A comparison of epithelial cell morphology and goblet cell densities between rigid gas permeable (RGP) wearers and non-contact lens wearers using impression cytology

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Abstract
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Methods: A comprehensive questionnaire and history were used to determine that all subjects had no ocular complaints and to determine the contact lens use of the RGP wearers. Subjects had normal ocular health as determined by anterior segment biomicroscopy exam. Impression cytology (IC) was performed on all subjects to obtain a representative sample of conjunctival epithelium. The samples were stained and then analyzed using light microscopy.

Results: There were no differences in conjunctival epithelial morphology and goblet cell densities among RGP wearers and non-contact lens wearers.

Conclusions: Impression cytology is a powerful and recommended diagnostic tool in assessing the conjunctival epithelium of all patients wearing contact lenses or those considering a transition into contacts of any kind. This comparison of RGP wearers and non-contact lens wearers suggests that RGP lenses may be preferable to soft contact lenses when there is concern about ocular health.

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Thesis

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A Comparison of Epithelial Cell Morphology and Goblet Cell Densities Between Rigid Gas Permeable (RGP) Wearers and Non-Contact Lens Wearers Using Impression Cytology.

By

Gary Cole
David Throneburg

A thesis submitted to the faculty of the College of Optometry Pacific University Forest Grove, Oregon For the degree of Doctor of Optometry May, 1997

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ABSTRACT

Background

Subjects who wear soft contact lenses (SCL) and polymethylmethacrylate (PMMA) lenses have morphological changes of their conjunctival epithelial cells along with decreased goblet cell densities compared to non-contact lens wearers. The goal of this study was to evaluate the conjunctival epithelium and goblet cell density among successful rigid gas permeable (RGP) wearers compared to non-contact lens wearers.

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Results

There were no differences in conjunctival epithelial morphology and goblet cell densities among RGP wearers and non-contact lens wearers.
Conclusions

Impression cytology is a powerful and recommended diagnostic tool in assessing the conjunctival epithelium of all patients wearing contact lenses or those considering a transition into contacts of any kind. This comparison of RGP wearers and non-contact lens wearers suggests that RGP lenses may be preferable to soft contact lenses when there is concern about ocular health.

Key Words
Impression Cytology, Rigid Gas Permeable Contact Lenses, Conjunctival Epithelium, Goblet Cells
INTRODUCTION

The majority of complications of contact lens wear are subjective symptoms and clinical changes of the ocular surface such as conjunctival hyperemia and/or edema, corneal neovascularization or infection. Patients without symptoms or signs are thought to be free of conjunctival and/or corneal surface changes. However, recent reports have suggested that contact lens wear can change the surface of the eye in asymptomatic wearers [2]. The technique used to detect these changes was impression cytology.

Impression cytology is a non-invasive means of obtaining a biopsy of the conjunctival surface using a small piece of filter paper to collect a one-layer epithelial sheet. Goblet cell density and epithelial cell morphology can be assessed. Since its introduction, the technique has proven useful in the diagnosis of a variety of external eye diseases, especially dry eye states.

Using impression cytology, clinically asymptomatic soft contact lens wearers showed evidence of early squamous metaplasia with enlarged, flattened epithelial cells with snake-like condensations of nuclear chromatin (snakes) [2]. The goblet cell density was also lower than non-lens wearers [2]. When soft lenses were applied to wearers of glasses with cytologically normal conjunctiva, the patients developed a rapidly increasing epithelial cell size with numerous nuclear alterations. There was also a decrease in goblet cell density.[2] Wearers of polymethylmethacrylate hard lenses also showed similar conjunctival changes when tested with impression cytology. They
too had flattened, enlarged epithelial cells with a decrease in goblet cell density. [5]

Since rigid gas permeable (RGP) contact lenses have been advocated as giving the best optical clarity while preserving ocular health, we compared the conjunctival epithelium using impression cytology in young adults who did not wear contact lenses with long-term, successful, asymptomatic RGP wearers.

METHODS

Subjects

Subjects were 21 young adults: 11 did not wear contact lenses and 10 were successful RGP wearers. The nature of the study was explained and informed consent was obtained. Each subject underwent extensive ocular health assessment including ocular and medical history, slit-lamp biomicroscopy, keratometry to observe distortion and fluroscein tear break up time (FTBUT). All subjects were required to meet the following criteria for the study: no history or presence of dry eye syndromes, allergy, any contact lens intolerance, no cornea or conjunctival abnormalities, no systemic disease, and no systemic or topical medications. A modified McMonnies dry eye questionnaire was given to eliminate any candidate based on subjective responses. Three potential subjects were not selected because two had keratoconjunctivitis sicca and the other had moderate papillary changes. The study was comprised of two experimental groups. Patient profiles of the control and study groups were comparable (Table 1). None of the subjects
wearing RGP's reported contact lens intolerance or demonstrated any evidence of ocular clinical changes.

Table 1. Subject Profiles of Non-Contact Lens Wearers and RGP Wearers

<table>
<thead>
<tr>
<th></th>
<th>RGP Wearers</th>
<th>Non-Contact Lens Wearers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.7</td>
<td>23.5</td>
</tr>
<tr>
<td>Gender</td>
<td>7 Males</td>
<td>6 Males</td>
</tr>
<tr>
<td></td>
<td>3 Females</td>
<td>5 Females</td>
</tr>
<tr>
<td>Length of Contact Lens Wear</td>
<td>7 (±3) years</td>
<td>0</td>
</tr>
<tr>
<td>Contact Lens Daily Wear</td>
<td>13(±2) hours</td>
<td>0</td>
</tr>
</tbody>
</table>

Impression Cytology

To examine the conjunctival epithelium, specimens were obtained from all subjects using impression cytology as modified by Nelson and Connor. [6] The patient was seated in a reclined position and a drop of topical anesthetic (0.5% proparacaine) was instilled into each eye about 1 minute prior to obtaining a sample. The anesthetic did not affect the quality of the sample. Millipore cellulose acetate filter (Millipore VSWP 0.025um) was applied to the bulbar conjunctiva 3mm posterior to the limbus inferiorly. The strips were held by hemostat or forceps and a sterile glass rod was used to apply pressure for 5-10 seconds. Multiple samples were taken and the best samples were obtained when the strips were applied to the same place on the bulbar surface as the previous
sample. The benefit of the first sample was to remove the primary layer of scattered columnar or squamous epithelium and mucus threads that interfere with the quality of the staining. Therefore it was essential that multiple samples be taken in order to maintain consistency and repeatable results.

Once the samples were obtained, they were applied to a glass slide cell side up and held to the slide by adhesive tape and allowed to air dry. The samples were stained using periodic acid-Schiff and hematoxylin. The filter was placed in 95% ethanol for 10 minutes, rinsed in distilled water 5 minutes and then placed in periodic acid for 5 minutes followed by another 5 minute rinse with distilled water. The sample was then immersed in Schiff's reagent 5 minutes and rinsed in tap water 5 minutes for development of a pink color. Hematoxylin stain was then applied for 1 minute. The strip was rinsed with tap water, decolorized 10 seconds with acid alcohol followed by a thorough rinse again with tap water. Saturated lithium carbonate was applied to transform the filter into a magenta blue color and air dried. The filter was covered with immersion oil, a glass cover slip was applied, and then examined under the microscope.

Cytological Analysis

The following data were recorded for each sample: epithelial cell morphology, epithelial cell nucleus to cytoplasm ratio (N/C), morphology of epithelial cell nuclei, goblet cell morphology and goblet cell density. Impression cytology grades (0-3) were
determined by comparing the results to a grading scale which is summarized in Table 2.

Table 2: Grading Scale (modified from Nelson and Cameron[3])

<table>
<thead>
<tr>
<th>Grade</th>
<th>Epithelial Cell Morphology</th>
<th>Nucleus/Cytoplasm Ratio</th>
<th>Goblet Cell Number</th>
<th>Goblet Cell Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Small, Round Cells, Large Nuclei</td>
<td>1:2</td>
<td>Abundant</td>
<td>Oval Cells Stain Intensely</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Enlarged, Polygonal Cells, Smaller Nuclei</td>
<td>1:3</td>
<td>Somewhat Reduced</td>
<td>Oval Cells Stain Intensely</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Enlarged, Polygonal Cells, Small Nuclei</td>
<td>1:4 to 1:5</td>
<td>Markedly Reduced</td>
<td>Smaller Cells Stain Less Intensely</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Large, Polygonal Cells, Small Nuclei, Nucleus May Be Absent</td>
<td>1:6</td>
<td>Absent</td>
<td>- -</td>
</tr>
</tbody>
</table>

RESULTS

This study compares the conjunctival epithelium of subjects wearing RGP contact lenses with non contact lens wearers. None of the subjects wearing RGP’s reported contact lens intolerance or demonstrated any evidence of ocular changes. The RGP group had an average wearing time of 7.1 years and daily wearing time of 13.2 hours. All subjects in the study were free of symptoms with clinically healthy conjunctivas.
The impression cytology procedure worked well with good cell yields from all subjects. Typically, a dense monolayer of epithelial cells was found with cells having round shapes, equal sizes, and nucleus to cytoplasm ratios of 1:2 (see Table 3). Significant numbers of intensely staining Goblet cells were typically found. Normal epithelial and goblet cell morphologies and densities were found for 20 of the 21 subjects. One subject, who had been a contact lens wearer for 20 years, showed Grade 1 conjunctival cell changes. None of the samples showed nuclear abnormalities. There was not a statistically significant difference between the Grades for the non-contact lens wearers as compared to the RGP lens wearers.

Obtaining multiple samples eliminated mucus remnants and cell clusters. This technique insured more reproducible results with each subject.

Table 3. Results of Impression Cytology Samples from RGP Contact Lens wearers and Non-contact Lens Wearers

<table>
<thead>
<tr>
<th></th>
<th>RGP Contact Lens Wearers</th>
<th>Non-Contact Lens Wearers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelial Cell</strong></td>
<td>Small, Round Cells</td>
<td>Small, Round Cells</td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td>Large Nuclei</td>
<td>Large Nuclei</td>
</tr>
<tr>
<td><strong>N/C Ratio</strong></td>
<td>1:2</td>
<td>1:2</td>
</tr>
<tr>
<td><strong>Goblet Cell Number</strong></td>
<td>Abundant</td>
<td>Abundant</td>
</tr>
<tr>
<td><strong>Goblet Cell</strong></td>
<td>Oval, Intensely stained</td>
<td>Oval, Intensely Stained</td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
DISCUSSION

No significant differences in conjunctival epithelial or goblet cell morphologies or in goblet cell densities were found between the RGP and non-contact lens groups. These results demonstrate that RGP contact lenses do not typically affect conjunctival epithelial and goblet cells in the same ways that PMMA and soft lenses do. The mild conjunctival cell changes found in a single subject who had been wearing contact lenses for 20 years were probably caused either by prior PMMA wear or the length of time he had been wearing lenses. The snake-like condensations of epithelial cell nuclear chromatin reported by Saini were not seen in any of the samples. RGP lenses seem to produce little or no damage to the conjunctival cells. A further investigation with a larger study population is needed to support the potential advantage of RGP contact lenses over soft or PMMA contact lenses when considering their impact on ocular health at the cellular level.

Also useful would be impression cytology performed at other regions of the bulbar conjunctiva. We know that morphology differs on the bulbar epithelium because cellular doubling can occur over a few millimeter area. Our samples were different from most studies in that we examined the inferior limbus rather than the superior limbus. Perhaps in future studies, samples should be taken more posterior, closer to the fornix where goblet cell numbers are known to be higher. Probing into the various regions of the bulbar conjunctival epithelium would give a better overall picture as to the effect of the contact lens on the conjunctival epithelium.
REFERENCES


