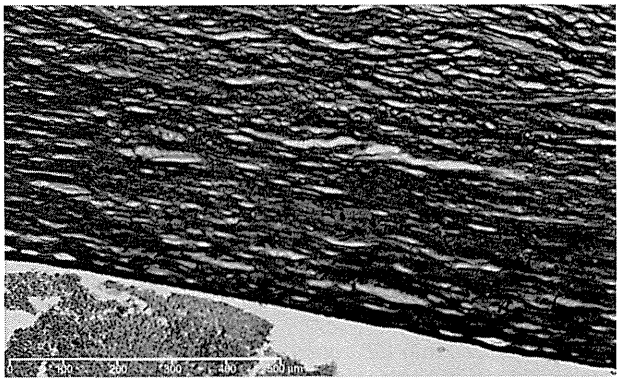

Figure 2 Case 4. Pathological findings of the removed cornea (Grocott's staining). A substantial amount of fungi are observed just above the Descemet's membrane.

suggested the presence of voriconazole at the top two-thirds or three-quarters of the cornea but it did not reach Descemet's membrane (Figure 3). It is important to note that the delivery of the drug is limited even by intrastromal injection.

A report[11] demonstrated that the aqueous humor concentration of voriconazole declines rapidly after topical application or intracameral injection, and also demonstrated that the decline of voriconazole shows an exponential decay with a half-life of 22 minutes. Although pharmacodynamics of intrastromal voriconazole is unknown, these results suggest that a sufficient level of voriconazole in the corneal stroma may not last long after the injection. Leakage of voriconazole liquid from the stroma to the epithelial side of the cornea can also reduce the amount of voriconazole after the injection.

In Case 5, the patient suffered from a peripheral corneal perforation caused by a plant injury (normal intra ocular pressure due to an incarcerated iris) (Figure 4). The keratitis around the perforation worsened and hypopyon gradually increased, even after three intrastromal injections of 1.0% voriconazole together with hourly application of 1.0% voriconazole eye drops and nightly use of natamycin ointment. The patient was keratoplastied with the frozen cornea followed by the topical steroid for 1.5 months. Two weeks after steroid use, keratitis recurred and progressed rapidly, even with three additional 1.0% voriconazole intrastromal injections.

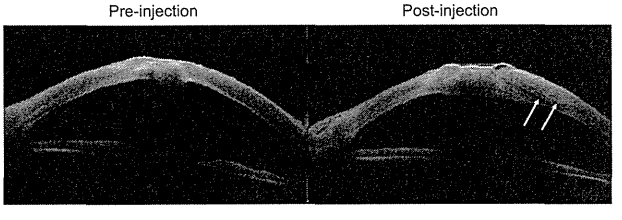

Figure 3 Optical coherence tomography images of Case 1 (pre- and post-injection).
Notes: Intrastromal fluid location just after the injection of voriconazole can be seen in the right picture. The high-density area is limited to the outermost two-thirds or three-fourths of the corneal stroma (white arrows).

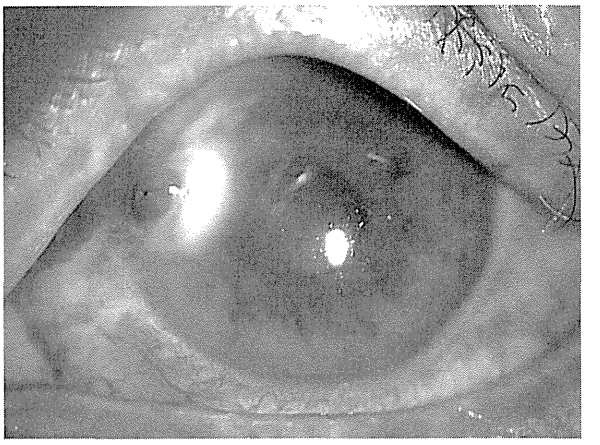

Figure 4 Case 5 (the anterior picture at the initial visit).
Note: The hyphate ulcer and the corneal perforation are observed.

Keratitis remission was finally achieved with administration of 3.0% voriconazole eye drops hourly, natamycin ointment five times daily, and intravenous injection of micafungin (100 mg/day) for 5 days. The removed cornea during optical keratoplasty revealed that hypha had infiltrated into the deep layer of the corneal stroma (Figure 5). Voriconazole delivered by intrastromal injection might not reach the infectious focus. Intrastromal injection of voriconazole was ineffective because of the rapid progress of *Fusarium* species keratitis[12,13] that might be related to the use of topical steroid in the early postoperative stage. The *Fusarium* keratitis patient (Case 6) had a history of foreign body sensation during her farm work. We initiated the treatment with 0.1% voriconazole intrastromal injection together with natamycin ointment four times a day. Although the steroid was not used in this case, the keratitis was refractory to multiple injections of voriconazole. Therefore, we believe that 0.05% voriconazole is not sufficient for *Fusarium*-related keratitis.

Marangon et al reported that MIC of voriconazole for clinical isolates of *Fusarium* species showed the highest

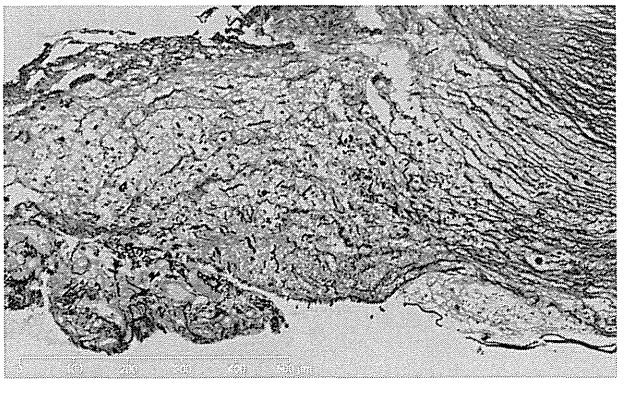

Figure 5 Case 5 (pathological findings of the removed cornea) (Grocott's staining).
Note: Hyphae is observed in the deep layers of the corneal stroma.

(8 μg/mL) value in all genera examined.[4] The MICs of voriconazole to *Fusarium* species that we detected in Cases 5 and 6 were also of a comparatively high value, 4 μg/mL. We injected between 0.3 mL and 0.5 mL of 1.0% voriconazole, which translates into 1,000 μg/mL, at the site. This was much higher than previous reports of success with voriconazole.[6–9] However, the effect of voriconazole on *Fusarium* keratitis is not enough for complete eradication, regardless of the concentration. In 2013, an article was published reporting that intrastromal injections of voriconazole do not offer any beneficial effect over topical 1% voriconazole therapy in a randomized clinical study.[14] Currently we are not sure how effective voriconazole intrastromal injection is in treating *Fusarium* keratitis.

Although keratitis caused by the *Candida* species might have been successfully treated with 0.05% voriconazole intrastromal injection, the same concentration as previous reports,[6–9] the optimal concentration for intrastromal injection remains to be determined. Moreover, all yeast described in this study is *Candida*. The response to the injection in other yeast fungi, such as *Cryptococcus* or *Trichosporon*, may differ. A recent animal study demonstrated that intracameral injection of ≥0.25% voriconazole could result in microstructural damage to corneal endothelial cells.[15] Further animal experiments relevant to voriconazole intrastromal injection are necessary to determine optimal concentration of voriconazole with minimal toxicity to corneal endothelial cells. A further prospective clinical study with fixed treatment protocol is necessary for redeeming the small sample size and the variety of treatment protocol in this study. Then, we should compare voriconazole intrastromal injection with the new treatment option, such as topical caspofungin, as to the effectiveness of fungal keratitis.[16]

In conclusion, 1.0% voriconazole intrastromal injection is effective in treating fungal keratitis caused by yeast. Even using lower concentrations of the voriconazole than the present study may be effective in some cases. However, a higher concentration of voriconazole, such as 1% solution, was not consistently effective in treating advanced fungal keratitis caused by filamentous fungi, especially the *Aspergillus* and *Fusarium* species.

Disclosure

The authors report no conflicts of interest in this work.

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New method for viewing Krehbiel flow by polymethylmethacrylate particles suspended in fluorescein solution

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ABSTRACT.

Purpose: **To investigate the changes in the tear flow velocities caused by ageing.**

Methods: **Ninety-nine subjects (41 men, mean age 48.3 ± 20.7 years) were recruited from the Department of Ophthalmology of the Ehime University Hospital. None of the subjects had serious abnormalities of the external surface of the eye. The Krehbiel flow of tears was determined by 40-μm polymethylmethacrylate (PMMA) beads suspended in a fluorescein sodium solution (PPF). The movement of the beads was video recorded through a slit-lamp during normal blinking. The flow of the beads was determined with a Motion ANALYZER® software (KEYENCE Co., Osaka, Japan). The velocity of the beads in young age, 20–40 years, middle age, 41–60 years and old age, ≥61 years, groups was determined.**

Results: **The equation describing the velocity (mm/second) of the PMMA particles as a function of age in the lower tear meniscus measured in the direction of the lacrimal punctum was $Y = 2.49 - 0.04X$, where Y = velocity and X = age ($r^2 = 0.214$; $p < 0.0001$). For the upper meniscus, the equation was $Y = 4.83 - 0.05X$ ($r^2 = 0.195$, $p < 0.0001$). The average velocity was 0.70 ± 1.66 mm/second in the lower and 2.16 ± 1.93 mm/second in the upper tear meniscus ($p < 0.0001$). The particle velocity decreased significantly with increasing age, but no significant difference between the male and female groups except for the lower tear meniscus when all subjects were analysed.**

Conclusion: **The PPF technique is a simple method of examining Krehbiel flow of tears and may be used for evaluating functional nasolacrimal duct obstruction quantitatively.**

Key words: fluorescein – Krehbiel flow – lacrimal duct – polymethylmethacrylate – tear clearance – tear meniscus

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Introduction

A functional nasolacrimal duct obstruction (FNDO) can arise from conjunctival laxity, punctal stenosis, nasolacrimal duct strictures and floppy eyelid syndrome. To determine the pathophysiology of FNDO, it is necessary to evaluate the clearance of tear fluid from the tear meniscus. A number of methods have been developed to analyse this (Jones 1962; Zappia & Milder 1972; Jordan & Baum 1980; Webber et al. 1987; Occhipinti et al. 1988; Shimizu et al. 1993; Hagele et al. 1994; Xu & Tsubota 1995; Sahlin & Chen 1996; Zheng et al. 2014); however, all of these are indirect methods. In addition, some methods require fluorescein dye to be used as a tracer (Jones 1962; Zappia & Milder 1972; Webber et al. 1987; Occhipinti et al. 1988; Shimizu et al. 1993; Hagele et al. 1994; Xu & Tsubota 1995), and another method, fluorophotometry, requires a special instrument (Webber et al. 1987; Occhipinti et al. 1988; Shimizu et al. 1993), and another uses anterior segment optical coherence tomography which also requires a special instrument (Zheng et al. 2014). It would be better if the dynamics of the tear flow in the tear meniscus can be examined by slit-lamp biomicroscopy because a slit-lamp is commonly available in the general clinic.

Earlier, Maurice prepared a lamp-black saline solution and applied this mixture topically and examined the tear flow dynamics by slit-lamp biomicroscopy (Maurice 1973). He observed a steady flow in the tear meniscus after blinking, a phenomenon called Krehbiel flow, and stated that Krehbiel flow resulted from the action of the lacrimal pump. Normal Krehbiel flow indicated a patency of the nasolacrimal duct. For some unknown reason, Klehbiel flow has not been commonly used to determine the tear flow and used to determine the pathophysiology of FNDO and epiphora.

Practically, the lampblack suspension is quite difficult to observe by slit-lamp examination due to its low contrast. Thus, the purpose of this study was to measure the rate of Krehbiel flow by a simple technique developed in our laboratory. Our technique consisted of suspending 40-μm polymethylmethacrylate (PMMA) particles in a fluorescein solution (PPF) which allowed us to observe Krehbiel flow. We were able to calculate the speed of the tear flow by video photography. The differences in the speed of Klehbiel flow were studied in normal individuals of different ages.

Materials and Methods

Subjects and experimental design

The purpose of this study and the procedures to be used were presented to all subjects, and a signed informed consent was obtained. The procedures were approved by the Institutional Review Board of Ehime University Hospital, and they conformed to the tenets of the Declaration of Helsinki.

Ninety-nine individuals (41 men and 58 women, mean ± SD age of 48.3 ± 20.7 years) were randomly recruited from patients without epihora who visited the Department of Ophthalmology, Ehime University Hospital, or from volunteers who had no symptoms or signs of epihora. To study age-related differences, subjects were placed into a young group consisting of 23 men (24.6 ± 2.8 years) and 15 women (26.5 ± 4.9 years), a middle age group consisting of 12 men (49.2 ± 4.7 years) and 13 women (52.4 ± 4.3 years) and an older group having six men (73.0 ± 2.7 years) and 29 women (71.2 ± 7.5 years). None of the subjects had nasolacrimal duct obstruction. No subject reported having epiphora, and the tear meniscus height was normal in these subjects as confirmed by slit-lamp examination, eyelid abnormalities such as entropion or extropion, blink dysfunction, severe conjunctivochalasis, cicatricial keratoconjunctival diseases such as Stevens–Johnson syndrome or ocular cicatricial pemphigoid, acute ocular surface disorders, trauma or infection.

Preparation of PMMA particles in fluorescein sodium solution (PPF)

Fine PMMA particles (Toughtic®AR650MX-W, $\varnothing$ = 40 μm; TOYOBO Co., Osaka, Japan) were prewashed with ethanol and acetone to remove monomers so that only cross-linked PMMA particles were used. Our pilot study showed that when the movements of the PMMA particles of diameters of 10, 40 and 100 μm (Toughtic®AR650MX-W, $\varnothing$ = 10, 40, 100 μm; TOYOBO Co.) were compared, the 10 μm particles tended to float at the top of the tear fluid, while the 100 μm particles sank to the bottom. On the other hand, the 40 μm particles moved freely in the tear film meniscus; therefore, they were chosen for this study. Next, a 5% PMMA concentration was chosen as the optimal concentration, because the particles remained in the tear meniscus during the 3–4 min observation period and could be easily identified. The optimal concentration of fluorescein was determined to be 0.2% because the PMMA particles were most easily observed at this concentration. An artificial tear solution (Epicatear™; Menicon Co., Nagoya, Japan) was used as the vehicle of this solution. After comparing PPF volumes of 2, 5, and 10 μl, 5 μl was chosen as the most suitable amount because it persisted in the eye throughout the observation period and did not overflow the lid margin. In the end, the PPF solution used consisted of a 0.2% fluorescein sodium solution with 5% PMMA beads of 40 μm sizes. The osmolarity of PPF was 286 mOsm/kg, pH was 7.30, and specific gravity was 1.20.

Observation of Krehbiel flow and velocity of PMMA particles

The PPF suspension was shaken vigorously to ensure that the fine PMMA beads were well distributed in the solution, and 5 μl of the PPF suspension was dropped into the conjunctival sac with a micropipette without topical anaesthesia. The movement of the fine PMMA particles in the lower and upper tear meniscus was video recorded through a slit-lamp microscope at 12× magnification. The video recordings were begun immediately after a blink, and the recordings were made for 5 seconds. The recordings were made with the patient in frontal gaze without blinking.

A video analysis software (MOTION ANALYZER®; KEYENCE Co., Osaka, Japan) was used to measure the velocity of the PMMA particles (Fig. 1). The video recording rate was 30 frames/second. To determine the bead velocity, one particle close to the lower or upper lacrimal punctum was randomly selected and followed for 0.337 seconds beginning 1 second after blinking. The particle velocity was calculated based on the distance between the starting and ending points measured on the video images. This protocol was repeated twice, and the average of the two values was used for the statistical analyses.

Repeatability of PPF measurements

Eight healthy volunteers, four men and four women with a mean ± SD age of 26.8 ± 3.5 years, were studied for the repeatability of the PPF measurements. One ophthalmologist (MY) performed all of the PPF recordings and analyses. On the first day, the movements of the PMMA beads in the lower tear meniscus of the right eye of each volunteer were recorded. Then, a second recording was made with at least a 30-min interval. On another day, the two recordings were carried out in a similar fashion. Finally, the interval repeatability of the PPF test measurements was determined statistically based on the four recordings.

In another set of experiments, 20 healthy volunteers, eight men and 12 women with a mean ± SD age of 47.3 ± 20.8 years, were studied for the repeatability analysis of interblinking. Similarly, one ophthalmologist (MY) performed all of the PPF recordings and analyses. The movements of the PMMA beads in the lower and upper tear meniscus of the right eye of each volunteer were recorded three times following a blink at an interval of 5 seconds. Finally, the interblinking repeatability of the PPF test measurements was determined statistically based on the three recordings each of the lower and upper meniscus.

Statistical analyses

The repeatability of the PPF measurements was determined by calculating the intraclass correlation coefficients (ICCs). Measurements were considered reliable when ICC was >0.7. The significance of differences between the velocities of the PMMA particles in the three age groups was determined by ANOVA and compared between groups using the Tukey–Kramer test. A

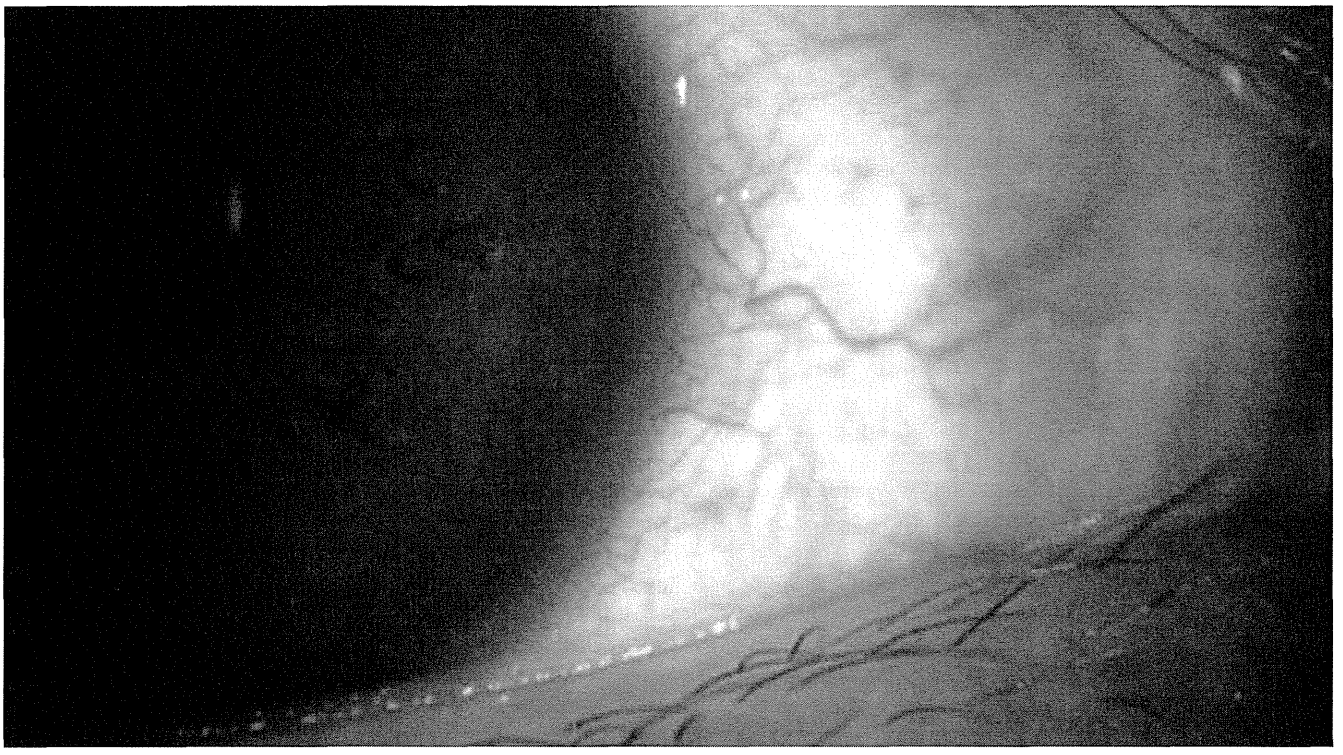

Fig. 1. Observation of tear flow in the lower tear meniscus with polymethylmethacrylate particles suspended in 2% fluorescein solution (PPF).

$p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using the JMP ver. 8 (SAS Institute, Cary, NC, USA).

Results

Repeatability of PPF measurements

The ICC for the velocities of the PMMA particles in the lower tear meniscus measured on different days and at different times was 0.928. The ICC for the interblinking was 0.828 for the lower meniscus and 0.689 for the upper meniscus. The interblinking ICC for the upper tear meniscus would have been 0.7 if the value had been rounded out. Thus, in spite of the fact that the interblinking repeatability of the upper was slightly lower than the other values, we still judged it to be in the good range.

Tear Krehbiel flow velocity

The equation describing the velocity (mm/second) of the PMMA particles as a function of age in the lower tear meniscus measured in the direction of the lacrimal punctum was $Y = 2.49-0.04X$, where Y = fluid velocity and X = age ($r^2 = 0.214$; $p < 0.0001$; Fig. 2A). For the upper meniscus, the equation was $Y = 4.83-0.05X$ ($r^2 = 0.195$, $p < 0.0001$; Fig. 2B). The PMMA particle velocity decreased significantly

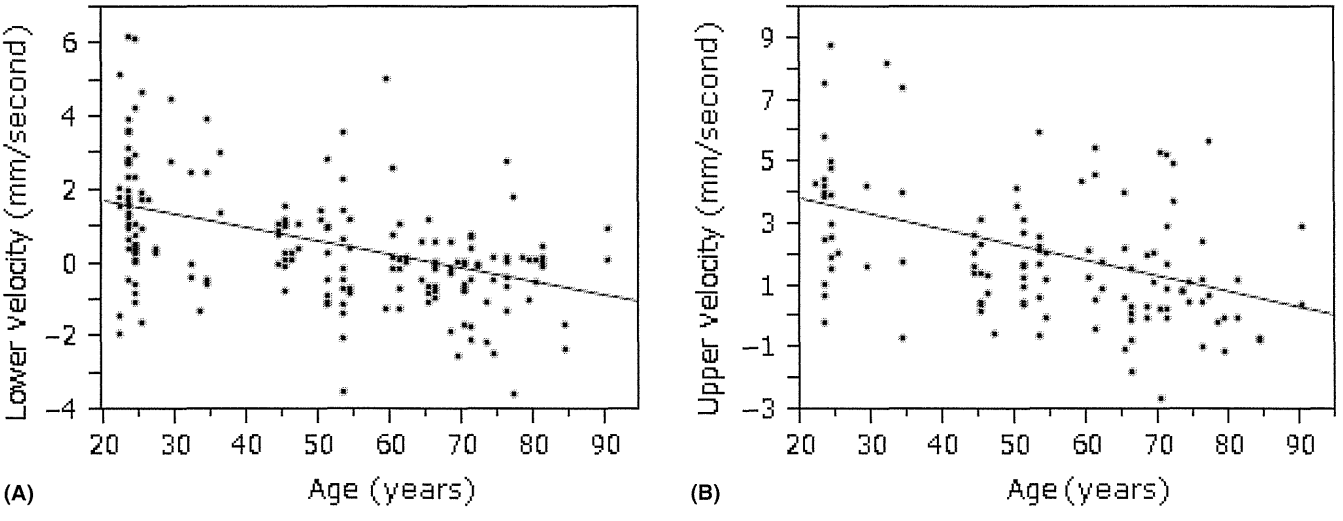

Fig. 2. Correlation between polymethylmethacrylate particle velocity and age. Equation describing the tear flow velocity as a function of age in lower (A) and upper (B). In lower, the equation was $y = 2.49-0.04x$, where y = fluid velocity and x = age ($r^2 = 0.214$; $p < 0.0001$). In upper, the equation was $y = 4.83-0.05x$ ($r^2 = 0.195$, $p < 0.0001$).

with increasing age, and the average velocity was 0.70 ± 1.66 mm/second in the lower and 2.16 ± 1.93 mm/second in the upper tear meniscus.

There was a significant difference between the mean velocities in the lower and upper tear meniscus for all age groups (all $p < 0.0001$), and no significant difference was noted between the male and female groups except for the lower tear meniscus when all subjects were analysed ($p = 0.0005$; Table 1).

Discussion

Maurice studied the movement of the lampblack particles in the lower tear meniscus immediately after blinking and also after a short period of continuous eye opening following a blink (Maurice 1973). He reported that immediately after blinking, the lampblack particles moved rapidly towards the lower lacrimal punctum, and the movement slowed after a period of continuous eye opening. In some cases, the particles were reported to flow in the opposite direction towards the temporal side of the eye due to the force of gravity on the inferior temporal lid margin. This study with a lampblack solution showed that it was a useful method to examine the dynamics of tear flow in the tear meniscus. However, the lampblack suspension has not been widely used clinically because lampblack particles are difficult to see, and a quantitative analysis method was not established. The lampblack solution was made based on Nagashima's formula (Nagashima 1976), which was an improved version of Maurice's lampblack suspension.

In our study, Krehbiel flow was clearly observed using PPF, and our results showed that there was a significant decrease in the average flow rate with increasing age. Also, the PPF flow rate was significantly faster in the upper meniscus than the lower meniscus. Our results showed that there was a good reproducibility of the measurement. Thus, our technique of using a suspension of PMMA particles in a fluorescein solution can obtain valid and repeatable measurements of the velocity of the tear flow.

We used 40-μm PMMA particles suspended in a 2% fluorescein solution to analyse the flow of tears in the lower meniscus. We believe that the PPF solution is an ideal solution for this. First, PMMA is safe and non-toxic to the human eye. PMMA is the material from which contact lenses, intra-ocular lenses, toothbrush bristles, dentures and syringes are made. The PMMA particles we used were coated with titanium oxide which is used as the basic ingredient in cosmetic foundations, UV protective cosmetics, toothpaste and the food dye in white chocolate.

Second, PMMA particles can be obtained in different sizes, and the 40-μm PMMA particles we used had a mean specific gravity of 1.20 which means that they neither floated nor sank in the tear fluid. Also, the PMMA particles are relatively uniform in diameter and do not tend to clump together as do lampblack particles. These properties make PMMA a better marker for observing tear flow in the tear meniscus.

Third, our PPF suspension was made with 0.2% fluorescein sodium which increased the contrast of the PMMA particles and facilitated an easy observation of pathologies such as conjunctivochalasis.

Fourth, the PPF solution was well tolerated by our subjects. Although our study was conducted without anaesthesia, none of the subjects reported intolerable foreign body sensation. When PPF test was carried out with or without eye drop anaesthesia in eight healthy volunteers, four men and four women, mean ± SD age of 26.8 ± 3.5 years, the PMMA particle velocities in the lower meniscus were not significantly different between two situations (data not shown). Thus, we decided to use this method without anaesthesia because of the advantage of minimal bias of tear overflow caused by the anaesthetic eye drop, and all subjects were under natural conditions of blinking during the examination.

These unique features of the PPF solution allowed us to directly examine the dynamics of tear flow in the tear meniscus with specific attention to Krehbiel flow and to investigate changes in tear flow dynamics due to ageing.

Interestingly, the average PPF flow rate was faster in the upper menisci than in the lower menisci which was not expected because it has generally been believed for many years that the main part of the tear fluid leaves the eye via the lower punctum. Thus, the drainage effect in the upper punctum may be higher than that in the lower. From these results, an upper punctum occlusion may be more effective than lower punctum occlusion during the punctum plug occlusion therapy. A further study should be conducted in this regard.

Also, particle movements in the lower tear meniscus appear to be affected by gravity. Although particles flowed towards the outer canthus (negative velocity) in 15.8% of the cases in the young group, this backward flow was seen more often in the older groups (M group: 30.0%, $p = 0.0659$, O group: 56.9%, $p < 0.0001$, respectively, vs. *Y* group, Pearson's chi-square test), suggesting that it is due to ageing. Because the lower lid tends to droop with ageing, this change increases

Table 1. Polymethylmethacrylate particle velocity in lower and upper meniscus.

	Male	Female	Both
Young			
Up	3.84 ± 2.17	4.29 ± 2.61	4.04 ± 2.34
Low	1.78 ± 1.67	1.45 ± 2.00	1.65 ± 1.80
Middle			
Up	1.89 ± 1.13	2.08 ± 1.63	1.99 ± 1.38
Low	0.66 ± 1.17	0.02 ± 1.73	0.33 ± 1.51
Old			
Up	1.45 ± 2.15	1.34 ± 1.89	1.36 ± 1.93
Low	−0.50 ± 0.83	−0.15 ± 1.07	−0.21 ± 1.04
All			
Up	2.46 ± 2.02	2.01 ± 2.21	2.18 ± 2.14
Low	1.20 ± 1.60	0.36 ± 1.61	0.71 ± 1.66
		*	

Low = lower meniscus; Up= upper meniscus; Young = young age group (20–40 years); Middle = middle age group (41–60 years); Old = old age group (≥61 years); All = all age; Both = male and female.
* $p = 0.0005$.

the effect of gravity on the PMMA particles; therefore, the negative flow of PPF may occur. Moreover, a conjunctivochalasis near the lower punctum with ageing may alter the PPF flow to the punctum. Further investigations are needed to examine this condition.

Our experiment has some limitations. For example, if the area is observed over a period of time, most of the particles will be cleared from the tear meniscus, and one may assume that these particles have drained through the punctum. However, the pseudo flow, in which particles flow past the punctum and towards the medial canthus, can be found in both normal subjects and in cases of lacrimal punctum obstruction. Therefore, tear fluid clearance cannot be evaluated based on particles flow medially, and it is best to perform a more comprehensive evaluation with a combination of tear fluid clearance evaluation methods (Jones 1962; Zappia & Milder 1972; Jordan & Baum 1980; Webber et al. 1987; Occhipinti et al. 1988; Shimizu et al. 1993; Hagele et al. 1994; Xu & Tsubota 1995; Sahlin & Chen 1996; Zheng et al. 2014).

In conclusion, the PPF technique can be used to evaluate Krehbiel flow in a relatively simple manner with good reproducibility. The findings showed that the velocity of PMMA particle flow into the lacrimal punctum slowed significantly with age. The PPF test should be useful for evaluating FNDO by quantifying the particle velocity. Future studies should evaluate the value of the PPF technique for evaluating diseases which may lead to FNDO, including conjunctival laxity syndrome, punctual stenosis, nasolacrimal duct strictures and floppy eyelid syndrome.

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Prevalence of Upper- and Lower-Lid-Wiper Epitheliopathy in Contact Lens Wearers and Non-wearers

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Objective: To report lid-wiper epitheliopathy (LWE)-like staining at the lower eyelid margin (lower-LWE) and to determine the prevalence of LWE (upper-LWE) and lower-LWE in contact lens (CL) wearers and non-CL wearers.

Methods: Four hundred forty-three eyes of 229 non-CL wearers, 405 eyes of 208 soft CL wearers, and 135 eyes of 71 rigid gas permeable CL wearers were studied. Lissamine green and fluorescein (FL) staining were used to assess the degree of LWEs, tear break-up time (BUT), and cornea-conjunctival staining (FL-S). The correlations between the prevalence of LWEs and the other factors were evaluated.

Results: The prevalence of lower-LWE was significantly higher (39.5%) than upper-LWE in non-CL wearers (upper-LWE; 12.0%; $P<0.001$). The prevalence of both upper- and lower-LWE were significantly correlated with age and FL-S, but not sex and BUT, in non-CL wearers. The prevalence of both upper- and lower-LWE was significantly higher in younger than older subjects ($P<0.001$). Upper- and lower-LWE were detected in a higher percentage of CL wearers than in non-CL wearers ($P<0.001$).

Conclusion: Our results indicate that examination of the lower eyelid margin would be preferable to that of the upper eyelid margin in studies of LWE.

Key Words: Lid-wiper epitheliopathy—Lower eyelid—Prevalence.

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Lid-wiper epitheliopathy (LWE) is defined as an epitheliopathy of a portion of the marginal conjunctiva of the upper eyelid.[1] Korb et al.[1] reported that the presence of LWE was correlated with dry eye symptoms in contact lens (CL) wearers. They studied LWE patients with or without dry eye intensively and showed that LWE occurs more frequently in patients with dry eye symptoms than without dry eyes.[2,3] In their recent report, they showed that the prevalence of LWE was higher in dry eye disease and suggested that LWE may be a diagnostic sign of dry eye disease.[2] However, their results also showed that LWE occurs in subjects without the conventional dry eye signs such as the Schirmer test and tear break-up time (BUT).[3] Although LWE may be caused by an alteration of the lubrication between the epithelium of the lid wiper and the ocular surface, the cause remains elusive.

It has been reported that the epithelial alterations occur only in upper eyelid as LWE, and Doughty et al.[4] demonstrated a similar staining with lissamine green (LG) at the lower eyelid margin. We also found a similar epitheliopathy at the lower eyelid margin.[5] Knop et al.[6,7] described the anatomical changes of the lid margins and showed that the characteristics of the lid-wiper region were similar for the upper and lower eyelids. Thus, the LWE-like staining in the lower eyelid margin may have the same or similar pathological basis as the LWE of the upper eyelid margin. However, the mechanism causing the LWE for either eyelid has not been definitively determined.

Thus, the purpose of this study was to determine the prevalence and degree of LWE (upper-LWE) and LWE-like staining in the lower eyelid (lower-LWE) in a large number of CL wearers and non-wearers. We also determined if there were significant correlations between the prevalence and degree of LWEs and the age, sex, corneal and conjunctival staining, and CL wear.

MATERIALS AND METHODS

The total number of the subjects was 508, including 229 non-CL wearers, 137 soft contact lens (SCL) wearers, and 71 rigid gas permeable contact lens (RGPCL) wearers. All were patients of the outpatient clinic of the Department of Ophthalmology, Ehime University Hospital. The presence of LWEs was determined in all patients who visited two experienced ophthalmologists (A.S. and M.Y.) on the same day. Subjects with acute inflammatory ocular surface disorders, eyelid closure failure, deformed eyelids, conjunctival concretions, abnormal blinking disorders, or a history of any type of eye surgery were excluded.

Fluorescein (FL; 1%) staining of the corneal and conjunctival staining with scores ranging from 0 to 9 (FL-S), and the tear film break-up time (BUT in sec) were measured according to the 2006 Japanese Dry Eye Diagnostic Criteria.[8] The measurements were made by the same two ophthalmologists (A.S. and M.Y.).

The presence of LWEs at the upper and lower eyelid margins was also determined by FL (1%) and LG staining (2%) (grades 0 to 3 after Korb et al.[1–3]). The correlations between the prevalence and the grade of LWEs, and age, sex, BUT, and FL-S were determined.

Statistical Analyses

All data are presented as the means±standard error of the means. Statistical analyses were performed with JMP for Windows, Version 8 (SAS Institute, Cary, NC). A P value less than 0.05 was considered statistically significant.

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RESULTS

Lid-Wiper Epitheliopathy in Non-Contact Lens Wearers

Four hundred forty-three eyes of 229 non-CL wearers (100 men and 129 women, age 3–94 years with a mean of 52.1±24.0 years [mean±SD]) were studied. The upper-LWE (grades 1–3) was detected in 55 (12.5%) of 443 eyes along the margin of the upper eyelid and in 174 (39.5%) of 443 eyes along the lower eyelid margin (lower-LWE) in non-CL wearers (Fig. 1).

The prevalence of lower-LWE was significantly higher in these non-CL wearers than that of upper-LWE ($P<0.001$; Table 1). The average LWE grade of the upper-LWE was 0.21±0.03, which was significantly lower than the lower-LWE score of 0.79±0.05 ($P<0.001$; Table 1).

All of the subjects were divided into those with LWE [LWE(+)] and those without LWE [LWE(−)], and the significance of the differences in the different parameters of the subjects was determined for these two groups. The LWE(+) group was significantly younger than that of LWE(−) group for both upper- and lower-LWE. No significant difference was detected for sex and BUT between the two groups. However, the FL staining scores were significantly higher in the LWE(+) group than in the LWE(−) group for both the upper- and lower-LWE (Table 2).

Because a significant difference was found in the ages between the LWE(+) and LWE(−) groups, the prevalence and average LWE scores of the eyes with LWE were examined in more detail by dividing the subjects into 20-year age groups. Both the prevalence and average LWE grading scores decreased significantly with increasing age ($P<0.0001$), and the most significant difference was detected between the 0 to 19 years and 20 to 39 years for the upper-LWE and between the 20 to 39 and 40 to 59 years for the lower-LWE (Fig. 2A, B). When the subjects were divided into younger (≤39 years) and older (>40 years) groups, both the prevalence and average LWE scores were significantly higher in the younger groups for both upper-LWE and lower-LWE (Fig. 2C, D).

Lid-Wiper Epitheliopathy in CL Wearers

Two hundred seventy eyes of 137 SCL wearers (35 men and 102 women, age 9–61 years, and mean 26.5±10.5 years) and 135 eyes of 71 RGPCL wearers (11 men and 60 women, age 14–61 year, and mean 37.0±11.8 years) were studied. The prevalence and grade of either type of LWE was significantly higher in the CL wearers than in the non-CL wearers. The prevalence and grade of upper-LWE was RGPCL>SCL while that of the lower-LWE was similar in RGPCL and SCL (Fig. 3).

The FL staining score was significantly higher in the LWE(+) group but not in the lower-LWE(+) group of the RGPCL wearers. No difference was detected in BUT scores in either the LWE(+) and LWE(−) groups, but the BUT score was higher in the upper-LWE group (−) of the SCL wearers (Table 3).

FIG. 1. Lissamine green staining to demonstrate lid-wiper epitheliopathy (LWE)-like staining along lower lid margin, that is, lower-LWE nasal margin in the vicinity of the punctum of lower lid margin is stained with lissamine green.

TABLE 1. *Prevalence and Grade of Lid-Wiper Epitheliopathy in Non–Contact lens Wearers*

	Prevalence	Grade
Upper-LWE	55 (12.5 %) of 443 eyes[a]	0.21±0.029[a]
Lower-LWE	174 (39.5 %) of 443 eyes[a]	0.79±0.052[a]

[a]$P<0.001$.

LWE, lid-wiper epitheliopathy.

DISCUSSION

Our results showed that LWE was relatively common in CL wearers and non-CL wearers. One of new findings was that LWE-like staining was found at the lower eyelid margins (lower-LWE) and not only in the upper lid margins. However, the prevalence of lower-LWE was higher than upper-LWE. Another new finding was that a higher prevalence of both upper- and lower-LWE was present in younger than older individuals, and the prevalence and severity of LWE decreased with increasing age. We found that upper-LWE was present in 12.5% of non-CL wearers. Because we did not question the subjects about having dry eye symptoms or having been diagnosed with the dry eye syndrome, the prevalence of upper-LWE in this study may be more comparable with that in nonsymptomatic subjects in the earlier studies.[2,3,9]

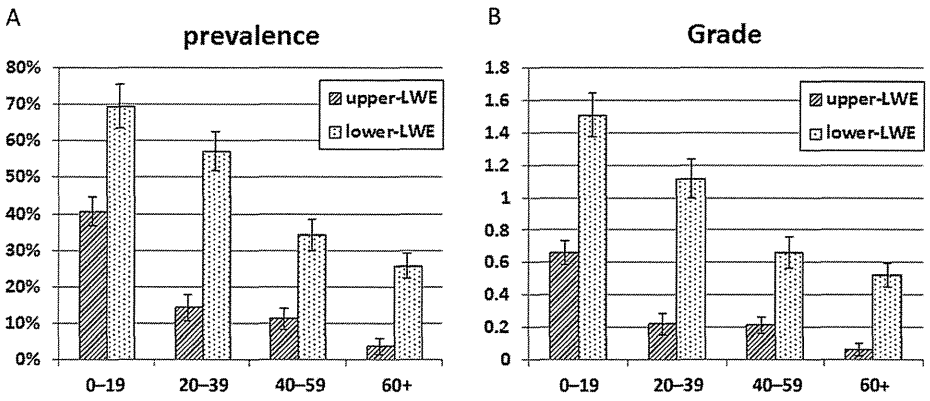
As Korb et al.[2,7] mentioned, the probable cause of LWE is a compromised tear film resulting from inadequate lubrication between the eyelid and ocular surface.[9] The higher prevalence of LWEs in CL wearers supports this suggestion because the tear film instability during CL wear has been well documented.[10–13] Another possibility of the higher prevalence of LWEs in CL wearers may be

TABLE 2. *Analysis of the Presence or Absence of Lid-Wiper Epitheliopathy in Non–Contact Lens Wearers*

Upper-LWE	Upper-LWE(−)	Upper-LWE(+)	Statistical Significance
Age, y	54.85±1.17	32.73±2.87	$P<0.001$
Sex	M 162:F 226	M 28:F 27	NS
BUT, sec	5.03±0.16	5.67±0.48	NS
FL-S (0–9)	1.03±0.08	1.62±0.30	$P<0.05$

Lower-LWE	Lower-LWE(−)	Lower-LWE(+)	Statistical Significance
Age, y	58.28±1.32	42.56±1.85	$P<0.01$
Sex	M 98:F 171	M 92:F 82	NS
BUT, sec	4.89±0.19	5.45±0.25	NS
FL-S (0–9)	0.93±0.09	1.37±0.14	$P<0.01$

BUT, break-up time; FL-S, fluorescein staining; LWE, lid-wiper epitheliopathy; NS, not significant.



C

Upper-LWE	prevalence		Grade	
Younger (< 39 years)	35 of 136 eyes (25.7%)	*	0.41 ± 0.078	*
Older (> 40 years)	20 of 307 eyes (6.5%)		0.12 ± 0.052	

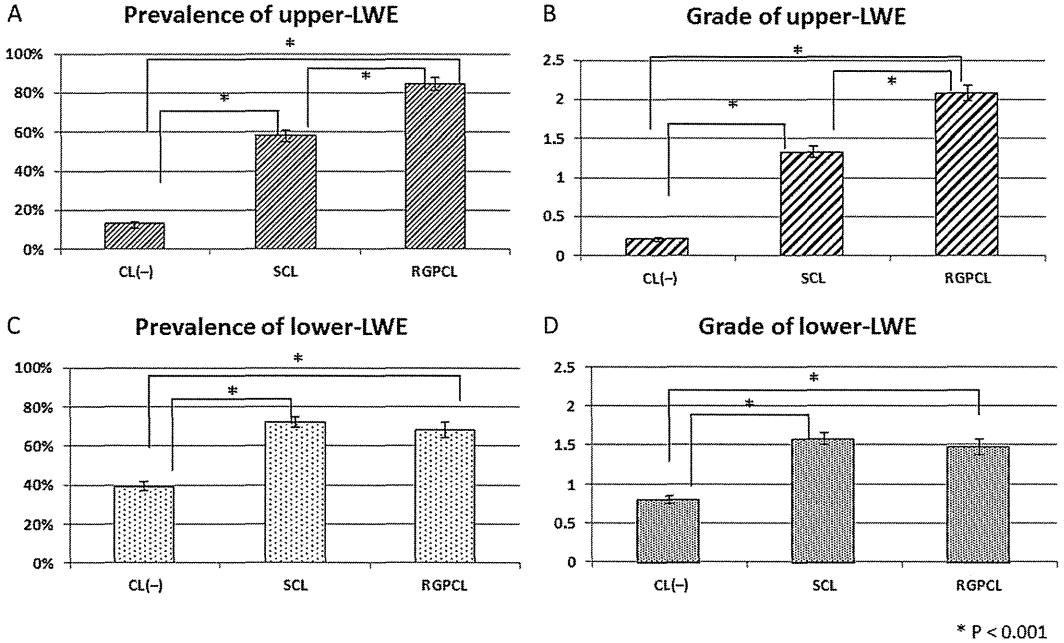
D

Lower-LWE	prevalence		Grade	
Younger (< 39 years)	85 of 136 eyes (62.5%)	*	1.28 ± 0.094	*
Older (> 40 years)	89 of 307 eyes (29.0%)		0.57 ± 0.063	

* $P < 0.001$

FIG. 2. Effect of age on prevalence and grade of lid-wiper epitheliopathy (LWE) in non–contact lens (non-CL) wearers. The prevalence (A) and average grades (B) of LWE was examined in 20-year age groups. Both the prevalence and average grades decrease with increasing age ($P<0.0001$). When the subjects were divided in younger (<39 years) and older (>40 years) groups, both of the prevalence (C) and average grades (D) of LWE were significantly higher in younger groups for both upper- and lower-LWE.

because of higher shear stress between the eyelids and CL because the surface of CL has more friction compared with corneas. The higher prevalence of LWEs in younger generations may support the hypothesis of higher shear stress. In a recent study, we demonstrated that the eyelid pressure was higher in younger individuals and decreased with increasing age. In addition, both the upper and lower eyelid pressures were significantly and negatively correlated with age.[14] Although eyelid pressure does not directly reflect the shear stress generated by the eyelids, it may partly be related to the share stress generated by the eyelids. These findings support the



* P < 0.001

FIG. 3. Prevalence and grade of lid-wiper epitheliopathy (LWE) in contact lens (CL) wearers. In soft contact lens (SCL) wearers, upper-LWE was detected in 157 (58.1%) of 270 eyes and lower-LWE in 184 (68.1%) of 270 eyes. This difference was not significant ($P>0.05$). (A) In rigid gas permeable contact lens (RGPCL) wearers, upper-LWE was detected in 114 (84.4%) of 135 eyes and lower-LWE in 97 (71.9%) of 135 eyes. This difference was not significant ($P>0.05$). (C) The prevalence and grade of either type of LWE was significantly higher in CL wearers than non-CL wearers. The prevalence and grade of upper-LWE was RGPCL>SCL while that of the lower-LWE was similar in RGPCL and SCL.

TABLE 3. *Analysis of the Presence and Absence of Lid-Wiper Epitheliopathy in Contact Lens Wearers*

SCL	Age	Sex	BUT	FL-S
Upper-LWE(−)	27.38±1.14	M 31:F 82	6.11±0.27	1.41±0.11[a]
Upper-LWE(+)	25.82±0.72	M 39:F 118	6.46±0.22	1.92±0.13[a]
Lower-LWE(−)	29.45±1.38[a]	M 25:F 51	5.72±0.09[b]	1.36±0.18[b]
Lower-LWE(+)	25.30±0.69[a]	M 45:F 149	6.54±0.20[b]	1.85±0.10[b]
RGPCL	**Age**	**Sex**	**BUT**	**FL-S**
Upper-LWE(−)	44.67±2.53[a]	M 3:F 18	4.14±0.48	0.95±0.21[a]
Upper-LWE(+)	35.59±1.06[a]	M 15:F 99	5.39±0.26	1.96±0.12[a]
Lower-LWE(−)	39.02±1.82	M 5:F 38	5.33±0.42	1.70±0.16
Lower-LWE(+)	36.05±1.22	M 13:F 79	5.13±0.26	1.85±0.14

[a]$P<0.01$.

[b]$P<0.05$.

BUT, break-up time; CL, contact lens; FL-S, fluorescein staining; LWE, lid-wiper epitheliopathy; RGPCL, rigid gas permeable contact lens; SCL, soft contact lens.

hypothesis that shear stress from the eyelids was involved in the development of LWEs.

One of the purposes of this study was to determine the cause of or the risk factors for LWE. Upper-LWE has been observed frequently in patients with dry eye or dry eye symptoms,[1–3,5,9] and thus, dry eye has been considered to be a risk factor of LWE. However, not all patients with LWE had tear deficiency in the earlier studies.[5,9] In our study, no difference was detected in the BUT values between LWE(+) and LWE(−) groups, and longer BUTs were detected in the lower-LWE(+) group than the LWE(−) groups among the SCL wearers. These results confirmed an earlier report that no difference was detected in any of dry eye tests, including the Shirmer test, BUT, and DR-1 among LWE(+) and LWE(−) groups.[5] Thus, these results indicate that dry eye may not be the only risk factor for LWEs although patients with LWEs can have dry eye. It will be necessary to examine and determine whether dry eye causes LWEs or LWEs cause the dry eye symptoms.

Interesting findings in this study were that the prevalence and grade of lower-LWE were significantly higher than those of upper-LWE. During a blink, the upper eyelid has a large vertical movement while the lower lid has a shorter horizontal nasalward movement.[15] Because of the large excursion of the upper eyelid, most investigators have paid more attention to the pressures generated by the upper eyelid movements. Among the limited number of studies that examined the effects of lower eyelid movements, Shore[16] reported that the decrease in lower eyelid movement with aging was closely correlated with the increase in eyelid laxity. However, it should also be remembered that the lower eyelid moves horizontally meaning that the eyelid margin rubs over the same area of the cornea and conjunctival surface. Thus, the friction of the eyelid movements on a restricted area of ocular surface might be greater by the lower eyelid than the upper eyelid. The lower-LWE is generally found at the nasal margin of the palpebral conjunctiva in the vicinity of the lower punctum (Fig. 1). This area moves against the surface of the bulbar conjunctiva, whereas the area of predilection of the upper-LWE is the middle of the eyelid that moves over a smooth corneal surface. These different locations of the movements may be another reason for the higher prevalence of lower-LWE. This hypothesis may be partly supported by some of the reports discussing lid-parallel conjunctival folds (LIPCOF), which are subclinical folds in the lateral, lower quadrant of the conjunctiva parallel to the lower lid margin, and are suggested to be the first mild stages of conjunctivochalasis. In a series of reports,[17,18] the presence of LIPCOF was significantly correlated with dry eye symptoms along with LWE (upper-LWE). Thus, it has been suggested that both clinical signs are related to mechanical

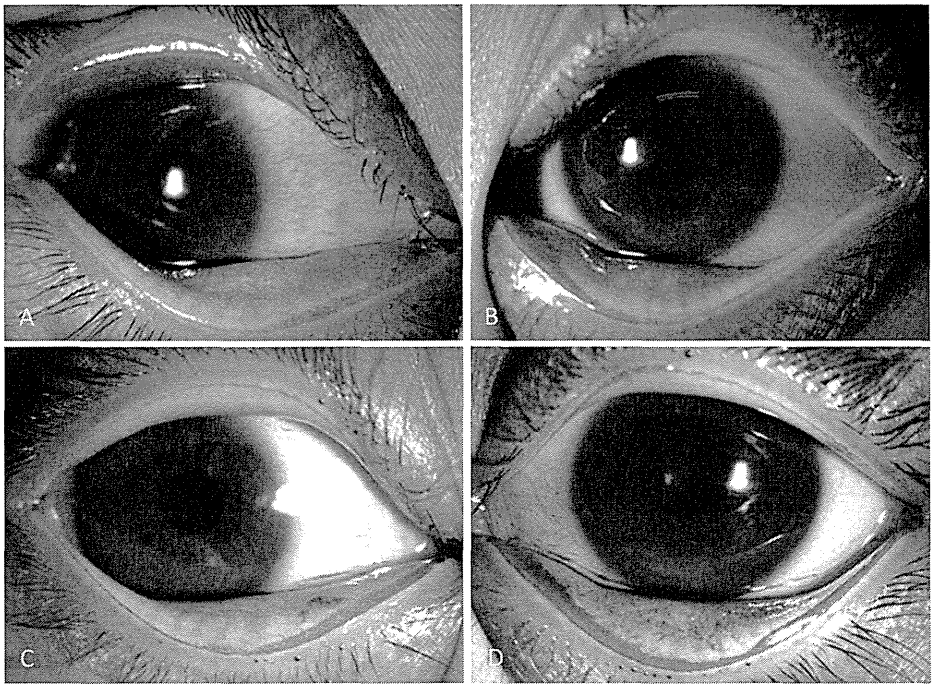


FIG. 4. Lid-wiper epitheliopathy in a case of unilateral soft contact lens (SCL) wearer. Slitlamp photograph of eye at the first visit, right eye (A) and left eye (B). Upper- and lower-LWEs are observed on the right eye, whereas only lower-LWE is observed on the left eye. Slitlamp photograph 1 month after discontinuing to wear SCL on the right eye: right eye (C) and left eye (D). Upper-LWE is not present, whereas the lower-LWE remains on her right eye. Lower-LWE was still observed on the left eye.

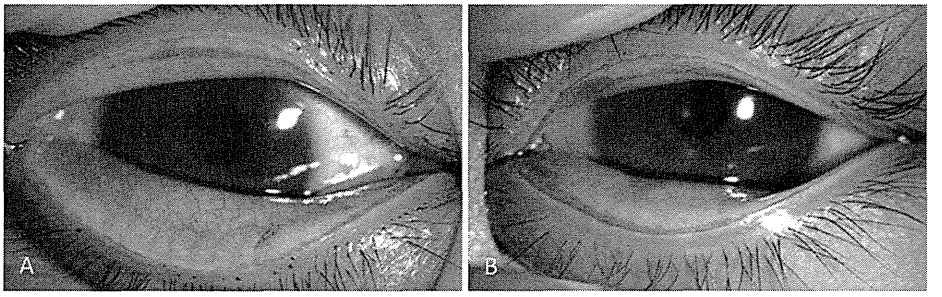


FIG. 5. Lid-wiper epitheliopathy by re-wearing soft contact lens (SCL). Slitlamp photograph of right eye (A) and left eye (B) after wearing SCL on both eyes 3 consecutive days, showing upper- and lower-LWE on the both eyes.

stress caused by rubbing.[18–20] Among those, Pult et al.[18] reported that nasal LIPCOF was related to dry eye symptoms. Although no report has reported on the relationship between lower-LWE and nasal LIPCOF, they may be significantly correlated. Future clinical studies including impression cytology are needed to understand the pathogenesis of LWE and LIPCOF.

Regarding the cause of LWE, we had a 33-year-old woman who presented with eye redness, discharge, asthenopia, and headaches. On her first visit, she wore SCL on her right eye but had not been worn SCL on left eye for 1 month because of severe eye pain. Slitlamp examination revealed upper-LWE on her right eye but not on her left eye (Fig. 4A, B), whereas lower-LWE was detected on both eyes (Fig. 4A, B). She was directed to stop wearing SCL and also to use 0.1% hyaluronic acid eye drops. These treatments resulted in the disappearance of the upper-LWE, but the lower-LWE remained on her right eye (Fig. 4C). In addition, most of the symptoms abated. Because she felt better, she expressed a desire to wear SCL again. Under informed consent that re-wearing SCL might induce LWE and the symptoms, she should be examined regularly if she chose to wear SCL again. Two months later she visited us after she had been wearing SCL on both eyes for 3 consecutive days. Slitlamp examination showed upper-LWE and unchanged lower-LWE on both eyes (Fig. 5A, B). The course of this case, that is, the disappearance of upper-LWE by discontinuing SCL on her right eye, and re-appearance of upper-LWE on both eyes by re-wearing SCL, proved the involvement of SCL as the cause of upper-LWE. An interesting finding in this case was the presence of lower-LWE regardless of SCL wear. It is likely that the palpebral conjunctival epithelium of the lower eyelid margin is more sensitive or fragile to the higher eyelid pressure and continuous friction. The presence of lower-LWE may be a risk factor of upper-LWE when wearing a CL.

In conclusion, we found that the prevalence of LWEs in randomly selected patients who visited the ophthalmological outpatient clinic was higher for lower-LWE than upper-LWE. The higher prevalence of lower-LWE may be caused by the continuous friction of the lower eyelid on the same region of the cornea during blinking. More attention should be paid to the lower eyelid margins and movements during blinking.

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Involved in design and conduct of study (A.S.); data collection (A.S., M.Y.); analysis and interpretation of the data (A.S); writing (A.S.); critical revision (Y.O.); and literature search (A.S.) of the manuscript. The study was approved by the Institutional Review Board of Ehime University. An informed consent for the examination was obtained from all subjects, and the procedures used conformed to the tenets of the Declaration of Helsinki.

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BMC Ophthalmology

RESEARCH ARTICLE Open Access

Evaluation of a new method of irrigation and aspiration for removal of ophthalmic viscoelastic device during cataract surgery in a porcine model

Arisa Mitani, Takashi Suzuki*, Yoshitaka Tasaka, Takahiro Uda, Yukako Hiramatsu, Shiro Kawasaki and Yuichi Ohashi

Abstract

Background: To determine if a method for irrigation and aspiration (I/A) during cataract surgery provides effective removal of ophthalmic viscoelastic device (OVD).

Methods: Japanese porcine eyes were used to evaluate I/A performance with Technique 1 (the I/A tip placed on the center of the anterior surface of the IOL), Technique 2 (the I/A tip alternately pressed near the edge of the IOL optic anterior surface on one side and then the other to tilt the IOL back and forth), and Technique 3 (the I/A tip inserted behind the IOL optic, between it and the posterior capsule). Techniques 1 and 2 were compared using the Miyake-Apple posterior view video technique to visualize the flow of irrigation fluid containing triamcinolone acetonide particles behind the IOL. To check the efficacy of OVD removal from behind the IOL for of all three I/A techniques, OVD with fluorescein beads were inserted inside the lens capsule before implantation of the IOL. After each I/A technique, eyes were prepared for Miyake–Apple viewing and pictures of the lens capsule were taken using fluorescent microscopy. Residual fluorescein beads in the capsular bag were analyzed.

Results: Technique 1 resulted in a straight flow of fluid behind the IOL, while Technique 2 resulted in a vortex flow. The average amount of OVD retained inside the capsule after using Technique 2 or 3 was significantly lower than after using Technique 1 ($p < 0.0001$).

Conclusions: Technique 2 proved to remove more effectively fluorescein bead-labelled OVD under the IOL than Technique 1.

Keywords: Intraocular lens, Ophthalmic viscoelastic device, Irrigation, Aspiration

Background

The ophthalmic viscoelastic device (OVD) is useful tool in modern cataract surgery. It coats and protects intraocular tissues and crates space. OVD which is used for insertion of the IOL into the lens capsule can be trapped behind the IOL. A common complication of OVDs after cataract surgery is an increase in postoperative intraocular pressure (IOP) because of OVD remaining in the lens capsule or the anterior chamber and obstructing the trabecular meshwork [1-6]. Since IOP spikes could cause the damage of the optic nerve and visual disturbance in the patients with glaucoma, ophthalmic surgeons should avoid IOP spikes after cataract surgery.

Along with IOP spikes, postoperative endophthalmitis is a complication of cataract surgery, and sometimes results in severe visual loss. The entry of external bacterial flora is often the cause of acute postoperative endophthalmitis. External bacterial flora probably enter the anterior chamber through the surgical wound; in fact contamination of the anterior chamber at the end of surgery has been noted to be as high as 5.7% to 21.1% [7-10]. Intraoperative or postoperative contamination of the anterior chamber seems to be the initial step of endophthalmitis. Some reports show that bacteria or the exoskeleton of bacteria are attached to intraocular lenses (IOL) which are explanted after either acute or late onset endophthalmitis [11,12]. Thus, one possibility is that bacteria contaminate the inside of the lens capsule, adhere to the IOL, and proliferate in the eye even without intraoperative

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complications. Along with clinical cases, we previously showed that *Enterococcus faecalis* inoculated into the lens capsule could lyse the lens capsule with neutrophils and spread into the posterior segment [13]. Since clearance of aqueous humor in the lens capsule seems to be less, microorganisms could grow and cause endophthalmitis.

Washing and cleaning the inside of the lens capsule at the end of surgery could reduce the incidence of IOP spikes and endophthalmitis. The anterior side of the IOL can be washed very well using the irrigation and aspiration (I/A) handpiece because the tip can easily access the anterior surface of the IOL. However, it is difficult to estimate the effectiveness of the I/A method for washing the inside of the lens capsule behind the IOL. Thus techniques which completely remove OVD and clean behind the IOL are needed. OVDs with different properties have been developed to cope with a variety of clinical situations [14-17]. There are several types of OVDs with different molecular weights and concentrations of sodium hyaluronate. A Cohesive OVD (sodium hyaluronate 1.0%) is often used for insertion of the IOL because it tends to hold together as a mass and is relatively easy to remove at the end of surgery [15,18]. Auffarth *et al.* have previously described the modified rock'n roll technique, in which circular movements of the I/A tip on the anterior surface of the IOL optic tilt and rock the IOL during I/A [19]. The technique was reported to be efficient in removing high molecular weight OVD (Healon 5) which leaves the eye with greater difficulty, behind the IOL. However rotation of the IOL could stress the lens capsule and zonular fibers. Since cohesive OVDs are easier to leave eye than high molecular weight OVD, rotation of the IOL could be unnecessary for removal of cohesive OVDs. To remove OVD from behind the IOL, insertion of the I/A tip under the IOL is an appropriate method. However it can occasionally induce complications such as aspiration of the lens capsule which can cause a tear. This technique can also be difficult for neophyte cataract surgeons. Thus, a possibly easier, safer and more effective I/A technique should be considered when a cohesive OVD is used. To check safe and effective I/A techniques, it is important to know dynamics of irrigation flow behind IOL during surgery. The porcine eyes were usually used for checking techniques because of similar anatomy of human and easy availability [20-24]. We recently observed the dynamic movements of posterior chamber-associated structures, e.g., the lens capsule, zonular fibers, and anterior hyaloid membrane during cataract surgery using view technique in bisected porcine eyes [24]. Furthermore we confirmed influence of eye bisection to anatomy were minimized [22-24]. Thus porcine model could be useful for checking I/A techniques.

In this study, we compared several I/A techniques. First techniques is that placement of the I/A tip on the anterior optic of the IOL with no further manipulation. That can be easily performed. Second techniques is the modified rock'n roll technique, in which alternately pressing the I/A tip near the edge of the IOL optic anterior surface on one side and then the other to tilt the IOL back and forth without rotation. Third techniques is placement of the I/A tip behind the IOL. We conducted two experiments. One is visualization of irrigation fluid flow behind the IOL during I/A techniques (Experiment 1). Another is checking effectiveness of I/A techniques for removal of fluorescein bead-labelled contaminated OVD (Experiment 2).

Methods

Porcine eyes

Twenty-eight porcine eyes were obtained from a local abattoir and were examined with a slit-lamp microscope and used within 24 hours of enucleation (3 eyes for experiment 1 and 25 eyes for experiment 2). Eyes with corneal trauma or other obvious abnormalities were not used.

I/A technique

We compared I/A techniques as follows; placement of the I/A tip on the anterior optic of the IOL with no further manipulation (Technique 1), alternately pressing the I/A tip near the edge of the IOL optic anterior surface on one side and then the other to tilt the IOL back and forth (Technique 2), and placement of the I/A tip behind the IOL (Technique 3) (Figure 1). All techniques used the same settings of 500 mmHg vacuum pressure, 26 mL/min aspiration rate, and bottle height of 60 cm.

Experiment 1 (visualization of irrigation fluid flow behind the IOL)

Three eyes were prepared using a modified Miyake-Apple method as follows [25-27]. Briefly, each eye was bisected diagonally to the equator using a razor-blade, and the

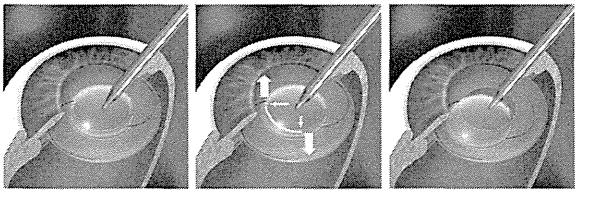

Figure 1 Removal techniques evaluated in the study. With Technique 1 (left), the I/A tip was placed on center of the anterior optic of the IOL. Technique 2 (center), alternately pressing the irrigation and aspiration (I/A) tip near the edge of the IOL optic anterior surface on one side and then the other to gently tilt the intraocular lens back and forth. Technique 3 (right), the I/A tip was inserted behind the optic of the IOL.

anterior segment was firmly attached to a plastic petri dish with superglue (Aron Alpha, Toagousei Co., LTD. Tokyo, Japan). After making a 2.8 mm corneal incision with a side port incision created at the 10 o'clock position, the anterior chamber was filled with a cohesive OVD (Sodium Hyaluronate 1%, Molecular Weight 1.5 – 3.9 × 10^6;Opegan Hi®, Santen Pharmaceutical, Osaka, Japan) and a continuous curvilinear capsulorrhexis (CCC) of 5.0 mm was performed. After hydrodissection, the lens nucleus was phacoemulsified using a Phacompo Phacoemulsificator® (Santen Pharmaceutical, Osaka, Japan) using balanced salt solution (BSS) (BSS plus; Alcon, Fort Worth, TX, US) for irrigation. Following phacoemulsification, residual cortical fibers was removed by I/A. And then we used an injector to implant an intraocular lens (Eternity X-60®, Santen Pharmaceutical, Osaka, Japan) into the capsular bag filled with a cohesive OVD. The Eternity X-60® is a monofocal 3-piece spherical hybrid acrylic IOL with a water content of 4.6%. Each IOL (Eternity X-60®, Santen Pharmaceutical, Osaka, Japan) was painted black using a permanent marker before being implanted into porcine eyes. After complete removal of OVD using technique 3, technique 1 or 2 was performed to check dynamics of irrigation flow behind IOL.

To help visualize the irrigation solution, 40 mg/ml triamcinolone acetonide (TA) (Bristol-Myers Squibb Company, New York, US) was added to the BSS. After starting I/A, 0.1 ml of TA was administered for 1 second via an irrigation tube. Dynamics of the irrigation were recorded by a 3CCD camera (DXC-C33, Sony, Tokyo, Japan). Video files, which had 30 frames per second, were converted to picture files using VirtualDub 1.9.11 (GNN General Public License). The whole IOL optic (6mm dia.) was used as the region of interest, and the volume of particles moving under the IOL in each frame was quantified as pixel intensity using image J software (National Institutes of Health, Bethesda, MD, US) [28]. The pixel intensity before irrigation was subtracted from the pixel intensity of each frame.

A grid line was overlaid on each picture frame. We calculated the distance particles moved across a 21 square grid for six successive frames, expressed as a drawing vector. We analyzed both methods in one eye, and repeated the testing in three different eyes.

Experiment 2 (removal of fluorescein bead-labelled OVD)

Twenty-five porcine eyes were used without dissection. Lensectomy was performed by phacoemulsification and I/A as describe previously. Following exchange to air in the anterior chamber, 0.1 ml of 5% 1.0 μm-fluorescein bead solution (Fluoresbrite™ Carboxylate YG 1.0 micron Microspheres; Polysciences Inc, Pennsylvania, US) was inserted into the lens capsule followed by 0.3 ml of Opegun Hi®. The IOL (Eternity X-60®, Santen Pharmaceutical, Osaka, Japan) was then implanted in the lens capsule. The eyes were then distributed into five groups according to I/A technique, I/A duration, and the location of the I/A tip during OVD removal as shown Table 1. Technique 1 was used in Group A and B, while Technique 3 was used in Group C. Technique 2 was used for Group D and Group E. In Group D, I/A was done for 10 seconds twice per side. In Group E, I/A was done for 5 seconds four times per side.

After the procedure, each eye was cut horizontally at the equatorial region using a razor-blade, and a picture of the lens capsule was taken using fluorescent microscopy (SteREO Lumar V12, Zeiss, Jena, Germany). Using image J software, the amount of residual fluorescein beads under the IOL were measured by pixel count and the results were analyzed [28]. Experiments were performed with five eyes per group.

Statistical analyses

Data in experiment of fluid dynamics were analyzed by Student's t-test for significance. Tukey-Kramer tests were used to compare the techniques in the experiment of removal of fluorescein bead-labelled OVD. Values of $p < 0.05$ were considered statistically significant.

Results

Experiment 1

Irrigation solutions behind the IOL in all tested eyes were visualized (Additional file 1). We checked flow of irrigation solution using both methods in three different eyes. Since flow pattern of particles in each technique were similar, a representative eye was estimated for movement of particles. Figure 2 shows a histogram of the pixel intensity at each time. We quantified pixel intensity of TA particles behind IOL in technique 1 or 2. The pixel intensity using Technique 2 increased and decreased in a shorter time span compared to Technique 1. To evaluate the dynamic flow of irrigation solution, we measured the distance and direction TA particles traveled for 6 frames (0.2 seconds). Figure 3 shows the vector in which particles crossed the grid lines as yellow arrows. The flow of the irrigation solution using Technique 1 was in one direction at an average distance of 0.66 mm (±0.23 mm) in the 0.2 seconds. For Technique 2 the flow was a focal vortex with an average distance of

Table 1 Groups for removal of OVD

Group	Technique	I/A duration
A	Technique 1	20 sec
B	Technique 1	40 sec
C	Technique 3	20 sec
D	Technique 2	20 sec (5 sec per side ×4)
E	Technique 2	20 sec (10 sec per side ×2)

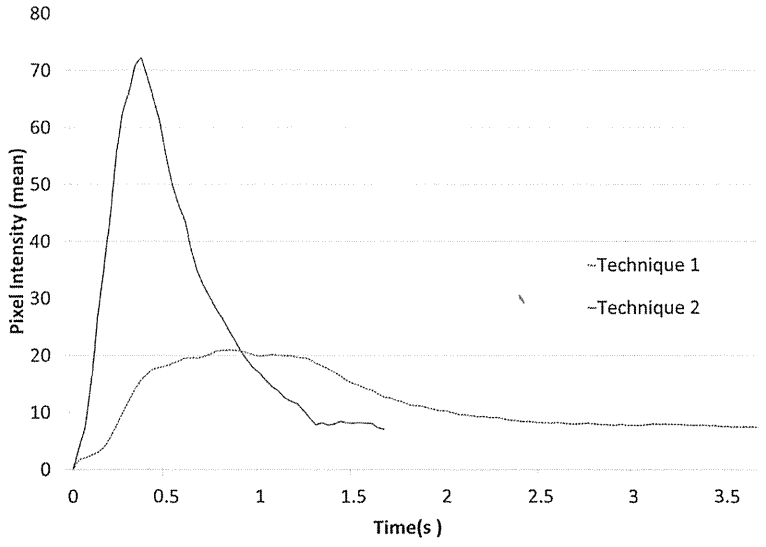

Figure 2 Histogram of pixel intensity in eye with Technique 1 (dashed line) and Technique 2 (solid line). Y-axis showing pixel intensity of TA, and X-axis showing time.

0.76 mm (±0.27 mm). There were no statistically significant differences between two methods.

Experiment 2

Fluorescent pictures were converted to white and black pictures using image J. Black pixel showed residual fluorescein beads. Figure 4 shows the pixel intensity of residual fluorescein beads for five eyes of each five group. Black pixels were found more in group A than in group B, C, D, or E. The average pixel intensity of fluorescein beads retained inside the capsule in group B, C, D and E was significantly lower than those of Group A ($p < 0.001$, Turkey-Kramer test) (Figure 5). There were no significant differences in residual fluorescein beads among Group B, Group C, Group D, and Group E.

Discussion

Removal of OVD from behind the IOL is critical to prevent IOP spikes, avoid shifts in centration of IOL as well as capsular block syndromes, and ensure sterility in the eye after surgery. If OVD behind the IOL has continuity to the anterior chamber, OVD could be removed by the I/A tip on the IOL. However it is difficult to remove OVD once its continuity to anterior chamber is lost. In that case, OVD should be displaced by the flow of irrigation solution and aspirated out of the eye. Although there are some reports which demonstrate irrigation fluid flow in the anterior chamber [21,29], little is known about irrigation fluid flow behind the IOL during I/A. Kaji *et al.* visualized irrigation fluid flow using 3-dimensional images and demonstrated the flow velocity decreased with increasing distance from the iris plan [21]. In this study, we could observe irrigation fluid flow behind the IOL during I/A using a blackened IOL and Miyake-Apple view method. We could not analyze flow using 3-dimensional images. However 2-dimensional images should suffice because of the minimal distance between the posterior capsule and the IOL. This study demonstrated different patterns of irrigation fluid flow due to the location of the I/A tip. The technique in which the I/A tip is held steady on the center of the anterior surface of the IOL optic appears to provide irrigation fluid flow parallel to the I/A handpiece. In this circumstance, fluid flow from the I/A tip could move to the equator of the lens capsule and repulse to area between IOL and posterior capsule. Technique 2, in which the I/A tip gently pressed down on alternate edges of the IOL optic anterior surface, had a vortex pattern of irrigation flow. The histogram of particle pixel intensity shows that the clearance of particles in this Technique 2 is more rapid than with the I/A tip simply centered on the IOL optic.

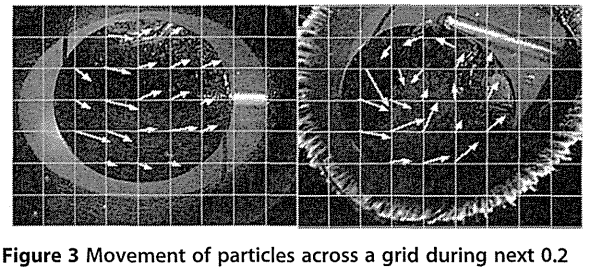

Figure 3 Movement of particles across a grid during next 0.2 seconds in a porcine eye showing Technique 1 (left) and 2 (right). The yellow arrows indicate the distance and direction in which particles moved for 0.2 seconds.

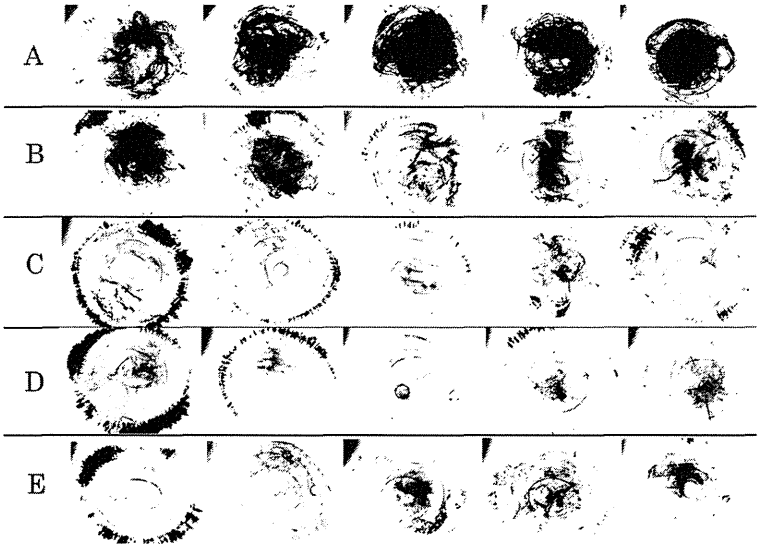

Figure 4 Photograph of fluorescent pixel after removal of the fluorescein bead-labelled OVD in all five eyes of each group (A, B, C, D, and E). Black pixel showing residual fluorescein beads.

Thus a vortex pattern of irrigation fluid induces a more rapid exchange of fluid and is therefore more effective in cleaning out the space between the IOL and the posterior capsule.

Along with visualizing and quantifying the flow of irrigation fluid behind the IOL, we also used 1.0 μm-fluorescein beads to determine the amount of residual fluorescein bead-labelled OVD behind the IOL after I/A. Because the diameter of gram positive cocci causing endophthalmitis, such as staphylococci, is about 1 μm, fluorescein beads ought to imitate bacterial contamination, as described previously [23]. This study demonstrated that the amount of residual fluorescein depends on how long the I/A tip is left on the center of the IOL; I/A for 40 seconds removed more beads than only 20 seconds, indicating that it might take longer to remove OVD from behind the IOL if the tip is only placed on the center of the anterior surface of the IOL. This was confirmed by the fact that the amount of residual fluorescence was less after cleaning with the I/A tip inserted between the IOL and posterior capsule. We reasonably conclude that doing I/A with the tip behind the IOL removes OVD more effectively. Importantly, this study demonstrated that technique 2 removed OVD as effectively as using the tip behind the IOL. To effectively remove OVD from behind the IOL, it is considered important to insert the tip behind the IOL. However this technique can induce complications such as aspiration

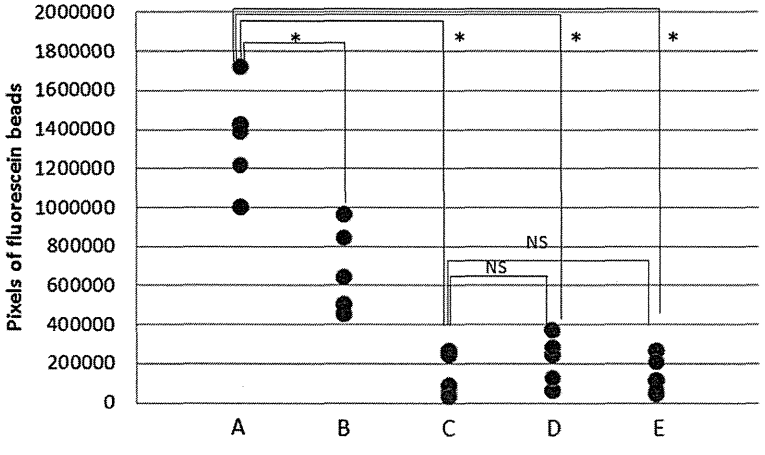

Figure 5 Pixel intensity after removal of the fluorescein bead-labelled OVD in each group. *P <0.001, (NS = not significant). Tukey–Kramer multiple comparison test, two-sided. Data represent individual values.

of the lens capsule, causing a tear. It can also be difficult neophyte cataract surgeons to learn and can be difficult for even experienced surgeons in cases where the CCC is small. In contrast, technique 2 should be easy for a beginner to learn. In previous reports, the rock n roll technique in which the I/A tip was moved in quick circular movements on top of the IOL along with Technique 2 could completely remove Healon 5 from the capsular bag [19]. Furthermore 'Judders' which are periodic, abrupt, horizontal displacements of the intraocular lens could remove OVD safely and effectively [30]. Thus it is critical to move the IOL for removal of OVD behind the IOL optic.

Some limitations exist in our porcine eye study. First, although the general trends we observed in porcine eyes are probably similar to those in humans, the IOP changes observed in our model may not exactly reflect the changes in human eyes due to the absence of aqueous flow. Second, the anatomic structure of the anterior segment, especially the zonules of Zinn in porcine eyes, are similar to that of human eyes, but the integrity of the tissue may be weakened in an enucleated porcine eye. Thus, further investigations are needed to check irrigation fluid flow behind the IOL during surgery.

Therefore this study demonstrated the importance of specific removal techniques for safe and complete removal of OVDs. Surgeons must be aware of the potential adherence of OVD to the posterior surface of the IOL and pay close attention to its complete removal in order to minimise bacterial contamination and elevated intraocular pressure after surgery.

Conclusions

This study demonstrated the importance of specific removal techniques for safe and complete removal of OVDs. Alternately pressing the I/A tip near the edge of the IOL optic anterior surface on one side and then the other to gently tilt the IOL back and forth, is an effective method for cleansing behind the IOL and for removing OVD from behind the IOL. Surgeons must be aware of the potential adherence of OVD to the posterior surface of the IOL and pay close attention to its complete removal in order to prevent bacterial contamination and elevated intraocular pressure after surgery.

Additional file

Additional file 1: Dynamics of irrigation fluid flow behind the IOL. Visualization of irrigation fluid flow behind the IOL in Technique 1 and 2. The techniques produced a view of the movement of particles during I/A.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AM and TS: Conception and design, acquisition, analysis and interpretation of data, drafting of manuscript, administrative and technical support. AM, TS, YT, TU, YH, and SK: Technical support and analysis and interpretation of data. TS and YO: Supervision. Read and approved the final manuscript. All authors approved the manuscript for submission.

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BMC Ophthalmology

CASE REPORT Open Access

Achromobacter buckle infection diagnosed by a 16S rDNA clone library analysis: a case report

Fumika Hotta[1†], Hiroshi Eguchi[1*], Takeshi Naito[1†], Yoshinori Mitamura[1†], Kohei Kusujima[2†] and Tomomi Kuwahara[3†]

Abstract

Background: In clinical settings, bacterial infections are usually diagnosed by isolation of colonies after laboratory cultivation followed by species identification with biochemical tests. However, biochemical tests result in misidentification due to similar phenotypes of closely related species. In such cases, 16S rDNA sequence analysis is useful. Herein, we report the first case of an *Achromobacter*-associated buckle infection that was diagnosed by 16S rDNA sequence analysis. This report highlights the significance of *Achromobacter* spp. in device-related ophthalmic infections.

Case presentation: A 56-year-old woman, who had received buckling surgery using a silicone solid tire for retinal detachment eighteen years prior to this study, presented purulent eye discharge and conjunctival hyperemia in her right eye. Buckle infection was suspected and the buckle material was removed. Isolates from cultures of preoperative discharge and from deposits on the operatively removed buckle material were initially identified as *Alcaligenes* and *Corynebacterium* species. However, sequence analysis of a 16S rDNA clone library using the DNA extracted from the deposits on the buckle material demonstrated that all of the 16S rDNA sequences most closely matched those of *Achromobacter* spp. We concluded that the initial misdiagnosis of this case as an *Alcaligenes* buckle infection was due to the unreliability of the biochemical test in discriminating *Achromobacter* and *Alcaligenes* species due to their close taxonomic positions and similar phenotypes. *Corynebacterium* species were found to be contaminants from the ocular surface.

Conclusions: *Achromobacter* spp. should be recognized as causative agents for device-related ophthalmic infections. Molecular species identification by 16S rDNA sequence analysis should be combined with conventional cultivation techniques to investigate the significance of *Achromobacter* spp. in ophthalmic infections.

Background

A 16S ribosomal DNA (rDNA) clone library analysis was performed for microbiological diagnosis in a clinical case of buckle infection. This type of analysis has previously been applied to a number of environmental samples to examine the microbial diversity within an ecological niche [1-6]. In clinical settings, it can be used to determine the microbial compositions of specimens, which would be beneficial to human health and would further our understanding of the pathological manifestations due to chronic infections [7-9]. In addition, in acute infections, causative bacteria are expected to be readily identified from the predominant sequences in specimens when a 16S rDNA clone library analysis is employed.

Buckle infection is a rare postoperative complication of retinal detachment. It generally occurs in the late stages of postoperative course. Although resident bacteria on the ocular surface, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, have been reported as the causative pathogens [10-12], environmental bacteria such as *Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia* can also cause infections [12-15]. Some of the previous articles describing device-related ophthalmic infections reported isolation of a single pathogen. Considering that we currently know relatively very little about the diversity of microorganisms in nature [16], culture-independent molecular approaches to detect the causative agents may be

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