The Use of Femtosecond Laser and Corneal Welding in the Surgery of Keratoconus

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24.1 Introduction

Since the beginning of the 2000s the femtosecond laser strongly entered the corneal surgery rooms. The major contribution of this technology is related to the enhanced cutting precision, speed of execution, and the ability to make a customized surgery in relation to specific patient's anatomy and pathology. All these characteristics effectively revolutionized the prognosis in corneal transplants surgery. The femtosecond laser is particularly suitable in the surgery of keratoconus patients, thanks to the opportunity to make customized intervention flaps, e.g., the femto DALK [1]. The technology evolution led to the development of high precision lasers, with increasing frequencies, so that surgeons have the ability to create accurate, reproducible incisions in a broad variety of shapes and patterns. The Prato Ophthalmic Department team, in collaboration with the researchers of the Institute of Applied Physics—Italian National Research Council of Florence designed and then realized the anvil profile in penetrating keratoplasty, showing self-sealing properties and mechanical resistance to external and internal loads [2]. Moreover, this profile is suitable to diode laser welding procedure. This original approach allows quick sealing of the surgical wounds and a rapid healing process. In the following paragraphs the characteristics of the anvil profile and of the laser welding processes are described. The results in clinical applications will be discussed in the final part of this chapter.

24.2 Femtosecond Laser Cutting Profile

The use of the femtosecond (FS) laser technology for corneal surgery has allowed great advances especially in penetrating keratoplasty (PK). Thanks to the FS laser, new variety of shapes and angulations in vertical and lamellar intrastromal incisions could be designed at a precise depth with minimal collateral tissue injury. Different incision patterns have been proposed in the past few years for the creation of a particular flap shape in PK, so that a watertight closuring effect of the surgical wound could be achieved together with a more biomechanically stable flap and an improved healing process. The best profile

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would be the one ensuring a wider wound incision, improving the fit and stability of the graft–host junction.

Variations from a vertical wound in PK include top hat, mushroom, zigzag, Christmas tree, and the more recent anvil.

Every FS-designed pattern has the purpose to create a more structurally stable and predictable wound configuration with the aim of a faster recovery of vision and higher optical quality in comparison with the conventional blade trephination.

In patients with keratoconus, selected to PK, it is important to preserve the largest part of patient's endothelial cells, because they are generally quite young and they have a good endothelium. Moreover, having a key-hole wound effect is crucial to have a good suture apposition and to decrease the astigmatism after surgery. Therefore, the use of FS technology could find a wide application in these patients: the anterior diameter of the donor cornea should be wider than the posterior one; a good centration of the cut can be easily achieved than manual trephination. In addition, the cutting pattern can be customized on the pachimetry in order to avoid misalignment between host and donor tissue.

Between the different FS-laser proposed shapes, mushroom pattern has been widely used for PK in keratoconus patients [3–5].

Recently, a new anvil-shaped laser incision called "anvil" has been proposed (Fig. 24.1): it is particularly suitable in keratoconus corneas because this graft shape provides a large contact surface in between donor and recipient corneas, improving the donor tissue stabilization in the recipient bed. This original profile also enables a perfect match with the laser welding procedure.

24.3 Laser Welding

Laser welding of biological tissues is a technique used to join tissues by inducing a photothermal effect within the wound walls. It has been proposed in several surgical fields over the last 30 years. The first successful test was reported at the end of the 70s, when a neodymium:YAG laser was used to join small blood vessels [6]. Since then, several experiments have been performed using a variety of lasers for sealing many tissue types, including blood vessels, nerves, skin, urethra, stomach, and colon (see also previous reviews [7, 8]). Laser welding has progressively gained relevance in the clinical setting, where it now appears as a valid alternative to standard surgical techniques.

Laser welding technique holds the promise to provide instantaneous, watertight seals, which is important in many critical surgeries, such as in ophthalmology, without the introduction of foreign materials (sutures). Other advantages over conventional suturing include reduced operation times; fewer skill requirements; decreased foreign body reaction; and therefore reduced inflammatory response, increased ability to induce regeneration of the original tissue architecture, and an improved cosmetic appearance. The final aim of this procedure is to improve the quality of life of patients by reduction of healing times and the risk of postoperative complications.

The laser–tissue interaction occurring during a laser-mediated welding of biological tissues is considered to be photothermal [7, 8]. This interaction is distinguished by the absorption of the light emitted by the laser source, which generates heat through a target volume. The thermal changes induced within the tissue about the lesion result, in turn, in a bond between its adjoining edges. The heat is produced through the absorption of the laser energy by endogenous or exogenous chromophores.

In the laser welding approach, optimized by our research team, we proposed the application of a photo-enhancing dye in the tissue and the use of a laser emitting in the near infrared region. The corneal tissue, as well as most of biological tissues, is transparent to the light in this wavelength region, while the stained tissue presents the optical absorbance peak at the laser wavelength. This means that when irradiating the corneal wounds, only the stained tissue is absorbing the laser light and the induced photothermal effect is confined in the stained region.

The result is the selective fusion of wound walls at low irradiation power per target area,

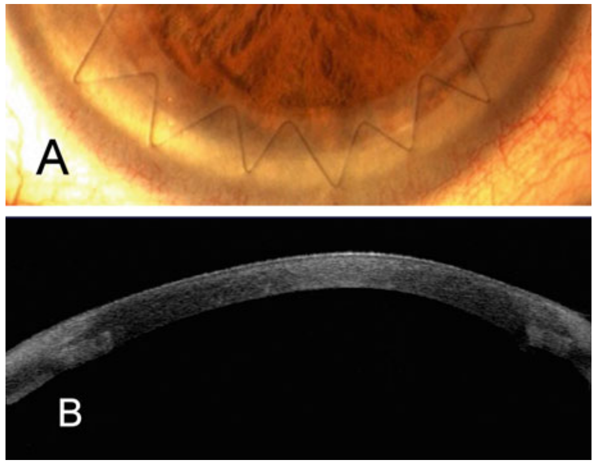

Fig. 24.1 The laser welded anvil profile at slit lamp (**a**) and OCT (**b**), 15 p.o. days

thus reducing the risk of thermal damage to surrounding tissues. The welding effect may be modulated in the depth of the transparent tissue, thus resulting in a more effective closure of the wound. Various chromophores have been employed as photo-enhancing dyes, including Indocyanine Green (ICG), fluorescein, basic fuchsin, and India ink [9]. A very popular setting of tissue laser welding includes the use of a near infrared laser, which is poorly absorbed by the biological tissue, in conjunction with the topical application of a chromophore absorbing in the same spectral region. Current examples of this modality are in the transplant of the cornea, in cataract surgery, in vascular tissue welding, in skin welding, and in laryngotracheal mucosa transplant [10, 11]. In all these cases, diode lasers emitting around 800 nm and the topical application of ICG have been used.

24.3.1 Surgical Applications of Thermal Laser Welding in Keratoplasty

To the best of our knowledge, the technique optimized and proposed by our research team is the only one laser welding application which has reached the preclinical and clinical phases. It is based on the use of a near infrared diode laser emitting at 810 nm and the topical application of the chromophore ICG, which shows high optical absorption at the laser wavelength emission [12–15]. The procedure consists in a preliminary staining phase with the chromophore, followed by an irradiation phase. ICG has been chosen because of its biocompatibility, which has already favored its exploitation in several biomedical applications. In practice, the chromophore is prepared in the form of an aqueous saturated solution of commercially available Indocyanine Green for biomedical applications (e.g., IC-GREEN Akorn, Buffalo Grove, IL or ICG-Pulsion Medical Systems AG, Germany). This solution is accurately positioned in the tissue area to be welded, using particular care to avoid the staining of surrounding tissues, and thus their accidental absorption of laser light. Then the wound edges are approximated and laser welding is performed under a surgical microscope.

The laser used in preclinical tests and in the clinical applications is typically an AlGaAs diode laser (e.g., Mod. WELD 800 by El.En. SpA, Italy) emitting at 810 nm and equipped with a fiber-optic delivery system.

We proposed two different protocols, to be used in penetrating keratoplasty [12, 13, 16] and in endothelial transplantation [17]. The first one is used in keratoconus patients, in combination with the femtosecond laser to cut donor and recipient tissues.

In penetrating keratoplasty, the technique has been named the continuous wave laser welding (CWLW). Noncontact, CW diode laser irradiation is used for the welding of corneal wounds, in substitution or in conjunction with traditional suturing procedures. The CWLW procedure developed to weld human corneal tissues in penetrating keratoplasty is as follows. The donor and recipient cornea are trephined by the use of a femtosecond laser. The donor cornea is then applied onto the patient's eye and secured by 8–16 interrupted stitches or continuous suturing. The ICG chromophore solution is prepared in sterile water (10 % w/w) in the surgery room, soon before its use. The surgeon places a small quantity of chromophore solution inside the corneal cut, using an anterior chamber cannula, in an attempt to stain the walls of the cut in depth. A bubble of air is injected into the anterior chamber prior to the application of the staining solution, so as to avoid perfusion of the dye. A few minutes after the application, the solution is washed out with abundant water. The stained walls of the cut appear greenish, indicating that ICG has been absorbed by the stroma. Lastly, the whole length of the cut is subjected to laser treatment. Laser energy is transferred to the tissue in a noncontact configuration, through a 300-μm core diameter fiber. The fiber is mounted in a handpiece and moved by the surgeon as a pencil. A typical value of the laser power density clinically used is around 10 W/cm^2, which results in a good welding effect. During irradiation, the fiber tip is kept at a working distance of about 1 mm, and at an angle of 20°–30° with respect to the corneal surface (side irradiation technique). This particular fiber position provides in-depth homogenous irradiation of the wound and prevents accidental irradiation of deeper ocular structures. The fiber tip is continuously moved over the tissue to be welded, with an overall laser irradiation time of about 120 s for a 360°. This procedure has been performed up to now on 300 patients with very satisfactory results [2, 12, 16]. The position of the apposed margins has been found to be stable over time, thus assuring optimal results in terms of postoperatively induced astigmatism after cataract and keratoplasty surgery. The lower number of stitches reduces the incidence of foreign body reactions, thus improving the healing process. Objective observations on treated patients have proved that the laser-welded tissues regain a good morphology (without scar formation) and pristine functionality (clarity and good mechanical load resistance, see Figs. 24.2 and 24.3).

In endothelium keratoplasty, the technique is called Pulsed Laser Welding (PLW). In this protocol, single laser spots (lasting tens of milliseconds) are delivered to the tissue, resulting in a photothermal effect localized within the spot dimension (a few hundreds of micrometers in diameter): the induced effect is a hard laser welding, consisting in a photocoagulation of the collagen confined at the donor/host interface. The result of the collagen denaturation at the welded site is a strong adhesion between the donor and host tissues, thus providing a suturing effect that is impossible to obtain with standard technique. The tissue regains his natural appearance in a short follow-up (1 month) and the adhesion between donor/host tissue is improved by the welding provided in the very early stage of the healing phase. However, this protocol is not used in keratoconus cases.

24.3.2 Mechanism of Thermal Laser Welding

The proposed approach has been characterized by the use of different experimental and theoretical studies, such as thermal modeling and microscopic analyses [11–15]. These include traditional methods as optical and fluorescence microscopy which allow for an investigation at the micron scale [18] and transmission and scanning electron microscopy (TEM and SEM, respectively) which are useful when studying nanometric structures [19–21]. Other used techniques are atomic force microscopy (AFM) and second-harmonic generation (SHG) microscopy which provide complementary information [22–24]. These studies have been helpful (although not exhaustive) in elucidating the different dynamics behind the sealing process.

The CWLW is based on the "soft" laser welding effect, as briefly described in this paragraph, while the PLW is based on "hard" laser welding.

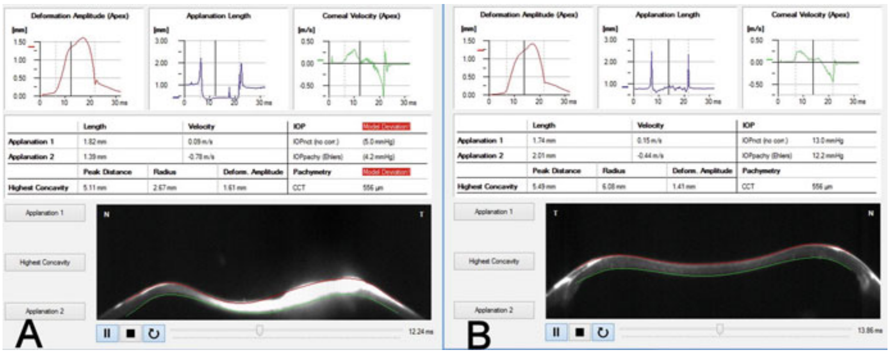

Fig. 24.2 Representative displays of the Corvis ST in a keratoconic eye (**a**) and in an eye of the same patient, following fs-penetrating keratoplasty (**b**). The deformation amplitude (DA) at the highest concavity in keratoconic eye (1.61 mm) is deeper than in fs-PK eye (1.41 mm)

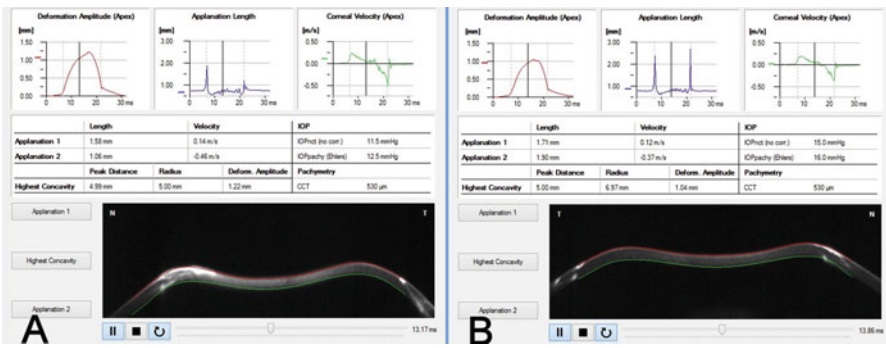

Fig. 24.3 Images from the Corvis ST at the highest concavity: simple femtosecond penetrating keratoplasty anvil profiled (**a**) versus laser welded fs-PK anvil profiled (**b**) of the same patient. Note the DA in laser welded fs-PK (1.04 mm) is smaller than in simple fs-PK (1.22 mm): this shows a greater biomechanical stability of the procedure. The anvil profile is evident in Scheimpflug image: a perfect synergy is remarkable at the host–graft junction

The main difference is the maximum induced temperature in the tissue and the treatment time duration, resulting in different biological effects. The "hard" photothermal effect results in the photocoagulation of the collagen content of the corneal stroma; the "moderate" effect consists in the "interdigitation" of collagen fibrils; and the "soft" effect results in the reorganization of the nonfibrillar components of the corneal stroma (mainly proteoglycans—PG- and glycosaminoglycans—GAGs).

As it has been pointed out, during hard laser welding the induced temperatures are higher than 70 °C. In these conditions there is a localized, strong loss of the regular appearance of the fibrillar collagen following laser welding. This is described as a full homogenization of the tissue (also called hyalinosis) [11, 25]; the appearance of fibrils fused together with a drastically altered morphology is the consequence of a complete denaturation of the collagen matrix occurring at high temperature values. Another frequently accompanying effect is the disruption of the cell membranes causing leakage of the cellular material in the extracellular space. In these cases, the wound sealing mechanism has been attributed to the photocoagulation of collagen and of other intracellular proteins, which act like micro-solders or endogenous glue on laser activation, thus forming new interactions between the tissue interfaces upon cooling [25].

In CWLW, it seems that the GAGs bridges connecting collagen fibrils in the native tissue are broken at the characteristic temperatures of diode laser welding (in the 50–65 °C range).

The individual GAG strands, freed upon heating, subsequently create new bonds with other free strands during the cooling phase. In practice, the interwoven fibrils observed at the weld site are supposed to be connected by several newly formed GAG bridges.

24.4 Clinical Results

A new dynamic Scheimpflug analyzer (Corvis ST, Oculus Optikgeräte, Wetzlar, Germany) has become available for clinical use. Because dynamic deformation of the cornea by a constant pressure air pulse is recorded through this ultra-high-speed Scheimpflug camera, not only the intraocular pressure and corneal thickness but also several parameters associated with the corneal biomechanics can be obtained. Between all these parameters (which not will be discussed on this occasion), deformation amplitude (DA; in mm) is defined as the displacement of the corneal apex from the original position at the highest concavity and it is significantly more useful and intuitive to demonstrate the biomechanical variations induced by transplant surgery.

In previous studies DA was found to be significantly greater in keratoconic eyes than in normal eyes and however, is widely accepted that the anterior segment surgery may change the biomechanical behavior of the cornea too.

Our findings showed that the DA in the keratoconic eyes underwent femtolaser penetrating keratoplasty (fs-PK) was significantly greater than in the control but not greater than in the fs-PK to which was applied the laser welding (see Figs. 24.2 and 24.3).

Some factors might have contributed to the changes in DA (and others parameters) after fs-PK: the biomechanical characteristics of the transplanted corneal button, the fibrotic wound healing of the host–graft junction site, and the application of laser welding have a positive recovering effect on DA, whereas the biomechanical characteristics of the corneoscleral rim of the residual recipient cornea may increase it, because the host cornea still has the weakened tissue characteristics of the keratoconic cornea.

In conclusion, penetrating keratoplasty has a beneficial effect on corneal biomechanics in keratoconus; as the severity of keratoconus increases, the viscoelastic properties of the cornea decrease but nearly return to normal levels with corneal transplantation. This beneficial effect is amplified when we may add the femtolaser technology (anvil profile) and the laser welding: both these elements are able to positively modulate the biomechanical behavior of the cornea [26–28].

24.5 Discussion and Conclusions

The intense collaboration with the physicists from the Institute of Applied Physics allowed us to develop and implement the welding process of the cornea. The advent of the femtosecond laser then has created new models for the surgical treatment of corneal transplants. The combination of these procedures, laser welding and femtosecond laser cutting profiles, has further increased the surgical success and the good quality of patient's life in postoperative treatment. Laser welding can definitely overcome the problems related to the restlessness of the incongruous surgical maneuvers, the risk of micro-traumas, and the possibility of reopening the wound walls at the time of stitches removal (even 16–18 months p.o.). The recovery of the work activities and sports is greatly encouraged by this procedure. The combination of these laser-based techniques can provide a quiet postoperative period for both the surgeon and the patient.

Compliance with Ethical Requirements Luca Menabuoni, Annalisa Canovetti, Alex Malandrini, Ivo Lenzetti, Roberto Pini, and Francesca Rossi declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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