratitis occurring without any predisposing factors. The estimation of relative risk was performed for all cases of microbial keratitis. Subsequent analyses were carried out where cases were divided into one of four mutually exclusive groups, according to the severity and position of the ulcer.

1. Culture positive ulcers. The rate of positive to negative corneal cultures was

low, hence for analysis this group was combined with severe culture negative ulcers.

2. Severe culture negative ulcers. Lesions greater than 2mm diameter within the central 4mm zone of the cornea.

3. Moderate culture negative ulcers. Either central lesions of less than 2mm diameter or peripheral lesions of greater than 2mm diameter outside the central 4mm zone.

4. Mild culture negative ulcers. Peripheral lesions of less than 2mm diameter.

In instances where numbers were small, an exact test was used to confirm probability values, based on Fishers exact test of trend and homogeneity (EGRET Package, Statistics and Epidemiology Research Corporation, Seattle).

Specific factors in the pathogenesis of lens related disease, such as lens hygiene, patients age, gender and social class were investigated using a Chi-square or Fisher's Exact Probability test[184], where applicable and multifactoral analysis. Misuse of daily wear lenses in the keratitis and control group was compared using a Fisher's Exact Probability test[184].

2.2. MICROBIOLOGICAL INVESTIGATION

An investigation of bacterial contamination of the lens care materials was carried out on lens wearers with presumed microbial keratitis, sterile non-progressive corneal infiltrates and control group C1.

2.2.1 Culture Techniques

2.2.1 (i) Corneal Cultures.

All microbial keratitis patients seen in casualty, had Gram stain investigation and corneal cultures carried out. The media used in each individual case depended on the size of the lesion and the preference of the casualty officer. A larger lesion provides more material for culture, so that either several different media may be

used, or similar plates may be incubated under aerobic, anaerobic and microaerophilic conditions.

In general, multiple solid and liquid phase media were used to isolate any causative agent. Broth cultures were found to be sucessful for small numbers of microbes, particularly where the patient had previously been treated with antibiotics

2.2.1 (ii) Lens Cases.

The solution in individual lens cells was stirred with a swab and the inside of the case lid swabbed, to investigate both the solution contents and any slime coating, before plating out on four types of media (see below). Where a heavy growth of organisms was found, a 10 microlitre aliquot was drawn from the lens cell for quantitative assessment of organisms by the Miles and Misra serial dilution technique[185].

2.2.1 (iii) Media Used.

All lens care materials and home environment samples collected for microbiological analysis were analysed on four types of media.

These were:

a. Blood Agar (Oxoid Ltd, UK) - a general purpose solid medium containing 10% horse blood.

b. Cetrimide Agar - a selective medium for the isolation of Pseudomonas species. This contains Pseudomonas agar (Oxoid Ltd, UK) plus CFC Supplement (Difco Ltd, UK) containing Cetrimide, Fucidin and Cefuroxime.

c. MacConkey Bile Salt Agar (Oxoid Ltd, UK) - a semi-selective medium containing an acid indicator for the identification of lactose fermenting organisms.

d. Sabouraud's Dextrose Agar (Oxoid Ltd, UK) - for the isolation of fungi and yeasts.

All samples collected from patients' homes were kept refrigerated overnight prior to analysis. Bacteriology samples sensitive to dessication were stored in Stuarts

transport media. Tubed swabs for amoeba analysis were stored dry at room temperature.

Bacteriology plates were first read after 18 hours incubation at 37^{O}C and finally after 48 hours. Biochemical typing, with API systems (Analytical Profile Index, France), was used to speciate organisms where applicable. For all lens care material and environmental bacteriology, growth of organisms was classified 0 - 4 as follows:

0 = No growth

1 = 1-5 cfu (colony forming units)

2 = 5-15 cfu

3 = >15 cfu

4 = confluent growth

2.2.1 (iv) Lenses.

Lenses from infected patients were bisected, with one half homogenised using a Griffiths tube, and cultured. The other half of the lens underwent parallel processing for electron microscopy to investigate the presence of a bacterial biofilm (Section 2.3).

Rigid lenses, lenses from control patients and from patients with sterile keratitis were either vortexed at high speed or manually shaken in 3ml of phosphate buffered saline (PBS) for several minutes. An aliquot of this solution was then cultured.

2.2.1 (v) Solutions.

3ml of any available commercial lens solutions were collected in a sterile vial and analysed as for lenses. For aerosol saline, the nozzle of the can was first swabbed, and a saturated swab was examined.

2.2.2 Investigation of The Epidemiology of *Pseudomonas aeruginosa* Infections.

Patients with lens related *P. aeruginosa* keratitis underwent further investigation

into the possible endogenous and exogenous sources of the organism. Corneal and lens care material cultures were taken as for section 3.2.1. Throat, finger nail, finger and toe webb swabs and stool samples were collected from 12 inpatients with *P. aeruginosa* keratitis, to investigate personal carriage of this organism. These samples were cultured on *Pseudomonas* selective agar (Section 2.2.1).

All species of *Pseudomonas* isolated from any source were purity plated, cultured in nutrient broth and stored in 25% glycerol broth at -20^{o}C. Home visits were carried out on 10 patients with *P. aeruginosa* keratitis and 45 control patients (Group C1).

Each sample was duplicated for separate bacteriology and amoeba analysis.

2.2.2 (i) Collection of Home Environment Samples.

Cold water taps from the bathroom and kitchen were initially sampled by inserting two swabs into each tap. Taps were not pre-heated, which is a standard public health technique, since the aim was to sample tap flora and not water distant in the pipe. Swabs were also taken from the bathroom drain and sink overflow. Samples were kept in transport medium and were cultured as described in Section 2.2.1.

2.2.3 Investigation of the Epidemiology of *Acanthamoeba* in Contact Lens Wearers.

In parallel with the study of the epidemiology of *P. aeruginosa*, environmental samples were duplicated and were analysed for the presence of amoebic cysts.

Dust in the bathroom was sampled with swabs behind the basin and in adjacent areas.

Water samples were collected from bathroom and kitchen cold water taps by drawing off 30ml of first drawn water into a sterile bottle. Tap water collected in this way was not run to waste, to simulate conditions in which lens wearers rinse

their lens cases.

Swabs were processed for amoebae by placing them onto non- nutrient agar (agar with no nutrient content to prevent growth of bacteria), seeded with live *Escherichia coli (E. coli)*, washed in PBS. The plate was then incubated at 37^{o}C in a humid enviroment for up to 7 days, and inspected under low power microscopy for both the vegetative state (trophozoites) and the cystic form. Trophozoites were seen to make tracks in the bacterial layer and were recognised by the presence of a contractile vacuole. Amoebic cysts were visible in the absence of a food supply and identification was made on the basis of cyst morphology. *Acanthamoebae* were recognised by their characteristic star shaped cysts with refractile double walls.

Water samples were checked visually for the presence of lime scale. Samples were spun at 2500 rpm for 10 minutes, inoculated onto non-nutrient agar seeded with live *E. coli* and incubated for 7 days.

Spun water samples were then further investigated using a more sensitive enrichment technique. This analysis was performed by P. Christy and S. Kilvington at the Public Health Laboratory in Bath. Spun samples were inoculated into a suspension of live *E. coli* prior to plating out. This enrichment technique proved to be more sucessful for isolation of small numbers of amoebae. All environmental amoebae isolated by this technique were speciated.

2.3 BACTERIAL ADHERENCE AND BIOFILM INVESTIGATION

2.3.1 Summary.

The formation of a bacterial biofilm on contact lenses and possibly on lens storage cases has been implicated in the pathogenesis of contact lens related keratitis. This study aimed to investigate initial bacterial adherence to lenses and biofilm formation on contact lenses and lens storage cases. In vitro and in vivo approaches were used:

1. Bacterial adherence to unworn hydrogel lenses was investigated using quantitative bacteriology and electron microscopy. Bacterial adherence to ionic and non-ionic hydrogel lenses was compared, and the effect of using washed or unwashed bacteria was assessed. Different techniques of washing lenses after bacterial incubation were compared. The rate of bacterial adherence with respect to time was investigated.

2. Bacterial adherence and biofilm formation on lenses and lens storage cases, from patients with culture proven keratitis, were evaluated.

2.3.2 In Vitro Experimental Design and Methods.

2.3.2 (i) Bacterial Culture and Quantification.

Bacterial adherence to unworn lenses was investigated using an existing strain of *P. aeruginosa*, which had been isolated from a corneal ulcer in a hydrogel lens wearer. This organism had been used for previous adherence experiments and in an animal model of lens related keratitis[175].

A stock of this organism had been previously purity plated, cultured in nutrient broth and aliquoted into 25% glycerol broth at -20^{O}C. Figure 2.1 shows *P. aeruginosa* plated onto blood agar.

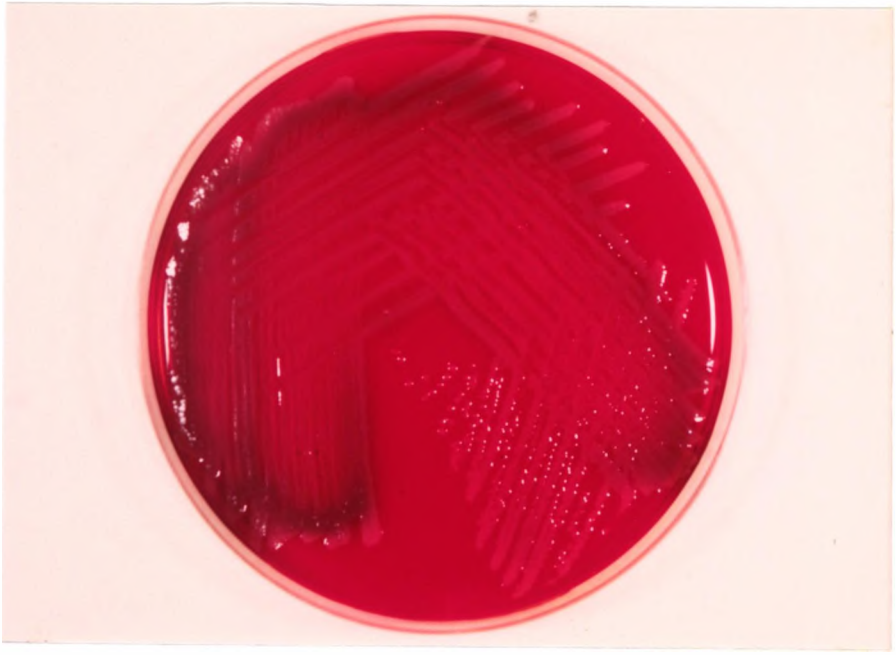
For each experiment, 10 microlitres of this frozen stock was transferred to nutrient broth, which was incubated under static conditions at 37^{O}C for 18 hours.

Where washed organisms were required, 10ml of the broth culture was spun at 3000 r.p.m. for 20 minutes to generate a pellet of organisms. The broth supernatant was removed and the pellet resuspended in PBS. Washing of organisms was repeated 3 times and the organisms were finally resuspended in 10 ml of PBS.

The bacterial culture was visually checked using MacFarland standard tubes (MacFarland, API, France) and quantification was performed using the Miles and Misra serial dilution technique[185]. This determined the number of colony forming units per ml of suspension (CFU/ml). Figure 2.2 demonstrates this technique on

Figure 2.1

Illustration shows strain of **P. aeruginosa** plated onto blood agar. Colonies show an elongated shape and metallic sheen, which is characteristic of this organism.



blood agar and Figure 2.3 on cetrimide agar.

2.3.2 (ii) Procedures for Transmission Electron Microscopy (TEM).

Preparation for TEM was performed by the following steps:

1. Fixed samples washed in three changes of 0.1M cacodylate buffer and 0.05% ruthenium red at pH 7.4 for 15 minutes at each change.
2. Post-fixation using 1% osmium tetroxide in 0.1M cacodylate buffer and 0.05% ruthenium red for 18-24 hours under constant agitation at room temperature.
3. Washing with 0.1M cacodylate buffer and 0.05% ruthenium red for 15 minutes, then three changes of distilled water at 10 minutes each change.
4. Dehydration through graded alcohols performed using 30%, 50%, 70%, 85% and 95% ethanol for 15 minutes at each change. Three changes at 15 minute intervals were carried out in 100% ethanol.
5. Embedding using propylene oxide as the transfer fluid. This involved replacing the ethanol with propylene oxide, using 100% propylene oxide for 2 changes at 15 minutes intervals. The propylene oxide was then gradually replaced with Araldite, using a ratio of 75:25 propylene oxide to Araldite for 2 hours, 50:50 for 2 hours, 25:75 for 2 hours and finally 100% Araldite overnight.
6. Samples were placed into resin moulds with fresh resin and were left at 60^{o}C for 24-48 hours. The temperature was reduced to 40^{o}C for a further 8 hours.
7. Once cooled, the specimens were removed from the moulds and allowed to harden for 1-2 days.

Specimens were then cut into thin sections* for examination in TEM at 75KV, using the Hitachi H-600 TEM System.

*Thin sections were cut by Stephen Davies at the Institute of Ophthalmology.

2.3.3 (iii) Procedures for Scanning Electron Microscopy (SEM).

Preparation for SEM was performed by treating the specimens in the following way:

Figure 2.2

Illustration shows Miles and Misra serial dilution technique on blood agar. Dilutions shown are clockwise from top 10^{-2}, 10^{-3} and 10^{-4}.

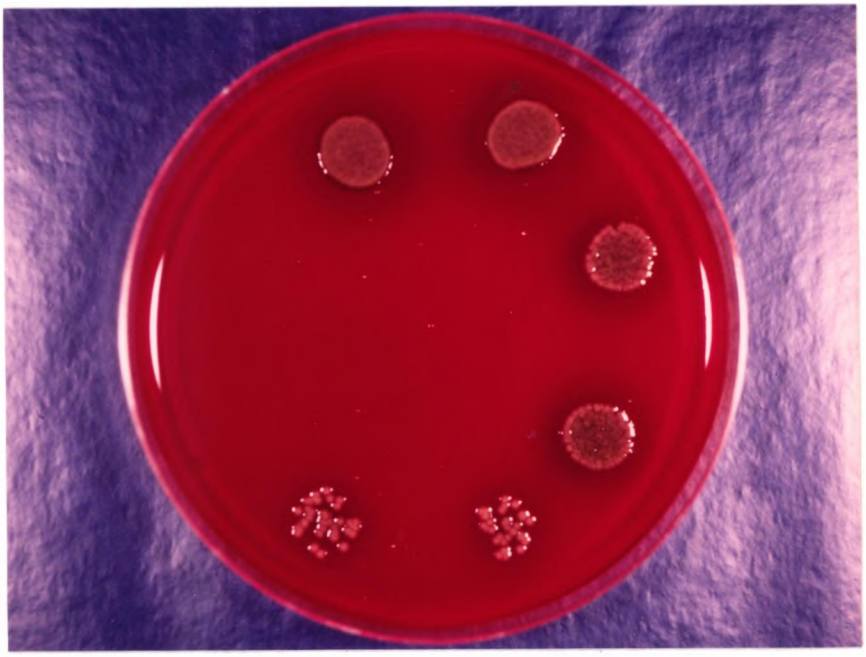
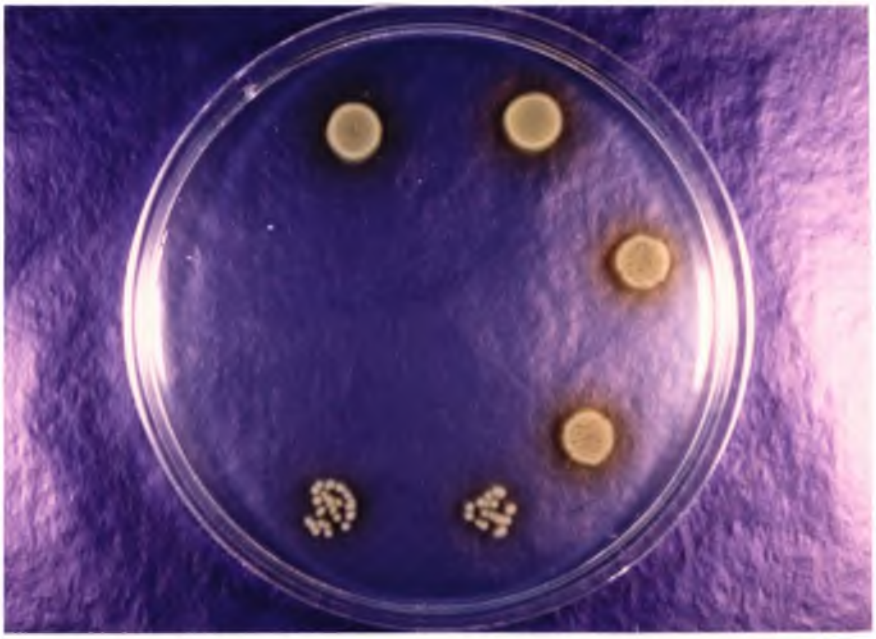


Figure 2.3

Illustration shows Miles and Misra serial dilution technique on cetrimide agar and demonstrates the pigment production by the organism.



1. Fixed samples washed in 0.1M cacodylate buffer** for three changes at 15 minute intervals.

2. Post-fixation using 1% osmium tetroxide in 0.1M cacodylate buffer**, pH 7.4 at room temperature for 1 hour.

3. Washed with 0.1M cacodylate buffer** for 15 minutes, then in distilled water for three changes at 10 minutes intervals.

4. Dehydration through graded alcohols performed as for TEM processing (Section 2.3.3 (ii)).

5. Critical point dried (CPD) using carbon dioxide with 100% alcohol as a transfer fluid. Other methods of drying, including air drying and use of a high molecular weight volatile solvent such as hexamethyldisilazane (HMDS), were carried out for comparison purposes. However, it was felt that despite CPD being a harsh technique, which may remove some material adherent to the surface, less lens surface disruption occurred using this method.

6. Mounted onto stubs either with silver paint or double sided adhesive. Two samples for each lens were generally processed to allow specimens to be mounted with both concave and convex side up on each stub.

7. Sputter coated with gold to a thickness of 20 microns and were examined in SEM at 20KV using the Hitachi S-520 SEM System.

**Early sample processing was carried out without the use of ruthenium red stain incorporated into the stock buffer solution. However as the methods evolved, ruthenium red was incorporated here at a concentration of 0.05%. This was carried out in an attempt to improve fixation of a bacterial biofilm, which may be present on the lens care materials.

2.3.3 (iv) Experimental Methods

Bacterial adherence to unworn ED4 lenses (a 75% water content non-ionic hydrogel material, supplied by Bymate Ltd, Gravesend, UK) was investigated under a range of different conditions. Lenses were lathed to produce a single cut lens of

BOZR 7.30mm, overall size 13.50mm and plano power, with centre thickness 0.15mm.

a. Effect of Increased Incubation Time on Adherence using Washed and Unwashed Organisms

Unworn contact lenses were submerged in 3ml of the unwashed bacterial suspension in sterile 30ml Universal bottles. Lenses and bacteria were incubated together at room temperature for 5, 15, 30, 45 and 60 minutes.

To remove organisms which were not irreversibly adhered, lenses were removed from the bacterial suspension and were transferred to 15ml of PBS. The PBS wash was vortexed at medium speed for 1 minute. The lenses were then transferred to fresh PBS and similarly vortexed. Three washes were performed in this way.

Lenses were removed from the final wash and were bisected. One half of the lens was immediately fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer with 0.05% ruthenium red, for subsequent electron microscopy. The remaining half was homogenised in a Griffiths tube with 0.9ml of PBS. Quantification of organisms was performed by the Miles and Misra serial dilution technique, by inoculating nutrient agar with log dilutions of this suspension.

This adherence assay was repeated 5 times using unwashed organisms and 5 times using washed organisms.

b. Effect of Washing or Rinsing Lenses on Bacterial Adherence

Unworn contact lenses were submerged in 3ml of the bacterial suspension and incubated at room temperature for 15, 30 and 60 minutes. To compare the effects of vortexing versus rinsing on bacterial adherence, lenses were either rinsed gently in 15ml of PBS, (the lenses were then transferred to fresh PBS and rinsed ten times in this manner), or lenses were vortexed in PBS for 1 minute at a medium speed, transferred to fresh PBS and washed three times in this manner.

Lenses were removed from the final rinse, bisected with one half fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer with 0.05% ruthenium red for electron microscopy. The remaining half was homogenised and adherent organisms quantified as in (a).

This assay was repeated 5 times using both washed and unwashed organisms.

c. Effect of Removing Water Film from Lens Surface

Unworn contact lenses were incubated with 3ml of the washed bacterial suspension for 60 minutes at room temperature. Lenses were rinsed gently, by dipping 3 times in PBS and were then removed and bisected. Half of the lens was immediately homogenised and adherent organisms were quantified as in (a). The remaining half was gently blotted on either side using sterile filter paper strips prior to homogenisation and bacterial quantification. Blotting of the lens was carried out to remove non- adherent organisms which may be carried across from the rinsing stage in the surface film of PBS on either side of the lens.

This assay was repeated 5 times.

d. Effect of Ionic Charge on Adherence

Unworn non-ionic lenses (Bausch and Lomb M3 Polymacon +3.00DS, 38% water content) and ionic lenses (Bausch and Lomb Etafilcon A -2.75DS, 58% water content), were incubated with 3ml of the washed bacterial suspension for 60 minutes at room temperature.

Lenses were removed, rinsed 3 times in PBS and bisected. Half of each lens was fixed for future electron microscopy and half homogenised with bacterial quantification carried out as in (a).

This was repeated for 10 ionic and 10 non-ionic lenses.

2.3.3 Statistical Analysis.

Data comparing adherence of washed and unwashed organisms was analysed using a one tailed Mann-Whitney U-test, where no assumption was made about the distribution of the data[184]. Differences in bacterial counts with time were assessed using the Kruskal-Wallis one way ANOVA[184]. Bacterial adherence to ionic and non-ionic materials was compared using a one tailed Mann Whitney U-test.

2.3.4 Patient Material.

Lenses and lens storage cases were collected from patients with clinically diagnosed microbial keratitis during the case control study. Semi-quantitative bacteriology was performed on these samples as described in Section 2.2.1.

Remaining lens samples were divided into segments which were treated in the following ways:

a. Segments were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for scanning electron microscopy.

b. Segments were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer with 0.05% ruthenium red for transmission electron microscopy.

c. Segments were stored in PBS at 4^{o}C, for future investigations.

d. Remaining segments were either fast or slow frozen in nitrogen based OCT (Tissue-Tek OCT Compound 4583) and were stored at -50^{o}C.

Methods c and d were aimed at evaluating viable methods for handling materials collected from patients.

Lens cases were either fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer with 0.05% ruthenium red or were stored at 4^{o}C.

A number of lenses (12) lens storage cases (3), taken from patients with presumed microbial keratitis, were processed and examined using scanning electron microscopy (Section 2.3.2 (iii)).

Lenses from patients with presumed microbial keratitis (4) were processed and examined using transmission electron microscopy (Section 2.3.2 (iv)).

CHAPTER 3. RESULTS

3.1 CASE CONTROL STUDY

3.1.1 Breakdown of Patients.

1884 patients were recruited from a total of 29,242 new casualty attenders. These were divided as follows:

TABLE 3.1

	TOTAL	MEAN AGE +/- 1SD	MALES:FEMALES
Group A	60	28.00 +/- 8.00	35:25
Group B	35	41.11 +/- 18.80	24:11
Group C	507	30.69 +/- 9.87	180:327
Group D	1044	29.06 +/- 8.56	392:652
Group E	238	42.10 +/- 31.63	141:97

(Group E included 1 in 100 of new patients attending the casualty department. Secondary referrals and existing hospital patients were excluded. Contact lens wearers (n=25), who were identified within this group, were included in Groups C and D according to their diagnosis).

3.1.2 Microbial Keratitis.

3.1.2 (i) Cases.

Of 95 new patients presenting with microbial keratitis. 60 were contact lens wearers; 28 wearing EWS lenses, 28 DWS lenses, 2 PMMA lenses and 2 GP lenses.

Of the non contact lens wearers, notes were unavailable in four cases. The remaining 31 patients were assigned to the following groups according to their respective predisposing factors.

1. Trauma in 22 cases, including:

    11 non-metallic foreign body injuries

    6 metallic foreign body injuries

    2 abrasions

    2 penetrating injuries.

2. Pre-existing ocular surface disorders (OSD) in 4 cases, including:

    2 neurotrophic corneas

    1 recurrent erosions

    1 recurrence of a previous infection which had been treated initially at another hospital.

3. No apparent predisdisposing factors (NONE) were evident in 5 cases, although 1 patient was diabetic.

3.1.2 (ii) 1 in 100 Controls.

Of the control patients identified (n=267), 25 wore contact lenses; 4 wore EWS, 11 wore DWS, 4 wore GP and 7 wore PMMA lenses. A further 4 patients presented with keratitis, 2 cases of which were lens related.

Of the remaining 238, predisposing factors represented in this group included:

1. Trauma in 53 cases, including:

    23 abrasions

    19 non-metallic foreign body injuries

    5 metal foreign body injuries

    3 non-specific trauma/penetrating injuries

    2 alkali/plaster burns

    1 traumatic iritis.

2. Ocular surface disorders (OSD) in 18 cases, including:

    5 cases of dry eyes

    4 Herpes Simplex keratitis

    3 chemical/toxic keratopathy

2 recurrent erosions

2 trichiasis

1 trachoma

1 Herpes Zoster keratitis.

3. No apparent predisposing conditions (NONE) were evident in 167 cases.

The complete diagnoses for the Group E controls are shown in Appendix 2.

Table 3.2 shows the total number of cases of microbial keratitis (cas) and controls (con) summarised according to their major risk factor and degree of severity. Risk factors have been ranked according to increasing risk. Table 3.3 shows the age and sex characteristics for the cases and controls in Table 3.2.

3.1.2 (iii) Relative Risk and Population Attributable Risk Percentage Data.
Data tables 3.4 to 3.25 are shown in Appendix 3, page 123-137.

Table 3.4 (page 123) shows the relative risks and population attributable risk percentages for all new cases of presumed microbial keratitis using a control group of 1 in 100 casualty attenders. 95 percent confidence intervals are shown in parentheses for the relative risk data.

These data show that ocular surface disorders, trauma and contact lens wear collectively were responsible for 91.3% of all cases of keratitis in this population.

The proportion of new keratitis cases attributable to contact lens wear, for the correction of low refractive errors, was 65%. Contact lens wear carried an 80.2x higher risk of keratitis compared with eyes with no apparent predisposing factor (None). Trauma was found to have a 13.9x higher risk and ocular surface disorders (OSD) a 7.4x higher risk of developing keratitis compared with eyes with no apparent predisposing factor.

Similar analyses have been performed for the different degrees of severity of

TABLE 3.2
TOTAL NUMBER OF CASES OF MICROBIAL KERATITIS TABULATED BY THEIR MAJOR ASSOCIATED RISK FACTOR AND DEGREE OF SEVERITY

RISK FACTOR	ALL		CULTURE POSITIVE		CULTURE NEGATIVE					
					Sev		Mod		Mil	
	Cas	Con	Cas	Con	Cas	Con	Cas	Con	Cas	Con
None	5	167	1	167	1	167	0	167	3	167
OSD	4	18	1	18	1	18	2	18	0	18
Trauma	22	53	6	54	1	55	8	54	7	55
CL Wear	60	25	9	26	2	27	11	26	38	27
Totals	91	263	17	265	5	267	21	265	48	267

TABLE 3.3
AGE AND GENDER CHARACTERISTICS FOR CASES AND CONTROLS IN TABLE 3.2

RISK FACTOR		ALL		CULTURE POSITIVE		CULTURE NEGATIVE					
						Sev		Mod		Mil	
		Cas	Con	Cas	Con	Cas	Con	Cas	Con	Cas	Con
None	M:F	0:5	91:76	0:1	-	0:1	-	0	-	0:3	-
	Mean	39.00	40.86	64.00	-	21.00	-	na	-	26.67	-
	SD	18.21	17.97	na	-	na	-	na	-	13.43	-
OSD	M:F	1:3	11:7	1:0	-	0:1	-	0:2	-	0	-
	Mean	38.00	50.78	36.00	-	31.00	-	42.50	-	na	-
	SD	17.64	21.43	na	-	na	-	na	-	na	-
Trauma	M:F	20:2	39:14	3:3	40:14	1:0	41:14	8:0	40:14	7:0	41:14
	Mean	39.05	35.49	48.5	35.53	40	35.47	32.5	35.53	38.29	35.47
	SD	18.66	13.34	30.7	13.24	na	13.12	7.46	13.24	14.81	13.12
CL Wear	M:F	35:25	9:16	6:3	9:17	1:1	10:17	6:5	10:16	23:15	10:17
	Mean	28.0	29.46	30.11	29.84	20.0	29.44	29.9	29.35	27.71	29.44
	SD	8.0	10.4	8.16	10.2	na	10.21	12.07	10.4	7.93	10.21

M:F = Male to Female ratio
Mean = Mean Age
SD = 1 standard deviation on mean age

keratitis for each major risk factor.

Table 3.5 (page 123) shows relative risks and population attributable risk percentages for all exposures of culture positive keratitis. A similar trend of increased risk is shown, with the relative risk for lens wear at 57.8x higher, trauma at 18.6x higher and OSD at 9.3x higher for keratitis than the risk for eyes with no apparent predisposing factor.

Table 3.6 (page 124) shows the risk data for severe culture negative keratitis. The trends of risk in this group differ, with the relative risk for lens wear at 12.4x higher, OSD at 9.3x and trauma at 3.0x higher than eyes with no apparent predisposing factor.

Table 3.7 (page 124) shows the risk data for moderate culture negative keratitis for all exposures. Due to there being small numbers of cases in this and the mild culture negative group, an exact test of trend, based on Fishers exact test of trend and homogeneity (EGRET) was used to confirm significance of trend. The relative risk for contact lens wear was found to be 145.4x higher, for trauma 52.2x higher and for OSD 45.3x higher for keratitis, compared with eyes with no apparent predisposing factor.

Table 3.8 (page 125) shows the risk data for mild culture negative keratitis for the different exposures. The trend of increasing risk differs for this group with the relative risk for contact lens wear at 78.3x higher, trauma at 7.1x higher and OSD at 0, compared with a relative risk of 1 for keratitis in eyes with no apparent predisposing factor.

Table 3.9 (page 125) shows the risk data for all degrees of keratitis for lens wearers compared with non-lens wearers. Lens wearers were found to have a risk of 18.4x higher of developing keratitis compared with non-lens wearers.

The population attributable risk percentage for all severities of keratitis associat-

The population attributable risk percentage for all severities of keratitis associated with contact lens wear ranged between 36-78%. Table 3.9 shows the overall population attributable risk estimate of 62%, comparing lens wear with no lens wear. If the risks for keratitis associated with contact lens wear could be eliminated, then the reduction in the new cases of keratitis would be 62% per year in this population.

3.1.2 (iv) Multivariate Analysis.

Controlling for confounding factors such as age, gender and socioeconomic class was not found to reduce the risks for the different exposures in microbial keratitis. The association between keratitis and the individual exposures of ocular surface disorders, trauma and contact lens wear persists despite taking into account age, gender and socioeconomic class as possible confounding factors. No higher order interactions, for example where lens wear is combined with the effect of age, gender or socioeconomic class, were found to cause effect modification. The strong association between lens wear and keratitis persists for males and females and for all age and socioeconomic strata.

3.1.2 (v) Causative Organisms.

Organisms were recovered from 17 of 91 corneal ulcers, with a breakdown as shown in Table 3.10.

TABLE 3.10

Causative Organisms for all Exposures of Microbial Keratitis.

MISC	OSD	TRAUMA		CL WEAR
0	0	1 Strep. species	GP	1 Acanthamoeba
		1 S. aureus	PMMA	0
		3 Moraxella	DWS	4 P. aerug
		3 S. epidermidis	EWS	1 P. aerug
				1 P. species
				1 S. epidermidis
				1 Moraxella

3.1.3 Microbial Keratitis in Contact Lens Users.

Table 3.11 shows the breakdown of lens related keratitis and controls, by lens type and severity of keratitis. Two groups of controls were used for this analysis. Group 1 is comprised of lens wearers without lens related disease (Group C), and group 2 comprises all lens users without keratitis (Group C + D). Table 3.12 shows the age and gender breakdown for cases and controls in Table 3.11.

Table 3.13 (page 126) shows the relative risk and population attributable risk data for all cases of lens associated microbial keratitis using control groups 1 and 2.

Using group 1 controls, comprising lens wearers without lens related disease, relative risks for EWSCL users were estimated at 36.8x higher, DWSCL users at 4.2x and PMMACL users at 1.3x higher risk of developing keratitis compared with that of GPCL users.

Using group 2 controls, comprising all lens wearers except those with keratitis, a similar trend of increasing risk was found. EWSCL users were found to have a 20.8x higher risk, DWSCL users a 3.6x higher risk and PMMACL users a 1.3x higher risk of developing keratitis compared with a risk of 1 for GPCL users.

Tables 3.14 to 3.17 show similar data for different severities of keratitis. Table 3.14 (page 127) shows the risk data for culture positive keratitis using group 1 and group 2 controls.

Using group 1 controls, EWSCL users were found to have a relative risk of 10.5x higher, DWSCL users 1.2x higher and PMMACL users 0, for culture positive keratitis, compared with a relative risk of 1 for GPCL users.

Using group 2 controls, comprising all lens users without culture positive keratitis, EWSCL users were found to have a relative risk of 5.2x higher, DWSCL users 1.0 and PMMACL users 0, compared with a risk of 1 for GPCL users. The trend of increased risk was found not to be significant.

TABLE 3.11

TOTAL NUMBER OF CASES OF MICROBIAL KERATITIS TABULATED BY LENS TYPE AND SEVERITY OF KERATITIS FOR GROUP 1 AND GROUP 2 CONTROLS.

LENS TYPE	ALL CASES			CULTURE POSITIVE		SEVERE or CULTURE POSITIVE		CULTURE NEGATIVE MOD		CULTURE NEGATIVE MILD	
	Cas	Cont Gp1	Cont Gp2	Cas	Cont Gp2	Cas	Cont Gp2	Cas	Cont Gp2	Cas	Cont Gp2
GP	2	92	245	1	246	1	246	1	246	0	247
PMMA	2	71	190	0	192	0	192	2	190	0	192
DWS	28	309	951	4	975	5	974	5	974	18	961
EWS	28	35	165	4	189	5	188	3	190	20	173
TOTALS	60	507	1551	9	1602	11	1600	11	1600	38	1573

TABLE 3.12

AGE AND SEX CHARACTERISTICS FOR CASES AND CONTROLS IN TABLE 3.11

LENS TYPE	ALL CASES			CULTURE POSITIVE		SEVERE or CULTURE POSITIVE		CULTURE NEGATIVE MOD		CULTURE NEGATIVE MILD	
	Cas	Cont Gp1	Cont Gp2	Cas	Cont Gp2	Cas	Cont Gp2	Cas	Cont Gp2	Cas	Cont Gp2
GP											
M:F	0:2	27:65	75:170	0:1	75:171	0:1	75:171	0:1	75:171	0	75:172
Mean	35.0	30.82	29.76	44.0	29.74	44.0	29.74	26.0	29.82	na	29.80
S.D	12.73	10.11	9.19	na	9.18	na	9.18	na	9.22	na	9.20
PMMA											
M:F	1:1	21:50	56:134	0	57:135	0	57:135	0:2	56:134	0	57:135
Mean	44.0	33.89	33.56	na	33.70	na	33.70	44.0	33.56	na	33.70
S.D	15.56	8.46	9.12	na	9.21	na	9.21	15.56	9.12	na	9.21
DWS											
M:F	16:12	192:117	597:354	3:1	610:365	4:1	609:365	3:2	610:364	9:9	604:357
Mean	25.61	29.79	28.50	27.5	28.42	25.8	28.43	23.2	28.45	26.22	28.46
S.D	4.82	9.54	8.47	3.12	8.41	4.66	8.41	5.63	8.40	4.71	8.45
EWS											
M:F	18.10	15:20	87:78	3:1	102:87	3:2	102:86	2:1	103:87	13:7	92:81
Mean	29.18	32.46	30.90	29.25	30.68	27.4	30.73	33.0	30.61	29.6	30.77
S.D	10.0	12.86	10.31	9.67	10.29	9.34	10.29	14.53	10.23	10.14	10.30

Table 3.15 (page 128) shows data for combined severe culture negative and culture positive keratitis, using group 1 and group 2 controls.

Using group 1 controls, EWSCL users were found to have a relative risk of 13.1x higher, DWSCL users 1.5x higher and PMMACL users 0, compared with a relative risk of 1 for GPCL users.

Using group 2 controls, EWSCL users were found to have a relative risk of 6.5x higher, DWSCL users 1.3x higher and PMMACL users 0, compared with 1 for GPCL users.

Table 3.16 (page 129) shows risk data for moderate culture negative keratitis using group 1 and group 2 controls.

Using group 1 controls, the relative risks for EWSCL users were 7.9x higher, DWSCL 1.5x higher and PMMACL 2.6x higher, for moderate culture negative keratitis, compared with a risk of 1 for GPCL users.

Using group 2 controls, the relative risks for EWSCL users were found to be 3.9x higher, DWSCL users 1.26x higher and PMMACL users 2.6x higher for moderate culture negative keratitis, compared with a relative risk of 1 for GPCL users.

The trend of increased risk was not found to be significant using either group 1 or group 2 controls, although the risks for EWSCL users were significantly higher than for other lens types.

Table 3.17 (page 130) shows the risk data for mild culture negative keratitis, for group 1 and group 2 controls.

Using group 1 controls, the relative risks for EWSCL users were found to be 106.8x, DWSCL users 11.1x and PMMACL users 1.3x, compared with a risk of 1 for GPCL users.

Using group 2 controls, the relative risks for EWSCL users were found to be 58.5x, DWSCL users 9.5x and PMMACL users at 1.3x for mild culture negative keratitis, compared with a risk of 1 for GPCL users. An EGRET exact test of trend confirmed significant differences between the relative risks for the different lens types, using both group 1 and group 2 controls.

Tables 3.18 (page 131) and 3.19 (page 132) show risk data for combined rigid lens users compared with daily and extended wear soft lens users. Rigid lens data was combined, since numbers of cases in rigid lens users with culture positive or severe culture negative keratitis were small. Miettinens test based approximate analysis was performed on all data. However, since numbers were small in some instances, an EGRET exact test was performed to confirm significant differences.

Using group 1 controls, the relative risks for EWSCL users was 18.6x higher and for DWSCL users 2.1x higher for culture positive keratitis, compared with a relative risk of 1 for rigid lens users.

Using group 2 controls, the relative risks for EWSCL users were found to be 9.3x higher and for DWSCL users 1.8x higher for culture positive keratitis, compared with a relative risk of 1 for rigid lens users.

Using group 1 controls, the relative risks for EWSCL users were found to be 23.3x higher and for DWSCL users 2.6x higher, for combined culture positive and severe culture negative keratitis, compared with a risk of 1 for GPCL users.

Using group 2 controls, the relative risks for EWSCL users were found to be 11.7x higher, for DWSCL users 2.2x higher for combined culture positive and severe culture negative keratitis, compared with a relative risk of 1 for rigid lens users.

3.1.3 (i) Multivariate Analysis.

Due to there being small numbers of patients wearing rigid lenses, it was not possible to include them in the multivariate model. Hydrogel lens users were

divided into daily and extended wear for this analysis. The model included age, gender, socioeconomic classification, for the patient and the head of household, lens age, wearing schedule, duration of lens wear, duration of symptoms, indication for lens wear, period since last lens check, lens hygiene level and type of hygiene regime.

a. EWSCL Wearers

For EWSCL users, the final model incorporated age as a continous variable, gender, socioeconomic classification, duration of symptoms, cycle time and enzyme cleaning frequency. Certain factors which were not individually significant were incorporated into the model, as they were found to be confounders.

Both a longer duration of symptoms (1 day<) and a longer cycle time (lens worn continuously for 6 days<) were significantly associated with keratitis, despite controlling for other factors. Patients' socioeconomic classification was an associated risk factor, with classifications of 5.2 and below (representing lower management/supervisory classifications to semi-skilled and manual workers), having a higher risk for keratitis compared with professionally qualified workers.

Enzyme cleaning frequency was found to be almost significant. Gender was found to be almost significant, with females having almost half the risk of developing keratitis as males.

Other hygiene and compliance factors, such as lens age and period since last lens check, were found not to be significant. However, the use of chlorine and heat disinfection systems could not be incorporated into the model, since small numbers of cases presented using these regimes. This is unlikely to be a major factor since only 12-15% of controls were found to use each of these types of care regimes.

b. DWSCL Wearers

For DWSCL lens users, the final model incorporated age, gender, socioeconomic

classification for the patient and head of household, duration of symptoms, duration of lens wear and disinfection frequency.

Gender was found to be a significant factor, with males having a higher risk of keratitis compared with females. A longer duration of symptoms was found to be associated with keratitis. Patients' age and socioeconomic classification were not found to be associated with keratitis. Frequency of lens disinfection was found to be a significant factor, with a lower frequency associated with keratitis. Duration of lens wear and other hygiene factors, were not found to be associated with keratitis. No differences were found between each of the different disinfection regimes.

3.1.3 (ii) Lens Hygiene.

Hygiene scores were compiled for lens wearers with keratitis (60) and controls without lens related disorders (507). Of the control group, hygiene data was available for 491/507. Questionnaires were incomplete in the remaining 16 cases. Scores were based on the frequency of lens cleaning, lens disinfection and enzyme use. Data was abstracted from the questionnaires completed by all lens wearers, and scores ranged from 0 (poor hygiene) to 18 (good hygiene).

Table 3.20 shows the overall breakdown by lens type and the mean hygiene scores for cases and controls for each lens type. The standard error of the scores for each group is also shown. Few rigid lens users presented with keratitis, hence the standard errors within these groups are large.

One way analysis of variance (ANOVA) performed on this data, shows that significant overall differences exist between the different groups. Analysis of 95% intervals on the means for each of these eight groups was performed to identify where these differences occurred (Figure 3.1). Poor hygiene was found to be associated with keratitis for DWSCL users only. No significant differences between cases and controls were found for EWSCL users. Amongst the control groups, significantly

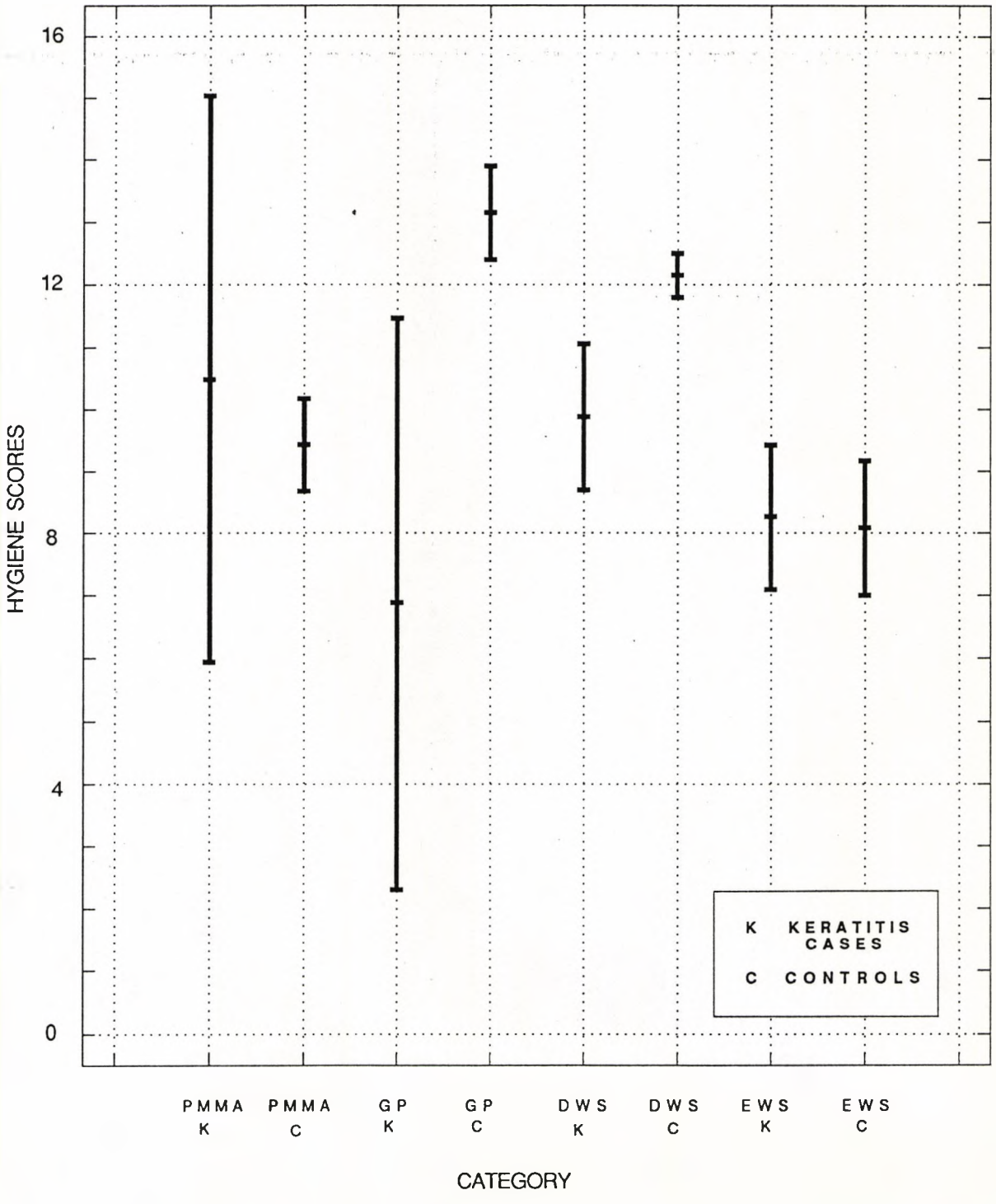
TABLE 3.20

Lens Hygiene Data for Wearers with Microbial Keratitis and Controls

	PMMA		GP		DWS		EWS	
	Cas	Con	Cas	Con	Cas	Con	Cas	Con
N	2	71	2	86	28	302	28	32
Mean	10.50	9.49	7.00	13.20	9.93	12.14	8.28	8.09
SE	3.50	0.57	7.00	0.45	1.05	0.26	0.96	1.01

ANOVA $p<0.001$

FIGURE 3.1 95 PERCENT INTERVALS ON MEAN HYGIENE SCORES



better hygiene was demonstrated by GPCL and DWSCL users compared with PMMACL and EWSCL users. No significant difference was found comparing DWSCL and GPCL users in the control group.

3.1.3 (iii) Lens Care Material Contamination.

Lens care materials were cultured from 49 patients with presumed microbial keratitis and from 44 control wearers without lens related disease, who were visited at home.

Bacterial contamination of the contact lens storage case occurred in 33/49 keratitis cases and in 16/44 control wearers. Bacterial contamination of the lens storage case was found to be significantly associated with microbial keratitis (Chi-Square Test $p=0.0055$). However, this association was only significant for DWSCL users, due to small numbers of wearers of other lens types.

Lens contamination was found in 16/30 wearers with keratitis, compared with 11/42 controls. Lens contamination was found to be significantly associated with keratitis (Chi-Square Test $p=0.033$).

Commercial solutions were found to be contaminated in 6/55 solutions collected from keratitis patients and from 10/108 control wearers. Rates of commercial solution contamination were not found to differ significantly between wearers with keratitis and those without.

For 16/49 wearers with keratitis, no organisms were cultured from any of the lens care materials.

Of the asymptomatic wearers, 35% were found to have bacterial contamination of the lens storage case. However, this group cultured fewer ocular pathogens compared with the keratitis group. No *P. aeruginosa* was isolated from any source from the control group.

Table 3.21 shows the breakdown of organisms isolated from the lenses, lens stor-

age cases and solutions from the keratitis and control groups.

No correlation could be found between poor hygiene and contamination and between lens case cleaning and contamination.

3.1.3 (iv) Misuse of Daily Wear Lenses.

Information on the misuse of daily wear lenses for overnight use, was sought for 28 patients with lens associated keratitis and 136 controls. Both keratitis patients and control wearers reported wearing their lenses overnight. The control group comprised 35 wearers with non-lens associated disease and 101 with lens related disease. Complete information on lens type and prescribed mode of wear was ascertained for 23 cases with keratitis and 86 controls. Of these 5/23 cases (22%) and 17/86 controls (20%) had been supplied with daily wear soft lenses, or had been prescribed strict daily wear only, and were using the lenses overnight. This difference between cases and controls was not found to be significant (Fisher Exact Probability, $p = 0.7006$).

A similar analysis was repeated using a selected control group, comprising EWSCL users without lens related disease. This was performed to exclude other lens related disorders for which misuse of daily wear lenses may be a factor, such as metabolic disorders. Misuse occurred in 3/21 users (14%) and the difference between cases and controls was not found to be significant (Fisher Exact Probability, $p = 0.4039$).

3.1.4 Contact Lens Related Disease.

1668 wearers using lenses for the correction of low refractive errors, were identified from a total of 29,242 new casualty attenders (5.7%). Lens type was unknown in 57 cases, which were excluded from the analysis. 1104 wearers (68.5%) presented with a lens related disorder and 507 (31.5%) presented with problems unrelated to lens wear. The breakdown by lens type is shown in Table 3.22. Of 1611 lens wearers identified, 1252 were fitted by 589 private practitioners in the UK. A

TABLE 3.21

Frequency of Organisms Isolated from the Lenses, Lens Storage Cases and Commercial Solutions (Soln) from Keratitis Patients and from Control Wearers without Lens Related Disease.

ORGANISM	KERATITIS			CONTROLS		
	Lens	Case	Soln	Lens	Case	Soln
Gram Negative Rods (Untypable non lactose fermenting GNR)	3	7	5	5	6	5
Coliforms	2	5	0	4	5	2
Coliforms + Gram negatives	2	2	1	0	1	0
Enterobacteria	0	1	0	1	1	1
Pseudomonas aeruginosa	4	8	0	0	0	0
P. aerug + E. coli	1	3	0	0	0	0
P. aerug + Gram negatives	1	2	0	0	0	0
P. aerug + Coliforms	1	1	0	0	0	0
Staphylococcus aureus	0	1	0	0	0	0
Enterobacteria + GNR	1	1	0	0	0	0
Enterobacteria + Coliforms	1	1	0	0	0	0
P. fluorescens + Coliforms	0	1	0	0	0	0
S. epid + Micrococci	0	0	0	0	1	0
S. epid + Enterobacteria	0	0	0	1	1	1
S. aureus + GNR	0	0	0	0	1	1
Total Positive Cultures	16	33	6	11	16	10
Total Sampled	30	49	55	42	44	108

P. aerug = Pseudomonas aeruginosa
S. epid = Staphylococcus epidermidis
S. aureus= Staphylococcus aureus

further 97 were fitted by practitioners outside the UK and insufficient practitioner information was obtained in 262 cases.

The relative risk and population attributable risk percentage have been estimated for any disorder occurring in contact lens users (Table 3.23, page 133). Cases were wearers presenting with a lens related disorder (Category D), and controls were those presenting without lens related disease (Category C).

EWSCL users were found to have the highest overall relative risk of developing any complication at 2.7x (95% confidence interval; 1.7-4.2x), compared with that of GPCL users. The trend of increased risk was found to be significant.

Diagnoses in Group C, for non-lens related diseases, are shown in Appendix 4, and for lens related disease in Appendix 5.

Relative risks and population attributable risk percentages were estimated for a range of lens related disease for two different control groups (Table 3.24 to Table 3.27). Group 1 consisted of lens users without lens related disease and Group 2 consisted of all lens wearers, except those with the specific complication for each analysis.

Table 3.24 (page 134) shows the risk data for toxic and hypersensitivity disorders, using two control groups.

Using group 1 controls (without lens related disorders), the relative risks for EWSCL users were 8.1x, DWSCL users 5.8x and PMMACL users 0.6x, compared with a relative risk of 1 for GPCL users.

Using group 2 controls (without toxic or hypersensitivity disorders), the relative risks for EWSCL users were 4.5x, DWSCL users 5.9x and PMMACL users 0.6x, compared with a relative risk of 1 for GPCL users. The trend of increasing risk was found to be significant using both sets of controls.

TABLE 3.22

Breakdown of Lens Wearers by Lens Type

LENS TYPE	LENS RELATED PROBLEM	NON-LENS RELATED PROBLEM	TOTAL
PMMA	121	71	192
GP	155	92	247
DWS	670	309	979
EWS	158	35	193
TOTALS	1104	507	1611

Table 3.25 (page 135) shows the risk data for abrasions.

Using group 1 controls, (without lens related disorders), the relative risks for EWSCL users were 0.6x, DWSCL users 0.5x and PMMACL users 1.1x, compared with a relative risk of 1 for GPCL users.

Using group 2 controls, (without abrasions), the relative risks for EWSCL users were 0.2x, DWSCL users 0.3x and PMMACL users 1.2x, compared with a relative risk of 1 for GPCL users. The trends of increasing risk were significant for both control groups.

Table 3.26 (page 136) shows the risk data for metabolic disorders.

Using group 1 controls, (without lens related disorders), the relative risks for EWSCL users were found to be 3.7x, for DWSCL users 1.2x and for PMMACL users 1.1x, compared with a relative risk of 1 for GPCL users.

Using group 2 controls, (without metabolic disorders), the relative risks for EWSCL users were found to be 2.1x, for DWSCL users 1.0x and PMMACL users 1.6x, compared with a relative risk of 1.0 for GPCL users. The trend of increasing risk was found to be significant for both control groups.

Table 3.27 (page 137) shows the risk data for sterile keratitis.

Using group 1 controls, (without lens related disorders), the relative risk for EWSCL users were found to be 4.7x, DWSCL users 2.3x and PMMACL users 1.0x, compared with a relative risk of 1 for GPCL users.

Using group 2 controls, (without sterile keratitis), the relative risk for EWSCL users were found to be 2.4x, DWSCL users 2.1x and PMMACL users 1.0x, compared with a relative risk of 1 for GPCL users. The trend of increasing risk was found to be significant for both control groups.

In summary, the relative risks were significantly largest for EWSCL users for metabolic disorders, sterile infiltrates and corneal infections at 2.1-3.7x, 2.4-4.7x and 20.1-36.8x respectively, compared with that of GPCL users. The highest relative risk for toxic and hypersensitivity disorders occurred for DWSCL lenses at 5.8-5.9x that of GPCL.

3.1.5 Epidemiology of *Pseudomonas aeruginosa* in Contact Lens Wearers.

Likely sources of bacterial contamination in the domestic water environment were sampled, for 10 lens wearers with culture proven *P. aeruginosa* keratitis and for 44 controls, without lens related disease.

P. aeruginosa was not isolated from any of the domestic water sites sampled. However, a proportion of cases and controls cultured similar non-lactose fermenting Gram negative organisms, from both the lens storage case and the domestic water environment. This correlation between the organisms from storage cases and domestic water environment was found in 3/10 cases and in 15/44 controls.

Personal carriage of *P. aeruginosa* was assessed in 12 lens wearers with culture proven infections. All sites were culture negative for *P. aeruginosa.*

P. aeruginosa with similar sensitivities to the strain isolated from the corneal ulcer, were identified from the lens storage case in 11/15 culture proven cases; 7 DWSCL lens users and 4 EWSCL users. *Pseudomonas fluorescens* was cultured from one lens storage case, and the remaining 3 were culture negative. 2/15 wearers used daily wear disposable lenses and 1/15 extended wear disposable lenses.

P. aeruginosa was isolated from 1/14 commercial lens solutions.

3.1.6 Epidemiology of *Acanthamoeba* in Contact Lens Wearers.

Tables 3.28 and 3.29 show the results and statistics for the amoebic investigation of the domestic water environment for 50 lens wearers, 6 with *P. aeruginosa* keratitis and 44 controls without lens related disease.

TABLE 3.28

Culture Results for Environmental Swabs

		BATHROOM		KITCHEN		STATISTICS
		Tap	Drain	Dust	Tap	
Gram Negative Rods.	Positive	32	40	-	27	p=0.5786 (C)
	Negative	18	10	-	23	(Not Significant)
Direct Culture Amoebae.	Positive	15	38	7	5	p=0.0114 (F)
	Negative	35	12	43	45	
Direct Culture Acanthamoebae	Positive	1	2	1	0	p=0.5000 (F)
	Negative	49	48	49	50	(Not significant, but numbers small)

TABLE 3.29

Culture Results for Bathroom and Kitchen Water Samples

		BATHROOM	KITCHEN	STATISTICS
Limescale	Present	32	21	p=0.0426 (C)
	Absent	18	29	
Direct Culture Amoebae	Present	24	12	p=0.0208 (C)
	Absent	26	38	
Enrichment Culture Amoebae	Present	42	33	p=0.0613 (C)
	Absent	8	17	(Not Significant)
Direct Culture Acanthamoebae	Present	1	0	p=0.5000 (F)
	Absent	49	50	(Not significant, but numbers small)
Enrichment Culture Acanthamoebae	Present	6	1	p=0.0559 (F)
	Absent	44	49	(*Small numbers test suggests significance)

Homes visited between August 1988 and March 1990 were mainly in North East London. Figure 3.2 shows the distribution of the 50 homes visited. Information was provided by the Thames Water Authority concerning the supply to many of these sites. These investigations showed that the 50 homes visited were supplied by at least 12 different water sources, and that sources corresponding to wearers with infections were represented in the control group.

Table 3.28 shows the results for bacterial and amoebic cultures using alginate swabs. A qualitative assessment only was performed; results were classified either positive or negative, for the presence or absence of organisms. Differences between kitchen and bathroom cultures were analysed using a Chi-Square test (C)[184], or Fisher Exact Test (F), where numbers were small.

No significant difference was found in the rate of contamination between the bathroom and kitchen cold water taps, with environmental Gram negative organisms. Bathroom taps showed a significantly higher rate of contamination with environmental amoebae ($p=0.01$), compared with kitchen taps. However, no significant difference was found between bathroom and kitchen tap contamination with *Acanthamoebae*.

Table 3.29 shows the results for bathroom and kitchen cold water tap samples. Differences between bathroom and kitchen data were analysed using a Chi-Square test using Yates correction (C), or Fisher Exact Test (F), where appropriate.

The presence or absence of limescale in the spun water samples was assessed microscopically. Limescale was found to be significantly associated with bathroom tap water ($p<0.05$). Figures 3.3a and 3.3b show limescale deposit on seeded non-nutrient agar at low and high magnification respectively, with amoebae moving away from the deposit, as the proximal food source becomes depleted. In Figure 3.3b, trophozoites with contractile vacuoles are clearly visible.

FIGURE 3.2 DISTRIBUTION OF HOMES VISITED IN THE LONDON AREA

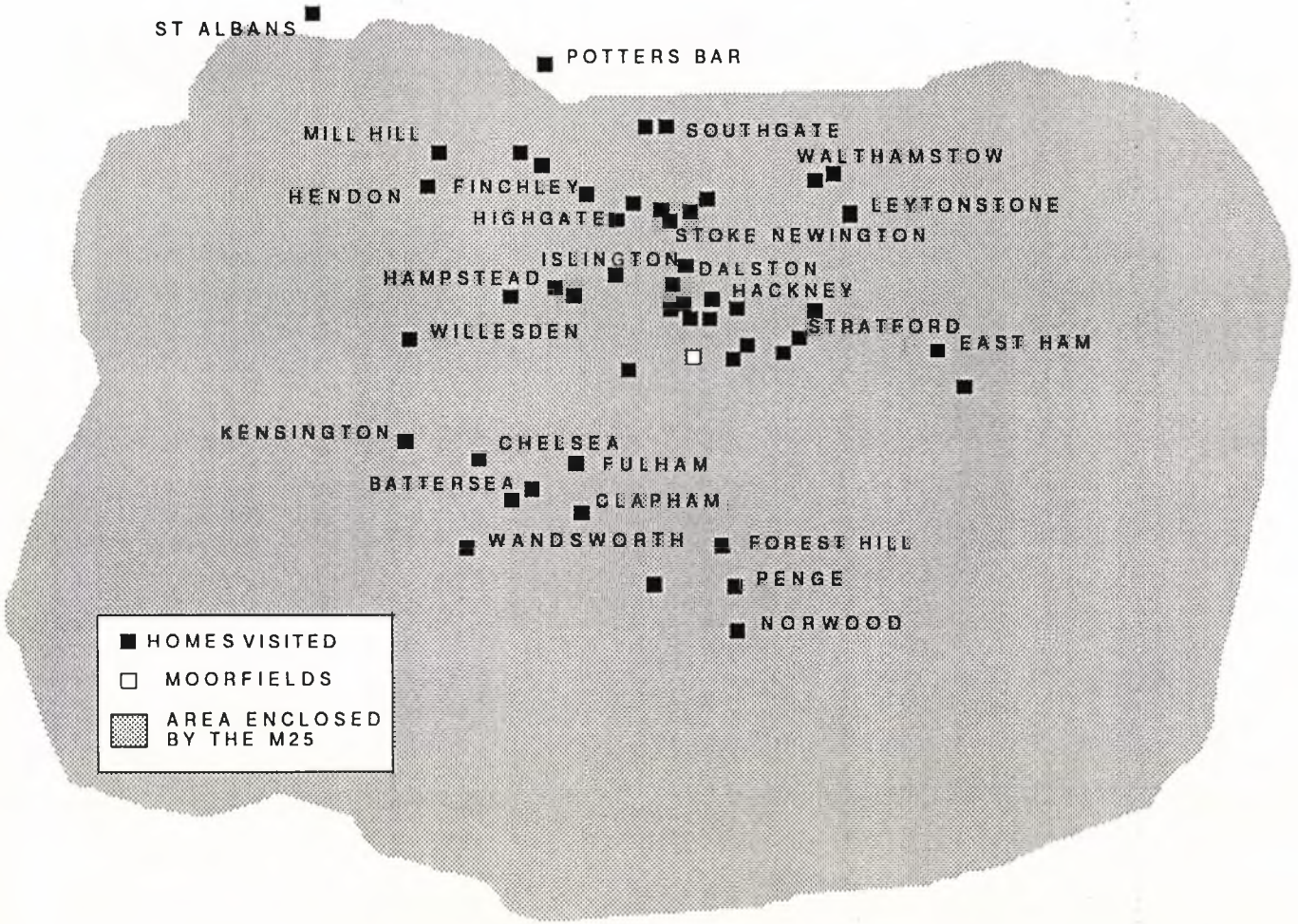
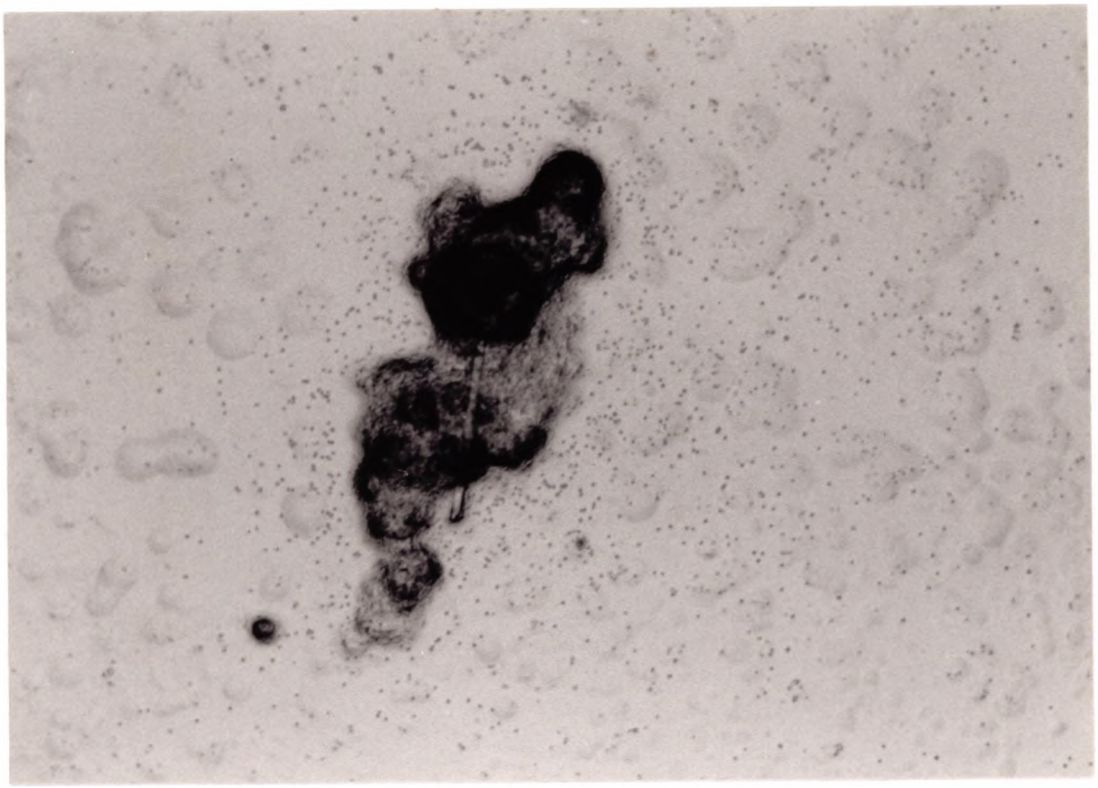


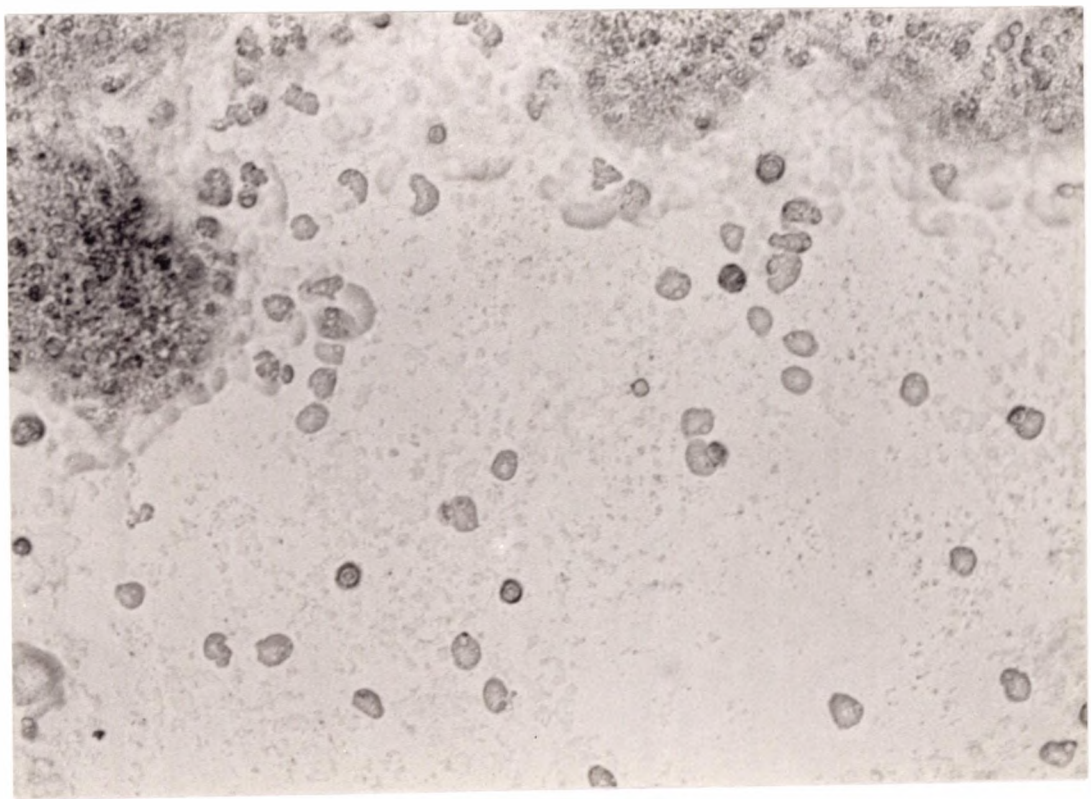
Figure 3.3

Light micrographs showing scale deposit on seeded non-nutrient agar at low (a) and high (b) magnification. Trophozoites of environmental amoebae are seen to move away from spun deposit towards food source.

a. Magnification 40x



b. Magnification 100x.



A direct culture technique, which identified the presence of large numbers of environmental amoebae, demonstrated a higher rate of amoebic contamination in bathroom compared with kitchen water samples. Direct culture of *Acanthameobae* showed no significant difference between bathroom and kitchen water samples. Fungi were also isolated from 2 bathroom and 2 kitchen water samples.

An enrichment culture technique to isolate small numbers of amoebae and *Acanthamoebae* in water samples, was performed by P. Christy and S. Kilvington at Bath Public Health Laboratory. Environmental amoebae were cultured from a higher proportion of water samples using an enrichment technique, compared with a direct culture technique. Amoebae were identified on the basis of cyst morphology, and included Hartmanella, Naegleria, Vahlkamfia, Vanella and Vexillifera species. Figure 3.4 shows *Acanthamoeba* cysts with characteristic star shapes.

Enrichment culture results for amoebae were not significantly different for bathroom and kitchen water samples, although the presence of *Acanthamoebae* was associated with bathroom water samples (From Table 3.29, *using a correction factor for small numbers of observations). Of the six bathroom water samples found to contain *Acanthamoebae*, 5 were also found to contain limescale.

Table 3.30 shows the results for direct amoebic culture and the presence of limescale for kitchen and bathroom water samples. The Fisher exact test was used to test for association between the presence of limescale and amoebae. Environmental amoebae were found to be positively associated with the presence of limescale for both kitchen and bathroom water samples.

3.2 BACTERIAL ADHERENCE TO UNWORN HYDROGEL LENSES

3.2.1 Incubation Time.

Table 3.31 and Figure 3.5, show the results for adherence with time using unwashed organisms. Adherence appears to increase with time reaching a maximum

Figure 3.4

Light micrograph of **Acanthamoeba** cysts showing characteristic star morphology.

Magnification 400x.

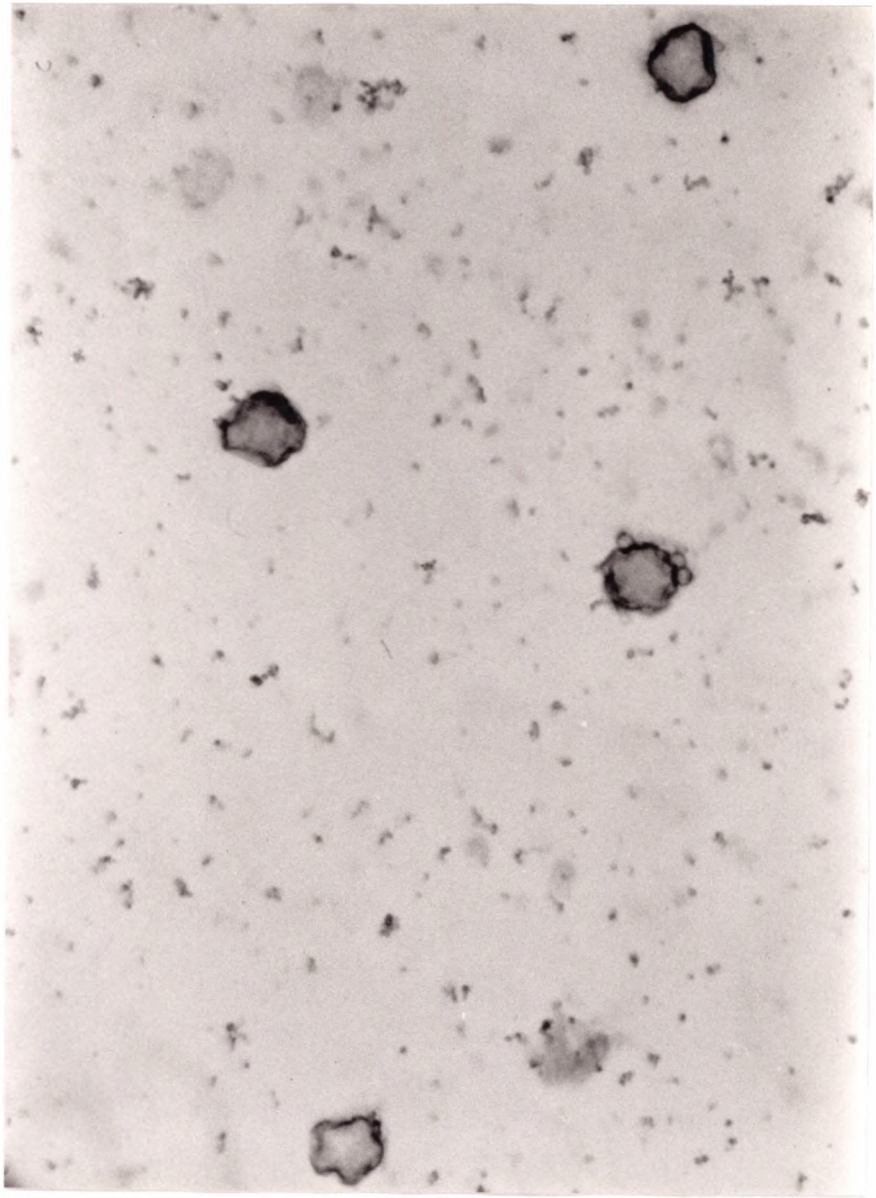


TABLE 3.30

Amoebic Contamination and Limescale Presence for Kitchen and Bathroom Cold Water Samples.

(i) Bathroom Water Samples

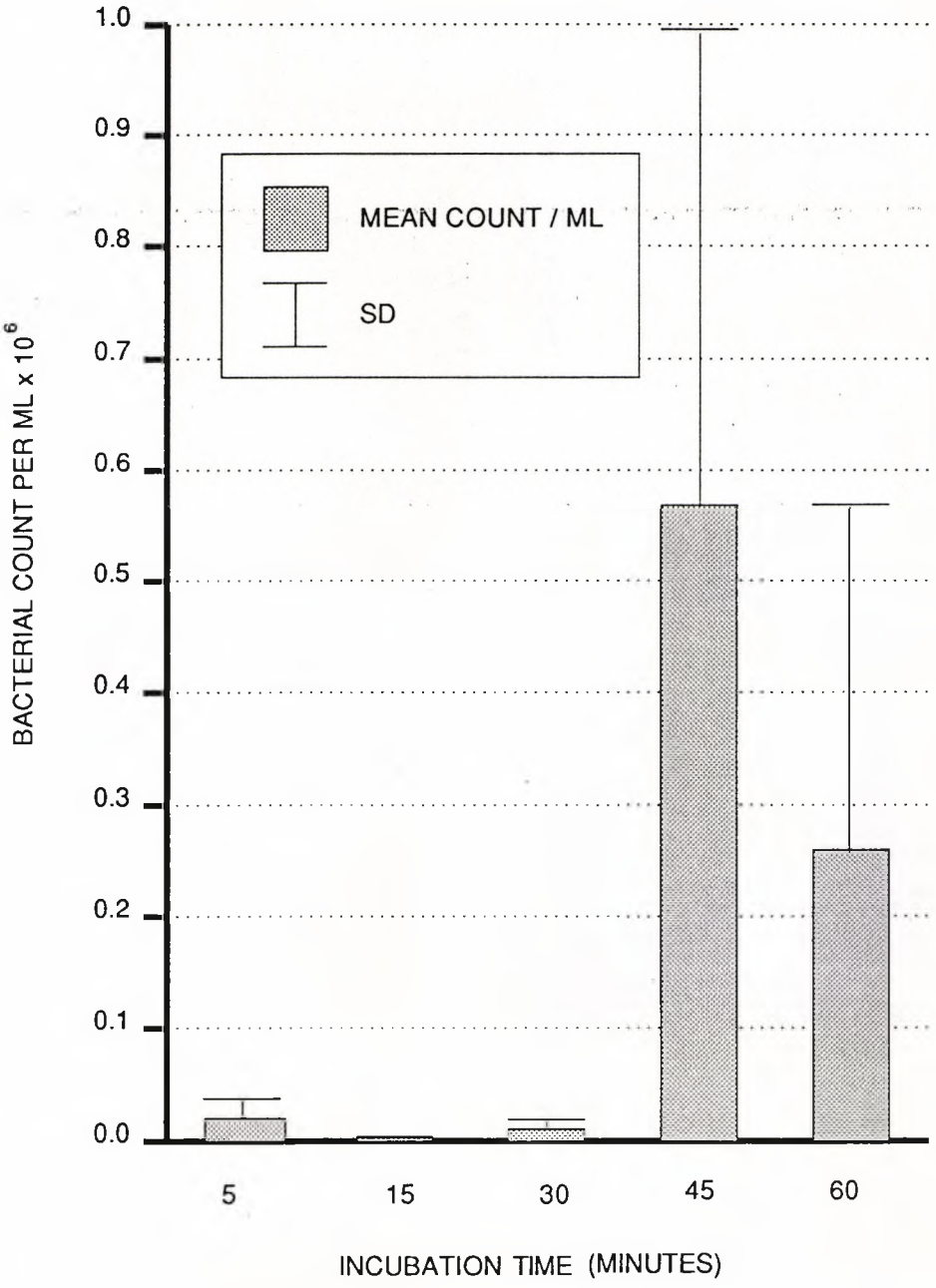
	AMOEBAE PRESENT	AMOEBAE ABSENT	STATISTICS
SCALE PRESENT	24	8	
			p<0.0001 (F)
SCALE ABSENT	0	18	
TOTALS	24	26	

(ii) Kitchen Water Samples

	AMOEBAE PRESENT	AMOEBAE ABSENT	STATISTICS
SCALE PRESENT	11	11	
			p=0.0002 (F)
SCALE ABSENT	1	27	
TOTALS	12	38	

FIGURE 3.5 BACTERIAL ADHERENCE WITH TIME

(VORTEXING TECHNIQUE)



at 45 minutes. Significant differences between counts for different incubation times were found (Kruskal-Wallis One-Way ANOVA, $p=0.001$). In a parallel experiment using washed organisms, no positive cultures were obtained at any incubation time.

Figure 3.6 shows adherent organisms in SEM (a). Front and back surfaces of unworn lenses showed similar levels of dehydration and surface disruption. Adherence was not seen to increase along the linear lathed surface marks (b).

3.2.2 Lens Washing.

Table 3.32 and Figure 3.7, show the data for adherence with time for washed and unwashed organisms, for 15, 30 and 60 minute incubation times. A similar trend of increased adherence with time was seen using this technique for unwashed organisms (Kruskal-Wallis One-Way ANOVA, $p=0.046$). However, differences in adherence with time were not found to be significant for washed organisms (Kruskal Wallis One-Way ANOVA, $p=0.5557$). Little difference in adherence was demonstrated between washed and unwashed organisms. Fewer, non-adherent organisms were removed using this gentle washing technique compared with a vortexing technique.

3.2.3 Lens Surface Film.

Table 3.33 and Figure 3.8, show the results for blotted compared with non-blotted specimens. The overall bacterial adherence counts for 60 minutes incubation time were not found to differ significantly (Mann-Whitney U Test, $p=0.8122$). However, counts for blotted lenses showed more variability compared with non-blotted lenses.

3.2.4 Lens Material Ionic Charge.

Table 3.34 and Figure 3.9, show the results for bacterial adherence to ionic compared with non-ionic lenses. Organisms were found to adhere in significantly higher numbers to non-ionic compared with ionic lenses (Mann- Whitney U Test, $p=0.0072$).

TABLE 3.31

Results for Bacterial Counts/ml with Incubation Time (Minutes) using Unwashed Organisms (Vortexing Technique).

Incubation Time	5	15	30	45	60	Culture
N	3	10	6	3	5	15
Mean Count/ml	2.02e4	2.88e3	1.07e4	5.68e5	2.61e5	4.42e7
SD	1.76e4	1.53e3	7.81e3	4.28e5	3.10e5	2.08e7

ANOVA p=0.001

TABLE 3.32

(a) Results for Bacterial Counts/ml with Incubation Time (Minutes) using Unwashed Organisms (Washing Technique).

Incubation Time (Minutes)	15	30	60	Culture (Unwashed)
N	7	4	4	6
Mean Count/ml	4.64e5	2.35e6	1.50e6	4.83e7
SD	1.98e5	1.15e6	3.17e5	2.11e7

ANOVA p=0.046

(b) Results for Bacterial Counts/ml with Incubation Time (Minutes) using Washed Organisms (Washing Technique).

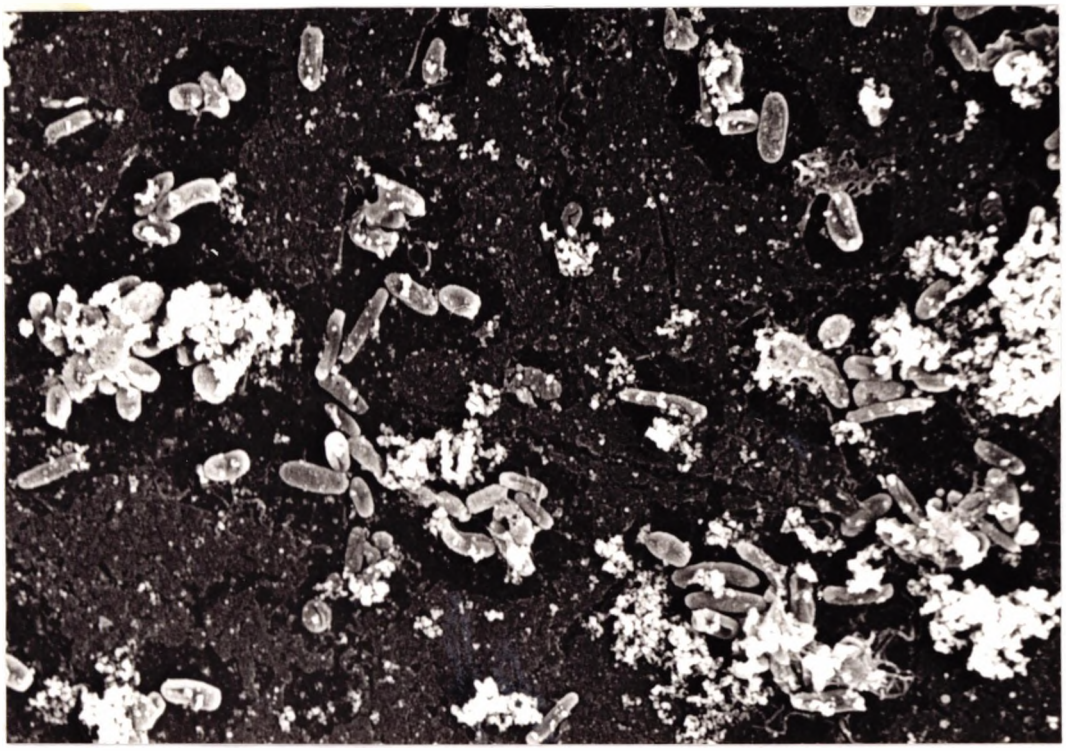
Incubation Time (Minutes)	15	30	60	Culture (Washed)
N	6	6	6	6
Mean Count/ml	1.26e6	3.19e6	1.92e6	3.33e7
SD	7.50e5	3.07e6	9.14e5	2.04e7

ANOVA p=0.5557 (Not Significant)

Figure 3.6

Scanning micrographs showing organisms adherent to an unworn ED4 lens in SEM (a) after an incubation time of 30 minutes with unwashed organisms. Front and back surfaces of unworn lenses showed similar levels of dehydration and surface disruption. Adherence was not seen to increase along the linear lathed surface marks (b).

a. Magnification 3500x, 1 cm bar represents 2um.



b. Magnification 3000x, 1 cm bar represents 2.3um.

TABLE 3.33

Results for Bacterial Count/ml using Blotted and Non-Blotted Lenses using Washed Organisms for 60 minutes Incubation Time.

Method	Blotted	Non-Blotted	Culture (Washed)
N	21	22	17
Mean Count/ml	1.81e6	1.72e6	2.41e7
SD	2.43e6	1.54e6	1.46e7

p=0.8122 (Not Significant)

TABLE 3.34

Results for Bacterial Counts/ml using Ionic and Non-Ionic Lens Materials for 60 minutes Incubation Time.

Method	Ionic	Non-Ionic	Culture (Washed)
N	18	15	17
Mean Count/ml	1.60e6	3.58e6	2.41e7
SD	1.71e6	1.87e6	1.46e7

p=0.0072

FIGURE 3.7 BACTERIAL ADHERENCE WITH TIME

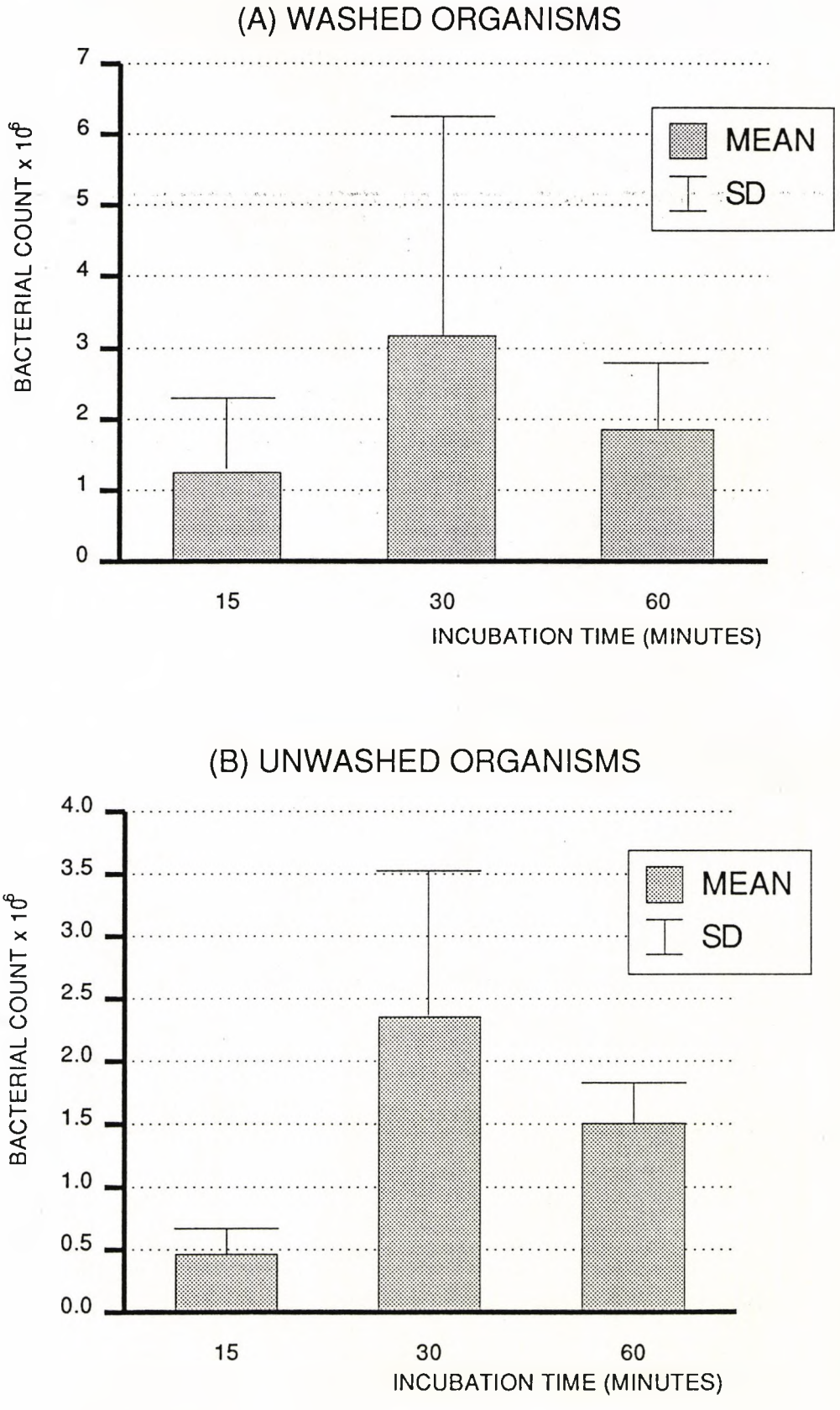


FIGURE 3.8 BACTERIAL ADHERENCE TO WASHED & BLOTTED LENSES

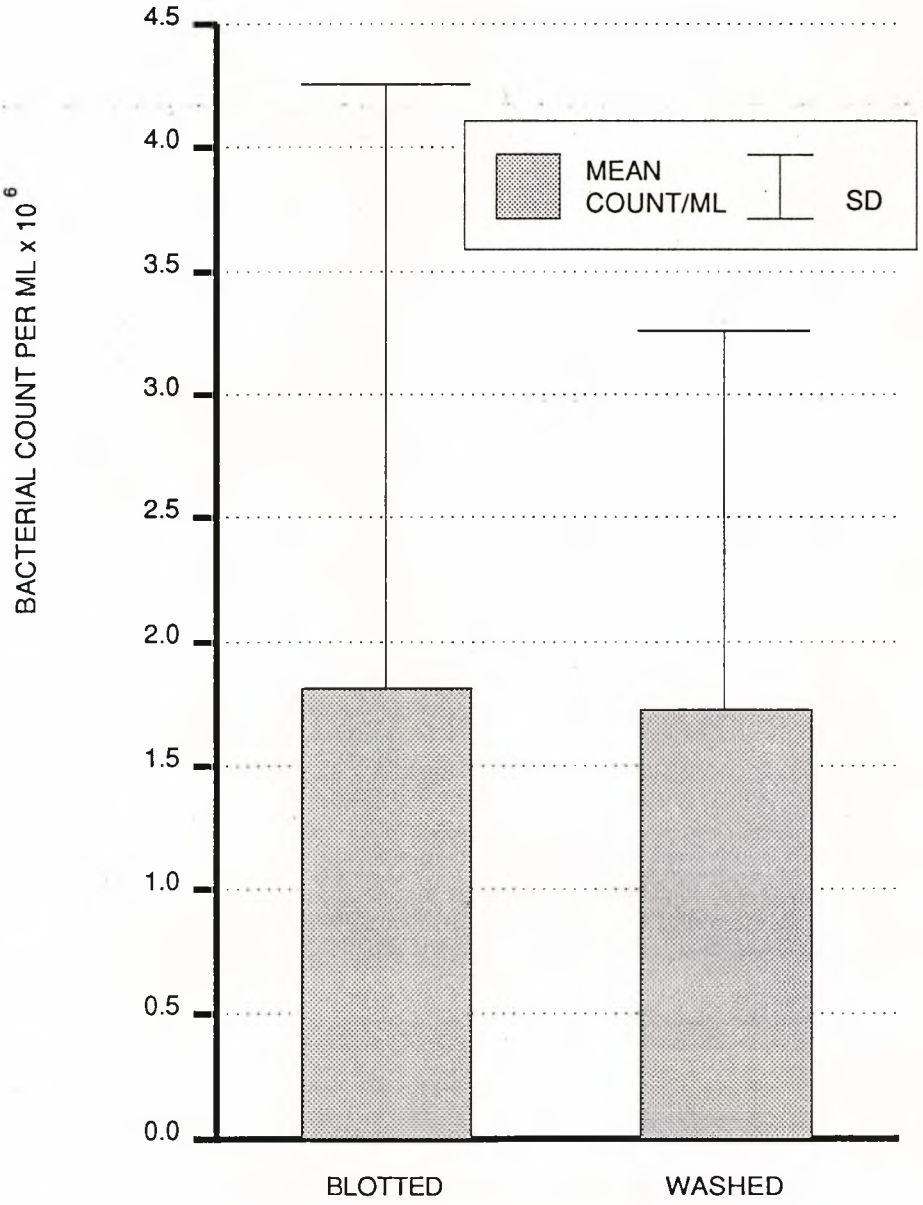
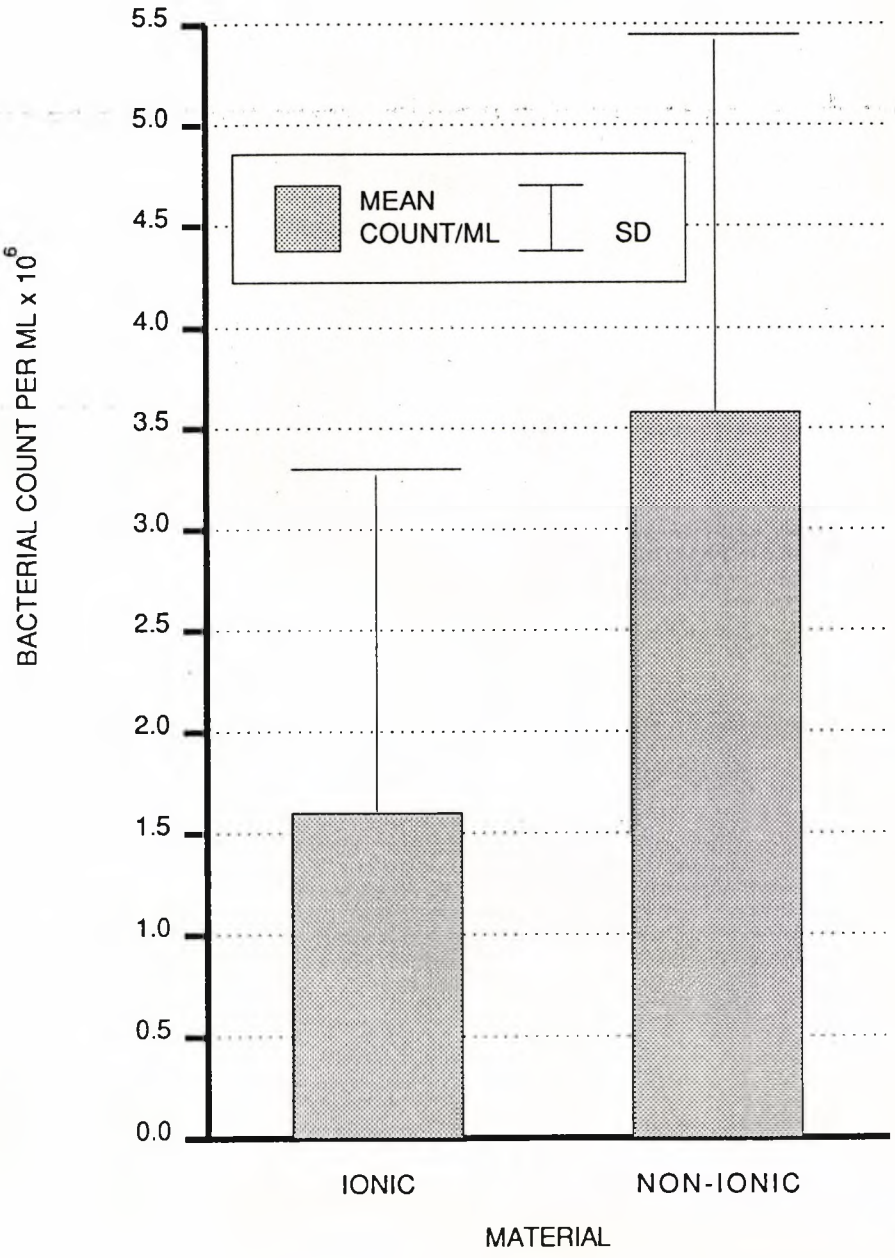


FIGURE 3.9 BACTERIAL ADHERENCE TO IONIC AND NON-IONIC LENSES



3.3 PATIENT MATERIAL

Evaluation of techniques for storage and processing of patient material was performed, using both new and worn ED4 hydrogel lenses, and new hydroxyethylmethacrylate (HEMA) lathed and spun cast lenses. This was performed to evaluate processing artefacts and to optimise the storage and fixation of patient material.

Air drying caused considerable irregular surface disruption. Fewer artefacts and less surface disruption occurred with critical point drying compared with either air drying or dehydration with HMDS. More disruption was apparent with samples which had been frozen slowly compared with snap frozen specimens. The least disruption and blistering was apparent with specimens which had been either fixed immediately, or stored in PBS at $4^{O}C$ prior to fixation. Specimen storage in OCT, followed by several rinses in PBS, resulted in minimal surface debris.

Less surface dehydration was apparent with HEMA lenses compared with ED4 lenses, irrespective of the method of processing. Front and back surfaces of unworn lenses showed similar levels of surface dehydration and disruption.

Figure 3.10 shows a comparison of each of the methods performed for different materials.

Lenses and lens cases from patients with lens related keratitis were retained where possible. Samples were investigated using quantitative or semiquantitative microbiology, scanning and transmission electron microscopy. Table 3.35 summarises these results for 9 cosmetic lens users and for 2 aphakic extended wear users. Examples of characteristic micrographs from the lenses and lens cases are shown in Figures 3.11 to 3.21.

Figure 3.10

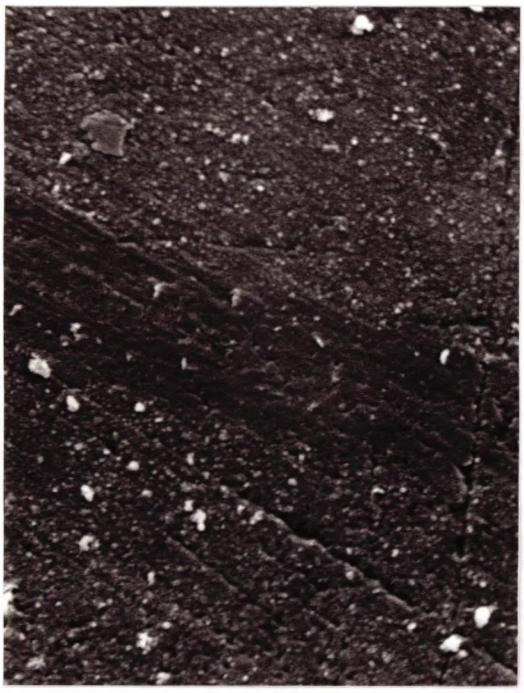
Scanning micrographs showing surface of an unworn ED4 hydrogel lens dehydrated using critical point drying (a) and HMDS dehydration (b). Each method was performed for snap frozen (i), slow frozen (ii) and fresh specimens (iii).

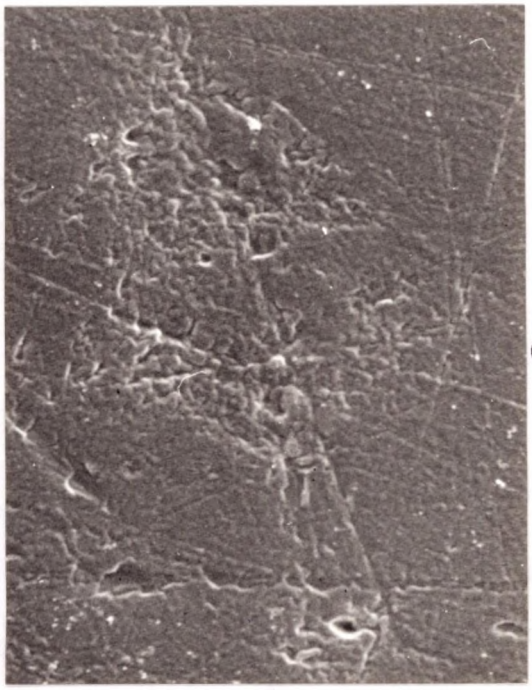
Surfaces for lathe cut Hema lenses (c) are shown for critical point drying (i) and HMDS dehydration (ii).

Surfaces Spun cast Hema lenses (d) are shown for critical point drying (i) and HMDS dehydration (ii).

a. Magnification 1500x, 1 cm bar represents 4.7um.

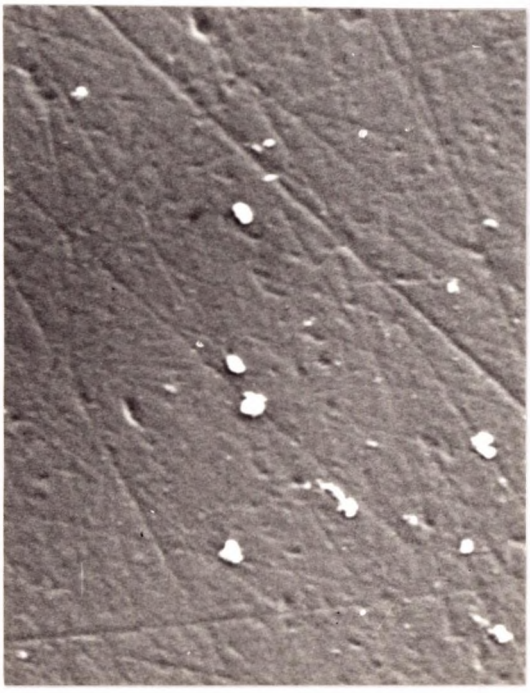
(i)





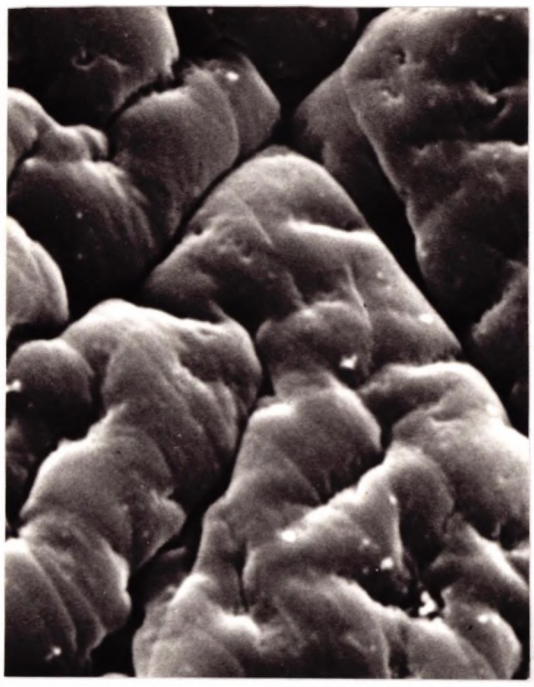
(ii)

(iii)

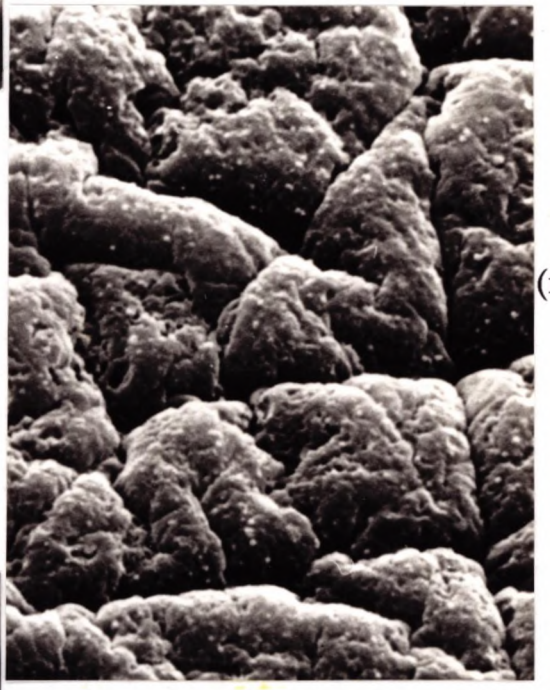


b. Magnification 1500x, 1 cm bar represents 4.7um.

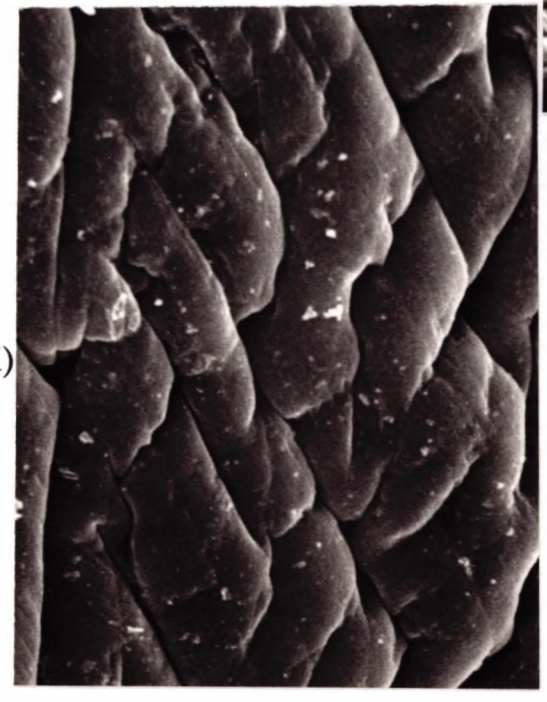
(i)



(ii)

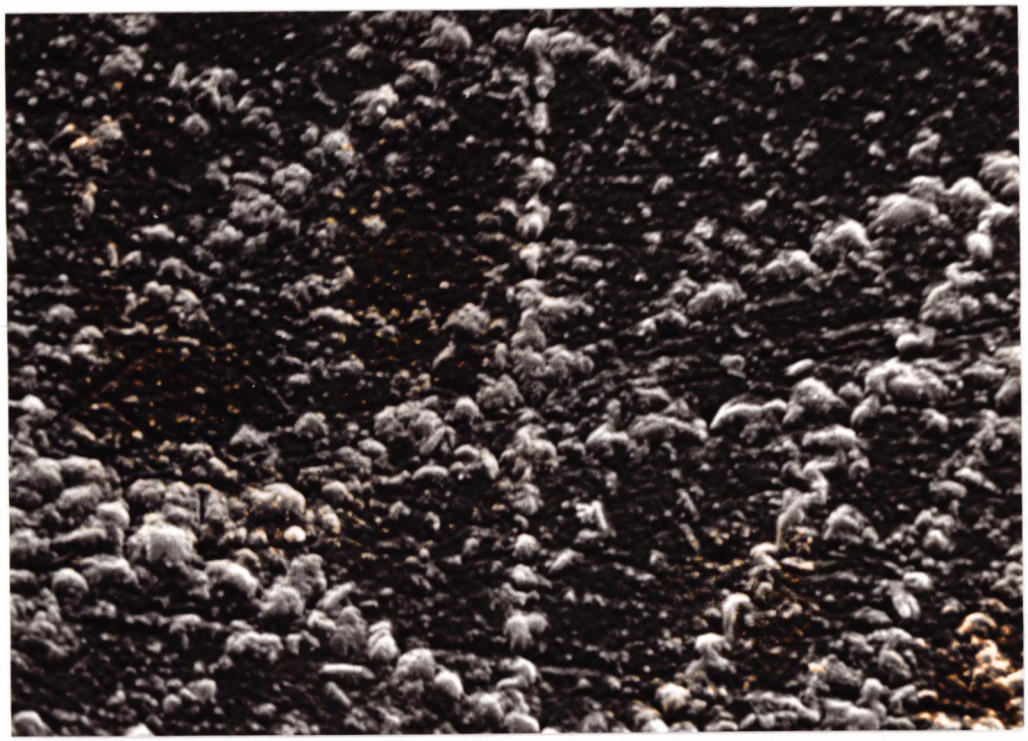


(iii)



c. Magnification 1500x, 1 cm bar represents 4.7um.

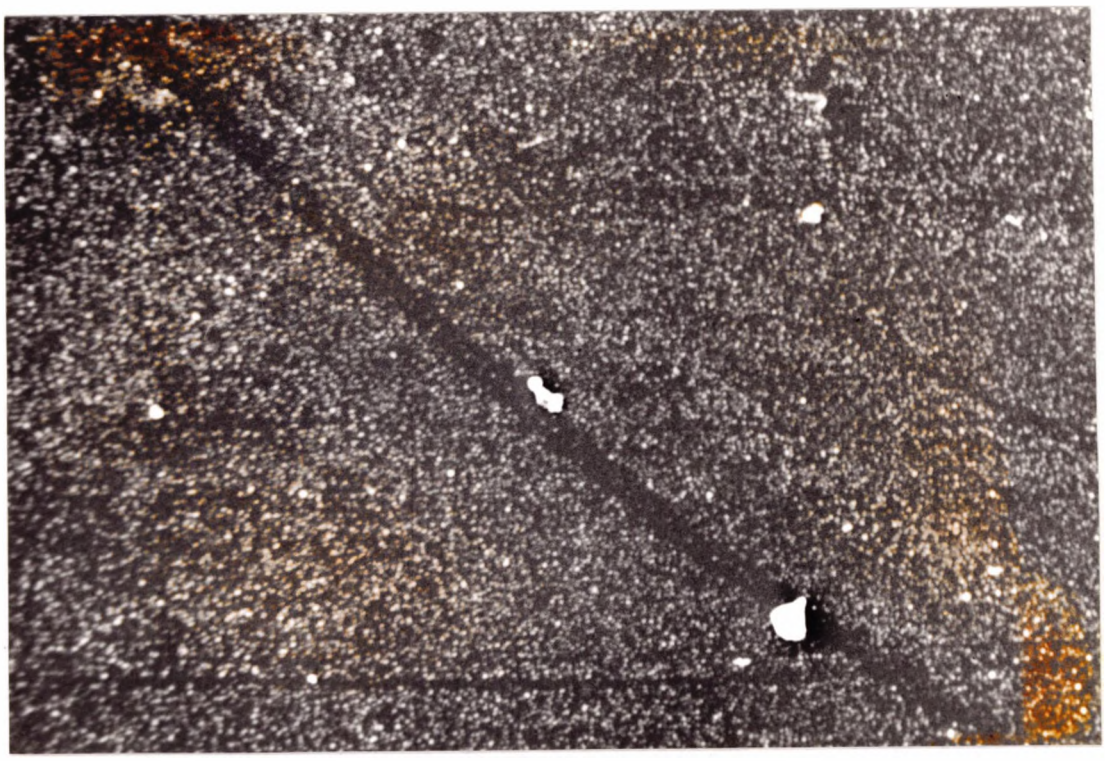
(i)



(ii)

d. Magnification 1000x, 1 cm bar represents 7.0um.

(i)



(ii)

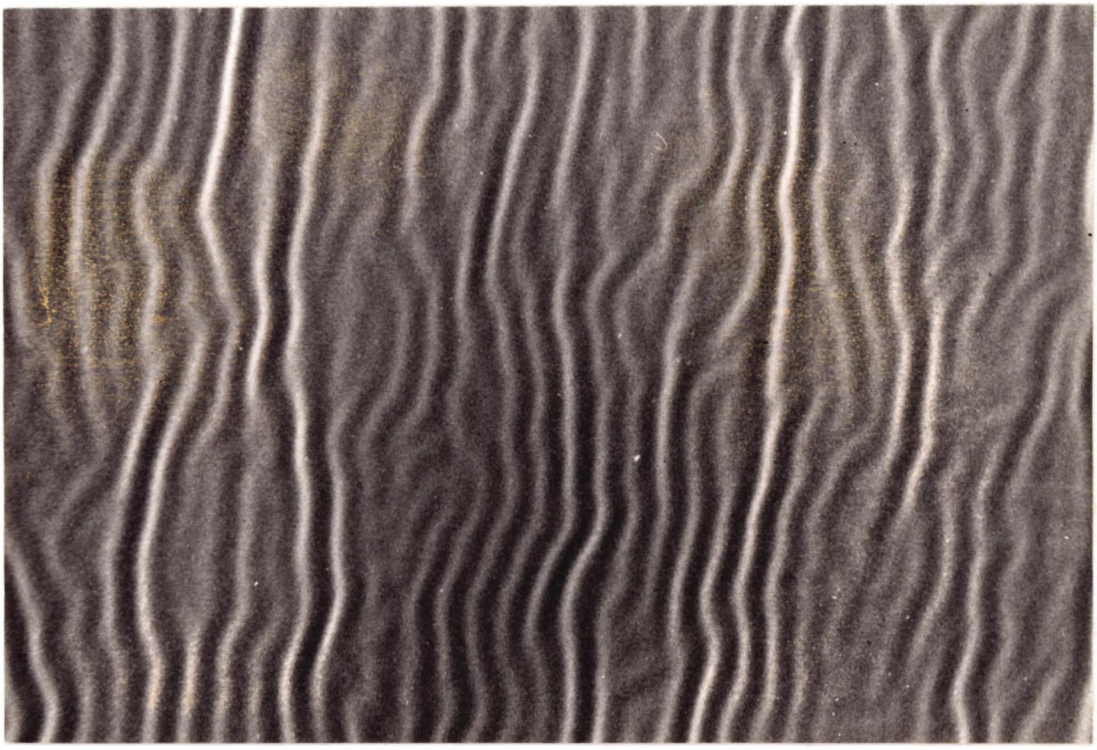


TABLE 3.35

NO.	LENS TYPE (AGE)	ULCER	LENS + STORAGE CASE*	SEM	TEM
1	EWS (12/12)	P.aerug	$1x10^9$ orgs/ml P.aerug + E.coli (case)	Fig 3.11	Fig 3.11
2	DWS (2/52 Disp)	P.aerug	$4x10^6$ P.aerug/ml (lens) $1.7x10^7$ P.aerug/ml (case)	Fig 3.12	Not done
3	DWS (36/12)	N/G	$2.65x10^7$ P.aerug/ml (case)	Fig 3.13	Fig 3.13
4	DWS (4/12)	P.aerug	$1.07x10^7$ P.aerug/ml (case) $6.08x10^5$ P.aerug/ml (lens)	Fig 3.14	Fig 3.14
5	DWS (4/52 Disp)	P.aerug	$2.5x10^5$ P.aerug/ml (lens) $3x10^8$ P.aerug/ml (case)	Fig 3.15	Not Done
6	DWS (? age)	N/G	$1.7x10^7$ P.aerug/ml (lens) Storage Case Discarded	Fig 3.16	Not Done
7	EWS (4/12)	N/G	N/G	Fig 3.17	Not Done
8	DWS (24/12)	N/G	(4) Coliforms	Fig 3.18	Not Done
9	EWS (? age)	N/G	No storage case used	Fig 3.19	Not Done
10	DWS (? age)	S.marc	N/G	Fig 3.20	Fig 3.20
11	EWS (? age)	N/G	No storage case used	Fig 3.21	Not Done

* Quantitative results shown represent the mean of 4 counts performed on nutrient agar.

P.aerug = Pseudomonas aeruginosa
S.marc = Serratia marcescens
Disp = Disposable lens

Figure 3.11

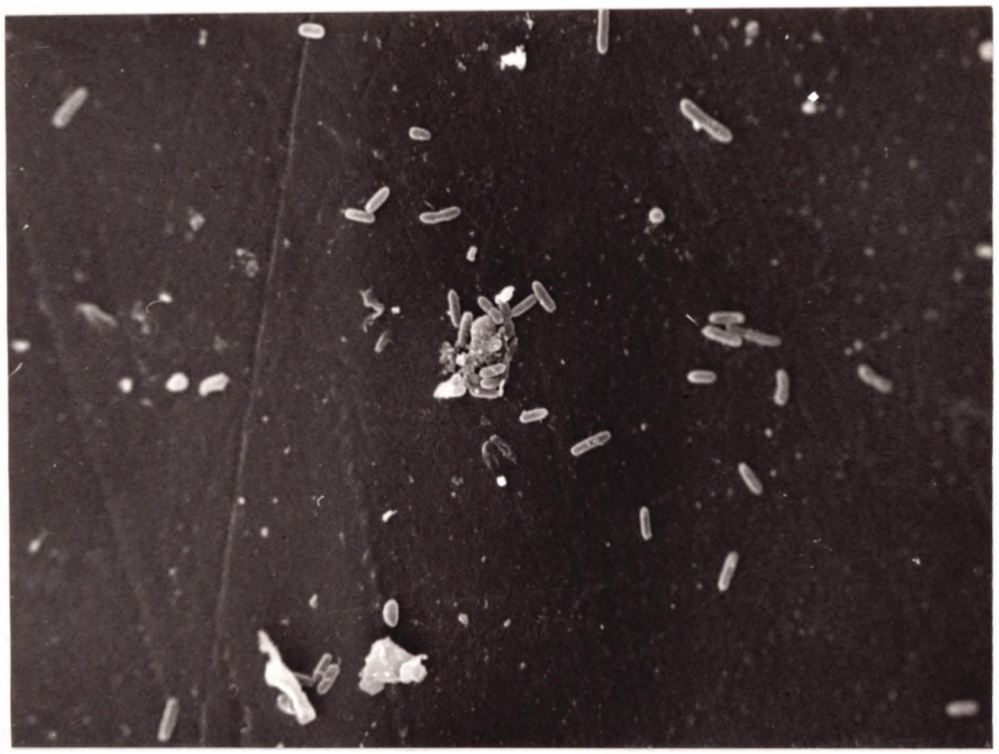
Scanning micrographs showing front and back surface of a 12 month old extended wear lens and storage case. *P. aeruginosa* was isolated from both ulcer and contact lens case. SEM at low and high magnification show rod shaped organisms adherent mainly to the back surface of the contact lens (a,b,d). Organisms were often associated with patchy surface biofilm (a). The front surface of the lens was found to show considerably more surface disruption compared with the back surface at the same magnification (c,e).

SEM of the lens storage case revealed a patchy surface film with isolated rod shaped organisms adherent to its surface (f).

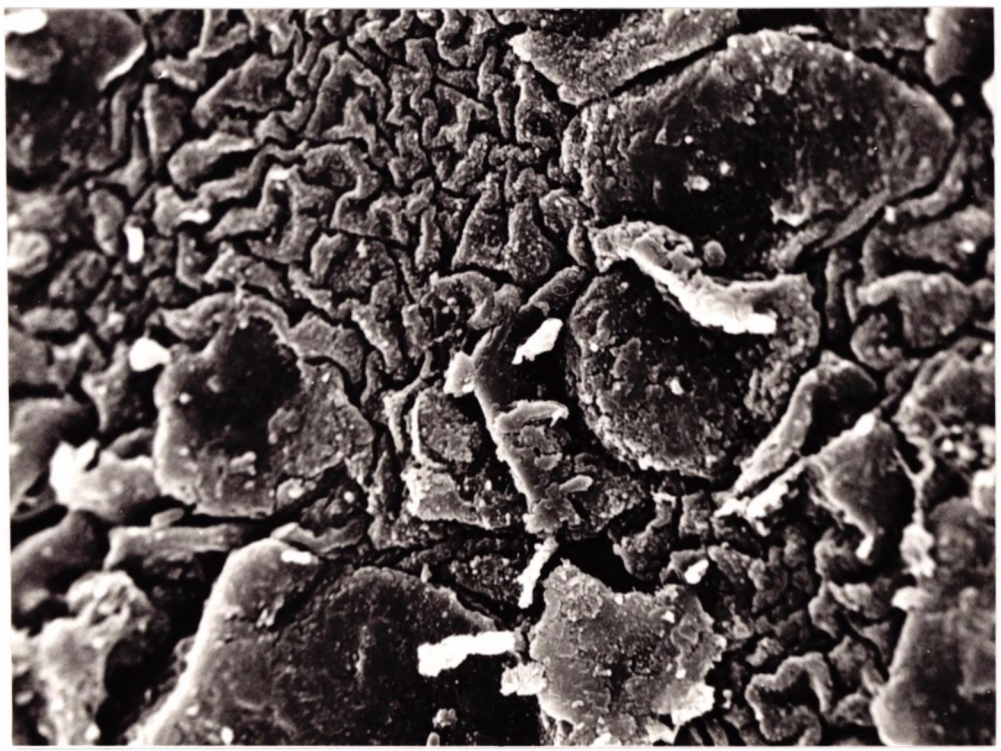
TEM of the contact lens surface (g-i) showed a ruthenium positive surface layer, thicker on the back compared with the front lens surface. Penetration of the lens surface by osmium tetroxide occurred, such that the lens appeared more dense than the surrounding resin. Organisms were present on the back surface of the lens and appeared to be associated with a more dense ruthenium staining layer (j,k). Splits visible in the lens surface were likely to have been induced during processing.

a. Back lens surface, magnification 5000x, 1 cm bar represents 1.4um.

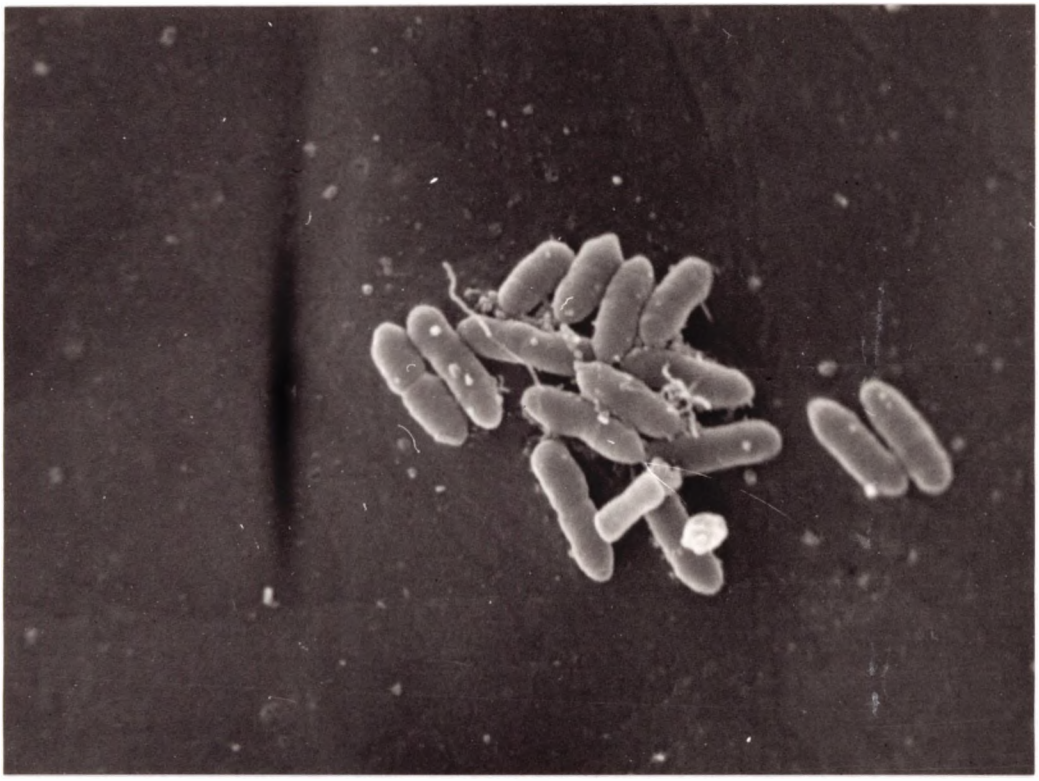
b. Back lens surface, magnification 2000x, 1 cm bar represents 3.5um.



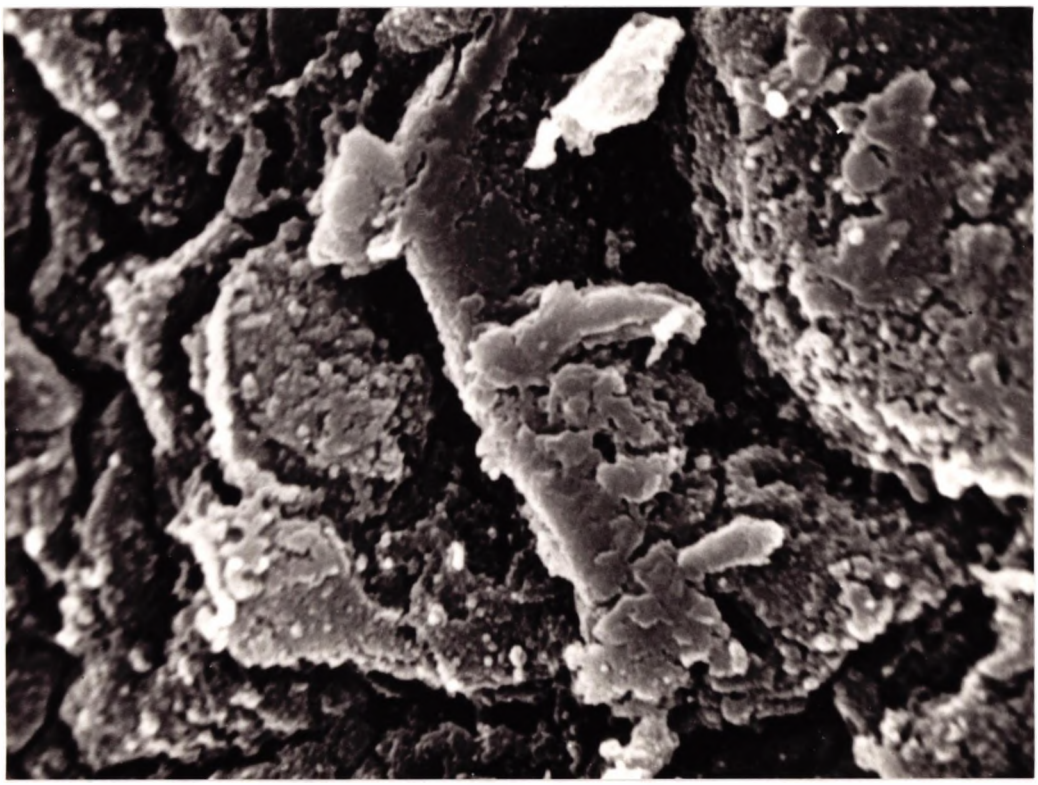
c. Front lens surface, magnification 2000x, 1 cm bar represents 3.5um.



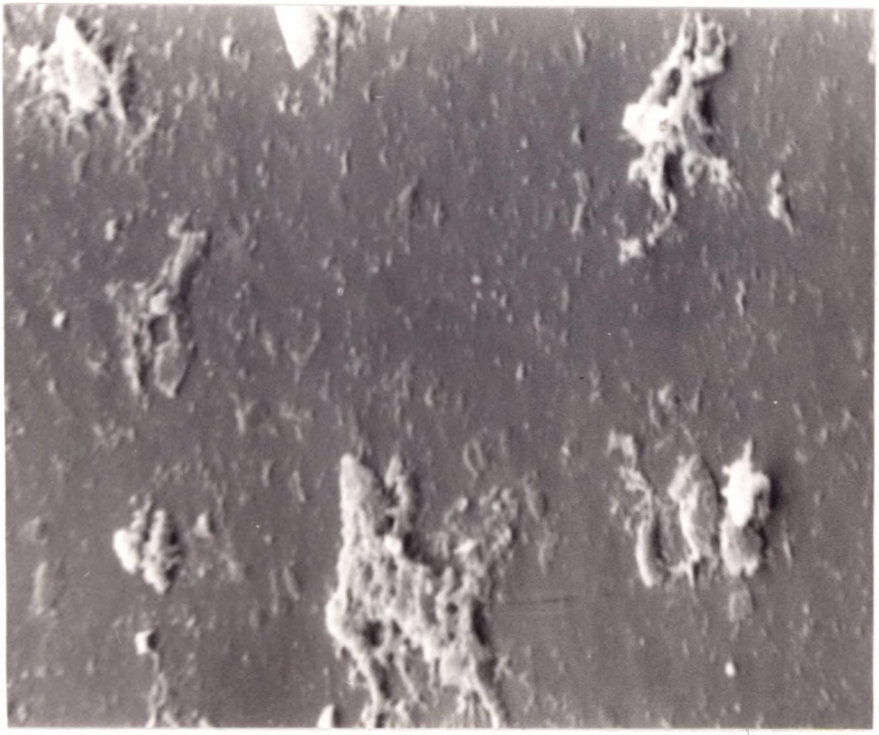
d. Back lens lens surface, magnification 8000x, 1 cm bar represents 0.9um.



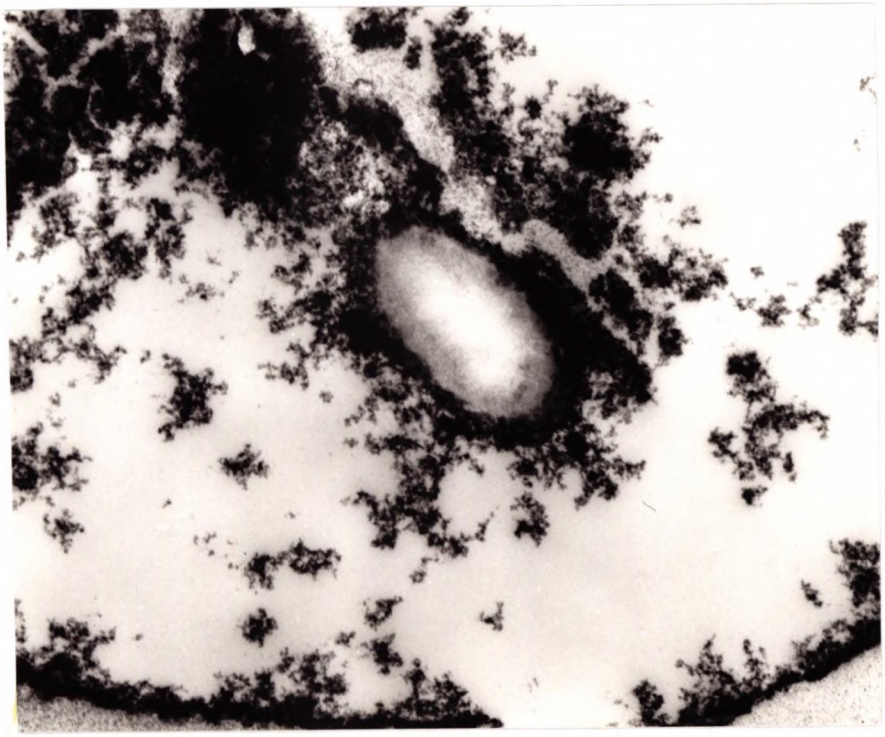
e. Back lens surface, magnification 8000x, 1 cm bar represents 0.9um.



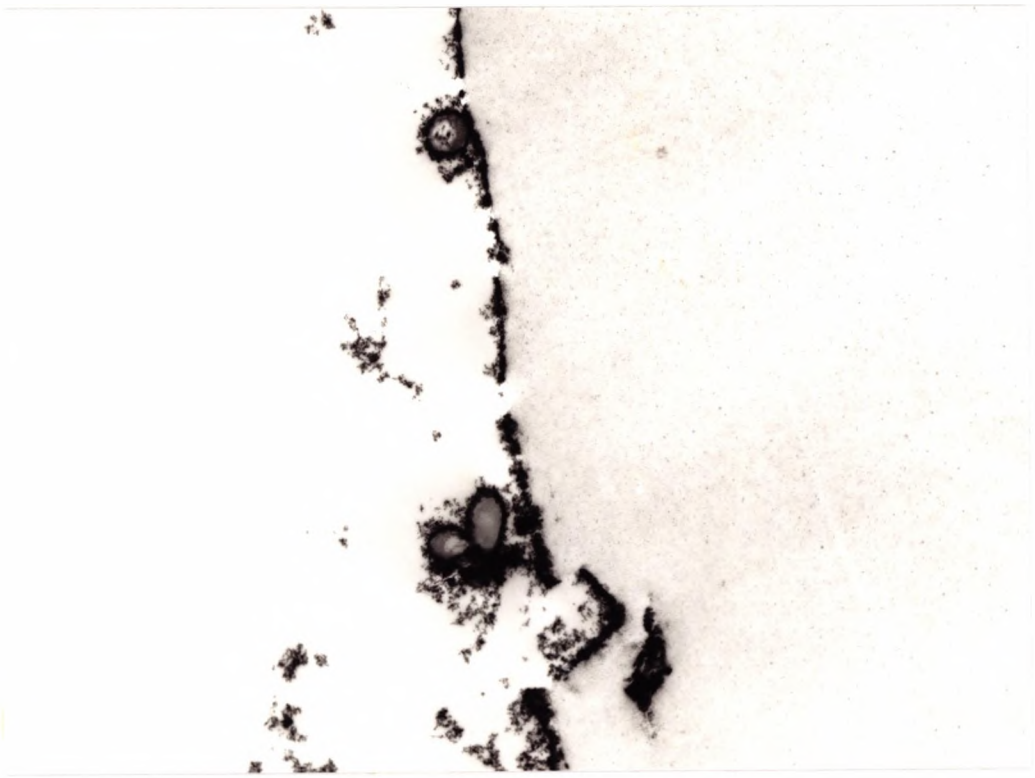
f. Lens storage case, magnification 5000x, 1 cm bar represents 1.6um.



g. Back lens surface, magnification 20000x, 1 cm bar represents 0.4um.



h. Back lens surface, magnification 6000x, 1 cm bar represents 1.2um.



i. Back lens surface, magnification 20000x, 1 cm bar represents 0.4um.

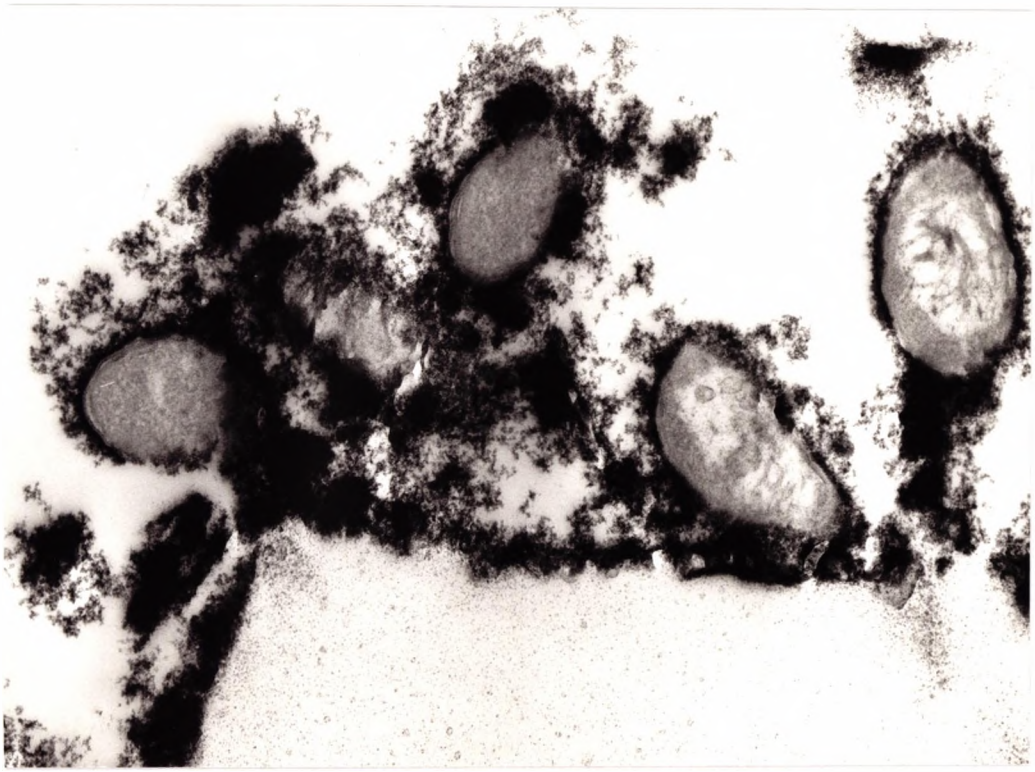
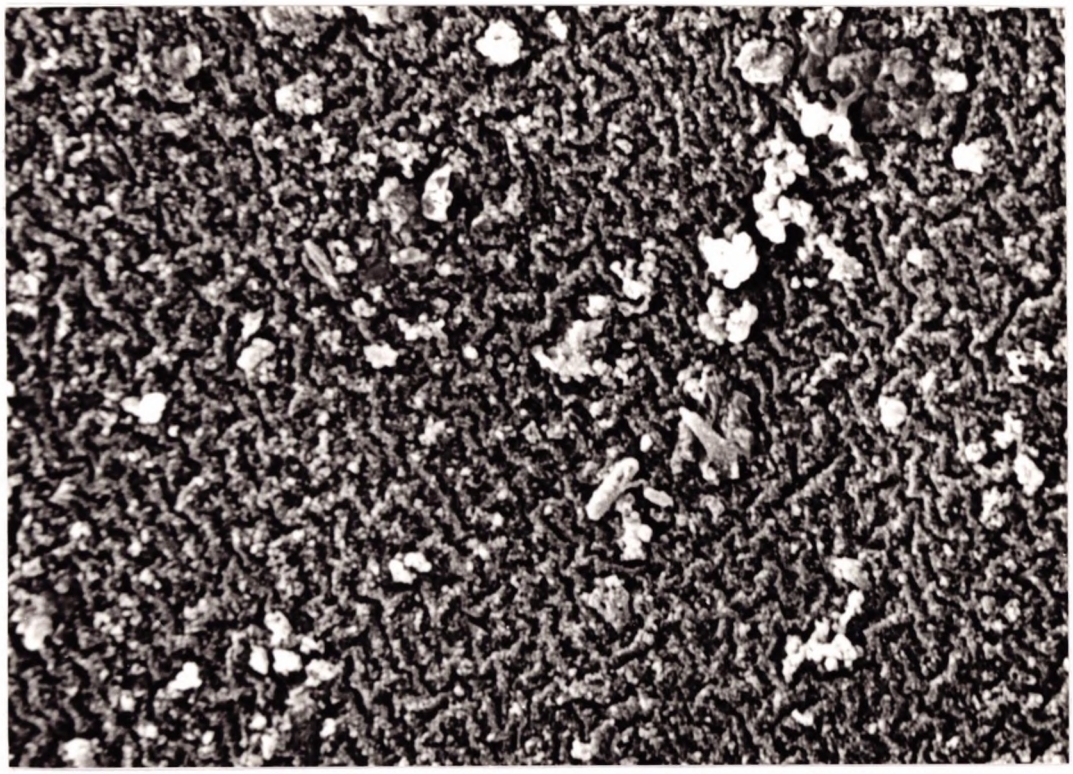


Figure 3.12

Scanning micrographs showing front and back surface of a 2 week old disposable lens worn on a daily wear basis. **P. aeruginosa** was isolated from both the ulcer and contact lens case and SEM showed rod shaped organisms adherent mainly to the lens back surface. Front (a) and back (b) lens surfaces showed similar levels of dehydration and surface disruption.

a. Magnification 2000x, 1 cm bar represents 3.5um.



b. Magnification 2000x, 1 cm bar represents 3.5um.

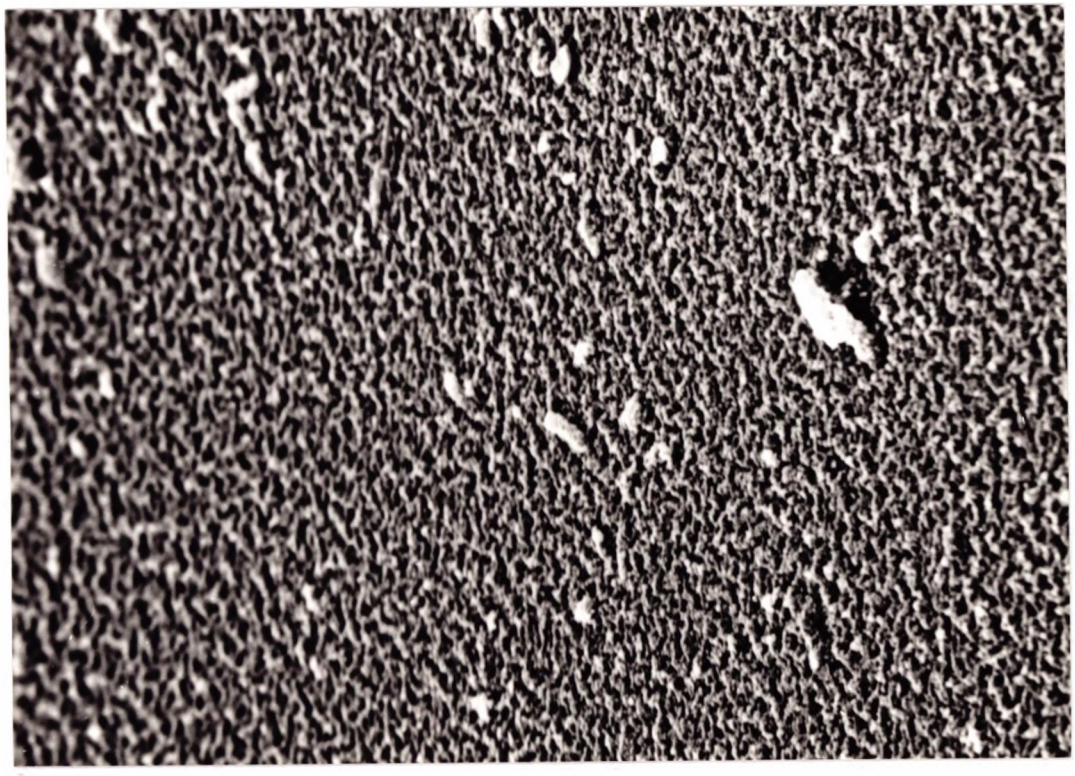


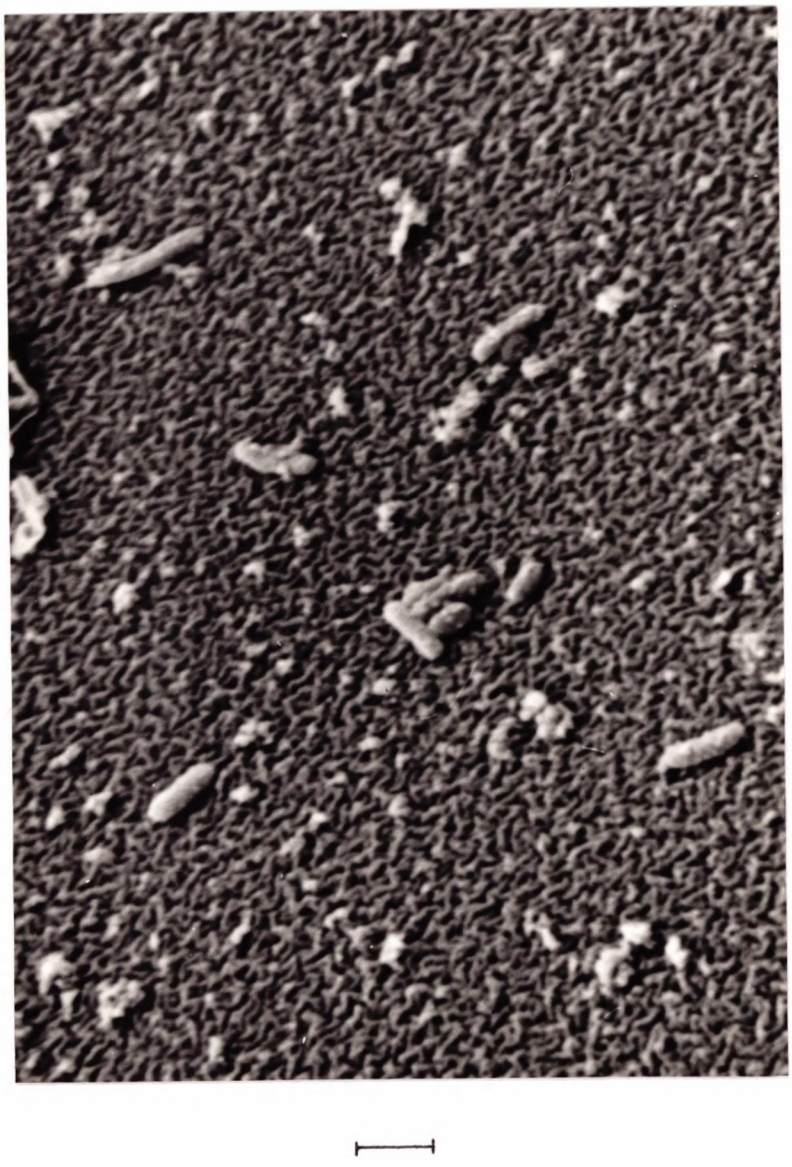
Figure 3.13

Scanning micrographs showing front and back surface of a 3 year old daily wear Hema lens. **P. aeruginosa** was isolated from the lens storage case, although no organisms were isolated from the ulcer. SEM of the lens surfaces demonstrated rod shaped organisms adherent mainly to the back surface (a). Similar levels of disruption and dehydration were visible on both surfaces.

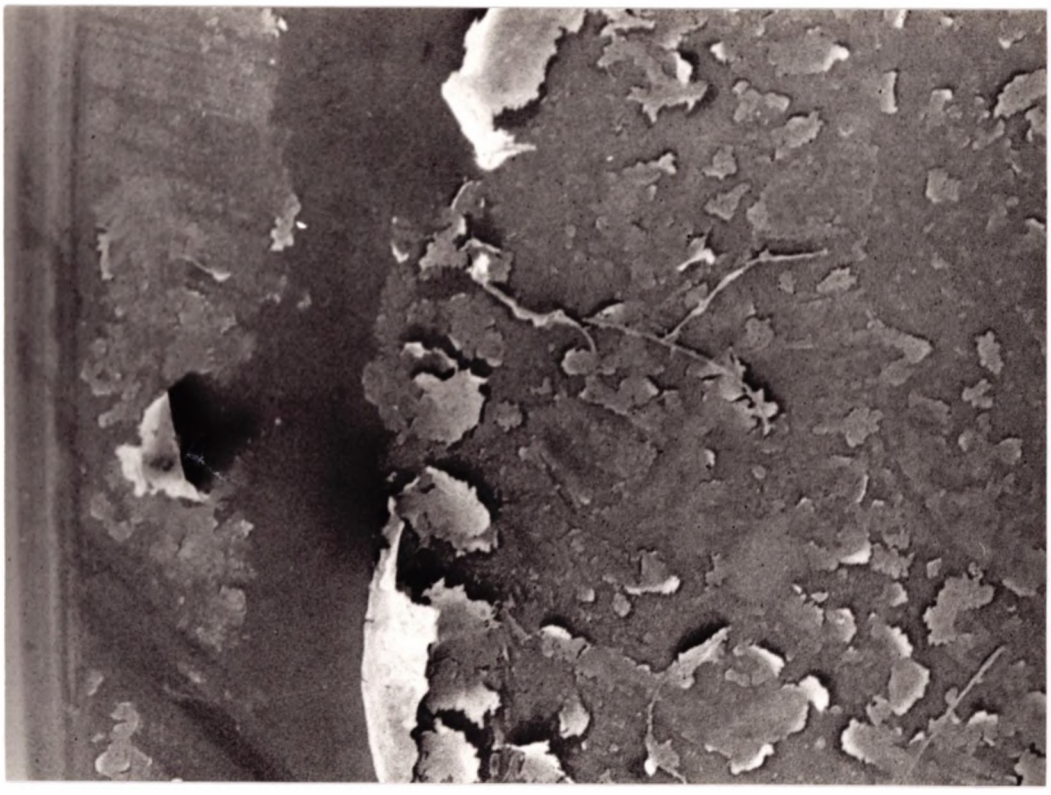
SEM of the lens storage case showed a dense film lining the case wells (b). At a higher magnification of 1200x, this thick film showed a mesh type structure trapping debris (c).

Transmission microscopy of the front and back surfaces of the contact lens showed a ruthenium positive layer of equal thickness on both surfaces. Low and high power micrographs of this film are shown (d,e respectively). No organisms associated with this film were seen.

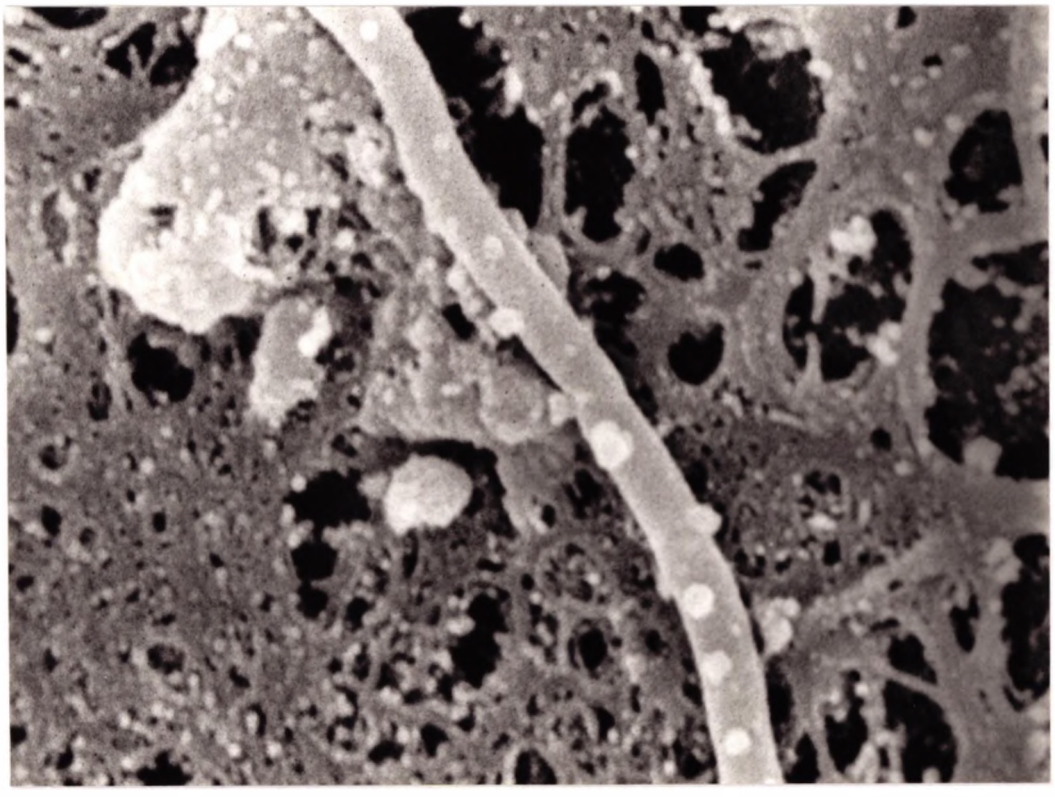
a. Back lens surface, magnification 4000x, 1 cm bar represents 1.7um.



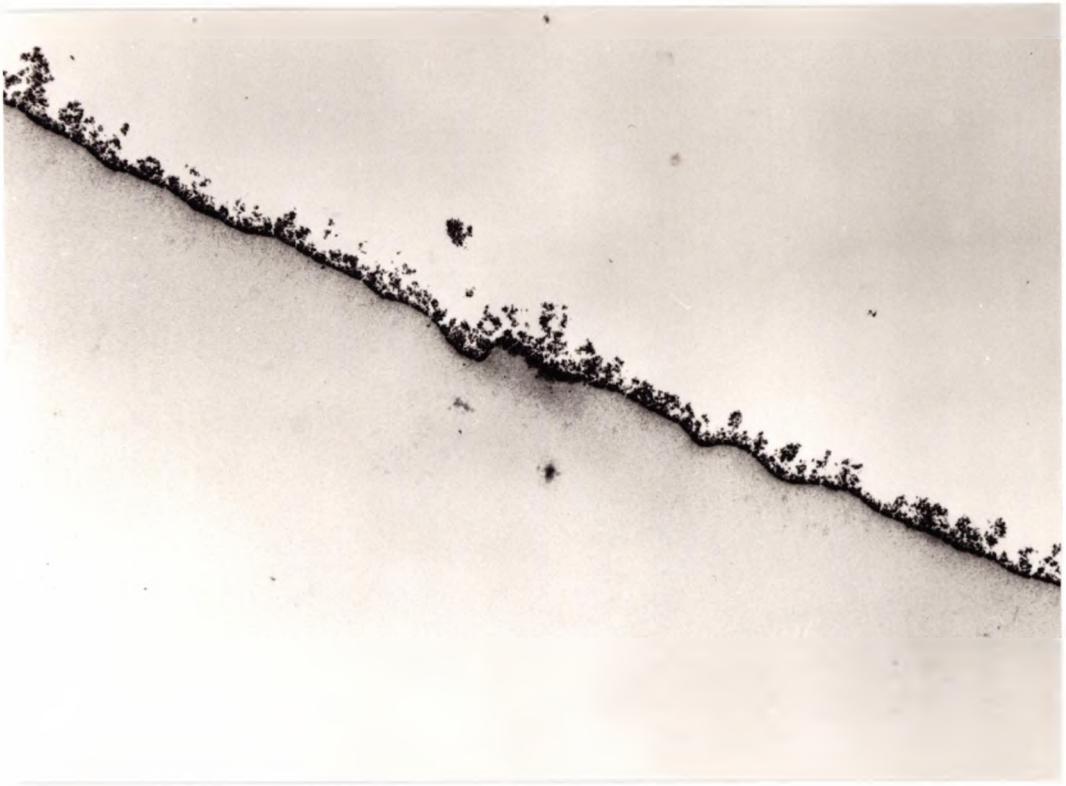
b. Lens storage case, magnification 20x, 1 cm bar represents 349um.



c. Lens storage case, magnification 1200x, 1 cm bar represents 5.8um.



d. Back lens surface, magnification 2000x, 1 cm bar represents 3.5um.



e. Back lens surface, magnfication 30000x, 1 cm bar represents 0.2um.

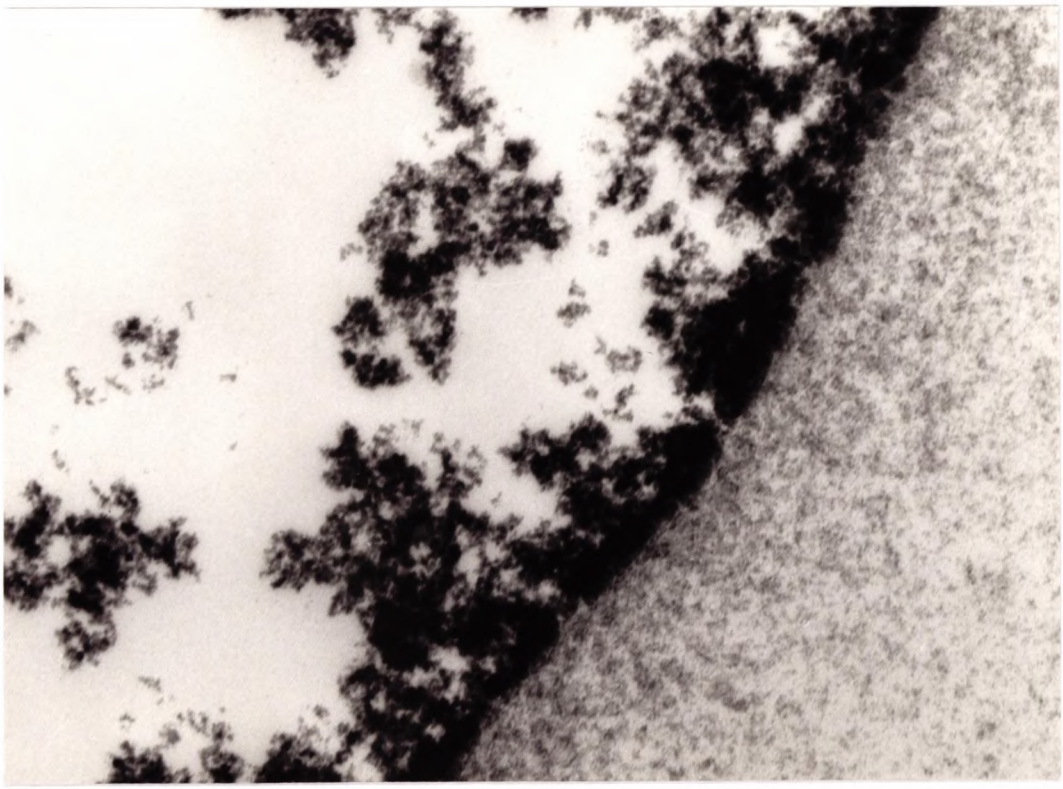
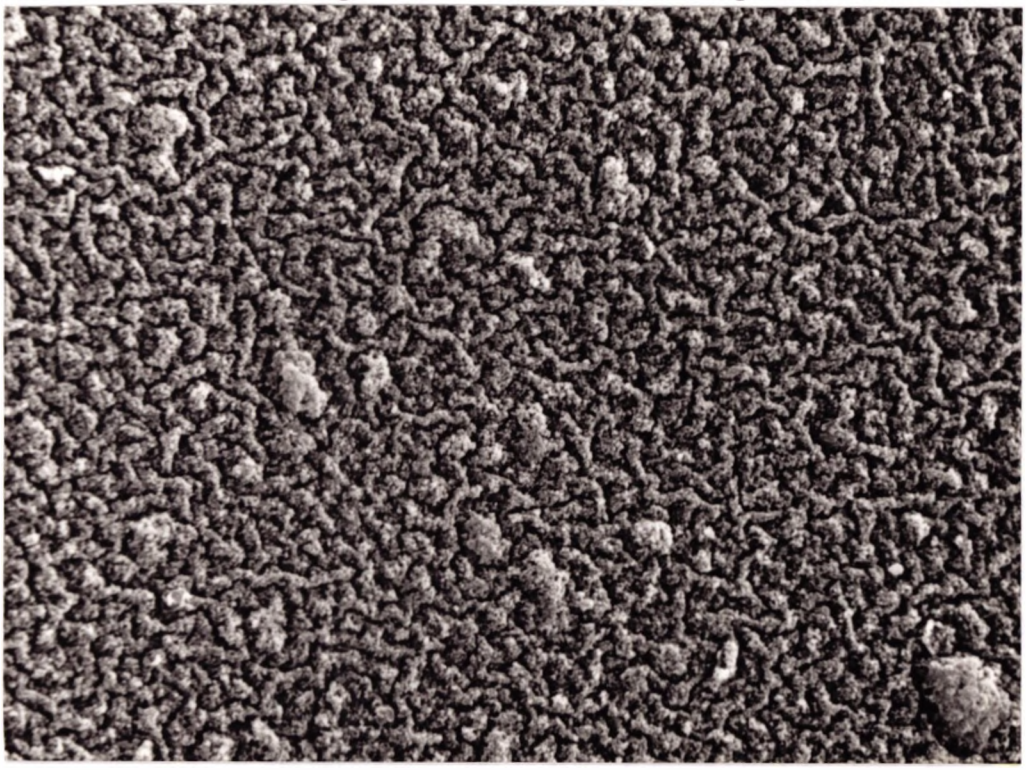


Figure 3.14

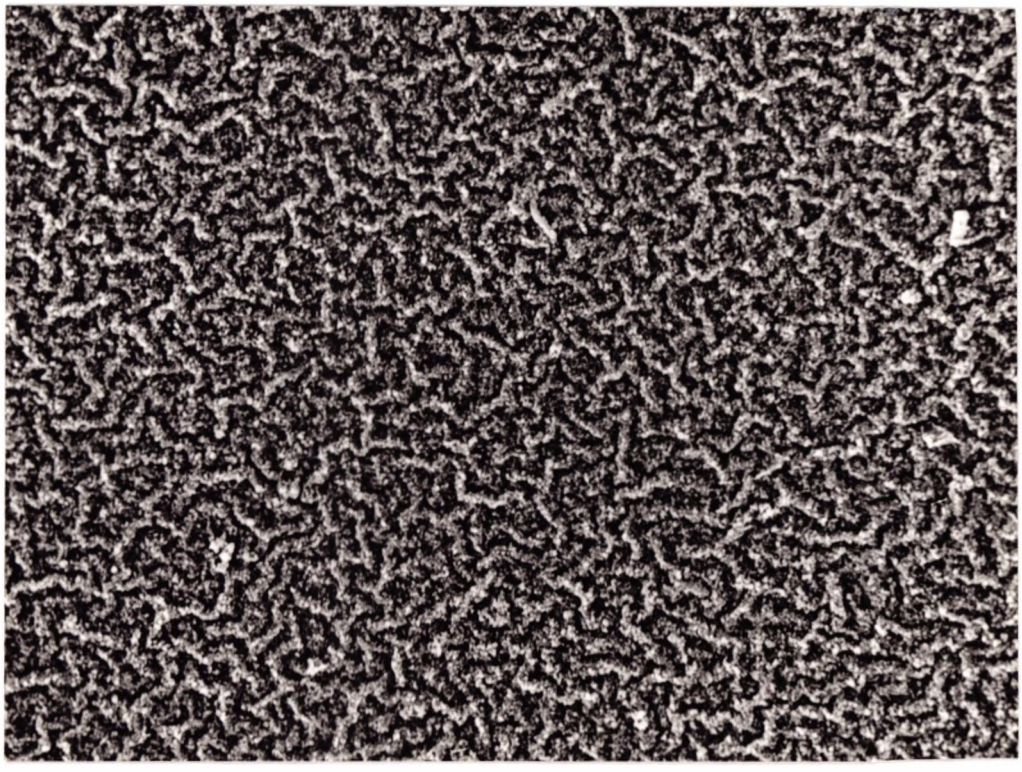
Scanning micrographs showing the back surface of a 4 months old Hema lens worn on a daily wear basis. **P. aeruginosa** was isolated from the ulcer and contact lens storage case. SEM showed minimal surface debris and disruption on the front and back surfaces (a). No organisms were found. An unworn lens of identical parameters is shown for comparison purposes (b), to assess the level of surface disruption occurring during processing.

Transmission microscopy showed a patchy ruthenium positive surface layer, thicker on the lens back surface (c), but no organisms were visible.

a. Lens back surface, magnification 1500x, 1 cm bar represents 4.7um.



b. Unworn hydrogel lens, magnfication 1500x, 1 cm bar represents 4.7um.



c. Lens back surface, magnification 17000x, 1 cm bar represents 0.4um.

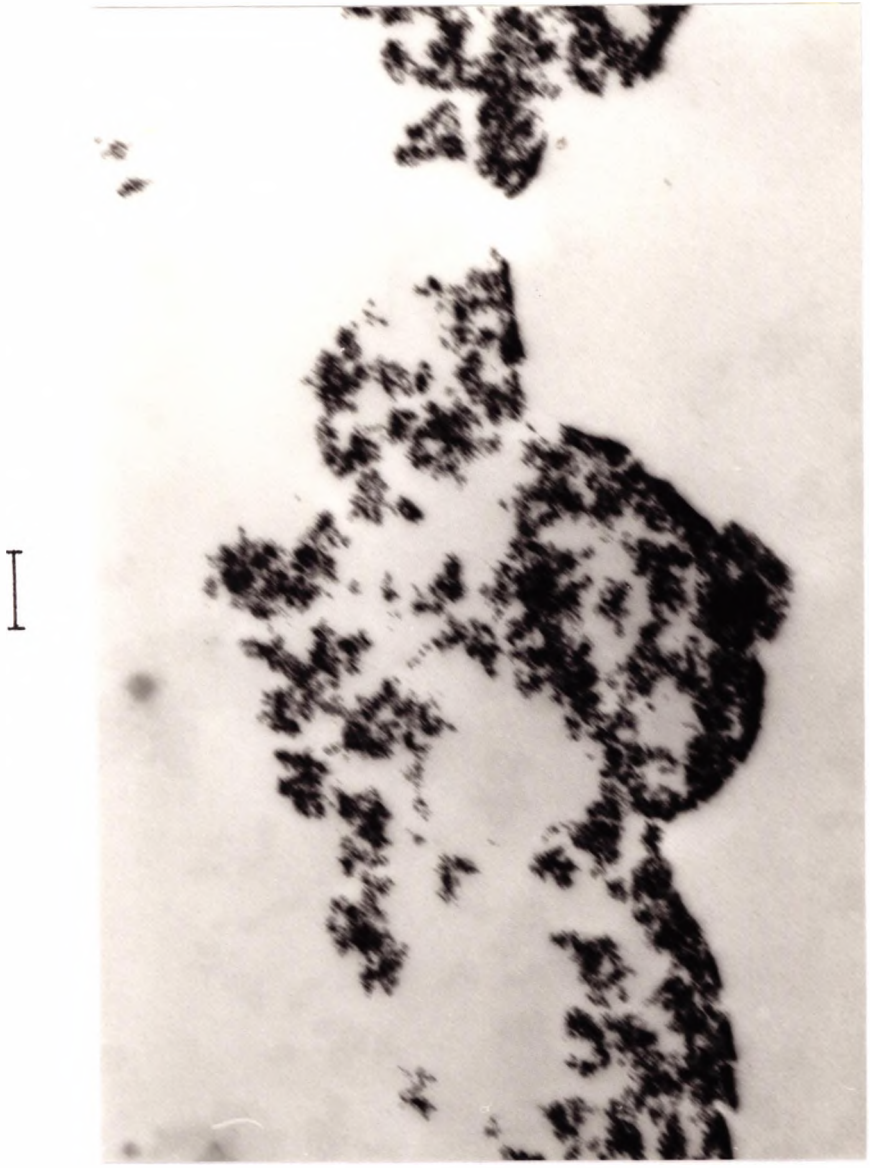
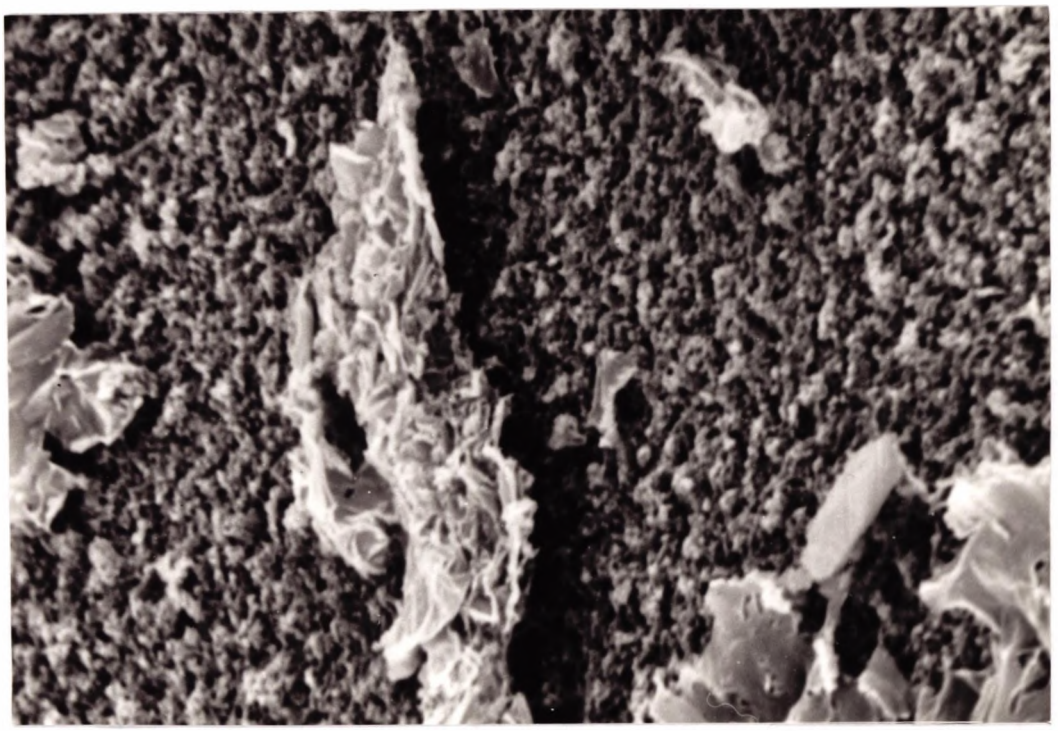


Figure 3.15

Scanning micrographs showing a month old disposable lens worn on a daily wear basis. **P. aeruginosa** was isolated from the ulcer and from the lens and lens storage case. SEM showed occasional isolated organisms adherent to the lens back surface (a) with a similar level of surface dehydration and disruption on the front surface (b).

a. Magnification 4000x, 1 cm bar represents 1.7um.



b. Magnification 4000x, 1cm bar represents 1.7um.

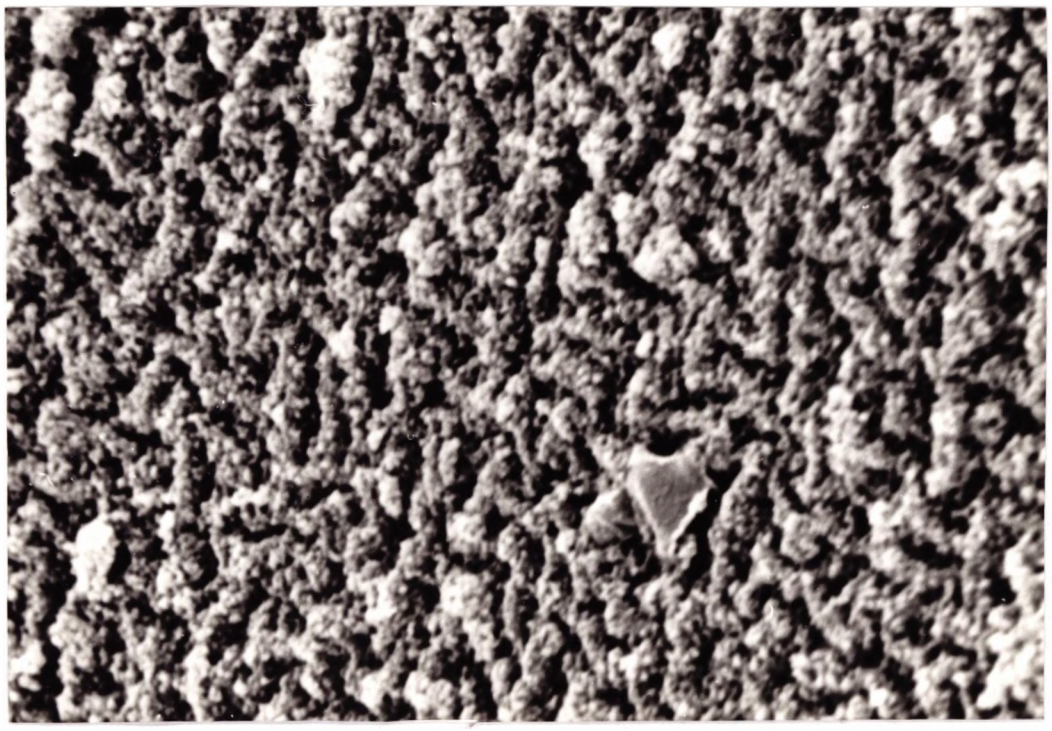
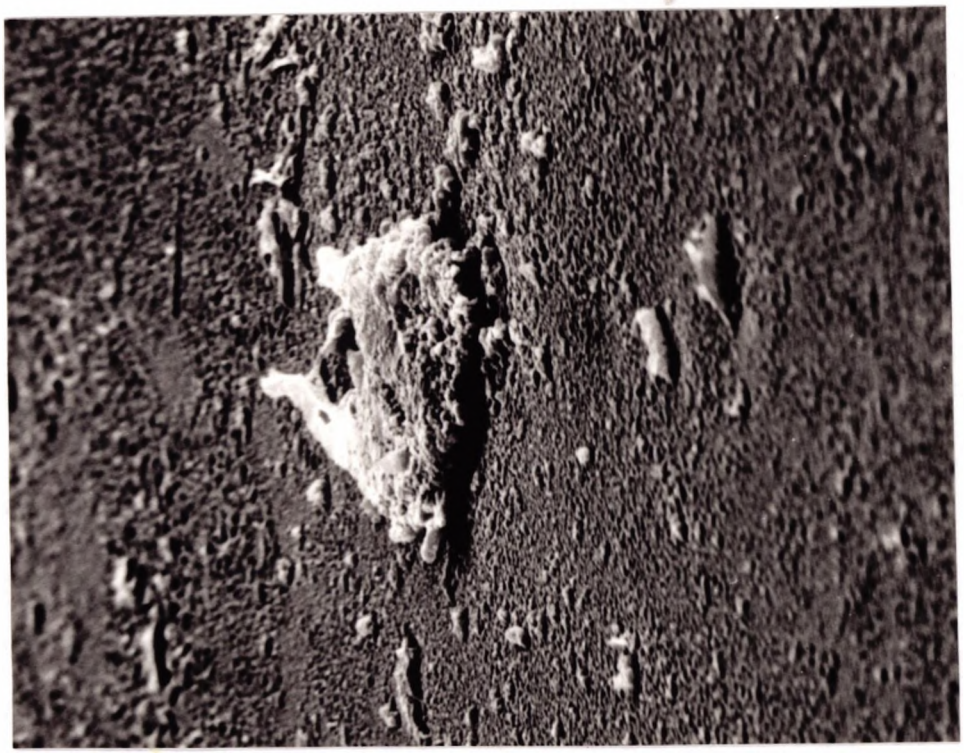


Figure 3.16

Scanning micrographs showing a daily wear soft lens. **P. aeruginosa** was isolated from the lens, although the ulcer was culture negative. SEM showed rod shaped organisms adherent to the back surface of the lens (a). The front surface of the lens (b) showed more surface disruption but less debris present compared with the back surface.

a. Magnification 2500x, 1cm bar represents 2.8um.



b. Magnification 2500x, 1 cm bar represents 2.8um.

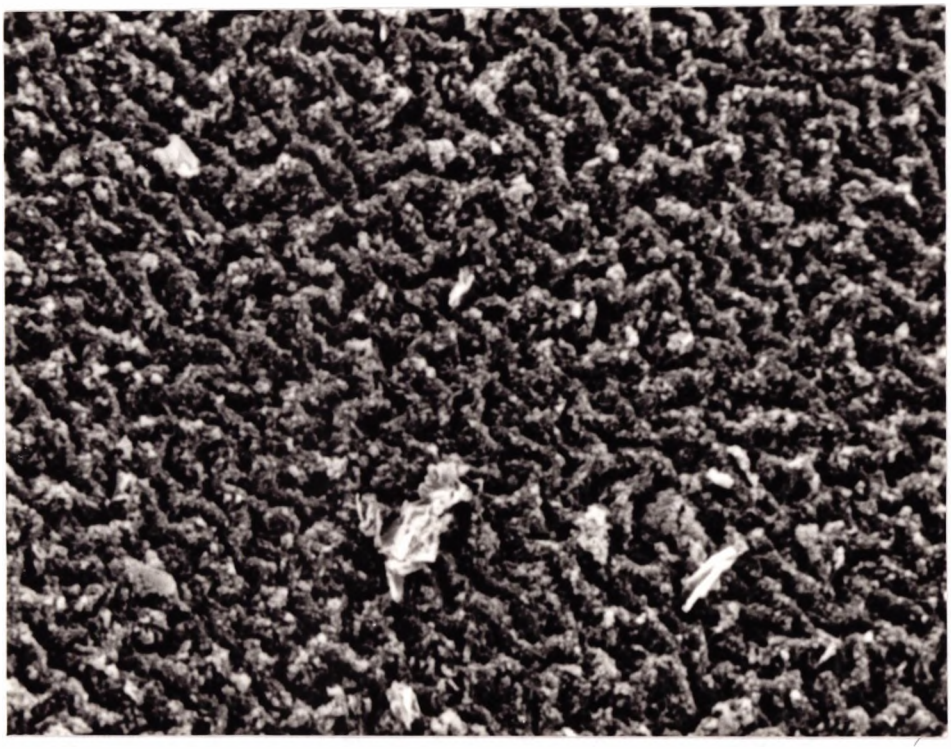
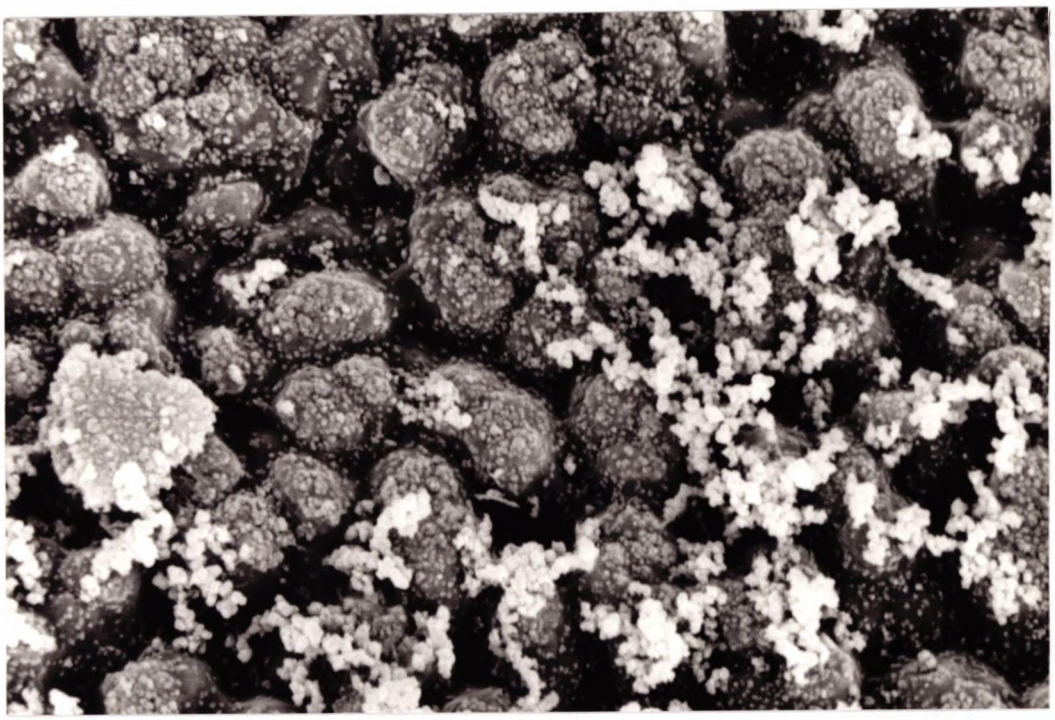


Figure 3.17

Scanning micrographs showing a 4 month old lens worn on an extended wear basis. No organisms were isolated either from the ulcer, lens or lens storage case. The lens sample was stored in OCT at -50^{0}C prior to processing for electron microscopy. SEM showed no organisms present on the lens, but flocculent debris was seen mainly on the lens back surface (a). Surface disruption had occurred to a similar extent on the front (b) and back surfaces.

a. Magnification 2000x, 1 cm bar represents 3.5um.

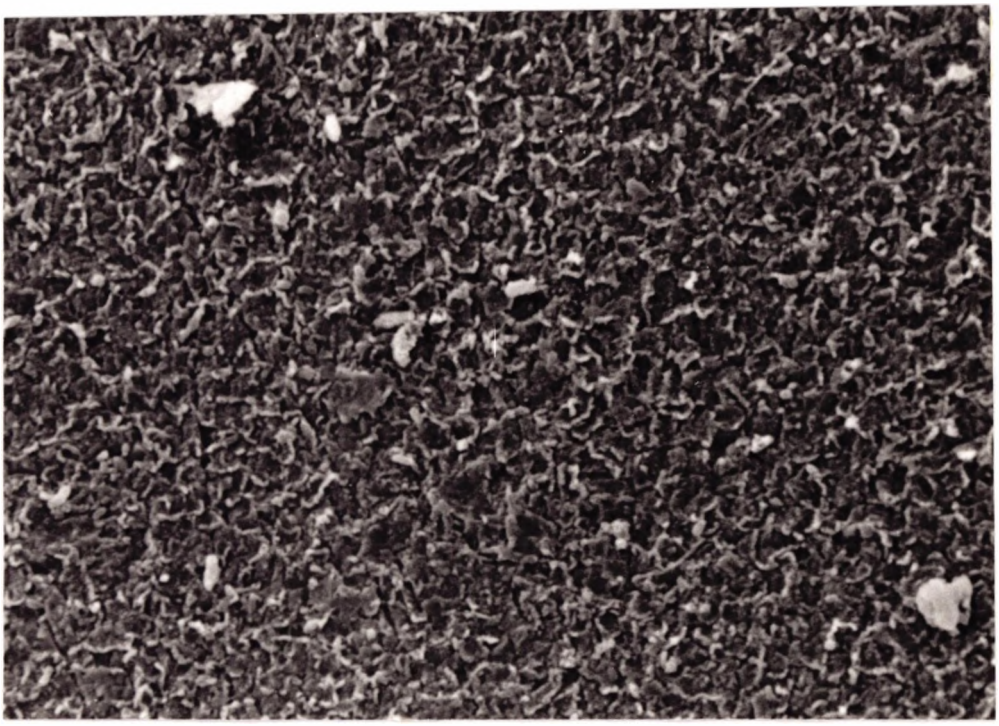


b. Magnification 3000x, 1 cm bar represents 2.3um.

Figure 3.18

Scanning micrographs showing a 2 year old Hema lens worn on a daily wear basis. No organisms were isolated from the ulcer, but confluent coliforms were cultured from the lens storage case. Occasional rod shaped organisms were visible on the lens front surface in SEM (a). More surface disruption was visible on the lens front surface (a) compared with the back surface (b). This lens had been stored in OCT prior to processing.

a. Magnification 1500x, 1 cm bar represents 4.6um.



b. Magnification 1500x, 1 cm bar represents 4.6um.

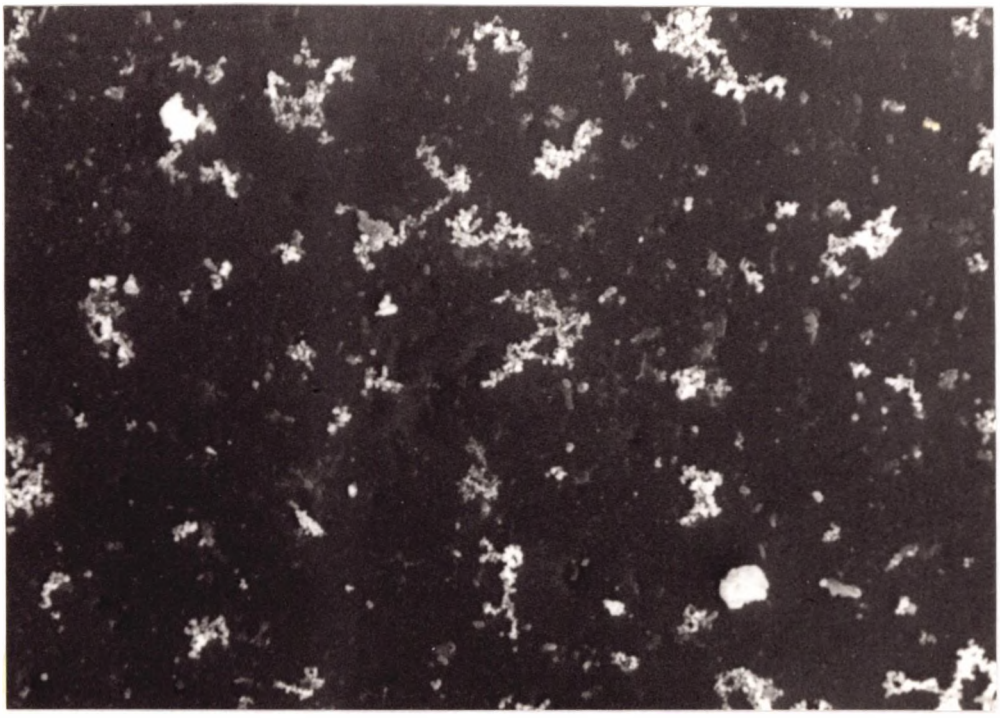
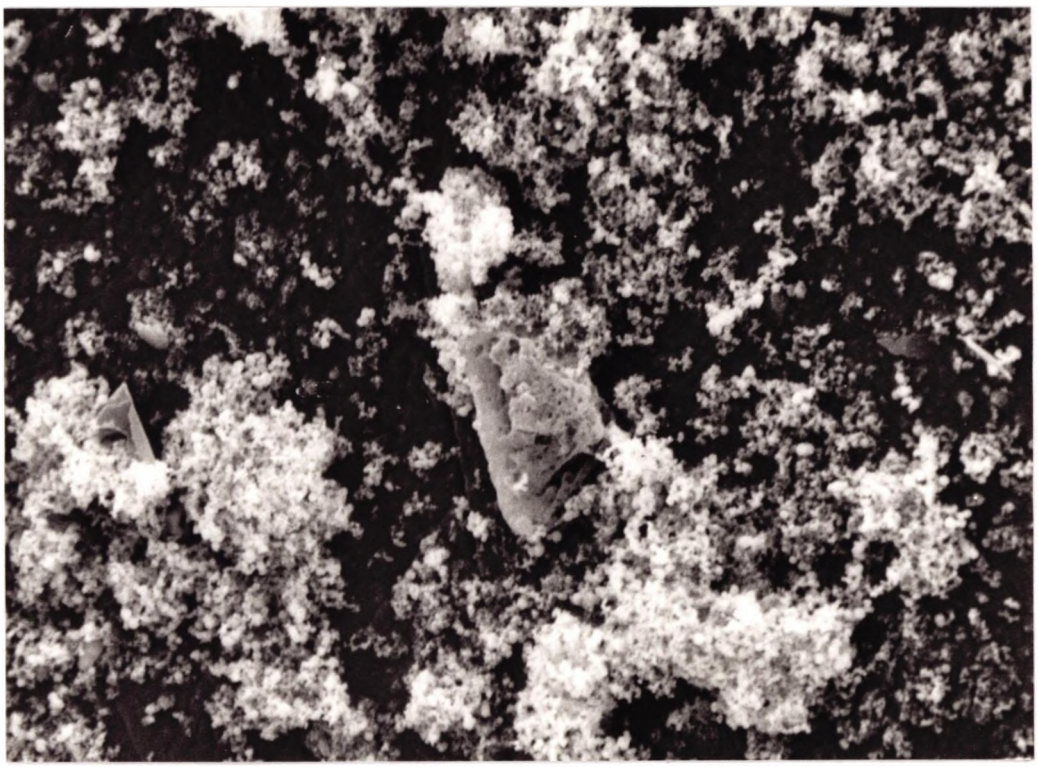


Figure 3.19

Scanning micrographs showing an aphakic extended wear soft lens. The lens age is unknown and no organisms were cultured from the ulcer. Front (a) and back (b) lens surfaces in SEM showed flocculent surface debris and similar surface disruption. No organisms were visible on either surface. This lens had been stored in OCT prior to fixation and processing.

a. Magnification 1000x, 1 cm bar represents 7.0um.



b. Magnification 1000x, 1cm bar represents 7.0um.

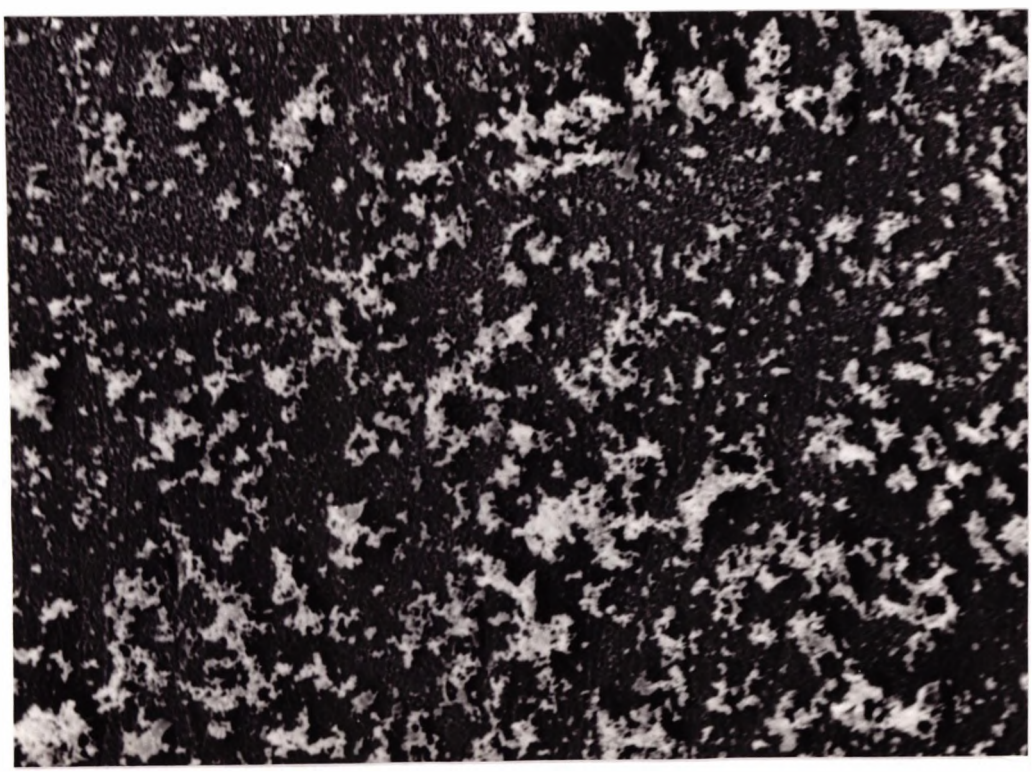
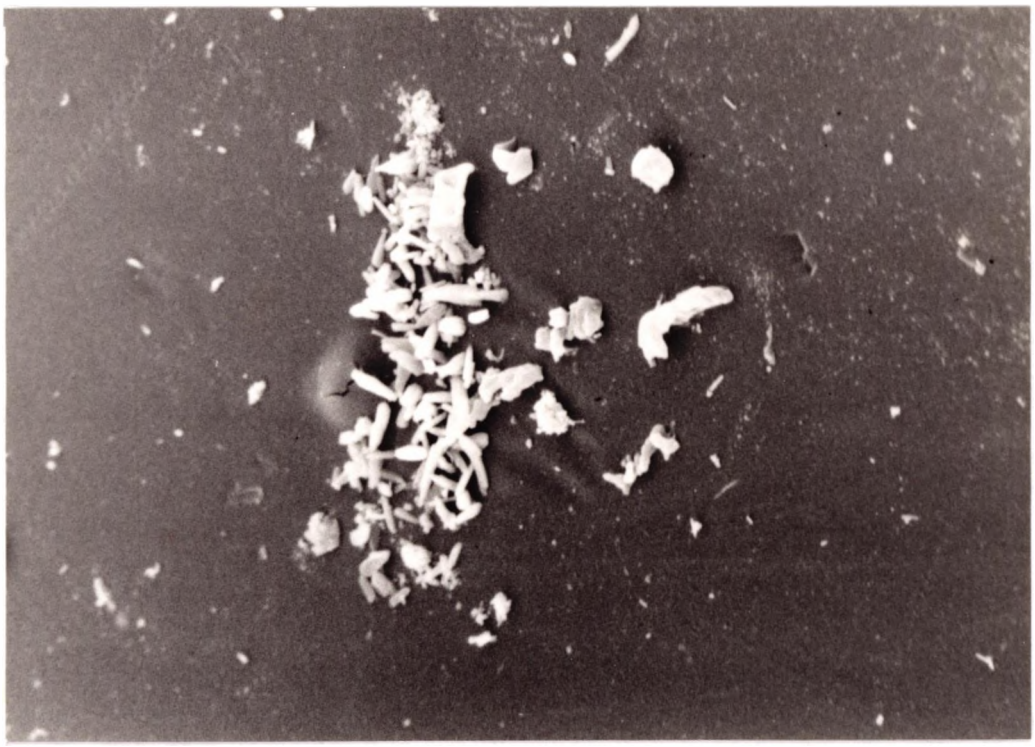


Figure 3.20

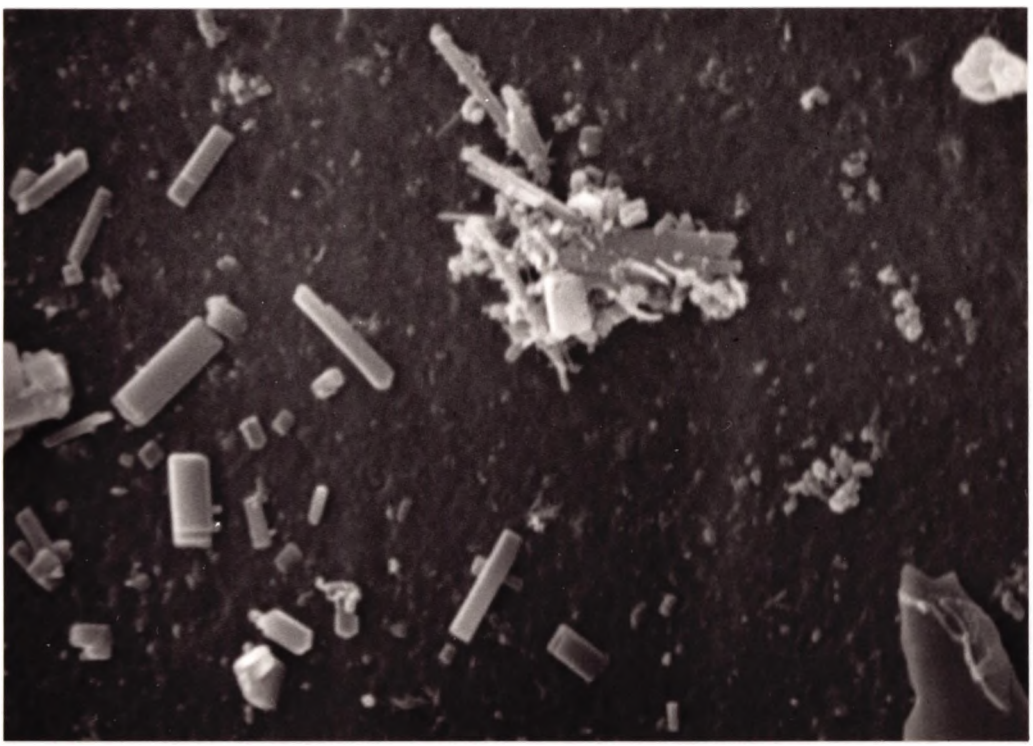
Scanning micrographs showing a Hema lens worn on a daily wear basis. *Serratia marcescens* was isolated from the ulcer but no organisms were isolated from the lens case. SEM of the back surface of the lens (a,b) showed no organisms but debris and possibly crystals from the storage solution. Little surface disruption was visible on either front or back lens surfaces and no organisms were visible.

TEM (c,d) showed a ruthenium positive surface layer present on both front and back surfaces of the lens. No organisms were found.

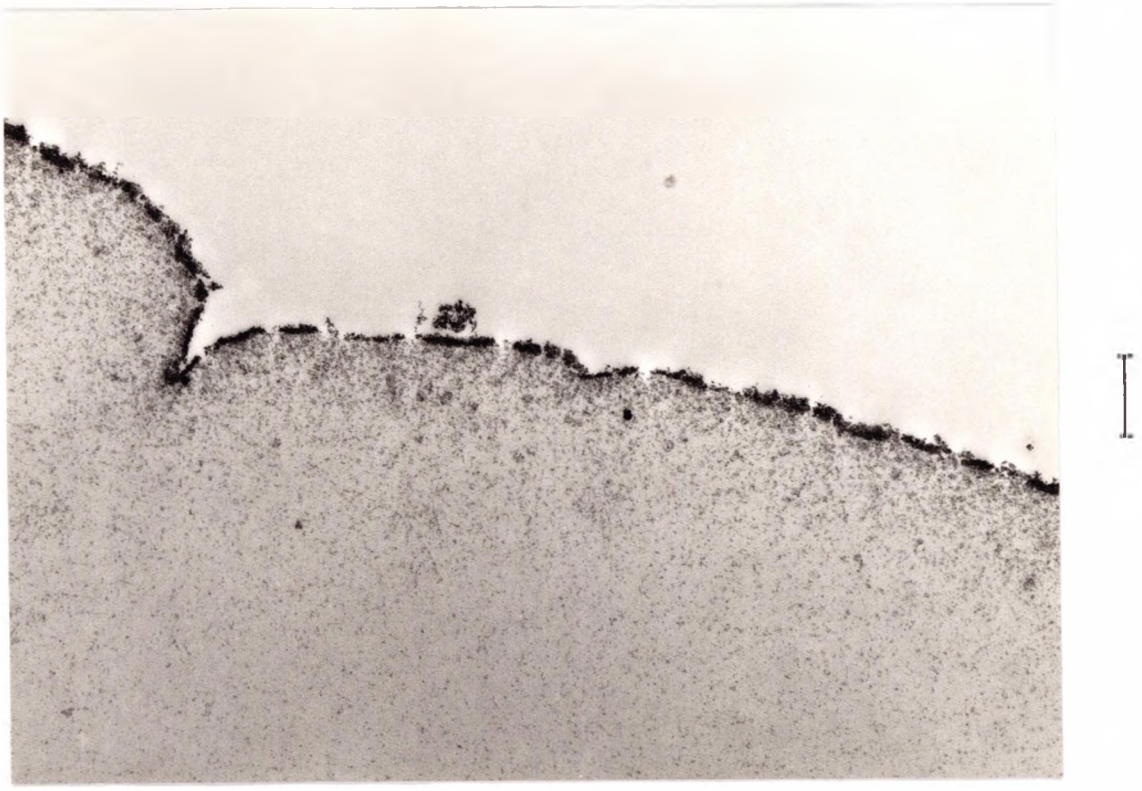
a. Magnification 600x, 1 cm bar represents 11.5um.



b. Magnification 8000x, 1 cm bar represents 0.9um.



c. Magnification 12000x, 1 cm bar represents 0.6um.



d. Magnification 17000x, 1 cm bar represents 0.4um

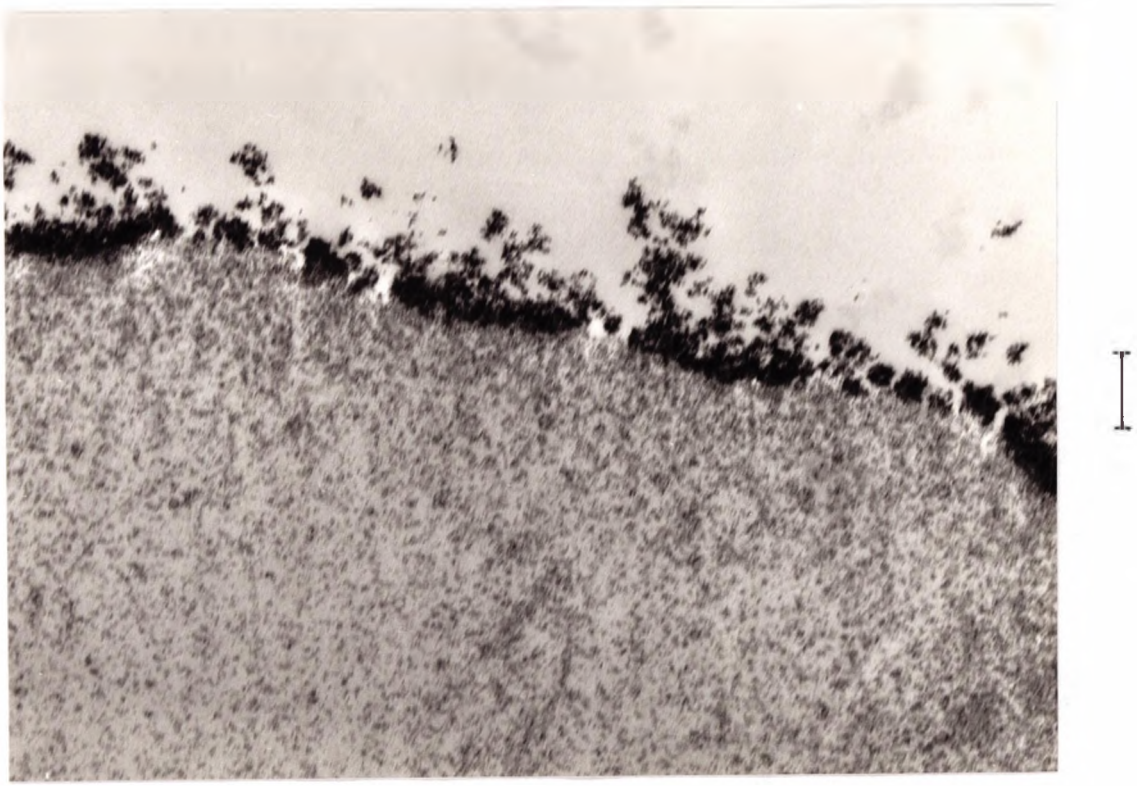
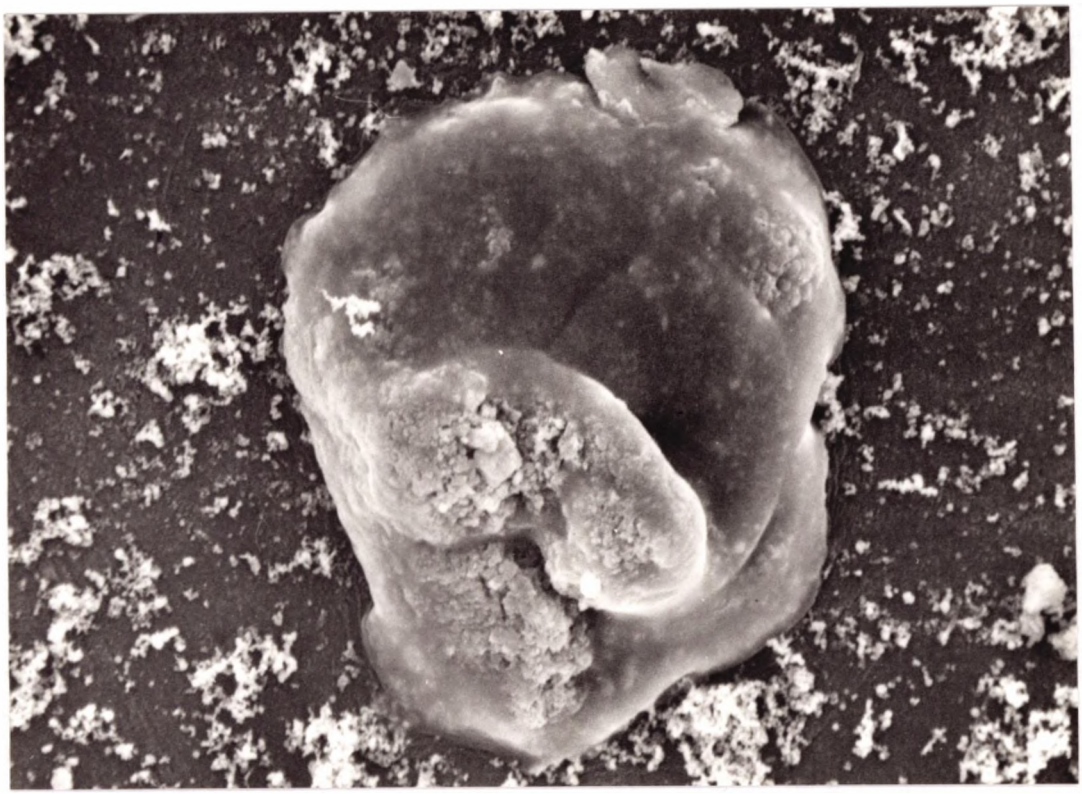


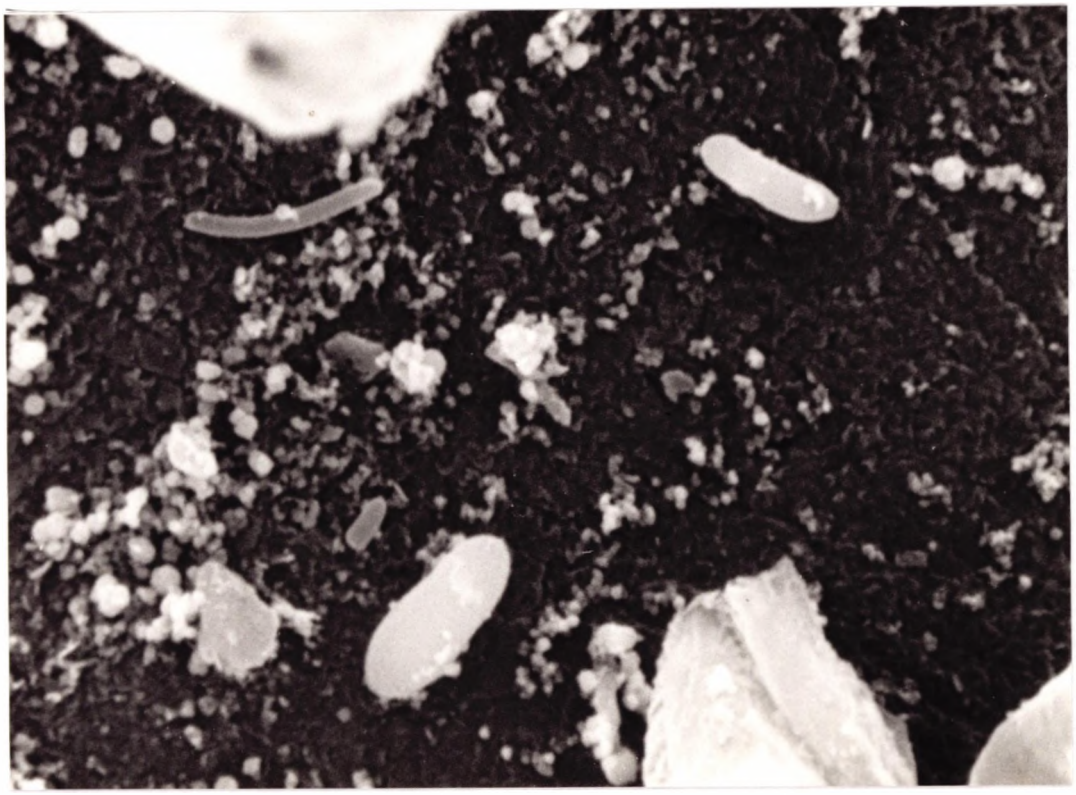
Figure 3.21

Scanning micrographs showing an aphakic extended wear soft lens. No organisms were cultured from the ulcer. SEM of the lens back surface showed flocculent debris, deposits (a) and isolated rod shaped organisms (b). Organisms were not found to be particularly associated with lens deposits.

a. Magnification 1000x, 1 cm bar represents 7.0um.



b. Magnification 1000x, 1 cm bar represents 7.0um.



CHAPTER 4. DISCUSSION

4.1 CASE CONTROL STUDY

4.1.1 Study Design.

A prospective case control study was performed to evaluate the relative and population attributable risks for all associations of presumed microbial keratitis. Also, to quantify the risks associated with a range of contact lens associated disease for different types of lenses.

This type of prospective study design within a well defined population of hospital casualty new attenders, has avoided many potential sources of bias often associated with case control studies.

The study population was restricted to new hospital attenders, which ensured that cases and controls were drawn from the same population. This methodology has avoided the problems associated with identifying a suitable control group for existing hospital patients, and ensured a high response rate, with increased accuracy. The feasibility of identifying new casualty attenders was demonstrated by a previous pilot study[19].

The hospital casualty department has a large catchment area, estimated at 2.5 million. Although the exact size is unknown, it is unlikely that using the population derived from one hospital will result in appreciable bias.

The effect of seasonal variations in the development of keratitis[53], and other lens related diseases have been eliminated by using a twelve month study duration.

Possible drawbacks to this approach include the use of hospital based controls which may not be representative of the lens wearing population. However, all lens wearers identified were fitted outside the hospital in private practice. Wearers using lenses for medical or therapeutic indications were excluded. Hospital at-

tenders with lens related disorders will not be representative of the entire spectrum of lens related complications. It is likely that mainly acute and sub-acute disorders would present, although some patients with chronic disorders may attend for an ophthalmic opinion, or if previous treatment has been ineffective.

The selection of several control groups to search for consistency of results, reduces potential bias, and the feasibility of using population derived controls to strengthen hospital based estimates, was considered. This would allow risk factors under investigation to be evaluated, free from some of the biases which may occur when using hospital controls. Population based controls would give some information about the proportion of different lens types within the community, although this would be limited due to the relatively low penetrance of lens wear in the population.

The extent and distribution of the hospital catchment population may be defined from the 1 in 100 casualty attenders identified. One proposal considered street interviews, conducted in town centres, with the quota of interviews derived from the known profile of the hospital population within the area. To provide at least 100 wearers of each type of lens, based on a penetrance of 3%, 18,000 subjects would need to be interviewed. Costing for this type of study was estimated at approximately 90,000 in 1988, and it was felt that this method of sampling is unlikely to produce an acceptable standard of data. More sophisticated sampling techniques would be necessary to ensure rigorous data collection, such as deriving controls from General Practitioner or Family Health Services Authority lists, based on the hospital casualty distribution. Other possible sources include using a post code or electoral register within the catchment area, and either interviewing a named member of the household, or screening the whole household by visit or by telephone. Street interviews would be unlikely to strengthen the hospital data, since subjects on town centre streets during daytime are determined by social circumstances, and may not be representative of particular age/sex or socioeconom-

ic strata. Therefore, a rigorous population based study would have been costly. However, to have attempted other means of population sampling would have provided a less valid estimate compared with the careful collection of hospital based control data.

The identification of additional risk factors or confounding factors was dependent on information derived from a questionnaire. Lens age and lens hygiene data is dependent on the subject's memory recall and truthful reporting. However, prospective identification of patients and the completion of a questionnaire at their first visit ensures a high response rate.

Observer variability in the diagnosis of contact lens related disease may be a source of bias. All casualty officers were informed of the study protocol and received written guides to the diagnosis (Table 2.1) and management of presumed microbial keratitis and other lens related disorders. Daily liasing with the nursing staff and casualty officers was carried out during the study to help maintain their cooperation, and to provide feedback on the study progress.

4.1.2 Age and Gender Breakdown.

Age and gender breakdown for the different groups of subjects was found to differ (Table 3.1). Contact lens wearers with keratitis were of a similar age to other lens wearers, but more males than females were affected, compared with the general trend amongst lens wearers. Results from a recently performed casualty study have shown a similar age trend amongst contact lens wearers, with the majority aged between 18-35 years old[186].

The age and gender breakdown for non-lens wearing new casualty attenders, is similar to that reported in other ophthalmic centres[187]. The preponderance of males between 30-50 years old may be attributable to the greater occupational risk of trauma. This age and sex trend is also reflected in the group of non-lens associated keratitis patients, of whom 22 of 31 had suffered prior trauma.

4.1.3 Microbial Keratitis.

A clinical case definition for keratitis was used, despite the possibility of observer variability. Microbial keratitis comprises a continuum of disease, ranging from small peripheral lesions with little anterior chamber involvement, to large, central, culture positive lesions associated with uveitis and hypopyon. Since small lesions may represent an early stage in the disease process and failure to isolate a causative organism does not exclude microbial keratitis[48], it is not appropriate to define strict parameters based on lesion size or culture results, for case inclusion. However, to eliminate bias which may be introduced by including a wide spectrum of keratitis, separate analyses were performed for different severities of keratitis.

The risks associated with contact lens wear for all degrees of severity of keratitis were found to be significantly higher compared with other risk factors. The relative risk for keratitis in lens wearers ranged from between 12.4x (95% confidence interval 1.8-85.4x) higher, for severe culture negative lesions, to 145x (36.8-574.2x) higher, for moderate culture negative lesions, compared with eyes with no predisposing factor. Trauma carried the next largest risk ranging between 3.0x (0.2-43.4x), for severe culture negative keratitis, to 52x (9.7-281.3x) for moderate culture negative keratitis, compared to no apparent predisposing factors. Of the three risk factors, ocular surface disorders carried the lowest relative risks for all degrees of severity of keratitis except for severe culture negative lesions. In this case, contact lens wear carried the highest risk at 12.4x (1.8-85.35x), followed by ocular surface disorders at 9.3x (0.9-95.8x), then trauma at 3.0x (0.2-43.4x).

The relative risk for contact lens wear was found to be 18x higher compared to no lens wear. The consistently higher risk for contact lens wear occurred despite a higher proportion of the cases associated with contact lens wear being less severe, than those associated with trauma. With prior knowledge of the association between contact lens wear and keratitis, there may have been a tendency for an increased number of corneal scrapes taken for mild lesions related to contact lens

wear. Persistence of the trend of increased risk to lens wearers for all degrees of severity of keratitis does not support this view.

The population attributable risk percentage (PAR%) for all severities of keratitis associated with lens wear was between 36-78%, with an overall estimate comparing lens wear with no lens wear (Table 3.9) of 62%. This implies that if the risks for keratitis associated with contact lens wear could be eliminated, then the reduction in new keratitis cases in this population would be 62% per year. Contact lens wear now represents the major avoidable risk factor in this population.

The low risk associated with ocular surface disorders, compared with trauma and contact lens wear, differs from previous hospital based surveys. These have included existing hospital patients with pre-existing disorders[33] and have tended to be biased towards in-patients[20,33]. More extreme, often culture positive, cases with greater morbidity have been considered in these previous studies[21].

The multivariate analysis has shown that differing relative risks persist for the different exposures in keratitis despite controlling for age, sex and socioeconomic class. Higher order interactions were also analysed and none of these were found to cause effect modification.

4.1.4 Lens Related Microbial Keratitis.

Extended wear hydrogel lenses were consistently found to have a significantly higher relative risk compared with other lens types, for all severities of keratitis. The relative risk for all cases of lens related keratitis for EWSCL users was found to range between 20.7x to 36.8x (depending on which control group was used), greater than the risk to GPCL users. The relative risk for DWSCL users was found to range between 3.6-4.1x greater than the risk to GPCL users. These results compare with the findings of a recently published case control study performed in the USA[54]. This study compared the relative risk of extended wear compared with strict daily wear, and estimated that overnight use carried a 10-15x greater risk

than daily wear only. However, due to small numbers of rigid lens wearers presenting, this USA study did not have the statistical power to estimate the risks associated with rigid lens wear. This UK study has enabled the risks associated with both DW and EW hydrogel lenses to be compared with the risks associated with GPCL. The greater risk of keratitis amongst EWSCL users compared with DWSCL users is reflected in both studies. This suggests that mode of lens use is the major factor determining the increased risk, and not the way in which the patient is managed.

Different severities of keratitis were analysed separately as discussed in Section 4.1.3. There may have been a tendency to perform a corneal scrape on a higher proportion of mild lesions in EWSCL users, due to a prior knowledge of the increased association. The relative risk for mild culture negative lesions in EWSCL users was found to be 58-106x higher than for GPCL users, dependent on which control group was used. However, in all degrees of severity, the trend of increased risk amongst hydrogel lens users was found to be significant at the 5% level.

Two control groups were used for comparison to assess any bias which may be introduced by using the Group 2 controls in the analysis. Group 2 controls comprised all lens wearers without keratitis. Some of this population of lens users may have had complications, which would be expected to predispose to keratitis. This would have the effect of reducing the relative risks for this larger control group. Group 1 controls, without lens related disease, were used to estimate whether this potential bias existed.

Greater relative and population attributable risks were found for extended wear lens users in all analyses, using Group 1 controls (without lens related disease). This difference suggests that lens type is also a factor in other disorders which may predispose to keratitis. Using a control group including lens wearers with disorders which predispose to keratitis, will reduce the relative risk. There was an overrepresentation of EWSCL users attending with lens related disorders, only 35

wearers attended with non lens related disease. With a small proportion exposed in control Group 1 (without lens related disorders), this will increase the relative risk and widen the confidence intervals. An improved estimate of relative risk is likely to be obtained by interpreting these risks from different control groups as an upper and lower estimate of the actual risk. For all degrees of severity of keratitis, the trend of higher risk with EWSCL persist using either Group 1 or Group 2 controls, except in the case of moderate culture negative lesions.

The PAR% estimations show that 45% of lens related keratitis is attributable to EWSCL and 35% to DWSCL. Hydrogel lens related keratitis accounts for 80% of all new cases of keratitis within this population. The risks associated with the use of rigid lenses for all severities of keratitis are small, whereas the risks associated with soft lens wear, particularly extended wear of lenses, are much greater.

4.1.4 (i) Multivariate Analysis.

The multivariate analysis was performed for EWSCL users with keratitis and a control group without keratitis. These data demonstrated that a lens worn continuously for longer than 6 days was significantly associated with keratitis. A duration of symptoms of longer than 1 day was also associated with keratitis. This conflicts with a previous study[125], which found no relationship between longer cycle times and any increase in incidence or severity of complications. In a review of 84 wearers with lens associated keratitis, Roth[188] showed an increase in frequency of corneal ulcers in wearers using lenses worn continuously for more than 14 days. The association of a longer cycle time in EWSCL users with keratitis justifies the reduction in recommended continuous wearing schedule to the 7 day limit, advised by the FDA in May 1989.

Other lens hygiene and compliance factors such as lens age, period since lenses were last checked, lens cleaning and disinfection frequency, were not found to be significantly associated with keratitis, although the association of enzyme cleaning frequency was found to be almost significant. The lack of significance amongst lens

hygiene factors in EWSCL users with keratitis, was confirmed by the overall analysis of cumulative hygiene scores, comparing lens wearers with keratitis to lens wearers without lens associated disease (Figure 3.1).

Patients' age was not found to be a significant risk factor, but lower socioeconomic class was associated. Males had a slightly increased risk compared with females.

These data from the multivariate analysis would imply that the use of disposable lenses on an extended wear basis is unlikely to reduce the risk of keratitis. Disposable lenses for EW, are intended for 1 or 2 weeks of continuous wear only. The increased risk of keratitis associated with overnight use of lenses does not appear to be associated with failure of compliance with a prescribed hygiene regime or increased lens age. These two factors, which represent the predominant advantages of disposable lenses over re- useable lenses, may reduce the risks associated with extended wear in other areas. A recent study of the oxygen transmissibility of currently available disposable lenses[189], has shown that complications related to hypoxia are unlikely to be alleviated using this type of lens.

The analysis for DWSCL users showed that less frequent lens disinfection was associated with keratitis. However, other compliance and hygiene factors, such as wearing schedule, lens age, time since lenses were last checked, were not associated. Males had a significantly increased risk of keratitis compared with females.

Duration of lens wear was not found to be a risk factor in either daily or extended wear lens use. However, data collected from 200 non-hospital based asymptomatic lens wearers sampled at a University, showed that compliance amongst lens wearers significantly reduces after 6 months of lens wear (Unpublished Data, Wilson and Woodward, 1990).

Detailed aspects of compliance were not included in the study questionnaire, but several previous studies have suggested a link between poor compliance and a

longer duration of lens wear. Compliance with a prescribed wearing schedule was documented in 30/50 daily wear lens users[190], and poor compliance was significantly associated with a duration of wear of longer than 2 years. A higher rate of bacterial contamination of the lens care materials was found to correlate with a duration of wear of longer than 1 year. Despite this suggestion that compliance reduces after 6 months to 2 years of lens wear, no association in this study between longer duration and keratitis was apparent.

Other aspects of compliance have been analysed using a health belief model[191]. Non-compliance with a prescribed regime was found to be associated with patient's age and cosmetic indication for lens wear. Sex and socioeconomic status were not found to be associated using this model.

4.1.4 (ii) Lens Hygiene and Bacterial Contamination Of Lens Care Materials. Bacterial contamination of the lens storage case and lenses themselves were significantly associated with microbial keratitis in daily wear soft lens users only. Commercial lens solutions were found to be contaminated in approximately 10% of cases, both for users with keratitis, and controls.

A link between bacterial contamination of lens care materials and corneal ulcer isolates was found in 4 of 7 of the culture proven lens related ulcers. Care materials were unavailable in 2/7 cases. In addition, 3/60 wearers with keratitis wore extended wear disposable lenses and used no lens care materials. Previous studies have reported similar findings, where sterile lens care materials are associated with culture proven ulcers[144,33]. Bacterial contamination of the lens care materials cannot sufficiently explain the mechanism for keratitis in EWSCL users, who have less exposure to solutions. However, since solutions are used less frequently in EW, care materials are more likely to be kept longer than the 28 day recommended limit, and may be contaminated.

Of the asymptomatic control wearers, 35% had bacterial contamination of the lens

storage case. However, these storage cases cultured fewer pathogens than those cultured from wearers with keratitis (Table 3.21). No *P. aeruginosa* was isolated from any care materials from the control group of wearers. This high rate of bacterial contamination of the lens storage case with environmental Gram negative organisms has been previously reported[82,81], and may be a factor in the development of sterile infiltrates[192] and contact lens related red eye (CLRRE). Bacterial contamination of lens care materials occurs in a high proportion of lens wearers, despite a low incidence of infections. This implies that additional factors are important in the pathogenesis of keratitis.

Poor lens hygiene appears to be associated with the development of keratitis in DWSCL users only. This association has not been shown for EWSCL users. Hygiene data, shown in Figure 3.1 also suggests an association between keratitis in GPCL users and poor hygiene. However, too few rigid lens users with keratitis presented to achieve statistically significant results. The hygiene study was limited, since data was derived from a simple questionnaire. Aspects of hygiene, such as handwashing and case cleaning, were omitted from the questionnaire due to difficulties in classification. Case cleaning information was sought for those patients who were interviewed. This data was obtained for patients with keratitis and a selected control group, although no correlation between case cleaning and bacterial contamination of the storage case was found. Bacterial contamination of the lens storage case occurred with reportedly good lens hygiene (hygiene score of 14 and above), in 16/33 keratitis cases compared with 5/16 controls.

These results confirm the findings of the recent case control study performed in the USA[54], that poor lens hygiene may not be as important in the development of keratitis as was previously thought. The findings imply that the mechansism of infection may differ in EWSCL and DWSCL.

Lens-eye-bacteria interactions are implicated in the pathogenesis of infections. Colonisation of the contact lens surface by bacteria, with the formation of a bacte-

rial biofilm, may explain infections arising in disposable extended wear lens users and in those wearers with sterile lens care materials, or reportedly good lens hygiene. Small numbers of pathogenic organisms may be derived from environmental sources which may adhere to and subsequently colonise the lens surface during wear. Extended wear of lenses may allow a greater opportunity for large numbers of organisms to colonise the lens surface.

Contact lenses alter the eye's defence mechanism in a variety of ways, by inducing anatomical, physiological and biomaterial mediated changes. Microbial keratitis is likely to result from a combination of these factors.

4.1.5 Lens Related Disorders.

The hospital casualty population has served as a control population for the estimation of relative risk. This group may not be representative of lens wearers in the community. However, the hospital has a large catchment population (estimated at 2.5 million) and of the 1611 lens wearers identified, 1252 were fitted by 589 different private practices within the catchment area. Bias from this source is therefore unlikely. A further 97 patients were fitted by practitioners outside the UK and insufficient information was available for 262 patients. Differences in relative risks are likely to be due to lens type rather than due to patient management.

The study is biased towards acute lens related disease requiring casualty attendance. Risks for chronic lens related disorders such as neovascularisation and endothelial polymegethism, could not be estimated from this type of study.

Lens related disorders differ from other external eye disorders, since contact lenses provide differing biological challenges to the eye. This unique spectrum of eye disease allows the straightforward diagnosis of lens related disorders (Table 2.1). Classification for analysis was based on a clinical case definition, despite the limitations to this approach, since the characteristics of lens related disorders are well defined.

The study has shown significant differences in relative risk for the different lens types for a range of acute lens related disease, for wearers using lenses for the correction of low refractive errors within this population.

The majority of wearers presenting, attended with problems relating to lens wear (1104/1611, 68.5%), with 507/1611 (31.5%) attending with problems thought to be unrelated to lens wear. Lens related problems accounted for 3.8% of new casualty attendances. This represents an increase in the incidence of lens related problems compared with a previous retrospective estimate of 2.5%[193] in 1980.

The overall relative risk for any complication occurring was found to be significantly highest for EWSCL users at 2.7x (1.7-4.2x) higher, than that of GPCL. There was an over-representation of EWSCL users in groups of severe complications.

A similar trend of increased risk for EWSCL use has been shown for aphakic lens users. A longitudinal study has shown a relative risk for EWSCL users developing any complication, at 2.5x that of GPCL users[24]. The relative risk of severe complications, including suppurative keratitis, overwear disorders and abrasions were found to be 9.9x higher with EWSCL compared with GPCL. The incidence of severe lens related complications is likely to be much higher in aphakic wearers compared with cosmetic lens users. Lens related microbial keratitis in aphakes has been estimated at 3-5% of extended wear lens users per year[49,50,24]. This compares with an estimate of between 1 in every 300 to 450 cosmetic EWSCL users per year[52]. However, with such large numbers of normal eyes exposed to cosmetic lens wear, there is potential for significant morbidity when considering other less severe, but more common complications.

Upper and lower estimates of relative risk for each group of complications result from the use of 2 control groups (Section 4.3). Group 2 controls comprised all

lens wearers except those with the specific complication being analysed. This control group may include lens users with complications which may predispose to the complication being analysed. Group 1 controls, without lens related disorders, were used to assess whether this potential bias existed. Similar trends of increasing risk were found with both control groups. All trends were found to be significant at the 5% level. The relative risks for EWSCL users for most disorders was greater using Group 1 controls. This may have occurred since few EWSCL users presented without lens related disorders and with a small proportion of the control group exposed to EWSCL wear. This may result in higher risks and wider confidence intervals.

Metabolic disorders, including overwear, tight lens syndrome and microcysts, showed the highest relative risk for EWSCL users, at 2.0-3.7x that of GPCL users. Differences in risk between DWSCL, PMMACL and GPCL users were not found to be significant, since the confidence intervals on the risk estimates overlap the referent. During overnight wear, metabolic complications may arise due to hypoxia, reduced exchange of tears and of metabolites. Oedema under such conditions of physiological stress, is due to several factors. These include hypoxia, causing stromal lactate accumulation[194], impaired carbon dioxide efflux (corneal acidosis)[195], mechanical trauma and tear hypotonicity.

Toxic and hypersensitivity responses, including giant papillary conjunctivitis, thiomersal keratopathy, toxic and enzyme keratopathy, showed the highest relative risk in DWSCL users at 5.8-5.9x that of GPCL users, and EWSCL users at 4.5-8.1x that of GPCL users. Differences in risk between GPCL and PMMACL users were not found to be significant. Population attributable risk percentage data showed that 67% of these complications could be attributed to DWSCL use, while only 10-11% could be attributed to EWSCL use.

These responses may arise as a result of exposure to compounds absorbed by or adsorbed onto lenses. Soft lenses particularly may act as reservoirs for solutions,

which elute from the lens onto the eye[130]. Preservatives or enzymes, may act as haptens, causing a local delayed hypersensitivity response[131]. Interactions between preservatives, contact lens surface and adsorbed surface mucoproteins may contribute to these responses[196]. Partly reversible binding of chlorhexidine[197] to hydrogel lenses has been reported. This preservative binding is thought to be enhanced by protein deposition on lenses[198] and an animal model has demonstrated toxic epithelial responses associated with chlorhexidine[199]. There is less binding of preservatives to rigid lenses and solution related disorders are less common. Toxic keratopathy as a result of misuse of peroxide systems, however, was more prevalent than presumed chlorhexidine toxicity in this study.

Papillary conjunctivitis is thought to arise as a result of several factors, an immunological response to protein deposited on lenses, which acts as an antigen[200], plus mechanical factors due to the lens edge[201]. Solution related reactions may compound the problem.

The relative risk for sterile keratitis was found to be 2.4-4.7x higher in EWSCL users compared with GPCL users. This differs significantly from the risk in presumed microbial keratitis at 21.8-36.8x higher in EWSCL users. This suggests different aetiologies in these two conditions, which supports the use of a clinical case definition to differentiate between presumed microbial and sterile lesions. None of the patients with presumed sterile lesions progressed to fulminating microbial keratitis. This may also imply a different aetiolgy in the two conditions, or it may be that early presentation and prompt treatment prevented an early microbial lesion from progressing to fulminating keratitis.

DWSCL users were also found to have a significantly higher relative risk for sterile infiltrates compared with GPCL users, at 2.1-2.3x greater. However the difference in risk between GPCL and PMMACL users was not found to be significant.

Sterile infiltrates represent an inflammatory response in the absence of any infect-

ing organism. The aetiology is not always clear, but factors may include delayed sensitivity to thiomersal in care materials[202,127], tight fitting lenses[127], a hypersensitivity response to bacteria[126], (which may be present in the lens care materials), or to bacterial toxins[129]. In extended wear lens users, an acute red eye reaction with peripheral infiltrates, is thought to be related to poor tear exchange behind the lens during the closed eye period. The build up of trapped cellular and metabolic debris may lead to an inflammatory response[203].

Population attributable risk percentage data shows that 35-39% of cases of sterile keratitis may be attributable to DWSCL use and 9-12% to EWSCL use. Although the relative risk of sterile keratitis is higher in EWSCL, the greater population attributable risk percentage in DWSCL users reflects the greater impact of DWSCL on the morbidity in this disorder.

Lens hygiene and storage case contamination data was analysed for a subset of these patients with sterile non- progressive infiltrates[192] (Appendix 6). An association was found between poor lens hygiene and sterile infiltrates for DWSCL users only. Lens case contamination results showed positive cultures in 10/15 wearers with infiltrates and 16/43 controls. These differences were found to be statistically significant, $p = 0.0471$ (Fisher Exact Test). Fewer pathogenic organisms were cultured from cases of control wearers, compared with *Serratia marcescens* (n=3) and *P. aeruginosa* (n=1), from wearers with infiltrates.

Abrasions and superficial punctate keratitis were found to have a significantly higher relative risk for rigid lenses, at approximately twice that of both DWSCL and EWSCL. This was to be expected since the likelihood of foreign bodies would be greater with rigid compared with hydrogel lenses, due to greater edge clearance. This clearance between the cornea and the edge of the contact lens allows foreign bodies to move behind the lens on blinking. Rigid lens users were found to be at a higher risk compared with soft lens users for abrasions only. Abrasions are

likely to represent the most minor group of complications considered here, since a RCL user with an abrasion will tend to remove the lens as a result of the foreign body sensation.

Lens eye interactions are probably important in the pathogenesis of lens related disease, since different lens types have varying effects on the anatomy and physiology of the eye. Contact lenses considered as biomaterials, also present different biological challenges to the eye in terms of their surface characteristics and antigenic load.

Contact lenses induce short and long term effects on the ocular surface, and these effects have been well documented for different modes of lens wear. Different relative risks for different lens types are likely to reflect these differing biomaterial effects.

Relative risks for a range of lens related disease have been shown to vary for different lens types and modes of wear. Extended wear soft lenses have been shown to have the highest overall relative risk at 2.7x that of GPCL. The relative risk was greatest for EWSCL users for microbial keratitis at 20.8-36.8x, for metabolic disorders at 2.0-3.7x and for sterile keratitis at 2.4-4.7x that of GPCL. Relative risks for toxic and hypersensitivity disorders were found to be 5.8-5.9x for DWSCL and 4.5-8.1x for EWSCL compared with GPCL.

Quantifying these relative risks and population attributable risk percentages, provides information regarding the morbidity associated with contact lens wear. This data also adds to our understanding of the pathogenesis of these biomaterial-mediated complications.

These data allow practitioners to make an informed decision when fitting new patients, based on the likelihood of developing lens related complications with each of the different lens types. Morbidity data for these common complications can be related to the time lost from work by wearers and the hospital casualty and

outpatient resources directed towards their management. The likelihood of developing any complication was 3x greater with an EWSCL compared with a GPCL. More severe complications, with greater morbidity, were significantly associated with the use of lenses on an overnight basis.

The study has also highlighted an increase in the proportion of ophthalmic casualty work related to lens related disorders within this population. This can be directly related to the increased use of lenses.

4.1.6 Epidemiology of *Pseudomonas aeruginosa* in Contact Lens Wearers.

P. aeruginosa was isolated from 11/15 storage cases from culture positive lens related ulcers. In 3 wearers, one of whom wore disposable extended wear lenses, there was no link between lens care material contamination and the isolate from the corneal ulcer. This group of patients, with either sterile or no lens care materials, lends support to the concept of a lens associated bacterial biofilm being important in the pathogenesis of these infections. Colonisation of a contact lens during wear may arise from low levels of pathogenic organisms derived from other sources, which may adhere to and subsequently proliferate on the surface of a contact lens. Another possibility which has not been considered previously is that case contamination may arise, when a lens contaminated and colonised during wear, is replaced into a sterile storage case.

P. aeruginosa was not isolated from any of the likely environmental sites or personal sites sampled for wearers with infections or controls. As this organism is widespread in the environment, it seems likely that causative organisms in lens wearers are derived from low levels of contamination from other environmental sources.

Direct culture may not be sensitive enough to detect small numbers of organisms which may be present in water. A more sensitive technique used in the examination of drinking water supplies involves the use of membrane filtration of the

water sample[204]. This technique is used by water authorities to determine the presence of *P. aeruginosa* in mains water.

4.1.7 Epidemiology of *Acanthamoeba* in Contact Lens Wearers.

Group II *Acanthamoebae*, thought to be similar to pathogenic strains which infect the cornea were isolated from 6/50 bathroom taps and from 1/50 kitchen taps.

Isolation of amoebae and *Acanthamoebae* from the taps and London water supply is to be expected from river water, treated by filtration techniques. Bacterial proliferation is likely in such an aquatic environment and amoebae will proliferate in the presence of bacteria. In addition, *Acanthamoeba* cysts are resistant to chlorine in mains water.

Differences between rates of culture of amoebae and *Acanthamoebae* from bathroom and kitchen taps may be attributable to several factors. The presence of limescale, found to a greater extent in bathroom taps, may provide a site of attachment for amoebae. This view is supported by microscopic examination of centrifuged scale samples plated directly onto seeded agar (Figure 3.3). Pressure differences between mains fed and tank fed supplies may allow greater scale build up in bathroom taps compared with kitchen taps. Bathroom taps were generally found to be tank fed and kitchen mains fed. Storage tanks are rarely drained and cleaned, therefore tank water may stagnate. It is also likely to be at a higher temperature than mains water, which will encourage bacterial regrowth.

No cases of *Acanthamoeba* keratitis have been reported in Scotland as yet. An interesting observation is that both bathroom and kitchen taps are supplied with mains water in Scotland.

Differentiation between environmental and pathogenic strains of Group II *Acanthamoebae* is not clear since no satisfactory animal model exists as yet for corneal infection which reproduces the human disease.

Contamination of lens storage cases with *Acanthamoebae* has been demonstrated for 7/102 asymptomatic lens wearers[81]. If lens storage cases are rinsed with freshly drawn bathroom tap water, scale containing bacteria, amoebae and *Acanthamoebae* may be deposited in the case. The presence of scale and debris may inactivate the chemical disinfection process used. Organic debris has been shown to inactivate chlorine based disinfection systems[205]. In addition, *Acanthamoebae* have been shown to be resistant to several lens cleaning and disinfection regimes (Section 1.3.1)

Since good disinfection management requires the removal of both bacteria and amoebae, the results of this study suggest that freshly drawn bathroom tap water should not be used to rinse contact lens storage cases. A previous study examining tap water rinsing of peroxide disinfected lenses has demonstrated 10^2-10^3 organisms/ml in running tap water[206]. Lens cases should be cleaned using a surfactant and mechanical cleaner then rinsed with boiled, cooled water from the domestic kettle. The case should be air dried prior to the next disinfection cycle. Dry storage will reduce proliferation of amoebae and bacteria.

The high proportion of asymptomatic lens users with bacterial and amoebic contamination of the lens storage case demonstrates that current use of lens care regimes is not as envisaged by manufacturers. The role of bacterial biofilm formation in lens case contamination in lens wearers is not clear, but amoebic proliferation requires bacterial presence. Ideally, good lens hygiene should involve removal of both vegetative organisms and cysts.

4.2 BACTERIAL ADHERENCE TO HYDROGEL LENSES

Epidemiological evidence has shown that hydrogel lens wear poses the greatest risk for microbial keratitis, which represents the most severe, sight threatening complication of cosmetic contact lens wear. Poor lens hygiene and/or lens care material contamination are not inevitably associated with the development of kerati-

tis. It seems likely that bacteria, lens and cornea interactions are implicated in the pathogenesis of keratitis. Bacterial adherence to hydrogel lenses with subsequent colonisation of the surface may be a factor in the development of lens related infections, and may explain infections arising in disposable lens users and in wearers with sterile lens care materials. Laboratory investigations to quantify the adherence of a strain of *P. aeruginosa* to unworn hydrogel lenses, were pursued to provide information on the initial time course of bacterial adherence and to validate a technique for quantification of adherent organisms. This enabled bacterial adherence to worn lenses to be quantified using a standardised technique.

Bacterial adherence to unworn hydrogel lenses has been demonstrated using a lens homogenisation technique, with bacterial quantification using a serial, log dilution method. This method compares well with results documented using other counting techniques, such as scintillation counting[157] and electron microscopy[150].

This method is also suitable for quantifying small numbers of adherent organisms, which may not be possible using scintillation counting[207]. However, homogenisation is not likely to be a suitable technique for quantifying organisms adherent to rigid lenses.

Bacterial adherence was found to increase up to an incubation period of 45 minutes. Differences in bacterial counts at 45 and 60 minutes were not found to be significantly different. These findings correlate with those of John et al. in 1989[150]. A similar trend of adherence with time was shown using both washed and unwashed organisms and with either a washing or vortexing technique.

Unwashed organisms appeared to adhere more strongly compared to washed organisms. Vortexing of incubated lenses reduced the count for washed bacteria to zero, whereas unwashed organisms adhered up to a level of 10^5 organisms/ml. This was to be expected, as a broth culture of unwashed organisms contains sticky proteinaceous material. Washing of the bacterial culture may partly remove any

bacterial glycocalyx, which may alter the adherence characteristics. However, using a gentle washing technique, bacterial counts for washed organisms were slightly, but not significantly less than for unwashed organisms. Bacterial adherence using washed organisms, was found to be very dependent on the washing technique used. It appears to be important to be able to specify the washing technique and that it is repeatable.

Removal of the lens surface water film prior to homogenisation was not found to affect the numbers of adherent organisms. The results for blotted lenses proved to be more variable than for non-blotted specimens, due to the variability of the capillary action of the blotting technique.

Significantly greater numbers of organisms were found to adhere to non-ionic compared with ionic lenses. This correlates well with findings from previous studies, using a scintillation counting technique[161].

The accuracy of this technique may be improved by initially using a spectrophotometer to quantify the bacterial culture. The concentration may then be adjusted either by dilution, or by respinning and resuspending the organisms. However, optical density is not an indicator of bacterial viability. The standard deviations on the viable bacterial cultures have remained constant throughout the series of experiments, and it may not be possible to improve on this using a photometric technique.

All adherence assays were performed using a single strain of *P. aeruginosa*, which was initially derived from a hydrogel lens related keratitis. Repeated subculture and storage of this organism may alter it's adherence characteristics. Studies on bacterial glycocalyces have shown that a glycocalyx evolves as a means of survival in natural habitat. Under laboratory conditions, the glycocalyx is lost[167]. However, for the purposes of these experiments, we have been able to demonstrate that quantifiable bacterial adherence to hydrogel materials, occurs using this strain.

It seems likely that adherence is also strain and species specific. A study evaluating bacterial adherence to corneal epithelial cells[163], found that adherence was greater with *Staphylococcus aureus*, *Streptococcus pneumoniae* and *P. aeruginosa* compared with other species tested. Adherence was also found to be strain specific, and pathogenicity was found to relate to the ability of the particular strain of the organism to adhere to epithelial cells.

4.3 PATIENT MATERIAL

It is recognised that bacterial adherence to and subsequent colonisation of unworn lenses in vitro, differs from the situation in vivo. Bacterial biofilm formation in vivo is likely to be mediated by host secretions, ocular surface defence mechanisms and a higher temperature, compared with in vitro. Lenses and lens storage cases from wearers with lens related infections were analysed using quantitative bacteriology, SEM and TEM to investigate the relationship between bacteria and contact lenses or lens storage cases in vivo.

Evaluation of storage and processing techniques using unworn lenses was initially performed to improve techniques for dealing with patient material. The least disruptive fixation and storage procedures used immediate fixation in glutaraldehyde with ruthenium red and phosphate buffer. Snap freezing specimens was found to be preferable to slow freezing, and critical point drying produced the least surface disruption. Surface abnormalities were seen to a greater extent in high water content lenses compared with low water content material. These findings have been previously reported for a variety of processing techniques[208], although different studies conflict as to the least disruptive processing technique[209,208].

Significant numbers of viable organisms were recovered from the lenses of patients with lens related microbial keratitis. The presence of adherent organisms has been demonstrated on 7/11 lenses in SEM. In 4/7 cases, no organism had been isolated

from the corneal ulcer. Bacteria, in the presence of ruthenium positive film, were found on the surface of 1/4 lenses. This bacteria containing biofilm was present on an extended wear hydrogel lens.

Differences in SEM appearance of front and back surfaces of worn lenses were consistently found. Front surfaces appeared to be more dehydrated and disrupted compared with the back lens surface, or compared with unworn lenses of similar parameters. This may have occurred as the result of lens surface drying during wear. This surface crenelation effect was not apparent with the two disposable lenses examined, which were both less than one month old. These differential dehydration effects on the front and back lens surfaces may be a function of lens age and individual tear resurfacing characteristics.

Fewer organisms and less debris appeared to adhere to the anterior surface compared with the posterior lens surface. This finding has been reported previously[209]. This may have been a processing artefact, possibly organisms were initially adherent, but may have become loose during processing. It may be that fewer organisms adhere to a more disrupted front lens surface or that blinking action and the pre-lens tear film prevent colonisation of the front surface. The back lens surface in vivo probably represents a more favourable environment for colonisation; it is more stable and possibly the higher temperature, particularly under extended wear conditions, may encourage bacterial proliferation.

Bacterial adherence to hydrogel lenses both in vitro and in vivo has been demonstrated. Large numbers of viable organisms were recovered from worn lenses. This implies that bacterial colonisation of the lens surfaces is occurring, and suggests a major failure in lens hygiene practices.

The licensing of lens care materials in the USA is moving towards antimicrobial testing of solutions against contaminated lenses. Whereas, in the UK, solutions are tested against planktonic organisms. Bacteria in their planktonic mode are con-

siderably more susceptible to antibiotics and antiseptics compared with bacteria in a microcolony mode of growth[174,210]. Testing of lens disinfection regimes against free bacteria in solution does not reproduce the in-use situation, which is likely to present a greater challenge to care systems.

There is controversy over whether bacterial adherence is greater to worn or unworn lenses. A recent study of in vivo bacterial adherence to new or worn rabbit lenses has shown that adherence is greater to new lenses[211]. This conflicts with previous studies, which claimed that adherence to worn/artificially deposited lenses was greater than to unworn[154,156,157]. A study by Klotz et al.[159], using lectins, has demonstrated carbohydrate moieties on the surface of worn hydrogel lenses. They speculate that such tear deposits may act as specific receptors for organisms. The presence of sialic acid on worn lenses was demonstrated. They also demonstrated that bacterial adherence is greater to worn lenses compared with unworn. Sialic acid is thought to be a receptor for *P. aeruginosa* from animal studies[103] and an in vitro study using human buccal epithelial cells[102]. It's presence on lenses may account for increased adherence to worn lenses. A recent SEM study has shown bacterial adherence is greater to new compared with worn disposable lenses[212]. It has been suggested that the rapid heavy proteinaceous coating of ionic lenses[213] during wear, inhibits attatchment of *P. aeruginosa*. If initial bacterial adherence is a major factor in the development of lens related infections, this may imply that if bacterial adherence to disposable lenses is inhibited by heavy deposition of tear proteins, that infectious keratitis may be less common with this type of lens material. However, the presence of polysaccharide biofilm on worn extended wear lenses, may imply that once bacterial colonisation, with production of a bacterial biofilm has occurred, that initial adherence is less important.

Electron microscopy is not a good technique for visualising biofilm, since dehydration during processing causes a collapse of the matrix of the extracellular glycocalyx surrounding the organisms. This technique can be improved upon by using

lectins as cross linking agents to stabilise the matrix[214].

In spite of these limitations, a bacterial biofilm on a worn extended wear hydrogel lens has been demonstrated. This has implications for the safety of any overnight wear. Little is known about the kinetics of bacterial biofilm development, but it's presence implies proliferation of bacteria on the back surface of the lens during wear. Initial bacterial adherence to a hydrogel lens may lead to subsequent biofilm formation in close proximity to the corneal surface. Swarmer or planktonic cells, free from the protective environment of the biofilm, may later be released from the body of the biofilm. The ability of the organism to cause epithelial damage via proteolytic or toxic products may partly explain the preponderance of *P. aeruginosa* related infections.

There is little doubt that the lens can act as a vector carrying organisms from contaminated care materials to the ocular surface. However, a further route may exist where the lens acts as a substratum for bacterial proliferation.

The presence of a corneal biofilm in animal contact lens wearing models has not been demonstrated in the absence of epithelial damage. However, it has been shown that organisms are cleared from a non-lens wearing rabbit eye within 4 hours[175]. In a lens wearing eye, greater numbers of organisms, compared with the initial inoculum, have been recovered from the lens. It has not been ascertained whether or not a corneal biofilm forms behind the contact lens. If lens wear alters the adherence characteristics of human corneal epithelial cells, it may allow a combined lens/epithelial/bacterial biofilm formation. It may be possible that lens removal disrupts this more recent corneal biofilm.

The formation of a bacterial biofilm within lens storage cases may allow a small number of organisms to proliferate within this protective environment. This may afford organisms a greater degree of resistance to antimicrobials in lens care regimes.

Bacterial existence within glycocalyx enclosed microcolonies is known to be the predominant mode of growth in nature. The presence of a bacterial biofilm has been implicated in infections involving biomaterials such as artificial joints, heart valves and catheters. Formation of a bacterial biofilm on a biomaterial, in intimate contact with the ocular surface, may be implicated in lens related infections.

CHAPTER 5. FURTHER WORK

5.1 EPIDEMIOLOGY

5.1.1 Disposable Lenses.

The present study has confirmed, using a case control study design, that the risks of lens related disease differ with different modes of lens wear. However, during the study period, disposable lenses were introduced into the UK market. These lenses were introduced to reduce the risks associated with lens related disease[215], particularly to reduce the risks associated with infections, deposition and solution related disorders.

Only 20 disposable lens wearers presented during the course of the study; 18 with lens related disease. From these small numbers it was not possible to quantify the relative risks.

Since disposable lenses were introduced, during 1988, there have been numerous case reports of microbial[58,216,59,60,217,218,61,219] and sterile keratitis[220] associated with disposable lens use.

Disposable lenses represent a new challenge for which the ocular risks need to be quantified. Evidence for risk factors in microbial keratitis from this study, has not confirmed any likely reduction in the risk of infection, using this mode of lens wear. Extended wear, of all hydrogel lenses, is known to cause corneal hypoxia, which results in many short and long term sequelae. A recent study has demonstrated that the oxygen transmissibility of existing disposable lenses is no improvement on currently available lenses[189].

There have been no prospective epidemiological studies carried out to date on disposable lenses. A preliminary pilot study performed in the Accident and Emergency department has determined that sufficient numbers of disposable lens users are presenting for treatment to allow a case control study to be performed. A

similar design of study with an improved hygiene questionnaire has been planned to quantify the risks associated with lens wear.

5.1.2 Case Control and Cohort Studies.

The relative risks associated with lens wear for microbial keratitis are similar to those estimated by a similar study design performed in the New England area[54]. Future studies need to be carried out in different centres to validate these findings for other lens related disorders. With the existing data, further analysis is planned to evaluate risk factors for the remaining complications of lens wear.

Assuming that resources were available, population based controls could be derived from the sources previously mentioned (Section 4.1). Estimates derived from rigorously collected population controls would identify sources of bias arising due to the use of hospital based controls.

Additional cohort studies would establish the incidence of lens related disease. This would provide valuable morbidity data, particularly for the more common lens related disorders.

5.2 IN VITRO STUDIES

The methods evolved, during this study, to enable quantification of bacterial adherence to hydrogel lenses, could be extended. This would involve using different bacterial incubation periods, different species and strains of organisms, and sizes of bacterial inocula. This information will allow assessment of the interaction of bacterial adherence, different lens materials and biofilm formation. The effect of different lens cleaning and disinfection techniques could then be evaluated in this model.

It would also be relevant to directly compare different methods to quantify adherent bacteria. Material has been processed during the study to enable SEM for counting of adherent organisms. This could then be compared with data already

derived from the colony counting techniques. This model is suitable to quantify adherent bacteria to lenses both in vitro and in vivo.

Additional methods have been considered to analyse and quantify bacterial biofilm. Variations of these techniques have been used previously in the analysis of lens deposits.

5.2.1 X-Ray Probe Analysis.

This would provide an initial elemental analysis which would indicate the presence of elements such as sulphur within the film. From this type of analysis, the presence of compounds such as sulphated glycoproteins can be ascertained. This technique is not quantitative.

5.2.2. Gel Electrophoresis.

This technique would provide semi quantitative information regarding the total protein content of the biofilm per lens. The surface film can be eluted from the lens by homogenisation in buffered saline, the salt concentration of which could be altered to vary the final yield of protein. Differing concentrations of polyacrylamide gel can be used to identify proteins of different molecular weights, depending on the composition of the film. A similar technique can be used to separate glycoproteins within biofilm and identify sulphated or non sulphated glycoproteins. The technique for glycoproteins would require different staining procedures. Bands of specific proteins can be cut from the gel and subsequently hydrolysed to produce amino acids.

Lipoproteins, where lipids are attatched to specific proteins, may also be similarly characterised by electrophoresis.

5.2.3. Mass Spectroscopy and Gas Phase Chromatography.

Mass spectroscopy provides a means of determining the mass of protein molecules within the biofilm. Gas chromatography may be used in conjunction with mass

spectroscopy to identify smaller molecules, such as amino acids or lipids. Thin layer chromatography may be used to determine the types of lipid present. Use of an organic solvent would be necessary to extract lipids from the biofilm, which could then be typed as phospholipids or neutral fats, and quantified. This technique could be used to differentiate between intracellular and extracellular lipids.

5.2.4. Radioactive Tracer Technique.

This technique may be useful as part of the investigation of the kinetics of bacterial biofilm formation and comparison of the efficacy of disinfection regimes. A radioactive tracer such as H-3 Glucosamine or S-35 (incorporated into chondroitin sulphate), which can be metabolised by bacteria, may be introduced into the lens case solution. The biofilm may then be removed using an organic solvent and the radioactivity measured. There are drawbacks with this type of technique, since the compounds are degradable, but a comparitive estimate of bacterial metabolism may be derived.

These four techniques may be applied sequentially to identify the basic components of bacterial biofilm formed in vitro, which may then be quantified.

5.3 PATIENT MATERIAL

Improved methods for dealing with patient material need to be evolved. Electron microscopy of patient samples could be performed, using prior treatment with lectins or antibodies[221], to stablilise the matrix of any biofilm present by cross-linkages. This would improve visualisation of the biofilm in EM.

An alternative to this, would involve the use of cold stage microscopy. Samples could be immediately frozen and viewed in a cold stage within the microscope. This would avoid fixing and processing which disrupts the biofilm, and would give an almost immediate image of any biofilm present.

Comparison of the materials from infected patients and controls, would help to

determine the role of biofilm in contamination of lens materials, and it's possible role in the pathogenesis of human keratitis. This work is fundamental to increasing our understanding of the pathogenesis of keratitis in contact lens wearers and the reasons for the high level of contamination of contact lens cases in both infected and asymptomatic lens users.

The development of an in vitro system would enable the differences between biofilm in this system and that developed in vivo to be examined. It would also provide a system in which the kinetics of bacterial biofilm build up can be investigated.

The high level of lens care material contamination, particularly lens storage cases, amongst asymptomatic lens wearers, is cause for concern. Development of such an in vitro model, would enable the effects of different hygiene and lens wearing regimes, on the development and persistence of bacterial biofilm, to be evaluated. This is relevant both in terms of contamination of CL and of lens storage cases.

CHAPTER 6. CONCLUSIONS

6.1 CASE CONTROL STUDY

6.1.1. Microbial Keratitis

This study has shown that contact lens wear for the correction of low refractive errors is currently the major avoidable cause of new keratitis cases within the study population. The population attributable risk percentage, for contact lens wearers,for all severities of keratitis was found to be 62%. This implies that if the risks for keratitis associated with contact lens wear could be eliminated, the reduction in new keratitis cases in this population could be up to 62% per year. The greater risk for keratitis associated with contact lens wear, compared with trauma and previous ocular surface disorders, was found to persist for all severities of keratitis. This supports the use of a clinical case definition. This increase over previously reported studies is likely to be a result of increased use of lenses, particularly extended wear lenses, within the population. Contact lens wearers were found to be at an 18x higher risk of developing microbial keratitis compared with non-lens wearers.

The relative risk for all severities of keratitis associated with contact lens wear was found to be 80.2x (38.5-166.9x) higher, compared with no apparent predisposing factor. The relative risk associated with trauma was found to be 13.9x (6.0-32.2x) higher, and for ocular surface disorders (OSD) 7.4x (2.2-25.3x) higher compared with no apparent predisposing factor.

Differing relative risks for different exposures associated with microbial keratitis were found to persist, despite controlling for age, gender and socioeconomic classification.

This study has enabled the relative risks for microbial keratitis, for rigid and hydrogel lenses, to be compared for the first time in a controlled study. The in-

creased risk to SCL wearers, particularly to EWSCL wearers has been confirmed and quantified.

Extended wear soft lens users were consistently found to have a significantly higher relative risk compared with other lens types, for all severities of keratitis. Compared with a risk of 1 for GPCL users, EWSCL users were found to have a relative risk for keratitis of 20.8-36.8x higher. The relative risk for DWSCL users, was found to be 3.6-4.1x greater than for GPCL users. Differences in risk between PMMA and GPCL users were not found to be significant. A previous study, performed in the USA, was not able to compare the risks associated with hydrogel lenses, to those associated with rigid lenses, due to a low penetrance of rigid lens wear in the study population. Since case reporting of keratitis has implied that the risk is lowest in rigid lens users, the ability to compare the risks associated with EWSCL and DWSCL to a baseline risk in GPCL use, has been a major strength of this study.

Keratitis in EWSCL users was found to be associated with wearing of a lens for longer than 6 days continuously. Various lens hygiene and compliance factors were not found to be associated with keratitis, neither were patient age or duration of lens wear. Males were found to have a slightly increased risk of keratitis and a lower socioeconomic classification was an associated factor. From these multivariate data, it appears that EW disposable lens use is unlikely to reduce the relative risk of infection compared with reusable EW lens use.

Keratitis in DWSCL users was found to be associated with less frequent lens disinfection, but that other hygiene and compliance factors were not associated factors. Age and socioeconomic classification were not found to be associated with keratitis, but males were found to have an increased risk compared with females.

Lens care material contamination was only found to be significantly associated with keratitis for DWSCL users only. However, numbers were small in the

EWSCL control group and no trend was apparent. Of lens users with keratitis, 16/49, including 3 EW disposable lens users, were found to have no link with bacterial contamination of the lens care materials. Similarly, good lens hygiene was reported by 16/33 wearers with keratitis in association with bacterial contamination of the lens storage case, compared with 5/16 controls.

Misuse of daily wear hydrogel lenses by wearing them overnight, was performed by a similar number of keratitis patients and asymptomatic controls.

The risks of keratitis associated with DWSCL use may be reduced by greater attention to lens hygiene and lens care material contamination. However, these factors appear to be less important in EWSCL use. The greatest reduction in risk occurs if overnight wear is avoided.

6.1.2 Lens Related Disorders

Lens related complications were found to acccount for 3.8% of all new casualty attenders. This compares with an incidence of 2.5%, estimated by a previous retrospective study in 1980. An increase in the proportion of ophthalmic casualty work related to lens related disorders reflects an increase in the penetrance of contact lens wear.

Numerous complications have been documented associated with the wearing of lenses. This study has enabled, for the first time in a controlled study, the relative and population attributable risks to be estimated for lens related complications in cosmetic lens users.

Significant differences in relative risk have been shown for different lens types for a range of acute lens related disorders. EWSCL users were found to be at an overall risk of developing any complication at 2.7x higher compared with GPCL users. This information is relevant to practitioners involved in initial fitting of lenses, and enables an informed decision to be made about the preferred lens type for management of wearers with low refractive errors. The morbidity of the more

common but less severe complications can be interpreted in terms of risk/benefit to patients. The benefits of an EW mode of use are seen as convenience, comfort, reduced lens handling and reduced expense in terms of care materials. These must be offset against increased likelihood of visual loss in severe complications, more frequent casualty visits, more frequent unscheduled practitioner visits and time taken from work. With more serious complications of lens wear, which were more frequently seen in EWSCL users, these often required multiple casualty attendances. In severe microbial keratitis, these wearers required hospital admission and intensive antibiotic therapy. Hospital and casualty resources are taken up with disorders which could be significantly reduced by selection of a more appropriate form of correction for low refractive errors.

Metabolic disorders and sterile infiltrates were found to have the highest risk in EWSCL users at 2.0-3.7x and 2.4-4.7x higher respectively, compared with that of GPCL users. Toxic and hypersensitivity disorders had a 5.8-5.9x higher risk in DWSCL users, and 4.5-8.1x higher in EWSCL users, compared with GPCL users.

The greatest risk of abrasions was found, as anticipated, amongst rigid lens users. The risk was found to be 2-3x higher than for hydrogel lens wearers. However, this group represented the least severe complication of lens wear.

6.1.3 Epidemiology of *Pseudomonas aeruginosa* in Contact Lens Wearers.

Pseudomonas aeruginosa is associated with contact lens related microbial keratitis more frequently than other organisms. A study was performed to evaluate *P. aeruginosa* contamination of lens care materials, personal carriage of *P. aeruginosa* and environmental contamination in a group of lens wearers with culture proven keratitis. These data were compared with results from control wearers without lens associated disease.

From a group of 15 hydrogel lens users with culture proven *P. aeruginosa* keratitis, 4 wearers showed no link between the lens care material contamination and the

corneal ulcer. These cases provide further support for the concept of the lens providing a surface for bacterial adherence and subsequent colonisation.

No *P. aeruginosa* was isolated from any of the domestic water sites sampled, and no personal carriage of *P. aeruginosa* was demonstrated. It seems possible that causative organisms may be derived from low levels of environmental contamination from other sources.

6.1.4 Epidemiology of *Acanthamoeba* in Contact Lens Wearers.

In the light of increased reporting on the association between *Acanthamoeba* keratitis and contact lens wear, a study was performed to evaluate the presence of Group II *Acanthamoebae* in the homes of contact lens wearers.

Group II *Acanthamoebae*, thought to be similar to pathogenic strains which infect the cornea, were isolated from 6/50 bathroom taps and 1/50 kitchen taps. Contamination with environmental Gram negative bacteria was found more frequently in bathroom taps, which tended to be tank fed compared with kitchen taps which tended to be mains fed. The presence of environmental amoebae in taps, was found to be significantly associated with the limescale. Amoebae and limescale were more frequently isolated from bathroom taps. These results suggest that, for disinfection to remove vegetative organisms, lens storage cases should not be rinsed in freshly drawn bathroom tap water. Cases should be cleaned using surfactant, rinsed with boiled water and air dried prior to the next lens disinfection cycle. Dry storage will inhibit the proliferation of amoebae and bacteria.

6.2 BACTERIAL ADHERENCE TO HYDROGEL LENSES

Bacterial adherence to unworn hydrogel lenses was demonstrated for a range of incubation times. The results correlate well with existing quantification techniques. This lens homogenisation and colony counting technique provides a reliable means for quantifying viable organisms, adherent to hydrogel lenses. Material has

capable of supporting bacterial colonisation. This would enable small numbers of environmental organisms to adhere to the lens and proliferate, with increased contact time at the corneal surface.

These findings add support to the theory that the formation of bacterial biofilm on hydrogel lenses is implicated in the pathogenesis of lens related infections. Any hypothesis of microbial keratitis in lens wearers must account for the greater risk of keratitis demonstrated for EWSCL users, the predominance of *P. aeruginosa* keratitis, and the group of wearers without care material contamination. *P. aeruginosa* is known to adhere to both worn and unworn hydrogel lenses. Formation of a bacterial biofilm, is known to provide a favourable mode of growth by enhancing adherence to a surface. This is likely to protect the enclosed organisms from ocular surface defences and from antimicrobials in lens care regimes. The presence of a bacterial biofilm on a worn hydrogel extended wear lens, implies bacterial proliferation on the back surface of the lens in close proximity to the corneal epithelium. *P. aeruginosa* has the ability to cause corneal damage through the release of proteolytic enzymes and toxins, which may cause an epithelial break. Epithelial damage may also arise as a result of physiological stress. An epithelial breach may expose specific receptor sites on corneal epithelial cells[222], which may promote adherence to free bacteria. Swarmer, or planktonic cells, released from the bacterial biofilm, may consequently adhere to damaged epithelial cells and cause corneal invasion.

Bacterial growth within adherent microcolonies, is known to be the predominant mode of growth in natural systems, and has been implicated in biomaterial mediated infections in other body sites. Such formation of a bacterial biofilm on human hydrogel lenses, may be implicated in the pathogenesis of lens related microbial keratitis. Future study is necessary to establish the kinetics of bacterial biofilm formation both in vitro and in vivo and to establish the effects of lens hygiene regimens on this new challenge.

been prepared for future scanning electron microscopy of lens samples, to allow colony counting methods to be correlated with direct counting of organisms from electron micrographs.

This technique of lens homogenisation and colony counting has been evolved to allow standardisation in the treatment of material collected from lens wearers. This will allow future comparison between lenses collected from wearers with infections and from asymptomatic controls.

6.3 PATIENT MATERIAL

Significant numbers of viable organisms were recovered from lenses and lens storage cases from wearers with microbial keratitis. This represents a major failure of disinfection systems in use.

Adherent organisms were demonstrated on the surface of 7/11 hydrogel lenses using SEM. In 4/7 cases, no organism had been isolated from the corneal ulcer. Bacteria in the presence of a polysaccharide rich biofilm, were demonstrated using TEM on 1/4 lenses.

Demonstration of such a bacterial biofilm on an extended wear lens has implications for the safety of any extended wear regime and for the efficacy of disinfection regimes.

Evidence from the case control study, implying a greater risk of keratitis in EWSCL users, plus the findings of bacteria within a biofilm on an EWSCL, suggest that the pathogenesis of microbial keratitis involves interaction between the lens, cornea and bacteria. Lens wear must somehow alter the susceptibility of the cornea to infection, with a greater alteration in EWSCL use. Previous studies have documented extensive corneal changes in EWSCL users, which may reduce the ocular resistance to infection. Lens wear may act as a vector for bacteria, carried from a contaminated lens care materials to the eye, or by providing a surface

The study has shown that contact lens wear, particularly hydrogel lens wear, for the correction of low refractive errors is the major cause of new keratitis cases within this population. Extended wear soft lens use carries overwhelmingly the highest relative risk of developing microbial keratitis, sterile keratitis and metabolic disorders. The overall relative risk of developing any complication is 3 times greater with an EWSCL compared with a GPCL. Lens related complications are associated with significant morbidity in terms of hospital resources, unscheduled practitioner visits, and time needed from work to attend for emergency treatment. The majority of more severe complications involving greater morbidity are seen more frequently in EWSCL use. This could be reduced by the selection of a more appropriate lens type for the correction of low refractive errors.

DATA SHEET A: LENS RELATED MICROBIAL KERATITIS

Name..... Cas. No..... Hosp. No.....
DOB..... Address.....
1st visit date..... .....
Age.... Admit Y/N .....
Sex M/F Postcode.....
Soc. class..... Tel. no.....

Diagnosis L/R Culture Pos Y/N Organism.....
.....
Indication..... Lens Type.....

Contact lens Refraction..... CL/Spectacle
Current type..... Age R.... L....
History [ ] DWHCL PMMA.... GP.... Unknown...
(Order and length) [ ] DWSCL....
[ ] EWSCL....
[ ] Other.....
EWSCL: Current cycle. Days in..... Days out.....
Time since lenses last removed
prior to onset of symptoms (days).....
Self handling Y/N
DWSCL: Days worn per week..... Av. hours per day.....
Suppliers name and address.....
.....
Time since last CL check.....
Hygiene regime:
..... Cleaning Y/N Frequency..... Soln age....
..... Disinfection Y/N Frequency.....Soln age....
Cold: Chemical Y/N Top-up/change
Peroxide Y/N
Chlorine Y/N
Heat: Saucepan/Unit/Thermos
..... Saline Y/N: Soln age....
Preserved Y/N
Home made Y/N
Aerosol/single dose Y/N
..... Other Y/N
..... Wetting Y/N Soln age....
..... Comfort Y/N Soln age....
..... Enzyme Y/N Frequency.....
Lens cases No..... Clean Y/N Frequency.....
..... Eye drops used Y/N

History of current episode.....
.....
Time from onset to diagnosis in days.....
Treatment before seen Y/N Optician/GP/Other.....
Antibiotic Y/N Type.....
Steroid Y/N Type.....
Other Y/N Type.....

Past History
Previous eye surgery Y/N Aphakia/other.....
Previous external disease Y/N.....
Previous CL problems Y/N.....
Systemic disease Y/N.....
Diabetes Y/N
Atopy Y/N [Eczema Y/N Asthma Y/N Hay fever Y/N
Food rash Y/N Other Y/N.....]

Examination of L eye

Lid and/or conjunctival disorder.....
.....
Corneal disorder.....
.....

Examination of R eye

Lid and/or conjunctival disorder.....
.....
Corneal disorder.....
.....

Examination of affected eye Draw extent of infiltrate
Visual axis involved Y/N
Infiltrate diameter (mm).....
Hypopyon Y/N
Code Mild/Moderate/Severe

Bacteriology

Gram stain.....
Cornea R.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
L.....
0 1-5 6-20 Semi-conf. Conf.
Conj. R.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
L.....
0 1-5 6-20 Semi-conf. Conf.
Lens R.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
L.....
0 1-5 6-20 Semi-conf. Conf.
Case Solution 1.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
2.....
0 1-5 6-20 Semi-conf. Conf.
Solutions Cleaning.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
Soaking.....
0 1-5 6-20 Semi-conf. Conf.
Saline.....
0 1-5 6-20 Semi-conf. Conf.
Wetting.....
0 1-5 6-20 Semi-conf. Conf.
Comfort.....
0 1-5 6-20 Semi-conf. Conf.
Eye drops.....
0 1-5 6-20 Semi-conf. Conf.

Outcome Two months post infection Y/N Other.....
Time in hosp..... One/+ admissions Y/N.....
No of OPD visits.....
Prior VA.....
Post VA Spectacles without pinhole.....
Spectacles with pinhole.....
Diagnostic CL.....
Surgical management Y/N Graft Y/N Other.....
Acute Y/N
Planned or offered Y/N

Examination of affected eye Draw extent of scar
Visual axis involved Y/N
Scar diameter (mm).....
Code Mild/Moderate/Severe

DATA SHEET B: NON-LENS RELATED MICROBIAL KERATITIS

Name..... Cas. No..... Hosp. No.....
DOB..... Address.....
1st visit date..... .....
Age.... Admit Y/N .....
Sex M/F Postcode.....
Soc. class..... Tel. no.....

Diagnosis L/R Culture Pos Y/N Organism.....
.....

History of current episode.....
.....
Time from onset to diagnosis in days.....
Treatment before seen Y/N Optician/GP/Other.....
Antibiotic Y/N Type.....
Steroid Y/N Type.....
Other Y/N Type.....

Past History
Previous eye surgery Y/N Aphakia/other.....
Previous external disease Y/N.....
Previous CL problems Y/N.....
Systemic disease Y/N.....
Diabetes Y/N
Atopy Y/N [Eczema Y/N Asthma Y/N Hay fever Y/N
Food rash Y/N Other Y/N.....]

Examination of L eye
Lid and/or conjunctival disorder.....
.....
Corneal disorder.....
.....
Examination of R eye
Lid and/or conjunctival disorder.....
.....
Corneal disorder.....
.....

Examination of affected eye Draw extent of infiltrate
Visual axis involved Y/N
Infiltrate diameter (mm).....
Hypopyon Y/N
Code Mild/Moderate/Severe

Bacteriology

Gram stain.....

Cornea R.....

Date..... 0 1-5 6-20 Semi-conf. Conf.

L.....

0 1-5 6-20 Semi-conf. Conf.

Conj. R.....

Date..... 0 1-5 6-20 Semi-conf. Conf.

L.....

0 1-5 6-20 Semi-conf. Conf.

Outcome Two months post infection Y/N Other.....

Time in hosp..... One/+ admissions Y/N.....

No of OPD visits.....

Prior VA.....

Post VA Spectacles without pinhole.....

Spectacles with pinhole.....

Diagnostic CL.....

Surgical management Y/N Graft Y/N Other.....

Acute Y/N

Planned or offered Y/N

Examination of affected eye Draw extent of scar

Visual axis involved Y/N

Scar diameter (mm).....

Code Mild/Moderate/Severe

DATA SHEET C: LENS USER WITHOUT ASSOCIATED DISEASE

Name..... Cas. No..... Hosp. No.....
DOB..... Address.....
1st visit date..... .....
Age.... Admit Y/N .....
Sex M/F Postcode.....
Soc. class..... Tel. no.....

Diagnosis L/R.....
.....
Indication..... Lens Type.....

Contact lens Refraction..... CL/Spectacle
Current type..... Age R.... L....
History [ ] DWHCL PMMA.... GP.... Unknown...
(Order and length) [ ] DWSCL....
[ ] EWSCL....
[ ] Other.....
EWSCL: Current cycle. Days in..... Days out.....
Time since lenses last removed
prior to onset of symptoms (days).....
Self handling Y/N
DWSCL: Days worn per week..... Av. hours per day.....
Suppliers name and address.....
.....
Time since last CL check.....
Hygiene regime:
..... Cleaning Y/N Frequency..... Soln age....
..... Disinfection Y/N Frequency.....Soln age....
Cold: Chemical Y/N Top-up/change
Peroxide Y/N
Chlorine Y/N
Heat: Saucepan/Unit/Thermos
..... Saline Y/N: Soln age....
Preserved Y/N
Home made Y/N
Aerosol/single dose Y/N
..... Other Y/N
..... Wetting Y/N Soln age....
..... Comfort Y/N Soln age....
..... Enzyme Y/N Frequency.....
Lens cases No..... Clean Y/N Frequency.....
..... Eye drops used Y/N

Past History
Previous eye surgery Y/N Aphakia/other.....
Previous external disease Y/N.....
Previous CL problems Y/N.....
Systemic disease Y/N.....
Diabetes Y/N
Atopy Y/N [Eczema Y/N Asthma Y/N Hay fever Y/N
Food rash Y/N Other Y/N.....]

Examination of L eye

Lid and/or conjunctival disorder.....
.....
Corneal disorder.....
.....

Examination of R eye

Lid and/or conjunctival disorder.....
.....
Corneal disorder.....
.....

Bacteriology

Lens R.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
L.....
0 1-5 6-20 Semi-conf. Conf.
Case Solution 1.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
2.....
0 1-5 6-20 Semi-conf. Conf.
Solutions Cleaning.....
Date..... 0 1-5 6-20 Semi-conf. Conf.
Soaking.....
0 1-5 6-20 Semi-conf. Conf.
Saline.....
0 1-5 6-20 Semi-conf. Conf.
Wetting.....
0 1-5 6-20 Semi-conf. Conf.
Comfort.....
0 1-5 6-20 Semi-conf. Conf.
Eye drops.....
0 1-5 6-20 Semi-conf. Conf.

DATA SHEET D: LENS USER WITH LENS RELATED DISEASE

Name..... Cas. No..... Hosp. No.....
DOB..... Address.....
1st visit date..... .....
Age.... Admit Y/N .....
Sex M/F Postcode.....
Soc. class..... Tel. no.....

Diagnosis L/R .....
.....
Indication..... Lens Type.....

Contact lens Refraction..... CL/Spectacle
Current type..... Age R.... L....
History [ ] DWHCL PMMA.... GP.... Unknown...
(Order and length) [ ] DWSCL....
[ ] EWSCL....
[ ] Other.....
EWSCL: Current cycle. Days in..... Days out.....
Time since lenses last removed
prior to onset of symptoms (days).....
Self handling Y/N
DWSCL: Days worn per week..... Av. hours per day.....
Suppliers name and address.....
.....
Time since last CL check.....
Hygiene regime:
..... Cleaning Y/N Frequency..... Soln age....
..... Disinfection Y/N Frequency.....Soln age....
Cold: Chemical Y/N Top-up/change
Peroxide Y/N
Chlorine Y/N
Heat: Saucepan/Unit/Thermos
..... Saline Y/N: Soln age....
Preserved Y/N
Home made Y/N
Aerosol/single dose Y/N
..... Other Y/N
..... Wetting Y/N Soln age....
..... Comfort Y/N Soln age....
..... Enzyme Y/N Frequency.....
Lens cases No..... Clean Y/N Frequency.....
..... Eye drops used Y/N

History of current episode.....
.....
Time from onset to diagnosis in days.....
Treatment before seen Y/N Optician/GP/Other.....
Antibiotic Y/N Type.....
Steroid Y/N Type.....
Other Y/N Type.....

Past History
Previous eye surgery Y/N Aphakia/other.....
Previous external disease Y/N.....
Previous CL problems Y/N.....
Systemic disease Y/N.....
Diabetes Y/N
Atopy Y/N [Eczema Y/N Asthma Y/N Hay fever Y/N
Food rash Y/N Other Y/N.....]

Examination of L eye

Lid and/or conjunctival disorder.....
.....

Corneal disorder.....
.....

Examination of R eye

Lid and/or conjunctival disorder.....
.....

Corneal disorder.....
.....

DATA SHEET E: CONTROLS (1 IN 100 CASUALTY ATTENDERS)

Name..... Cas. No..... Hosp. No.....
DOB..... Address.....
1st visit date..... .....
Age.... Admit Y/N .....
Sex M/F Postcode.....
Soc. class..... Tel. no.....

Diagnosis L/R .....
.....

If Patient is a Contact Lens User:

Indication..... Lens type.....

Contact lens Refraction..... CL/Spectacle
Current type..... Age R.... L....
History [ ] DWHCL PMMA.... GP.... Unknown...
(Order and length) [ ] DWSCL....
[ ] EWSCL....
[ ] Other.....
EWSCL: Current cycle. Days in..... Days out.....
Time since lenses last removed
prior to onset of symptoms (days).....
Self handling Y/N
DWSCL: Days worn per week..... Av. hours per day.....
Suppliers name and address.....
.....
Time since last CL check.....
Hygiene regime:
..... Cleaning Y/N Frequency..... Soln age....
..... Disinfection Y/N Frequency.....Soln age....
Cold: Chemical Y/N Top-up/change
Peroxide Y/N
Chlorine Y/N
Heat: Saucepan/Unit/Thermos
..... Saline Y/N: Soln age....
Preserved Y/N
Home made Y/N
Aerosol/single dose Y/N
..... Other Y/N
..... Wetting Y/N Soln age....
..... Comfort Y/N Soln age....
..... Enzyme Y/N Frequency.....
Lens cases No..... Clean Y/N Frequency.....
..... Eye drops used Y/N

CONTACT LENS USER QUESTIONNAIRE

Complications of contact lens wear are increasing and this questionnaire is part of a study to assess the risks associated with lens wear. Both parts of this questionnaire are confidential. In Part 1, it is very important to have the information regarding your head of household for us to be able to make comparisons within the study. Please ask the nursing staff for help if you have difficulty completing this part of the form. Part 2 deals with details of your contact lens wear.

Please answer both parts an hand this questionnaire back to the casualty nurse. Your help in this is very much appreciated.

JK Dart FRCS (Consultant Ophthalmologist) and F Stapleton (Optometrist).

Full Name..... Casualty Number.....

Address..... Date of Birth.....

..... Postcode.....

Telephone No..... Todays Date.....

Occupation.....

Part 1 - Occupation of Head of Household

If the head of your household (see note 1) is unemployed or is in receipt of a state pension, please give these details for the chief wage earner (see note 2) in the household.

If the head of your household is in receipt of a job related pension, please give these details for their previous job.

a. What is the occupation of the head of your household?.....
.....

b. What position, rank or grade does that person hold?.....
.....

c. In what industry or type of firm?.....
.....

d. Does that person have any qualifications, degree or apprenticeship?.....
.....

e. How many staff is that person responsible for?.....
.....

Note 1 The head of the household is the person who is responsible for the rent or mortgage payments. In the case of joint responsibility, this refers to the male, or the elder person if both are of the same sex.

Note 2 The chief wage earner is the closest relative of the head of the household who is working, in this order; spouse, oldest related male or oldest related female.

PTO

Part 2 - Details of Contact Lens Wear

Please ring the appropriate response for each question, where necessary.

a. For what reason do you wear contact lenses? Are you short sighted/long sighted/ keratoconic/dont know?
Have you had a cataract removed/a corneal graft/other medical condition which requires contact lenses?

b. Which type of contact lenses do you wear?
Hard/Gas permeable/Soft?

c. How long have you been wearing this type of lens?.....
.....

d. How old are your current lenses?
Right..... Left.....

e. Do you wear your lenses overnight? Yes or No.

If yes, how many days do you leave your lenses in for?.....
How many days do you leave your lenses out for?.....

If no, how many hours per day do you wear your lenses for?.....
How many days per week do you wear your lenses for?.....

f. Do you use a lens cleaning solution? Yes or No.
If yes, what is the name of the solution?.....
How many time per week do you clean your lenses?.....

g. Do you use a lens disinfection or soaking solution? Yes or No.
If yes, what is the name of the solution?.....
How many times per week do you disinfect/soak your lenses?.....

For soft lens users, do you use any other method of lens disinfection, eg heating or peroxide (10:10 or Oxysept) or chlorine (Softab or Aerotab)? If so, which do you use?.....

h. Do you use protein remover tablets with your lenses? Yes or No.
If yes, how many times per month?.....

i. Do you use a lens wetting solution? Yes or No.
If yes, what is the name of the solution?.....

j. Do you use saline? Yes or No.
If yes, is it an aerosol/preserved/single dose units/home made or other form?.....

k. Do you use any other eye drops? Yes or No.
If yes, what are they called and how often do you use them?.....
.....

l. Where did you get your contact lenses from? Please give the name and address of optician/hospital/other supplier.....
.....

m. How long ago did you last have your eyes checked by your contact lens practitioner?.....

CASUALTY PATIENT QUESTIONNAIRE

This questionnaire is part of a study to assess the various eye problems which are seen in this casualty department. It is very important to have the information regarding the occupation of your head of household, for us to be able to make comparisons within our study. This questionnaire is completely confidential.

Please ask the nursing staff if you have any difficulty in completing this questionnaire.

Please answer these questions and hand this questionnaire back to the casualty nurse. Your help in this is very much appreciated.

JK Dart FRCS (Consultant Ophthalmologist) and F Stapleton (Optometrist).

Full Name..... Casualty Number.....

Address..... Date of Birth.....

..... Postcode.....

Telephone No..... Todays Date.....

Occupation.....

Occupation of Head of Household

If the head of your household (see note 1) is unemployed or is in receipt of a state pension, please give these details for the chief wage earner (see note 2) in the household.

If the head of your household is in receipt of a job related pension, please give these details for their previous job.

a. What is the occupation of the head of your household?.....
.....

b. What position, rank or grade does that person hold?.....
.....

c. In what industry or type of firm?.....
.....

d. Does that person have any qualifications, degree or apprenticeship?.....
.....

e. How many staff is that person responsible for?.....
.....

Note 1 The head of the household is the person who is responsible for the rent or mortgage payments. In the case of joint responsibility, this refers to the male, or the elder person if both are of the same sex.

Note 2 The chief wage earner is the closest relative of the head of the household who is working, in this order; spouse, oldest related male or oldest related female.

APPENDIX 2

DIAGNOSES FOR 1 IN 100 CONTROL GROUP (Group E)

DIAGNOSIS	FREQUENCY
Abrasion	23
Foreign Body	19
Conjunctivitis	17
Chalazion, Meibomian cyst, stye	14
Viral/Adenoviral/Follicular Conjunctivitis	11
Glaucoma	7
NAD	7
Anterior Uveitis	6
Sub-conjunctival Haemorrhage	6
Sub-tarsal Foreign Body	6
Dry Eyes	5
Lens Opacities	5
Metal Foreign Body	5
Toxic/Chemical Keratopathy/Conjunctivitis	5
Blepharoconjunctivitis	4
Episcleritis	4
Herpes Simplex Keratitis	4
Meibomian Gland Dysfunction	4
Posterior Vitreous Detatchment	4
Limbal Vernal/Hay Fever Conjunctivitis	3
Photokeratitis	3
Retinal Detatchment	3
Trauma (Non-specific)/Penetrating Injury	3
Alkali/Plaster Burn	2
Blepharitis	2
Conjunctival Cyst	2
Lid Eczema/Skin Rash	2
Recurrent Erosions	2
Scleritis	2
Trichiasis	2
Vitreous Floaters	2
Adenovirus Keratitis	1
Central Retinal Vein Occlusion	1
Concretion	1
Conjunctival Abrasion	1
Dendritic Keratitis	1
Entropion	1
Epiphora	1
Filamentary Keratitis	1
V Nerve Palsy	1
Headache	1
Herpes Zoster Keratitis	1
Hypertropia	1
Hysterical Blindness	1
Lateral Rectus Palsy	1
Macular Scar	1
Marginal Keratitis	1

Ocular Hypertensive	1
Panuveitis	1
Posterior Lid Margin Disease	1
Retinal Tuft	1
Retrobulbar Neuritis	1
Sebaceous Cyst	1
Thumb Laceration	1
Trachoma	1
Traumatic Iritis	1
Upper lid papilloma	1
Vitreous Syneresis	1
Unknown Diagnosis or missing notes	26
Total	238

(Further 25 contact lens wearers not included in this breakdown)

APPENDIX 3

DATA TABLES 3.4 TO 3.27

TABLE 3.4

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR ALL EXPOSURES OF MICROBIAL KERATITIS

EXPOSURE	CASES	CONTROLS	RELATIVE RISKS	PAR%
None	5	167	1.000 (referent)	
OSD	4	18	7.422 (2.17-25.34)	3.80%
Trauma	22	53	13.864 (5.96-32.23)	22.43%
CL Wear	60	25	80.16 (38.51-166.86)	65.11%
Totals	91	263		91.34%

Chi squared test of trend = 127.62 p=<0.01

Degrees of freedom = 1

TABLE 3.5

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR ALL EXPOSURES OF CULTURE POSITIVE KERATITIS

EXPOSURE	CASES	CONTROLS	RELATIVE RISKS	PAR%
None	1	167	1.000 (referent)	
OSD	1	18	9.278 (0.90-95.83)	5.25%
Trauma	6	54	18.556 (3.81-90.33)	33.39%
CL Wear	9	26	57.808 (16.14-207.10)	52.03%
Totals	17	265		90.67%

Chi squared test of trend = 31.00 p=<0.01

Degrees of freedom = 1

TABLE 3.6

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR ALL EXPOSURES OF SEVERE CULTURE NEGATIVE KERATITIS

EXPOSURE	CASES	CONTROLS	RELATIVE RISKS	PAR%
None	1	167	1.000 (referent)	
OSD	1	18	9.278 (0.90-95.83)	17.84%
Trauma	1	55	3.036 (0.21-43.42)	13.41%
CL Wear	2	27	12.370 (1.79-85.35)	36.77%
Totals	5	267		68.02%

Chi squared test of trend = 4.18 p=<0.05

Degrees of freedom = 1

TABLE 3.7

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR ALL EXPOSURES OF MODERATE CULTURE NEGATIVE KERATITIS

EXPOSURE	CASES	CONTROLS	RELATIVE RISKS	PAR%
None	0	167	1.000 (referent)	
OSD	2	18	45.270 (7.00-292.96)	10.63%
Trauma	8	54	52.248 (9.70-281.34)	36.25%
CL Wear	11	26	145.377 (36.81-574.20)	49.66%
Totals	23	267		96.54%

Chi squared test of trend = 39.82 p=<0.01

Degrees of freedom = 1

For Miettinens test, 0.5 added to each cell for analysis, but Egret exact test confirms significant differences using unaltered data.

TABLE 3.8

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR ALL EXPOSURES OF MILD CULTURE NEGATIVE KERATITIS

EXPOSURE	CASES	CONTROLS	RELATIVE RISKS	PAR%
None	3	167	1.000 (referent)	
OSD	0	18	0.000 (0.00-1.00)	-0.67%
Trauma	7	55	7.085 (2.10-23.91)	12.53%
CL Wear	38	27	78.346 (33.95-180.79)	78.16%
Totals	48	267		90.02%

Chi squared test of trend = 92.44 p=<0.01

Degrees of freedom = 1

For Miettinens test, 0.5 added to each cell for analysis, but Egret exact test confirms significant differences using unaltered data.

TABLE 3.9

RELATIVE RISK AND POPULATION ATTRIBUTABLE RISK PERCENTAGE FOR ALL DEGREES OF KERATITIS FOR LENS WEARERS AND NON-LENS WEARERS

EXPOSURE	CASES	CONTROLS	RELATIVE RISKS	PAR%
NO LENS	31	238	1.000 (Referent)	
LENS WEAR	60	25	18.426 (10.88-31.20)	62.36%
TOTALS	91	263		

Chi squared test of trend = 117.65 p=<0.001

Degrees of freedom = 1

TABLE 3.13

THE RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR ALL CASES OF CONTACT LENS ASSOCIATED MICROBIAL KERATITIS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	2	92	1.000 (Referent)	
PMMA	2	71	1.296 (0.18-9.43)	0.76%
DWS	28	309	4.168 (1.09-16.00)	35.47%
EWS	28	35	36.800 (12.59-107.55)	45.40%
TOTALS	60	507		81.53%

Chi squared test of trend = 43.95 p<0.01

Degrees of Freedom (df) = 1

Exact test confirms significant differences

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	2	245	1.000 (Referent)	
PMMA	2	190	1.289 (0.18-9.21)	0.80%
DWS	28	951	3.607 (0.52-4.44)	33.70%
EWS	28	165	20.788 (7.26-59.56)	44.40%
TOTALS	60	1551		78.90%

Chi squared test of trend = 37.15 p<0.05

Degrees of Freedom (df) = 1

TABLE 3.14

THE RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS RELATED CULTURE POSITIVE KERATITIS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	1	92	1.000 (Referent)	
PMMA	0	71	0.000 (Not applicable)	
DWS	4	309	1.191 (0.13-10.79)	7.13%
EWS	4	35	10.514 (1.68-65.95)	40.22%
TOTALS	9	507		47.35%

Chi squared test of trend = 5.03 p<0.025

Degrees of Freedom (df) = 1

Exact test confirms significant differences.

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	1	246	1.000 (Referent)	
PMMA	0	192	0.000 (Not applicable)	
DWS	4	975	1.009 (0.11-9.08)	0.41%
EWS	4	189	5.206 (0.72-37.59)	35.91%
TOTALS	9	1602		36.32%

Chi squared test of trend = 3.33 p>0.05

Degrees of Freedom (df) = 1

TABLE 3.15

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS RELATED CULTURE POSITIVE AND SEVERE CULTURE NEGATIVE MICROBIAL KERATITIS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	1	92	1.000 (Referent)	
PMMA	0	71	0.000 (Not applicable)	
DWS	5	309	1.489 (0.17-12.76)	14.92%
EWS	5	35	13.143 (2.30-79.94)	42.00%
TOTALS	11	507		56.92%

Chi squared test of trend = 7.09 p<0.01

Degrees of Freedom (df) = 1

Exact test confirms significant differences. (nb exact test using severe culture negative cases only failed to find significant differences)

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	1	246	1.000 (Referent)	
PMMA	0	192	0.000 (Not applicable)	
DWS	5	974	1.263 (0.15-10.82)	9.46%
EWS	5	188	6.543 (1.00-42.82)	38.51%
TOTALS	11	1600		47.97%

Chi squared test of trend = 4.89 p<0.05

Degrees of Freedom (df) = 1

TABLE 3.16

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS ASSOCIATED MODERATE CULTURE NEGATIVE KERATITIS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	1	92	1.000 (Referent)	
PMMA	2	71	2.592 (0.25-26.98)	11.17%
DWS	5	309	1.489 (0.17-12.76)	14.92%
EWS	3	35	7.886 (1.10-56.75)	23.81%
TOTALS	11	507		49.90%

Chi squared test of trend = 1.68 p>0.05

Degrees of Freedom (df) = 1

Exact test failed to find significant differences.

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	1	246	1.000 (Referent)	
PMMA	2	190	2.589 (0.25-26.45)	11.16%
DWS	5	974	1.263 (0.15-10.82)	9.46%
EWS	3	190	3.884 (0.47-32.10)	20.25%
TOTALS	11	1600		41.57%

Chi squared test of trend = 0.65 p>0.05

Degrees of Freedom (df) = 1

TABLE 3.17

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS ASSOCIATED MILD CULTURE NEGATIVE KERATITIS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	0	92	1.000 (Referent)	
PMMA	0	71	1.294 (0.03-65.67)	0.28%
DWS	18	309	11.058 (1.15-104.61)	42.02%
EWS	20	35	106.831 (19.21-413.00)	50.67%
TOTALS	38	507		92.97%

Chi squared test of trend = 42.16 p<0.01

Degrees of Freedom (df) = 1

For Miettinens test, 0.5 added to each cell for analysis, but Egret exact test confirms significant differences using unaltered data.

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	0	247	1.000 (Referent)	
PMMA	0	192	1.286 (0.03-64.72)	0.28%
DWS	18	961	9.524 (0.96-94.94)	41.39%
EWS	20	173	58.487 (12.16-281.27)	50.37%
TOTALS	38	1573		92.04%

Chi squared test of trend = 32.94 p<0.01

Degrees of Freedom (df) = 1

For Miettinens test, 0.5 added to each cell for analysis, but Egret exact test confirms significant differences using unaltered data.

TABLE 3.18

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS RELATED CULTURE POSITIVE KERATITIS COMPARING RIGID LENSES WITH DAILY AND EXTENDED WEAR SOFT LENSES

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
RIGID	1	163	1.000 (Referent)	
DWS	4	309	2.110 (0.25-18.16)	23.38%
EWS	4	35	18.629 (3.60-96.51)	42.06%
TOTALS	9	507		65.44%

Chi squared test of trend = 9.05 $p<0.01$

Degrees of Freedom (df) = 1

Exact test confirms significant differences.

Rigid lens users have been combined for this analysis since the numbers of cases are small in this group and can be combined for comparison with soft lens users.

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
RIGID	1	438	1.000 (Referent)	
DWS	4	975	1.797 (0.21-15.65)	19.71%
EWS	4	189	9.270 (1.51-56.74)	39.65%
TOTALS	9	1602		59.36%

Chi squared test of trend = 5.79 $p<0.05$

Degrees of Freedom (df) = 1

TABLE 3.19

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS ASSOCIATED CULTURE POSTIVE AND SEVERE CULTURE NEGATIVE KERATITIS COMPARING RIGID LENSES WITH DAILY AND EXTENDED WEAR SOFT LENSES

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
RIGID	1	163	1.000 (Referent)	
DWS	5	309	2.638 (0.33-21.05)	28.22%
EWS	5	35	23.286 (4.94-109.69)	43.50%
TOTALS	11	507		71.72%

Chi squared test of trend = 12.12 $p<0.01$

Degrees of Freedom (df) = 1

Exact test confirms significant differences.

Rigid lens users have been combined in this analysis since numbers of cases in the rigid lens groups are small, and can be combined for comparison with soft lens users.

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
RIGID	1	438	1.000 (Referent)	
DWS	5	974	2.248 (0.28-18.24)	25.24%
EWS	5	188	11.649 (2.11-64.22)	41.55%
TOTALS	11	1600		66.79%

Chi squared test of trend = 8.00 $p<0.05$

Degrees of Freedom (df) = 1

TABLE 3.23

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGE FOR ANY COMPLICATION OCCURRING FOR EACH DIFFERENT LENS TYPE. CASES ARE ALL USERS WITH LENS RELATED DISEASE (CATEGORY D) AND CONTROLS ARE USERS WITHOUT LENS RELATED DISEASE (CATEGORY C)

LENS TYPE	CASES	CONTROLS	RELATIVE RISKS	PAR%
GP	155	92	1.000 (referent)	
PMMA	121	71	1.012 (0.68-1.50)	0.13%
DWS	670	309	1.287 (0.96-1.72)	13.53%
EWS	158	35	2.679 (1.73-4.16)	8.97%
TOTALS	1104	507		22.63%

Chi squared test of trend = 15.94 $p<0.05$

Degrees of Freedom (df) = 1

TABLE 3.24

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGE FOR LENS WEARERS WITH TOXIC OR HYPERSENSITIVITY RESPONSES FOR GROUP 1 AND GROUP 2 CONTROLS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	10	92	1.000 (Referent)	
PMMA	5	71	0.648 (0.21-1.973)	-1.13%
DWS	194	309	5.776 (3.13-10.67)	66.84%
EWS	31	35	8.149 (3.84-17.30)	11.33%
TOTALS	240	507		77.04%

Chi squared test of trend = 52.37 p<0.05

Degrees of Freedom (df) = 1

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	10	237	1.000 (referent)	
PMMA	5	187	0.634 (0.22-1.87)	-1.20%
DWS	194	785	5.857 (3.27-10.49)	67.03%
EWS	31	162	4.535 (2.28-9.04)	10.07%
TOTALS	240	1371		75.90%

Chi squared test of trend = 40.71 p<0.05

Degrees of Freedom (df) = 1

TABLE 3.25

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGE FOR LENS WEARERS WITH ABRASIONS OR POOR LENS FITTING FOR GROUP 1 AND GROUP 2 CONTROLS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	67	92	1.000 (Referent)	
PMMA	59	71	1.141 (0.72-1.822)	2.91%
DWS	110	309	0.489 (0.33-0.71)	-45.83%
EWS	15	35	0.588 (0.30-1.16)	-4.18%
TOTALS	251	507		-47.10%

Chi squared test of trend = 15.86 p<0.05

Degrees of Freedom (df) = 1

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	67	180	1.000 (referent)	
PMMA	59	133	1.192 (0.79-1.81)	3.78%
DWS	110	869	0.340 (0.24-0.47)	-85.04%
EWS	15	178	0.226 (0.13-0.40)	-20.42%
TOTALS	251	1360		-101.68%

Chi squared test of trend = 63.66 p<0.05

Degrees of Freedom (df) = 1

TABLE 3.26

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGE FOR LENS WEARERS WITH METABOLIC DISORDERS FOR GROUP 1 AND GROUP 2 CONTROLS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	33	92	1.000 (Referent)	
PMMA	29	71	1.139 (0.63-2.053)	1.48%
DWS	130	309	1.173 (0.75-1.84)	8.02%
EWS	47	35	3.744 (2.10-6.69)	14.41%
TOTALS	239	507		23.91%

Chi squared test of trend = 11.23 $p<0.05$

Degrees of Freedom (df) = 1

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	33	214	1.000 (referent)	
PMMA	29	163	1.154 (0.67-1.99)	1.62%
DWS	130	849	0.993 (0.66-1.50)	-0.39%
EWS	47	146	2.088 (1.28-3.40)	10.25%
TOTALS	239	1372		11.48%

Chi squared test of trend = 4.09 $p<0.05$

Degrees of Freedom (df) = 1

TABLE 3.27

RELATIVE RISKS AND POPULATION ATTRIBUTABLE RISK PERCENTAGES FOR LENS USERS WITH STERILE KERATITIS FOR GROUP 1 AND GROUP 2 CONTROLS

LENS TYPE	CASES	CONTROLS Group 1	RELATIVE RISKS	PAR%
GP	13	92	1.000 (referent)	
PMMA	10	71	0.997 (0.41-2.41)	-0.02%
DWS	101	309	2.313 (1.26-4.26)	39.00%
EWS	23	35	4.651 (2.19-9.86)	12.28%
TOTALS	147	507		41.26%

Chi squared test of trend = 18.66 $p<0.05$

Degrees of Freedom (df) = 1

LENS TYPE	CASES	CONTROLS Group 2	RELATIVE RISKS	PAR%
GP	13	233	1.000 (referent)	
PMMA	10	182	0.985 (0.42-2.30)	-0.11%
DWS	101	878	2.062 (1.15-3.70)	35.38%
EWS	23	170	2.425 (1.22-4.84)	9.19%
TOTALS	147	1463		44.46%

Chi squared test of trend = 10.01 $p<0.05$

Degrees of Freedom (df) = 1

APPENDIX 4

DIAGNOSES FOR CONTACT LENS WEARERS WITHOUT LENS RELATED DISEASE (GROUP C)

DIAGNOSIS	FREQUENCY
Viral/Adenoviral/Follicular Conjunctivitis	76
Chalazion/Meibomian Cyst/Stye	42
Blepharitis	35
NAD	35
Anterior/Posterior Uveitis	32
Episcleritis	18
Herpes Simplex Keratitis	12
Adenovirus Keratitis	11
Adenovirus Keratoconjunctivitis	11
Sub-conjunctival Haemorrhage	11
Trichiasis	11
Blepharoconjunctivitis	9
Meibomian Gland Dysfunction	9
Posterior Vitreous Detatchment	9
Sub-tarsal Foreign Body	9
Toxic/Chemical Keratopathy/Conjunctivitis	9
Dry Eyes	8
Viral Keroconjunctivitis	8
Allergic Conjunctivitis	7
Foreign Body (Non-lens associated)	7
Retinal Detatchment	7
Retinal/Lattice/Pigment degeneration	7
Abrasion (Non-lens associated)	6
Pingueculae	6
Conjunctival Cyst	5
Posterior Lid Margin Disease	5
Viral Keratitis	5
Amblyopia/Uncorrected Refractive Error	4
Filamentary Keratitis	4
Lagophthalmos	4
Lid Eczema/Skin Rash	4
Limbal Vernal/Hay Fever Conjunctivitis	4
Trauma (Non-specific)/Penetrating Injury	4
Phlyctenular Conjunctivitis	4
Concretion	3
Conjunctival Abrasion	3
Phlycten	3
Retinal Hole	3
Retrobulbar Neuritis/Optic Neuritis	3
Visual Migraine	3
Chorioretinitis	2
Commotio Retinae	2
Congenital Corneal Scarring	2
Headache	2
Herpes Zoster Keratitis	2

Herpes Zoster Lid Disease	2
Marginal Keratitis (Non-lens associated)	2
Metal Foreign Body	2
Keratoconus	2
Photokeratitis	2
Retention Cyst	2
Sebaceous Cyst	2
Traumatic Iritis	2
TRIC Conjunctivitis	2
Alkali/Plaster Burn	1
Anisocoria	1
Conjunctival Melanosis	1
Conjunctivitis (Non-lens associated)	1
Corneal Dystrophy	1
V Nerve Palsy	1
Foster-Fuchs Haemorrhage	1
Glaucoma	1
Herpes Simplex Lid Disease	1
Insect Sting on Lid	1
Lid Naevus	1
Malignant Melanoma	1
Myalgia (unknown cause)	1
Panuveitis	1
Pharyngeoconjunctival Fever	1
Preseptal Cellulitis	1
Pseudomembranous Conjunctivitis	1
Retinal Embolism	1
Tarsal Conjunctival Bleeding	1
Unknown Diagnosis	1
Vitreous Haemorrhage	1
Vitreous Floaters	1
Vitreous Syneresis	1
Total	507

APPENDIX 5

DIAGNOSES FOR LENS RELATED DISORDERS (GROUP D)

Metabolic Disorders

Overwear	187
Hypoxia/Oedema	32
Microcystic Epitheliopathy	11
Tight Lens Syndrome	9
Total	239

Toxic and Hypersensitivity Disorders

Toxic Keratopathy/Conjunctivitis	74
Thiomersal Keratopathy/Conj.	67
Contact Lens Related Red Eye	37
Giant/Papillary Conjunctivitis	34
Enzyme Keratopathy	12
Limbal Hypersensitivity	7
Superior Limbic Keratopathy	5
Atopic Kerato/Conjunctivitis	4
Total	245

Abrasions/Mechanical Disorders

Corneal Abrasion	167
Superficial Punctate Keratitis	38
Poor Lens Fitting	19
Corneal FB	17
Conjunctival Abrasion	10
Total	255

Sterile Keratitis

Sterile Infiltrates	147

Conjunctivitis

Presumed Bacterial Conjunctivitis	81

Tear Resurfacing Disorders

Inferior Closure Staining	8
3+9 O'Clock Staining	7
Drying/Exposure	6
Dellen	2
Total	23

Miscellaneous

Lens Intolerance/Discomfort	29
Unknown Diagnosis	12
Lost Contact Lens	8
Old Scarring	5
Follicular Conjunctivitis	3
Epithelial Thickening	2
Conjunctival Injection	1
Limbal Lesion	1
Superior Epithelial Arcuate Lesion	1
Total	51

Total Lens Related Disorders = 1044

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'Sterile' Corneal Infiltrates in Contact Lens Wearers

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Summary

Ninety four patients with 'sterile' keratitis presenting consecutively over a nine month period to the Accident and Emergency Department of Moorfields Eye Hospital were studied. This condition was found to account for 0.49% of all new casualties. A significant association was found in these patients, compared with controls, with contact lens hygiene, particularly for daily wear soft contact lenses, and contact lens case contamination by bacteria suggesting that these may be important factors in the aetiology of 'sterile' keratitis. Compared to gas permeable hard contact lenses the relative risk of developing 'sterile' keratitis in our patients was found to be 2.3 times higher with extended wear soft contact lenses, 1.56 times higher with daily wear soft contact lenses and 0.509 with polymethylmethacrylate lenses (test of trend p-value <0.05). The results indicate that 'sterile' corneal infiltrates are related to contact lens hygiene and in part to contact lens case contamination by bacteria and also to the type of lens worn.

Complications from contact lenses have increased in this country as contact lens wear has become popular, in recent studies at this hospital contact lens wearers accounted for 2.5% of all new attendances to the Casualty Department[1] and figures as high as 10% have been reported.[2]

Suppurative keratitis is the most serious complication associated with contact lens wear. The spectrum of suppurative keratitis extends from the small infiltrate with or without an epithelial defect to the large necrotic corneal abscess, with hypopyon, of fulminating microbial keratitis. Corneal infiltrates may be 'sterile' or microbial, we have used the term 'sterile' to describe non-progressive keratitis because of the uncertain pathogenesis of these lesions some of which may be sterile and others early or spontaneously resolving microbial infiltrates. The distinction between the two in the early stages is difficult on clinical grounds[3] and is of considerable importance since the latter constitute a serious and potentially sight threatening complication. A group of patients were studied who on clinical grounds had 'sterile' corneal infiltrates. We performed a prospective study to establish the number of patients affected amongst casualty attenders, clinical features of the lesions, the role of contact lens hygiene and contact lens case contamination in the pathogenesis of these lesions, and the relative risk of developing sterile keratitis for the different lens types.

Patients and Methods

All new patients with a current history of contact lens wear who attended Moorfields Eye Hospital from 21 April 1989 for a nine month period with a clinical diagnosis of 'sterile' keratitis were included in the study.

Inclusion criteria for patients with 'sterile' keratitis were discomfort rather than pain, small lesions with limited or no epithelial

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involvement and mild or no anterior chamber reaction. Exclusion criteria were painful lesions with well defined epithelial defects and anterior chamber activity. Patients who were already on treatment were also excluded.

The duration and nature of symptoms prior to attendance was recorded. Clinical features of the corneal infiltrates on slitlamp examination were documented; including site, size and number of infiltrates, area of corneal staining and degree of anterior chamber activity. Infiltrates were considered to be peripheral if any part of them was within 2 mm of the limbus (Fig. 1), others were described as central (Fig. 2). Arcuate infiltrates were defined as those peripheral lesions which extended over more than one clock hour (Fig. 3).

Patients were followed until symptoms resolved, the infiltrate was epithelialised and reduced in size. The time from presentation to resolution was noted.

Patients were treated with topical G. chloramphenicol 0.5% (preservative: phenylmercuric acetate 0.002%) or G. gentamicin 0.3% (preservative: benzalkonium chloride 0.02%) either four, six or twelve times a day with or without topical G. prednisolone 0.3% (preservative: benzalkonium chloride 0.01%) four times a day.

Assessment of hygiene

Patients completed a questionnaire which included details of the indication for contact lens wear, the type and age of the lens, the cleaning and disinfection regimes used and the pattern of lens wear. In order to evaluate the degree of lens care, each patient was given a hygiene score based on the frequency of lens cleaning and disinfection, and the frequency of use of enzyme tablets. The scores ranged from 0 (poor) to 18 (good). A maximum score of 18 for daily lens wear users was achieved with daily cleaning (7), daily disinfection (7) and weekly use of enzyme tablets (4). To allow extended wear lens users to be compared directly with other wearers, the hygiene scores were based on the level of cleaning and disinfection which occurred each time the lenses were removed. These data were compared with that of a control group of contact lens wearers presenting to the casualty department over the same period of time as the patients with sterile infiltrates, but without keratitis. Hygiene scores for patients with sterile corneal infiltrates and controls were

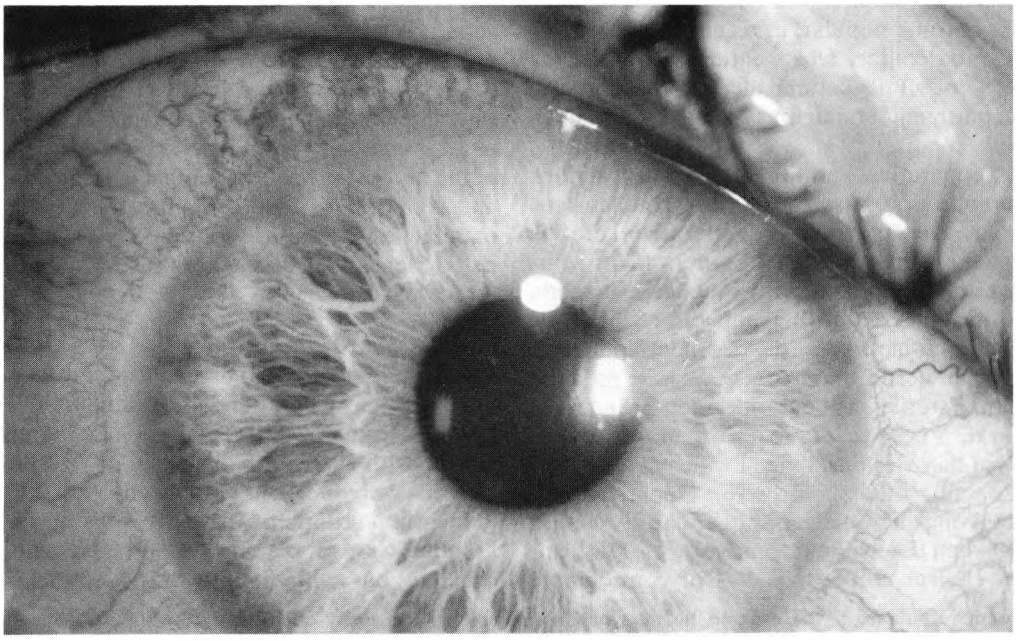

Fig. 1. *Peripheral corneal infiltrate.*

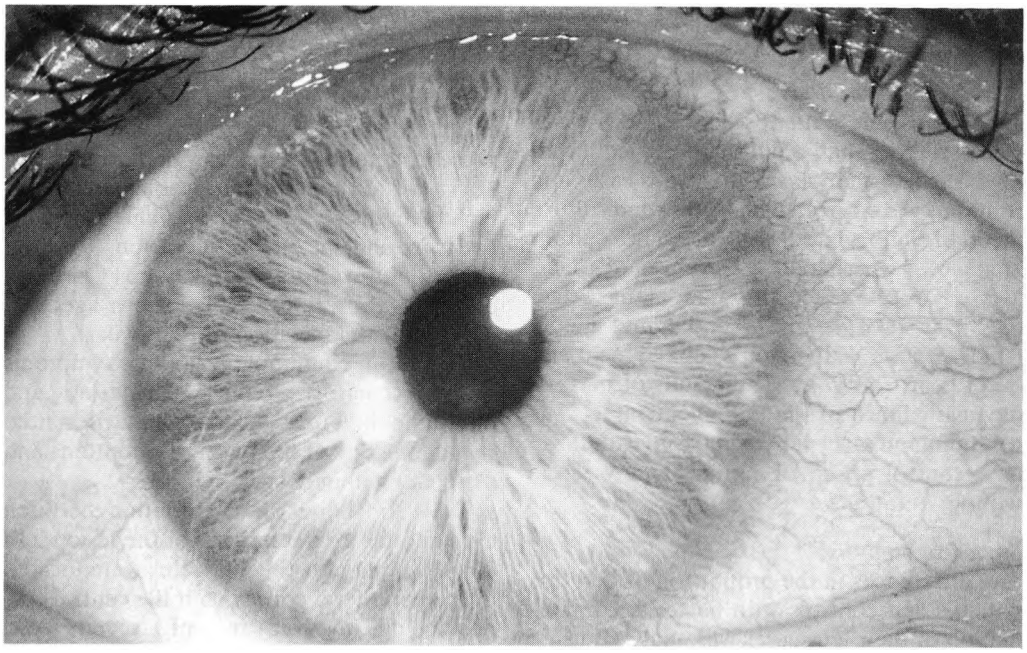

Fig. 2. *Central corneal infiltrate.*

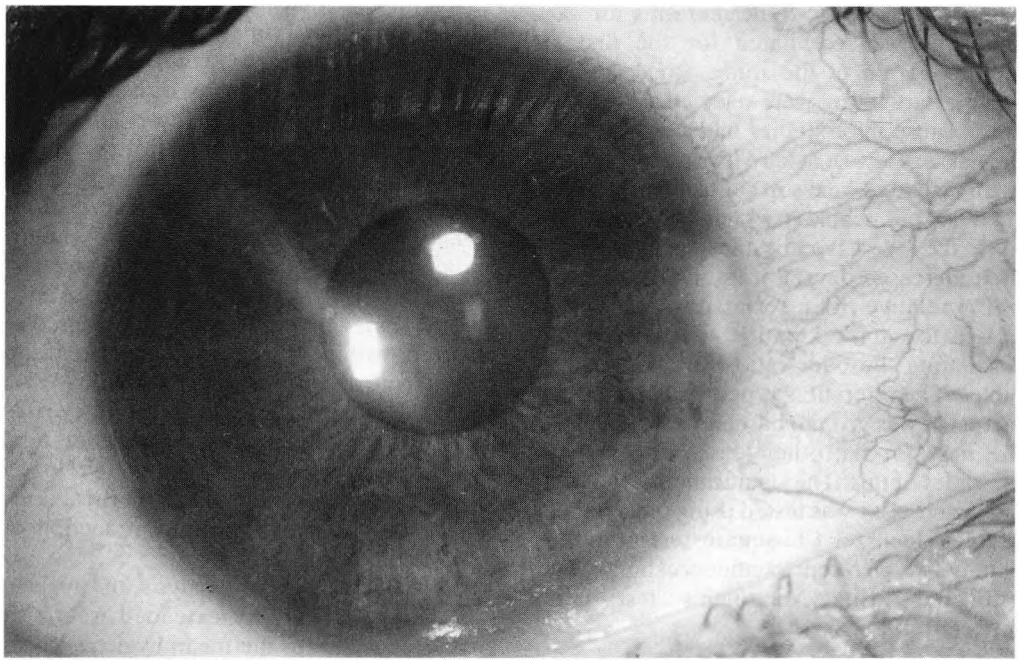

Fig. 3. *Arcuate corneal infiltrate.*

compared for each different type of contact lens.

Lens case bacteriology

Where possible patient's contact lens cases were swabbed and solutions were collected for microbial analysis. Contact lens cases and solutions were stirred with a swab and culture medium innoculated directly. The culture media used were blood agar, Saboraud's agar, McConkey bile salt agar, pseudomonas selective agar (Oxoid CFC), and liquid media (thioglycolate, Robertson's cooked meat and brain heart infusion). The results of bacteriological culture of the patient's contact lens solutions and cases were compared with those of a control group of contact lens wearers without keratitis.

Statistical analysis

The differences in the proportion of keratitis patients and controls with bacterial contamination of their lens cases was analysed using the Chi square test.

The overall differences in the hygiene scores between the different groups of wearers were analysed using a multifactor analysis of variance (multiway ANOVA). The relative risk of developing 'sterile' keratitis for each lens type was calculated for the first six months period of the study. Rigid gas permeable hard lenses were selected as the referent because pilot studies had suggested that the risk was lowest in hard lens users and highest in extended wear soft contact lens wearers. Also gas permeable hard lenses are assumed to be the safest type of hard lens because of their increased oxygen transmission.

The relative risk associated with each type of lens for sterile keratitis was estimated by calculating the odds ratio from contingency tables. The referent, gas permeable hard contact lens, was given a baseline risk of 1.0 and the risks for the other lenses calculated as multiples of this. The significance of the trend in relative risks was tested using the extended Mantel-Haenszel Chi-square test of trend.[4] Ninety-five per cent confidence limits were estimated using Miettinen's test based procedure.[5]

Results

During the period of the study 19,281 new patients attended the Casualty Department 1,125 (5.8%) of whom were contact lens wearers. Ninety-four patients had 'sterile' keratitis which accounted for 0.49% of all new casualties, 8.4% of all contact lens wearers presenting to the emergency department and 12.5% of all lens related problems. Eighty-eight patients presented on a single occasion, five twice and one on three occasions. Twenty-two patients were lost to follow-up before resolution of the lesions. Table I shows age, sex, lens type and indication for lens wear.

The most common presenting symptoms were discomfort, redness, watering and photophobia. Forty (43%) patients presented within one day of the onset of symptoms and 81 (86%) within a week.

Table II shows the distribution between eyes and the characteristics of the lesions. In 87 (92%) patients the infiltrates were located in the peripheral cornea with the central cornea being involved in only seven (8%) patients: arcuate infiltrates were seen in 13 (14%) patients. There was no predilection for any quadrant of the cornea. Fifty one (54%) patients had a solitary corneal infiltrate and 43 (46%) multiple corneal infiltrates.

An epithelial defect was present in 46 cases but was limited to a superficial punctate keratitis or a defect smaller than the lesion itself; in the remaining cases there was no breach of the epithelium. Eighty-three cases had no anterior chamber reaction and in eleven there was minimal anterior chamber activity.

Forty-eight patients were treated with antibiotics alone, 41 with a combination of antibiotics and steroids and five patients received no treatment. In the group treated with antibiotics alone 31 (65%) of the lesions had resolved within a week and in the group treated with antibiotics and steroids 25 (61%) had resolved within a week. In 79 (84%) cases complete resolution occurred without residual corneal scarring and although in 15 (16%) there was some residual corneal scarring in no case was there any reduction of visual acuity.

Table III shows an analysis of hygiene scores. Apart from the extended wear soft contact lens group, the mean hygiene scores were consistently lower in the keratitis cases compared to controls. The results of a multi-

Table I *Study patients: age, sex, lens type and indication for lens wear*

	No	(%)
Age		
Range 19–53 yrs		
Mean 28.98 yrs		
Sex		
Male	36	(38.3)
Female	58	(61.7)
Total	94	
Lens Type		
DW-SCL[a]	79	(84.0)
EW-SCL[b]	15	(20.0)
GP-HCL[c]	11	(11.7)
PMMA-HCL[d]	4	(4.3)
Indication for lens wear		
Myopia	88	(93.6)
Hypermetropia	5	(4.3)
Aphakia	1	(1.1)

[a] DW-SCL: Daily wear soft contact lens.
[b] EW-SCL: Extended wear soft contact lens.
[c] GP-HCL: Gas permeable hard contact lens.
[d] PMMA-HCL: Polymethylmethacrylate hard contact lens.

Table II *Distribution and characteristics of lesions*

	No	(%)
Side		
Right eye	43	(46)
Left eye	39	(41)
Both eyes	12	(13)
Site		
Peripheral	87	(92)
Central	7	(8)
Arcuate	13	(14)
Number		
Single	51	(54)
Multiple	43	(46)
Epithelial defect		
Present	46	(49)
Absent	48	(51)
Anterior chamber activity		
Present	11	(12)
Absent	83	(88)

factor analysis of variance are shown in Table IV and indicate that the keratitis cases had significantly lower hygiene scores compared to controls in the three lens-type groups ($F = 5.34$, $p = 0.02$). Homogenicity of this phenomenon across all three groups of lens types is indicated by the absence of a significant 2-factor interaction ($p = 0.79$), which suggests that the differences in hygiene scores between keratitis cases and controls, are independent of the three lens types. Further analysis of variance including the extended wear soft contact lens group, showed a significant association between lens type and hygiene score, manifesting mainly as lower hygiene scores in the extended wear group compared to wearers of other lens types ($F = 4.41$, $p = 0.005$).

The results of microbial cultures of contact lens cases are shown in Table V. In the study group 15 cases were obtained for culture and in the control group 43 cases. Ten (66.7%) cases in the study group were found to be contaminated by bacteria and 16 (37.2%) in the control group. These differences were significant $p = 0.048$.

The relative risk of developing sterile keratitis for the different lenses are shown in Table VI. Compared to gas permeable hard contact lenses this was highest for soft contact lenses particularly for extended wear soft contact lenses at 2.33 and lowest with polymethylmethacrylate hard contact lenses at 0.509. However, the trend of increasing risk for soft contact lenses was significant ($p<0.05$).

Discussion

Suppurative keratitis is a non-specific descriptive term for corneal infiltrates of any cause. Clinically three main types of infiltrates may be identified. (1) Small (less than 1 mm) peripheral infiltrates with no epithelial defect

Table III *Analysis of contact lens hygiene scores*

Contact Lens type	*Number of patients*	*Mean hygiene score*	*Standard error*
GP-HCL[a]			
Cases	4	9.00	1.68
Controls	25	10.64	0.70
PMMA-HCL[b]			
Cases	11	12.18	1.00
Controls	39	12.70	0.72
DW-SCL[c]			
Cases	64	10.48	0.56
Controls	82	12.10	0.47
EW-SCL[d]			
Cases	15	9.27	1.30
Controls	8	7.13	1.42
Total	248		

[a] GP-HCL: Gas permeable hard contact lens.
[b] PMMA-HCL: Polymethylmethacrylate hard contact lens.
[c] DW-SCL: Daily wear soft contact lens.
[d] EW-SCL: Extended wear soft contact lens.

Table IV *Multifactor analysis of variance—comparison of mean hygiene scores of cases and controls in GP-HCL[a], PMMA-HCL[b] and DW-SCL[c] (summary of results)*

Source	*Degrees of freedom*	*Mean sum of squares*	*F-ratio*	*P-value*
Keratitis (Case/Control)	1	95.208	5.35	0.02
Lens type	2	47.775	2.69	0.07
2-Factor interaction lens type × Keratitis	2	4.218	0.237	0.79
Residuals	219	17.781		
Totals	224			

[a] GP-HCL: Gas permeable hard contact lens.
[b] PMMA-HCL: Polymethylmethacrylate hard contact lens.
[c] DW-SCL: Daily wear soft contact lens.

Table V *Contact lens case contamination by bacteria*

	Study group No	(%)	*Controls* No	(%)	*p value* (χ^2)
Negative cultures	5		27		
Positive cultures	10	(66.7%)	16	(37.2%)	0.048 (3.90)
Non-lactose fermenting Gram negative organisms (NLF GNR)	5		12		
Serratia marcescens	3		0		
Pseudomonas aerogenosa	1		0		
Enterobacteria	1		1		
Mixed cultures	0		3*		

* 2 mixed cultures of staphylococcus, enterobacteria and micrococci.
1 mixed culture of staphylococcus aureus and NLF GNR's.

and no anterior chamber reaction, which are not associated with pain or discharge. This group is thought to be sterile (2) Larger (greater than 2 mm) infiltrates with epithelial defects and anterior chamber activity, which are associated with pain and discharge. This group is thought to be infected even though in some cases the organism cannot be cultured. (3) Between these groups is a spectrum of lesions which may or may not be infected.

Infiltrates which are central, associated with pain, discharge, epithelial staining or anterior chamber reaction suggest infection.[3] Sterile infiltrates, less commonly associated with these features, are usually smaller and may be multiple or arcuate. However, the dis-

Table VI *The relative risk of different contact lenses for "sterile" keratitis*

Type of lens	*Cases*	*Controls*	*Relative risk*	*95% confidence limits*
GP-HCL[a]	9	126	1.0 (referent)	
PMMA-HCL[b]	4	110	0.509	0.2–1.7
DW-SCL[c]	50	448	1.56	0.8–3.2
EW-SCL[d]	14	84	2.33	1.0–5.5

Extended Mantel-Haenszel Test of Trend $\chi^2 = 6.005$
P-value <0.005
[a] GP HCL: Gas permeable hard contact lens.
[b] PMMA-HCL: Polymethylmethacrylate hard contact lens.
[c] DW-SCL: Daily wear soft contact lens.
[d] EW-SCL: Extended wear soft contact lens.

tinction between sterile and infected corneal infiltrates may be difficult on clinical grounds alone and since the consequences of untreated microbial keratitis may be devastating it is prudent to treat lesions as infected if any doubt exists.

Patients included in this study had on clinical grounds a 'sterile' keratitis with minimal symptoms, little discharge, small corneal lesions with limited epithelial involvement and little or no anterior chamber activity. Ninety-four patients presented with these features and accounted for 0.49% of all new casualties. Although it has been well documented that contact lens wear is a major predisposing factor for microbial keratitis,[6-9] we are not aware of other reports showing this high incidence of 'sterile' keratitis in a contact lens wearing population.

In the majority of patients infiltrates were peripheral (92.3%) with arcuate defects accounting for 13.8% of cases. In 51% of cases there was no epithelial defect and in the remainder there was some breach of the epithelial surface. In only eleven cases was there any anterior chamber activity. There was no difference in the time to resolution of the lesions treated with antibiotics alone or with steroids and antibiotics. Due to the design of the study, however, it was not possible to determine the relative efficacy of different treatments in the resolution of the lesions. None of the patients progressed to fulminating microbial keratitis despite the potential difficulty in distinguishing between sterile and microbial keratitis in some patients. Two possible explanations of this are: firstly the aetiology of sterile infiltrates is different and secondly it is possible that some of our cases did have an early infection but that early presentation and prompt treatment with topical antibiotics prevented progression to fulminating keratitis.

The aetiology of 'sterile' keratitis is not well established although several theories have been proposed. It is likely that it is an immunologically mediated reaction to the lens material itself[10] or a toxicity reaction to the solutions used in cleaning and sterilisation.[11-13] Alternatively bacteria or toxins adherent to the surface of the lens may present an antigenic load.[14] In some cases the infiltrates may represent a hypersensitivity reaction to staphylococci.[15] Other cases may represent a spontaneously resolving infection or an early infection.

Little information is available on the importance of various risk factors relevant to these theories.[16] The relationship of lens hygiene, microbial contamination of the lens cases, lens type and the pattern of contact lens wear has been investigated in this study.

We found a statistically significant difference in hygiene scores between those patients with 'sterile' corneal infiltrates and controls. When each lens type is analysed separately the difference is most marked in the daily wear soft contact lens group. However, in the extended wear soft contact lens group there is no significant difference and we believe that this may be related to the small numbers of controls in this group. The results also show an association between lens types and hygiene scores with significantly lower hygiene scores in the extended wear group compared to wearers of other lenses. These findings suggest that this complication of contact lens wear is related to the degree of contact lens hygiene practised by patients, but because the association is relatively weak it is likely to be one of several factors. To our knowledge this is the first time that such an association has been shown. This evidence is supported by the finding of a significant difference in the microbial contamination of lens cases between the study group and controls. However, it is notable that 37.2% of controls had contaminated lenses, a finding that has also been described in asymptomatic contact lens wearers.[17,18]

The relative risks of different contact lens types for all complications of contact lens wear has been reported and showed higher risks for soft contact lens wear.[2] Using polymethylmethacrylate lenses as the referent the risk (with 95% confidence limits) for daily wear soft contact lenses was 2.0 (1.1–3.3) and for extended wear lenses 6.8 (1.8–25.6). Also several retrospective studies have shown that contact lenses are likely to be a major predisposing factor in microbial keratitis.[6-9] To our knowledge the relative risks of these different lenses for sterile keratitis has not been reported. This study shows that there was a significantly increased trend of risk associated with soft contact lens wear, being greatest for extended wear soft contact lens wear: poly-

methylmethacrylate hard contact lenses had the lowest risk. These differences may be the results of the interactions of different lens materials with the ocular surface, antigens and bacteria, different preservatives in hard and soft contact lens solutions and different patterns of wearing time.

Our results suggest that contact lens hygiene, lens case contamination and the type of lens worn are significant factors in the aetiology of 'sterile' infiltrates. Patients considering wearing contact lenses should be advised of the increased risks for complications associated with soft contact lenses and particularly extended wear contact lenses. Those presenting with corneal infiltrates should be counselled by the clinician that the risk of further episodes is related to contact lens hygiene, contact lens case contamination and the type of lens worn and that improved lens hygiene may reduce the risk of further episodes.

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