Effects of Rigid and Soft Contact Lens Daily Wear on Corneal Epithelium, Tear Lactate Dehydrogenase, and Bacterial Binding to Exfoliated Epithelial Cells

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Objective: To determine the effects of lens type and oxygen transmissibility on human corneal epithelium during daily lens wear (DW).

Design: Prospective, randomized, double-masked, single-center, parallel treatment groups clinical trial.

Participants: Two hundred forty-six patients fitted with: (1) high oxygen-transmissible soft lenses (n = 36), (2) hyper oxygen-transmissible soft lenses (n = 135), and (3) hyper oxygen-transmissible rigid gas-permeable (RGP) lenses (n = 75).

Intervention: Irrigation chamber to collect exfoliated epithelial surface cells, confocal microscopy, and tear collection at baseline, 2 weeks, and 4 weeks of DW.

Main Outcome Measures: (1) *Pseudomonas aeruginosa* (PA) binding to exfoliated corneal epithelial surface cells, (2) central epithelial thickness, (3) superficial epithelial cell area, (4) epithelial surface cell exfoliation, and (5) tear lactate dehydrogenase (LDH).

Results: Four weeks of DW with the high oxygen-transmissible soft lens significantly increased PA binding from baseline 6.55 ± 3.01 to 8.75 ± 3.05 bacteria per epithelial cell ($P < 0.01$). By contrast, hyper oxygen-transmissible soft lens wear increased binding significantly less (6.13 ± 2.45 to 7.62 ± 3.06; $P < 0.01$), whereas hyper oxygen-transmissible RGP lens wear demonstrated no significant changes (5.91 ± 2.40 to 6.13 ± 2.17; $P = 0.533$). No significant change in central epithelial thickness was found after 4 weeks of DW in either soft lens; however, the epithelial thickness decreased by 9.8% ($P < 0.001$) with RGP lens wear. Epithelial cell surface area increased 3.3% and 4.1% with the high and hyper oxygen-transmissible soft lenses, respectively, and 10.5% with the hyper oxygen-transmissible RGP lens ($P < 0.001$). Epithelial desquamation significantly decreased in all groups ($P < 0.001$). Tear LDH levels increased for all test lenses ($P < 0.001$).

Conclusions: Increased PA binding induced by wear of a conventional soft lens material is significantly greater than that induced by the new hyper oxygen-transmissible soft silicone hydrogel lens during DW. However, both soft materials showed significant increases in PA binding as compared with baseline controls. By contrast, hyper oxygen-transmissible RGP lens DW did not increase PA binding significantly. Taken together, these findings suggest for the first time both an oxygen effect as well as a difference between soft and rigid lens types on PA binding in DW. *Ophthalmology 2001;108:1279–1288 © 2001 by the American Academy of Ophthalmology.*

It has long been recognized by clinicians that the most severe complication of contact lens wear is infectious ulcerative keratitis. A decade ago, seminal case-control studies by Poggio et al[1] and Schein et al[2] first established the annualized incidence of infectious ulcerative keratitis with soft contact lens use as 4.1 per 10,000 persons for daily wear lenses (DW), rising to 20.9 per 10,000 persons for extended wear lenses (EW). Patients who used soft contact lenses overnight or for longer periods had an increased risk of 10 to 15 times that of those in DW alone. A singular strength of these benchmark studies was the establishment of relative risk estimates for soft contact lens-related keratitis before the introduction of disposable soft lenses into general clinical practice. Unfortunately, neither study ad-

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dressed the issue of risk estimates by lens type (rigid vs. soft). In both of these reports, however, overnight wear was identified as the most significant risk factor for development of lens-related infectious ulcerative keratitis.

Additional clinical epidemiologic studies have confirmed and extended these results, demonstrating that contact lens wear itself (DW and EW) has surpassed trauma as the main risk factor for ulcerative infectious keratitis in humans.[3,4] An additional study by Dart et al[4] further stratified relative risk estimates by lens types and wearing schedules.

Most recently, Cheng et al[5] reexamined the incidence of infectious ulcerative keratitis in the entire contact lens wearing population of the Netherlands for both soft and rigid materials in both DW and EW. Unfortunately, despite the widespread introduction of disposable soft contact lenses into general clinical practice in the late 1980s, their findings reconfirm an incidence of infectious ulcerative keratitis of 3.5 per 10,000 persons in soft contact lens DW. Importantly, the study also confirms the previous findings of Dart et al[4] that DW of rigid gas-permeable (RGP) contact lenses provides significantly reduced risk for the development of infectious ulcerative keratitis (1.48 per 10,000).[5] The demonstrated failure of disposable soft lenses to reduce significantly the risk of DW-related ulcerative keratitis[5] has made use of contact lenses a continuing concern as the numbers of millions of patients using these devices to correct refractive errors continues to grow throughout the world. Even if all EW contact lenses were discontinued, one half to two thirds of cases of lens-related infectious ulcerative keratitis would still occur as a result of DW. In the United States alone, a possible annual incidence of 27,000 cases has been reported.[3]

Recognition of this important fact led us to propose a testable clinical hypothesis that: (1) corneal infection can not occur in the absence of initial microbial binding to surface epithelial cells and (2) the amount of lens-induced surface binding is inversely proportional to the oxygen transmission of the contact lens itself. In 1993, we selected a fully infectious strain of *Pseudomonas aeruginosa* (PA) as a standard test organism because PA is the most important and most devastating causative agent isolated from lens-related infectious ulcerative keratitis in humans.[6–10] Important initial support for this approach was derived from earlier studies demonstrating an increase in PA binding to the rabbit cornea after overnight lens wear[8] and the clinical observations of Fleiszig et al,[9] who were the first to show greater adherence of PA bacteria to human exfoliated epithelial cells from the corneas of 10 general EW soft contact lens wearers as compared with nonlens-exposed controls. In 1994, extensive studies in the rabbit model demonstrated for the first time a significant correlation between lens oxygen transmissibility, induced corneal surface epithelial damage, and increased PA binding[10]; and recently, we have extended and confirmed similar results in an extensive clinical trial with EW.[11] In both animals[10] and humans,[11] test lens wear with novel hyper oxygen-transmissible lens materials for both rigid and soft test lenses did not increase PA binding significantly above baseline (no lens) control values, suggesting that this new generation of contact lenses may offer a significant potential advance in safety for EW. No studies, however, have been reported that demonstrate differences in PA binding during DW of conventional high oxygen-transmissible soft lenses compared with the new hyper oxygen-transmissible soft and RGP lens materials. Using previously established methods,[10,11] the primary purpose of this study was to examine prospectively in a double-masked, randomized (soft lens study) parallel group clinical trial, the effects of conventional soft high oxygen-transmissible and the new hyper oxygen-transmissible soft and RGP lenses on the binding of PA to exfoliated human corneal epithelial cells during DW. To evaluate further effects of contact lens wear, we used concomitantly an independent assessment of the corneal surface by in vivo confocal microscopy, which permits noninvasive measurement of lens-related changes in epithelial surface cell area and central epithelial thickness. Additionally, we monitored surface cell exfoliation rates as measured by the standardized irrigation chamber as well as changes in tear lactate dehydrogenase (LDH) levels.[11]

Table 1. Inclusion and Exclusion Criteria

Soft lenses	1. Age 21–38 years
	2. Myopia: −0.50 to −6.00 diopters (D) with degree of regular astigmatism <0.75 D
Rigid lenses	1. Age 18–60 years
	2. Myopia: −1.00 to −20.00 D with any degree of regular astigmatism
All lenses	3. Any gender, race, or national origin accepted
	4. No history of ocular allergies
	5. No lens wear 1 month before entry into the study
	6. Visual acuity of 20/30 or higher with the test lenses
	7. Normal ocular surface, cornea, conjunctiva
	8. Schirmer test above 5 mm/5 min
	9. No blepharitis or other eyelid problems
	10. Intraocular pressure less than 21 mmHg
	11. Normal crystalline lens
	12. Normal-appearing retina and optic nerve
	13. No current use of ocular or systemic medication
	14. Females not pregnant or with plans to become pregnant

Materials and Methods

Study Population

Two hundred ninety patients were enrolled initially, after undergoing a comprehensive ocular examination to screen for eligibility; 246 patients completed all study procedures. Table 1 shows the inclusion and exclusion criteria for all test lenses; patient characteristics are presented in Table 2 for each test lens. All test groups were similar for age, gender, and refractive error, and no effects of these parameters were found on any outcome measure in the data analysis. All patients signed a consent form as approved by the Institutional Review Board of The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas. Patients were required not to wear any contact lenses for at least 1 month before starting the study. Nineteen enrolled patients wearing soft test lenses and 25 RGP lens wearers left the study prematurely. Most of the reasons for early withdrawal involved patient disinterest (lost to follow-up) and discomfort (RGP lens study), as shown in Table 3. No significant adverse patient reactions, such as corneal ulceration, were encountered in the overall study.

Table 2. Patient Characteristics

	High Oxygen-transmissible Soft Lens	Hyper Oxygen-transmissible Soft Lens	Hyper Oxygen-transmissible Rigid Gas-permeable Lens
Age (yrs)			
Mean ± standard deviation	30.3 ± 4.3	29.2 ± 4.3	34.5 ± 7.9
Range	23–38	20–38	22–55
Gender			
Female	26	86	56
Male	10	49	19
Race			
Asian	2	14	7
Black	12	23	18
Hispanic	2	17	7
White	20	81	43
Contact lens power (diopters)			
Mean ± standard deviation	−3.10 ± 1.4	−2.79 ± 1.8	−3.82 ± 2.21*
Range	−0.50 to −5.50	−0.75 to −5.50	Plano to −12.25

*The following five patients with cylindrical lenses were not included in the average: (1) S+0.50 C−1.00 right eye; plano C−2.00 left eye; (2) S−5.25 C−6.75 right eye; S−5.75 C−6.50 left eye; (3) S+0.25 C−3.00 right eye; (4) S−1.75 C−3.50 right eye; S−2.25 C−4.25 left eye; (5) S−1.50 C−3.00 right eye; S−0.50 C−2.00 left eye; S = sphere; C = cylinder.

Study Protocol

Table 4 outlines the study design. Patients were examined at two (RGP lens wearers) and three time points (soft lens wearers) for data collection to assess five outcome measures: (1) PA binding to exfoliated epithelial cells, (2) epithelial thickness, (3) superficial epithelial cell area, (4) superficial epithelial cell exfoliation, and (5) tear LDH. The data collection visits were performed at day 0 (prelens baseline), day 14 (2 weeks of DW), and day 30 (4 weeks of DW).

Contact Lenses. Sample size calculations to detect significant changes in the outcome measures were derived from preliminary data of a previous study[11]; to detect a significant increase in PA binding of one half the magnitude as compared with baseline control with a power of 0.8, a minimum number of 20 subjects per lens group was required. Additional subjects were added to compensate for potential dropouts and to satisfy the requirements of a subsequent 12-month EW phase of the study requiring division of the silicone hydrogel groups into a 6- and 30-night EW schedule (data not reported here). Patients in the soft lens study were randomized in compliance with a random number table generated by a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA); 40 patients were randomly assigned to the test group wearing a high oxygen-transmissible soft contact lens and 150 patients were randomized to the group with a hyper oxygen-transmissible soft contact lens (silicone hydrogel). The material specifications of the test contact lenses are given in Table 5. Both soft lenses were dispensed with Renu Multipurpose Solution and rewetting drops (Bausch & Lomb, Rochester, NY). A parallel group of 100 patients were similarly assigned to the test wear of a hyper oxygen-transmissible RGP contact lens (Table 5). Rigid gas-permeable lens wearers received Claris soaking and cleaning solution, Lens Plus Saline, and Claris rewetting drops (Allergan, Irvine, CA). Enzyme tablets were not used in this study. All study patients were instructed to insert the contact lenses in the morning on awakening and to take them out in the evening before going to sleep (10–16 hours lens wear per day).

Contact Lens Fitting. After acceptance into the study, all patients were individually trial fitted with the test lenses assigned to them. It should be noted that the RGP contact lenses in this study were only available in 0.10-mm steps instead of the more common 0.05-mm intervals.

Outcome Measures. The observers in charge of clinical data collection were masked from the knowledge of test lens type and examination time point (baseline, 2 weeks, and 4 weeks DW). On clinical collection of the outcome measures, the cytologic specimens, tear samples, and images were coded with a combination of letters and numbers that masked laboratory personnel for visit number or test lens. At the conclusion of the study, the code was broken to organize the data in a spreadsheet and to perform statistical analyses. All samples were taken in the morning between 9 and 12 AM to control for daily circadian fluctuations in the exfoliation rate.[12]

Table 3. Exit List

	High Oxygen-transmissible Soft Lens	Hyper Oxygen-transmissible Soft Lens	Hyper Oxygen-transmissible Rigid Gas-permeable Lens
Patient disinterest (lost on follow-up)	2 (5%)	8 (5.3%)	15 (15%)
Pregnancy	1 (2.5%)	1 (0.7%)	1 (1%)
Discomfort		3 (2%)	9 (9%)
Heavy deposits		2 (1.3%)	
Tear problems	1 (2.5%)	1 (0.7%)	
Total exit	4 (10%)	15 (10%)	25 (25%)
Total enrolled	40	150	100
Total completed daily lens wear	36	135	75

Table 4. Study Design

Visit	Time Interval	Procedure
1	0	Comprehensive eye examination; patients accepted/rejected Contact lens trial fitting and ordering contact lenses At least 1 month without lens wear
2	0	Data collection visit no. 1: prelens baseline of the five outcome measures
3	1 day	Contact lens + solutions dispensed
4	2 wks	Data collection visit no. 2 after 2 wks daily wear (except rigid gas-permeable lens wearers)
5	4 wks	Data collection visit no. 3 after 4 wks daily wear

Data collection visit: (1) right eye: lens removal and corneal surface irrigation to collect corneal epithelial cells; (2) left eye: lens removal followed by tear collection and tandem scanning confocal microscopy.

Cell Exfoliation Rates and Bacterial Binding

Corneal epithelial cells were collected noninvasively in vivo with a special irrigation chamber by previously established methods.[12] *Pseudomonas aeruginosa* strain ATCC 27853 (American Type Culture Collection, Rockville, MD) is fully infectious to the cornea and has been established as the standard test organism in both experimental rabbit[8] and previous human bacterial adherence studies.[11] Validity of the correlation between total surface corneal epithelial PA binding and PA binding to exfoliated cells has previously been published.[11,13] A Leica fluorescent microscope (Deerfield, IL) with an epifluorescence attachment was used (1) to examine the total number of PA bacteria per corneal epithelial cell (PA binding), and (2) to count the total number of collected corneal epithelial cells under oil immersion at ×630 magnification (exfoliation rate/minute).[11,13] We did not include epithelial cells attached to the contact lens in the analysis because this number was shown in a previous study[14] to be extremely small.

Corneal Epithelial Thickness and Cell Surface Area

The thickness of the central corneal epithelium and the area of the surface epithelial cells was determined by in vivo tandem scanning confocal microscopy performed on the left eye of each patient by previously published methods.[15] To measure epithelial cell surface area, four different images (475 × 350 μm) of the central corneal surface epithelium were selected from the video and digitized on a computer (Fig 1). Approximately 120 to 150 superficial epithelial cell areas per patient were outlined with specialized software that calculated the mean cell surface area, as performed in previous studies.[11,16] All measurements were recorded by the same masked technician.

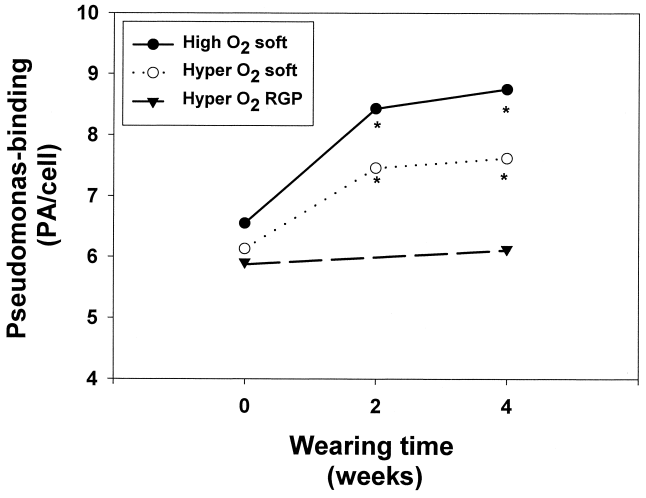

Figure 1. The relationship between *Pseudomonas aeruginosa* binding and time for the three lens groups at baseline, 2 weeks, and 4 weeks of daily wear. Table 6 contains the means, standard deviation, and the details of the statistical analysis (*statistically significant).

Tear Lactate Dehydrogenase

In prior studies, changes in tear LDH levels have been established as an objective assessment of the status of the corneal epithelial surface. Dead, damaged, or dying cells release LDH after toxic, mechanical, or contact lens-mediated injury. Tear LDH was assayed by previously published methods.[11]

Statistical Methods

The results of all outcome measures were compared for each contact lens group separately to the prelens, baseline values with a

Table 5. Test Lens Parameters

Lens Type and Classification	Commercial Name	Material	Water Content	Oxygen transmissibility*	Equivalent Oxygen Percentage†	Base Curve (mm)	Diameter (mm)	Thickness‡ (mm)
High oxygen-transmissible soft lens	Acuvue§	Etafilcon A	58%	32.5	12.42	8.4/8.8	14.00	0.07
Hyper oxygen-transmissible soft lens	Pure Vision‖	Balafilcon A	35%	110	19.90	8.6	14.00	0.09
Hyper oxygen-transmissible RGP lens	Menicon Z¶	Tisilfocon A	<0.5%	97	19.13	7.00–8.00	9.00–9.80	0.13

*Measured in saline at 35°C by the polarographic method, edge effect correction measured at −3.00 diopters (D) lens power (International Standards Organization no. 9913). 10^{-9}(cm/sec)(ml O_2/ml mmHg).

†21% = normal at sea level.

‡Center thickness measured at −3.00 D lens power.

§Vistakon Inc., Jacksonville, FL.

‖Bausch & Lomb, Inc., Rochester, NY.

¶Menicon Ltd., Nagoya, Japan.

Table 6. *Pseudomonas aeruginosa* Binding to Exfoliated Corneal Epithelial Cells

	Baseline	2 Wks of Daily Lens Wear	4 Wks of Daily Lens Wear
High oxygen-transmissible soft lens*	6.55 ± 3.01 PA/cell, n = 36	8.43 ± 3.46 PA/cell†, n = 36	8.75 ± 3.05 PA/cell†, n = 35
Hyper oxygen-transmissible soft lens‡	6.13 ± 2.45 PA/cell, n = 135	7.46 ± 2.74 PA/cell†, n = 132	7.62 ± 3.06 PA/cell†, n = 134
Hyper oxygen-transmissible RGP lens§	5.91 ± 2.4 PA/cell, n = 75	NA	6.13 ± 2.17 PA/cell, n = 75

ANOVA = analysis of variance; NA = not available; PA = *Pseudomonas aerginosa*; RGP = rigid gas permeable.

*Significant increase over time: $P = 0.0080$ (one-way RM ANOVA).

†Statistically significant, one-way repeated-measure ANOVA to baseline and Student-Newman-Keuls multiple comparisons test.

‡Significant increase over time: $P < 0.0001$ (one-way RM ANOVA).

High oxygen-transmissible vs. hyper oxygen-transmissible soft: $P = 0.0122$, power 0.93 (two-way ANOVA); significant difference.

§No significant change: $P = 0.533$ (paired *t*-test).

one-way repeated-measure analysis of variance (ANOVA; soft lenses) or the paired *t*-test (RGP lenses) and subsequently with a post hoc multiple comparison test (Student-Newman-Keuls test; soft lenses) with SAS statistical software (SAS Institute Inc., Cary, NC). In addition, the high and hyper oxygen-transmissible soft contact lenses were compared with each other using a two-way repeated-measure ANOVA, holding lens type and time as independent variables. A poststudy power analysis was conducted for each of the five variables with PASS 2000 software (NCSS Statistical Software, Kaysville, UT). The results are represented as means ± standard deviation. The data collected from epithelial cell exfoliation and tear LDH were found not to have a normal distribution. To ensure the correct application of the ANOVA test, the data were transformed into a natural logarithmic scale to obtain a normal distribution before statistical analysis. Correlation between variables was determined with Pearson product moment correlation. If patients missed the 4 weeks of DW visit, their data were deleted from the analysis. An appropriate general linear model was used otherwise to handle the rare missing 2-week data points for the soft contact lens subjects.

Results

Pseudomonas aeruginosa Binding

Figure 1 and Table 6 show the results of PA ATCC 27853 binding to exfoliated corneal epithelial cells for all three test lenses at each interval sampled. *Pseudomonas aeruginosa* binding to exfoliated epithelial cells increased significantly from 6.55 ± 3.01 at baseline to 8.43 ± 3.46 after 2 weeks of DW in the high oxygen-transmissible soft lens group (27% increase; $P = 0.0097$) and to 8.75 ± 3.05 (34% increase; $P = 0.0046$) after 4 weeks of DW. The hyper oxygen-transmissible soft group demonstrated baseline binding of 6.13 ± 2.45 and increased 22% to 7.46 ± 2.74 after 2 weeks of DW and 24% to 7.62 ± 3.06 after 4 weeks of DW ($P < 0.0001$). *Pseudomonas aeruginosa* binding in the high oxygen-transmissible soft lens group was significantly higher ($P = 0.0122$) than the group with hyper oxygen-transmissible soft lenses (two-way repeated-measure ANOVA). By contrast, the parallel test study with the hyper oxygen-transmissible RGP contact lens wear did not show a statistically significant increase in PA binding from baseline: 5.91 ± 2.40 to 6.13 ± 2.17 at 4 weeks (4% increase; $P = 0.533$).

Corneal Epithelial Thickness

Figure 2 and Table 7 show no apparent change in central epithelial thickness during DW of both soft lens test groups. Rigid gas-permeable lens wearers, however, underwent a striking and significant decrease in central corneal epithelial thickness of approximately 9.8% after 1 month of DW: from 49.79 ± 3.25 μm at baseline to 44.92 ± 4.32 μm at 1 month ($P < 0.0001$).

Superficial Corneal Epithelial Cell Surface Area

The average superficial cell area of the central epithelium enlarged with wear of all test contact lenses (Fig 3 and Table 8). Soft test lenses increased similarly: 3.3% (high oxygen transmissibility) and 4.1% (hyper oxygen transmissibility) at 4 weeks. Although there is no statistically significant difference between the two soft lenses (Table 8), the larger sample size contributes to the statistical significance of the increase for the hyper oxygen-transmissible test lens. By contrast, RGP test lens wearers demonstrated a highly significant increase of 10.5% ($P < 0.0001$). A strong inverse correlation between the central thickness of the corneal epithelium and the area of the surface epithelial cells was found in the RGP group ($r = 0.42$; $P < 0.0001$).

Corneal Epithelial Cell Exfoliation Rates

Figure 4 and Table 9 display the observed corneal epithelial surface cell exfoliation rates. All three test contact lenses signifi-

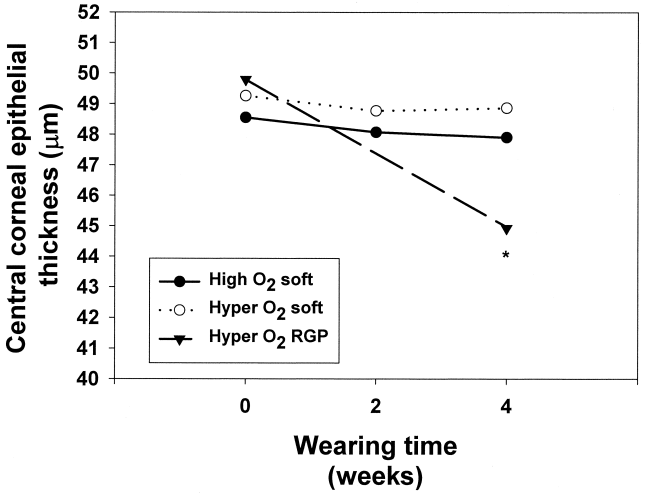

Figure 2. The central corneal epithelial thickness as measured with tandem scanning confocal microscopy. Table 7 contains the means, standard deviation, and the details of the statistical analysis (*statistically significant).

Table 7. Central Corneal Epithelial Thickness

	Baseline	2 Wks of Daily Lens Wear	4 Wks of Daily Lens Wear
High oxygen-transmissible soft lens*	48.55 ± 3.2 μm, n = 36	48.07 ± 3.04 μm, n = 36	47.9 ± 3.25 μm, n = 36
Hyper oxygen-transmissible soft lens†	49.26 ± 3.44 μm, n = 135	48.77 ± 3.48 μm, n = 131	48.86 ± 3.35 μm, n = 134
Hyper oxygen-transmissible RGP lens‡	49.79 ± 3.25 μm, n = 75	NA	44.92 ± 4.32 μm, n = 75

ANOVA = analysis of variance; NA = not available; RGP = rigid gas-permeable.

*No significant change over time: $P = 0.3072$ (one-way RM ANOVA).

†No significant change over time: $P = 0.1949$ (one-way RM ANOVA).

High oxygen-transmissible soft vs. hyper oxygen-transmissible soft: $P = 0.1352$, power 0.43 (two-way RM ANOVA); no significant difference.

‡Significant decrease: $P < 0.0001$ (paired *t*-test).

cantly downregulated the number of exfoliated corneal epithelial cells as collected with the irrigation chamber during DW ($P < 0.0001$), but there was no significant difference between the soft test lenses (two-way ANOVA, $P = 0.5573$).

Tear Lactate Dehydrogenase

Tear LDH significantly increased in all lens groups (Fig 5 and Table 10; $P < 0.0001$). No significant changes, however, were noted in tear LDH between DW of the two soft test lenses ($P = 0.7179$).

Discussion

Our previous animal[10] and clinical[11] studies have established that contact lens oxygen transmissibility determines susceptibility to binding of PA to exfoliated corneal epithelial cells during EW. Because short-term hypoxia alone will not increase PA binding, the presence of a contact lens is required.[17] The current study, however, is the first to demonstrate increased PA binding to exfoliated epithelial cells during DW of soft contact lenses and to establish a significant difference between conventional, high oxygen- and new, hyper oxygen-transmissible soft lens materials. It is of considerable clinical interest, however, that both soft materials induced significant increases in PA binding when compared with baseline (prelens) levels. In contrast, DW of the hyper oxygen-transmissible RGP lens did not induce a significant increase in PA binding compared with baseline levels.

The clinical significance of these results derives from the established observations that topical application of even high concentrations of PA to normal rabbit corneas fails to produce infection; likewise, in the absence of trauma, spontaneous PA infection has not been reported clinically in normal human eyes. Therefore, if the generally accepted hypothesis is valid that initial binding is required for in vivo infection to be initiated, any significant lens-mediated increase in PA binding over normal nonlens exposed levels will increase the relative risks for infection to occur. Conversely, contact lenses that can be worn in DW or EW that do not produce significant increases in PA binding should be associated with a decreased clinical risk of ulcerative infectious keratitis. Unfortunately, however, although we cannot yet translate the small but significant increases in lens-associated PA binding observed in DW into relative risk values, it is doubtful if such increased binding is a linear process. Most microbiologic effects on infectious processes are geometric, and small increases in PA binding may yield highly magnified risks.

Taken together, these new results offer the first quantitative, prospective, objective data that agree exactly with previously reported relative risk stratification for contact lens-related infectious ulcerative keratitis in humans. Multiple high-quality epidemiologic studies have firmly established that the relative risk rank order for lens-related infectious keratitis in humans is: extended conventional soft lens wear > daily conventional soft lens wear > daily RGP lens wear.[4,5,18] The results of PA binding studies reported here agree fully with these results and would predict that RGP lens wear should promote the lowest risk for ulcerative infectious keratitis. Most importantly, however, the results of this study support an important prospective, testable, epidemiologic prediction: as a new generation of hyper oxygen-transmissible soft contact lenses replace conventional lower oxygen-transmissible lenses in clinical practice, for the first time the incidence of infectious ulcerative keratitis should significantly decrease. Because half to two

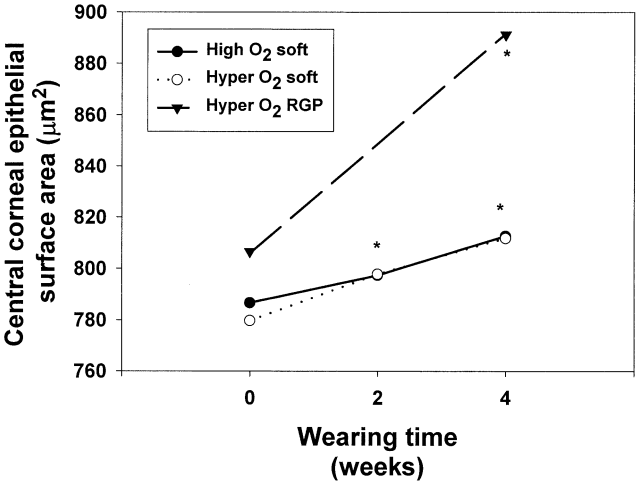

Figure 3. The surface area of the superficial corneal epithelial cells as observed with tandem scanning confocal microscopy. Table 8 contains the means, standard deviation, and the details of the statistical analysis (*statistically significant).

Table 8. Superficial Epithelial Cell Area of the Central Cornea

	Baseline	2 Wks of Daily Lens Wear	4 Wks of Daily Lens Wear
High oxygen-transmissible soft lens*	786.6 ± 80.2 μm^2, n = 36	797.35 ± 72.7 μm^2, n = 36	812.6 ± 77.3 μm^2, n = 36
Hyper oxygen-transmissible soft lens†	779.7 ± 90.4 μm^2, n = 135	797.8 ± 83.5 μm^2, n = 131	811.8 ± 83.4 $\mu m^{2‡}$, n = 134
Hyper oxygen-transmissible RGP lens§	806.5 ± 109.4 μm^2, n = 75	NA	891.2 ± 144.7 μm^2, n = 75

ANOVA = analysis of variance; NA = not available; RGP = rigid gas permeable.

*No significant change over time: $P = 0.3206$ (one-way RM ANOVA).

†Statistically significant, one-way repeated-measure ANOVA to baseline and Student-Newman-Keuls multiple comparisons test.

‡Significant increase over time: $P = 0.0011$ (one-way RM ANOVA).

High oxygen-transmissible soft vs. hyper oxygen-transmissible soft: $P = 0.1352$, power 0.05 (two-way RM ANOVA); no significant difference.

§Significant increase: $P < 0.0001$ (paired *t*-test).

thirds of all reported cases of infectious ulcerative keratitis occur in DW,[3] successful introduction of these new hyper oxygen-transmissible materials should increase the clinical safety of contact lens wear for millions of patients.

Consistent demonstration of a lens-related oxygen transmission effect on PA binding EW[9,11] suggests induction of specific epithelial, surface-binding receptors by lens-related hypoxia. Such receptors would mediate adherence to the corneal epithelial surface as a prerequisite for infection.[19,20] *Pseudomonas aeruginosa* adherence or binding to a cell appears to be mediated by lectin-like ligands on the bacterial cell surface and specific receptors on the target cell membrane, in the cell glycocalyx as well as in the cytoplasm.[19] These cell membrane receptors are lipase-sensitive glycoproteins[21] that can be identified and localized with high specificity using gold-label electron microscopy. Recently, Latkovic and Nilsson[22] used gold-labeled wheat germ agglutinin (WGA) to recognize sialic acid and N-acetylglucosamine receptors for PA on the rabbit corneal epithelium and to assess the effect of soft lens oxygen transmissibility on WGA cell membrane binding. After 24 hours of closed-eye test lens wear, significantly larger numbers of WGA receptors were exposed after soft lens wear than without a contact lens ($P < 0.001$), and significantly more lectin receptors were expressed after wear of a low oxygen-transmissible soft test lens than a conventional, high oxygen-transmissible soft lens material ($P < 0.01$). Additionally, soft lens wear with either test lens also appeared to thin or compress the corneal epithelial cell glycocalyx layer, reflected as an increase in WGA receptors.[22]

The striking finding in the current study that hyper oxygen-transmissible RGP lens DW did not increase PA binding significantly above baseline (no lens wear) values, whereas DW of both soft lenses elevated PA binding levels, albeit significantly less for the hyper oxygen-transmissible soft material, suggests, for the first time, both a lens-related oxygen effect as well as an important biologic difference between soft and rigid lens types in DW. Overall, these data are consistent with human epidemiologic risk by lens type for development of ulcerative infectious keratitis. Importantly, these human clinical results are also consistent with previous observations in the rabbit model for EW[10] that, at the same oxygen-transmission values, soft lenses also produced greater PA binding values than rigid lenses. Taken together, these results suggest that there may be additional factor(s) associated with the interactive fit of soft lenses with the epithelial surface that result in greater bacterial binding per unit area of lens-exposed cornea than produced by rigid lenses. Furthermore, rigid lenses used in this study were fit for higher degrees of myopia in older patients than for soft lens wearers (Table 2).

An obvious explanation for these results may be soft lens-mediated stagnation of the postlens tear film, which is only flushed 1% to 2% with blinking.[23] By contrast, the rigid lens covers a smaller area of the cornea, and depending on diameter, geometry, and movement dynamics, mediates postlens tear exchange more efficiently.[24] Because one critical component of the postlens tear film, the mucus layer, is capable of inhibiting PA binding to the underlying corneal epithelium, Fleiszig et al[25] have hypothesized that soft lens-associated stagnation of the of the postlens tear film may alter the ability of the mucus layer to protect the surface epithelium from PA adherence. To minimize further the risk of lens-related infectious keratitis and reduce PA binding in DW as observed in this study, it thus may still be important for clinical lens designers to enhance tear exchange under all soft contact lenses.

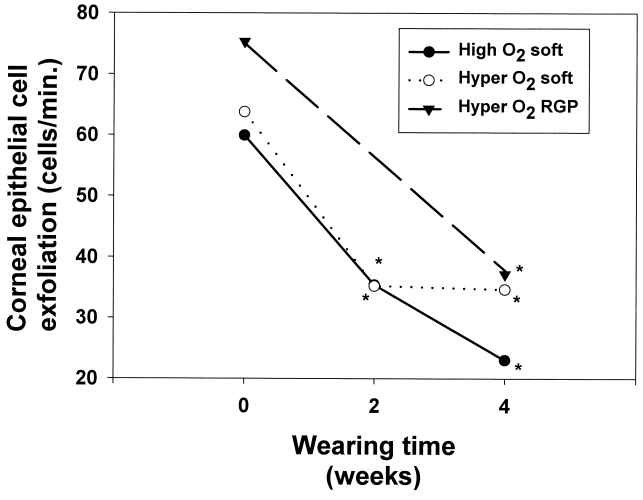

Figure 4. Superficial epithelial cell exfoliation as measured with the irrigation chamber. Table 9 contains the means, standard deviation, and the details of the statistical analysis (*statistically significant).

Table 9. Corneal Epithelial Surface Cell Exfoliation

	Baseline	2 Wks of Daily Lens Wear	4 Wks of Daily Lens Wear
High oxygen-transmissible soft lens*	59.94 ± 52.11 cells/min, n = 36	35.42 ± 27.5 cells/min†, n = 36	23.03 ± 17.74 cells/min†, n = 36
Hyper oxygen-transmissible soft lens‡	63.81 ± 65.01 cells/min, n = 135	34.35 ± 29.97 cells/min†, n = 131	34.67 ± 37.81 cells/min†, n = 134
Hyper oxygen-transmissible RGP lens§	75.32 ± 69.58 cells/min, n = 75	NA	37.2 ± 35.14 cells/min, n = 75

ANOVA = analysis of variance; NA = not available; RGP = rigid gas-permeable.

*Significant decrease over time: $P < 0.0001$ (one-way RM ANOVA).

†Statistically significant, one-way repeated-measure ANOVA to baseline and Student-Newman-Keuls multiple comparisons test.

‡Significant decrease over time: $P < 0.0001$ (one-way RM ANOVA).

High oxygen-transmissible soft vs. hyper oxygen-transmissible soft: $P = 0.5573$, power 0.59 (two-way RM ANOVA); no significant difference.

§Significant decrease: $P < 0.0001$ (paired *t*-test).

There are two limitations of the present study. The first is that lens-related PA binding has only been studied for 1 month, and long-term DW adaptive effects in PA binding levels may occur at later time intervals. The second study limitation is the concomitant use of potentially surface-active cleansing, wetting, and disinfecting solutions that may also affect PA binding.[16] Study conditions, however, were deliberately selected to mirror typical current clinical care for and use of DW lenses. Further studies are also necessary to explore these areas.

Thinning of the central corneal epithelium has been reported previously with EW of both rigid and soft contact lenses.[11,26] By contrast, results of this study do not demonstrate significant central corneal epithelial thinning with the soft contact lenses during DW, however, the rigid lens produced a 9.8% central thinning after 1 month of DW. The rapid central epithelial thinning produced by RGP wear may be explained by a dramatic loss of surface corneal epithelial cells, a significant decrease in basal epithelial cell proliferation or upward movement (or both), or by compression of surface corneal epithelial cell layers with possibly centrifugal redistribution of corneal epithelial cells (midperipheral corneal epithelial layer thickening; "orthokeratology" effect).[27] A dramatic loss of total epithelial surface layers has been observed in the rabbit model[28] with hypoxic EW of rigid polymethyl methacrylate materials (oxygen transmission almost 0). Such wear produces sloughing of surface cells, exposing underlying wing cells with significantly smaller cell surface areas.[28] By contrast, wear of higher oxygen RGP materials produced enlargement of surface corneal epithelial cells in both rabbits[10] and humans.[11] In this current clinical human study, hyper oxygen-transmissible lens DW produced not only larger surface corneal epithelial cells, but was also accompanied by decreased surface cell exfoliation. Taken together, these observations are incompatible with rigid lens-increased desquamation as the cause for observed central epithelial layer thinning.

A second explanation for central epithelial thinning associated with RGP DW is a greater overall decrease in central epithelial basal cell proliferation or upward movement to replace exfoliated surface cells (decreased replacement) than the observed reduction in surface cell shedding with larger surface cell residence times producing larger surface cell areas. In support of this possibility, Hamano and Hori[29] demonstrated for the first time that EW with soft lenses decreased the mitotic division rate in the corneal epithelium of the rabbit; Ren et al[30] also found a relationship between oxygen transmissibility of the RGP lens and basal epithelial proliferation in the rabbit cornea. A low oxygen-transmissible RGP lens inhibited central basal cell proliferation by 80% after 48 hours of EW, whereas the hyper oxygen-transmissible lens used in the current study produced only 20% inhibition in central basal cell proliferation.[30] Thus, epithelial thinning could partly be produced by an oxygen-related (hypoxic) decrease in central basal cell proliferation; unfortunately, there are no comparable reports as yet on the impact of DW on proliferation rates. The observation that no central epithelial thinning was detected with DW of both high and hyper oxygen-transmissible soft contact lenses, however, is most consistent with the hypothesis that the rapid central thinning of the epithelium produced by RGP DW is primarily caused by a mechanical rigid lens effect (epithelial redistribution). Such a mechanical effect is further consistent with the recent observations of Swarbrick et al[27] of central corneal epithelial thinning associated with concomitant midperipheral thick-

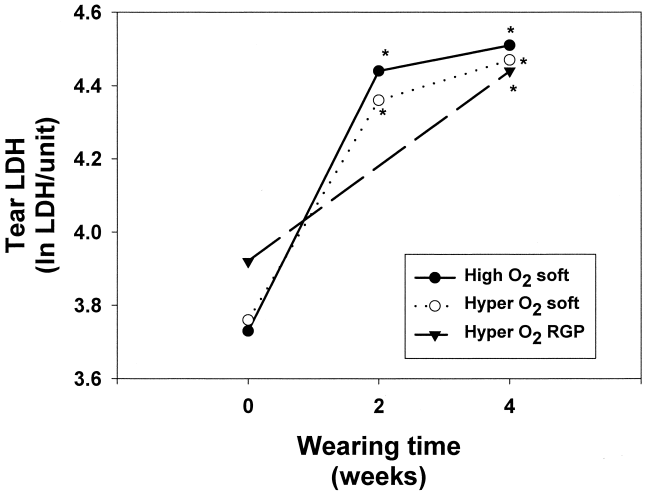

Figure 5. Tear lactate dehydrogenase activity. Table 10 contains the means, standard deviation, and details of the statistical analysis (*statistically significant).

Table 10. Tear Lactate Dehydrogenase Levels*

	Baseline	2 Wks of Daily Lens Wear	4 Wks of Daily Lens Wear
High oxygen-transmissible soft lens[†]	3.73 ± 0.56, n = 36	4.44 ± 0.8[‡], n = 36	4.51 ± 0.69[‡], n = 36
Hyper oxygen-transmissible soft lens[§]	3.76 ± 0.55, n = 135	4.36 ± 0.82[‡], n = 131	4.47 ± 0.79[‡], n = 134
Hyper oxygen-transmissible RGP lens[‖]	3.92 ± 0.69, n = 75	NA	4.44 ± 0.85, n = 74

ANOVA = analysis of variance; NA = not available; RGP = rigid gas permeable.

*Reported as ln LDH/unit (see Methods).

[†]Significant increase over time: $P < 0.0001$ (one-way RM ANOVA).

[‡]Statistically significant, one-way repeated-measure ANOVA to baseline and Student-Newman-Keuls multiple comparisons test.

[§]Significant increase over time: $P < 0.0001$ (one-way RM ANOVA).

High oxygen-transmissible soft vs. hyper oxygen-transmissible soft: $P = 0.7179$, power 0.07 (two-way RM ANOVA); no significant difference.

[‖]Significant increase: $P < 0.0001$ (paired *t*-test).

ening at the edge of the central flattened zone in EW RGP orthokeratologic trials.

Previous studies of surface cell exfoliation in human EW[11,17] have demonstrated that either hypoxia alone or rigid and soft lens wear decreases surface cell exfoliation rates, and in EW, there is an inverse correlation between lens oxygen transmissibility and observed cell shedding ($P = 0.0072$; $r = -0.265$).[11] O'Leary et al,[14] however, were the first to show that DW decreases surface cell shedding, and the current study confirms that the total number of exfoliated surface corneal epithelial cells collected in DW with the irrigation chamber decreases and further establishes that this DW effect appears independent of lens oxygen transmissibility.

The underlying mechanism controlling superficial cell exfoliation in the normal and contact lens-wearing eye, however, has not yet been identified. Perhaps the soft lens serves as a barrier protecting against the strong shear forces of the eyelids during blinking; however, RGP wear may be expected to add an extra element of mechanical surface rubbing. Nevertheless, in both DW and EW, all lens wear appears to decrease surface cell exfoliation rates.

Previous studies have shown for the first time that cell death precedes cell removal from the corneal surface and that shedding is an apoptotic process.[31] Similarly, under hypoxic conditions in an in vivo rabbit corneal perfusion model, the surface epithelial cell shedding rate is decreased without contact lens exposure.[32] Taken together with the observed decreased cell shedding seen in the current DW and past EW study,[11] these results suggest that when energy is reduced by hypoxia, the apoptotic desquamation process is downregulated and that the decreased cell shedding rate appears to be both oxygen dependent and lens dependent. The critical physiologic regulatory mechanisms underlying these observations remain to be established.

Numerous previous studies have reported an increase in area of corneal epithelial surface cells in EW.[11,33,34] Taken together with the finding of lens-induced decreases in superficial cell exfoliation rates, these results may indicate a stagnation of the corneal surface with prolonged cell residence times on the postlens epithelium. It is clear from the current study that contact lens DW also increases superficial cell area of the central corneal epithelium.

Tear LDH activity was measured in the current study as an indicator of contact lens-induced hypoxic or mechanical damage, or both, to postlens surface[35–37] epithelial cells; however, beyond a generalized increase associated with all test lens wear, no effect of lens oxygen transmission or type was observed on tear LDH levels in DW.

Overall, the DW studies reported here both confirm and significantly extend results from previous EW human clinical trials concerning the effects of contact lens wear on the corneal epithelial surface.[11] The five outcome measures studied may be important objective biologic predictors for postmarketing contact lens safety. In particular, if the hypothesis is valid that no lens-related ulcerative infectious keratitis can occur without initial lens-induced increased bacterial binding, introduction of a new generation of hyper oxygen-transmissible lens materials into widespread clinical practice may finally improve the safety of contact lens wear for millions of patients in both DW and EW.

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