

Evaluation of Femtosecond Laser Clear Corneal Incision: An Experimental Study

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ABSTRACT

PURPOSE: To evaluate the changes of corneal topography following femtosecond laser and manual clear corneal incision (CCI).

METHODS: Inflation testing was performed in 14 human eye globes to evaluate the topographic response of the cornea to CCIs. In seven samples (femtosecond laser group), a 2.75-mm three-plane CCI was created using the iFS femtosecond laser (Abbott Medical Optics, Inc., Santa Ana, CA); the remaining seven samples (control group) received a 2.75-mm manual CCI using disposable angled knives. Topographic maps of the anterior and posterior cornea were acquired using a Scheimpflug topographer Pentacam HR (Oculus Optikgeräte GmbH, Wetzlar, Germany). Keratometric data were used to analyze the curvature changes of the cornea. The changes of corneal astigmatism were analyzed by vector analysis.

RESULTS: After CCI, the mean change of the anterior keratometric power was 0.04 ± 0.39 and 0.05 ± 0.51 diopters (D) (analysis of variance, $P > .05$) in the femtosecond laser and control groups, respectively. The mean change ($P > .05$) of the posterior corneal keratometric power was 0.16 ± 0.19 and 0.15 ± 0.18 D, respectively. The average change of the anterior and posterior corneal astigmatism vector magnitude was 0.17 D or less in both groups ($P > .05$). A slight against-the-rule astigmatic change of the anterior and posterior corneal interfaces was found after both CCI techniques.

CONCLUSION: The 2.75-mm three-plane CCI created with femtosecond laser showed minimal changes of the anterior and posterior corneal topography, comparable with those of single-plane angled manual incision.

[J Refract Surg. 2013;29(6):418-424.]

Femtosecond laser cataract surgery is a relatively new technique that uses commercial femtosecond laser platforms to perform clear corneal incision (CCI), capsulorrhexis, and cataract nucleus fragmentation.^{1,2} Although femtosecond laser CCI can be theoretically performed with high accuracy through personalized design of the intrastromal tunnel, there is still no knowledge about its effect on corneal topography.

In the current experimental study, we aimed to evaluate the topographic changes of the anterior and posterior cornea in femtosecond laser-assisted three-plane CCI compared with single-plane angled CCI created with surgical disposable knives.

MATERIALS AND METHODS

Fourteen human eye globes, not suitable for transplantation, were obtained from the Veneto Eye Bank Foundation (Venezia Zelarino, Italy). The eye globes were explanted between 3 and 21 hours after death and immediately preserved at 4°C in a corneal storage medium (Eusol C; Alchimia, Padova, Italy) and enriched with 15% dextran. All samples were used for experimentation within 48 hours. The experimental protocol was approved by the internal scientific board and the local ethical committee was notified.

The eye globes were randomized, using the allocation scheme 1:1, in two groups: the femtosecond laser group

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Submitted: September 12, 2012; Accepted: March 6, 2013; Posted online: May 3, 2013

Supported in part by an unrestricted educational grant by Abbott Medical Optics, Inc.

The authors have no financial or proprietary interest in the materials presented herein.

The authors thank Giovanni Desiderio and Salvatore Abate at CNR-IPCF Unit of Support of Cosenza and DeltaE S.r.l for their technical assistance in the development of the Ocular Biomechanics Modulator apparatus and for developing the mask used for femtosecond laser clear corneal incision.

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doi:10.3928/1081597X-20130430-01

TABLE 1
Femtosecond Laser Clear Corneal Incision Settings

Cut	Length (mm)	Side Cut Angle (degrees)	Energy (μ J)	Spot Separation (μ m)	Layer Separation (μ m)
Anterior cut	0.70 \pm 0.14 ^a	30	1.25	4	3
Lamellar cut	1.00	0	0.85	4	4
Posterior cut	0.65 \pm 0.10 ^a	40	1.20	4	3

^aThe length of the anterior and posterior cuts were related to the corneal thickness of the individual sample treated. Values are expressed as mean \pm standard deviation.

(n = 7), in which the CCI was performed using a commercial femtosecond laser platform (iFS Femtosecond laser 150 KHz; Abbott Medical Optics, Inc., Santa Ana, CA), and the control group (n = 7), in which the CCI was manually performed using a disposable 2.75-mm angled knife (Clear Cut; Alcon Laboratories, Inc., Fort Worth, TX). The corneal incisions were located at the 3-o'clock position in all samples and were performed by two experienced cataract surgeons (SS performed the laser CCI and ML the manual CCI).

The corneal topography maps of the anterior and posterior corneal interfaces were obtained using a Scheimpflug-based Pentacam HR (Oculus Optikgeräte GmbH, Wetzlar, Germany).

EXPERIMENTAL APPARATUS

A whole eye globe inflation test was designed to perform ex vivo measurements of the anterior and posterior corneal topography. An experimental apparatus, the Ocular Biomechanics Modulator (OBM) (Figure A, available as supplemental material in the PDF version of this article), was used for the scope. The OBM was designed and developed to evaluate the corneal deformation into a controlled environment, and the temperature and humidity of the moist chamber were continuously monitored and recorded. The IOP of the eye globe was monitored by means of a water column and a pressure transducer (DS27C002A1; Valcom, Milano, Italy), and modified by infusing saline solution into the posterior segment of the eye through a needle by an automated pumping system (Genie Plus syringe pump; Kent Scientific Corp., Torrington, CT). The needle was gently inserted into the eye globe through the optic nerve head. The OBM apparatus was controlled by dedicated software written in LabView (National Instruments Corporation, Austin, TX).

EXPERIMENTAL PROTOCOL

Before the experiment, each eye globe was gently mounted into a specially designed holder to guarantee proper centration during the topography measurements. The eye holder was placed in an acrylate box, where an

ultrasound humidifier (JC 380; Life Tool Technologies, Ancona, Italy) provided a constant humid atmosphere. A commercial air conditioner heat pump was used to maintain a constant room temperature. Within the OBM apparatus, the IOP of the eye globe was kept constant at 18 mm Hg for 10 minutes to achieve the unique pre-conditioning reference state of the corneal tissue before testing (both before and after CCI).³⁻⁵ The IOP was then kept constant at 18 mm Hg during the experiment.

Three Pentacam HR measurements were taken for each eye before and after CCI. The CCI length was measured on the Scheimpflug image using the caliper tool of the Pentacam HR software. Measurements were done, by two independent observers, along the horizontal scan passing through the CCI (0° to 180° meridian); the CCI length was calculated as the linear distance between the most anterior and posterior incision sites.

FEMTOSECOND SURGICAL PROCEDURE

The eye globe was kept in the specially designed holder to guarantee proper centration during surgery. In the femtosecond laser group, a three-plane CCI was programmed: the anterior and posterior cuts intersected the lamellar cut at 30 μ m from its anterior and posterior edges, respectively. The femtosecond laser CCI was personalized to each sample taking into account the corneal thickness at the site of incision: the lamellar cut was placed at 50% stromal depth (Figure B, available as supplemental material in the PDF version of this article). The laser parameters are summarized in Table 1. Each eye globe was applanated using the disposable flat interface contact lens of the system, without using the suction ring provided by the manufacturer. This procedure was used to maintain the IOP at 18 mm Hg during surgery, thus avoiding the effect of an unpredictable IOP increase to the postoperative corneal deformation. A custom mask (Figure B) was placed on the superior side of the interface contact lens. This approach was required because the Intralase iFS software did not permit modification of the posterior and lamellar cut positions and angles. The maximum diameter of the mask's aperture was 2.75 mm.

TABLE 2

Mean ± Standard Deviation Curvature Values of the Anterior and Posterior Corneal Interfaces

Group	Curvature Data	Anterior Cornea		Posterior Cornea	
		Before Corneal Cut	After Corneal Cut	Before Corneal Cut	After Corneal Cut
Femtosecond laser	simK (D)	43.62 ± 1.81	43.66 ± 1.71	-6.70 ± 0.56	-6.54 ± 0.18
	Vector astigmatism	0.96 ± 0.91 at 96°	1.11 ± 0.67 at 61° ^a	0.76 ± 0.50 at 61°	0.81 ± 0.57 at 117°
Control	simK (D)	42.02 ± 0.99	42.08 ± 1.10	-6.82 ± 0.53	-6.67 ± 0.66
	Vector astigmatism	1.36 ± 0.43 at 102°	1.30 ± 0.80 at 146° ^a	0.62 ± 0.08 at 54°	0.78 ± 0.23 at 92°

simK = simulated keratometry; D = diopters

^aP values of .05 or less was the difference in the induced direction of astigmatism vector axis between femtosecond laser and control groups.

After both femtosecond laser and manual CCI, the samples were kept in the eye holder at 18 mm Hg and Pentacam HR measurements were taken within 1 hour.

STATISTICS

Pentacam HR data were expressed as mean ± standard deviation. The mean of three Pentacam HR measurements was used for statistics. The curvature of the anterior and posterior corneal interfaces was expressed using the simulated keratometry (simK) value exported by the topographer, which was calculated as the mean of the steepest and flattest keratometry indices.

The accuracy of the Pentacam HR instrument to evaluate linear distances and radii of curvature was determined by imaging a corneal model (acrylic material, LE-124; Eyeteck Ltd., Morton Grove, IL) with known dimension. The experimental error was defined as the difference between a set of three repeated experimental measurements and the nominal values of the corneal model. The percent error, reported as a percentage, was used to determine accuracy of the instrument and was defined as the ratio of the error to the nominal value.

Repeatability of Pentacam HR measurements was determined using the coefficient of repeatability and the coefficient of variation values both before and after CCI. The coefficient of repeatability was calculated as 1.96 × the within-sample standard deviation of the simK value. The coefficient of variation, expressed in percentage, was calculated from the intrasession standard deviations for the three independent consecutive measurements.

Vector analysis of corneal astigmatism for both the anterior and posterior corneal interfaces was performed to determine its change in both magnitude and direction after CCI. The analysis of variance was used to test the significance of topographic changes induced by the method of CCI and the differences between the femtosecond laser and control groups.

The minimum difference detectable by the test has been measured to understand the achieved power of

the statistical analysis (β). Statistical analysis was performed using SPSS software version 17 (SPSS, Inc., Chicago, IL). Differences with a P value of .05 or less were considered statistically significant.

RESULTS

The mean donor age was 66.42 ± 6.47 years, with no difference between the samples of the femtosecond laser and control groups (P > .05). The pre-cut anterior corneal topographies showed with-the-rule astigmatism in all samples. During the experiments, the room temperature and humidity of the moist chamber were 32°C ± 1°C and 70% ± 3%, respectively.

The time duration of the femtosecond laser CCI ranged between 28 and 31 seconds. After femtosecond laser CCI, the eye globe was placed under the operating microscope and the residual bridges of stromal collagen fibers were dissected with a spatula.

The experimental errors of the Pentacam HR instrument were estimated to be 0.04 mm for linear distances and 0.06 mm for radii of curvature, with a percent error of 3.0% and 0.8% with respect to the nominal values of the reference cornea, respectively. In this study, the intended length of the femtosecond CCI was set to an average 2.29 mm (Table 1). The average length measured using the caliper tool of the Pentacam HR software was 2.21 ± 0.12 mm. The average length measured in the manual CCI group was 1.89 ± 0.20 mm.

Before CCI, the coefficient of repeatability was 0.35 and 0.22 diopters (D) for the anterior and posterior simK values, respectively. The coefficient of variation of the anterior and posterior Pentacam HR measurements was 4% and 8%, respectively. After CCI, the coefficient of repeatability values were 0.29 and 0.14 D and the coefficient of variation values were 4% and 7%, respectively. There was no difference in the repeatability statistics of Pentacam HR measurements between groups.

Table 2 summarizes the corneal curvature data of the femtosecond laser and control groups before and after

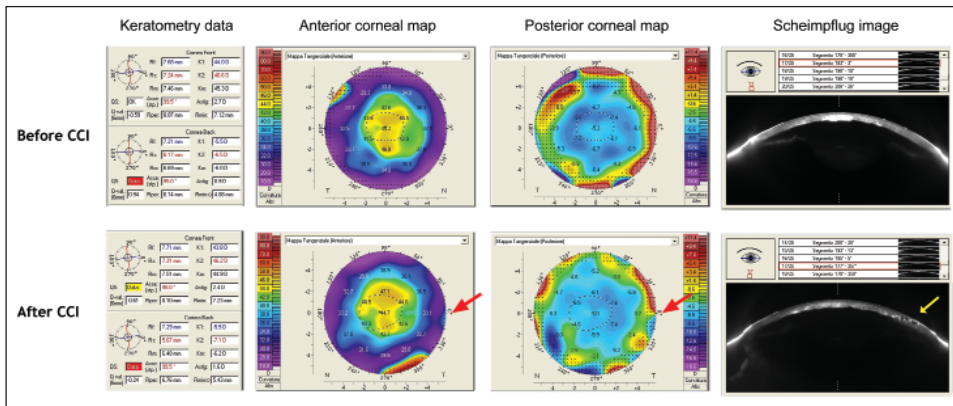


Figure 1. Corneal topography maps of the anterior and posterior interfaces (upper row) before and (lower row) after femtosecond laser clear corneal incision (CCI). The red arrows highlight the site of CCI, at the 3-o'clock position in all samples. After CCI, the mean changes of the anterior keratometric values were 0.5 D or less. The Scheimpflug image shows the femtosecond laser CCI after manual dissection of residual stromal bridges (yellow arrow).

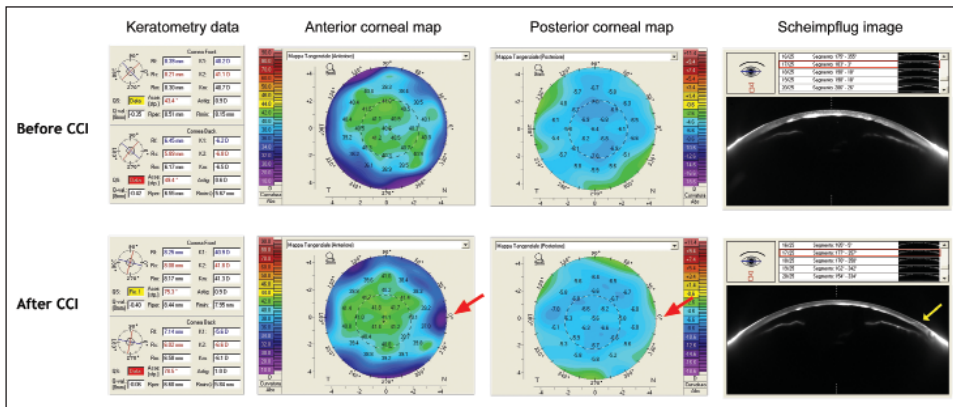


Figure 2. Corneal topography maps of the anterior and posterior interfaces (upper row) before and (lower row) after manual clear corneal incision (CCI). The red arrows highlight the site of CCI. After incision, the mean changes of the anterior keratometric values were 1.0 D or less. The Scheimpflug image shows the single-plane angled CCI (yellow arrow).

CCI. After CCI, there were no statistically significant changes of the average simK values of the anterior and posterior cornea: the mean change of the anterior corneal keratometric power ($P > .05$, $\beta = 89\%$) was 0.04 ± 0.39 and 0.05 ± 0.51 D in the femtosecond laser and control groups, respectively. The mean change of the posterior corneal keratometric power ($P > .05$, $\beta = 90\%$) was 0.16 ± 0.19 and 0.15 ± 0.18 D, respectively. The analysis of variance indicated no significant influence of the precut anterior ($P = .16$) and posterior ($P = .40$) corneal curvature values on the anterior and posterior topographic changes induced by the two CCI techniques, respectively. **Figures 1** and **2** show the corneal maps and Scheimpflug images of two samples before and after femtosecond laser and manual CCI, respectively.

The change of magnitude of the anterior corneal astigmatism vector was lower than 0.16 D in both groups ($P > .05$; $\beta = 83\%$), with no differences between groups ($P = .39$; $\beta = 99\%$). The femtosecond laser CCI induced a clockwise change of corneal astigmatism axis, contrary to the manual incision that induced a counter-clockwise change ($P = .06$, $\beta = 88\%$). The mean surgically induced astigmatism (SIA) of the anterior cornea was 0.78 ± 0.36 D at 112° and 0.92 ± 0.46 D at 48° in the femtosecond laser and control groups, respectively. The mean SIA of the posterior cornea was 0.79 ± 0.44 D at 57° and 0.61 ± 0.31 D at 66° in the

femtosecond laser and control groups, respectively. The change of magnitude of the posterior corneal astigmatism vector was lower than 0.17 D in both groups ($P = .51$, $\beta = 85\%$). There was a counter-clockwise change of the astigmatism vector axis in both groups. There were no statistically significant differences in the induced changes of magnitude and direction of the posterior corneal astigmatism between groups ($P > .05$, $\beta > 80\%$). The vector change of corneal astigmatism has been plotted using a double-angle format, as illustrated in **Figure 3**.

DISCUSSION

Both the 2.75-mm femtosecond laser and manual CCI showed minimal impact on the central corneal topography. The average corneal keratometric change was 0.06 D or less in both groups, although the standard deviation from the mean was lower in the femtosecond laser than in the control group. The SIA was analyzed by vector analysis from anterior and posterior keratometric data. The change of magnitude of the astigmatism vector was relatively low and comparable between groups. The femtosecond laser incision induced a clockwise change of corneal astigmatism, contrary to the manual incision, which induced a counter-clockwise change. However, the net topographic effect was comparable between methods showing an against-the-rule astigmatic

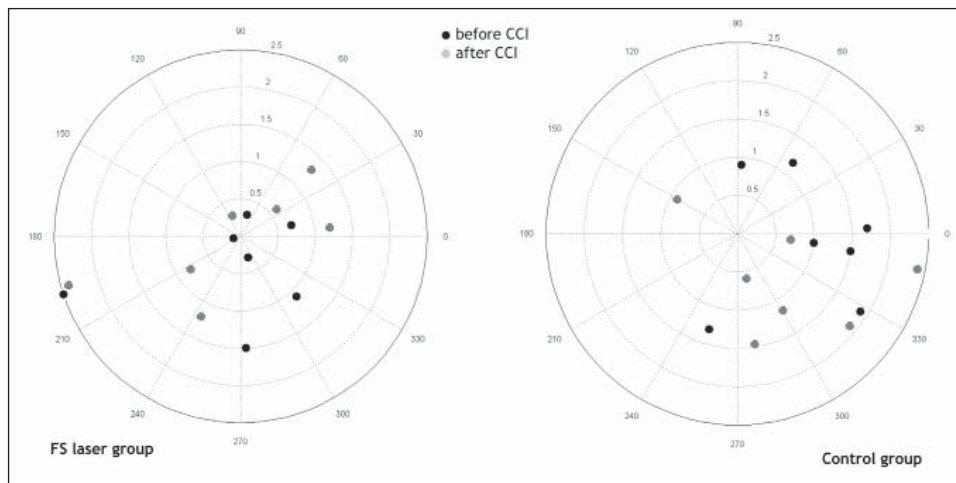


Figure 3. Double-angle plot of the anterior corneal astigmatism before (black circles) and after (gray crosses) clear corneal incision (CCI) in the femtosecond laser (FS) and control groups. Each point represents a single sample astigmatism uniquely characterized by a pair of values in the x- and y-coordinates. After corneal incision, the change of vector astigmatism direction was between 0° and 60° in 5 of 7 cases (71%) in the femtosecond laser group and in 4 of 7 cases (57%) in the control group. Eighty-six percent of samples (6 of 7) in the femtosecond laser group and 71% (5 of 7) in the control group were within 1.5 D from the origin both before and after corneal incision.

change, although with lower-standard variation from the mean in the femtosecond laser than in the control group. We hypothesized that the change of the anterior corneal astigmatism axis direction may depend on the different tunnel incision geometry between groups. In the femtosecond laser group, we performed 1.00 mm interlamellar dissection at 50% corneal depth, whereas in the control group we performed a single-plane posterior-angled incision. Moreover, the femtosecond CCI was on average 300- μ m longer than the manual CCI.⁶⁻⁸

We programmed a three-plane CCI using a commercial femtosecond laser platform not yet designed to perform femtosecond laser cataract surgery and a customized mask. A set of preliminary measurements (unpublished data) was performed to choose the optimal CCI design by comparing two-plane and three-plane corneal incisions. We found that, for a 2.75-mm size, a three-plane CCI had the optimal characteristics in terms of length and self-sealing.

To preliminarily evaluate the effect of femtosecond laser CCI for cataract surgery, we performed inflation experiments on enucleated human eye globes. Whole eye globe inflation testing could be considered the most accurate ex vivo method to evaluate corneal deformation, although care must be taken to control tissue hydration if the results aim to mimic in vivo biomechanical responses.⁹⁻¹⁶ Therefore, it should be required to monitor and maintain the temperature and humidity of the room constant during the experiment, as done in this study. The use of an intact eye is closer to the in vivo situation than the use of a corneoscleral button: the limbal junction is indeed allowed to move, which is thought to be crucial to establish a biomechanical model mimicking the function of a real cornea.⁶⁻⁸ Preconditioning is widely used in the mechanical testing of soft tissues^{3,4}; the procedure is required to attain a unique, pretesting reference state and ensure reproducibility of the corneal strain measurements.

Previous clinical studies demonstrated that the SIA after CCI, analyzed from keratometric data, was related primarily to the size and secondarily to the location of the CCI itself.¹⁷⁻²² The SIA was found to decrease postoperatively. Olsen et al.¹⁸ showed that the mean SIA was 1.41 ± 0.66 D 1 day postoperatively and decreased to 0.72 ± 0.35 D 6 months postoperatively. Ozkurt et al.²² showed that although the corneal astigmatism decreased during a 6-week follow-up, temporal CCI yielded less SIA than nasal CCI. The lower induction of SIA by temporal CCIs in comparison with superior or nasal CCIs was confirmed by other studies.²⁰⁻²⁶ However, the effect of incision site on astigmatism induction was shown to be minimal in CCIs of 2.75 mm or less.^{27,28} Although differences are generally expected between ex vivo and in vivo studies, the results shown in this study are in agreement with previous clinical data.¹⁷⁻³⁰ The limit of ex vivo studies is that wound healing is not taken into account, so the definite effect of CCI on corneal curvature and astigmatism could be even lower than that found immediately after cutting, as previously shown.^{18,22,29,30}

The repeatability of our Pentacam HR measurements in ex vivo ocular tissues was high and comparable to what was shown in clinical studies.³¹⁻³⁵ In this study, the coefficient of repeatability ranged between 0.29 and 0.35 and 0.14 and 0.22 D for the anterior and posterior simK values, respectively. The coefficient of variation of the anterior and posterior Pentacam HR measurements was 4% and 8% or less, respectively. Averaging three readings from one session, as done in this study, has improved repeatability and generated precise results for analysis.³² Obtaining reproducible data is fundamental in any mechanical characterization system to verify the reliability of results.

Only one study³⁶ investigated the geometry of corneal tunnel incisions created with a femtosecond laser platform. Researchers used a 15-KHz femtosecond

laser platform (IntraLase model 1; Abbott Medical Optics, Inc.) to create single-plane angled CCIs, 3.00-mm wide and with variable lengths ranging from 1.00 to 2.00 mm. They found that a 3.00 × 2.00 mm CCI was more stable than narrower incisions, showing less leakage at various levels of indentation pressure performed with an ophthalmodynamometer. A three-plane CCI, as done in the current study, could theoretically bring additional advantages in terms of safety and stability in comparison with a single-plane angled CCI.^{36,37} The high reproducibility of femtosecond laser CCI, as shown in the current study, could further minimize the rate of complications related to the CCI early after cataract surgery.^{38,39} Clinical studies are needed to confirm this hypothesis.

In the current study, we showed comparable results between three-plane femtosecond laser and manual single-plane angled CCIs for cataract surgery. The central topography of the anterior and posterior corneal interfaces did not show significant changes of the mean keratometric data or the SIA between laser and manual techniques, except for a different change of anterior SIA direction. Further evaluation of femtosecond laser clear corneal mini-incision could add valuable information to the improvement of corneal tunnel design for femtosecond laser-assisted cataract surgery.

AUTHOR CONTRIBUTIONS

Study concept and design (SS, PD, ML); data collection (MR); analysis and interpretation of data (SS, GL, ML); drafting of the manuscript (SS, GL, MR, ML); critical revision of the manuscript (SS, GL, PD); statistical expertise (ML); obtained funding (SS, ML); administrative, technical, or material support (GL, PD, MR); supervision (PD)

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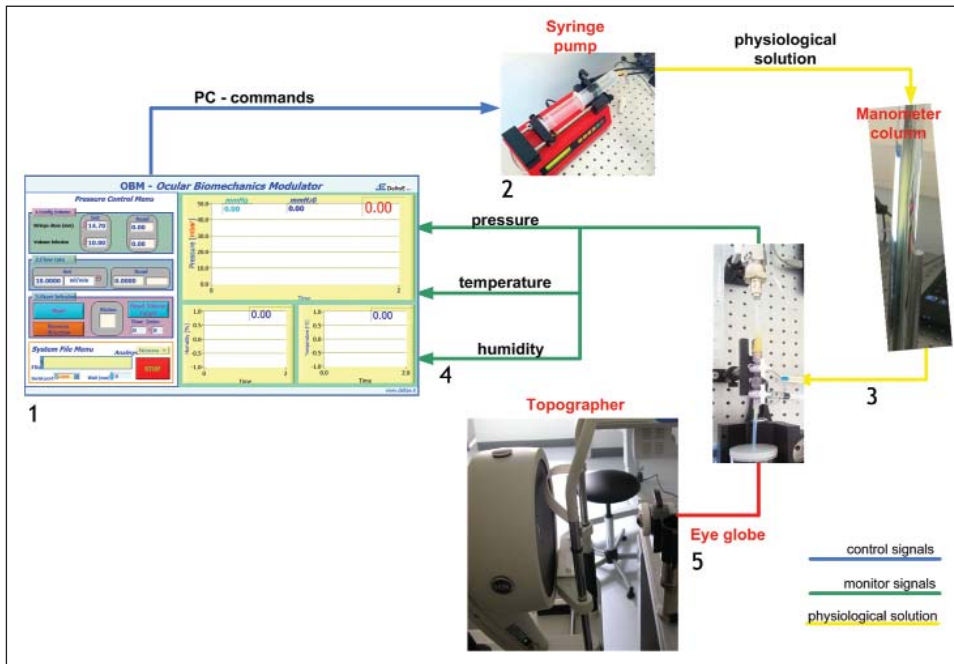


Figure A. Schematic set-up of the Ocular Biomechanics Modulator (OBM). The OBM was designed and developed to perform inflation testing on human eye globes with the intent to evaluate the topographic response of the cornea to femtosecond laser corneal incisions, mimicking an in vivo situation as much as possible. (1) A computer data acquisition system was developed and controlled by a LabView (National Instruments Corporation, Austin, TX) algorithm to control a (2) pumping and (3) pressure system, while continuously (4) monitoring the environmental variables. Within the OBM, the intraocular pressure was monitored by means of a water column and a pressure transducer and modified by infusing saline solution into (5) the posterior segment of the eye through a needle by an automated pumping system.

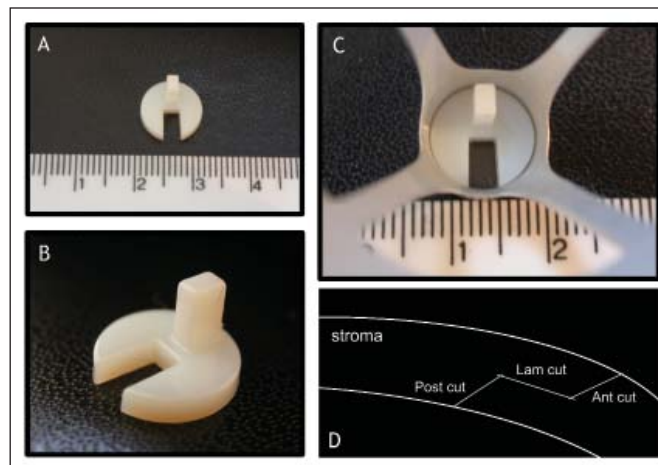


Figure B. (A and B) A purpose-designed mask was developed to create a 2.75-mm three-plane clear corneal incision using the Intralase iFS laser platform. Currently, the Intralase iFS (Abbott Medical Optics, Inc., Santa Ana, CA) does not permit the design of lamellar (Lam cut) and posterior (Post cut) dissection with adjustable angles. The custom mask was gently placed, using forceps, over the contact lens interface of the laser (C) immediately after applanation of the eye globe. (D) A scheme of the three-plane clear corneal incision is shown: the anterior and posterior cuts intersect the lamellar cut, positioned at 50% stromal depth 30 μm from its anterior and posterior edges, respectively.