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CORNEAL WOUND HEALING PROTEOMICS IN FEMTOSECOND LASER SURGERY

Faculty of Medicine and Health Technology
Bachelor's thesis in medicine
December 2020

Contents

ABSTRACT

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Refractive errors are very common among healthy, working age people. Typically, eyeglasses are used to correct them. In 1990's UV laser light based Laser in situ keratomileusis (LASIK) technique was developed to reshape the cornea for better vision. In early 2000 femto-LASIK, where corneal flap is opened with a pulsed femtosecond laser instead of mechanical blade. A decade later, small incision lenticule extraction, SMILE procedure appeared. In SMILE the whole surgery is done with a femtosecond laser and the cut lenticule inside the corneal stroma is pulled out with tweezers from a small opening in the side. This leaves the corneal center area intact. However, such procedures are harsh on the cornea and cause an immediate, complicated wound healing process with inflammation and sometimes infection. These processes cause changes in tear fluid and corneal surface proteomic content. Some changes are temporary, and some are permanent. Studying these changes may help to predict the operation of the eye and prevent side effects.

This report is part of advanced studies in medicine / medical bachelor's thesis, where as a part of an ophthalmic research group human eye tear film protein changes were studied in femtosecond laser refractive surgery. Research results have been accepted for publication in a peer reviewed international scientific journal, Clinical proteomics. [1] This report concentrates on a supplementary systematic literature review on the topic and reports the search process and results.

Normal eye has a thin layer of watery fluid, tear film, on top of cornea and conjunctiva. Tear film can be easily and non-invasively collected from under the eyelids with a small capillary test tube. Modern proteomic tools enable the analysis of hundreds of proteins simultaneously from small sample. In this study we collected tear film samples from over hundred LASIK and SMILE patients with 2–3 µl capillary test tubes. The final analysis was based on 70 LASIK patient data and samples. Tear film samples were collected before the operation and 1.5 h after surgery and one month later. Samples were measured with a mass spectrometer and results were analyzed with bioinformatic methods. A systematic literature review was made to supplement the understanding in femtosecond laser refractive surgery and proteomics. Search was made in OvidMedline-database and 25 search results were obtained. Some results were excluded from the final analysis based on the title and abstracts when they were clearly outside the scope of the literature review. 13 peer reviewed articles were included in the final qualitative analysis. These publications mostly reported LASIK operation related complications and protein changes in them but also newer SMILE operation related problems.

In the analyzed publications tens of proteins were discussed, and most results were related to matrix metalloproteinase-9 (MMP-9), which changes appeared in, for example, keratoconus, ectasia, dry eye disease and in refractive laser surgery in general. However, the research results were mostly concentrated on histopathological and immunohistochemical studies of corneal tissue and less on tear film samples. A typical limitation of a study was the lack of before and after procedure samples for comparison and instead studies were comparing operated patients to a control group and not the patient's protein changes due to the surgery. This problem is addressed by use of modern mass spectrometers that bring significant advantage to studying proteomic changes. This report's most important finding may be the notice of development of research methods in refractive surgery and proteomics. Therefore, it is likely that tear film analysis will develop rapidly in the 2020's and the predictability of operation related complications will improve significantly, despite the already very low level of occurrence.

Keywords: LASIK, SMILE, proteomics, wound healing, corneal, femtosecond laser surgery

TIIVISTELMÄ

Lasse Orsila: Sarveiskalvon haavan paranemisen proteomiikka femtosekuntilaserkirurgiassa
Lääketieteen syventävät opinnot, kirjallinen työ
Tampereen yliopisto
Lääketieteen lisensiaatin tutkinto-ohjelma
Joulukuu 2020

Ohjaajat: lääketieteen lisensiaatti Petri Mäkinen ja professori Hannu Uusitalo

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Erilaiset silmän taittovirheet ovat hyvin yleisiä. Niiden korjaamiseen on perinteisesti käytetty silmälaseja. 1990-luvulla kehitettiin UV-laservaloon perustuva laseravusteinen paikallinen sarveiskalvon muokkausleikkaus (Laser in situ keratomileusis, LASIK). 2000-luvun alussa alkoi yleistyä femto-LASIK, jossa sarveiskalvon pintaan avattava läppä leikataan auki pulssitetulla femtosekuntilaserilla mekaanisen leikkuuterän sijaan. Noin kymmenen vuotta myöhemmin taittovirheen korjauskirurgiassa alettiin käyttää SMILE-leikkausta eli 'small incision lenticule extraction' -toimenpidettä, jossa koko sarveiskalvon taittovoimakkuuden korjaus tehdään femtosekuntilaserilla ja poistamalla sarveiskalvon sisältä toimenpiteessä irtoava sarveiskalvon pala. Tällaiset leikkaukset ovat sarveiskalvolle kuormittavia toimenpiteitä ja aiheuttavat välittömän, monimutkaisen haavanparanemisreaktion, inflammaatiota ja joskus infektioita. Nämä tapahtumat heijastuvat muutoksina silmän pintaosien ja kyynelnesteen proteiinipitoisuuksissa. Muutoksista toiset ovat väliaikaisia ja toiset pitkäkestoisempia. Tällaisten silmän pinnalla ja kyynelnesteessä tapahtuvien muutosten tutkiminen voi auttaa ennustamaan silmän toimintaa jatkossa ja ehkäistä haittavaikutuksia.

Tämä kirjallinen työ on osa lääketieteen syventäviä opintoja, joissa osana tieteellistä tutkimusryhmää tutkittiin silmän kyynelnesteiden proteomiikan muutoksista femtosekuntitaittovirhekirurgiassa. Tutkimustuloksista on laadittu julkaisu, joka on vertaisarvioinnin jälkeen hyväksytty julkaistavaksi kansainväisessä biolääketieteen alan tiedelehdessä. [1] Tässä kirjallisessa työssä on raportoitu julkaisun aihepiiristä laaditun systemaattisen kirjallisuuskatsauksen hakuprosessi ja tulokset.

Kyynelnestettä voidaan kerätä helposti ja hellävaraisesti silmän luomiraosta. Nykyaikainen proteomiikka mahdollistaa satojen proteiinien samanaikaisen analysoinnin hyvin pienestä määrästä kyynelnestettä. Tutkimustyössä kerättiin yli sadan LASIK- ja SMILE-leikkauksen yhteydessä potilaiden kyynelnesteitä 2–3 µl kapillaariputkiin. Lopullinen analyysi perustui 70 tutkimuspotilaan tietoihin ja näytteisiin. Kyynelnestenäytteitä kerättiin ennen leikkausta ja 1,5 tuntia leikkauksen jälkeen sekä kuukauden kuluttua leikkauksesta. Näytteet analysoitiin massaspektrometrillä. Tulokset analysoitiin bioinformaattisin keinoin. Aihepiiristä laadittiin systemaattinen kirjallisuuskatsaus syventämään tietämystä jo julkaistusta aineistosta silmien proteomiikasta femtosekuntilasertaittovirhekirurgiassa. Julkaisuhaut tehtiin OvidMedline-tietokannasta. Haussa löytyi 25 julkaisua. Hakutuloksesta seulottiin otsikoiden ja tiivistelmien perusteella pois selvästi aihepiirin ulkopuolella olevat julkaisut ja kirjallisuuskatsauksen kvalitatiiviseen analyysiin hyväksyttiin 13 vertaisarvioitua julkaisua. Näissä julkaisuissa käsiteltiin erityisesti LASIK-leikkausten komplikaatioiden yhteydessä havaittuja proteiinimuutoksia sekä myös uudempaan SMILE-leikkaukseen liittyviä ongelmia.

Arvioiduissa julkaisuissa esiintyi kymmeniä eri proteiineja, joista eniten tutkimustuloksia liittyi matriksin metalloproteinaasi 9:ään (MMP-9), jonka muutoksia esiintyi muun muassa sarveiskalvon kartiopullistumassa (keratokonus), sarveiskalvon pullistumassa (ektasia), kuivasilmäisyydessä ja yleisesti lasertaittovirhekirurgiassa. Keskeinen puute tutkimustuloksissa oli proteomiikkatutkimusten keskittyminen histologisiin värjäyksiin eikä niinkään kyynelnestenäytteisiin. Menetelmissä ei yleensä ole ennen ja jälkeen toimenpiteen vertailukohtia. Lisäksi suurin osa tuloksista perustui kontrolliryhmiin vertailuihin eikä saman potilaan proteomiikan muutosten seuraamiseen toimenpiteissä. Tähän ongelmaan uudenaikaiset massaspektrometrimenetelmät tuovat merkittävää hyötyä, kuten tämän lääketieteen syventävän työn tutkimusjulkaisussa osoitetaan. Tämän kirjallisen työn merkittävin hyöty lienee tutkimusmenetelmien kehityksen huomaaminen taittovirhekirurgiassa ja proteomiikassa. Näin ollen on todennäköistä, että silmän kyynelnesteiden analysoinnissa tapahtuu merkittävää edistystä 2020-luvulla ja leikkauskomplikaatioiden todennäisyyksien arviointi tulee kehittymään.

Avainsanat: LASIK, SMILE, proteomics, wound healing, corneal, femtosecond laser surgery

INTRODUCTION

FemtoLasik surgery [2–5] is based on Laser in situ keratomileusis (LASIK) eye surgery that was first introduced 30 years ago in 1990. In LASIK, the cornea shape is modified with a pulsed ultraviolet (UV) laser light, typically an EXCIMER laser, which is short for excited dimer laser. These gas lasers [6] contain a noble gas, e.g., Ar, Kr, Xe, and a halogen, e.g., F or Cl, in He or Ne buffer gas. An excimer gain medium is pumped with short (nanosecond) current pulses in a high-voltage electric discharge. The laser wavelength is determined based on the used gas and typically varies in the range 157–351 nm; in refractive surgery, Ar-F-gas is used, which gives 193 nm operational wavelength. In human eye, the shorter the wavelength, the smaller distance the light pulses penetrate in the cornea. Cornea has a high absorption to the UV and hence the light will not travel to the retina and damage the visual nerve cells. In LASIK, the laser light is focused into the cornea's interior part, stroma, to reshape the light refracting properties to minimize the aberrations and improve the patient's visual acuity. However, because of the high absorption of the UV light to the cornea, and that we don't want to damage the surface epithelium, a flap needs to be cut to the cornea's surface during the procedure; this is done with a femtosecond laser in FemtoLasik. Conventional LASIK uses a special blade to cut the flap. The flap is carefully folded away before the EXIMER laser illumination and then turned back after the stroma shape has been corrected with UV laser pulses. A femtosecond laser is a solid-state laser with a broad-spectrum mode-locked to produce ultrashort laser pulses. Different wavelengths of light propagate in phase locked wavefront and in time domain hit the target with some femtoseconds (fs). In FemtoLasik the pulse durations are typically in the order of 100 fs to hundreds of fs. This is such a short time interval that the laser pulse electric field rips a part the material and the eye tissue does not have the time to warm up. This happens in a very controlled manner and produces a clear-cut surface to the stroma. In *in vitro* experiments, it has been observed that reducing the pulse duration reduces the amount of collateral tissue damage [7].

SMILE (Small Incision Lenticule Extraction) is the latest laser based refractive surgery method. [8] In SMILE procedure fs-laser pulses are focused to the corneal interior and with a tight focusing lens, a good precision in depth direction can be achieved and the surgery can be done in two different layers. The operated area is disk-shaped, and the eye surgeon removes a small disk-shaped piece of tissue, a lenticule. In order to extract the lenticule, a 3–4 mm long opening is made with a femtosecond laser and the lenticule is pulled out with tweezers from the opening. SMILE has the

advantage over FemtoLasik that the cornea's surface remains mostly undisturbed and corneas epithelium is much less damaged.

Corneal wound healing is individual, and, in this study, we analyze the changes in laser based refractive surgery in the tear fluid markers, i.e., biomarkers, proteins. [9–11] Biomarker changes [12], [13] are believed to show corneal wound healing individual differences. The objective is to find out which of the tear fluid proteins best predict different aspects of corneal wound healing. The different stages of corneal wound healing are illustrated in Fig. 1. The 12 main phases of this process are described by Wilson et. al [14]: keratocyte apoptosis is the first detectable event following an epithelial injury. This happens almost instantaneously and is followed by a complex cascade of events in the stroma and epithelium. Fig. 1 summarized this process in 6 simplified main phases.

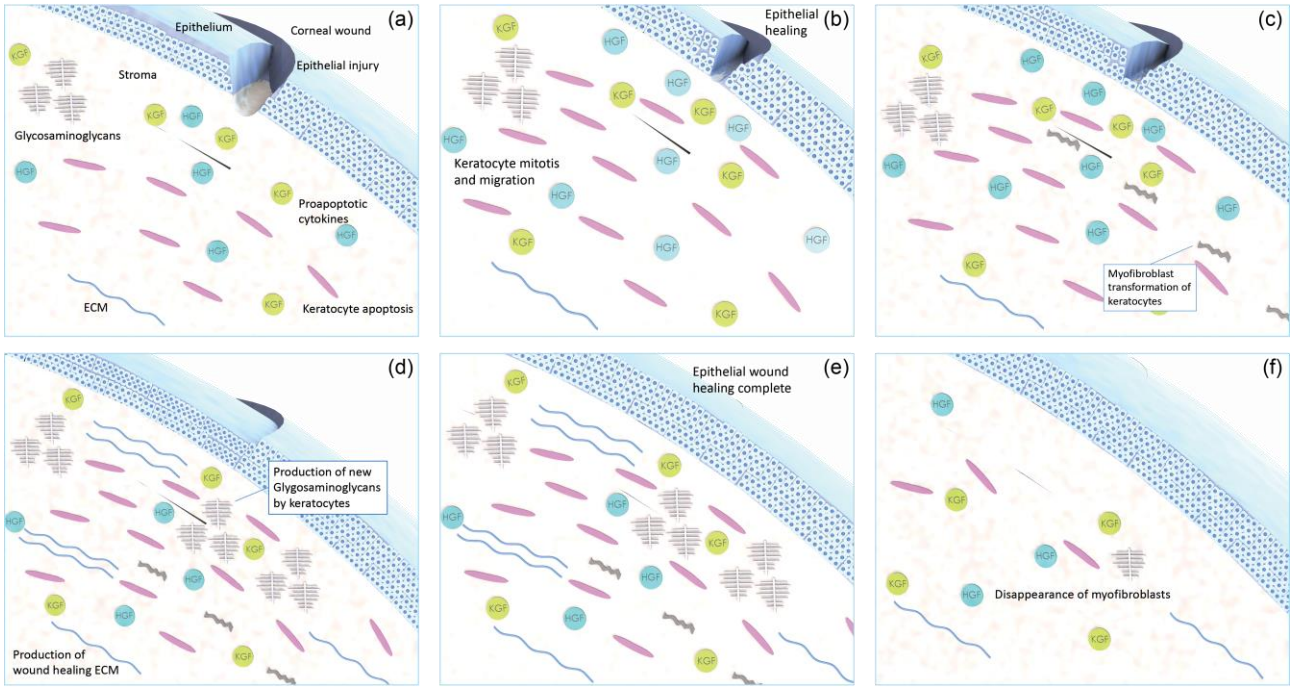

Fig. 1 (a) Immediately after the injury, various cytokines and growth factors are released to the stroma inducing keratocyte apoptosis. Keratinocyte (KGF) and hepatocyte (HGF) growth factors displayed as green and blue spheres. (b) After the initial phase, the inflammation response is also initiated, lasting several hours. Cell migration is also initiated in both epithelial and stromal layers. (c-d) The migration phase is followed by a proliferation phase, where keratocytes further from the injury being to proliferate and are transformed into myofibroblasts and fibroblasts. In the epithelium, the migrated cells begin to cover the wound area, and this is facilitated by a temporary wound healing extracellular matrix (ECM). Cell differentiation is also taking place at the same stage. (e-f) The initial wound in the epithelial layer is healed relatively quickly, although the layer's complete re-attachment to underlying layers through hemidesmosomes as well as the underlying remodeling may last years.

OBJECTIVES

The objective of the study was to find FemtoLasik and SMILE operation related biomarkers that predict successful procedure and/or possible complications. The main focus is in fs-LASIK ja SMILE operation tear fluid proteomic changes. The research hypothesis is that certain human eye protein profiles vary from average and this predicts laser assisted refractive surgery induced dry eyes and possibly other complications. The study brings new information about predicting refractive surgery complications and improving operation outcomes. This report is a systematic literature review on the topic to supplement the work.

ETHICAL CONSIDERATIONS

Pirkanmaa hospital district's ethical board has reviewed the research plan and given a recommendation for approval.

MATERIAL AND METHODS

Tear fluid samples were collected from laser surgery patients before and after the operation and 1 month's post-operation during a control visit. Tear fluid was collected with 2-3 µl capillary tube by placing the capillary between the eye and the lower eyelid. Research material was formed of tear fluid samples from about 70 FemtoLasik operations and 50 SMILE operations. Samples are stored in Eppendorf-tubes and frozen for later analysis. Sample analysis was done with high resolution mass spectrometer, Sciex Triple TOF 5600+, that can separate over 1000 proteins at once. Measurement only requires a few microliters of tear fluid to determine the about 1500 proteins in them. Samples were analyzed in Tampere University Center for Proteomics and Personalized Medicine (PPM). Protein data was analyzed with bioinformation methods. The results were submitted to Clinical Proteomics Journal, manuscript titled "Early changes in tear film protein profiles after femtosecond LASIK surgery." The article [1] has been accepted for publications on 11 October 2020. This academic report is written prior to publishing the article and hence article results are not discussed here, and this academic report concentrates on a systematic literature review of the topic.

Collecting literature review material

Material is collected primarily from Medline-database and OvidMedline search tool was used for the purpose. Ovid MEDLINE(R) ALL resource was selected for the purpose and advanced search tool was used. Searches were limited to English language and studies on humans.

Search in OvidMedline

OvidMedline searches were implemented with *advanced search* function and the keywords were written one like per sub-topic. The founding idea for the search was to find all the laser based refractive surgery related articles with proteomics or biomarkers mentioned. Studies were then narrowed down to English language articles were studies were concluded on humans. 11th October 2020 search structure is shown in Fig. 2.

# ▲	Searches	Results
1	lasik.mp. or Keratomileusis, Laser In Situ/	6655
2	smile.mp. or Small Incision Lenticule Extraction/	5074
3	refractive surgery.mp. or Refractive Surgical Procedures/	5150
4	femtolasik.mp.	15
5	1 or 2 or 3 or 4	14884
6	proteomics.mp. or Proteomics/	81172
7	Biomarkers, Pharmacological/ or Biomarkers/	283676
8	6 or 7	358613
9	limit 8 to english language	341145
10	limit 9 to humans	255727
11	5 and 8	33
12	9 and 11	32
13	10 and 12	25
14	5 and 8 and 9 and 10	25

Fig. 2 Ovid Medline search keywords and related Boolean operations.

The search yielded 25 results and they were screened for relevance. After going through the titles and abstracts, articles that did not mention refractive surgery were excluded. Also commentaries and case studies and studies that were not conducted on humans were excluded. After this phase 13 articles remained. Results are listed in Table 1.

Table 1. List of article search results. Articles included in the search results are labeled with green and those that were excluded are labeled with red.

#	Title	Source	Authors	Year	Included
1	Bilaterally Asymmetric Corneal Ectasia Following SMILE With Asymmetrically Reduced Stromal Molecular Markers.	Journal of Refractive Surgery. 35(1):6-14, 2019 Jan 01.	Shetty R; Kumar NR; Khamar P; Francis M; Sethu S; Randleman JB; Krueger RR; Sinha Roy A; Ghosh A	2019	Yes
3	Tear Martix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 in Post-Lasik Ectasia.	International Ophthalmology. 39(3):631-637, 2019 Mar.	Elmohamady MN; Abdelghaffar W; Salem TI	2019	Yes
5	Wound Healing, Inflammation, and Corneal Ultrastructure After SMILE and Femtosecond Laser-Assisted LASIK: A Human Ex Vivo Study.	Journal of Refractive Surgery. 34(6):393-399, 2018 Jun 01.	Luft N; Schumann RG; Dirisamer M; Kook D; Siedlecki J; Wertheimer C; Priglinger SG; Mayer WJ	2018	Yes
7	Biomarkers of ocular surface disease using impression cytology. [Review]	Biomarkers in Medicine. 11(12):1135-1147, 2017 Dec.	Hagan S	2017	Yes
10	The Growing Need for Validated Biomarkers and Endpoints for Dry Eye Clinical Research. [Review]	Investigative Ophthalmology & Visual Science. 58(6):BIO1-BIO19, 2017 05 01.	Roy NS; Wei Y; Kuklinski E; Asbell PA	2017	Yes
12	Evaluation of point-of-care test for elevated tear matrix metalloproteinase 9 in post-LASIK dry eyes.	British Journal of Ophthalmology. 100(9):1188-91, 2016 09.	Chan TC; Ye C; Chan KP; Chu KO; Jhanji V	2016	Yes
13	Comparative analysis of two femtosecond LASIK platforms using iTRAQ quantitative proteomics.	Investigative Ophthalmology & Visual Science. 55(6):3396-402, 2014 May 06.	D'Souza S; Petznick A; Tong L; Hall RC; Rosman M; Chan C; Koh SK; Beuerman RW; Zhou L; Mehta JS	2014	Yes
15	Long-term changes in corneal structure and tear inflammatory mediators after orthokeratology and LASIK.	Investigative Ophthalmology & Visual Science. 53(9):5301-11, 2012 Aug 07.	Gonzalez-Perez J; Villa-Collar C; Gonzalez-Meijome JM; Porta NG; Parafita MA	2012	Yes
16	Epithelial ingrowth cells after LASIK/ALTK (automated lamellar therapeutic keratoplasty): are they corneal epithelial stem cells?.	British Journal of Ophthalmology. 96(7):1043-6, 2012 Jul.	Nicolas M; Abouzeid H; Deprez M; Hafezi F; Munier FL; Varga Z; Majo F	2012	Yes
17	Oxidative stress in keratoconus?.	Investigative Ophthalmology & Visual Science. 52(12):8592-7, 2011 Nov 04.	Arnal E; Peris-Martinez C; Menezo JL; Johnsen-Soriano S; Romero FJ	2011	Yes
20	Keratectasia after laser in situ keratomileusis: a histopathologic and immunohistochemical study.	Archives of Ophthalmology. 126(12):1655-63, 2008 Dec.	Meghpara B; Nakamura H; Macsai M; Sugar J; Hidayat A; Yue BY; Edward DP	2008	Yes
24	Immunohistochemical evaluation of two corneal buttons with post-LASIK keratectasia.	Cornea. 26(8):983-91, 2007 Sep.	Maguen E; Maguen B; Regev L; Ljubimov AV	2007	Yes
25	Risk factors for epithelial erosions in laser in situ keratomileusis.	Journal of Cataract & Refractive Surgery. 28(10):1780-8, 2002 Oct.	Bashour M	2002	Yes
2	Invasive stratified mucin-producing carcinoma (i-SMILE) of the uterine cervix: report of a case series and review of the literature indicating poor prognostic subtype of cervical adenocarcinoma. [Review]	Journal of Cancer Research & Clinical Oncology. 145(10):2573-2582, 2019 Oct.	Horn LC; Handzel R; Borte G; Siebolts U; Haak A; Brambs CE	2019	No
4	Study of Anti-Malarials in Incomplete Lupus Erythematosus (SMILE): study protocol for a randomized controlled trial.	Trials [Electronic Resource]. 19(1):694, 2018 Dec 20.	Olsen NJ; James JA; Arriens C; Ishimori ML; Wallace DJ; Kamen DL; Chong BF; Liao D; Chinchilli VM; Karp DR	2018	No
6	A Feasibility Study of Autism Behavioral Markers in Spontaneous Facial, Visual, and Hand Movement Response Data.	IEEE Transactions on Neural Systems & Rehabilitation Engineering. 26(2):353-361, 2018 02.	Samad MD; Diawara N; Bobzien JL; Harrington JW; Witherow MA; Iftekharuddin KM	2018	No
8	Comparison of femtosecond laser-assisted corneal intrastromal xenotransplantation and the allotransplantation in rhesus monkeys.	BMC Ophthalmology. 17(1):202, 2017 Nov 09.	Jin H; Liu L; Ding H; He M; Zhang C; Zhong X	2017	No

9	Ursodeoxycholic acid attenuates experimental autoimmune arthritis by targeting Th17 and inducing pAMPK and transcriptional corepressor SMILE.	Immunology Letters. 188:1-8, 2017 08.	Lee EJKwon JE; Park MJ; Jung KA; Kim DS; Kim EK; Lee SH; Choi JY; Park SH; Cho ML	2017	No
11	Midline posterior glossectomy and lingual tonsillectomy in obese and nonobese children with down syndrome: Biomarkers for success.	Laryngoscope. 127(3):757-763, 2017 03.	Propst EJ; Amin R; Talwar N; Zaman M; Zweerink A; Blaser S; Zaarour C; Luginbuehl I; Karsli C; Aziza A; Forrest C; Drake J; Narang I	2017	No
14	Epstein-Barr virus-associated natural killer/T-cell lymphomas. [Review]	Bailliere's Best Practice in Clinical Haematology. 26(1):15-21, 2013 Mar.	Asano N; Kato S; Nakamura S	2013	No
18	Intraepithelial flap creation during epi-LASIK.	Journal of Cataract & Refractive Surgery. 36(4):702-3, 2010 Apr.	Tandon R; Padmanabhan P; Gujar P	2010	No
19	Histological evaluation of mechanical epithelial separation in epithelial laser in situ keratomileusis.	Journal of Cataract & Refractive Surgery. 35(7):1251-9, 2009 Jul.	Soma T; Nishida K; Yamato M; Kosaka S; Yang J; Hayashi R; Sugiyama H; Maeda N; Okano T; Tano Y	2009	No
21	Different epithelial cleavage planes produced by various epikeratomes in epithelial laser in situ keratomileusis.	Journal of Cataract & Refractive Surgery. 34(12):2079-84, 2008 Dec.	Choi SK; Kim JH; Lee D; Lee JB; Kim HM; Tchah HW; Hahn TW; Joo M; Ha CI	2008	No
22	Epi-laser in situ keratomileusis: comparative evaluation of epithelial separation with 3 microkeratomes.	Journal of Cataract & Refractive Surgery. 34(10):1761-6, 2008 Oct.	Herrmann WA; Hillenkamp J; Hufendiek K; Prahs P; Lohmann CP; Helbig H; Kobuch K	2008	No
23	Clinical pharmacology in the molecular era.	Clinical Pharmacology & Therapeutics. 83(2):220-5, 2008 Feb.	Dollery CT	2008	No

RESULTS

Search results that were included in the results, listed in Table 1, are analyzed regarding the topic of the report: Corneal would healing proteomics in femtosecond laser surgery. This is interpreted in a way that while reading the search result articles, relevant information is collected from the articles that give new information about refractive surgery and wound healing, particularly article's proteomic information. The set of articles is not meant to be a full coverage of the topic but to give the reader a review of literature on this topic that is relevant on the article that this project report in based on. The main findings of each article are listed in the following subsections.

Bilaterally Asymmetric Corneal Ectasia Following SMILE With Asymmetrically Reduced Stromal Molecular Markers

Shetty et al. [15] have studied 178 patients in a retrospective study regarding SMILE surgery and corneal ectasia. Corneal ectasia [16] occurs when the inner layers of your cornea become weak, causing the cornea to change shape, protrude forward and distort your vision. [15] In rare cases, it can be a complication of LASIK. Shetty et al. analyzed the proteins found in extracted lenticules and

tear samples. Tear sample ratio of cytokines, chemokines, cell adhesion molecules, and soluble receptors profiles with ectasia after SMILE had higher cytokine profiles that the suspect-ectatic eye, for example with interleukin (IL) 8,9 and 10, but the results were not statistically significant and hence the tear sample results were not informative. For the extracted lenticules, total levels of lysyl oxidase (LOX), MMP9, IL-6, IL-10, bone morphogenetic protein 7 (BMP7), TNF-alpha, TIMP1, collagen type I alpha 1 (COLIA1), collagen type IV alpha 1 chain (COLIVA1), CD68, cathepsin K, ITGbeta-1, signal transducer and activator of transcription 3 (STAT3), transforming growth factor beta (TGF-beta), and transforming growth factor beta receptor 2 (TGFbetaR2) were measured. The main finding of the study was that LOX was significantly reduced in ectatic eyes and TGF-beta and BMP7 were elevated. The finding is consistent with the fact that LOX is a natural collagen cross-linking enzyme produced in the extracellular matrix in the cornea. The study also cites other works where LOX concentrations have been observed to be linked to poorer clinical outcomes of corneal cross-linking and severity of keratoconus.

Tear Martix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 in Post-Lasik Ectasia

Elmohamady et al. studied the concentrations of matrix metalloproteinase-9 (MMP-9) and tissue inhibitors of metalloproteinase-1 (TIMP-1) in the tear films with post-Lasik ectasia (PLE). The target was to determine the levels of these proteins and to detect if they affected the development of the disease. Research material had 92 eyes from 92 patients divided in PLE group, keratoconus (KC) group, Lasik group with no complications and a control group. [17] It was found out that the MMP-9 levels were significantly lower and TIMP-1 significantly higher in Lasik and control group tear films compared to PLE and KC groups. However, only a limited amount of the studied eyes were operated with a femtosecond laser for flap creations; only 2 out of 12 PLE cases were done with Visumax and 6 were operated with Moria microkeratome and in 4 cases this was not known. Moreover, the study lacked pre-Lasik MMP-9 and TIMP-1 concentration values and hence the causality of the effect remains questionable as well as if these results can be applied to femtosecond laser surgery.

Wound Healing, Inflammation, and Corneal Ultrastructure After SMILE and Femtosecond Laser-Assisted LASIK: A Human Ex Vivo Study

Luft et al. report on immunofluorescence and scanning electron microscope (SEM) results on SMILE and fs-LASIK operated eyes. A total of 16 donor eyes were obtained for the study from a tissue bank and 5 underwent a conventional SMILE operation with VisuMax 500-kHz fs-laser and 3 eyes were treated with SMILE without extracting the lenticule. 5 eyes received fs-LASIK treatment and 3 eyes remained unoperated as a control group. [18] The article concentrates on the immunofluorescence data on the samples but also reports valuable information about surface properties of SMILE operated eyes with SEM: the surface texture of the stromal lenticule bed in SMILE corneas were more irregular, had distortions of stromal collagen lamellae and collagen fibers had more fringed appearance. The main conclusion of the study was that on the extracellular level, fibronectin expression, which is a marker for fibrosis, at the location of the laser injury was higher level after FS-LASIK than after SMILE. On the other hand, fibronectin, which is an extracellular matrix glycoprotein that is produced by activated fibroblasts in the corneal stroma, is important for corneal stromal wound healing since it provides a provisional extracellular matrix, hence enabling the migration of fibroblasts.

Biomarkers of ocular surface disease using impression cytology

S. Hagan has written a review article on biomarkers of ocular surface diseases that utilize impression cytology techniques which permits the retrieval of outermost layer of ocular surface cells. This enables conjunctival epithelial cell morphology analysis in diagnosis of dry eye disease (DED), which is liked to ageing, contact lens wear, autoimmune disease and LASIK surgery. [19] However, the article does not focus on femtosecond laser surgery and is hence outside the scope of this literature review. It is still interesting to note from this literature review that there are inflammatory markers related to DED even without refractive surgery; these include p38MAPK-α, IL-1β, IL-8, MCP-1 and MMP-9 that were all upregulated in subjects with DED in comparison with normal eyes.

The Growing Need for Validated Biomarkers and Endpoints for Dry Eye Clinical Research

Roy et al. have collected a vast amount of biomarker data about dry eyes disease (DED) from over 200 articles. [20] DED is analyzed due to is common occurrence and lack of objective diagnostic criteria. The analysis covers a broad range of biomarkers related to DED but only a few related to femtosecond refractive surgery; these LASIK related results are from a publication by Chan et al. [21] that is included in the search results of this literature review and discussed later on. Roy et al. list TNF-a, IL-6, IL-17a, and IL-8 as the most consistent cytokines/chemokines to occur at consistently elevated levels in DED tears compared to non-DED eyes.

Evaluation of point-of-care test for elevated tear matrix metalloproteinase 9 in post-LASIK dry eyes

Chan et al. have evaluated the use of matrix metalloproteinase 9 (MMP-9) level in post-LASIK dry eyes detection. They evaluated 14 post-LASIK dry eyes and 34 normal eyes. The tear film MMP-9 concentration for post-LASIK dry eyes was 52.7 +/- 32.5 ng/ml and 4.1 +/- 2.1 ng/ml. However, only half of post-LASIK eyes had significant inflammation associated with increased MMP-9. [21]

Comparative analysis of two femtosecond LASIK platforms using iTRAQ quantitative proteomics

D’Souza et al. compare two femtosecond LASIK methods in terms of tear film protein content. The comparison is between 500-kHz Visumax femtosecond laser (Carl Zeiss Meditec) and 60-kHz Intralase femtosecond laser (Abott Medical Optics, Inc.). Visumax had pulse energy of energy 0.16 to 0.165 μJ and Intralase 0.92–1.08 μJ [22]; neither system’s laser pulse width was disclosed in the study so the evaluation of the thermal and ablation properties cannot be reasonably estimated. One can calculate that the average power for Visumax is 80 mW and for Intralase 57 mW, therefore heating effect should be in a similar magnitude but somewhat more pronounced for Visumax. The

study analyzed 22 fs-LASIK surgeries for each method and the study groups were of similar age and spherical equivalent refraction with not underlaying eye diseases. A total of 1594 unique proteins were identified and 824 proteins with at least 2 peptides were quantified and the tear protein ratios were differentially regulated between the eyes treated with different lasers. The secretoglobulins Lipophilin A (1.80-fold) and Lipophilin C (1.77) were significantly upregulated ($p < 0.05$) at 1 week postoperatively in Visumax but not in Intralase-treated eyes. At 1 week, orosomucoid1 was upregulated (1.78) in Intralase but not Visumax-treated eyes. In the same eyes, lysozyme, cathepsin B, and lipo-oxygenase were downregulated. Transglutaminase-2 was downregulated in both groups of eyes. [22] The most relevant proteins in the study were lacrimal gland protein lacritin (LACRT), Clusterin (CLU), and Pre-pro-megakaryocyte potentiating factor (MSLN), Lactotransferrin (LTF), Alpha-2-glycoprotein 1 zinc (AZGP1), Lysozyme (LYZ), and Lactoperoxidase (LPO). Regarding this literature review, D'Souza et al. claim that they are the first to publish team film protein change result after LASIK surgery. In conclusion, the study shows that femtosecond laser surgery induces changes of tear proteins with a profile different from idiopathic dry eye.

Long-term changes in corneal structure and tear inflammatory mediators after orthokeratology and LASIK

Gonzalez-Perez et al. report on tear film inflammatory mediators after orthokeratology and LASIK. They collected tears and assayed using ELISA for IL-6, IL-8, matrix metalloproteinase-9 (MMP-9), and epidermal growth factor (EGF). Only MMP-9 (1.3 times) and EGF (1.4 times) were increased for LASIK compared to the control group and even then, concentrations were more associated with high degree of myopia. [4] However, the study reported results on conventional LASIK with Hansatome microkeratome and not femtosecond LASIK. Therefore, the results are of limited applicability to this the literature review. Still, epithelial cells and keratocytes have receptors for different cytokines, chemokines, and growth factors and thus, the activity of inflammatory mediators in these cells may be important for the response of corneal tissue to wounding and tissue redistribution. These results may obviously be useful to differentiate the results between fs-LASIK and conventional LASIK.

Epithelial ingrowth cells after LASIK/ALTK (automated lamellar therapeutic keratoplasty): are they corneal epithelial stem cells?

Nicolas et al. discuss about post-LASIK epithelial ingrowth (EI) and they have performed immunohistochemistry on 4 patients with EI but only one of them with femtoLASIK. [23] They reported strong expression of CK3 (differentiation marker) and in majority of cell nuclear expression of BM11, p63 and C/EBP. However, due to small sample size and majority of patients not being femtoLASIK patients, the reported results cannot be considered to be relevant to this literature review.

Oxidative stress in keratoconus?

Arnal et al. have studied oxidative stress biomarkers in keratoconus. They determined the total antioxidant capacity and total nitrites present in the samples as well as lipid peroxidation products and the glutathione content 4-hydroxynonenal (4-HNE) levels. [24] The study had 6 healthy corneas, 7 keratoconus corneas and 4 post-LASIK corneas, i.e., no femto-LASIK surgery was studied. They noticed, e.g., that antioxidant capacity in ectasia associated with LASIK patients was decreased significantly and oxidative stress products like nitrides were accumulated in the corneal tissues.

Keratectasia after laser in situ keratomileusis: a histopathologic and immunohistochemical study

Meghpara et al. have investigated histopathologically and immunohistochemically keratectasia after LASIK, healthy corneas and keratoconus corneas. [25] Sample size was 5 corneas from post-LASIK keratectasia patiens, 2 keratoconus patiens and 2 healthy donor corneas. The investigation's LASIK procedure was conventional LASIK with microkeratome incision for flap formation. Therefore, results are not directly within the scope of this literature review. However, similar protein markers are mentioned compared to other search results. For example, Meghpara et al. report that the healthy corneal epithelium and corneas with keratectasia displayed nearly no staining for MMP-1, whereas

strong staining was observed in the corneal epithelium of keratoconus cases. Both MMP-2 and MMP-3 immunolabeling were at the background level or absent in all corneal specimens. By utilizing also tunneling electron microscopy (TEM) imaging, the article concludes that after LASIK the collagen fibrils in the residual stromal bed of corneas with keratectasia may be mechanically stretched while the surrounding extracellular matrix is concurrently compressed because of loss of structural resistance to intraocular pressure.

Immunohistochemical evaluation of two corneal buttons with post-LASIK keratectasia

Maguen et al. have studied two post-LASIK keratectasia samples and compared them with two normal post-LASIK donor samples. They performed immunohistological staining for over 30 proteins and found out several differences between the control samples and the keratectasia corneas. For example, the area around the flap interface in ectatic corneas had decreased expression of nidogen-1 and nidoge-2 compared to control samples. [26] MMP-3 was seen in scattered keratocytes and MMP-10 appeared strongly in the ectatic corneas compared to control samples. They also reported that MMP-1, MMP-2, MMP-7, MMP-9, urokinase, cathepsins H and L did not show any changes. A limitation of this study is obviously an unusually small sample size and the fact that a conventional LASIK was used and not femto-LASIK. Yet, quite a few proteins were analyzed in the study and they found differences in the protein distribution over the corneal relative to the operated flap.

Risk factors for epithelial erosions in laser in situ keratomileusis

M. Bashour published in 2002 an article on risk factors for epithelial erosion in LASIK. The study consists of 962 patients (1852 eyes) operated with conventional LASIK. [27] He observed that lighter skin types are a risk factor for epithelial defects, with almost all the epithelial defects occurring in patients with Fitzpatrick Skin Type (FST) I or II and no defects in those with FST V or VI. In patients with FST I or II or Lancer Ethnicity Scale (LES) 1 or 2, the relative risk of an epithelial defect was 10 times greater than in other patients. Moreover, people 40 years or older were at 6 times greater risk than young patients. Similarly, light hair or blue/green eyes patients had 2 to 3 times greater than in patients with darker hair or eyes. [27] The article does not cover proteomic changes in eyes and measurements were mainly visual inspections and questionnaires. However, the study sample size is quite extensive, and some results are backed by 130 000 LASIK operations in the same clinic by the author and his colleagues.

DISCUSSION AND CONCLUSIONS

A large number of articles has been published on femtosecond refractive surgery, on the order thousands of articles, and hundreds of thousands related to biomarkers. However, these topics are seldom combined and only a much smaller number of articles are published that combine these topics. After analyzing the search results, it became clear that some additional keywords could have yielded a better outcome and the limited knowledge of the field beforehand could be the main limiting factor of this systematic literature review. On the other hand, literature review process has been followed without much bias and the outcome represents objectively the report topic “Corneal wound healing proteomics in femtosecond laser surgery.”

The systematic literature review process yielded 13 articles that matched the search criteria. They reported mostly LASIK related results, and some published before femtosecond refractive surgery was first introduces, i.e., flap creation of the surgery was done with a conventional microkeratome instead of a pulsed femtosecond laser system. Also SMILE related articles were found and several laser surgery related proteomic changes were identified. For example, lysyl oxidase (LOX) was significantly reduced in ectatic eyes and TGF-beta and BMP7 were elevated. [15] Such information could be useful in evaluating treatments in ectatic or keratoconus eyes. Matrix metalloproteinase-9

(MMP-9) and tissue inhibitors of metalloproteinase-1 (TIMP-1) were often reported in the studies, particularly the MMP-9. This is probably because of the available analysis methods like ELISA. Clearly, analysis methods have become more powerful during the 21st century, which stands out from the selection of articles and is even more pronounced if out article on the topic would be included in the comparison. Elmohamady et al. reported that the MMP-9 levels were significantly lower and TIMP-1 significantly higher in Lasik and control group tear films compared to PLE and KC groups [17]. On the other hand, in dry eyes disease MMP-9 were found to be upregulated [19]. This was also reported by Chan et al. when they studied post-LASIK dry eyes and found increased levels of MMP-9 [21] and by Gonzalez-Perez [Gonzalez]. Another category of important proteins for femtosecond laser surgery is fibronectin, which is an extracellular matrix glycoprotein, is important for corneal stromal wound healing [18].

Many other proteins were also mentioned in the studies but with less significance to femtosecond laser surgery. Clearly biomarkers and proteomics is a rapidly developing area and has only recently been adopted to use in refractive surgery research. This should enable plenty of new research topics and quite possibly enable the development of predictive protein markers for femtosecond laser surgery that have clinical relevance. In the found studies the protein analysis was only concentrated on analyzing some target group and a control group but to a major surprise, the studies were lacking systematic before and after comparison of protein; main reason for this is evidently the fact that analysis methods were heavily relying on old staining techniques that are destructive in nature and could only be applied *ex vivo*. Now modern mass spectrometer enable a much more detailed view on the protein spectrum in tear fluids and we can expect a significant progress in that front in the next 10 years.

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