·Clinical Research·

Confocal microscopic evaluation of cornea after AquaLase liquefaction cataract extraction

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Abstract

• AIM: The most recent and innovative AquaLase liquefaction technology has offered an alternative to lens extraction. Many studies have investigated its functions and advantages. This article focuses on evaluating the *in vivo* microscopic cornea changes after AquaLase liquefaction by using a laser confocal microscope.

• METHODS: In this perspective, randomized case study, 37 eyes of 35 patients submitted to cataract surgery were chosen to undergo AquaLase liquefaction cataract extraction. Each patient was assessed before the operation, on the 1st, 7th, and 30th postoperative days, and 6 months after the cataract extraction. The morphologies and quantitative comparisons of corneal cells and corneal nerves layer by layer were evaluated in vivo with the Heidelberg Retina Tomograph III-Rostock Cornea Module (HRT-III RCM) confocal microscope. ANOVA and Post-Hoc Bonferroni test were carried out to compare the results pre- and post-operation.

• RESULTS: ANOVA results indicated no post-operation changes for epithelium and anterior stroma cells. Irregular segments of sub-basal nerve fiber were most pronounced seven days post-operation. In the mid and posterior stroma, keratocytes were obvious compared with the preoperative condition. Corneal endothelium cells became obviously swollen in cytoplasm and nucleus. The mid and posterior stroma cell density decreased from the 1st to 7th postoperative days (P <0.05). The corneal endothelium cell density decreased (P<0.05) and did not revert to the preoperative level after six months (P<0.05).

• CONCLUSION: Slight microstructural abnormalities were identified in the corneal recovery process after AquaLase liquefaction. AquaLase liquefaction cataract extraction is safe for cornea.

• KEYWORDS:aquaLase liquefaction;cataract;cornea changes

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INTRODUCTION

With the improvement of ultrasonic phacoemulsification cataract surgery, smaller incisions and shorter intraocular operations have virtually eliminated astigmatism and inflammation as factors impairing early visual restoration [1-2]. Transient or permanent corneal edema, however, has become a major component delaying visual recovery in the early postoperative period [3]. Ultrasonic energy is one of the relatively amenable factors contributing to corneal edema. AquaLase liquefaction device is a new modality available for lens extraction on the Infiniti Vision System (Alcon Laboratories, Fort Worth, Texas). It is a non-thermal, fluid-based cataract extraction system that does not employ ultrasound energy. It generates and heats micropulses of 4μL bolus of balanced salt solution (BSS) to approximately 57℃ . The micropulses are propelled within the AquaLase handpiece to liquefy the lens [4,5]. There is virtually no possibility of an incision burn from AquaLase energy [4]. In terms of postoperative complications in the cornea, many studies focused on endothelial cell changes, since they are critical to corneal decompensation [6-8]. A specular microscope has enabled studies of endothelial cell counts to be conducted on the cornea *in vivo*, but until recently, studies of other layers and innervations have been limited to animal tests. In consideration of safety and efficiency of the device, the analysis of the cornea layer by layer after surgery has become more significant. The Heidelberg Retina Tomograph III system (HRT III; Heidelberg Engineering GmbH Heidelberg, Germany) is the latest generation of such a confocal microscope equipped with a corneal module [9]. It provides a non-invasive method

of examining the living human cornea in a healthy and pathological state, making it a powerful clinical and research tool. With this technique, it is possible to assess the cornea under more physiological conditions than before. In the present study, we focus on the in vivo central cornea microscopic changes after AquaLase liquefaction cataract extraction and observe the dynamic recovery process of cornea layer by layer.

MATERIALS AND METHODS

Subjects Inclusion criteria consisted of a dilated pupil of 7.0mm or larger, a corneal endothelial cell count greater than 2000 cells/mm^2 and lens nucleus density grade Ⅱ-III, according to the Lens Opacities Classification System III (LOCS III). Exclusion criteria were previous intraocular inflammation, or other ocular and physical illness that could affect corneal recovery, including corneal diseases, glaucoma, previous intraocular surgery history and systematic diseases such as diabetes.

Veteran surgeon (JS Zhang), who had conducted more than 250 cases using the AquaLase device prior to this study, performed all of the surgeries. HRT III was utilized to observe the in vivo corneal changes preoperation and on the 1st, 7th, and 30th days and the 6th month preoperation. The AquaLase liquefaction system was used on the Infiniti Vision System (Alcon, USA). Proparacaine hydrochloride 5g/L eye drops (Alcaine, Alcon, USA) were used as topical anesthesia before the surgery. A 3.0mm self-sealing clear corneal incision was made on the nasal or temporal side. Sodium hyaluronate 3.0% -chondroitin sulfate 4.0% (Viscoat, Alcon, USA) was used to reform and stabilize the surgical planes and protect the corneal endothelium. A 5.5-6.0mm continuous curvilinear capsulorhexis was performed. After hydrodissection, a standard pre-chop technique was used with AquaLase liquefaction. Acrysof SN60AT or SA60AT IOL (Alcon, USA) was injected into the capsule. TobraDex eye drops were prescribed three times daily starting on the first postoperative day. All patients experienced no intraoperative or postoperative complications, as assessed by repeated slit lamp examinations. A total of 37 eyes of 35 cataract patients (18 men, 17 women) who underwent age-related cataract extraction were systematically examined from September 2006 to March 2008. The mean age of the patients was 56.7±8.37 years (ranging from 49 to 68 years). There were 4 eyes with grade I cataract, 24 eyes with grade II cataract and 9 eyes with grade III cataract. The median AquaLase time was 3.3 (ranging from 1.3 to 4.3) seconds. The number of pulses ranged from 213 (very soft lens) to 4530 (grade 3). The median number of pulses was 2325. Fixed flow and vacuum were used in all cases, with a dynamic rise of 2. The mean peak vacuum was 517 ±37 (ranging from 328 to 586) mmHg.

Methods A randomized study was conducted at the Eye Institute of China Medical University, according to the tenets of the Declaration of Helsinki. The research ethics committee approved the study design, and written consents were obtained from all patients. Standard preoperative examinations were performed after patients' enrollment. Before examination, one drop of a topical anesthetic, proparacaine hydrochloride 5g/L, and one drop of a gel tear substitute, carbomer 2g/L (Bausch & Lomb, US), were instilled in the lower conjunctiva fornix. The examination was performed in the sagittal axis, so that as the operation proceeded, each layer of cornea was successively examined. For all eyes, several confocal microscopic images were taken at each corneal layer in order to have at least 3 images in one calculative layer. The results of corneal morphology were compared after surgery in all layers of central cornea. All HRT III images were analyzed in a masked manner by an examiner. Comparisons were made of the qualitative condition of the following aspects: corneal epithelium, sub-basal corneal nerves, and nerve fiber bundles in the corneal stroma, Bowman's layer condition, corneal stroma, and corneal endothelium. Quantitative comparisons were made of the following: wing and basal epithelium cell densities, Langerhans cell density, stroma keratocyte densities, and corneal endothelium cell density.

Statistical Analysis A power calculation was performed for the sample size. The calculation showed that a sample size of 30 would have a 99% power to detect a difference with a significance level of 0.05 (2 tailed). The normal distribution of the variables was determined using the Kolmogorov-Smirnov test. ANOVA was performed together with a post-hoc Bonferroni test. $P<0.05$ was considered statistically significant.

RESULTS

Corneal Epithelium Central corneal epithelium cells appear as polygonal cells of various sizes forming a regular mosaic with dark cell bodies and bright cell borders. Some cell nuclei indicated a bright dot in the body of cells. In four cases (10.8%), the border of wing cells blurred by the first day of postoperative examination with two or three cells which diminished within one day. The detailed changes of basal epithelium cells were difficult to distinguish. There were no statistically significant mean cell density changes at any time postoperatively for epithelium cell (Table 1). Preoperatively, endothelial cells appear on the image as a regular array of predominantly hexagonal cells that exhibit bright cell bodies and dark cell borders. The cell's nucleus was not obvious. Postoperatively, corneal endothelium cells lose their normal hexagonal appearance by becoming irregularly shaped and become obviously swollen in both

Table 1 Cell densities after AquaLase liquefaction Mean±SD

	Pre-operation	Day 1	Day 7	Day 30	Month 6
Superficial epithelium	1225±161	1197±101	1203±95	1238±100	1216±113
Wing cell	5334±218	5286±195	5288±255	5278±237	5393±259
Basal epithelium	11423±802	11268±937	11289±985	11538±899	11417±1005
Anterior stroma	536±22	528±20	529±26	533±31	542±29
Mid stroma	242±25	224±19[a]	227±23[a]	237±24	243±32
Posterior stroma	221±27	187±21[a]	205±31[a]	218±19	217±21
Endothelium cell	2664±162	2413±175[a]	2487±149[a]	2563±193[a]	2513±145[a]

[a]P<0.05 *vs* properation

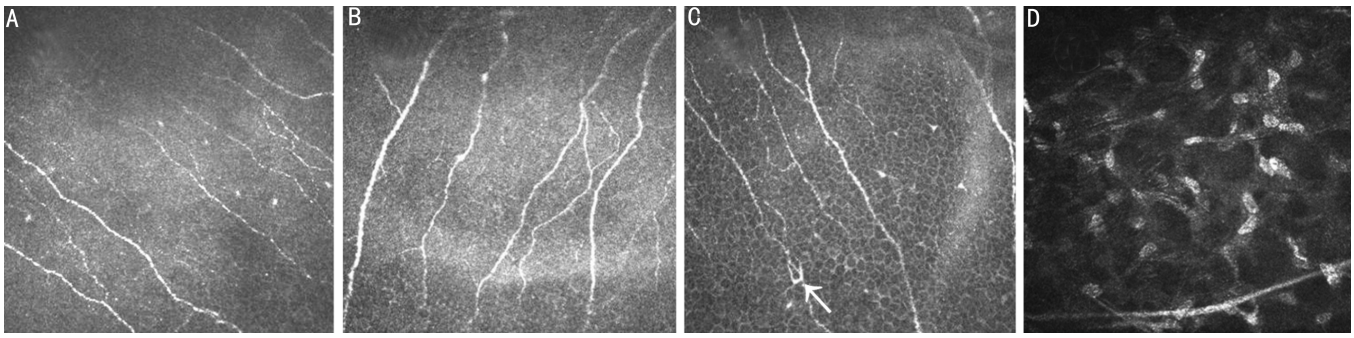

Figure 1 Normal central corneal morphology of preoperation (380μm×380μm) A: Sub-basal corneal nerves; B: Sub-basal corneal nerve fiber; C: Day 7 post-operation; D: Langerhans cells

the cell's body and nucleus. The cell nucleus was visible. These conditions were severe enough that they did not recover completely at one month postoperatively. Morphology improved by the one month postoperative exam, but the effects on the cell nucleus were still identifiable. Compared with the preoperative condition, cell density change had a statistically significant difference at all postoperative exams (P<0.05). Cell density decreased most dramatically at the one-day and seven days postoperative exams. At six months, cell morphology recovered, but cell density did not increase to preoperative levels (P <0.05).

Corneal Nerves Sub-basal corneal nerves are located between Bowman's layer and the basal epithelium. Preoperatively, sub-basal nerves appeared as well-defined linear structures with homogeneous reflectivity. Dichotomous branches (Y shape) and thinner connecting nerve fiber bundles (H shape) are observed (Figure 1A). We observed some irregular segments of nerve fiber, most pronounced seven days post-operation (Figure 1B). The nerve fibers became thicker and curvier, with non-homogeneous reflectivity in some areas. Some nerve fibers looked discontinuous, and the frequency of abnormal branches increased. Conditions did not appear to improve by the 30 day post-operative examination, but recovered at 6 months. With the current confocal microscopy imaging technique, nerve fiber bundles in the corneal stroma appeared as thick, highly reflective, straight structures of various orientations preoperatively (Figure 1D). There was no obvious change after AquaLase liquefaction was observed.

Bowman's Layer and Langerhans Cell In the normal cornea, Bowman's layer appears as an amorphous homogenous layer. A study by Patel *et al* [9] revealed the presence of cells at the level of Bowman's layer called the Langerhans cell. In the images taken for this study, the Langerhans cells appeared as bright corpuscular particles and individual cell bodies with or without processes, or cells bearing dendrites (Figure 1C). The cell morphology and cell densities did not change at any time during the study.

Corneal Stroma Keratocytes in the anterior stroma (Figure 2A) are imaged in this study as clearly demarcated, highly reflective, oval keratocyte nuclei with varying orientation both pre- and postoperatively. Cell bodies and stromal collagen are not usually visible in the normal cornea with the HRT III. Morphology of the anterior stroma keratocyte did not show obvious modification during the entire postoperative examination. Cell density decreased at the first day post-operation, but was not statistically significant (P>0.05) (Table 1). In the mid and posterior stroma (Figure 2B), keratocytes have a regular oval or swollen shape with less cell density compared with the anterior stroma layer. Keratocyte density is lowest in the posterior stroma. Postoperatively, the cell nucleus morphology did not change noticeably, but was more obvious compared with the preoperative condition, especially in the posterior stroma.

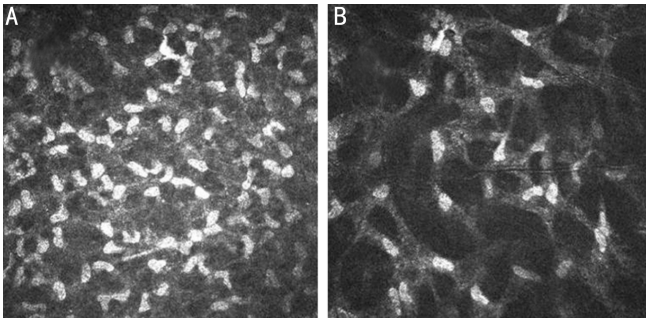

Figure 2 Normal corneal stroma preoperation (380μm × 380μm) A: Anterior; B: Mid

Cell density of mid- and posterior stroma were decreased compared with the preoperative condition (P<0.05), and recovered at thirty days post-operation (Table 1).

DISCUSSION

Although safety and efficiency profiles of modern ultrasonic phacoemulsification cataract surgery have improved dramatically compared to the traditional extra capsular approach, some postoperative complications impairing visual recovery such as cystoids macular edema, posterior lens capsule rupture, cornea wound burn, corneal edema, and posterior capsule opacification are still unavoidable[11-13]. Ultrasound phacoemulsification uses a sharp tip, and methods of energy application were mechanical cutting and a cave effect. Therefore, it is hard to avoid cornea damage because of ultrasound energy, and mechanical cutting by a sharp tip was the one of the reasons for posterior lens capsule rupture [14]. AquaLase is a new surgical method of cataract removal which operates by the utilization of water force with a soft silicone tip. It is a non-thermal, fluid-based cataract extraction system that does not employ ultrasound energy [5,14]. In comparison with ultrasonic phacoemulsification, which has been widely used, AquaLase has some proposed advantages, including reduced posterior capsule rupture risk with a soft silicone tip and thermal injury to the corneal endothelium and the corneal incision *et al.* AquaLase is excellent for polishing the capsule and removing lens epithelial cells, and is good for reducing PCO risk [14-16]. Therefore, it is especially well suited to refractive lens procedures and pediatric cataracts.

Considering safety and efficiency, some studies have already been performed for AquaLase liquefaction. They showed AquaLase cataract extraction to be safe for the endothelium, even in older patients with harder cataracts and a lower ECC preoperatively. Nada showed that the differences in postoperative changes in ECC and pachymetry between the AquaLase and NeoSoniX were not statistically significant in patients younger than age eighty. But for patients aged eighty years or older, AquaLase had better results than phacoemulsification [14]. Up to now, research addressing postoperative corneal complications is mainly focused on corneal endothelium cell loss and corneal thickness by using technologies such as a corneal pachymetric, non-contact specular endothelium microscope or other methods. Solid examination methods are limited with regard to observing the change of each layer of cornea during the recovery process after AquaLase *in vivo*. *In vivo*, non-invasive laser confocal microscope has recently become available for laboratory and clinical use. It has permitted more detailed, layer-by-layer observations of corneal microstructures. It provides high-resolution images, which allow observation of the corneal microscopic changes and aspects of the corneal interface. Cell density counting software can provide the cell density layer by layer.

We have been using the Infiniti Vision System since July 2005. In the present study, AquaLase was a safe and efficient method of cataract removal. We found that AquaLase liquefaction is performed more efficiently with prechopping of the nucleus. There were minimal loss of endothelial cells and fewer changes in the corneal stroma and corneal nerve fibers after surgery. Corneal nerves appeared discontinuous and less linear than in their preoperative condition, but these recovered starting from 30 days of post-operation. This was consistent with the other results that surgical manipulation induced neurogenic inflammation at the cellular level within minutes after the start of surgery. The changes of cornea nerve fiber might affect cornea sensation and ocular surface stability at the early stage of post-operation. Langerhans cell's morphology and densities did not change at any time during the study. Studies indicated that HLA-DR antigens occur on Langerhans cells in the normal conjunctiva and cornea. Langerhans cell may function as antigen presenting cells and stimulating cells to allogenic lymphocytes. Thus, there was no immuno-activity involved in the post-operation recovery process.

Corneal stroma keratocyte swelled and cell density decreased after surgery. This was most pronounced in the posterior stroma, especially at the first week postoperatively. Statistically significantly postoperative stroma keratocyte changes occurred only in a short time after AquaLase cataract removal. The reason we believe was cornea edema after surgery induced increased cornea thickness and decreased cell density. Research had already proved that postoperative corneal swelling correlates strongly to corneal endothelial cell loss after phacoemulsification cataract surgery [8]. Corneal endothelium cells swelled in both the cytoplasm and nucleus postoperatively. Endothelium cell density decreased soon after surgery and increased slowly

beginning from 7 days postoperatively. By the sixth month, corneal endothelium cell morphology recovered but cell density still had statistical significance compared with the pre-operation level. Corneal endothelium cell loss ratio of ultrasound phacoemulsification was 4-25% [17], but for AquaLase in this research, the cell loss ratio was only less than 6%. The results suggest AquaLase is "friendly" to the corneal stroma and corneal endothelium cell.

Operative factors associated with endothelial cell loss during AquaLase liquefaction include age, high nucleus grade, direct mechanical trauma by lens fragments or instruments, increased infusion and the effects of the irrigating solution and time used for AquaLase liquefaction [18,19]. Ophthalmic viscosurgical devices play an important role by protecting endothelial cells and maintaining space in the anterior chamber or capsular bag. Limitations of this study include a relatively small sample size. However, the repeatability and accuracy of HRT-III in the determination of cornea morphology and cell density suggest that in our initial power calculation, a large sample size was not required. Another limitation of the study is the relatively short follow-up period. And the software of HRT-III was not good enough for calculating the nerve fiber density changes. Further randomized controlled studies with greater numbers of patients that compare AquaLase liquefaction with ultrasound phacoemulsification or other kinds of emulsification methods particularly at risk for all layer corneal damage postoperatively are necessary. In conclusion, AquaLase is a promising new technology. Based on the results of our research, we consider AquaLase to be safe for the cornea, although slight microstructural abnormalities were identified in the cornea recovery process via confocal microscopy. However, considering this is a small series study, a larger series study in the future would be beneficial.

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