Effect of povidone iodine contact lens disinfecting solution on orthokeratology lens and lens case contamination and organisms in the microbiome of the conjunctiva

Keywords. Povidone iodine, contamination, colonisation, lens case, orthokeratology

Abstract

Purpose. To compare lens cleaning routines using a povidone iodine-based rigid lens disinfecting solution and its effect on conjunctival colonisation, and lens and lens case contamination.

Methods. Participants, aged 6-10 years, receiving orthokeratology treatment were randomised to four lens cleaning routines: with and without the use of daily and/or weekly cleaners, which were performed by their parents. Conjunctival colonisation was compared before lens wear and at 1-, 3-, and 6-month after commencement of lens wear. Contamination of lenses and lens cases was investigated at these times. Organisms were identified using MALDI-TOF mass spectrometry.

Results. Of the 76 participants who completed the study, conjunctival colonization was present in 24 (32%) at baseline. Of the remaining 52 participants, 34 consistently yielded no growth. Participants positive at baseline were statistically more likely to be colonized after commencement of lens wear (p=0.020). Overall, colonization rate was reduced to 15% (11/72) after 6-month lens wear, which reached significance for initially colonized participants ($p < 0.001$). Few cultures yielded potential ocular pathogens, with notably no *Pseudomonas aeruginosa*. Contamination rates of both lenses and lens cases were also low,

with few isolations of ocular pathogens. No significant differences were observed between cleaning regimes for conjunctival colonization or contamination of lenses or cases.

Conclusions. Disinfection for rigid and ortho-k lens wearers may be effectively achieved with the use of povidone iodine-based solution, apparently regardless of cleaning routine adopted in the current study. The absence of pathogens in the conjunctiva, lenses, and lens cases in the great majority of samples indicates that it can improve the safety of overnight lens wear.

Introduction

Multipurpose solutions (MPS) are the most commonly prescribed regimen to clean, disinfect, and rinse rigid contact lenses. Traditionally, these solutions are based on quaternary ammonium compounds (QAC) (e.g. polyquad) and biguanides (e.g. polyhexamethylene biguanide (PHMB)). Hydrogen peroxide (H_2O_2) can also be used to disinfect contact lenses. In recent years, solutions containing povidone iodine (PI) have been introduced, initially for soft lenses,[1-5] and later expanded to include rigid lenses.[6-7] PI has been safely used as a disinfectant for ophthalmic operations and prophylactic eyedrops for neonates for many years.[8] PI-based soft and rigid lens solutions have been reported to be as effective against *Pseudomonas aeruginosa* as other MPS and H_2O_2-based systems.[5,6,9] These solutions were also reported to be effective against *Acanthamoeba*.[2,6] The more recently introduced formulation for RGP lenses consists of an anionic surfactant and PI solution, to which neutralizing tablets containing sodium sulphite and proteolytic enzyme are added.

Major problems affecting reusable contact lens wear are compliance with disinfecting regime and care of the lens case. Numerous studies have reported high levels of contamination of lens cases, ranging from 30% to over 80%.[3,10-12] This is partly due to the build-up of organisms into a biofilm in the case, which are not as easily killed as planktonic organisms.[14,15] In contrast to QACs and biguanides, both PI and H_2O_2 are able to kill organisms in biofilms formed in lens cases.[7] In addition, oxidizing disinfecting solutions use specialized cases and obvious changes in the colour (e.g. PI becoming colourless) or characteristic (e.g. Bubbling with H_2O_2) of the solutions discourage topping up. Topping up of solutions leads to dilution of active agents, reducing microbiocidal activity and increasing biofilm formation.[16] Failing to clean the lens case daily and replace it regularly (preferably

monthly) is also a major factor in contamination.[11,17] Investigation of contact lens contamination has shown somewhat lower rates compared to lens cases, but few of these studies have investigated rigid lenses.[11,13] This may be attributable to the fall in popularity of rigid lenses until the development of modern orthokeratology (ortho-k) for myopia control and use of scleral lenses.[18,19] Lower contamination has been suggested to be linked with patients' awareness that the lens will be in contact with the cornea.[11] Prevention of lens contamination is of particular importance for ortho-k users as this is an overnight modality, in which lenses are reused for up to a year.[20,21]

In recent years, there has been an upsurge in interest in the microbiome present in various sites of the human body.[22] Much of this attention has been focused on the gut and the mouth, with fewer studies addressing the eye. A study investigating the ocular microbiome of lens wearers showed an increased risk for ocular pathogens, in particular, gram-negative bacteria.[23] It has been suggested that use of MPS and other disinfecting solutions for contact lens care leads to transfer of the active agents into the eye, which may select for a more limited microbiome in the conjunctiva.[12,13] Some practitioners advise the use of saline to rinse the lenses before insertion to avoid irritation and other effects of residual MPS in the eye.[24,25] However, partly to streamlining of the lens care process, manufacturers generally do not suggest the use of saline for rinsing. MPS at low concentration may still kill or retard the growth of organisms, changing the balance of the microbiome.[26] More importantly, low levels of disinfectant lead to tolerance or development of resistance in bacteria to the active agents, in particular, QACs and biguanides.[27,28] This phenomenon has been observed in both cross-sectional and longitudinal comparisons of spectacle wearers and ortho-k patients.[29,30] Survival of such organisms may explain the increased levels of coagulase-negative staphylococci (CNS) in the conjunctiva of contact lens wearers.[13,31]

However, the effects of the use of PI-based disinfecting solutions on the conjunctival microbiome have not been reported. As PI is an oxidising agent, development of resistance to PI-based disinfecting solutions is unlikely, but selection or removal of species may occur if introduced into the eye.

The current study aimed to investigate the effect of various cleaning routines on the contamination rate of lenses and cases of ortho-k participants using a PI disinfecting solution over six months of lens wear. In addition, the effect on colonization of the lower palpebral conjunctiva in children before and after ortho-k lens wear was determined.

Methods

In this 6-month prospective study, eligible participants were randomly assigned into one of the four study groups, with variations in lens care procedures, as shown in Table 1. They were required to carefully follow their instructions for lens care. Each participant was required to attend on five occasions, which included two pre-lens visits to determine the baseline microbiome of the conjunctiva and three visits after 1, 3, and 6 months of lens wear. At the latter visits, samples were collected from the lenses and lens cases to determine levels of contamination and organisms present. Colonization in the conjunctiva was also determined at each of these visits.

Participants

Eighty participants with low myopia (-4.00D to -0.75D), aged 6-10 years, were randomly assigned to one of the four study groups before commencing ortho-k lens wear. All

participants were concurrently participating in two myopia control studies (ClinicalTrials.gov registration numbers: NCT02955927 and NCT03191942) using Katt BE free ortho-k lenses (Precision Technology Services, Vancouver, B.C., Canada) made from Boston XO material (Dk 100 units) They were invited to participate in study when they had learnt lens handling and pending for lens delivery. Informed consent for this study were obtained from the parents prior to the commencement of the study. Tenets of the Declaration of Helsinki revised in 2013 were followed. The study was approved by the Departmental Research Committee of the School of Optometry, The Hong Kong Polytechnic University (approval number: HSEARS20170430002) and registered at ClinicalTrials.gov (registration number: NCT03193255).

All lens procedures, including insertion, removal, and cleaning, were performed by the parents. All lenses were rinsed with saline (cleadew rinsing solution, Ophtecs Japan Inc., Tokyo, Japan), followed by putting a drop of unpreserved artificial tears (Teare, Ophtecs Japan Inc., Tokyo, Japan) on the back surface of the lens before insertion. All participants were instructed to use PI-based solution (cleadewGP, Ophtecs Inc., Japan) for disinfection after lens removal in the morning, using the prescribed method for their group as shown in Table 1.

All solutions and accessories were replaced monthly and upon presentation of their used bottles at follow up visits, to ensure compliance. Parents were also required to disinfect the lens cases weekly by soaking in freshly boiled water for 10 mins, before following the daily routine of air-drying before sleep. Participants were required to use and care for their lenses as instructed and attend the aftercare visits. Compliance with handling procedures were

reviewed by asking the parents/participants to describe the routine procedures for lens insertion at night and for lens removal and cleaning in the morning.

Lens surface deposits and scratches, contamination of lenses and cases, and colonization of the conjunctiva were determined for each participant at the post lens-wear visits. To ensure safe ortho-k lens wear, any participant whose lenses displayed significant surface deposition was excluded from the study and advised to follow a more stringent care routine.

Samples

Samples from the conjunctiva and the lens were collected from the left eye of each participant, using the sterile Remel BactiSwabs (Thermo Fisher Scientific, Massachusetts, US). Before sampling, swabs were moistened with sterile phosphate buffered saline (PBS) and then immediately placed in sterile tubes containing Amies agar before refrigeration at 0-4^{o}C. Conjunctival samples were taken by gently rolling the swabs on the lower palpebral conjunctiva. Participants were asked to look up and their lower eyelid was gently reverted to avoid touching the cornea or the eyelashes. The ortho-k lenses, after disinfection for at least four hours, were removed from the lens cases with sterile tweezers. Lens samples were taken by gently swabbing the concave surface of the lens. The lens cases containing solution were sealed, labelled, and refrigerated together with the conjunctival and lens swabs. They were transferred to the laboratory within 48 hours.

Swabs were moistened with the solution in the lens case and the remaining solution poured away, before samples were collected from all inner parts of one compartment of the lens case. Two separate swabs were used, one for the inner lens cap and holder and one for the

main body, then combined in a bijou bottle containing brain heart infusion medium for incubation and subsequent culture.

The lens case was then gently rinsed twice with PBS to remove planktonic bacteria before draining for 10 minutes. A 250-µL aliquot of 0.4% crystal violet was added to the unsampled compartment of the case and gently dispersed. The stain was gently poured away after two minutes and the compartment gently rinsed twice with PBS to remove excess crystal violet. After drying for 30 minutes at room temperature, the stained biofilm was dissolved in 200 µL of absolute alcohol, transferred to a microlitre plate, and the optical density (OD) determined spectrophotometrically (Genesys 20, Thermo Scientific) at 600 nm.

Microbial assessment

All swabs were placed in bijou bottles containing sterile brain heart infusion broth. They were vortexed for 10 seconds and incubated overnight. Conjunctival samples were sub-cultured onto blood agar (37°C aerobically and anaerobically) and chocolate agar (37°C in 5% CO_2). Lens samples were cultured on blood agar (37°C aerobically and anaerobically), whereas samples from lens cases were cultured on blood agar (37°C aerobically and anaerobically), chocolate agar (37°C in 5% CO_2), and MacConkey agar (37°C aerobically). Individual colonies were analysed using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Bruker Microflex LT/SH system; Bruker Corp, Billerica, MA) for bacterial identification.

Treatment of data

Baseline age and sex differences among the four study groups were tested using one-way ANOVA and chi-square test, respectively. Differences in levels of colonization in the lower conjunctiva before and after lens wear were determined by McNemar's tests. Friedman tests were performed to evaluate changes in colonization and contamination levels over time, whereas repeated measures ANOVA (RM ANOVA) was used to evaluate the changes in OD after ortho-k lens wear for each of the four study groups. Binary logistic regression (enter method) was performed to determine the effect of factors on the risk of baseline colonization, and the risks of contamination of lenses and lens cases after ortho-k lens wear.

Results

Of the 80 participants who completed the baseline visits, one participant from Group 3 was excluded before lens delivery, because of recurrent corneal staining. The demographic data of the 79 participants are shown in Table 2. There were no significant differences in age and sex among the four groups of participants. The mean ± SD age was 9.1 ± 1.1 years and 66% were female. Three participants developed adverse events after the 3-month visit and were terminated from the study and did not attend the 6-month visit. Colonization and contamination rate of these three participants were not included in the 6-month analysis.

Conjunctival colonization

Baseline samples collected for three participants, one from Group 3 and two from Group 4, were discarded due to delayed laboratory processing. Conjunctival colonization was detected in 24 participants (32%), before lens wear (Figure 1). Result of the binary logistic

187 regression model investigating the effect of age, sex and study groups on the risk of baseline
188 colonization was statistically insignificant ($\chi 2(5) = 4.60$, $p = 0.466$).

189 After commencement of lens wear, samples from 34 of 52 participants negative at baseline,
190 consistently yielded no growth. Positive cultures were obtained from the remaining 18.
191 Figure 1 shows results of 42 participants with positive cultures. Twenty-four (32%)
192 participants yielded positive cultures at baseline and 15 (63%) had at least one positive
193 culture after commencement of ortho-k: 10 at one visit only; four at two visits; and only one
194 at all visits. Fifty-two participants were negative at baseline, of whom 18 subsequently
195 yielded positive cultures (15 on one visit only) (see Figure 1). Overall, colonization rate was
196 somewhat reduced from 32% at baseline to 15% after six months of lens wear, but this
197 change did not reach significance (Friedman test, $p = 0.079$). If only initially colonised
198 participants are considered, only 42% were colonized after one month and 25% on the two
199 later visits. Colonization was not affected by ortho-k lens wear (McNemar's test; $p = 0.122$),
200 i.e. participants with colonization before lens wear were more likely to continue to yield
201 positive cultures after lens wear (OR: 3.1; 95% CI: 1.1 to 8.6; $p = 0.027$) (Table 3).

202 At baseline, only four participants yielded a potential ocular pathogen, *Streptococcus*
203 *pneumoniae*, *Staphylococcus aureus*, and *Acinetobacter.* After commencement of lens wear,
204 cultures from five participants yielded an ocular pathogen on one occasion only, one at 1-
205 month visit, and two at each of the subsequent visits. Three of these isolates were *S. aureus*,
206 the remaining one being *S. pneumoniae.* A wide range of opportunistic or non-pathogenic
207 organisms were identified from the conjunctival swabs among the 39 participants with
208 positive colonization. *Micrococcus luteus* was the most commonly isolated organism (28%;
209 21/76), both before and after lens wear.

Contamination

Some participants failed to bring their lenses and lens cases with them at the three scheduled visits, so only 74, 76, and 72 lenses and cases were analysed. Measurements of OD to indicate presence of biofilm in the lens cases yielded very similar results at all three post-wear visits and between groups (RM-ANOVA, time: $p = 0.116$; interaction: $p = 0.84$).

Contamination rates of the lenses were fairly low and constant during the first 6 months (1-month: 16%; 3-month: 5%; 6-month: 10%) (Friedman test, $p = 0.113$). The most frequently isolated organism was *M. luteus*. Ocular pathogens were only isolated from lenses of two participants at the 1-month visit: one with *Acinetobacter* and the other with *S. pneumoniae*. There were no ocular pathogens observed on lenses at 3-month and 6-month visits.

The contamination rates of the lens cases were slightly higher than that of lenses (1-month: 24%; 3-month: 13%; 6-month: 19%), but again the change in the contamination rate over time was insignificant (Friedman test, $p = 0.674$). Only one case yielded a potential pathogen (*Acinetobacter*) at the 6-month visit. All other isolates were opportunists. Binary logistic regressions were performed to evaluate the effect of age, sex, study group, baseline colonization and OD on contaminations of lenses and lens cases. It was revealed that contamination of lenses was not associated with these factors ($\chi2(9) = 10.33$, $p = 0.324$). However, the regression model was significant for contamination of lens cases ($\chi2(9) = 23.64$, $p = 0.005$, Nagelkerke $R^2 = 0.412$). Contamination of lens cases was associated with increasing age ($p = 0.021$) and assignment to Group 4 ($p = 0.011$), but not with the other variables ($p > 0.056$). Participants with contaminated lens cases were statistically older but the difference was clinically insignificant (9.3 ± 1.0 years vs. 8.8 ± 1.2 years). The odds of

contamination of the lens cases was higher in Group 4 than in Group 1 (OR = 12.45; 95% CI: 1.79-87.01), but differences between other groups did not reach significance ($p > 0.254$). The contamination rates varied substantially between visits for all groups (see Table 4).

Discussion

This study demonstrated that the rate of conjunctival colonization decreased after commencing lens wear and contamination rates of lenses and lens cases were low, indicating that the PI-based disinfecting solution offers a good alternative for rigid lens care.

Pre-lens wear colonization was observed in 31.6% of participants, which is in agreement with the reports by Sankaridurg et al.[31] (36%) and Iskeleli et al.[32] (30%). Colonization was not affected by sex, age, or cleaning regime. At baseline, opportunistic organisms were carried by 27.6% of participants, while pathogens were only present in 5.3%. Following lens wear, rates of colonization with opportunists showed a downward trend, falling to 20.3%, 15.8%, and 12.5% at 1-, 3-, and 6-month visits, respectively, although this trend did not reach significance (Friedman test, $p = 0.131$). This decrease in positive cultures after commencing lens wear has been reported by previous studies.[33,34] However, it was observed, whilst colonization was not associated with lens wear, those who were colonized at baseline were significantly more likely to be colonized after commencing ortho-k treatment (see Figure 1). A study on extended wear RGP lenses has shown that the number of participants with positive conjunctival cultures decreased after 2-month of lens wear compared to baseline, although there was an increase in isolates of potential pathogens.[35] However, other studies have reported conflicting results. Zhang et al.[36] reported no differences between contact lens wearers and non-wearers, although the abundance of some organisms was reduced in

ortho-k wearers. In contrast, Stapleton et al.[37] found that a decrease in colonization only occurred after a considerable period extended soft contact lens wear.

In the current study, pathogens remained rare after commencement of lens wear, with only three incidences of colonization with *S. aureus* and two with *S. pneumoniae*. This low rate of colonization with ocular pathogens is encouraging, as it indicates no increase in risk of infection associated with ortho-k lens wear. Interestingly, colonization with pathogens was transient, with no participant being colonized on more than one occasion. The presence of pathogens was much lower than in an earlier study investigating effects of ortho-k lens wear on periorbital colonization, in which 39% of participants (9/23) were colonized with potentially pathogenic organisms.[13] This study was also conducted in Hong Kong, but lens disinfection was performed using a PHMB-based solution. It is possible that colonization of the conjunctiva in the current study was reduced as a result of use of a PI-based disinfecting solution. The pathogens isolated from the conjunctiva are those frequently present in the nasal cavity or naso-pharynx[13,38] and may be transferred to the eye directly by fingers or contaminated lens. It is therefore vital for practitioners to stress the importance of hand washing before lens handling and to remind patients to avoid touching their eyes.

The rate of lens contamination was low, in comparison to previous studies,[35,39] although there do not appear to have been reports on rigid lenses in recent years. Notably there was no difference between the groups, indicating that the cleaning regime had little impact on the contamination level within six months of lens wear. Although this reflects adequate disinfection by the solutions, it is also possible that participants and/or their parents may pay particular attention to lens hygiene, as they are aware that the lens will be inserted directly into the eye. Some parents of Group 1 (no-rub) participants questioned this cleaning

strategy as they thought failure to rub would result in lenses not being properly cleaned. Although they were instructed to continue with their allocated strategy as this was the manufacturer's recommended cleaning procedure, this may have resulted in some participants in Group 1 not complying with their designated procedures. The lack of differences between cleaning groups may also be attributable to the presence of an anionic surfactant and proteolytic enzyme in the system, which may eliminate the need for rubbing and use of additional cleaning products. However, as ortho-k lenses are used for a prolonged period of up to one year, rubbing and protein treatment may be required to remove more stubborn deposits, which can build up over time.[40]

It was encouraging to note that only two participants' lenses yielded potential pathogens at the 1-month visit and no further isolates occurred at the 3- or 6-month visits. This reflects both adequate cleaning and disinfection of the lenses. Although most studies have reported limited transient contamination of contact lenses with pathogens, there are some reports of high levels of contamination with such organisms in soft lenses.[41,42] This was attributed to the presence of biofilm, which may not be easily eliminated by MPS. Presence of pathogens was associated with corneal infiltrative events, but whether this was causative or a consequence remains unresolved.

As with lenses, overall contamination of lens cases, at 18.9%, was much lower than previously reported in rigid lens cases.[12,43] In a review, Szczotka-Flynn et al.[12] reported an overall contamination rate of greater than 50% in lens cases. The review includes studies by Donzis et al.[44] and Devonshire et al.,[45] who reported contamination rates of 41% and 78%, respectively.

However, a previous study, comparing lens case designs used conventional flat cases and a cylindrical case, noted that the inner surface contamination was much lower in cylindrical than in flat cases.[43] The cases provided by the manufacturer of the PI-based solution is a cylindrical case with a slightly smaller diameter, which would prevent insertion of fingers into the chambers. The lens holder, which is attached to the lid, is inserted into the inter-connected chambers after these are filled with the solution. The results indicated that the lens case was disinfected simultaneously with the lenses. However, a recent study[3] of use of a PI-based solution for soft lenses reported a similar overall contamination rate (70%) to findings with MPS disinfecting solutions.[46,47] Notably, the level of contamination in the PI study (soft lens) was very low and considered insignificant for 73% of these cases and gram-positive and fungal contamination was lower than for other disinfecting solutions. It is possible that the high rate of contamination, albeit low level, may be partly attributable to the rather complex lens case design that does require considerable finger contact to insert or withdraw the lenses. It has been recently reported that the use of the PI-solution in the cylindrical lens case, provided for the RGP version of the solution, resulted in complete loss of viability of both *S. aureus* and *Pseudomonas aeruginosa* biofilms.[7] With respect to biofilm, only the PI-based solution and hydrogen peroxide system were able to achieve such results. It is important to note that biofilm must be assessed by culture or viability as measurements of OD, following crystal violet staining, can lead to false positive results due to presence of dead organisms or residual stain in complex lens case designs.[7] It is notable that very few incidences of isolation of pathogens were noted in lens cases, with only one participant having a case with *Acinetobacter* at the 6-month visit. Most pathogens are strong biofilm producers[42] and the ability of this PI-based solution to kill organisms in the

biofilm can prevent their survival in the lens case. Notably none of the lens cases in this study yielded *P. aeruginosa*.

Both hydrogen peroxide system and PI-based solutions are strongly microbiocidal and require neutralization following disinfection, which must be done correctly to ensure safety. There is a clear colour change when neutralization has been achieved with PI and some hydrogen peroxide systems. However, other hydrogen peroxide systems, relying on a neutralizing disc within the case, may fail if used beyond the recommended period of use, leading to potential ocular damage.[48] In addition to its strong disinfecting ability, use of PI-based solution has other advantages, including no report of bacterial resistance due to genetic changes, i.e., *qac* genes[49] and improved comfort for day wear lenses.[3]

The main limitation of this study is the inability to determine complete compliance with cleaning instructions provided to each group, correct rinsing procedures, and intermittent cleaning of lens cases. As solutions were provided free of charge to participants, it is unlikely that they would have substituted an alternative disinfecting solution or continued use of a lens case beyond a month. Caution must also be applied for comparison of studies of contamination of lens cases with earlier reports: active agents and their concentrations have changed considerably in the last decades for soft contact lens solutions; manufacturers have paid most attention to effectiveness against *Pseudomonas*, as it was the leading of microbial keratitis; and in contrast with current practice, a new lens case was not provided with the purchase of each bottle of disinfecting solution. Therefore, the age of cases tested in previous reports of lens case contamination may be variable and include some that may have been used for several months (or even longer!). In addition to changing of lens cases, some participants had to replace their lenses during the study period due to fitting issues,

breakages, or loss. Both of these problems could potentially have resulted in lower rates of contamination.

In conclusion, PI-based solution offers a viable alternative system of disinfection for rigid and ortho-k lens wearers. Unexpectedly, there was no difference in contamination rates among the cleaning routines used. The absence of pathogens in the great majority of samples cultured and the dramatically reduced contamination rate compared with those previously reported indicate that use of PI-based solution can improve the safety of overnight lens wear.

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Figure Legend

Figure 1. Conjunctival colonization of participants in the four study groups at different visits. Only one participant (ID #19) had both pathogenic and opportunistic pathogens in baseline visit

493 **Table 1. Lens case cleaning regimes**

Group 1	Group 2	Group 3	Group 4
No rub	Rub with cleadewGP	Rub with daily cleaner	Rub with daily cleaner and weekly protein removal
Disinfect lenses without cleaning after removal every morning	Rub lenses with cleadew GP solution and rinse with saline after removal every morning before disinfection	Rub the lenses with daily cleaner* and rinse with saline after removal every morning before disinfection	Rub lenses with daily cleaner* and rinse with saline after removal every morning before disinfection and use P-AB weekly

494 * O2 Daily Care Solution, Ophtecs Inc., Japan

495 P-AB: Progent A+B, Menicon Co, Japan

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Table 2. The mean (±SD) of baseline age and the number (percentage) of female participants at baseline

	All	Group 1	Group 2	Group 3	Group 4	p-value
N	79	19	20	20	20	--
Age (years)	9.1±1.1	9.1±1.5	8.9±1.1	9.3±0.8	8.9±1.2	0.623^
Female	52 (66%)	11(58%)	14 (70%)	13 (65%)	14 (70%)	0.839[#]

^ One-way ANOVA; [#] Chi-squared tests

503 **Table 3. Association of conjunctiva colonization before and after orthokeratology wear**

	Negative after ortho-k (n=43)	**Positive after ortho-k (n=33)**
Negative before ortho-k (n=52)	34 (65.4%)	18 (34.6%)
Positive before ortho-k (n=24)	9 (37.5%)	15 (62.5%)

504 McNemar's test; p = 0.122

505 **Table 4. Contamination rates in lens and lens cases**

		Overall contamination rate	Post lens wear visit		
			1-month	3-month	6-month
Number of valid samples		**222**	**74**	**76**	**72**
Lens contamination		**23/222 (10.4%)**	**12 (16%)**	**4 (5%)**	**7 (10%)**
	Group 1	4/53 (8%)	3/17 (18%)	0/19 (0%)	1/17 (6%)
	Group 2	7/57 (12%)	4/19 (21%)	1/20 (5%)	2/18 (11%)
	Group 3	4/55 (7%)	2/19 (11%)	0/18 (0%)	2/18 (11%)
	Group 4	8/57 (14%)	3/19 (16%)	3/19 (16%)	2/19 (11%)
Case contamination		**42/222 (18.9%)**	**18 (24%)**	**10 (13%)**	**14 (19%)**
	Group 1	7/53 (13%)	2/17 (12%)	1/19 (5%)	4/17 (24%)
	Group 2	10/57 (18%)	6/19 (32%)	0/20 (0%)	4/18 (22%)
	Group 3	9/55 (16%)	4/19 (21%)	3/18 (17%)	2/18 (11%)
	Group 4	16/57 (28%)	6/19 (32%)	6/19 (32%)	4/19 (21%)

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507

		Visit			
No	**Group**	**Baseline**	**1-month**	**3-month**	**6-month**
1	1				
2	1				
3	1				
4	2				
5	2				
6	2				
7	2				
8	2				
9	2				
10	2				
11	2				
12	3				
13	3				
14	3				
15	3				
16	3				
17	3				
18	4				
19	4				
20	4				
21	4				
22	4				
23	4				
24	4				
25	1				
26	1				
27	1				
28	1				
29	1				
30	1				
31	1				
32	2				
33	2				
34	2				
35	3				
36	3				
37	3				
38	3				
39	4				
40	4				
41	4				
42	4				
43-76 were **negative** for all cultures					

508

Staphylococcus aureus
Coagulase-negative staphylococci and micrococci
Acinetobacter
Streptococcus. pneumoniae
No growth

509
510

511 **Figure 1.**