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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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

Today, several sophisticated excimer laser systems are available for laser refractive surgery, with laser-assisted in situ keratomileusis (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.[1] 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.[2] However, apart from being a fast one-cut procedure with less healing response compared to femto-LASIK,[3] 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)[4] introduced by Sekundo in 2008 and published in 2011.[5] Meanwhile, 5-year follow-up results of SmILE have been published.[2] 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 (Muehlfoff D, unpublished observations, 2016) had been performed worldwide, with numbers steadily increasing. There is also a large body of peer-reviewed literature, reviews,[6] and textbooks[7] 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 keratectasia. 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

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surgical outcomes and manage postoperative complications. As SmILE supposedly is superior in preserving corneal integrity when compared to flap-based procedures like FLEx or LASIK, it is reasonable to assume that SmILE may also show more biomechanical stability. Reinstein et al.[8] further elaborated this hypothesis with a mathematical model estimating the relative differences in postoperative stromal tensile strength following PRK (photorefractive keratectomy), LASIK, and SmILE procedures. A numerical study by Roy et al.[9] suggests an increase in residual stromal stress after LASIK, but not after SmILE procedures. Only few experimental studies have analyzed differences in the in vivo corneal deformation response following an air puff,[10-12] but they did not find a significant difference between FLEx and SmILE procedures. It should be noted that the corneal deformation response following an air puff is very sensitive to changes in corneal thickness,[13] which might have masked the subtle differences between FLEx and SmILE in this setup. In addition, the strain during air-puff deformation is induced very rapidly; this makes it impossible to retrieve long-term viscoelastic properties, which are particularly important for the stability after refractive surgery. Therefore, air-puff deformation may not be a good indicator for long-term resistance after refractive surgery.

Although several systems (including air-puff tonometers and Brillouin microscopy) have been developed to estimate the corneal biomechanical properties in vivo, the most accurate tests are destructive, in terms of measuring the actual corneal stress–strain relationship and hence the calculation of the Young's modulus, and can be performed only in ex vivo tissue. One-dimensional stress–strain testing is the gold standard in mechanical engineering. In ex vivo measurements of the corneal tissue it faces the problem that the stress distribution is not representative of the natural stress situation in the eye implied by the intraocular pressure (IOP). While inflation tests[14-16] overcome this problem, they cannot separate corneal from scleral deformation and hence are rather a measure for overall ocular biomechanics. Hammer et al.[17] have recently developed a setup for two-dimensional (2D) stress–strain testing to overcome this issue. A spherical indenter is used to apply the test load similar to the IOP, while simultaneously, the vertical displacement of the corneal sample is recorded. Given that the corneal button is circumferentially fixed at 10-mm diameter, the load applied by the indenter is translated into tensile and compressive stress. As the cornea is assumed to be nearly incompressible,[18] we did consider only tensile strain and not changes in corneal thickness. Although the corneal swelling pressure[19,20] might still induce minor thickness changes during the stress–relaxation test, its expected contribution to the vertical displacement was considered negligible for the extent of the load that was applied in this study. This setup allows elastic and viscoelastic soft tissue characterization and was applied in the current study to determine, to our knowledge for the first time, the experimental difference between FLEx and SmILE refractive procedures.

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 applanation pack) was used to applanate 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 mm.[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 (Tisseel 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'Argentière, France) until the biomechanical measurements were performed.

Biomechanical Characterization

For 2D biomechanical measurements, corneoscleral buttons were excised and mounted circumferentially within a customized holder[17] 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 ε is defined as the relative amount of material deformation (i.e., extension) resulting from the applied stress[29] and was calculated with

$$\varepsilon = \frac{\Delta^2 + R^2}{2\Delta R} \cdot \sin^{-1}\left(\frac{2\Delta R}{\Delta^2 + R^2}\right) - 1. \qquad (1)$$

Stress σ is defined as the externally applied force per cross-sectional area[29] and was calculated with
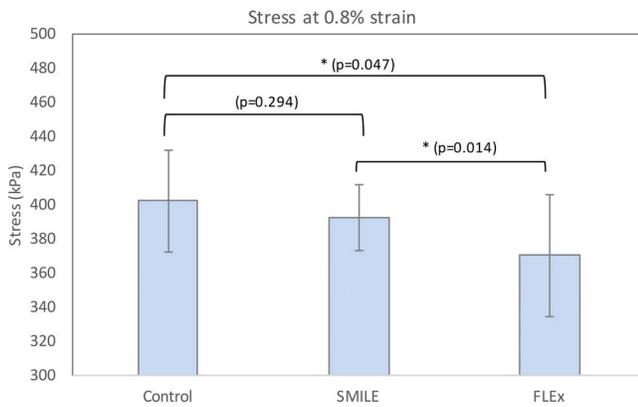
FIGURE 1. Corneal stress at 0.8% of strain. Significant differences were found between control and FLEx corneas as well as SmILE and FLEx corneas. *Error bars* indicate standard deviation.

$$\sigma = \frac{F}{2 \cdot \pi \cdot R \cdot \text{th}} \qquad (2)$$

where F is the applied force, R is the radius of the central opening in the corneal holder, th = 700 μm is the corneal thickness, and Δ 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× 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 (σ) by strain (ε). In our setup the bidirectional Young's modulus was calculated with

$$E_{\text{Young}} = \frac{\sigma}{\epsilon}. \qquad (3)$$

E_{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_∞).[31]

$$\frac{\sigma(t)}{\varepsilon_0} = E_\infty + \sum_{i=1}^{2} E_i \cdot e^{\frac{1}{\tau_i}} \qquad (4)$$

where t represents time and τ_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).

Statistical Analysis

The data showed a normal distribution. The student's t-test (Excel 2016 for Mac; Natick, MA, USA) was used to determine significant differences between treatment groups. Confidence intervals of 95% were set.
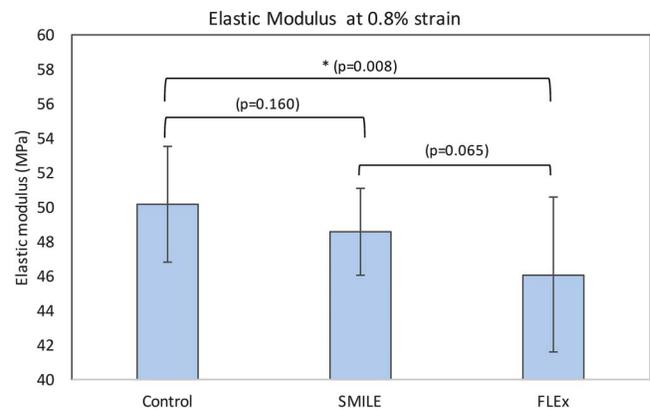
FIGURE 2. Young's moduli at 0.8% of strain obtained from stress–strain extensometry. Significant differences were found between control and FLEx corneas. *Error bars* indicate standard deviation.

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

TABLE. Viscoelastic Material Properties Obtained From Fitting a Two-Element Prony Series to the Stress Relaxation Curve

	E1	E2	E∞	E0
Control				
Modulus (MPa)	1.71	1.47	0.47	3.65
SD (MPa)	0.39	0.13	0.12	0.28
SmILE				
Modulus (MPa)	1.6	1.58	0.52	3.69
SD (MPa)	0.3	0.11	0.09	0.28
FLEx				
Modulus (MPa)	1.53	1.51	0.51	3.54
SD (MPa)	0.29	0.86	0.11	0.21

None of the parameters showed statistically significant differences between conditions.

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weaken the cornea less than flap-based procedures,[9] because the creation of a flap severs both the Bowman's layer and the anterior, biomechanically stronger, lamellae of the human cornea.[32] A recent finite element method (FEM) study[9] also could show 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,[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 + 235-μm correction, in SmILE only by 235-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.[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 (−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 3.5×3.5-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[11–13,17,33] 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 1.04%. 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.[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.,[8] thicker corneal caps for SmILE result in a better preservation of remaining tensile strength, while thinner flaps are preferred for LASIK or FLEx in order to less weaken the cornea. The effect of different cap and flap thicknesses may be evaluated in future experimental studies.

Clinically, SmILE cannot completely prevent corneal ectasia[35]; nevertheless, most of the cases reporting iatrogenic ectasia included patients at risk (forme fruste keratoconus).[36,37] It is important to point out that biomechanically suspect cases should not undergo any refractive laser surgery and that the potential biomechanical advantage a new procedure like SmILE might offer should not be used to enlarge the spectrum of corneas eligible to surgery by performing surgery on borderline cases. In such cases, rather an entirely different approach to refractive correction should be considered, such as phakic IOLs, refractive lens exchange, or no surgery at all. For eligible corneas, however, any procedure that offers a biomechanical advantage over current techniques is a step forward toward a safer overall procedure. Our results confirm that SmILE better preserves the corneal stress resistance. While directly after surgery the stress in the cap may be reduced due to the extraction of the lenticule, the cap can contract with time and contribute to resist the IOP.

In conclusion, we provide experimental evidence supporting the mathematical model approaches published previously[8,9] with post-SmILE corneas being more stress resistant as opposed to flap-based FLEx procedure in ex vivo porcine eyes This finding did not apply to the dynamic material properties, though. Once more sensitive in vivo techniques for the measurement of corneal biomechanics (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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