Refractive Correction and Biomechanical Strength Following SMILE With a 110- or 160-µm Cap Thickness, Evaluated Ex Vivo by Inflation Test

Iben Bach Damgaard, Anders Ivarsen, and Jesper Hjortdal
Department of Ophthalmology, Aarhus University Hospital, Aarhus, Denmark

PURPOSE. To examine the refractive correction and corneal biomechanical strength after small incision lenticule extraction (SMILE) by using a 110- or 160-µm cap thickness.

METHODS. Thirty-two human donor corneas were allocated into 4 groups, combining one of two cap thicknesses (110 and 160 µm) with one of two spherical corrections (−4 D and −8 D). Each cornea was mounted on an artificial anterior chamber. The chamber pressure was adjusted by an attached 8% dextran media column.

RESULTS. A 110-µm cap thickness caused more anterior flattening ($\Delta r_{15\mu}\cap, 1.02 \pm 0.08 \text{ mm versus } 0.60 \pm 0.17 \text{ mm}$), less posterior steepening ($\Delta r_{15\mu}\cap, -0.19 \pm 0.11 \text{ mm versus } -0.45 \pm 0.20 \text{ mm}$), and more myopic correction ($\Delta TCRP_{15\mu, apex,zone} -6.30 \pm 0.96 D$ versus $-4.55 \pm 1.66 D$) than a 160-µm cap thickness for $-8 \text{ D SMILE (} P < 0.034\text{)}$, but not for $-4 \text{ D SMILE (} \Delta TCRP_{15\mu, -4D, 110\mu}\cap, -3.86 \pm 1.31 D$ versus $\Delta TCRP_{15\mu, -4D,160\mu}\cap, -3.57 \pm 1.27 D$, $P = 0.718\text{)}$.

After SMILE, increased chamber pressure caused anterior steepening ($P < 0.014\text{)}$, which was similar at cap thicknesses of 110 and 160 µm ($\Delta r_{15\mu}\cap, -0.13 \pm 0.14 \text{ mm versus } -0.09 \pm 0.05\text{mm}$, $P = 0.431\text{)}$.

CONCLUSIONS. For high myopic corrections, a 160-µm cap caused less anterior curvature flattening and more posterior steepening than a 110-µm cap, and consequently less myopic correction. The inflation test revealed a reduction in the biomechanical strength after SMILE; this was similar when using a 110- or 160-µm cap thickness.

Keywords: small incision lenticule extraction, SMILE, cap thickness, inflation test, corneal biomechanical properties

Since its introduction in 2011, small incision lenticule extraction (SMILE) for myopia or myopic astigmatism has been commonly performed by refractive surgeons worldwide.1,2 With a femtosecond laser, an intrastromal lenticule is created and sequentially removed through a minor incision to flatten the anterior corneal surface.3 The intrastromal lenticule can be created in the desired corneal depth and leaves the anterior stromal layer (the cap) intact. Mathematical4 and computational modelling analyses5,6 suggest that SMILE may be better at preserving the biomechanical integrity than other flap-based laser refractive procedures such as laser-assisted in situ keratomileusis (LASIK) and femtosecond lenticule extraction (FLEX). In vivo biomechanical studies evaluating the corneal deformation response with noncontact tonometry have been inconsistent, and SMILE has been equal or superior to LASIK.7–15 Furthermore, iatrogenic ectasia has been reported after both LASIK16 and SMILE,17–20 which is characterized by corneal biomechanical weakening, severe protrusion, and decreased visual acuity.

Although a considerable amount of the literature has assessed the dependency of cap thickness on the postoperative biomechanical strength,4,12,22,23 the anterior one-third of the corneal stroma consists of an interwoven arrangement of collagen fibers, while the collagen fibers in the posterior two-thirds of the corneal stroma are arranged in distinct lamellae with a predominant vertical and horizontal arrangement.24 The collagen arrangement seems to be responsible for the depth-dependent exponential decrease in the corneal tensile strength, with the 40% anterior stroma being the strongest region, and the 60% posterior stroma being at least 50% weaker.25

In SMILE, a cap thickness of 110 to 130 µm is commonly used for myopic correction. Consequently, the stromal lenticule is usually removed in the stronger part of the cornea. The lamellar cut during cap creation may cause only a minor reduction in the corneal biomechanical strength,26 but the shape of the removed lenticule still requires that several collagen fibers are damaged during the procedure. Hence, removal of the lenticule in the deeper layers (thicker cap) may better preserve the corneal biomechanical strength than removal in the superficial layer. Furthermore, a thicker cap...
may also have other possible advantages, such as less disruption of the nerve integrity and better options for postoperative enhancements in the cap.\textsuperscript{27-29}

The biomechanical strength after lamellar and side-cut incisions has previously been examined ex vivo in human donor corneas.\textsuperscript{30} However, no studies have assessed the effect of lenticule removal in the deeper versus superficial corneal layers by ex vivo inflation. Lenticule removal in the deeper layers preserves the anterior stroma, but it also requires a deeper incision for lenticule extraction, which damages the corneal lamellae. Hence, the benefits of lenticule removal in the deeper corneal layers may counteract the disadvantage of a deeper incision in terms of the corneal biomechanical strength after SMILE.

This ex vivo study on human donor corneas aimed to examine the corneal biomechanical properties and refractive correction following SMILE using a 110- or a 160-µm cap thickness. An inflation test was performed to describe the corneal biomechanical strength after surgery by evaluating the anterior and posterior corneal curvature steepening during increased chamber pressure. Based on previous studies of the corneal tensile strength distribution,\textsuperscript{25,30} this study tested the following two hypotheses: (1) SMILE performed in the deeper corneal layers (160-µm cap thickness) better preserves the biomechanical strength than SMILE performed in the superficial corneal layers (110-µm cap thickness), and (2) SMILE performed in the deeper corneal layers causes less myopic correction than SMILE performed in the superficial corneal layers due to less flattening of the anterior corneal surface.

**METHODS**

A total of 32 human donor corneas were allocated into 4 groups by combining one of two cap thicknesses (110 µm and 160 µm) with one of two myopic spherical corrections (−4 D and −8 D). The donor corneas were deemed unsuitable for patient treatment due to low endothelial cell count. It was ensured that none of the donor corneas came from the same donor or had any scars, opacities, dystrophies, or severe arcus senilis. The tissue was received from the Veneto Eye Bank Foundation (Venice, Italy) in a storage medium (CorneaMax; Eurobio, Les Ulis, France), and transferred to a dehydrating organ culture medium with 8% dextran for a minimum of 24 hours. Each donor cornea was mounted on an artificial anterior chamber (AAC; Barron; Kadena Products, Inc., Denville, NJ, USA) containing transport medium. The epithelium was removed to the rim with a blunt spatula. The donor cornea was then placed upside down in organ culture medium with 8% dextran for 30 minutes to control the hydration before preoperative measurements. The humidity and room temperature were kept stable at 45% and 21°C during pre- and postoperative measurements.

The AAC with the mounted cornea was placed in a custom-made holder to ensure the same orientation during the tomographic measurements pre- and postoperatively. The chamber pressure was adjusted with a column containing transport medium attached to the infusion port and monitored with a pressure transducer (Compass compartment pressure; Centurion, Williamston, MI, USA). After adjusting the height of the column, the chamber pressure was kept stable for two minutes before measurements were performed.

SMILE was performed with the 500-kHz Visumax femtosecond laser (Carl Zeiss Meditec, Jena, Germany) as previously described.\textsuperscript{3} The chamber pressure was set to 23 mm Hg, and the contact glass was adjusted to the center of the AAC. The corneal curvature was set to 7.8 mm. The lenticule diameter was 6.5 mm with a minimum lenticule thickness of 30 µm. Hence, the maximum lenticule thickness calculated by the Visumax software was 93 µm for the −4 D groups, and 150 µm for the −8 D groups, respectively. The 7.3-mm-diameter cap was cut with a 2.55-mm incision positioned at 90°, with a cap thickness of either 110 µm or 160 µm. After laser application, the remaining tissue bridges were broken with a blunt spatula, and the lenticule grasped and removed through the incision with a pair of forceps.

The mounted corneas were stored in a moist chamber (100% humidity and 21°C) until the interface air bubbles had dissolved. The corneal tomography was measured before and after SMILE at a chamber pressure of 15 and 40 mm Hg (Pentacam; Oculus, Wetzlar, Germany). The main outcomes were the radii of the anterior and posterior corneal curvatures for the sagittal 3-mm-diameter zone (sag 3 mm) and the total corneal refractive power (TCRP$_{\text{inmm, apex, zone}}$).\textsuperscript{31} The average changes in the variables after operation (Δ = postoperative – preoperative values) and at increased chamber pressure (Δ = 40 mm Hg – 15 mm Hg values) were compared between groups. The central corneal thickness (CCT) and cap thickness after SMILE were evaluated by optical coherence tomography (OCT; Heidelberg Engineering GmbH, Heidelberg, Germany).

The CCT was used to monitor the corneal hydration during the study. Before SMILE, the first preoperative CCT measurement from the Pentacam HR was used as a reference value for hydration. After SMILE, the reference value was set to the preoperative CCT minus the calculated maximum lenticule thickness from the Visumax software. The CCT was kept stable by moisturizing the corneal surface with a semiwet sponge containing isotonic saline.

Statistical analyses were performed using Stata (v12.0; Stata Corporation, TX, USA), GraphPad Prism for MAC OS X (v6.0; GraphPad Software Inc., La Jolla, CA, USA), and G’power (Heinrich Heine University, Düsseldorf, Germany). Mixed model ANOVA was used to take into account both paired datasets (before-after SMILE and low-high chamber pressure), and unpaired datasets (refractive correction and cap thickness). Preoperative characteristics were compared with 1-way ANOVA and unpaired t-test. A P value less than 0.05 was considered statistically significant.

The study was evaluated by the Central Denmark Region on Health Research Ethics. No ethical approval was needed, as anonymous donor tissue was used for the study (request 185/2015). The next of kin of the donors gave written consent that the donor tissue could be used for research purposes.

**RESULTS**

Donor characteristics and thickness measurements are found in Table 1 and Figure 1. Donor age, time in dextran, and preoperative CCT measured after epithelial removal were similar between groups (P > 0.170). The postoperative cap thicknesses were comparable in both the 110-µm groups (−4 D, 119 ± 14 µm versus −8 D, 117 ± 15 µm; P = 0.804) and the 160-µm groups (−4 D, 155 ± 18 µm versus −8 D, 157 ± 4 µm; P = 0.675). There was no significant difference in postoperative CCT measured with the Pentacam HR and the Heidelberg OCT (P = 0.151). The average time the donor corneas were mounted in the chamber was 134 ± 38 minutes.

**Corneal Tomography**

The average changes in anterior and posterior curvature (Δr) at 15 and 40 mm Hg are shown in Table 2 and Figures 2A and 2B. For the −8 D groups, a 110-µm cap thickness caused significantly more anterior curvature flattening (Δr$_{\text{15 mm Hg}, \text{1.02}}$ ≥ 0.09 mm versus 0.60 ± 0.17 mm) and less posterior
### DISCUSSION

The present ex vivo study showed a tendency toward undercorrection after SMILE when using a cap thickness of 160 μm. The average percentage of undercorrection using a 160-μm cap was 11% and 4% for a −4 D correction and a −8 D correction, respectively. After SMILE, the inflation test revealed less corneal biomechanical strength for all combinations of cap thicknesses and refractive corrections when the chamber pressure was increased from 15 to 40 mm Hg. SMILE using a 110-μm cap thickness seemed to cause a more pronounced, though nonsignificant, impact on the biomechanical strength than SMILE using a 160-μm cap thickness.

The dependency of cap thickness on the achieved refractive correction has previously been examined in vivo in myopic SMILE-treated patients. Study 1 evaluated the refractive outcome using cap thicknesses of 130, 140, 150, or 160 μm in patients with a mean preoperative spherical equivalent of −4.89 ± 1.48 D. The authors reported a 12-month postoperative undercorrection of −0.10 ± 0.60 D (2%) and −0.17 ± 0.25 D (3.5%) at a cap thickness of 130 or 160 μm, respectively. However, an intended overcorrection of 10% was performed in patients with a 160-μm cap thickness, because of possible energy loss due to a thicker cap. Another prospective contralateral eye study of SMILE evaluated the refractive error when using a cap thickness of 100 or 160 μm (preoperative sphere of −4.05 ± 1.50 D and −3.84 ± 1.51 D, respectively). The average spherical error one month after SMILE was comparable between 100- and 160-μm cap thickness (100 μm, −0.03 ± 0.35 D versus 160 μm, 0.02 ± 0.35 D), but no intended overcorrection was performed in the 160-μm group. The current ex vivo study also found an

### Table 1. Preoperative Characteristics and Thickness Measurements Before and After SMILE, Mean ± SD and Range

<table>
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<tr>
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<th>4 D SMILE</th>
<th>8 D SMILE</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td></td>
<td>110-μm cap, n = 8</td>
<td>160-μm cap, n = 8</td>
</tr>
<tr>
<td>Donor age, y</td>
<td>63 ± 10 (47 to 74)</td>
<td>67 ± 11 (45 to 78)</td>
</tr>
<tr>
<td>Time in Dextran, h</td>
<td>55 ± 16 (24 to 70)</td>
<td>39 ± 14 (24 to 63)</td>
</tr>
<tr>
<td>CCT before SMILE, Pentacam HR, μm</td>
<td>469 ± 12 (449 to 485)</td>
<td>461 ± 19 (441 to 500)</td>
</tr>
<tr>
<td>CCT after SMILE, Pentacam HR, μm</td>
<td>578 ± 15 (559 to 395)</td>
<td>573 ± 25 (551 to 417)</td>
</tr>
<tr>
<td>CCT after SMILE, Heidelberg OCT, μm</td>
<td>373 ± 18 (349 to 393)</td>
<td>367 ± 27 (332 to 408)</td>
</tr>
<tr>
<td>Cap thickness, Heidelberg OCT, μm</td>
<td>119 ± 14 (104 to 151)</td>
<td>155 ± 18 (112 to 160)</td>
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</table>

Thickness measurements were performed after removal of the epithelial layer.
undercorrection after $-4 \text{D}$ SMILE correction with an average undercorrection (TCRP) percentage of $3.5\%$ ($-3.86 \pm 1.31 \text{D}$) and $11\%$ ($-3.57 \pm 1.27 \text{D}$) when using a cap thickness of 110 μm or 160 μm, respectively. Thus, we observed a tendency, although nonsignificant, toward a higher degree of undercorrection when SMILE was performed in the deeper corneal layers.

Surprisingly, we observed an undercorrection of $43\%$ in the $-8 \text{D}$ group, with a 160-μm cap thickness, more than 4 times higher than in the $-4 \text{D}$ group. Thus, we observed a tendency, although nonsignificant, toward a higher degree of undercorrection when SMILE was performed in the deeper corneal layers.

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Previous in vivo studies have evaluated the refractive error in SMILE by using a 160-μm cap thickness in patients with low to moderate myopia, but the refractive error after high myopic correction remains unknown. It may be questioned if the amount of undercorrection is positively correlated with the intended correction when the stromal lenticule is removed in the deeper corneal layers. However, this question has previously been examined in 670 SMILE-treated eyes for high myopia and a cap thickness between 110 and 120 μm; however, no dependency between postoperative error and attempted refraction was found.

The ex vivo inflation test revealed a biomechanical weakening after SMILE in all 4 groups, with an average steepening of the anterior curvature between 0.09 and 0.31 mm at increased chamber pressure, whereas no significant
FIGURE 2. (A–C) The average change after SMILE (Δ = postoperative and preoperative values) for the anterior and posterior corneal curvature and the TCRP at a chamber pressure of 15 and 40 mm Hg. a markings: Significant difference between −4 D and −8 D correction at identical cap thickness. b markings: Significant difference between 110 μm and 160 μm cap at identical refractive correction. (D–F) The average change at increased chamber pressure (Δ = 15 mm Hg - 40 mm Hg values) for the anterior and posterior corneal curvature and TCRP after before and after SMILE. *P < 0.05, mixed model ANOVA. Bars represent standard deviations.
changes were seen in the posterior curvature. The 110-μm cap thickness seemed to cause more pronounced corneal weakening than the 160-μm cap thickness for −8 D corrections, although the difference was marginally insignificant (P = 0.051; Table 4).

The sample size was determined by corneal curvature data from our previous study, where a similar experimental setup was used to evaluate the biomechanical strength after intrastromal lenticule implantation. The power analysis suggested that 32 donor corneas were needed to detect an average difference of 0.12 mm in Δ between 110- and 160-μm cap thicknesses, with a standard deviation of 0.08 mm, 80% power, and a 5% significance level. Thus, the number of included corneas would, with a 95% probability, be sufficient to show a relevant difference between the two cap thicknesses, assuming that our previous observations could be applied to this study.

Vertical and lamellar stromal cuts have previously been shown to weaken the biomechanical strength when tested ex vivo in human donor corneas. By using the inflation test, the authors demonstrated comparable biomechanical weakening after corneal lamellar cuts at 90- and 160-μm depths, with a 5% corneal apical displacement at increased chamber pressure. However, the study did not assess the impact of tissue removal, but solely the lamellar and vertical cuts. Hence, this may explain the discrepancy with our study, where the difference between 110- and 160-μm cap thicknesses may be predominantly caused by removal of the stromal lenticule rather than the lamellar cut between the cap and the lenticule. Future studies using inflation tests after SMILE may consider comparing corneal tomography before SMILE, after incision, and after lenticule removal to determine the effect of tissue removal on the biomechanical properties.

In vivo assessment of the corneal biomechanical properties is possible with commercially available devices evaluating the corneal deformation during an air-pulse, including the Ocular Response Analyzer (ORA; Reichert Inc., Dephew, NY) and the Corvis ST (Oculus, Wetzlar, Germany). Corneal viscoelasticity may be described by the corneal hysteresis (CH) and the corneal resistance factor (CRF) with the ORA, although they may not be optimal for a distinct description of the corneal viscosity and elasticity. A contralateral eye study of myopic SMILE using a 100- or 160-μm cap thickness reported significantly lower values of CH and CRF in the 100-μm cap group; this may suggest a more pronounced decrease in the postoperative viscoelasticity. However, the average cylindrical and spherical correction was slightly higher in the group with a 100-μm cap thickness, although no significant differences were reported between groups. The positive correlation between lenticule thickness and the average decrease in CH and CRF may explain the difference reported between the 100- and 160-μm cap groups. The in vivo biomechanical strength has also been examined with the Corvis ST in SMILE-treated rabbits, comparing 100- and 160-μm cap thicknesses. After adjusting for intraocular pressure (IOP), the study found no significant differences in the Corvis ST parameters at 4 months, suggesting a similar biomechanical performance at 100- and 160-μm cap thickness, when tested under such circumstances. However, the study included a small sample size (n = 12), which may be too small to detect a difference.

Table 4. The Average Difference in the Anterior and Posterior Corneal Curvature (Δr = 40 mm Hg - 15 mm Hg Values) and ΔTCRP During Increased Chamber Pressure

<table>
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<tr>
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<th>−4 D SMILE</th>
<th>−8 D SMILE</th>
<th>P Value</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<td></td>
<td>110-μm cap, n = 8</td>
<td>160-μm cap, n = 8</td>
<td>110 μm cap, n = 8</td>
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<tr>
<td>Anterior r sag, mm</td>
<td>0.01 ± 0.04</td>
<td>0.02 ± 0.08</td>
<td>−0.01 ± 0.03</td>
</tr>
<tr>
<td>Δr postop</td>
<td>−0.15 ± 0.14*</td>
<td>−0.09 ± 0.05*</td>
<td>−0.31 ± 0.21*</td>
</tr>
<tr>
<td>Posterior r sag, mm</td>
<td>0.00 ± 0.04</td>
<td>0.01 ± 0.03</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>Δr postop</td>
<td>0.07 ± 0.05</td>
<td>0.01 ± 0.03</td>
<td>0.07 ± 0.09</td>
</tr>
<tr>
<td>TCRP 15 mm Hg</td>
<td>0.12 ± 0.43</td>
<td>−0.14 ± 0.73</td>
<td>0.11 ± 0.28</td>
</tr>
<tr>
<td>ΔTCRP postop</td>
<td>0.90 ± 1.42*</td>
<td>0.86 ± 0.50*</td>
<td>1.90 ± 1.23*</td>
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</table>

* P < 0.05.
Strip extensiometry is commonly used for measurement of corneal biomechanical properties. An obvious drawback is the uneven stress distribution in the corneal strips, which will be relatively compressed on the epithelial side and elongated on the endothelial side. Hence, inflation tests may be preferable as the chamber pressure, at least in an intact cornea, will cause similar in-plane meridional and circumferential stresses within the layers of the corneal stroma. Lenticule extraction may cause relaxation of the collagen fibers in the cap, and stress distribution may thus not be identical in the front and back part of the corneal stroma.

Still, there may be tension, although reduced, in the cap after SMILE. The arc-length of the cap before SMILE at 15 mm Hg was between 6719 and 6723 μm, calculated from the preoperative anterior radii and a chord length of 6.5 mm (corresponding to the diameter of the lenticule). The relationship between strain and increased IOP has previously been evaluated in normo-hydrated human corneas, where a pressure increase from 2 to 15 mm Hg caused a strain of approximately 0.190%. Based on the calculated arc-length of the cap, 15 μm (0.190%) of the arc-length was due to elongation of the tissue when the chamber pressure was 15 mm Hg. Therefore, shortening of arc-length after SMILE would be between 11 and 38 μm (calculated from the postoperative anterior curvature radii) when we subtract the 13-μm elongation caused by the 15 mm Hg chamber pressure. However, we did not take into account that Young’s modulus is low at low IOP. Hence, increasing the chamber pressure from 0 to 2 mm Hg may cause an elongation that exceeds 11 to 38 μm. Furthermore, collagen remodulation after SMILE may also contribute to stress in the cap, as the severed collagen fibers may recoil and cause a minor peripheral stromal expansion. However, the expansion is less compared to the expansion after LASIK. The results in this study seem to support that there may still be tension in the cap after lenticule extraction, as lenticule removal in the deeper corneal layers seemed to cause less reduction in the biomechanical strength. If there was no tension in the cap, we would expect a more pronounced reduction in the biomechanical strength when increasing cap thickness.

Our model used for testing corneal biomechanical properties has some limitations. Postoperative follow-up was not possible, although it would have been interesting to evaluate the stromal modulation and endothelial layer damage after deep lenticule removal. The epithelial layer was removed before the preoperative measurements, as it caused inadequate topography measurements and insufficient laser cutting. Thus, cap thickness was calculated from the stromal surface after removal of the epithelial layer. The postoperative weakening of the biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelium. The biomechanical response during inflation may be different between intact eyes and mounted removal of the epithelium. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The postoperative weakening of the biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer. The biomechanical strength after SMILE may, therefore, be less severe in all 4 groups than if SMILE was performed prior to removal of the epithelial layer.
Cap Thickness and Biomechanical Strength After SMILE


