In vitro Evaluation of the Location of Cholesteryl Ester Deposits on Monthly Replacement Silicone Hydrogel Contact Lens Materials

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Purpose: The deposition profile of cholesteryl ester on the surface and throughout the matrix of silicone hydrogel contact lens (CL) materials was determined under conditions that mimic a daily wear regimen.

Methods: In this in vitro study, four SiHy CL materials (senofilcon C, lotrafilcon B, comfilcon A and samfilcon A) were incubated in an artificial tear solution (ATS) for up to 30 days. CL incubation was alternated between the ATS (16 hours) and a multipurpose care regimen (8 hours). The ATS included fluorescently tagged cholesteryl ester (5-cholesten-3β-ol 6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]caproate; CE-NBD) and confocal laser scanning microscopy visualized the distribution of the lipid through the CLs.

Results: The distribution of CE-NBD was homogenous from the anterior to posterior surface in senofilcon C and comfilcon A, at all time points. For lotrafilcon B and samfilcon A, CE-NBD localization was heterogeneous, with greater amounts on the surfaces on Day 1 and Day 14 compared to the lens matrix; however, differences in concentration between the surface and bulk diminished by Day 30.

Conclusion: The distribution of the non-polar lipid CE-NBD varied with lens material chemistry. While some lens materials deposited the lipid primarily on the surface after 16 hours of exposure, all materials exhibited a homogenous distribution after one month.

Keywords: lipid distribution, silicone hydrogel contact lenses, cholesteryl ester, artificial tear solution

Introduction

Discomfort experienced during contact lens (CL) wear continues to be an issue, particularly towards the end of the day,1,2 and has been linked closely to CL discontinuation.3,4 The causes attributed to CL discomfort are multifactorial, with lens deposition being one of the potential factors.5 The degree to which tear film components deposit on CLs is primarily driven by the lens material and frequency of replacement, but other factors such as ocular physiology, tear film composition, wear modality, cleaning regimen, and environmental factors can all play a role in controlling the amount of deposition.6–8 Although protein deposition has been extensively studied,8 more attention over the past decade has been given to studying lipid deposition, particularly on silicone hydrogel materials.7,9–11 The composition and amount of lipid deposition have been characterized from both in vivo and in vitro studies.7,10–15 However, the location of this lipid deposition in terms of whether it is surface located or occurs within the bulk of the material is not fully understood.9,16
The location of protein deposition has been previously studied using confocal microscopy and sectioning methods.\textsuperscript{17} Accurately locating the lipid deposition profile throughout the lens may help to better understand its role in CL discomfort, because lipids deposited on the lens surfaces interact directly with the ocular surface and may be more prone to degradation through oxidative processes, which could contribute to downstream pathways leading to discomfort.\textsuperscript{18–20} Previous clinical studies have demonstrated little to no correlation between the quantity of lipid deposition and levels of discomfort.\textsuperscript{18–20} It is therefore beneficial to study the location of lipid deposition in addition to the quantity and composition of lipid deposition. The nonpolar phase of the lipid layer of the tear film is composed of cholesteryl esters, wax esters, free cholesterol, hydrocarbons and triglycerides.\textsuperscript{21} A representative lipid that makes up an abundant amount of the lipid layer that is believed to be vital in maintaining the structural integrity of the tear film is cholesterol oleate, which is part of the cholesteryl ester family.\textsuperscript{22} Large quantities of hydrophobic chains of cholesteryl esters allow for self-assembly of liquid-crystal-like structures that contribute to the stability of the tear film and will therefore be the focus of this study.\textsuperscript{21}

Reusable daily wear silicone hydrogel lenses, which include biweekly and monthly replacement lenses, account for approximately 52% of fits today.\textsuperscript{23} In the current study, the lipid deposition profile throughout the lens matrix was determined over a period of 30 days for four contemporary frequent replacement silicone hydrogel lens materials (Table 1). An in vitro approach was used that further included a daily cleaning cycle, using a commonly prescribed multipurpose solution.

### Materials and Methods

Four monthly replacement silicone hydrogel lenses were examined: Lotrafilcon B [Air Optix\textsuperscript{®} Aqua, Alcon, Fort Worth, TX], comfilcon A [Biofinity\textsuperscript{®}, CooperVision, Pleasanton, CA], samfilcon A [Ultra\textsuperscript{®}, Bausch + Lomb, Rochester, NY], and senofilcon C [Acuvue\textsuperscript{®} Vita\textsuperscript{TM}, Johnson & Johnson Vision, Jacksonville, FL). All lenses were obtained from the manufacturer in the original packaging and had a dioptic power of −3.00D. The lens properties are outlined in Table 1.

An artificial tear solution (ATS) was prepared using a slightly modified composition as described previously to incorporate the fluorescently tagged cholesteryl ester.\textsuperscript{24} The ATS included various physiological tear film components, such as different proteins, lipids and salts (Table 2). In comparison to the previously used ATS a slightly higher concentration of polar lipids was used by adding phosphatidylethanolamine. This was done to mimic the ratio of approximately 30:70\textsuperscript{25} for polar vs non-polar lipids in the tear film a concentration of 0.0171mg/mL for the combined polar lipids and 0.0394mg/mL for the combined non-polar lipids was used. The ratio of Phosphatidylecholine to Phosphatidylethanolamine was 2:1 (comparable to Rantamäki,\textsuperscript{26} ratio 3.5:1) and the ratio of Cholesteryl oleate to Cholesterol was 13:1 (comparable to McMahon,\textsuperscript{27} ratio 10:1). To detect the lipid of interest, 0.1% of fluorescently

| Table 1 Properties of Silicone Hydrogel Contact Lenses |
|---------------------------------------------|
| **Acuvue® Vita™** | **Air Optix® Aqua** | **Biofinity®** | **Ultra®** |
| United States Adopted Name (USAN) | Senofilcon C | Lotrafilcon B | Comfilcon A | Samfilcon A |
| Manufacturer | Johnson & Johnson | Alcon | CooperVision | Bausch + Lomb |
| Centre Thickness (mm) | 0.07 | 0.08 | 0.08 | 0.07 |
| Water Content (%) | 41 | 33 | 48 | 46 |
| Oxygen Permeability (x10\textsuperscript{-11}) | 122 | 110 | 128 | 114 |
| Oxygen Transmissibility (x10\textsuperscript{-9}) | 147 | 138 | 160 | 163 |
| Surface Treatment | None | Plasma Coating | None | None |
| Wetting Agent | PVP | Wetting agent in packaging solution (1% copolymer 845) | None stated | PVP |
tagged cholesteryl ester (5-cholesten-3β-ol 6-[[7-nitro-2-1,3-benzoxadiazol-4-yl]amino]caproate - CE-NBD) was added. The biocide ProClin 300 was included in the ATS to prevent bacterial contamination. The osmolality and surface tension of the ATS used in this study were within the same ranges as previously reported (osmolality: 304–306 mmol/kg, surface tension: 40–46 dynes/cm).

Daily lens wear was simulated for 1 day, 14 days and 30 days. Prior to the incubation in ATS, lenses were removed from the original manufacturer’s packaging and soaked in 2 mL of phosphate buffer saline (PBS) (EMD-Millipore, Billerica, MA) for 17 hours using a 24 well polystyrene plate (Corning®, Corning, NY) to remove residual blister pack solution. This procedure was followed since the composition of the blister solution is unknown, but will differ from company to company, and thus could potentially make the interpretation of the results more complex and potentially confounding. Lenses were then blotted on lens paper and transferred into a 6 mL glass vial (Wheaton, Millville, NJ) containing 1 mL of ATS for 16 hrs in a 37°C incubator, while rotated at 60 rpm. One milliliter of ATS was required to submerge the lens in the vial study. A moderate rotation setting of 60 rpm was selected to simulate the non-stagnant wear experienced when a patient is wearing lenses. Day 1 lenses were removed from the ATS and prepared for imaging as described below, while day 14 and day 30 lenses were transferred to a 24 well plate containing 2 mL of OPTI-FREE® PureMoist® care solution (OPTI-FREE PM, Alcon, Fort Worth, TX) to simulate a daily cleaning regime without applying any manual lens rubbing to reduce variability. After 8 hours at room temperature, lenses were placed back into freshly prepared ATS. This lens cycling was repeated between the care solution and ATS on each weekday for 14 and 30 days, respectively. Over the weekends, lenses remained incubated in ATS without cycling. Control CLs were incubated in ATS without CE-NBD. To reduce transfer of excess ATS and care

### Table 2 Composition of Artificial Tear Solution

| Organic/Inorganic Components | Supplier | Catalogue Number | Lipid Components | Supplier | Catalogue Number |
|------------------------------|----------|------------------|------------------|----------|------------------|
| Sodium chloride              | EMD      | SX90420-3        | Cholesteryl Olate | Sigma-Aldrich | C9253            |
| Potassium chloride           | Sigma-Aldrich | P5405          | Cholesteryl ester NBD | Avanti Polar Lipids | 810251P         |
| Sodium carbonate             | Sigma-Aldrich | 2234B4         | Cholesterol       | Sigma-Aldrich | C8667            |
| Potassium hydrogen carbonate | Sigma-Aldrich | 237205        | Phosphatidylethanolamine | Sigma-Aldrich | 76548            |
| Trisodium citrate dihydrate  | Sigma-Aldrich | S1804          | Phosphatidylcholine | Sigma-Aldrich | P3556            |
| Calcium chloride dihydrate   | Sigma-Aldrich | C7902          | Protein components | Supplier | Catalogue number |
| Urea                         | Sigma-Aldrich | U5378          | Bovine albumin    | Sigma-Aldrich | A7888            |
| Glucose                      | Sigma-Aldrich | G7021          | Hen Egg Lysozyme  | Sigma-Aldrich | L6876            |
| Sodium phosphate dibasic     | Sigma-Aldrich | S7907          | Bovine mucin      | Sigma-Aldrich | M3895            |
| Hydrochloric acid            | Sigma-Aldrich | H9892          | Recombinant Human Lactoferrin | InVitria | 777LACD15        |
| Bacteriostatic Agent         | V/V %    | Supplier | Catalogue Number |
| ProClin 300                  | 0.02     | Sigma-Aldrich | 48912-U           |

Abbreviation: PVP, polyvinyl pyrrolidone.
solution, lenses were rinsed in 2 mL of PBS three times after each incubation. At the respective time points, lenses were removed from ATS and prepared for CLSM imaging. A 5 mm disk was punched out from the centre of the lens and fixed onto a microscope slide (Goldline, VWR, Radnor, PA) as described previously. Four replicates were analyzed for each lens type at each time point.

The lens samples were imaged using a Zeiss LSM 510 Meta, Axiovert 200 confocal microscope (ZEISS Inc., Toronto, Canada) from the anterior to posterior surface of the lens at three different locations (Figure 1). To visualize the CE-NBD deposited in the lens, the excitation was set to 488 nm on an Argon laser and the emission wavelength was set to 505 nm using a long-pass filter. The lens samples were viewed under a C-Apochromat 40X/1.2 corr objective lens, which magnified the lens such that each visible plane was 224.56µm². The laser setting (gain setting on the photomultiplier detector) was adjusted if necessary to capture the dynamic range of the fluorescence intensity over four lens types at three incubation time points. The deposition profile at each plane along the depth of the lens was scanned at 2µm intervals using z-stacking and were rendered into a cross-sectional image of the lens using ZEN Lite software (ZEISS Inc., Germany).

For the image analysis, the average relative intensity of fluorescence (RIF) value at each plane of the lens was plotted against the depth of the lens using ImageJ software (Bethesda, MS, USA) to graph the corresponding deposition profile curve (Figure 1). Since each lens profile had a slightly different thickness, all scans were normalized to the equal number of data points using piecewise linear interpolation. The RIF values imaged at the three locations were averaged and the values were then averaged with the four replicates of each lens type at each time point. The endogenous fluorescence intensity of the negative control CLs was subtracted from the test CLs. In addition, to compare the data between the different incubation periods, a scaling factor was calculated to account for the different gain settings used to capture the images on day 30. The scaling factor was determined by imaging the 14-day CLs at both the 14-day gain setting as well as the 30-day gain setting. A linear relationship between gain and fluorescence intensity for the range of gain settings was confirmed.

Statements on comparisons between the different lens deposition profiles and time points are intended to allow for easier interpretation of the data and are not based on inferential statistical analysis. Since the aim of the study was to visualize CE-NBD throughout the lens and was not intended to quantify the amount deposited, additional statistical analysis was not conducted.

**Results**

All materials accumulated CE-NBD over time and the deposition profile varied depending on the duration of incubation and the lens type (Figure 2A–D).
After 1 day of incubation, the deposition profile was uniform in senofilcon C and comfilcon A materials, whereas lotrafilcon B and samfilcon A had higher RIF values on the surfaces than the core of the lens (Figure 3A–D). In samfilcon A, the RIF value became gradually lower towards the centre of the lens, while lotrafilcon B exhibited distinct fluorescent peaks on both surfaces with uniformly lower RIF values distributed throughout the matrix.

After 14 days of incubation, lenses exhibited a higher RIF signal in the lens matrix, indicating more lipid uptake compared to Day 1 (Figure 3A–D). The uptake pattern for senofilcon C and comfilcon was similar compared to day 1. For samfilcon A, higher RIF values were observed at the anterior and posterior surfaces of the lens compared to the center of the lens. Moreover, the RIF value at the posterior surface of samfilcon A was lower than the anterior surface.
The gradual decrease of the RIF signal when traveling through the lens material and resulting asymmetry between surfaces is due to laser attenuating as shown previously29 and not an indicator of lower deposition rates on the back surface compared to front surface. For lotrafilcon B, the peaks at both surfaces were slightly less pronounced compared to Day 1.

After 30 days of incubation, the adjusted RIF signal was significantly higher for all lenses on Day 30 compared to Day 14 after the scaling factor was applied, confirming a continuous increase in CE-NBD accumulation throughout the 30 days. The uniform deposition curves suggest that CE-NBD was evenly distributed in all lens types, with the exception of samfilcon A (Figure 3A–D). Although the curve suggests a more homogeneous distribution pattern than at the previous incubation periods, samfilcon A maintained a slightly higher RIF value at the anterior and posterior surfaces compared to the center of the lens after 30 days of incubation.

All CE-NBD deposition profile curves exhibited an apparent asymmetry between the anterior and posterior surfaces, with higher RIF values on the anterior surfaces than the posterior surfaces. This phenomenon was also observed in previous studies conducted using the same confocal technique.17 To determine whether this was a true effect, additional experiments were conducted, which imaged the lenses in the reverse orientation from the posterior to anterior surface, at the three time points (data not shown). The same reduction in intensity was noted the deeper the laser traveled through the lens material. This suggests that the asymmetry in the RIF values between the two surfaces is due to depth-dependent attenuation of the laser through the thickness of the CL.29

### Discussion

Upon the insertion of a CL, various tear components readily deposit onto the lens.7,30 These deposits can alter the lens property and its interaction with the ocular surface and tear film,31,32 which may impact the lens performance on eye.19 Previous studies have visualized surface and bulk lipid deposition using various lipid stains, such as Nile Red and oil red O.16,33 However, contrasting results were observed in the stain behavior between different studies16,33 and it has been suggested that lipid stains may produce inconsistent staining that is not reflective of actual lipid deposits within the lens.34 In the current study, CE-NBD was used as a spectroscopically traceable analogue to determine CO uptake. CE-NBD and CO have a similar molecular weight (MW 662.9 vs 651.1) and the NBD moiety and oleic acid are both esterified to cholesterol at C3.35,36 CE-NBD-cholesterol is only slightly less lipophilic than CO, but more lipophilic than cholesterol, as defined by computed partition coefficients (10.9, 16.6, 8.7, respectively).35,36

Regardless of lens type, the amount of CE-NBD deposition increased with time, as suggested by an increased RIF value (Figure 3A–D). Although a cleaning regimen was used, lipid deposition continued to increase with time. The location of the cholesteryl ester deposits was highly influenced by the lens material. The lenses containing NVP (comfilcon A) and PVP (senofilcon C) showed a strong attraction of cholesteryl ester into the lens matrix, which may suggest that NVP within a lens can enhance lipid incorporation, by increasing lipid solubility within the matrix.37 Samfilcon A also incorporates PVP monomers; however, a more gradual increase in lipid accumulation towards the lens centre was seen in this lens type, suggesting that either the PVP distributed within the lens material is different from that seen in senofilcon A or the concentration relative to the other material components differs between the two materials. Lotrafilcon B, which has a plasma surface treatment, deterred cholesteryl ester deposition to the bulk of the lens for the first 14 days and mainly deposited on the surfaces.38 This observation may be compared to the slightly lower amount of lipid deposited in lotrafilcon B than other CLs seen in previous studies.12,14 This suggests that surface properties may play a bigger role in reducing overall lipid deposition than bulk polymeric composition.

However, regardless of the type of silicone hydrogel tested, the amount of lipids deposited increased over time, which is in agreement with studies investigating the quantitative uptake to silicone hydrogel lenses.7,15 Whether or not higher lipid uptake over time has any impact on CL comfort is still unclear and requires further clinical investigation.18,20

CE-NBD was imaged using confocal microscopy, which is a useful tool to detect tear film component deposition at different depths within the lens material. However, a few limitations should be mentioned. In this study, the range of fluorescence intensity over the course of 30 days was too large and required the adjustment of gain settings, and scaling factors had to be used to encompass both the low RIF at Day 1 and high RIF at Day 30. In addition, it is unclear if the quantum yield of the fluorophore may differ depending on the lens material or if the sorption properties of NBD-CE may be slightly different compared to unconjugated CE.39 For this reason, the RIF
values cannot be used to determine the relative amount of deposition between different lens materials.

This was the first study to determine the location of non-polar lipids throughout the matrix of contact lenses using a complex ATS and a daily cleaning cycle to better mimic typical wear conditions over one month. However, since a static vial with a set volume of solution was used during the incubation phases, lenses were permanently immersed in ATS. This does not accurately reflect the interactions between the lens and the ocular environment, including factors such as tear flow, tear exchange, intermittent air exposure, and friction due to blinking. Whether this has an impact on the lipid uptake was recently investigated by Walther et al., who compared the lipid accumulation on daily disposable lenses. The authors concluded that the vial method may result in a faster uptake of the fluorescently labeled lipid for certain lens materials compared to an in vitro eye-blink instrument, which simulates physiologic tear flow (2 mL/24 hours). Moreover, the cleaning regime did not incorporate a rubbing step to minimize variability. Even though previous studies on tear film deposits have found no effect or only a minor difference in uptake between lenses that were cleaned with and without lens rubbing, there is the potential that this may have impacted the distribution of lipids on the lenses over time. Future in vitro studies on contact lens deposition may therefore benefit if additional features of the ocular environment/anatomy and lens care regime can be simulated.

**Conclusion**

As seen with tear film proteins, the lipid cholesteryl ester deposited on the surface and within the lens matrix of all four silicone hydrogel lens materials over time and varied depending on the lens material. These different lipid deposition profiles may have varying effects, such that lens surface deposits interact more readily with the ocular surface, versus a limited interaction of lipids sequestered within the bulk of the lens and further studies on the clinical relevance of this is warranted.

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