Assessment of conjunctival epithelium after laser in situ keratomileusis by impression cytology

Richard C. Lee
*Pacific University*

**Recommended Citation**
https://commons.pacificu.edu/opt/1377

This Thesis is brought to you for free and open access by the Theses, Dissertations and Capstone Projects at CommonKnowledge. It has been accepted for inclusion in College of Optometry by an authorized administrator of CommonKnowledge. For more information, please contact CommonKnowledge@pacificu.edu.
Assessment of conjunctival epithelium after laser in situ keratomileusis by impression cytology

Abstract
Purpose: To assess the effect of laser in situ keratomileusis (LASIK) on the conjunctival epithelium and its possible relationship to dry eye complications following the LASIK procedure.

Methods: Impression cytology samples of 15 patients were analyzed pre and post operatively. Impression samples were collected from the temporal bulbar conjunctiva utilizing Millipore cellulose acetate filter paper. These specimens were fixed with 95% ethanol, stained with periodic acid Schiff technique, analyzed by light microscopy and graded based on representative photomicrographs.

Results: At two weeks post LASIK, 13 patients (86.7%) demonstrated a reduction in goblet cell density and epithelial cell hypertrophy, while 2 patients remained unchanged (p

Conclusion: The appositional force against the bulbar conjunctival epithelium generated by the microkeratome suction ring during laser in situ keratomileusis causes a significant decrease in the conjunctival goblet cell density and significantly affects epithelial cell morphology in otherwise healthy eyes. These changes in the ocular surface may be implicated in the dry eye complications commonly found after LASIK.

Degree Type
Thesis

Degree Name
Master of Science in Vision Science

Committee Chair
Patrick Caroline

Keywords
laser in situ keratomileusis, impression cytology, microkeratome, dry eye, goblet cell

Subject Categories
Optometry

This thesis is available at CommonKnowledge: https://commons.pacificu.edu/opt/1377
Copyright and terms of use

If you have downloaded this document directly from the web or from CommonKnowledge, see the “Rights” section on the previous page for the terms of use.

If you have received this document through an interlibrary loan/document delivery service, the following terms of use apply:

Copyright in this work is held by the author(s). You may download or print any portion of this document for personal use only, or for any use that is allowed by fair use (Title 17, §107 U.S.C.). Except for personal or fair use, you or your borrowing library may not reproduce, remix, republish, post, transmit, or distribute this document, or any portion thereof, without the permission of the copyright owner. [Note: If this document is licensed under a Creative Commons license (see “Rights” on the previous page) which allows broader usage rights, your use is governed by the terms of that license.]

Inquiries regarding further use of these materials should be addressed to: CommonKnowledge Rights, Pacific University Library, 2043 College Way, Forest Grove, OR 97116, (503) 352-7209. Email inquiries may be directed to: copyright@pacificu.edu
ASSESSMENT OF CONJUNCTIVAL EPITHELIUM AFTER LASER IN SITU KERATOMILEUSIS BY IMPRESSION CYTOLOGY

By

RICHARD C. LEE, B.S.

A thesis submitted to the faculty of the
College of Optometry
Pacific University
Forest Grove, Oregon
For the degree of
Doctor of Optometry
May 2001

Advisor
Patrick Caroline
Richard C. Lee, Author

Patrick Caroline, Advisor
Grade Page

Assessment of Conjunctival Epithelium
After LASIK
By Impression Cytology

Richard C. Lee, Author

Patrick Caroline, Advisor
Richard will complete his Doctor of Optometry degree in the spring of 2001. After graduation, he plans to complete a residency and fellowship in Seattle, Washington. He previously attended the University of Washington, where he acquired his Bachelor of Science in cellular and molecular biology.
Assessment of conjunctival epithelium after laser in situ keratomileusis by impression cytology

Abstract

Purpose: To assess the effect of laser in situ keratomileusis (LASIK) on the conjunctival epithelium and its possible relationship to dry eye complications following the LASIK procedure.

Methods: Impression cytology samples of 15 patients were analyzed pre and post operatively. Impression samples were collected from the temporal bulbar conjunctiva utilizing Millipore cellulose acetate filter paper. These specimens were fixed with 95% ethanol, stained with periodic acid Schiff technique, analyzed by light microscopy and graded based on representative photomicrographs.

Results: At two weeks post LASIK, 13 patients (86.7%) demonstrated a reduction in goblet cell density and epithelial cell hypertrophy, while 2 patients remained unchanged (p<0.01). At four weeks post LASIK, 9 of 14 patients (64%) retained a higher cytology grade(p<0.25). A statistically significant difference between the control samples and the post-operative samples was demonstrated.

Conclusion: The appositional force against the bulbar conjunctival epithelium generated by the microkeratome suction ring during laser in situ keratomileusis causes a significant decrease in the conjunctival goblet cell density and significantly affects epithelial cell morphology in otherwise healthy eyes. These changes in the ocular surface may be implicated in the dry eye complications commonly found after LASIK.

Key Words
Laser in situ keratomileusis
Impression cytology
Microkeratome
Dry eye
Goblet cell
Acknowledgements

I would like to thank Patrick Caroline for providing the resources, insight and patience necessary to complete this project.
Introduction

Laser in situ keratomileusis (LASIK) has become the most widely accepted refractive surgery method used for the safe and effective correction of myopia and astigmatism.\textsuperscript{1-4} The incidence of complications from this procedure has a reported occurrence of approximately 5%.\textsuperscript{5} Common complications found with the LASIK procedure include intraoperative bleeding, intraoperative epithelial defect, transient surface debris, significant dry eye, blood in the flap interface, irregular astigmatism, decentration of the flap, corneal haze, and epithelial ingrowth.\textsuperscript{5} Most complications arise from creation of the corneal flap by the microkeratome.

Dry eye following LASIK has become a nemesis to the refractive surgery community. Though several speculative theories exist, the exact etiology of this condition remains unknown. This study investigates the adverse effects to the conjunctival epithelium that may occur as a result of trauma induced during LASIK. The safe and simple technique of impression cytology was utilized to investigate the conjunctival epithelium pre- and postoperatively to determine the effects, if any, that may be induced by LASIK.

Materials and Methods

Fifteen myopic and/or astigmatic patients, 7 males and 8 females whose ages ranged from 21-30 years, underwent the bilateral LASIK procedure. Impression cytology samples were collected from the temporal bulbar conjunctiva at 1 week pre-operatively, 2 weeks post-LASIK, and 4 weeks post-LASIK. Samples were collected upon cellulose acetate filter discs (Millipore cat no. HAWP01300) applied by an ophthalmodynamometer under
a force of 60 grams for three seconds duration. The samples were fixed in 95% ethanol, stained with periodic acid Schiff (PAS) technique, mounted upon microscopic slides and representative photomicrographs were taken for analysis. Each sample was graded according to goblet cell density and epithelial cell morphology on a scale of 0-3 as per Nelson’s classification.

**Statistics**

The impression cytology control grades were compared with the post-operative grades utilizing paired sample statistical analysis.

**Results**

*Pre-operative:*

Microscopic evaluation and projected grid screen analysis of the cytology samples revealed relatively normal goblet cell densities and epithelial cell morphologies in all subjects. The median pre-operative cytology score was grade 0. Figures 1 and 2 demonstrate the typical appearance of the epithelial cell morphology and goblet cell density.

*Post operative (2 weeks):*

Histologic examination of the impression samples from 2 weeks postoperative demonstrated epithelial cell hypertrophy and a decrease in goblet cell density in 13 of 15 subjects (86%). The statistical significance demonstrated was $p<0.01$. The median cytology score of these samples was grade 2. Representative photomicrographs are
found in figures 3 and 4. Two patients showed no cytological changes of the ocular surface. In addition, superficial punctate keratitis was demonstrated in five patients at this postoperative visit.

**Postoperative (4 weeks):**

Of the thirteen patients who demonstrated histopathological changes at two weeks, three patients demonstrated improved goblet cell densities and a slight regression in morphological epithelial cell changes. The statistical significance, as compared to the pre-operative values, for these impression samples was $p<0.25$. The median score of cytology samples was grade 1. One patient did not return for follow-up testing.

**Discussion**

Goblet cell density reflects the overall health of the ocular surface and the stability of the tear film. A loss or decrease in goblet cell density will reduce the mucin component of the tear film and a secondary tear disruption will occur. Subsequently, the conjunctival epithelial cells undergo a process of hypertrophy, and in cases of longstanding goblet cell depletion, (e.g. cicatrical pemphigoid), squamous metaplasia or keratinization occurs.11

We utilized the technique of impression cytology to study the changes in goblet cell density and the conjunctival epithelial cells in patients who underwent the LASIK procedure.

In our study, a statistically significant change occurred from pre-operative cytology grade values as compared to postoperative values. The highest degree of significance was
present at the two-week postoperative sampling. An improvement was demonstrated in
the health of the ocular surface at four weeks, but cytology grades still remained
significantly changed.

Many studies investigating the etiology of dry eye following LASIK have revolved
around corneal sensitivity. The cutting of the corneal nerves by the microkeratome blade
has been implicated in reducing the overall sensitivity of the cornea, thus reducing reflex
blinking and tear secretion.2,12-14 However, a recent study by Chuck and coauthors,
concludes that corneal sensitivity returns to 95% within baseline sensitivity by three
weeks post-LASIK.15 This would suggest that the dry eye symptomatology experienced
by LASIK patients would be the result of another underlying process causing disruption
of the tear film.

During the creation of the corneal flap with LASIK, the microkeratome blade apparatus is
stabilized upon the globe by the vacuum ring plate. In order to achieve mechanical
stabilization, the vacuum ring applies an appositional suction force against the bulbar
conjunctiva greater than 65 mmHG.16 The force raises the intraocular pressure
respectively, which has been reported to cause ischemic damage to the optic nerve.17 It
is this appositional force that may also cause damage to the ocular surface, significantly
reducing the goblet cell density, causing dry eye symptomatology.

Our results demonstrate significant changes in conjunctival goblet cell density and
epithelial cell morphology. These changes occur as a result of damage to the epithelial
tissue during LASIK. In the analysis of the procedure, only the suction ring plate and the
topical intraoperative/postoperative lubricants and medications could affect the ocular
surface adversely. We believe that it is the strong appositional counterforce placed upon
the bulbar conjunctiva by the suction ring that is responsible for the reduction of goblet
cell density. Correspondingly, a disruption of tear film stability produces the dry eye
symptomatology associated with LASIK. Given that the majority of LASIK recipients
have a normal and healthy ocular surface prior to surgery, a full recovery of the goblet
cell density is expected but at an interval greater than 4 weeks postoperatively. It is
advisable to supplement the ocular tear film with artificial lubricants until such recovery
occurs to prevent squamous metaplasia of the conjunctival epithelial cells.
References


Figure Legend

Figure 1: Preoperative Impression cytology sample, Grade 0. Patient AB. Note the ratio of nucleoplasm to cytoplasm is 1:2. Numerous goblet cells present (pink).

Figure 2: Preoperative impression cytology sample, grade 0. Patient CD. Note numerous goblet cells (pink). Nucleoplasm to cytoplasm ratio is 1:2.

Figure 3: 2 week postoperative sample, grade 2. Patient AB. Note the nucleoplasm to cytoplasm ratio is 1:6 and lack of goblet cells.

Figure 4: 2 week postoperative sample, grade 2. Patient CD. Note loss of goblet cell density and nucleoplasm/cytoplasm ratio is 1:4.
Figures

Figure 1. Patient AB

Figure 2. Patient CD

Figure 3. Patient AB

Figure 4. Patient CD