1. INTRODUCTION

1.1. Motivation and Objective

Clear corneal and temporal incisions have gained increased popularity in cataract extraction surgery, although this technique has serious risks. Lack of suture-induced astigmatism, lack of conjunctival trauma, which means less discomfort and bleeding, and faster visual recovery are advantages of leaving corneal cut sutureless [1,2]. A recent report showed that 57% of cataract surgeons prefer clear corneal incisions, and 92% of these surgeons favor no-suture closure [3]. A number of authors have reported significant increase in endophthalmitis with clear corneal approach compared to scleral tunnel incisions [4-10]. Lertsumitkul *et al* [4] reported a 3.5-fold, Nagaki *et al* [5] noted a 5.6-fold increased risk of postoperative endophthalmitis with clear corneal temporal incisions compared with sclerocorneal incisions. According to those studies there is an increased risk of acute endophthalmitis with self-sealing clear corneal incisions, this suggestion raises the question whether it might be possible for surface bacteria to traverse these clear corneal incisions during the postoperative period. Thus, recently laser welding techniques are under investigation for closing corneal cuts.

Objective of this study is to investigate the potential of NIR to MIR lasers for the cornea welding purposes. Different wavelengths and laser delivery conditions were applied to corneal cuts *in vitro* and thermal effects of laser welding process were examined by transmission light microscopy.

1.2. Outline

By writing this thesis, I have provided thorough background of the cornea anatomy and histology which are followed by corneal laser welding experiments; first preliminary study for finding optimum parameters of 809-nm, 980-nm, 1070-nm and 1985-nm corneal laser welding and secondly, a comparative study of 1070-nm vs 1985-nm was carried out.

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

Chapter 3 focuses on experimental set up, experimental protocol and histologic evaluation. It introduces the subject numbers and the parameters of lasers.

Chapter 4 shows the results of preliminary experiment and the results of comparative study of 1070-nm vs 1985-nm.

Finally Chapter 5 and Chapter 6 provides a discussion on the work done. In the discussion section firstly it discusses preliminary results and secondly discusses comparative study 1070-nm vs 1985-nm and compares the results with other studies. It provides recommendations for future work in corneal laser welding with specific wavelengths of lasers.

2. BACKGROUND

2.1. Lasers in Ophthalmology

The eye is like a camera. Light comes in through the cornea, a clear cover that is like the glass of a camera's aperture. The amount of light coming in is controlled by the pupil, an opening that opens and closes a little like a camera shutter. The light focuses on the retina, a series of light-sensitive cells lining the back of the eye. The retina acts like camera film, reacting to the incoming light and sending a record of it via the optic nerve to the brain.

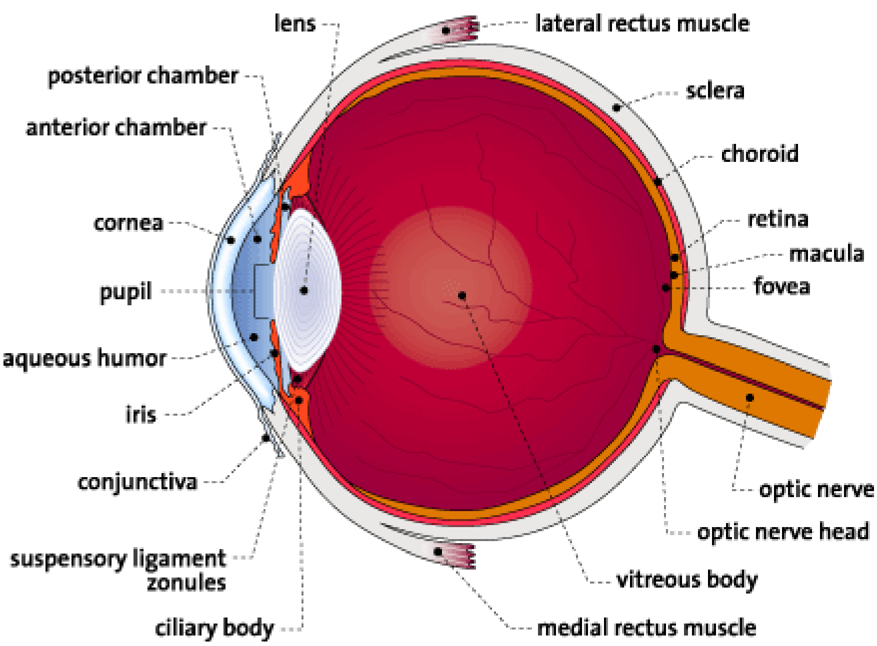


Figure 2.1. Anatomy of eye [11]

When looking into someone's eyes, we can easily see several structures:

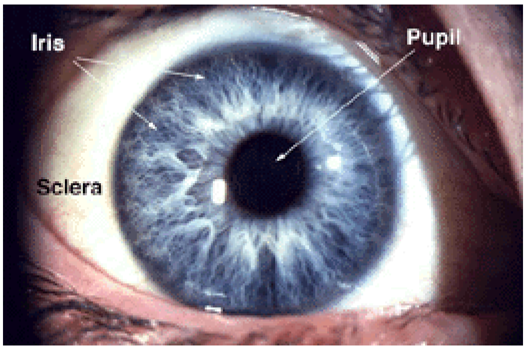


Figure 2.2. Picture of a human eye [12]

A black-looking aperture, the pupil, that allows light to enter the eye (it appears dark because of the absorbing pigments in the retina).

A colored circular muscle**,** the iris, which is beautifully pigmented giving us our eye's color (the central aperture of the iris is the pupil). This circular muscle controls the size of the pupil so that more or less light, depending on conditions is allowed to enter the eye. Eye color, or more correctly, iris color is due to variable amounts of eumelanin (brown/black melanins) and pheomelanin (red/yellow melanins) produced by melanocytes.

A transparent external surface**,** the cornea that covers both the pupil and the iris. This is the first and most powerful lens of the optical system of the eye and allows, together with the crystalline lens the production of a sharp image at the retinal photoreceptor level.

Lasers provide a convenient source of focused light energy that can be delivered to a target and, specifically, can do one of four effects in the eye:

1) Photothermal effect
2) Photodisruption effect
3) Photoablation effect
4) Photochemical effect

1) Photothermal effect is used in corneal tissue welding procedure. In the case of the use of a monochromatic laser beam, the choice of wavelengths (absorption strength) and the duration of laser beam irradiance determine how the thermal effect manifests. The two important parameters are the peak value of the tissue temperature reached and the spread of

the heating zone area in the tissue. The heating of an area in a tissue can produce four effects:

i)Hyperthermia: meaning a moderate rise in temperature, corresponding to temperatures of 41ºC to 44ºC for some tens of minutes and resulting in cell death due to changes in enzymatic processes. ii) Coagulation: refers to an irreversible necrosis without immediate tissue destruction. The temperature reached (50º to 100º C) for around a second, produces desiccation, blanching, and a shrinking of the tissues by denaturation of proteins and collagen. iii) Volatilization: means a loss of material. The various constituents of tissue disappear in smoke at above 100º C, in a relatively short time of around one tenth of a second. When the local temperature of a tissue reaches 100º C, water starts converting into steam, producing thermal ablation (photothermal ablation) of the tissue. This ablation is a purely thermo mechanical effect produced by the pressure buildup due to steam formation. At the edges of the volatilization zone there is a region of coagulation necrosis, there is a gradual transition between the volatilization and healthy zones. iv) Carbonization: occurs when the tissue temperature reaches above 150º C, at which tissue chars, converting its organic constituents into carbon. This process has to be avoided because it is of no benefit and leads to irreparable damage of a tissue.

Lasers can create a thermal lesion, that is, a burn, in the same way that light from the sun that is focused with a magnifying glass will burn paper. This thermal lesion can create a scar or hole in the target tissue. In photocoagulation techniques depend on the thermal effect, i.e., the absorption characteristics of the tissues to be coagulated indicate the wavelength to be chosen. The three important ocular light absorbers are melanin, hemoglobin and xanthophyll. For anterior segment work, melanin in the iris and trabecular meshwork is the most important absorber and hemoglobin in blood is the second most important. In retinal work, hemoglobin and xanthophyll absorption are the most important absorbers. Use of visible or near infrared laser wavelengths to treat retinal disease or glaucoma. Examples are : i) diabetic retinopathy associated with capillary nonperfusion or swelling caused by leaking microaneurysms, ii) retinal vein occlusions that block ocular blood drainage causing retinal hemorrhage, ischemia, and swelling, iii) age-related macular degeneration, which, in the wet-type neovascular tissue, invades normal retina, producing, macular edema and hemorrhage, iv) retinal tears which can occur as a part of aging, as a complication following catarct surgery or from an eye injury, and v) glaucoma, which may be treated by producing a channel in iris structures or shrinkage of drainage tissues in order to facilitate lowering of eye pressure.

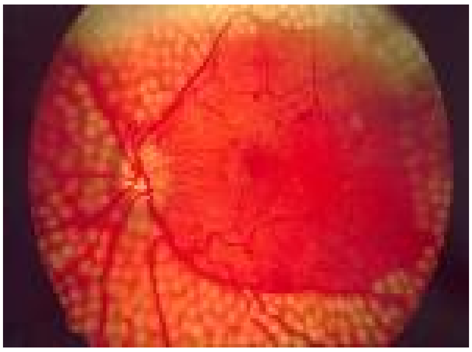


Figure 2.3. Laser treatment for diabetic retinopathy [13]

Figure 2.4. Retinal vein occlusion [14]

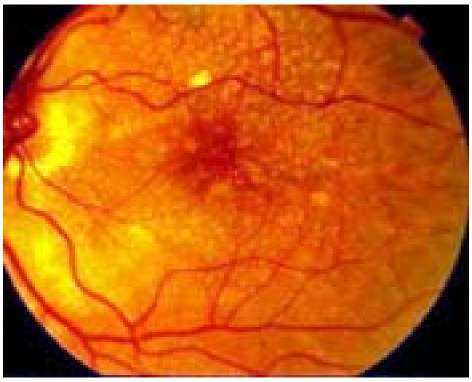


Figure 2.5. Age related macular Degeneration [15]

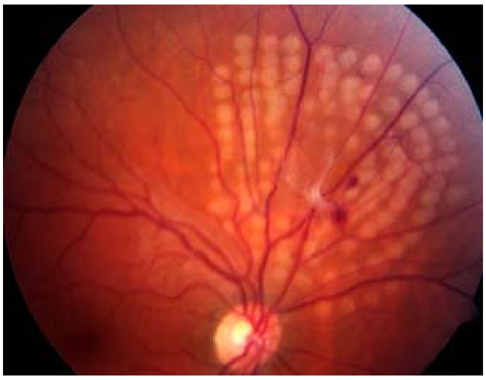


Figure 2.6. Retinal tear surrounded with laser spots [16]

2) Photodisruption effect is achieved with very short duration, high-powered lasers such as the Q-switched neodymium YAG. Non-thermal effects cause disruption of any target tissue either transparent or opaque.

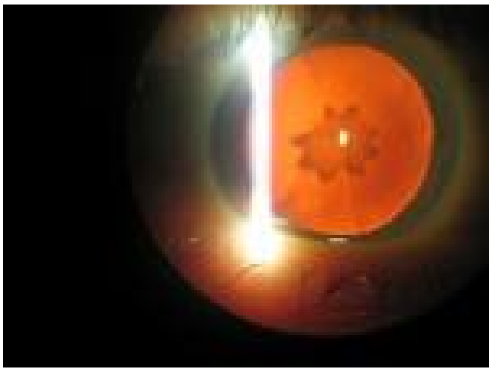


Figure 2.7. Posterior capsulotomy [17]

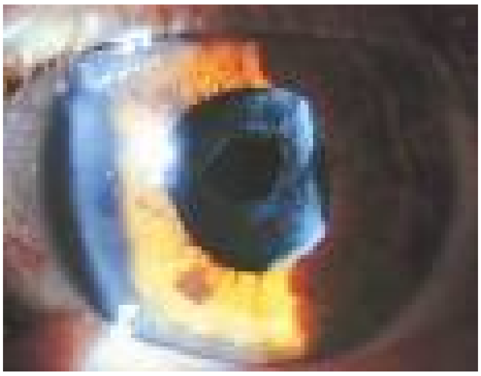


Figure 2.8. Posterior capsulotomy [18]

3) Photoablative effect; is direct breaking of cellular structure. It is performed by high energy UV radiation. Refractive surgery is a rapidly expanding area in ophthalmology, partly due to the introduction of excimer laser which was a huge step forward and is now well established. Lasers are now routinely used to correct for myopia (near- sightedness) and for hyperopia (far-sightedness) with two techniques: photorefractive keratectomy (PRK) and laser insitu keratomileusis (LASIK). In these procedures, a pulsed laser beam flattens the cornea by removing more tissue from the center of the cornea than from its midzone. The result of flattening of the cornea is that the focus of the eye moves farther back toward its desired spot on the retina and corrects the vision for distance. In PRK, the outer epithelial layer of the cornea first is removed by a mechanical (soft brush) or chemical (alcohol) means or even by using a laser beam (transepithelial ablation). The laser beam then is used to ablate and reshape the cornea. In LASIK, the ophthalmologist creates a hinged flap of the cornea approximately 125 μm in thickness using a specialized cutting blade mounted on a vacuum device. The cutting tool, known as a microkeratome, is then removed, thereby exposing the underlying corneal tissue to ultraviolet ablation of the desired degree. Finally, the corneal flap is returned to its original position. Another method is laser thermal keratoplasty (LTK). With LTK, the laser is utilized to shrink the cornea, causing its central part to become steeper. It utilizes the application of concentric rings of laser energy to gently heat the cornea and steepen its curvature.

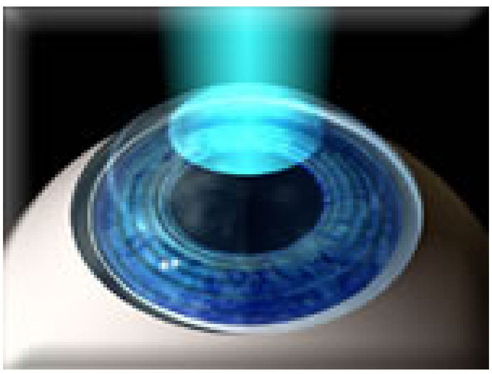


Figure 2.9. PRK [19]

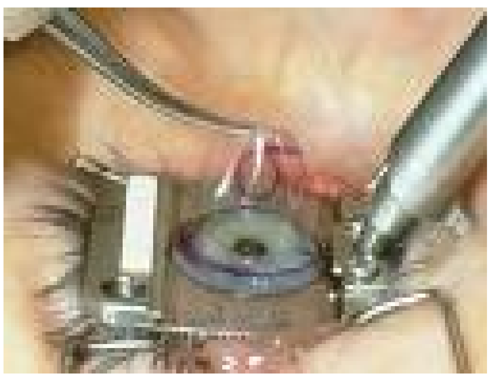


Figure 2.10. LASIK [20]

The fourth laser effect is photochemical and is called Photodynamic therapy (PDT). PDT involves the use of photochemical reactions mediated through the interaction of photosensitizing agents, light and oxygen. Photodynamic therapy (PDT) is a treatment for wet age-related macular degeneration (wet AMD). It is not used to treat dry AMD. In photodynamic therapy, a light-sensitive medicine called verteporfin (Visudyne) is injected into the bloodstream. The medicine collects in the abnormal blood vessels under the macula. Laser light is then shone into the eye, which activates the medicine and causes it to create blood clots that block the abnormal blood vessels.

Table 2.1. Laser applications in opthalmology

Procedure	Laser Photocoagulation			Laser Thermal Keratoplasty (LTK)	Laser-Assisted In Situ Keratomileusis (LASIK)
Commonly used lasers	Argon ion laser	Krypton ion laser	Laser diode	Ho:Yag laser	ArF excimer laser
Wavelength	514.5 nm	647 nm	810 nm	2.1 µm	193 nm
Operation regime (pulse duration)	CW (0.1-1.0 sec.)	CW (up to10sec.)	CW (up to 2sec.)	Pulse (0.25-1 sec.)	Pulse (15-25 nsec.)
Power (energy)	0.05-0.2W	0.3-0.5W	2W	20mJ	50-250mJ
General references and websites	21, 22			23-25	26-28

2.2. Tissue welding

Tissue engineering is a field of bioengineering that recently has seen an immense amount of growth. It covers a broad spectrum including biocompatible artificial implants, tissue regeneration, tissue welding and soldering, and tissue restructuring and contouring. Tissue contouring and restructuring; is use of lasers to ablate, shape or change pigmentation of a tissue. Tissue generation; is laser activation or incision to stimulate new tissue generation.

Laser tissue welding is a developing biotechnology that looks promising for applications in practically all surgeries [29]. Laser tissue welding utilizes the energy from the laser beam to join or bond tissues. The absorbed laser energy can produce alterations in the molecular structure of the tissues to induce soldering between neighboring tissue structures. Since the laser tissue-welding process is a noncontact and nonmechanical method, it is ideally suited for cases where suturing and stapling is difficult. The surgical requirements for tissue welding are to produce stronger welding strength while minimizing tissue thermal injury. To achieve these goals, current efforts are focused on developing new techniques using low laser energy, and reduced energy absorption to produce localized thermal effect. The approaches to join or bond tissues are;

- Direct Welding of Tissues: Local heating to 60°C-80°C by laser energy absorption (photothermolysis) to denature collagen, uncoiling their native triple helical structure and producing collagen bonding
- Laser Soldering: Use of proteineous solder at the surfaces to be joined followed by application of laser light to selectively heat the solder and seal it to the surrounding tissue
- Dye-enhanced Laser Soldering: A dye absorbing at the laser wavelength of soldering added to the solder to enhance selective absorption and subsequent heating of the solder and not of the nontarget tissue

Laser tissue welding was first demonstrated by Jain and Gorisch (1979), who used Nd:YAG laser light to seal rat arteries [30]. Subsequent studies suggested that laser interaction could be used to heat a tissue sufficiently to denature collagens in the tissue surfaces to form new connecting structures [31-32]. Early studies of laser tissue welding employe CO_2 lasers. The use of CO_2 laser relied on water the largest constituent of most tissues, absorbing strongly at its wavelength (10.6μm). This strong absorption limiting its use to extremely thin tissues. Other lasers employed for laser tissue welding are argon-ion and Nd:YAG laser , which produce deeper and more uniform tissue heating than that achieved by using a CO_2 laser. Pulsed lasers have the appeal that they can minimize collateral thermal damage. However, the choice of laser wavelengths and exposure parameters (energy, pulse duration, etc.) is clearly dependent on the tissue absorption, optical penetration depth, and the thermal relaxation time in the tissues to be welded. The optical penetration depth clearly has to be matched with the extent of the thickness to be welded to provide uniform heating.

The laser soldering technique utilizes laser light to fuse a proteineous solder to the tissue surface, thereby providing greater bond strength with less collateral damage compared to direct welding. Blood was the first material used as a solder. Subsequently, egg-white albumin followed by other proteins such as those derived from blood fibrinogen and other albumins were used a solder substitutes.

Dye- enhanced soldering was introduced to take advantage of the strong absorption of light by the selected dye and the efficient conversion of light into heat by the dye dispersed in the solder. This method also provided the benefit that an appropriate dye can be selected to match its absorption peak with the particular laser wavelength utilized. This method has allowed the ability to use the more common and relatively inexpensive 808-nm diode laser with the help of a biocompatible dye, indocyanine green (ICG) [33]. In yet another approach, a polymer scaffold doped with serum albumin and ICG was used [34]. They found that addition of the polymer membrane improved the weld strength and provided better flexibility compared to the use of albumin protein solder alone. The polymer scaffold makes the solder sufficiently flexible, allowing it to wrap around the tissue. Solders can be used for applications other than tissue bonding. Laser-assisted tissue sealing (LATS) can be used to seal bleeding surfaces for homeostasis (blood clotting).

Applications of laser welding and soldering have been diverse [35]:

- Cardiovascular surgery: Primary vascular anastomosis; sealing to reduce blood lose in vascular surgery
- Thoracic surgery: Sealing of air leaks after lung biopsy or wedge resection; sealing of the bronchial stump
- Dermatology: Skin closure with improved cosmetic and faster healing
- Gynecology: Repair of fallopian tubes
- Neurosurgery: Welding and repair of peripheral nerves
- Ophthalmology: Laser solder closure of incisions in the sclera and cornea
- Urology: Closure of ureter, ureteoneocystostomy, urethra and bladder

2.3. Cornea Welding

The cornea is the outermost glass-like part of the eye. This is the part that is irritated upon dust in the eye and where we place the contact lenses on. It is approximately half a millimeter (550µm) thick and has a diameter of 12 mm. It is like a section of a sphere. This is the part that refracts the light rays the most. Therefore, the cornea is of utmost importance in the focusing ability of the eye. A minimal change in the structure of the cornea makes a big change in the focusing function. Therefore, refractive surgery, which has been developed to solve the refractive errors of the eye by changing the shape of the cornea.

The cornea is the front window of the eye. It protects the interior of the eye much like the watch crystal protects the inter workings of a watch. It is clear and lets light through as it helps focus the images on the retina or film of the eye. The cornea has 5 layers (listed out side to inside) the epithelium (the outer skin, 7 cell layers thick), Bowman's layer (20 µm thick), stroma (majority of the cornea), Descements Membrane (10 µm thick) and the endothelium (inner skin). A microscopic drawing and light microscope view shows all 5 layers.

Epithelium

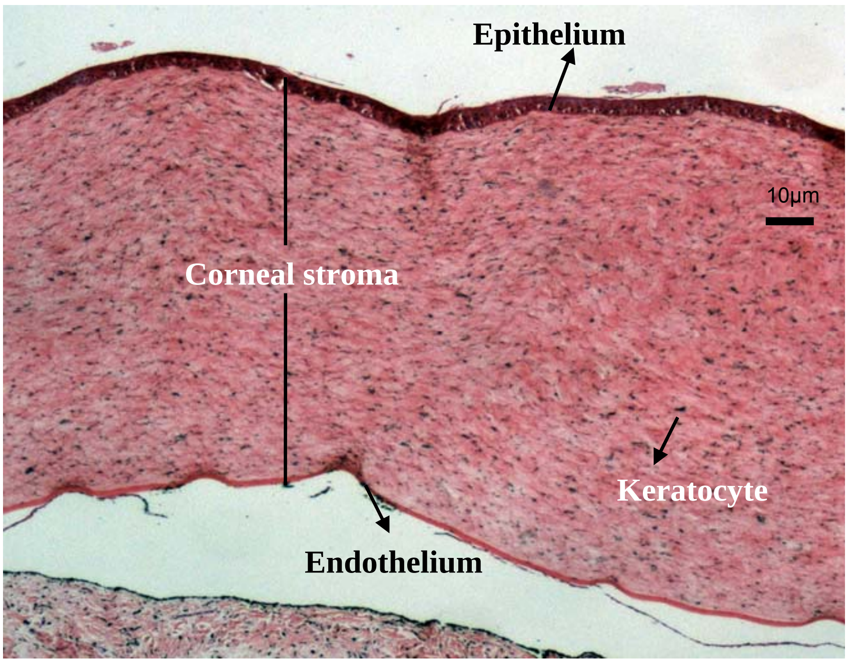


Figure 2.11. Histology of cornea (Boğaziçi University Institute of Biomedical Engineering, Biophotonics Lab), magnification: 4X

The vast majority of the corneal stroma consists of 200 to 500 layers of flattened collagenous lamellae extending from limbus to limbus, some crossing the apex (center) of the corneal dome and others which do not . In the anterior one third of the stroma, collagen lamellae are thin (about 0.2 to 1.2 μm thick and 0.5 to 30 μm wide), run obliquely to the corneal surface, and sometimes split into two to three sub layers that become interwoven. In the posterior stroma, collagen lamellae tend to be arranged parallel to the surface and are thicker (1.0 to 2.5 μm thick and 100 to 250 μm wide).

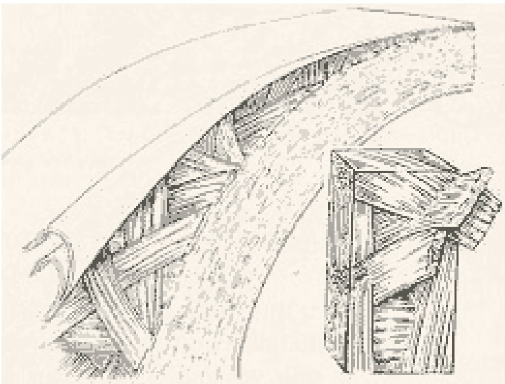


Figure 2.12. Collagen arrangement [36]

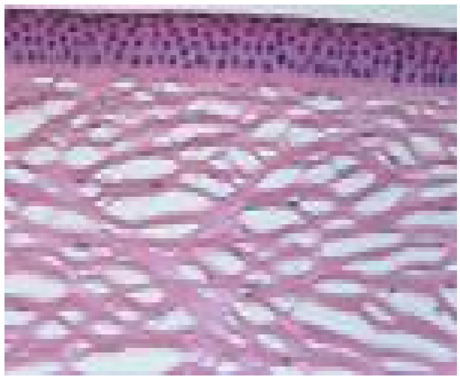


Figure 2.13. Stromal collagen [37]

About 60% of the glycosaminoglycans in the stroma consist of keratan sulphate and the remainder dermatan sulphate. There is a correlation between the increase of collagen fibril diameter in the peripheral cornea and the decrease in keratan sulphate. The normal corneal collagen fibers and its lattice is tighter centrally and looser peripherally. The central corneal collagen fibrils extend to the periphery where the fibrils weave into the limbal collagen imparting considerable strength. The normal cornea consists of 78% water. About 12-15% and 1-3% of the net weight of the tissue is composed of collagen and glycosaminoglycans respectively.

The corneal lamella is thinner centrally (2 μm) than peripherally (3 μm). This molecular lattice arrangement also holds true for the anterior to posterior corneal stroma. At any given point on the cornea, the anterior lamella are smaller then the posterior lamella.

When we go from periphery of the cornea to the central cornea, the lattice structure of the collagen tightens up and the collagen lamellae become smaller. The ground substance (glycosaminoglycans) of the central cornea is predominately keratan sulphate while the periphery is predominately dermatan sulphate. The ground substance is the packing material (filler) between the collagen lattice structure and between the collagen lamellae. Both dermatan sulphate and keratan sulphate bind to the collagen fibers at specific binding sites (cross-linking). These sites are essential to the spacing of the fibrils and to the width of the interfibrillar space and thus the size of the hexagonal array. The corneal stroma is unusual in that it contains no hyaluronic acid except at the limbus where there is a gradual increase in hyaluronic acid (ground substance) concentration towards the sclera.

A cataract is a clouding of the natural focusing lens in your eye. It is usually a part of the normal aging process and most people over the age of 70 have some signs of cataract. Light cannot pass through a cataract easily, so the retina only receives blurred and distorted images. Modern cataract surgery (Figure 2.14) begins with a very small clear corneal incision, approximately 3mm wide in the eye (Figure 2.13). No stitches are used, and normal daily activities can be resumed soon after surgery.

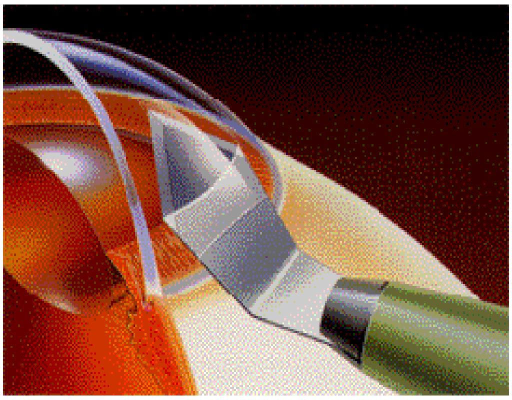


Figure 2.14. Clear corneal incision [38]

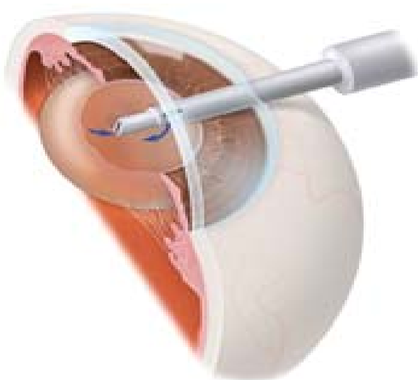


Figure 2.15. Phacoemulsification surgery [39]

Corneal tissue welding can achieve leak proof, full thickness closure of corneal incisions. The potential advantages which are expected by this technique are: simplification of the surgical technique and reduction of the intervention time, suppression of suture materials and thus of foreign body reaction, reduction of postoperative intraocular infection (Figure 2.14-2.16), reduction and control of post-operative astigmatism. Welding activates leukocytic and fibroblastic infiltration uniformly across the wound in many tissues, including enzymatic degradation of damaged tissue and de novo collagen synthesis. Thus, the stimulation of homogenous wound healing by welding could reduce postoperative astigmatism. It may also lead to more rapid wound closure as well as faster healing.

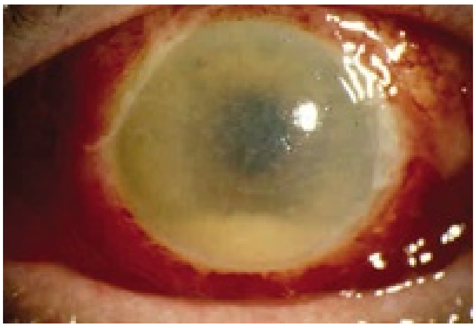


Figure 2.16. Endophthalmitis [40]

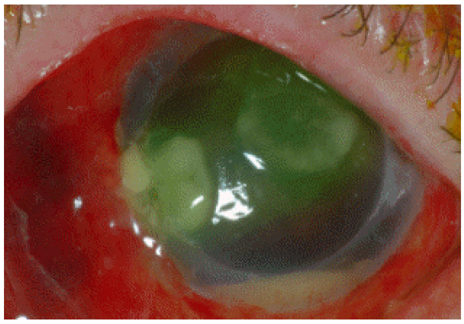


Figure 2.17. Endopthalmitis [41]

Postoperative intraocular pressure is known to vary, frequently dropping to less than 5 mmHg,[42] and in response to blinking, with the help of telemetric intraocular pressure monitoring devices it is shown that there is large fluctuations in intraocular pressure occur in individual eyes [43-44]. The possibility of imbibition of surface fluid through the self-sealing clear corneal wound into the anterior chamber in the early postoperative period is suggested by an apparently increased occurrence of endophthalmitis in up to 0.29% of cases [4–10].

Ahlberg and associates [45] have reported that the average particle size of India ink is 10micron in diameter. Therefore, when India ink is detected in the anterior chamber, there is the possibility that bacterial particles of similar size may enter the anterior chamber through the incisions. Sarayba *et al* study [46] showed that the light micrographs of clear corneal incisions show penetration of India ink into all of the corneal wounds.

Another disadvantage of clear corneal cut was shown by Tam *et al* [47]; Three patients who had uneventful phacoemulsification through a clear corneal incision were identified because of a postoperative wound leak. In phacoemulsification with corneal incisions, an everted flap of posterior corneal tissue, a corneal tongue, may prevent normal anatomical apposition of the surgical wound edges leading to potential wound incompetence.

Laser welding of corneal tissue is an alternative technique to conventional suturing procedures in ophthalmic surgery [48]. Laser tissue welding is a non-contact method aiming to bond biological tissue with the laser energy delivery.

Laser tissue welding utilizes the energy from the laser beam to join or bond tissues. The absorbed laser energy can produce alterations in the molecular structure of the tissues to induce bonding between neighboring tissue structures. During welding, water in the tissue absorbs the laser energy with wavelengths between 800-nm and 2000-nm that used in this thesis and subsequently heats the collagen helix. When the collagen tissue temperature rises above 60ºC, bonding is disrupted and partial dissociation occurs, followed by covalent and/or non-covalent bonding of the tissue protein molecules as the tissue cools. Welding of collagen containing tissue at low temperature probably occurs due to a morphological change in collagen fibers with disaggregation, interdigitation and reformation of these fibers [32]. Other mechanisms may involve protein cross linking as well as denaturation and random renaturation of proteins. Fibrin polymerization may also play a role as may covalent crosslinks in extracellular matrix proteins [49]. As a result, indirect photothermal activation of the stromal collagen is thus induced by laser radiation, resulting welding effect, which produces an immediate sealing of wound edges and good mechanical strength. The surgical requirements for tissue welding are to produce stronger welding strength while minimizing tissue thermal injury. An understanding of the underlying molecular mechanisms is necessary to optimize the welding process and minimize collateral damage such as molecular denaturation, buckling, and gaps in the weld. Successful welding therefore requires precise control of laser power and exposure times to control tissue temperature and dehydration. Water is the key molecule in welding via overtone vibrational absorption.

The choice of the laser emission parameters (wavelength and energy dose) as well as of the principal absorber of the tissue targeted by laser radiation is critical factors. Since the major component of biological tissue is water, earlier reported experiments have been based on the use of water as an endogenous choromophore to absorb laser light. Various laser types with wavelengths exhibiting high optical absorption in water have been used for this task, such as CO_2 (10.6 μm), holmium:YAG (2100-nm), erbium: YAG (2940-nm), and diode lasers. ICG is commonly used in laser welding or laser soldering as a cromophore [50, 51] in order to induce differential absorption between the dyed region and the surrounding tissue. This dye is characterized by high optical absorption around 800 nm so it used in association with 810 nm laser radiation to the corneal wound to be repaired.

In ophthalmology, experimental studies of laser-induced suturing of corneal tissue on animal models have been reported since 1992 by various authors [52–64], based on the use of near- and far-infrared lasers, directly absorbed by the water content of the cornea.

Keates *et al.* used a carbon dioxide laser (10.6 μm) to weld human scleral and corneal eye bank tissue and albino rabbit eye tissue, but achieved no fusion of the tissues [52]. Burstein *et al.* describe the first successful fusion of corneal tissue from porcine cadaver eyes using a fundamental hydrogen fluoride (HF) wavelength of 2,560 nm at 30 mW and a HF overtone wavelength of 1,340 nm at 320mW produced from a HF chemical laser [53]. Barak *et al.* used the temperature-controlled pulsed CO_2 laser to weld corneal and corneoscleral wounds in bovine in vitro eyes and in vivo rabbit eyes [54, 55]. Trabucchi *et al.* investigated the tissue fusion, attempted both with direct absorption of radiation at 1950-nm radiation and with ICG dye-enhanced technique at 810-nm. Their results; the group of corneal wounds treated with the ICG-enhanced technique revealed tissue welding in 70% of treated wounds and using 1950-nm tissue fusion was observed in 50% of treated wounds. They investigated that macroscopic evaluation revealed a pronounced thermal damage of the epithelium in the samples treated with 1950-nm radiation [56]. Savage *et al* concluded that 1455-nm NIR laser welding system provides strong, full thickness welds and does not require the use of extrinsic dyes, chromophores, or solders [57]. Pini *et al.* used a low power diode laser (805 nm) in combination with the photoenhancing dye indocyanine green applied to the incision to perform the first in vivo human corneal weld [58]. Menabuoni *et al* used 810-nm with association of ICG for welding in cataract surgery in humans and concluded that the laser-assisted corneal sealing procedure was rapid and safe and could serve as an alternative to corneal suturing, with significant potential applications for the closure of longer incisions [59].

Corneal laser welding is a relatively new technique still under investigation and the present thesis study will be the first comparative investigation covering a range of 800-2000 nm wavelength.

3. METHOD

3.1.Subjects

A total of 40 freshly enucleated bovine eyes, 38 eyes for tissue welding with 4 type of laser, 2 eyes for control, were used for the determination of welding parameters optimal for corneal wound closure. Twenty three treated cornea and 2 control cornea, totally 25 cornea excised and submitted for microscopic evaulation. 10 eyes for 1070-nm and 10 eyes for 1985-nm sampling were excised and again submitted for microscopic evaluation.

3.2.Experimental Set up

The 980-nm diode laser (OPC-D010-980-FCPS, OptoPower, Tuscon, AZ, USA, courtesy of Prof.Dr.Çilesiz) was controlled by a microcomputer-based instrument, which was designed and manufactured by the group at Biophotonics Laboratory at Institute of Biomedical Engineering, Bogazici University. Laser was delivered to the target tissue with a 400-μm optical fiber (Spindler-Hoyer, Göttingen, Germany). The parameters (power, exposure time, number of cycles, and on–off duration of pulses) of the laser were adjusted via LabView interface.

1070-nm YLF laser (YLM-20-9C IPG Laser GmbH) was delivered to cornea with an optical fiber. Radiating light wave was in continuous type (CW) and YLF laser's maximum power was 20 W.

809-nm laser is a handmade which was designed and manufactured at Biophotonics Laboratory at Institute of Biomedical Engineering, Bogazici University. Output power was 0-35 A diode current and its maximum power was 10 W.

A diode-pumped and fiber-coupled Tm:YAP (Tm3+:YAlO3) laser system emitting at 1980-nm is developed for medical applications. The laser system is designed as fiber-coupled and with modulation control by PC. It was designed and manufactured by our group at Biophotonics Laboratory at Institute of Biomedical Engineering, Bogazici University and Koç university, Physics department, Laser Research Laboratory. Maximum power of this laser was 1.14 W with a current of 17 A.

3.3.Protocol

In the study, four types of laser corneal welding; 1070-nm diode laser corneal welding, 809-nm diode laser welding with indocyanine green (ICG) as an absorptive dye, 1.9-nm Tm:YAP laser, 980-nm diode laser corneal welding were examined comparatively *in vitro* in bovine eyes for the study.

A total of 60 freshly enucleated bovine eyes, 38 eyes for tissue welding with 4 type of laser, 20 eyes for sampling with two different laser wave lengths, 2 eyes for control, were used for the determination of welding parameters optimal for corneal wound closure. Forty three treated cornea and 2 control cornea, totally 45 cornea excised and submitted for microscopic evaluation, other 15 corneas were not submitted for microscopic evaluation due to macroscopic opacification and photocoagulation signs (Figure 3.1.).

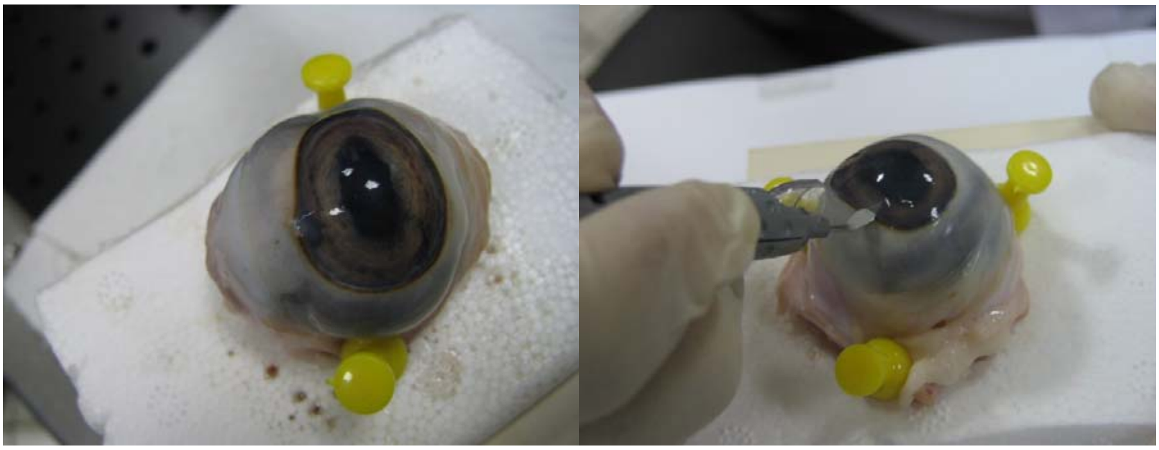


Figure 3.1. and Figure 3.2. Macroscopic opacification was observed. Laser power was decreased until macroscopic whitening was lost. Clear corneal cut was done with precalibrated knife which is used in phacoemulsification cataract surgery in human eye (Boğaziçi University Institute of Biomedical Engineering Biophotonics Lab) [59-60]

The eyes were used for the experiments within 2 hours. Full thickness, one-plane cut 3.2 mm in length limbal corneal cuts were produced using a pre-calibrated knife (Figure 3.2.).

Eyes were then prepared for the laser welding procedure. The laser beam was focused in the middle of the tissue (Figure 3.3.). Optimal power, duration and modulation parameters of all 4 laser types were investigated.

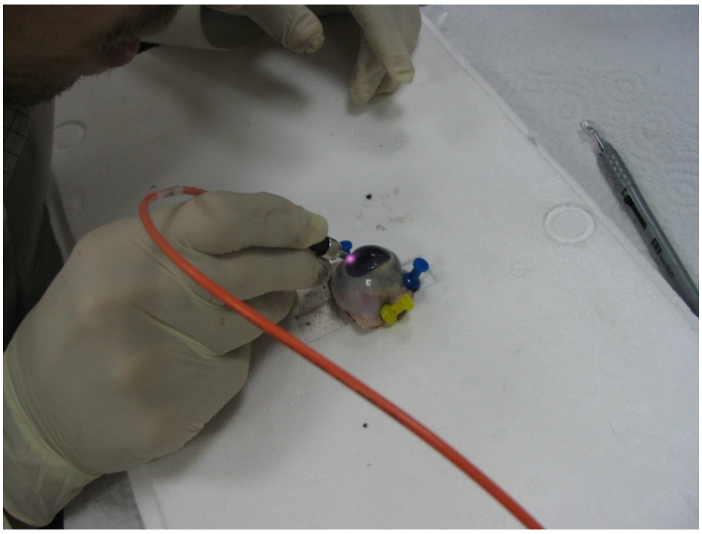


Figure 3.3. Laser irradiation of clear corneal cut (Boğaziçi University Institute of Biomedical Engineering Biophotonics Lab) [61]

7 freshly enucleated bovine corneas welded with 809-nm diode laser. The fiber tip was kept at a constant distance between 2-4 mm from the external surface of the cornea. A 12% w/w in sterile water concentration solution of ICG was placed inside the corneal cut of 4 eyes and a 6% w/w in sterile water concentration solution of ICG was placed inside the corneal cut of 3 eyes , using an anterior chamber cannula, and then washed out after a few minutes with water. The laser power emission was 200 mW on the corneal surface of all 7 eyes. Laser light was delivered to the cut by means of contiguous spot, vary between 3- 20 seconds. All parameters used in 809-nm diode laser were listed in table 4.1.

7 freshly enucleated bovine corneas welded with 980-nm diode laser. The laser spot size on the external surface of the cornea was 2 mm. The laser power emitted to corneal surface was between 1W- 3W. Laser light was delivered to the cut by means of contiguous spot; vary between 5- 30 seconds. All parameters used in 980-nm diode laser were listed in table 4.2.

3 freshly enucleated bovine corneas welded with 1070-nm YLF fiber laser. The laser spot size on the external surface of the cornea was 1.6 mm. The laser power emitted to corneal surface were between 1.5W- 2W. Laser light was delivered to the cut by means

of contiguous spot, vary between 5- 10 seconds. All parameters used in 1070-nm YLF fiber laser were listed in table 4.3.

6 freshly enucleated bovine corneas welded with 1985-nm Tm:YAP laser. The laser spot size on the external surface of the cornea was 0.61 mm. The laser power emitted to corneal surface were between 0.38W – 0.46 W. Laser light was delivered to the cut by means of contiguous spot, vary between 2- 30 seconds. All parameters used in 1985-nm Tm:YAP laser were listed in table 4.4.

According to the preliminary results additional 10 eyes for 1070-nm and 10 eyes for 1985-nm were used with constant parameters for further investigation.

10 bovine corneas welded with 1070-nm diode laser. The laser spot size on the external surface of the cornea was 1.6 mm. The laser power emitted to corneal surface was 2W. Delivered laser light to the cut by means of contiguous spot was 5 seconds.

10 bovine corneas welded with 1985-nm diode laser. The laser spot size on the external surface of the cornea was 0.61 mm. The laser power emitted to corneal surface was 0.46 W. Delivered laser light to the cut by means of contiguous spot was 2 seconds.

3.4.Histologic evaluation

Twenty six treated corneas, 20 comparative study corneas and 2 control corneas, totally 48 corneas were subsequently cut into 1 to 1 mm rectangular samples including the laser-treated 3.2 mm and submitted for microscopic evaluation. For histological evaluation incision area of the cornea was dissected and fixed in 10% neutral formalin for at least 48 hours. The fixed samples were dehydrated in ethanol and cleared in xylene. The cornea specimens were than embedded in paraffin cut in to 5 µm sections and stained with hematoxylin and eosin (H&E) for histopathological examination by light microscopy.

4. RESULTS

A total of 60 freshly enucleated bovine eyes, 38 eyes for tissue welding with 4 type of laser, 20 eyes for sampling, 2 eyes for control, were used for the determination of welding parameters optimal for corneal wound closure. Forty three treated cornea and 2 control cornea, totally 45 cornea excised and submitted for microscopic evaluation. Laser welded corneal cuts, control corneal cut without any laser procedure (Figure 1) and control cornea without any cut were examined histologically with hematoxylin-eosin. No opacification or photocoagulation signs were observed macroscopically at the welded site of 45 corneas.

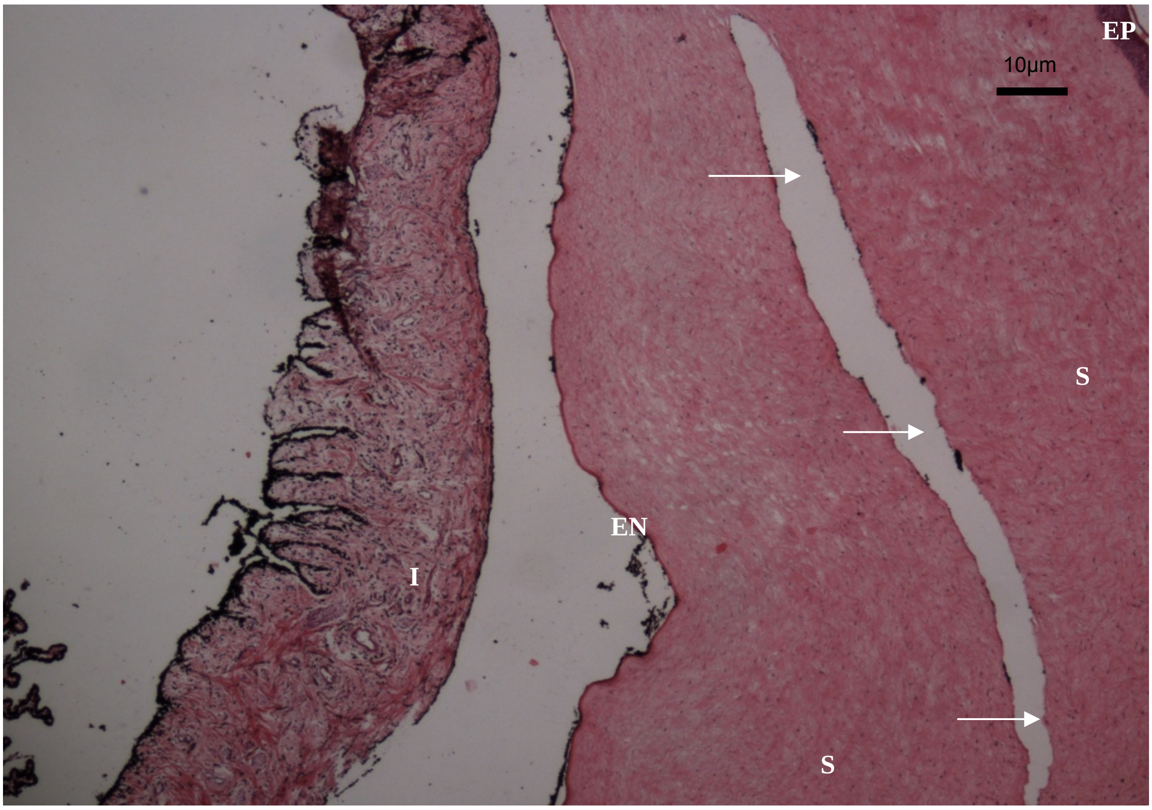


Figure 4.1. Clear corneal cut. EP: Epithelium, S: Stroma, EN: Endothelium, I: Iris, Clear Corneal Cut is shown with white arrows. Magnification: 4X, stained with hematoxylin and eosin (H&E)

4.1.Preliminary Results

4.1.1. 809-nm Diode Laser

7 freshly enucleated bovine corneas welded with 809-nm diode laser. The fiber tip was kept at a constant distance between 2-4 mm from the external surface of the cornea. 809-nm diode laser beam with the power of 200mW and the fiber tip at 3mm after 5 seconds caused photocoagulation and opacification at cornea without any ICG. 2 corneas full- length welded and 5 corneas not welded with these parameters of 809-nm diode laser. (Figure 2-3) No opacity of the corneas was seen visually after the welding. All parameters used in 809-nm diode laser and their results were listed in table 4.1.

Table 4.1. Parameters and results for 809-nm corneal welding

809-nm	**Spot size**	**Power**	**Energy density (J/cm^{2})**	**Duration (Sec)**	**ICG (%)**	**results**	**Histology**
Eye 1	2mm	200mW	19.35	3.	6	Not welded	Small hole
Eye 2	4mm	200mW	16	10	6	Welded	Epithelium degeneration no carbonization less eosinophil
Eye 3	4mm	200mW	32	20	6	Not welded	Less eosinophil
Eye 4	3mm	200mW	8.55	3	12	Welded	Epithelium degeneration carbonization less eosinophil
Eye 5	4mm	200mW	4.8	3	12	Not welded	No alteration
Eye 6	4mm	200mW	8	5	12	Not welded	Less eosinophil
Eye 7	4mm	200mW	16	10 sec.	12	Not welded	No alteration

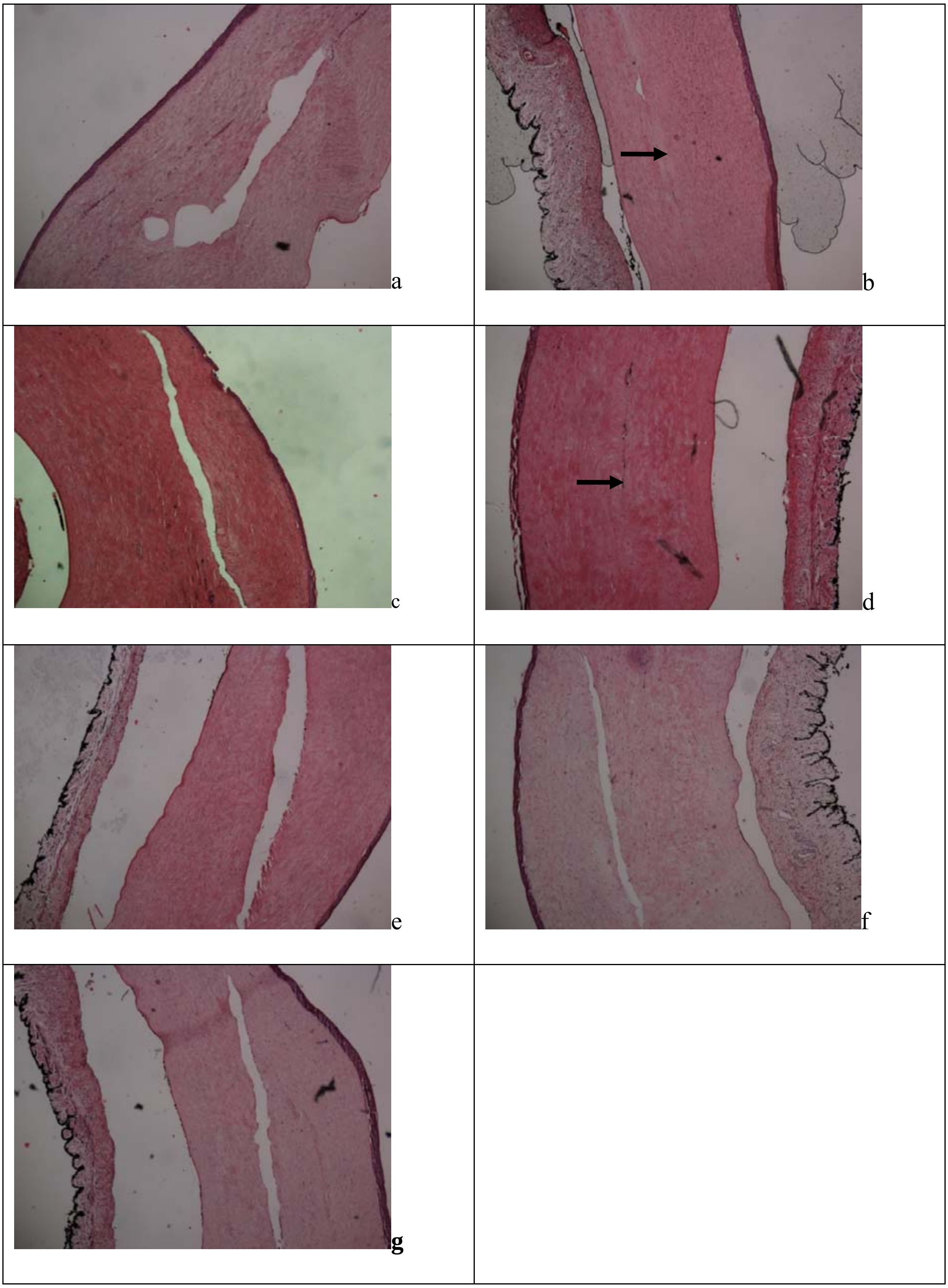


Figure 4.2. a) Eye 1, b) Eye 2, c) Eye 3, d) Eye 4, e) Eye 5, f) Eye 6, g) Eye 7. Welded corneal cuts were shown with black arrows. Parameters and histological observations are summarized in Table 4.1

According to these preliminary results; total welding effects was seen both with 12% ICG and 6% ICG before 809-nm laser irradiation. In the histological observation of welded eyes, Eye 2; degeneration was observed on the front aspect epithelium of cornea, a tiny incision area was observed at the area where laser had been applied. The nearby region of incision area in substantia propria cornea, less eosinophilic presence and no carbonization was observed. Eye 4; degeneration was observed on the front face epithelium of cornea, no pathology evidence at the incision area.

These results showed that it is possible to use 809-nm laser for corneal welding with less ICG concentrations but without using ICG, laser irradiation was not absorbed in cornea and iris was affected. Results were not consistent, with 6% ICG, cornea was welded with 16 J/cm^2 energy density but cornea was not welded with 32 J/cm^2 energy density. The same results were seen with 12% ICG also, cornea was welded with 8.55 J/cm^2 energy density but cornea was not welded with 16 J/cm^2 energy density. Because of this inconsistency, 810-nm was not chosen for further investigation.

4.1.2. 980-nm Diode Laser

7 freshly enucleated bovine corneas welded with 980-nm diode laser. The fiber tip was kept at a constant distance of 2 mm from the external surface of the cornea. 980-nm diode laser beam with the power of 3 W after 24 seconds caused photocoagulation and opacification at cornea. 2 corneas full- length welded and 6 corneas not welded with these parameters of 980-nm diode laser. (Figure 4-6) Figure 4 illustrates the welded bovine cornea and mild carbonization in stroma but no opacity of the cornea was seen visually after the welding. All parameters used in 980-nm diode laser and their results were listed in table 4.2.

Table 4.2. Parameters and results for 980-nm corneal welding

980-nm	Spot size	power	Energy density (J/cm^2)	duration (sec.)	results	Histology
Eye 1	2mm	1W	322.58	10	Not welded	No alteration
Eye 2	2mm	1W	645.16	20	Not welded	Less eosinophil
Eye 3	2mm	1W	967.74	30	Not welded	Collagen fibrils not parallel
Eye 4	2mm	2W	322.58	5	Not welded	No alteration
Eye 5	2mm	2W	645.16	10	Not welded	Less eosinophil
Eye 6	2mm	2W	1290.32	20	Welded	Low carbonization Less eosinophil
Eye 7	2mm	3W	483.87	5	Welded	High carbonization Less eosinophil

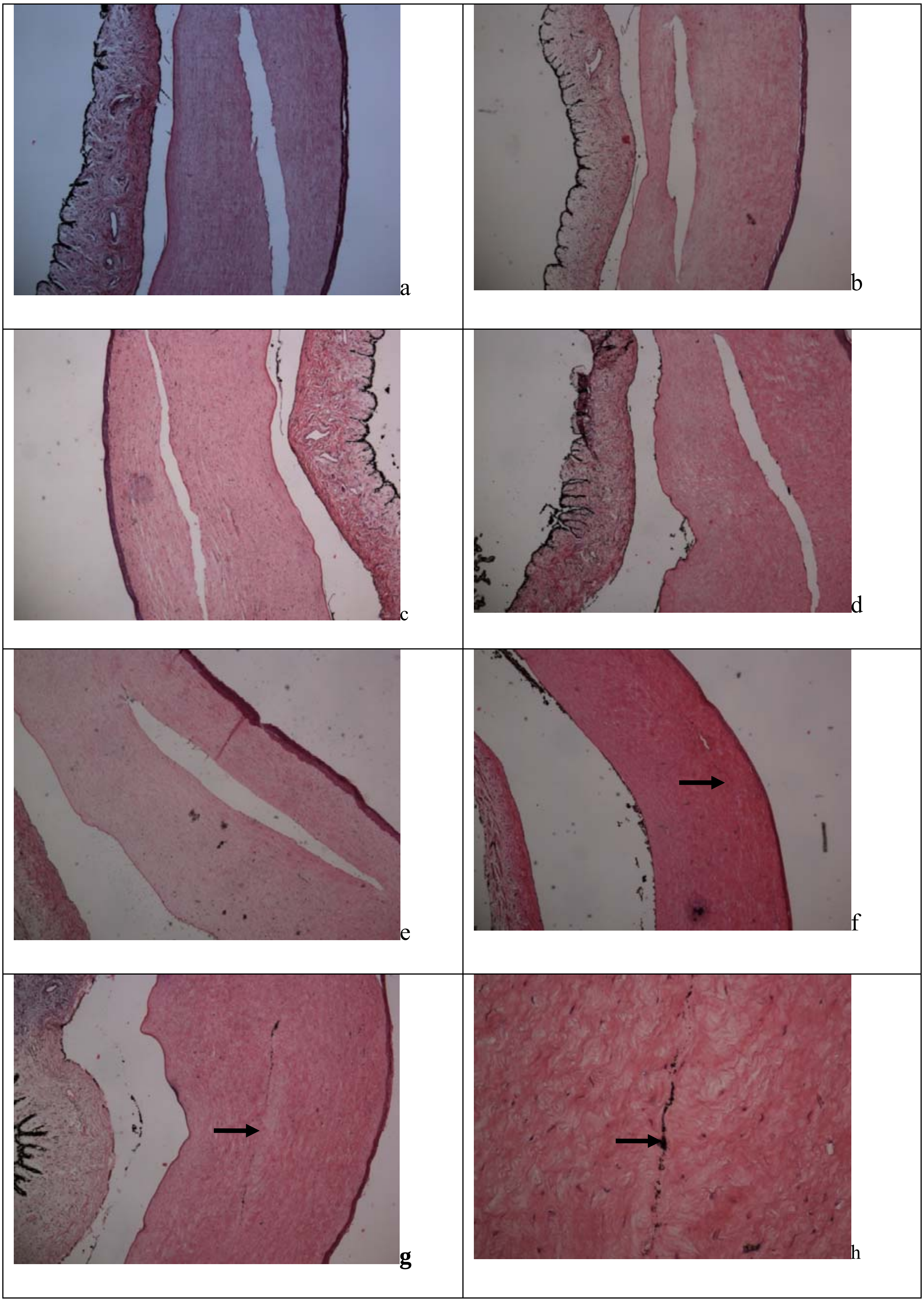


Figure 4.3. a) Eye 1, b) Eye 2, c) Eye 3, d) Eye 4, e) Eye 5, f) Eye 6, g) Eye 7, h) Eye 7, zoom 10. Welded corneal cuts were shown with black arrows. Parameters and histological observations are summarized in Table 4.2

According to 980-nm corneal welding preliminary results; firstly 1W power was used with increasing energy density values, secondly 2W power was used and with 1290.32 J/cm^2 energy density, cornea was full thickness welded. Total welding effect was seen also with 3W power and 483.87 J/cm^2 energy density. In the histological observation of welded eyes, Eye 6; less eosinophil and less carbonization presence were observed considering to environment tissue. Eye 7; any incision hole was not observed. At the incision area only a long linear formation with carbonization was observed. Nearby area to this formation less eosinophilic was observed. By extra zoom, incision area with many carbonizations was observed. At the nearby zone, collagen fiber bundles were observed which have less eosinophilic feature.

Results were consistent but because of carbonization in histological examination 980-nm was not chosen for further investigation. Less carbonization can be seen with optimal laser duration an 980-nm can be another option for corneal laser welding for future investigations.

4.1.3. 1070-nm YLF Fiber Laser

3 freshly enucleated bovine corneas welded with 1070-nm Tm: YAP laser. The fiber tip was kept at a constant distance of 2 mm from the external surface of the cornea. 1070-nm Tm: YAP laser beam with the power of 2 W after 15 seconds caused photocoagulation and opacification at cornea. 2 corneas full- length welded (Figure 7-9) and 1 cornea a full-thickness weld was not achieved but cut edges of two pieces of cornea incision was narrowed with these welding parameters of 1070-nm Tm: YAP laser. (Figure 10) All parameters used in 1070-nm diode laser and their results were listed in table 4.3.

Table 4.3. Parameters used for 1070-nm corneal welding

1070-nm	Spot size	power	Energy density (J/cm^2)	duration (sec.)	results	Histology
Eye 1	1.6mm	1.5W	750	10	Incision narrowed	Less eosinophil
Eye 2	1.6mm	2W	500	5	Welded	not able to distinguish collagen fibers
Eye 3	1.6mm	2W	1000	10	Welded	no hole or carbonization

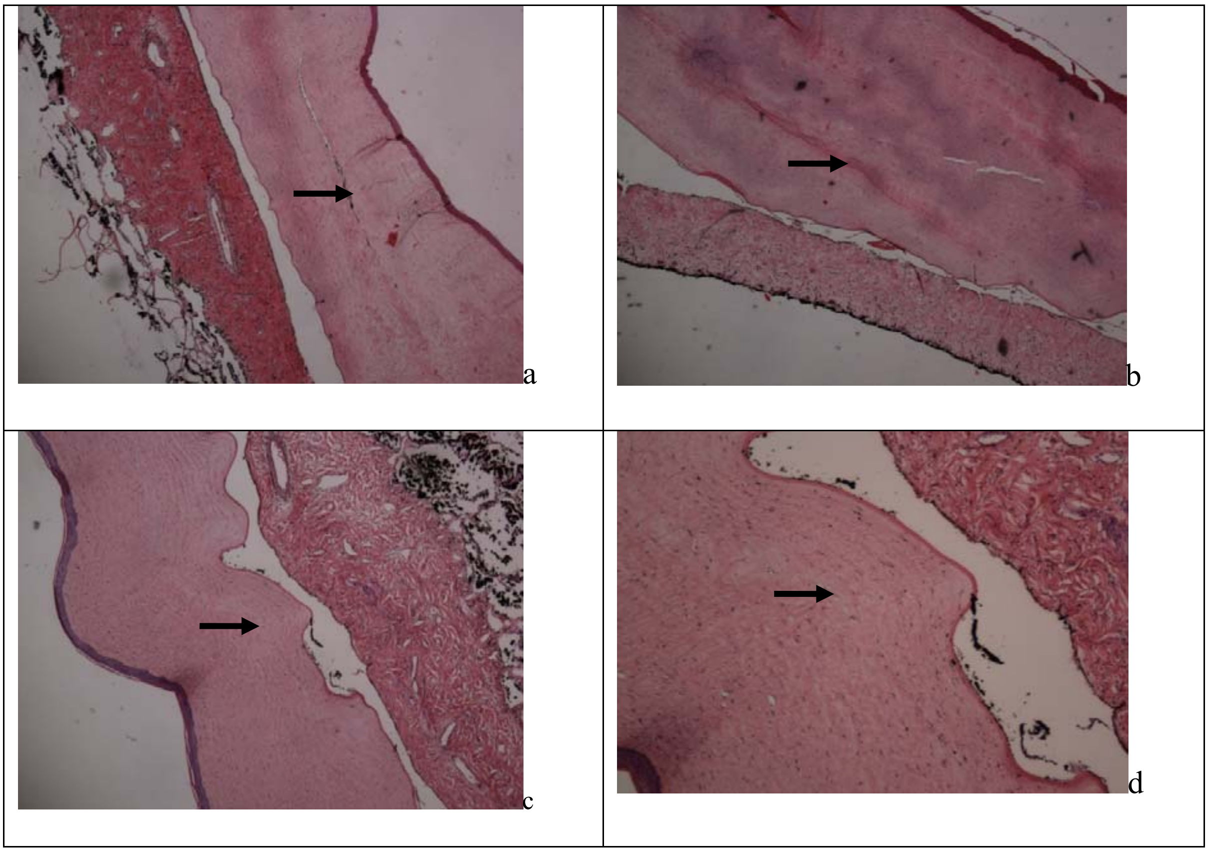


Figure 4.4. a) Eye 1, b) Eye 2, c) Eye 3, d) Eye 3, zoom 10. Welded corneal cuts were shown with black arrows. Parameters and histological observations are summarized in Table 4.3.

According to these preliminary results; results were found consistent with 1.5 W and 750 J/cm^2 incision was narrowed, power increased to 2 W and with 500 J/cm^2 and 1000 J/cm^2 energy densities corneas were full thickness welded. In the histological observation of welded eyes, Eye 1; an incision area was observed, which is appropriate to the tendency of collagen fibers and incision area is on linear form and many carbonization were observed at the covered areas. Eye 2; two prosecuting linear incisions, one is short and one is long, were observed and at the nearby areas of incision, more pathology was observed. Eye 3; at the incision area, no hole or carbonization were not observed, in this area more eosinophilic image was observed. Collagen fibers which exist nearby area of the incision, were changed at the direction of incision. Likewise, ondulation at the direction of incision was observed in the internal epithelium. Because of consistency and no carbonization effect on histological examination, 1070-nm was chosen for further investigation.

4.1.4. 1985-nm Tm: YAP Laser

6 freshly enucleated bovine corneas welded with 1985-nm Tm: YAP laser. The fiber tip was kept at a constant distance of 2 mm from the external surface of the cornea. 1985-nm Tm: YAP laser beam with the power of 0.46 W after 1 second caused photocoagulation and opacification at cornea. 4 corneas full- length welded and at 2 corneas not welded with these parameters of 1985-nm Tm: YAP laser. (Figure 11-18) No opacity of the cornea was seen visually after the welding. All parameters used in 1985-nm Tm: YAP laser and their results were listed in table 4.4.

Table 4.4. Parameters used for 1985-nm corneal welding

1985-nm	Spot size	power	Energy density (J/cm^2)	duration (sec.)	results	Histology
Eye 1	0.61mm	0.38 W	1317	10	Partially welded	Very low carbonization, small holes
Eye 2	0.61mm	0.38 W	2634	20	Welded	Small holes Very low carbonization
Eye 3	0.61mm	0.38 W	3951	30	Welded	Small holes No carbonization
Eye 4	0.61mm	0.46 W	319	2	Welded	No carbonization Collagens are not parallel
Eye 5	0.61mm	0.46 W	478.5	3	Not welded	Less eosinophil
Eye 6	0.61mm	0.46 W	638	4	Welded	Degenerative area at the epithelium no carbonization

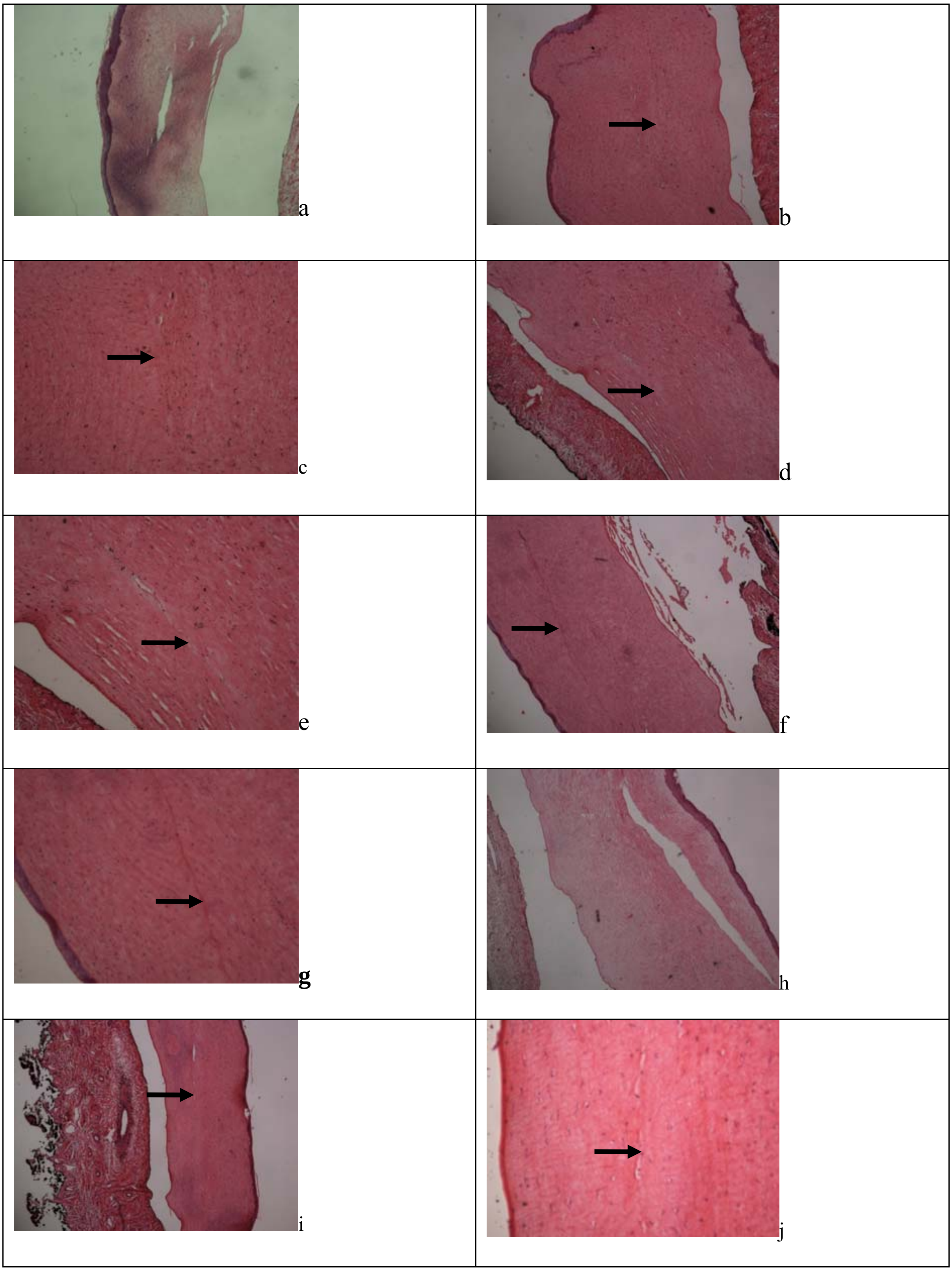


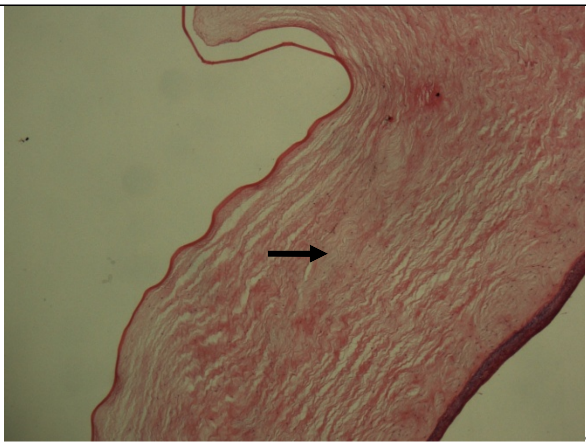
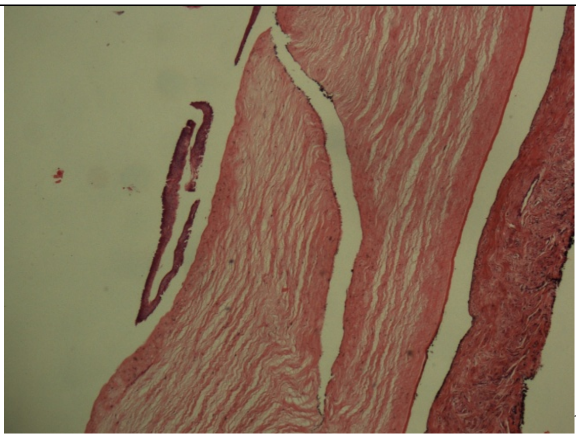
Figure 4.3. a) Eye 1, b) Eye 2, c) Eye 2, zoom 20 d) Eye 3, e) Eye 3, zoom 20 f) Eye 4, g) Eye 4, zoom 20 h) Eye 5, i) Eye 6, j)Eye 6, zoom 20. Welded corneal cuts were shown with black arrows. Parameters and histological observations are summarized in Table 4.4.

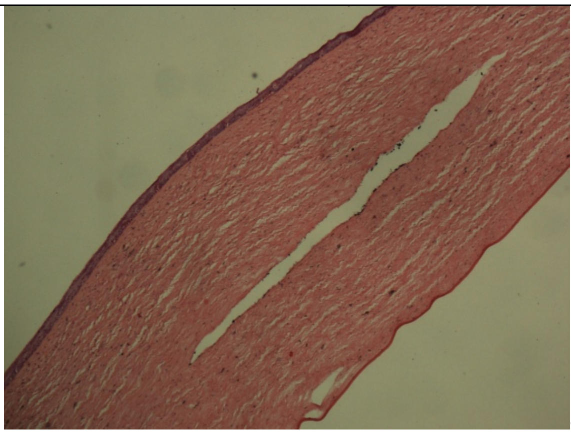
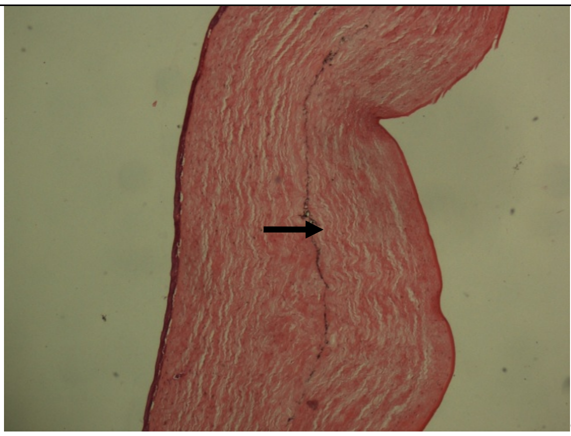
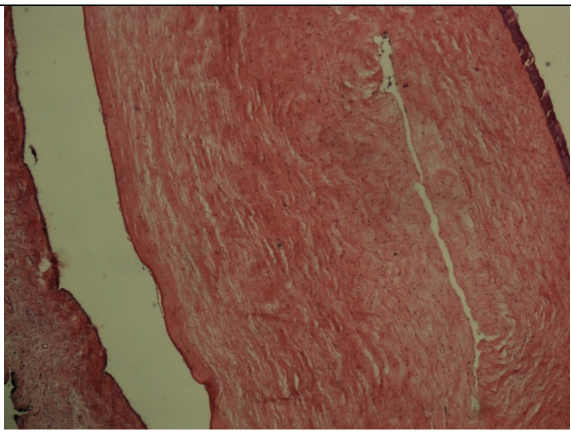
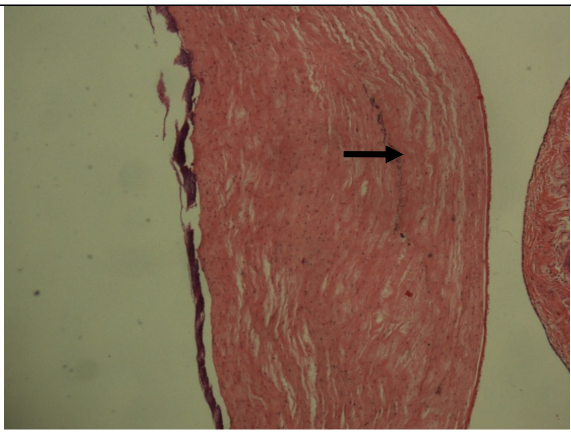
According to 1985-nm preliminary results; results were consistent except one result. Firstly 0.38 W power used with increasing energy density, cornea was not welded with 1317 J/cm^2 but with 2634 J/cm^2 and higher energy densities, full thickness welded. Same welding effect was seen with 0.46 W power and 319 J/cm^2 and 638 J/cm^2 but cornea was not welded with same power and 478.5 J/cm^2 energy density. In the histological observation of welded eyes, Eye 2; incision line of cornea substantia propria was not parallel to the direction of collagen fibers, an appropriate form of collagen fibers to this situation was observed. Occasionally small holes were observed on the incision line. In many areas along the incision line, fully closure and rarely 1-2 small holes were observed. On the nearby areas, an increase of eozinophilia and collagen fiber tendency to the direction of incision line was observed. In extra zoom, very less carbonization on the area with holes was observed. Eye 3; along the incision line, closure of a big area was observed. Also rarely linear and round shaped holes were observed. Eozinophilia was observed frequently on the nearby area to the incision line. On the substantia propria cornea area especially nearby to internal epithelium, some breaches were observed which are close together. No carbonization was observed in the incision area. Eye 4; no carbonization was observed in this area. Eosinophilia of the area had no difference in respect of environment tissue. Collagen fibers had tendency to the line of incision on the areas where the direction of incision line is not parallel to the direction of collagen fibers exist in substantia proproa cornea. Eye 6; incision area was observed which is linear and about to parallel to the tendency of collagen fibers with occasionally has different sizes of holes. At this area, more pathology was observed when the hole size was big. Also degenerative area was observed at the front aspect epithelium of cornea. Less eosinophilic image was existed on the nearby area to the incision line. Because of no carbonization in histological results and four corneas were full thickness welded, 1985-nm was chosen for further investigation even there is an inconsistency in one corneal result.

4.2.Comparative study: 1070-nm vs 1985-nm

According to these results; 1070-nm with the parameters of 1.6mm spot size, 2 W power, 500 J/cm^2 energy density, 5 seconds duration and 1985-nm with the parameters of 0.61 mm spot size, 0.46 W power, 319 J/cm^2 energy density, 2 seconds duration were chosen for sampling. The result of totally 20 corneas of sampling is listed in Table 5 and Table 6.

Ten eyes were radiated with 1070-nm diode laser. Five corneal incision were full thickness welded, 2 incision were narrowed and 3 incision were not welded with 5 seconds of 1070-nm laser radiation. Small holes and low carbonization was seen in microscopic examination of welded corneas. Collagen fibers were not parallel even though incisions were not welded. Less eosinophil was seen in all 10 corneas histology.

	Figures	Results	Histology
Eye 1 1070nm	 a	Welded	small holes, low carbonization, less eosinophil
Eye 2 1070nm	 b	Not welded	collagen fibers not parallel, less eosinophil

Eye 3 1070nm	 c	Not welded	less eosinophil
Eye 4 1070nm	 d	Welded	small holes, high carbonization, less eosinophil, collagen fibers not parallel
Eye 5 1070nm	 e	Narrowed	less eosinophil, collagen fibers not parallel
Eye 6 1070nm	 f	Welded	small holes, high carbonization, less eosinophil,

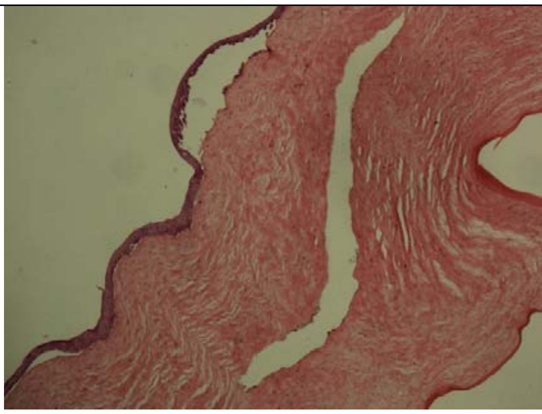
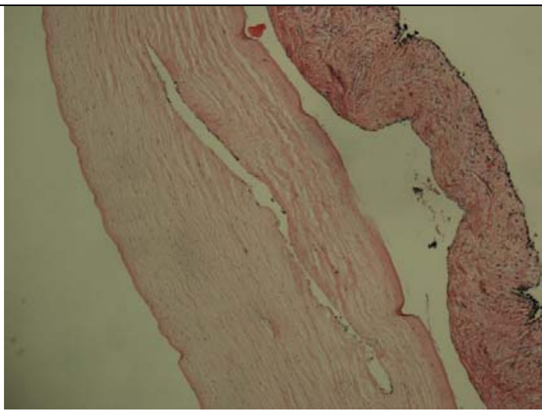
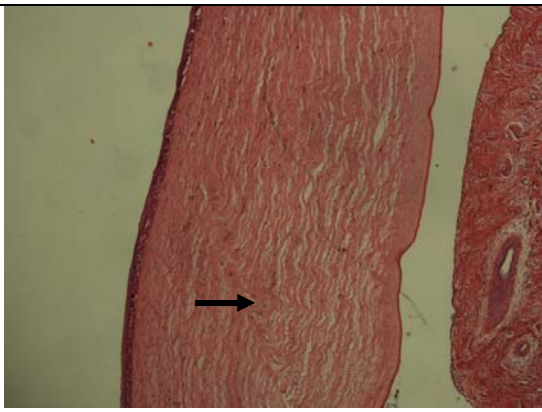
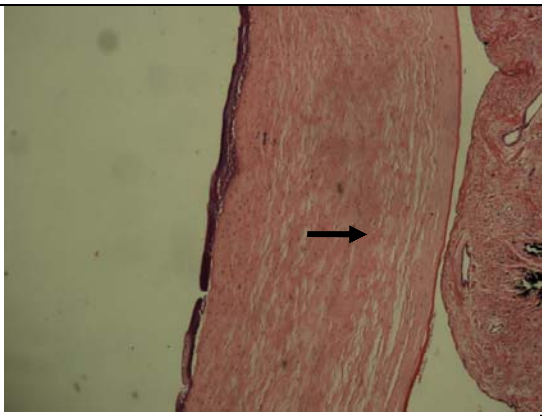
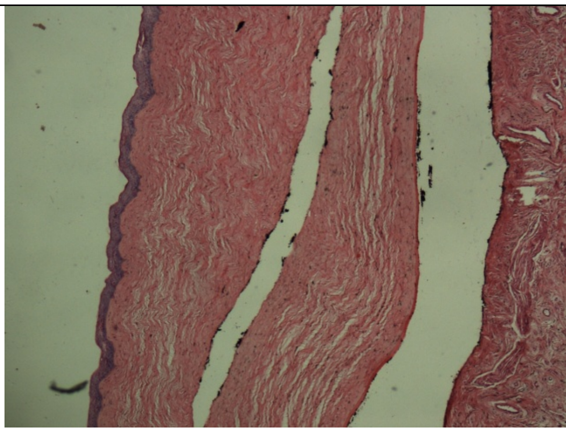
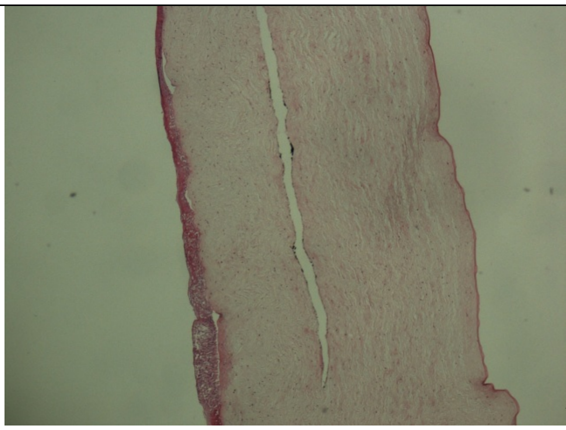
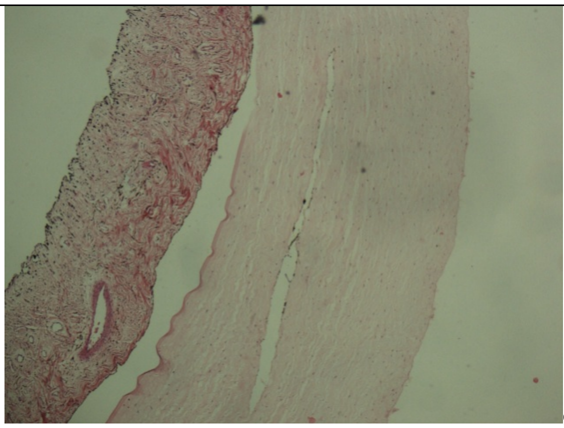
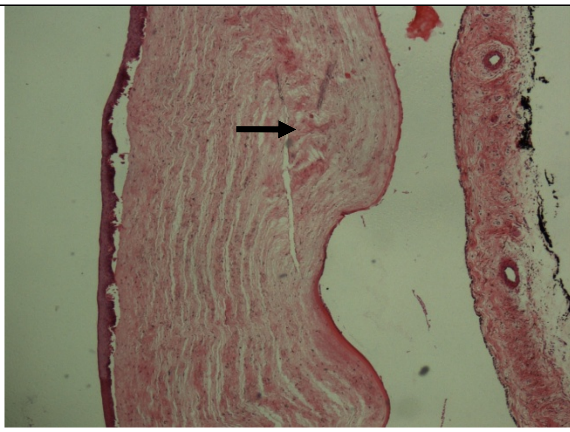
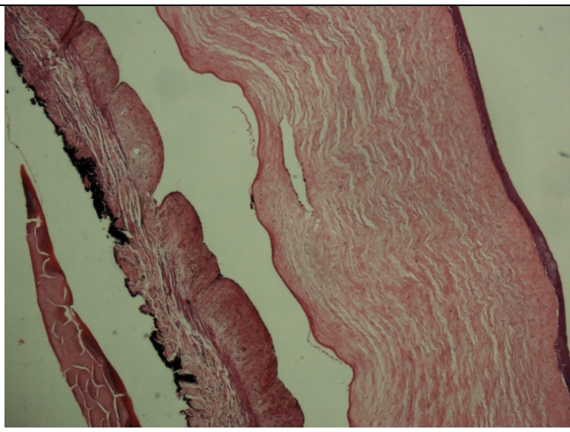
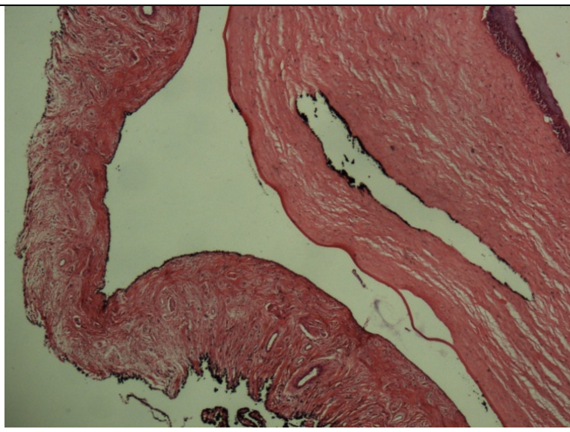
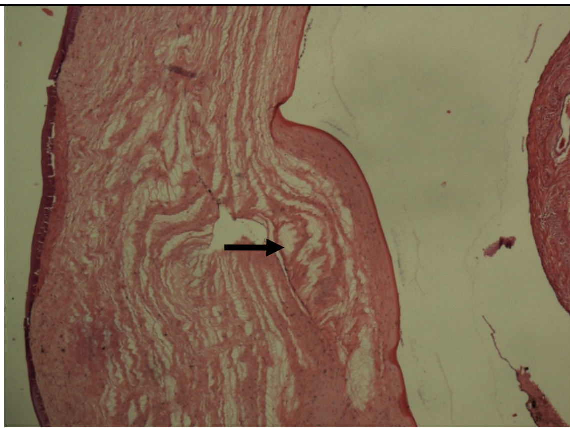
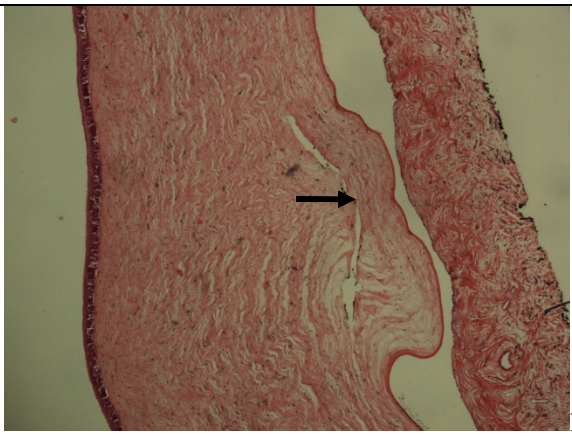
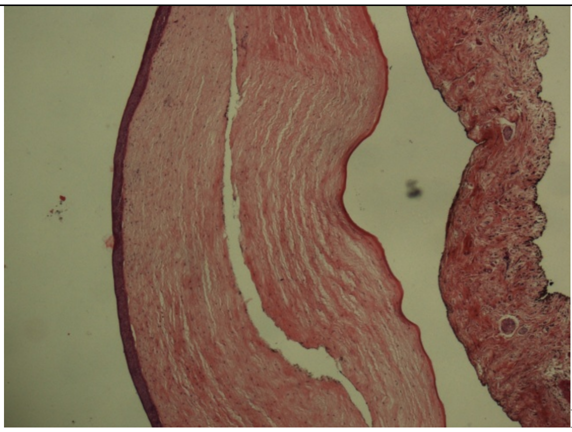
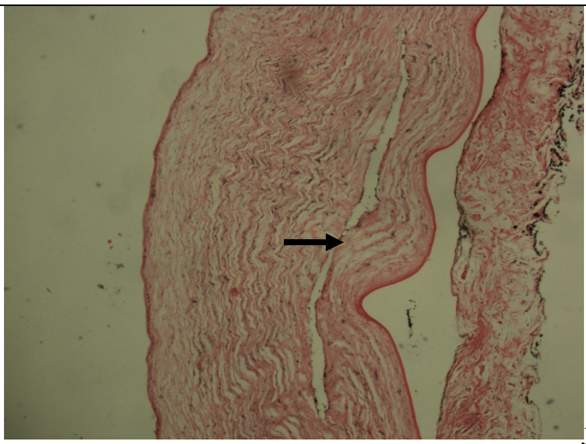
Eye 7 1070nm	 g	Not welded	collagen fibers not parallel, less eosinophil
Eye 8 1070nm	 h	Narrowed	less eosinophil, , low carbonization
Eye 9 1070nm	 I	Welded	no carbonization, less eosinophil, collagen fibers not parallel
Eye 10 1070nm	 j	Welded	small holes, low carbonization, less eosinophil

Figure 4.6. 1070-nm results. Welded corneal cuts were shown with black arrows.

Ten eyes were radiated with 1985-nm for the study. Four corneal incision were partially welded, 4 incision were narrowed and 2 incision were not welded with 2 seconds of 1985-nm laser radiation. As 1070-nm laser radiation small holes and low carbonization was seen in microscopic examination of welded corneas. Collagen fibers were not parallel in all 10 eyes. Less eosinophil was seen if incisions were welded or narrowed but eosinophils were not affected if incisions were not welded.

	Figures	Results	Histology
Eye 1 1985nm	 a	Not welded	collagen fibers not parallel
Eye 2 1985nm	 b	Narrowed	Low carbonization, collagen fibers not parallel
Eye 3 1985nm	 c	Narrowed	Low carbonization, collagen fibers not parallel

Eye 4 1985nm	 d	Partially welded	No carbonization, less eosinophil, collagen fibers not parallel
Eye 5 1985nm	 e	Narrowed	Low carbonization, collagen fibers not parallel
Eye 6 1985nm	 f	Not welded	collagen fibers not parallel
Eye 7 1985nm	 g	Partially welded	a hole on the incision, low carbonization, less eosinophil, collagen fibers not parallel

Eye 8 1985nm	 h	Partially welded	Low carbonization, less eosinophil, collagen fibers not parallel
Eye 9 1985nm	 I	Narrowed	Low carbonization, collagen fibers not parallel, less eosinophil
Eye 10 1985nm	 j	Partially welded	Low carbonization, collagen fibers not parallel, less eosinophil

Figures 4.7. 1985-nm results. Welded corneal cuts were shown with black arrows.

5. DISCUSSION

According to our results; 1985-nm and 1070-nm lasers were found better wavelengths compared to others which means histologic results showed less or no carbonization, parallelism of collagens and full thickness welding. 1985-nm with 0.38 W power and with minimum duration of 20 seconds welded corneal tissue full thickness and without any microscopic carbonization. 1070-nm with power of 2W and minimum duration of 5 seconds also welded corneal tissue full thickness and without any carbonization.

980-nm results also satisfactory with power of 2W and 20 seconds but mild carbonization of the adjacent corneal tissue were seen in the microscopic view. Duration between 10 seconds which the cornea was not welded and 20 seconds should be tested and it is important to find minimum duration time for a clear welded cornea without carbonization.

809-nm results are divided according to concentration of ICG, used as chromophores. 809-nm combined with 6% ICG concentration, 200mW power and 30 seconds duration parameters, results is same with 809-nm combined with 12% ICG, 200 mW and 3 seconds duration. The difference is with 6% ICG, fiber tip was kept 1mm height and with 12% ICG, fiber tip was kept 2mm height. Corneal tissue welded full thickness with both two concentrations. There are no study about toxicity of ICG application before welding procedure but Chang *et al* study showed the toxicity for the intracamaral usage, instead of 809 nm diode laser using 980-nm, 1070-nm or 1985-nm laser types for corneal welding procedure may be safer because there is no need for ICG or any chromophores for welding. On the other hand, it is very important to find the minimum ICG concentration in order to minimize the risk of ICG toxicity on cornea endothelium or epithelium.

Laser duration was used continuously without any pulse. Clear corneal cut width is 3.2 mm and the intra stromal length is approximately 1mm which means laser should be targeted to 1mm length of cornea. It is possible to miss that 1mm area with continuous laser duration instead of using several small pulses. This is the potential cause of inconsistency of 809-nm and 1985-nm diode laser results. 809-nm with the parameters of

6% ICG concentration, 200mW, 3mm height and 10 seconds resulted with welded cornea but with same parameters and 20 seconds resulted with not welded. Similar results for 1985-nm, with parameters of 0.46 W power and duration of 2 seconds, cornea was full thickness welded but with same parameters but 3 seconds, cornea was not welded.

The shortest exposure time and the lowest power parameters which welded the cornea totally were chosen for 1070-nm and 1985-nm comparative study. In 1070-nm laser welding it was seen that with 5 seconds of duration, 5 of 10 corneas were welded totally and it is not an adequate duration of laser to weld all corneas. Instead of 5 seconds, with between 5-10 seconds, there would be better welding results. The situation was same with 1985-nm sampling corneas. The 1985-nm 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 while preliminary study for all 4 types of laser, 3 seconds also was not enough to weld cornea with 1985-nm laser welding. According to these results 4 seconds of duration would be the best option for welding sampling corneas safely and totally.

Cornea incision has a healing process almost three months which happens with some signals, transmitters and cells accumulation in incision area. In an *in vitro* bovine eye study like my thesis, there is no process for cornea healing after clear corneal incision and after laser application but in an *in vivo* animal study there are some factors which may effect histologic results after a clear corneal cut and laser application. In an animal eye, there is an intraocular pressure change which may be more than tensile strength of the welded incision. Eye blinking also another mechanical factor which may make a tension to the incision area. Further in vivo study is needed to show these effects on laser welded clear corneal cut.

Whereas persistent clinically recognized hypotony is very uncommon after clear corneal phacoemulsification, transient hypotony in the first few hours after surgery is actually common. In addition, after eye rubbing or blinking, the intraocular pressure can spike and then transiently undershoot the baseline pressure. Thus, we believe the possibility does, indeed, exist for bacteria to traverse the corneal incisions in some eyes during this early postoperative period when intraocular pressures might be low, and the wound morphology is so labile. This postoperative entry of microorganisms into the eye would explain the development of staphylococcal endophthalmitis in an eye after clear corneal surgery despite an aqueous aspirate at the conclusion of surgery that was culture negative [62]. If transiently low intraocular pressure does allow delayed inoculation of the anterior chamber from organisms on the ocular surface, the ideal prophylactic antibiotic would have a longer half-life within the anterior chamber than does vancomycin. Another option would be the laser welding of clear corneal cut.

In Sarayba *et al.* study [46], application of manual pressure was a simulation of the force generated by the eyelid during a blink or a hard squeeze and the sudden release of force as would be seen when opening the eyelid in a blink cycle. Postoperative phacoemulsification patients who rub their eyes or who apply pressure on their eyelids during application of medication may generate the same or even higher stress to the wound. Our corneal welding results especially 1070-nm and 1985-nm wavelengths are chance to prevent endophthalmitis due to vacuum effect that allowed microorganism from the outside to flow into the intraocular space through clear corneal incisions.

Chang *et al.* investigate the corneal endothelial cytotoxicity of dyes for capsule staining in cataract surgery [66]. Indocyanine green 0.25% did not induce significant damage to corneal endothelial cells. Significant cytotoxicity was observed with ICG 0.50% and exposure to ICG 0.25% for 1 to 10 minutes showed a trend toward cytotoxicity after 10 minutes. On TEM, corneal endothelial cells that had been exposed to ICG 0.50% showed remarkable organelle swelling and disruption, electron-dense granules, and cell lysis.

Our study suggested that 809-nm diode laser welding in association with the topical application of ICG is a valid method for the closure of corneal tissues but 980-nm, 1070-nm diode laser and 1985-nm Tm: YAP laser welding without topical application of any cromophores are other safe options. On the other hand another interesting result of our study is the same full-thickness welding results of 809-nm with 6% ICG and 12% ICG which means reduced toxicity risk with lower concentration of ICG usage.

6. CONCLUSION

As a conclusion, our study suggested that 809nm diode laser welding in association with the topical application of ICG is a valid method for the closure of corneal tissues but 908-nm, 1070-nm diode laser and 1985 Tm: YAP laser welding without topical application of any cromophores are other safe options. According to results of comparative study, 1070-nm and 1985-nm wave lengths have a great potential. On the other hand another interesting result of our study is the same full-thickness welding results of 809-nm with 6% ICG and 12% ICG which means reduced toxicity risk with lower concentration of ICG usage. Further *in vivo* studies are needed in order to investigate the effect of 809-nm, 980-nm,1070-nm, and 1985-nm lasers on living organism cornea.

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INFRARED LASERS FOR CORNEAL TISSUE WELDING

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ABSTRACT

INFRARED LASERS FOR CORNEAL TISSUE WELDING

Objective of this study is to investigate the potential of infrared lasers for cornea welding in order to seal corneal cuts done during cataract surgery. 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. Corneal welding is rather a new application area in laser medicine, and few studies reported successful welding dose for different infrared wavelengths. Different laser wavelengths were studied comparatively in the present research. Diode lasers (809-nm and 980-nm), a fiber laser (1070-nm) and a Tm: YAP lasers (1985-nm) were used in a power range of 200mW-3W. *In vitro* experiments were performed on a total of 60 freshly enucleated bovine eyes. Full thickness, one-plane 3.2 mm long clear corneal cuts were done using a pre-calibrated knife. Laser power, irradiation duration, energy density and spot size were the parameters used and histological indicators of photothermal effect were observed. According to preliminary results; 1070-nm YLF laser and 1985-nm Tm:YAP laser were selected for further investigation. Histological examination of hematoxylin-eosin stained samples revealed that 1070-nm and 1985-nm laser wavelengths have a great potential for corneal welding.

Keywords: cornea laser tissue welding, clear corneal cut, 809-nm diode laser, 980-nm diode laser, 1070-nm fiber laser, 1985 Tm: Yap laser

ÖZET

KORNEAL DOKU KAYNAĞINDA INFRARED LASERLER

Bu çalışmanın amacı kızıl altı laserlerin katarkt cerrahisi sırasında oluşturulan kornea kesisini yapıştırma özelliklerinin incelenmesi ve karşılaştırılmasıdır. Kızıl altı laserler yumuşak dokuları yapıştırmak için kullanılabilirler. Su moleküllerinin ve kollajen gibi protein mollekülerinin kızıl altı enerjiyi absorbe etmesi ile uygulama alanında ısı farkı oluşturulur. Laserle kornea doku yapıştırması bu alanda yeni sayılabilecek bir konudur ve çok az çalışmada farklı kızıl altı dalga boyları için başarılı yapıştırma dozları rapor edilmiştir. Bu tez çalışmasında farklı dalga boyları karşılaştırmalı olarak araştırılmıştır. Bu amaçla 809-nm ve 980-nm diyot laserler, 1070-nm fiber laser ve 1985-nm Tm: YAP laser 200mW ile 3W güç değerleri arasında kullanılmıştır. Çalışma *in vitro* olarak toplam 60 adet yeni eniküle edilmiş koyun gözünde yapılmıştır. Daha önceden kalibre edilmiş 3.2 mm genişliğinde bıçak ile tam kat korneal kesiler oluşturuldu. Laser gücü, uygulama süresi, enerji yoğunluğu ve laser demet çapı kullanılan parametrelerdi ve fototermal etkinin histolololojik belirleyicileri incelendi. Bu sonuçlar ışığında; 1985-nm Tm:YAP laser ve 1070-nm YLF laser daha ileri araştırma için seçildi. Hematoksilen eosin ile boyanmış histolojik incelemeler 1070-nm ve 1985-nm dalga boylarının kornea yapıştırılmasında potansiyeli olduğunu göstermiştir.

Anahtar Sözcükler: kornea laser doku yapıştırılması, korneal kesi, 809-nm diyot laser, 980-nm diyot laser, 1070-nm fiber laser, 1985 Tm: Yap laser

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..III

ABSTRACT……………………………………………………………………………….IV

ÖZET………………………………………………………………………………………V

LIST OF FIGURES……………………………………………………………………VII

LIST OF TABLES..XI

LIST OF ABBREVIATIONS…………………………………………………………….XII

LIST OF SYMBOLS……………………………………………………………………..XIII

1. INTRODUCTION……………………………………………………………………..1

1.1. Motivation Objective............……………………………………………............1

1.2. Outline.....……………………………………………………………............….1

2. BACKGROUND………………………………………………………………….....3

2.1. Lasers in Ophthalmology……………………………………………………….3

2.2. Tissue Welding…………………………………………………………………8

2.3. Cornea Welding……………………………………………………………….11

3. METHOD & MATERIALS…………………………………………………………18

3.1. Subjects………………………………………………………………………….18

3.2. Experimental Set up……………………………………………………………..18

3.3. Protocol…………………………………………………………………………19

3.4. Histologic Evaluation……………………………………………………………21

4. RESULTS & DISCUSSION……………………………………………………...22

4.1. Preliminary Results……………………………………………………………23

4.1.1. 809-nm diode laser…...………………………………………………....23

4.1.2. 980-nm diode laser…...…………………………………………………26

4.1.3.1070-nm YLF fiber laser……………………………………………...28

4.1.4. 1985-nm Tm: YAP laser………………………………………………..30

4.2. Comparative Study: 1070-nm vs 1985-nm.............……………………….........33

5. DISCUSSION………………………………………………………………….39

6. CONCLUSION…………………………………………………………………42

REFERENCES……………………………………………………………………………43

LIST OF FIGURES

Figure 2.1. Anatomy of eye..3

Figure 2.2. Picture of a human eye 4

Figure 2.3. Laser treatment for diabetic retinopathy 6

Figure 2.4. Retinal vein occlusion 6

Figure 2.5. Age related macular degeneration 6

Figure 2.6. Retinal tear surrounded with laser spots 6

Figure 2.7. Posterior capsulotomy 6

Figure 2.8. Posterior capsulotomy 6

Figure 2.9. PRK 7

Figure 2.10. LASIK 7

Figure 2.11. Histology of cornea (biophotonics lab picture)...........................11

Figure 2.12. Collagen arrangement 12

Figure 2.13. Stromal collagen..12

Figure 2.14. Clear corneal incision..13

Figure 2.15. Phacoemulsification surgery...13

Figure 2.16. Endopthalmitis..14

Figure 2.17. Endopthalmitis..14

Figure 3.1. Macroscopic opacification (Biophotonics Lab)..............................19

Figure 3.2. Clear corneal cut (Biophotonics Lab)......................................19

Figure 3.3. Laser irradiation (Biophotonics Lab).......................................20

Figure 4.1. Clear corneal cut...22

Figure 4.2. a) Eye 1. 809-nm- 200mW- 2mm- 3 seconds- %6 ICG.............................24

Figure 4.2. b) Eye 2. 809-nm- 200mW- 4mm- 10 seconds- %6 ICG............................24

Figure 4.2. c) Eye 3. 809-nm- 200mW- 4mm- 20 seconds- %6 ICG............................24

Figure 4.2. d) Eye 4. 809-nm- 200mW- 3mm- 3 seconds- %12 ICG............................24

Figure 4.2. e) Eye 5. 809-nm- 200mW- 4mm- 3 seconds- %12 ICG............................24

Figure 4.2. f) Eye 6. 809-nm- 200mW- 4mm- 5 seconds- %12 ICG............................24

Figure 4.2. g) Eye 7. 809-nm- 200mW- 4mm- 10 seconds- %12 ICG...........................24

Figure 4.3. a) Eye 1. 980nm- 1W-10 seconds...27

Figure 4.3. b) Eye 2. 980nm- 1W-20 seconds...27

Figure 4.3. c) Eye 3. 980nm- 1W-30 seconds...27

Figure 4.3. d) Eye 4. 980nm- 2W-5 seconds...27

Figure 4.3. e) Eye 5. 980nm- 2W-10 seconds...27

Figure 4.3. f) Eye 6. 980nm- 2W-20 seconds...27

Figure 4.3. g) Eye 7. 980nm- 3W-5 seconds...27

Figure 4.3. h) Eye 7. 980nm- 3W-5 seconds- 10 zoom...27

Figure 4.4. a) Eye 1. 1070-nm- 1.5W-30 seconds...29

Figure 4.4. b) Eye 2. 1070-nm- 2W- 5 seconds...29

Figure 4.4. c) Eye 3. 1070-nm- 2W- 10 seconds...29

Figure 4.4. d) Eye 3. 1070-nm- 2W- 10 seconds-10 zoom...29

Figure 4.5. a) Eye 1. 1985-nm- 0.38W- 10 seconds...31

Figure 4.5. b) Eye 2. 1985-nm- 0.38W- 20 seconds...31

Figure 4.5. c) Eye 2. 1985-nm- 0.38W- 20 seconds-20 zoom...31

Figure 4.5. d) Eye 3. 1985-nm- 0.38W- 30 seconds...31

Figure 4.5. e) Eye 3. 1985-nm- 0.38W- 30 seconds- 20 zoom...31

Figure 4.5. f) Eye 4. 1985-nm- 0.46W- 2 seconds...31

Figure 4.5. g) Eye 4. 1985-nm- 0.46W- 2 seconds-20 zoom...31

Figure 4.5. h) Eye 5. 1985-nm- 0.46W- 3 seconds...33

Figure 4.5. i) Eye 6. 1985-nm- 0.46W- 4 seconds...31

Figure 4.5. j) Eye 6. 1985-nm- 0.46W- 4 seconds-20 zoom...31

Figure 4.6. a) Eye 1. 1070-nm sampling...33

Figure 4.6. b) Eye 2. 1070-nm sampling...33

Figure 4.6. c) Eye 3. 1070-nm sampling...34

Figure 4.6. d) Eye 4. 1070-nm sampling...34

Figure 4.6. e) Eye 5. 1070-nm sampling...34

Figure 4.6. f) Eye 6. 1070-nm sampling...34

Figure 4.6. g) Eye 7. 1070-nm sampling...35

Figure 4.6. h) Eye 8. 1070-nm sampling...35

Figure 4.6. i) Eye 9. 1070-nm sampling...35

Figure 4.6. j) Eye 10. 1070-nm sampling...35

Figure 4.7. a) Eye 1. 1985-nm sampling...36

Figure 4.7. b) Eye 2. 1985-nm sampling...36

Figure 4.7. c) Eye 3. 1985-nm sampling...36

Figure 4.7. d) Eye 4. 1985-nm sampling...37

Figure 4.7. e) Eye 5. 1985-nm sampling…………………………………………….....37
Figure 4.7. f) Eye 6. 1985-nm sampling…………………………………………….....37
Figure 4.7. g) Eye 7. 1985-nm sampling……………………………………..….........37
Figure 4.7. h) Eye 8. 1985-nm sampling……………………………………..….........38
Figure 4.7. i) Eye 9. 1985-nm sampling……………………………………..….........38
Figure 4.7. j) Eye 10. 1985-nm sampling…………………………………..……......38

LIST OF TABLES

Table 2.1. Laser applications in ophthalmology…………………………………………...8

Table 4.1. Parameters and results for 809-nm corneal welding………………………….23

Table 4.2. Parameters and results for 980-nm corneal welding………………………….26

Table 4.3. Parameters and results for 1070-nm corneal welding………………………...29

Table 4.4. Parameters and results for 19854-nm corneal welding……………………….30

LIST OF ABBREVIATIONS

A	Ampere
AMD	Age-related macular degeneration
CW	Continuous wave
HF	Hydrogen floride
ICG	Indocyanine-green
LASIK	Laser insitu keratomileusis
LATS	Laser-assisted tissue sealing
LTK	Laser thermal keratoplasty
µm	Micrometer
mJ	Millijoule
mm	Millimeter
nm	Nanometer
NIR	Near-infrared
PDT	Photodynamic therapy
PRK	Photorefractive keratectomy
TEM	Transmission electron microscopy
YAG	Yittrium Aliminium Garnet

LIST OF SYMBOLS

CO_2	Carbon dioxide
^oC	Degree celcius
Tm: YAP	Thulium yttrium aliminium perovskite
Tm^{3+}: $YAIO_3$	Thulium yttrium aliminium perovskite
w/w	Mass of a substance/total mass of a solution or mixture