Biomechanical Differences Between Femtosecond Lenticule Extraction (FLEx) and Small Incision Lenticule Extraction (SmILE) Tested by 2D-Extensometry in Ex Vivo Porcine Eyes

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Today, several sophisticated excimer laser systems are available for laser refractive surgery, with laser-assisted in situ keratectomelesis (LASIK) being the most popular procedure. In 2006, Sekundo and coworkers presented a procedure called femtosecond lenticule extraction (FLEx) with first results published in 2008. In FLEx, both the flap and the refractive lenticule are created in a one-cut procedure using a femtosecond laser. To date, 5-year results are available showing a remarkable stability of the achieved refractive outcome. However, apart from being a fast one-cut procedure with less healing response compared to femto-LASIK, FLEx did not offer any additional benefits compared to the widespread femto-LASIK since it also required the creation of a flap, hence weakening the anterior cornea. The next development stage of pure femtosecond laser corneal refractive surgery was the small incision lenticule extraction (SmILE) introduced by Sekundo in 2008 and published in 2011. Meanwhile, 5-year follow-up results of SmILE have been published. Unlike in FLEx, SmILE does not require a flap and the lenticule is extracted via a 2- to 3-mm incision leaving the remaining anterior stroma and Bowman’s layer untouched. Per manufacturer of the VisuMax laser, by the end of 2016 over 600,000 procedures (Muehlhoff D, unpublished observations, 2016) had been performed worldwide, with numbers steadily increasing. There is also a large body of peer-reviewed literature, reviews, and textbooks available. The reason for the wide use of SmILE is its minimally invasive approach that offers several advantages such as more postoperative comfort, less neurotrophic keratopathy, and a presumed better preservation of biomechanical stability, among others.

Corneal biomechanical properties are key elements in the development of disease states such as keratoconus and keratocataracts. Also, the success of corneal surgeries depends not only on biological, but also on biomechanical factors. Therefore, the better we understand the biomechanical response of corneal tissue, the more precisely we may predict

**Purpose.** To evaluate the biomechanical stability of ex vivo porcine corneas after femtosecond lenticule extraction (FLEx) and small incision lenticule extraction (SmILE) refractive surgeries.

**Methods.** Forty-five porcine eyes were equally divided into three groups: Groups 1 and 2 were treated with FLEX and SmILE procedure, respectively. Group 3 served as control. A refractive correction of −14 diopters (D) with a 7-mm zone using either a 160-μm flap (FLEx) or a 160-μm cap (SmILE) was performed. For two-dimensional (2D) elastic and viscoelastic biomechanical characterization, two testing cycles (preconditioning stress–strain curve from 1.27 to 12.5 N, stress-relaxation at 12.5 N during 120 seconds) were conducted. Young’s modulus and Prony constants were calculated.

**Results.** At 0.8% of strain, FLEx (370 ± 36 kPa) could resist a significantly lower stress than SmILE (392 ± 19 kPa, P = 0.046) and the control group (402 ± 30 kPa, P = 0.013). Also, FLEx (46.1 ± 4.5 MPa) had a significantly lower Young’s modulus than the control group (50.2 ± 3.4 MPa, P = 0.008). The Young’s modulus of SmILE (48.6 ± 2.5 MPa) had values situated between untreated corneas and FLEx-treated corneas. When compared to untreated controls, the stress resistance decreased by 8.0% with FLEx and 2.5% with SmILE; Young’s modulus decreased by 5.1% with FLEX and 1.04% with SmILE. With a cap-based procedure, both anterior cap and stromal bed carry the intraocular pressure, while in a flap-based procedure, only the stromal bed does.

**Conclusions.** Compared to flap-based procedures like FLEx, the cap-based technique SmILE can be considered superior in terms of biomechanical stability, when measured experimentally in ex vivo porcine corneas.

Keywords: SmILE, flap, cap, extensometry, biomechanic
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Methods

Specimens

Forty-five porcine eyes were collected from the local slaughter house in Marburg. All pigs were of similar age (7–9 months) and were slaughtered at once within 24 hours (not steamed) prior to the experiment, the eyes enucleated and kept in water at 6°C. They showed a good quality of epithelium. As we cannot exclude permanent changes induced by the biomechanical measurement, an untreated control group was used instead of performing pre- and postsurgical measurements, similar to previous studies in which the biomechanical stiffening of corneal cross-linking was analyzed.21,22

Refractive Procedure

In order to prevent an unequal degradation of the corneal tissue, all refractive manipulations were performed within one working day. Eyes were equally divided into three groups: Group 1 (n = 15) was treated with the FLEX procedure, group 2 (n = 15) with the SmILE procedure. Group 3 (n = 15) was left untreated (controls).

The globes were mounted into a custom-designed holder and the IOP was adjusted to approximately 20 mm Hg. For further standardization of the refractive procedure, and given that the epithelium hardly contributes to the mechanical corneal properties,23 the epithelium was scraped off from all corneas prior to the suction, ensuring that differences in epithelial transparency would not interfere with the laser beam. The M-size contact glass (treatment application pack) was used to apply the cornea by the VisuMax 500-kHz femtosecond laser (Carl Zeiss Meditec AG, Jena, Germany) with the following energy settings: 160 nJ and 4.5-µm track/spot distance. Because porcine corneas are substantially thicker than human corneas (on average by 110–150 µm, Faber et al.24), groups 1 and 2 received a refractive correction of −14 diopter (D) sphere with the 7-mm zone using either a 160-µm flap (FLEX) or a 160-µm cap (SmILE). The typical flap/cap thickness in humans, including the epithelium, is approximately 110 µm.25–28 We used a proportionally slightly higher flap/cap thickness in the ex vivo porcine eyes in order to compensate for postmortem corneal swelling. The minimal lenticule thickness was set to 15 µm. At the end of the FLEX procedure the flap was sealed using fibrin glue (Tissel 2 mL; Baxter, Deerfield, IL, USA) to imitate epithelialization. Sealing the flap had two functions: first, to imitate epithelialization, and second, to prevent the stroma from getting swollen in the time until the biomechanical measurements were performed. To our knowledge, this is the first experimental study about corneal biomechanics using this sealing technique. Directly after the surgical intervention, corneal buttons were excised and preserved in Stem Alpha.1 (Stem Alpha, Saint-Genis-l’Argentièr, France) until the biomechanical measurements were performed.

Biomechanical Characterization

For 2D biomechanical measurements, corneoscleral buttons were excised and mounted circumferentially within a customized holder7 on a commercial stress-strain extensometer/indenter (Z0.5; Zwick GmbH & Co., Ulm, Germany). The load was applied three-dimensionally on the posterior cornea by means of a 10-mm-diameter indenter, representing the IOP. This way, the indentation mode of the extensometer could be used to apply a tensile stress. A model assuming a homogeneous tissue was applied to quantify the resulting corneal deformation. The central displacement in vertical direction was measured as a function of stress and converted into strain. Strain \( \varepsilon \) is defined as the relative amount of material deformation (i.e., extension) resulting from the applied stress29 and was calculated with

\[
\varepsilon = \frac{\Delta l^2 + R^4}{2AR^2} \sin^{-1}\left(\frac{2AR}{\Delta l^2 + R^4}\right) - 1. 
\]
where \( F \) is the applied force, \( R \) is the radius of the central opening in the corneal holder, \( \text{th} = 700 \mu\text{m} \) is the corneal thickness, and \( \Delta \) is the vertical indentation. To quantify the overall decrease in the mechanical stress resistance, a constant corneal thickness was assumed. In consequence, the global deformation response of the corneal tissue is a measure of its stability. A more detailed description of how stress and strain were calculated can be found in our recent publication.\(^{17}\) Two testing cycles were performed in order to analyze the elastic and viscoelastic material properties: \( 2\times \) preconditioning—stress–strain curve from 1.27 to 12.5 N and stress–relaxation at 12.5 N during 120 seconds. The entire biomechanical measurement did not take longer than 2.5 minutes per corneal sample and hence did not cause relevant dehydration.

The Young’s modulus of elasticity (\( E \)) is a measure of the intrinsic stiffness of a material and was calculated by dividing corneal stress (\( \sigma \)) by strain (\( \epsilon \)). In our setup the bidirectional Young’s modulus was calculated with

\[
E_{\text{Young}} = \frac{\sigma}{\epsilon}.
\]

\( E_{\text{Young}} \) was determined at 0.8% of strain. This amount of strain still allowed full recovery of the induced deformation in pure collagen hydrogels\(^{30}\) and therefore did not involve plastic strain.

Prony constants describe the dynamic material properties and were calculated by decomposing the stress–relaxation curve \( \sigma(t) \) into short-term (\( E_1, E_2 \)) and long-term moduli (\( E_\infty \)).\(^{31}\)

\[
\frac{\sigma(t)}{\sigma_0} = E_\infty + \sum_{i=1}^{2} E_i \cdot e^{-t/t_i}
\]

where \( t \) represents time and \( t_i \) the time constants for the short-term moduli. The fitting procedure was performed with custom programs written in Matlab software (Matlab R2015a; Mathworks, Natick, MA, USA).

**RESULTS**

**Stress–Strain**

At 0.8% of strain (Fig. 1), FLEX (370 ± 36 kPa) could resist a significantly lower stress than SmILE (392 ± 19 kPa, \( P = 0.046 \)) and the control group (402 ± 30 kPa, \( P = 0.013 \)). Also, FLEX (46.1 ± 4.5 MPa) had a significantly lower Young’s modulus (Fig. 2) than the control group (50.2 ± 3.4 MPa, \( P = 0.008 \)). The Young’s modulus of SmILE (48.6 ± 2.5 MPa) had values situated between untreated corneas and FLEX-treated corneas, but the difference did not reach the level of statistical significance in comparison to FLEX (\( P = 0.065 \)) or controls (\( P = 0.159 \)). Compared to untreated controls, the stress resistance decreased by 8.0% with FLEX and 2.5% with SmILE; the Young’s modulus decreased by 5.1% with FLEX and 1.04% with SmILE.

**Stress–Relaxation**

The Table presents the short- and long-term moduli obtained from viscoelastic parameter fitting. \( P \) values were corrected with the Bonferroni method for multiple comparison. No statistically significant differences were observed.

**DISCUSSION**

There is a widespread presumption in the refractive community that cap-based refractive procedures such as SmILE
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Weaken the cornea less than flap-based procedures,\textsuperscript{9} because the creation of a flap severs both the Bowman's layer and the anterior, biomechanically stronger, lamellae of the human cornea.\textsuperscript{3,2} A recent finite element method (FEM) study\textsuperscript{9} also showed that the mechanical stress distribution after SmILE remains similar to the geometry analog control, while after LASIK the stress in the flap is reduced, and the stress in the residual stromal bed is increased, respectively. Hence, this suggests that after flap-based procedures such as FLEX, the flap does not contribute to support the IOP any longer and therefore, the thicker the flap and the higher the correction, the stronger the mechanical weakening. Also, according to the FEM simulations,\textsuperscript{9} after SmILE, the anterior part of the cap is still supporting the remaining cornea and able to take up mechanical stress. Therefore, we may assume that with cap-based surgery, the mechanical weakening depends solely on the thickness of the lenticule. Our results are in line with these assumptions, showing a stronger mechanical weakening after FLEX than SmILE, given that in FLEX the effective stromal thickness that provides mechanical resistance is reduced by 100-µm flap + 255-µm correction, in SmILE only by 255-µm correction.

In this regard, also no difference is to be expected between FLEX and femto-LASIK since in both procedures a flap is cut and the same amount of tissue (lenticule in FLEX and "ablation" in femto-LASIK) is removed. We chose to perform FLEX as a representative for a flap-based procedure simply due to practical reasons. To date, the presumption of different biomechanical behavior between cap- and flap-based techniques is not sufficiently backed up by experimental evidence in the literature.

Reinstein et al.\textsuperscript{8} calculated the remaining tensile strength of the postoperative human cornea using a mathematical model. They estimated 54% remaining tensile strength after LASIK as compared with 75% after SmILE assuming a 110-µm flap and a 130-µm cap and 110 µm of stromal tissue removal. A recent ex vivo study in human eyes (Gapsis BC, et al. IOVS 2016;57;ARVO E-Abstract 2395) reported a similar corneal strength reduction after SmILE and LASIK with high refractive corrections (\textasciitilde 8 D). However, it is important to note that the refractive correction was performed over an optical zone of 6.5 mm, while the mechanical test was performed only in the central 3.5 \times 3.5 mm area. This implies that flap and cap were clamped to the stromal bed, which is not comparable to the condition in the patient. In this case no mechanical difference can be expected. Also, in vivo measurements of corneal biomechanics after SmILE and LASIK surgery using air-puff deformation systems are inconclusive\textsuperscript{11-13,51,55} potentially because they do not measure long-term deformation, which is essential for maintaining the corneal shape over time. In our experimental setting we applied the load gradually from the posterior surface and then measured its relaxation behavior under constant load. During the mechanical characterization, a spherical indenter applies the test load in a similar way onto the endothelium as the IOP acts in vivo, allowing for a more natural stress distribution compared to air-puff deformation measurements. Moreover, it brings the advantage that stress–strain curves of the corneal tissue can be directly recorded and separated from other ocular tissues such as the sclera. We could show that the flap-based refractive procedure FLEX, in contrast to the cap-based procedure SmILE, induced a significant corneal weakening when compared to controls. With FLEX, the Young's modulus decreased by 5.1%, with SmILE only by 1.0%. As expected, the Young's modulus of SmILE showed values situated between untreated corneas and FLEX-treated corneas, but the difference did not reach the level of statistical significance. This may be due to the fact that the differences were not large enough for the number of eyes treated (the observed statistical power was 75.4% for the stress comparison and 79.8% for the modulus of elasticity). Also, probably the treatment range of –14 D was not large enough to show the differences in (swollen) ex vivo porcine corneas. In a preliminary test, we evaluated a treatment range of –10 D, in which 10 porcine eyes were treated with FLEX and 10 with SmILE and compared to 10 untreated corneas. Although some differences in corneal biomechanics were noticeable between the groups, no statistical significance was reached. We attribute the lack of significant difference to the sensitivity of the stress–strain measurements, rather than to the fact that there is no difference between SmILE and FLEX for smaller refractive corrections and therefore have increased the refractive correction to –14 D for the current study.

A limitation of this study is that although porcine corneas show a tensile strength and stress–strain relation similar to human corneas, their stress–relaxation behavior is significantly different.\textsuperscript{34} Hence, porcine corneas may not appropriately represent potential viscoelastic modifications in human corneas post refractive surgery and the results from this study cannot be directly extrapolated to the behavior of a living human cornea. Another limitation was that we did not measure and account for differences in preoperative corneal pachymetry.

We decided to use the same cap and flap thicknesses in order to make the results comparable. However, as shown by Reinstein et al.\textsuperscript{8} thicker corneal caps for SmILE result in a higher ablation and account for differences in preoperative corneal pachymetry (e.g., Brillouin microscopy) become widely available, prospective noninferiority clinical studies matched by refraction, treatment zone, age, and sex may be envisaged.

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References


