Association between conjunctival goblet cells and corneal resident dendritic cell density changes in new contact lens wearers

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Results:

Despite their many advantages, contact lenses can alter the anterior ocular surface, leading to symptoms of dryness and discomfort. Part of the aetiology of these symptoms is attributed to the osmotic stress caused by the lens on the ocular surface, leading to an inflammatory (adaptive) response via activating signalling pathways in a variety of cell types located on the ocular surface epithelium. Resident corneal dendritic and conjunctival goblet cells play an important role in mediating this response by not only unleashing complex biochemical reactions and signalling pathways, but also changing cell morphology and density. Density changes of epithelial immune cells have been associated with contact lens wear. The authors have previously demonstrated that contact lens wear symptomatology is linked with increased dendritic cell density in the lid wiper, conjunctiva and central cornea and decreased conjunctival goblet cell density. Goblet cells are known to produce and secrete the immunoregulatory molecule transforming growth factor-β that has been reported to suppress activation of dendritic cells on the ocular surface. Resident dendritic cell density increases in the central cornea due to the epithelial cell secretion factors released into the tears under inflammatory response to osmotic stress. Factors such as interleukins and tumour necrosis are known to activate immature resident corneal dendritic cells and recruit them into the central cornea through upregulation of chemokine receptors and major histocompatibility complex II antigen. Based on these studies, it would be reasonable to assume that conjunctival goblet cells could indirectly reduce dendritic cell activation and migration to the central cornea in lens wear, facilitating lens comfort and adaptation.

The association between conjunctival goblet and corneal resident dendritic cell densities in contact lens wearers has not been investigated concurrently in a controlled, prospective evaluation. Understanding this relationship may provide new insights into cellular behaviour during contact lens wear. Therefore, to explore the relationship between conjunctival goblet and resident corneal dendritic cells in new contact lens wearers, existing data from a prospective six-month observational study were analysed.

Methods

This study was conducted in accordance with the Declaration of Helsinki, approved by Queensland University of Technology.
The prospective, randomised, controlled six-month observational study has been described elsewhere. Non-contact lens wearers were recruited from the general population in Brisbane, Australia through the QUT community via recruitment email and were assessed for the presence of any corneal compromise. Participants were excluded if they had a history of contact lens wear in the previous six months, recent history of ocular inflammation, ocular trauma or surgery, long-term topical ocular medication or any systemic disease/condition that may affect the cornea or conjunctiva as well as dry eye symptoms (scores of ≥ 7 points) on the five-item dry eye questionnaire (DEQ-5) and corneal staining (> grade 2) on the Efron grading scales. Sample size calculation (G*Power 3.1) to detect a difference of 620 goblet cells/mm² at a significance level of p ≤ 0.05, assuming a measurement standard deviation of ±154 goblet cells/mm² calculated that a minimum sample size of 23 participants was required per group, allowing for 20 per cent attrition. This analysis gave 90 per cent power, with a type I error of five per cent to detect a difference in goblet cell and dendritic cell and densities between the two groups (wearers and non-lens wearers) after six months of lens wear. The cohort of 60 participants was assigned to the contact lens-wearing group and 23 non-lens wearers were assigned to the control group. The contact lens-wearing group was fitted with the Biomedics 1-day Extra daily disposable soft contact lens (CooperVision, Scottville, NY, USA). Refractive limitations of subjects were myopia > 7.00 D, hyperopia > 2.00 D and astigmatism > 1.50 D. At one week, the contact lens-wearing group was assigned to the symptomatic group according to the following criteria: eight-item Contact Lens Dry Eye Questionnaire (CLDEQ-8) ≥ 17 points; and either a non-invasive break-up time ≤ 10 seconds, phenol red thread ≤ 10 mm or corneal staining > grade 2.

**Corneal confocal microscopy (CCM)**

The methods used during the present study to quantify conjunctival goblet and corneal dendritic cells using CCM have been reported previously and are provided briefly here. CCM was conducted using a Heidelberg Retina Tomograph (HRT 3) with Rostock Corneal Module (Heidelberg Engineering GmbH, Heidelberg, Germany). While fixation was maintained on a near target attached to the CCM instrument, set on section capture, multiple non-overlapping images were captured from the nasal bulbar conjunctiva, approximately 2–4 mm from the limbus and at approximately 15 microns depth and at the central cornea at the level of Bowman’s layer (~50 μm depth) (Figure 1). Dendritic cells with and without visible dendrites were observed and counted at the level of the basal epithelium and sub-basal nerve plexus of the cornea. Goblet cells were identified as cell size of 25 to 30 μm in diameter, hyperreflective, bigger than surrounding cells, round to oval in shape, and sometimes with a visible nucleus. Goblet cells at the nasal bulbar conjunctiva were scanned while moving the applanating lens at nine different locations (approximating a 3 × 3 grid) and at approximately three different depths. Eleven images from the conjunctival epithelium and eight images of the central cornea were selected and analysed in a randomised, masked fashion using a semi-automated tool of the instrument to quantify cell densities. Goblet and dendritic cell density changes over time were reported as cells/mm².

**Statistical analysis**

The Shapiro-Wilk test was conducted to determine normality of the data (α = 0.05). An absolute change in cell density of both cell types from baseline to six months was calculated for each participant. Differences between groups was then analysed using analysis of variance. Since absolute values of cell density reflect cross-sectional associations, Pearson’s correlation was used to determine associations between the total change of cell densities after six months of lens wear. In order to predict the number of resident corneal dendritic cells that migrate to the central cornea (cells/mm²) based on the conjunctival goblet cell loss, after six months of lens wear, linear regression analysis was conducted. Statistical analysis was conducted with SPSS software (SPSS v26.0; IBM, Armonk, NY, USA). Statistical significance level was set at p < 0.05.

**Results**

Of the 83 enrolled individuals, 14 discontinued: six due to contact lens discomfort, four were lost to follow-up, and four discontinued for personal reasons unrelated to lens wear. After one week of contact lens wear, the contact lens group was re-assigned to symptomatic and asymptomatic groups. The symptomatic group reported mean CLDEQ-8 values of 21 ± 4 points, whereas the asymptomatic values were 12 ± 3 points. This range of scores was maintained in each contact lens group at the six-month visit (20 ± 9 versus 11 ± 3), with significant differences at baseline and final visit (p < 0.001).

**Cell density changes**

At baseline, there were no differences of goblet cell densities between the groups. The average goblet cell density in the total lens wear cohort was initially 476 ± 41 cells/mm² and decreased by 28 per cent in the symptomatic group and 14 per cent in the asymptomatic group after six months of contact lens wear. The control group also showed a decrease of two per cent at the six-month follow-up visit (Figure 2). Conversely, symptomatic contact lens wearers showed the most profound changes over six months – a 59 per cent increase in resident corneal dendritic cell density. The asymptomatic group showed an increase in resident corneal dendritic cell density of 19 per cent (Table 1). The absolute changes of both cell types were significantly different between the three groups (control, symptomatic and asymptomatic) after six months of lens wear, except for changes in corneal dendritic cell density in the asymptomatic and control groups, which was not significant (Table 1).

**Associations between resident corneal dendritic and conjunctival goblet cell densities after six months of lens wear**

There was an inverse association between changes in conjunctival goblet cell density and resident corneal dendritic cell density in all participants regardless of the group (r = −0.34, p = 0.03). No significant associations were found independently in each group, including the control non-lens-wearing group.

In order to observe the main effect of change after six months of contact lens...
wear and to predict resident dendritic cell migration to the central cornea based on conjunctival goblet cell loss, a regression analysis was conducted using the contact lens cohort, regardless of group (Figure 3).

The regression equation was $F(1,46) = 5.352$, $p = 0.03$, with an $R$ of 0.34, indicating that predicted change in dendritic cell density is equal to $77 \pm 1.5$ goblet cells/mm$^2$. Dendritic cell density in the central cornea increased by 1.5 cells/mm$^2$ for every decrease of 1 cell/mm$^2$ in conjunctival goblet cell density.

**Discussion**

Marked changes in goblet and dendritic cell density were observed with the introduction of contact lens wear, particularly in symptomatic wearers. This is the first study to demonstrate that this increase in dendritic cells in the cornea after six months of wear can be predicted based on the degree of goblet cell loss in the conjunctiva.

Similar to the results of this study, the vast majority of research studies on conjunctival goblet and corneal dendritic cell densities in lens wearers suggest a loss in goblet cells$^{18-24}$ and increased number of dendritic cells in the central cornea$^{25-27}$ as a result of lens wear adaptation, regardless of lens type (rigid, soft hydrogel or conventional) or measuring technique (in vivo or ex vivo). However, there has been no previous demonstration of an interlink between these cells in response to contact lens wear using
the same cohort in a longitudinal study. The present study, therefore, demonstrates not only an association between the cell densities, but also a prediction of migratory resident dendritic cells to the central cornea based on conjunctival goblet cell loss.

While contact lens comfort is a major determinant of successful lens wear, the associations found between changes in cell density after six months of wear occurred in the total lens wear population rather than the individual groups (symptomatic and asymptomatic). These results indicate that goblet and dendritic cell densities adapt in contact lens wearers, regardless of symptoms of contact lens discomfort, in order to compensate for the effect of the contact lens-induced stress on the ocular surface. Contact lens symptomatology has been recently associated with neuropathic corneal pain by way of a model involving the interlink between the nervous and immune systems for dry eye and ocular discomfort. Recent work also suggests a role of dendritic cell numbers in the central cornea as part of this immune ‘crosstalk’.

Goblet cells are also known to support immune tolerance of the ocular surface, as cholinergic stimulated goblet cells. Cell antigen passes into the stroma through goblet cell-associated passages and increased antigen binding by mucins degranulation during homeostasis. Immuno-modulatory crosstalk between goblet and dendritic cells in the conjunctiva have been proposed recently using mice cultured tissue, demonstrating that goblet cell-derived soluble factors can alter dendritic cell phenotype by downregulating expression of major histocompatibility complex class II. Although this was demonstrated only in conjunctival epithelium, it is reasonable to assume that the releasing factors by conjunctival goblet cells to protect the ocular surface are likely to be distributed by the tears over the corneal surface, mirroring the same effect on corneal dendritic cells.

Migration of resident dendritic cells to the central cornea in response to contact lens wear adaptation has been described in humans, suggesting that corneal dendritic cells increase by two-fold within the first two hours of lens wear, indicating a rapid, subclinical inflammatory response. Although this is a short time frame approach to evaluate resident dendritic cell migration to the central cornea in humans, a more sophisticated approach using time-lapsed imaging has been proposed recently, demonstrating that immature dendritic cells at the sub-basal nerve plexus move at a speed of 1 μm/minute. This indicates that increased dendritic cell density in the central cornea is likely to occur within minutes rather than hours.

Table 1. Percentage of cell density change in contact and non-contact lens wearers from baseline to the six-month follow-up visit. Multiple comparisons of absolute change of cell density between non-lens wearers and lens-wearing groups after six months of lens wear.
hours or days after contact lens insertion. Further research to observe real-time migration rate of dendritic cells in contact lens wear is needed.

There are limitations of using CCM in humans to assess cells based on morphologi- cal appearance as there is the uncertainty of cell phenotype which could provide more insights into the role of these cells in contact lens adaptation and comfort. Additional limitations to the study would be morphological cell changes, especially during inflammatory acti- vation of dendritic cells where enlargement of dendritic cell density. However, these associ- ations between cell density change were not morphological cell densities: a pilot study. Clin Exp Ophthalmol 2012; 4: 1201–1207.

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