Visualizing Hydrophobic Domains in Silicone Hydrogel Lenses with Sudan IV

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Purpose. A lipophilic dye is used to investigate the degree to which the surface and bulk hydrophobic domains of the lenses can be imaged and to identify specific changes in the availability of those domains after in vitro wear and cleaning conditions. The effect of a multipurpose solution (MPS), OPTI-FREE RepleniSH, on lens hydrophobic domains was also investigated.

Methods. Hydrophobic domains were determined using a saturated solution of Sudan IV. Staining periods of 30 minutes and 16 hours were used to determine surface versus bulk hydrophobic domains. Four types of silicone hydrogel lens materials were tested. The degree of staining was visually documented by photography and quantitatively determined by extraction and analysis of the total amount of dye adsorbed.

Results. Specific differences in staining were found for all control lenses. Exposure to in vitro wear conditions significantly decreased the staining response for all lens types as compared with unworn lenses ($P = 0.001$). However, the trend of staining remained the same: balafilcon A > galyfilcon A > senofilcon A > lotrafilcon B. MPS decreased the extent of staining; the degree of its effect varied with lens type.

Conclusions. Hydrophobic staining with Sudan IV visualized domains on and within silicone hydrogel lenses. Differences in staining response after exposure to wear and cleaning conditions indicate the potential for protein and lipid deposition on the different lens types and the ability of MPS to affect that deposition. Hydrophobic staining may be useful for determining differences in surface modification and lipophilicity of silicone hydrogel lenses. (*Invest Ophthalmol Vis Sci.* 2012;53:3473–3480) DOI:10.1167/iovs.11-9104

Silicone hydrogel contact lenses provide the wearer with an improved level of oxygen permeability compared with conventional hydrogel lenses. The integration of polysiloxane-based elastomers into the hydrogel allows an increase in ion permeability.[1–3] However, these lenses also possess surface properties that lead to complications different from conventional hydrogels.[4] In general, pure silicones have properties of low surface-free energy, hydrophobic and lipophilic behavior, and poor wettability.[5] Incorporation of silicone elastomers adds these properties to the lens surface. The siloxane moieties in the lens migrate to the lens surface, causing molecular domains of hydrophobicity on the area of the lens that interacts the most with the surrounding environment.[6,7] These properties have a direct effect on the biofouling of the lenses, which has been linked to various biocompatibility issues and health concerns such as heightened inflammatory responses and reduced comfort and visual acuity.[8–10]

Biofouling of silicone hydrogel lenses, initially dictated by the Vroman effect, is ultimately controlled by such properties as the hydrophobic, hydrophilic, water content, and surface-free energy properties of the material.[11–13] Increased variation in lens surface silicone content creates hydrophobic domains (HDs), areas of higher hydrophobicity with an increased vulnerability to lipid deposition.[14] Tear film components can also penetrate into the bulk of the lens depending on the properties of these components and the hydrogel; therefore, variation in bulk properties of the lens is also of concern.[15–17] With conventional hydrogels which are predominately hydrophilic in nature, protein deposition was the major factor in biofouling. However, the lipophilic nature of silicone hydrogels has changed the deposition profile such that lipids are the major factor in the biofouling of these lens types.[18–20]

In order to combat the hydrophobic and lipophilic nature of the lens surface caused by the introduction of silicone into the hydrogel, manufacturers employ three approaches, surface treatment, internal wetting agents, and hydrophilic polymers, to make the surface more hydrophilic. Balafilcon A's (PureVision; Bausch & Lomb Inc., Rochester, NY) surface, for example, is modified by a plasma oxidation technique that results in glassy, silicate islands that bridge the more hydrophobic areas on the surface.[1] Lotrafilcon A, on the other hand, uses a plasma coating treatment that coats the lens surface with a 25-nm thick hydrophilic polymer.[21,22] Galyfilcon A (Acuvue Advance; Johnson & Johnson Vision Care, Jacksonville, FL) and senofilcon A (Acuvue Oasys; Johnson & Johnson Vision Care) do not utilize such techniques, but rather use internal wetting agents to increase hydrophilicity.[21]

Current methodologies to determine the hydrophobicity of contact lens surfaces are limited to surface energy measurements such as captive bubble, sessile drop, and Wilhelmy plate contact angles.[23] Contact angle measurements provide overall degrees of hydrophobicity/hydrophilicity, but do not determine the specific spatial variations on a molecular domain scale across the lens surface and within the lens bulk. However, the proteins and lipids associated with biofouling of the lenses interact at the molecular domain level. In order to determine the performance issues of a lens type, a more specific type of analysis is needed.

In this study, lens hydrophobic domains were visualized and imaged through the use of a hydrophobic, lipophilic stain, Sudan IV (Sigma-Aldrich, Inc., St. Louis, MO). Sudan IV (Sigma-Aldrich, Inc.), also known as scarlet red, is a diazo dye and a

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Table 1. Properties of Lens Types Used in the Study

	% Water	Material	Surface Treatment	Dk (barrers)	Principal Monomers	Contact Angle‖
Acuvue Advance*	47	Galyfilcon A	None (internal wetting agent)	60	MPDMS, DMA, HEMA, EGDMA, PVP	65.6 ± 6.4
Acuvue Oasys*	38	Senofilcon A	None (internal wetting agent)	103	MPDMS, DMA, HEMA	78.7 ± 9.5
PureVision†	36	Balafilcon A	Plasma oxidation	91	TEGDMA, PVP, NVP, TPVC, NCVE, PBVC	93.6 ± 2.9
O2Optix‡	33	Lotrafilcon B	Plasma coating	110	DMA, TRIS, siloxane monomer	44.4 ± 6.4
Acuvue 2*	58	Etafilcon A	None	19	HEMA, MAA, EGDMA	18 ± 4.2
Biomedics 38§	38	Polymacon	None	8.4	HEMA, EGDMA	24 ± 5.1
Biomedics XC§	60	Omafilcon A	Surface active side chain	44	HEMA, MA PC, TEGMA	13 ± 3.8

* Johnson & Johnson Vision Care, Jacksonville, FL.
† Bausch & Lomb Inc., Rochester, NY.
‡ Ciba Vision, Inc., Duluth, GA.
§ CooperVision, Inc., Fairport, NY.
‖ Per manufacturer's data and (23).

lysochrome.[24,25] Sudan IV (Sigma-Aldrich, Inc.) staining allows visualization of potential biofouling areas and areas of lipid deposition.[26] Through this visualization, this study investigated the ability of hydrophobic domain staining to differentiate between lens types in terms of both surface treatments and bulk properties. The effect of both in vitro wear and cleaning conditions on the lens hydrophobic domain staining response was also determined.

Methods

A saturated solution of Sudan IV (Sigma-Aldrich, Inc.) in silicone oil was created.[26] For this, 2.0 g of Sudan IV (Sigma-Aldrich, Inc.) powder was mixed in 96.3 g of silicone oil (Sigma-Aldrich, Inc.) with a magnetic stirrer for 30 minutes. The solution then was centrifuged for 20 minutes at 13,500 rpm. Afterwards, 69.45 g of supernatant was removed and brought up to 150 mL by adding silicone oil. A standard curve was performed on each dye preparation.

Four silicone hydrogel lens types, with properties listed in Table 1, were used throughout this study: Galyfilcon A (Johnson & Johnson Vision Care), senofilcon A (Johnson & Johnson Vision Care), lotrafilcon B (O2Optix; Ciba Vision, Inc., Duluth, GA), and balafilcon A (Bausch & Lomb Inc.). Additionally, three conventional hydrogel lenses were used as controls to measure the effectiveness of Sudan IV (Sigma-Aldrich, Inc.) to stain hydrophobic versus hydrophilic domains specifically: Etafilcon A (Acuvue 2, Johnson & Johnson Vision Care), polymacon (Biomedics 38; CooperVision, Inc., Fairport, NY), and omafilcon A (Biomedics XC; CooperVision, Inc.). Their properties are also listed in Table 1. All lenses were −2.00 diopters in power and of the same base curve. Before use, all lenses were individually soaked in clean, labeled contact lens cases containing 5 mL of saline (Unisol 4; Alcon Research Laboratories, Inc., Fort Worth, TX) for 24 hours at room temperature. All lenses were tested in replicates of six.

Silicone hydrogel lenses were stained with Sudan IV (Sigma-Aldrich, Inc.) dye after exposure to one of five conditions—three control and two experimental: saline control, artificial tear fluid (ATF) control, MPS control, MPS before ATF, and MPS after ATF. Conventional hydrogel lenses were stained with Sudan IV (Sigma-Aldrich, Inc.) dye after exposure to only saline control conditions. The saline control group lenses were exposed to Sudan IV (Sigma-Aldrich, Inc.) directly after removal from their saline-soak contact lens cases. The ATF control group lenses were placed in ATF for 12 hours as described below after the saline soak. The MPS control group lenses were placed individually in contact lenses case wells with 5 mL of MPS overnight (12 hours) at room temperature after 24 hours in saline prior to dye exposure and analysis. The two experimental groups of lenses were tested as described in Figure 1.

The MPS used in this study's experiments was OPTI-FREE RepleniSH (Alcon Research Laboratories), a commercially available MPS. It was used as suggested by the manufacturer. No rubbing of the lenses with MPS was performed.

ATF was used to simulate the tear film in the eye during these in vitro studies. The components of the ATF are shown in Table 2. The dry lipid layer components of the ATF were prepared in appropriate ratios and stored in the freezer until use. Individual lipid layer mixtures were solubilized in 200 μL of chloroform just prior to use. The aqueous buffer was made using the concentrations shown in deionized (DI) water. The proteins were then added to the buffer solution.

ATF lens exposure simulated wear conditions using a published technique.[27] Individual lenses were added concave-side down on a bed of 32 silanized 2-mm glass beads in a silanized 16-mL scintillation vial containing 1 mL of ATF solution. The ATF lipid layer was added to the top of the ATF solution by pipetting on the 200-μL chloroform/layer mixture. Care was taken to ensure that the lens within the ATF solution was not directly exposed to the chloroform/lipid mixture. The chloroform of the chloroform/lipid mixture was allowed to evaporate for 5 minutes. The lens vials were then capped and placed in a 34°C rocking water bath for 12 hours. The rocking of the vials allowed the ATF solution within to break over the anterior surface of the lens, creating an air/tear film/lens interface, simulating day wear conditions. At the end of the in vitro wear period, lenses were stained for hydrophobic domain analysis as described below.

For staining purposes, each lens was lightly rinsed with saline, blotted against a fiberglass tissue (KIMWIPE; Kimberly Clark, Roswell, GA), and placed into a standard lens case well containing 5 mL of Sudan IV (Sigma-Aldrich, Inc.) dye solution for either 30 minutes or 16 hours at room temperature, depending upon the type of staining required, surface or bulk. Preliminary studies were performed to determine the appropriate dye exposure times which would provide only surface staining and bulk staining. Various exposure times ranging between 5 minutes to 24 hours were tested (data not presented). Consistent reproducible results for surface staining were not found

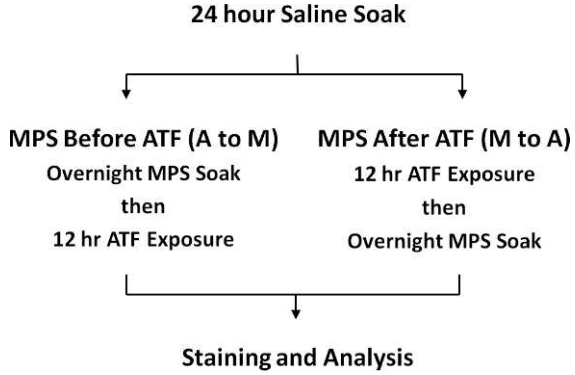

Figure 1. Schematic of experiments investigating the effects of MPS on lens Sudan IV dye binding and biofouling.

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Table 2. Artificial Tear Fluid Components

Component	Concentration (mg/mL)	Manufacturer
Aqueous buffer		
NaCl (crystal)	6.626	Fisher Scientific
MOPS	4.18	Sigma-Aldrich, Inc.
KCl (crystal)	1.716	J.T. Baker
Na bicarbonate (powder)	1.376	Mallinckrodt
Lactic acid (dens. 1.2)	0.24	Sigma-Aldrich, Inc.
CaCl, dihydrate	0.147	Sigma-Aldrich, Inc.
NaH_2PO_4	0.1	Sigma-Aldrich, Inc.
Proteins in solution		
Lysozyme	1.9	Sigma-Aldrich, Inc.
Lactoferrin (20%)	0.36	Sigma-Aldrich, Inc.
α-Acid glycoprotein (50%)	0.25	Sigma-Aldrich, Inc.
Albumin	0.2	Sigma-Aldrich, Inc.
Mucin	0.15	Sigma-Aldrich, Inc.
γ-Globulins	0.1	Sigma-Aldrich, Inc.
IgG	0.0067	Sigma-Aldrich, Inc.
Lipid layer		
Cholesterol	0.068	Sigma-Aldrich, Inc.
Cholesterol stearate	0.024	Sigma-Aldrich, Inc.
Sphingomyelin	0.004	Sigma-Aldrich, Inc.
Galactocerebrosides	0.004	Sigma-Aldrich, Inc.
Phosphatidylcholine	0.004	Sigma-Aldrich, Inc.
Chloroform	50 μL/mL	EM Science

until after 30 minutes of dye exposure. Likewise, consistent results for bulk staining were not found until after 16 hours of dye exposure. After dye exposure, lenses were rinsed twice in sequential saline-filled scintillation vials and then placed in a clean lens case with only enough saline to wet the lens until staining was imaged and quantified.

The distribution of staining on the lens was documented by photography through a microscope. Specifically, the stained lens was placed on a clear lens holder with the same curvature as the lenses and then photographed using a digital camera (Nikon COOLPIX 950; Nikon, Melville, NY) mounted onto the lens of a dissecting light microscope (Bausch & Lomb, Rochester, NY) under low ambient light conditions.

The degree of staining was quantified by dye extraction and analysis. Dye extraction was performed by placing each lens into a scintillation vial with 1 mL of dimethyl sulfoxide. The vials were placed in an ultrasonic water bath for 45 minutes at room temperature. The lens was removed from the extracted fluid, and the fluid was analyzed with a UV spectrophotometer at 522 nm. In the initial trials, nondye-exposed control lenses and the extracted lenses were also measured at 522 nm to confirm that all of the dye was removed from the lenses with this procedure.

Statistical analysis of all the numerical data was performed using an analysis of variance (ANOVA) method and statistical and data analysis software (Statistical Analysis Systems; SAS Institute Inc., Cary, NC).[28]

Results

Visualization of Control Staining of Hydrophobic Domains

Image analysis of the saline control silicone hydrogel lenses exposed to the Sudan IV (Sigma-Aldrich, Inc.) solution consistently showed specific degrees and reproducible patterns of staining amongst lens types while the staining of the conventional hydrogel lenses was not visually distinguishable. Visually, the extent of 30-minute surface staining for the silicone hydrogel lenses followed the overall trend of balafilcon A > galyfilcon A > lotrafilcon B > senofilcon A (Fig. 2). In contrast, images of the 16-hour stained lenses showed more extensive, widespread staining throughout the lens (Fig. 3). The extent of staining visualized in the 16-hour images followed a trend similar to those with a 30-minute Sudan IV (Sigma-Aldrich, Inc.) soak with balafilcon A > galyfilcon A > both senofilcon A and lotrafilcon B, which had similar amounts of visible staining. However, the differences between the images of the lens types were not as apparent as seen with surface staining. In general, the periphery stained more deeply than the center for all lens types.

Quantification of Control Staining of Hydrophobic Domains

Quantification of the amount of Sudan IV (Sigma-Aldrich, Inc.) for the 30-minute and 16-hour stained saline control lenses showed significantly less dye in the conventional lenses than the silicone hydrogel lenses, confirming the specificity of the dye for the HDs (Fig. 4). The amount of staining found for each 30-minute stained silicone hydrogel lens type followed the same general trend as seen with the visual analysis of the lens images ($P < 0.0001$ between all). For the 16-hour bulk staining, the only statistically significant difference in response between the silicone hydrogel lenses was for the lotrafilcon B lenses.

Staining Response after ATF Exposure (In Vitro Wear Conditions)

Images taken of the lenses exposed to ATF (in vitro wear conditions) all visually and quantitatively showed a decrease in degree of staining compared with the saline controls, except for the surface staining of senofilcon A lenses, implying that the deposition of ATF components onto the lens surfaces blocked the surface HDs (images not shown). Significant decreases in absorbance values were seen for all lens types compared with the respective saline control lenses ($P < 0.01$) except for senofilcon A lenses, which had an increased absorbance value ($P < 0.0001$). Similarly, ATF exposed lenses with a 16-hour Sudan IV (Sigma-Aldrich, Inc.) soak showed significant decreases in absorbance data for all lens types compared with the respective saline control lenses ($P < 0.001$), except for balafilcon A, which did not show a significant change.

Staining Response after MPS Exposure

Imaging of MPS control lenses also showed a visually noticeable decrease in staining as compared with saline control lenses, suggesting that components of the MPS strongly associate with the HDs on the lens surface, blocking the dye's ability to bind to them (data not shown). Surface staining of the MPS control lenses resulted in decreased staining for all lens types ($P < 0.001$) except for senofilcon A, which had no significant change from saline control values. The bulk staining significantly decreased for all lens types ($P < 0.001$, Fig. 5).

Statistically significantly lower staining was found for the surfaces of the MPS control lenses compared with ATF control lenses in both balafilcon A ($P = 0.01$) and senofilcon A ($P < 0.001$), indicating that the MPS components associated more strongly with those lenses than the ATF components (Fig. 5). A decrease in staining was found across all lens types for the bulk of MPS control lenses compared with ATF control lenses.

Experimental Trial 1: MPS before ATF Exposure (M to A)

Overall, for both staining periods, analyses of surface stained lenses treated with MPS prior to ATF exposure (simulated wear

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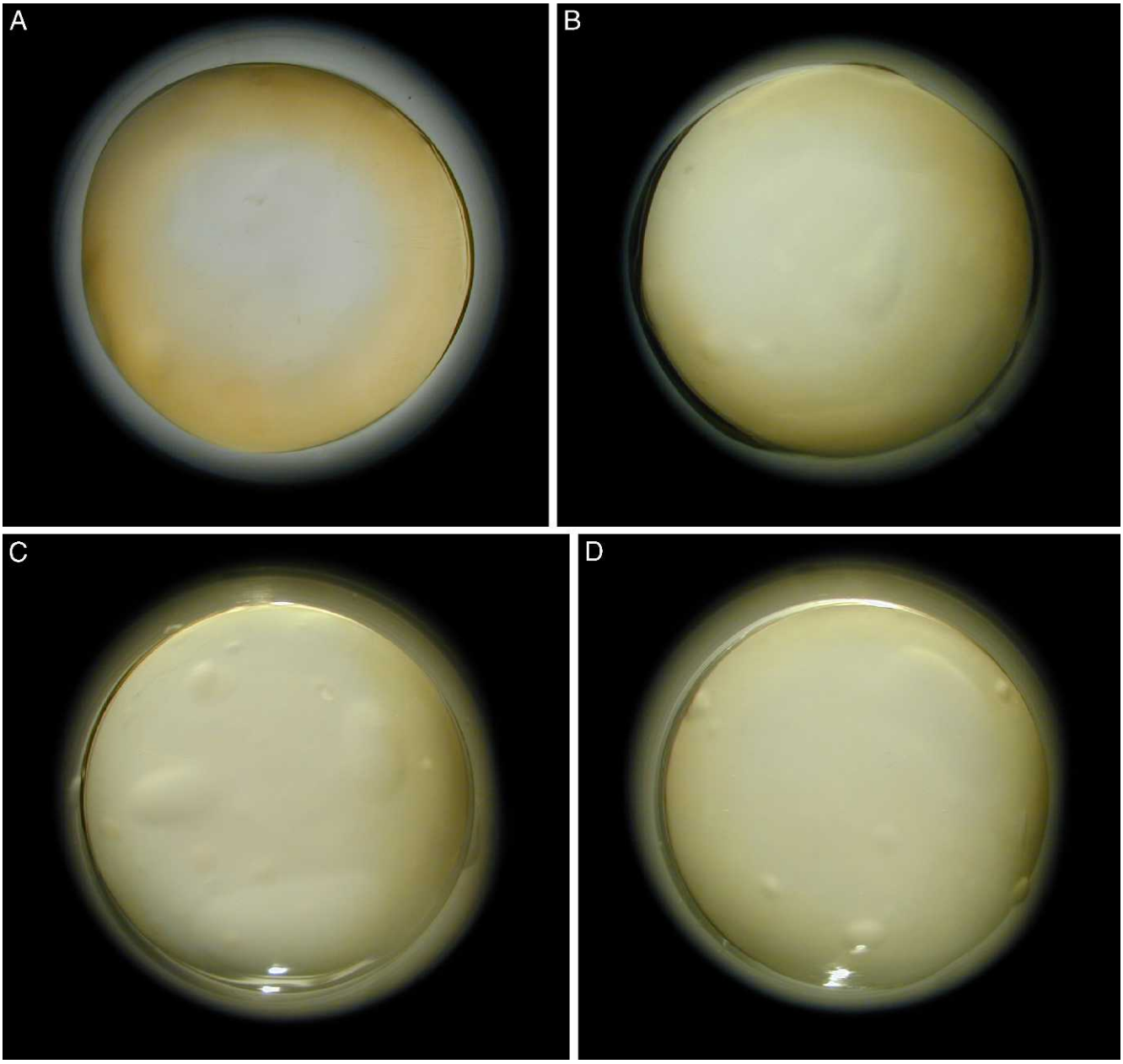

Figure 2. Saline control silicone hydrogel lens images for 30-minute Sudan IV soaks of: (**A**) Balafilcon A. (**B**) Galyfilcon A. (**C**) Senofilcon A. (**D**) Lotrafilcon B.

conditions) resulted in similar findings as the lenses exposed to ATF alone (Fig. 5). This suggests that when exposed to competitive ATF components, the ATF has more affinity to the HDs than MPS. Balafilcon A had the greatest degree of surface staining of any lens type ($P \leq 0.01$) and also, along with senofilcon A, had the greatest amount of bulk staining ($P \leq 0.05$).

Experimental Trial 2: MPS after ATF Exposure (A to M)

MPS-after-ATF exposure significantly decreased staining on the lens surface, 30-minute staining period, compared with the ATF controls for balafilcon A and senofilcon A ($P < 0.001$), indicating that the MPS components had an effect on the lens surface components and HDs available (Fig. 5). However, the decrease in surface staining response for galyfilcon A was not statistically significant and an increase was seen for lotrafilcon B. For bulk staining, a significant decrease was seen for all lens types ($P < 0.001$). MPS has a reversible mechanism. Overall, balafilcon A showed the most significant results and greatest percent change after the MPS was allowed to affect the lens surface.

Discussion

The use of Sudan IV (Sigma-Aldrich, Inc.) dye demonstrated significant differential staining between the saline control lens materials (hydrogels). Since the dye migrates to the hydrophobic areas of the lens, the degree of staining reflects the varying amount of HDs available for binding and thus varying potential

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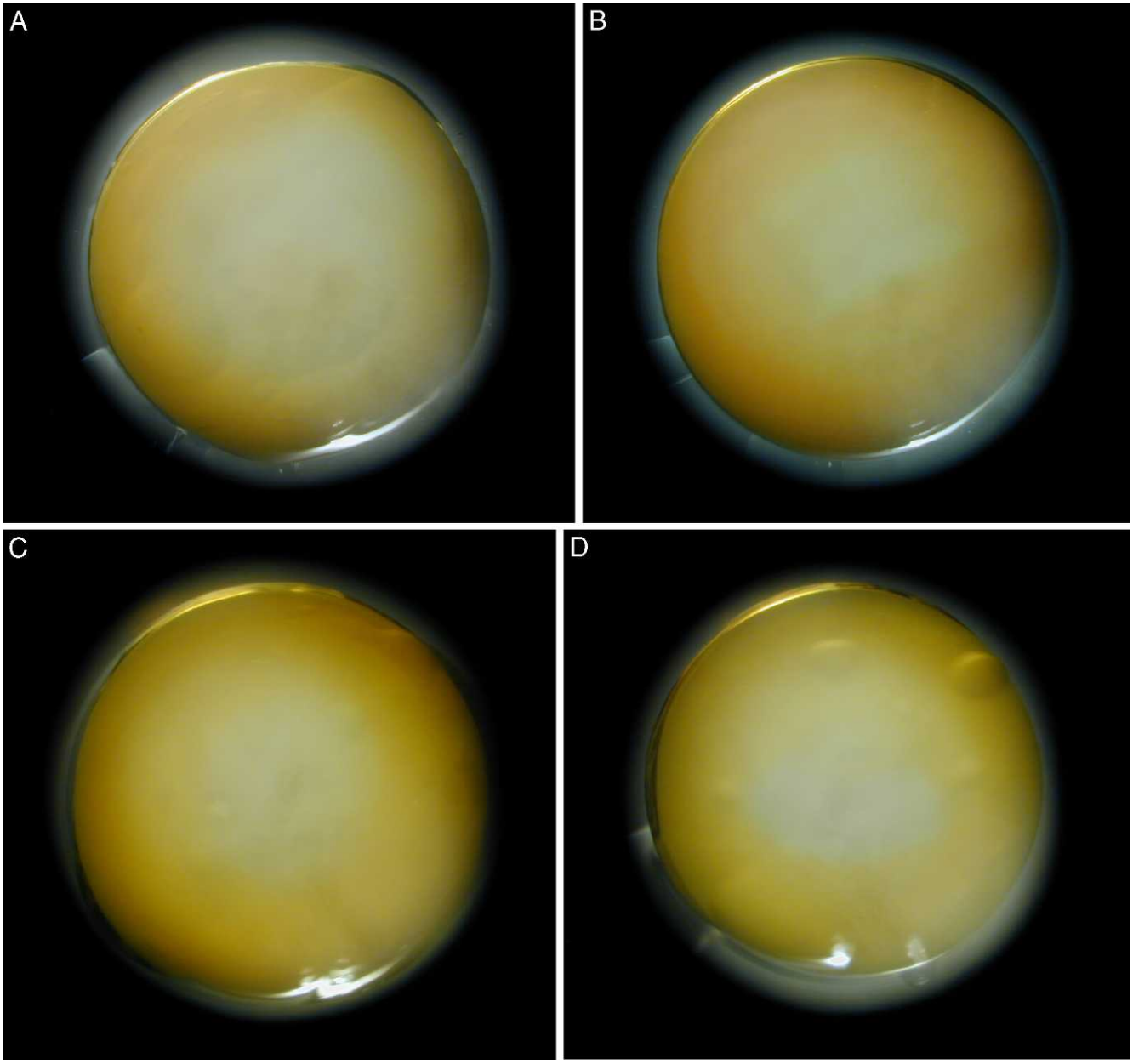

Figure 3. Saline control silicone hydrogel lens images for 16-hour Sudan IV soaks. (**A**) Balafilcon A. (**B**) Galyfilcon A. (**C**) Senofilcon A. (**D**) Lotrafilcon B.

degrees for natural lipid biofouling. Additionally, by varying the length of dye exposure, study authors were able to distinguish between the surface and bulk HD characteristics of each tested lens type. With the 30-minute Sudan IV (Sigma-Aldrich, Inc.) soak, the dye only penetrated the surface of the lenses and gave distinctly different stain patterns on the varying lens types. However, the overall trend was for the peripheral regions of the lens to stain more deeply than the central regions, implying that the dye staining was independent of lens thickness. If the staining was dependent on the thickness and, therefore, diffusion dependent, the central portions of the minus power lenses would have stained more deeply than their outer peripheral rims. The general trend of HD domain exposed on the surface of the saline controls was shown to be balafilcon A > galyfilcon A > lotrafilcon A > senofilcon A. This trend suggests that senofilcon A would have the lowest amount of potential hydrophobically driven biofouling.

When exposed to in vitro wear conditions, the ATF provides proteins and lipids that deposit on the lens, decreasing areas on the lens for possible staining. In general, all lens types had a decrease of over 15% or more in the amount of HDs available for staining after ATF exposure when compared with control lenses. Interestingly, the surface staining of senofilcon A increased after ATF exposure, indicating an increase in surface hydrophobicity. The 30-minute stained senofilcon A control lenses showed very little surface staining, suggesting very few HDs available for dye binding. However, once ATF components deposit on the surface, the components themselves can have exposed HDs to which the dye binds.[29] In general, contribution of the adsorbed components to the HD structure is negligible when compared

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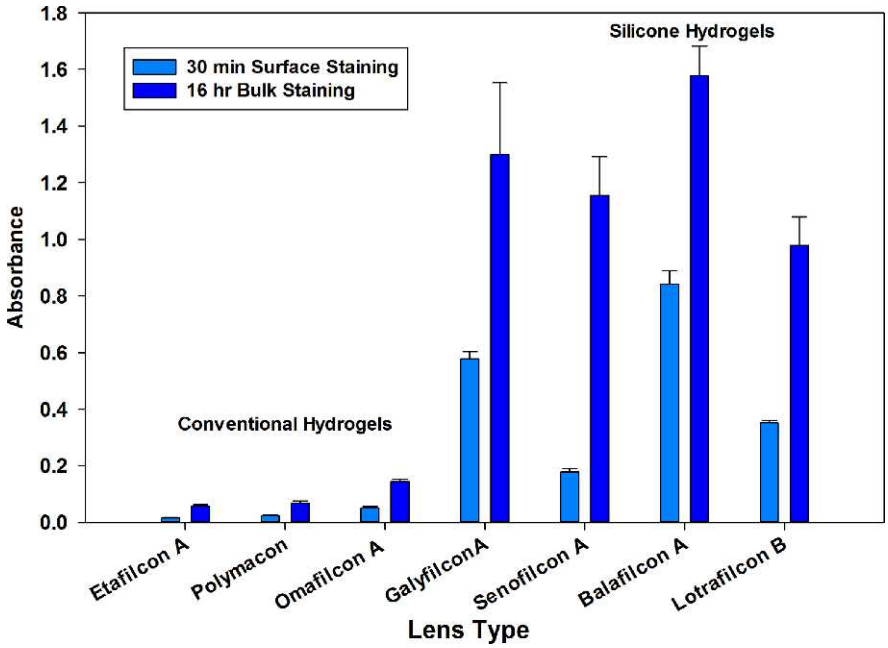

FIGURE 4. The mean Sudan IV dye binding response in conventional and silicone hydrogels for saline control lenses. All silicone hydrogel lenses had statistically more (at least three times or more) staining than conventional lenses in both the surface and bulk ($P = 0.0001$). The difference in amount of surface staining found for each silicone hydrogel lens type was Balafilcon A > Galyfilcon A > Lotrafilcon B > Senofilcon A, ($P < 0.0001$ between all). For the bulk staining, the Lotrafilcon B lens response was significantly lower than both Balafilcon A ($P = 0.0104$) and Galyfilcon A ($P = 0.005$).

with the lens surface; but when the lens surface has very low HDs to begin, with the adsorbed components can play a significant role. Therefore, for most lenses including the lenses tested in this study, the least amount of staining results from the most biofouling—except in unusual cases such as for the senofilcon A lenses.

MPSs are used to disinfect lenses and remove lens deposits that are adsorbed from the tear film. Additionally, some solutions contain polymers that specifically associate with the hydrophobic or hydrophilic areas of the surface as a preventative measure and barrier to biofouling and to increase lens comfort. MPSs can vary in their efficiency with differences in their composition, manufacturer, and cleaning and storage times.[22,30] While the staining results for the MPS control lenses were similar to those found after exposure to ATF, the rationale for the results is very different. In this instance, MPS components associate with the lens HDs in an attempt to block biofouling, thus also decreasing the binding sites for staining. The variance in the quantification results represents the effectiveness of the MPS in blocking the HDs from biofouling for each lens type. The lower the absorbance value, the more effective the barrier the MPS provided. Therefore, in this part of the study, a lower degree of staining is more appealing. For all lens types, a decrease in staining of the MPS controls compared with the saline controls demonstrates that it was effective on all lenses in blocking hydrophobic domains. Lotrafilcon B resulted in the least staining and thus has the greatest MPS association with this lens; however, the MPS caused the most significant change in the balafilcon A lenses, the lens type with the greatest biofouling potential.

Since quantification of staining was able to successfully demonstrate the effects of ATF and MPS individually on the HDs of the lenses, the ability of the MPS to remove and/or prevent ATF biofouling was investigated. The MPS-before-ATF trials investigated the MPS's effectiveness in blocking ATF component deposition. Likewise, the MPS-after-ATF trials investigated the MPS's capacity to remove ATF components already deposited on the lens. Differences in the MPS's effectiveness across lens types were found for both trials. In general, the MPS-before-ATF trials showed similar amounts of staining as the ATF control lenses. This suggests that the MPS was not effective in changing the nature of the lens surface and the attraction of the ATF components to the surface was stronger than the MPS's barrier effect to block them.

On the other hand, the trials investigating the cleaning qualities of the MPS did show significant effectiveness. The MPS-after-ATF trials showed significantly decreased staining for most lenses compared with the ATF control lenses both at the surface and in the bulk. Since it is most likely the ATF control lenses' staining represents HDs that have not been blocked by deposited components, the decrease seen in the MPS-after-ATF trials may indicate the MPS's effectiveness in blocking these domains from further biofouling and/or cleaning of the deposited material. The lotrafilcon B's greater amount of surface staining in the MPS-after-ATF trials than the ATF control lenses could be explained by the MPS having a better cleaning ability and a less saturated barrier blocking of the HDs.

An in vitro model was successfully developed that allows for differentiation of lenses based on the accessibility of the hydrophobic domains on the surface and within the lens. Hydrophobic staining with Sudan IV (Sigma-Aldrich, Inc.) reliably visualized domains on and within differing silicone hydrogel lenses. The pattern of staining was specific for each lens type. Differences in staining response after exposure to wear and cleaning conditions indicate the potential for increased lipid deposition on the different lens types and the ability of MPS to affect that deposition. This hydrophobic staining technique may be useful for determining differences in the surface modification techniques and lipophilicity of silicone hydrogel lens types. As both surface modification techniques and lipid deposition have been linked to contact lens comfort and biocompatibility,[31,32] this staining technique may also be useful in determining the wearability of silicone hydrogel lenses.

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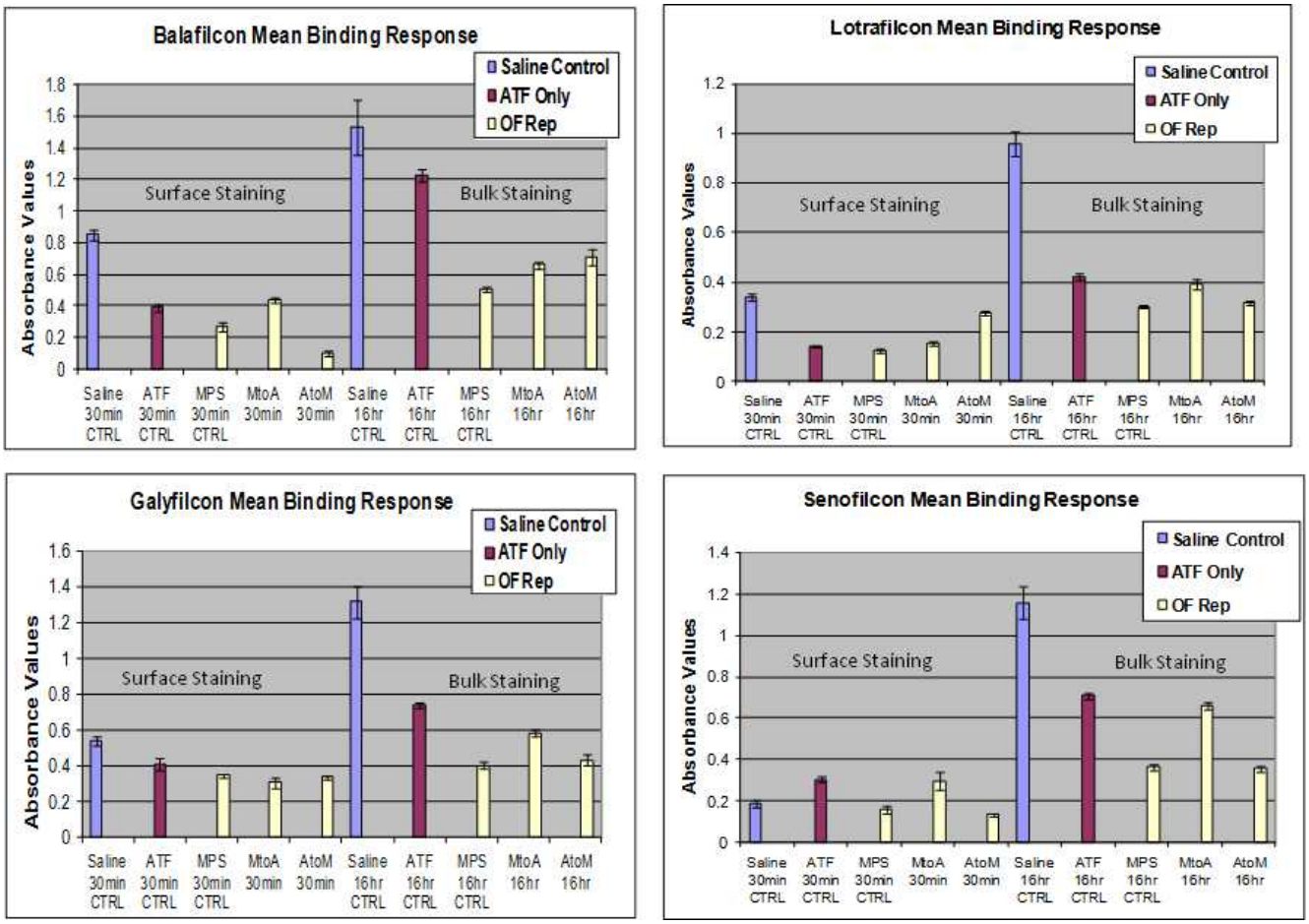

Figure 5. The mean Sudan IV dye binding response to the hydrophobic domains of silicone hydrogel lenses; 30-minute surface binding and 16-hour bulk binding. Groups of lenses: Saline control lenses; ATF alone exposure; MPS alone exposure; M to A, MPS-before-ATF; A to M, MPS-after-ATF.

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