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An imaging-based analysis of lipid deposits on contact lens surfaces

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ABSTRACT

Purpose: To evaluate whether LipidTOX could stain lipid deposits on contact lens (CL) surfaces and compare lipid deposition patterns on various CL surfaces using an imaging method.

Methods: Ten CLs each of six silicone hydrogel materials and one hydrogel material were incubated in cholesteryl oleate solution (5.60 mg/ml) for 12 hours. The CLs were then separately stained with Oil Red O and LipidTOX and imaged under a fluorescence microscope. Twenty worn senofilcon A CLs from both eyes of ten participants were also stained similarly. The area of deposition was calculated using Fiji software. Median deposition areas were compared between various materials using the Kruskal-Wallis test. The Mann-Whitney U test was used to compare the median deposition areas in the same material using the two dyes and also to compare the median deposition areas on worn and doped senofilcon A CLs.

Results: LipidTOX staining needed fewer steps than Oil Red O for staining the deposits. There was no statistically significant difference between the median areas of lipid deposition among the CL materials using either Oil Red O ($p = 0.42$) or LipidTOX ($p = 0.21$). However, significantly different median deposition areas were found between in vitro and ex vivo stained senofilcon A CLs using both Oil Red O ($p = 0.002$) and LipidTOX ($p = 0.029$).

Conclusions: LipidTOX can stain lipid deposits on contact lens surfaces and is simpler to use than Oil Red O. Lipid deposition in the central 2 mm diameter zone did not significantly differ between various commercial CL materials.

1. Introduction

There are approximately 130 million contact lens (CL) wearers worldwide and 38.5 million in the US [1]. Contact lenses are regulated as medical devices that need to be biocompatible with the human tissues with which they come in contact. According to the United States Food and Drug Administration (FDA), a device is considered biocompatible if its component materials do not either directly or through the release of their material constituents: (i) produce adverse local or systemic effects; (ii) act as carcinogens; or (iii) produce adverse reproductive and developmental effects [2]. Therefore, the FDA requires data from systemic testing of the device to confim that the benefits provided by the final product exceed any potential risks produced by device materials before clearing the device for marketing. Even after becoming commercially available, devices may exhibit biocompatibility issues. For example, CL can develop protein and lipid deposition on their surfaces and/or matrix, which can be a concern for biocompatibility with the ocular surface, as deposits have been associated with adverse reactions including symptomatology, inflammation and infection [3–9].

Silicone hydrogels (SiHys) are the most widely prescribed soft contact lens materials in most parts of the world [10]. Silicone hydrogels, although highly permeable to oxygen, still exhibit deposition and there is debate as to whether SiHy materials deposit more lipids than hydrogel materials [11–16]. It has been reported in some studies that SiHy materials attract more lipids from the tears, which is proposed to be due to their inherent hydrophobicity, and can lead to subsequent dewetting of the CL surface and tear film instability [14,16,17]. Begley et al. in 2001 reported frequencies of 77% and 73% of CL wearers with discomfort and dryness, respectively [18]. Nichols et al. in 2005 reported that 52% of CL wearers experienced increased dryness and discomfort when compared to spectacle wearers (23.9%) and emmetropes (7.1%) [19]. Dryness and discomfort have been reported to be the major causes for permanent discontinuation of CL wear. Giannoni and Nichols reported that 40% of permanent discontinuations were due to dryness and discomfort [20]. Similarly, Pritchard et al. also reported 40% of CL wearer discontinuations to be due to discomfort [21]. Therefore, with such a large proportion of CL wearing population affected with the problem of discomfort, there is a great need to understand the etiology of CL discomfort, including the role of deposition.

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Lipid deposition on CLs has also been associated with CL discomfort [22]. The lipids that deposit on CLs come from the meibum, which makes its way into the tear film from the meibomian gland orifices. Meibum is produced in the meibomian glands located in the upper and lower eyelids. The major lipids present in the meibum include wax (25–68%) and cholesterol esters (0–65%), although there are many other types of lipids present [23–25]. These and other ocular surface lipids can oxidize and degrade to products like malondialdehyde, which may be toxic to ocular surface tissues [26]. Together, this may lead to dryness and discomfort [27,28] as such products have been found to be increased in intolerant CL wearers [29].

There are various methods of studying lipid deposits on the surfaces and in the matrix of CLs [30]. They can mainly be categorized into observational techniques and assays. Observational techniques include imaging through microscopy (light, confocal, scanning electron, transmission electron, atomic force etc.) and techniques like chromatography. Assays include specific CL lipid analyses like a total lipid assay, and cholesterol and phospholipid assays [31]. Any one of the above-mentioned techniques or assays cannot provide a full description of lipid deposition alone. Observational methods generally show the morphology of deposits while assays provide more quantitative insights, although many assays require extraction from the material using organic solvents, which may lead to contamination of the sample with polymer from the CL material. Imaging techniques capable of quantifying lipids without extraction (and potential contamination) avoid this issue, and concurrently provide the morphology and distribution pattern of the deposition.

Previously, imaging studies on CL materials have generally used Oil Red O and Nile Red to stain lipids [32,33]. LipidTOX is a neutral lipid stain that can be detected by fluorescence microscopy or a high-content screening reader. LipidTOX has been used previously to stain lipids in rat cortical neurons [34], human hepatoma cells [35], adipocytes [36] macrophages [37] and in immortalized human meibomian gland epithelial cells [38]. It appears that this dye has not been used to stain lipid deposits on CL materials. Thus, the purpose of this research was to evaluate whether LipidTOX could stain lipids on contemporary contact lens materials and compare lipid deposition patterns qualitatively and quantitatively using this dye and an imaging method.

2. Methods

2.1. In vitro arm

2.1.1. CL materials

Six commercially available unworn SiHy materials and one hydrogel material (10 total for each type of material) were included in the study (Table 1). All lenses had an optical power of ≤ -2.00.

2.1.2. Doping

A cholesteryl oleate stock solution was prepared at a concentration of 8.60 mM (5.60 mg/ml) as directed by the manufacturer [39]. In brief, 56.00 mg of cholesteryl oleate (C9253, Sigma Aldrich, St. Louis, MO) was dissolved in 1.00 mL of nonaethylene glycol monododecyl ether (P-9641, Sigma-Aldrich) followed by the addition of 9.00 mL of hot 0.10 M, pH 7.0 phosphate buffer. The solution was then stored at room temperature. For a positive control, one microliter of olive oil was added to 99.00 µL of stock phosphate buffered saline (PBS) solution (0.10 M, pH 7.0) to prepare 1% olive oil in PBS. Eighty-five µL of propylene glycol (100%) (P4347, Sigma-Aldrich) was added to 15 µL distilled water giving 85% solution of propylene glycol in distilled water.

To make sure that cholesteryl oleate dissolved completely in the solvent and did not suspend as small droplets, the cholesteryl oleate solution was mixed with both the dyes separately and examined under a microscope at 10× magnification. Cholesteryl oleate was observed to have dissolved completely and no suspended droplets were visible. Ten unworn CLs of each of the seven materials were then placed in 10 wells of closed CL cases with 1.00 mL of 5.60 mg/ml cholesteryl oleate solution. Similarly, two unworn CL of each material were doped in 1.00 mL of 1% olive oil in PBS as positive controls and another two unworn CL of each material were doped in 1.00 mL of PBS as negative controls. The CL cases were then placed on a rocker table with gentle agitation for 12 hrs at room temperature. Contact lenses were then removed from the incubation solution with clean metal forceps and were lightly blotted with a Kim Wipe. The CLs were then stained with Oil Red O and LipidTOX as described below.

2.1.3. Oil Red O and LipidTOX staining

Oil Red O was prepared just prior to staining the CLs. In brief, 0.5 g of powdered Oil Red O (P O0625, Sigma Aldrich) was added to 100 mL propylene glycol (100%) and heated at 100 °C for 10 min. It was then allowed to stand at room temperature and was filtered with a 0.20 µm syringe filter. Contact lenses were rinsed with PBS, stained with Oil Red O for two minutes in a contact lens case, placed on a rocker and were then differentiated with 85% propylene glycol in distilled water for one minute. Lastly, the CLs were rinsed with PBS for 20 min and lightly blotted with a Kim Wipe.

LipidTOX was also prepared just prior to staining the CLs. In brief, 10 µL LipidTOX (H34475, Thermo Fisher Scientific) was added to 990 µL of PBS to prepare a 1% solution in PBS per ml in room temperature. Contact lenses were first rinsed with PBS and then incubated in 1 mL of LipidTOX solution for 30 min in a CL case for optimal staining of the lipids. They were then rinsed with PBS for 20 min and lightly blotted with Kim Wipes.

In order to make sure that both the dyes were staining the same lipids on the contact lens surfaces, an unworn senofilcon A (Acuvue Oasys) CL was stained with both the dyes simultaneously and then imaged through a tetramethylrhodamine (TRITC) filter for Oil Red O, through a fluorescein isothiocynate (FITC) filter for LipidTOX, and finally through a combined filter (Fig. 1) for comparison of fluorescence

Table 1
Contact lens material information.

Trade Name (Manufacturer)	USAN[a]	FDA[b] Group	% Water Content	Lens Type
Air Optix Night & Day (Alcon Inc., Fort Worth, TX)	Lotrafilcon A	V	24	SiHy[c]
Aviara (Coopervision Inc., Pleasanton, CA)	Enfilcon A	V	46	SiHy
PureVision (Bausch & Lomb Inc., Rochester, NY)	Balafilcon A	V	36	SiHy
1-day Acuvue True Eye (Vistakon Inc., Jacksonville, FL)	Narafilcon A	V	46	SiHy
Dailies Total 1 (Alcon Inc., Fort Worth, TX)	Delefilcon A	V	33	SiHy
Acuvue Oasys (Vistakon Inc., Jacksonville, FL)	Senofilcon A	V	38	SiHy
Acuvue 2 (Vistakon Inc., Jacksonville, FL)	Etafilcon A	IV	58%	Hy[d]

[a] USAN = U.S. Adopted Name.
[b] FDA = Food and Drug Administration.
[c] SiHy = Silicone Hydrogel.
[d] Hy = Hydrogel.