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Research Article

Comparison of the Foreign Matter Removal Effect of Different Over-thecounter Eye Washing Methods

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Abstract

Purpose: Eye washing with artificial tears using over-the-counter (OTC) eyewash is a widespread method for preventing allergic conjunctival disease. More specifically, OTC eyewash is widely used to remove foreign matter from the surface of the eye and is expected to neutralise allergic symptomology in the eye, although confirmatory evidence is missing. While there are many methods for eye washing, only a handful of reports have examined the efficacy of each method to date. Within this context, our study aimed to compare the effects of cup-type and eye drop-type eyewash on the efficacy of foreign matter removal.

Methods: Insoluble and soluble types of pseudo-foreign matter, imitating $PM_{2.5}$ and pollen, were instilled into the eyes of rabbits and humans for the verification tests conducted herein. After the pseudo-foreign matter was instilled, eye washing was performed with both methods, and the residual amount of foreign matter associated with each methodology was measured.

Results: In the washing test using insoluble foreign matter in rabbits, the concentration of foreign matter in the recovered solution was statistically significantly lower in the eye washing groups than in the non-eye washing group, regardless of the size of the foreign matter. When applying the eye drop-type eyewash, the mean foreign matter concentration decreased as the volume of the drops increased. In the washing test using water-soluble foreign matter conducted in humans, the percentage of residual foreign matter after washing the anterior eye decreased as the volume of the eye drops was increased in the eye drop-type eyewash group. This was similar to the test conducted with insoluble foreign matter in rabbits; however, in the lower eyelid conjunctiva, the residual rate was statistically significantly lower in the cup-type eye washing group than in the six eye drop washing group (38.0% ± 30.8% and 73.0% ± 33.6%).

Conclusion: These results indicate that cup-type eyewash is more effective than eye drops in removing foreign matter from the conjunctiva of the lower eyelid in humans. Pending confirmation in future research, these results suggest that cup-type eyewash is highly effective in removing foreign matter regardless of particle size.

Keywords: Cup-type Eyewash; Drop-type Eyewash; Foreign Matter Removal; Over-the-counter Drugs; $PM_{2.5}$; Pollen

Abbreviations

ANOVA: Analysis of Variance; FSC: Forward Scatter; OTC: Over-the-counter; $PM_{2.5}$: Fine Particulate Matter; SSC: Side Scatter

Introduction

Approximately 30% of the Japanese population is predisposed to allergies [1]. This number has been increasing in recent years due to increasing Asian dust, fine particulate matter (i.e., particles with an aerodynamic diameter of ≤2.5 μm; $PM_{2.5}$), and pollen exposures [2]. Although the entire human body is exposed to these allergenic substances, the eyes come into direct contact with the outside world more frequently and are the likely sites for symptom occurrence. The prevalence of allergic conjunctival disease in Japan has been as high as 48.7% [3].

Avoiding and removing antigens to prevent allergic symptoms in the eyes, such as itching, is considered the most important self-care [4]. More specifically, eye washing is a self-care method that removes antigens using a large amount of chemical solution. The widespread use of over-the-counter (OTC) eyewash has become common among general consumers, especially in contact lens wearers prone to unpleasant symptoms (such as itching eyes and a foreign matter sensation), which are typically the motivation for using OTC eyewash. In fact, approximately 60% of contact lens wearers have experienced using an eyewash. Those who have used OTC eyewash mainly use it to remove foreign matter from the eyes, and its effectiveness in doing that matters the most to them [5].

Among OTC eyewash options, Eyebon (Kobayashi Pharmaceutical Co., Ltd., Osaka, Japan) has been the top eyewash in terms of usage in Japan. Eyebon is a cup-type eyewash with a special cup used for washing the eyes. Cup-type eyewash is the most common eyewash available in Japan, and there is strong evidence supporting the high efficacy of this method in ameliorating allergic symptomology in the eyes in prior studies [6-8]. However, nowadays, eye washing methods are evolving. A case in point is the emergence of the drop-type eyewash that does not have a dedicated cup, and it is indicated specifically for contact lens wearers.

To the best of our knowledge, only a few reports have discussed the differences in the effectiveness of removing foreign matter across eyewashes. However, this is one of the most important functions of eyewash. Within this context, the current study aimed to examine differences in cleaning effects between two major types of eyewash widely available in the market: cup-type and eye drop-type eyewashes.

Materials and Methods

Verification test for evaluating the effects of foreign matter removal

To verify the effects of foreign matter removal in different eyewash methods, we conducted a study wherein we washed the eyes of both rabbits and humans after instilling pseudo-foreign matter. Since $PM_{2.5}$, Asian dust, and pollen are known to cause and exacerbate allergy symptoms in the eye [9], we used two types of insoluble fluorescent beads that are approximately the same size as $PM_{2.5}$ and pollen. Since insoluble fine particles have a size similar to that of foreign substances, there is an advantage that the test can be performed under conditions closer to actual conditions. However, the insoluble fluorescent beads used in this study were flow cytometer calibration beads, which are not supposed to be applied to humans, as there is no safety data on the effects of dispersions of insoluble fluorescent beads on the human body at present. Therefore, water-soluble fluorescein was used in tests on humans because insoluble fluorescent beads can also damage the ocular surface. Fluorescein has been commonly used as a diagnostic agent for dry eye and an ophthalmic contrast agent. Water-soluble fluorescein has been used widely in clinical trials.

Test agent and eye washing method: Eyebon

Eyebon (Kobayashi Pharmaceutical Co., Ltd) is a commercially available cup-type eyewash commonly sold in Japan. Initially, the habitual use of Eyebon was found to cause a safety issue because the repeated use of eyewash containing a specific preservative (benzalkonium chloride) was reported to damage the corneal epithelium [10]. After the latter study was published, Eyebon was modified to a preservative-free composition, and various reports on its safety have since been published [11-13]. As of 2021, a total of seven cup-type Eyebon eyewashes are on the market in Japan, none of which contain preservatives. Therefore, there are a few safety concerns regarding the use of this eyewash in this study, which was approved by the ethics review committee at our institution. Figure 1 illustrates the eye washing procedure used in this study.

Test agent and eye washing method: Well Wash Eye

Well Wash Eye (Santen Pharmaceutical Co., Ltd., Osaka, Japan) is a commercially available eye drop-type eyewash commonly sold

Figure 1: Instructions for the use of Eyebon eyewash (Kobayashi Pharmaceutical Co., Ltd.). (1) Pour the solution up to the line (5 mL) inside the attached eyewash cup. (2) Press the cup firmly to the eye and tilt the head backward, careful not to spill the solution. (3) Wash the eye for 30 s, blinking several times. *Before administration, remove contact lenses, if worn, and wipe off cosmetics and blots around the eyes.

in Japan. It does not come with a special cup and has the shape of a common eye-drop container. Several drops are administered in succession and onto a paper to prevent the chemical solution from overflowing from the conjunctiva.

Test using insoluble foreign matter

Study participants and design

Rabbits were used for this portion of the investigation because they have been widely used in pharmacological testing, and background data are available. The acclimation period was set to ≥7 days, and the rabbits with good weight gain and no abnormalities detected in the evaluation of their general condition were used for the current study. The aforementioned cup-type (Eyebon) and eye drop-type (Well Wash Eye) eyewashes were used in this test. In the test evaluating cup-type eyewash, eye washing was performed with 5 mL of solution administered for 30 s (n = 6). On the other hand, in evaluating the eye drop-type eyewash, eye washing was performed using a micropipette that was used to administer 20 μL, 40 μL, 80 μL, and 120 μL of eyewash, corresponding to one, two, four, and six drops (n = 6 each), respectively. In this study, a control group (n = 12) that did not undergo either cuptype or eye drop-type eye washing (i.e., the non-eye washing group) was established as the common referent.

Selection of pseudo-foreign matter

Two types of insoluble fluorescent beads were used as pseudo-foreign matter in the current study. The first was a blue laser calibration bead used for flow cytometry with a diameter of 2.5 μm; this substance imitates $PM_{2.5}$. The second type of insoluble fluorescent bead used in this study was a calibration bead with a diameter ranging from 25 to 35 μm; this substance imitates pollen. Each pseudo-foreign substance was instilled at 30 μL.

Quantification of the cleaning effect of each method

After instilling the pseudo-foreign matter and washing the eyes using each method, we collected the drug solution held in the conjunctival sac using a capillary tube. The concentration of pseudo-foreign matter in the recovered liquid was measured using a flow cytometer (AccuriC6; Nippon Becton Dickinson Co., Ltd., Tokyo, Japan). The 2.5 μm diameter beads were examined in terms of forward scatter (FSC) and side scatter (SSC), and the single fractions were analysed. Since the particle size of the 25-35 μm diameter beads varied within the specified range, the fraction with the highest fluorescence intensity and the widest FSC was analysed.

Test using soluble foreign matter

Study participants and design

This portion of the study was conducted in accordance with the principles delineated in the Declaration of Helsinki regarding ethical guidelines for medical research conducted in humans. This study was approved by the Ethics Review Board of SOUKENCo, Ltd. (Tokyo, Japan) and was conducted between December 2020 and January 2021. The subjects had previously agreed to participate in this study, which was conducted at our testing institute (Kobayashi Pharmaceutical Co., Ltd. Central Research Institute), and the health condition of their eyes was confirmed by pre-screening. For pre-screening, the dry eye quality of life questionnaire was used. Subjects were asked to answer a questionnaire, which was tabulated by the investigators. Patients were excluded if they scored≧33 points and were suspected of having dry eye. The scores were determined based on the study by Sakane., *et al*. [14].

The study's exclusion criteria were as follows: (1) predisposition to allergies to Eyebon ingredients; (2) severe eye pain; and (3) the use of eyewash and eye drops outside of the study setting on the date of the study. Individuals who met one or more of these criteria were excluded from the study. A total of 22 participants (13 men, nine women; average age, 27.3 ± 1.8 years) were selected for study inclusion. Cup-type eyewash (Eyebon) and eye drop-type eyewash (Well Wash Eye) were used in this evaluation. In the cup-type eyewash, 5 mL of solution was applied for 30 s (16 eyes of eight participants). In evaluating the eye drop-type eyewash, two to six drops were administered (28 eyes of 14 participants).

Selection of pseudo-foreign matter

Fluorescein, a water-soluble fluorescent substance, was used as the pseudo-foreign matter in this portion of the study. Fluorescein is used in diagnosing dry eye and was selected because it is highly safe for use in humans. FLUORESCITE (Novartis Pharma Co., Ltd., Basel, Switzerland) was diluted 10-fold with physiological saline and was instilled into the subjects' eyes.

Quantification of cleaning effects

The enrolled subjects were instructed to clean the area around their eyes with a damp cotton pad prior to the test in order to keep the skin surrounding the eyes clean. After the instillation of the pseudo-foreign matter, a slit lamp (Kowa Company, Ltd., Aichi, Japan) was used to photograph the anterior eye and the conjunctiva of the lower eyelid. Thereafter, eye washing was performed using the method described above, followed by lightly wiping off excess water in the periphery of the eye with paper and photographing the anterior segment of the eye and the conjunctiva of the lower eyelid using a slit lamp. The fluorescence area value of the pseudo-foreign matter in the image was quantified using WinROOF image analysis software (Mitani Corporation, Hukui, Japan). The percentage of the residual pseudo-foreign matter was calculated as the fluorescence area value after eye washing compared to before.

Statistical analysis

All statistical analyses were conducted using Microsoft Excel 2013 for Windows (Microsoft Corporation, Seattle, WA, USA). The specific statistical methodologies employed in this investigation are described below.

Test using insoluble foreign matter

Concentrations of foreign matter in the collected tears were evaluated using a one-way analysis of variance (ANOVA), followed by Dunnett's test as a *post hoc* multiple comparison test. A comparison of the residual rate of foreign matter in regard to particle size was conducted using unpaired t-tests. In each analysis, a two-sided p-value of <0.05 was considered the threshold for statistical significance.

Test using soluble foreign matter

Foreign matter residual rates in the cup-type and eye drop-type eyewash groups were analysed using one-way ANOVA followed by Dunnett's test as a *post hoc* multiple comparison test, as well as via unpaired t-tests. In each analysis, a two-sided p-value of <0.05 was considered the threshold for statistical significance.

Results and Discussion

The results reported herein demonstrate that cup-type eyewash was more effective than eye drop-type eyewash in removing foreign matter from the conjunctiva of the lower eyelid in humans. Moreover, our results suggest that cup-type eyewash was highly effective in removing foreign matter regardless of particle size. To our knowledge, only a few prior reports have evaluated differences in the effectiveness of removing foreign matter, which is one of the most important functions of eyewash. Further, to the best of our knowledge, this study is the first to quantify the effect of eye washing in terms of foreign matter removal.

In the washing test using insoluble foreign matter conducted in rabbits, the concentration of foreign matter in the recovered solution was statistically significantly lower in the cup-type eyewash group as well as in the 40 μL, 80 μL, and 120 μL eye drop groups compared with the non-eye washing group (i.e., the common referent), regardless of foreign matter size. When evaluating the eye drop-type eyewash, the mean foreign matter concentration was found to decrease as the volume of the drops was increased (Figure 2). No statistically significant difference in the percentage of residual foreign matter between the 2.5 μm diameter and 25-35 μm diameter particle instillations was observed in the cup-type eyewash group (4.8% ± 2.1% and 3.7% ± 2.4%, respectively [$p = 0.48$]). On the other hand, the percentage of residual foreign matter in the 120 μL eye drop group was statistically significantly higher for the 2.5 μm diameter particle instillation (15.7% ± 8.1%) than for the 25-35 μm diameter particle instillation (5.7% ± 6.0%) (Student t-test: $p = 0.05$) (Figure 3). In addition, based on fluorescent stereomicroscopic imaging of the rabbit eye (Figure 4), the detection range of the foreign matter (shown in grey) was narrower in the eye washing group than in the non-eye washing group. Foreign matter was rarely detected in the cup-type eyewash group (Figure 4 (B)), with only a slight detection level in the eyelids.

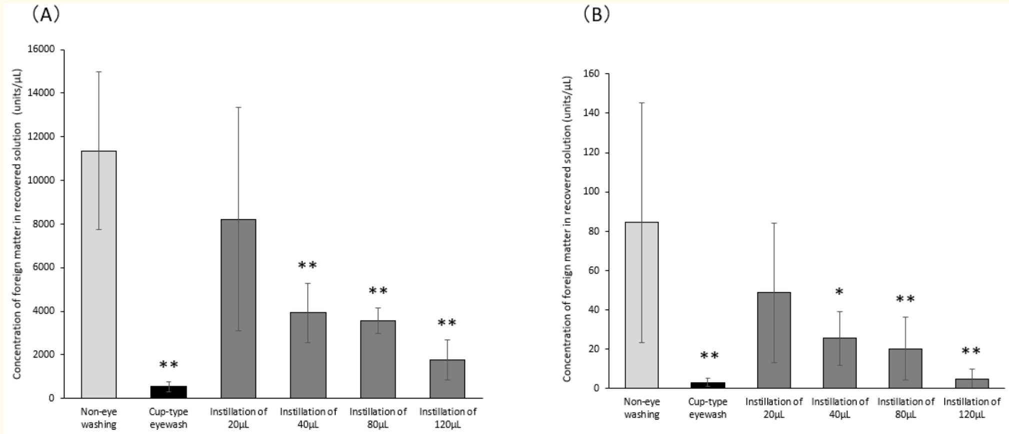

Figure 2: Foreign matter concentrations before and after eye washing in the (A) 2.5 μm diameter foreign matter instillation group and (B) the 25-35 μm diameter foreign matter instillation group. Our results reflect the concentration of foreign matter in the recovered solution. When compared with the non-eye washing (i.e., referent) group, the concentration was found to be statistically significantly decreased after cup-type eye washing as well as after administering 40 μL, 80 μL, and 120 μL of eye drop-type eyewash. No statistically significant difference was observed after administering the 20 μL eye drop-type eyewash.

Results are presented as means ± standard deviations (SD); non-eye washing group, n = 12; eye washing groups, n = 6 each. Dunnett's test findings: *p < 0.05 and **p < 0.01, for the eye washing groups vs. in the non-eye washing group.

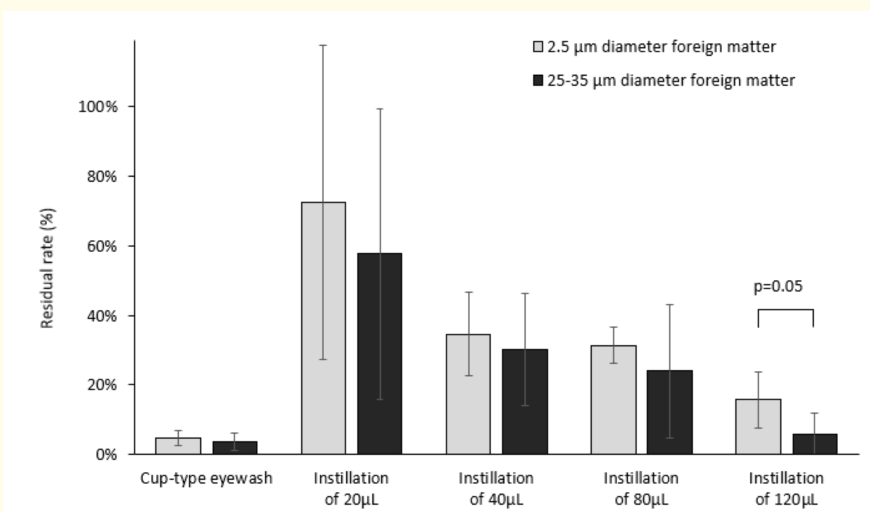

Figure 3: Residual rate of insoluble foreign matter after eye washing. When applying the cup-type eyewash, there was no statistically significant difference in the residual rate regardless of the particle size. However, in the group evaluating the administration of 120 μL of eyewash, the residual rate tended to be higher after instilling the smaller particle size.

Results are presented as the average variance extracted (AVE) ± standard deviation (SD) and as Student's t-test findings.

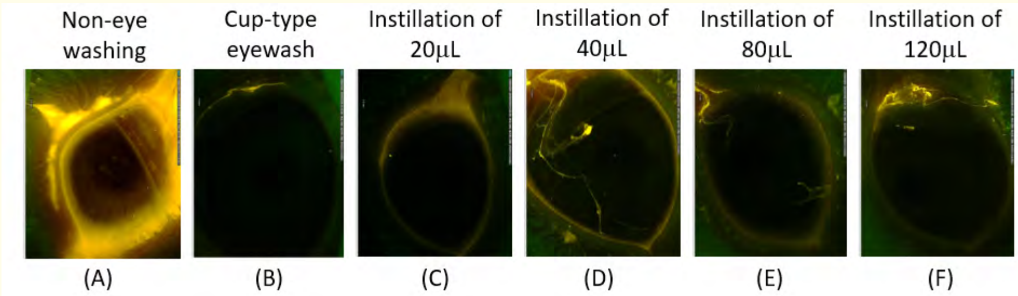

Figure 4: Distribution of foreign matter with a diameter of 2.5 μm before and after eye washing in the (A) non-eye washing (i.e., referent), (B) cup-type eyewash, (C) 20 μL eye drop, (D) 40 μL eye drop, (E) 80 μL eye drop, and (F) 120 μL eye drop groups. The images were taken with a fluorescent stereomicroscope (Science Eye Co., Ltd, Saitama, Japan.) so that the rabbit eye could fit in one field of view. Foreign matter is shown in yellow.

In the washing test following water-soluble foreign matter instillation conducted in humans, the percentage of residual foreign matter after washing the anterior eye decreased as the volume of eye drops was increased in the eye drop-type eyewash group, as evidenced in the test above with the insoluble foreign substance. When compared with six drops, which is equivalent to the maximum dosage of both the cup-type eyewash and the eye drop-type eyewash, the residual rates were 19.4% ± 14.6% and 32.6% ± 16.3% after two and four drops, respectively, and no statistically significant difference was evident (Figure 5 and 6). However, in the lower eyelid conjunctiva, a statistically significant difference was found in the residual rate between cup-type and six eye drop-type eye washing groups (38.0% ± 30.8% and 73.0% ± 33.6% [p = 0.01]), demonstrating that the residual rate was lower when using the cup-type eyewash (Figure 7 and 8).

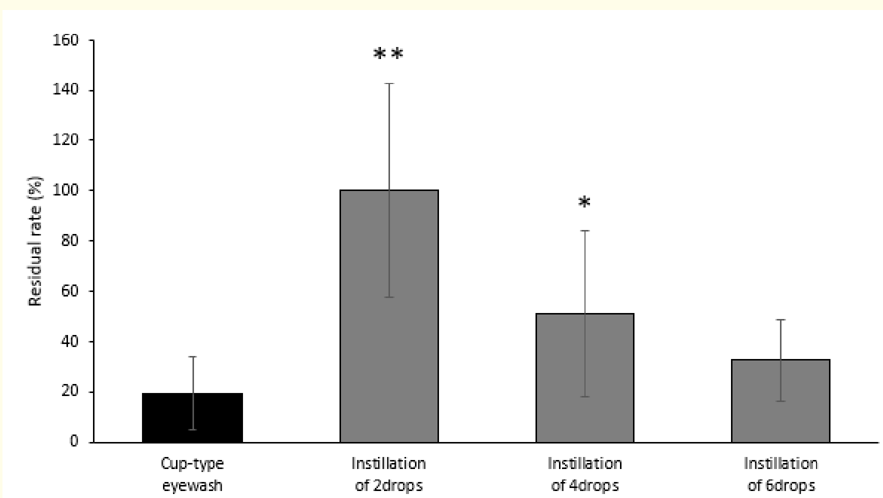

Figure 5: Percentage of residual soluble foreign matter in the anterior segment after eye washing. The residual rates after administering two and four drops were statistically significantly higher than those observed after cup-type eye washing. Still, no statistically significant difference was observed after administering six eye drops.

Results are presented as means ± standard deviations (SD); cup-type eyewash, n = 16; two eye drops, n = 16; four eye drops, n = 16; six eye drops, n = 16. Dunnett's test findings: *$p < 0.05$ and **$p < 0.01$, for the eye drop groups vs. the cup-type eye washing group.

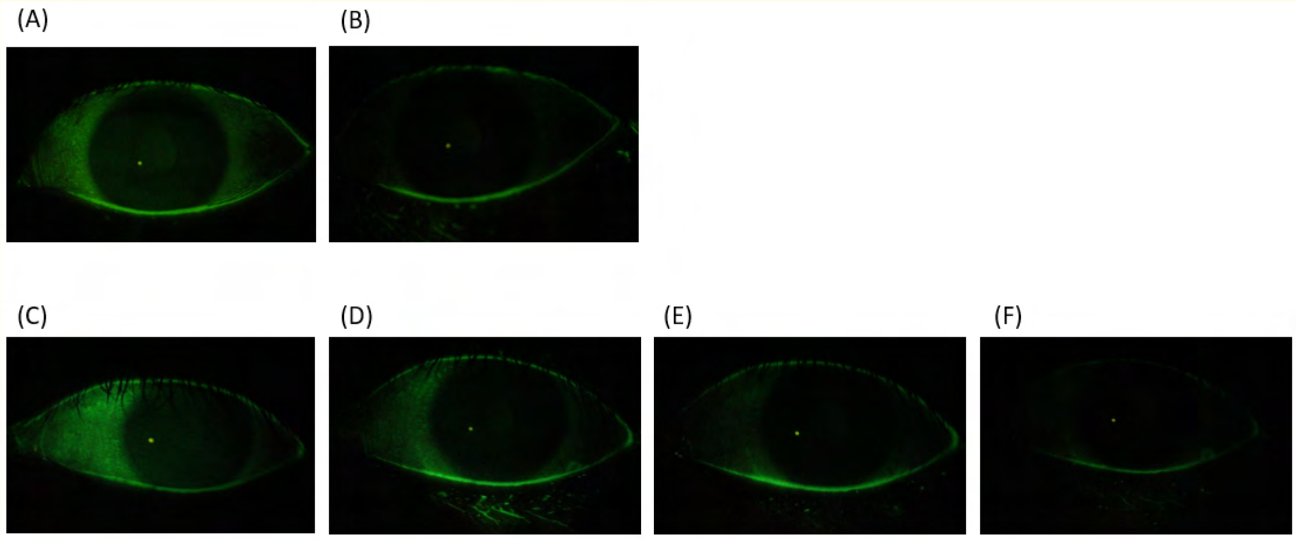

Figure 6: Distribution of water-soluble foreign substances in the anterior eye area before and after eye washing: (A) before applying cup-type eyewash, (B) after applying cup-type eyewash, (C) before applying eye drop-type eyewash, (D) after applying two drops of eyewash, (E) after applying four drops of eyewash, and (F) after applying six drops of eyewash. Herein, we provide an example in which the anterior eye area was photographed with a slit lamp before and after each eye washing. The fluorescence range decreased after eye washing, and the foreign matter was washed away. Foreign matter is indicated using green fluorescence.

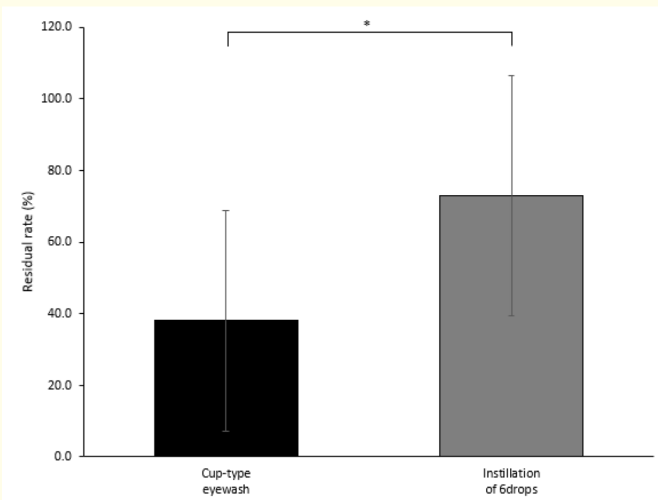

Figure 7: Residual rate of water-soluble foreign substances in the conjunctiva of the lower eyelid after eye washing. The percentage of residual water-soluble foreign substances in the lower eyelid conjunctiva after eye washing was statistically significantly lower in the cup-type eye washing group than after applying six eye drops using the evaluated eye drop solution.

Results are presented as means ± standard deviations (SD); cup-type eyewash, n = 16; two eye drops, n = 16; four eye drops, n = 16; six eye drops, n = 16. Student t-test findings: * $p < 0.05$.

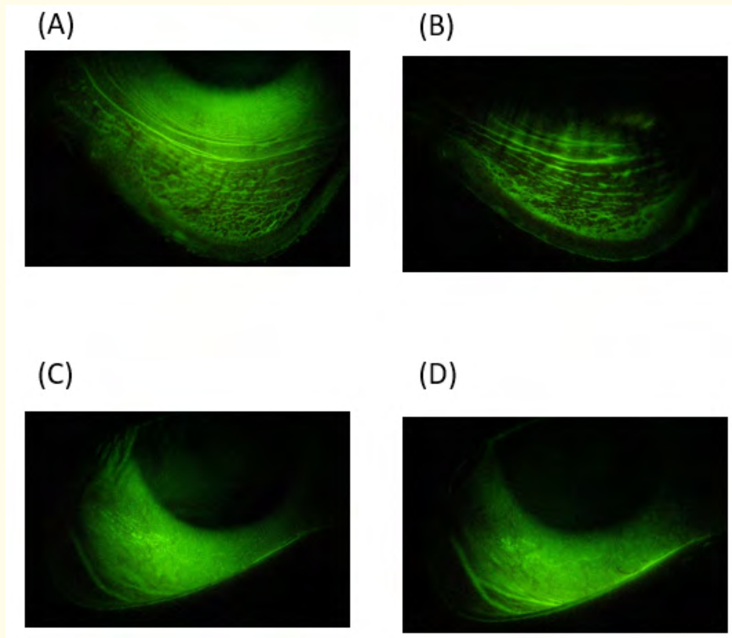

Figure 8: Distribution of water-soluble foreign substances in the lower eyelid conjunctiva before and after eye washing: (A) before applying cup-type eyewash, (B) after applying cup-type eyewash, (C) before applying eye drop-type eyewash, and (D) after applying six eye drops. This figure shows an example in which the anterior eye area was photographed with a slit lamp before and after each eye washing. The fluorescence range decreased when applying the cup-type eyewash, but the change was small when applying the eye drop-type eyewash. Foreign matter is indicated by green fluorescence.

Allergic conjunctivitis caused by pollen, $PM_{2.5}$, and other allergens present in the air is accompanied by characteristic symptoms such as itching, redness, foreign matter sensation, and excessive production of tears. This may result in a substantial deterioration in the quality of life. The basic principle of prevention and treatment of allergic diseases is the removal of the causative antigen, and the same theory applies to ocular allergic diseases. Japanese guidelines for the clinical management of allergic conjunctival diseases recommend self-care with respect to effective antigen removal by various means, as specified in the referenced guidelines [4]. The present study verified the foreign matter removal effect of different eye washing methods (i.e., self-care methods) after foreign substances were instilled onto the surface of the eye in a scenario mimicking real-life exposure. To the best of our knowledge, this study is the first to quantify the foreign matter removal effects of eye washing. Statistical studies of conjunctival foreign bodies have been performed in the past, analysing the types of foreign bodies that enter the eyes in daily life [15]. However, information on how to deal with these has not been described. Therefore, in this study, the significance of removing the foreign body by eye washing was assessed.

This study clarified that a greater volume of solution needed to be in contact with the eye in order to exert an optimal effect, as the residual rate decreased as the volume of the administered solution increased for both the 2.5 μm diameter foreign matter instillation and the 25-35 μm diameter foreign matter instillation. The foreign matter removal effect differed depending on the particle size of the foreign matter, and our results suggest that finer foreign matter tends to be more difficult to clean. On the other hand, our study demonstrated that the cup-type eyewash showed a stronger foreign matter removal effect regardless of the particle size of the foreign matter. Since the percentage of residual foreign matter in the lower eyelid conjunctiva was also lower when using the cup-type eyewash, we can conclude that its associated foreign matter removal effect was high. However, researchers have yet to clarify the effect of cup-type eyewash on the upper eyelid conjunctiva and the inferior tarsal sulcus, which are regions presumed to contain much of the foreign matter in the conjunctiva. Hence, additional verification is required in future research.

From the viewpoint of antigen removal, regular eye washing is recommended during the pollen season according to standard ophthalmology and allergy care guidelines. Although tears have a self-cleaning effect, they easily rupture the outer wall of Japanese cedar pollen, and tears may likely exert a similar effect on other allergens [16]. Hence, it is most desirable to wash the entirety of the foreign particle off before the main antigen elutes due to the risk of the rupture of the outer wall. We have observed that some OTC eyewash solutions have been reported to be effective in suppressing pollen rupture [17]. As eyewash is used during the pollen season, it is better to select a solution that is highly effective in suppressing the rupture of the outer wall of pollen particles, as well as solutions that are demonstrably effective in removing pollen. We believe that doing so will lead to the mitigation and prevention of allergy symptoms in the eye, thereby leading to a greatly improved quality of life.

Although it became possible to quantify the cleaning effects, this study had some limitations. For safety purposes, studies with insoluble foreign bodies cannot be conducted in humans and will use animals. Although rabbits are often used in studies of ocular relationships, they blink much less than humans. Since blinking during eye washing is considered an effective practice for the expulsion of foreign bodies, further validation is required for more near actual studies to be conducted in animal models.

Conclusion

In this study, we quantified and compared the effects of foreign matter removal in a comparative evaluation of the cup-type and eye drop-type eyewash methods. We clarified that the cup-type eyewash was highly effective in removing foreign matter imitating $PM_{2.5}$ and pollen on the surface of the eyes. The eye drop-type eyewash was as effective as the cup-type eye wash in removing foreign matter and imitating pollen on the surface of the eye. However, its foreign matter removal effect on the conjunctiva of the lower eyelid was significantly inferior to that of the cup-type eyewash. This study focused on the cleaning effect, i.e., the foreign body removal rate. However, it was not possible to investigate how much allergic symptoms, such as eye itching, were alleviated according to the removal rate. Therefore, future studies can be conducted to that effect.

Conflicts of Interest

The authors declare that they have no actual or potential conflicts of interest to declare.

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