Real-Time Optical Coherence Tomography-Guided Femtosecond Laser Sub-Bowman Keratomileusis on Human Donor Eyes

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• PURPOSE: **To determine the usefulness of optical coherence tomography (OCT) as a tool in guiding the femtosecond (fs) laser in the creation of a sub-Bowman keratomileusis (SBK) flap in human eyes.**

• DESIGN: **A nonrandomized case series.**

• METHODS: **In a private research laboratory setting, we performed an in vitro investigation on human autopsy eyes. Five human cadaver eyes, unsuitable for transplantation, underwent flap creation with a fs laser. The laser procedure was controlled in real-time with an OCT system (Thorlabs HL AG, Luebeck, Germany) to ensure that the cut was placed just underneath the Bowman layer. The fs laser worked at a repetition rate of 10 MHz with a single-pulse duration of < 400 fs (pulse energy in the nanoJoule range). As a control, all eyes underwent histologic dissection (toluidine blue) and were examined using light microscopy (LM).**

• RESULTS: **Video monitoring of the flap creation supported the feasibility of real-time OCT monitoring of the fs laser flap creation process. A clear distinction of the corneal epithelium was possible in all eyes. The Bowman membrane was not identified in all donor eyes at the given resolution of the OCT used in this study. Still, LM examination confirmed that the real-time monitoring assured a positioning of the cutting plane at minimum distance underneath the Bowman layer.**

• CONCLUSION: **This small laboratory test offers evidence that real-time OCT monitoring of creation of a SBK flap using a fs laser is possible, thus ensuring that the flap is created at the proper depth. (Am J Ophthalmol 2008; 146:42–45. © 2008 by Elsevier Inc. All rights reserved.)**

THE BIOMECHANICAL STABILITY OF THE CORNEA FOLlowing excimer laser ablation depends on the thickness of the residual corneal bed. Therefore, biomechanical stability is believed to be better after photorefractive keratectomy (PRK).[1] Laser in situ keratomileusis (LASIK) has the advantage of being less painful, faster in visual recovery, and associated with mild wound healing activity in comparison to PRK, since the corneal epithelium with its basal membrane is preserved during the operation and remains vital afterwards (Marshall J. Wound healing and biomechanics of corneal flap creation, presented at the European Society of Cataract and Refractive Surgeons Annual Meeting, London, United Kingdom, September 9 to 13, 2006, unpublished data). Marshall supported the concept of LASIK with a thin flap, possibly performed as a sub-Bowman keratomileusis (SBK), as proposed by Daniel S. Durrie and Stephen Slade (Durrie D. Prospective, randomized, contralateral study comparing femtosecond Sub-Bowman's Keratomileusis and PRK, presented at the Intralase User Meeting, Las Vegas, Nevada, November 9, 2006, unpublished data; Slade S. Clinical results of SBK vs surface ablation, presented at the Sixth International Congress on Advanced Surface Ablation and SBK, Cleveland Clinic, Fort Lauderdale, Florida, May 5, 2007, unpublished data).

In this experimental study on human donor eyes, an optical coherence tomography (OCT) system was combined with a femtosecond (fs) laser in one setup in an attempt to determine if real-time OCT could be used to control LASIK flap creation.

The experimental fs laser system used in this study delivers pulses in the nanoJoule (nJ) energy range to the eye and uses Mhz repetition rates. Based on the laser parameters, the nature of the cutting processes of this laser and the IntraLase FS laser (Advanced Medical Optics, Santa Ana, California, USA) are different. In a "high-pulse energy laser," such as the IntraLase, the cutting process is driven by mechanical forces that are applied by the expanding bubbles disrupting the tissue. This cutting process is very efficient because the radius of disrupted tissue is larger than the laser spot itself. Hence, the spot separation of the scanned laser pulses can be larger than the spot diameter. At its current energy setting, the IntraLase is unable to cut flaps thinner than 90 μm. This is because of the standard deviation of the device and manufacturing differences in the docking equipment through which the cornea is applanated and the laser is focused.

With the experimental laser used here, low pulse energies were used for the cutting process that is confined by the focal spot size of the laser pulse. Because of this, the laser can be set at a closer distance to the Bowman layer. The resulting gas bubbles are smaller and do not merge, which reduces the likelihood of a vertical breakthrough, even when the cutting plane is set below the Bowman layer. Another difference is

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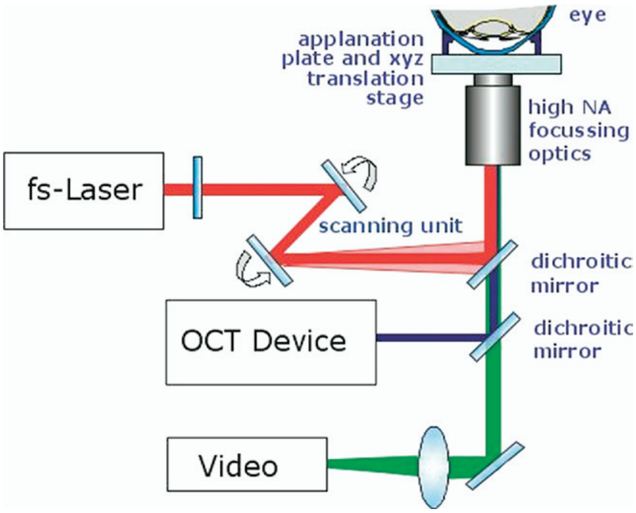

FIGURE 1. Experimental setup for real-time optical coherence tomography (OCT)-guided femtosecond (fs)-laser flap creation. The donor eye is mounted on the applanation plate (x-y-z translation). Once the corneal layers are identified by the OCT device the cutting plane for the fs laser is adjusted and the laser is scanned and focused with high numerical aperture. The cutting process can be controlled on the video monitor during the operation by switching the dichroitic mirrors.

that more pulses are needed to create a flap of similar depth and diameter. To keep the total operation time at the same level, higher pulse repetition rates are required. Both laser systems are similar with regard to operating time.

METHODS

IN A PRIVATE RESEARCH LABORATORY, AN IN VITRO INVESTIGATION on human autopsy eyes was performed. Five human donor eyes, unsuitable for transplantation, underwent fs laser LASIK flap creation. The autopsy eyes were stored in Kaufman solution and kept at a temperature of 4 C. After removal from the storage medium, the eyes were rinsed with balanced salt solution (BSS; Alcon Laboratories, Fort Worth, Texas, USA). All eyes were checked for defects at the slit-lamp to ensure that there were no corneal scars or pathology present. All eyes had some stromal and epithelial swelling present, but the details of the anterior chamber structures were visible.

The setup for experimental surgery is depicted in Figure 1. The eyes were fixated by placing them cornea-downward on the applanation window and then secured by a ring-like holder. Applanation was achieved by applying slight downward pressure on the posterior of the globe. Once the eye was fixed on the applanation mask, it was ensured that the OCT (Thorlabs HL AG, Luebeck, Germany) and the video camera delivered an adequate image of the cornea. The OCT system operates at a wavelength of 930 nm and has a spatial resolution of 6.2 μm axial and 9.2 μm lateral. The acquisition time of the OCT system was 10 frames per second at maximum speed.

The fs laser used (T-Pulse 200; Amplitude Systems, Bordeaux, France) had a wavelength of 1030 nm and worked at a repetition rate of 10 MHz with a single pulse duration of 250 to 400 fs, an average power of 200 to 400 mW, and a single pulse energy in the range of 10 to 50 nJ at the sample.

All the beam paths, the fs laser, the OCT signal, and the video signal were aligned collinearly by dichroitic mirrors. Because of the narrowness in this experimental setup, the mirror for the fs laser and for the OCT device could not be used simultaneously in this configuration. As a result, it was necessary to switch the mirrors between the two devices. The switch between the two modes was done at preset intervals during the flap creation process, enabling real-time imaging of the laser-tissue interaction. In OCT mode, the interface of the computer control enables the surgeon to preset the laser-cutting plane at any desired position within the anterior stroma. For the purpose of this study, the fs laser cut was set underneath the interface of epithelium and stroma at a distance of 15 μm. With an epithelium thickness of approximately 40 μm, measured with the OCT system, and a supposed thickness of the Bowman membrane of 10 μm, the cutting depth was chosen to be 65 μm. Flaps were created using a raster pattern. It was necessary to adjust the depth of the flap only once at the beginning of the cutting process. No other adjustments were necessary.

After flap creation, the cutting plane could be identified based on the bubble layer. This induced bubble layer was less pronounced compared to bubble layers seen with commercially available fs lasers and dissipated within one to three minutes. Flap lift was done with a pinpoint, blunt spatula and then repositioned. The histologic sections were taken near the greatest diameter of the flap, perpendicular to the cutting plane. Although the fs laser pulses were applied at a very high repetition rate (10 Mhz), there were no signs of thermal or mechanical damage in the tissue layers adjacent to the cutting plane.

Once the flaps were cut, the eyes were fixated in 4.0% paraformaldehyde, 2.5% glutaraldehyde, 1.0% natriumcacodylate, pH 7.2 glutaraldehyde and sent to the Department of Pathology of the Institute of Veterinary Medicine, University of Hannover, Germany. An 0.1% concentration of toluidine blue was warmed up with the 4-micron tissue samples, and after 60 minutes staining time in the heat chamber, the tissue was rinsed and then dried on a heat plate at 70 C. Histopathologic examination was then performed with light microscopy (LM). The flap was opened in all eyes and repositioned to the bed prior to fixation.

RESULTS

THE VIDEO MONITORING OF THE LASER PROCESS SUPPORTED the feasibility of the concept of real-time monitoring via OCT. A clear distinction of the corneal epithelium was

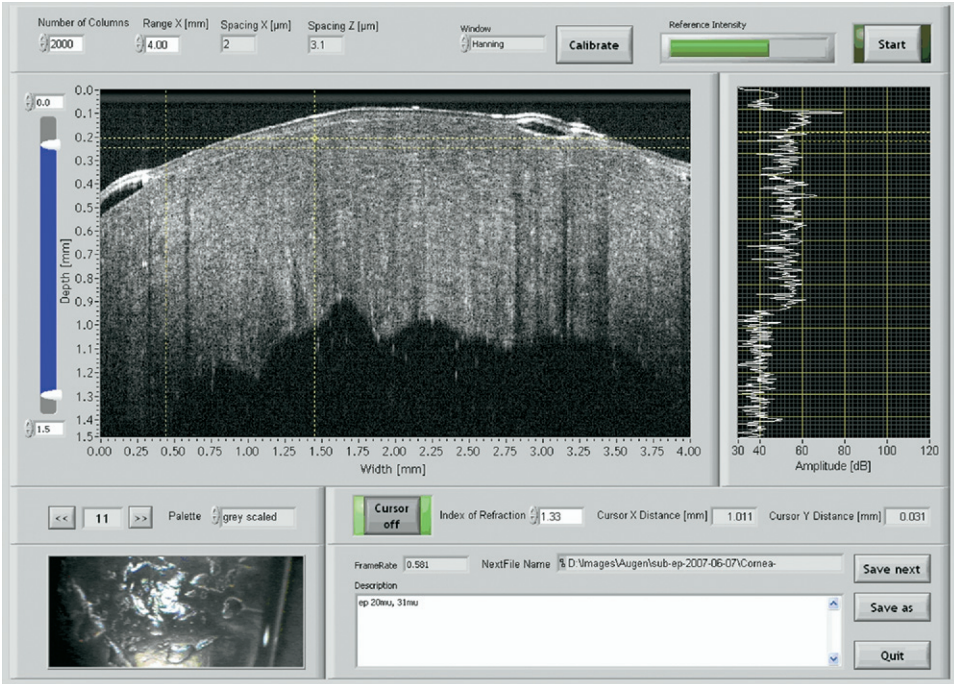

FIGURE 2. This is the control interface for monitoring the surgical procedure with both the OCT and the cornea with the real-time proceeding of the laser cut depicted.

possible in all eyes. In some eyes, the Bowman layer could not be clearly visualized because of the previously mentioned edema. However, we were able to clearly identify the transition between epithelium and the stroma (Bowman) layer, which provides sufficient evidence for the cutting plane.

Once the corneal layers were identified on the system's OCT image, the cutting plane could be adjusted. The cutting process could be monitored in real-time on the OCT image. This ensured that the preset flap thickness was maintained and that no perforation had occurred during the flap cut. The merging gas bubbles, induced by the fs laser interaction, were noted in all procedures. In no eyes did the gas bubbles break through the anterior corneal lamellae and Bowman layer. The gas underneath the flap led to a clear distinction of the stage of the surgical procedure and was also monitored in real-time during the surgical procedure (Figure 2).

Dissection and the lifting of the flap were possible in all eyes. All flaps were intact; no perforation occurred in any of the procedures. LM of the histologic sections (taken from a mid-periphery section of the flap) confirmed the position of the cutting plane at minimum distance beneath Bowman in the five eyes. Flap thickness was controlled on the histologic sections and confirmed the operative settings: 50 (± 5) microns (Figure 3). Setting the system at 50 µm leads to an actual cutting depth of 65 µm because of the refractive index of 1.3 of the cornea. This results in a focus shift of 15 µm. The cutting depth of 65 µm was measured both in the OCT images and in the histologic section.

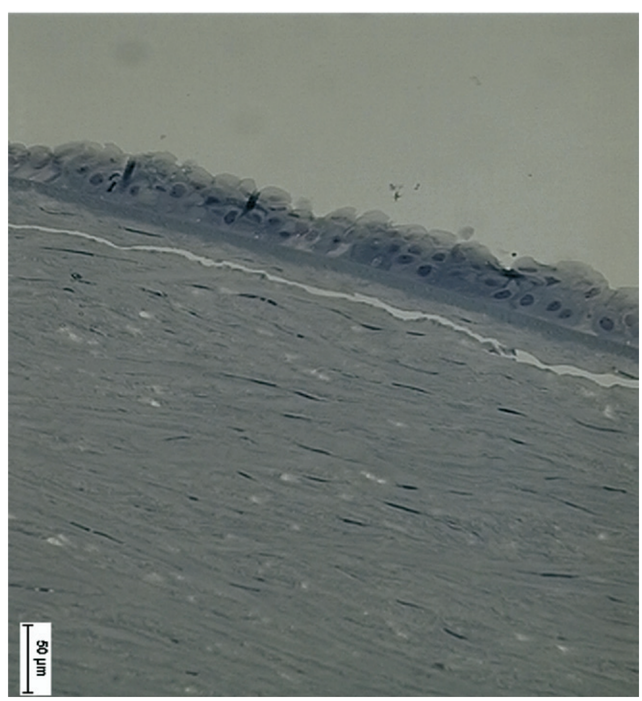

FIGURE 3. A histologic section (toluidine blue) of a human donor eye after creation of a sub-Bowman keratomileusis (SBK) flap using an OCT-guided fs laser. The fs laser cut is positioned to be 15 µm below the Bowman layer. Total flap thickness is approximately 65 µm.

DISCUSSION

COMMERCIALLY AVAILABLE FS LASERS, SUCH AS THE IntraLase FS laser (Advanced Medical Optics [AMO], Irvine, California, USA) have demonstrated that it is possible to cut corneal flaps with a higher degree of precision compared to mechanical microkeratomes.[2,3] With a standard deviation in the range of approximately ± 5 to 10 μm, the IntraLase enables the surgeon to safely and predictably cut flaps as thin as 90 to 100 μm[4] (Kermani O, Oberheide U, Gerten G. Intralase (60 kHz) femto-lasik with 90μm flap, presented at the 25th Congress of the ESCRS, Stockholm, Sweden, September 8 to 12, 2007, unpublished data). In fs laser application the interaction process is based on nonlinear absorption and consecutive disruption of the tissue. Nonlinear absorption means that the corneal tissue is transparent for the infrared laser radiation at moderate intensities where no absorption takes place. Only at very high intensities will multiple (low-energy) infrared photons act the same as one (high-energy) ultra-violet (UV) photon and be absorbed by the tissue. Because this happens only at the focal point of the laser beam, it gives the user the advantage of 3-dimensional tissue processing. The absorption process is not limited to the surface anymore.[5–7]

The precision of fs laser tissue interaction scales inversely with the applied pulse energy and pulse width.[8] In order to safely cut flaps even thinner than 100 μm, a fs laser would optimally work with laser pulses below 400-fs pulse width and with a single pulse energy in the nJ range.[9]

Previously published work has shown that the OCT can be used to visualize the corneal microstructure at high resolution in the micrometer range.[10] The OCT can be used for visualization of corneal pathologies.[11] The OCT can also be used to measure the flap thickness after LASIK.[12] To date, there have been no published studies into the use of the OCT in real-time control of a surgical procedure such as creation of a fs laser corneal flap.

This is a first proof-of-principle study on human cadaver eyes. Prior to the experiments on human eyes, two years of work was necessary to develop the experimental setup and extensive work was done on fresh porcine eyes. In this trial, we could measure the thickness of the epithelium layer but determination of the Bowman membrane thickness was difficult because of the axial resolution of 6 μm compared to the typical thickness of 10 to 20 μm for the Bowman membrane. Although one could add the typical thickness of Bowman to the epithelium layer when defining the cutting depth, a higher axial resolution of the OCT would be helpful to determine the Bowman layer more accurately. The spatial resolution of OCT measurements depends on the width of the spectrum of the light source, the numerical aperture of the focusing optics, and the measurement time. Recent laboratory-based OCT systems have a resolution of better than 3 μm and a data acquisition time of better than 100 Hz per frame.[13] This shows that, in principle, there is no technical limitation in order to have sufficient control of the laser cut with OCT technology.

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