CLINICAL AND EXPERIMENTAL
OPTOMETRY

RESEARCH PAPER

Effect of three months of soft contact lens wear on conjunctival cytology

Clin Exp Optom 2016; 99: 336–341 DOI:10.1111/cxo.12373

Kishor Sapkota*† MOptom FIACLE
Sandra Franco* PhD
Paula Sampaio† PhD
Madalena Lira* PhD

*Centre of Physics, University of Minho, Braga, Portugal
†Centre of Molecular and Environmental Biology (CBMA), University of Minho, Braga, Portugal
E-mail: kishorsapkota@gmail.com

Submitted: 20 May 2015
Revised: 30 June 2015
Accepted for publication: 24 July 2015

Background: The purpose of this study was to investigate the effect of three months of soft contact lens wear on conjunctival goblet cell density and epithelial cell morphology.
Methods: This was a longitudinal clinical trial. Conjunctival impression cytology was performed on the superior palpebral conjunctiva in fifty-four eyes of twenty-seven neophyte contact lens wearers before and after three months of contact lens wear. Goblet cell density was determined by optical microscopy and epithelial cell morphology was classified according to the Tseng classification. Changes in goblet cell density as well as epithelial cell grading were determined. The effects of lens material and wearing modality on cytological changes were also investigated.
Results: Goblet cell density reduced significantly by 85 ± 151 cells/mm^2 ($p < 0.001$) after three months of contact lens wear. Reduction in goblet cell density was associated with lens materials; it was higher in conventional hydrogel lenses in comparison to silicone-hydrogel lenses ($p = 0.008$). The highest reduction in goblet cell density was found with Nelfilcon A lens wear ($p = 0.002$) and the lowest with Comfilcon A lens wear ($p = 0.414$). There was no statistically significant difference in grading of epithelial metaplasia before and after three months of contact lens wear ($p = 0.075$). Age was not correlated with the reduction in goblet cell density ($r = -0.196$, $p = 0.160$) but it was associated with the change in epithelial cell morphology ($p = 0.036$).
Conclusion: Three months of soft contact lens wear statistically significantly reduced goblet cell density; however, no significant changes were found in the grading of epithelial metaplasia. Contact lenses with lower oxygen permeability, higher Young modulus and higher thickness highly affected the conjunctival cytology.

Key words: conjunctival cytology, dry eye, epithelial metaplasia, goblet cell density, soft contact lens

The popularity of contact lenses is increasing and it is estimated that 140 million people use this mode of refractive error correction in the world.[1] Contact lenses are also worn for therapeutic purposes in certain ocular surface diseases to relieve pain or improve prognosis[2] and for aesthetic purposes to enhance the appearance.[3] Recent studies have shown a possible use of contact lenses on continuous drug delivery into the eyes[4] and monitoring the tear glucose level.[5] Because contact lenses are worn directly on the ocular surface, they may lead to adverse effects on the morphologic, metabolic, cytological and immunologic states of the ocular surface[6] and these effects are significantly higher in extended lens wearers.[7] Dry eye is the most common complaint found in contact lens wearers.[8,9] Soft contact lens wear disrupts normal tear physiology by thinning and breaking up the tear film, interrupting tear film reformation and rupturing the lipid layer, increasing the evaporation rate.[10] Contact lens wear also alters the secretion of aqueous, lipid and mucin components of the tear film as well as changes in their biochemistry.[11] Many researchers found a decrease in tear mucin level with contact lens wear.[12,13] It may change the morphology of conjunctival epithelial cells and/or the number of goblet cells.[14,15]

Conjunctival cytological examination reveals early, subclinical, cytotoxic effects attributable to contact lens wear as well as to the preservatives and chelating agents in soft contact lens care systems.[16] Conjunctival impression cytology is a minimally invasive method to assess the ocular surface with no side effects.[17] Conjunctival impression cytology involves the collection of cells from the conjunctival surface with the help of a special type of filter paper by impression on the surface and examination of the superficial layer(s) of conjunctival epithelium with different types of staining. It can be done with or without anaesthesia.[18] It can be used in wide range of techniques from simple light microscopic examinations to polymerase chain reactions. With optical microscopy, epithelial cell morphology, goblet cell density and the presence or absence of any inflammatory cells can be examined.[19] Many recent studies conducted on conjunctival surface applying conjunctival impression cytology found increased levels of epithelial squamous metaplasia and loss of goblet cell density in pathological conditions of the eyes.[17,18]

The majority of the previous studies concluded that contact lens wear reduces the number of goblet cells and increases epithelial cell metaplasia;[20–22] however, most of these studies were cross-sectional and they involved comparison of cytological data in contact lens wearers and with non-contact lens wearers. None of them evaluated the effect of the lens materials on conjunctival cytology. Recently, many companies have introduced highly biocompatible lens materials, which might behave differently on the ocular surface. The present longitudinal clinical trial was designed to investigate the changes in

conjunctival cytology after three months of soft contact lens wear. Goblet cell density as well as the changes in epithelial cell morphology were determined. The effects of lens materials and wearing modality on the conjunctival cytology were also analysed. As earlier studies found that the effects of contact lens wear on conjunctival cytology start within a few weeks, occurring rapidly during the initial period of lens wear and reaching maximum at about three months,[21,23,24] this trial was conducted for a period of three months.

METHODS

Study design

This was a longitudinal contra-lateral clinical trial conducted at the University of Minho, Portugal. Ethical approval was obtained from Ethical Committee of University of Minho. Each subject signed a consent form after the explanation of study procedures and its possible consequences and the tenets of Declaration of Helsinki were followed.

Subjects

Twenty-seven myopic subjects with normal ocular/systemic health, no previous history of contact lens wear or ocular surgery and aged between 18 and 35 years were included in the study. Subjects with visual acuity less than 6/6 in one eye, refractive astigmatism higher than 0.75 D and pregnant women were excluded from the study.

Procedure

During the baseline visit, conjunctival impression cytology was performed on the superior bulbar conjunctiva on both eyes of each subject.[18] Nitrocellulose Millipore[17] MF-Membrane filter (MILLIPORE, Ireland) with pore size 0.45 µm was used without application of topical anaesthesia.[25,26] Briefly, a semi-circular piece of filter paper with diameter 13 mm, touched the superior bulbar conjunctiva for a few seconds and was removed in a gentle peeling motion. The paper was then stained with PAS, haemotoxylin and eosin[27] and the slides were observed by an optical microscope with total magnification 100× and 400×. Goblet cells were counted in the higher power field (with total magnification of 400×) and goblet cell density was calculated as the number of cells per square millimetre. Morphological changes in epithelial cells including shape, size and characteristics of the nucleus were noted and graded according to the Tseng classification (Table 1).[28] This procedure was repeated in three random fields of area and the average was used in analysis.

Lenses and solutions

Subjects were fitted with a daily disposable lens (Nelfilcon A or Stenofilcon A) on one eye and a monthly disposable (Lotrafilcon B or Comifilcon A) on the other eye. Contact lens details are presented in Table 2. Post-lens fitting evaluation was performed and refitting with another type of contact lens among the study lenses was done where fitting was unacceptable. Subjects were well trained for contact lens usage, care and maintenance. In the dispensing time, contact lenses, lens case and solution were provided for each subject for the coming month. Moreover, a paper with information about types and lens care methods was provided, where they should indicate the number of wearing hours every day. This helped participants to wear the lenses correctly in the recommended eye. Subjects were informed to wear lenses at least five days in a week and a minimum of eight hours per day, except during the first week, when the number of wearing hours per day was flexible. There was no limit on the number of days or hours when the lenses could be worn; however, they were not allowed to wear lenses during sleep, swimming or bathing. At the end of every month, participants should visit the office to monitor the adequacy of lens and solution use and to provide new lenses and lens care products for the next month. For the first and second months, all the subjects used OPTI-FREE puremoist solution (Polyquad 0.001% and Aldox 0.0006%, Alcon Laboratories, Texas, USA) while for the third month, 16 subjects used OPTI-FREE puremoist and 11 subjects used AOSEPT PLUS (Hydrogen peroxide 3%, Alcon Laboratories, Texas, USA). These two types of solution were provided for each subject, since another objective of the study was to investigate the effect of different types of solutions in contact lens wearers' comfort. There was no significant difference in reduction of goblet cell density and epithelial cell metaplasia between the eyes with these solutions ($p > 0.05$). Subjects were advised to contact the researcher at any time if they felt adverse events with the necessary management. Conjunctival impression cytology was repeated after three months of contact lens wear.

Statistical analysis

Data were analysed with Statistical Package for Social Science (SPSS 22, IBM Corp, Armonk, New York, USA). Descriptive statistics were expressed in mean ± standard deviation (SD). Kolmogorov–Smirnov test was performed to examine the normality of the variables. Parametric tests were applied for normally distributed variables, while non-parametric tests were applied for others. One-way analysis of variance with *post hoc* testing was applied to compare the reduction in goblet cell density between different lens materials. Spearman's rho was used to test the correlation of changes in goblet cell density with the age of the subjects. Chi-square test was applied to examine the proportion in changes in epithelial cell metaplasia. Statistically significance was considered when p values were less than 0.05.

RESULTS

Fifty-four eyes of 27 subjects (63.0 per cent female) were included in this longitudinal clinical trial. The mean age of the subjects was 23.5 ± 3.0 years (range 20 to 33 years). Contact lens were worn as follows: Lotrafilcon B (n = 12), Nelfilcon A (n = 12), Comfilcon A (n = 15), Stenofilcon A (n = 15) and the mean power of the lenses used was -1.98 ± 1.60 D (range -0.50 to -5.50 D, 95% CI, -1.60 to -2.45 D). All subjects completed the study without any significant adverse event except for one case that showed a contact lens-induced peripheral ulcer in the right eye wearing a Lotrafilcon B lens. This complication was resolved after 10 days of lens wear discontinuation without any additional treatment and this subject continued the study.

Figure 1 shows the representative images of the cytology with 100× total magnification. Goblet cell density was significantly reduced by 85 ± 151 cells per mm^2 ($p < 0.001$) (Figure 2). Figure 3 shows the Bland–Altman graph showing the change in goblet cell density with the initial goblet cell density. Reduction in goblet cell density was strongly correlated with the baseline goblet cell density ($r = 0.846$, $p < 0.001$).

There was no significant difference in the baseline goblet cell density among the eyes with different lenses ($p = 0.069$). As shown in Table 3, the magnitude of the reduction in goblet cell density was significantly associated with contact lens materials ($p = 0.034$); the reduction was the greatest with Nelfilcon A lens wear (166 ± 147 cells/mm^2), while it was least with Comfilcon A lens wear (32 ± 137

Classification	Goblet cells	Epithelial cells	Nucleus cytoplasm ratio
Grade 0	Moderate density	Uniform size/form	1:1
Grade 1	Decreased density	Mild enlargement	1:2-1:3
Grade 2	Absent	Moderate enlargement, flattened (squamoid)	1:4
Grade 3	Absent	Markedly squamoid	1:6
Grade 4	Absent	Markedly squamoid, large	1:8
Grade 5	Absent	Shrunken cytoplasm	Nucleus may be absent

Table 1. Conjunctival epithelial cell metaplasia classification

	Lotrafilcon B	Nelfilcon A	Comfilcon A	Stenofilcon A
Company	Alcon	Alcon	Cooper Vision	Cooper Vision
Brand name	AirOptix Aqua	AquaComfort	Biofinity	MyDay
Water content (%)	34	69	48	54
Thickness (mm)	0.08	0.10	0.08	0.07
Base curve /diameter (mm)	8.6/14.2	8.7/14	8.7/14.5	8.4/14.2
Oxygen Permeability (barrer)	110	26	128	80
Modulus (MPa)	1.2	0.89	0.75	0.4
Transmissibility (barrer/cm)	137.5	26	160	100

Table 2. Characteristics of the lenses used in the study

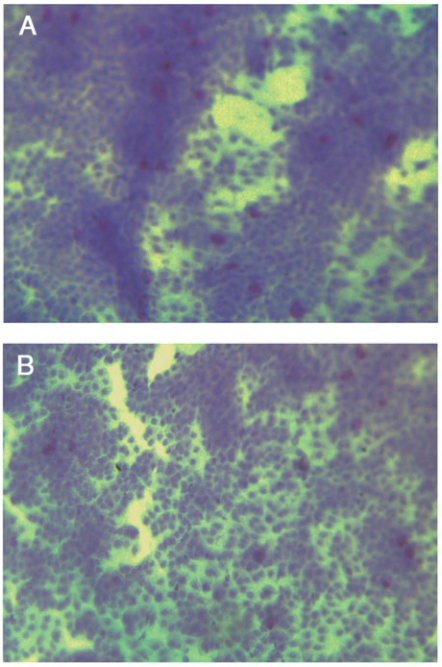

Figure 1. Representative images of the conjunctival impression cytology (100× total magnification) of subject: A. Before starting to wear contact lenses, B. After three months of contact lens wear.

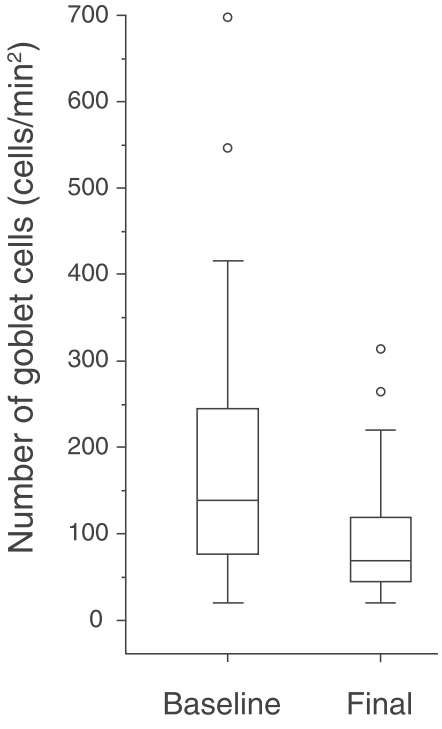

Figure 2. Goblet cell density before (baseline) and after contact lens wear (final) (n = 54)

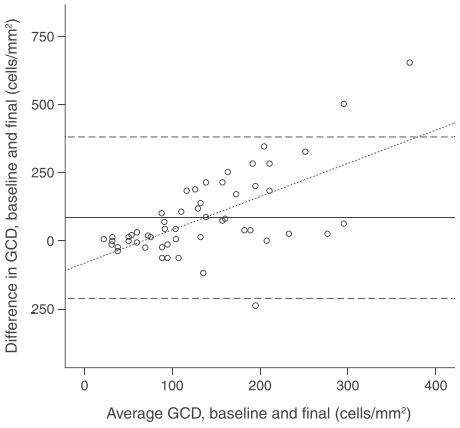

Figure 3. Bland–Altman plotting showing the relation between changes in goblet cell density (GCD) with the average goblet cell density (n = 54)

cells per mm^2). Multiple comparisons showed that Nelfilcon A lens wear had a higher reduction than Comfilcon A and Stenofilcon A lens wear ($p < 0.05$). There was no significant difference between Nelfilcon A and Lotrafilcon B, between Lotrafilcon B and Comfilcon A or Stenofilcon A and between Comfilcon A and Stenofilcon A lens wear ($p > 0.05$). The reduction of goblet cell density was not associated with the wearing modality of the lenses; it was similar with daily disposable and monthly disposable lenses ($p = 0.332$). There was no significant correlation between the decrease in goblet cell density and age ($p = 0.160$) of the subjects.

During that period, no significant change in epithelial cell metaplasia was observed ($p = 0.075$); however, as shown in Table 4, epithelial cell metaplasia grading increased in 74 per cent of the eyes by at least one grade due to contact lens wear. The change in epithelial cell morphology was correlated with the age of the subjects (Spearman's rho = 0.286, $p = 0.036$). It was not associated with wearing

	Goblet cell density (cells/mm^2)			
	Baseline	Final	Reduction	p values
Lotrafilcon B	239 ± 164	132 ± 79	107 ± 187	0.018
Nelfilcon A	254 ± 122	89 ± 61	166 ± 147	0.002
Comfilcon A	219 ± 106	188 ± 76	32 ± 137	0.414
Stenofilcon A	219 ± 108	164 ± 30	53 ± 113	0.362
Total (n = 54)	233 ± 137	148 ± 67	85 ± 151	<0.001

Table 3. Reduction in goblet cell density with different types of study contact lenses

		Final			Total
		Grade 0	Grade 1	Grade 2	
Baseline	Grade 0	8 (19.0%)	27 (64.3%)	7 (16.7%)	42 (100.0%)
	Grade 1	0 (0.0%)	5 (45.5%)	6 (54.5%)	11 (100.0%)
	Grade 2	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (100.0%)
Total		8 (14.8%)	33 (61.1%)	13 (24.1%)	54 (100.0%)

Table 4. Changes in conjunctival epithelial cell morphology after three months of soft contact lens wear

modality of the lenses (p = 0.850). With silicone lens wear, 69.0 per cent of the tested eyes changed at least one grade while for non-silicone hydrogel lens wear the value found was 91.7 per cent; however, changes in epithelial cell metaplasia were not significantly related to the lens material (p = 0.158).

DISCUSSION

In this study, the effects of three months of soft contact lens wear on conjunctival cytology were evaluated. Subjects were neophyte contact lens wearers and different types of contact lens (one conventional hydrogel – Nelfilcon A, one silicone hydrogel lens with surface plasma treatment - Lotrafilcon B, one silicone lens without surface treatment – Comfilcon A and one new contact lens recently available in the market with smart silicone chemistry– Stenofilcon A) were used. Two of the lenses (Nelfilcon A, Stenofilcon A) are daily disposable lenses, while the other two (Lotrafilcon B, Comfilcon A) are monthly disposable lenses. Lenses were worn in a contra-lateral manner such that one eye was fitted with a daily disposable and the other with a monthly disposable lens.

In this study, we found a significant reduction in goblet cell density after three months of contact lens wear. This reduction in goblet cell density may explain the origins of contact lens-induced dry eye.[29] As depicted in Figure 2, the initial goblet cell densities were highly varied and so were the changes in goblet cell density. Consistent with our findings, Doughty[20] using meta-analysis, found a high variation in goblet cell density ranging between 10.5 ± 1.1 and 152.85 ± 29 cells per mm^2 in soft contact lens wearers. Eyes with higher baseline goblet cell density were found to suffer higher changes. The effect of contact lens wear on conjunctival goblet cell density may be due to the physical and mechanical effects of the lens. Each blink induces contact lens movement, as well as some friction of the upper lid on the superior bulbar conjunctiva, the part which was used for conjunctival impression cytology. Our results are consistent with studies that report a reduction in goblet cell density induced by soft contact lens wear.[20] Simon et al[22] found a significant decrease in goblet cell density following six-months of soft contact lens wear, while Knop and Brewitt[21] found a decrease in goblet cell density after three to six month of soft contact lens wear and the degradation of conjunctival cytology started within the first few weeks. In another comparative study of conjunctival cytology in contact lens wearing subjects and non-contact lens wearing controls, Cakmak et al[30] found significant degradation of epithelial cell morphology and goblet cell density. Contrary to our findings, some studies found an increase in goblet cell density after soft contact lens wear. Lievens, Connor and Murphy[31] found an increase in goblet cell density following six months wear of Acuvue 2 and PureVision contact lenses. Connor et al[32] also found a nearly two-fold increase in goblet cell density after six months of soft contact lens wear. These authors speculated that this increase in goblet cell density may be due to an adaptive response of the ocular surface.

Hirji, Scott and Sabel[16] suggested that lens care solution plays a role in the changes in conjunctival cytology associated with contact lens wear; however, in the current study, the reduction in goblet cell density was not associated with the wearing modality of the lenses (daily disposable versus monthly disposable). This suggests that there is no link between reduction in goblet cell density and lens care solution.

As shown in Table 3, the number of goblet cells changes differently when different lens materials are used: the reduction was maximal for the Nelfilcon A lens wearers (p = 0.002), followed by Lotrafilcon B (0.018) but there was no significant reduction in Comfilcon A (p = 0.414) and Stenofilcon A lens wearers (p = 0.362). The Nelfilcon A lens, which was the non-silicone hydrogel lens used in the study, was the thickest lens with the highest water content among the contact lenses studied. This material has the lowest oxygen permeability and a high Young's modulus (only less than Lotrafilcon B). On the other hand, Comfilcon A lens has high oxygen transmissibility but low Young's modulus. So low oxygen permeability, high Young's modulus and high centre thickness might be important factors that can affect conjunctival goblet cells. Although, Lotrafilcon B has high oxygen permeability, it significantly reduced the goblet cell density. It is the only silicone lens used in this study having plasma treatment on its surface. Besides oxygen permeability, surface treatment may also affect goblet cell density. On multiple comparisons, only Nelfilcon A lens wear showed a difference in goblet cell density reduction compared to Comfilcon A and Stenofilcon A lens wear. Lievens, Connor and Murphy[31] found that non-silicone hydrogel lenses create more irritation than silicone lenses. A higher reduction in goblet cell density by Nelfilcon A lens wear may be due to the higher irritation on the ocular surface. It is interesting that the lenses, which were manufactured by the same company had

similar effects on conjunctival cytology. This highlights the effect of lens characteristics and designs on conjunctival goblet cells. Contrary to the present findings, Simon et al[22] did not show a statistical difference in cytological changes with different lens materials. Similarly, Lievens, Connor and Murphy[31] did not find a difference in goblet cell density in Acuvue 2 or PureVision contact lens wearers. The reduction in goblet cell density associated with contact lens material in the present study might be due to the different characteristics of the lens used (Table 2).

There was no significant correlation between the decrease in goblet cell density and the age of the subjects. Zhu et al[33] concluded that goblet cell density does not change with age but the function of the goblet cells decreases with age. This may be the case but testing of the function of the goblet cells was out of the scope of this study. Moreover, age-related variation may not take place over the narrow range of age featured in the current study.

There was no significant change in conjunctival epithelial cell metaplasia with contact lens wear; however, as shown in Table 3, it was increased by few grades in the majority of the eyes. Before wearing contact lenses, 'grade 0' it was observed in 78 per cent of the eyes but it was found in only 15 per cent of the eyes after contact lens wear. Epithelial metaplasia in contact lens wearers is thought to be due to the mechanical influence of the lens on the ocular surface.[34] Increase in epithelial cell metaplasia may be one of the causes of contact lens-induced dry eye because increased severity of dry eye and conjunctival epithelial cell metaplasia are associated with one other.[35] Grade change did not reach a significant level probably due to the fact that the change was only a single grade in the majority of the eyes (61per cent). Epithelial metaplasia was not associated with lens wearing modality. Silicone lens wear was less associated with changes in epithelial metaplasia by comparison with non-silicone hydrogel lenses, which might be due to the higher oxygen permeability of silicone lenses. Both the aforementioned lens materials used in our study had similar modulus of rigidity and so the mechanical influence of the lens on the ocular surface is thought to be similar.[34]

Previous studies have found that aging does not alter the epithelial cell morphology;[33] however, a significant correlation of the change in epithelial metaplasia with age was observed in the current study, indicating that adult lens wearers are more susceptible to epithelial metaplasia. An already compromised ocular surface due to age may be more susceptible to damage by contact lens wear.

Contrary to the findings of the current study, several studies have shown significant differences in conjunctival epithelial metaplasia in contact lens wearers and non-contact lens wearers. Tomatir, Erda and Gürlü found significant differences in conjunctival impression cytology with contact lens wear for a period of 6.9 ± 2.6 months (range four to 12 months);[36] however, they used different lens materials: soft hydroxyethylmethacrylate (vinyl pyrrolidone copolymer) lenses in 40 eyes, polymacon lenses in 70 eyes and rigid gas-permeable (RGP) lenses in 40 eyes. Similarly, Simon et al[22] found significant changes in epithelial squamous metaplasia after six-month soft contact lens wear. They also found that the magnitude of changes correlated with the duration of lens wear and was significantly higher in symptomatic wearers compared to non-symptomatic wearers; however in that study, the number of soft contact lens wearers was small as only 11 subjects completed the study. Moreover, the significant change in that study may be due to the longer duration (six months) in comparison to the present study. Doughty and Naase[37] found significant differences in epithelial cell size in non-contact lens wearers and successful daily contact lens wearers (with duration of four to nine years). Munshi, Sathe and Ganar[24] found a significant increase on epithelial squamous metaplasia grade in subjects, who wear soft or RGP lenses in comparison to the control group[24] but they did not find any association of epithelial squamous metaplasia with the duration of contact lens wear. In a recent cross-sectional study conducted in European women, Doughty[38] found significantly higher epithelial squamous metaplasia in soft contact lens wearers than in non-lens wearers. In this same study,[38] in addition to cell enlargement, a larger nucleus was observed in contact lens wearers.

In the current study, a highly significant reduction in goblet cell density was observed after three months of soft contact lens wear but the change in conjunctival epithelial cell morphology was not significant. This suggests that contact lens wear probably affects the number of goblet cells before there are observable changes to epithelial cell morphology.

Consistent with the study by Simon et al,[22] we did not find any snakelike chromatin due to soft contact lens wear. Moreover, we did not encounter any neutrophils or lymphocytes in the sample, contrary to a study conducted by Hirji, Scott and Sable.[16] This may be due to the short duration of contact lens wear by the subjects in this study.

There are some limitations in this study. We used a small sampling area (high power field of view with 400 times total magnification) to count the goblet cells and it is likely to make some error because of the variability in goblet cells;[39] however, observations were made in three random areas and the average was used for analysis to minimise this error.

From this study, it can be concluded that soft contact lens wear reduces goblet cell density, which is dependent upon the lens materials but may not significantly change the conjunctival epithelial cell morphology. Oxygen permeability, material strength (Young's modulus), surface treatment and thickness of the contact lenses are important factors that can induce conjunctival cytological changes. Conjunctival impression cytology may help to detect early changes on contact lens wearers; however, as suggested by Munshi, Sathe and Ganar[24] cytological changes, which occur during early periods of contact lens wear may be due to the adaptive changes of ocular surface. To confirm the findings of this study, a long term, longitudinal study may be helpful.

ACKNOWLEDGEMENTS

A preliminary report of this study was presented in American Academy of Optometry Meeting, Denver 2014.

The authors would like to thank Alcon Vision Care and CooperVision for providing contact lenses used in this study. We would like to thank Professor Brendan Barrett for his help in the proof-reading of this manuscript.

REFERENCES

1. Stapleton F, Keay L, Jalbert I, Cole N. The epidemiology of contact lens related infiltrates. *Optom Vis Sci* 2007; 84: 257–272.
2. Ambroziak AM, Szaflik JP, Szaflik J. Therapeutic use of a silicone hydrogel contact lens in selected clinical cases. *Eye Contact Lens* 2004; 30: 63–67.
3. Zhang L, Chan O, Roy L, Barr JT. A meta-analysis of studies on cosmetically tinted soft contact lenses. *Clin Ophthalmol* 2013; 7: 2037–2042.
4. Garhwal R, Shady SF, Ellis EJ, Ellis JY, Leahy CD, McCarthy SP et al. Sustained ocular delivery of ciprofloxacin using nanospheres and conventional contact lens materials. *Invest Ophthalml Vis Sci* 2012; 53: 1341–1352.
5. Yao H, Shum AJ, Cowan M, Lähdesmäki I, Parviz BA. A contact lens with embedded sensor for monitoring tear glucose level. *Biosens Bioelectron* 2011; 26: 3290–3296.
6. Stapleton F, Stretton S, Papas E, Skotnitsky C, Sweeney DF. silicone hydrogel contact lenses and the ocular surface. *Ocul Surf* 2006; 4: 24–43.

7. Efron N, Morgan PB, Hill EA, Raynor MK, Tullo AB. Incidence and morbidity of hospital-presenting corneal infiltrative events associated with contact lens wear. *Clin Exp Optom* 2005; 88: 232–239.
8. Begley CG, Chalmers RL, Mitchell GL, Nichols KK, Caffrey B, Simpson T et al. Characterization of ocular surface symptoms from optometric practices in North America. *Cornea* 2001; 20: 610–618.
9. Kastelan S, Lukenda A, Salopek-Rabatić J, Pavan J, Gotovac M. Dry eye symptoms and signs in long-term contact lens wearers. *Coll Antropol* 2013; 37 (Suppl 1): 199–203.
10. Thai LC, Tomlinson A, Doane MG. Effect of contact lens materials on tear physiology. *Optom Vis Sci* 2004; 81: 194–204.
11. Mann A, Tighe B. Contact lens interactions with the tear film. *Exp Eye Res* 2013; 117: 88–98.
12. Yasueda S, Yamakawa K, Nakanishi Y, Kinoshita M, Kakehi K. Decreased mucin concentrations in tear fluids of contact lens wearers. *J Pharm Biomed Anal* 2005; 39: 187–195.
13. Pisella PJ, Malet F, Lejeune S, Brignole F, Debbasch C, Bara J et al. Ocular surface changes induced by contact lens wear. *Cornea* 2001; 20: 820–285.
14. Greiner J V, Covington HI, Korb DR, Allansmith MR. Conjunctiva in asymptomatic contact lens wearers. *Am J Ophthalmol* 1978; 86: 403–413.
15. Greiner J V, Allansmith MR. Effect of contact lens wear on the conjunctival mucous system. *Ophthalmology* 1981; 88: 821–832.
16. Hirji N, Scott J, Sabel A. Conjunctival cytology in hard and soft contact lens wear. *Ophthalmic Physiol Opt* 1985; 5: 333–335.
17. Calonge M, Diebold Y, Enrıquez de Salamanca A, García-Vázquez C, Corrales RM, Herreras JM. Impression cytology of the ocular surfac: a review. *Exp Eye Res* 2004; 78: 457–472.
18. Egbert PR, Lauber S MD. A simple conjunctival biopsy. *Am J Ophthalmol* 1977; 84: 798–801.
19. Lopin E, Deveney T, Asbell PA. Impression cytology: recent advances and applications in dry eye disease. *Ocul Surf* 2009; 7: 93–110.
20. Doughty MJ. Contact lens wear and the goblet cells of the human conjunctiva—A review. *Cont Lens Anterior Eye* 2011; 34: 157–163.
21. Knop E, Brewitt H. Induction of conjunctival epithelial alterations by contact lens wearing. *Ger J Ophthalmol* 1992; 1: 125–134.
22. Simon P, Jaison SG, Chopra SK, Jacob S. conjunctival impression cytology in contact lens wearers. *Indian J Ophthalmol* 2002; 50: 301–306.
23. Doughty MJ. Contact lens wear and the development of squamous metaplasia of the surface cells of the conjunctiva. *Eye Contact Lens* 2011; 37: 274–281.
24. Munshi MM, Sathe V, Ganar A. Conjunctival impression cytology in contact lens wearers. *Cytopathology* 2001; 12: 314–320.
25. Blades K, Doughty MJ, Patel S. Pilot study on the use of impression cytology specimens for quantitative assessment of the surface area of bulbar conjunctival cells. *Optom Vis Sci* 1998; 75: 591–599.
26. Thatcher RW, Darougar SJB. Conjunctival impression cytology. *Arch Ophthalmol* 1977; 95: 678–681.
27. Rivas L, Oroza MA, Perez-Esteban A, Murube-del-Castillo J. Topographical distribution of ocular surface cells by the use of impression cytology. *Acta Ophthalmol* 1991; 69: 371–376.
28. Tseng S. Staging of conjunctival squamous metaplasia by impression cytology. *Ophthalmology* 1985; 92: 728–733.
29. Shimazaki-Den S, Dogru M, Higa K, Shimazaki J. Symptoms, visual function and mucin expression of eyes with tear film instability. *Cornea* 2013; 32: 1211–1218.
30. Cakmak SS, Unlü MK, Karaca C, Nergiz Y, Ipek S. Effects of soft contact lenses on conjunctival surface. *Eye Contact Lens* 2003; 29: 230–233.
31. Lievens CW, Connor CG, Murphy H. Comparing goblet cell densities in patients wearing disposable hydrogel contact lenses versus silicone hydrogel contact lenses in an extended-wear modality. *Eye Contact Lens* 2003; 29: 241–244.
32. Connor CG, Campbell JB, Steel SA, Burke JH. The effects of daily wear contact lenses on goblet cell density. *J Am Optom Assoc* 1994; 65: 792–794.
33. Zhu W, Hong J, Zheng T, Le Q, Xu J, Sun X. Age-related changes of human conjunctiva on in vivo confocal microscopy. *Br J Ophthalmol* 2010; 94: 1448–1453.
34. Albietz J. Conjunctival histologic findings of dry eye and non-dry eye contact lens wearing subjects. *CLAO J* 2001; 27: 35–40.
35. Murube J, Rivas L. Impression cytology on conjunctiva and cornea in dry eye patients establishes a correlation between squamous metaplasia and dry eye clinical severity. *Eur J Ophthalmol* 2003; 13: 115–127.
36. Tomatir DK, Erda N, Gürlü VP. Effects of different contact lens materials and contact lens-wearing periods on conjunctival cytology in asymptomatic contact lens wearers. *Eye Contact Lens* 2008; 34: 166–168.
37. Doughty MJ, Naase T. Nucleus and cell size changes in human bulbar conjunctival cells after soft contact lens wear, as assessed by impression cytology. *Cont Lens Anterior Eye* 2008; 31: 131–140.
38. Doughty MJ. objective assessment of squamous metaplasia of conjunctival epithelial cells as associated with soft contact lens wear versus non-lens wearers. *Cornea* 2014; 33: 1095–1102.
39. Doughty MJ. Sampling area selection for the assessment of goblet cell density from conjunctival impression cytology specimens. *Eye Contact Lens* 2012; 38: 122–129.