CORNEAL WELDING VIA INFRARED LASERS: IN VITRO & IN VIVO STUDIES

by

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ABSTRACT

CORNEAL WELDING VIA INFRARED LASERS: IN VITRO & IN VIVO STUDIES

Infrared lasers can be used to weld soft tissues. Water molecules and also protein molecules such as collagen absorb the infrared energy and a temperature gradient can be created at the application site. Objective of this PhD thesis is to investigate the potential of infrared lasers for welding tissue to contact lens and also for cornea welding in order to seal corneal cuts done during cataract surgery. One of the new application in the field is our study about amniotic membrane welded to contact lens by 1470-nm diode laser: a novel method for sutureless amniotic membrane transplantation. This study showed a new method for laser welding of a tissue to contact lens for ophthalmologic application. Corneal welding is rather a new application area in laser medicine, and few studies reported successful welding dose for different infrared wavelengths. Full thickness, one-plane 3.2 mm long clear corneal cuts were done using a pre-calibrated knife. Laser power and irradiation duration were the parameters used and histological indicators of photothermal effect were observed. In the field of corneal laser welding we made experiments which 809-nm, 908-nm, 1070-nm and 1980-nm laser used to weld clear corneal incisions. According to these in-vitro studies and their histologic results, another experiment was planned to compare 1070-nm and 1890-nm wavelengths which we found the best results in previous studies. In this thesis, *in vitro* cornea laser welding experiments were performed also with 1470-nm diode laser which have high water absorption. According to preliminary results; in-vivo rabbit study was planned with the best two energy parameters options of 1470-nm diod laser which we had with the in-vitro study. Full thickness, one-plane 3.2 mm long clear corneal cuts were done using a pre-calibrated knife under anesthesia. After irradiation, rabbit cornea's were observed in postoperative first day, first week, second week and forth week. In this thesis it is possible to find a wide range of studies and their results about laser welding. In the light of the findings of these studies it may be predicted that laser welding applications will have much more place in all biomedical applications.

Keywords: clear corneal cut, infrared lasers, laser tissue welding, rabbit

ÖZET

KIZILÖTESİ LASERLER İLE KORNEAL DOKU KAYNAĞI: IN VITRO & IN VIVO ÇALIŞMALAR

Kızılötesi laserler yumuşak dokuların yapıştırılması için kullanılabilir. Su molekülleri ve kollajen gibi protein molekülleri kızılötesi enerjiyi absorbe edebilir ve uygulama alnında ısı farkı oluşturularak etki sağlanalabilir. Bu doktora tezinin amacı kızılötesi laserlerin doku – kontakt lens kaynağında ve katarakt ameliyatında yapılan korneal kesilerin kapatılmasında kornea doku kaynağının potansiyelini araştırmaktır. Laser kaynağı konusunda yeni bir uygulama olan çalışmamızda 1470-nm laser ile amniyotik membran – kontakt lens kaynağı: amniyotik membran transplantasyonu için dikiş gerektirmeyen yeni bir metoddur. Bu çalışma oftalmolojik uygulamalarda yeni bir metodu dokunun kontakt lense laser ile kaynak yapılması metodunu sunmuş oldu. Kornea doku kaynağı laser işlemleri arasında nispeten yeni bir uygulamadır, değişik dalga boylarında kızılötesi laserler ile başarılı doku kaynağı rapor eden az sayıda makale vardır. Deneylerimizde kalibre edilmiş bıçak ile korneada tam kat tek plan 3.2mm genişliğinde kesiler yapıldı. Laser gücü, laser süresi incelenen parametrelerdi ve histolojik belirleyici olan fototermal etkiler incelendi. Korneal laser doku kaynağı alanında 809-nm, 908-nm, 1070-nm and 1980-nm laser ile deneyler yapıldı. Bu in-vitro deneylerin sonuçlarına gore elde ettiğimiz sonuçlar arasında en iyi iki sonuç aldığımız 1070-nm ve 1890-nm laseri karşılaştırmak için yeni bir deney planlandı ve çalışma yapıldı. Bu tezde ayrıca suyun absorbe etme seviyesi yüksek olan 1470-nm laser ile de in-vitro kornea laser doku kaynağı deneyleri yapıldı. In-vitro çalışmada en iyi sonuçları elde ettiğimiz iki güç ve süre seçeneği seçildi ve 1470-nm diyot laser ile in-vivo tavşan çalışması planlandı. Anestezi altında kornea kesisi kalibre edilmiş tam kat 3.2mm genişliğinde bıçak ile yapıldı. Laser uygulamasından sonra tavşan korneaları birinci gün, birinci hafta, ikinci hafta ve dördüncü hafta takip edildi. Bu tezde laser kaynağı konusunda birçok çalışmayı ve bu çalışmaların sonuçlarını birarada görme imkanı sağladı. Bu sonuçlar ışığında laser kaynağının biyomedikal uygulamalar arasında ileride daha fazla yer alabileceği öngörüsü yapılabilir.

Anahtar Sözcükler: korneal kesi, kızılötesi laserler, laser doku yapıştırılması, tavşan

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LIST OF ABBREVIATIONS

A	Ampere
AM	Amniotic membrane
AMD	Age-related macular degeneration
CL	Contact lens
CW	Continuous wave
H&E	Hematoxylin and eosin
HF	Hydrogen floride
ICG	Indocyanine-green
LASIK	Laser insitu keratomileusis
LATS	Laser-assisted tissue sealing
LTK	Laser thermal keratoplasty
µm	Micrometer
mw	Milliwatt
mm	Millimeter
nm	Nanometer
NIR	Near-infrared
PDT	Photodynamic therapy
PRK	Photorefractive keratectomy
Sec.	Second
S.C.	Subcutan
TEM	Transmission electron microscopy
YAG	Yittrium Aliminium Garnet
YLF	Yttrium Lithium Fluoride

LIST OF SYMBOLS

CO_2	Carbon dioxide
^oC	Degree celcius
Tm: YAP	Thulium yttrium aliminium perovskite
Tm^{3+}: $YAlO_3$	Thulium yttrium aliminium perovskite
w/w	Mass of a substance/total mass of a solution or mixture

1. INTRODUCTION

1.1.Motivation and Objective

The use of lasers in treating a wide spectrum of illnesses including front and back layers of the eye is provoked by a rising realization of laser-tissue interferences in ophthalmology. As knowledge of operating characteristics and curation limitations of lasers rises, as a result the use of lasers in the treatment of other ophthalmologic issues will be increased. Even though it has serious risks, clear corneal and not permanent incisions has increased their popularity. (Figure 1.1. – 1.2). Having not enough suture-induced astigmatism, having no conjunctival trauma, in other wordsfewer uncomfortableness and bleeding, and faster visual recovery are advantages of leaving corneal cut sutureless [1,2]. A growing biotechnology, Laser tissue welding, is up and coming for the applications in almost every operations. [3]. Energy that comes from the laser beam is utilized by laser tissue welding to connect or stick the tissues. The absorved laser power is able to generate variences in the molecular mould of the fibers to be the reason of soldering in between close tissue moulds. As the laser fiber welding period has no contact and unmechanized procedure, with the events where stiching and pricking is challenging it is best used. With the help of corneal tissue welding, closure of corneal incisions are impermiable and the thickness is full. By using this technique, there will be gain of potential advantages. Surgical techniques will be simplier and the intervention time will reduce and there will be suppress with suture materials, thereby foreign body reaction and less postoperative intracular infection. After astigmatism operation control and reduction. Across the wound in many tissues, welding enables leukocytic and fibroblastic filtration on an equal basis. Enzymatic degradation of wrecked tissue and de novo collagen synthesis are some of those tissues across the wound. Consequently, with the help of welding, the stimulation of homogenous wound healing occurred and it is concluded that could reduce the postoperative astigmatism. It brings on faster healing and quicker wound closure.

Figure 1.1. Clear corneal incision [4]

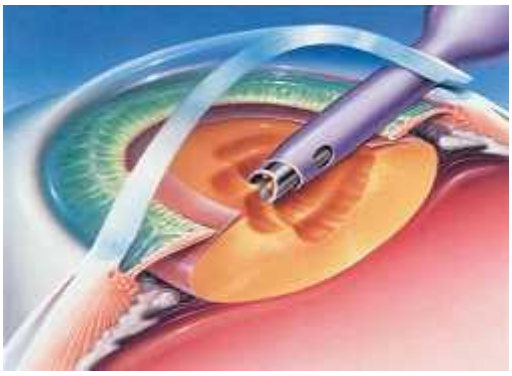


Figure 1.2. Cataract surgery [5]

The goal of this recommended thesis is to search for:

- an unusual way for amniotic pellicle graft without suture; Contact lens and amniotic membrane were bound to each other by 1470-nm diode laser.
- the main parameters of corneal welding with infrared lasers (809nm, 980nm,1070nm, 1470nm, 1980nm)
- the effects of 1470-nm laser cornea welding *in vivo* during laser procedure and during postoperative one-month follow-up.
- the observation of leakage in the welded corneal incision of rabbits *in vivo*.

1.2.Outline

By writing this thesis, I have provided thorough background of the cornea anatomy and histology which are followed by laser welding experiments; *in-vitro* and *in-vivo* study in rabbit eyes was carried out.

Chapter 2 provides information on applications of lasers in ophthalmology, cornea anatomy and histology, laser tissue welding and corneal laser welding.

Chapter 3 explains our study; by 1470-nm diode laser, amniotic membrane and contact lens were welded to each other: an unusual way for amniotic pellicle graf without suture. Amniotic membrane connected to contact lens with 1470-nm diode laser: a genuine way for amniotic pellicle graft without suture.

Chapter 4 shows our in-vitro studies and their histologic results of corneal tissue welding with 809nm, 908nm, 1070nm and 1890 nm lasers. According to the results of 809-

nm, 908-nm, 1070-nm and 1890-nm laser irridiation, another study was planned to compare 1070-nm and 1890-nm wavelengths which we found the best results in previous studies. These experiments were presented in the MSc thesis of Rifat Rasier were added to this PhD thesis to show and compare all of our study results about laser welding.

Chapter 9 shows the 1470-nm laser cornea welding results of in-vitro bovine eye and in-vivo rabbit eye experiments.

Finally Chapter 10 and Chapter 11 provides a discussion on the works done. In the discussion section firstly it discusses corneal laser welding experiments with 809nm, 908nm, 1070nm and 1890nm. Secondly in-vitro and in-vivo cornea welding results of 1470-nm and compares the results with other studies. It provides recommendations for future work in corneal laser welding with 1470-nm laser which has a specific water absorption. Lastly it discusses one of the new application method of laser welding is tissue and material welding. In our 1470-nm contact lens-amniotic membrane welding study the objective was to succeed the achievment of the conventional AMT with the help of simplicity of applying a contact lens.

2. BACKGROUND

2.1.Anatomy of The Eye

The eyes contain many layers:

In the front part of the eye, there is cornea and this transparent external spherical membrane covers the pupil and the iris also. This is the initial and the most significant refractive tissue of the eye. Additionally it helps the lively image producing and crystalline lens at retinal photoreceptor.

The eyes color of ours are given by Iris that is a nicely pigmented colored circular muscle. Melanocytes generates the color that is based on variable quantity of eumelanin (black and brown melanins) and pheomelanin (red and yellow melanins. The size of the pupil controled by this circular muscle in order that is more or less light if the conditions aresuitable to enter the eye. This cyclical muscle goes over the pupil's dimension hence little or much light, subjected to the circumstances is permitted to go into the eye.

Pupil is a black-looking apreture and, due to the absorbing pigments in the retina, it looks dark. Light enter to the eye by allowence of the pupil. transparent jelly-like proteinaceous material is used to make convew lens that is the crystalline lens.

The inner back area of the eye ball is called retina and it is a half transparent membrane which is ligt sensitive and is equivalent to the screen of a camera.

Many structures can be easily seen in case looking into someone's eyes:

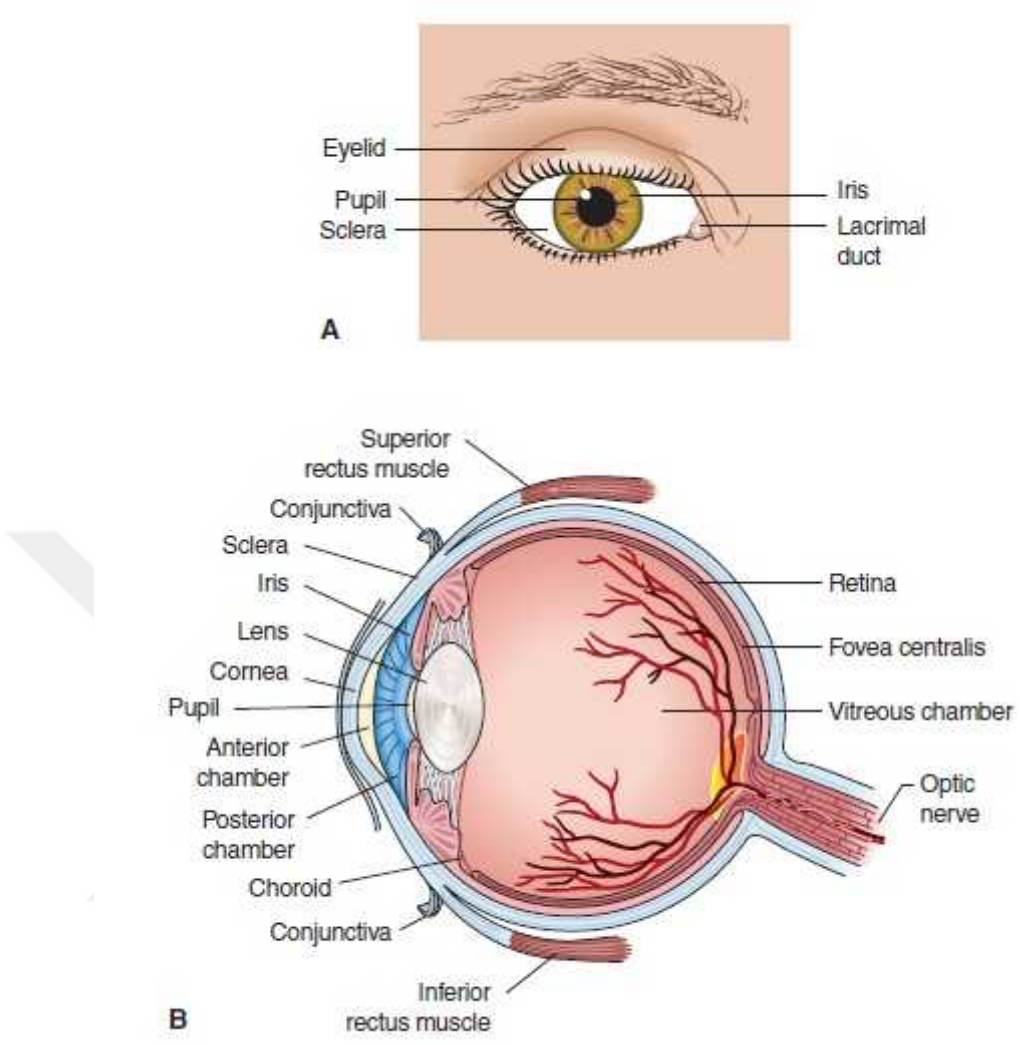


Figure 2.1. A. Picture of a human eye **B.** [6] Anatomy of eye

Through the crystalline tissue light comes in, similar to the camera glass, cornea is a transparent cover..(Figure 2.2) The pupil controls how much light comes in to the eye and it is like an opening which opens and closes similar to the camera. At the eye's back part, a range of cells that are fragile to the light line where the light focuses on the retina. By changing light into electrical impulses and sending a record of it with the help of optic nerve to the brain, layer retina acts like camera film.

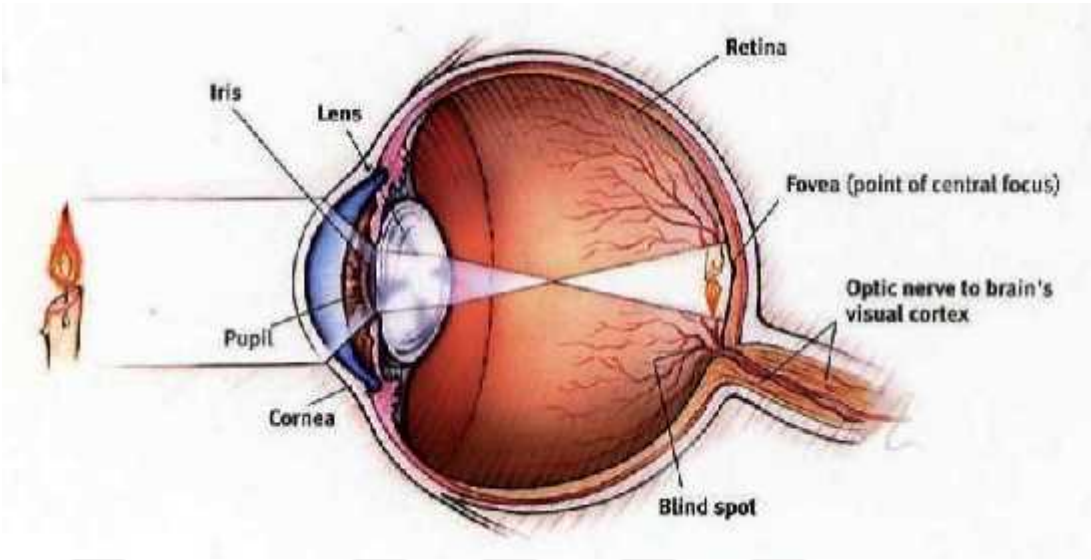


Figure 2.2. Focusing Light rays [7]

2.2.Laser

Laser is the shortened version of Light Amplification by Stimulated Emission of Radiation. An active medium in a resonant cavity with two mirrors at the opposite ends forms the fundamental laser cavity. One of the mirrors which is transmissive allows partial transmission of laser light out of the laser cavity. A pump source introduces energy in to the active medium and excites a number of atoms. In this manner reinforced, compatible and aligned light power is let go as laser power by the mirror that partly conveys to the aimed tissue. The various lasers differ mainly in the characteristic of active medium and the way this active medium is pumped.

Tissue effects of laser:

1) Ionizing effect:
   - Photodisruption
2) Photochemical effect
   - Photoablation
   - Photoradiation
3) Photothermal effect:
   - Photocoagulation
   - Photovaporization

2.3.Lasers in Ophthalmology

Procedure	Commonly used lasers	Wavelength	Power
Laser photocoagulation	Argon ion laser	514.5 nm	0.05-0.2W
Laser photocoagulation	Laser diode	810 nm	2W
Laser stand for therm. keratoplasty (LTK)	Ho: Yag L.	2.1 μm	20 mJ
Laser-Assisted In Situ Keratomileusis (L.A.S.I.K)	ArF excimer laser	193 nm	50-250mJ
Femtosecond LASIK flap creation	Infrared laser	1053 nm	1 μJ

Table 2.1. Laser applications in opthalmology

Lasers in ophthalmology will be classified according to effect:

1) Q-switched neodymium YAG laser effect the targeted tissue by ionizing effect which is achieved with very short duration, high-power laser pulses. No matter if it is transparent or opaque, the effect of it is non-thermal and it leads to deterioration of any target tissue. Posterior laser capsulotomy is the application of this effect in ophthalmology. (Figure 2.3.- 2.4.)

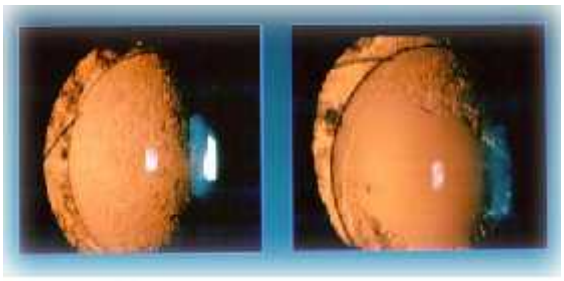


Figure 2.3. Posterior capsulotomy [8]

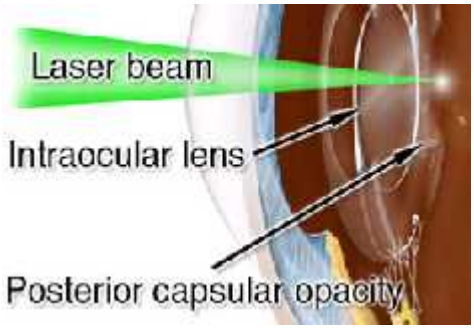


Figure 2.4. Posterior capsulotomy [9]

Femtosecond laser flap formation: Production of photodistraction or photoionization impact of transparent tissue, for instance cornea and the Nd: YAG laser processed by the same effect, is how the Femtosecond laser works.[10] Femtosecond laser as well as Nd: Y.A.G laser application culminate in production of a bunch of independent electrons which is expanding quickly and ionized molecules. The result is disruption of the targeted tissue with the effect of the acoustic shock wave.[11] However, differ a considerably effect in the amount of collateral damage which they provoke and the pulse duration. While femtosecond laser has pulse continuity in the femtosecond differs (10-15 second.), Nd: Y.A.G laser also has a pulse continuity in nanosecond between (10-9 second.) Nd: Y.A.G laser is 106 times more than collateral damage with FS laser, decreasing the pulse duration minimises the collateral tissue damage amount. [12,13] This makes Nd: Y.A.G laser is less safer than femtosecond laser for corneal surgeries usage that needs exquisite and less damage. Femtosecond laser flap formation is the application in ophthalmology.(Figure 2.5.)

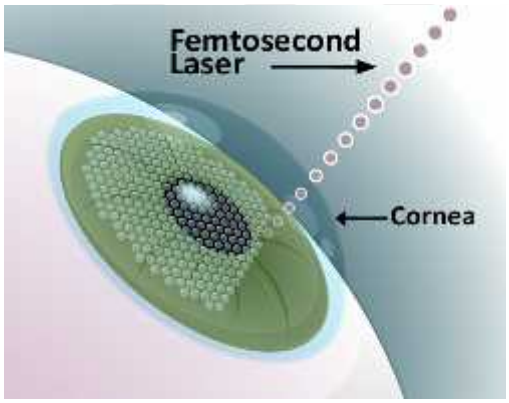


Figure 2.5. Femtosecond laser flap formation [14]

2) Photochemical effect: Laser refractive surgery is the photoablation application. Cellular structure is broken directly by this effect. A 193 nm high UV radiation performs it. Refractive surgery is a rapidly expanding area in the correction of refractive errors, with the usage of excimer laser there was a huge step forward and is now well established. For myopia correction (in other words near-sightedness), lasers are used and now techniques used for correction of hyperopia(far-sightedness): photo refractive keratectomy (P.R.K) and laser insitukeratomileusis (L.A.S.I.K) (Figure 2.6. - 2.7.). By taking out more tissue from the acentric area of cornea or center of cornea above mentioned methods, a intensive laser ray forms the cornea. For myopia, by flattening the cornea the eye focus moves

farther back toward its desired focus of the eye acts far back to its aimed point on retina which fixes the seeing. In PRK, the cornea's epithelial layer is taken out by a mechanic (soften brush) or chemicalized (alcoholic) ways or by the use of laser ray (trans epithelial ablation) instead of a flap creation. After that, for ablating and reshaping the cornea, laser beam is used. In L.A.S.I.K, the opthalmologist makes superior or temporal collapsible cornea clap whose thickness is rougly 90-120 μm by using a microkeratome which is a privatized cutting edge fixed on an underpressure tool. Afterwards, microkeratome is removed, flap opened, consequently exposing the underlying stroma layer of corneal tissue to excimer laser the wanted degree ablation. In the end, it was achieved to return the corneal flap to its original position. Another method is laser thermal keratoplasty (LTK) which is an older technique. To shrink the peripheral zone of the cornea, the laser is used, with LTK central . It makes useful the concentric application circles of laser power to warm up the peripheric cornea and to draw up its center warp.

Figure 2.6. PRK [15]

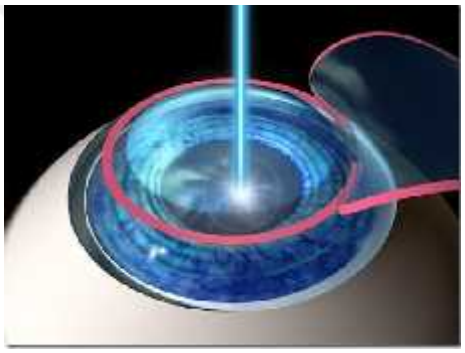


Figure 2.7. LASIK [16]

For macular degeneration which related to age, Photodynamic therapy (PDT): PDT effect is photochemical and a verteporfin, which is a drug that fragile to light that is administered into the circulation of the blood. Photochemical reactions which are mediated via the interaction of agents which are sensitive to lights, oxygen and lights are involved in PDT. Curation for wet- macular degeneration relevant to age (wet-A.M.D) is PDT. For treating the dry AMD, it is not used. The medicine gathers in the not normal choroidal reg below macula after it was administered. By activating the agent, laser light makes it to create blood coagulations and it tampon the anti normal vessel under the macula.

3) Photothermal effect : The thermal effect of, According to the wavelenghts choice (absorption strenght) a mono chromatic laser ray manifests thereby laser's duration ray irradiance. There are two significant parameters that highest worth of the tissue warmth that come up to and sprawl of the tissue's thermal effect region. Inside a fiber, warming of an area may provoke four impressions (Figure 2.8.):

1)Hyperthermia

2) Coagulation

3) Volatilization

4) Carbonization

Hypertermia corresponds to temperatures of 41ºC to 44ºC for some tens of minutes which is a moderate rise in temperature, and it results in death of cell because of the alterations in enzymatic processes. Coagulation equal to temperature reached 60º-100º C C for in about a second, it produces blanching, and by denuturation of proteins and collagen, a shrinking of the tissues occurs. Coagulation means to an irrevocable necrosis with no urgent tissue wrecking. Volatilization corresponds to temperature 100º C in one tenth of a second which is a relatively short time. The various constituents of tissue fade away in smoke at above 100º C, and that results material loss. When the local temperature of a tissue reaches 100º C, water becomes steam by producing tissue thermal ablation (photo thermal ablation). Because of steam formation, this tissue is produced by the pressure build up and is a purely thermo mechanical effect. At the edges of the targeted tissue volatilization zone a stepped transition happens between the volatilization and healthy zones. At the edge there is coagulation necrosis region and then the healthy zone there is a gradual transition between the volatilization and healthy zones. In case the warmth of the tissue goes up to 150º C, carbonization happens, where fibers charts, transform into carbon. We should avoid this process as it causes an irrecoverable tissue damage.

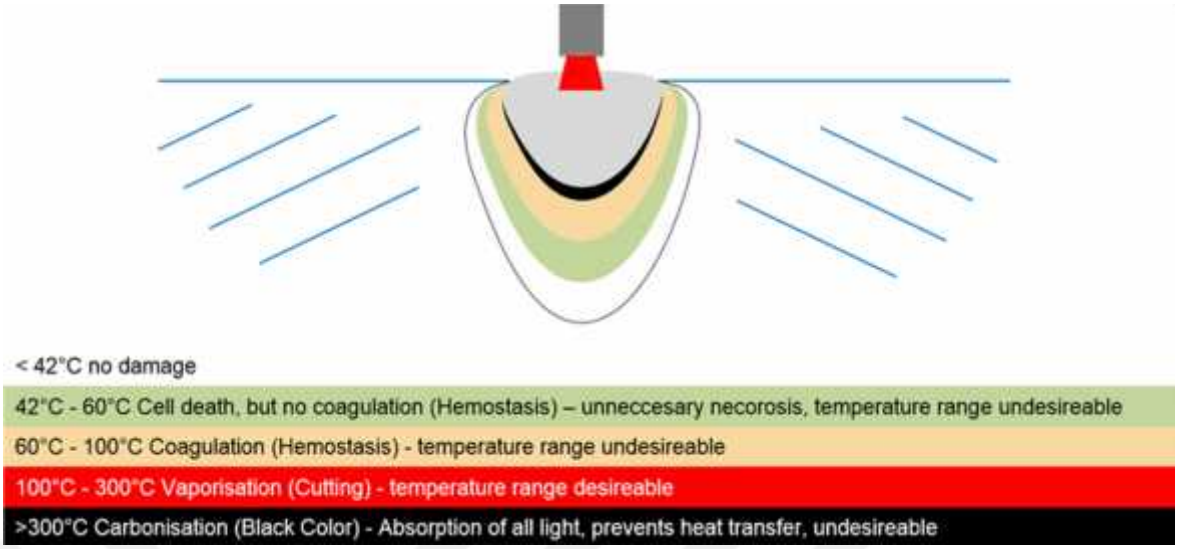


Figure 2.8. Photothermal effect [17]

Photocoagulation techniques is a technique that uses the thermal effect, The absorption chracteristics of the targeted tissues to be coagulated should be used while choosing the wavelenght of the laser. Inside the eyes, there are 3 significant light suckings called melanin, hemoglobin and xanthophyll. Just like a light comes form the sun which is focused with a magnifying piece of glass will burn a paper, lasers are able to do a thermal effect just as a burn. A scar or hole in the target tissue can be a result of this thermal effect.

Glaucoma is one of the illnesses for the ophthalmological applications of photothermal effect for anterior segment. The most significant absorber is the melanin in the iris and in the trabecular meshwork, the second most significant absorber is hemoglobin in blood in the eye. (Figure 2.7.). By generating a canal in iris forms or by depreciation of effusion fibers, called trabecular meshwork, Glaucoma can be cured. This application causes to lowering of eye pressure with the help of laser.

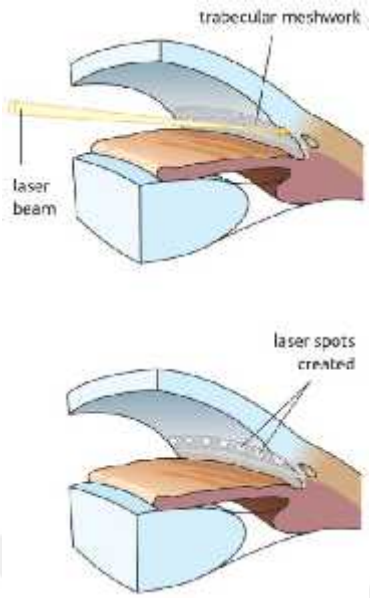


Figure 2.9. Laser treatment for glaucoma [18]

In retina, hemoglobin and xanthophyll are the most significant absorbers in retina. Apparent or close infra red ray usage lenghts of wave to heal eye disorders. Samples are: 1) due to the leaking microaneurysms diabetic retino pathy connected to vas capulare not perfusion or tumefaction. 2-) Retinal hemorrhage, ischemia, and swelling caused by retinal vessel blockage that closes occular blood defection. 3-) macular degeneration that related to age ,which, invading into the normal retine by the wet type choroidal vessels and it is causes macular edema and hemorrhage. 4) After a cataract operation, after an eye insult retinal teardrops can occur as a complication trauma.

Figure 2.10. Laser treatment for laser diabetic retinopathy [19]

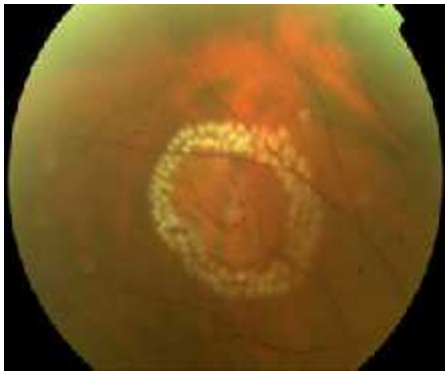


Figure 2.11. Retinal tear surrounded with laser spots[20]

2.4. Laser Tissue Welding

As well as being used as a therapeutic tool, it has ben cleared that rays are very useful in several medicinal and chirurgical implementations. Promising for all type of surgical applications, laser tissue welding is seen as a developing biotechnology. [21]. Because of the laser, forced alterations inside the target fiber protein is special attention for the compounding of many fibers. To create a bond between the 2 adjasent points proteins within the target tissue are coagulated. It is best used for the cases where stapling and suturling is difficult the laser fiber-bonding procedure is no contacted and not mechanic way. To produce strong welding and minimum thermal injury is the main chirurgical necessities for fiber bonding. The fundamental objectives in order to get strong bonding area with minimum thermal energy, not high laser power use as well as decreased power sucking to minimize the forced effect. First demonstration of laser tissue welding was a study that utilized N.d:Y.A.G laser light for blocking mouse's vessels [22]. Following searchings claimed laser coaction might be utilized to warm up a fiber adequaetly to unnature collagens in the fiber faces to structure a novice bonding moulds. [23, 24]. Previous searches of ray fiber welding CO2 rays were used. Absorbing strongly at its wavelength, the use of C.O.2 ray depended on liquid that the biggest component of many fibers.(10.6μm). Argonion and N.d:Y.A.G are different rays that are used for ray fiber bonding, has some advantages compare to CO_2 laser usage which are hollower and drabber fiber warming up product. Pulsed lasers can reduce collateral thermal damage. Parameters which are connected to laser wavelenghts choice and exposure parameters such as energy, pulse duration, etc. are the fiber absorb, visional diffusion deepen, and the forced relaxaing period in the fibers to provide uniform heating visional diffusion deepen obviously must be coupled with size of the bonded dodism.

2.4.1.Procedure of Laser Tissue Bonding

Aim for utilizing energy for laser tissue welding from ray to connect or compound fibers. Changes in molecular structure of the target tissue because of the absorbed laser energy can induce soldering between neighboring tissue structures. There are many procedures for laser fiber bonding that was suggested as collagen-to-collagen fusion, the tissue proteins of equivalent cruciate-ligamenting, fiber proteins' unnaturation, expediting

of connatural fibrinogen poly merization, forming non covalent welding inbetween collagen molecules, and interdigitation as well as collagen fibers' bending similar to rope.[25-27]

In course of welding, power of laser is sucked by liquid in fiber with wavelength 1470-nm that used in this thesis and afterwards warms up the collagen helix. The time the collagen tissue temperature goes up to 60ºC, 2 welding is hindered and partly dispersal occurs. Afterwards, equivalent and/or non-equivalent welding of fiber protein molecules like the fiber happens. Collagen bonding which contains fiber at not high heat quite probably happens because of a stylistic alteration in disaggregated collagen fibers, inter digitation and interchange of mentioned tissues [28]. Along with proteins' denaturation and random renaturation, some other mechanisms may have proteing cross linking also. Fibrine poly merization can show a performance like equivalent cruciate ligament in extra cellular matrix proteins [29]. As a result, laser radiation cause photothermal activation of the stromal collagen, resulting welding effect, welding effect that creates a fast which makes seals of wound edges and good mechanical strength. To produce strong welding and minimum thermal injury is the main surgical requirements for tissue welding. It is essential to understand the underlying molecular mechanisms to enhance the welding process and reduce collateral best use of the bonding period and make it minimum the cognate fiber harm like molecul unnaturation, flexion and spaces in the bond. To achieve an accomplished welding, there must be an accurate checking for ray energy and exposing periods to control the warmth of the fiber liquid loss. By way in overtone vibrational absorption, the key molecule in welding is water.

The crucial factors of the ray extraction arguments' choosings are; along with length of wave and power dosage, tissue's principal absorber which is targeted by laser radiation. Previous reported experiments have been relied on the water usage as an endogenous choromophore in order to absorb laser light as the main component of lots of biological tissue is water. CO2, erbium: Y.A.G(2940-nm) as well as diode rays are some of the laser types with lenghts of waves that shows high visual absorb in liquid and they were utilized for this task like CO_2 (10.6 μm), holmium:YAG (2100-nm), erbium: Y.A.G (2940-nm), as well as diode rays. In order to reduce different absorbs inbetween the colored area and the surrounding fibers, ICG is prevalently used in fiber bonding or ray plumbing as a cromophore. [30, 31) Above mentioned color is symbolized by eminent visual absorb

about 800 nm hence it is used in relation of with 800 nm laser radiation to the cornea injury that would be fixed.

2.4.2. Approaches for Tissue Bonding

The 3 dealings utilized for fiber bonding;

1) direct bonding of fibers
2) ray brazing
3) color-increased brazing

Unmediated tissue laser bonding usages, are the three approaches that are used for tissue welding. During this interaction lasers heat endogenous components, like water to disrupt bonding, partial dissociation and coagulate tissue proteins with direct laser beam. Local heating on the target tissue is around 60°C with the help of ray power absorp to unnature collagen, revealing three decker indigenous spiral form and by production of collagenious bond.

The ray brazing utilizes a chromophore-enhanced protein solder which is augment the welding procedure. When it is compared to the direct welding the laser soldering provides stronger connection power with not more cognate harm since a proteineous solder is fused by laser light to the tissue surface. Acute wound strength is improved, use of solders allow using different laser wavelengths due to different absorption. Initially, blood is used as a solder. Later on, other proteins like some proteins derived from blood fibrinogen and other albumins following the egg-white albumin, were used as a solder substitute. Other solders include the biodegradable polymer polylactic-co-glycolic acid thin film, which improves acute weld strength with liquid albumin solder.

Dye- enhanced soldering put to good use to color sucking at the ray length of wave brazing included to the weld to widen elective absorp of target tissue as well as subsequent heating for solder. Two benefits from this technique; begin with the powerful sucking of light by color that is elected, secondly, effective light conversion towards warmth of color fallen apart in braze. With that technique, chance of selecting a suitable dye is given to. This technique gave a chance to select an appropriate dye to pair with its highest point of absorb with the special ray length of wave that is used. By using that way, it is permitted using the more popular and cheap eight-zero-eight -n.m diode ray with help of

biocompatible dye indocyanine green (I.C.G) [32]. A consecutive scaffoldage filled with IV drip albumin and I.C.G was utilized in other dealing.(33) It has been revealed that by adding the polymer membrane weld strength is enhanced and better flexibilityis provided when we compare to the using only albumin protein plummet. Consecutive scaffoldage creates an adequately flexible solder by letting it surround the fiber. Different from the tissue bonding, solders can be used for applications. For bleeding surfaces for homeostasis, ray helped fiber wafering shall be utilized.

There are various applications for laser welding and soldering; main vascular anastomosis; sealing for the reduction of blood lose, cardiovascular operation, healing of air passage following a lung biopsy or taking part of a cotter out of body; healing of bronchial suffers, closing of skin with progressed cosmeticals and quicker recovering in dermatology, healing of oviduct tubes in gynecology, bonding and fixing of acentric nerve in neurosurgery, ureter closing ureteroneocystostomy , urethra and bladder in urology, sclera and cornea incisions closure of laser solder s in ophthalmology.

2.5.Laser Cornea Welding

Cornea is the front part of the eye is covered with a slick, convex outer surface of transparent avascular tissue and inner surface concave, which is similar to small watch-lass. (Figure 2.14) Cornea must be transparent to meet the diverse functional demands, refract light and provide a protective interface. It is averagely 550μm thick and has a diameter of 12 mm. Cornea is the most refractive part that refracts the light rays. Both the pupil and the iris are in the transparent cornea as an external surface. This is the eye's optical system's strongest refractive part and lets, together with crystalloid lens the sharpened sight producing on the retina photo receptor. Accordingly, cornea is in utmost significance in focusing ability of eye.

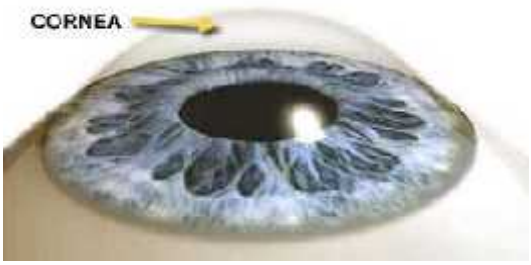


Figure 2.14. Cornea [34]

We can call the cornea as eye's front window. The cornea works as the watch that crystal protects the inner workings of a watch and it also protects the eye interior. Similar to the film of the camera it enables focusing the images on the retina's photoreceptors because it is clear and enables the lights come through. A minimal change in the shape or structure of the cornea makes a big refractive change in the focusing of ray light on the retina. Therefore, refractive surgery works by changing the shape of the cornea.

There are 5 layers in the cornea (listed from outside to inside) the epithelium (the outer skin, 7 cell layers thickness), overlay of Bowman (20 μm thickness), stroma (general of cornea), Descements Membrane (10 μm thick) and the endothelium (inner skin). All five layers can be seen by a microscopic drawing and light microscope view. Human cornea consist of 78% water, 15% collagen, 5% other protein, 0.7% keratin sulphate, 0.3% chondroitin/dermatan sulphate.

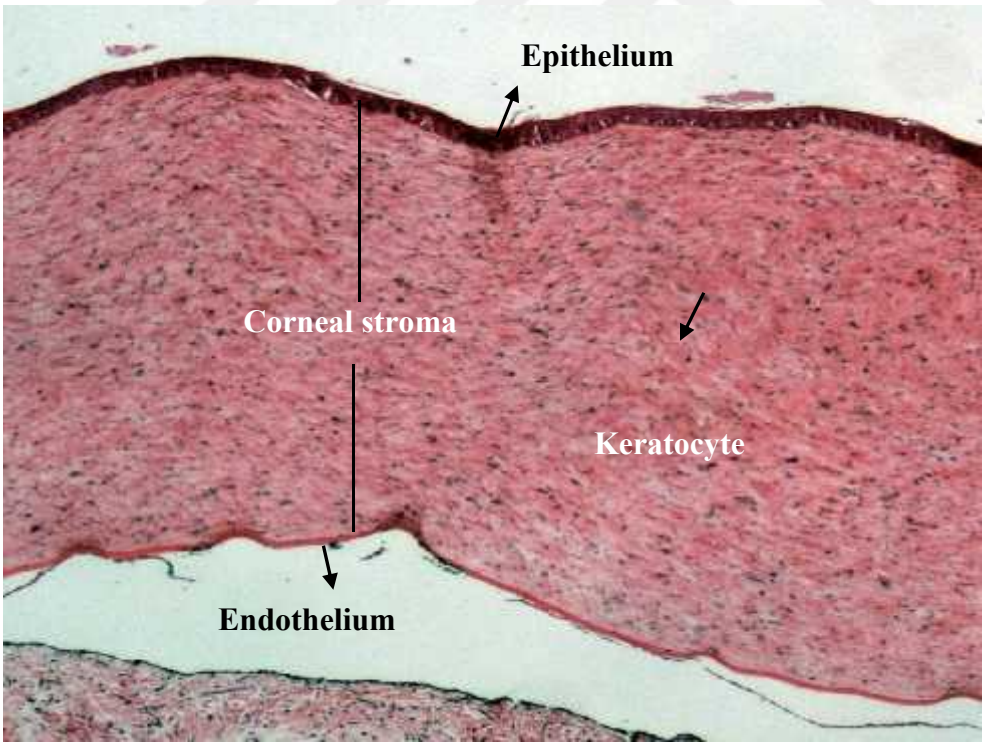


Figure 2.15. Histology of cornea (Boğazici University Institute of Biomedical Engineering., Biophotonics Lab), magnification: 4X [35]

Corneal epithelium' histology is stratified, squamous and non-keratinized. Basal cells replace with on a weekly basis and the surface's oldest shed. The modified region of anterior stroma is the overlay of Bowman, by this layer, the front junction between cornea and limbus is delineated. In a travma situation there is no regeneration in Bowman's layer and replaced by scar tissue. Stroma is the main part of the cornea that the laser welding process take place and nearly 90% of thickness of cornea which formed by collagen bundles that regularly arranged lamellae and lie in proteoglycan essential item. Descement's pellicle is basic layer of cornea endothel, strong resistant sheet. This membrane's thickens with in some corneal degenerative conditions and with age also. Endothelium, the relatively firmed count of corneal endothel cells production is nearly 500000 and that's one hexagonal layer, cuboidal cells attached back aspect for descement's membrane. Endotheial cell density is 6000 cells/mm^2 at birth, it is about 2500 cells/mm^2 around middle age, rate of cell loss slows in that ages. In case the density of cells go down to 500 cells/ mm2 corneal edema occurs and tranperancy diminishes.

Consisting of from 200 up to 500 layers of collagenous lamellae, the corneal stroma extends from one side to other. In stroma's 30% anterior, collagen lamellae run deviously to the corneal surface and are (about 0.2- 1.2 μm thickness and 0.5 - 30 μm wideness), Collagen lamellae is prone to organized parallelized to face and grossed (thickness - 1.0 to 2.5 μm and wideness- 100 to 250 μm) in the posterior stroma. 12-15% and 1-3% of the net weight of the tissue form from collagen and glycosaminoglycans and 78% of the normal cornea is water. (Figure 2.16. - 2.17).

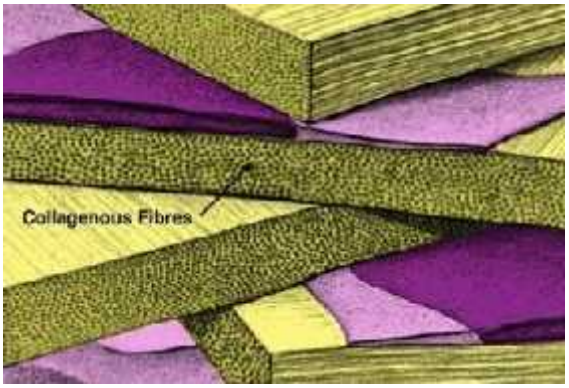


Figure 2.16. Collagen arrangement [36]

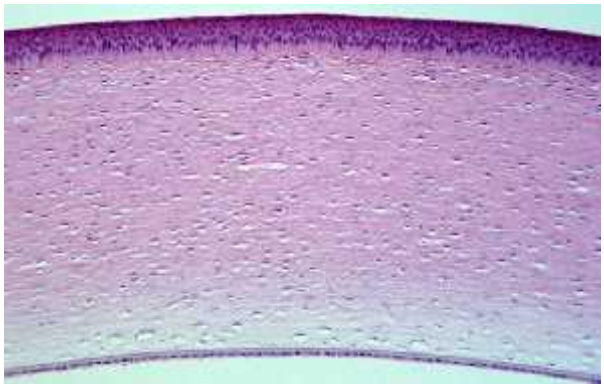


Figure 2.17. Stromal collagen [37]

By cornea's physical characteristics and the hydration that is controlled, corneal transparency is obtanined. The stroma has the uniform collagen fibrils which are

positioned in a regular lattice so that scattered light is destroyed by the mutual interference. The cornea will remain transparent as long as the lattice positioned fibrils are separated by less than 400-700nm which is a wavelength of light. If this regularly arranged lattice structure changed by stromal oedema or mechanical stress, it will cause to transparency deprivation. The following factor for transperancy of cornea is the corneal hydration and corneal epithelium and endothelium is in control of it. The layers of corneal epithelium and endothelium have functions as being barrier and doing metabolic pumping. Stroma hydration is identified by proteoglycans. Nearly 78% is sustained by physiological hydration of cornea, if 5% swell of this value is allowed the cornea, it start to scatter remarkable light quantities. Other factors maintain corneal transparency is avascular cornea, unmyelinated corneal nerve fibers, integrity of all layers of cornea.

Clouding of the ingenious ocular in the eye is named cataract. (Figure 2.18) Cataract can develop in one or both eyes. It is usually a part of the normal aging process and it is the fundamental reason for visual lost of individuals who are above 40. A normally clear lens allows light to pass through to the retina (back of the eye), so that the patient can see images clearly. Lens with cataract do not let light to pass easily, so the back part of the eye only receives blurred and distorted images. When the lens becomes more dens or opaque (cloudier), the light is more scattered and the person's vision will be distorted.

If the patient’s vision is mildly affected surgical treatment may not be needed. During its early stages, changing glasses diopters and brighter lights (more contrast) may help improve vision. When the cataracts are severe the only effective treatment is surgery. Cataract surgery is performed by taking out the cloudy lens from the eye and an artificial clear lens is put in its place - an intraocular implant (intraocular lens). Surgery begins with a very small clear corneal incision, approximately 3mm wide in the eye (Figure 2.19) Figure 2.18). The artificial lens is then inserted through the cut and no stitches are used at the end of operation.

Figure 2.18. Cataract [38]

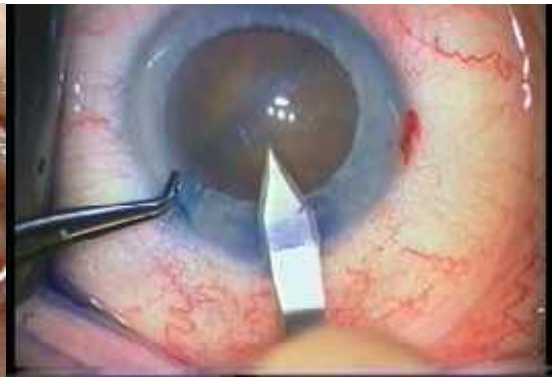


Figure 2.19. Clear corneal incision [39]

For joining or bonding the tissues, laser fiber bolding uses the power out of laser ray. During welding, the laser energy with wavelenght of 809-nm, 980-nm, 1070-nm, 1890-nm and 1470-nm which used in this thesis subsequently heats the collagen helix is sucked by liquid in the fiber. Hence, a prospering bonding necessitates a certain controlling over the laser energy, as to check the fiber warmth and dehydrationing exposure times are needed. For welding by overtone vibrational absorption Water is the crucial molecule. There are critical factors such as choosing laser emission parameters like wavelength and energy dose together with the tissue's principal absorber which is targeted by laser radiation. Since the 78% of cornea is water and the water in the content of cornea is an endogenous choromophore to absorb laser light.

At the end of a standard cataract surgery clear corneal incision is not closed with any technique if there is not any leakage. Leak proof, corneal incisions of complete thickness closure can be achieved by corneal laser and that was made for reaching pathologic lens. The potential advantages which are expected by cornea laser welding are; reduction of surgery time, simplification of the surgical technique, suppression of reaction related to suture material, reduction of postoperative endopthalmitis (intraocular infection) (Figure 2.20-2.21), reduction of post-operative astigmatism. Equal Infiltration of leukocytic and fibroblastic activates the laser tissue welding and that is the reason of enzymatic degradation of harmed tissue across the incision. When it is compared to suture the stimulation of indiscrete corneal incision closure which is same with wound healing by welding could decrease the astigmatism after operation.

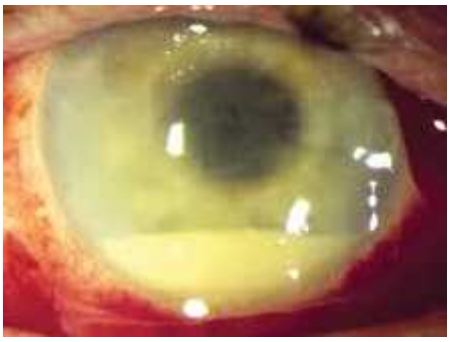


Figure 2.20. Endophthalmitis [40]

Figure 2.21. Endopthalmitis [41]

Following the cataract surgery, it is known that intraocular pressure varies, typically it falls to less than 5 mmHg,[42] and as a response to blinking, it is revealed by telemetric with the help of interior of eye compression following up tools , there are big undulations in the interior of eye pressure happens in each sights. [43,44]. Up to 0.29 cases, probability of absorption of face liquid via the sealed by itslef- clean corneal damage in the front part in previous after operative process is advised by apparent rising for endophthalmitis. [45–51].

Ahlberg and associates [52] informed about approximate particle India ink's extent is 10micron as diameter. Hence, in case the India ink is specified in front part, for bacterial particles of similar sizes, it is likely to get in the front part by cuttings. Sarayba *et al* study [53] showed that around all the corneal wounds, there are India ink penetration that is revealed by the light micrographs of clear corneal incisions.

Another disadvantage of clear corneal cut was shown by Tam *et al* [54]; As a result of wound leak that occurs after operation, 3 ill individuals with eventless phacoemulsification via clear corneal cut were specified. In phacoemulsification with corneal incisions, posterior corneal tissue's reversed flap, cornea related tongue, might hinder structural supplementation of the injuries that need surgery caused a probable injury deficiency.

In ophtalmic surgery, corneal tissue laser welding is another optional technique to conventional suturing procedures.[55] Laser tissue welding is a method with no contact and its objective is to unite biological tissue with the laser energy delivery.

Ophthalmology, probatory works for laser-caused stiching of cornea fiber on labarotary animals were revealed from the 1992 by different searchers[56–68], depending on close- and further- infra red lasers using that are sucked by liquid substance of corneal part of eye straightly. Laser cornea bonding is a relatively new technique still under investigation and the present thesis study will be the first comparative investigation of the wide range of 800-2000-nm wavelengths for different kind of welding studies.

3. AMNIOTIC MEMBRANE WELDED TO CONTACT LENS BY 1470-NM DIODE LASER: A NOVEL METHOD FOR SUTURELESS AMNIOTIC MEMBRANE TRANSPLANTATION

3.1. Introduction

Our study published in 2014 described a new branch in laser welding applications especially in the field of ophthalmology. The study showed a new method for laser welding of a tissue to contact lens for ophthalmologic application.[69] Mitotic division of corneal epithelium's basal cell layer normally renewed in 7-10 days on a regular basis. Epithelial cells on the surface shed to precorneal tear film. The basal layer cells are constantly replaced by multiplying the cells. Corneal wound healing takes place in three stages these are: migration of healthy epithelial cells to this area, regeneration and stromal wound healing to close the defect [70, 71].

If corneal epithelial injury is minor, after injury damage is tries to be closed by cell migration as soon as possible. Epithelial migration begins within minutes there may be delays up to 4-5h of a wider damage. This delay time prior to the rapid cell division required for anatomical, physiological and biochemical preparation. Clinically, corneal epithelial migration is sufficient to close many lesion. [70,71]

Human being's amniotic membrane (A.M) is reproduced from the fatal layers. AM is most interior placenta's layer. Throughout history, there are three layers of AM, one is single layer, the other is cubic epithelium basement membrane and lastly stroma. Macroscopically one side of the membrane is smooth, shiny, transparent and non-adhesive epithelial face and the other is matte and adhesive stromal face. Epithelial and stromal side can be determined with the help of triangle sponge. When sponge touched to the membrane it is the stromal side that attached. The thickest basement membrane of the

human body is AM. Clinically this allows the storage of frozen membrane at -80°C so epithelium cells can be protected for a long time and stay alive. Histochemical examinations on the basal membrane of AM show that it is similar to the conjunctiva. [72]

AM accelerates epithelialization, maintains normal epithelial phenotype and inflammation, cicatrisation, reduces neovascularization. These effects depends on suppression of inflammation with the effect of AM antiprotease, suppression of lipopolysaccharide that increase IL-1α and IL-1β, reducing TGF-β signaling system and scar system by reducing the conversion of myofibroblastsand fibroblast, the creation of epithelial progenitor cells and colony survival by being basal membrane and increasing growth factors carring within[70,71, 73–76].

AM acting to preserve the epithelium from the eyelid movemenets used as a bandage CL. It is claimed that there is growth factors in AM needed for treatment of deep ulcerations and desmatocels in patients at risk of perforation of the cornea, inflammation will suppressed with the use of AM, also increases the success of future penetrating or lamellar keratoplasty through containing basal membrane and collagen but not allogenic cells. [77] Gris *et al* study showed that the AM implant stayed in place for approximately 12.5days (range, 3-34)[78]. In 11 out of the 20 patients, in the first 8 days, the AM implant became wide apart. The time cornea implantation was coated with softened CL following the operation. Therapeutic CLs are used to increase the regeneration of corneal epithelial healing and lenses. Therapeutic CLs cover elements that help in reducing pain, hydration and protection of the cornea to reduce oedema, corneal epithelial healing maintaining the mechanical disorders of the cornea. [79] With sufficient oxygen permeability of therapeutic CLs help to improve the surface of the cornea. Additionally, it can be used as a mechanical barrier to preserve the surface of the eye from influences. Therapeutic CLs can be use after AMT for decreasing inflammation until completion of reepithelialization. [80]

3.2.Materials&Methods

AM was taken from seronegative pregnant women for HIV, Hepatitis B, C and syphilis during elective caesarean section under laminar flow and sterile conditions. With sterilized phosphat-tamponed physiologic salty dispersion including the penicillin, AM

was cleaned from blood clots, fifty m.g/mL, streptomycin fifty m.g/m.L; neomycin 100 mg/mL amphotericin B 2.5 mg/mL. By using blunt dissection, rest of chorion was seperated from Amnion.

AM was aseptically stored in the same saline solution for 1h and then used in the laboratory. AM can be used in three techniques. Placed into the area to cover defects “inlay” (graft) technique, placed on the cornea and limbuscover to cover “overlay” (patch) technique and to close the thin region “filling” (gag) technique. In this study, sutureless overlay (patch) technique was used. In this procedure, the upper face was stromal side and epithelial side was in touch with the ocular surface. In this sutureless overlay technique with CL, it is obvious that the patient vision will be clear immediately after epithelialization under the AM and after the CL removal. The longer the duration of the AM on ocular surface, epithelialization will be more successful.

3.2.1. Experimental Set up

Experiments were done at Bogazici University, Department of Biomedical Engineering, Biophotonics Lab.

1470-nm Diode Laser: Designed and produced by DILAS (DILAS, Dioden laser GmbH Mainz-Hechtsheim, Germany) and controlled by software. (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)
SMA connector: Opto Power OPC-OC-01, Opto Power Corporation, Tuscon, AZ, USA. (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)
Optical fiber: Spindler–Hoyer, Göttingen, Germany, 400 μm silica. (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)
Laser Goggles: 630-1320nm (Laser Vision, USA) 190-380nm, 840-950nm, 950-1070nm (LG1, Thorlabs Inc., Newton, NJ, USA) (available at Boğaziçi Üniversitesi, Biomedical Engineering Institute, Medical Lasers Laboratory)
Powermeter: Newport 1918-C, USA (available at Boğaziçi Üniversitesi, Biomedical Engineering Institute, Medical Lasers Laboratory)

Opto-mechanic devices: Thorlabs, USA (available at Boğaziçi Üniversitesi, Biomedical Engineering Institute, Medical Lasers Laboratory)

3.3.Results

Primarily, AM was spread on ocular surface to understand which side of the AM is epithelium and which side is stroma. Epithelial face was bright and stromal face was more matte when touched with the tip of forceps to the epithelium, gel-like vitreous elongation was formed. Following the decision of epithelial surface of this method, membrane's stromal side was spread inside of the CL with the specifications of 14 mm diameter, base curve 8.6, -0.25D refraction, 36% water content silicone hidrogel [PureVision (balafilcon) Bausch&Lomb, Rochester, NY, USA] and it was bonded to CL by the 1470 nm diode laser (DILAS Mini Diode Laser System, Meinz, Germany) which was especially customized production for our biophotonics laboratory. From four aparted points 600 micron diameter fiber tip of the laser was connected with the epithelial side of the AM. (figure 2.10). Following the membrane welding to the CL excess AM near the CL was removed with a scalpel.

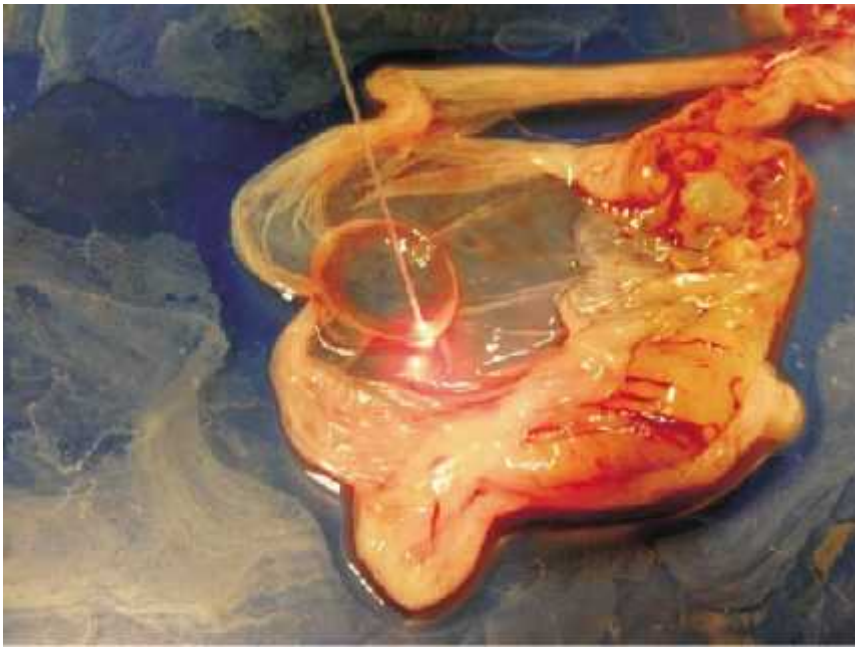


Figure 3.1. The connection with AM's epithelial side occurred with 600 µm diameter fiber tip of the laser.

Inside the CL, AM's was spread and that CL has the specifications of 14 mm diameter, base curve 8.6, -0.25D refraction, 36% water content silicone hidrogel [PureVision (balafilcon) Bausch&Lomb, Rochester, NY, USA]. Then with the 600 µm diameter fiber, laser applied with the power varying from 200mW to 800mW on the layer of the epithelium of the AM by contacting with fiber tip. Different power levels and exposure times were tested and for seven seconds, 340mW was found suitable. CL and AM linked with the welding effect in 4 point spot 4 welding by touching fiber tip with no extreme contraction or burn effect of AM. After the application of the laser with the appropriate dose and duration, It was seen that while holding AM portions, AM and CL were together which goes beyond CL with the assist of two forceps. (**Figure 3.2.**). When the AM was hold in the air with the help of two forceps, 4 point of spot welding points which was done with laser fiber, visualized clearly on the transparent surface of the CL (Figure 2.12.). When laser applied with higher energy (450 mW for 5s, 600 mW for 5s, 800 mW for 3s), burning of the AM, contraction of the membrane tissue or while holding AM portions AM and CL were together which goes beyond CL with the assist of two forceps. If the laser applied with lower energy (150 mW for 15s, 250 mW for 10s) than specified values, which are 340 mW for 7s, when tissue was hold from sides of the excessive membrane, it was viewed that membrane did not weld to CL and separated from the CL. After the optimum dose application when it was observed that CL and AM was welded, the excessive tissue which was extended beyond the CL was cut with a scalpel (Figure 2.13).

Figure 3.2. AM and CL were not separated from each other while holding AM portions that extend beyond the CL with the help of two forceps

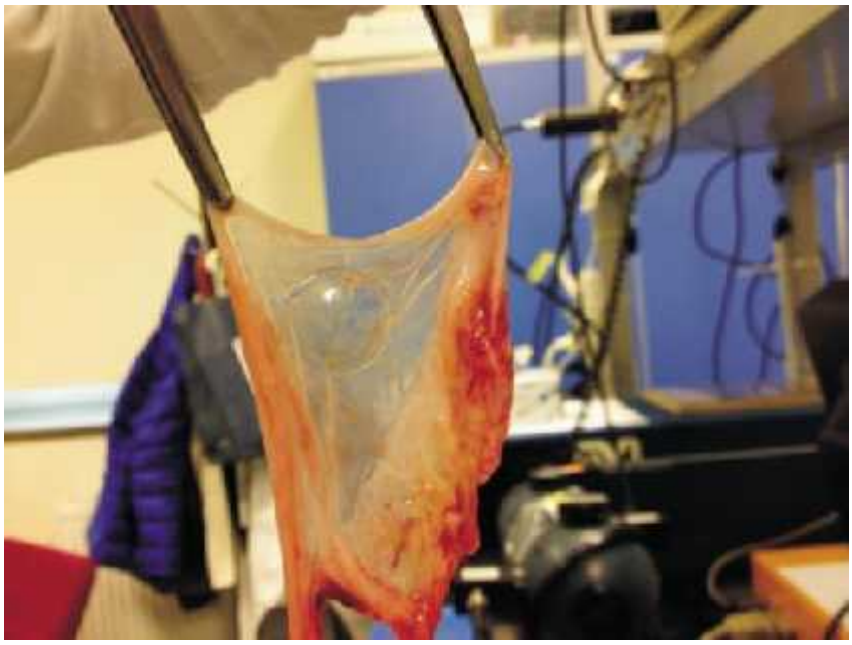


Figure 3.3. When the AM was hold in the air, 4 point of spot welding points visualized clearly on the transparent surface of the CL.

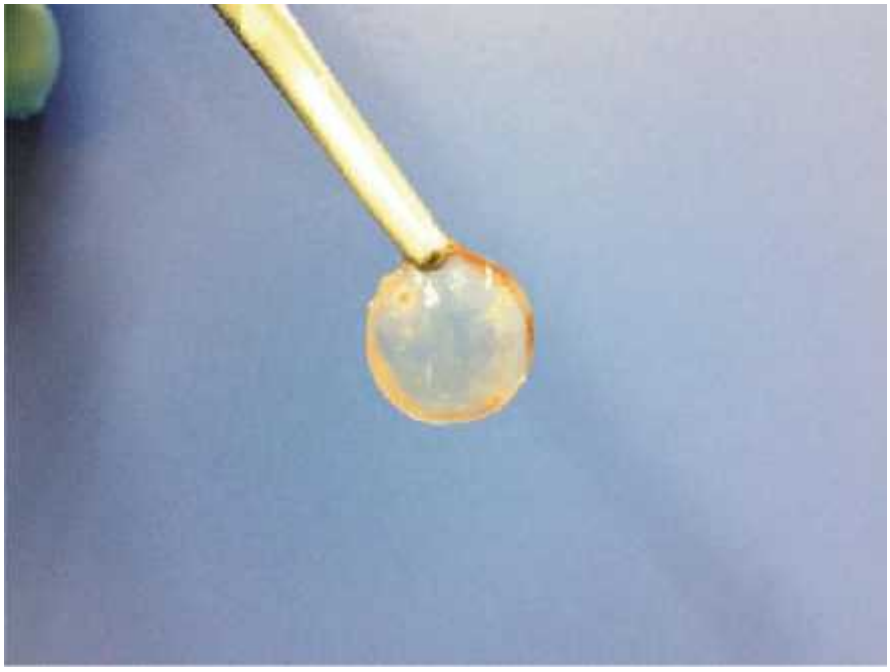


Figure 3.4. After the application the excessive tissue which was extended beyond the CL was cut with a scalpel.

4. IN-VITRO CORNEAL TISSUE WELDING WITH 809-NM, 980-NM, 1070-NM AND 1980-NM DIODE LASERS

4.1.Introduction

At the end of a standard cataract surgery clear corneal incision is not closed with any technique if there is not any leakage. If there is leakage sutur is used to close the corneal incision. In ophtalmic surgery, corneal tissue laser welding is another optional technique to conventional suturing procedures.[55] Laser cornea bonding is a relatively new technique still under investigation and in this study 809-nm, 980-nm, 1070-nm and 1980-nm wavelengths was investigated comparatively.

4.2.Material&Methods

A total of 40 freshly enucleated bovine eyes, 38 eyes for tissue-welding experiments and 2 eyes for control group, were used in the first part of study. Fifteen eyes were excluded because of macroscopic carbonization, opacification, or corneal shrinkage. Experiments were done within 2 hours after enucleation of bovine eyes. Full-thickness, one-plane, 3.2-mm length, limbal corneal cuts were done using a precalibrated knife. (Figure 2.22) Laser beam was foculized in the centre of the clear corneal cut. (Figure 2.23) 7 eyes used for 809-nm diode laser (manufactured in BU photonics laboratory) welding with indocyanine green (ICG) as an absorptive dye was examined in vitro in bovine eyes. For 809-nm diode laser applications, a chromophore (ICG) was added to the target tissue to localize the optical absorption and to yield a selective photothermal effect. Different concentrations of ICG (6% and 12% wt/wt in sterile water) were applied. It is easy to place these dense preparations inside the corneal cut, these preparations are viscous enough and are placed by using a front store cannula. A little time following the procedure, the dispersion has been cleaned with abundant water. 980-nm diodial laser (OPC-D010-980-FCPS; OptoPower, Tuscon, AZ) welding has been examined in vitro in 7 bovine eyes. 1070-nm diode laser (YLM-20-9C IPG Laser GmbH) welding was examined in vitro in 3 bovine

eyes. 1.98-mm Tm:YAP laser system (a diode-pumped 1980-nm Tm:YAP laser developed in Biophotonics Laboratory at Bogazici University by collaboration with Laser Research Laboratory at Koc University, Istanbul, Turkey) welding was examined in vitro in bovine eyes. 6 eyes used for 1980-nm wavelength. Optimal power, duration, and energy density were investigated

Figure 4.1. Clear corneal cut was done with precalibrated knife which is used in phacoemulsification cataract surgery in human eye (Boğaziçi University Institute of Biomedical Engineering Biophotonics Lab) **Figure 4.2.** Laser irradiation of clear corneal cut (Boğaziçi University Institute of Biomedical Engineering Biophotonics Lab)

According to the results of 809-nm, 908-nm, 1070-nm and 1890-nm laser irridiation, another study was planned to compare 1070-nm and 1890-nm wavelengths which we found the best results in previous studies.

10 eyes for 1070-nm laser and 10 eyes for 1980-nm laser were used with constant parameters for this study. The eye samples were exposed for 5 seconds to 1.5 W of 1070-nm laser irradiation. Exposure to 0.46 W of 1980-nm laser irradiation was performed for 2 seconds. For histological evaluation, incision area of the cornea was dissected and repaired in 10% neutral formalin for at minimum 48 hours. Dehydration in ethanol and clearing in xylene have been the repaired samples. Paraffin-embedded corneal specimens were ramified for 5-mm thickness and for histopathological inspection via light microscopies they have been pointed to hematoxylin and eosin.

4.3.Results

Over 7 eyes, two eyes of successful full-length welding were reported with less eosinophils and epithelium degeneration with 809-nm laser both doses of ICG (Figure 4.3 – 4.4). Application of 809-nm diode laser with either dose of ICG did not provide a satisfactory welding result. Corresponding power and durations were 200mW - 10 seconds for 6% ICG and 200mW - 3 seconds for 12% ICG. Not any bleardness from 2 corneas were detected folowing the bonding. However, in the absence of ICG, poorly absorbed laser energy can propagate into deeper parts and iris can be damaged. According to those preliminary results, 809-nm diode laser was not figured like promising laser for cornea welding purposes.

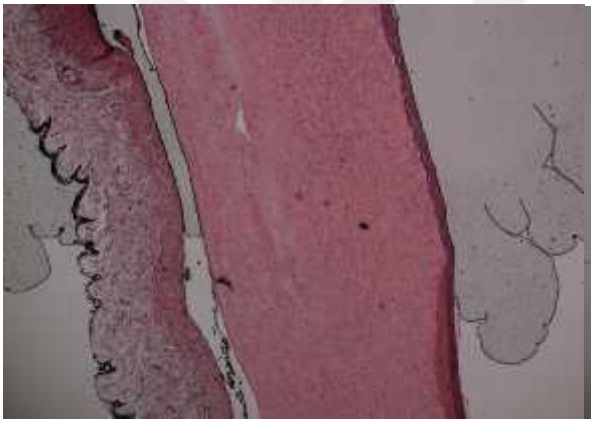


Figure 4.3. 809nm 200mW-10sec.6%ICG **Figure 4.4.** 809nm 200mW-3sec. 12%ICG

Over 7 eyes, two cases of welding were reported with carbonization and less eosinophils for 2 W- 20 seconds and 3 W–5 seconds of 980-nm irradiation (Figure 2.26-2.27). Three Watts of 980-nm irradiation for 24 seconds resulted in photocoagulation and opacification at cornea. According to the preliminary results, 980-nm diode laser has not been figured out as promising wavelength of cornea bonding purposes.

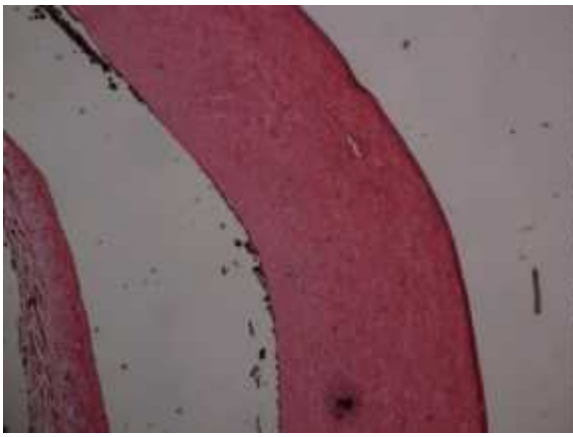


Figure 4.5. 980nm 2W-20sec**.**

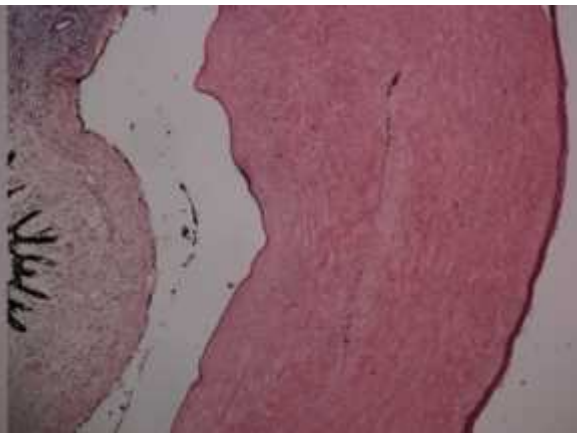


Figure 4.6. 980nm 3W-5sec

The 1.5 W of 1070-nm YLF laser exposure for 15 seconds caused photocoagulation and opacification at cornea; thus, 10 and 5 seconds of exposure time were investigated. Two full-length welds were obtained with 1.5 W-10seconds duration irridiation with 1070-nm. (Figure 2.28-2.29) Power of 0.75 W was not found sufficient for successful welding, even though a significant narrowing was noted at this power level. According to the preliminary results, 1070-nm YLF laser was found to be a promising wavelength, which deserved further investigation.

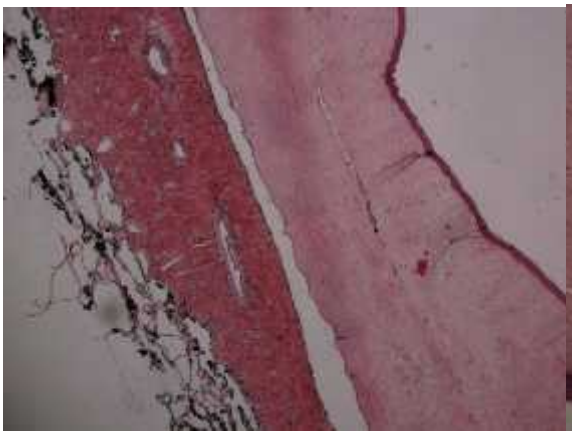


Figure 4.7. 1070nm 1,5W-10sec**.**

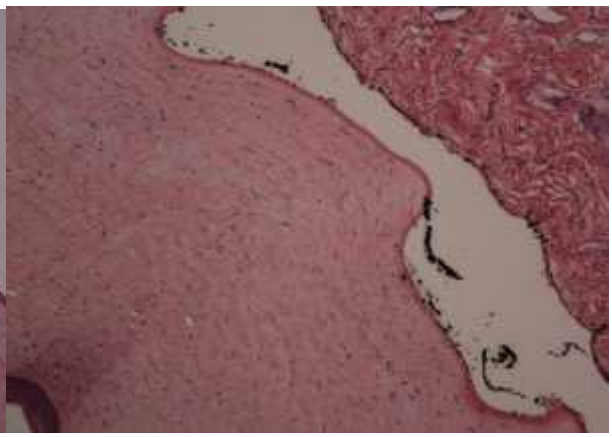


Figure 4.8. 1070nm 2W-10sec.

Over 6 corneas, 4 were welded in full thickness without or very little carbonization with 1980-nm Tm:YAP laser. (Figure 2.30-2.31) The 1980-nm Tm:YAP laser irradiation caused immediate welding with very low powers (380mW and 460 mW) and moderate energy densities. Not any turbidity of corneal was determined following the bonding. Results are summed up in table 7.1.

Figure 4.9. 1980nm 0,38W-20sec**.**

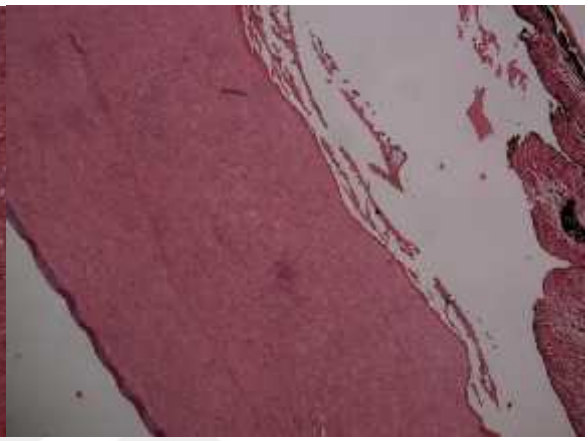


Figure 4.10. 1980nm 0,46W-2sec.

According to these results best two wavelengths was chosen (1070-nm vs 1980-nm) and best parameters were determined with reference to the previous results. Ten corneal tissues were irradiated with 1070-nm YLF laser; histological examinations showed that 5 of them were successfully welded; 2 incisions were narrowed. Small holes and low carbonization were observed in microscopic examination of welded corneas. On the other hand, 1980-nm Tm:YAP laser group of 10 corneal tissues were found less successful. Neither of them was found as full-thickness welded, but 4 of them were partially welded, 4 corneal cuts were found narrowed and 2 incisions were not welded. (Figure 2.32-2.33) As in the case of 1070-nm laser welds, small holes and low carbonization were also detected in microscopic examinationof 1980-nm Tm:YAP welds. Collagen fibers were not found parallel in all eyes.

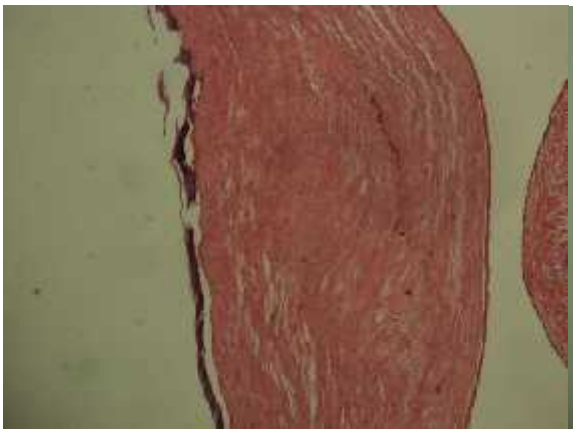


Figure 4.11. 1070nm 1,5W-5sec**.**

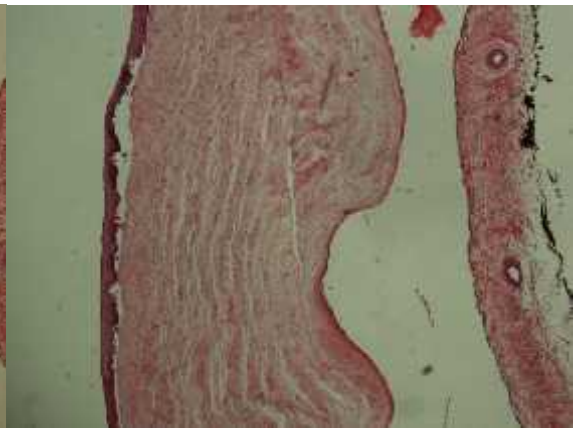


Figure 4.12. 1980nm 0,46W-2sec.

Further experiments were needed to shed light on the welding qualities of those promising wavelengths of 1070- and 1980-nm lasers.

Laser_Eyes	Power	Exposure Time (Sec)	ICG (%)	Results
809_Eye 1	200mW	3	6	Not welded
809_Eye 2	200mW	10	6	Welded
809_Eye 3	200mW	20	6	Not welded
809_Eye 4	200mW	3	12	Welded
809_Eye 5	200mW	3	12	Not welded
809_Eye 6	200mW	5	12	Not welded
809_Eye 7	200mW	10	12	Not welded
980_Eye 1	1W	10	NA	Not welded
980_Eye 2	1W	20	NA	Not welded
980_Eye 3	1W	30	NA	Not welded
980_Eye 4	2W	5	NA	Not welded
980_Eye 5	2W	10	NA	Not welded
980_Eye 6	2W	20	NA	Welded
980_Eye 7	3W	5	NA	Welded
1070_Eye 1	1W	10	NA	Incision narrowed
1070_Eye 2	1.5W	5	NA	Welded
1070_Eye 3	1.5W	10	NA	Welded
1980_Eye 1	0.38 W	10	NA	Partially welded
1980_Eye 2	0.38 W	20	NA	Welded
1980_Eye 3	0.38 W	30	NA	Welded
1980_Eye 4	0.46 W	2	NA	Welded
1980_Eye 5	0.46 W	3	NA	Not welded
1980_Eye 6	0.46 W	4	NA	Welded

Table 4.1. Results of 809-nm, 980-nm, 1070-nm and 1980-nm laser corneal welding

	1070-nm (1.5 W - 5 sec.)	**1980-nm (0.46 W- 2 sec.)**
Eye 1	Welded	Not welded
Eye 2	Not welded	Narrowed
Eye 3	Not welded	Narrowed
Eye 4	Welded	Partially welded
Eye 5	Narrowed	Narrowed
Eye 6	Welded	Not welded
Eye 7	Not welded	Partially welded
Eye 8	Narrowed	Partially welded
Eye 9	Welded	Narrowed
Eye 10	Welded	Partially welded

Table 4.2. Results of comperative study: 1070-nm vs 1980-nm laser corneal welding

5. IN-VITRO AND IN-VIVO CORNEAL TISSUE WELDING WITH 1470-NM DIODE LASER

5.1. Introduction

Main aim of corneal tissue welding is to minimize the risk of endophthalmitis and suture related infection, bleeding and refractive error after modern cataract surgery. One of the essential objective of this reseacrh, *in-vivo* part for showing that 1470-nm laser *in-vivo* cornea welding eliminate the risk of postoperative wound leak through the clear corneal cut which was shown by many previous studies. [81-85] Although there are some studies about laser tissue welding of cornea original aspect of this study according to our knowledge this is the first study that uses 1470-nm laser for cornea welding and also for medical usage. Laser fiber-welding procedure is best suitable for situations where stiching and pricking is challenging and it is a noncontact and nonmechanical method.

5.2. Materials&Methods

5.2.1. Subjects

In this PhD research work, 1470-nm diod laser corneal welding were examined in vitro in bovine eyes. A total of 13 freshly enucleated bovine eyes, 12 eyes for tissue welding with experiments and 1 eye for control goup, were used. After the results of this in-vitro study, in in-vivo study 6 eyes of 3 rabbits were used for each group. First 3 rabbits (6 eyes) group was in 170 mW-7 seconds parameters, second 3 (6 eyes) rabbits group was in 320 mW-2 seconds parameters and 2 eyes of 1 rabbit was control group. In in-vivo study 215mW-3,5 seconds group was excluded because of 2 eyes were not welded over 4 eyes and high carbonization, changes in distribution of undulation in H&E and Sirius red staining of histologic examination. Control group will have clear corneal incision as all cataract surgeries and laser welding was not performed and was left without any sutures as the cataract surgeries performed in today technology**.**

5.2.2. Experimental Set up

In vitro experiments were done in the Department of Molecular Biology and Genetics at Bogazici University, Biophotonics Lab.

In vivo experiments on New Zealand rabbits will be done at Bogazici University, Department of Molecular Biology and Genetics.

In vivo experiments will be implemented through a protocol which is confirmed by the Institutional Animal Research and Care Ethic Committee at Bogazici University.

5.2.2.1. Equipments:

1470-nm Diode Laser: Designed and produced by DILAS (DILAS, Dioden laser GmbH Mainz-Hechtsheim, Germany) and controlled by software. (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)

SMA connector: Opto Power OPC-OC-01, Opto Power Corporation, Tuscon, AZ, USA. (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)

Optical fiber: Spindler–Hoyer, Göttingen, Germany, 400 μm silica. (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)

Laser Goggles: 630-1320nm (Laser Vision, USA) 190-380m, 840-950nm, 950-1070nm (LG1, Thorlabs Inc., Newton, NJ, USA) (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)

Powermeter: Newport 1918-C, USA (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)

Opto-mechanic devices: Thorlabs, USA (available at Bogazici University, Biomedical Engineering Institute, Medical Lasers Laboratory)

5.2.2.2. Animals:

New Zealand Rabbits (available at Bogazici University, Dept. of Moleculer Biology and Genetics, Animal Lab)

5.2.2.3. Anesthetics:

Isoflurane: Forane (Baxte, USA)

Proparacaine hydrochloride %0.5: Alcaine (Alcon, Switzerland)

Carprofen: Novox (Norbrook Lab, UK)

5.2.2.4. Histology Equipments:

Tissue Processor: TP 1020, Leica Microsystems Nussloch GmbH, Nussloch, Germany (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Paraffin Embedding System: Leica EG 1150 H, Leica Microsystems Nussloch GmbH, Nussloch, Germany (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Rotary Microtome: Leica RM2255, Leica Microsystems Nussloch GmbH, Nussloch, Germany (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Cold Plate: Leica EG1150 C, Leica Microsystems Nussloch GmbH, Nussloch, Germany (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Slide Staining Set: Bio-Optica Strumentazioni Scientifiche Slide Staining Set, Code, Bio Optica Milano s.p.a. Italy. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Etuve: Nüve EN 025, Nüvesanayi Malzemeleri İmalat ve Ticaret AŞ, Ankara, Türkiye (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Digital Biological Microscope: DMWB1-223, Motic China Group Co., Ltd., Xiamen P.R.C, China. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Light Microscope with 5 MP Digital Camera: Nikon, Japan (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

5.2.2.5. Chemicals & Disposables:

Haematoxylin and Eosin: Bio Optica, Milano, Italy. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Xylene: Riedel-de Haën, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Formaldehyde Solution: Fluka & Riedel-de Haën, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Acetone: Riedel-de Haën, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Ethanol Absolut: Riedel-de Haën, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Lithium Carbonate: Riedel-de Haën, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Sodium Dihydrogen Phosphate Dihydrate: Merck KGaA, Darmstadt, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

Entellan: Merck KGaA, Darmstadt, Germany. (available at Bogazici University, Biomedical Engineering Institute, Tissue Laboratory)

5.2.3.Protocol

5.2.3.1. Predosimetry & Dosimetry, *in vitro* study

In this study, 1470-nm diod laser corneal welding were examined in vitro in bovine eyes. A total of 13 freshly enucleated bovine eyes, 12 eyes for tissue welding with experiments and 1 eye for control goup, were used. Experiments were done within 2 hours after enucleation. Full thickness one-plane cut 3.2 mm long limbal corneal cuts were done using a pre-calibrated knife.(Figure 3.1) Laser beam stayed focused in the center of the corneal cut. Optimal power, duration and energy density were investigated.

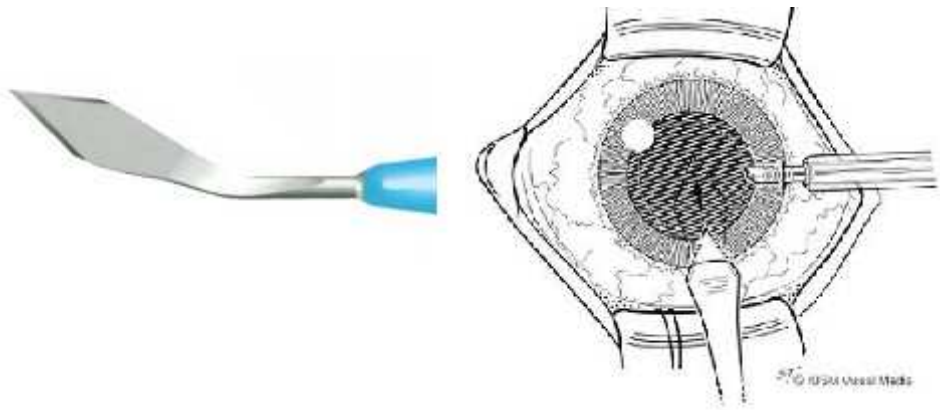


Figure 5.1. Pre-calibrated knife and clear corneal incision

For histological evaluation, incision area of the cornea was dissected and stabilized in 10% neutral formalin for no less than 48 hours. The stabilized samples went through a dehydration process with ethanol and purged in xylene. Cornea samples which were put in paraffin were divided into parts for their thickness with 5 µm and colored by hematoxylin-

eosine (H&E) for histopathological analysing with the help of light microscopy. Sirius Red staining is introduced as a technique for determination of collagen, which allows quantitative morphometric measurements to be implemented in locally described tissue zones. It stained the finer collagen fibers more intensely and increased their birefringence spectacularly. The advantage of this method is to show undulating distribution of collagen fibers. Sirius red protocole is same with H&E until sectioning of paraffin embedded cornea specimens. Nuclei were stained with Weigert's haematoxylin for 8 minutes, and then for 10 minutes the slides were washed in the tap water which was running. Stained with picro-sirius red for 60 minutes. Washed acid-containing water by changing twice. Discard the greater part of the water from the slides by shaking vigorously. Dehydrate in 100% ethanol by changing three times. Purge in xylene and put in a resinous area. The sections were examined by utilizing a microscope which was endowed with filters in order to provide illumination which was polarized in a circular way. Tissue images were gained with a 10X, 20X and 40X objective lens, recorded on a digital camera.

12 bovine eyes divided into three groups according to the parameters used: First group parameters are 170 mW and 7 seconds duration, second group's are 215 mW and 3,5 seconds and third group's are 320 mW and 2 seconds. In all three groups continous wave used and the distance between laser prob and corneal incision area was 3 mm.

Eyes were then prepared for the laser welding procedure. The laser beam stayed focused in the center of the tissue. Optimal power, duration and modulation parameters of all 4 laser types were investigated.

5.2.3.2. Surgical procedures, *in vivo study:*

After the results of *in-vitro* study, rabbits were grouped for *in-vivo* study. Healthy adult male New Zealand rabbits with body weighing 300-350 grams, randomly selected from Vivarium Laboratory of Bogazici University. Anesthesia method is summarized in Table 1.

Agents	Doses	Method of application and volume	Frequency of application	Efficacy duration
Isoflurane	%5	inhalation	During operation	During operation
Topical Anesthetic Proparacaine hydrochloride %0.5	% 0,5 Alcaine	drop	1 time	During operation
Carprofen	4mg/kg	s.c.	1time(second dose if needed)	24 hours

Table 5.1. Anesthesia agents and method details

Clear corneal incisions were done with single use precalibrated 3.2 mm knives.(Figure 3.2., 3.3 and 3.4)Laser beam with 400 um diameter spot size was applied to the center of the corneal cut.

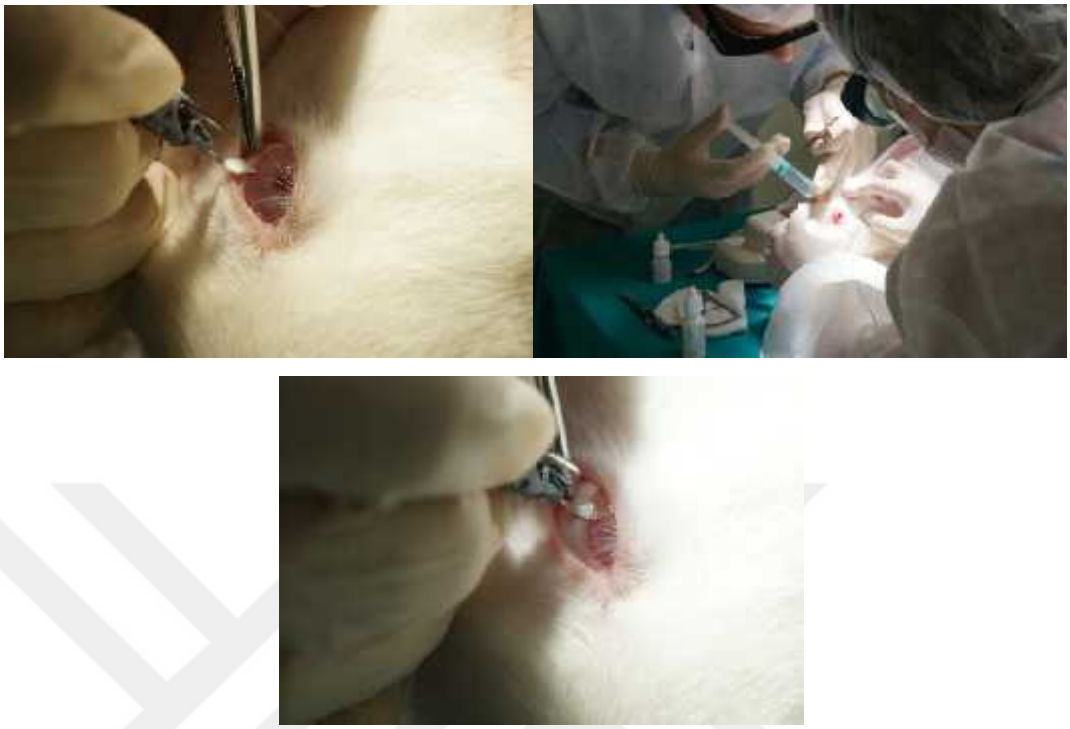
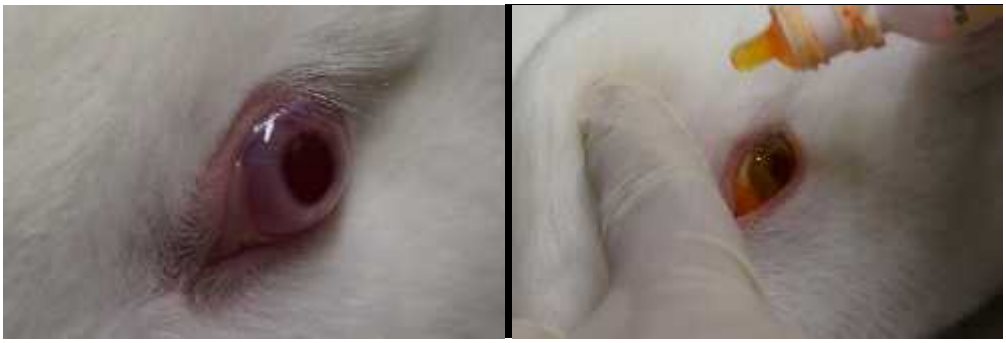


Figure 5.2. , 5.3. and 5.4. In-vivo rabbit clear corneal incision, operation

6 eyes of 3 rabbits were used for each group. First group was 3 rabbits (6 eyes) and the parameters were 170 mW-7 seconds, second group was 3 rabbits (6 eyes) and the parameters were 320 mW-2, control group was 1 rabbit (2 eyes). Control group will have clear corneal incision as all cataract surgeries and laser welding was not performed and was left without any sutures as the cataract surgeries performed in today's technology. In *in-vivo* study 215mW-3,5 seconds group was excluded because 2 eyes were not welded over 4 eyes and high carbonization, changes in distribution of undulation in H&E and Sirius red staining of histologic examination in *in-vitro* study.

After laser irradiation, rabbit corneas were observed in postoperative first day, first week, second week and forth week. During this 4 week period infection signs, anterior segment depth and leakage from the anterior segment was observed in the 1st day, 1st week and 1st month. Leakage from the anterior segment was tested with seidel test. (Figure 3.5, 3.6, 3.7.) Post-operative 30 days antibiotic and steroid drops were used.



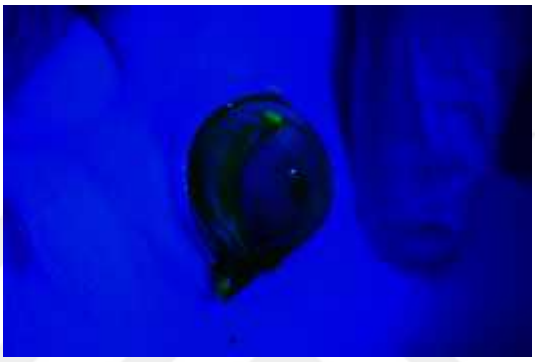


Figure 5.5. 5.6. and 5.7. Post-operative first day anterior depth and leakage control

Figure5.8. Post-operative 30 days antibiotic and steroid drops were used

5.2.4.Histologic Evaluation

For histological evaluation, eyes are enucleated, incision area of the cornea was dissected and stabilized in 10% neutral formalin for no less than 48 hours. (Figure 3.9, 3.10, 3.11) The fixed samples were dehydrated in ethanol and then purged in xylene. Cornea samples which are put in paraffin were divided into parts for their thickness with 5

μm and colored with hematoxylin-eosine (H&E) for histopathological examination by means of light microscopy. Sirius Red staining is introduced as a technique for determination of collagen in locally described tissue zones. It stained the finer collagen fibers more intensely and increased their birefringence spectacularly. The advantage of this method is to show undulating distribution of collagen fibers. Sirius red protocol is used in in-vitro corneas and the protocole was same with H&E. Nuclei were stained with Weigert'shaematoxylin for 8 minutes, and then for 10 minutes, the slides were washed in tap water which was running. Stained with picro-sirius red for 60 minutes. Washed in acid containing water by changing twice. Dehydrate in 100% ethanol by changing three times. Purge in xylene and put in a resinous area. The sections were examined by utilizing a microscope which was endowed with filters in order to provide illumination which was polarized in a circular way. Tissue images were gained with a 10X, 20X and 40X objective lens, recorded on a digital camera. Histologically; increment of closure along the incision line, less carbonization existence at the welding areas are the markers of better results under microscope. The damage of laser beam determined by the existence of big holes and blackened carbonization on the incision area.

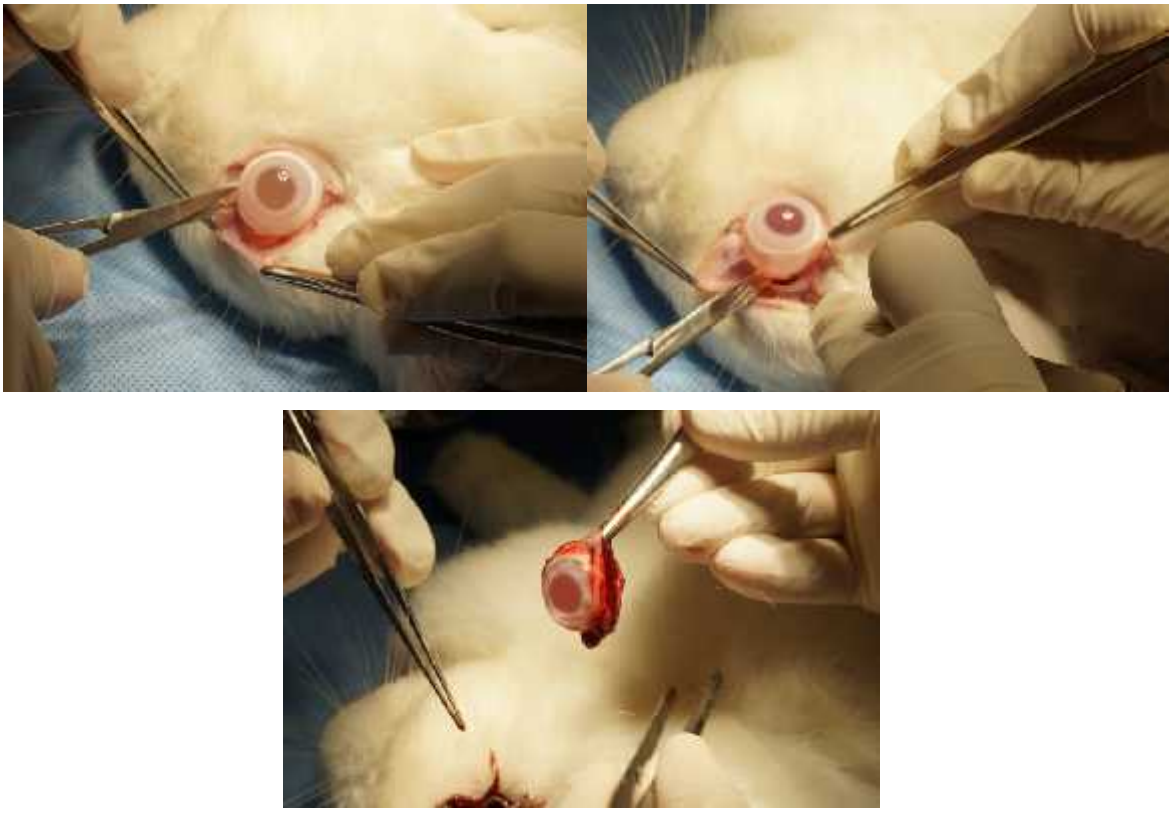


Figure 5.9., 5.10. and 5.11. In-vivo rabbit eye enucleation

5.3. Results

A total of 12 freshly enucleated bovine eyes and 14 rabbit eyes used for tissue welding with 1470-nm diode laser. No opacification or photocoagulation signs were examined macroscopically at the welded site of 26 corneas.

5.3.1.*In-vitro* Study

12 freshly enucleated tissue sections of bovine eyes on slides were colored with haematoxylin-eosin and picro-sirius for the examination by means of light microscopy. (Figure 4.1. – 4.2. – 4.3)

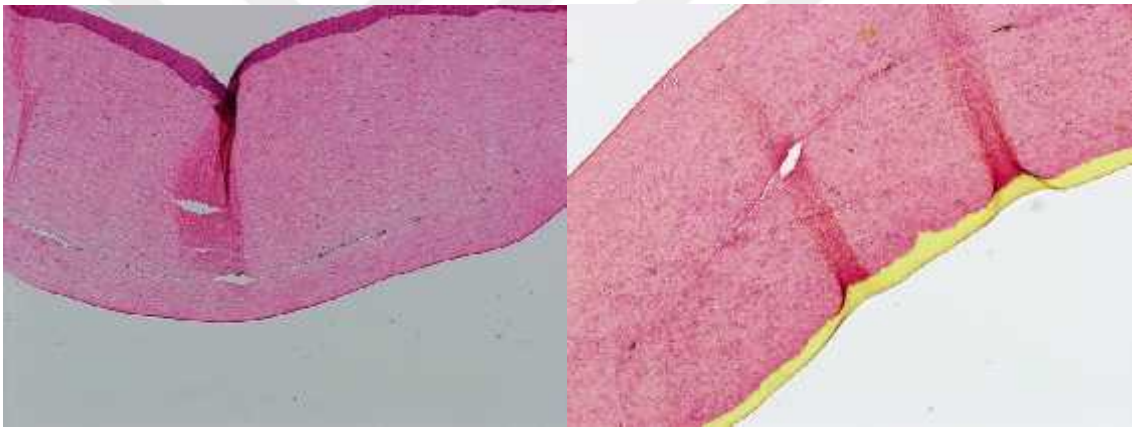


Figure 5.12. 170mW-7sec histologic section stained with A. H&E and B. Sirius red

Figure 5.13. 215mW-3,5sec histologic section stained with A. H&E and B. Sirius red

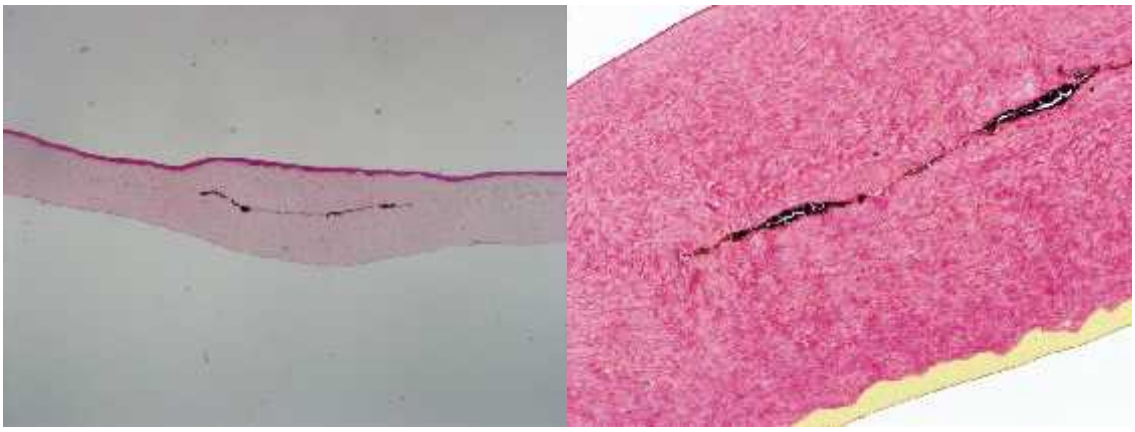


Figure 5.14. 320mW-2sec histologic section stained with A. H&E and B. Sirius red

Results of the 3 group are summarized in Table 2.

1470-nm	Eye 1	Eye 2	Eye 3	Eye 4
170 mW-7 sec	Welded	Welded	Welded	Welded
215mW-3,5 sec	Welded	Not welded	Welded	Not welded
320mW-2 sec	Not welded	welded	Welded	Welded

Table 5.2. *In-vitro* study parameters and welding result

5.3.2. *In vivo* study

Rabbit corneas were observed in postoperative first day, first week, second week and forth week. During this 4 weeks period there were no infection signs, anterior segment depths were normal and no leakage through the clear corneal incision was observed. Seidel test was done for the leakage control.(Figure 2)

Eyes was enucleated at the end of 4.week for histological assessments. 12 eyes and 2 control eyes, totally 14 eyes tissue sections on slides were colored with haematoxylin-eosin for the analsing with the help of light microscopy.(Figure2)

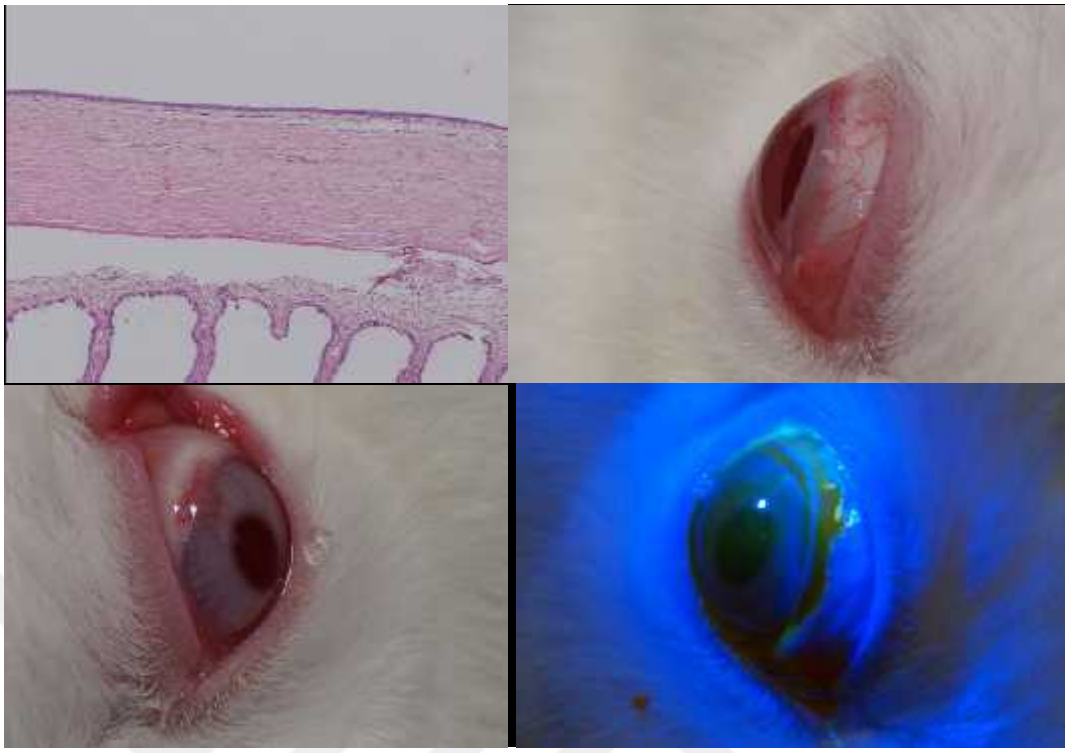


Figure 5.15. 320mW-2 sec histologic section A. welded, stained with H&E B. anterior camara depth is normal C. no infection sign D. no leakage sign, tested with seidel

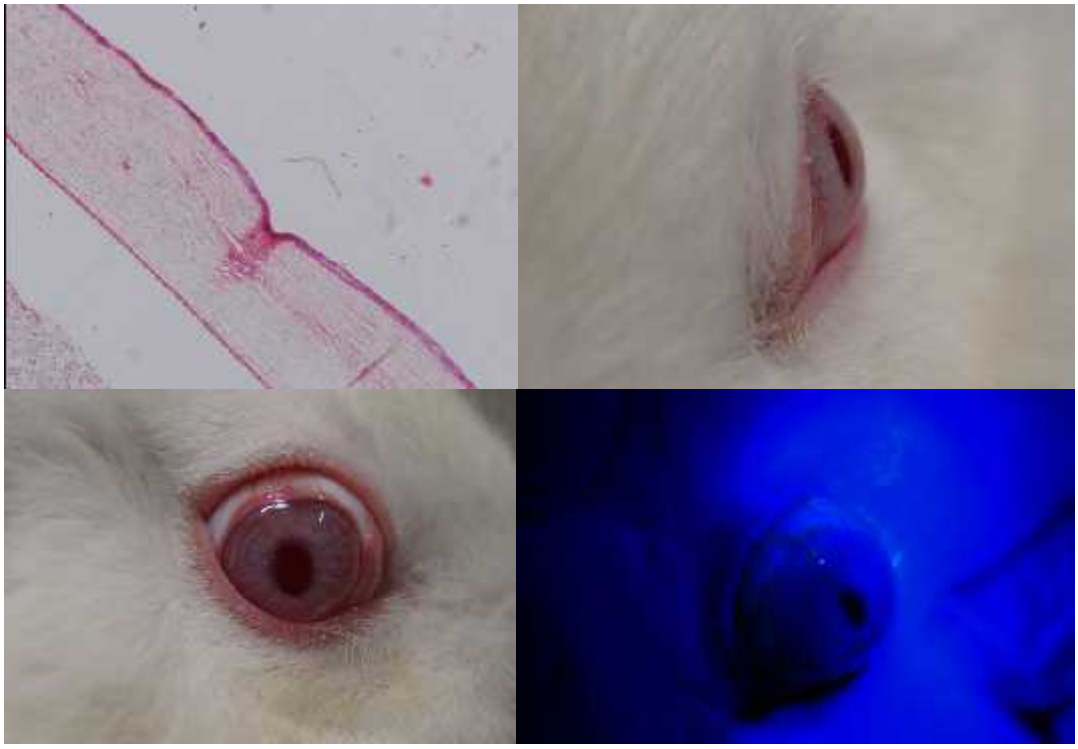


Figure 5.16. 170mW-7 sec histologic section A. welded, stained with H&E B. anterior camara depth is normal C. no infection sign D. no leakage sign, tested with seidel

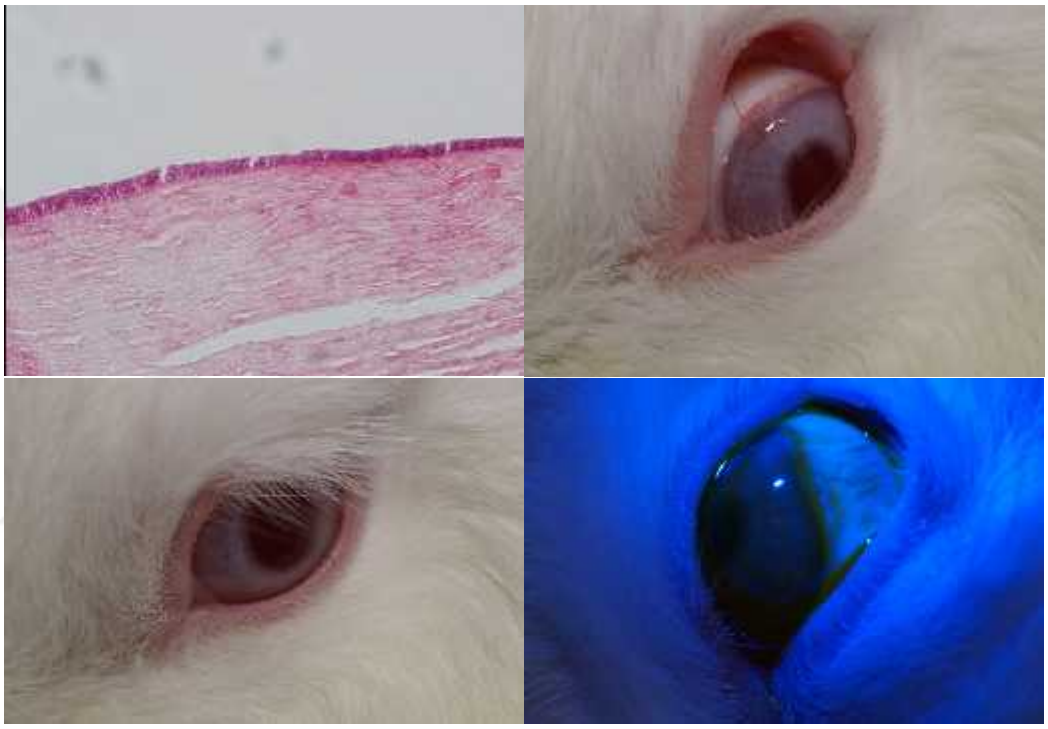


Figure 5.17. 320mW-2 sec histologic section A. not welded, stained with H&E B. anterior camara depth is normal C. no infection sign D. no leakage sign, tested with seidel

The histologic examination results were summarized in Table 3.

Eye	Power	Duration (sec.)	Results	Postop leakage	Postop infection signs
1 Right	170 mW	7 seconds	Not welded	No leakage	No infection
1 Left	170 mW	7 seconds	Welded	No leakage	No infection
2 Right	170 mW	7 seconds	Corneal cut cannot be determined	No leakage	No infection
2 Left	170 mW	7 seconds	Corneal cut cannot be determined	No leakage	No infection
3 Right	170 mW	7 seconds	Not welded	No leakage	No infection
3 Left	170 mW	7 seconds	Welded	No leakage	No infection
4 Right	320 mW-	2 seconds	Corneal cut cannot be determined	No leakage	No infection
4 Left	320 mW	2 seconds	Not welded	No leakage	No infection
5 Right	320 mW	2 seconds	Welded	No leakage	No infection
5 Left	320 mW	2 seconds	Welded	No leakage	No infection
6 Right	320 mW	2 seconds	Corneal cut cannot be determined	No leakage	No infection
6 Left	320 mW	2 seconds	Welded	No leakage	No infection
Control Right	-	-	Not Welded	No leakage	No infection
Control Left	-	-	Not Welded	No leakage	No infection

Table 5.3. *In-vivo* study parameters, welding results, infection and leakage results

6. DISCUSSION

This PhD study is a series of experiments about tissue welding via laser with a range between 809-nm to 1980-nm.

In cataract surgery sutureless clear corneal incisions created a revolution impact. Although clear corneal incisions have many advantages, many studies showed the existence of the leakage of the wound or having a gap in the beginning of the period of postoperation.

Mehran*et al.*andSarayba *et al*. placed India ink on the wound surface to assess the self-sealing features of incisions of the basic clear corneal cataract while the intraocular pressure was changed, in order to imitate the intraocular pressure variations secondary to squeezing of the eye or blinking. [82,83] From aqueous specimens of globes, the optical densities were measured both at the beginning and end of India ink implementation by utilizing a spectrophotometer. The globes having sutureless clear corneal wounds showed an important rise in spectrophotometric readings. Herretes *et al*.,monitored inflow of blood-tinged tear fluid into the anterior chamber through the wound was by utilizing digital video in following phacoemulsification with the usage of sutureless corneal incisions. Inflow of extraocular fluid was examined in all eyes once the cannula was removed, even after hydrosealing of the wound. Two patients demonstrated spontaneous inflow of fluid. [84]

Behrens *et al*., examined the clear corneal incision wound with non-contact optical coherence tomography to evaluate dynamics of the wound in the instant period of postoperation after phacoemulsification surgery. One patient over eight patients showed partial spontaneous gaping in the incision, another patient demonstrated localized gaping of the internal condition of the wound. Four other incisions demonstrated localized Descemet's membrane detachment. All in these patients this situations are undetected by slit-lamp evaluation. [85]

Thomas *et al*.reported one of the largest study which is comparing the incidence of postoperative endophthalmitis in clear corneal cataract surgeries implemented by having and having no suture closure in 815 consecutive eyes that had cataract surgery by way of a

single surgeon over a 5-year phase. Five cases improved culture-positive postoperative endophthalmitis in the group which was unsutured and nothing in the group which was sutured ($P = 0.022$). [86]

In Sarayba *et al*. study, implementation of manual pressure was a imitation of the force which was caused by the eyelid during a hard squeeze or a blink and the immediate force release as would be seen during the time of opening the eyelid in a blink cycle. [66] Postoperative phacoemulsification patients rubbing their eyes or applying pressure on their eyelids throughout implementation of medication may cause the same or even greater stress to the wound.

These studies results showed that there is an inflow in to the anterior camara after the phacoemulsification operations through sutureless clear corneal incisions. Corneal welding method is a chance to prevent inflow in to the anterior camara that allowed microorganism from the outer side to flow into the anterior camara through clear corneal incisions. Some studies showed that corneal tissue welding may be an important alternative to suture in clear cornal incisions. In ophthalmology, empiric studies of suturing which is laser-induced of corneal tissue on animal models have been stated since 1992 by many different authors, based on the usage of near- and far-infrared lasers. [57-64]

Burstein *et al*. describe the initial successful integration of corneal tissue from porcine cadaver eyes by utilizing an essential hydrogen fluoride (HF) wavelength of 2,560 nm at 30 mW and a HF overtone wavelength of 1,340 nm at 320 mW produced from a HF chemical laser. [57] Barak *et al*. utilized the temperature-controlled pulsed CO_2 laser so as to integrate corneal and corneoscleral wounds in bovine *in-vitro* eyes and in vivo rabbit eyes. [58,59] Keates *et al*. utilized a carbon dioxide laser (10.6 μm) in order to integrate human scleral and corneal eye bank tissue and tissue of the albino rabbit eye, but accomplished no integration of the tissues. [56] Trabucchi*et al*. investigated the tissue fusion, attempted both with direct radiation absorption at 1950-nm radiation and with ICG dye-enhanced technique at 810-nm. Their results; the unity of corneal wounds which were cured with the ICG-enhanced technique showed tissue welding in 70% of cured wounds and utilizing 1950-nm tissue integration was examined in 50% of cured wounds. They investigated that macroscopic assessment demonstrated a salient thermal damage of the epithelium in the samples which were treated with 1950-nm radiation. [60] Savage *et al*

concluded that 1455-nm NIR laser welding system ensures strong and full thickness welds and does not entail the usage of extrinsic dyes, chromophores, or solders. [61] Pini*et al*. utilized a low power diode laser (805 nm) in integration with the photoenhancing dye indocyanine green which was implemented to the incision in order to carry out the first in vivo human corneal weld. [62] Menabuoni*et al* used 810-nm with association of ICG for welding in cataract surgery in humans and concluded that the corneal sealing technique which was laser-assisted was fast and safe and could provide an option to suturing of corneal, with important possible implementations for the longer incisions' closure. [63]

In 2013 Strassman*et al*. examined the effectiveness and ability to be reproduced of corneal cuts' CO2 laser soldering utilizing real-time infrared fiber- optic radiometric control of tissue temperature in bovine eyes and to assess the time of this method in rabbit eyes. [64] As a method; both in *in-vitro* and in vivo experiments corneal cuts were sealed with a laser of CO_2 , having or having no albumin soldering, a fiber-optic radiometric temperature control system for the CO_2 laser was utilized. Pressure of leaking and histological results were examined and made comparison between groups. They concluded that CO_2 laser soldering which is integrated with the fiber-optic radiometer is an efficient, reliable, and fast tool for the closure of corneal wounds, and has some advantages over conventional suturing with regards to pressure of leaking and surgical time.

Latest study in 2013, Buzonetti*et al*. evaluated the effectivitiy of diode laser welding to close corneal wounds in diffusing keratoplasty (PKP) and cataract surgery in pediatric patients. [87] Patients had surgery for congenital cataract or femtosecond laser–assisted corneal transplantation. The surgery was followed by corneal wound closure using diode laser welding of the stroma with in combination with the photoenhancing dye indocyanine green. Buzonetti *et al* concluded that laser welding of corneal tissue seemed to be secure and reliable and efficient in children for whom a sutureless surgical procedure is significant to decrease the usage of anesthesia for suture management, avoid endophthalmitis, and develop the antiamblyopic impact.

Kate Xie presented a study to to assess the impact of welding with diode laser on strength of the wound in corneas cut via femtosecond laser. [88] Conclusion of that study was uptake of ICG in cadaver corneas pre-cut via the femtosecond Zig-Zag incision, it does not supplement important mechanical stableness. Ilan Gabay presented a study, about

developing a system for sealing clear corneal incision by means of soldering through a semiconductor disk laser (λ=1.9μm), under the control of close temperature. [89] The system was examined in the corneas of 15 eyes of pigs, *in-vivo*. Optical Coherent Tomography (OCT) and histopathologic analysing demonstrated little thermal harm and fine apposition. Yishai Porat demonstrated the potentiality of an combined laser tissue bonding equipment with an optical coherency tomography probe, which will ensure, first of all, a real time feedback of the variation of the tissue structure, and demonstrate the progress of bonding. [90] Rossi *et al*. presented a study that they called"all laser" endothelial keratoplasty performed in human subjects. [91] After the stromal layer of the donor flap was stained with a liquid ICG solution, the donor flap was put into the eye of the recipient and laser-welded to the eye of the recipient. A transplanted flap insertion was examined in all eyes.

Convetti at al introduce a new penetrating keratoplasty method, laser-asssested, which combines a femtosecond anvil as trephination pattern with the procedure of laser welding. [92] 24 eyes of 22 patients experienced penetrating keratoplasty. A femtosecond laser was utilized in order to create anvil-profiled cuts in donor and corneas of the recipient. Welding of diode laser was achieved, supporting basic suturing. To conclude, the integration of the femtosecond laser cutting technique, providing the anvil profile, with the help of the diode laser welding tehcnique, sealing the injuries, evolved the total results of corneal surgery, as applied to different kinds of pathologic situations.

In our *in-vitro* studies infrared (809-nm diode, 980-nm diode, 1070-nm YLF, and 1980-nm Tm:YAP) lasers irradiated full-thickness corneal cuts on freshly removed bovine eyes to get immediate laser welding. [93] According to our results, 1980- and 1070-nm lasers were found as better wavelengths compared with others. The 1980-nm laser with 0.38 W of power and with minimum duration of 20 seconds welded corneal tissue full thickness and without any microscopic carbonization. The 1070-nm laser with power of 2 Wand minimum duration of 5 seconds also welded corneal tissue full thickness and without any carbonization.

Results of 980-nm laser are also satisfactory with power of 2 W and minimum duration of 20 seconds, but mild carbonization of the adjacent corneal tissue was seen in the microscopic view. Duration between 10 seconds, in which the cornea was not welded, and

20 seconds should be tested, and it is important to find the minimum duration for a clear welded cornea without carbonization.

Results of 809-nm laser varied according to concentrations of ICG, used as chromophores. The result of 809-nm laser combined with 6% ICG concentration, 200-mW power, and 30-second duration parameters is the same with 809-nm laser combined with 12% of ICG concentration, 200 mW of power, and 3 seconds of duration. There are no studies about toxicity of ICG application before welding procedure, but Chang et al investigated the corneal endothelial cytotoxicity of dyes for capsule coloring in cataract surgery. ICG 0.25% did not cause important harm to corneal endothelial cells. Important cytotoxicity was examined with ICG 0.50%, and being exposed to ICG 0.25% for 1–10 minutes demonstrate a tendency to cytotoxicity after 10 minutes. On transmission electron microscopy, corneal endothelial cells that had been subjected to ICG 0.50% demonstrated salient organelle swelling and disruption, electron-dense granules, and cell lysis. Instead of 809-nm diode laser, using 980-nm, 1070-nm, or 1980-nm laser types for corneal welding procedure may be safer because there is no need for ICG or any other chromophores for welding. On the other hand, it is very important to find the minimum ICG concentration to minimize the risk of ICG toxicity on corneal endothelium or epithelium.

The shortest exposure time and the lowest power parameters, which welded the cornea totally, were chosen for 1070-nm laser and 1980-nm laser comparative study. In 1070-nm laser welding, it was seen that with 5 seconds of duration, 5 of 10 corneas were welded totally and that it is not an adequate duration of laser to weld all corneas. Instead of 5 seconds, with between 5 and 10 seconds, there would be better welding results. The situation was the same with 1980-nm laser sampling corneas. The 1980-nm laser results showed that 2 seconds of duration is not an adequate duration to weld corneas. Four corneas were partially welded and 6 corneas were not welded with 2 seconds of duration, and it was seen that, although preliminary study for all 4 types of laser, 3 seconds also was not enough to weld cornea with 1980-nm laser welding. According to these results, 4 seconds of duration would be the best option for welding sampling corneas safely and totally.

Histologically, increment of closure along the incision line, less carbonization existence at the welded areas, regularity of substantia propria of the cornea without disruption and eosinophils to periphery tissue are the markers of better results under microscope. The damage of laser beam was determined by the existence of big holes and blackened carbonization on the incision area and also by the decline of eosinophilis at substantia propria cornea. Collagen fibers tended to the incision line, and collagen fiber eosinophils of the nearby area have no difference with the area at periphery, and no carbonization was observed in the incision area when full-thickness closure occurred after laser welding. In the light of the foregoing, 1070-nm and 1980-nm wavelengths have a great potential for corneal welding. According to this study we planned our PhD thesis study with 1470-nm which has better water absorbtion.

In our in-vitro and in-vivo study 1470-nm wavelength chosen because of higher water absorption in this wavelength compared to other wavelengths such as 809-nm which is used mostly in other studies. Water absorption is very important for our study because approximately 12-15% and 1-3% of the net weight of the tissue is made up of collagen and glycosaminoglycans respectively and the normal cornea consists of 78% water. To find most suitable wavelength for in vivo study, in our previous studies different wavelengths was tested *in-vitro* in Biophotonics Laboratory Bogazici University. As a result; cornea is a transparent tissue without any laser absorber like photoenhancing dye indocyanine green usage, laser beam could be absorbed difficultly by the water content of cornea and it may targeted in iris tissue which has highly melanin content. This was lead to damage in iris tissue and in human eye this may cause to deformation of pupil shape and aberration in vision, increase in eye pressure because of pigment discharge inside to anterior chamber of the eye and finally fail of cataract surgery. Most studies with longer-term follow-up are required to use 1470-nm laser for corneal welding of clear corneal incision.

One of the new aplication method of laser welding is tissue and material welding. Our study with 1470-nm is the first aplication of amniotic mebrane welding to a contact lens. Mitotic division of the basis cell layer of the corneal epithelium is normally renewed in 7-10 days on a regular basis. Epithelial cells on the surface shed to precorneal tear film. The basal layer cells are constantly replaced by multiplying the cells. Corneal wound

healing takes place in three stages these are: migration of healthy epithelial cells to this area, regeneration and stromal wound healing to close the defect. [94,95]

If corneal epithelial injury is minor, after injury damage is tries to be closed by cell migration as soon as possible. Epithelial migration begins within minutes, there may be delays up to 4-5 hours of a wider damage. This delay time prior to the rapid cell division required for anatomical, physiological and biochemical preparation. Clinically, corneal epithelial migration is sufficient to close many lesion. [94, 95] AM accelerates epithelialization, maintains normal epithelial phenotype and inflammation, cicatrisation, reduces neovascularization. These effects depends on suppression of inflammation with the effect of AM antiprotease, suppression of lipopolysaccharide that increase IL-1α and IL-1β, reducing TGF- β signaling system and scar system by reducing the conversion of myofibroblastsand fibroblast, the creation of epithelial progenitor cells and colony survival by being basal membrane and increasing growth factors carring within. [94-101]

AM acting as a bandage CL saves the epithelium from the eyelid movements. it is claimed that there is growth factors in AM needed for treatment of deep ulcerations and desmatocels in patients at risk of perforation of the cornea, inflammation will suppressed with the use of AM, also increases the success of future penetrating or lamellar keratoplasty through containing basal membrane and collagen but not allogenic cells. [77]

AMT in the cornea therapeutic CL can be used in almost all applications. Gris et al study showed that the AM implant kept in place for 12.5 days-mean (range, 3-34). [78] In eleven of the twenty patients, the AM implant separated within the first 8 days. Once the corneal implant was in a postoperative way wrapped with a soft CL, duration of attachment was increased. Therapeutic CLs are used to increase the regeneration of corneal epithelial healing and lenses. Therapeutic CLs cover elements that help in reducing pain, hydration and protection of the cornea to reduce oedema, corneal epithelial healing maintaining the mechanical disorders of the cornea. [79] With sufficient oxygen permeability of therapeutic CLs help to improve the surface of the cornea. Also be used as a mechanical obstacle to save the surface of the eye from external influences. Therapeutic CLs can be use after AMT for decreasing inflammation until completion of reepithelialization. [80]

Today, that has been practiced cover (overlay) technique; biological AM acts as a CL and at the end separates from the receiving tissue. In the cover technique AM applied to cover limbus. [102,103] In this technique stromal face should be above and epithelial side should be in contact with the ocular surface. In the cover technique result of separation of AM from ocular surface after epithelisation under AM, patients immediately reach to clear vision. Separation of CL- AM complex from ocular surface can be easily done by removing the CL. The longer the duration of AM in the ocular surface epithelialization are more successful. However, in this method in some patients, AM may leave before corneal epithelialization is complete. [104] Stay on the surface can be controlled much more comfortable with our welded CL - AM complex. While CL is on the surface of the cornea, AM cannot be separated easily before epithelisation is completed. AM is attached to the peripheral conjunctiva and episclera with 8-0 polyglactin sutures. CL - AM welded complex do not require any sutures and eliminates the many problems resulting from suture such as suture abscess, subconjunctival haemorrhage, infection due to sutures, irritation of the eye lids.

Adhering AM to cornea or conjunctiva with tissue adhesive is an another method that can be applied except suturing AM to conjonctiva and episclera. In the present study, instead of welding AM to CL with laser, AM could be pasted to CL with these adhesives but very serious side effects of these adhesives can be seen. Cyanoacrylate-based glues have been the most widely utilized glues for ophthalmic surgery in a traditional way. [105] The major disadvantage of cyanoacrylate glue is that they form a firm, resistant mass *in situ*. This continues as a foreign body which causes inflamed reactions such as giant papillary conjunctivitis and corneal neovascularization. [106] They are also resistant to fluids and metabolites. Though these drawbacks prevent its intraocular usage, they are not very important if the glue is implemented in a superficial way. [107]

Fibrin glue is another substance to adhere tissues.Hick *et al*. and Kheirkhah *et al*. stated that fibrin glue use in AMT and it was found out to be secure and reliable and efficient in fixing the AM to the ocular surface. [108,109] Sekiyama *et al*. assessed the effectiveness and reliability of transplantation of fibrin glue coated freeze dried AM for ocular surface reconstruction. [110] They figured out that the freeze dried AM maintained most of its biological features which indicated that it was reliable and effective for ocular surface reconstruction. Szurman et al indicated that general applicability of being

reproduced and safe sutureless AM fixation onto the corneal surface by fibrin glue in rabbits, and also they demonstrated several advantageous characteristics as increase in, better epithelialization pattern, biocompatibility and having no membrane shrinkage. [111] But the major disadvantage to fibrin glue usege is the danger of conveyed disease from pooled and single-donor blood donors. [112-114] This problem can be minimized by gaining the blood from screened healthy donors but the mst secure preparation is by utilizing the patient's own blood to form fibrin glue. [112, 113] It is autologous and expensive donation needs no less than 24 hours for processing. The final product often has changeable concentrations thereby bringing about a performance which is unpredictable. Furthermore, tensile strength of fibrin glue has not been determined enough and prevents quantification, being dependent on different kinds of external factors, too. Hence laser welding does not contain any chemical or biological substance, the laser welding procedure we used in our study is not supposed to contain any side effects like risk of transmitted disease or inflammatory reactions which can be seen in tissue glues.

The fact that the welding procedure has been applied to a standard CL is considered to be the weak point of our study. The CL to be weld should cover the perilimbal area as well, so the procedure has to be applied also with a wider lens. The scleral CLs seem appropriate.

Scleral CLs are large-diameter GP lenses with diameters ranging from 13mm to more than 20mm. Unlike standard GP lenses, scleral CLs completely wrap the cornea and expand onto the sclera. Corneo-scleral lenses may ensure better first comfort, centration, and stableness when they are compared to a corneal GP lens for many patients. Almost everyone who can wear a corneal GP could be wearing a corneo-scleral GP instead. Following a fitting guide, these lenses are simple to fit and cheap. The material of the scleral CLs is similar to that of Bausch&LombCL, that we used in this project. Because of that the AM is thought to be weld to the scleral lenses as well. Many corneo-scleral CLs are available including Semi-scleral 13.5 (ABBA Optical); SO_2Clear (Dakota Sciences); Perimeter (Essilor Contact Lenses); and DigiForm (TruForm Optics). Because of the difficulty of obtaining these optionel lenses in our country we designed our work according to a normal diameter CL. In addition to that a CL with 14 mm diameter can provide sufficient corneal- amnion contact for pathologies on corneal surface.

7. CONCLUSIONS

As a conclusion according to these studies, results showed that there is a inflow in to the anterior camara and there is a higher rate of endopthalmitis after the phacoemulsification operations with use of sutureless clear corneal incisions. Corneal welding method is a chance to prevent endophthalmitis due to vacuum impact that enabled microorganism from the outer side to flow into the intraocular space via clear corneal incisions. In our 1470-nm CL-AM welding study it was aimed to accomplish the achievement of the conventional AMT with the simpliness of implementing a CL and to aviod dangers and side effects of corneal or conjunctival suturing. Especially to children the necessity of general anaesthesia during AMT is a risk by itself. The results demonstrated that the implementation of the CL–AM complex will be as easy as the implementation of a CL and lasts short and will not need anaesthesia for the children patients. This study is a beginner study for this application. An in vivo animal study should be designed as the continuation. The long term effects of the CL – AM complex to the corneal surface need to be investigated with future studies.

REFERENCES

1. Agapitos PJ. Cataract surgical techniques. Curr Opinion Ophthalmol 1993; 4:39–43.

2. Lyle WA, Jin GJ. Prospective evaluation of early visual and refractive effects with small clear corneal incision for cataract surgery. J Cataract Refract Surg 1996; 22:1456–1460.

3. Leaming DV. Practice styles and preferences of ASCRS members—2002 survey. J Cataract Refract Surg 2003;29: 1412–1420.

4.https://www.pfmmedical.com/en/productcatalogue/featherR_micro_scalpels/clear_cornea_scalpel/index.html

5. http://www.akaleyehospital.com/cataract/

6. http://body-disease.com/eye-and-ear-anatomy-and-physiology-review/

7. http://www.slideshare.net/thelawofscience/human-eye

8. http://avclinic.com/eye-conditions/cataracts/yag-capsulotomy/

9. http://aktis.com.cy/technology-equipment/ellex-super-q-yag-laser/

10. Chung SH, Mazur E. Surgical applications of femtosecond laser. J Biophotonics. 2009;2(10):557-572.

11. Soong HK et al. Femtosecond Lasers in Ophthalmology. Am J Ophthalmol 2009; 147:189-197.

12. Stern D, Schoenlein RW, Puliafito CA et al. Corneal ablation by nanosecond, picosecond, and femtosecond lasers at 532 and 625 nm. Arch Ophthalmol. 1989;107(4):587-592.

13. Ratkay-Traub I, Ferincz IE, Juhasz T, et al. First clinical results with the femtosecond neodymium-glass laser in refractive surgery. J Refract Surg 2003; 19:94 –103.

14. http://www.doutoralencar.com/?p=1838&lang=en

15. http://www.californialasikcenter.com/prk/

16.https://www.quora.com/What-are-the-differences-between-PRK-and-LASIK-refractive-eye-surgeries-and-which-is-better

17. http://www.rolleundrolle.at/lang1/basics.html

18. https://www.glaucoma-association.com/about-glaucoma/treatments/laser/

19. http://diabetesreducer.com/spi/?aff_id=1619&subid=thecorner/diabetic-retinopathy/

20. http://www.retinaeye.com/posteriorvitreous.html

21. Bass LS, Treat MR. Laser Tissue welding: A comprehensive review of current and future clinical applications in C.A. Puliafito ed. Laser surgery and Medicine, principals and practice, Wiley-Liss New York, 1996 pp. 381-415

22.Jain KK,Gorisch W. Microvascular repair with Neodmiyum YAG laser. Acta Neurochirurgical Suppl 1979; 28,1 ; 260-262

23.Jain KK. Sutureless extra-intracranial anastomoses by laser. Lancet 1984; 8046, 817-817

24. Schober R, Ulrich F, Sander T, Drusclen H, Hessel S. Laser induced alteration of collagen substurcture allow microsurgical tissue welding. Science 1986; 232, 1421- 1422

25.Dew DK., Supik I, Darrow C, Price GF. Tisuue repair using laser: a review, Orthopedics, 1993; 16:581.

26. Vale BH, Frenkel A, Trenka-Benthin S, Matlaga BF. Microsurgical anastomoses of rat carotid arteries with the CO2 laser. Plast. Reconstr. Surg. 1986;77: 759.

27. Schober R, Ulrich F, Sander T, Druselen H, Hessel S. Laser induced alteration of collagen substucture allows microsurgical tissue welding . Science 1986; 232: 1421.

28. Schober, R., Ulrich, F., Sander T., Dürsclen, H., and Hessel, S., Laser induced alteration of collagen substructure allows microsurgical tissue welding, Science 1989; 232, 1421-1422.

29. Siobhan A. C, Laura L, Carole L. W, and Jean E. S. Covalent Cross-linking of Fibronectin to Fibrin Is Required for Maximal Cell Adhesion to a Fibronectin-Fibrin Matrix. The journal of biological chemistry. Vol. 272, No. 40, Issue of October 3, pp. 24999–25005, 1997

30. McNally-Heintzelman K. M., ‘‘Laser tissue welding,’’ Chap. 39 in Biomedical Photonics Handbook, pp. 39-1/39-45, 2003; T. Vo-Dinh, Ed., CRC Press, Boca Raton.

31. Bass L. S. and Treat M. R., ‘‘Laser tissue welding: a comprehensive review of current and future clinical applications,’’ Lasers Surg. Med. 1995; 17, 315–349.

32. Oz, M.C., Chuck, R.S., Johnson, J.P., Parangi, S., Bass, L.S., NOwygrod, R., and Treat, M.R. Indocynanine green dye enhanced vascular welding with the near infrared diode laser, Vasc. Surg. 1990; 24, 564-570.

33. McNally K.M., Song B., Hammer D., Heintzelman D.L., Hodges D.E., Welch A.J. Improved laser-assisted vascular tissue fusion using solder-doped polymer membranes on a cynanine model, Proc SPIE 2000; 3907, 65-73.

34. https://www.microchirurgiaoculare.com/en/eye-conditions/corneal-diseases/corneal-diseases-causes-and-symptoms/

35. Rasier R. Infrared laser for corneal tissue welding. Master Thesis 2009

36. http://www.slideshare.net/OptoIhsan/corneal-physiology

37. http://www.corneabank.hu/corn=5.php

38. http://www.arnotteye.com/english/conditions-and-treatments-cataract.php

39. https://endmyopia.org/no-more-surgery-a-new-eye-drop-dissolves-cataracts/

40. http://www.vision-and-eye-health.com/endophthalmitis.html

41. https://www.funscrape.com/Search/endophthalmitis.html

42. Shingleton BJ, Wadhwani RA, O'Donoghue MW, et al. Evaluation of intraocular pressure in the immediate period after phacoemulsification. J Cataract Refract Surg 2001; 27:524–7.

43. Percicot CL, Schnell CR, Debon C, Hariton C. Continuous intraocular pressure measurement by telemetry in alpha-chymotrypsin- induced glaucoma model in the rabbit: effects of timolol, dorzolamide, and epinephrine. J Pharmacol Toxicol Methods 1996; 36:223–8.

44. Coleman DJ, Trokel SL. Direct-recorded intraocular pressure variations in a human subject. Arch Ophthalmol. 1969; Nov; 82(5):637-40.

45. Lertsumitkul S, Myers PC, O'Rourke MT, Chandra J. Endophthalmitis in the western Sydney region: a case-control study. Clin Exp Ophthalmol 2001; 29:400–5.

46. Nagaki Y, Hayasaka S, Kadoi C, et al. Bacterial endophthalmitis after small-incision cataract surgery: effect of incision placement and intraocular lens type. J Cataract Refract Surg 2003; 29:20–26.

47. John ME, Noblitt R. Endophthalmitis. Scleral tunnel vs. clear corneal incision. In: Buzard KA, Friedlander MH, Febbraro McDonnell et al _ Clear Corneal Cataract Incision

Morphology 2347 JL, eds. The Blue Line Incision and Refractive Phacoemulsification. Thorofare, NJ: Slack, Inc.; 2001:53–56.

48. Colleaux KM, Hamilton WK. Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. Can J Ophthalmol 2000; 35:373–8.

49. Pını R, Basıle V, Ambrosını S, Vannellı G, Rossı F, Menabuonı L, Pratesı R, Monıcı M. Healing process stıudy of laser-welded corneal tissue by Multispectral Imaging Autoflorescence Microscopy (MIAM) Progr. Biomed. Opt. İmaging

50. Bleustein CB, Walker CN, Felsen D, Poppas DP. Semisolid albumin solder improved mechanical properties for laser tissue welding. Lasers Surg Med 2000; 27:140- 146.

51. Pini R, Menabuoni L.Laser welding of biological tissue: experimental studies in ophthalmology. Istituto di Fisica Applicata CNR, 2005.

52. Ahlberg KM, Assavanop P, Tay WM. A comparison of the apical dye penetration patterns shown by methylene blue and India ink in root-filled teeth. Int Endod J 1995; 28:30–34.

53. Sarayba M, Taban M, Ignacıo S, Behrens A, Mcdonnell P. Inflow of Ocular Surface Fluid Through Clear Corneal Cataract Incisions: A Laboratory Model. Am J Ophthalmol 2004;138:206-10

54. Tam Y., MD, Vagefi M. Reza, MD, and Naseri Ayman, MD The Clear Corneal Tongue: A Mechanism for Wound Incompetence After Phacoemulsification Diamond Am J Ophthalmol 2007; 143:526–528.

55. DeCoste SD, Farinelli W, Flotte T, Anderson RR. Dye enhanced laser welding for skin closure. Lasers Surg Med 1992; 12(1):25–32.

56. Keates RH, Levy SN, Fried S, Morris JR. Carbon dioxide laser use in wound sealing and epikeratophakia. J Cataract Refract Surg 1987; 13(3):290–295.

57. Burnstein N. L., Williams J. M., Nowicki M. J., Johnson D. E., and Jeffers W. Q., ‘‘Corneal welding using hydrogen fluoride laser,’’ Arch. Ophthalmol. 1992; (Chicago) 110, 12–13.

58. Barak A., Ma-Naim T., Belkin M., and Katzir A., ‘‘Temperaturecontrolled CO2 laser tissue welding of ocular tissues,’’ Proc. SPIE 1997; 2971, 103–105.

59. Barak A., Eyal O., Rosner M, Belotserkousky E., Solomon A., Belkin M., and Katzir A., ‘‘Temperature-controlled CO2 laser tissue welding of ocular tissues,’’ Surv. Ophthalmol. 1997; 42, S77–81.

60. Trabucchi G, Gobbi PG, Brancato R, Carones F, Resti A, Jansen A, Pini R. Laser welding of corneal tissue: Preliminary experiences using 810 nm and 1950 nm diode lasers. Proc Soc Photo Opt Instrum Eng 1996;2623:380–387.

61. Savage HE, Halder RK, Kartazayeu U, Rosen RB, Gayen T, McCormick SA, Patel NS, Katz A, Perry HD, Paul M, Alfano RR. NIR laser tissue welding of in vitro porcine cornea and sclera tissue. Lasers Surg Med 2004; 35(4):293–303.

62. Pini R, Menabuoni L, Starnotti L. First application of laser welding in clinical transplantation of the cornea. Proc SPIE 2001;4244:266–271.

63. Menabuoni L, Pini R, Rossi F, Lenzetti I, Yoo SH, Parel JM. Laser-assisted corneal welding in cataract surgery: retrospective study. J Cataract Refract Surg. 2007 Sep;33(9):1608-12

64. Strassmann E., Loya N., Gaton D. D., Ravid A., Kariv N., Weinberger D., and Katzir A, ‘‘Temperature controlled CO2 laser soldering of pig cornea,’’ Proc. SPIE 2002; 4609, 222–228.

65. Strassmann E, Loya N, Gaton DD, Ravid A, Kariv N, Weinberger D, Katzir A. Laser soldering of the cornea in a rabbit model using a controlled-temperature CO2 laser system. Proc Soc Photo Opt Instrum Eng 2001; 4244:253–265.

66. Burstein NL, Williams JM, Nowicki MJ, Johnson DE, Jeffers WQ. Corneal welding using hydrogen fluoride laser. Arch Ophthalmol 1992; 110(1):12–13.

67. Desmettre TJ, Mordon SR, Mitchell V. Tissue welding for corneal wound suture with CW 1.9 micro diode laser: An in vivo preliminary study. Proc Soc Photo Opt Instrum Eng 1996; 2623:372–379.

68. Strassman E., Loya N., Gaton D. D., Ravid A., Kariv N., Weinberger D., and Katzir A., ‘‘Laser soldering of the cornea in a rabbit model using a controlled-temperature CO2 laser system,’’ Proc. SPIE 2001; 4244, 253–265.

69. Rasier R, Gulsoy M. Amniotic membrane welded to contact lens by 1470-nm diode laser: a novel method for sutureless amniotic membrane transplantation. Int J Ophthalmol. 2014; 7(6): 996-1000.

70. Yu FS, Yin J, Xu K, Huang J. Growth factors and corneal epithelial wound healing. Brain Res Bull. 2010; 81(2-3):229–235.

71. Sun TT, Tseng SC, Lavker RM. Location of corneal epithelial stem cells. Nature. 2010; 463(7284):250–254.

72. Luanratanakorn P, Ratanapakorn T, Suwan-apihcon O, Chuck RS. Randomised controlled study of conjunctival autograft versus amniotic membrane graft in ptergyum excision. Br J Ophthalmology. 2006; 90(12):1476–1480.

73. Tseng SCG. Amniotic membrane transplantation for Ocular Surface Recostruction. Biosence Reports. 2001; 21(4):481–489.

74. Tosi GM, Massaro-Giordano M, Caporossi A, Toti P. Amniotic membrane transplantation in ocular surface disorders. J Cell Physiol. 2005; 202(3):849–851.

75. Willoughby CE, Batterbury M, Kaye SB. Collagen corneal shields. Surv Ophthalmol. 2002; 47(2):174–182.

76. Tseng SC, Espana EM, Kawakita T, Di Pascuale MA, Li W, He H, Liu TS, Cho TH, Gao YY, Yeh LK, Liu CY. How does amniotic membrane work? Ocul Surf. 2004; 2(3):177–187.

77. Solomon A, Meller D, Prabhasawat P, John T, Espana EM, Steuhl KP, Tseng SC. Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers. Ophthalmology. 2002; 109(4):694–703.

78. Gris O, del Campo Z, Wolley-Dod C, Güell JL, Bruix A, Calatayud M, Adán A. Amniotic membrane implantation as a therapeutic contact lens for the treatment of epithelial disorders. Cornea. 2002; 21(1):22–27.

79. Satıcı A, Güzey M, Çam V, Gürler B. Therapeutic use of disposable soft contact lenses in corneal diseases. Türkiye Klinikleri Ophthalmology Journal. 2000; 9(2):102–106.

80. Feiz V, Schwab IR. Surgical Ocular Surface Reconstruction. In: Yanoff M, Duker JS, editors. Ophthalmology. 3rd. England: Mosby Elsevier; 2009. p. 375.

81. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am J Ophthalmol. 1997; 123(3):303–312.

82. Taban M, Sarayba MA, Ignacio TS, Behrens A, McDonnell PJ. Ingress of India Ink into the anterior chamber through sutureless clear corneal cataract wounds. Arch Ophthalmol. 2005; 123:643–8.

83.Sarayba MA, Taban M, Ignacio TS, Behrens A, McDonell PH. Inflow of extraocular fluid into the eye through clear cornea cataract incisions: A laboratory model. Am J Ophthalmol. 2004; 138:206–10.

84. Herretes S, Stark WJ, Pirouzmanesh A, Reyes JM, McDonnell PJ, Behrens A. Inflow of ocular surface fluid into the anterior chamber after phacoemulsification through sutureless corneal cataract wounds. Am J Ophthalmol. 2005; 140:737–40.

85. Behrens A, Stark WJ, Pratzer K, McDonnell PJ. Dynamics of clear corneal incisions after phacoemulsification surgery in the early postoperative period using optical coherence tomography. J Refract Surg. 2008;24:46–9.

86. Thomas SS, Musch DC, Soong HK. Postoperative endophthalmitis associated with sutured versus unsutured clear corneal cataract incisions. Br J Ophthalmol. 2007;91:728–30.

87. Buzonetti L, Capozzi P, Petrocelli G, Valente P, Petroni S, Menabuoni L, Rossi F, Pini R. Laser welding in penetrating keratoplasty and cataract surgery in pediatric patients:early results. J Cataract Refract Surg. 2013 Dec; 39(12):1829-1834

88. Xie K. Diode laser welding in femtosecond laser-anbledkeratoplasty: Microscopic characterization and evaluation of wound strength. ASCRSD symposium 2015

89.Gabay I. Closure of incision in cataract surgery *in-vivo* using a temperature controlled laser soldering system based on 1.9 micrometer semiconductor laser. Proc. SPIE 9702, Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XVI, 97020B (March 7, 2016); doi:10.1117/12.2209133

90. Porat Y. Optical coherence tomography (OCT) in laser tissue bonding of incisions in the cornea. Proc. SPIE 9317, Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications XV, 93170M (March 5, 2015); doi:10.1117/12.2182287

91. Rossi F, Menabuonş L, MAlandrini A, Canovetti A, Lenzetti I, Pini R. All-laser endothelial corneal transplant in human patients . Proc. SPIE 8209, Ophthalmic Technologies XXII, 82091O (March 8, 2012); doi:10.1117/12.907941

92. Canovetti A, Malandrini A, Lenzetti I, Rossi F, Pini R, Menabuoni L. Laser-Assisted Penetrating Keratoplasty: 1-Year Results in Patients Using a Laser-Welded Anvil-Profiled Graft .Am J Ophthalmol 2014;158:664–670.

93. Rasier R, Ozeren M, Ozgur A, Bahcecioglu H, Seckin I, Kalaycıoglu H, Kurt A, Sennaroglu A, Gulsoy M. Corneal Tissue Welding With Infrared Laser Irradiation After Clear Corneal Incision. Cornea. 2010 Sep; 29(9):985-90.

94. Amenta PS, Martinez-Hernandez A, Treistad RL. Repair and regeneration. In: Damjanov I, Linder J (eds). Anderson's Pathology, 10th ed, St. Louis: Mosby-Year Book, Inc, pp. 416-447, 1996.

95. Kruse FE. Stem cells and corneal epithelial regeneration. Eye 8: pp. 170-183, 1994.

96. Tseng SCG: Amniotic membrane transplantation for Ocular Surface Recostruction. Biosence Reports, Vol. 21, No. 4, August 2001

97. Sato H, Shimazaki J, Shimazaki N, et al: Role of growth factors for ocular surface reconstruction after amniotic membrane transplantation. Invest Ophthalmol Vis Sci 1998;39: 428.

98. Aquavell JV, Del Caro M, Musco PS, Ueda S, De Paolis M. The effect of a collagen bandage lens on corneal wound healing.A preliminary report. Ophthalmic Surg 1987; 8: 570-573

99. Sorsby A, Hythorne J, Reed H. Futher experience with amniotic membrane grafts in coustic burns of the eye. Br J Ophthalmol 1947; 31: 409-418

100. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am J Ophthalmol 1997; 123: 303-312

101. Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic

102. Letko E, Stechschulte SU, Kenyon KR, Sadeq N, Romero TR, Samson CM, et al. Amniotic membrane inlay and overlay grafting for for corneal epithelial defects and stromal ulcers. Arch Ophthalmol 2001; 119: 659-663.

103. Yaycıoğlu RA, Akova YA. Amnion Membran Transplantasyonunun Oftalmolojide Yeri. T Klin J Ophthalmol 2003,12: 227-234.

104. Khodadoust AA, Silverstein AM, Kenyon KR, Dowling je: Adhesion of regenerating corneal epithelium: the role of basement membrane. Am J Ophthalmology 1968;65:339-48.

105. Trott AT. Cyanoacrylate tissue adhesives. An advance in wound care. JAMA. 1997;277:1559–60.

106. Carlson AN, Wilhelmus KR. Giant papillary conjunctivitis associated with cyanoacrylate glue. Am J Ophthalmol. 1987;104:437–8.

107. Tseng YC, Hyon SH, Ikada Y, Shimizu Y, Tamura K, Hitomi S. *In vivo* evaluation of 2-cyanoacrylates as surgical adhesives. J ApplBiomater. 1990;1:111–9.

108. Hick S, Demers PE, Brunette I, La C, Mabon M, Duchesne B. Amniotic membrane transplantation and fibrin glue in the management of corneal ulcers and perforations: A review of 33 cases. Cornea. 2005;24:369–77.

109. Kheirkhah (LCT)A, Casas V, Raju VK, Tseng SC. Sutureless amniotic membrane transplantation for partial limbal stem cell deficiency. Am J Ophthalmol.2008;145:787–94. [PMCID: PMC2840647]

110. Sekiyama E, Nakamura T, Kurihara E, Cooper LJ, Fullwood NJ, Takaoka M, et al. Novel sutureless transplantation of bioadhesive-coated, freeze-dried amniotic membrane for ocular surface reconstruction. Invest Ophthalmol Vis Sci. 2007;48:1528–34.

111. Szurman P, Warga M, Grisanti S, Roters S, Rohrbach JM, Aisenbrey S, Kaczmarek RT, Bartz-Schmidt KU. Sutureless amniotic membrane fixation using fibrin glue for ocular surface reconstruction in a rabbit model.Cornea. 2006 May;25(4):460-6.

112. Everts PA, Knape JT, Weibrich G, Schonberger JP, Hoffman J, Overdevest EP, et al. Platelet-rich plasma and platelet gel; a review. J Extra Corpor Technol.2006;38:174–87.

113. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyl J, et al. Platelet-rich fibribn (PRF); a second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral RadiolEndod. 2006;101:37–44.

114. Aizawa P, Winge S, Karlsson G. Large-scale preparation of thrombin from human plasma. Thromb Res. 2008;122:560–7.