SURFACE PIGMENTS ON COSMETIC CONTACT LENSES AND IMPLICATIONS ON SAFE CONTACT LENS WEAR

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Surface pigments on cosmetic contact lenses and implications on safe contact lens wear

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Ka Yin Chan (Candidate)

Abstract

Title of thesis: **Surface pigments on cosmetic contact lenses and implications on safe contact lens wear**

Chief Supervisor: **Prof. Pauline Cho**

Background

The use of cosmetic contact lenses (CCL) has become increasingly popular especially in Asian countries such as Korea, Taiwan, Singapore and China. The public can easily purchase CCL online, at cabinet stores, flea markets, department stores, and accessories stores. CCL not prescribed and dispensed from optometric practices are just commodities to the salespersons who have no proper training in contact lens care and handling. Lack of training poses a threat to wearers who are not provided with any eye examination, aftercare services, or advice on proper lens usage and care. The quality of these CCL is also an issue as there is a lack of information on the manufacturer, the pigments used, manufacturing process, and the colour printing process. With huge demand for CCL in the market and lack of regulations of the sale of CCL, there is a need to review the safety of CCL. To date, research on CCL is scarce. This is probably due to the relatively low popularity of CCL, particularly in Caucasian countries. It was not until recent years that CCL regained attention due to increasing popularity in Asian countries and reports of microbial keratitis cases related to CCL. There was therefore a need to investigate the characteristics of CCL, particularly surface pigment CCL, and their implications on safe CCL wear.

Objectives

The objectives of this PhD study were to:

1. develop a method to determine the location and permanency of pigments on CCL
2. investigate the effect of surface pigments of CCL on microbial adherence
3. investigate cytotoxic effect of surface pigment of CCL on porcine corneal epithelial cells using the new porcine eye model (PEM)
4. investigate the effect of surface pigments of CCL on protein deposition

Methods

Experiment 1: The permanency of pigments of five brands of CCL was tested using a cotton bud rub-off test. Each lens was removed from its blister pack and placed on the cleaned surface of an electronic scale to allow monitoring of the force applied when each lens was rubbed to ensure consistency of force applied (applied force between 110 – 230g) for all lenses. Any pigment coming off the lens surface was determined by examining the tip of the cotton bud for pigment transfer after every rub. The procedures were repeated on both front and back surface.

Experiment 2: Fifteen brands of new CCL (five lenses of each brand) were challenged with *Pseudomonas aeruginosa* ATCC 9027. Three brands of lenses and their clear counterparts were also challenged with *Staphylococcus aureus* ATCC 6538 and *Serratia marcescens* ATCC 13880. Lenses were incubated in bacterial suspension immediately after they were removed from the blister packs or storage vials. After 24 hours, the lenses

were removed aseptically and rinsed gently with phosphate buffered saline to remove loosely attached micro-organisms and the viable organisms adhered to the lenses were enumerated using an automated colony counter after plating.

Experiment 3: In order to test the cytotoxic effects of CCL in an *ex vivo* model, improvements were needed to the existing porcine eye model. These modifications were required because the current model only allows two porcine eyes set up each time and there was no strict control of the surrounding temperature or humidity. A total of 57 porcine eyes were used and they were mounted on four test PEM with blinking and lacrimation simulation. The nictitating membrane of the porcine eyes was held by a movable arm connecting to a motor to simulate blinking. An infusion wing was set right above the cornea so that Dulbecco's phosphate buffered saline (DPBS) could be applied to the superior limbal region regularly to simulate lacrimation. Viability of the corneal epithelial cells was assessed with 0.4% trypan blue solutions three hours after the commencement of the experiment. Two controls were set up: control A was to assess cell viability immediately without any treatment and control B was to assess cell viability on PEM without blinking and lacrimation simulation after three hours.

Back surface pigment CCL and the clear contact lens clear counterparts were pre-soaked in different multipurpose solutions (MPS) and hydrogen peroxide system for 24 hours. The CCL were then placed on the porcine eyes on PEM with blinking and lacrimation simulation. Cell viability was assessed after three hours of experiment using Annexin V-FITC/7-AAD kit.

Experiment 4: Ten young adults aged 18-35 years old were recruited. Subjects were required to wear the contact lenses (two brands of CCL and one clear contact lenses of the same lens material) for eight to ten hours. At the end of a day's wear, the subjects returned to the clinic and the contact lenses were removed and collected for protein quantification.

Results

Experiment 1: Only one brand of CCLs was found to have no pigment coming off after repeated rubs with a wetted cotton bud. The other CCL all had pigment transferred to the cotton bud after two rubs (Range: 1-7).

Experiment 2: Surface pigment CCL showed significantly higher amounts of microbial colonization than their clear counterparts for all bacterial species tested ($p<0.028$). No significant differences in the amount of microbial adherence were observed between the sandwich design CCL lenses and their clear counterparts for all strains of micro-organisms ($p>0.402$).

Experiment 3: No significant difference was found in the number of dead cells between the four test PEMs in both central ($p=0.53$) and peripheral cornea ($p=0.19$). There were significantly more dead cells (central and periphery) in the test PEMs compared to control A ($p<0.01$) but significantly less when compared to control B ($p<0.01$).

The results showed that all MPS showed no significant difference in the percentages of healthy cells between the CCL and clear contact lenses ($p>0.05$). The number of early necrotic, late necrotic and apoptotic cells between CCL and clear contact lenses in all tested solutions were also not significantly different ($p>0.05$).

Experiment 4: The results showed no significant differences in protein deposition between sandwich CCL (Median: 583 [Range: 362 - 980]) or surface pigment CCL (Median: 600 [Range: 483 - 892]) and clear contact lens (Median: 639 [Range: 347 - 731]).

Conclusions

The rub-off test provided an indirect and simple method to determine the pigment location of CCL. Our study showed that CCL with pigments printed on the surface resulted in significantly higher bacterial adhesion. However, using the improved PEM showed that the cytotoxic effects of leachates from surface pigments CCL were not significantly different compared to those of clear contact lenses after three hours of exposure. Protein deposition on CCL, either sandwiched or surface pigments, after one day of lens wear, was also not different from those on clear contact lenses worn by the same subject.

Publications arising from the thesis

Journal articles

1. Chan K,Y, Cho P, Boost M.V. Corneal epithelial cell viability of an *ex vivo* porcine eye model. *Clinical and Experimental Optometry*. 2014; 97(3): 337-340
2. Chan K.Y, Cho P, Boost M.V. Microbial adherence to cosmetic contact lenses. *Contact Lens & Anterior Eye*. 2014; 37(4): 267-272

Conference papers

1. Cho P, Chan K.Y. Permanency of pigments on colored contact lenses – a pilot study. Poster presentation at *The 8th Asia Cornea & Contact Lens Conference*, 26-27 April 2012, Hong Kong, China (Abstract book P.35)
2. Chan K.Y, Cho P, Boost M.V. Corneal epithelial cell viability of an *ex vivo* porcine eye model. Poster presentation at *The American Academy of Optometry*, 24-27 October 2012, Phoenix, United States of America (Poster #61, Abstract book P.79)
3. Chan K.Y, Cho P, Boost M.V. Microbial adherence to cosmetic contact lenses. Paper presentation at *The 37th British Contact Lens Association Clinical Conference & Exhibition*, 6-9 June 2013, at Manchester, United Kingdom (Abstract book P.46)
4. Chan K.Y, Cho P, Boost M.V. Cytotoxicity of leachates from cosmetic contact lenses on a porcine eye model. Poster presentation at *The*

9th Asia Cornea & Contact Lens Conference 2014 at Kaohsiung, Taiwan (Poster #15; Abstract book P.64)

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List of abbreviations

AFM	Atomic Force Microscopy
BBS	Borate buffered saline
CCL	Cosmetic contact lenses
CFU	Colony forming unit
Dk	Oxygen permeability
DPBS	Dulbecco's phosphate buffered saline
EOP	Equivalent oxygen percentage
FDA	Food and Drug Administration
H_2O_2	Hydrogen peroxide
HCEC	Human corneal epithelial cell
HEMA	Hydroxyethyl methacrylate
MPS	Multipurpose solution
MTT	3-(4-,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCAC	National Consumer Affairs Centre
PBS	Phosphate buffered saline
PEM	Porcine eye model
PHMB	Polyhexamethylene biguanide
RGP	Rigid gas permeable
SEM	Scanning electron microscopy
SICS	Solution induced corneal staining
UV	Ultra-violet

Chapter 1

Background of Study

1.1 Cosmetic contact lenses

There are three types of tint in commercially-available contact lenses and they are cosmetic tint, prosthetic tint and handling tint. Cosmetic tint lenses, also known as cosmetic contact lenses (CCL), beauty contact lenses, or decorative contact lenses, are designed to beautify the wearer's appearance by enhancing the eye colour with the tint whereas prosthetic tint lenses are designed to normalize abnormal appearance due to cornea or iris deformities (Efron, 2002). Handling tint, also commonly named as visibility tint, is usually incorporated into the lens to help in lens handling, in case of lens dislocation or locating a dropped lens (Efron, 2002). Most soft lenses include handling tint for better visibility, both on-the-eye and off-the-eye.

Among the three types of tinted contact lenses, CCL are more frequently reported in the literature in terms of their comfort, vision and impact on ocular health (Chapter 1.2). However, most of these studies were performed in the 1990s and the CCL investigated have since been replaced with CCL of improved lens material and more natural colour variants and designs. However, to our knowledge, there are limited studies reporting on the more

recently introduced CCL. In the following chapters, only CCL will be discussed unless otherwise stated.

1.1.1 Types of cosmetic contact lenses

Modern CCL include coloured lenses and limbal-ring lenses (Figure 1.1) whereas in the past only coloured lenses were available. Coloured lenses can be used to change the iris colour so as to achieve an enhancement effect. Limbal-ring lenses are larger and the extra definition between the iris and the sclera makes the eye appear larger and more defined (Lorenz et al., 2014).

Many such lenses are of plano power, being used only for cosmetic or dramatic purposes rather than to improve vision (Singh et al., 2012) but the trend is changing as the demand for CCL as fashion accessories increases and many brands of CCL are now powered. CCL (non-limbal-ring) have a similar lens diameter to clear contact lenses, but the pigments are located para-centrally, leaving the central pupil area colour-free.

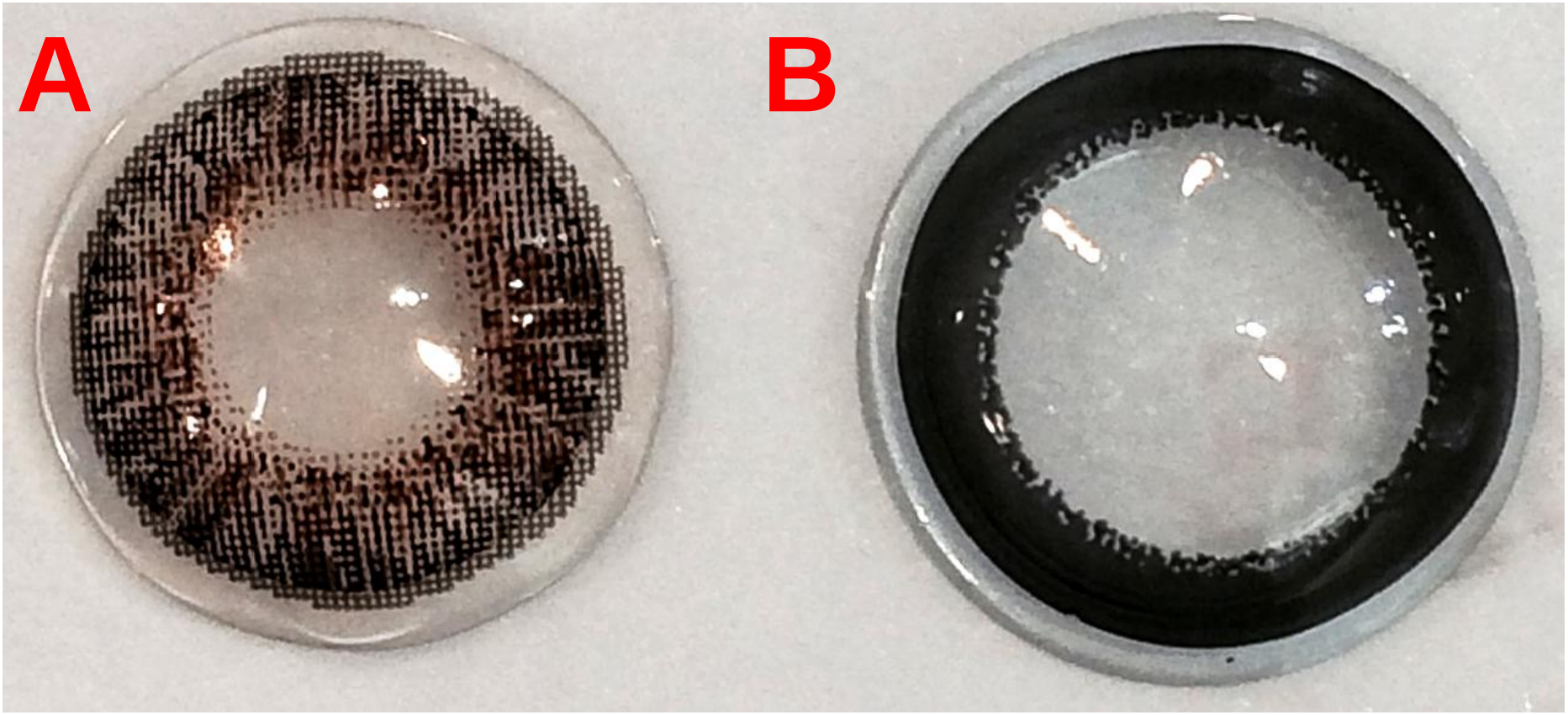


Figure 1.1 Two main types of cosmetic contact lenses (A) coloured (B) limbal-ring

1.1.2 Manufacturing process

The manufacturing process of modern CCL is similar to that of clear contact lenses except that an additional colour pigment processing step is incorporated. A number of tinting technologies are available and they are classified according to the tint, either translucent or opaque.

Translucent tints can be applied by vat dye tinting, chemical bond tinting and printing while opaque tints can be applied by dot matrix printing, opaque backing and laminate constructions (Efron, 2002). Vat dye tinting is performed by soaking a finished lens in a water-soluble dye and then exposing it to air so that the dye can be trapped within the matrix. Chemical bond tinting is similar and achieved by soaking the lens in dye solution with the presence of a catalyst to form a strong covalent chemical bond with the lens polymer (Efron, 2002). Both these methods allow a stable and uniform tint to be attained. However, current CCL usually comes with dramatic colour effects and the translucent tinting resulted from these two methods may not be able to achieve a significant iris enhancement effect.

In CCL, the colour pigment processing is surface printing, embedded pigment, or sandwiching of the pigments in the lens material. Surface printing is achieved by dot matrix printing (Knapp, 1986) or opaque backing (Efron, 2002) on the lens surface to create an iris pattern. Dot matrix printing works by creating a bonding between the dye and the

lens surface with the presence of binding polymer (Efron, 2002). It allows a combination cosmetic effect from both the colour dots and individual's natural iris colour. This method is usually applied to the front lens surface. Opaque backing is similar to dot matrix printing but the printing is on the back surface and the light will be reflected off by the opaque dye (Efron, 2002). However, the use of surface printing technology has created concern because the pigments are in contact with the eye. Potential problems, such as comfort (Section 1.2.6) and vision (Section 1.2.7), have been identified.

The laminate construction method allows the dye to be incorporated in the lens matrix and avoids direct contact of the pigments with the cornea or conjunctiva (Efron, 2002). Laminate constructions can be achieved either by sandwiching the dye between two layers of polymers so that the dyes are encapsulated (sandwich process) or by copolymerizing the dye to the polymers (embedded) (Efron, 2002) (Figure 1.2). Spaulding and Herrin (2005) improved the sandwich processing technique and patented the method to reduce lens deformation during the process. Kunzler and co-workers (2006) patented another method which involves wrapping the surface pigments with a coating layer composed of a material different from the lens-forming polymer. Laminate constructions allow the pigments to be encapsulated in the lens matrix by different methods and direct contact of colorants with the cornea or palpebral conjunctiva is avoided.

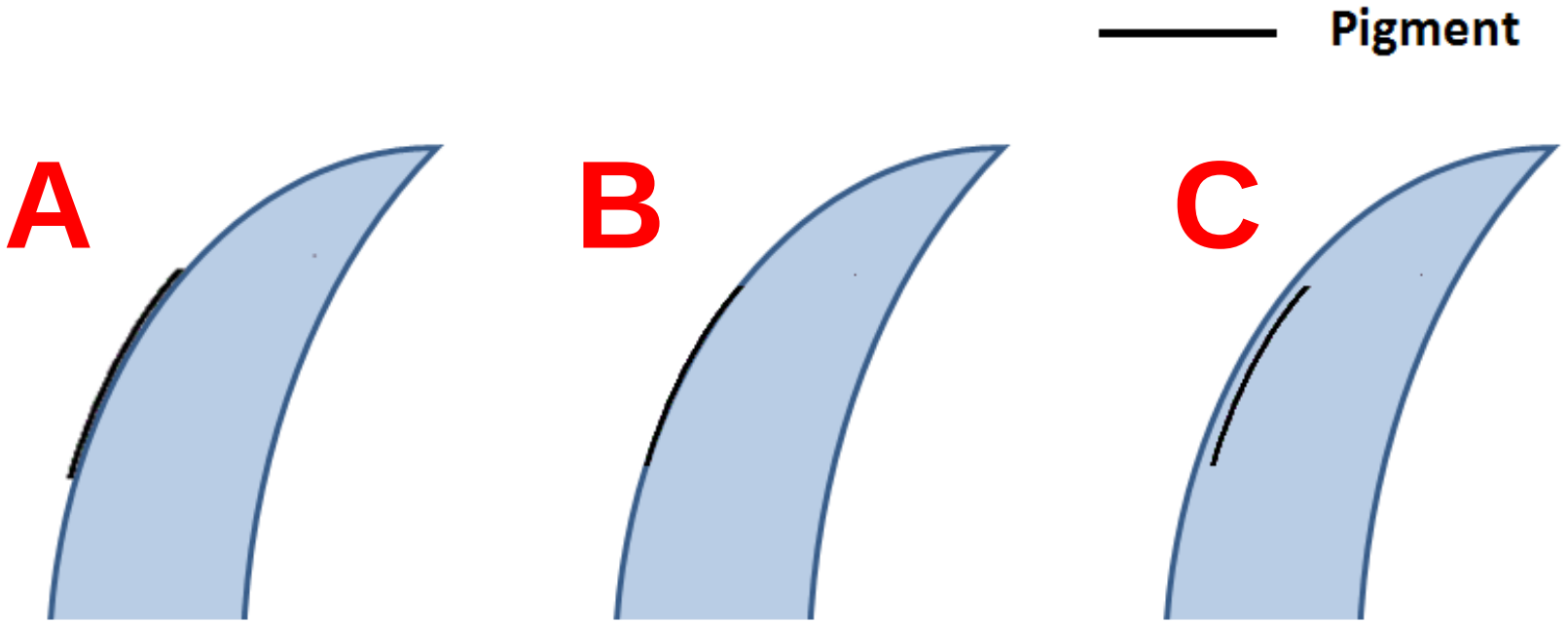


Figure 1.2 Schematic diagram showing three different colour tinting methods (A) surface printing (B) embedded (C) sandwich

1.1.3 Popularity of cosmetic contact lenses

The use of CCL has become increasingly popular especially in Asian countries such as Korea, Taiwan, Singapore and China (Morgan et al., 2012, 2013, 2014). Morgan and co-workers (2014) conducted a practitioners survey about worldwide contact lens prescribing in 2013. They reported that CCL accounted for 16% and 41% of prescribing of soft contact lenses in China and Korea respectively. In comparison with Asian countries, the prescribing trend of CCL in United Kingdom and United States remained low. Table 1.1 shows the prescribing trends of CCL worldwide from 2003-2013.

Morgan and Efron (2009) examined the data of annual contact lens surveys in United Kingdom between 1997 and 2008 and found that significantly more females were fitted with CCL. Two thirds of the CCL wearers were also fitted for part time wear (one to three times per week) and 68% of the prescribed CCL were for wearers with no prior experience of contact lens wear. The wearers of CCL were usually teenagers and adolescents (Morgan and Efron, 2009; Singh et al., 2012).

Table 1.1 Prescribing trend of cosmetic contact lenses in soft contact lens category from 2003-2013

Authors (Published year)	Year of survey	China	Korea	Taiwan	Singapore	Japan	United Kingdom	United States
Morgan *et al.* (2004)	2003	-	-	-	6%	3%	1%	4%
Morgan *et al.* (2005)	2004	-	-	-	1%	11%	1%	5%
Morgan *et al.* (2006)	2005	-	-	-	7%	2%	1%	4%
Morgan *et al.* (2008)	2007	6%	-	-	-	1%	3%	0%
Morgan *et al.* (2011)	2010	0%	15%	24%	-	1%	1%	1%
Morgan *et al.* (2012)	2011	20%	20%	60%	-	2%	0%	1%
Morgan *et al.* (2013)	2012	-	40%	56%	32%	2%	1%	1%
Morgan *et al.* (2014)	2013	16%	41%	0%	-	4%	1%	1%

1.1.4 Sale of cosmetic lenses in Hong Kong

Currently, in Hong Kong, there is no legislation controlling the sale of CCL (Hong Kong's Information Services Department, 2010). The public can easily purchase CCL online, at cabinet stores, flea markets, department stores, and accessories stores. Cabinet stores are shops with glass compartment cabinets which sell a range of different products (Figure 1.3), including beauty accessories, spectacles frames, watches, computer accessories, and CCL. CCL not prescribed and dispensed from optometric practices are just commodities to the salespersons who have no proper training in contact lens care and handling. This lack of training poses a threat to wearers who are not provided with any eye examination or aftercare services or advice on proper lens usage and care. The quality of these CCL is also an issue as there is a lack of information on the manufacturer, the pigments used and the colour printing process.

As is the case with prescription contact lenses, improper usage and care of these lenses can lead to significant complications such as microbial keratitis (Sauer et al., 2011) (Chapter 1.3).

Figure 1.3 Cabinet store selling cosmetic contact lenses over-the-counter

1.2 Concerns on cosmetic contact lenses

To date, research on CCL is limited. This is probably due to the relatively low popularity of CCL, particularly in Caucasian countries. It was not until recent years that CCL regained attention due to increased popularity in Asian countries (Section 1.1.4) and microbial keratitis cases related to CCL were reported (Chapter 1.3). The results of these CCL studies included data concerning non-compliance, oxygen permeability (Dk), comfort, ocular health, visual function, visual field and surface roughness and these are summarized in the following sections.

1.2.1 Patient compliance

One of the major concerns of practitioners with CCL is patient compliance. As discussed in Section 1.1.4, purchasing lenses without having proper eye examination and education poses a risk of complications due to the absence of proper care. In addition, sharing and overwear of CCL can also lead to an increased risk of microbial keratitis.

Steinemann and co-workers (2005) reported 12 cases of CCL related microbial keratitis in a retrospective study over an 8-week period. Seven patients admitted overnight and continuous wear, with the longest one period extending to six weeks. Three of the 12 patients admitted to sharing CCL with friends or relatives.

Singh and co-workers (2012) also reported 13 cases of CCL related microbial keratitis. Five of these patients had shared lenses with friends or relatives.

Such non-compliant behaviors could be attributable to the wearers not obtaining their lenses from optometric practices and therefore not receiving proper lens care and maintenance instructions (Steinemann et al., 2003, 2005; Singh et al., 2012).

1.2.2 Oxygen permeability

An early study performed by Benjamin and Rasmussen (1986) reported that the pigments used in CCL did not affect the oxygen performance of these lenses. Two brands of CCL (two colorants for each), Ciba Vision Care and Bausch & Lomb (brand name not mentioned), and their clear control lenses (same material as CCL) were placed on subjects' eyes (all these brands have been phased out). The designs of these two brands of CCL were not mentioned. As four lenses were used for each brand and colour, a total of 24 lenses were used. Five subjects were recruited. The subjects inserted the lenses in both eyes in pairs and the equivalent oxygen percentage (EOP) measurement was performed for static wear of each lens with a micro-polarographic electrode (25μm in diameter). The exposure time was four minutes and a drop of saline was instilled every 30 seconds to avoid lens dehydration. Ten readings were taken for each lens.

Four minutes of wash out period was allowed in between each pair. The procedures were repeated until 24 measurements with different lenses were performed. The order of lens wear was not mentioned. They reported that the oxygen uptake rate of CCL (both brands) were not significantly different from their clear control lenses. The authors concluded that wearing of CCL did not affect the oxygen transmission and that the EOP of CCL and clear lenses were the same.

Gauthier and co-workers (1992) evaluated the Dk of two new (unused) opaque tinted CCL. Wesley-Jessen (now Alcon) Durasoft 3 Colour lens (phemfilcon A) (phased out), which had a dot matrix of colour applied to the anterior surface of the lens and Cooper Vision Permaflex Mystique lens (polymacon) (phased out) with copolymerized pigment sandwiched the between the two layers of lens polymer. Durasoft 3 Colour lens was compared with a control lens of the same material and water content while Permaflex Mystique was compared with the closest matched clear lens (different material and water content) from the same manufacturer (both control lenses are no longer available in Hong Kong). The Dk of these CCL and their clear control lenses were measured using a polarographic technique. Measurements were made on the clear central zone and the periphery (pigmented region of CCL) of these lenses. Four of each brand of CCL and two of each clear control lenses were measured. No differences were found between readings taken centrally and peripherally for both CCL lens types, compared with their clear control lenses. Therefore, the authors concluded that the opaque tint in these CCL did not affect

their oxygen permeability. However, since the control (clear) lenses compared to Permaflex Mystique (made of polymacon) were made of different material, it is not known if this difference would have affected their results. The authors also did not mention how they could precisely locate the cathode (diameter not mentioned) when measuring the peripheral pigmented area (distance from central clear zone). Based on previous reports, the size of the measuring cathode is usually 4mm in diameter (Gonzalez-Meijome et al., 2008). If the area for measuring the pigment is too small, the Dk of the clear zone may also influence the Dk measurement at the periphery (pigmented area of CCL).

Bucci and co-workers (1997) compared the Dk of CLT CCL (two colorants: Blue Sapphire and Grown Cocoa) with clear control lenses of the same material (all these lenses have been phased out). The manufacturing method of these CCL, the number of lenses tested, and the measuring method were not mentioned. The Dk of the pigmented areas of the CCL were measured and were compared with the clear lenses. They reported that the Dk was not significantly different between the pigmented and non-pigmented areas. However, they did not mention whether they were comparing the peripheral pigmented area of the CCL with the peripheral area of the clear control lenses or if they were comparing the peripheral CCL area with the central area of the clear control lenses. This study provided little information on the methodology employed.

Mayers and Lorenz (2013) used the same technique as Gauthier and co-workers (1992) to compare the Dk between etafilcon A CCL (sandwich process) and its clear counterparts (same material). To ensure that the measurement was unaffected by the clear optical zone of CCL, a specially made CCL with pigments covering both the central and peripheral portions were used and only the central portion was measured. The number of lenses tested was not mentioned. They reported that the Dk of the CCL was 19.7 x 10^{-11}(cm^2/sec /mL O_2 x mmHg) whereas that of the clear control lens was 21.4 x 10^{-11}(cm^2/sec /mL O_2 x mmHg). No significant difference in Dk was found between the CCL and their clear counterparts. The authors concluded that the Dk of the tested CCL were not different from the clear control lenses. The use of a specially made CCL with pigments at both central and peripheral portion allowed more reliable results because there is no need to locate the measuring cathode on the pigmented area (usually peripheral portion of the lens), and hence, errors could be minimized.

Since there are limited publications on the impact of pigments on CCL on oxygen permeability, it cannot be assumed that all pigments approved by United States Food and Drug Administration (FDA) for CCL do not affect oxygen permeability. Also, there is a wide range of CCL of unknown sources currently on the market, as discussed earlier, from different countries and it is unclear whether the manufacturers of these products used United States FDA approved pigments as information on many of these brands of CCL is not available. It is

important to remember that Dk may be affected not only by the type of pigments, but also by the density of the pigments used on the CCL. All Dk values, if available, provided by the manufacturers are Dk of the lens materials and it is unclear if or how the pigments affect the oxygen transmissibility of the CCL.

1.2.3 Ocular health

Many studies involving the assessment of ocular integrity to evaluate the effect of CCL on ocular health have employed fluorescein staining (Gauthier et al., 1992; Fisher and Comstock, 1996; Rah et al., 2013; Mayers and Lorenz, 2013).

Gauthier and co-workers (1992) (See Section 1.2.2) evaluated ocular health by means of corneal staining after wearing CCL. Twenty two subjects, aged 19-43 years old, were recruited and asked to wear the Durasoft 3 Colour and its clear control lens (material same as CCL) on the first day for eight hours and Permaflex Mystique CCL and its clear control lens (material not the same as CCL) on another day (same wearing time for both CCL). The order of wear of the two pairs was randomly selected. A wash out period of at least one day was allowed between pairs. Corneal oedema was determined by change of thickness using Holden-Payor micro-pachometer and the presence of striae by slit lamp biomicroscopy. The Permaflex Mystique CCL was found to result in a higher level of corneal oedema (4.7±2.4% oedema and 40% incidence of striae) than Durasoft 3 Colour (1.8±2.5%

oedema and 2% incidence of striae) and its own control lens (2.7±2.2% oedema and 14% incidence of striae) after eight hours of wear. In terms of corneal staining, they found no significant difference in corneal staining between CCL (both Durasoft 3 Colour and Permaflex Mystique) and their clear control lenses after wear. They concluded that the clinical performance of these two brands of CCL on corneal physiology varies. However, the determination of corneal oedema maybe questionable as the authors reported the striae number as a percentage and did not clearly state how they determined these percentages.

Fisher and Comstock (1996) studied ocular integrity after wearing CCL. Five brands of opaque tinted CCL were investigated. They were PBH Natural Touch in baby blue colour (polymacon; 38% water content), Wesley-Jessen (now Alcon) Durasoft 2 Colour in baby blue (phemfilcon; 37% water content), Wesley-Jessen (now Ciba Vision) Durasoft 3 Colour in baby blue and in Complement (phemfilcon; 55% water content) and Ciba Illusions (tefilcon; 37.5% water content) (all these CCL have been phased out). The manufacturing methods of these CCL were not specified. Ten subjects were recruited and they all wore PBH Natural Touch in one eye (randomly assigned) and one of the other four CCL in the contralateral eye. Four day sessions were arranged and the sessions were separated by 24-48 hours. All CCL were worn for 4-6 hours on each day. The examination was performed with a slit lamp biomicroscope after application of fluorescein to the

eye. Only one of the variants (Complement) of Durasoft 3 Colour lens was significantly associated with corneal staining while the other CCL were not. All CCL showed no change in conjunctival staining. In terms of corneal swelling (determined by Holden-Payor micro-pachometer), Ciba Illusions, Natural Touch and Durasoft 2 Colour lenses resulted in 2.5%, 1.7% and 1.3% corneal thickening. However, the differences were not statistically significant. They concluded that these CCL did not differ from one another in short term wear.

Mayers and Lorenz (2013) evaluated conjunctival injection, and bulbar and limbal redness of 100 subjects after wearing sandwich design CCL (etafilcon A) for seven to nine days (days of wear and number of hours not mentioned). Ocular conditions after wearing CCL were compared with the ocular integrity after wearing their own habitual contact lenses (did not mention whether they were CCL or clear lenses). Slit lamp biomicroscope was used to evaluate the ocular health of the subjects. No significant differences between the study CCL and their own habitual lenses were observed. However, the authors did not mention whether the wearing time and pattern of the CCL and the habitual lenses were the same or not.

Rah and co-workers (2013) also investigated the ocular integrity of subjects after wearing CCL by analyzing data collected from six studies. These six studies, with duration from one week to three months of CCL wear, were carried out in different parts of Asia. Five

brands of Bausch & Lomb plano CCL (Planned replacement: Lacelle, Lacelle Colours, Naturelle and annual replacement: Alamode) were studied. The designs for each CCL were not described. The average wearing time for these lenses was 10.1±2.8 hours. Slit lamp evaluation was performed at each visit to assess the ocular integrity, including epithelial oedema, microcysts, corneal staining, bulbar and limbal injection, neovascularization, infiltrates, and tarsal abnormalities. Results from all six studies were pooled and analyzed. A total of 1742 eyes (871 subjects aged 26.8±6.6 years old) were examined during the study period. Using a scale (no name or reference given) from 0 (no finding) to 4 (severe finding), they reported that corneal staining with Grade 2 or higher was only noted in four eyes whereas other slit lamp signs were Grade 1 or lower in all eyes. The authors concluded that these CCL were safe to wear.

Recently, the National Consumer Affairs Centre (NCAC) of Japan issued a press release regarding the safety of CCL (Japan National Customer Affairs Center, 2014). Sixteen brands of CCL (modality varied from daily disposable lenses to annual replacement lenses) were evaluated after eight hours of wear, in terms of corneal oedema, corneal staining and limbal redness. These 16 brands CCL were chosen because they were the most popular brands and were approved in Japan. The centre found that 10 of these brands of CCL were surface pigment lenses although six of them claimed to be non-surface pigmented. Twenty eyes were assessed for each brand.

Among the 16 brands, 12 brands of CCL, all made of 2-Hydroxyethyl methacrylate (HEMA) with low water content (~38%), were found to result in Grade 2 or higher corneal oedema (Efron scale (Efron, 1998)). In terms of corneal staining, 12 brands CCL (including some of the brands as above) resulted in Grade 3 or higher corneal staining. One of them was associated with Grade 4 corneal staining in 30% of the eyes tested. Among these 12 brands, seven were confirmed to have the surface pigment on the back surface which would be in contact with the cornea when worn. Thirteen of the 16 brands were associated with Grade 3 or higher conjunctival staining and 10 brands with limbal redness. The center concluded that only one brand (1 Day ACUVUE® DEFINE™ by Johnson & Johnson Ltd) was free from any form of compromise in ocular integrity.

Comparison of these studies reveals that there are contradictory results reported in terms of the effect of CCL on ocular integrity. This could be due to the use of different brands of CCL because the designs, material, water content, as well as the pigments used, are different. Corneal staining can be a result of various factors, such as dry eye, abrasion, mechanical or toxic response (Efron, 2013). However, these studies did not describe the pattern of the corneal staining observed, except for Japan's NCAC study which included some sample photos of corneal staining in an arc shape at the mid peripheral cornea, suggesting that the staining was due to contact with

the pigments. Hence, the cytotoxic effect of pigments in CCL remains uncertain.

1.2.4 Surface roughness and location of pigment

Steffen and Barr (1993) compared the comfort of wearing CCL (Wesley-Jessen Durasoft 3 Colour) and its clear control lens counterpart (same material and water content). The CCL used dot matrix surface printing in manufacturing. Twenty subjects were recruited and the lenses were inserted in the right eye in random order. Lenses were worn in the dark for five minutes each and the subjects were asked to rate the comfort of the two lenses. A five-minute wash out period was allowed in between the two lenses. They found that CCL was rated less comfortable than the clear lenses and it was speculated that the roughness of the raised pigment areas or a wettability difference on the lens surface could be possible causes of lens discomfort. However, the short adaptation time was also challenged but it was believed that this had little impact on the experiment as both CCL and clear lenses had the same adaptation period.

Lorenz and co-workers (2014) studied the pigment location and surface roughness of CCL using scanning electron microscopy (SEM) to capture cross-sectional images of several brands of CCL to reveal the location of the pigment. Seven commercially available CCL (1 Day ACUVUE® DEFINE^{TM}, Naturelle by Bausch and Lomb, Freshlook

Colour by Alcon, One Day Delight MAX2 by Woods HK Ltd, CAMAX Colour daily disposable lenses by CAMAX, Eye Coffret 1 day UV by SEED and TICON Cosmetic Daily by TICON) (12 lenses for each brand) were tested. Only one brand (1 Day ACUVUE® DEFINE^{TM}) was found to have lenses with pigments sandwiched 7.6-9.1μm below the front surface. The remaining brands were found to be surface pigmented either on front (0.0-4.3μm from lens surface) or back surface (0.0-4.1μm from lens surface). The surface roughness of the pigmented area of these CCL was also tested with atomic force microscopy (AFM) and it was found that CCL with surface pigments had statistically rougher surfaces at the pigmented areas than the non-pigmented areas. CCL with sandwiched pigments were found to have consistent roughness values between the pigmented and non-pigmented areas.

The results from Lorenz's group provided important information on the relationship between pigments and surface roughness. The combined use of SEM and AFM allowed quantification of location of pigments (in terms of distance from the lens surface) and of the surface roughness.

1.2.5 Permanency of pigments

Since some of the commercially available CCL are using surface printing designs, it is important to confirm that such pigments do not come off from the lenses. To date, there is only one report studying the permanency of pigments.

Lutzi and co-workers (1985a) studied five brands of CCL and examined the spectral transmittance of the CCL before and after two weeks of cleaning procedures (by cleaner and heat disinfection or cleaner and Hydrogen peroxide systems).The five brands of CCL studied were from Ciba Vision (now Alcon), Freflex, N&N, PCL and Truflex (no details about the company or brand names of CCL were provided; all these CCL have been phased out). The designs of these CCL were not mentioned. Thirty six lenses from Ciba Vision and four lenses each for the remaining four companies were used. They found that the colour came off in CCL from N&N with both cleaning systems and in CCL from PCL and Truflex with heat disinfection only (heat disinfection was not recommended by the manufacturers but the authors studied its effect on permanency of pigments in this study). Results with CCL from Freflex were disregarded by the authors due to questionable validity of the results obtained as a striated colour pattern was used in these lenses. Only one brand of CCL (Ciba Vision; now Alcon) had no significant change in colour over two weeks of cleaning with both cleaning procedures. The results from this study suggested that the pigments were not stable in some brands of CCL. This study allowed quantitative measurement of pigment permanency. However, the limitations of this study included unknown designs of the CCL investigated and the colour stability was tested using a non-recommended heat disinfection procedures. In addition, the number of lenses tested of each brand was not the same (36 lenses from one

company and four lenses of each brand from the other three companies). The inconsistent results from Freflex indicated that the pattern of CCL may also influence the results. Lutzi and co-workers (1985a) suggested that a larger scale experiment was warranted. This is especially important in view of the huge advancement in CCL manufacturing process and the increased popularity of CCL in recent years.

The NCAC report of Japan (Japan National Customer Affairs Center, 2014) (See Section 1.2.3) also reported fading of colour in two of 16 brands of CCL tested after 10 cycles of lens care using multipurpose solution (MPS) with rubbing. Both brands of lenses were confirmed to be of surface pigment design using SEM. In their study, there were other brands of lenses which had surface pigments but they did not demonstrate colour fading after repeated disinfection procedures. This suggests that the tinting methods may also influence the permanency of pigments.

1.2.6 Comfort

There have been different opinions expressed with respect to the impact of pigments on lens comfort, CCL have been reported to be less comfortable than both clear contact lens with the same material and design (Steffen and Barr, 1993), as well as those made of the same material but by different manufacturers (Spraul et al., 1998).

Steffen and Barr (1993) (See Section 1.2.4) conducted a single blind trial on 20 subjects wearing CCL and clear lens (same material) on the same eye consecutively (five minutes interval between each lens) in a dark room. CCL was found to be less comfortable compared with the clear lens. However, Steffen and Barr (1993) commented that the difference may not be clinically significant as no subject ranked CCL as 'discomfort' in their study.

Spraul and co-workers (1998) studied the influence of a special effect CCL (a type of Halloween lenses called Crazy Lens (Bach Optic, Germany); phased out) on visual function. The pigment printing method of the CCL was not mentioned. They recruited nine subjects to wear the CCL and a clear lens (same material and power; other parameters not mentioned) consecutively. One eye of each subject was randomly chosen for the study and each lens was worn for 30 minutes. The order of lenses worn first was randomized. Subjects were asked to rate the comfort of these lenses on a scale from 1 (very good) to 10 (very bad). The Halloween lenses were found to be significantly less comfortable (mean=5.7) when compared to the clear lenses (mean=2.8). However, the authors commented that the decreased visual function (See Sections1.2.7 and 1.2.11) may have biased the results.

Daniels and co-workers (1989) investigated the effects of a dot matrix printed CCL (Wesley-Jessen D3X4 lens; phased out) on comfort and

visual field. They surveyed 68 subjects who were fitted with CCL and 72% of them rated the overall comfort of D3X4 lens as excellent or good. These subjects were also asked to compare the D3X4 lens with their habitual clear lenses. The results showed that 55% of them reported that their previous non-pigmented lenses were more comfortable whereas 45% of the subjects gave the opposite rating. Thirty-eight percent reported dryness with the CCL. However, the duration of wearing these lenses was not mentioned. Unmasked experimental design and comparison with habitual lenses were also the limitations of this study.

In Gauthier and co-workers study (1992) (See Section 1.2.3), comfort was assessed after 15 minutes and eight hours of lens wear using a visual analogue scale. No difference in overall comfort was found between the two tested CCL (Durasoft 3 Colour and Permaflex Mystique) at either time interval, compared with their control lenses.

Similar to the effects of CCL on ocular health, study reports are not in agreement. Again, this is probably due to the use of different CCL, with different water content, material and designs. Moreover, most of these studies, except for Steffen and Barr (1993), were not masked. Since most of these studies evaluated comfort in terms of subjective rating, it is unknown if the subjective evaluation was affected by wearers knowing the type of lenses (CCL or clear lens) they were wearing. To date, no long-term study has reported on CCL comfort.

1.2.7 Visual acuity

In terms of visual acuity, Gauthier and co-workers (1992) (See Section 1.2.3) compared high and low contrast visual acuities under high and low room illumination for two CCL (Durasoft 3 Colour and Permaflex Mystique) and their clear control lenses. The visual assessment was performed before and after 15 minutes and eight hours of wear. They found no significant difference in visual acuities (both luminance conditions) between CCL (both Durasoft 3 Colour and Permaflex Mystique) and clear control lenses.

Fisher and Comstock (1996) (See Section 1.2.3) compared high and low contrast visual acuities under ambient illumination. None of the tested CCL (PBH Natural, Durasoft 2 Colour, Durasoft 3 Colour in baby blue and in Complements and Ciba Illusions) showed any difference, compared with spectacles correction at baseline, in both high and low contrast visual acuities after lens wear for 4-6 hours.

Bucci and co-workers (1997) reported five cases who presented to their clinic because of blurred vision with CCL wear (CCL involved included Vantage Accents, Softmate and CSI) (all these CCL have been phased out). The vision of these patients with CCL varied from 20/25 to 20/70 whereas their visual acuity with clear lenses or spectacles was 20/20.They speculated that the drop of vision was due

to the irregular astigmatism induced by CCL wear. However, since this was a case series and various brands and types of CCL were involved, no firm conclusion can be drawn.

Spraul and co-workers (1998) (See Section 1.2.6) compared visual acuity between Crazy Lens (Bach Optic, Germany) and a clear control lens (same material and power; other parameters not mentioned) after 30 minutes wear. They found a decrease in decimal visual acuity from 1.20±0.13 (clear lenses) to 0.90±0.23 (Crazy Lens). However, it is not known if such decrease was statistically significant as the authors did not report the statistical values.

Voetz and co-workers (2004) investigated effects of three brands of CCL and a control lens (Bausch & Lomb Soflens Comfort) on vision and corneal topography. The CCL tested were Cooper Vision Crazy Lenses, Ciba Vision (now Alcon) WildEyes and Ciba Vision (now Alcon) Freshlook Colours (only Freshlook Colours are still commercially available; the other CCL have been phased out). They classified the CCL into three levels of tinting (minimal, intermediate and heavy tinting) by comparing the visible light absorbance at the pigmented areas. The manufacturing methods of these lenses were not mentioned. Seven subjects, aged 22-29 years old, were recruited and they wore two of the four brands of lenses in right and left eyes at the same time for one hour at each visit. The order of the brand of lenses to wear first was randomized. The remaining two brands of

lenses were worn on a second day. Visual acuities in photopic and scotopic conditions were measured. Freshlook Colours, WildEyes, and Crazy Lenses were classified as minimal, intermediate, and heavily tinted respectively. They found that the visual acuity with Crazy Lenses dropped significantly to 0.29 logMAR under both high and low luminance during lens wear. A drop of 0.09 logMAR acuity (photopic condition) was also found immediately after removal of this CCL but visual acuity returned to normal within two hours of lens removal. No statistically significant changes in visual acuity were found with the other two brands of CCL and clear lenses during or after lens removal. The drop in visual acuity when wearing Crazy Lenses could be explained by the change in corneal profile (See Section 1.2.8). However, the major limitation of their study is that the subjects were of different refractive power (emmetropic, myopic or myopic astigmatism; detailed demographical data not presented) but they were all wearing plano CCL (all three brands) and a -0.50D clear control lenses. Such differences may have affected the results although the authors also measured the baseline visual acuities.

In most of the studies reported (except for Bucci and co-workers(1997) which was a case series), the subjects wore CCL for only a short period of time (from 15 minutes to six hours). Contradictory results were found and it appeared that different CCL may give different results as Voetz and co-workers (2004) only found reduced vision in the heavily tinted CCL.

1.2.8 Irregular astigmatism

There have also been reports that CCL induced irregular astigmatism (Schanzer et al., 1989; Bucci et al., 1997; Voetz et al., 2004).

Schanzer and co-workers (1989) reported a case series concerning three patients who complained about blurred vision with CCL (CTL, United States, (phased out)) wear. All three patients had been wearing CCL on a daily basis for 1.5 to 3 years. Corneal topography after removal of the lenses was measured and the measurement was repeated after having discontinued CCL wear for one to three weeks. Corneal topographical irregularities were observed immediately after lenses remove, but the irregularities disappeared after the end of the lens cessation period (one to three weeks). However, the authors did not report the parameters of the CCL, such as thickness, because that may help to explain the induced astigmatism after wearing this particularly brand of CCL.

Bucci and co-workers (1997) (See Section 1.2.7) also reported five cases of blurred vision with CCL wear (CCL involved included Vantage Accents, Softmate and CSI).They evaluated the corneal topography and revealed irregular astigmatism post CCL wear. The wearing time of these subjects was not mentioned. These two reports were only case reports and not much information was provided by the authors. They speculated that the pigmented area on the CCL caused the irregularities because characteristic ring-shaped irregularities were

found at the peripheral cornea where the pigmented area of CCL rested on the cornea.

Voetz and co-workers (2004) (See Section 1.2.7) compared corneal changes after wearing three different brands of CCL. Corneal topography was measured before lens wear and immediately after lens removal. The lenses were worn for one hour and corneal recovery was measured 10, 20, 30, 60, 90, 120 and 150 minutes after lens removal. They found that wearing CCL with high pigment density led to more significant corneal changes than CCL with less pigment density. Corneal distortions were revealed on the cornea at the junction of the clear optical zone and the tinted annular zone of the CCL. The corneal changes took at least two hours to return to normal. This study revealed that corneal topography can be significantly affected even after one hour of CCL wear. However, this is not applicable to all kinds of CCL as the designs of the CCL used were not mentioned. It is unclear if the pigments on these CCL were on the surface, back or front, and whether the irregularities were due to the presence of pigments, irrespective of their location. However, unlike previous studies (Schanzer et al., 1989; Bucci et al., 1997), this prospective study provided information on the corneal changes before and after CCL removal.

1.2.9 Peripheral vision blur

Peripheral vision blur ("Hazy" vision, halos and ghosting) had been reported in CCL wearers and a significantly higher incidence of hazy vision with CCL than with clear lenses has been described in various studies (Gauthier et al., 1992; Fisher and Comstock, 1996; Albarrán Diego et al., 2001; McCarthy and Schnider, 2003; Voetz et al., 2004).

In Gauthier and co-workers study (1992) (See Section 1.2.3), 59% and 72% of the subjects wearing Durasoft 3 Colour and Permaflex Mystique CCL reported hazy vision, whereas only 27% and 14% reported hazy vision with corresponding control lenses. However, the difference in frequencies of reports of hazy vision was not significantly different between the two brands of CCL. The authors suggested that the greater incidence of hazy vision experience with Permaflex Mystique CCL could be due to the smaller clear pupil zone of this CCL (4.7mm) than another brand (5.0mm).

Fisher and Comstock (1996) (See Section 1.2.3) reported that subjects complained of hazy vision with CCL. However, the visual acuity after wearing the CCL was not different from the baseline best correction. The authors also did not give additional information on the number of subjects who complained of hazy vision and did not explain if this complaint applied to all the five brands of CCL.

Albarrán Diego and co-workers (2001) studied the effect of CCL (Bausch & Lomb Optima Colours) on visual performance. The design

of the CCL was not mentioned but seven different variants were used. Sixteen subjects, aged 18-24 years old, were recruited. Each subject was fitted with a pair of CCL (randomly selected) and was required to wear each pair for a week. A wash out period of 24 hours was allowed before another pair was tested. All the measurements, including contrast sensitivity (See Section 1.2.12), colour vision (See Section 1.2.10) and visual field (See Section 1.2.13), were conducted at baseline (no lens wear) and on the last day after wearing each pair of lenses. All subjects were also required to rate their vision with each pair of lenses. All subjects reported hazy peripheral vision with all colour variants CCL and the effect of haziness increased with reduced illumination. However, no details (e.g. Grading, statistics) about these subjective reports from subjects were provided.

McCarthy and Schnider (2003) evaluated peripheral vision of two brands of CCL (ACUVUE®2 COLOURS by Johnson and Johnson Ltd and Freshlook Colorblends by Ciba Vision, now Alcon). Designs of these two CCL were not mentioned. A hundred and five subjects were recruited and they were asked to wear these two brands of CCL for two weeks each. The order of brand of lenses worn was randomly assigned. The subjects were asked to rate peripheral haziness using a scale from zero (very bad) to 50 (very good). They found significantly better performance on peripheral vision with ACUVUE®2 COLOURS (39.6±9.4) than Freshlook Colourblends (34.0±12.0) and suggested

that this could be due to the larger clear optical zone in ACUVUE®2 COLOURS (5.4mm) than in Freshlook Colorblends (5.0mm).
Voetz and co-workers (2004) (See Section 1.2.7) also evaluated presence of haloes when using CCL by subjective rating from subjects. Seven subjects rated the presence of haloes from zero (none) to 50 (very bad) before lens wear, 30 and 60 minutes after lens wear. CCL tested were Cooper Vision Crazy Lenses, Ciba Vision (now Alcon) WildEyes and Ciba Vision (now Alcon) Freshlook Colors (only Freshlook Colors are still commercially available; the other CCL have been phased out). Significant increases in haloes were reported in subjects after wearing Crazy Lenses. WildEyes and Freshlook Colours and the control lens (Bausch & Lomb Soflens Comfort) did not show any increase in haloes. They suggested that the halo and ghosting could be a residual optical effect of pigments on CCL as Crazy Lenses have a smaller clear optical zone (4mm) compared with WildEyes (5mm) and Freshlook Colours (5.5mm).

All these studies evaluated peripheral vision based on subjects' report. None of them used quantitative measurements or a grading system. Only two studies (McCarthy and Schnider, 2003; Voetz et al., 2004) considered the small diameter of the clear pupil zone as a potential cause of the peripheral blur but the relationship was not investigated.

1.2.10 Colour vision

Few studies have investigated the effect of CCL on colour vision (Tan et al., 1987; Albarrán Diego et al., 2001).

Tan and co-workers (1987) investigated four variants (Blue, green, aqua, amber) of a front surface printed CCL (Ciba Softcolor) and compared the color vision performance with a clear lens (parameters not mentioned) using 20 subjects aged between 18 and 24 years old with normal colour vision. Lanthony New Colour Test was used and no significant differences between all CCL and clear lenses were found. Albarrán Diego and co-workers (2001) (See Section 1.2.9) also found no difference in colour vision discrimination with or without CCL. Their subjects were also aged between 18 and 24 years old and with normal colour vision and they were asked to wear seven variants of Bausch & Lomb Optima Colours in both eyes consecutively. Colour vision discrimination was assessed using Farnsworth-Munsell 100-hue test under four luminance conditions (two photopic and two mesopic conditions). No difference in colour discrimination was found at all luminance levels with or without CCL. They concluded that colour vision was not affected by wearing CCL.

1.2.11 Glare sensitivity

Only one study has investigated the effect of CCL on glare sensitivity. Lutzi and co-workers (1985b) assessed blue and amber CCL (Ciba

Softcolor) with low, medium, and high tinting intensity on glare sensitivity. Glare sensitivity was assessed using the Alpascope (company and country not mentioned). Seven subjects were enrolled and they were first assessed by wearing clear lenses in both eyes before the lenses were returned to the manufacturer for tinting into blue or amber colour (of different tinting intensities). Five subjects each wore a pair of clear/light tint and a pair of dark tint contact lenses (random pair) (total 20 lenses) and two subjects wore clear/light tint lenses only (total four lenses). In total, twenty four lenses, of three intensity (light, medium and dark) and two colours (amber and blue colours) were tested. Each subject was required to complete the nine sets of data collection on glare sensitivity. The glare sensitivity between various color and intensity CCL and clear lenses was compared. No difference in glare sensitivity was found between the CCL (both colours) with different colour intensities and their clear counterparts. Although this study allowed a quantitative measure of glare sensitivity, the authors mentioned some doubts about the repeatability of the measurements owing to the design and testing procedures of the Alpascope which did not take into account the influence of retinal adaptation, reaction time, and the colour changes in target with increasing intensity.

1.2.12 Contrast sensitivity

Several studies have evaluated contrast sensitivity with and without CCL wear. Spraul and co-workers (1998) (See Section 1.2.6)

compared the contrast sensitivity between Crazy Lens (Bach Optic, Germany) and a clear lens (same material and power; other parameters not mentioned) on visual function. The pigment printing method of the CCL was not mentioned. They recruited nine subjects to wear CCL and a clear lens consecutively. One eye of each subject was randomly chosen for the study and each lens was worn for 30 minutes. Contrast sensitivity measurement, in both photopic and scotopic conditions, was performed using MCT 8000 from Vistech Instrument (Texas, US). They found a drop in contrast sensitivity under scotopic conditions for middle spatial frequencies and an increase in contrast sensitivity under scotopic conditions for high spatial frequencies with Crazy Lens wear.

Albarrán Diego and co-workers (2001) (See Section 1.2.9) studied the effect of CCL (Bausch & Lomb Optima Colours) on visual performance. The design of the CCL was not mentioned, but seven different variants were used. Sixteen subjects were randomly fitted with seven pairs of CCL and they were required to wear each pair for a week. A wash out period of 24 hours was allowed before the next pair was tested. Comparison was made with no lens wear and after lens wear. Measurements were performed using Vistech 6000 test at different spatial frequencies. They found no difference in contrast sensitivity with or without CCL (all variants).

Özkagnici and co-workers (2003) compared the contrast sensitivity of 48 individuals, aged between 19 and 28 years old, after wearing

opaquely tinted CCL (Ciba Vision Illusions; now Alcon) and clear lenses (New Vues) for six hours. The CCL and clear lenses were of different materials, water content, and optical zone sizes. The designs of the CCL were not mentioned. Subjects were evenly divided into two groups with one group wearing CCL while another group wore clear lenses (different material and water content). The contrast sensitivity (measured using Pelli-Robson chart) was significantly lower both monocularly and binocularly in the CCL group after six hours of wear, whereas no difference was found in the clear lens group. The authors concluded that CCL were associated with reduced contrast sensitivity function. The study had a limitation in that the comparison was not on the same subjects but on two different groups of subjects. It is not known if the ocular parameters of two groups of subjects were closely matched as the authors did not report the demographical data of the subjects. The study was also limited by the use of a control lens of different material and water content from the CCL. The clear lens had a smaller optical zone size (7.8mm) than the CCL (8.55mm) and the clear zone size of the CCL was not mentioned. There was also no ocular assessment after lens wear and it is not known if tears or lens quality or ocular integrity changed after lens wear, which may contribute to a drop in contrast sensitivity.

Hiraoka and co-workers (2009) investigated the effect of CCL on contrast sensitivity. Twenty two subjects wore a sandwich design CCL (1 Day ACUVUE® COLOURS) in both eyes for at least 30 minutes

and contrast sensitivity was measured under both photopic and mesopic conditions using the CSV-1000E chart (Vector Vision Co, United States). They found a significant decrease in log contrast sensitivity (both photopic and mesopic conditions) at all spatial frequencies wearing CCL, compared with no lens wear. They also investigated the influence of the CCL on higher-order aberrations using a Hartmann-Shack wavefront analyzer (Topcon Co, Japan). They found a significant increase in coma, spherical and total higher-order aberrations with CCL wear and increase in total higher-order aberrations was found to show a significant negative correlation with contrast sensitivity function. The authors hypothesized that higher-order aberrations may be a good predictors of the photopic visual performance in CCL.

1.2.13 Visual field

Visual field restriction associated with the use of CCL has also been frequently reported (Josephson and Caffery, 1987; Insler et al., 1988; Lee et al., 1990; Trick and Egan, 1990; Albarrán Diego et al., 2001).

Josephson and Caffery (1987) measured the perimetry of ten subjects who were asked to wear CCL (Wesley-Jessen D3X4 lens) and clear control lenses (subjects' own habitual lenses) on two separate days. Half of the subjects performed the visual field assessment with their habitual lenses first while the other half with the CCL first. They found at least 10 to 45 degree peripheral restriction in the horizontal and

vertical fields, respectively in all subjects when wearing CCL, in comparison with clear lens wear (no standard deviations were reported by the authors). The authors suggested that slight decentration of the lenses may be associated with visual field loss, but no data on lens centration was reported.

Insler and co-workers (1988) also investigated the effect of a dot matrix surface printing CCL (Durasoft 3 Colour) on perimetry. Ten subjects were recruited and divided into two groups. One group had the assessment with the CCL first, followed by no lens, and the other group had the assessment with no lens first, followed by wearing CCL. The assessment was performed after wearing the lenses for an hour but the authors did not mention if there was any resting interval in between. Nine subjects demonstrated five to 10 degrees peripheral visual field loss with the CCL. The authors concluded that this small visual field constriction could be due to the small 5mm clear zone of the CCL. Unlike other studies (Josephson and Caffery, 1987; Lee et al., 1990; Trick and Egan, 1990), Goldmann perimetry, instead of automated perimetry, was used by the authors. Potential bias may be induced. Direct comparison of this study with other studies using automated perimetry may not be appropriate, but these two instruments have been reported to be comparable in detecting glaucomatous visual field defects (Trope and Britton, 1987).

In contrast, several studies reported no visual field restriction with CCL (Lee et al., 1990; Trick and Egan, 1990; Gauthier et al., 1992;

Albarrán Diego et al., 2001). Lee and co-workers (1990) investigated the visual field restriction of ten subjects (aged 20-30 years old) who wore CCL (Durasoft 3 Colour) and a clear control lens (same material and water content as CCL). Both CCL and clear lens were worn on the same eye in random order and visual field assessment, using an automated perimeter, was performed 25 minutes after each lens insertion. The authors did not mention if there was any resting interval in between lenses. They found no specific pattern of any visual field defects but a slight decrease in retinal sensitivity. The authors speculated that this may be due to glare.

Trick and Egan (1990) recruited 17subjects and one eye was selected for the assessment. Perimetry, using a Humphrey visual field analyzer, was assessed after wearing CCL (Durasoft 3 Colour) and clear lens (parameters not mentioned) alternatively. The order of testing was randomized. The authors did not mention if there was any resting interval in between. No difference in perimetry was found between CCL and clear lenses.

Gauthier and co-workers (1992) (See Section 1.2.2) assessed the perimetry with CCL (Durasoft 3 colour lens and Permaflex Mystique) and clear lenses (matched material for Durasoft 3 Colour lens but different material for Permaflex Mystique). One eye wore CCL and the other eye wore clear lenses. Assessment, using the Humphrey Autoperimeter, was performed after wearing the lenses for four hours. They also found no difference in visual field between wearing CCL (both brands) and the clear lenses.

Albarrán Diego and co-workers (2001) (See Section 1.2.9) compared the effect of CCL (Bausch & Lomb Optima Colours) on visual field with no lens wear. The design of the CCL was not mentioned, but seven different variants were used. Visual field assessment, using Goldmann perimeter, was performed after 90 minutes of lens wear. The visual field assessment without lens wear was performed on another day. They found a decrease in contrast threshold, but no effect on visual field, in eccentricity greater than 30 degrees with CCL wear. The authors hypothesized that the reduction of contrast threshold in this eccentricity may be associated with peripheral hazy vision reported by the subjects.

Similar to the findings on other visual functions and comfort of CCL, different opinions on the impact of CCL on visual field have been reported. These could be due to the use of different CCL in various studies as the design of the CCL, size of the clear optical zone and density and colour of the pigments also play an important role in the amount of visual restriction.

1.3 Cosmetic contact lens related microbial keratitis

There are many reports of infectious keratitis in the literature associated with the use of CCL (Johns and O'Day, 1988; Snyder et al., 1991; Steinemann et al., 2003, 2005; Sauer et al., 2011; Singh et al., 2012) but they are limited to case reports only. Most of these patients obtained their contact lenses

without having any proper contact lens fitting procedures, or receiving any contact lens usage and care instructions from licensed eye care professionals.

Johns and O'Day (1988) reported a case of microbial keratitis in a 14 year-old girl, who had worn CCL occasionally. The visual acuity dropped to finger counting in her right eye after wearing the CCL for two days. A large corneal infiltrate was found in the para-central cornea with reports of pain and discharge. The patient's visual acuity in the affected eye was reduced to 20/40 after treatment because of the corneal scar.

Snyder and co-workers (1991) reported five cases of microbial keratitis associated with the use of CCL. All patients were intermittent CCL users and three of five patients did not perform routine lens cleaning and disinfection properly. Each case was associated with plano CCL wear. Two patients had no loss of visual acuity after treatment, but two patients had visual acuity reduced to 20/30 and 20/50 while the final patient only retained light perception after treatment.

Steinemann and co-workers (2003) reported six cases of microbial keratitis in which all patients were new CCL wearers and obtained their lenses from unlicensed vendors. In 2005, Steinemann and co-workers reported another 12 cases of microbial keratitis associated with the use of CCL. Similar to previous reports, none of the CCLs were dispensed by eye care professionals and all patients were new CCL wearers and had no idea of proper conduct of lens care procedures. Hospitalization was required for intensive treatment in one third of the reported cases.

Singh and co-workers (2012) studied cases with symptoms of pain and redness after use of CCL at an Eye Institute between November 2009 to February 2010. Thirteen cases of microbial keratitis associated with CCL use (mean age 19±3.8) were identified. Eight cases had corneal ulcer in the visual axis and the visual acuity of these patients was 6/24 or less after treatment.

Sauer and co-workers (2011) conducted a prospective multi-centre study in 12 French University hospitals between 2007 and 2009. There were 256 patients presenting to the hospital diagnosed with microbial keratitis. Of these, 12.5% were CCL wearers. They reported that patients who had worn CCL and developed microbial keratitis were usually relatively young and new to contact lens wear. Most of them did not obtain their CCL from eye care practitioners and the relative risk of microbial keratitis in these patients was significantly higher.

1.4 Microbial adherence to contact lenses

A number of contact lens complications like microbial keratitis, contact lens-induced red eye, contact lens-induced peripheral ulcer are related to the colonization of micro-organisms on contact lenses (Holden et al., 1996; Sankaridurg et al., 1996, 1999, 2000). Contact lenses can be in contact with several other media including lens cases, care solutions, lens accessories and hands. If any of these is contaminated, contact lenses may become a vector to deliver the pathogens to the eye. Therefore, the ability of micro-

organisms to adhere to the contact lenses is an important aspect requiring attention in contact lens wear.

Numerous studies have been conducted to investigate the adherence of micro-organisms to contact lenses (Dart and Badenoch, 1986; Miller and Ahearn, 1987; Miller et al., 1988; John et al., 1989; Boles et al., 1992; Gorlin et al., 1996; Taylor et al., 1998; Williams et al., 1998; Bruinsma et al., 2002; Dang et al., 2003; George et al., 2003; Williams et al., 2003; Henriques et al., 2005; Vermeltfoort et al., 2006; Kodjikian et al., 2008; Santos et al., 2008; Choo et al., 2009; Giraldez et al., 2010; Onurdağ et al., 2011; Subbaraman et al., 2011; Babaei Omali et al., 2012; Burnham et al., 2012; Vijay et al., 2012). These studies involved hydrogel, silicone hydrogel, unworn and worn contact lenses, and lipid or protein coated lenses and investigations included bacterial, fungal and *Acanthamoeba* adherence.

Pseudomonas aeruginosa is the most commonly used bacterial strain in adherence studies. One of the reasons is because it is responsible for the majority of the microbial keratitis cases reported in contact lens wear. Generally, the adherence of *P. aeruginosa*is is greater than *Staphylococcus aureus* to unworn hydrogel and silicone hydrogel lenses (Ahanotu et al., 2001; Bruinsma et al., 2001; George et al., 2003; Bandara et al., 2004; Borazjani et al., 2004; Henriques et al., 2005; Kodjikian et al., 2008). This can be explained by the characteristics of *P. aeruginosa*. This organism has both pili and flagella on its surface which are involved in the adhesion

process (Sato and Okinaga, 1987; Hahn, 1997). It also has a greater surface hydrophobicity than *Staphylococci* (Klotz et al., 1989).

In experiments on bacterial adhesion, two methods, static and dynamic adhesion methods, have been employed (An and Friedman, 1997; Bos et al., 1999). The static method is performed by soaking the lenses in a known concentration of bacteria for a period of time. The dynamic adhesion method allows an accurate washing procedure in a flow chamber instead of manual rinsing to remove the loosely bound bacteria. The latter method allows adhesion to be performed in a more controlled environment and provides more information such as the initial adhesion rate and removal percentage (Bos et al., 1999). However, Cerca and co-workers (2004) also commented that the static method can be as effective as the dynamic method if performed accurately.

A number of factors which may influence bacterial adhesion to contact lenses have been investigated and these include lens material as well as surface hydrophobicity, water content, contact lens surface smoothness, and deposition of tears mucus, proteins and lipids. Unless specified, only the adhesion of viable *P. aeruginosa*, *S. aureus* and *Staphylococcus epidermidis* will be discussed in the following sections.

1.4.1 Lens material

Table 1.2 summarizes the studies of microbial adhesion to unworn hydrogel and silicone hydrogel lenses. Willcox and co-workers (2001)

investigated the adherence of *P. aeruginosa* (Paer1 and 6294) to new contact lenses. Both silicone hydrogel lenses (balafilcon A) and hydrogel lenses (etafilcon A, polymacon) were studied. Three lenses were used for each lens material and for each strain of bacteria. Adhesion was allowed by exposing the lenses to the bacteria for 10 minutes (static method). Unworn balafilcon A lenses resulted in significantly higher adherence of *P. aeruginosa* (both strains) than unworn etafilcon A lenses. The authors concluded that the bacterial adhesion varies with the type of lens polymer and with bacterial strains.

Kodjikian and co-workers (2008) investigated *P. aeruginosa* (clinical strain) and *S. epidermidis* (N890074 and a clinical strain) adherence to silicone hydrogel lenses (balafilcon A, lotrafilcon B and galyfilcon A) and hydrogel lenses (etafilcon A). Ninety six unworn lenses (distribution not mentioned) were used in their study and adhesion was allowed by exposing the lenses to the bacteria for four hours (static method). They found that *P. aeruginosa* adherence to silicone hydrogel lenses (all three brands) was significantly higher than to etafilcon A lenses. *S. epidermidis* (N890074) adherence to lotrafilcon B lenses was also higher than to etafilcon A lenses while *S. epidermidis* (clinical strain) adherence to balafilcon A and lotrafilcon B lenses were higher than to etafilcon A lenses.

Subbaraman and co-workers (2011) investigated the adherence of *P. aeruginosa* (Paer 6294 and 6206) and *S. aureus* (Saur 31) to three

brands of unworn silicone hydrogel (balafilicon A, senofilcon A and lotrafilcon B) and a brand of hydrogel (etafilcon A) lenses. Three lenses of each brand were used. All lenses were soaked in bacterial suspension for 24 hours (static method). They found significantly higher adhesion (both strains of *P. aeruginosa* and *S. aureus*) in unworn silicone hydrogel lenses than unworn hydrogel lenses.

These studies generally showed that adhesion of bacteria was higher in silicone hydrogel lenses, despite the different soaking time in the bacterial suspension. However, they did not look into the physio-chemical properties, such as hydrophobicity, of the lenses. Therefore, these authors only suggested that the hydrophobic nature of silicone hydrogel lenses was the cause.

Table 1.2 Summary of studies of bacterial adhesion to unworn hydrogel and silicone hydrogel lenses

Authors	Static / dynamic (incubation)	Lenses	Bacteria	Results
Willcox *et al*. (2001)	Static (10 minutes)	Hydrogel (etafilcon A) Silicone hydrogel (balafilcon A)	*P. aeruginosa* (Paer1, 6294)	• balafilcon A lenses had higher bacterial adherence (both strains) than etafilcon A
Kodjikian *et al*. (2008)	Static (4 hours)	Hydrogel (etafilcon A) Silicone hydrogel (balafilcon A, galyfilcon A,lotrafilcon B)	*P. aeruginosa* (clinical strain) *S. epidermidis* (N890074, clinical strain)	• Higher *P. aeruginosa* adhesion on silicone hydrogel lenses than etafilcon A • Higher *S. epidermidis* (N890074) adhesion in lotrafilcon B than etafilcon A • Higher *S. epidermidis* (clinical strain) adhesion in lotrafilcon B and balafilcon A than etafilcon A
Subbaraman *et al*. (2011)	Static (24 hours)	Hydrogel (etafilcon A) Silicone hydrogel (balafilcon A, lotrafilcon B, senofilcon A)	*P. aeruginosa* (Paer 6294, 6206) *S. aureus* (Saur 31)	• Higher bacterial adhesion (all strains) on the silicone hydrogel lenses than etafilcon A

1.4.2 Hydrophobicity

Since all commercially available contact lenses have to be hydrophilic to be wearable, either inherently or after treatment (e.g. in some brands of silicone hydrogel lenses), hydrophobic lenses in this thesis refer to 'relatively less hydrophilic' lenses.

A number of studies have been performed to investigate the relationship between bacterial adhesion and hydrophobicity of contact lenses (Bruinsma et al., 2001, 2002; Henriques et al., 2005; Vermeltfoort et al., 2006; Santos et al., 2007, 2008; Giraldez et al., 2010; Vijay et al., 2012). Hydrophobicity appeared to be a crucial factor accounting for differences in microbial adhesion to the lenses (Dutta et al., 2012). Table 1.3 summarizes the studies of the effect of hydrophobicity on microbial adherence.

Bruinsma and co-workers (2001) compared the adhesion of *P. aeruginosa* (clinical isolate) and *S. aureus* (799) to hydrophobic and hydrophilic ionic hydrogel contact lenses. The number of each brand of lenses used in the experiment was not mentioned. Hydrophobicity was determined by measuring the contact angle of unworn lenses. Sessile drop advancing technique (the liquid used was not mentioned) was employed to measure the water contact angle on the convex side of the lenses at room temperature. Adhesion was allowed by circulating the bacterial suspension for two hours in the flow chamber (dynamic method). They found no significant difference in adherence

of *P. aeruginosa* (clinical isolate) and *S. aureus* (799) between two brands of lenses. However, the authors found a significantly higher initial adhesion rate of cell surface damaged *P. aeruginosa* (clinical isolate), which became less hydrophobic on the cell surface, to the hydrophilic lenses (1142cm^2/second) than to the relative hydrophobic contact lenses (80cm^2/second). However, the authors only mentioned that they were comparing lenses of FDA group three and group four lenses (both ionic in nature) and did not mention whether they were of the same lens material. The lenses were also of different water content (58% and 36%). The effect of the proportion of water content on adhesion is not known.

Henriques and co-workers (2005) investigated the adherence of *P. aeruginosa* (ATCC10145) and *S. epidermidis* (ATCC 12228 and 9142) to unworn silicone hydrogel lenses (balafilcon A, lotrafilcon A and galyfilcon A) and hydrogel lenses (etafilcon A), as well as the hydrophobicity of these lenses. The number of lenses used was not mentioned. Hydrophobicity was determined by measuring the contact angle of the convex side of the unworn lenses using video-based optical contact angle measuring system OCA 20 (Data-physics, Germany). The liquid used for measurement was not mentioned. Lenses were soaked in bacterial suspension for two hours. The water contact angle of these lenses showed that balafilcon A and lotrafilcon A lenses were relatively more hydrophobic than galyfilcon A and etafilcon A lenses. Both balafilcon A and lotrafilcon A lenses were

found to have higher adherence of *P. aeruginosa* and *S. epidermidis* (9142) than the other two brands. The authors suggested that silicone hydrogel lenses which have relatively more hydrophobic surface were more prone to bacterial adherence and that genetic differences between *S. epidermidis* strains (12228 and 9142) may also contribute to adherence ability.

Giraldez and co-workers (2010) assessed the effect of hydrophobicity on *S. epidermidis* (CECT 4184) adhesion. They studied two unworn silicone hydrogel lenses (senofilcon A and comfilcon A) and three brands of unworn hydrogel lenses (omafilcon A, ocufilcon B and nelfilcon A). The number of lenses for each brand was not mentioned. Hydrophobicity was determined by measuring the contact angle of unworn lenses using the same method employed by Henriques and co-workers (2005). Lenses were incubated in the bacterial suspension for two hours. Both brands of silicone hydrogel lenses were found to be more hydrophobic and displayed higher adhesion of *S. epidermidis* than the hydrogel lenses. The authors concluded that hydrophobic contact lenses were more prone to *S. epidermidis* adhesion.

However, some studies reported conflicting findings. Vemeltfoort and co-workers (2006) compared *P. aeruginosa* (clinical strain) and *S. aureus* (835) adhesion in two brands of unworn silicone hydrogel lenses (balafilcon A and lotrafilcon A), using three pairs of lenses of each brand. Hydrophobicity was determined by measuring the contact

angle of the concave side of the contact lenses using sessile drop advancing technique in an enclosed chamber with 100% humidity. The liquid used for measurement was not mentioned. Adhesion was allowed by circulating the bacterial suspension for two hours in the flow chamber. Lotrafilcon A lenses were found to be relatively more hydrophilic while balafilcon A lenses relatively more hydrophobic. *P. aeruginosa* (clinical strain) adhesion was not significantly different between the two brands but a greater initial rate of *S. aureus* (835) adhesion was found in lotrafilcon A lenses than balafilcon A lenses. Vijay and co-workers (2012) also investigated *P. aeruginosa* (6294, 6206 and GSU-3) and *S. aureus* (31, 38 and 6538) adhesion to unworn silicone hydrogel lenses, using three lenses of each of 10 commercially available brands of lenses. The hydrophobicity values of the lenses were historical data from several studies using different methods to measure the water contact angle, including captive bubble receding, captive bubble advancing, sessile drop advancing, and Wilhelmy balance. Lenses were incubated in bacterial suspension for 18 hours and the correlation of hydrophobicity and bacterial adhesion determined. These results were contradictory to other studies (Bruinsma et al., 2001; Henriques et al., 2005; Giraldez et al., 2010). However, the hydrophobicity data of studies were determined using different methods. Each method yielded different results on hydrophobicity and these variations may affect the results of correlation.

Santos and co-workers (2008) also compared the adhesion of *S. epidermidis* (9142) to four brands of unworn silicone hydrogel lenses (balafilcon A, lotrafilcon A, lotrafilcon B and galyfilcon A) and one brand of hydrogel lens (etafilcon A), using seven lenses of each brand. Hydrophobicity was determined by measuring the contact angle of unworn lenses using the same method employed by Henriques and co-workers (2005). Adhesion was allowed by circulating the bacterial suspension for two hours in the flow chamber. Etafilcon A lenses was the only brand found to be hydrophilic. In terms of *S. epidermidis* (9142) adherence, the authors found no significant difference among the five brands of unworn contact lenses, although balafilcon A and galyficon A lenses were found to be more hydrophobic than the other brands.

The above *in vitro* studies generally suggested that exposure of silicone hydrogel lenses resulted in higher bacterial adherence and that hydrophobicity is a contributing factor accounting for this higher adherence. *In vivo* studies have also given similar results.

In Santos and co-workers (2008) experiment on worn contact lenses, 31 subjects wore silicone hydrogel lenses in one eye and hydrogel lenses in the other eye. They were divided into four groups and each group wore a specific brand of silicone hydrogel lenses and hydrogel lenses. All silicone hydrogel lenses were worn for 30 days (including galyfilcon A lenses which are recommended by manufacturer to only

be worn for 14 days) and the hydrogel lenses were worn for 15 days. After each wearing period, the worn lenses were collected for analysis and the procedures were repeated for the remaining five brands of silicone hydrogel lenses. There was no information on the inclusion of a wash out period. The study period lasted for six months during which six silicone hydrogel lenses of each brand and 12 hydrogel lenses were collected from each subject. Hydrophobicity was determined by measuring the contact angle of unworn lenses using the same method employed by Henriques and co-workers (2005). Adherence to worn lenses was evaluated by circulating a known bacterial concentration on worn and unworn lenses for two hours and the effect of wear on adhesion was compared. It was found that hydrophobicity changes with lens wear and the pattern of change varied between silicone hydrogel and hydrogel lenses. In general, worn etafilcon A lenses became significantly more hydrophobic, compared to unworn lenses while worn silicone hydrogel lenses become less hydrophobic compared to unworn counterparts. Changes in hydrophobicity were more significant in galyfilcon A and balafilcon A lenses. However, the difference in days of wear between silicone hydrogel lenses and hydrogel lenses may also affect the adherence results but the authors did not attempt to explain the reason.

Bruinsma and co-workers (2002) compared unworn etafilcon A lenses with etafilcon A lenses worn for 10 and 50 days. Ten subjects were recruited and required to wear lenses on both eyes for 10 and 50 days

(10 hours per day). The worn lenses were collected and adhesion was allowed by circulating the bacterial suspension for two hours in the flow chamber. The lenses become more hydrophilic after 50 days of wear, but the initial adhesion rate was lower in lenses after wearing for 50 days, in comparison with unworn lenses. The authors suggested that changes in lens surface physio-chemical properties may have affected the adhesion of *P. aeruginosa* (clinical strain).

Santos and co-workers (2007) compared microbial adhesion to four types of silicone hydrogen lenses (balafilcon A, lotrafilcon A, lotrafilcon B and galyfilcon A) and to hydrogel lenses (etafilcon A). Thirty one subjects were recruited. Each type of silicone hydrogel lenses was worn by seven or eight subjects while the hydrogel lenses were worn by all 31 subjects. All silicone hydrogel lenses were worn for 30 days (including galyfilcon A which should only be worn for 14 days as recommended by manufacturer) and the hydrogel lenses were worn for 15 days, the lenses being collected for analysis after each wearing period. The study lasted for six months during which six silicone hydrogel lenses for each brand and 12 hydrogel lenses were collected from each subject. Hydrophobicity was determined by measuring the contact angle of unworn lenses using OCA 20 (DataPhysics, Germany) at room temperature and three standard liquids of different polarities (Millipore water, formamide and 1-bromonaphtalene). Hydrophobicity, in the form of surface tension, was calculated using a mathematical equation. The collected lenses were analyzed for microbial adherence

by enumerating the viable bacteria on the lens. Amongst these lenses, only etafilcon A lenses were found to be hydrophilic while all the silicone hydrogel lenses were hydrophobic. Microbial adherence was found to be highest in balafilcon A lenses, followed by etafilcon A, lotrafilcon B, galyfilcon A and lotrafilcon A lenses, despite etafilcon A lenses being found to absorb the greatest amount of proteins. The authors suggested that the higher microbial adherence in balafilcon A lenses was due to its superior hydrophobicity. However, the study only presented general bacterial adherence to the lenses not for specific types of bacteria. The hydrophobicity was also measured on new lenses instead of the worn lenses. Their results agreed with the later study of this group (Santos et al., 2008), on hydrophobicity changes with lens wear. This *in vivo* study design may introduce other factors, such as protein deposition and interaction of MPS, which may affect the hydrophobicity and, in turn, bacterial adherence.

Table 1.3 Summary of studies on bacterial adhesion in terms of hydrophobicity

Authors	Static / dynamic (incubation)	Unworn / worn	Lenses		Contact Angle (Mean±standard deviation or just mean or just range) * / [surface tension] (Mean)	Bacteria	Results
Bruinsma *et al*. (2001)	Dynamic (2 hours)	Unworn	Hydrophobic** hydrogel (36% water content)		106°	*P. aeruginosa* (clinical isolate) Cell surface damaged *P. aeruginosa* (clinical isolate) *S. aureus* (799)	• No difference in *P. aeruginosa* and *S. aureus* strains between more hydrophobic and hydrophilic contact lenses • *P. aeruginosa* became less hydrophobic after cell surface damaged • Higher adhesion of cell surface damaged *P. aeruginosa* to more hydrophilic contact lenses
			Hydrophilic hydrogel (58% water content)		57°		
Bruinsma *et al*. (2002)	Dynamic (2 hours)	Unworn	Hydrogel	etafilcon A	45° ± 10°	*P. aeruginosa* (clinical strain)	• Lenses became more hydrophobic after 10 days of wear, and became more hydrophilic after 50 days of wear • Initial adhesion rate decreased after 10 days of wear and dropped even lower after 50 days of wear
		Worn (10 days)			61° ±25°		
		Worn (50 days)			27° ±14°		
Henriques *et al*. (2005)	Static (2 hours)	Unworn	Hydrogel	etafilcon A	20° - 30°	*P. aeruginosa* (ATCC 10145) *S. epidermidis* (ATCC 12228, 9142)	• balafilcon A and lotrafilcon A lenses were more hydrophobic while etafilcon A and galyfilcon A lenses was more hydrophilic • Hydrophobic surface (>50°) were more prone to *P. aeruginosa* and *S. epidermidis* (9142) adhesion
			Silicone hydrogel	ealafilcon A	70° - 80°		
				galyfilcon A	30° - 40 °		
				lotrafilcon A	50° - 60°		

* Different measuring methods involved

** Relatively less hydrophobic lenses

Table 1.3 Summary of studies on bacterial adhesion in terms of hydrophobicity (Con't)

Authors	Static / dynamic (incubation)	Unworn / worn	Lenses		Contact Angle (Mean±standard deviation or just mean or just range) * / [surface tension] (Mean)	Bacteria	Results
Vermeltfoort *et al*. (2006)	Dynamic	Unworn	Silicone hydrogel	balafilcon A	$59^o \pm 17^o$	*P. aeruginosa* (clinical strain) *S. aureus* (835)	• lotrafilcon A lenses was more hydrophilic while balafilcon A lenses was more hydrophobic • *P. aeruginosa* (clinical strain) were not significantly different in both types of unworn lenses • Greater initial adhesion rate of *S. aureus* (835) to unworn lotrafilcon A lenses than to balafilcon A lenses
					$20^o \pm 6^o$		
					$20^o \pm 5^o$		
			Silicone hydrogel	lotrafilcon A	$34^o \pm 3^o$		
					$21^o \pm 7^o$		
					$20^o \pm 5^o$		
Santos *et al*. (2007)	N/A (*ex vivo*)	Worn	Hydrogel	etafilcon A	[+23.14]	Non-specific	• Silicone hydrogel lenses were more hydrophobic while etafilcon A lenses were more hydrophilic • Highest bacterial adhesion to balafilcon A lenses was found because of the higher hydrophobicity • Greatest amount of protein adhered to etafilcon A lenses was found
			Silicone hydrogel	balafilcon A	[-39.40]		
				galyfilcon A	[-36.17]		
				lotrafilcon A	[-27.10]		
				lotrafilcon B	[-34.24]		

* Different measuring methods involved

** Relatively less hydrophobic lenses

Table 1.3 Summary of studies on bacterial adhesion in terms of hydrophobicity (Con't)

Authors	Static / dynamic (incubation)	Unworn / worn	Lenses		Contact Angle (Mean±standard deviation or just mean or just range) * / [surface tension] (Mean)	Bacteria	Results
Santos *et al*. (2008)	Dynamic (2 hours)	Unworn	Hydrogel	etafilcon A	40^o - 50^o	*S. epidermidis* (9142)	• Unworn silicone hydrogel lenses were more hydrophobic than unworn etafilcon A lenses • Amount of bacterial adhesion was not different among all lenses
			Silicone hydrogel	balafilcon A	100^o - 110^o		
				galyfilcon A	90^o - 100^o		
				lotrafilcon A	50^o - 60^o		
				lotrafilcon B	60^o - 70^o		
		Worn	Hydrogel	etafilcon A	80^o - 90^o		• Silicone hydrogel lenses became less hydrophobic after worn • etafilcon A lenses became more hydrophobic after worn • Worn silicone hydrogel lenses were less prone to adhesion than worn etafilcon A lenses
			Silicone hydrogel	balafilcon A	80^o - 90^o		
				galyfilcon A	70^o- 80^o		
				lotrafilcon A	50^o- 60^o		
				lotrafilcon B	50^o - 60^o		
Giraldez *et al*. (2010)	Static (2 hours)	Unworn	Hydrogel	nelfilcon A	$34.49^o \pm 3.31^o$	*S. epidermidis* (CECT 4184)	• Silicone hydrogel lenses were significantly more hydrophobic than hydrogel lenses • Higher amount of adhesion were found in both brands of silicone hydrogel lenses, compared with hydrogel lenses
				ocufilcon B	$34.97^o \pm 6.38^o$		
				omafilcon A	$35.30^o \pm 4.44^o$		
			Silicone hydrogel	comfilcon A	$48.38^o \pm 7.07^o$		
				senofilcon A	$55.15^o \pm 6.87^o$		

* Different measuring methods involved
** Relatively less hydrophobic lenses

Table 1.3 Summary of studies on bacterial adhesion in terms of hydrophobicity (Con't)

Authors	Static / dynamic (incubation)	Unworn / worn	Lenses		Contact Angle (Mean±standard deviation or just mean or just range) * / [surface tension] (Mean)	Bacteria	Results
Vijay *et al*. (2012)	Static (18 hours)	Unworn	Silicone hydrogel	asmofilcon A	20^{o} - 94^{o}	*P. aeruginosa* (6294, 6206, GSU-3) *S. aureus* (031, 038, ATCC 6538)	• More hydrophobic surface attracted less adhesion • Moderate to strong correlations between *P. aeruginosa* or *S. aureus* adhesion and surface hydrophobicity of the lenses
				balafilcon A	23^{o} - 95^{o}		
				comfilcon A	19^{o} - 58^{o}		
				enfilcon A	20^{o} - 68^{o}		
				filcon II 3	18^{o} - 42^{o}		
				galyfilcon A	22^{o} - 111^{o}		
				lotrafilcon A	49^{o}		
				lotrafilcon B	20^{o} - 41^{o}		
				narafilcon A	22^{o} - 37^{o}		
				senofilcon A	25^{o} - 92^{o}		

* Different measuring methods involved
** Relatively less hydrophobic lenses

1.4.3 Water content and ionicity

Several studies have investigated the effect of water content and ionic charge on microbial adhesion (Miller and Ahearn, 1987; Lawin-Brussel et al., 1991; Cook et al., 1993; Arciola et al., 1995; Dang et al., 2003), most studies revealing an inverse relationship between water content and adhesion (Miller and Ahearn, 1987; Cook et al., 1993; Garcia-Saenz et al., 2002). Table 1.4 summarizes the studies of the effect of water content and ionicity on microbial adherence.

Miller and Ahearn (1987) investigated adherence of *P. aeruginosa* (20 clinical isolates) to different FDA group contact lenses. Twelve types of lenses (three from each FDA group) were studied. The number of lenses used for each strain varied from three to five (distribution) not specified. Lenses were soaked in bacterial suspension for 72 hours. Higher levels of adherence of non-viable *P. aeruginosa* on non-ionic hydrogel lenses (both high and low water content) were found when compared to ionic hydrogel lenses. Decreased amounts of *P. aeruginosa* (20 clinical isolates) adherence with higher water content lenses were also reported. However, the authors stated that no correlation between adherence and the percentage of water content was found. Comparison was also made in terms of the ionicity of the lenses, and despite water content levels, non-ionic lenses showed higher adhesion rates than ionic lenses.

Cook and co-workers (1993) investigated the adhesion of *P. aeruginosa* (unknown strain) to 10 hydrogel lenses (HEMA), either protein-coated or uncoated with different water contents. The number of lenses for each type was not mentioned. Adhesion was estimated by the dynamic method for two hours. They found a decreased adhesion rate as water content increased, whether the lenses were protein-coated or not. However, they did not mention the ionicity of the lenses therefore it is not known whether ionicity affected the results.

Arciola and co-workers (1995) investigated *S. aureus* (unknown strain) adhesion to two types of hydrogel (ionic/high water content lenses and non-ionic/low water content lenses). New unworn lenses were soaked in a bacterial suspension for 24 hours and the total bacterial adhesion enumerated. The experiment was carried out 22 times. Higher numbers of *S. aureus* were found to adhere to the ionic/high water content than to non-ionic/low water content lenses.

However, contradictory results had been reported by Lawin-Brussel and co-workers (1991) who investigated *P. aeruginosa* (clinical strain) adhesion to seven brands of unworn hydrogel lenses (FDA Groups I, II and IV). Using three sets of lenses for each lens type, they found a higher total number of *P. aeruginosa* attaching to non-ionic/high water content than non-ionic/low water content lenses.

Dang and co-workers (2003) also quantified *P. aeruginosa* (PA01) adhesion to contact lenses from each FDA group (three lenses for

each group) which were soaked in a known concentration of bacteria for four hours and the total bacterial number was enumerated. The results showed that non-ionic/high water content contact lenses had the highest number of adherent bacteria.

Table 1.4 Summary of studies on bacterial adhesion in terms of water content and ionicity

Authors	Static / dynamic (incubation)	Unworn / worn	Lenses	Water content (%)	Ionicity	Bacteria	Results
Miller and Ahearn(1987)	Static (72 hours)	Unworn	tefilcon	37.5	Non-ionic	*P. aeruginosa* (20 clinical strains)	• In general, bacteria adhesion decreased as water content increased • Non-ionic hydrogel lenses had higher bacterial adhesion to both high and low water content lenses
			polymacon	38.6			
			tetrafilcon A	42.5			
			vifilcon A	55			
			lidofilcon A	70			
			surfilcon A	74			
			phemfilcon A	38	Ionic		
			etafilcon A	43			
			bufilcon A	45			
			phemfilcon A	55			
			etafilcon A	58			
			bufilcon A	55			
Lawin-Brussel *et al*. (1991)	Static (1 hour)	Unworn	polymacon	38.6	Non-ionic	*P. aeruginosa* (clinical strain)	• Higher total number of *P. aeruginosa* attachment to non-ionic/high water content non-ionic lenses than to non-ionic/low water content lenses
			crofilcon A	39			
			bufilcon A	55			
			etafilcon A	58	Ionic		
			perfilcon A	71			
			bufilcon A	55			
Cook *et al*. (1993)	Dynamic (2 hours)	Unworn	10 types of HEMA lenses (exact material name not mentioned)	33 - 69	Not mentioned	*P. aeruginosa* (unknown strain)	• Bacteria adhesion decreased as water content increased
Arciola *et al*. (1995)	Static (24 hours)	Unworn	etafilcon A	58	Ionic	*S. aureus* (unknown strain)	• Ionic/high water content lenses had higher total *S. aureus* (unknown strain) adhesion
			polyHEMA	38.6	Non-ionic		

Table 1.4 Summary of studies on bacterial adhesion in terms of water content (Con't)

Authors	Static / dynamic (incubation)	Unworn / worn / coated	Lenses	Water content (%)	Ionicity	Bacteria	Results
Dang *et al*. (2003)	Static (4 hours)	Unworn	polymacon	38.6	Non-ionic	*P. aeruginosa* (PA01)	• Non-ionic/high water content lenses showed the highest total bacterial adhesion • Non-ionic/low water content lenses have the lowest total bacterial adhesion
			alphafilcon A	66			
			phemfilcon A	38	Ionic		
			phemfilcon A	55			

1.4.4 Surface roughness

The surface roughness of both hydrogel and silicone hydrogel were found to be higher after being worn but the degree of roughness varied between different lenses (Bhatia et al., 1997; Lira et al., 2008; Santos et al., 2008). Surface roughness is associated with contact lens wear because of the deposition of protein derived from tears (Dutta et al., 2012) (Section 1.5). Table 1.5 summarizes the studies of the effect of surface roughness on microbial adherence.

Bruinsma and co-workers (2001) (See Section 1.4.2) investigated the surface roughness of two contact lenses and found that the unworn hydrophobic lens had a rougher surface than the hydrophilic lens but the surface roughness of the two lenses became almost the same after lens wear. Although they found higher cell surface damaged *P. aeruginosa* adhesion to the hydrophobic lens, they did not evaluate the influence of surface roughness on this observation. They only speculated that the increase in adhesion was due to the change of hydrophobicity of the bacterial surface.

Giraldez and co-workers (2010) (Section 1.4.2) investigated the effect of surface roughness on *S. epidermidis* (CECT 4184) adherence. The surface roughness was determined using AFM. *S. epidermidis* (CECT 4184) adherence to both hydrogel and silicone hydrogel lenses. Omafilcon A and comfilcon A lenses, which had lower surface roughness, were found to have less bacterial adhesion. A significant

correlation was also found between roughness and *S. epidermidis* adherence in both hydrogel and silicone hydrogel lenses. However, the authors also stressed that the influence of roughness on bacterial adhesion remains inconclusive because not all roughness parameters were correlated with adherence.

However, contradictory results to those described above were reported by Santos and co-workers (2008) (See Section 1.4.2). They determined the surface roughness of silicone hydrogel lenses using AFM. Unworn galyfilcon A lenses were significantly less rough compared with unworn balafilcon A and lotrafilcon B lenses, but bacterial adhesion did not differ between these unworn lenses. The results also indicated that after wear both galyfilcon A and balafilcon A lenses were significantly rougher than the unworn lenses of the same material. However, an equivalent change was not found in lotrafilcon B lenses. With respect to bacterial adhesion, only used balafilcon A lenses were found to have less *S. epidermidis* (9142) adherence in comparison with the unworn lenses. Worn lenses of other materials were not different from their unworn counterparts. Higher surface roughness did not seem to lead to adherence of more *S. epidermidis*. The authors suggested that surface roughness changed during wear, but this change did not have a great impact on lens bacterial adhesion. Their study has a limitation in that the area measured by AFM was not the same in all lenses. Galyfilcon A and lotrafilcon B lenses were analyzed within a 25μm^2 frame, while balafilcon A lenses were

analyzed within a 100μm^2 frame because some surface details were only visible in this frame (Henriques et al., 2005). Giraldez and co-workers (2010) and Santos and co-workers (2008) used different strains of *S. epidermidis*. The differences in their results could be due to the use of different contact lenses or different strains.

Bruinsma and co-workers (2002) (See Section 1.4.2) compared unworn etafilcon A lenses with these lenses worn for 10 and 50 days, using AFM to determine the surface roughness. The lens surface after 50 days of wear was significantly rougher than unworn lenses, but the increase in surface roughness did not result in higher *P. aeruginosa* (clinical strain) adhesion.

Vermeltfoort and co-workers (2006) (See Section 1.4.2) also investigated the relationship of surface roughness and bacterial adhesion. Unworn lotrafilcon A lenses had rougher surfaces than balafilcon A lenses. Greater initial adhesion rates of *S. aureus* (835) were found in lotrafilcon A lenses than balafilcon B lenses but the initial adhesion rates of *P. aeruginosa* (clinical strain) did not differ between the two brands of unworn silicone hydrogel contact lenses.

These study imply a rougher lens surface may not always lead to higher microbial adherence, but their finding may be not be reliable as they did not control for other factors (e.g. hydrophobicity) which might affect adhesion. Overall, studies indicate that adhesion of bacteria to

contact lenses varies between different bacterial strains and can be affected by the characteristics of the lenses.

Table 1.5 Summary of studies on bacterial adhesion in terms of surface smoothness

Authors	Static / dynamic (incubation)	unworn / worn	Lenses		Mean roughness (nm)	Bacteria	Results
Bruinsma *et al*. (2001)	Dynamic (2 hours)	Unworn	Hydrophobic** hydrogel (36% water content)		13	*P. aeruginosa* (clinical isolate) Cell surface damaged *P. aeruginosa* (clinical isolate) *S. aureus* (799)	• No difference in levels of adhesion of *P. aeruginosa* and *S. aureus* strains between more hydrophobic and hydrophilic contact lenses • *P. aeruginosa* became less hydrophobic after cell surface damage • Higher adhesion of cell surface damaged *P. aeruginosa* to more hydrophilic contact lenses
			Hydrophilic hydrogel (58% water content)		4		
		Worn	Hydrophobic hydrogel (36% water content)		13 -16		• Adhesion of *P. aeruginosa*(clinical isolate) to both worn hydrophobic and hydrophilic contact lenses decreased compared to their unworn counterparts
			Hydrophilic hydrogel (58% water content)		13 -16		
Bruinsma *et al*. (2002)	Dynamic (2 hours)	Unworn	Hydrogel	etafilcon A	4	*P. aeruginosa* (clinical strain)	• Lens surface after 50 days of wear was significantly rougher than unworn lenses • Change in surface roughness did not result in higher bacterial adhesion
		Worn (10 days)			5 ± 2		
		Worn (50 days)			10 ± 7		
Vermeltfoort *et al*. (2006)	Dynamic (2 hours)	Unworn	Silicone hydrogel	balafilcon A	6 ± 1	*P. aeruginosa* (clinical strain) *S. aureus* (835)	• Unworn lotrafilcon A lenses had higher surface roughness than balafilcon A lenses • Greater initial adhesion rates of *S. aureus* (835) to unworn lotrafilcon A lenses than unworn balafilcon A lenses • *P. aeruginosa* (clinical strain) were not significantly different in both unworn lenses
				lotrafilcon A	12 ± 2		

** Relatively less hydrophobic lenses

Table 1.5 Summary of studies on bacterial adhesion in terms of surface smoothness (Con't)

Authors	Static / dynamic (incubation)	unworn / worn	Lenses		Mean roughness (nm)	Bacteria	Results
Santos *et al*. (2008)	Dynamic (2 hours)	Unworn	Silicone hydrogel	balafilcon A	7.04 ± 0.66	*S. epidermidis* (9142)	• Unworn balafilcon A and lotrafilcon B lenses showed higher roughness than galyfilcon A lenses • No difference in bacterial adhesion between the unworn lenses
				galyficon A	2.32 ± 0.085		
				lotrafilcon B	4.51 ± 2.83		
		Worn	Silicone hydrogel	balafilcon A	17.63 ± 14.78		• Worn galyficon A and balafilcon A lenses exhibited higher roughness than the unworn lenses • Worn balafilcon A lenses adhered significantly less bacteria than unworn lenses • *S. epidermidis* adhesion of worn galyficon A and lotrafilcon B lenses were not significantly different from their unworn lenses
				galyficon A	30.09 ± 11.27		
				lotrafilcon B	4.96 ± 4.12		
Giraldez *et al*. (2010)	Static (2 hours)	Unworn	Hydrogel	nefilcon A	11.25 ± 0.38	*S. epidermidis* (CECT 4184)	• nelficon A and ocufilcon B lenses exhibited higher roughness than omafilcon A • nelficon A and ocufilcon B lenses showed higher number of bacteria adhesion than omafilcon A
				ocufilcon B	11.01 ± 1.79		
				omafilcon A	1.90 ± 0.39		
			Silicone hydrogel	comfilcon A	1.56 ± 037		• senofilcon A lenses showed significantly higher roughness than comfilcon A lenses • senofilcon A lenses showed higher number of bacteria adhesion than comfilcon A lenses
				senofilcon A	3.34 ± 0.28		

** Relatively less hydrophobic lenses

1.4.5 Lens depositions

In contact lens wear, proteins, lipids and mucin can easily be deposited on the lens surface and they have a significant impact on the surface properties of the lenses, allowing pathogens to adhere more easily (Maïssa et al., 1998; Berry et al., 2008). Table 1.6 summarizes the studies of the effect of lens deposition on microbial adherence.

Willcox and co-workers (2001) investigated the adherence of *P. aeruginosa* (Paer1 and 6294) to worn and unworn contact lenses. Both silicone hydrogel lenses (balafilcon A) and hydrogel lenses (etafilcon A and polymacon) were used. Five subjects wore the lenses in both eyes for six hours on different days. Unworn lenses were used as a control. Measurement of bacterial adhesion was performed by soaking the lenses in a bacterial suspension for 10 minutes. Worn balafilcon A and polymacon lenses had significantly higher adherence of *P. aeruginosa* (Paer1 and 6294), compared to their unworn control. In contrast, less *P. aeruginosa* (Paer1 and 6294) adhered to worn etafilcon A lenses, compared to their unworn control.

Santos and co-workers (2008) (See Section 1.4.2) compared *S. epidermidis* (9142) adhesion to worn and unworn silicone hydrogel lenses (balafilcon A, lotrafilcon A, lotrafilcon B and galyfilcon A) and hydrogel lenses (etafilcon A). Worn etafilcon A lenses became more

hydrophobic than the unworn etafilcon A lenses. Their results suggested that the change in hydrophobicity may account for the increase of adherence. The authors also found that worn etafilcon A lenses had higher *S. epidermidis* (9142) adherence than other worn silicone hydrogel lenses.

The effect of lens deposition on microbial adherence has also been investigated. An increased bacterial adherence was found in albumin-coated hydrogel (Cook et al., 1993; Taylor et al., 1998; Subbaraman et al., 2011) and silicone hydrogel lenses (Subbaraman et al., 2011).

Cook and co-workers (1993) (See Section 1.4.3) investigated the adhesion of *P. aeruginosa* (unknown strain) to 10 different protein-coated or uncoated water content hydrogel lenses (HEMA). They found that protein-coated (albumin, fibrinogen and mucin) lenses resulted in more adherence of *P. aeruginosa*.

Taylor and co-workers (1998) measured the adhesion of *P. aeruginosa* (RT1 and Paer1) and *S. epidermidis* (NCTC 11047) to albumin-coated polymacon and etafilcon A hydrogel lenses. The lenses (six lenses for each combination) were incubated in the bacterial suspension for one hour. They found an increased number of adherent bacteria with increasing albumin concentration in both lens types but the adherence of all three strains to polymacon was significantly higher than to etafilcon A lenses.

Williams and co-workers (2003) investigated the effects of unworn, patient worn lenses (daily wear for six hours, overnight wear and continuous wear), lactoferrin-coated, and lysozyme-coated hydrogel lenses (etafilcon A) on adhesion of *P. aeruginosa* (Paer 1). Five lenses were collected for each sample and soaked in bacterial suspension for 10 minutes. They found that bacterial adherence to the worn lenses, despite variations in wearing time, were not significantly different compared to the unworn lenses. Lactoferrin or lysozyme-coating on the lenses were also found to have no effect on adhesion of *P. aeruginosa*.

Subbaraman and co-workers (2011) (See Section 1.4.1) investigated the adherence of *P. aeruginosa* (Paer 6294 and 6206) and *S. aureus* (Saur 31) in three types of silicone hydrogel (balafilicon A, senofilcon A and lotrafilcon B) and one type of hydrogel (etafilcon A) lenses. Uncoated lenses and lenses coated with lysozyme, lactoferrin or albumin were investigated by soaking them in a known concentration of bacteria suspension for 24 hours. Compared with uncoated lenses, viable *S. aureus* and *P. aeruginosa* adhesion were found to increase in albumin-coated lenses (all four lens types). However, for lysozyme-coated lenses, there was increased viable *S. aureus* adhesion (all four lens types) but no increase in *P. aeruginosa* adhesion to any of the lens types when compared with uncoated lenses. A significant increase in viable *S. aureus* adherence was also found for all lactoferrin-coated lenses but there was a decrease in viable *P.*

aeruginosa adherence on all lactoferrin-coated lenses. Unlike Taylor and co-workers (1998) and Subbaraman and co-workers (2011), Williams and co-workers (2003) did not find a difference in *P. aeruginosa* adherence to etafilcon A lenses which may be due to the different bacterial strains or incubation time differences.

Boles and co-workers (1992) reported conflicting results concerning bacterial adhesion. They investigated the adhesion of *P. aeruginosa* (clinical strain) to etafilcon A lenses after seven days of continuous wear. Four subjects were recruited and their worn and unworn lenses were soaked in bacterial suspension for one hour. Significantly less protein deposits and adhesion was found on the worn lenses than the unworn lenses. They suggested that the some bacteria may be removed from the lenses into the saline during transport to the laboratory.

Although different proteins may result in different patterns of microbial adherence, most *in vivo* and *in vitro* studies suggested that protein deposition lead to a higher bacterial adherence in both hydrogel and silicone hydrogel lenses.

Table 1.6 Summary of studies on bacterial adhesion in terms of lens deposition

Authors	Static / dynamic (incubation)	Wearing time	Protein coated	Lenses		Bacteria	Results (compared with uncoated/unworn lenses)
Boles *et al.* (1992)	Static (1 hour)	7 days (continuous wear)	N/A (*in vivo*)	Hydrogel	etafilcon A	*P. aeruginosa* (clinical strain)	• Less bacterial adhesion found in worn lenses
Cook *et al.* (1993)	Dynamic (2 hours)	N/A (*in-vitro*)	Albumin	10 types of HEMA lenses (exact material name not mentioned)		*P. aeruginosa* (unknown strain)	• Higher adhesion on all protein coated contact lenses, when compared to unworn lenses
			Fibrinogen				
			Mucin				
Taylor *et al.* (1998)	Static (1 hour)	N/A (*in-vitro*)	Albumin	Hydrogel	etafilcon A	*P. aeruginosa* (RT1, Paer 1) *S. epidermidis* (NCTC 11047)	• Higher albumin coated lenses had higher amount of bacterial adhesion • Adherence to albumin-coated polymacon lenses was significantly higher than to albumin-coated etafilcon A lenses
					polymacon		
Willcox *et al*. (2001)	Static (10 minutes)	6 hours	N/A (*in vivo*)	Hydrogel	etafilcon A	*P. aeruginosa* (Paer 1, 6294)	• Significantly higher adhesion to worn balafilcon A and polymacon lenses • Significantly lower adhesion to worn etafilcon A lenses
					polymacon		
				Silicone hydrogel	balfilcon A		

Table 1.6 Summary of studies on bacterial adhesion in terms of lens deposition (Con't)

Authors	Static / dynamic (incubation)	Wearing time	Protein coated	Lenses		Bacteria	Results (compared with uncoated / unworn lenses)
Williams *et al.* (2003)	Static (10 minutes)	6 hours	N/A (*in vivo*)	Hydrogel	etafilcon A	*P. aeruginosa* (Paer 1)	• No differences noted in worn and unworn lenses in any wear modality
		6 - 8 hours (overnight wear)					
		Continuous wear (duration not mentioned)					
		N/A (*in-vitro*)	Lactoferrin				• No increase in viable bacterial adherence to both lactoferrin and lysozyme-coated lenses
			Lysozyme				
Santos *et al.* (2008)	Dynamic (2 hours)	15 days (12 - 14 hours per day)	N/A (*in vivo*)	Hydrogel	etafilcon A	*S. epidermidis* (9142)	• Bacterial adhesion to worn etafilcon A lenses was significantly than unworn lenses • etafilcon A became more hydrophobic after worn
		30 days (12 - 14 hours per day)		Silicone hydrogel	balafilcon A		• balafilcon A was less prone to adhesion after being worn, but not in other silicone hydrogel lenses • Both balafilcon A and lotrafilcon B lenses became less hydrophobic after being worn • In general, more bacteria adhered to worn etafilcon A lenses than other worn silicone hydrogel lenses
					galyfilcon A		
					lotrafilcon A		
					lotrafilcon B		

Table 1.6 Summary of studies on bacterial adhesion in terms of lens deposition (Con't)

Authors	Static / dynamic (incubation)	Wearing time	Protein coated	Lenses		Bacteria	Results (compared with uncoated / unworn lenses)
Subbaraman *et al.* (2011)	Static (24 hours)	N/A (*in vitro*)	Albumin	Hydrogel	etafilcon A	*P. aeruginosa* (Paer 6294, 6206) *S. aureus* (Saur 31)	• Increased *P. aeruginosa* (Paer 6294, 6206) and *S. aureus* (Saur 31) adhesion to all four types of lenses
				Silicone hydrogel	balafilcon A		
					lotrafilcon B		
					senofilcon A		
			Lactoferrin	Hydrogel	etafilcon A		• Decreased *P. aeruginosa* (Paer 6294, 6206) adhesion to all four types of lenses • Increased *S. aureus* (Saur 31) adhesion to all four types of lenses
				Silicone hydrogel	balafilcon A		
					lotrafilcon B		
					senofilcon A		
			Lysozyme	Hydrogel	etafilcon A		• No difference in *P. aeruginosa* adhesion (Paer 6294, 6206) to all four types of lenses • Increased *S. aureus*(Saur 31) adhesion to all four types of lenses
				Silicone hydrogel	balafilcon A		
					lotrafilcon B		
					senofilcon A		

Hydrophobicity, ionicity, water content, surface roughness and lens deposition all appear to play a role in microbial adherence. To a certain extent, these variables are inter-correlated and it is impossible to separate one from the others. In summary, it appears that bacterial adherence is most influenced by the hydrophobicity of the lens. Adherence also varies between different strains of bacteria and different proteins also give differing bacterial adherence patterns. It was noted that *in vitro* and *in vivo* experiments yielded different microbial adherence results because of the changes in hydrophobicity and surface roughness after wear. However, it is generally agreed that hydrophobic content lenses and lenses with higher roughness and deposition are more susceptible to bacterial adherence.

Although many studies have investigated microbial adherence to hydrogel and silicone hydrogel lenses, to date, there is no in depth investigation of microbial adherence to CCL. As described in Section 1.1.2, there are different methods of applying pigments to lenses when manufacturing CCL, including surface pigment. Giraldez and co-workers (2010) showed that a rougher lens surface may attract more adhesion and Lorenz and co-workers (2014) have reported that surface pigment CCL have a rougher lens surface. It is not known if such pigments, if exposed on the lens surface, will affect microbial adherence.

1.5 Protein deposition on contact lens wear

The tear film plays an important role in maintaining optical clarity, acting as a lubricant, protecting the eyes against micro-organisms acting as a medium of oxygen transmission and maintaining metabolism (Holly and Lemp, 1977; Holly, 1980; Bron, 1985; Boot et al., 1989). It is composed of electrolytes, proteins, lipids, mucus and peptides (Holly and Lemp, 1977; van Haeringen, 1981). Protein is one of the major components of the aqueous layer of the tear film with more than 90 types of proteins identified in human tears. Of these, lysozyme, lactoferrin, and albumin are most important and are present in relatively high quantities (Sitaramamma et al., 1998; de Souza et al., 2006; Zhou et al., 2006). Proteins in the tear film can result in high protein deposition on worn contact lenses (Taylor et al., 1998) and are the primary source of deposition in contact lenses (Willcox et al., 2002). They can accumulate on the lens material over the time of lens wear resulting in high levels of deposition (Castillo et al., 1984; Lin et al., 1991; Maïssa et al., 1998). The tear proteins which frequently deposit on hydrogel lenses are albumin, lysozyme, and lactoferrin (Wedler et al., 1987; Green-Church and Nichols, 2008; Zhao et al., 2008). Of these proteins, lysozyme is the most widely studied (Garrett et al., 1998, 1999; Subbaraman et al., 2009) and is the deposit which accounts for most problems in contact lens wear (Karageozian, 1976; Leahy et al., 1990; Bontempo and Rapp, 2001; Zhao et al., 2008).

1.5.1 Effect of protein deposition on comfort

Nilsson and Lindh (1988) reported significant correlation between deposition on the lens and discomfort. Sixty-six subjects wearing hydrogel lenses with a daily cleaning regime of cleaner and hydrogen peroxide were recruited. An additional step of the use of weekly enzymatic cleaning was introduced for the right lens only. The multi-enzyme system was capable of removing protein, lipids, and mucin from the lens surface. After six months of wear, the authors examined both lenses using a slit lamp biomicroscope and compared the deposition rates. Subjects were also asked to rate the comfort of wearing their lenses. Lens comfort was significantly better in the right eye (wearing lenses with weekly enzymatic cleaning) than the left eye (wearing lenses without the use of weekly enzymatic system). Less deposition was also observed on the right lens than the left lens. Use of enzymatic cleaners was associated with reduced contact lens discomfort and lens deposition. The authors concluded that enzymatic removal of lens deposits was beneficial for contact lens wear. However, the limitation of this study was that the deposition was determined by subjective grading from slit lamp examination on the lenses. Different kinds of deposition may be involved but it was not possible to identify whether the deposition consisted of proteins. The use of enzymatic cleaners was also limited to the removal of protein deposit s but not other organic deposits.

Brennan and Efron (1989) surveyed 104 symptomatic hydrogel lens wearers visiting the University Optometry Clinic. They found that more

than 30% of patients wearing lenses with lens age older than six months reported dryness whereas only 12% of patients who wore lenses with lens age less than six months experienced dryness. The authors proposed that dryness may be due to the deposition of proteins which lead to decreased tear stability and faster tear break up and subsequently dryness. However, the level of protein deposition was not determined in this study and the conclusion of a relationship between deposition and lens comfort was only a speculation of the authors.

Lever and co-workers (1995) evaluated the relationship between comfort and protein deposition. They collected 977 replacement lenses from 29 clinical sites over a period of ten months. There were two inclusion criteria for eligibility – either the patient complained of discomfort with their lenses or there were surface deposits on the lenses as determined by the investigators. Only lenses which fulfilled either one of the two criteria were collected but these lenses (replaced lenses) must have been worn for at least three months. The patients were also asked to rate the comfort of lens wear at the time of replacement and the investigators evaluated the protein deposition in the laboratory using a chemical assay. Different lens types were collected and analysis was done by comparing the replaced lenses with the worn lenses without complaint (no complaint lenses) from the same FDA group (historical data). They found no difference in the total lens protein between the replaced lenses and the no complaint lenses.

Also, there was no correlation between total lens protein deposition and patient subjective comfort ratings in all FDA group lenses. The authors concluded that protein deposition was not the sole factor in determining lens comfort.

Jones and co-workers (1996) compared vision, comfort, lipid, and protein deposition between two groups of contact lens wearers. Twelve subjects were recruited and fitted with Precision UV lenses (Pilkington Barnes-Hind, United States). Six subjects were instructed to replace their lenses every month for three months (1/12 replacement), while the remaining six subjects were asked to wear the same pair for three months before replacement (3/12 replacement). The wearing time per day was not mentioned. Subjects were asked to rate the overall satisfaction subjectively from one (least comfortable) to ten (very comfortable). The level of protein deposition was determined quantitatively using ultra-violet (UV) spectroscopy. The authors found that the protein deposition levels on the 3/12 replacement lenses (106±16μg/lens) were significantly higher than on the 1/12 replacement lenses (42± 7μg/lens), but the levels of comfort between the two groups after one week, one month and three months of wear did not differ significantly. The authors concluded that monthly replacement was better than quarterly replacement because they attracted less protein.

Although no firm conclusion can be drawn from these studies, these reports now have less relevance as there has been a huge

advancement in technology in recent years with much improved lens materials which may lessen the issue of discomfort. These studies were also limited by the fact that masking was not possible. While lens deposition may be a factor leading to lens discomfort, other factors such as lens modulus and coefficient of friction of the lens surface also contribute to lens comfort.

1.5.2 Effect of protein deposition on vision

Jones and co-workers (1996) (See Section 1.5.1) also evaluated the high and low contrast visual acuity of their two groups of subjects (1/12 and 3/12 replacement) and found both high and low contrast visual acuities did not differ between groups at all visits.

Michaud and Glaude (2002) studied the effect of extended replacement of etafilcon A lenses on protein deposition and visual acuity. Lenses were worn on a daily wear basis (at least eight hours a day). Lenses in one eye were replaced biweekly (as recommended by the manufacturer; on-time replacement) but in the other eye, lenses were worn for a maximum of 30 days before replacement (extended-replacement). The actual days of wear for the extended-replacement lenses varied as the investigator stopped lens wear if symptoms such as redness, worsened vision, or discomfort arose. The visual acuity of the eye wearing the extended-replacement lenses was compared with the other eye. Protein deposition on the lenses was determined using a protein assay. Seventeen subjects completed the study and the amount of protein deposited on the extended-replacement lenses was

found to be significantly higher. However, the visual acuity did not differ significantly between replacement times. The authors concluded that extended-replacement of contact lenses led to higher protein accumulation on the lens but did not affect vision.

Some studies have reported reduced vision in subjects whose lenses had protein deposits. Gellatly and co-workers (1988) evaluated the relationship between high and low contrast visual acuity and lens deposition. Lenses from 51 subjects who had worn the lenses from zero to 45 months (exact time of wear per day for each lens was not mentioned) were assessed. Visual acuity measurement was performed with the lenses on the eye and the protein deposition of the lenses was graded using Rudko classification (Rudko and Proby, 1974) under a 15x magnification slit lamp biomicroscope. They found decreases in both high and low contrast visual acuities and increased deposition on older contact lenses. They concluded that increased deposition was associated with reduced visual acuities. Unlike other studies (Lever et al., 1995; Jones et al., 1996), Gellatly and co-workers (1988) used Rudko classification to grade the level of deposition which may include types of deposits other than protein. Therefore, the reduced visual acuity may not be solely due to protein deposition.

Lens deposition of various proteins on contact lenses has been extensively reported (Castillo et al., 1984; Lin et al., 1991; Maïssa et al., 1998) and effect

on microbial adherence has been discussed in Section 1.4.4. In a review of tinted (coloured) lenses, Lowther (1987) pointed out that some tinting processes (Vat dye tinting and chemical bond tinting) may alter the charge on the lens surface which could facilitate protein deposition. Therefore, a higher protein deposition is possible in reusable CCL. However, to date, there is no investigation of protein deposition on CCL.

1.6 Cytotoxic effect of care solutions

Contact lens solutions, although required for disinfection of contact lenses, may create problems of ocular allergy and toxic reactions, and this has been observed, especially with the first generation of disinfectants (Mondino and Groden, 1980; Mondino et al., 1982). Despite the use of higher molecular weight biocides in more recently available MPS, studies have also shown that they can still be potentially adsorbed onto the lens and be subsequently released onto the cornea after insertion (Rosenthal et al., 2006; Powell et al., 2010; Willcox et al., 2010a; Gorbet et al., 2011).

Corneal staining is commonly assessed in clinical practice to determine the integrity of the cornea (Morgan and Maldonado-Codina, 2009). Several studies have tried to evaluate the toxicity of preservatives in care solutions by corneal staining using fluorescein (Jones et al., 1997, 2002; Andrasko and Ryen, 2008). However, hyper-fluorescence can result due to various causes ranging from compromised corneal integrity due to corneal abrasion to a normal phenomenon attributable to dimple veiling and mucin balls

(Dumbleton et al., 2000). Because of this, biochemical tests can be employed to evaluate the corneal cell condition at cellular level.

Several investigations of ocular toxicity of care solutions at the cellular level have been reported (Mowrey-McKee et al., 2002; Santodomingo-Rubido et al., 2006; Chuang et al., 2008; McCanna et al., 2008; Choy et al., 2009; Dutot et al., 2010; Gorbet et al., 2011; Tanti et al., 2011; Choy et al., 2012, 2013). The majority of these studies were *in vitro* studies performed on animal cell lines or more recently, on human corneal epithelial cells (HCEC). All work reported with contact lenses were performed on clear contact lenses.

1.6.1 Solutions

Mowrey-McKee and co-workers (2002) investigated the cytotoxic effects of MPS and hydrogen peroxide by various methods (United States Pharmacopeia direct contact test and three modifications of elution test - trypan blue uptake test, regrowth of cells after exposure test and quantitation of viable cells after exposure test) using mouse fibroblast cells. Five brands of MPS and a brand of hydrogen peroxide system were studied, together with a control using sodium chloride. The five brands of MPS were Solo-care (Ciba Vision Ltd, United States), Optifree Express (Alcon Ltd, United States), ReNu (Bausch & Lomb Ltd, United States), ReNu Multiplus (Bausch & Lomb Ltd, United States) and Complete Comfort Plus (Abbott Medical Optics Inc, United States). The hydrogen peroxide system was AOSept (Ciba Vision Ltd, United States). Three lenses were used for each MPS in the direct

contact test and three replicates were used for each MPS in the three modified elution tests. The authors found that Solo-care, Complete Comfort Plus and neutralized AOSept exhibited no cytotoxic effects in all four tests. The two MPS from Bausch & Lomb were found to inhibit the growth of the mouse fibroblast cells after exposure. Optifree Express exhibited higher cytotoxic effects on cells, with evidence of cell lysis, inhibition of cell regrowth, and decreased proportion of viable cells. The authors concluded that the cytotoxic effects varied between different MPS and Optifree Express was shown to have higher potential to cause a cytotoxic response.

Cytotoxic effects of MPS at different concentrations on Chinese hamster lung fibroblasts cells were also investigated (Santodomingo-Rubido et al., 2006). The cytotoxic effects of six commercially available MPS (MeniCare Soft by Menicon Co Ltd, Japan, Complete Moisture Plus (Abbott Medical Optics Inc, United States), Solo-care, Optifree Express, ReNu Multiplus and ReNu MoistureLoc (Bausch & Lomb Ltd, United States)) and a physiological saline (control) on the cell line were studied. The cells were exposed to the MPS for six days and the cytotoxicity of diluted MPS at different concentrations (1.25%, 2.5%, 5% and 10%) was evaluated using a colony-forming assay. However, the number of samples for each MPS and the concentration were not mentioned. The results revealed that ReNu MoistureLoc was the most cytotoxic (toxic at all concentrations), followed by ReNu Multiplus (slightly toxic at 1.25% and at all higher concentrations), and

Optifree Express and Solo-care (cytotoxic at 5% and 10% only). Both MeniCare Soft and Complete Moisture Plus were found not to be cytotoxic at all concentrations. The authors concluded that different MPS had different levels of cytotoxic effect. MPS with identical concentrations of polyhexamethylene biguanide (PHMB) could also lead to different amounts of cytotoxicity.

Chuang and co-workers (2008) also investigated the cytotoxic effects of different MPS (ReNu Multiplus, Complete Easy Rub by Abbott Medical Optics Inc, Optifree Express and Optifree Replenish by Alcon Ltd) on HCEC using 3-(4-,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay (exposure for 30 minutes, one, two, four, six and 24 hours), DNA Fragmentation assay (exposure for 30 minutes), fluorescein permeability assay (exposure for a hour) and tight junction protein staining method (exposure for two or six hours). The number of replicates for each MPS and method was not mentioned. Only Complete Easy Rub was found to have no difference (at all exposure times) in cell survival compared with balanced salt solution (control). The other MPS were found to reduce cell survival after exposure for six hours. Both cell apoptosis and epithelial permeability of cells soaked in Complete Easy Rub were found to be no different from the control whereas the other MPS showed some cytotoxic effects. The tight junction proteins were also not disturbed after exposure to Complete Easy Rub but other MPS were found to exhibit effects on the integrity of the tight junction. They concluded that the cytotoxic effect varied between MPS. Similar

to Santodomingo-Rubido and co-workers (2006), they also reported that MPS with identical concentrations of PHMB gave different results on cytotoxicity.

McCanna and co-workers (2008) investigated the effect of MPS on HCEC by evaluating their sodium fluorescein permeability and alamarBlue activity. Five MPS (ReNu Multiplus, Optifree Express, Solo-care, Complete Moisture Plus and AQuify 5 minute from Ciba Vision Ltd, United States) were investigated and HCEC were exposed to these solutions for 15 minutes (three and four replicates for each MPS for sodium fluorescein permeability assay and alamarBlue assay, respectively). They reported a significant loss of tight junctions (indicating cell damage), increased permeability to sodium fluorescein (indicating membrane damage) and reduced metabolism in HCEC treated with Optifree Express but not in other MPS. The authors concluded that Optifree Express was more cytotoxic than the other tested MPS.

Choy and co-workers (2009) investigated the cytotoxic effects of MPS on porcine corneal cells. Three MPS (MPS A containing polyquaternium-1, MPS B containing PHMB and MPS C containing polyhexanide) were studied. The corneal epithelial cells of porcine corneas were first digested using 0.25% trypsin/EDTA and the dissociated cells were exposed to MPS for 10 seconds, 30 seconds, one minute, 5 minutes, or 10 minutes. A total of 20 samples were

used for each MPS and control (DPBS) under these five conditions. Cell viability was determined using Annexin V-FITC/7-AAD kit. The results indicated a significantly higher number of early necrotic cells (at all exposure times) in MPS A than other MPS and control. A significantly higher number of late necrotic cells were observed in MPS A (after five and 10 minutes of exposure) than other MPS and control. The authors concluded that MPS A had greater cytotoxic effect on porcine corneal epithelial cells, compared with other MPS and control.

Choy and co-workers (2012) also studied the cytotoxic effects of MPS on HCEC using MTT cell viability assay and Annexin V-FITC/7-AAD kit. MTT cell viability assay and Annexin V-FITC/7-AAD kit were used to evaluate the metabolic rate of HCEC and the cell viability and membrane integrity respectively. Three MPS (same as previously used by Choy and co-workers (2009) used) were investigated. In assessing the cell viability and membrane integrity, the HCEC were exposed to these solutions for periods of one, five, 10 and 15 minutes (four samples for each) while cell metabolic activity was evaluated by exposing to different concentrations (10%, 20%, 30% and 40%) of MPS for 12 hours and 96 hours (four samples for each). The cytotoxic effects were determined by comparing to the controls exposed to DPBS. Exposures to MPS A for 10 and 15 minutes were found to have the highest percentages of late necrotic cells compared to the control. The MTT assay also revealed that all MPS showed significant inhibition of cell metabolism at three different concentrations (except

10%) after exposure for both 12 and 96 hours. This indicated that long-term exposure to diluted MPS might interfere with the cell metabolism, even though it did not cause cell damage. The authors concluded that MPS A had the greatest effects on cell viability and metabolic activity. Both studies by Choy and co-workers (2009, 2012) on porcine epithelial cells and HCEC gave the same conclusion in that higher late necrotic cells were observed after 10 minutes of exposure to MPS A and MPS A was the most toxic among three MPS tested.

Choy and co-workers (2013) also investigated the cytotoxic effects of MPS for rigid gas permeable (RGP) lenses. The same methods as for their study on soft lens MPS (Annexin V-FITC/7-AAD kit and MTT assay) were used to evaluate HCEC exposed to RGP lens MPS (MPS A containing 0.003% chlorhexidine gluconate and 0.0005% PHMB, MPB B containing 0.0005% PHMB, and MPS C containing 0.0001% PHMB). The experimental protocol was the same as their study on soft lens MPS (Choy et al., 2012). They found that MPS A showed highest percentage of early and late necrotic cells at all exposure times than other MPS and control (DPBS). Inhibition of cell metabolism was found in MPS A (at all test concentrations) after 12 hours of exposure. The authors concluded that chlorhexidine gluconate appeared to have caused higher cytotoxicity. They also suggested rinsing lenses (both soft lenses and RGP) with saline prior to lens insertion after soaking in MPS.

Despite using different assessment methods and cell lines, most of these studies indicated that Optifree Express exhibited higher cytotoxic effects. Several studies also found that MPS with identical concentrations of PHMB had different cytotoxic effects on animal cells or HCEC. This suggests that, other than the disinfecting agent, the isotonic and surfactant agents in the formulae may also play a role in cytotoxicity of care solutions. Although these studies only evaluated the overall cytotoxic effect of the MPS as a whole, patients using the MPS will be using the MPS rather than the specific ingredients.

1.6.2 Lens-solution combination

Research studies investigating ocular toxicity often only evaluate the cytotoxic effects of care solutions alone with the absence of contact lenses whereas biocompatibility studies usually involve both. Because of the porous nature of soft contact lenses, they have the potential to uptake the components of ophthalmic solutions, including disinfecting agents or other chemical agents (Chapman et al., 1990; Rosenthal et al., 2006). This uptake may subsequently be released during lens wear. Several studies have been conducted to evaluate the cytotoxic effect of lens packaging solution (Gorbet et al., 2010) and MPS release (Gorbet et al., 2011; Tanti et al., 2011) from contact lenses on HCEC.

Gorbet and co-workers (2011) investigated the interaction of five MPS (Optifree Express, Optifree Replenish, Complete Moisture Plus, ReNu

Fresh by Bausch & Lomb and Solo-care) with six types of silicone hydrogel lenses (balafilcon A, lotrafilcon A, lotrafilcon B, comfilcon A and galyfilcon A) on HCEC using MTT assay, integrin expression, and caspase activation methods to determine cell viability. All lenses (three to five lenses per MPS for each method) were pre-soaked in the MPS or control (Phosphate buffered saline (PBS)) for 18-24 hours before being placed in contact with HCEC for 24 hours. Significant reductions in cell viability in lenses soaked in ReNu Fresh (all lenses except comfilcon A) and Optifree Express (all lenses) were observed. These two MPS were also found to lead to a significant reduction of integrin expression, indicating a disruption in cell-cell adhesion. Significant caspase activation was also observed with the lenses (except balafilcon A) soaked in Optifree Express, indicating an initiation of apoptosis. Contact with lenses made of lotrafilcon A material were also found to result in significantly lower viability compared with lenses of the other three silicone hydrogel materials. This suggested that MPS uptake and release differed between lenses due to difference in the chemistry of the lens and the surface treatment (Powell et al., 2010; Willcox et al., 2010a). The authors concluded that cytotoxic effects were affected by types of lenses, suggesting that lenses themselves also have an impact on the cytotoxic effect of MPS.

Tanti and co-workers (2011) used the same methods as Gorbet and co-workers (Gorbet et al., 2010, 2011) to investigate the effect of release of MPS (Optifree Express, ReNu Multiplus and Complete

Moisture Plus) from two silicone hydrogel lenses (balafilcon A and lotrafilcon A) on HCEC. The lenses (three to six lenses per MPS for each method) were pre-soaked in MPS or controls (PBS or borate buffered saline (BBS)) for 18-24 hours before exposure to HCEC for eight or 24 hours. One more control was also prepared with MPS exposed to HCEC (without contact lenses) directly for eight or 24 hours. Cell viability of MPS soaked lenses were compared with their respective control (Optifree Express and ReNu Multiplus were compared with BBS while Complete Moisture Plus were compared with PBS). No significant difference in cell viability (eight hours of exposure) was found between MPS-soaked lenses (both balafilcon A and lotrafilcon A). Significantly higher reductions in cell viability (24 hours of exposure) were observed in lenses (both balafilcon A and lotrafilcon A) soaked in Optifree Express and ReNu Multiplus, compared with lenses soaked in Complete Moisture Plus. Cell viability (24 hours of exposure) in balafilcon A soaked in Optifree Express was found to be higher than that in lotrafilcon A soaked in Optifree Express, suggesting that different physical properties of the lenses may affect the uptake and release of MPS. Integrin expression was found to be reduced with both silicone hydrogel lenses soaked in ReNu Multiplus and Optifree Express at eight and 24 hours while caspase activation was only found to be increased in lotrafilcon A lenses soaked in Optifree Express at 24 hours. The authors concluded that disinfecting agents played a role in cytotoxicity. The lens-solution combination also resulted in different cytotoxic effect.

Similar to studies evaluating the cytotoxicity of MPS alone (Mowrey-McKee et al., 2002; McCanna et al., 2008; Choy et al., 2012), both Gorbot and co-workers (2011) and Tanti and co-workers (2011) found that Optifree Express has higher cytotoxic effects. The cytotoxicity effect was different between lens types suggesting that lens type was also a factor that affected the uptake and release of certain components in the care solutions.

Studies have generally agreed that MPS exposure, alone or in combination with contact lenses, resulted in cytotoxic effects on epithelial cells. To date, these studies were all performed on hydrogel or silicone hydrogel lenses and there is no investigation on CCL. It is unknown if leachates of MPS from CCL are different from those of clear lenses. It is also unknown if the pigment of the CCL, if in direct contact with the cornea, will cause any cytotoxicity. These questions warrant further studies.

1.7 Animal eye models

Animal eye models, using rabbit, mice, chick or monkey, have been extensively used in different aspects of eye research including dry eye (Gilbard et al., 1987, 1988; McLaughlin et al., 1988; Kaswan et al., 1989; Fujihara et al., 1995, 1998; Maitchouk and Beuerman, 2000; Moore et al., 2001; Choy et al., 2004, 2006, 2008), myopia (Wiesel and Raviola, 1977) and keratitis (Wilson, 1970; Kessler et al., 1977; Moreira et al., 1991; Moreau

et al., 2002; Barequet et al., 2004; Mah et al., 2007). Because of the undesirable and irreversible results that experiments may cause, human eyes cannot be considered in such experiments.

1.7.1 Porcine eye model

In the past, rabbit eyes were commonly used as a model to study corneal and dry eye research. (Doughty, 1994) The *in vivo* rabbit eye model allowed investigation of dry eye by closing the lacrimal gland excretory duct and removing the nictitating membrane and harderian gland. (Gilbard et al., 1987, 1988)

In the last decade, porcine eyes have been used to develop a dry eye model to investigate exposure keratitis. Owing to the similarity of porcine eyes to human eyes in size (Eklund et al., 2003) and composition (Camber et al., 1987), porcine corneas are commonly used as a substitute for human cornea to study drug permeation (Camber, 1985; Reichl et al., 2004), tonometry (Eklund et al., 2003; Hallberg et al., 2006) and corneal diseases (He et al., 1992). Kampmeier and co-workers (2000) also studied the thermal and biochemical properties of porcine cornea and found these parameters were not too different from those of humans. Hence, the porcine cornea is a good substitute for human corneal models. Pig organs are also more readily available because pork is consumed as meat and pig breeding is easy. Although porcine eyes show similarities in many aspects to human eyes, the porcine eye model (PEM) is limited to *ex*

vivo study as the pig is large and high maintenance costs would be required for *in vivo* study.

Choy and co-workers (2004) first proposed a porcine dry eye model with lacrimation and blinking systems incorporated. The viability of the porcine eyes were compared between eyes examined immediately after enucleation and eyes mounted on PEM with lacrimation and blinking simulation for four hours. Trypan blue solution was used to examine the corneal viability. The experiment showed that the porcine corneal epithelial cells remained viable for four hours *ex vivo* if the eyes were maintained with moisture. The PEM were further used to experiment the possibility of simulating different severity of dry eye (Choy et al., 2008) and to investigate the effect of various commercially available artificial tears in a simulated severe dry eye condition (Choy et al., 2006).

1.7.2 Pros and cons of Porcine eye model

There are a lot of arguments regarding the use of animal experimentation (Coleman, 1991; Michael Conn and Parker, 2008). Of these, *in vivo* experiments are the most challenged as the animal is sometimes purpose-bred and sacrificed after the experiment. Researchers have supported the need for animal research as the interactions of molecules and cells are situations that cannot be manipulated even with the use of sophisticated computer systems. Opponents have argued that animal experiments were not necessary

and could be misleading because of the differences in mechanisms and structures from human beings (Coleman, 1991). Monkeys are rarely used in research largely due to the ethical controversy of the use of non-human primates, like monkeys, gorillas, and chimpanzees, in experiments which involve invasive procedures. This controversy arises because of the argument that non-human primates also experience pain like humans. PEM has an advantage that it is of less ethical concerned because it is an *ex vivo* design. The pigs were not killed purely for experimental purpose. The cost for an *in vivo* design experiment is also higher as a large space and a high maintenance cost are required.

However, PEM is also limited by the fact that it cannot be used to study long term dry eye effect because *ex vivo* design has a limitation that the eyes cannot be maintained vital. Although the size and composition of porcine eyes are similar to human eyes, monkey eyes are much more similar to human eyes anatomically than porcine as human eyes do not have the nictitating membrane (the third eyelid) that is present in porcine eyes.

1.8 Summary

The popularity of CCL is increasing, particularly in Asian countries in recent years (Morgan et al., 2012, 2013, 2014). However, there are increasing concerns about over-the-counter sale of CCL as the end users of these

lenses are usually adolescents, who treat them as fashion accessories instead of medical devices. There have been reports of microbial keratitis related to the use of CCL, but the majority of these adverse events were considered to be due to non-compliant behaviors such as sharing and overwear of contact lenses. With huge demand of CCL in the market and lack of regulations of the sale of CCL, there is a need to review the safety of CCL. A recent report by Japan NCAC raised concerns as some manufacturers made false claims on the design of their CCL (Japan National Customer Affairs Center, 2014). Eleven brands claimed they were using embedded pigments or a sandwich design but only two brands were found to be truly embedded whereas the rest were found to have pigments tinted on the lens surface. Although some of these pigments were FDA approved, their effects on microbial adherence, protein deposition and cytotoxicity was not known. There is, therefore, a need to investigate the surface pigment CCL and their implication on safe CCL wear.

Chapter 2

Knowledge gaps and objectives

2.1 New porcine eye model

Animal eye models are mainly used in optometry for investigation of the pathophysiology of dry eye (Gilbard et al., 1987, 1988; McLaughlin et al., 1988; Kaswan et al., 1989; Fujihara et al., 1995, 1998; Maitchouk and Beuerman, 2000; Moore et al., 2001; Choy et al., 2004, 2006, 2008) and keratitis (Wilson, 1970; Kessler et al., 1977; Moreira et al., 1991; Moreau et al., 2002; Barequet et al., 2004; Mah et al., 2007). This is related to the potentially severe consequences of dry eye and keratitis to the cornea and *in vivo* human studies are limited. Animal eyes are reasonable for human eyes substitute in *in vivo* studies.

Choy and colleagues (2004) first developed a PEM to investigate evaporative dry eye and the effects of various artificial tears on dry eye. However, the use of the original PEM was limited because only two porcine eyes could be set up each time and there was no strict control of the surrounding temperature or humidity. To resolve these limitations, a modified PEM was developed so that up to four porcine eyes could be set up simultaneously, together with a use of chamber made of acrylic which allows better control of temperature and humidity. The previous model has only been used in the study of dry eye

whereas areas other than dry eye such as cytotoxicity have not been explored.

2.1 Safety of cosmetic contact lenses

Use of CCL has become popular in recent years (Morgan et al., 2012, 2013, 2014) (Section 1.1.3), but most studies involving CCL were limited to case reports. The designs of these lenses, as well as the pigments used, are factors that can affect the safety of patients using these lenses. However, to date, there is no formal report regarding the safety of such lenses. The pigment used for CCL may be coated on the surface or may be embedded or sandwiched in the lens. Many manufacturers claim that their CCL have the pigments embedded or sandwiched in the material and their claims have not been substantiated.

Cytotoxicity of the pigments used in CCL has also not been investigated. Although some CCL manufacturers claimed to have used pigments approved by FDA for ocular use, the wide variety of CCL that can be purchased from different retail outlets such as flea markets, beauty salons, department stores, the internet, and other unlicensed vendors (Section 1.1.4) poses a serious threat to the safety of the use of CCL. This is of particular concern in countries where the sale of CCL is increasing in popularity and sales of such lenses from unlicensed vendors are common (Personal communication). The Draize eye test is the United States FDA endorsed standard toxicity test for evaluating the safety of materials for use in or around eyes, including

pigments used in CCL (Wilhelmus, 2001).The pigments are applied to animal eyes (usually albino rabbit) and then the eyes are monitored for abnormal signs for 14 days. However, the test has become controversial in recent years owing to the subjectivity in the test (Prinsen, 2006).The test also involves no microscopic investigations. In addition, aspects other than toxicity, such as comfort, delayed response and systemic effects, are not taken into account. In view of these problems with the Draize test, biochemical tests involving microscopic and cellular investigations have more commonly been used in studies to evaluate cytotoxic response of the eye to test articles. Researchers commonly use effects on HCEC and biochemical test such as MTT to investigate cytotoxic effects of contact lens solutions towards corneal epithelial cells. Several recent studies have examined the effects of contact lenses interaction with MPS when evaluating the cytotoxicity (Powell et al., 2010; Gorbet et al., 2011; Tanti et al., 2011). However, there is no published report investigating the cytotoxic effect of pigments and solution uptake and release due to the presence of surface pigments in CCL.

Previous literature has shown that hydrophobicity, water content, ionicity, surface roughness and protein concentration in the tear film play a role in deposition on contact lenses (Section 1.5). Protein deposition on contact lens can cause discomfort, visual disturbance and complications such as contact lens papillary conjunctivitis (Section 1.5). The surface pigment of CCL was found to have impact on the surface roughness (Mayers et al., 2013) and it is not known if this affects protein deposition.

As mentioned in Section 1.4.1, the surface hydrophobicity is a crucial factor which affects microbial adherence (Dutta et al., 2012). However, published reports only used clear contact lenses and CCL have not, to our knowledge, been tested. It is not known if the additional factors such as surface pigments on contact lens surface affect microbial adherence. Cytotoxicity of pigments, protein deposition and microbial adherence are important considerations in determining the safety of CCL. With the increasing popularity of CCL especially in Asian countries, it is essential that these issues are addressed.

2.2 Objectives

To address the above unknown issues, a series of studies were conducted in this PhD study and the objectives were to:

- develop a method to determine the location and permanency of pigments on CCL
- investigate the effect of surface pigments of CCL on microbial adherence
- investigate cytotoxic effect of surface pigment of CCL on porcine corneal epithelial cells using the new PEM
- investigate the effect of surface pigments of CCL on protein deposition

Chapter 3

Permanency of pigments of cosmetic contact lenses – a pilot study

3.1 Introduction

The use of CCL has become increasingly popular especially in Asian countries including Korea, Taiwan, Singapore and China (Morgan et al., 2012, 2013) (Section 1.1.3). The wearers of these contact lenses are usually teenagers and adolescents (Singh et al., 2012). CCL include coloured lenses and limbal-ring lenses which are used to change the colour or the normal appearance of the eye (Section1.1.1). However, like conventional contact lenses worn for correction of refractive errors, use of these lenses can also cause significant complications such as microbial keratitis, if they are not handled properly (Sauer et al., 2011) (Section 1.3).

Purchasing CCL from unlicensed vendors could pose a health threat to the wearers as the manufacturer, as well as the parameters of the CCL, are unknown. In Hong Kong and many Asian countries, CCL can be purchased over the internet and from retail outlets, such as cabinet stores (Section 1.1.4) and, flea markets, where the salespersons have not received proper training

on contact lens handling and care. They are therefore unable to provide any proper instruction and advice on proper lens wear and care.

As discussed in Section 1.2.4, pigments coated onto a lens surface may roughen the surface and may pose other problems to contact lens wear. Many manufacturers or contact lens distributors claim that their contact lenses are of embedded or sandwiched designs. To confirm this, a study was designed to investigate the location of pigments in samples of CCL available from different retail sources in Hong Kong.

3.2 Experimental design

3.2.1 Contact lenses

Five brands of CCL were tested. Four out of these five brands were purchased online or from a cabinet store and one from an optical store (Table 3.1). They were chosen because most (except lens A) did not provide any information on the pigment design and were sold in retail sources frequented by teenagers. The colour of these lenses was limited to Brown or Hazel if available as this appears to be the most popular colour used by Asian wearers (Personal communication).

3.2.2 Rub-off test

The permanency of pigments of the five brands of CCL was tested using a cotton bud rub-off test. Ten lenses of each brand of lenses were used. Each lens was removed from its blister pack and placed

on the cleaned surface of an electronic scale to allow monitoring of the force applied when each lens was rubbed to ensure consistency of force applied (applied force between 110 – 230g) for all lenses (Figure 3.1). The applied force was determined by simulating the force which would be applied when cleaning the contact lenses with a finger. Any pigment coming off the lens surface was determined by examining the tip of the cotton bud for pigment transfer after every rub. A maximum of 20 rubs was applied to a lens for each rub-off test.

The front surface of each lens was rubbed first. If there was pigment transfer before 20 rubs, the lens was recorded as failing the rub-off test and the number of rubs recorded. If there was no pigment transfer to the cotton bud after 20 rubs, the procedures were repeated on the back surface. Any lens which did not show pigment transfer to the cotton bud after 20 rubs on either surface was recorded as passing the rub-off test.

Table 3.1 Cosmetic contact lenses used for the rub-off test

Lens	Brand	Company	Manufacturing origin	Purchased from	Colour printing / process*
A	1 Day ACUVUE® DEFINE™	Johnson and Johnson	United States	Retail store	Sandwiched
B	Barbie Eye	Distributor: Star Plus Co.	Korea	Internet	INA
C	Sweety Eye	Unknown	Korea	Cabinet store	INA
D	Tutti Circle	Bescon	Korea	Internet	INA
E	Magic Color	GEO Medical	Korea	Cabinet store	INA

* – according to information provided by the manufacturer or distributor; INA – information not available

Figure 3.1 Rubbing the front surface of a cosmetic contact lens with a wetted cotton bud on an electronic balance

3.3 Results

The results of the rub-off tests are shown in Table 3.2. Only one CCL was found to have no pigment coming off after repeated rubs with a wetted cotton

bud. The other CCL all showed pigment had been transferred to the cotton bud with multiple rubs.

Table 3.2 Rub-off test results

Lens	Surface	Number of rubs before pigment came off	Number of lenses
A	N/A	No pigment came off	10
B	Back	1 2 3	3 4 3
C	Back	1 2 3	3 3 4
D	Front	1 2 3	4 5 1
E	Back	2 5 6 7	4 4 1 1

3.4 Discussion

This pilot study utilized a simple standardized method to determine the location of the pigments in CCL. Although the technique does have a limitation in that the cotton bud is rougher than human skin, the primary aim of the experiment was to determine if the pigments were present on the surface of the lenses. The force applied by the cotton bud was gentle enough, to mimic the force that patients may apply when rubbing contact lenses when cleaning their lenses and was monitored by use of an electronic scale to provide standardization. The results indicated that not all CCL were of

sandwich design or had the pigments embedded in the material. For most lenses which failed the rub-off test, pigment transfer to the cotton bud was noted after less than 10 gentle rubs on the surface (Figure 3.2). This suggests that the pigment is not firmly attached to the lens material and could detach into the eye during wear potentially causing problems and sensitivity.

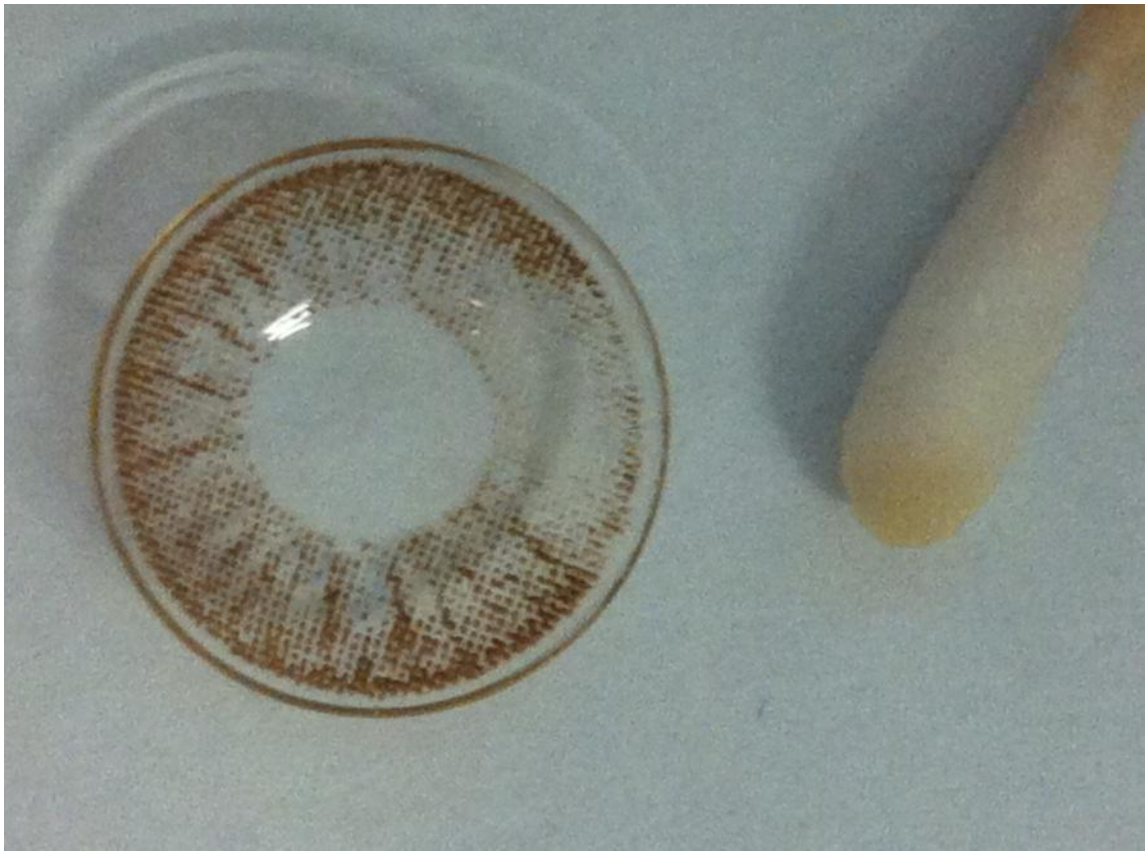


Figure 3.2 Pigments of a cosmetic contact lens transferred to a cotton bud during the rub-off test

Pigment location and the surface smoothness of CCL have been investigated by Mayers and co-workers (2013) (Section1.2.4). They used scanning electron microscopy to capture cross-sectional images of CCL to reveal the

location of the pigment. Lens A used in this study was also tested in their study and showed that the pigment was sandwiched inside the lens material. The result of our pilot study is in agreement with their findings as Lens A passed our rub-off test. They also tested the surface roughness of CCL with atomic force microscopy and reported that CCL with surface pigments have a rougher surface than CCL with embedded or sandwiched pigments. The five CCL brands used comprised three annual replacement type, one each daily disposable and monthly disposable lenses. With repeated use, CCL behave similarly to other contact lenses in that the denatured proteins from the tears become deposits and accumulate on the lens surface (Bhatia et al., 1997; Lira et al., 2008; Santos et al., 2008), which in turn affect the lens comfort (Jones et al., 1996; Pritchard et al., 1996).

Our results suggested that some CCL have the pigments printed on the surface of the lens, either on the front or back surface. The pigments would be in direct contact with the papillary conjunctiva if they are on the front surface, or the cornea if the pigments are on the back surface. This may lead to issues of the comfort and ocular irritation during CCL wear. The pigments on the surface may also affect the surface smoothness which affects both comfort (Jones et al., 1996; Pritchard et al., 1996) and microbial adhesion (Bruinsma et al., 2002; Giraldez et al., 2010; Vermeltfoort et al., 2004, 2006; Tran et al., 2012; Bos et al., 1999; Packham, 2003) as found in clear contact lenses (Section 1.4). Thorough investigations on surface pigments of CCL with respect to these issues are warranted.

3.5 Conclusion

This pilot study provided a simple and indirect method to determine the pigment location of CCL.

Conference presentation

Cho P, Chan KY. Permanency of pigments on colored contact lenses – A pilot study. Poster presentation at *The 8th Asia Cornea & Contact Lens Conference* 2012 at Hong Kong, China *(Abstract book P.35)*

Chapter 4

Microbial adherence to cosmetic contact lenses

4.1 Introduction

In recent years, CCL have become increasingly popular in Asian countries and the compliance of CCL users with care regimes has become a concern to practitioners (Section 1.1.3). Many CCL users choose to purchase their CCL over the internet or from other unlicensed retail outlets and are either not given the appropriate advice or neglect the importance of contact lens care (Section 1.1.4). As a consequence of this, even plano CCL are now classified as medical devices by Medicines and Healthcare Products Regulatory Agency in the United Kingdom (British Contact Lens Association, 2013), United States (US Food and Drug Administration, 2013), China (State Food and Drug Administration, 2013) and Korea (Personal communication). The safety and the effectiveness of these lenses are overseen by the FDA or the equivalent in other countries. However, in Hong Kong, contact lenses are not classified as medical devices and are not subject to registration prior to marketing. Whilst, practitioners in Hong Kong are not allowed to supply contact lenses to patients without conducting eye examinations or without valid prescriptions, the law only applies to licensed optometrists. Patients

may purchase contact lenses, including CCL, from the internet, in other retail outlets or from cabinet stores (Section 1.1.4). There are a number of reports of infectious keratitis in the literature associated with the use of CCL (Singh et al., 2012; Sauer et al., 2011; Snyder et al., 1991; Steinemann et al., 2003, 2005) (Section 1.3) and most of the patients in these reports obtained their contact lenses without having any proper contact lens fitting procedures, or receiving any contact lens handling guidelines from licensed eye care professionals. Sauer and co-workers (2011) reported that patients who had worn CCL and developed microbial keratitis were usually relatively young and new to contact lens wear. Patients who purchased contact lenses (not exclusively CCL) via the internet have been shown to be less compliant with regard to the use and care of contact lenses such as having eye examinations at least once a year (Fogel and Zidile, 2008). Stapleton and co-workers (2008) also found an increased risk of microbial keratitis in patients purchasing lenses on the internet.

The quality of CCL is another concern which requires attention. The pilot study in Chapter 3, which aimed to determine the location of the pigments using a standardized rub-off test, showed that only one of five commercially available brands tested demonstrated permanency of pigment on the lens. Lenses with pigments easily rubbed off were obtained either from cabinet stores or the internet and the only brand that passed the test was purchased from an optical shop.

Because of the increasing popularity of CCL, there is an urgent need to determine the safety of these lenses. As discussed in Section 1.4, to date, most of the literature on microbial adherence to contact lenses concerns adherence to hydrogel (Dart and Badenoch, 1986; Miller and Ahearn, 1987; Miller et al., 1988; John et al., 1989; Boles et al., 1992; Taylor et al., 1998; Williams et al., 1998; Bruinsma et al., 2001, 2002; Garcia-Saenz et al., 2002; Williams et al., 2003; Kodjikian et al., 2008; Giraldez et al., 2010) or silicone hydrogel contact lenses (Kodjikian et al., 2008; Vermeltfoort et al., 2004; Zhang et al., 2005; Henriques et al., 2005; Vermeltfoort et al., 2006; Santos et al., 2007, 2008; Subbaraman et al., 2011; Babaei Omali et al., 2012; Burnham et al., 2012; Tran et al., 2012; Vijay et al., 2012). The aim of the study reported in this chapter was to investigate microbial adherence to new unused CCL.

4.2 Experimental design

4.2.1 Contact lenses

Fifteen brands of CCL (Lenses A – Q) were tested. Samples of 12 brands were purchased from optical shops from registered optometrists, two were purchased from cabinet stores (Section 1.1.4) and one was purchased on the internet (Table 4.1).The colour of these lenses was limited to Brown or Hazel if available. Clear counterparts of Brands A, B, and C were also studied. These were of the same material and water content as Brands A, B, and C (not taking the manufacturing process into account).

Table 4.1 Properties of the contact lenses

Purchased from	Lens	Product Name	Company	FDA group	Material	Dk	Water content (%)	Color	Color printing/ Process*
Optical shop	A1	Tutti Circle Color	Bescon	I	Polymacon	8.4	38	Brown	INA
	A2	Ultraflex 38	Cooper Vision	I	Polymacon	8.4	38	Clear	N/A
	B1	Freshlook Illuminate	Ciba Vision (now Alcon)	II	Nelfilcon A	26	69	Rich Brown	Embedded
	B2	Dailies AquaComfort Plus	Ciba Vision (now Alcon)	II	Nelfilcon A	26	69	Clear	N/A
	C1	1 Day ACUVUE® Define	Johnson & Johnson	IV	Etafilcon A	28	58	Vivid	Sandwich process
	C2	1 Day ACUVUE® Moist	Johnson & Johnson	IV	Etafilcon A	28	58	Clear	N/A
	D	Perfect Eyes Big Eye Color	Unicon Company	I	HEMA/ MAA	20.5	42	Party Brown	INA
	E	One Day Delight Max HydrationPlus	St Shine Optical	I	Filcon I	12.8	42	Brown	Embedded
	F	One Day Delight Max2 HydrationPlus	St Shine Optical	I	Filcon I	12.8	42	Hazel	Embedded

Table 4.1 Properties of the contact lenses (Con't)

Purchased from	Lens	Product Name	Company	FDA group	Material	Dk	Water content (%)	Color	Color printing/ Process*
Optical shop	G	One Day Delight Max3 HydrationPlus	St Shine Optical	I	Filcon I	12.8	42	Chestnut Brown	Embedded
	H	Lacelle	St Shine Optical	I	Hefilcon A	11	42	Tender Brown	Embedded
	J	Lacelle Color	St Shine Optical	I	Hefilcon A	11	42	Sparkling Gold	Embedded
	K	Freshlook One-day	Ciba Vision (now Alcon)	II	Nelfilcon A	26	69	Pure Hazel	Embedded
	L	aquaSoft Color 1 Day	Unicon Optical	II	HEMA/ MMA	21	55	Brown	INA
	M	Crystal-i 1 Day	E & E Optics (HK)	II	HEMA	8.4	38	Brown	INA
Cabinet store	N	Magic Color	GEO Medical	I	pHEMA	INA	42	Brown	INA
	P	Neo Cosmo	Neo Vision Co. Ltd	I	pHEMA	INA	45	Brown	INA
Internet	Q	Freaky	INA	INA	INA	INA	INA	UV Glowing Blue	INA

* – according to information provided by the manufacturer or distributor; NA – not applicable; INA – information not available

4.2.2 Rub-off test

Before the commencement of the study, the standardized rub-off test described in Chapter 3 was performed and used to determine if the pigments of a CCL were coated on the lens surface or sandwiched. Five lenses of each brand of lenses were used. The rub-off test was performed on all CCL.

4.2.3 Bacterial suspension

A new set of each of the 15 brands of lenses (five lenses of each brand) was challenged with *Pseudomonas aeruginosa* ATCC 9027. A1, B1, and C1 lenses and their clear counterparts (A2, B2, C2) were also challenged with *Staphylococcus aureus* ATCC 6538 and *Serratia marcescens* ATCC 13880. The bacterial strains were those recommended by the ISO14729 to be used for testing efficacy of contact lens disinfection solutions. Nutrient agar plates were used for the cultivation of bacterial strains. A single bacterial colony from the agar plate was cultured in 10mL Tryptone Soya Broth overnight at 37^{o}C in ambient air for 24 hours. The cells were then harvested by centrifugation (CR 4-12, Jouan Inc, Winchester, VA) for 10 minutes (2000g at room temperature). The supernatant was discarded and the pellet was washed in sterile PBS twice before they were resuspended in PBS. The concentration of each inoculum was adjusted spectrophotometrically (Spectronic 20 Gensys Visible Spectrophotometer, Spectronic Instruments Inc, Rochester, NY)

to give an optical density of 0.10 at 660nm which is approximately equivalent to 10^8 colony forming unit (CFU) mL^{-1}.

4.2.4 Bacterial adherence

Lenses were incubated in bacterial suspension immediately after they were removed from the blister packs or storage vials. The new, sterile contact lenses were transferred with sterile forceps and into 2mL of 10^8 CFU mL^{-1} suspension and incubated at 37^{o}C for 24 hours on a plate shaker at 125rpm.

4.2.5 Enumeration of viable micro-organisms

After 24 hours, the lenses were removed aseptically and rinsed gently with 4mL PBS to remove loosely attached micro-organisms before being transferred to bijou bottles containing 10mL sterile PBS. Each lens was then vortexed vigorously for one minute to remove the adhered micro-organisms and a 0.1mL aliquots of neat and diluted extracts were plated out on nutrient agar plates and spread evenly using a sterile glass hockey stick. All plates were incubated at 37^{o}C for 24 hours and the organisms which had adhered to the lenses were enumerated using an automated colony counter (aCOLyte colony counter, Synbiosis, Frederick, MD, USA) with the plate giving a count between 30 and 300 colonies being used.

4.2.6 Treatment of data

Kruskal-Wallis tests, followed by Mann-Whitney U tests with corrections for multiple comparisons, were used to evaluate differences in microbial adherence among CCL. Mann-Whitney U tests were used to assess differences in microbial adherence between A1, B1, and C1 lenses and their own clear counterparts. A p-value of less than 0.05 was considered statistically significant. Friedman test, followed by post-hoc Wilcoxon signed rank tests, were used to compare the microbial adherence between the three bacterial strains in A1 and A2 lenses. A p-value of less than 0.017 was considered statistically significant.

4.3 Results

Figure 4.1 shows the microbial adherence of all CCL challenged with *P. aeruginosa*. The amount of bacterial adherence varied between different brands of lenses. CCL which failed the rub-off test showed significantly higher levels of *P. aeruginosa* adherence ($8.7x10^5$ - $1.9x10^6$ CFU/lens) than CCL which passed this test ($p<0.01$). Microbial adherences to B1 and C1 lenses were at least six times less than those of other lenses tested.

The results of the rub-off tests are shown in Table 4.2. Only B1 and C1 lenses had pigments that did not rub off easily.

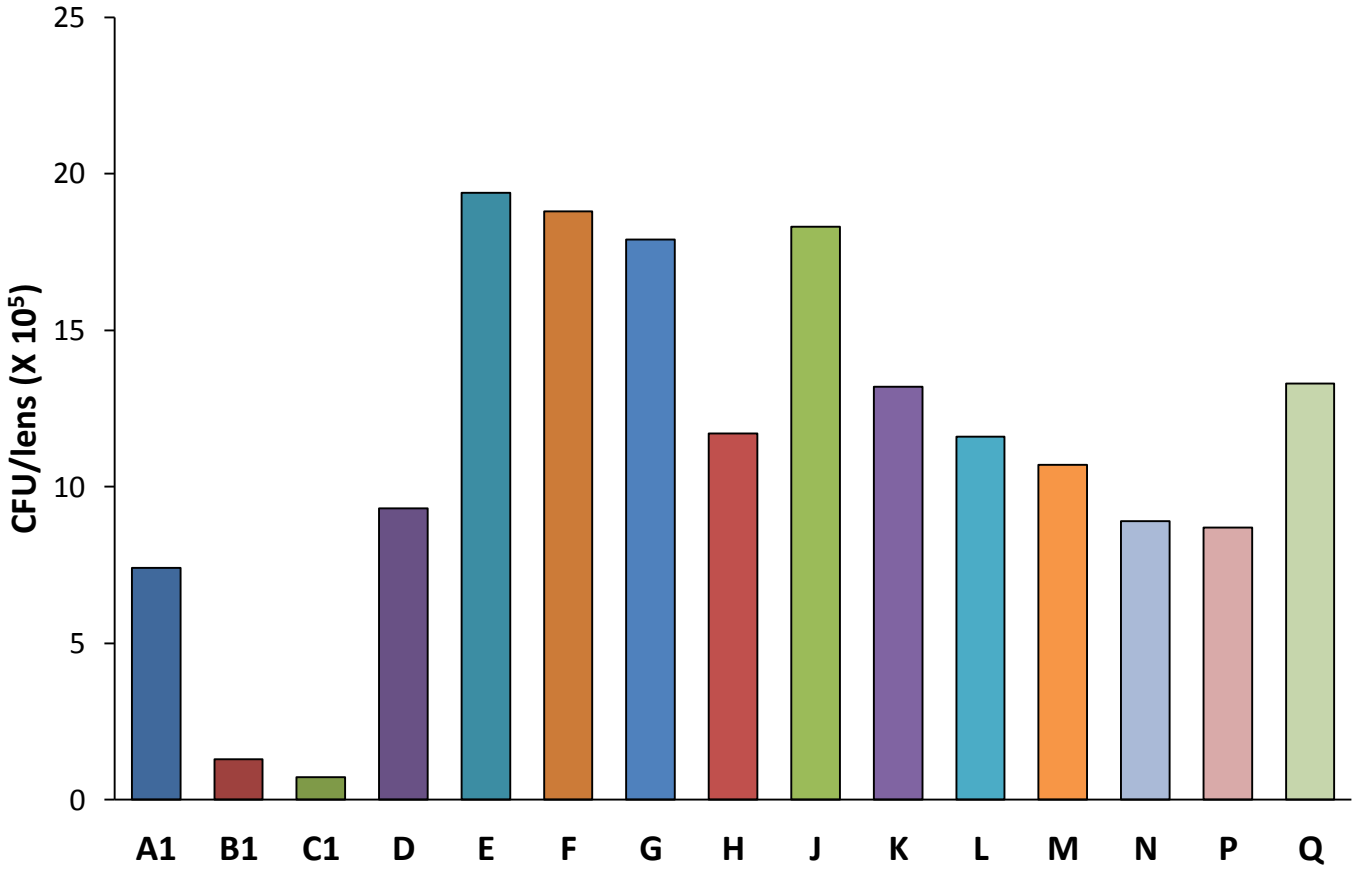


Figure 4.1 Adherence of *Pseudomonas aeruginosa* to different types of cosmetic contact lenses

Table 4.2 Results of the rub-off test on cosmetic contact lenses

Lenses	A1	B1	C1	D	E	F	G	H	J	K	L	M	N	P	Q
Pass		x	x												
Fail	x			x	x	x	x	x	x	x	x	x	x	x	x
Pigment	F	--	--	F	B	B	B	B	B	F	F	F	B	F	F

F – Front surface; B – Back surface

Figures 4.2, 4.3 and 4.4 show the results of microbial adherences of *P. aeruginosa*, *S. aureus,* and *S. marcescens* to A1, B1, and C1 lenses and their clear counterparts respectively. A1 lenses showed significantly higher amounts of microbial colonization than A2 lenses, their clear counterparts, for all bacterial species. However, no significant differences in the amount of microbial adherence were observed between B1 and C1 lenses and their clear counterparts (B2, C2) for all strains of micro-organisms (Table 4.3). Significant differences in adherence of the three bacterial strains to A1 lenses were observed ($p=0.015$). Post-hoc tests indicated that the amount of adhesion of *S. marcescens* on A1 lenses was significantly higher than for the other two bacteria ($p=0.014$).

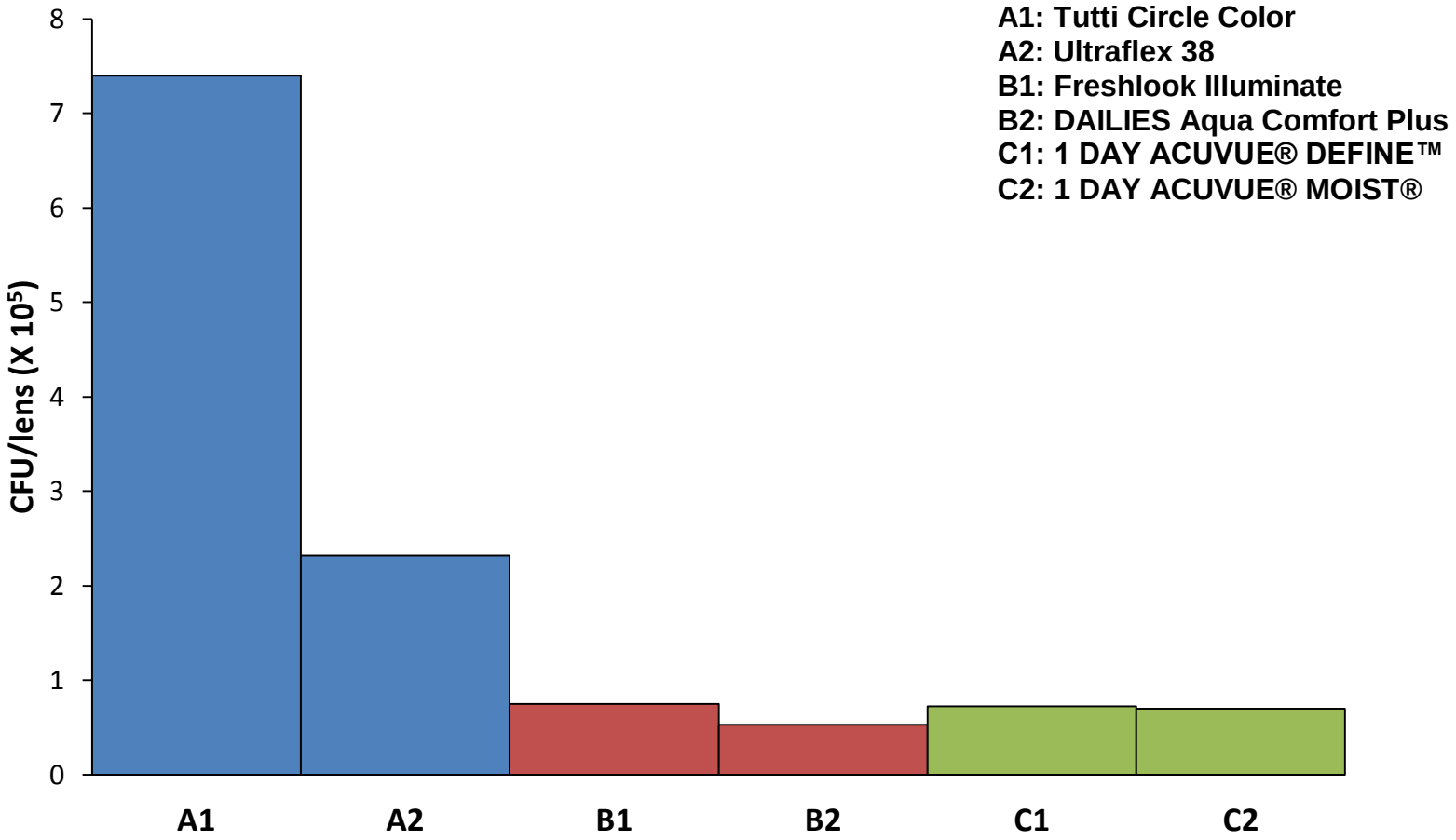


Figure 4.2 Adherence of *Pseudomonas aeruginosa* to three brands of cosmetic contact lenses and their clear counterparts

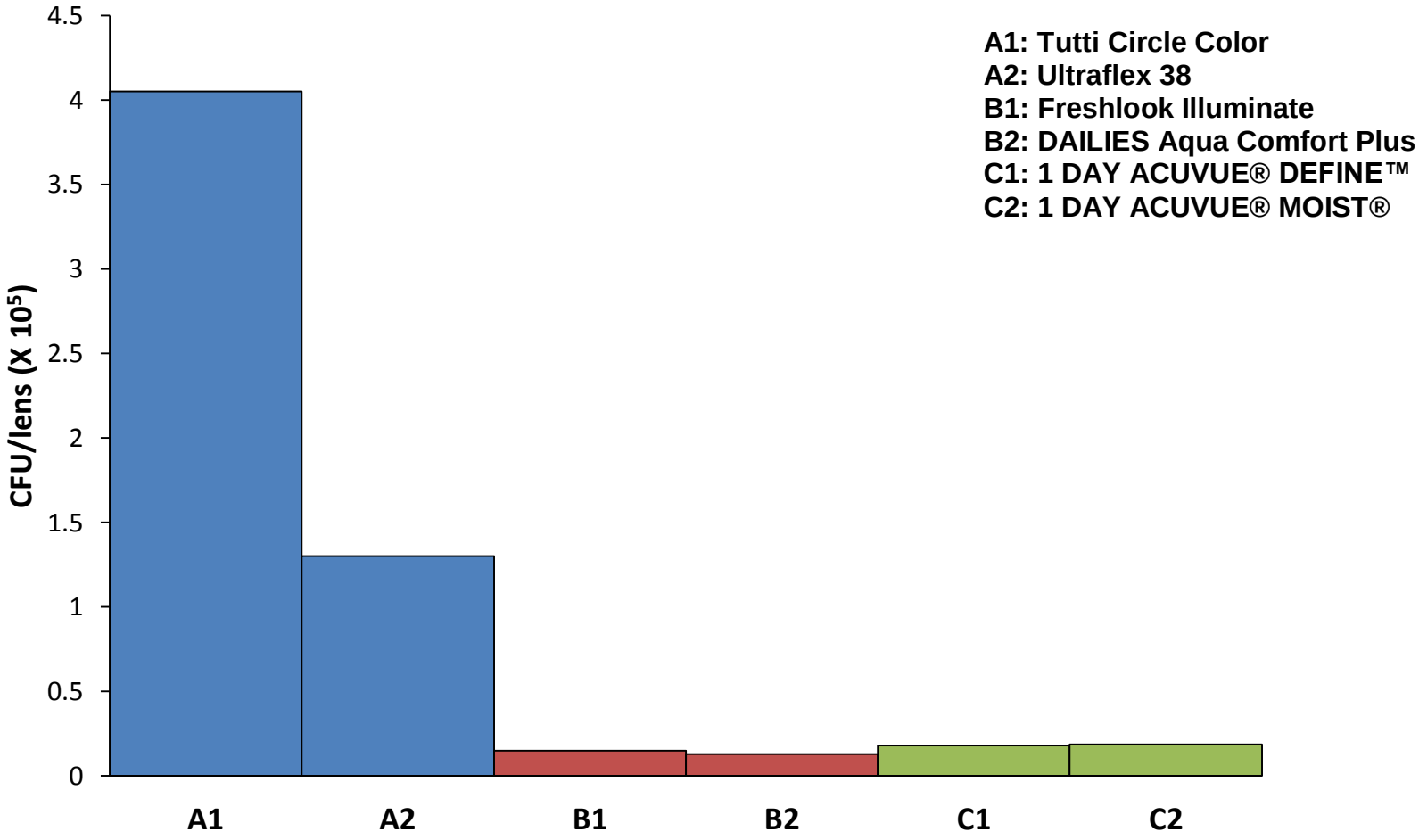


Figure 4.3 Adherence of *Staphylococcus aureus* to three brands of cosmetic contact lenses and their clear counterparts

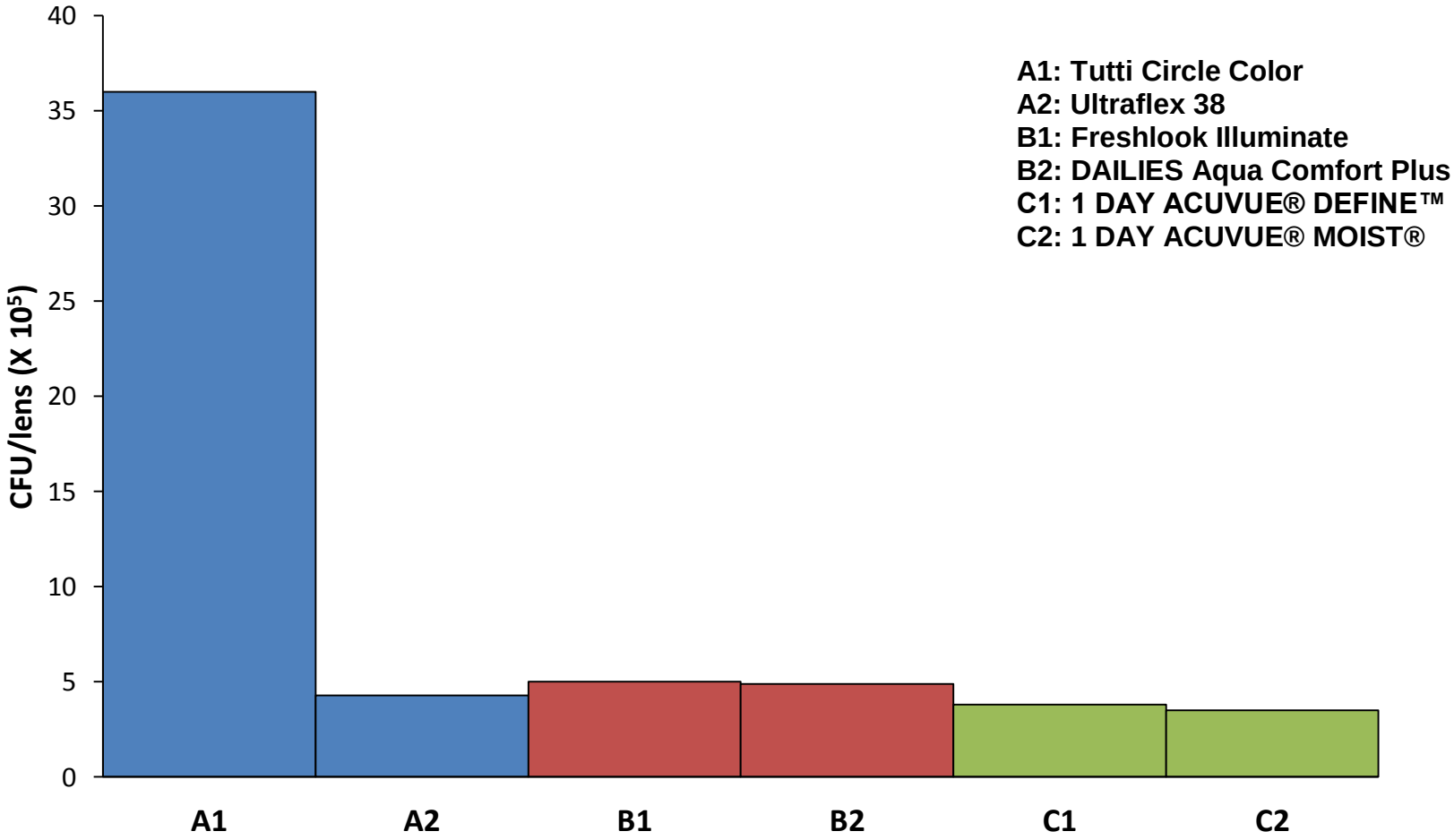


Figure 4.4 Adherence of *Serratia marcescens* to three brands of cosmetic contact lenses and their clear counterparts

Table 4.3 Microbial adherence of micro-organisms to three brands of cosmetic contact lenses and their clear counterparts

Micro-organisms	Contact lenses		CFU/lens (Median [Range]) ($x10^5$)	P value
ATCC 9027 *Pseudomonas aeruginosa*	Group A A1	Tutti Circle Color	7.40 [5.8 – 25.80]	**0.009***
	Group A A2	Ultraflex 38	2.32 [1.04 – 2.96]	
	Group B B1	Freshlook Illuminate	1.29 [0.75 – 1.69]	0.602
	Group B B2	DAILIES Aqua Comfort Plus	1.17 [0.53 – 1.41]	
	Group C C1	1 DAY ACUVUE® DEFINE™	0.72 [0.51 – 1.23]	0.600
	Group C C2	1 DAY ACUVUE® MOIST®	0.70 [0.46 – 1.52]	
ATCC 6538 *Staphylococcus aureus*	Group A A1	Tutti Circle Color	4.05 [2.15 – 8.50]	**0.028***
	Group A A2	Ultraflex 38	1.30 [0.29 – 2.50]	
	Group B B1	Freshlook Illuminate	0.15 [0.06 – 0.23]	0.402
	Group B B2	DAILIES Aqua Comfort Plus	0.13 [0.10 – 0.19]	
	Group C C1	1 DAY ACUVUE® DEFINE™	0.18 [0.07 – 0.37]	0.917
	Group C C2	1 DAY ACUVUE® MOIST®	0.19 [0.15 – 0.47]	

Table 4.3 Microbial adherence of micro-organisms to three brands of cosmetic contact lenses and their clear counterparts (Con't)

Micro-organisms	Contact lenses			CFU/lens (Median [Range]) ($x10^5$)	P value
ATCC 13880 *Serratia marcescens*	Group A	A1	Tutti Circle Color	36.00 [30.00 – 42.00]	**0.009***
		A2	Ultraflex 38	4.30 [4.10 – 6.40]	
	Group B	B1	Freshlook Illuminate	5.00 [3.50 – 11.60]	0.602
		B2	DAILIES Aqua Comfort Plus	4.90 [4.20 – 6.30]	
	Group C	C1	1 DAY ACUVUE® DEFINE™	3.80 [3.00 – 4.70]	1.000
		C2	1 DAY ACUVUE® MOIST®	3.50 [3.00 – 7.10]	

P value - Probability values of Mann-Whitney tests for differences between cosmetic contact lenses and their clear counterparts

* indicates significance

4.4 Discussion

Because of the easy accessibility of CCL, they are now commonly used by adolescents to change their eye colour or appearance (Section 1.1.1). Although there are reports of infectious keratitis associated with wearing over-the-counter CCL (Singh et al., 2012; Snyder et al., 1991; Steinemann et al., 2003, 2005), no detailed evaluation of the safety of CCL has been published (Section 1.3). As mentioned earlier in this chapter, reports of severe complications associated with CCL in the last few years have resulted in some countries, including United Kingdom and China, stepping up their regulation on CCL (British Contact Lens Association, 2013; State Food and Drug Administration, 2013).

Microbial adherence is a method to evaluate the susceptibility of a contact lens to microbial colonization (Section 1.4). Microbial adherence between CCL and clear hydrogel lenses should not be different if the pigments in a CCL are embedded or sandwiched in the material. However, in our pilot study (Chapter 3), we demonstrated that the pigments of many CCL could be easily rubbed off using wetted cotton buds and most of these lenses were sold in cabinet stores or on the internet. Many CCL, including Halloween contact lenses (CCL used at Halloween to produce dramatic eye effects), can be purchased on the internet and most of these lenses are not daily disposable lenses and are therefore likely to be re-used. It is also of particular concern that users of such lenses are relatively less compliant (Fogel and Zidile, 2008). The presence of the pigment in these lenses may

increase the ease of attachment or adherence of deposits and micro-organisms. It may also increase the mechanical irritation to the palpebral conjunctiva if the pigment is on the front surface of the lens, hence, increasing the risk of contact lens associated complications. Willcox and co-workers (2010b) also suggested that corneal erosion and bacteria on contact lenses may contribute to the development of microbial keratitis. Awareness of increased propensity for microbial adherence of such lenses would be useful for practitioners and users. To our knowledge, this is the first study conducted on microbial adherence of CCL.

The rub-off test was used as an indirect method used to confirm whether the pigment of the CCL was embedded in the lens material. The concept of the embedded or sandwich design of CCL is to avoid direct contact of the pigment with the cornea or the eyelid (Section 1.1.2). Most brands of CCL tested failed the rub-off test in the current study. The results of the rub-off test did not support some manufacturers' claim of embedded or sandwich design (see Table 4.2). Peeling off of the pigment layer (Figure 4.5) and pigment transfer during the rub-off test (Figure 4.6) were observed for some of the lenses which claimed to have used an embedded design.

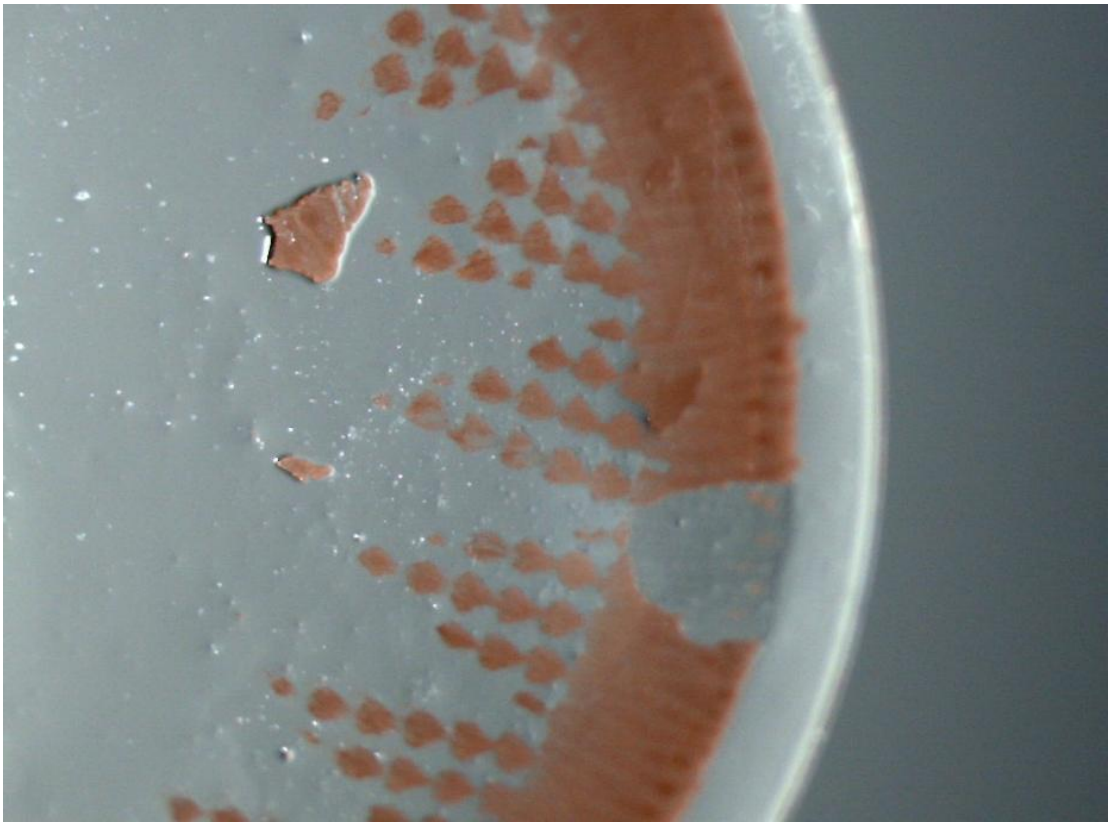


Figure 4.5 Colour pigment peeling from a cosmetic contact lens after gentle rubbing with a wetted cotton bud

Figure 4.6 Pigments transferred to the wetted cotton bud after rubbing 20 times

Microbial keratitis can be caused by various pathogenic micro-organisms. Coagulase-negative *Staphylococcus* and *P. aeruginosa* are the two most frequently isolated organisms, followed by *S. aureus* (Houang et al., 2001; Green et al., 2008; Wong et al., 2011). In this study, it was observed that lenses which failed the rub-off test had higher adherence levels than lenses which passed the test. The replacement frequency of most of the CCL tested in this study was daily (except for A, N, P and Q lenses) and it may be argued that the adherence of bacteria to daily disposable lenses is not of concern as they should be discarded after use. However, previous studies have reported that daily disposable contact lenses did not reduce the risk of

contact lens associated microbial keratitis (Stapleton et al., 2008; Dart et al., 2008) and that many wearers will reuse their lenses (Boost et al., 2011). There have been several reports of microbial keratitis with CCL associated with reuse, improper or overwear of CCL (Singh et al., 2012; Steinemann et al., 2003, 2005). Hence, the importance of microbial adherence to daily disposable CCL should not be underestimated.

Our results highlight the importance of embedded or sandwich design for the colour additives of CCL. These designs avoid direct contact of the colour additives with the cornea or the lids and also provide a smoother surface which can reduce microbial adherence.

Comparing the levels of microbial adherence of A1, B1, and C1 lenses to their clear counterparts demonstrated that microbial adherences of all bacterial strains studied were higher in lenses having the pigments on the lens surface (i.e. failed the rub-off test) whereas CCL with embedded pigments demonstrated no significant difference compared to their clear counterparts. Among the three bacterial strains, *S. marcescens* showed the highest adherence level to CCL with surface pigments, followed by *P. aeruginosa*. Since lens material and water content level were controlled, this indicates that the pigments were most likely the factor leading to the higher microbial adherence.

As mentioned above, surface pigments could increase the surface roughness of the CCL. Previous studies have suggested that surface roughness may

have an effect on bacterial adhesion (Bruinsma et al., 2002; Giraldez et al., 2010; Vermeltfoort et al., 2004, 2006; Tran et al., 2012; Bos et al., 1999; Packham, 2003) (Section 1.4.3). Although most reported that lenses with higher surface roughness would increase bacterial adherence, Vijay and co-workers (2012) found an inverse correlation between surface roughness and *P. aeruginosa* adherence. The role of some roughness parameters of a contact lens still remains unclear (Giraldez et al., 2010).

In the current study, all the CCL tested were new, unused, hydrophilic hydrogel lenses. The replacement frequency of some of these CCL is monthly or yearly. The adherence rate of micro-organisms to worn CCL is not known but it has been suggested that protein deposition increases after repeated use of contact lenses (Solomon et al., 1996; Ilhan et al., 1998). A correlation between bacterial colony counts and protein concentration has been reported (Barr et al., 1988) and lenses soaked in artificial tear fluid also showed increased bacterial adherence (Willcox et al., 2001). Deposits have been found to decrease the lens wettability (Jones et al., 1996) and the addition of a surface coat of pigment can render the originally smooth surface rough (Mayers et al., 2013). Protein deposition on lens surfaces favours the growth of bacteria and enhances microbial adherence (Miller et al., 1988; Taylor et al., 1998; Santos et al., 2008; Subbaraman et al., 2011; Butrus and Klotz, 2009). Further investigation of microbial adherence to worn CCL and adherences of fungi and *Acanthamoeba* to CCL are warranted.

4.5 Conclusion

Our study showed that CCL with pigments printed on the surface resulted in significantly higher bacterial adhesion.

Journal article

Chan KY, Cho P, Boost MV. Microbial adherence to cosmetic contact lenses. *Contact Lens & Anterior Eye*. 2014; 37(4): 267-272

Conference presentation

Chan KY, Cho P, Boost MV. Microbial adherence to cosmetic contact lenses. Paper presentation at *The 37th British Contact Lens Association Clinical Conference & Exhibition*, 6-9 June 2013, at Manchester, United Kingdom

Award received

Da Vinci Award 2013, British Contact Lens Association

Chapter 5

Evaluation of repeatability of corneal epithelial cell viability of the porcine eye model and cytotoxic effects of leachates from cosmetic contact lenses on porcine eyes

5.1 Introduction

MPS system is the most common choice of contact lens disinfection method (Morgan et al., 2011). Corneal epithelial damage has been reported after using MPS suggesting that these solutions exhibit cytotoxic effects on cells. Some clinical studies have shown significantly more corneal staining (SICS) in patients using particular MPS and hypothesized that the active ingredients caused toxic effects, resulting in corneal staining (Jones et al., 1997, 2002; Andrasko and Ryen, 2008) (Section 1.6). Some studies have reported that the corneal staining observed in these patients may not be a true desiccation of cells but due to the high affinity association between fluorescein and the active ingredients (Bright et al., 2012) (Section 1.6).The phenomenon is known as ‘preservatives associated transient hyper-fluorescence (Efron,

2013). However, Gorbet and co-workers (2014) disagreed and found an association between increased epithelial cell shedding and SICS in a lens-solution combination, suggesting that the staining observed was not transient in nature as previous studies had suggested (Bright et al., 2012; Efron, 2013). There is still much debate regarding the relevance of SICS and no firm conclusion has been drawn yet.

Owing to the subjectivity of clinical assessment of corneal integrity, a more sophisticated and objective method to evaluate ocular toxicology is required. Biochemical methods with the use of animal eyes, animal cells, human conjunctival cell line or HCEC have been employed to investigate the cytotoxic effect of MPS alone (Section 1.6.1) or of contact lenses pre-soaked in MPS (Section 1.6.2) (Mowrey-McKee et al., 2002; Santodomingo-Rubido et al., 2006; McCanna et al., 2008; Chuang et al., 2008; Choy et al., 2009; Dutot et al., 2010; Gorbet et al., 2010; Powell et al., 2010; Tanti et al., 2011; Choy et al., 2012, 2013). Most of these studies revealed higher cytotoxic effects of polyquaternium-1 and Aldox-based MPS on corneal epithelial cells.

Some studies have included contact lenses in their investigation of solution cytotoxicity because different lens materials can have different solution uptake rates during the daily disinfecting process and release rates during lens wear (Powell et al., 2010; Vaughan and Porter, 1993). The porous nature of soft contact lenses means that they have the potential to uptake solution preservatives or other buffering ingredients and subsequently release them into the eye upon lens wear (Chapman et al., 1990; Rosenthal

et al., 2006). Tanti and co-workers (2011) found a lens effect in the mechanism of solution cytotoxicity. However, as discussed previously (Section 1.6), the effect of blinking and lacrimation could not be taken into consideration in these studies as they used an *in vitro* model. This chapter described a study using *ex vivo* PEM which incorporates simulated blinking and lacrimation, to investigate the effect of solution cytotoxicity released from CCL pre-soaked in MPS.

However, in order to compare CCL and clear lenses under the same conditions and to include simulated lacrimation and blinking, the PEM developed by Choy and co-workers (2004) needed modification. They tested the repeatability of a PEM based on fluorescein staining. Although the viability of the epithelial cells using fluorescein was found to be repeatable, the sensitivity of fluorescein grading was subject to examiner bias. Later, Choy and co-workers (2009) reported that fluorescein staining did not correlate well with the results from flow cytometry in their study of cytotoxicity of ophthalmic solutions. In view of this, trypan blue exclusion test, a fast and inexpensive standard test, was used for identification of dead cells in this experiment. A major limitation in the PEM developed by Choy and co-workers (2004, 2006, 2008) is the limited number of PEM that could be set up at any one time. Due to the particular parts used, it was only possible to set up two PEMs at any one time and some of the integral parts were longer in production preventing the development of additional testing set ups. Hence, it was necessary to develop a new PEM design that would allow more PEM to be set up at any one time, and with better control of the ambient

temperature and humidity to allow the PEM to be used for study of cytotoxic effects of CCL.

Before the use with CCL it was necessary to investigate the repeatability of the cell viability of the new PEM using trypan blue exclusion test.

5.2 Experimental design

5.2.1 Porcine eye preparation

Porcine eyes were obtained from a local abattoir in Hong Kong. When the pigs were killed, the eyes were enucleated with the lids and conjunctiva intact. The lids were closed and taped so that the corneas were not exposed to the atmosphere during the transportation from the abattoir to the laboratory. The eyes were kept in a cool environment and transported to the laboratory within an hour. Upon arrival, the eyes were rinsed with DPBS (Sigma-Aldrich Co., St. Louis, USA) and the integrity of the corneas was examined with the slit lamp biomicroscope (Topcon SL-2D, Tokyo, Japan) after instillation of fluorescein from saline-wetted fluorescein strips (Contacare Ophthalmics & Diagnostics, Vadodara, Gujarat,India). Corneas with more than Grade 1 corneal staining or with any corneal abnormality such as abrasion were discarded.

Part 1: Validation

5.2.2 Porcine eye model set up

A total of 57 eyes were used (11 eyes for each test PEM and controls). Nine eyes were discarded because of poor corneal condition such as infiltrates or >Grade 2 (Efron scale(1998)) staining. The porcine eyes were then prepared by removing the lids and the surrounding tissues of the eyeball (except for the nictitating membrane and the bulbar conjunctiva). The eyes were mounted on a plastic platform with the cornea facing up. The nictitating membrane was held by a movable arm connecting to a motor to simulate blinking. An infusion wing was set directly above the cornea (Figure 5.1) so that DPBS could be regularly applied to the superior limbal region to simulate lacrimation. Both the blinking rate and the DPBS administration time were adjustable and controlled by a software program, so that they worked simultaneously in a default manner, lacrimation followed by blinking. The lacrimation-blink interval was set at 60s.

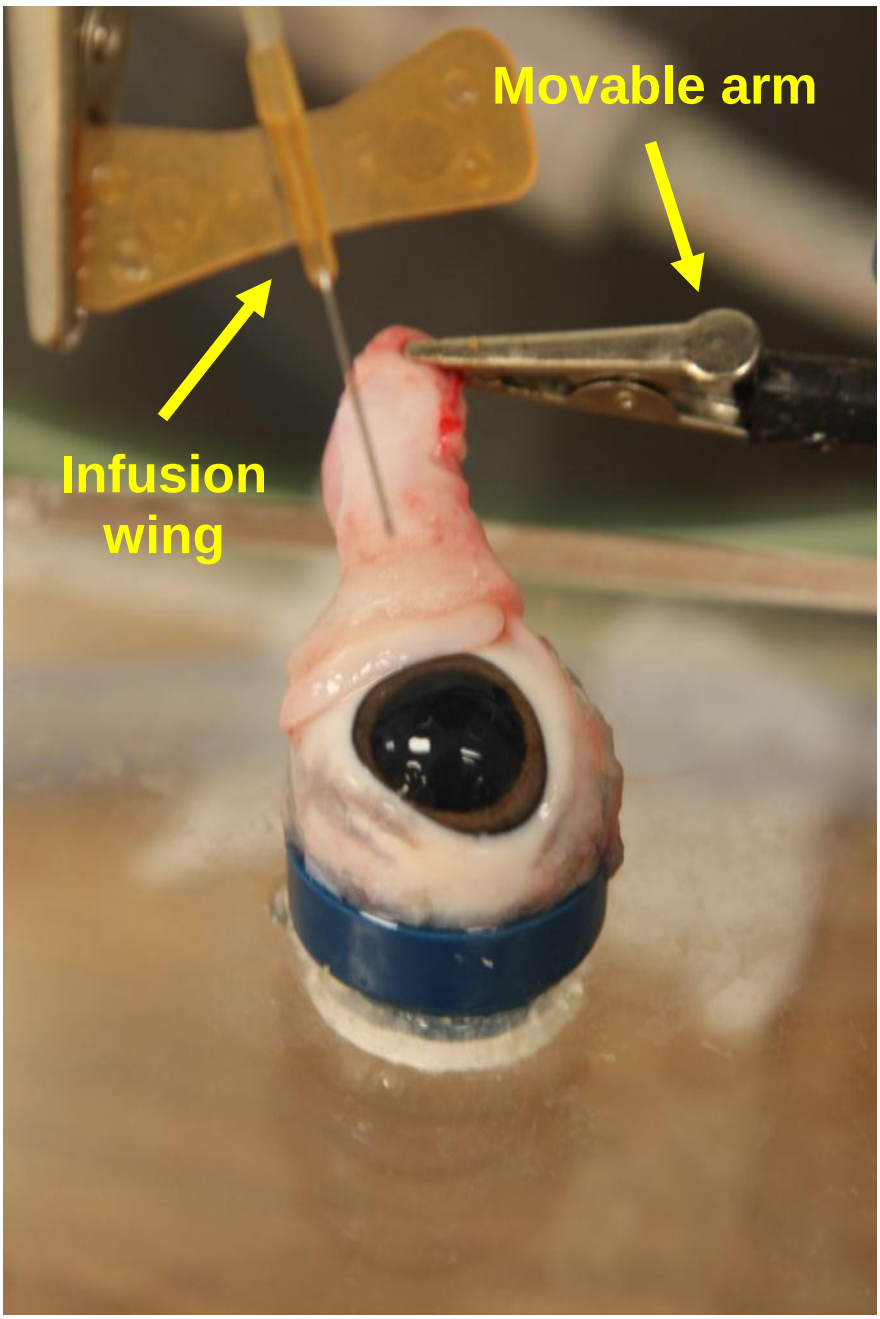


Figure 5.1 Porcine eye model with simulation of lacrimation and blinking

Six eyes were used to set up the test PEMs and controls each day and experiments were conducted on eleven days over one month. One eye was dissected immediately on arrival (‘Immediate’ control)

and assessed with trypan blue solution for cell viability using the same procedures as the test eyes (described in Section 5.2.3). Four eyes were used to set up the test PEMs (Sets A-D) and they were mounted with the set up allowing simulation of 'blinking' and 'lacrimation'. The remaining eye was left exposed to the air (i.e. no treatment) until the end of the experiment ('Delayed' control). The four test PEMs and the Control A PEM were kept in an air-sealed/closed acrylic container to maintain a constant temperature and humidity level (Figure 5.2). An air pump was put inside the chamber to maintain constant humidity. The temperature was kept within 22-24°C and the humidity within 42-52% during the experiment.

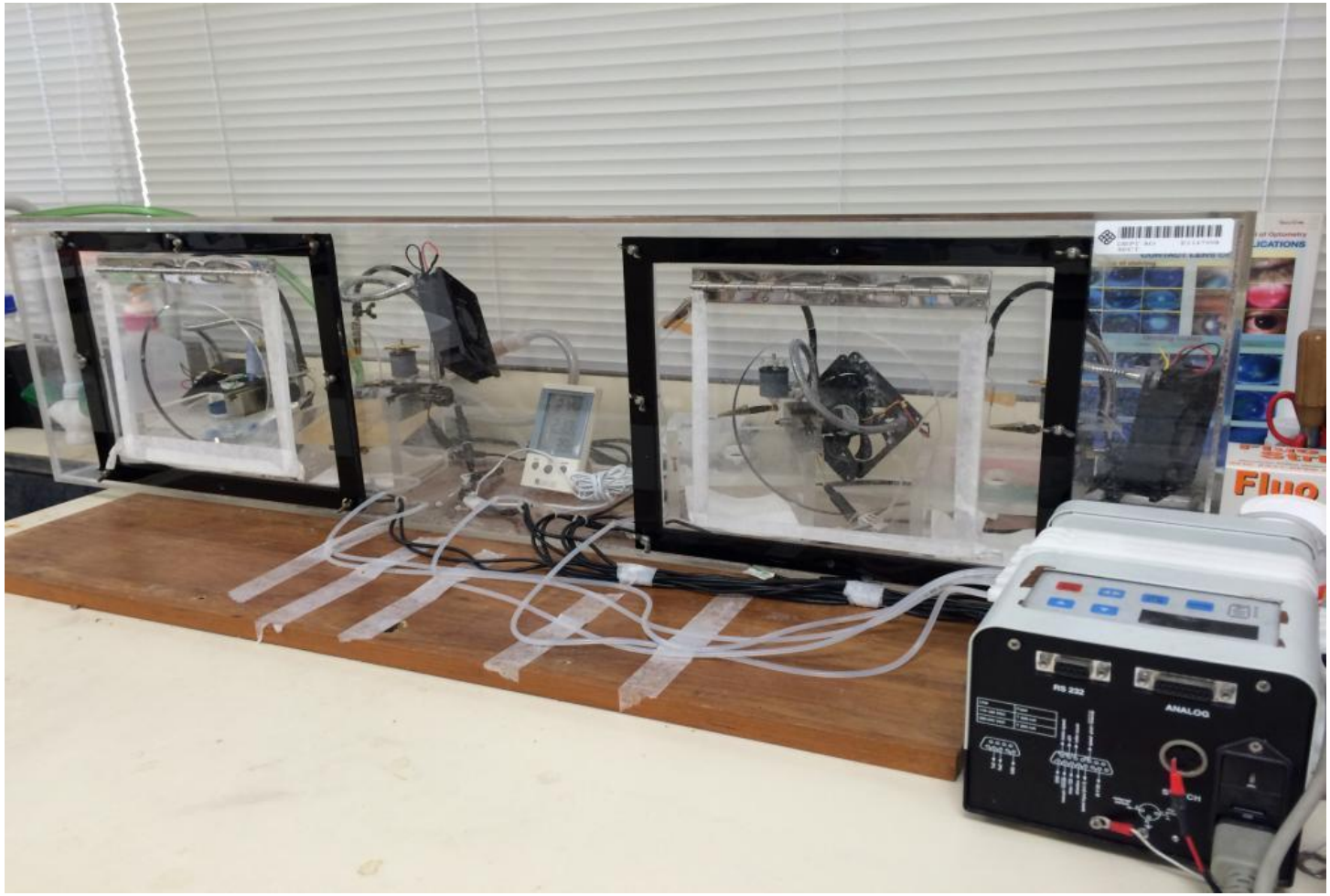


Figure 5.2 Porcine eye models in closed chamber with constant temperature and humidity

5.2.3 Assessment of the viability of porcine corneal epithelial cells

Three hours after the commencement of the experiment, the viability of the porcine corneal epithelium was assessed with 0.4% trypan blue solution (Sigma-Aldrich Co.) (except for 'immediate' control which was assessed immediately before the commencement of the experiment). The whole corneas were immersed in 0.4% trypan blue solution for two minutes. The eyes were then rinsed with DPBS, the corneas dissected out and the number of stained cells in the central (central 5mm) and peripheral corneas counted under a microscope (10x eyepiece with a 10x objective) (Olympics CH-2, Tokyo, Japan) within a 5mm x 5mm grid (final field size is 0.25mm^2). Three different locations within the central 5mm of the cornea and three different locations at the periphery were counted and averaged. The number of stained cells between the four test PEMs, between the test PEMs and 'immediate' control and 'delayed' control, and the differences between the central and peripheral cornea were determined. The above procedures were repeated 11 times over one month.

Part 2: Cytotoxic effects of leachates

5.2.4 Contact lens disinfection solutions

The cytotoxic effects of four MPS and a hydrogen peroxide (H_2O_2) system on PEM were investigated. The active ingredients of these solutions are shown in Table 5.1. The contact lenses were pre-soaked

in these solutions for 12 hours before the experiment. An additional step of neutralization following manufacturer's recommendation was performed for the H_2O_2 system. A control was also set up by soaking the lenses in PBS at pH 7.6 which resembles the pH of human tears.

Table 5.1 Active ingredients of contact lens disinfecting solutions used

	Name	Company	Active ingredients
MPS A	Biotrue	Bausch + Lomb	0.00013%polyaminopropyl biguanide 0.0001%polyquaternium
MPS B	Optifree Pure Moist	Alcon	0.001%polyquaternium-1 0.0006%myristamidopropyl dimethylamine (ALDOX)
MPS C	Complete Easy Rub	Abbott Medical Optics	0.0001% polyhexamethylene biguanide
MPS D	Revita Lens	Abbott Medical Optics	0.00016% alexidine dihydrochloride 0.0003% polyquaternium-1
H_2O_2	Oxysept	Abbott Medical Optics	3% hydrogen peroxide
PBS	Phosphate buffered saline	N/A	N/A

5.2.5 Contact Lenses

Both CCL and clear contact lenses were used in this study (Table 5.2). Since most CCL do not have clear counterparts in terms of lens material and water content, for comparison between CCL and clear contact lenses, clear contact lenses of the same FDA group as the

CCL were selected to minimize the influence of lens material on solution release. To determine the pigment location of the CCL, the standardized rub-off test developed and described in Chapter 3 was used. The CCL used in this experiment failed the rub-off test and the pigments were found to be on the back surface of the lenses, so in direct contact with the cornea when worn (The results are reported in Chapter 4).

Table 5.2 Properties of the cosmetic contact lenses (CCL) and clear contact lenses used

	Product Name	Company	FDA group	Material	Dk	Water content (%)	Color
CCL	Lacelle	St Shine Optical	I	Hefilcon A	11	42	Tender Brown
Clear lens	Soflens 38	Bausch + Lomb	I	Polymacon	24	38	N/A

N/A – not applicable

5.2.6 Porcine eye model set up

A total of 36 eyes were used and three samples were collected for each contact lens and solution combination. Four PEMs with simulation of 'blinking' and 'lacrimation' were set up each time as described above. The blinking frequency was set at 15 times per minute with a drop of DPBS (lacrimation) after each blink. Pre-soaked CCL were applied to two PEMs while two pre-soaked clear contact lenses were applied to the other two PEMs (Figure 5.3). Before

applying to the PEM, the MPS pre-soaked contact lenses were first rinsed with 10mL DPBS (Sigma-Aldrich Co., St. Louis, USA).

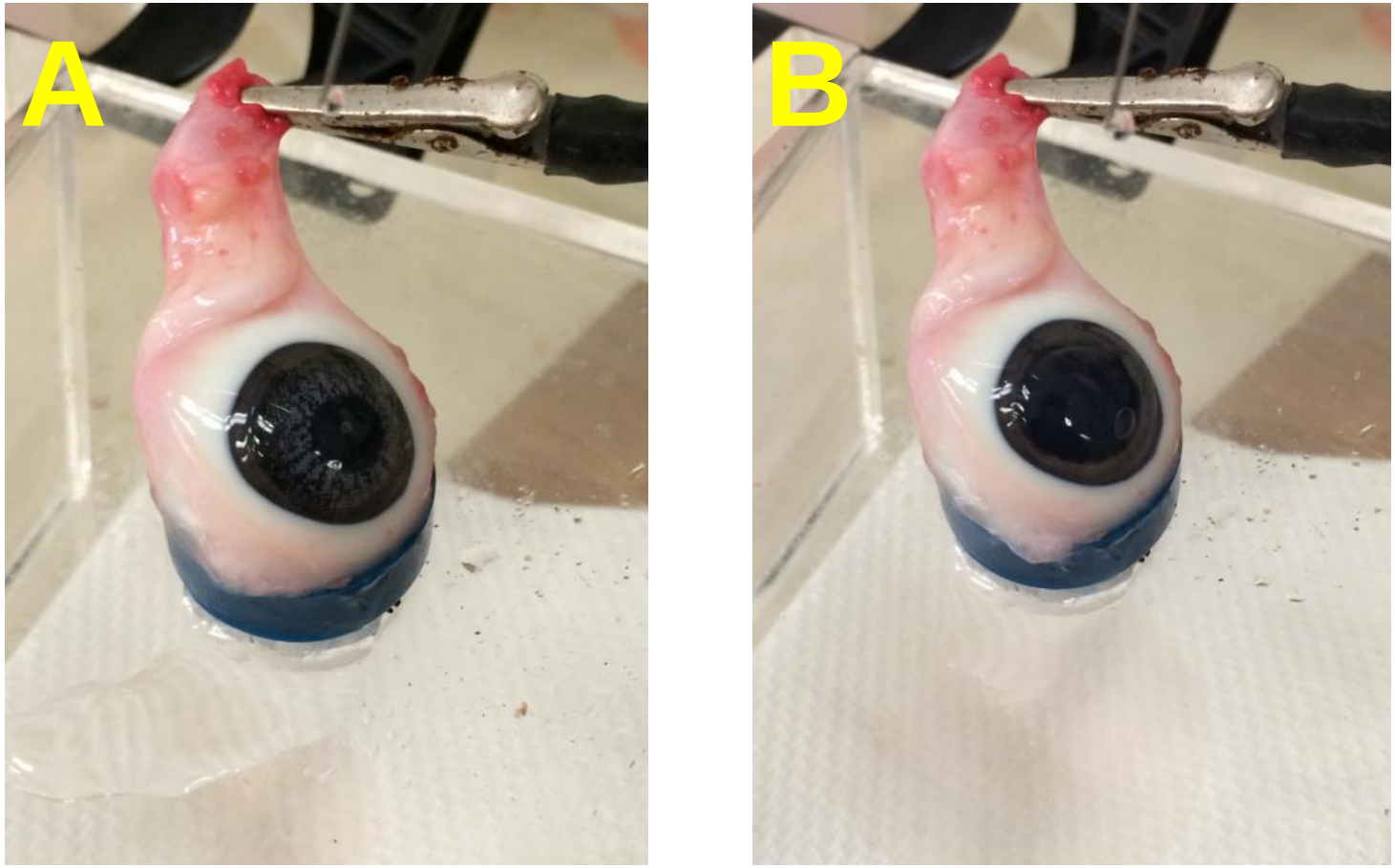


Figure 5.3 Cosmetic contact lens (A) and a clear lens (B) on the porcine eye model

5.2.7 Assessment of the corneal epithelial cells viability

After three hours, the lenses were removed and the cornea was rinsed with DPBS and dissected. Each cornea was carefully dissected just posterior to the limbal area and was immersed in 0.25% trypsin/EDTA (Gibco/Invitrogen, California, US) at 37^{o}C for 60minutes in a humidified carbon dioxide incubator (Steri-Cycle CO2 incubator, Thermo Scientific, US) to release the corneal epithelial cells. The corneas were gently rubbed with a blunt blade to remove the adhered epithelial cells, followed by rinsing with the soaked 0.25% trypsin/EDTA. The suspension of the shed cells was then centrifuged at 800rpm for eight minutes at 21^{o}C (Heraeus Multifuge X1R, Thermo Scientific, US). The cells were re-suspended in DPBS and the enumeration of cells was performed microscopically to ensure sufficient cells for analysis. Annexin V-FITC/7-AAD kit (Beckman Coulter, Brea, CA, USA) was used for cell viability assessment. Cell pellets were re-suspended in 200µL of binding buffer and 20µL of Annexin V-FITC solution and 40µL 7-AAD viability dye were added to the suspension. The suspension was kept on ice and incubated for 15 minutes in the dark. The suspension was then diluted with 800µL binding buffer before performing flow cytometry using Beckman Coulter flow cytometer (Beckman Coulter, Harbor Boulevard, Fullerton, CA).

5.2.8 Treatment of data

Since the number of eyes in each group was small, non-parametric tests were used for statistical analysis.

Part 1: Validation

Kruskal-Wallis test was used to test for the consistency between the four test PEMs. Since the four test PEMs showed no significant difference in cell viability on different days, the results were pooled. Results of 'immediate' control and 'delayed' control across different days were also pooled respectively. Mann Whitney U tests were used to test for the difference in number of dead cells between the test PEMs (pooled) and 'immediate' control and 'delayed' control. Wilcoxon signed rank test was used to test for differences in number of stained cells between the central and peripheral cornea and differences between test and control PEM.

Part 2: Cytotoxic effects of leachates

Mann Whitney U tests were used to test for the difference in percentages of damaged viable cells, healthy cells, necrotic cells and apoptotic cells between CCL and clear lenses in each solution.

5.3 Results

Part 1: Validation

No significant difference was found in the number of dead cells between the four test PEMs in both central ($p=0.53$) and peripheral cornea ($p=0.19$). There were significantly more dead cells (central and periphery) in the test PEMs compared to 'immediate' control ($p<0.01$) but significantly less when compared to 'delayed' control ($p<0.01$). The number of dead cells in the central and peripheral cornea of each test and control PEM are shown in Table 5.3. Significantly higher numbers of dead cells were found between the central and peripheral corneas in the test PEMs while no differences were found in both 'immediate' control PEM and 'delayed' control PEM.

Table 5.3 Number (Median [Range]) of stained dead cells in test and control porcine eye models

	No. of eyes	Central cornea	Peripheral cornea
'Immediate' control	10	111 [97 – 164]	109 [88 – 143]
'Delayed' control	10	307 [276 – 315]	296 [276 – 313]
Set A	9	211 [168 – 227]*	145 [120 – 168]*#
Set B	8	205 [189 – 241]*	144 [128 – 184]*#
Set C	9	207 [181 – 239]*	158 [133 – 191]*#
Set D	11	198 [182 – 228]*	152 [135 – 174]*#

*Significantly different in number of dead cells from both 'immediate' control and 'delayed' control (Mann-Whitney U tests; $p<0.01$)

#Significantly different in number of dead cells between central and peripheral cornea (Wilcoxon signed rank tests; $p<0.001$)

Part 2: Cytotoxic effects of leachates

Following staining with the use of Annexin V-FITC and 7-AAD kit, the cell viability determined and presented in a figure divided into four quadrants, labelled B1 to B4 (Figure 5.4). B1 represents damaged viable cells which are stained with 7-AAD only whereas B2 represents necrotic cells which are stained with both 7-AAD and V-FITC dyes. B3 are healthy viable cells which do not stain with either of the dyes; and B4 are apoptotic cells which are stained with V-FITC dye only. Necrotic and apoptotic cells were both dead cells but they were of different mechanism.

The cell viability between CCL and clear lens with each solution tested was compared. The results showed that all MPS showed no significant difference in the percentages of healthy cells between the CCL and clear contact lenses (Table 5.4). The number of damaged, necrotic and apoptotic cells between CCL and clear contact lenses in all tested solutions were also not significantly different. Figures 5.5a – 5.5f show the distribution of epithelial cells in various states for all the solutions tested.

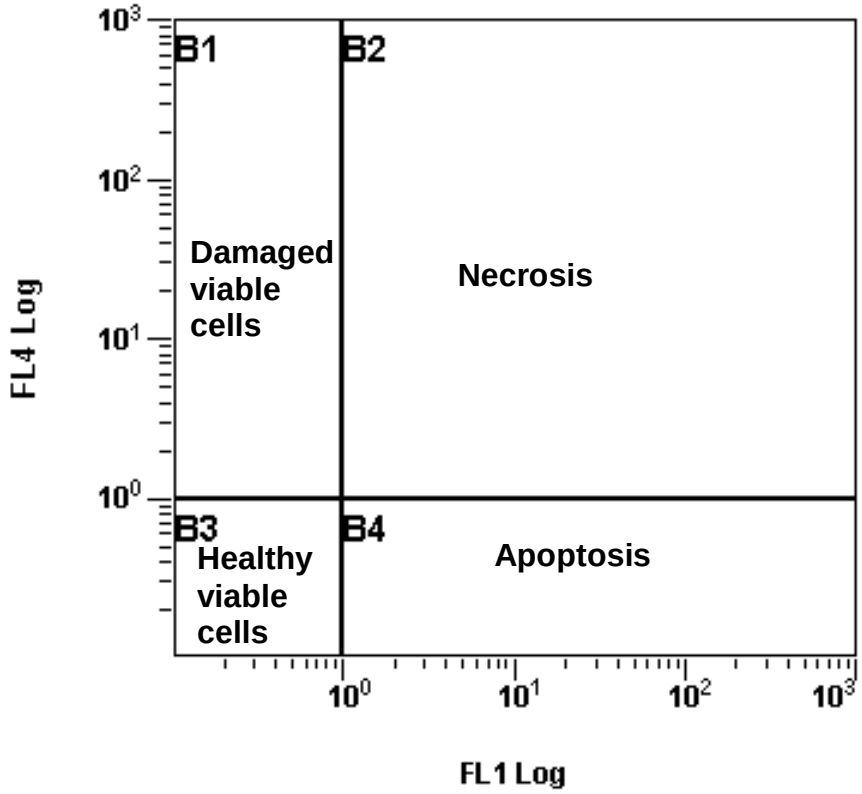


Figure 5.4 Flow cytometric analysis plot of result with Annexin V-FITC and 7-AAD kit

Table 5.4 Porcine epithelial cells cytotoxic effects (Median [Range]) after three hours of contact with cosmetic contact lenses (CCL) and clear contact lenses pre-soaked in different test solutions

	Damaged viable cells (B1)		P value	Necrotic cells (B2)		P value
	Clear contact lenses	CCL		Clear contact lenses	CCL	
MPS A	29.07 [28.96 – 30.74]	23.02 [17.13 – 23.96]	0.050	2.38 (2.03 – 3.18]	4.06 (1.25 – 5.17]	0.513
MPS B	19.25 [17.92 – 25.23]	24.32 [23.02 – 25.71]	0.275	4.44 (1.99 – 6.00]	3.39 (2.01 – 3.96]	0.513
MPS C	22.00 [18.43 – 27.70]	17.57 [14.29 – 25.61]	0.275	2.05 (0.59 – 2.95]	1.03 (0.51 – 1.38]	0.275
MPS D	17.55 [16.43 – 18.61]	15.83 [15.78 – 15.97]	0.050	2.58 (0.44 – 2.71]	1.97 (0.99 – 2.52]	0.513
H_2O_2	20.98 [18.86 – 23.16]	25.81 [19.92 – 29.10]	0.275	0.46 (0.35 – 1.55]	0.55 (0.52 – 2.14]	0.275
PBS	20.37 [18.16 – 23.17]	22.76 [18.79 – 25.01]	0.827	1.48 (1.45 – 1.50]	1.85 (0.79 – 2.06]	0.050
	Healthy viable cells (B3)		**P value**	**Apoptotic cells (B4)**		**P value**
	Clear contact lenses	**CCL**		**Clear contact lenses**	**CCL**	
MPS A	74.26 [71.59 – 78.97]	64.73 [64.37 – 69.51]	0.050	1.27 [0.70 – 1.51]	0.83 [0.17 – 1.13]	0.275
MPS B	69.41 [67.62 – 72.11]	74.33 [71.55 – 76.87]	0.127	1.83 [0.19 – 3.36]	1.81 [1.20 – 2.45]	0.827
MPS C	77.13 [69.91 – 77.16]	81.63 [73.11 – 83.35]	0.275	0.33 [0.25 – 1.49]	0.29 [0.25 – 0.98]	0.658
MPS D	79.67 [79.30 – 80.74]	81.00 [80.90 – 82.78]	0.050	0.57 [0.21 – 1.20]	0.75 [0.45 – 1.06]	0.827
H_2O_2	78.33 [76.45 – 79.09]	71.57 [70.33 – 79.24]	0.513	0.23 [0.04 – 0.50]	0.31 [0.02 – 0.48]	0.827
PBS	77.77 [74.27 – 79.72]	75.00 [74.12 – 77.40]	0.513	0.64 [0.35 – 1.11]	0.38 [0.08 – 1.75]	0.275

P value: Probability values of Mann Whitney U tests for differences between CCL and their clear counterparts

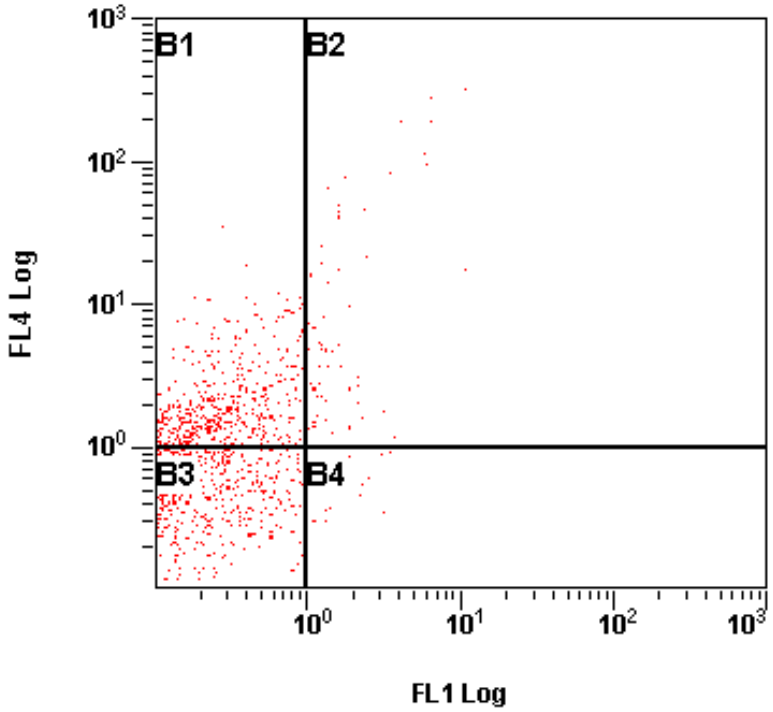
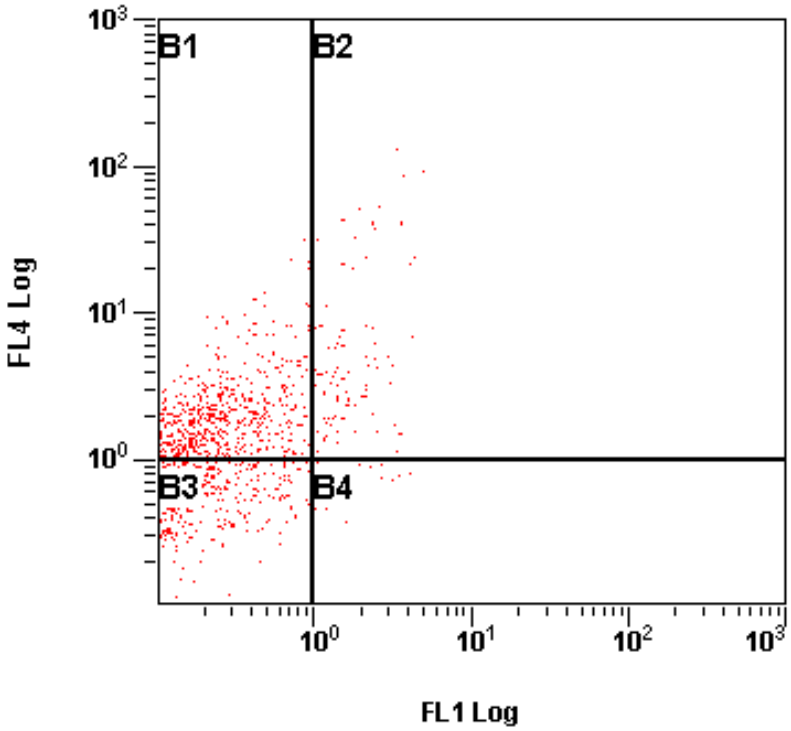
	Clear contact lens	Cosmetic contact lenses
MPS A		

Figure 5.5a Flow cytometric analysis plot of epithelial cells with clear and cosmetic contact lenses pre-soaked in multipurpose solution A (MPS A) using Annexin V-FITC and 7-AAD kit

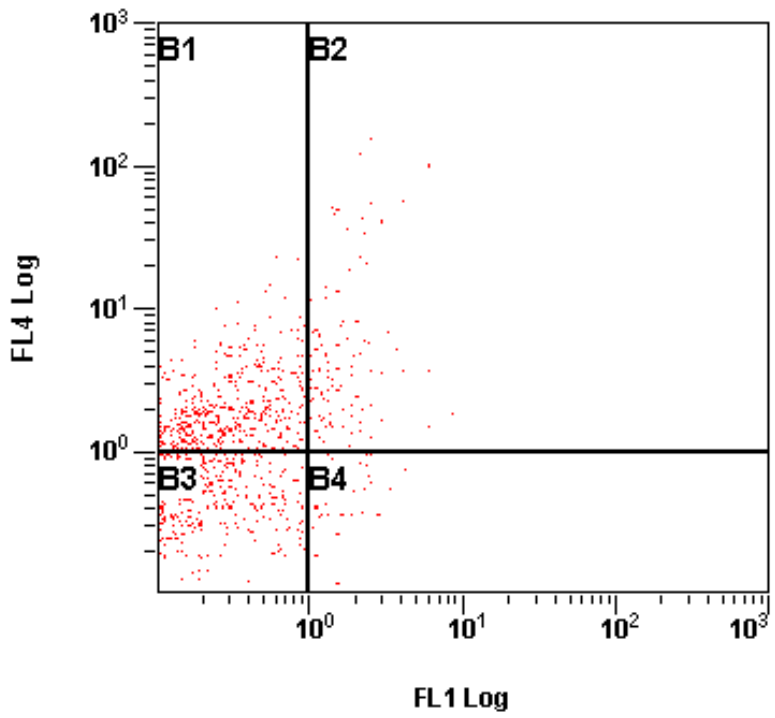
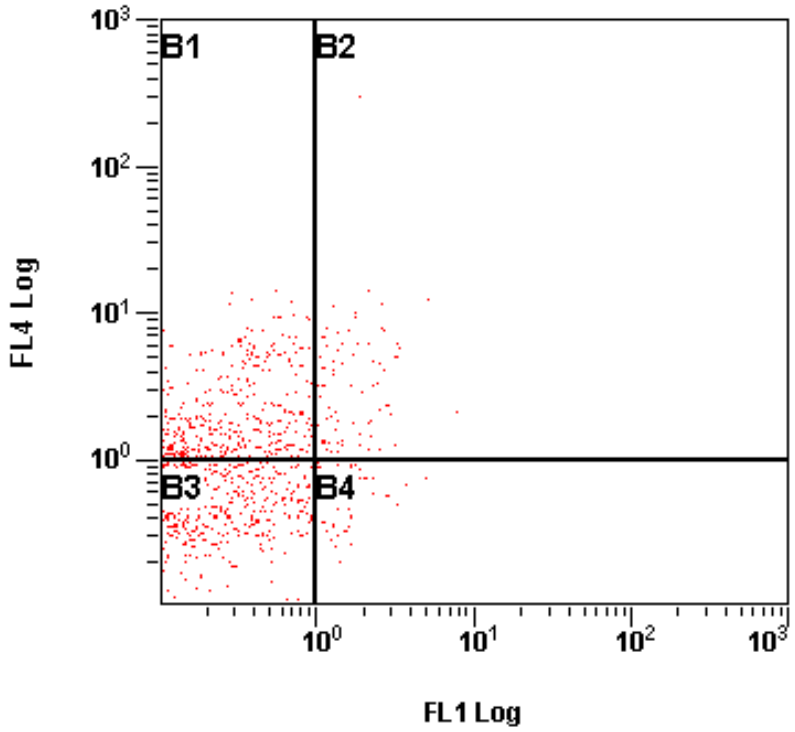
	Clear contact lens	Cosmetic contact lenses
MPS B		

Figure 5.5b Flow cytometric analysis plot of epithelial cells with clear and cosmetic contact lenses pre-soaked in multipurpose solution B (MPS B) using Annexin V-FITC and 7-AAD kit

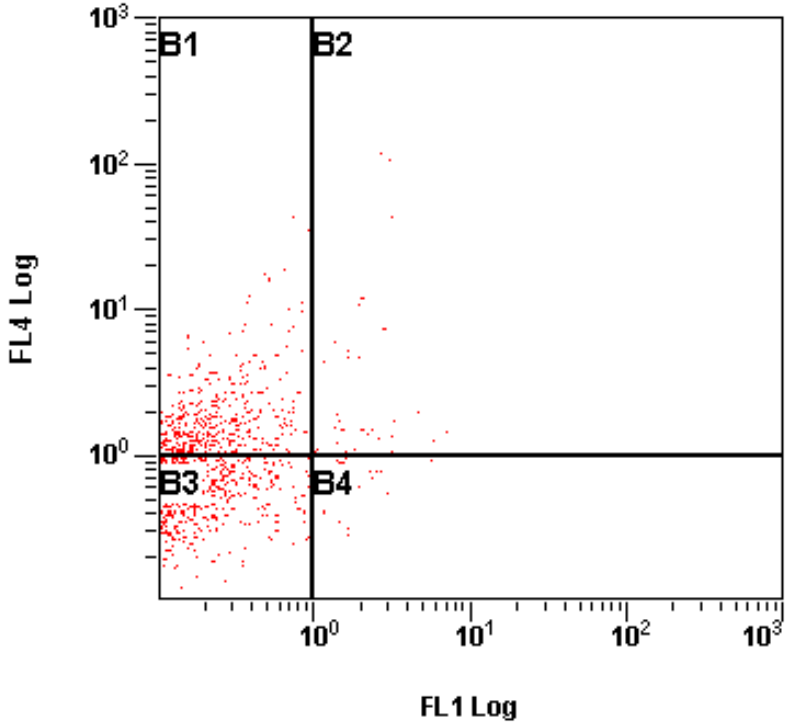
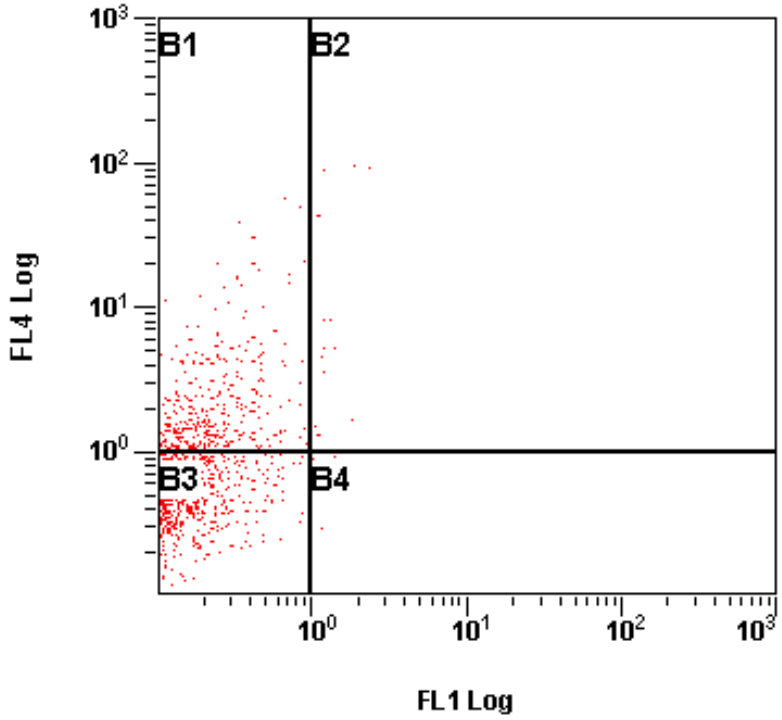
	Clear contact lens	Cosmetic contact lenses
MPS C		

Figure 5.5c Flow cytometric analysis plot of epithelial cells with clear and cosmetic contact lenses pre-soaked in multipurpose solution C (MPS C) using Annexin V-FITC and 7-AAD kit

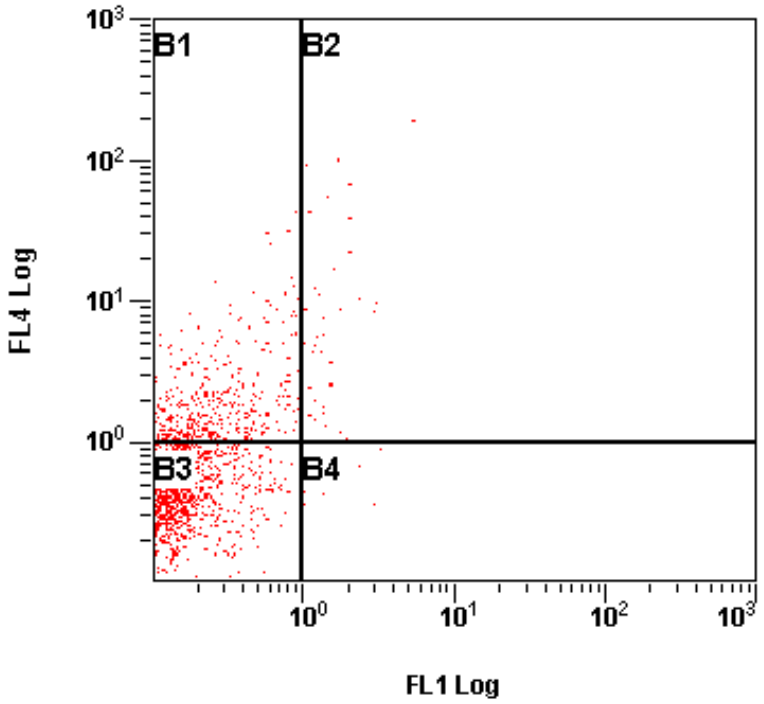
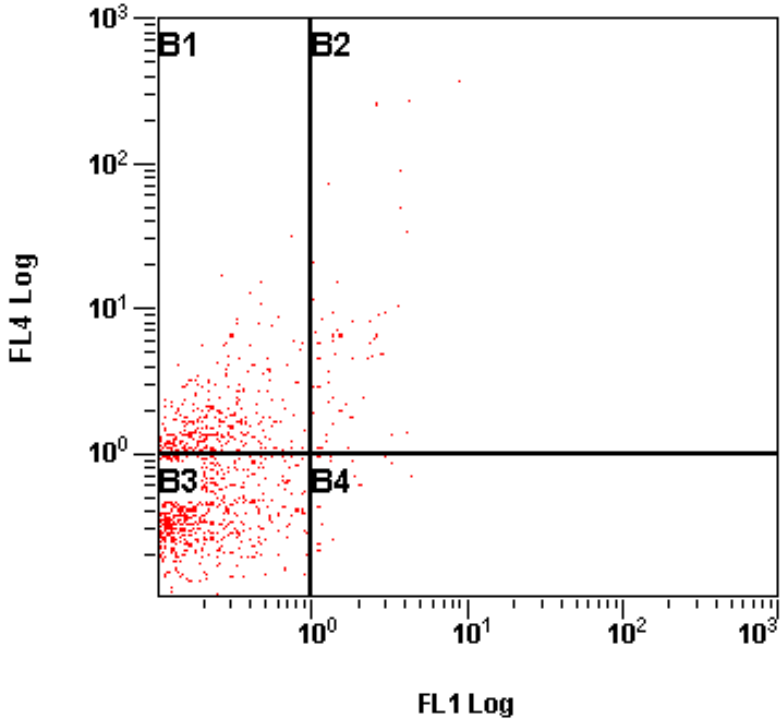
	Clear contact lens	Cosmetic contact lenses
MPS D		

Figure 5.5d Flow cytometric analysis plot of epithelial cells with clear and cosmetic contact lenses pre-soaked in multipurpose solution D (MPS D) using Annexin V-FITC and 7-AAD kit

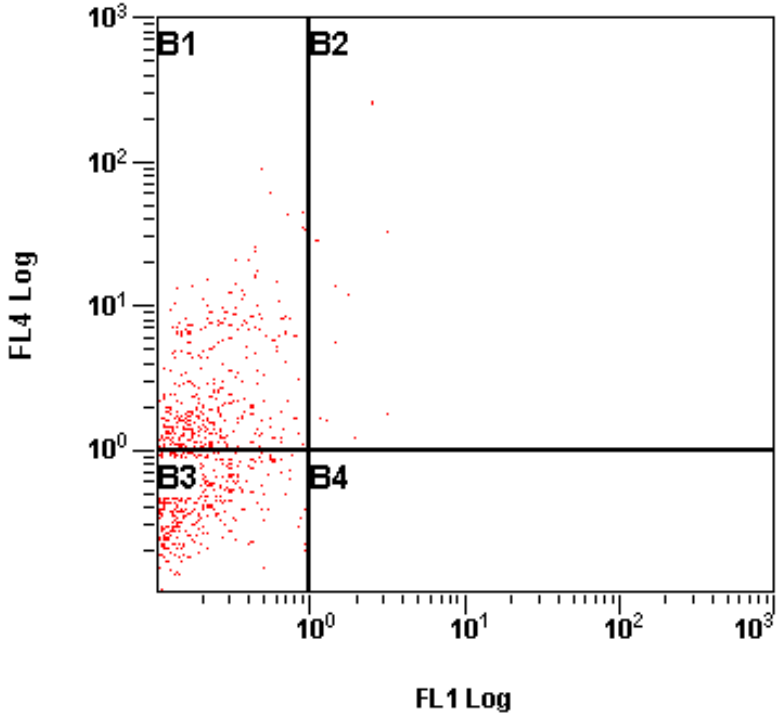
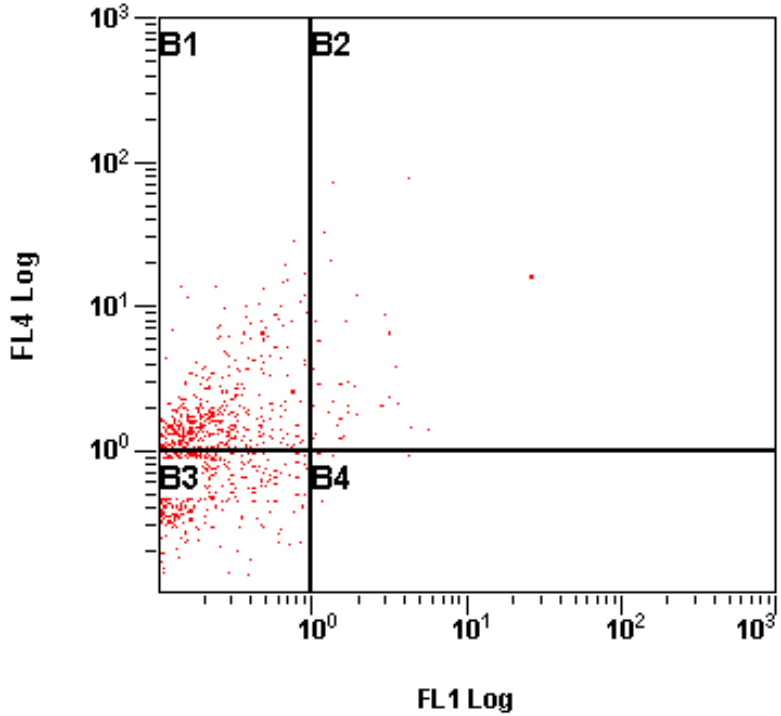
	Clear contact lens	Cosmetic contact lenses
H_2O_2		

Figure 5.5e Flow cytometric analysis plot of epithelial cells with clear and cosmetic contact lenses pre-soaked in hydrogen peroxide (H_2O_2) solution using Annexin V-FITC and 7-AAD kit

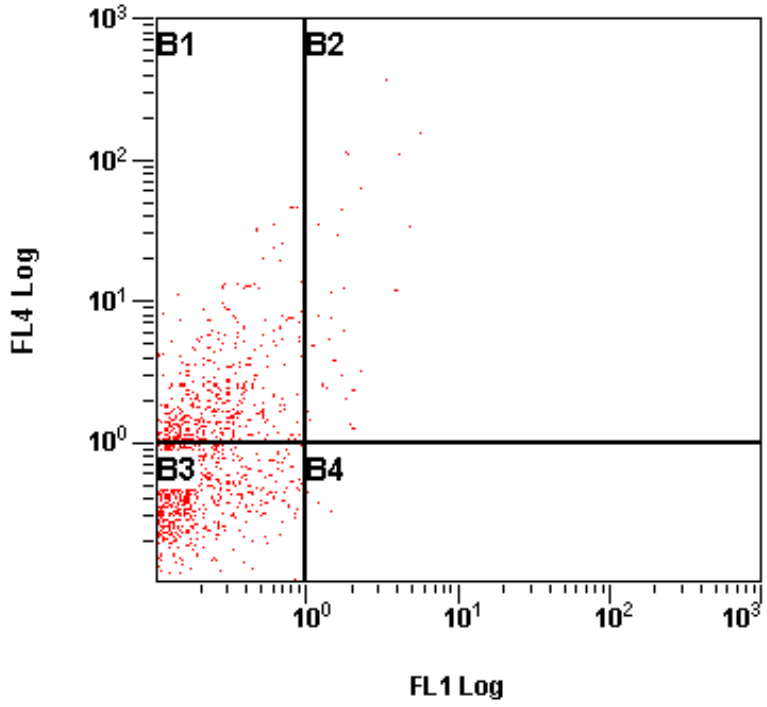
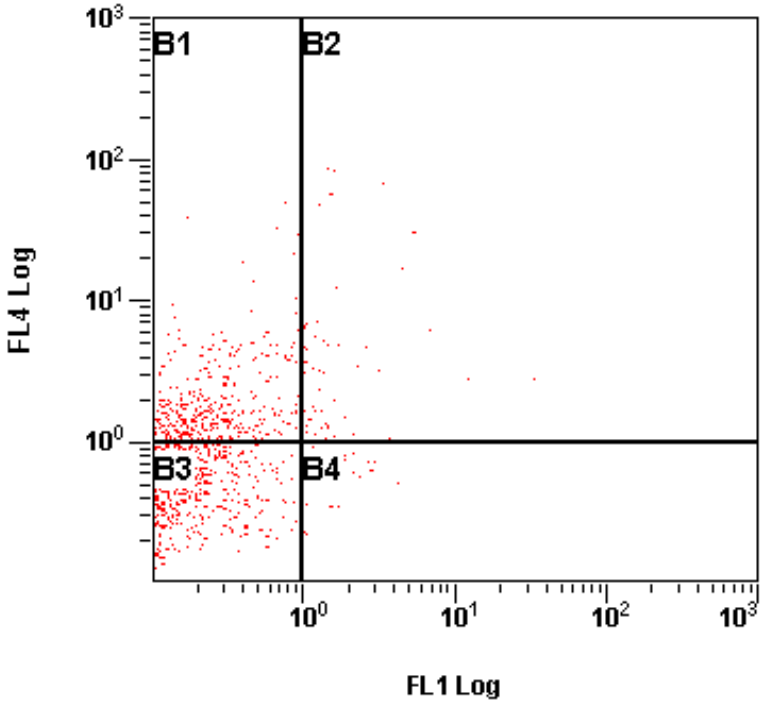
	Clear contact lens	Cosmetic contact lenses
PBS		

Figure 5.5f Flow cytometric analysis plot of epithelial cells with clear and cosmetic contact lenses pre-soaked in phosphate buffered saline (PBS) using Annexin V-FITC and 7-AAD kit

5.4 Discussion

The design of the PEM was successfully modified and validated to allow the setting up of six PEMs at each time. Instead of using fluorescein grading, trypan blue was employed to investigate the viability of epithelial cells and to assess consistency between PEM. The enumeration of trypan blue staining allows direct comparison of number of dead cells observed between PEM.

The result with 'immediate' control showed that most of the epithelial cells were viable before the experiment. No significant differences in the number of dead cells were found among the four test PEMs, either in the central or peripheral cornea. Our results indicated that the epithelial cell viability among the four PEMs was consistent. The new set up allows six models to run at the same time so that the efficiency of experiments can be increased. Test PEMs showed significantly less dead cells when compared with 'delayed' control, suggesting that the simulated blinking and lacrimation were effective in improving the exposure conditions as more epithelial cells remained viable.

A higher number of dead cells were observed in central cornea than the peripheral cornea in the test PEM, which was in agreement with a previous study (Choy et al., 2004). This could be due to the higher friction force in the central cornea or because of the cell renewal movement. Newly differentiated cells may move from the periphery to the central cornea to replace sloughed off or damaged cells (Thoft and Friend, 1983), suggesting that the cells in the periphery are more 'healthy'. However, studies have also reported that

porcine corneas contain oligopotent stem cells which can generate individual colonies of corneal cells (Majo et al., 2008).

In this study, DPBS was used to as a 'lacrimal' fluid. It should be noted that DPBS cannot mimic the more complex structure of the tear film and its adherence to the cornea may be limited. However, the major purpose of the DPBS in this study was to maintain the cornea in a hydrated condition.

Our results showed that porcine epithelial cell viability was not significantly different among CCL and clear contact lenses for all the solution tested. The results suggested that the surface pigments themselves may not be cytotoxic to the corneal epithelial cells because, otherwise, cytotoxic effects should have been observed in porcine eyes with CCL pre-soaked in PBS. The surface pigments on the CCL did not demonstrate a higher uptake of solution during disinfection and subsequent release during 'wear' (lens-corneal contact). However, in our experiment, only one type of CCL was used. It is not known if other pigment dyes will give similar results. This study was also limited in that the lenses were only pre-soaked for 12 hours and the lenses were 'worn' for three hours only. Cosmetic contact lenses are more popular nowadays and patients tend to wear them for a longer period of time. Because of the limitations of *ex vivo* PEM, in which cell viability is limited to four hours, it is not known if cytotoxic effects will occur or not if other CCL were used or if the CCL were 'worn' for a longer time.

To our knowledge, this is the first report of the use of an *ex vivo* model to study the effect of MPS release from contact lenses on corneal epithelial cells. The PEMs used in this study were validated in pilot studies and found to give consistent results. Prior to this study, most solution toxicity studies used HCEC (Chuang et al., 2008; McCanna et al., 2008; Gorbet et al., 2010, 2011; Tanti et al., 2011; Choy et al., 2012, 2013; Erdinest et al., 2013) or animal cell lines (Mowrey-McKee et al., 2002; Santodomingo-Rubido et al., 2006) (Section 1.6) to evaluate the potential cytotoxic effects of MPS alone or as lens-solution combinations on epithelial cells. Without the consideration of the effects of blinking and lacrimation, most of these studies found a higher cytotoxic effect of a particular MPS and certain combinations of lens and solutions (Santodomingo-Rubido et al., 2006; Chuang et al., 2008; Dutot et al., 2008; McCanna et al., 2008; Gorbet et al., 2010, 2011; Tanti et al., 2011; Choy et al., 2012, 2013; Dutot et al., 2013; Erdinest et al., 2013; Gorbet et al., 2014). In this study, both blinking and lacrimation effects were taken into consideration.

Jones and Powell (2013) suggested that water content, ionic charge and the degree of hydrophilicity were the major factors impacting the uptake and release of active biocides (Section 1.6). Although the CCL and clear lenses used in this experiment were not of the same material due to non-availability, the experimental variation was minimized by choosing a clear lens of similar water content and in the same FDA category. The CCL used in this experiment was studied in Chapter 3 on the determination of the location of the pigment using our standardized rub-off test and was found to have

pigments printed on the back surface. This brand was chosen because it has the pigments are on the back surface of the lenses so to be in contact with the corneal epithelial cells when being worn. Lorenz and co-workers (2014) evaluated the surface roughness of CCL and revealed that CCL with pigments on the surface gave significantly higher roughness. With the presence of pigments, the uptake or release of biocides may be different.

In this study, the cytotoxic effect of the leachates from cosmetic contact lenses was studied. Although some of the solutions used the same active ingredients for disinfection, the difference in concentration, as well as the presence of the surfactants, lubricants or buffer in formulation, may also influence the uptake and release of the solutions. Therefore, only the overall effect of the leachates towards the epithelial cells can be evaluated.

Lenses used in this study were all new unused lenses. Lens material, together with contact lens solution, has been shown to play a role in cytotoxicity. A previous study revealed a change in surface characterization in worn contact lenses (Bruinsma et al., 2002), with surface roughness being increased in over worn contact lenses. It is reasonable to speculate that lens deposition resulting from lens wear may also influence the cytotoxic effect. Further investigation is warranted to confirm this.

The small number of samples in this study is another limitation. The small sample size may not be adequate to detect significant differences among solutions. A larger group of samples is needed for better evaluation. In the

current experiment, it was also found that humidity (when relative humidity rose to over 90%) affected the result even when porcine eyes were dissected immediately upon arrival of the laboratory without applying the contact lenses. In view of this, all experiments were conducted with environmental humidity controlled within the range of 50% to 70% to minimize the influence from humidity. However, relative humidity is an external factor that cannot be controlled before the eyes were collected and may seriously affect the results of the experiment. Therefore, the *ex vivo* PEM may not be an appropriate model to be used in studying the cytotoxicity of leachates from cosmetic contact lenses. HCEC could be a better substitute because the environmental conditions can be carefully controlled.

5.5 Conclusion

Our results confirmed the consistency of results using the improved PEM and suggested that this PEM can be used in future studies where human eyes cannot be used, and in particular for studying the effects of CCL on an intact eye model. The cytotoxic effects of leachates from surface pigments CCL were not significantly different to clear contact lenses. However, the *ex vivo* PEM may not be a good model to study the cytotoxic effect because of the potential influence from environmental factors.

Journal article

Chan KY, Cho P, Boost M. Corneal epithelial cell viability with an *ex vivo* porcine eye model. *Clinical and Experimental Optometry*. 2014; 97(3): 337-340

Conference presentation

Chan KY, Cho P, Boost M. Corneal epithelial cell viability with an *ex vivo* porcine eye model. Poster presentation at *The American Academy of Optometry*, 24-27 October 2012, US

Chan K.Y, Cho P, Boost M.V. Cytotoxicity of leachates from cosmetic contact lenses on a porcine eye model. *The 9th Asia Cornea & Contact Lens Conference 2014* at Kaohsiung, Taiwan

Chapter 6

Protein deposition to cosmetic contact lenses – a pilot study

6.1 Introduction

Protein deposition has been reported to be a concern for contact lens wear in terms of vision (See Section 1.5.1) and comfort (See Section 1.5.2). Similar to microbial adherence and cytotoxicity of leachates, this aspect has been studied extensively with clear contact lenses, made from either hydrogel or silicone hydrogel lenses. However, little is known with respect to CCL, in particular surface pigmented CCL.

This chapter describes a study investigating the protein deposition between CCL of various types: either sandwiched pigment design or surface pigment design, and clear contact lenses.

6.2 Experimental design

6.2.1 Subject enrollment

Ten young adults aged 18-35 years old were recruited. Subjects with a history of ocular trauma, refractive surgery and rigid lens wear were

excluded. All subjects were occasional soft contact lens wearers and did not wear contact lenses immediately prior to the experiment. Informed consent was obtained from each subject before commencement of the study. Ethics approval for the project was obtained from the Departmental Research Committee of the School of Optometry, The Hong Kong Polytechnic University, and all the procedures in the study followed the tenets of Declaration of Helsinki in 2002.

Subjects were required to come to our clinic for an eye examination to ensure there were no contraindications for contact lens wear. They were required to attend the Optometry Clinic twice on two occasions separated by not more than a week. Each subject was required to wear two pairs of lenses (two CCL and two clear contact lenses) on two separate days. Each subject was fitted with a CCL in one eye and a clear lens in the other eye (screening visit). If successful (routine fitting assessment), the contact lenses were dispensed to the subjects (CCL for one eye and clear lens for the other eye).

Subjects were required to wear the contact lenses for eight to ten hours. At the end of a day's wear, the subjects returned to the clinic where the contact lenses were removed and collected for protein analysis. The ocular health was assessed again to check corneal integrity. The above procedures were repeated at visit two with another pair of contact lenses (CCL used for the eye which previously

wore the clear lens, and clear for the other eye which previously wore CCL).

All lenses used were commercially available in Hong Kong and both CCL and the clear contact lenses were made of the same material and of the same water content. Figure 6.1 shows the procedures of the experiment.

6.2.2 Contact lenses

Three types of contact lenses, comprising two types of CCL and one clear contact lenses, were used in this experiment (Table 6.1). The colour of the CCL was Brown or Hazel as available. The clear counterparts of these CCL were of the same material and water content (not taking manufacturing process into account).To determine the pigment location of the CCL, the standardized rub-off test was as described in Chapter 3 was used. Five lenses of each brand were used in the rub-off test.

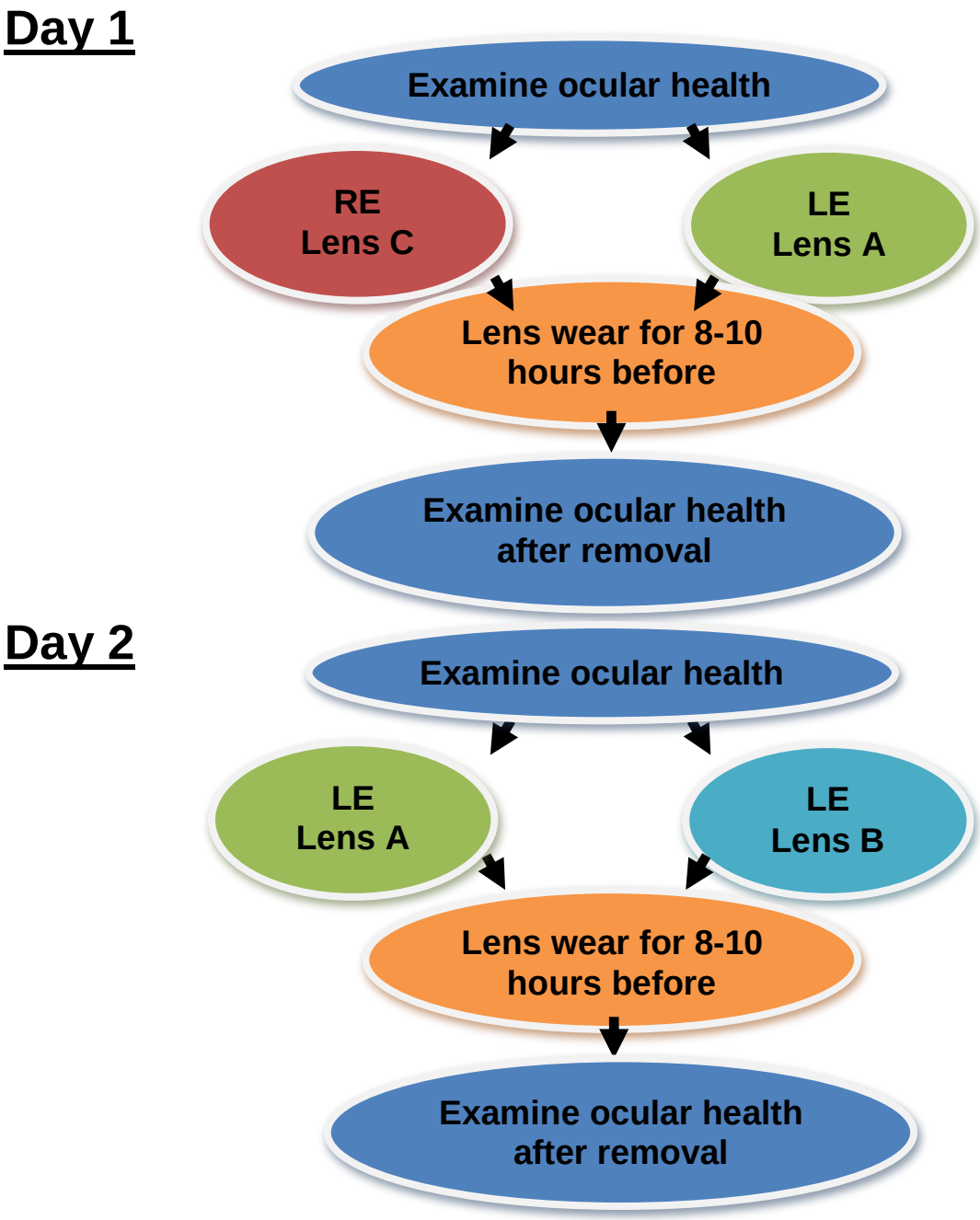


Figure 6.1 Flowchart of the experiment procedures (Lens A – Clear lens; Lenses B & C- Cosmetic contact lenses)

Table 6.1 Properties of the contact lenses used

Lens	Product Name	Company	FDA group	Material	Dk	Water content (%)	Color	Color printing/ process*
A	1 Day ACUVUE® MOIST®	Johnson & Johnson	IV	Etafilcon A	28	58	N/A	N/A
B	1 Day ACUVUE® DEFINE™	Johnson & Johnson	IV	Etafilcon A	28	58	Vivid	Sandwich
C	Freshkon Alluring Eyes 1 Day	Oculus Optical	IV	Etafilcon A	28	58	Winsome Brown	Embedded process on front surface

* – according to information provided by the manufacturer or distributor; N/A – not applicable

6.2.3 Protein extraction

Worn lenses were placed in sealed vials containing 1mL of 1:1 mixture of 0.2% trifluoroacetic acid (Sigma-Aldrich Co., St. Louis, USA) and acetonitrile (Thermo Fisher Scientific Ltd, USA). The vials were incubated with shaking in the dark at room temperature for 24 hours. The extraction solution was then analyzed.

7.2.4 Protein quantification

Bradford assay was used to quantify the total protein deposits from the CCL and their clear lenses counterparts. During the quantification, 20µL of the each extracted sample was used. These were neutralized by adding 25µL 0.5M phosphate at pH 7.2, 145µL water and 10µL 500mM calcium chloride. This resulted in the total volume for each sample being 200µL, following which they were mixed and vortexed. The samples were allowed to precipitate for five minutes and 1mL of 99.9% ethanol was added. The samples were vortexed and centrifuged (15000g for 90 seconds) and the supernatant was then discarded by aspiration. A further 1mL of 70% ethanol was added and the above procedures repeated until aspiration. The samples were then dried in the CentriVap micro IR vacuum concentrator (Labonco, Kansas city, MO) for 15 minutes. A 0.25µL aliquot of sample was mixed with 0.75mL Bradford reagent (Sigma-Aldrich Co., St. Louis, USA) and incubated at room temperature for 10 minutes. The

absorbance was then measured with a spectrophotometer (Spectronic 20 Gensys Visible Spectrophotometer, Spectronic Instruments Inc, Rochester, NY) at 595nm. A standard curve (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20µg) was prepared using Bovine serum albumin (Sigma-Aldrich Co., St. Louis, USA) and the total amount of protein for each sample was then calculated by comparing against the curve.

6.2.5 Treatment of data

Since the number of lenses in each sample was small, non-parametric tests were used for statistical analysis. Wilcoxon signed rank test was used to test for the difference in the total amount of protein deposition between CCL and clear lenses on each day.

6.3 Results

The results of the rub-off test are shown in Table 6.2. No pigments on lens B could be rubbed off whereas lens C failed the rub-off test with pigments transferred to the cotton bud when the front surface was rubbed. The pigments of lens C were on the front surface which was in direct contact with the palpebral conjunctiva when worn.

Nine subjects aged between 22 and 29 years old completed the study, one subject being excluded because of failure to comply with the wearing schedule. The protein deposition levels on CCL and clear lens were compared. Clear lenses had been worn for both days as a control. The protein deposition on the clear lens on the first day was not significantly

different to that on lenses worn on the second day (p=0.086). The results showed no significant differences in protein deposition between CCL (sandwich or surface pigment) and clear contact lens (Table 6.3).

Table 6.2 Results of the rub-off tests

Lens	Brands	Material / water	Rub-off tests
A	1 Day ACUVUE® Moist®	Etafilcon A / 58%	N/A
B	1 Day ACUVUE® Define™	Etafilcon A / 58%	Pass
C	Freshkon Alluring Eyes 1 Day	Etafilcon A / 58%	Fail (front surface)

N/A – not applicable

Table 6.3 Total protein deposition (Median [Range]) on cosmetic contact lenses (CCL) and clear lenses

Day	Lens	Total protein deposition (mg/lens)	P value
1	A	639 [347 – 731]	0.859
	C	583 [362 – 980]	
2	A	658 [427 – 806]	0.678
	B	600 [483 – 892]	

P value – Probability values of Wilcoxon signed rank test for differences between CCL and clear lens

6.4 Discussion

In this experiment, the CCL involved were daily disposable, as clear counterparts for biweekly/monthly lenses are not available. Although it is arguable that the protein deposition of daily disposable lenses is of lesser concern owing to its modality, published literature shows that protein deposition occurs within minutes of lens insertion (Leahy et al., 1990). There have been several reports of microbial keratitis with CCL associated with reuse, improper, or overwear of CCL (Singh et al., 2012; Steinemann et al., 2003, 2005). Similar to its microbial adherence, the protein deposition of daily disposable CCL should not be underestimated.

In this experiment, CCL, either surface pigment or sandwiched pigment, did not result in higher deposition in comparison with clear contact lenses. There was also no statistical difference in protein deposition of surface pigment CCL and sandwiched pigment CCL. The result indicated that the amounts of protein deposition on CCL, surface or sandwiched pigment, did not differ from clear contact lenses after being worn for a day. This suggested that both surface pigment and sandwiched pigment are suitable option, in terms of protein deposition, to be worn in daily disposable modality. However, other factors such as comfort have to be considered when recommending these CCL to patients as it is unknown if surface pigment CCL can lead to more discomfort as CCL have been reported to have higher surface irregularities in surface pigment CCL (Lorenz et al., 2014).

Since this is only a pilot study, the result only indicated that there was no difference in protein deposition between surface pigment CCL and sandwiched pigment CCL after one day of wear. The subjects participating in this study had only worn the lenses for a maximum of 10 hours, without overwear of contact lenses. In real life scenario, CCL patients may not be compliant with wearing or replacement schedule as previous studies have reported that CCL users may overwear their contact lenses (Singh et al., 2012; Steinemann et al., 2003, 2005). With clear lenses, it has been shown that longer time of wear (non-compliant to manufacturer's recommended replacement schedule) results in higher protein deposition (Michaud and Giasson, 2002). It is not known if the surface pigment in reusable CCL would attract more protein deposition with repeated use. Further investigation on this is warranted.

6.5 Conclusion

Protein deposition on CCL, with either sandwiched or surface pigments, after one day of lens wear was not different from those on clear contact lenses worn by the same subject.

Chapter 7

Summary and the way forward

7.1 Main conclusions

CCL has become increasingly popular particularly in Asian countries such as Hong Kong and Korea (Section 1.1.3). Because of the lack of regulations on the selling of CCL in Hong Kong, CCL are sold over the internet or via other unlicensed retail outlets (Section 1.1.4). There are a number of reports of infectious keratitis associated with the use of CCL, owing to the improper use of these lenses (Section 1.3). In view of this, it is essential to determine if CCL can be safely worn. Laboratory methods have been employed in this PhD study to investigate the effect of pigments of CCL on microbial adherence and solution uptake and release.

A standardized rub-off test was developed to determine the location of pigments on CCL. Manufacturers have claimed that their CCL utilize embedded or sandwich designs, in which the pigments are not in direct contact with the cornea or the palpebral conjunctiva. However, such claims have previously not been verified and the rub-off test can help to determine the location of the pigments. Of the five CCL tested, only one was found to have no pigment detaching after repeated rubs with a wetted cotton bud (Chapter 3).

In the investigation of microbial adherence of CCL (Chapter 4), 15 brands of CCL commercially available in Hong Kong were investigated. Rub-off tests showed that only two brands passed the test, indicating that they are either of embedded or sandwich designs. All these CCL were incubated in suspension of known concentration of *P. aeruginosa* and results showed that surface pigment CCL, which failed the rub-off test, had significantly higher *P. aeruginosa* adherence (at least six times more) than the CCL which passed the rub-off test. Significantly higher adherence of *P. aeruginosa*, *S. aureus* and *S. marcescens* to surface pigment CCL than their clear counterparts of same material and water content were found, suggesting that the difference in microbial adherence was due to the pigments on the CCL surface.

Because of the potential adverse events that may result, there was a need to employ an animal eye model to investigate the effects of, solution and contact lens on corneal epithelial cells. Cytotoxicity of leachates from CCL was investigated with the use of PEM (Chapter 5). To complete this investigation, a new PEM was developed with improved efficiency that allowed four PEMs to run at the same time, together with better control of environmental factors like temperature and humidity in a closed chamber. The new PEM was validated (and gave repeatable and consistent results for the four PEMs). CCL with back surface pigment and the clear contact lenses of the same FDA category as the test CCL were used to evaluate the influence of leachates from CCL pre-soaked in solutions, including four MPS and one H_2O_2system. Results showed that the cytotoxic effects did not differ

between the CCL and CL in all solutions tested. The results suggested that the surface pigments of CCL were not cytotoxic and their presence on the lens surface did not interfere with the uptake and release of MPS. However, the *ex vivo* PEM may not be a good model to study the cytotoxic effect because of the potential influence from environmental factors.

In the experiment on protein deposition (Chapter 6), two designs of CCL (surface pigment and sandwiched pigment) and clear contact lenses, having the same material and water content, were used. Subjects were recruited to wear these three types of contact lenses for eight to ten hours. Results showed no difference in the total amount of protein deposited on CCL (both designs) and clear contact lenses. This suggested that both designs were suitable for daily disposable wear as they did not show higher deposition than clear lenses.

7.2 Limitations of the studies

As with most research studies, there are some limitations in both animal model used and the scope of the experiments. The PEM does provide an alternative method to study different condition of corneal diseases such as dry eye and to assess the cytotoxicty of contact lens solution, and effects of presence of contact lenses on corneal epithelial cells. However, because of the *ex-vivo* design which has a limited viability, only short-term studies can be conducted. Cell lines such as HCEC may be necessary for the evaluation of long term effects of cytotoxicity. Cell lines also allow investigation of cytotoxic effects of leachates at cellular level. Although the PEM has the

advantages that both blinking and lacrimation can be simulated, PEM is unable to simulate the 'real' effect of lacrimation as the composition of DPBS is different to real human tears which have higher viscosity.

In the solution toxicity study, because of the unavailability of clear contact lenses of same material and water content as the test CCL, only clear contact lenses of the same FDA category and of similar water content and Dk were chosen for comparison. The difference of lens material, as well as surface hydrophobicity, may also influence uptake and release of MPS. Small sample size in this part of the study was another limitation.

The use of the rub-off test to determine the location of pigments is an indirect method. In microbial adherence experiment, rub-off test can be performed by a masked examiner so that it could ensure the examiner studying microbial adherence would not be affected by the results of rub-off test. Owing to the limitation of equipment, the cross-sectional imaging of the CCL, which is completely objective, is more appropriate to ascertain the location of pigments

7.3 Future direction

There are many other potential studies that could be performed to address the other aspects of CCL wear, such as

- **Potential effects of front surface pigments CCL on eyelids**

Studies have shown that surface pigmented CCL have a rougher lens surface. Further study is warranted to investigate if lid wiper epitheliopathy would be resulted with long term use of front surface pigment CCL

- **Bacterial adherence on used CCL**

  Deposits are known to affect the surface smoothness of contact lenses and enhance micro-organisms adherence. Further study is warranted especially for reusable CCL.

- **Fungal and *Acanthamoeba* adherence on new and used CCL**

  Apart from bacteria, both fungal and *Acanthamoeba* require more attention because of their adverse effects on ocular health and vision.

- **Cytotoxicity of MPS on unused and used CCL using HCEC**

  Current study showed no difference in cytotoxic effects of leachates of MPS from unused CCL and clear contact lenses using the PEM. Further study is warranted on the cytotoxic effects of leachates on unused and used CCL, using HCEC which allows longer term studies, to determine the influence of depositions on uptake and release of MPS

- **Longer term of CCL wear on protein deposition**

The current study found no difference on protein deposition between surface pigment CCL and clear contact lenses. Effects of longer term CCL wear, such as wearing CCL for two weeks, on protein deposition could be investigated.

- **Proteome analysis of the adhered proteins on CCL**

  The proteome profiling of the adhered proteins between surface pigment CCL and clear contact lenses could also be investigated. This allows comprehensive information of the deposition of various tear proteins on the lenses.

Apart from CCL research, the PEM could be used in other vision research including:

- **Cytotoxicity of Chinese herbal medicine**

  The application of Chinese herbal medicine for the treatment of dry eye and glaucoma has been advocated in recent years. Apart from their effectiveness on disease treatment, it is important to evaluate if they are safe to be used and study the potential adverse effects of different dosages.

- **Ability of UV blockage contact lenses**

  More contact lenses equipped with an additional feature of UV blockage are being marketed. Little is known about their ability of UV

blockage, as well as the effect of UV exposure on corneal epithelial cells.

With the advancement of technology, it is anticipated that the design and the material of contact lenses, as well as CCL, will improve. Thus, more research is needed to ensure safe and healthy contact lens wear.

Appendix A: Information Sheet

Research Study Information Sheet

Title of Project:
Protein deposition on cosmetic contact lenses

Project Leader:
Prof. Pauline Cho, SO (Tel: 2766 6100)

Project member:
Mr. Ka Yin Chan, SO (Tel: 2766 4462)

Why is the study being performed?
This study aims at comparing protein depositions between worn cosmetic lenses and clear contact lenses.

What do volunteers for the study have to do?
1. If you would like to volunteer for the study, you will be asked to sign an informed consent form that you understand the information presented on this sheet.
2. You will be invited to attend two visits. Both vision and corneal health will be assessed to ensure no contraindication of contact lens wear. A pair of contact lenses (cosmetic lens on one eye and clear lens on the other eye) will be fitted and dispensed if successful.
3. You will be required to wear contact lenses (cosmetic lens on one eye and clear lens on the other eye) for 8-10 hours for two days. At the end of each day's wear, your eyes will be examined again and the worn lenses will be collected for analysis. A new pair of lenses will be dispensed and the procedures repeated.

Is there any benefit or risk if I participate in the study?
Risk: Risks associated with contact lens wear include corneal staining and infection. Infection risk is low if the lenses are used according to the optometrist instruction.

Benefit: The study gives no specific benefits to the participants.

Can a volunteer withdraw from the study?
Yes, you can stop from participating in the study at any time with no penalty.

Can I get more information on the study?
Yes, contact *Mr. Ka Yin Chan* and he will try to answer any questions you may have.

Appendix B: Consent form

Title of Study

Protein deposition on cosmetic contact lenses

Informed Consent Form

Have you read the information sheet provided?	Yes / No
Have you had an opportunity to ask questions and discuss this study?	Yes / No
Have your received satisfactory answers to all of your questions?	Yes / No
Have you received enough information about the study?	Yes / No
Do you understand that participation is entirely voluntary?	Yes / No

Do you understand that you are free to withdraw from the study

- at any time **Yes / No**
- without having to give a reason **Yes / No**
- without affecting your future care **Yes / No**

Do you agree to take part in this study? Yes / No

……
Signature of participant

……
Name of participant

……
Date

Appendix C: Ethics approval letter

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

To	Wong Hie Hua (School of Optometry)		
From	GUGGENHEIM Jeremy Andrew, Chair, Departmental Research Committee		
Email	jagugge@polyu.edu.hk	Date	28-May-2014

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 19-May-2014 to 31-Jul-2014:

Project Title:	Protein deposition on used cosmetic contact lenses
Department:	School of Optometry
Principal Investigator:	Wong Hie Hua
Reference Number:	HSEARS20140508002

Please note that you will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In the case of the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Departmental Research Committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

You will receive separate email notification should you be required to obtain fresh approval.

GUGGENHEIM Jeremy Andrew

Chair

Departmental Research Committee

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