

# Angled Mushroom Pattern Femtosecond Laser Lamellar Keratoplasty for Krumeich Ring Insertion: A Laboratory Study

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## Abstract

**Background and objective:** The aim of this laboratory study was to investigate an alternative corneal trephination technique, femtosecond laser-enabled keratoplasty (FLEK), for inserting and stabilizing the depth of a metallic alloy intrastromal corneal ring (ISCR) for penetrating keratoplasty (PKP).

**Patients and methods:** The FLEK procedures were performed on eye bank corneas mounted on an artificial anterior chamber. An angled mushroom pattern cut formed an elbow to support 7.5- and 8.0-mm ISCRs at 300  $\mu$ m depth. Optical coherence tomography (OCT) was used to visualize the position of the inserted ISCR.

**Results:** OCT images showed that the diameters of the femtosecond laser cut patterns matched the size of the respective ISCRs. Both rings rested stably on the corneal rims even prior to suturing. After suturing, the depths for the 7.5-mm and 8.0-mm ISCRs were measured by OCT to be 301.0  $\mu$ m and 299.5  $\mu$ m, respectively.

**Conclusion:** The angled mushroom pattern cut by femtosecond laser may improve the predictability and stability of the ISCR position within the wound in PKP performed by FLEK.

**Keywords:** Krumeich ring; Femtosecond laser; Lamellar keratoplasty

**Abbreviations:** FLEK: Femtosecond Laser-Enabled Keratoplasty; ISCR: Intrastromal Corneal Ring; PKP: Penetrating Keratoplasty; OCT: Optical Coherence Tomography; IOP: Intraocular Pressure; H & E: Hematoxylin and Eosin

## Introduction

In 1999, Krumeich and Daniels designed an intrastromal corneal ring (ISCR) with the intent of providing intraoperative and postoperative corneal wound stability and decreasing postoperative astigmatism [1]. The ISCR was to be placed in the interface between a donor button and a recipient bed during a standard penetrating keratoplasty (PKP). While the original rings did not significantly correct postoperative astigmatism, they did prevent distortion of the donor button related to peripheral changes or suture traction. Moreover, the immune rejection rate was reduced by about two thirds in cases of PKP with ISCR insertion compared to PKP alone [2]. In those studies, the donor and recipient corneas were cut with straight incisions, allowing the ISCR to migrate within the wound during the surgery and in the early postoperative period. This freedom of movement may be the reason why postoperative corneal astigmatism was not significantly corrected with ISCR insertion.

Femtosecond Laser Enabled keratoplasty (FLEK) is an alternative to traditional PKP that produces shaped incisions in the corneal stroma [3,4]. The purpose of this study was to evaluate the feasibility of utilizing FLEK for a more targeted insertion of ISCRs.

## Patients and Methods

The ISCR, or Krumeich ring, is a closed ring (Figure 1) composed of a titanium-cobalt-chrome-molybdenum alloy (cobalt 69.5%, chrome 24.0%, molybdenum 4.5%, and titanium 2%). Two rings of different diameters were used in our experiment. The smaller one had an inner diameter of 7.5 mm, an outer diameter of 7.8 mm, and a thickness of 0.15 mm ( $\pm$  0.02 mm standard deviation). The larger ISCR ring had an

inner diameter of 8.0 mm, an outer diameter of 8.3 mm, and a thickness of 0.15 mm ( $\pm$  0.02 mm).

Two eye bank corneas unsuitable for human transplantation were obtained from Tissue Bank International (Memphis, TN, USA). They were stored at 4°C in Optisol-GS storage medium (Bausch & Lomb, Irvine, CA, USA). Both corneas were inspected by slit lamp to ensure no previous radial keratotomy scar or LASIK flap were present and that the central stroma was free of scarring. The storage time from the death of the donor prior to the experiments was less than 10 days.

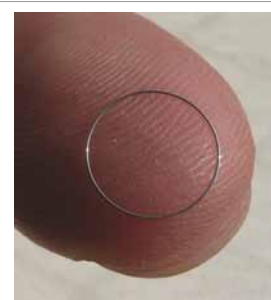


Figure 1: The Krumeich intrastromal corneal ring

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Received June 06, 2012; Accepted July 09, 2012; Published July 15, 2012

**Citation:** Hsia T, Tang M, Pan B, Krumeich JH, Li Y, et al. (2012) Angled Mushroom Pattern Femtosecond Laser Lamellar Keratoplasty for Krumeich Ring Insertion: A Laboratory Study. J Clin Exp Ophthalmol 3:232. doi:10.4172/2155-9570.1000232

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The corneas were mounted on Barron artificial anterior chambers (Katena Products, Inc., Denville, NJ, USA) which were filled with Optisol-GS through a side port. Saline bottles on an IV pole were used to maintain intraocular pressure (IOP). The corneal epithelium was removed from all donors and recipients for insertion of the 7.5-mm ISCR.

The center of the cornea and a 6-mm ring were marked with Gentian violet. Corneal incisions were made with a 60 kHz Intralase femtosecond laser system (Abbot Medical Optics, Inc., Santa Ana, CA, USA). An angled mushroom pattern was programmed for inserting the 7.5-mm ISCR (Figure 2A and Figure 2B). The anterior side cut diameter, 7.8 mm, was set to match the inner diameter of the ring, taking into consideration that the chord length is shorter than the arc length [5]. The outer diameter of the ring lamellar cut, 8.2 mm, was set to match the outer diameter of the ring. The anterior side cut angle was set to 117° and the posterior side cut angle was set to 135°. The anterior cut depth was set to 300 μm. The femtosecond laser settings for the 7.5-mm ISCR are listed in Table 1.

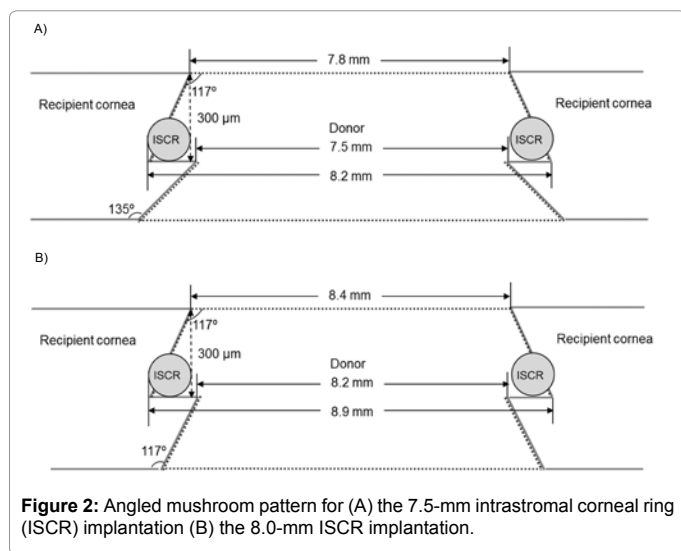


Figure 2: Angled mushroom pattern for (A) the 7.5-mm intrastromal corneal ring (ISCR) implantation (B) the 8.0-mm ISCR implantation.

	Range	Donor/Host for 7.5 mm Ring	Donor/Host for 8.0 mm Ring
<b>Posterior Side Cut</b>			
Anterior depth (μm)	90-1200	270	270
Diameter (mm)	3-9.5	7.5	8.2
Posterior depth (μm)	90-1200	1200	1200
Side cut angle (degree)	30-150	135	117
Energy (μJ)	0.3-2.85	2.4	2.4
<b>Ring Lamellar Cut</b>			
Depth in cornea (μm)	90-1200	300	300
Outer diameter (mm)	3-9.5	8.2	8.9
Inner diameter (mm)	3-9.5	7.4	8.1
Spiral start	IN or OUT	OUT	OUT
<b>Anterior Side Cut</b>			
Posterior depth (μm)	90-1200	330	330
Diameter (mm)	3-9.5	7.8	8.4
Side cut angle (degree)	30-150	117	117
Energy (μJ)	0.3-2.85	2.4	2.4
<b>Alignment Incisions</b>			
Marks On/Off	On or Off	On	On

Table 1: Femtosecond laser settings for 7.5 mm and 8.0 mm Krumeich intrastromal corneal rings

The laser cut was opened with 0.12-mm Colibri forceps and a Sinsky hook. Viscoelastic material (Healon, Advanced Medical Optics, Santa Ana, CA, USA) was then injected into the anterior chamber. Two cardinal sutures were placed and left untied. The ISCR was then inserted into the elbow of the angled mushroom pattern (Figures 3A and 3B). A Sinsky hook was used to adjust the position of the ISCR as needed.

The button excised from the recipient cornea was put on the elbow of the angled mushroom pattern created in the recipient cornea, and the pre-placed sutures were tied. The wound was sutured using an additional two cardinal sutures and a 16-bite 10-0 nylon running suture. The cardinal sutures were then removed.

### Insertion of the 8.0-mm ISCR

The same procedures were followed for insertion of the 8.0-mm ISCR. All laser settings were based on the same design for inserting the 7.5-mm ring but were adjusted for the larger ring diameter. The anterior side cut diameter, 8.4 mm, was set to match the inner diameter of the ring, taking into consideration that the chord length is shorter than arc length [5]. The outer diameter of the ring lamellar cut, 8.9 mm, was set to match the outer diameter of the ring. The anterior cut depth was set to 300 μm. However, one major adjustment was made in the angled mushroom pattern cut for inserting the 8.0 mm ISCR. The angle of the posterior side cut was decreased from 135° to 117° to create a less angled mushroom base pattern. The femtosecond laser settings for the 8.0-mm ISCR are listed in Table 1.

### Optical coherence tomography

A high resolution Fourier-domain optical coherence tomography (OCT) system (RTVue, Optovue Inc., Fremont, CA, USA) was used to image the wound junction immediately after the final sutures were placed. The system operated at a wavelength of 830 nm, a scanning speed of 26,000 axial scans per second, and an axial resolution of 5 μm (full-width-half-maximum). Radial line scans were used to image the graft junction at the 3-, 6-, 9-, and 12-o'clock positions. The line scans were 6 mm in length and consisted of 1024 axial scans. The depth of the ISCR was measured perpendicularly from the anterior corneal surface to the top of the ring in each OCT image.

### Histology

The sutures and ISCRs were removed after OCT scans. Histology using hematoxylin and eosin (H&E) staining was used to confirm the edge configuration of the femtosecond laser cuts. The corneal buttons and recipient corneas were fixed using 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). The tissues were then dehydrated and embedded in paraffin wax. Five micrometer thick sections were prepared for light microscopy.

### Results

#### 7.5-mm ISCR

The ISCR placed on the recipient cornea was in a stable position

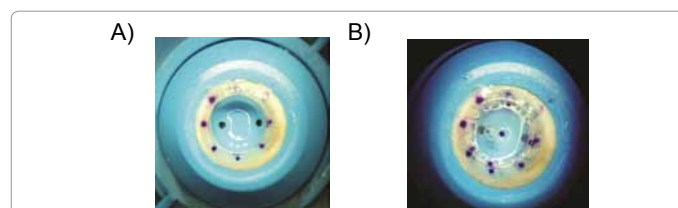


Figure 3: An intrastromal corneal ring (7.5 mm) sits on the recipient corneal rim (A) without suturing (B) with suturing.

without the support of donor button or sutures (Figure 3A). Its position remained stable after suturing (Figure 3B). OCT imaging for the 7.5-mm ISCR showed that the ring fit well in the elbow of the angled mushroom pattern in all four quadrants. Wound apposition was excellent except for a gap in the posterior aspect in one quadrant (Figure 4A) that may have been associated with a shallow suture bite and the 135° posterior side cut angle. Because the scan position was between 2 suture bites, a small anterior wound gap was also observed (Figure 4A). The ring depths were measured to be 325 μm, 300 μm, 288 μm, and 291 μm at the 3-, 6-, 9-, and 12-o'clock positions, respectively. The average ring depth was 301.0 μm ± 16.8 μm.

### 8.0-mm ISCR

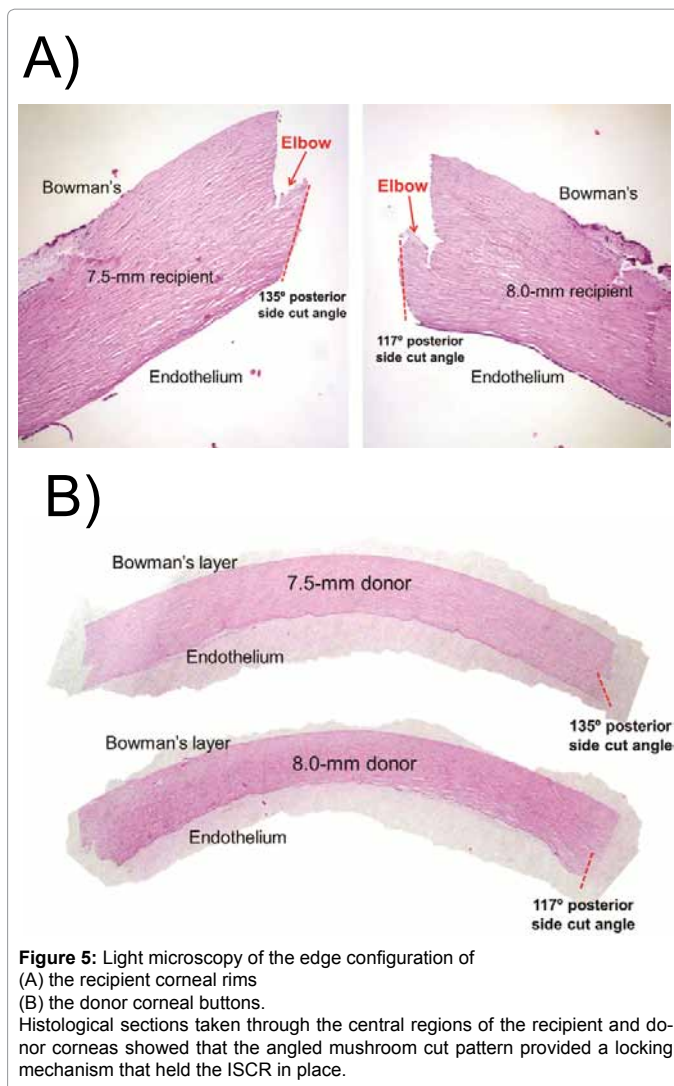
The 8.0-mm ISCR was implanted using a femtosecond laser cut that had a similar shape to the pattern for 7.5-mm ISCR with one exception – the posterior side cut angle was reduced from 135° to 117° to help prevent posterior wound gaping. The surgeon also took care to maintain sufficient suture depth so that the bite included the posterior base of the mushroom-shaped graft. These changes improved posterior wound apposition. In all 4 imaged quadrants, OCT showed good wound apposition after suturing (Figure 4B). No gap was apparent in either the anterior or posterior aspect of the wound. The ISCR fit well and was in a stable position in the elbow of the angled mushroom pattern in three of the four quadrants. At the 9-o'clock position, it tilted anteriorly. The ring depths were 314 μm, 312 μm, 198 μm, and 374 μm at the 3-, 6-, 9-, and 12-o'clock positions respectively. The mean depth was 299.5 ± 73.5 μm.

### Histology

Histological sections confirmed the edge configuration of the angled mushroom pattern for the recipient (Figure 5A) and the complementary pattern for the donor button (Figure 5B).

### Discussion

This study investigated the possibility of using FLEK, which can make shaped incisions in the corneal stroma, to create a cut that stabilizes the placement of an ISCR. For our experiment, we modified

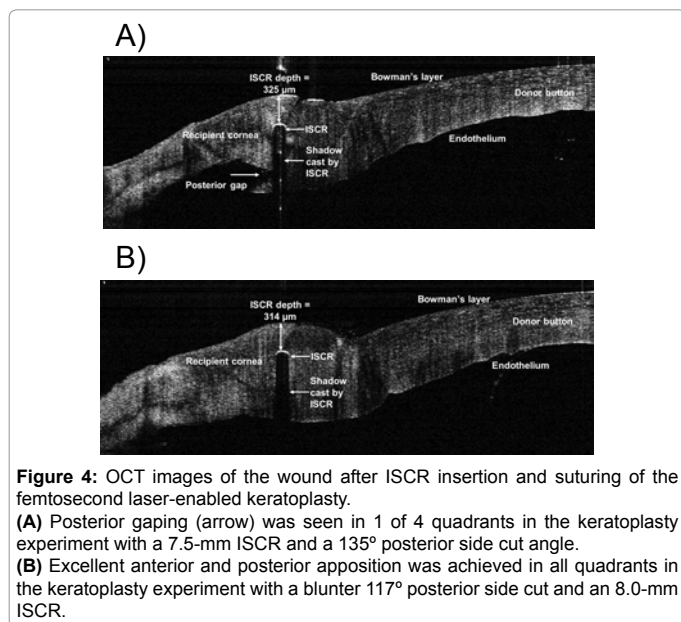


**Figure 5:** Light microscopy of the edge configuration of (A) the recipient corneal rims (B) the donor corneal buttons. Histological sections taken through the central regions of the recipient and donor corneas showed that the angled mushroom cut pattern provided a locking mechanism that held the ISCR in place.

the usual mushroom cut into an angled mushroom pattern so as to create a recess into which the ISCR could be wedged. For the 7.5-mm ring, all four quadrants had consistent depth and placement within the elbow. For the 8.0-mm ring, three of the four quadrants were well-placed, but the fourth had a slight anterior tilt that brought it closer to the surface. Upon closing the wound, we noticed that the ISCR naturally fell into position in the elbow of the cut as the suturing progressed. These observations suggest that the geometry of the angled mushroom cut provides a good fit for ISCR placement. However, it is important to note that the ring can shift towards the anterior side of the wound junction, as was the case in one of the eight sites examined by OCT in our study. Thus, surgeons need to ensure that the ring is tucked into the elbow of the anterior side cut after the cardinal sutures are placed.

A drawback of a flared posterior side-cut angle is that if the suture does not go deep enough, the posterior graft edge can drop into the anterior chamber. We noticed this problem with the 7.5-mm ring graft and solved it by making the posterior side cut more perpendicular and by conscientiously passing the suture deeper to bite into the posterior graft side cut.

This study demonstrates an alternative method to current techniques for inserting ISCRs. Using femtosecond laser and OCT



**Figure 4:** OCT images of the wound after ISCR insertion and suturing of the femtosecond laser-enabled keratoplasty. (A) Posterior gaping (arrow) was seen in 1 of 4 quadrants in the keratoplasty experiment with a 7.5-mm ISCR and a 135° posterior side cut angle. (B) Excellent anterior and posterior apposition was achieved in all quadrants in the keratoplasty experiment with a blunter 117° posterior side cut and an 8.0-mm ISCR.

in ISCR implantation has profound implications on current clinical concepts. Since OCT can assess pachymetry and cut depths over the cornea, the outcome of new laser settings with various diameters and cut depths can be evaluated. It will be interesting to assess in future investigations the outcomes of ring placements in other locations near Descemet's membrane.

Femtosecond laser-enabled keratoplasty provides faster and stronger wound healing over traditional PKP because of the ability to make shaped incisions in the corneal stroma [3,4]. However, immune rejection remains a leading cause of graft failure in FLEK [6,7]. The Krumeich ring can reduce corneal transplant complications by reducing immune rejection and blocking neovascularization [2]. The technical difficulty of ISCR insertion using the mechanical trephine system may have prevented its widespread use. Our method of femtosecond laser shaped trephination provides for easier insertion of the ISCR at the desired depth, and may lead to more rapid adaptation and utilization of the Krumeich ring. As such, it will be important to develop a standardized method for inserting Krumeich rings in a safe and stable manner, allowing control of the ring depth to limit neovascularization and prevent graft failure.

Lastly, our study demonstrated that an angled mushroom pattern cut with its characteristic elbow allows the Krumeich ring to be wedged naturally. Such a design provides greater stability for the ring than straight cut incisions. This may allow the Krumeich ring to correct postoperative corneal astigmatism by rigidly supporting a round graft shape. This technique may help achieve Krumeich and Daniel's initial clinical aim of using ISCRs to decrease postoperative corneal astigmatism [1]. Future clinical studies will be needed to assess this.

Several limitations are worth noting. Although the diameters of the femtosecond laser cuts used in our experiments appeared to fit the ISCRs well, as judged by the ease of insertion and positioning, it is possible that slightly smaller diameters of femtosecond laser cuts will also fit the ISCRs well. In this study, we assumed the corneal stroma to be incompressible and rigid along the radial direction, so that the arc length does not change before and after applanation. As such, the

anterior side cut diameter was set to the arc length equivalent of the inner ring diameter, and the anterior side cut angle was set to create a vertical side wall after the cornea recovered from the applanated state to its natural dome shape. An investigation of FLEK by Heur et al. [5] showed that the femtosecond laser cut diameter setting is between the actual measured arc length and chord length. Thus the optimal diameter setting may be slightly smaller than the settings used in our study. Given the flexibility of corneal tissue, it is likely that this technique would work well over a range of laser settings. Further studies are needed to confirm whether or not other settings will also work well.

In summary, we demonstrated that ISCRs can be easily positioned at an exact depth using penetrating angled mushroom pattern femtosecond laser cuts in both graft and host corneas. Clinical studies are needed to compare the efficacy of ISCR implantation in cuts prepared by femtosecond laser trephination versus mechanical trephination. In addition, clinical studies are needed to determine if ISCRs implanted with this laser technique can help reduce postoperative astigmatism.

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