

Journal of Optometry

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ORIGINAL ARTICLE

Biochemical analyses of lipids deposited on silicone hydrogel lenses

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Received 28 March 2010; accepted: 23 July 2010

KEYWORDS

Contact lens;
Lipids;
Phospholipids;
Silicone hydrogel lenses;
Tears

Abstract

Purpose: This study was performed to determine the levels of lipids deposited on *in vivo* worn silicone hydrogel lenses.

Methods: Three silicone hydrogel materials, galyfilcon A, senofilcon A, and asmofilcon A, were worn for 2 weeks by 35 normal subjects. Total lipid deposition was determined by the sulfo-phospho-vanillin reaction. Cholesterol was estimated by a colorimetric probe through enzymatic oxidation. Phospholipid level was estimated by determining phosphorus with ammonium molybdate through enzymatic digestion.

Results: The total lipid content recovered from galyfilcon A, senofilcon A, and asmofilcon A was 32.9 ± 33.8 , 42.1 ± 14.0 , and 36.6 ± 31.9 $\mu\text{g}/\text{lens}$, respectively. The cholesterol content recovered from galyfilcon A, senofilcon A, and asmofilcon A was 26.2 ± 26.9 , 28.6 ± 19.4 , and 31.1 ± 21.1 $\mu\text{g}/\text{lens}$, respectively. There were no statistically significant differences in total lipids and cholesterol among the contact lens types. However, the quantity of phospholipid recovered from the asmofilcon A (7.0 ± 5.5 $\mu\text{g}/\text{lens}$) lenses was significantly higher than from galyfilcon A (1.1 ± 0.8 $\mu\text{g}/\text{lens}$) and senofilcon A (2.4 ± 0.8 $\mu\text{g}/\text{lens}$) lenses ($p < 0.05$, Mann-Whitney test).

Conclusions: The quantity of total lipid and cholesterol deposited on the 3 silicone hydrogel lenses tested did not differ. However, there were significant differences in the amounts of phospholipid deposited among the 3 silicone hydrogel lenses, of which clinical significance should be explored in the future study.

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PALABRAS CLAVE

Lentes de contacto;
Lípidos;
Fosfolípidos;
Lentes de hidrogel
de silicona;
Lágrimas

Análisis bioquímico de los lípidos depositados en las lentes de hidrogel de silicona**Resumen**

Objetivos: Se realizó este estudio para determinar la concentración de lípidos depositados en lentes de hidrogel de silicona utilizadas *in vivo*.

Métodos: Tres materiales de hidrogel de silicona (galyfilcon A, senofilcon A y asmoofilcon A) fueron utilizados durante 2 semanas por 35 sujetos normales. El depósito total de lípidos se determinó mediante la reacción de sulfofosovanilina. Se estimó el colesterol mediante una sonda colorimétrica mediante oxidación enzimática. El nivel de fosfolípidos se calculó mediante la determinación del fósforo con molibdato amónico mediante digestión enzimática.

Resultados: El contenido de lípidos totales recuperados en galyfilcon A, senofilcon A y asmoofilcon A fue de $32,9 \pm 33,8$, $42,1 \pm 14,0$ y $36,6 \pm 31,9$ $\mu\text{g/lente}$, respectivamente. El contenido de colesterol recuperado en galyfilcon A, senofilcon A y asmoofilcon A fue de $26,2 \pm 26,9$; $28,6 \pm 19,4$ y $31,1 \pm 21,1$ $\mu\text{g/lente}$, respectivamente. No hubo diferencias estadísticamente significativas en el contenido de lípidos y colesterol totales entre los distintos tipos de lentes de contacto. Sin embargo, la cantidad de fosfolípidos recuperados en las lentes de asmoofilcon A ($7,0 \pm 5,5$ $\mu\text{g/lente}$) fue significativamente superior que en las de galyfilcon A ($1,1 \pm 0,8$ $\mu\text{g/lente}$) y de senofilcon A ($2,4 \pm 0,8$ $\mu\text{g/lente}$) ($p < 0,05$, prueba de Mann-Whitney).

Conclusiones: La cantidad de lípidos y colesterol totales depositados en las 3 lentes de hidrogel de silicona era la misma. Sin embargo, hubo diferencias significativas en las cantidades de fosfolípidos depositados entre las 3 lentes de hidrogel de silicona, y la importancia clínica de este hecho debe explorarse en un estudio futuro.

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Introduction

Silicone hydrogel contact lenses have been available in clinical use since 1999 in the United States and 2004 in Japan. These lenses appear to overcome many of the lens-induced hypoxic problems associated with contact lens wear, whereas several clinical complications have been reported to occur as a result of mechanical disturbance, infection, and deposition.¹⁻³

Deposits that occur on silicone hydrogel lenses is a well-known clinical problem and may result in reduced comfort, visual performance, and lens wettability.³ Clinically significant levels of deposition occur in about 10-15% of silicone hydrogel wearers who clean their lenses without a digital rub.^{4,5} Although the addition of a rub and rinse step can reduce the deposits rate, certain patients still have significant deposits on their silicone hydrogel lenses.⁵⁻⁷

It is widely recognized that the adsorption of proteins and lipids onto contact lens is a complex process influenced by many variables including material surface charge, water content, degree of hydrophilicity, use schedule, and tear film composition.^{3,8-12} Many studies indicate that silicone hydrogel lenses adsorb a minimal amount of protein;¹³⁻¹⁶ however, the degree of lipid deposition on these lenses is under debate.^{13,17-19} Using high-performance liquid chromatography (HPLC), Jones et al. reported that 300-600 $\mu\text{g/lens}$ of cholesterol (CH), oleic acid (OA), and oleic acid methyl ester (OAME) were deposited on worn balafilcon A and lotrafilcon A lenses.¹³ However, Mariarz et al. used similar HPLC methods to quantify CH, OA, and OAME on worn balafilcon A lenses,¹⁷ and found that both OA and OAME were below the level of quantification (< 1.5 $\mu\text{g/lens}$), although up to 37 $\mu\text{g/lens}$ of CH was detected. Carney et al. used

fluorophotometric probes to measure *in vitro* adsorption of CH and phosphatidylethanolamine (PE) on various silicone hydrogel lenses.¹⁸ They found that the adsorption of CH (non-polar lipid) was greater than that of PE (polar lipid) for all lens types tested, whereas the degree of lipid adsorption varied based on the lens material. They concluded that lipid deposition on silicone hydrogel lenses was not different than conventional hydrogel lenses.

In our previous studies, we successfully measured the levels of protein and lipid deposited on conventional hydrogel lenses.^{20, 21} We determined the levels of lipids deposited on silicone hydrogel lenses by different analytical lipid quantification methods from those of previous studies.

Materials and methods**Subjects and contact lenses**

Forty-five experienced asymptomatic contact lens wearers (16 men and 29 women) ranging in age from 14 to 55 years participated in the study. Fifteen subjects were galyfilcon A lenses (Acuvue Advance; Johnson & Johnson Japan, Tokyo, Japan) wearers, 15 subjects were senofilcon A lenses (Acuvue Oasys; Johnson & Johnson) wearers, and 15 subjects were asmoofilcon A lenses (Premio; Menicon, Nagoya, Japan) wearers. The subjects were asked to wear their lenses for 12-14 hours a day for 2 weeks at the next visit. They were instructed to use multi-purpose solutions with a rub and rinse procedure. The type of MPS was not specified. At the next visit, however, 10 subjects were excluded from the study because of various reasons (no visit, not wearing lenses everyday, or wearing new lenses at the

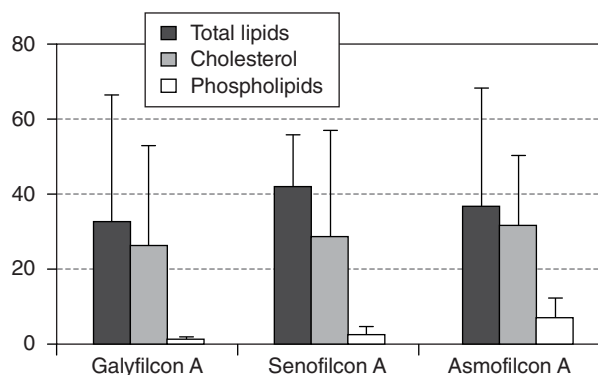


Figure 1 Total lipid, phospholipid, and cholesterol deposited on galyfilcon A, senofilcon A, and asmofilcon A contact lenses. There were no significant differences in total lipid or cholesterol among the groups (Mann-Whitney test). The quantity of phospholipid recovered from asmofilcon A ($7.0 \pm 5.5 \mu\text{g}/\text{lens}$) was significantly higher than from galyfilcon A ($1.1 \pm 0.8 \mu\text{g}/\text{lens}$) and senofilcon A ($2.4 \pm 0.8 \mu\text{g}/\text{lens}$) lenses ($p < 0.05$, Mann-Whitney test).

visit). Consequently, galyfilcon A lenses were collected from 13 subjects, senofilcon A from 10 subjects, and asmofilcon A from 12 subjects. The contact lenses were collected from subjects using metal tweezers, and then stored in a glass vial with nitrogen gas at -80°C for 2-6 weeks until analysis.

The principles of the World Medical Association and Declaration of Helsinki were followed. The subjects received a full explanation of the procedures and provided their informed consent for participation prior to the experiment. The protocol was approved by our Institutional Review Board, and all subjects provided written informed consent.

Lipid analysis

Lipids were extracted by the modified Bligh and Dyer procedure.²² In brief, whole contact lens samples were placed in a test tube with 1.0 mL of a 2:1 chloroform:methanol extraction solvent (Wako Inc., Osaka, Japan) for 16 hour at 4°C . After adding 0.2 mL of water, the tubes were vortexed for 30 seconds. The aqueous layer was discarded, and the organic solvent layer was divided into three parts, then used for analysis. Assays were also performed on new unworn sample of galyfilcon A, senofilcon A, and asmofilcon A lenses that served as the control.

Following extraction, total lipids were measured by the sulfo-phospho-vanillin reaction.^{20, 21} The lipid extracts were evaporated to dryness under nitrogen gas and reconstituted in 50 μL of distilled water. After adding 100 μL of 95% concentrated sulfuric acid (Wako), the samples were boiled at 100°C for 10 min, placed in a 96-well microplate, and mixed with 150 μL of the working reagent containing 1.2 mg/mL vanillin (Kokusai Shiyaku Co., Tokyo, Japan). The absorbance of the solution was measured at 655 nm using a spectrophotometer.

CH levels were estimated using the cholesterol/cholesterol ester quantitation kit (BioVision, Mountain View, CA, USA) according to the manufacturer's instructions. The evaporated lipid extracts were reconstituted in 50 μL of

reaction buffer provided by the manufacturer. Samples were then placed in a 96-well microplate and mixed with 50 μL of the working reagent containing cholesterol oxidase and a probe to produce resorufin. After incubating at 37°C for 60 min, the absorbance of the solution was measured at 570 nm using a spectrophotometer.

Phospholipid levels were estimated by determining phosphorus with ammonium molybdate through enzymatic digestion.^{20, 21} After the lipid extracts were evaporated to dryness under nitrogen gas, 50 μL of 10 mM TRIS hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) buffer, pH 7.8, containing 2.0 U/mL phospholipase C (from *Bacillus cereus*, Sigma) was added and the sample was incubated at 37°C for 20 min. The samples were then incubated at 37°C for an additional 30 min after adding 50 μL of 175 mM diethanolamine hydrochloride (Sigma-Aldrich) buffer, pH 9.6, containing 2.0 U/mL alkaline phosphatase (human placental origin, Sigma). Fifty μL of each sample was placed in a 96-well microplate and mixed with the molybdate-malachite green reagent (BIOMOL Inc., Plymouth Meeting, PA, USA). The absorbance of the solution was measured at 620 nm using a spectrophotometer.

Results

The results of the lipid analysis are shown in Figure 1. In all three assays used in the study, there were no differences in the samples from unworn galyfilcon A, senofilcon A, asmofilcon A lenses, and blank test tubes. These background measures were subtracted from the measures of each tested sample.

There were no significant differences in the total lipid content recovered from galyfilcon A ($32.9 \pm 33.8 \mu\text{g}/\text{lens}$), senofilcon A ($42.1 \pm 14.0 \mu\text{g}/\text{lens}$), and asmofilcon A ($36.6 \pm 31.9 \mu\text{g}/\text{lens}$) lenses (Mann-Whitney test).

In addition, there were no statistically significant differences in the CH content recovered from galyfilcon A ($26.2 \pm 26.9 \mu\text{g}/\text{lens}$), senofilcon A ($28.6 \pm 19.4 \mu\text{g}/\text{lens}$), and asmofilcon A ($31.1 \pm 21.1 \mu\text{g}/\text{lens}$) lenses (Mann-Whitney test).

However, phospholipid content recovered from asmofilcon A ($7.0 \pm 5.5 \mu\text{g}/\text{lens}$) was higher than that recovered from galyfilcon A ($1.1 \pm 0.8 \mu\text{g}/\text{lens}$) and senofilcon A ($2.4 \pm 0.8 \mu\text{g}/\text{lens}$) lenses ($p < 0.05$, Mann-Whitney test). The quantity of phospholipid recovered from senofilcon A was also significantly higher than from the galyfilcon A lenses ($p < 0.05$, Mann-Whitney test).

Discussion

In the present study, we estimated the quantity of total lipid, CH, and phospholipid deposited on 3 different silicone hydrogel lens materials. Our results suggest that cholesterol is the major class of lipids that forms deposits on silicone hydrogel lenses as reported by Maziarz et al.¹⁷ and Carney et al.¹⁸ These results are noteworthy because each study used a different analytical method to quantify the lipid. The deposition of OA and/or OAME appeared to be minimal because the amount of CH was equal to 70-80% of total lipid in our study.

The quantity of total lipid and CH did not differ significantly among the 3 lenses tested and were similar to the quantity of total lipid deposited on polyacon and etafilcon A lenses in our previous studies.^{20,21} Our results confirm that the quantity of lipid deposited on silicone hydrogel lenses is similar to conventional hydrogel lenses.¹⁹

The interesting finding of our study was that there were significant differences in the quantity of phospholipid recovered from the 3 silicone hydrogel lenses. Although phospholipid comprised only a small part of the lipid deposited, asmoofilcon A had more phospholipid than galyfilcon A and senofilcon A lenses. The water content of asmoofilcon A (40%) is similar to garyfilcon A (47%) and senofilcon A (38%). Phospholipids were not detected from worn polyacon and etafilcon A lenses in our previous studies.^{20,21} Therefore, factors other than water content might be responsible. Although the significance of phospholipids deposited on contact lenses remains to be established, it may have a beneficial effect on wettability of silicone hydrogel lenses. Goda and Ishihara reported that the synthetic phospholipids-polymer coating on silicone hydrogel lenses improves their wettability and biocompatibility, while maintaining high oxygen permeability compared with the original silicone hydrogel material.²³ However, whether dryness and discomfort are improved by refitting conventional hydrogel lenses to silicone hydrogel lenses remains controversial.^{24,25} Further clinical and experimental studies are required to clarify this issue.

Some patients develop significant deposition on their silicone hydrogel lenses.³⁻⁷ The exact mechanism and profile of deposition on silicone hydrogel lenses should be explored in further studies. A lipidomic approach using micro HPLC and mass spectroscopy to exhaustively analyze all classes of lipids may give us a new insight into this issue.²⁶ Because tear fluids contain proteins that possess lipid-binding properties, such as tear lipocalin and phospholipid-transfer protein, there may be a biochemical interaction between proteins and lipids associated with the deposition of lipids on silicone hydrogel lenses.²⁷⁻²⁹ All silicone hydrogel lenses analyzed in the present study were collected from asymptomatic contact lens wearers. Therefore, analyses of silicone hydrogel lenses with clinically significant deposits might give a different result. Such studies are in progress in our laboratory.

Acknowledgment

Supported by a grant from Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Conflict of interest

The authors have no conflict of interest in any materials in this manuscript.

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