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IMPACT OF A RINSE STEP ON PROTEIN REMOVAL FROM SILICONE HYDROGEL CONTACT LENSES

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Abstract

PURPOSE—To determine the impact of the rinse step in “no rub” contact lens care systems relative to its ability to assist in removing loosely associated and bound tear film proteins from a worn silicone hydrogel lens.

METHODS—After informed consent, subjects were fitted with lotrafilcon B contact lenses (CIBA Vision, Inc). If the fit was acceptable, subjects were asked to wear the lenses on a daily wear basis for 5 (+2, −0) days for an outcome visit. Subjects were instructed to use AQuify Multi-Purpose Disinfecting Solution (CIBA Vision, Inc) following the manufacturer's “no rub” instructions. At the outcome visit, contact lenses were then collected by a gloved examiner, with a sterile metal forceps, who rinsed the right lens but did not rinse the left lens upon removal from the eyes. Protein was extracted with a 50:50 0.2% trifluoroacetic acid-acetonitrile solution and quantified using a Bradford analyses.

RESULTS—Twenty contact lens wearers were enrolled in this study. For the non-rinsed lenses, the first extraction yielded 13.4 ± 9.2 µg/lens of protein, while the second extraction yielded 5.8 ± 2.8 µg/lens of protein. For the rinsed lenses, first extraction yielded an average of 3.0 ± 1.9 µg/lens of protein, while the second extraction yielded an average of 4.0 ± 2.3 µg/lens. Repeated measures ANOVA showed a significant interaction (F-statistic = 18.9, $p < 0.0001$) between the rinse of a lens and extraction number.

CONCLUSIONS—Rinsing a contact lens following removal from the eye removes well over one-half of the protein associated with it. Further, in order to biochemically recover all protein from a silicone hydrogel lens, it may be important to perform more than one chemical extraction from it.

Keywords

silicone hydrogel; contact lenses; tear film; rinse solution; protein quantification

INTRODUCTION

Contact lens care systems have numerous possible functions including disinfection, imparting wetting and/or lubrication, and cleaning.¹ Each function is considered important, as each will together determine the ultimate success of the contact lens wearer. For instance, it is critical the contact lenses are adequately “cleaned” as tear film and other debris can

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quickly bind to and accumulate on contact lens polymers possibly leading to discomfort, altered surface wetting, altered visual performance, and even impaired immune responses of the ocular surface.^{2–5} It is well documented that the tear film contains numerous proteins such as human serum albumin, lysozyme, lactoferrin, and lipocalin all of which can potentially be deposit on silicone hydrogel contact lenses and in doing so can cause adverse affects to the contact lens and patient.^{6–9} A contact lenses' material composition, water content, poor size, charge, surface roughness, and care in concert with the tear film protein's charge, size, tear film concentration, and duration of the contact lenses' exposure to the tear film all affect the amount and degree of protein binding to the contact lens.^{7–17}

With the existence of “no rub” contact lens care solutions, it is important to evaluate both the care solution relative to its ability to chemically clean lens materials, in addition to each step of the care regimen (i.e., the rinse step, the overnight soak step). We previously demonstrated that a care regimen including a rub step was better able to remove more visible deposition than the same care regimen without a rub step.¹⁸ However, there remains somewhat of a paucity of scientific information on the efficacy of each step in the regimen relative to their contributions to overall cleaning efficacy. One critical element of the “no rub” lens care cleaning regimens is the rinse step following removal of the contact lens after its daily wear. While it is recommended that patients rinse their contact lenses following their removal from the ocular surface to help remove loosely associated and/or chemically bound tear film proteins before storing the lenses, the vast majority of wearers probably fail to do so. Patients rinse their contact lenses to remove loosely associated (and bound) proteins before storing the lenses in care solutions—this is part of clinical scenario and the rationale for this study. However, none this literature has specifically addressed how the rinse step may be a critical one in terms of its overall contribution to the cleanliness and removal of protein from a contact lens. Thus, the purpose of this work was to determine if the impact of the rinse step on overall removal of loosely associated and bound tear film proteins from a silicone hydrogel contact lens.

MATERIALS AND METHODS

Study Design and Patient Sample

This was a prospective, open-label, dispensing study. This project's protocol was approved by The Ohio State University Biomedical Sciences Institutional Review Board in accordance with the Declaration of Helsinki. Enrolled subjects were asked to sign an informed consent and an institutionally approved HIPPA form. Only subjects who were 18 years or older and current silicone hydrogel contact lens wearers with prescriptions between –10.00 DS and +6.00 DS with less than 0.75 DC of astigmatism were included in the study. Subjects were excluded from the study if they were currently suffering from an ocular disease, pregnant, breast-feeding, using any ocular medications, rewetting drops, or had a hypersensitivity to AQuify Multi-Purpose Disinfecting Solution (CIBA Vision, Inc). Further, subjects were only dispensed lenses if they had a clinically acceptable fit in lotrafilcon B silicone hydrogel contact lenses (O2 Optix, CIBA Vision, Inc) at the first visit.

Visit One

Each subject's visual acuity was taken with their habitual contact lenses. The subjects were then asked to remove their current contact lenses, and a slit-lamp biomicrope was used to evaluate ocular health, followed by a refraction to determine contact lens power. The subject was then fitted with a new pair of lotrafilcon B contact lenses and was asked to wait for about 5 minutes for the lenses to settle on the ocular surface before the fit was evaluated (centration, coverage, and movement). If the fit was clinically acceptable and vision was better than 20/30 in each eye, they were dispensed lenses, AQuify Multi-Purpose

Disinfecting Solution and a case (CIBA Vision, Inc). Subjects were then provided instructions on appropriate daily use of the care solution as directed by the manufacturer's "no rub" indication (i.e., do not rub lenses, rinse each side of the lens 5 seconds upon removal after each day of wear). Subjects were asked not to sleep in the study lenses for the duration of the study, and not to use any other care solutions or rewetting drops/artificial tears while they were enrolled in the study. The subjects were finally instructed to wear their contact lenses to the follow up visit scheduled 5 to 7 day following the first visit.

Visit Two

At the second visit, study instruction compliance was assessed, followed by examination of visual acuity and ocular health. The right contact lens was then removed by a gloved examiner with a sterile metal forceps, rinsed for 5 seconds with the AQuify Multi-Purpose Disinfecting Solution, and placed in a 4 mL glass vial. The left contact lens was then removed by the gloved examiner with the same metal forceps and placed in a different 4 mL glass vial (but not rinsed). Both contact lenses were then stored dry in the 4 mL glass vials at -80°C until all subjects completed the study, at which time protein analyses was conducted. Each vial used in the study was pre-labeled with a subject ID and identified as right or left eye. The subjects were then exited from the study.

Protein Analysis

All contact lenses were removed from storage and allowed to briefly thaw. A 1.5 mL solution of 50:50 0.2% trifluoroacetic acid-acetonitrile (TFA) was added to each lens. The 50:50 0.2% TFA solution was created by first adding 1mL trifluoroacetic acid (Fisher, 04902) to 499 mL of water and then adding 500 mL acetonitrile (Fisher, A998). All contact lenses were then allowed to sit undisturbed for 18 hours at -20°C while they were undergoing the extraction process. The contact lenses were then transferred to another glass vial and an additional 1.5 mL of extraction buffer was added. The samples derived from the first extraction buffer were stored at -20°C for 4 days before a Bradford analysis was performed.¹⁹ The second set of glass vials (containing the original contact lenses) were then allowed to sit for another 18 hours at -20°C for the duration of the second extraction. The contact lenses were removed after the second extraction, and placed in a new 4 mL glass vial and stored at -80°C for possible later use. The second set of extraction solutions were then stored at -20°C for three days.

The extraction solutions were removed from storage and allowed to reach room temperature before being quantified with the Bradford analysis (Sigma-Aldrich, B6916).^{7, 19} This was conducted by adding 60 μL of contact lens extraction solution, 20 μL of water, and 200 μL of Bradford reagent to each 96-Well MicroPlate (Life Science Products, Inc., MP8117-R) well. The bovine serum albumin (BSA) (Sigma, A7638) standard curve wells contained various amounts of BSA ranging from 8.0 to 0.5 μg of BSA suspended in 20 μL of water. Each standard curve well then received 60 μL of TFA and 200 μL of Bradford reagent solution to account for any variations that could arise from different solutions in the reaction. The analytical blank wells for the standard curve were created in the same manner as the other standard curve wells; however, water containing no BSA was added to these wells. An average of the analytical blank wells was then subtracted from each experimental and standard curve responses to account for any possible interaction between the contact lens material, TFA, and Bradford solution. Each unknown sample was analyzed in triplicate to increase the accuracy of the final results. The 96-well microplate was then read in the Infinite 200 plate reader (TECAN) at 595 nm. The contact lens samples were then compared to the BSA standard curve, and the protein amounts recovered from the extraction solutions were reported in $\mu\text{g}/\text{lens}$.

Sample Size and Statistical Analyses

The sample size for the study was determined by paired sample size calculations based on the comparison of protein extraction quantities (in $\mu\text{g}/\text{lens}$) between eyes. It was found that 20 subjects would be sufficient to detect a difference in protein between the rinsed and non-rinsed contact lens samples as small as $0.70 \mu\text{g}/\text{lens}$ (standard deviation of $1.75 \mu\text{g}/\text{lens}$, power = 80% and $\alpha = 0.05$). As previous studies have showed that typical quantities of proteins extracted from lotrafilcon B range from 10 - $15 \mu\text{g}$ ^{7, 19}, the effect size associated with the completion of 20 subjects would be less than 10%, if the effect size truly exists.

Following completion of protein analyses, a repeated measures analysis of variance (ANOVA) was used to examine the data relative to predicting protein concentration. Predictors considered in the model included lens rinse (upon lens removal, by the examiner), extraction (1 vs. 2), and an interaction of these terms. In addition to descriptive statistics, F-statistics and p-values are reported from the model. Spearman's correlation coefficients were used to examine the relation between proteins extracted from each lens when comparing rinsed and non-rinsed lenses and the number of extractions.

RESULTS

There were 20 subjects who completed the study. The average age of the subjects was 29.6 ± 6.4 years, and the sample was 90% female. The first TFA extraction from the non-rinsed lenses yielded an average of $13.4 \pm 9.2 \mu\text{g}/\text{lens}$ of protein, while the second extraction yielded $5.8 \pm 2.8 \mu\text{g}/\text{lens}$ of protein (Figure 1). The first extraction from the rinsed lenses yielded an average of $3.0 \pm 1.9 \mu\text{g}/\text{lens}$ of protein, while the second extraction yielded an average of $4.0 \pm 2.3 \mu\text{g}/\text{lens}$ (Figure 1). Repeated measures ANOVA showed a significant interaction (F-statistic = 18.9, $p < 0.0001$) between the rinse of a lens and extraction. In this regard, the interaction indicates that the second extraction was associated with more protein recovery than the first extraction step from the rinsed lens, while more protein was associated with the first extraction for the non-rinsed lens than from the second extraction. Overall, there was less protein associated with a rinsed lens than with a non-rinsed lens.

For the first extraction, there was a strong correlation between the rinse and non-rinsed lenses ($r = 0.56$, $p = 0.01$), which can be observed schematically in Figure 2. For the second extraction, there was also a strong correlation between the non-rinsed and rinsed lenses ($r = 0.66$, $p = 0.002$), which can be observed schematically in Figure 3. There was no correlation between the first and second extraction for the non-rinsed lenses ($r = 0.07$, $p = 0.77$) or for the rinsed lenses ($r = 0.32$, $p = 0.16$).

DISCUSSION

Successful contact lens wear with a planned replacement modality is associated with the use of an appropriately selected and use of a contact lens care system. One of the major functions of contact lens care systems is cleaning (i.e., debris removal) and cleaning may occur either through chemical (e.g., surfactants) or physical means (e.g., rubbing and/or rinsing). It is thought that lenses free from excessive tear film debris may be less attractive to microbial contamination, although there is also evidence suggesting that certain bound proteins (e.g., lactoferrin) may also be toxic to bacteria.^{5, 20, 21} Further, microbial binding may also be associated with material chemistry as it has been demonstrated that *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* differentially bind etafilcon A (Vistakon, Inc), galyfilcon A (Vistakon, Inc), balafilcon A (Bausch & Lomb, Inc), and lotrafilcon B (CIBA Vision, Inc) contact lenses in vitro binding to the later to the greatest degree.²²

Our data show that a rinse step (following removal of contact lenses, but before a soak step) with AQuify Multi-Purpose Disinfecting Solution significantly reduces the amount of loosely associated and/or chemically bound tear film protein associated with a worn silicone hydrogel material. This care solution's main surfactant agent is Pluronic F127, which works by solubilizing debris (e.g., protein). It is worth noting that there are multiple surfactants used in the various multipurpose solutions; however, to date there has not been a direct head-to-head comparison of the efficacy of the various surfactants used in care solutions per se (at least in an in-vitro sense). There have, however, been several studies that have evaluated the effectiveness of entire contact lens care solutions as cleaning agents. In a previous study, Mok and coworkers evaluated the effectiveness of four different commercially available "no rub" contact lens care systems at removing proteins deposited on etafilcon A contact lenses.²³ Of the four systems, which used a rinse and soak step, it was found that the Opti-Free Express No Rub Lasting Comfort and Opti-Free Express (Alcon Laboratories, Inc) contact lens care solutions were more effective at removing protein from etafilcon A contact lenses than ReNu MultiPlus (Bausch & Lomb) and Solo Care Plus (CIBA Vision, Inc) contact lens care solutions. In a related study, Emch and Nichols collected lotrafilcon B and galyfilcon A contact lenses worn by patients for about 8 hours and placed them directly into different contact lens care solutions and allowed them to soak for approximately 24 hours.¹⁰ The contact lenses were then removed, discarded, and the protein was extracted from the contact lens care solutions. The effectiveness of each care solution was evaluated by determining how much total protein was in the care solutions (after lens removal), showing that Opti-Free Express (Alcon Laboratories, Inc) was associated with the most protein. The study also showed that the lotrafilcon B material (CIBA Vision, Inc) released more protein during the soaking step than the galyfilcon A material (Vistakon, Inc), which is consistent with other reports.⁷

The role of the rinse step has been previously investigated concerning microbial removal from contact lenses. For instance, Rosenthal and coworkers demonstrated that removing the rinse step (with a soak step) from the contact lens cleaning regimen was associated with an increase in the number of surviving bacteria after the cleaning regimen.²⁵ They also showed that the longer the duration of the rinse step (greater volume of solution used), the greater the number of bacteria that were removed from the contact lens surface. There does not appear to be much in the literature on the evaluation of physical processes (e.g., rinsing and/or rubbing) associated with the cleaning functions of contact lens care solutions. Thus, the purpose of this study was to examine the effect of a rinse on the total protein load associated with a silicone hydrogel lens. Our data show that a rinse step (following removal of contact lenses, but before a soak step) significantly reduces the amount of loosely associated and/or chemically bound tear film protein associated with a worn silicone hydrogel material after normal daily use. It is important to note that this study did not attempt to differentiate between how much loosely associated and chemically bound tear film protein was removed from each contact lens and it may be of interest to the scientific community to address this question in the future. The total overall protein extracted (from both extractions) from the non-rinsed lenses was about 19 $\mu\text{g}/\text{lens}$, whereas the total overall protein extracted (from both extractions) from the rinsed lenses was about 7 $\mu\text{g}/\text{lens}$. Another way to look at these data is that the first extraction yielded over 10 μg more protein extracted from the non-rinsed lens compared to the rinsed lens (Figure 1). Thus, for this type of material, the rinse step substantially contributes to the total tear film protein removed. It remains critical that practitioners continue to educate patients about the importance of the rinse step in the lens care regimen, not only for disinfection purposes, but also relative to helping maintain a clean lens during the wear cycle.

To the best of our knowledge, this appears to be the first report demonstrating the impact of a second extraction on worn silicone hydrogel contact lenses. Compared to the first

extraction, a similar trend was observed from the second extraction, whereby just over 2 μg more protein was extracted from the non-rinsed lenses compared to the rinsed lenses (Figure 1). It was interesting to note, however, that there was a difference in the trends observed for the rinsed and non-rinsed lenses relative to the first and second extractions. In this regard, the second extraction from the rinsed lenses yielded more protein than did the first extraction (although it was still nearly 2 μg less in magnitude than the second extraction associated with the non-rinsed lenses). A possible explanation for this observation could be that the extra extraction degraded the contact lenses releasing additional small proteins (e.g., lysozyme) bound within the matrix of the lenses. In this regard, it could be that the first extraction recovers proteins primarily associated with the surface of a lens. This phenomenon may not have been observed with the non-rinsed lenses as there was so much more protein loosely associated with the surface of the lenses. Thus, from an analytical stand-point, total protein removed by extraction buffers may be optimized by conducting more than one extraction.

In summary, this study shows that practitioners should instruct and emphasize to their patients to rinse their contact lenses upon removal as indicated by the manufacturers in order to maintain low levels of protein deposition.

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REFERENCES

1. Rosenthal RA, Henry CL, Stone RP, Schlech BA. Anatomy of a regimen: consideration of multipurpose solutions during non-compliant use. *Cont Lens Anterior Eye* 2003;26:17–26. [PubMed: 16303493]
2. Allansmith MR, Korb DR, Greiner JV, Henriquez AS, Simon MA, Finnemore VM. Giant papillary conjunctivitis in contact lens wearers. *Am J Ophthalmol* 1977;83:697–708. [PubMed: 868969]
3. Gellatly KW, Brennan NA, Efron N. Visual decrement with deposit accumulation of HEMA contact lenses. *Am J Optom Physiol Opt* 1988;65:937–941. [PubMed: 3265595]
4. Nilsson SE, Andersson L. Contact lens wear in dry environments. *Acta Ophthalmol (Copenh)* 1986;64:221–225. [PubMed: 3727964]
5. Taylor RL, Willcox MD, Williams TJ, Verran J. Modulation of bacterial adhesion to hydrogel contact lenses by albumin. *Optom Vis Sci* 1998;75:23–29. [PubMed: 9460783]
6. Zhao Z, Wei X, Aliwarga Y, Carnt NA, Garrett Q, Willcox MD. Proteomic analysis of protein deposits on worn daily wear silicone hydrogel contact lenses. *Mol Vis* 2008;14:2016–2024. [PubMed: 18989384]
7. Green-Church KB, Nichols JJ. Mass spectrometry-based proteomic analyses of contact lens deposition. *Mol Vis* 2008;14:291–297. [PubMed: 18334948]
8. Suwala M, Glasier MA, Subbaraman LN, Jones L. Quantity and conformation of lysozyme deposited on conventional and silicone hydrogel contact lens materials using an in vitro model. *Eye Contact Lens* 2007;33:138–143. [PubMed: 17502748]
9. Senchyna M, Jones L, Louie D, May C, Forbes I, Glasier MA. Quantitative and conformational characterization of lysozyme deposited on balafilcon and etafilcon contact lens materials. *Curr Eye Res* 2004;28:25–36. [PubMed: 14704911]
10. Emch AJ, Nichols JJ. Proteins Identified From Care Solution Extractions of Silicone Hydrogels. *Optom Vis Sci*. 2009
11. Santos L, Rodrigues D, Lira M, et al. The influence of surface treatment on hydrophobicity, protein adsorption and microbial colonisation of silicone hydrogel contact lenses. *Cont Lens Anterior Eye* 2007;30:183–188. [PubMed: 17291818]

12. Subbaraman LN, Glasier MA, Senchyna M, Sheardown H, Jones L. Kinetics of in vitro lysozyme deposition on silicone hydrogel, PMMA, and FDA groups I, II, and IV contact lens materials. *Curr Eye Res* 2006;31:787–796. [PubMed: 17050272]
13. Lord MS, Stenzel MH, Simmons A, Milthorpe BK. The effect of charged groups on protein interactions with poly(HEMA) hydrogels. *Biomaterials* 2006;27:567–575. [PubMed: 16054682]
14. Soltys-Robitaille CE, Ammon DM Jr, Valint PL Jr, Grobe GL 3rd. The relationship between contact lens surface charge and in-vitro protein deposition levels. *Biomaterials* 2001;22:3257–3260. [PubMed: 11700797]
15. Jones L, Mann A, Evans K, Franklin V, Tighe B. An in vivo comparison of the kinetics of protein and lipid deposition on group II and group IV frequent-replacement contact lenses. *Optom Vis Sci* 2000;77:503–510. [PubMed: 11100888]
16. Hart DE. Influence of contact lens material surface characteristics and replacement frequency on protein and lipid deposition. *Optom Vis Sci* 1999;76:616–617. [PubMed: 10498001]
17. Jones L, Evans K, Sariri R, Franklin V, Tighe B. Lipid and protein deposition of N-vinyl pyrrolidone-containing group II and group IV frequent replacement contact lenses. *Clao J* 1997;23:122–126. [PubMed: 9108978]
18. Nichols JJ. Deposition rates and lens care influence on galyfilcon A silicone hydrogel lenses. *Optom Vis Sci* 2006;83:751–757. [PubMed: 17041321]
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254. [PubMed: 942051]
20. Williams TJ, Willcox MD, Schneider RP. Interactions of bacteria with contact lenses: the effect of soluble protein and carbohydrate on bacterial adhesion to contact lenses. *Optom Vis Sci* 1998;75:266–271. [PubMed: 9586751]
21. Bruinsma GM, van der Mei HC, Busscher HJ. Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. *Biomaterials* 2001;22:3217–3224. [PubMed: 11700793]
22. Kodjikian L, Casoli-Bergeron E, Malet F, et al. Bacterial adhesion to conventional hydrogel and new silicone-hydrogel contact lens materials. *Graefes Arch Clin Exp Ophthalmol* 2008;246:267–273. [PubMed: 17987309]
23. Mok KH, Cheung RW, Wong BK, Yip KK, Lee VW. Effectiveness of no-rub contact lens cleaning on protein removal: A pilot study. *Optom Vis Sci* 2004;81:468–470. [PubMed: 15201721]
24. Rosenthal RA, Henry CL, Schlech BA. Contribution of regimen steps to disinfection of hydrophilic contact lenses. *Cont Lens Anterior Eye* 2004;27:149–156. [PubMed: 16303537]

Figure 1.
The average ($\pm$ SD) total protein recovered from the rinsed and non-rinsed, after each extraction step.

Figure 2.
Protein recovered from the rinsed vs. non-rinsed lenses following the first extraction.

Figure 3.
Protein recovered from the rinsed vs. non-rinsed lenses following the second extraction.