



Femtosecond Laser for Deep Lamellar Endothelial Keratoplasty in the Porcine Model

Thesis

Submitted for partial fulfillment of the MD degree in
Ophthalmology

By

Ossama Tarek Sayed Nada

M.B., B.Ch., M.Sc. (Ophthalmology)

Ain Shams University

Supervised by

Prof. Dr. Shaker Ahmed Khedr

Professor of Ophthalmology

Faculty of Medicine - Ain Shams University

Prof. Dr. Ossama AbdelKader Salem

Professor of Ophthalmology

Faculty of Medicine - Ain Shams University

Prof. Dr. Mamdouh Hamdy El-Kafrawy

Professor of Ophthalmology

Faculty of Medicine - Ain Shams University

Prof. Dr. Isabelle Brunette

Professor of Ophthalmology

Director of Research and Post-graduate Studies

University of Montreal

Faculty of Medicine

Ain Shams University

Cairo – 2010



استخدام الفمتوسيكوند ليزر فى ترقيع القرنية
الطبقي العميق المصحوب بالخلايا المبطنه للقرنية فى نموذج قرنية
الخنزير
رسالة

توطئة للحصول على درجة الدكتوراه
فى طب وجراحة العيون

مقدمة من

الطبيب / أسامة طارق سيد ندا
بكالوريوس الطب والجراحة، ماجستير طب وجراحة العيون
جامعة عين شمس

تحت إشراف

الأستاذ الدكتور / شاكراً أحمد خضر
أستاذ طب وجراحة العيون
كلية الطب - جامعة عين شمس
الأستاذ الدكتور / أسامة عبد القادر سالم
أستاذ طب وجراحة العيون
كلية الطب - جامعة عين شمس
الأستاذ الدكتور / ممدوح حمدي الكفراوي
أستاذ طب وجراحة العيون
كلية الطب - جامعة عين شمس
الأستاذة الدكتورة / إيزابيل برونيت
أستاذ طب وجراحة العيون
كلية الطب - جامعة مونتريال

كلية الطب - جامعة عين شمس

القاهرة - ٢٠١٠

ACKNOWLEDGEMENT

First and foremost I thank **GOD** for helping me so much and granting me the power to accomplish this work, words can never describe my gratitude for the great support without which, this work would not be accomplished.

I would like to express my sincere thanks to Professor Dr. **Shaker Khedr**, Professor of Ophthalmology, Faculty of Medicine, Ain Shams University, for his great support, help, his valuable advices, his wise guidance and enthusiastic encouragement.

My sincere thanks and deep appreciation goes to Professor Dr. **Ossama Salem**, Professor of Ophthalmology, Faculty of Medicine, Ain Shams University, for his faithful guidance, careful supervision. His generous contributions and meticulous revisions helped to clarify this study.

My ultimate thanks go to Prof. Dr. **Mamdouh El-Kafrawy**, Professor of Ophthalmology, Faculty of Medicine, Ain Shams University, for his great help and guidance. He taught me and helped so much to accomplish this work.

Many thanks to Prof. Dr. **Isabelle Brunette**, Professor of Ophthalmology, Montreal University, for her valuable assistance and effort in performing my research project.

I would like to express my extreme thanks to **my mother, father and my sister** for their help, patience, care, support understanding and encouragement.

Last but not least, many Thanks to:

- Dr. Michél Podteteney.
- Dr. Jean-Claude Kieffer.
- Dr. Michael Bauschmann.
- Predrag Corluka.
- Nicolas Tran-Ankh.
- Michaela Anca Marian.
- Thouria Bensaoula.
- Solmaz Mogadaszadeh.
- Christophe Bernard.
- Marie-Éve Choronzey.
- Stéphanie Proulx.
- All Eye Bank technicians in Québec Eye Bank – Canada.

For their great help in different stages of my project.

CONTENTS

	Page
Protocol.....	-
Introduction.....	1
Aim of the Work.....	3
Review of literature.....	6
Corneal Anatomy and Physiology.....	6
Endothelial keratoplasty.....	17
Femtosecond laser in corneal surgery	41
Materials and Methods.....	53
Results.....	71
Discussion.....	95
Summary	102
Conclusion	104
Recommendations	105
References	106

LIST OF TABLES

Table No.	Title	Page
(4.1)	All the study parameters	94

LIST OF FIGURES

Figure No.	Title	Page
(2.1)	A histological section of a normal human cornea stained with Heamatoxylin and Eosin.....	6
(2.2)	Location of corneal endothelial metabolic pump and barrier.....	12
(2.3)	Factors that affect barrier functions.....	13
(2.4)	Diagrammatic representation of the posterior lamellar keratoplasty procedure.....	19
(2.5)	Slit-lamp photograph 1 month after posterior lamellar keratoplasty.....	20
(2.6)	The surgical technique of DLEK.....	21
(2.7)	Postoperative photos of DLEK.....	22
(2.8)	Original DSEK technique as described by Melles and colleagues 7,8 for selective endothelial replacement consists of 6 surgical steps.....	25
(2.9)	Slit lamp photo of a DSEK on the first day postoperatively.....	26
(2.10)	Slitlamp photograph of a typical DSEK grft 6 months after surgery.....	26
(2.11)	Scanning electron microscopy at about 350X magnification of paired cadaver eyes.....	27
(2.12)	Slit lamp photograph of donor tissue detachment 1 day after DSEK.	36
(2.13)	Mechanism of corneal femtodissection.....	43
(2.14)	Decreasing tissue bridges by decreasing the distance between laser pulses.....	44

(2.15)	Decreasing tissue bridges by overlapping laser pulses.....	45
(2.16)	Decreasing tissue bridges by the “double pass” technique.	46
(2.17)	Distance between laser pulses and laser pulse energy.....	46
(2.18)	LASIK flap creation with femtosecond laser	49
(2.19)	Schematic diagram of LASIK flap creation with raster pattern, while cornea is flattened by applanating lens.....	49
(2.20)	Schematic diagram of posterior lamellar button creation for deep lamellar endothelial keratoplasty and donor cut in Descemet stripping with automated endothelial keratoplasty.....	50
(2.21)	Some examples of shaped trephination configurations for femtosecond laser–assisted penetrating keratoplasty.	51
(3.1)	Pig eye	54
(3.2)	Haag-Streit international slit lamp model.....	56
(3.3)	Tonovet.....	57
(3.4)	Ultrasound pachymeter instrument Tomey SP 3000.....	58
(3.5)	Ablation setup installed at (Maisonneuve-Rosemont hospital)	60
(3.6)	Sample slit lamp photos.....	60
(3.7)	Optical Coherence Tomography.....	61
(3.8)	Central horizontal and vertical scans.....	62
(3.9)	A) Stereomicroscope; B) Deep laser cut as seen with a stereomicroscope with magnification 12X.....	62
(3.10)	A human globe placed in a plastic holder attached to the mechanical tester by an 8/0 stitch.....	67
(3.11)	Gold coating by resistive thermal evaporation	69

(3.12)	Scanning Electron Microscopy (SEM).....	69
(3.13)	A SEM of a Femtosecond laser cut corneal stroma (Magnification 1000X) with a surface plot performed by Image J software.....	70
(4.1)	Circular pattern of laser ablation not perfectly aligned on the surface of a glass slide	72
(4.2)	Craters are less evident when the plane of ablation is below the surface	73
(4.3)	Laser ablation pattern at the surface of the microscope slide.....	73
(4.4)	Image obtained with 1000X magnification showing debris around the craters.....	73
(4.5)	Random pig eye OCT image	74
(4.6)	Non symmetric cut thickness due to misalignment of the beam.....	74
(4.7)	The example of laser cut not perfectly parallel to endothelium due to alignment	75
(4.8)	OCT image of Laser cut deeper than the attempted depth .	76
(4.9)	Cut thickness becomes thinner as pulse energy is reduced	77
(4.10)	Microcavitation size versus femtosecond laser pulse energy graph	77
(4.11)	The size of microcavitation bubbles as a function of spot spacing.....	78
(4.12)	Cross-sectional view of cornea post-operatively using stereomicroscope	79
(4.13)	Illustration of flap separation using forceps	80
(4.14)	Flap separation using forceps, pulse energy 11.3 μ J for spot separation 2-5 μ m	81

(4.15)	Appearance of tissue bridges as the pulse energy is lowered to 9 μ J	82
(4.16)	Tissue bridges as a result of low pulse energy of 6.8 μ J	82
(4.17)	Flap surface smoothness for constant pulse energy 11.3 μ J, spot separation 2-5 μ m, magnification 50X.....	83
(4.18)	Slit lamp image showing large bubbles formation	84
(4.19)	Flap surface pulse energy 9.1 μ J, spacing 4-6 μ m.....	84
(4.20)	Cooling Vs Ouabaine as a model of stromal hydration of the human cornea over a 48 hours interval.....	86
(4.21)	CCT (U/S) Vs CCT (OCT).....	88
(4.22)	Ablation depth error (%).....	89
(4.23)	Ablation depth error showing values of absolute difference of the actual from the targeted ablation depth...	89
(4.24)	Flap separability	90
(4.25)	Surface roughness.....	91
(4.26)	SEM image of a rough stromal bed with the white arrows pointing to torn tissue briges	91
(4.27)	SEM image of a smooth stromal bed.....	91
(4.28)	A summarized representation of the femtodissection followed by cut evaluation processes	93

LIST OF ABBREVIATIONS

ARVO	: Association for research in Vision and Ophthalmology
BSS	: Balanced Salt Solution
CH	: Central Horizontal
CV	: Central Vertical
DLEK	: Deep Lamellar Endothelial Keratoplasty
DSEK	: Descemets Stripping Endothelial Keratoplasty
DSAEK	: Descemets Stripping Automated Endothelial Keratoplasty
DMEK	: Descemets Membrane Endothelial Keratoplasty
EK	: Endothelial Keratoplasty
EKG	: Endothelial Keratoplasty Group
FS	: Femtosecond
FLEx	: Femtosecond Laser lenticular Extraction
HMDS	: Hexamethyl disilazine
HMR	: Hopital Maisonneure Rosemont
LASIK	: Laser in Situ Keratomilieusis
IOL	: Intra Ocular Lens
IOP	: Intra Ocular Pressure
IP	: Imbibition pressure
OCT	: Optical Coherence Tomography
PK	: Penetrating Keratoplasty
PLK	: Posterior Lamellar Keratoplasty
PLD	: Posterior Lamellar Disk
SEM	: Scanning Electron Microscope
SP	: Swelling pressure

Introduction

Pseudophakic bullous keratopathy and Fuch's endothelial dystrophy are among the leading indications of penetrating keratoplasty (PK). Both diseases share in common an altered endothelial function with progressive failure which ultimately leads to severe and painful keratopathy and blindness. And for many decades, full thickness PK was the only available treatment to restore sight in these eyes (42.4% of all grafts in the US and Canada)^[1]. Recently, Melles et al.^[2] have designed a new surgical technique for posterior lamellar keratoplasty (PLK) that allows replacement of the endothelial layer through a limbal incision along a deep lamellar plane; it consists of manually dissecting the recipient and donor corneas at 80% to 90% stromal depth. Their technique obviates the need for surface corneal incisions or sutures and thus has the potential of virtually eliminating the cardinal problems associated with wound healing. Later, Terry and Ousley^[3], performed deep lamellar endothelial keratoplasty (DLEK), using a modified instrumentation and reported outcomes similar to PLK. Subsequently, Melles reported a technique for stripping Descemet's membrane from the recipient cornea to remove the diseased or dysfunctional endothelial layer, thus eliminating the need to perform the intracorneal lamellar dissection and recipient button excision^[4] called Descemet's stripping with endothelial keratoplasty (DSEK) for manual stripping and automated stripping (DSAEK) in-case of using a mechanical micro-keratome.

In parallel to the progress made in manual/mechanical microsurgery techniques, the advent of ultra short pulsed lasers, such as the femtosecond laser increases our expectations with regards to the precision and least complications in the field of partial thickness keratoplasty. The femtosecond laser allows non thermal laser- tissue interaction; thus cuts can be made with very little thermal and mechanical damage leading to minimal collateral damage^[5]. The high precision of the femtosecond laser effects is certainly related to the fact that the energy threshold for femtosecond optical breakdown is very low^[6].

Although the femtosecond laser is already used for flap creation in lasik surgery and in other fields of ophthalmic surgery as intra-corneal segments implantation^[7], experimental studies in corneal^[8] and glaucoma surgeries^[9], it is still considered in a process of continuous development. More work is needed to optimize the laser settings in order to provide even smoother interface surfaces^[8], to minimize the collateral effects resulting from non-linear absorption, and to improve its focusing and performance in case of diminished corneal transparency^[10].

Justification

Corneal transparency relies heavily on the arrangement of collagen fibers which are the main structural component of corneal stroma. It is well known that there is a local ordering of the fibrils and that distribution of the fibrils is adequately uniform to account for the transparency of the cornea. To maintain this transparency, it is required that the distance between the collagen fibrils are less than one half the wavelength of visible light. In normal corneas, collagen fibres are responsible for a slight light scattering causing the light transmittance through the cornea to decrease slightly as the wavelength decreases from 700 to 400 nm^[11].

This dependence of the corneal transparency on the distribution and size of collagen fibrils is supported by observations of the swollen corneas and by the structure of the opaque sclera. When the epithelial barrier or endothelial barrier of the cornea is damaged, the stroma imbibes water and swells, leading to a loss of corneal transparency. This uptake of water causes corneal stromal hydration causes the formation of “lakes” devoid of collagen fibres within the stroma. This leads to an increase in the distance between collagen fibrils and increased corneal thickness, leading to a wavelength- dependent loss of light transmittance that increases with the amount of corneal swelling^[12].

It was also proved that corneal hydration levels affect the efficiency of laser ablation in LASIK procedures. With less hydrated corneal surfaces, ablation effects were greater than for corneas not blotted during the procedure but these patients appear to undergo greater myopic regression^[13].

Consequently as deep corneal stromal dissection in a pathologically hydrated cornea is an important step of endothelial transplantation surgery (DLEK, DSEK and DSAEK); the behaviour of the femtosecond laser in hydrated corneas needs to be investigated prior to any possible use in these types of deep lamellar corneal surgeries.

Aim of the work:

The main objective of our research work was to optimize the femtosecond laser parameters in order to achieve deep lamellar ablation required for posterior lamellar disk preparation which, in turn, is needed for posterior lamellar corneal surgeries.

As a second objective we investigated the effect of corneal stromal hydration on the femtosecond laser cut qualities, namely: depth precision, flap separability and flap surface smoothness.

Clinical impact:

The clinical use of femtosecond laser in refractive surgery has shown considerable superiority over the mechanical microkeratome not only in the better quality and precision of the cut¹⁰ but only in the final visual outcome in the form of better uncorrected visual acuity and less optical aberrations¹⁴, consequently we expect that success in developing the optimum parameters for the femtosecond laser in clear and oedematous corneas will be a revolutionary step improving the outcome of lamellar corneal surgeries by making it faster, easier, more accurate and predictable and hence, better clinical results.

Our protocol aims at three main objectives:

1. Reaching the optimal laser parameters for deep stromal ablation 320 μm of the total central corneal thickness in clear corneas.
2. Developing a reproducible and controlled model for stromal hydration.
3. Using the previously obtained laser parameters (i.e. in step 1) to reach an ablation depth of 320 μm of the total central corneal thickness, we will study the effect of corneal hydration on the quality of the FSL ablation (depth precision, cut precision, collateral damage, ablation profile).

Materials and Methods:

Exclusion / inclusion criteria:

A total number of 80 post mortem adult pig eyes will be used in this study due to anatomical similarities with the human corneas and 42 postmortem human globes unsuitable for transplantation.

On the day of the experiment, eyes are collected from adult pigs (to obtain eyes nearly of the same size) at the nearest possible slaughterhouse. They are excised together with the lids and extra-ocular muscles in order not to cause any damage to the epithelium, and then transported in a plastic bag together with an ice pack inside a plastic container.

All eyes included in this study should have fully transparent corneas (on enucleation) and no visible corneal pathology as opacity, vascularization, and infiltration (evident by the slit lamp).

Pre-laser study parameters:

A-Slit lamp examination:

- Model: Haag Streit international, BQ900, equipped with a digital camera Sony 3ccD Ex-wave HAD, viewed with a Zeiss visupak program.
- Justification: To exclude any corneal abnormality pre-operatively and to detect the effect of laser post operatively regarding the size of ablation (diffuse illumination low power), size of the bubbles (diffuse illumination high power) and the depth of ablation (slit illumination low and high power).
- Method: The eye is put on a specific metallic arm handle mounted on the slit lamp frame. A central photo with diffuse illumination showing the whole cornea is taken at different degrees of magnification. Slit lamp photos are then with slit illumination in different parts of the cornea and with different degrees of magnification.
- Duration of the exam is 5 minutes.

B-Intra-ocular pressure measurement (IOP):

- Model: Tonovet, TV01, Tiolat Oy, Helsinki, Finland.
- Justification: IOP should be kept between 12 and 22 mmHg by injection of BSS into the vitreous cavity through the optic nerve, as this is the normal range of intra-ocular pressure.

C-Central corneal pachymetry :

- Model: Tomey SP 3000.
- Justification: To measure the corneal thickness which is a main study parameter, we use an ultra-sound pachemeter as it is the conventionally used standard method for pachymetry.
- Method: The pachymetry probe is disinfected with alcohol tampon then dried with a 4X4 inches gauze. The corneal surface is moistened with BSS and the pachymetry

probe is applied to the geometrical centre of the cornea. Three consecutive measurements are obtained and the mean is used to put each eye in its correct group.

- Pre- and post-test (laser) measurements
- Duration of the exam: 5 minutes
- Parameters extracted: Corneal thickness measured in microns.

Post-laser study parameters:

A-Slit lamp examination:

To detect the uniformity of ablation.

B-Central corneal pachymetry :

To compare with pre-laser values

C-Optical coherence tomography:

- Model: OCT III, Carl Zeiss Meditec Inc-Dublin,CA
- Justification: To document the corneal pachymetry, to exclude any major corneal curvature abnormalities and to characterize the ablation profile postoperatively.
- Method: The eye is put on a specific metallic arm handle mount on the OCT frame. The cornea is kept moist by BSS drops. The cornea is scanned in the following scans with the same sequence: central horizontal (CH), central vertical (CV), each scan is 5 mm in length, where one photo is taken for each position.
- Done only Post-Laser
- Duration of the exam: 10 minutes.
- Parameters extracted:
 - The depth of ablation at the centre of the image.
 - Continuity and thickness of the ablation line.
 - The plane of surface ablation regarding its parallelism to the descemet's membrane.

Throughout the procedure, the eye is placed in a special holder and corneal surface is moistened with BSS eye drops unto the entire corneal surface every two minutes to avoid dryness. Furthermore the eye is transported from one apparatus to the next one in a moist chamber.

E-Stereomicroscopy:

- Justification:
 - Detect the precision of alignment.
 - Detect possible collateral damage either direct or indirect secondary to bullae diffusion.
 - Detect the surface smoothness.