# *Collagen Cross Linking in Post Laser In situ Kerato Mileusis (Lasik) Keratectasia*

**Essay**
Submitted for partial fulfillment of
Master Degree in Ophthalmalogy

**By**
***Aziza Ahmed Hassan Aly***
M.B.B., Ch.

**Supervisors**
Prof. Dr. Yehia Mahmoud Salah El-Din
Professor of Ophthalmology
Faculty of Medicine
Cairo University

Prof. Dr. Ibrahim Mohi El-Din Taher
Professor of Ophthalmology
N.I.L.E.S
Cairo University

Faculty of Medicine
Cairo University

2008

# Acknowledgement

First and foremost thanks to ALLAH, the most kind, the most merciful , who gives me every thing and allows this work to be done.

With great pleasure, I'm honored to express my deepest gratitude and thank to my Prof Dr/ Yehia Mahmoud Salah El- Din professor of ophthalmology faculty of medicine, Cairo University for his support, supervision guidance and continuous encouragement. Wards are not enough

I am grateful to Prof Dr/ Ibrahim Mohi El-Din Taher professor of ophthalmology N.I.L.E.S faculty of medicine, Cairo University for unlimited helps, and for his time, efforts that devoted for supervision of this work, honest assistance and cooperation he offered through the study.

Also, I'm more indebted to Prof Dr/Sayed Saif El-Din who was the spiritual father for me, the mercy of ALLAH upon him.

Also I present this work to the soule of Dr/Mai Ahmed Al Ray the mercey of ALLAH upon her.

Finally I'm more indebted and grateful than words can possibly express my feelings to my father, mother and all the members of my family.

# Abstract

Keratectasia is one of the most sever complications after refractive laser surgery. Usually penetrating keratoplasty is the treatment of choice to achieve an optical rehabilitation in such cases. A new technique of collagen cross linking leads to stiffening of the anterior parts of the corneal stroma and increasing the biomechanical stability of the cornea. It is now used in treating post lasik keratectasia by the use of combined riboflavin and ultra violet-A radiation. It arrests the progression of ectasia by increasing the stiffness of the cornea that will be advised prior to lasik. It is a minimally invasive procedure with little side effects.

It is now under trial for infectious keratitis with corneal melting, prevention or reduction of immunological reactions in cases of corneal transplantation.

**Key Word**:

Collagen Cross Linking in Post Laser In Situ Kerato Mileusis Keratectatsia

.

# List of contents


| Title | Page |
|---|---|
| List of abbreviations | II |
| List of figures | IV |
| List of tables | VI |
| Introduction | 1 |
| Anatomy of the cornea | 4 |
| Biochemistry of the cornea | 13 |
| Iatrogenic keratectasia | 20 |
| Collagen crosslinking | 37 |
| Corneal elasticity and crosslinking | 54 |
| Laboratory studies on collagen crosslinking | 57 |
| Summary | 72 |
| References<br>Arabic Summary | 78 |

## List of abbreviations

| | |
|---|---|
| **AGEs** | Advanced glycation end products |
| **BSCVA** | Best spectacle corrected visual acuity |
| **TCA** | Tricarboxylic acid cycle |
| **DNA** | Deoxy ribonucleic acid |
| **TGFB** | Transforming growth factor -B |
| **LASIK** | Laser assisted in situ keratomileusis |
| **IOP** | Intra ocular pressure |
| **RK** | Radial keratotomy |
| **PERK** | Prospective evaluation of Radial keratotomy |
| **RSB** | Residual stromal bed |
| **FFKC** | Forme fruste keratoconus |
| **RGP** | Rigid gas permeable contact lens |
| **INTACS** | Intra stromal corneal segments |
| **UVA** | Ultra violet-A |
| **C3R** | Corneal collagen cross linking Riboflavin |
| **AGES** | Advanced glycation end products |
| **ROS** | Reactive oxygen species |
| **CXL** | Collagen cross linking |
| **BSS** | Balanced salt solution |
| **IRSS** | Italian refractive surgery socitey |
| **UCVA** | Uncorrected visual acuity |
| **MRSE** | Manifest refraction of spherical equivalent |
| **SE** | Spherical equivalent |
| **OCT** | Optical coherent tomography |
| **SD** | Standard deviation |
| **TUNEL** | Terminal deoxynulceotidyl transferase deoxy-UTP-nick-end labeling |
| **LED** | Light-emitting diodes |

# List of figures


| Number | Title | Page |
|---|---|---|
| **Figure 1** | Histological view of the corneal layer | **6** |
| **Figure 2** | Asymmetric bow-tie patterns with skewed steep radial axes above and below the horizontal meridian(AB/SRAX) | **26** |
| **Figure 3** | No skewing | **26** |
| **Figure 4** | Skewed 30 degree from the vertical meridian | **26** |
| **Figure5& 6** | ORBSCAN showed keratectasia after lasik in a 3-D pattern | **27** |
| **Figure 7&8** | Difference in strength between crosslinked& non crosslinked collagen matrix | **27** |
| **Figure 9** | Difference in stiffening effect with preserved curvature between crosslinked & non crosslinked cornea | **39** |
| **Figure 10** | Mechanism of collagen cross-linking using UVA and photosensitizer riboflavin | **40** |
| **Figure 11** | Corneal topography showed 10 diopters of corneal flattening in one patient before and after C3R | **41** |
| **Figure 12** | Two views of the collagen cross-linking machine | **43** |
| **Figure 13** | The energy of the UVA light source is checked prior to treatment | **43** |
| **Figure 14** | UVA:300mw/cm2for 30 minutes | **44** |
| **Figure 15** | Treatment of the central 7mm of the centrally abraded cornea with riboflavin drops and UVA | **44** |
| **Figure 16** | Device used in C3-riboflavine treatment to provide a combined application of UVA and riboflavin | **45** |
| **Figure 17** | Fluoroscopy showed riboflavin absorption | **46** |
| **Figure 18** | Comparison between non disepithelalized and disepithelalized | **47** |
| **Figure 19&20** | Orbscan showed difference between de-depithelized and non - depithelized | **48** |
| **Figure 21** | Stress strain measurements in porcine corneal flaps | **51** |
| **Figure 22** | Cross linking is the introduction of covalent chemical bonds between corneal lamellae perpendicular to tangential tension | **55** |
| **Figure 23** | Depth dependent keratocytes loss according to the irradiance level | **60** |

# List of tables


| Number | Title | Page |
|---|---|---|
| **Table 1** | Pre-operative grading system for detection of patients who are at risk for ectasia | **28** |
| **Table 2** | Residual stromal bed thickness safety index | **29** |

# Introduction

Collagen, which makes up to 71% of the dry weight of the cornea, provides the structural back bone of many tissues (cornea, cartilage, skin….) In the cornea, collagen is present in the subepithelial basement membrane, Bowman's layer, the lamellae of the stroma and Descemet's membrane, Keratectasia is one of the most sever complications after refractive laser surgery, that may need lamellar or penetrating keratoplasty as the treatment of choice (1).

A new technique of collagen cross linking by photo sensitizering riboflavin and UVA similar to photo- polymerization in polymers has been developed by Theo Seiler. (II) Corneal cross linking is a method to mechanically strengthen the cornea by increasing the number of molecular bonds between collagen fibrils, and thus be atherapeutic means to arrest or partially reverse the progression of lasik induced keratectasia.( III)

Physiologically, this process occurs with increasing age. ( IV)

Cross -linking is an out patient procedure requiring a session per eye. The epithelium is partially removed to enable penetration of the then instilled riboflavin (Vitamin B2) and dextran eye drops (10mg of riboflavin -5- phosphate in 10 ml of dextran-T 500 20% solution). Riboflavin is applied every two minutes for half an hour followed by UVA irradiation (370nm) at five minute intervals for 30 minutes. It is critical that the cornea has a minimum thickness of 400µm.If the cornea is thinner, it needs to be swollen by means of hypotonic distilled water or a specifically formulated version of (10 mg of riboflavin -5-phosphate in 10 ml of dextran –T500 20% solution) eye drops is better used. (V)

Respecting this method is important because the function of the Riboflavin is two–fold. For once, it serves as a shield against the applied UVA

light of 370 nm that the eye is exposed to for half an hour (irradiance: 3mw/cm2) with out shielding endothelial damage, induction of cataract and or retinal damage would be possible. Riboflavin's key role is its function as a photo sensitizer. After about three months cross linked cornea tend to become flatter. (VI)

Collagen cross linking makes possible to prevent or reduce immunological reactions and the risk of graft rejection by pretreatment of the donor cornea, ultimately prolonging graft survival.(VII)

Collagen cross linking is a promising option for treating patients with therapy-refractory infectious keratitis to avoid emergency keratoplasty. (VIII)

Collagen cross-Linking is a new modality to prevent or treat the increasingly significant ectasia following corneal laser refractive surgery particularly LASIK.

# Aim of the work

To review the recent literature regarding the use of corneal collagen cross linking as a recent modality in the treatment of cases of post lasik keratectasia.

**(1) Anatomy of the cornea**

# Anatomy of the cornea

The cornea is a transparent avascular tissue with a smooth, convex outer surface and concave inner surface, which resembles a small watch-glass.The main function of the cornea is optical; it forms the principal refractive surface, accounting for 70% (40- 45diopters) of the total refractive power of the eye. Refractive requirements are met by the regular anterior curvature of the cornea and the optically smooth quality of the overlying tear film. The resistance of the cornea is due to the collagenous components of the stroma, which provides a protective layer of the cornea. (1) Transparency of the cornea is achieved by the regularity and fineness of its collagen fibrils, the closeness and homogeneity of their packing.Water is constantly pumped out of the cornea by its posterior layer, (the endothelium). This maintains the optical homogeneity of the corneal layers and prevents swelling and clouding. (2)

The cornea is thus an evolutionary compromise, being a multicomponent, thick, tough avascular tissue with a smooth surface and uniform curvature. (1)

## Dimensions

In front the cornea appears elliptical, being 11.7mm wide in horizontal meridian and 10.6mm in the vertical in adults.The posterior surface of the cornea appears circular, about 11.7mm in diameter, this difference is due to the overlap of sclera and conjunctiva above and below than laterally.The axial thickness of the cornea is 0.52mm with a peripheral thickness of the cornea is 0.67mm. (1)

The cornea formes part of what is almost a sphere, but it is usually more curved in the vertical than the horizontal meridian, giving rise to astigmatism (with the rule) in its central third, the optical zone, the radius of curvature of the

anterior surface is 7.8mm and that of the posterior 6.5mm in adult males. The natural and normal cornea is generally prolate, with steeper curvature centrally and relatively flatter peripherally. (1)

## Surface zones of the cornea

The corneal surface can be divided in to four anatomical zones:

1-Central zone: also called optical zone of the cornea, it is2.4mm in diameter and overlies the entrance of the pupil where it represents the most spherical area of the cornea and determines the high-resolution image formation on the fovea. (3)

2-Para central zone: also called mid, intermediate or mid peripheral zone, it is 6-8 mm in diameter. (3)

3-Peripheral zone: also called transitional zone, it is 7-11 mm in diameter. (3)

4-Limbal zone: it is 11.5-12 mm in diameter; it is the ring of cornea about 0.5 mm wide that contains the capillary arcade and stem cells. (3)

## Structure of the cornea

Behind the precorneal tear film are five tissue layers:

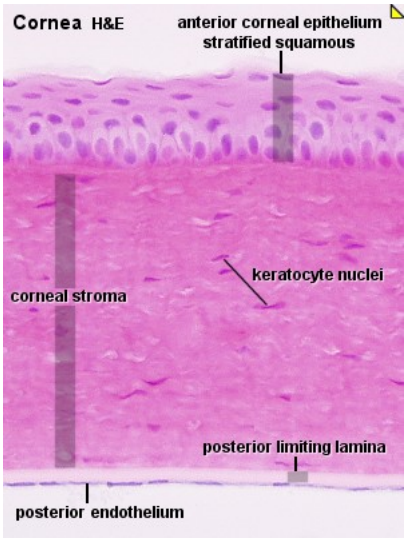
1-Epithelium

2-Bowman`s layer

3-Stroma

4-Descemet`s membrane

5-Endothelium



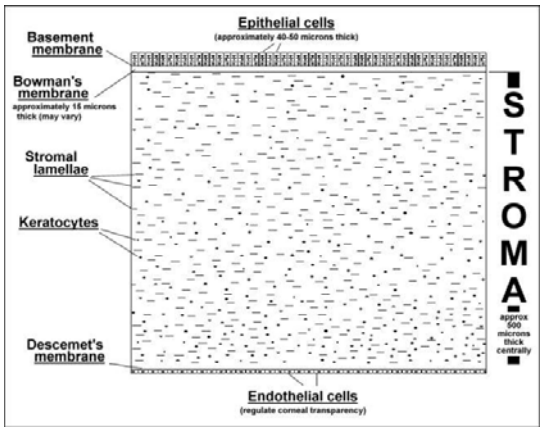




Figure (1) Histological view of the corneal layer

Br, j opthalmo.2001 (4)

## 1-Epithelium

The corneal epithelium is stratified, squamous and none keratinized. It is continuous with that of the conjunctiva at the corneal limbus, but differs in possessing no goblet cells. The epithelium is 50-90µm, thick and consists of five or six layers of nucleated cells. Thickness has been measured accurately by high-frequency ultrasound at 50.7 µm. (5)

The deepest of these, the basal cells, arranged in a palisad like manner in perfect alignment on a basal lamina. They form the germinative layer of the epithelium, continuous peripherally with that of the limbus. These basal cells are columinar (10 µm wide and 15 µm tall), with flat bases and rounded heads, each nucleus is oval and orinted parallel to the long axis of the cells. (6)

The second epithelial layer (the wing or umbrella cells) consists of polyhedral cells, convex anteriorly, which cap the basal cells and send processes between them, the long axes of their oval nuclei are parallel to the corneal surface. (7)

The next two or three layers are also polyhedral and become wider and increasingly flattened towards the surface. The surface cells have the largest surface area and this is greater in periphery (e.g. 850 µm above) compared to (560 µm centrally).The most superficial cells may be as wide as 50 µm and 4 µm in depth; they retain their nuclei and do not show keratinization. Their flattened nuclei project backwards leaving the surface perfectly smooth. (8)

The most superficial cells of the epithelium are mostly hexagonal and firmly attached to each other at relatively straight cell boundaries; they exhibit surface microvilli or microplicae. (8)

The plasma membranes of contiguous cells interdigitate with their neighbours with an intervening space of no more than 20 nm. Adhesion is achieved by numerous desmosomes; they are sparse between the superficial and wing cells.The basal cells are connected to one another by desmosomes and to the underlying basal lamina by hemidesmosomes (8).

The entry of water and small molecules from the stroma is not restricted, which permits the occurrence of epithelial oedema, with widening of the intercellular spaces, for example when the endothelial pump fails. (9)

Basal lamina:

The basal lamina is secreted by the basal cells, which also synthesize the hemidesmosomal strctures concerned in attachment of epithelium to the lamina.The basal lamina is an irregular zone (0.5- 1µm wide)of granulomorphous and filamentary material. A deep osmophilic lamina densa (30-60nm) and a superficial lamina lucida (24nm) are distinguished ultrastructurally. (10)

It is traversed by electron-dense anchoring fibrils which form narrow bundles insert into the subjacent stroma or bowmans layer and terminating in anchoring

plaques.This arrangement accounts for the tight adherence of the basal epithelium to subjacent cornea. (11)

## 2-Bowman`s layer (anterior limiting lamina)

Before electron microscopy was developed, Bowman`s layer was thought to be a specialized corneal membrane, but it is now described as a modified region of the anterior stroma. (1)

Bowman`s layer is a narrow, a cellular homogeneous zone (8-14 μm) thick, immediately subjacent to the basal lamina of the corneal epithelium. The anterior surface is smooth and parallel to that of the cornea; though sharply defined from the overlying epithelium anteriorly it is infiltrated by the lamina densa and merges into the stroma behind.The perimeter of Bowman`s layer, which has a rounded border, delineates the anterior junction between cornea and limbus and is marked clinically by summits of the marginal arcades of the limbal capillaries. (1)

Ultrastructurally Bowman`s layer consists of a meshwork of fine collagen fibrils of uniform size, lying in a ground substance. Fibril diameter (24-27nm) is less than that of substantia propria. In the posterior region of this layer the fibrils become progressively more orderly in their orientation, blending and interweaving with the fibrils of the anterior stroma. Here and there anteriorly, bundles of the stromal lamella insert into the Bowman`s layer. The compacted arrangement of the collagen confers great strength, to this zone. Bowman`s layer is relatively resistant to trauma, both mechanical and infective; once destroyed it is not renewed but is replaced by a coarse scar tissue. It is perforated in many places by unmyelinated nerves in transit to the corneal epithelium. (12)