

Video Article

An “All-laser” Endothelial Transplant

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Abstract

The “all laser” assisted endothelial keratoplasty is a procedure that is performed with a femtosecond laser used to cut the donor tissue at an intended depth, and a near infrared diode laser to weld the corneal tissue. The proposed technique enables to reach the three main goals in endothelial keratoplasty: a precise control in the thickness of the donor tissue; its easy insertion in the recipient bed and a reduced risk of donor lenticule dislocation. The donor cornea thickness is measured in the surgery room with optical coherence tomography (OCT), in order to correctly design the donor tissue dimensions. A femtosecond laser is used to cut the donor cornea. The recipient eye is prepared by manual stripping of the descemet membrane. The donor endothelium is inserted into a Busin-injector, the peripheral inner side is stained with a proper chromophore (a water solution of Indocyanine Green) and then it is pulled in the anterior chamber. The transplanted tissue is placed in the final and correct location and then diode laser welding is induced from outside the eyeball. The procedure has been performed on more than 15 patients evidencing an improvement in surgery performances, with a good recovery of visual acuity and a reduced donor lenticule dislocation event.

Video Link

The video component of this article can be found at <http://www.jove.com/video/52939/>

Introduction

In this work we present an original approach to endothelial keratoplasty, based on the use of a femtosecond laser to prepare donor tissue and a near infrared diode laser to weld it onto the recipient bed. Intraoperative measurement of the donor cornea is necessary to correctly design the donor tissue dimensions. Endothelial keratoplasty has been proposed in the recent years to replace penetrating keratoplasty in treating endothelial disease^{1,2}. The main advantage of this technique is a faster visual recovery, with respect to penetrating keratoplasty, reduced anesthesia during surgery, a decreased risk of graft rejection and the preservation of eye integrity. The main risk factor is postoperative donor lenticule dislocation. The standard technique is performed by inserting the donor endothelium in its final position where it is maintained by the injection of an air bubble: no sutures are used because of the mechanical, biophysical and dimensional characteristics of the endothelium. Moreover, visual acuity recovery can be limited mainly because of a mismatch between donor and recipient tissues due to a thick tissue transplanted.

Here we present a procedure in performing endothelial keratoplasty that can overcome those main problems. The donor endothelium can be assured in its final position by the use of the laser welding technique. This is a controlled and localized photothermal process: it can be induced at the donor/recipient interface. It has been studied in the last ten years and proposed in penetrating keratoplasty and in the transplant of endothelium³⁻⁵. The near infrared light (wavelength: 810 nm) emitted by a low power diode laser is delivered towards the biological tissue at the wound site. The cornea is naturally transparent to this wavelength: in order to make this tissue to absorb the laser light, it is necessary to stain it with a chromophore. The proposed dye is a sterile saturated water solution of Indocyanine Green (ICG). We demonstrated that when corneal tissue is properly stained with this ICG preparation, it shows an absorption peak at 810 nm⁶. Moreover, ICG is widely used in clinical diagnostics and its safety has been already demonstrated in human subjects. The stained cornea absorbs the diode laser light energy and the main resulting effect is a controlled temperature rise at the welding site. No thermal effects are induced in the unstained tissues. The temperature enhancement induces reversible thermal denaturation in the stromal collagen, with an immediate closing of the wound walls upon cooling. This laser welding effect was firstly demonstrated in cataract surgery^{7,8} and penetrating keratoplasty^{9,10}. An optimized approach that we are presenting in this paper has been studied for application in endothelial keratoplasty.

In the proposed surgery, single laser spots (lasting tens of msec) are delivered to the tissue, resulting in a photothermal effect localized within the spot dimension (a few hundreds of μm in diameter): the induced effect is a hard laser welding, consisting of 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 (stitches). The tissue regains his natural appearance

in a short follow up (1 month) and the adhesion between donor/host tissues is improved by the welding provided in the very early stage of the healing phase.

To avoid the other main risk of the endothelial keratoplasty, *i.e.* the transplant of a thick donor tissue, intrasurgical optical coherence tomography (OCT) is used: a commercial device measures the thickness of donor cornea, so that a correct cut profile can be designed with the femtosec laser. The proposed "all laser" endothelial transplant thus seems to improve the clinical results of this minimally invasive surgery.

Protocol

The study was conducted with the prospectively approval of the hospital's Ethics Committee; informed consent was obtained. The study was in adherence to the tenets of the Declaration of Helsinki.

1. Donor Endothelium Preparation

1. Use a donor cornea as prepared by the local eye bank at room temperature.
2. In the surgery room, pull out the donor cornea from its delivery container and set apart the solution for tissue preservation and nutrition that is used for corneal transport.
3. Place the donor corneo-scleral rim over an artificial anterior chamber, into which a liquid can flow and create a variable pressure; cover the cornea with the tissue retaining head.
4. Connect the artificial anterior chamber (AAC) through a 3-way connector to a syringe filled with the cornea preservation and nutrition liquid.
5. Use the compression ring to hold the retainer securely in place.
6. Use the syringe connected to the AAC to maintain the intracameral pressure: fill the anterior chamber with the tissue preservation solution until reaching the optimal pressure (see 1.7). Close the connector.
7. Test the pressure inside the anterior chamber with a finger. Change the inside pressure until reaching the right internal pressure (in the range 60–70 mmHg). Close the 3-way connector.
8. Measure the thickness of the donor cornea with Optical Coherence Tomography (OCT). Maintain the anterior chamber with that cornea in a fixed position in front of the instrument optics and then take a full thickness-OCT acquisition.

2. Femtosecond Laser Preparation of Donor Endothelium

1. Perform appplanation of the donor cornea under the femtosecond laser device. Use the femtosec laser to cut the donor tissue with three subsequent cuts: posterior side cut, full lamellar and anterior side cut. Set the following parameters for the three cuts.
 1. For the full lamellar cut (raster pattern, start out), set the cut depth corresponding to the thinnest point of the donor cornea (measured with the OCT as described in 1.8), always subtracting 95 μm . Set the pulse energy in the range 0.8-0.9 μJ (depending on the working depth). Set the diameter to 8.7 mm. Set the tangential spot separation to 2 μm . Set the radial spot separation to 2 μm .
 2. For the anterior side cut, set the posterior depth 30 μm deeper than the previous full lamellar cut. Set the pulse energy to 2.10 μJ ; the diameter to 8.6 mm; the spot separation to 3 μm , while the layer separation to 3 μm .
 3. For the posterior side cut, locate the anterior depth of the posterior side cut 30 μm anteriorly than full lamellar cut. Set the posterior depth to 900 μm . Set the pulse energy to 2.10 μJ ; the diameter to 8.3 mm; the spot separation to 2 μm ; the layer separation to 2 μm .

3. Recipient Eye Preparation

1. Prepare the patient for surgery. Make one 4.00 mm corneal incision at 12 o'clock with a 4.0 mm precalibrated blade. Make one limbal paracentesis with a 1.2 mm precalibrated blade, placed at 2 o'clock. Make another one limbal paracentesis at 6 o'clock with a 30° stab knife.
2. Insert an anterior chamber maintainer in the patient's anterior chamber, through the 2 o'clock paracentesis.
3. Perform one 8.2 mm diameter circular descemetorexis with the dedicated hook.
4. Strip the Descemet membrane and endothelium from the posterior stroma and remove the tissues with a Descemet hook.

4. Chromophore Preparation

1. Put 1 mg of Indocyanine Green powder in a 1.5 ml microcentrifuge tube.
2. Add 9 mg of sterile water (the ICG water solution is 10% w/w).
3. Manually mix the ICG powder and the water with a metal stirrer.

5. Staining of Donor Endothelium

1. Put the donor endothelium onto the wider part of a Busin-injector, with the inner side in contact with the injector surface.
2. Stain the inner side of donor endothelium with the chromophore solution, in its peripheral part, using a spatula. The correctly stained tissue has a homogeneous greenish color. Wait 3 min before starting step 5.3.
3. Pull the donor endothelium on the anterior part of the Busin-injector, rolled.

6. Inserting the Donor Endothelium

1. Grasp and insert the folded donor endothelium using an atraumatic coaxial forceps for endothelial lenticule.
2. Remove the anterior chamber maintainer.

3. Suture the corneal incision and the paracentesis (performed in step 3.1) with a Nylon 10.0 single stich.
4. Inject an air bubble to unfold the donor tissue and to press it against the recipient cornea. The air bubble must completely fill the anterior chamber space.

7. Laser Welding

1. Place the donor lenticule in the center of the recipient cornea, from inside with a hook or moving the air-bubble from the outside of the cornea with a spatula.
2. Use a diode laser emitting at 810 nm, equipped with a 300 μm core diameter sterile fiber optic, with 0.22 as a numerical aperture (NA).
3. Keep the fiber tip outside the eyeball, and deliver the laser light towards the stained endothelium, through the transparent corneal tissue. The fiber tip is in non-contact configuration.
4. Use the following settings for the laser: single spot emission mode, 70–80 msec pulse duration, 35–40 mJ per pulse. He-Ne aiming beam on.
5. Deliver single laser spots at the stained periphery of the donor endothelium. Deliver the spots sequentially: the final aspect is a ring of spots in the periphery of the donor lenticule. The distance between two adjacent spots center is the double of a spot diameter.
6. Apply a contact lens on the patient eye, along with 0.3% tobramycin and 0.1% dexamethasone ophthalmic suspension.

Representative Results

The “all laser” surgical procedure is proposed to perform minimally invasive corneal transplantation. The procedure is easy to perform (see **Figure 1**): with respect to a standard endothelial transplant only the steps of measuring the corneal thickness, staining the donor tissue and delivering the laser light are added. The achieved advantages largely compensate an increased surgical time of a few min. The use of intraoperative OCT to measure the donor cornea thickness and the femtosecond laser used to customize the donor lenticule dimensions enables the improvement of the donor/host interface adhesion (see **Figure 2**). In doing so, the surgery is designed following the needs and morphological characteristics of the single patient. The laser welding procedure provides an immediate closure of the donor/host interfaces⁴. In a standard technique, it is not possible to suture the donor tissue in any way, because of its biomechanical characteristics and location. The common postoperative risk is the donor lenticule dislocation. In our experience, donor endothelium dislocation did not occur in any of the 15 treated patients. To reach this goal it is important to deliver a complete ring of spots, covering the external diameter of the donor/recipient interface. At the beginning of the clinical trials, we performed a semicircular welding trajectory in selected patients. In one of these patients suffering from Fuch’s dystrophy with corneal deficit, a partial dislocation of the lenticule was observed (see **Figure 3**): interface adhesion was evident only at the welded site. For this reason, we experimented the procedure delivering a complete ring of spots, with optimized results.

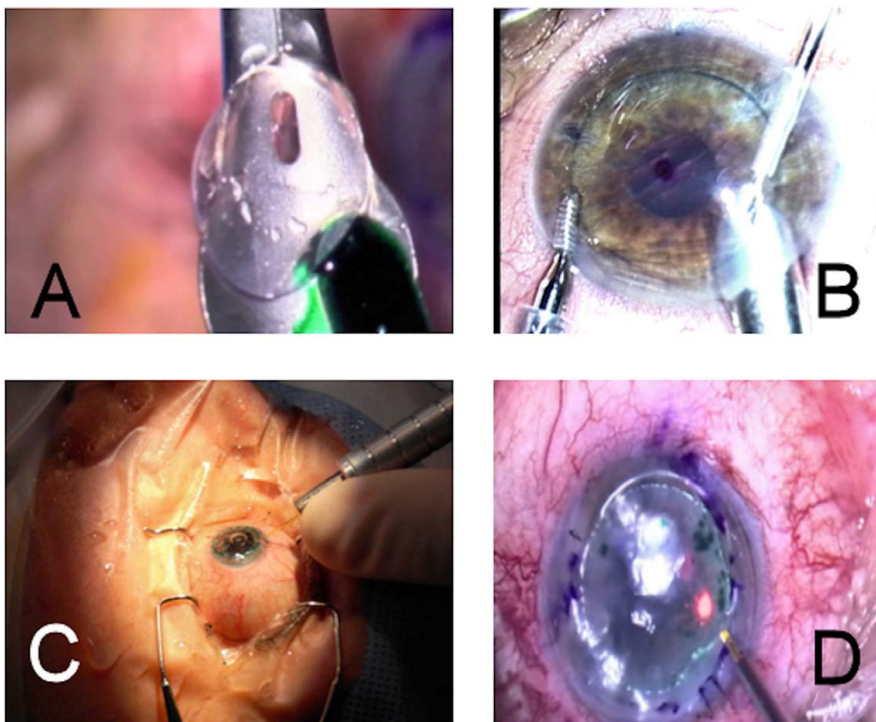


Figure 1: Endothelial Transplant. (A) The donor endothelium is put onto the injector and its inner surface is stained with a water solution of Indocyanine Green. (B) The endothelium is inserted inside the patient’s eye and positioned in its final and correct location. (C and D) Laser welding is provided from the outside, delivering single spots with a 300 μm core diameter optical fiber, mounted on a hand piece.

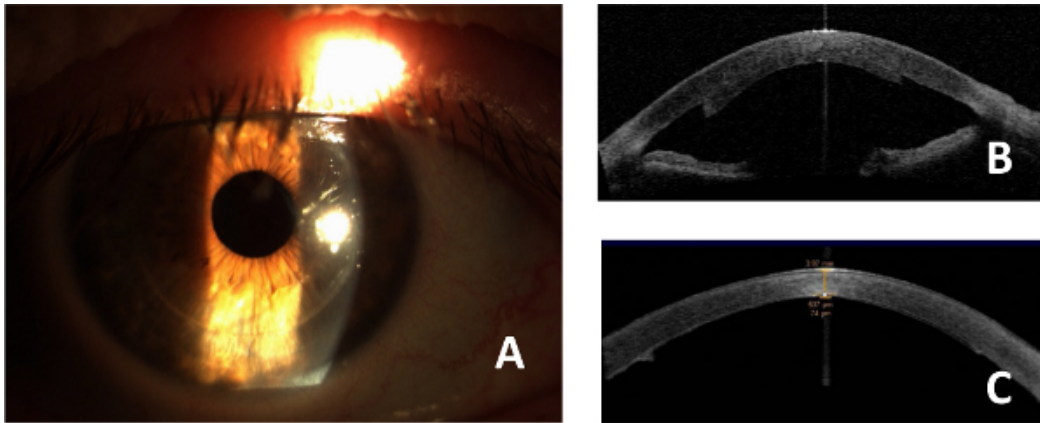


Figure 2: Postoperative results. (A) Slit lamp image of a transplanted eye, 1 week after surgery. No residual ICG is present, photothermal damage at the donor/host interface is not evident. (B) OCT image of a transplanted endothelium with the proposed “all laser” technique and without performing donor cornea thickness measurement (1 week after surgery). The transplanted endothelium is thick, with poor adhesion at the periphery. (C) OCT image of a transplanted endothelium with the proposed “all laser” technique and OCT donor cornea thickness measurement (1 week after surgery). The thickness lenticule is regular and the adhesion is good.

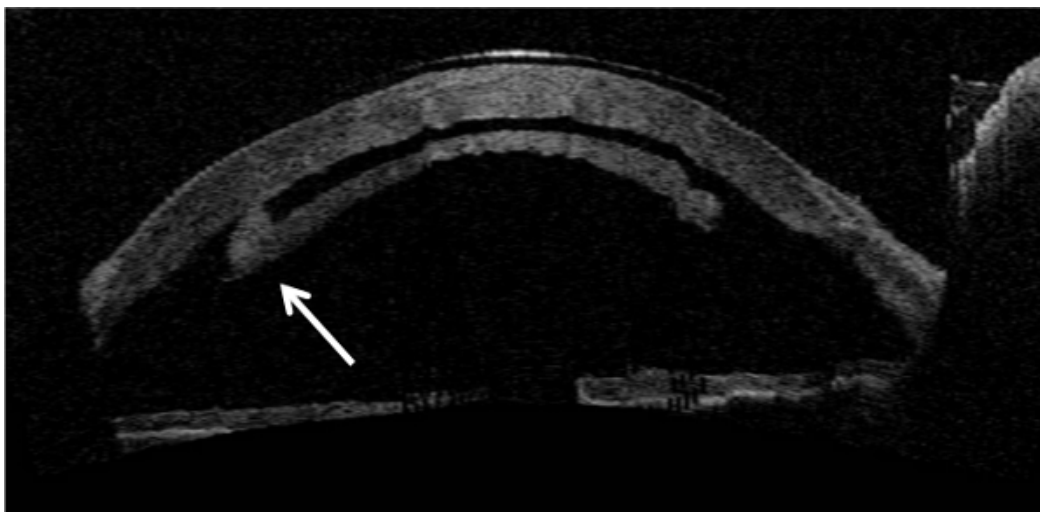


Figure 3: Laser welding efficiency. OCT image of a partially welded endothelium in a patient with Fuch's dystrophy, 1 day after surgery. In this patient, only a portion of the endothelium was welded onto the recipient's stroma: donor lenticule dislocation was observed the first day after surgery; this image shows the evidence that the adhesive effect was present only at the weld sites (white arrow).

Discussion

The “all laser” endothelial transplant is an original approach to minimally invasive corneal transplantation.

All the procedures described within the protocol were performed in the surgery room, observing the hygienic and sterilization procedure that are common practices during surgeries, such as the use of sterilized gloves, gown, mask and cap. The ICG solution was prepared in the surgery room, soon before its application in staining the donor endothelium. The ICG powder, the water and all the tools used to prepare the staining solution were sterile and commercially available for the use in human subjects. The laser fiber optic was sterilized and it was mounted on a particular hand piece, so that it could be used under the surgical microscope.

In the proposed approach, the use of an intraoperative OCT provides a correct measurement of the donor tissue thickness. This information is used to design a personalized cut profile, with desired and reduced donor lenticule thickness, by the use of a femtosecond laser to cut the donor tissue. Laser welding procedure is provided, thus stabilizing the donor lenticule position in the recipient bed. This technique enables to reduce the donor tissue dislocation risk: to the best of our knowledge, it is the only way to suture the donor endothelium to the recipient bed.

A critical aspect of this procedure is that the ICG solution has to be prepared in the surgery room, soon before its use. This is due to the optical properties of ICG that quickly degrades. An improvement could be the realization of a ready-to-use kit. Another critical aspect is that the staining procedure introduces a difficulty in the endothelial transplant procedure, because the surgeon has to stain the inner side of the donor lenticule. Moreover, the laser welding procedure cannot be performed in donor tissue that is thinner than 50 μm .

However, the postoperative results are encouraging the dissemination of the procedure and further exploitation in other surgical fields. As it provides a method to suture thin tissues that are located in inaccessible sites, a possible application of the same procedure is in microvascular

anastomosis or in the closing of the lens capsule bag. To reach these goals, the next step in the research activities will be the standardization of the procedure, designing a platform integrating a vision system and an automated delivery system for the laser light to weld the tissue that can be adapted to different surgical scenes and target tissue.

Disclosures

The authors declare that they have no competing financial interests.

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References

1. Tan, D. T., Dart, J. K., Holland, E. J., Kinoshita, S. Corneal transplantation. *Lancet*. **379**, (9827), 1749-1761 (2012).
2. El Hussein, M. A., Manero, F., Gris, O., Elies, D. Historical Review and Update of Surgical Treatment for Corneal Endothelial Diseases. *Ophthalmol Ther*. (2014).
3. Rossi, F., et al. Laser tissue welding in ophthalmic surgery. *J Biophotonics*. **1**, (4), 331-342 (2008).
4. Pini, R., et al. Combining femtosecond laser ablation and diode laser welding in lamellar and endothelial corneal transplants. *Proc. SPIE Ophthalmic Technologies XVIII*. **6**, (844), 684411-1-684411-7 (2008).
5. Rossi, F., et al. All-laser endothelial corneal transplant in human patients. *Proc. SPIE Ophthalmic Technologies XXII*. **8209**, 82091O-1-82091O-3 (2012).
6. Rossi, F., Pini, R., Menabuoni, L. Experimental and model analysis on the temperature dynamics during diode laser welding of the cornea. *J Biomed Opt*. **12**, (1), 014031-1-014031-7 (2007).
7. Menabuoni, L., et al. Laser-assisted corneal welding in cataract surgery: retrospective study. *J Cataract Refract Surg*. **33**, (9), 1608-1612 (2007).
8. Buzzonetti, L., et al. Laser Welding in Penetrating Keratoplasty and Cataract Surgery in Pediatric Patients. Early Results. *J Cataract Refract Surg*. **39**, (12), 1829-1834 (2013).
9. Menabuoni, L., et al. The 'anvil' profile in femtosecond laser-assisted penetrating keratoplasty. *Acta Ophthalmol*. **91**, (6), e494-e495 (2013).
10. Canovetti, A., et al. Laser-assisted penetrating keratoplasty: one year's results in patients, using a laser-welded "anvil"-profiled graft. *Am J Ophthalmol*. **158**, (4), 664-670 (2014).