Corneal Epithelial Remodeling in a 6-month Follow-up Period in Myopic Corneal Refractive Surgeries

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Conflict of Interest

The authors indicate no financial conflict of interest

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Highlights

In the early postoperative period, the epithelial remodeling of FS-LASIK, SMILE and tPRK showed different trends, while the eipthelial distribution tended to be consistent in the later stage.

Author contrubutions

Study concept and design (QMW, YFY, SHC, FJB, AE); data collection (MMZ, YX, ZW, AMW, CW, DTZ); analysis and interpretation of data (MMZ, YX, RV, ZW, AMW, CW, DTZ, ZXQ, PW, XBZ, JJW, FJB); writing the manuscript (MMZ, YX, RV, ZW, AMW, CW, DTZ, ZXQ, PW, XBZ, JJW, QMW, YFY, SHC, FJB, AE); critical revision of the manuscript (QMW, SHC, FJB, AE); statistical expertise (MMZ, YX); administrative, technical, or material support (SHC, FJB); supervision (QMW, SHC, FJB, AE).

Abstract

Purpose: To investigate changes in corneal epithelial thickness profile during a 6-month follow-up period after transepithelial photorefractive keratectomy (tPRK), femtosecond laser–assisted excimer laser in situ keratomileusis (FS-LASIK), and small incision lenticule extraction (SMILE).

Methods: This prospective study included 76 eyes of 76 participants who underwent refractive surgery for myopia with or without astigmatism (23 FS-LASIK, 22 SMILE and 31 tPRK). Corneal epithelial thickness and corneal anterior curvature were individually measured by anterior segment spectral-domain optical coherence tomography and a Scheimpflug-based tomographic system before the operation (pre) and at 1 or 3 days (pos1-3d), 1 week (pos1w), 1 month (pos1m), 3 months (pos3m) and 6 months (pos6m) postoperatively. The thickness and curvature measurements were averaged over 25 areas bounded by the temporal-nasal and inferior-superior meridians and the circles separating the central, paracentral, midperipheral and peripheral regions.

Results: The epithelium thickness was similar in the 3 surgery groups and in all four corneal quadrants in both the pre and pos6m stages (all $P > 0.05$). The epithelial thickness after tPRK fluctuated the most during the 6-month follow-up period, compared with FS-LASIK and SMILE. The largest increase in epithelial thickness in all three groups over the 6-month follow-up period was in the inferior-temporal paracentral area (7.25±2.58μm for FS-LASIK; 5.79±2.41μm for SMILE; 4.88±5.84μm for tPRK; all $P < 0.001$). Only the epithelium thickness of tPRK group increased from pos3m to pos6m ($P < 0.05$), while all changes in the FS-ASLIK and SMILE groups were not significant ($P > 0.05$). Furthermore, there was a positive correlation between epithelial thickness changes and curvature gradient changes in the paracentral region of tPRK corneas ($r = -0.549$, $P = 0.018$), but not in other corneal regions in all three surgery groups.

Conclusions: The epithelial thickness remodeling followed different trends after FS-LASIK, SMILE and tPRK from the early postoperative stage onwards, but the thickness distributions arrived at 6 months after the three surgery forms were similar. While epithelial remodeling after FS-LASIK and SMILE stabilized by, and beyond, pos3m, it did not stabilize by pos6m after tPRK. These changes can affect corneal profile after surgery and may lead to deviation from the intended surgical outcome.

Keywords: corneal epithelial remodeling, FS-LASIK, SMILE, tPRK, OCT

INTRODUCTION

The corneal epithelial cells are known to migrate inwards from stem cells located in the limbus and compensate irregularities that may exist in the stromal anterior surface.[1, 2]. This compensation activity, called epithelial remodeling, has been observed after keratoconus [3], keratoplasty, cataract surgery [4], collagen cross-linking [5] and refractive surgery [6-8]. Previous studies showed that epithelial remodeling modified the epithelial thickness profile following the surface ablation conducted in refractive surgeries, potentially leading to reduced predictability of surgical outcomes [9], possibly leading to reduced predictability of refractive surgery outcome [10, 11] . For this reason, it is important to quantify the epithelium's remodeling patterns in order to improve the predictability of the long-term outcome of refractive surgery.

There are currently three most common and relatively safe myopia-correction refractive surgeries - femtosecond assisted-laser in situ keratomileusis (FS-LASIK), small incision lenticule extraction (SMILE) and transepithelial photorefractive keratectomy (tPRK) [12]. Earlier studies found that the epithelium thickened to varying degrees after FS-LASIK [11], SMILE [8, 13] and tPRK [7, 14], but assessment of that change was limited to the central 6mm diameter region and did not include comparisons between the three surgeries. A comparative study that considers epithelial thickness changes over a larger region may help in applications including the selection of surgical methods and parameters.

At present, the mainstream measure of epithelial thickness is spectral-domain optical coherence tomography (SD-OCT) [15-17]. As a non-contact method, SD-OCT can measure epithelial thickness over an area with 9mm diameter and avoid potential disadvantages, such as corneal abrasion, infection risk and patient discomfort [18]. SD-OCT technology has also shown excellent repeatability in measuring epithelial thickness after refractive surgery in previous studies [19].

For these reasons, this report seeks to present the results of a comprehensive study in which corneas undergoing the three common forms of refractive surgery are compared in terms of the epithelial

thickness changes during 6 months follow up within the central 9mm diameter region by using SD-OCT.

PATIENTS AND METHODS

Seventy-six patients with myopia and astigmatism, who had undergone corneal refractive surgery at the Eye Hospital of Wenzhou Medical University (WMU) were included in this prospective study. Among these patients, 23 received FS-LASIK, 22 underwent SMILE and 31 underwent tPRK. Each participant received the same surgery on both eyes. One eye from each patient was selected for use in the study based on a random number generated by Microsoft Excel (Microsoft Corp, Redmond, Washington). This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Eye Hospital, WMU. After explaining the nature of the study and possible results, written informed consent was obtained from all participants to use their data in research.

Preoperative and postoperative assessment

All participants underwent a detailed clinical evaluation, including age, gender, ocular medical history and family history of keratoconus. Preoperative evaluation included uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), non-contact tonometry to measure intraocular pressure, IOP (TX-F, Tomey company, Japan), anterior segment slit-lamp biomicroscopy (SLll5, Zeiss, Germany), fundus photography and dry eye screening. UCVA and BSCVA were measured with the Chinese standard logarithm visual chart at five metres distance and then converted into Logarithm of minimal angle of resolution (LogMAR) unit. Corneal topography (Pentacam HR, Oculus Optikgerate, Germany) and the corneal epithelial thickness map (SD-OCT, RTVue-XR; Optovue Inc., Fremont, CA) were also recorded. All patients were examined before surgery (pre), and 1 day (or 3 days after tPRK due to wear of bandage contact lens post-surgery for this period [20], pos 1-3d), 1 week (pos1w), 1 month (pos1m), 3 months (pos3m) and 6 months (pos6m) after surgery. All examinations were performed by the same experienced technician (NJL).

Surgical procedures

All surgeries were performed by the same experienced surgeon (SHC). In FS-LASIK surgery, a 95 to 110 μm thick, 8.5 to 9.0 mm diameter flap with a superior hinge was created using an Intralase iFS150 femtosecond laser (150 HZ frequency, Abbott Medical Optics, Inc, USA). Laser ablation was then performed with an optical zone diameter set at 6.1 to 7.1 mm using an Amaris 750 excimer laser (750 HZ frequency, Schwind eye-tech-solutions GmbH & Co.KG, Germany) in the aberration-free mode. In SMILE surgery, a VisuMax femtosecond laser (500 HZ frequency, Carl Zeiss Meditec AG, Jena, Germany) with lenticule diameter of 6.0 to 6.9 mm, intended cap thickness of 120 μm, and incision length of 2.0 mm was used. In tPRK surgery, the epithelium and stroma were ablated in a single step using the aberration-free mode of the Amaris 750 excimer laser, the central ablated epithelium thickness was set at 55 μm. The optical zone diameter ranged from 6.0 to 7.2 mm. Following tPRK ablation in high myopia cases (in cases with refractive error ≤-6.00 D), 0.02% mitomycin-C (Sigma-Aldrich, St Louis, MO), a cotton strip with a diameter of 6mm was applied to the corneal stroma for 15-20s.

After three surgery procedures, a drop of Tobramycin & Dexamethasone (Tobradex; Alcon, TX, USA) was instilled at the surgical site. A bandage contact lens (Acuvue Oasys; Johnson & Johnson, FL, USA) was then placed on the cornea for 1 day after FS-LASIK or 3-7 days after tPRK – or until complete corneal re-epithelization. Topical levofloxacin 0.5% (Cravit; Santen, Osaka, Japan) was then used for 7-10 days after all three surgeries. A postoperative tapering dose of fluorometholone (0.1%, Flumetholon; Santen, Osaka, Japan) and sodium hyaluronate (0.3%, Hialid; Santen, Osaka, Japan) were given 4 times per day, with a tapering period of 1 month in the FS-LASIK and SMILE groups, and 2–3 months in the tPRK group.

Epithelium thickness profiles

The RTVue-XR SD-OCT was used to measure the thickness of the epithelium and stroma with the "Pachymetry Wide" scan mode, which included evenly-spaced, 9-mm long, A-scans in eight, meridional directions. The measurements were repeated three times, and maps depicting the average thickness were generated using the device's auto-fitting algorithm. The maps were subsequently

divided into 25 areas, shown in **Figure 1**, including a central 2 mm diameter area, eight paracentral areas with 2-5 mm diameter range, eight midperipheral areas with 5-7 mm diameter range and eight peripheral areas with 7-9 mm diameter range. Within each area, the average epithelial thicknesses were determined. The OCT imaging was performareaed prior to clinical examination of the eye at each clinic visit. In order to minimize the effect of tear film thickness, the use of tear substitutes and other topical eye medications were prohibited over the 2 hours preceding the OCT scans.

Corneal curvature gradient

In a corneal tangential curvature map, each point has its own defined curvature, and the difference between the curvatures of two adjacent points denotes the curvature gradient. Furthermore, the Radial Curvature Gradient (CG) describes the rate of curvature change over a specific distance, along a one half-meridian. That half meridian starts at the vertex and spans radially towards the limbus. Its unit is defined as the refractive power change (D) per millimetre distance (mm), or D/mm.

The Pentacam software calculates the minimum and maximum Curvature Gradient, derived in an 8mm zone around the corneal vertex. For a more specific analysis, the average Curvature Gradient is displayed in 3 zones, with diameters of 3, 5 and 7mm around the vertex. **Figure 2** shows a common tangential curvature map on the left and the corresponding Radial Curvature Gradient map of a patient after myopic LASIK surgery. In the Radial Curvature Map on the right, the minimum (-16 D/mm) and maximum (26.7 D/mm), as well as the average Curvature Gradients in the specific zones are displayed, 3mm: 0.1 D/mm, 5mm: 5.0 D/mm, 7mm: 4.5 D/mm.

In our study, the corneal anterior elevation data obtained with the Pentacam within the central 7 mm diameter region was analyzed to determine the curvature and its first derivative with respect to distance away from apex (i.e. the curvature gradient) along the 8 main principal meridians (S, ST, T, IT, I, IN, N, SN). Numerical integration was also used to determine the mean values of curvature

gradient over the three corneal subregions, namely the central, paracentral and midperipheral regions.

Statistical Analysis

SPSS Statistics 26.0 (IBM Corp, Armonk, NY) was used for statistical analysis. All quantitative data were expressed as means ± standard deviation. Normality was tested with the Shapiro–Wilk normality test, and non-parametric statistics were used for variables with non-Gaussian distribution. To compare the epithelial thickness changes before and after surgery, a paired t test or a Wilcoxon matched test was performed for data obtained with the same surgical procedure. One-way analysis of variance (one-way ANOVA) was used to compare the difference in epithelial thickness in different surgery groups. The power of the sample size was determined using Gpower statistical software (Version 3.1.2, Franz Faul, University Kiel, Germany) (see Supplementary **Table S1**). Pearson correlations examined relationships among different parameters. In all tests, a P value of less than 0.05 was indicative of statistical significance. Bonferroni test was used for post-hoc pairwise comparison.

RESULTS

The demographics of participants are presented in **Table 1.** The three surgery groups were matched in age, best corrected visual acuity (BCVA), spherical equivalent (SE) and optical zone diameter (OZD).

The epithelial thickness and their trends in the central, paracentral, midperipheral and peripheral regions as shown in **Table 2.** There was no significant difference in epithelial thickness in pre stage between the central, paracentral and midperiphery regions, while the peripheral epithelial thickness was significantly smaller than in the other three regions ($P < 0.05$). During all follow-up stages, the changes in epithelial thickness compared with the pre stage were similar in the FS-LASIK and SMILE groups, in all regions except in the midperipheral region at the pos3m stage. Meanwhile, in all follow-up stages, except pos6m, the changes in epithelial thickness compared with the pre stage

in tPRK were higher than in FS-LASIK and SMILE in the four corneal regions. Furthermore, the epithelium thickness of tPRK groups was significantly thickened from pos3m to pos6m in all regions (all P<0.05), the changes were not found in the FS-ASLIK and SMILE group (all P>0.05). Nonetheless, at pos6m, the epithelium thickness became similar in all three surgery groups and in all four corneal regions.

Epithelial thickness after tPRK fluctuated the most during the 6-month follow-up period, compared with FS-LASIK and SMILE (**Figure 3**). In the FS-LASIK group, the epithelial thickness changes at pos1d stage were the highest ($p < 0.05$ in all 25 areas except T5, ST5, S5, I5, T7, I7, IT7), but the differences between thickness changes became successively smaller in later follow-up stages. It was also observed that the epithelial thickness was larger in all central and paracentral areas (all $P < 0.05$) than the pre stage over the whole 6-month follow-up period. In contrast, the midperipheral and peripheral areas maintained similar thickness levels (all $P > 0.05$) during all follow up stages beyond pos1-3d. Furthermore, the SMILE group demonstrated stable epithelial thickness values in all 25 areas during all follow-up stages ($P > 0.05$), while the tPRK group showed the largest changes in epithelial thickness between pre and pos3d all areas (all $P < 0.05$), but these changes became successively smaller over the rest of the follow-up period. At the final pos6m stage, the epithelial thickness was still higher in the central and paracentral areas than at the pre stage ($P < 0.05$, except in S2, SN2, N2), but this trend did not exist in the midperipheral and peripheral areas ($P > 0.05$).

In the FS-LASIK group, most areas within the central and paracentral areas had thicker epithelium during follow-up, and a similar, but less consistent, phenomenon could be observed in the SMILE group. In these two groups, the largest increase in epithelial thickness over the 6-month follow-up was in the inferior-temporal paracentral area (IT2). In this area, the thickness change (pos6m vs pre) was 7.25±2.58μm ($P < 0.001$) in FS-LASIK and 5.79±2.41μm ($P < 0.001$) in SMILE, while it was 4.88±5.84μm ($P < 0.001$) in tPRK. However, these thickness changes did not differ significantly between the three surgery groups ($P = 0.138$). In contrast, the midperipheral and peripheral areas showed the largest thinning with a value of 3.14±1.94μm in area T7 after FS-

LASIK ($P < 0.001$) and 2.96±2.76μm in area IT7 after SMILE ($P = 0.005$). Of the 25 areas considered, the superior-peripheral area (S7) was the thinnest both before and after surgery in all three groups.

The differences in epithelial thickness between tPRK and FS-LASIK or SMILE in all 25 areas were highest at the pos1-3d stage (all $P < 0.05$, **Figure 4**), then the gap became gradually narrower in later follow-up stages. At the final pos6m stage, the epithelial thickness was similar in the three surgery groups (all $P > 0.05$).

The study also considered the curvature gradient results shown in **Table 3**. There were no significant differences in preoperative curvature gradient between the three surgeries ($P = 0.487$ in the central region; $P = 0.106$ in the paracentral region; $P = 0.705$ in the midperipheral region). The curvature gradient in all regions was significantly altered at pos1-3d compared with pre stage (all $P < 0.05$) except in the central region of the FS-ASLIK group ($P = 0.073$). There were minor fluctuations in the curvature gradient until pos1w then the results became stable over the rest of the follow-up period. Compared with the pre stage, paracentral and midperipheral curvature gradients increased significantly and became positive in the three surgery groups at pos1w (all $P < 0.001$), but this trend was not apparent in the central region ($P = 0.831$ in FS-LASIK; $P = 0.434$ in SMILE; $P = 0.295$ in tPRK). From pos1w to pos6m, the curvature gradient in the midperipheral region was significantly reduced in FS-LASIK ($P = 0.004$) and SMILE ($P = 0.028$), and the gradient in the central and paracentral regions decreased significantly in the tPRK group ($P = 0.009$, 0.012). No other significant changes were observed in other regions.

To analyze the effect of curvature gradient on epithelial thickness changes, the correlation analysis was performed between the curvature gradient (at pos1d in FS-LASIK and SMILE, pos1w in tPRK) and the changes in epithelial thickness and corneal thickness (from pos1d to pos6m in FS-LASIK and SMILE, from pos1w to pos6m in tPRK). – To avoid the effect of corneal bandage lens removal in tPRK (carried out 3 days after surgery) on epithelial thickness, thickness changes at pos1w were

used in this analysis. While the curvature gradient at pos1w was positively correlated with the epithelial thickness changes in the paracentral region of tPRK (r = -0.549, P = 0.018), no other regional correlations were detected in all surgery groups. There were also no correlations between curvature gradient and corneal thickness changes in all regions.

DISCUSSION

Understanding the characteristics of corneal epithelium, and the remodeling it commonly undergoes following refractive surgery, is of great significance in planning the surgical procedures and analyzing the postoperative refractive regression. This study provides detailed analysis of epithelial remodeling in patients undergoing the three most common refractive surgeries, FS-LASIK, SMILE and tPRK. The results demonstrated clear differences in epithelial remodeling among the three patient groups in the early postoperative stage, and the time taken to restore the preoperative epithelial thickness.

Corneal wound healing may be essential to early epithelium remodeling, which begins a few hours after injury and continues for at least 10 days [21], this may explain the significant differences in early postoperative epithelial thick ness changes demonstrated in our results in the three surgery groups. In support of this potential link, the epithelial injury after FS-LASIK and SMILE, which is neither obvious nor substantial compared to tPRK, has led in our study to a mild epithelial healing response in the early stages after operation.

In this study, we found that epithelial remodeling following FS-LASIK and SMILE was stable at pos3m; but was not stable after tPRK; a result which may be attributed to the different surgical procedures. In tPRK, the corneal epithelium is completely ablated and the epithelium in the ablation zone needs to regrowafter surgery [12]; as such, an extended time period is required to achieve epithelial stability. There have been several studies indicating that the central epithelial remodeling following LASIK tends to reach stability at pos1w[22], pos1m[15] or pos3m[23], and 1 month postoperatively following FS-LASIK in the region with diameter between 4 and 8mm[24]. Similarly, earlier data suggested that epithelial remodeling stabilized following SMILE 3 months postoperatively.[8] However, corneal epithelial thickness after myopic LASIK showed a progressive

255 epithelial thinning from 1 to 6 months of follow‐up in Díaz‐Bernal's study [25]. As for tPRK,
256 continuous epithelial hyperplasia was observed within 6 months in this study, which was consistent
257 with Shetty's study [14] and different from Chen's observation of epithelial stability from pos3m to
258 pos6m[7]. Differences in the mean pre-surgery SE between Chen's study and ours may be behind the
259 inconsistent results (-2.82±1.54D in their study) especially that previous studies showed significant
260 correlation between the amount of correction and epithelial remodeling.[6, 26, 27] Therefore, refraction
261 regression caused by epithelial remodeling may be later in tPRK. Latifi et al. have reported that the
262 epithelial hyperplasia became stable in the central region at 12 months, and it continued to increase
263 in the paracentral region even after 18 months after surgery. [28]
264
265 The present study found that the epithelial thickness changes after tPRK had the maximum standard
266 deviation (compared with FS-LASIK and SMILE), which indicated that epithelium remodeling with
267 tPRK was the most unstable among the three operations. On the other hand, epithelium remodeling
268 with SMILE was the most stable, which was consistent with the results of previous research.[17].
269 This may be due in part to the improved corneal biomechanical properties and the mild dry-eye
270 disease (DED) after SMILE [29, 30]. Abou et al. reported that DED patients had a highly irregular
271 corneal epithelial surface [31], whether the treatment of dry eye symptoms after tPRK contribute to
272 the stability of epithelial thickness warrant further study in subsequent research.
273
274 The epithelium thickness distribution was similar at pos6m in the three surgery groups (**Figure 4**).
275 Canto-Cerdan [11] and Kanellopoulos et al.[32] also found similar epithelial thickness variations
276 following FS-LASIK and SMILE after 6-month and 2-year follow-up, respectively. Despite the
277 similar behavior obserat the end of the follow-up period, our study found significant differences in
278 the mid-peripheral region at pos3m after FS-LASIK and SMILE – and similar results were reported
279 by Ryu et al [17]. These differences could be related to the diameter of the treatment zone being
280 significantly larger in SMILE than in LASIK despite the fact that the planned optical zone size was
281 similar in the two procedures.
282

Previous studies found that the epithelium in normal eyes was thickest in the superior region and thinnest in the inferior region, which is potentially related to the application of eyelid pressure [33]; similar epithelial distribution characteristics were found in our study before refractive surgery. Our results also confirmed that the corneal epithelium underwent regional variations in thickness remodeling after refractive surgery. For example, not all epithelial thickness changes in the paracentral areas exceeded the thickness changes in the central region; a trend that was also identified by Fan et al.[34] Yet still, the changes in epithelial thickness were most marked in the paracentral inferotemporal area, followed by the central region and then the paracentral supra-nasal area for all surgeries in this study. The reasons behind the epithelial non-uniform remodeling are currently unknown. In connection with this observation, Mathers et al. noted that the shear forces imposed by eyelids during blinking resulted in regional variations in the epithelial changes in mean cell area, perimeter, and shape factor[35]. However, no quantitative data for eyelid-related shear forces has yet been provided for different areas on the corneal surface. We inferred in this study that the relatively flat cornea after refractive surgery may result in an anatomical mismatch between the eyelid and the corneal surface, resulting in the emergence of uneven shear and a concentration of corneal epithelial cells in the inferotemporal areas. In addition, it should be noted that SD-OCT cannot rule out the influence of tear film thickness when measuring epithelial thickness. In this regard, gravity may cause an inferior-vs-superior change in tear film thickness, which may in turn contribute to the vertical asymmetry exhibited in this study in the epithelial thickness profile. When using corneal wavefront treatment with small ablation amounts for correcting high order aberrations, uneven distribution of epithelium may not completely eliminate the corneal irregularity, thus affecting the predictability and efficacy of surgery [36]. Also, epithelial distribution characteristics after refractive surgery should also be fully considered during tPRK enhancement.

The corneal epithelium has the ability to vary its thickness in order to reconstruct a smooth optical surface [37], reducing the curvature of the ablation zone following myopic refractive surgery. Dierick et al proposed a hypothesis of migration inhibition of corneal epithelium, and suggested that a certain tension between adjacent epithelial cells existed and allowed the epithelium to proliferate easily in relatively flat regions [38]. Zhou et al. showed that higher keratometry values, astigmatism,

asphericity, and corneal higher-order aberrations on the stromal surface compared to the anterior corneal surface [36]. Vinciguerra et al further proposed a curvature gradient hypothesis by analyzing the correlation between postoperative immediate curvature gradient and long-term changes in corneal curvature and suggested that the curvature gradient determines the epithelial remodeling behavior and the subsequent changes in corneal curvature[39]. Based on this hypothesis, the epithelial remodeling after refractive surgery may be mainly related to the curvature gradient in the stromal surface. The hypothesis indicated that in order to smooth out the areas with high curvature gradients (along the edges of optical zones), the epithelial thickness changes were different inside and outside these areas. Our research confirms the curvature gradient hypothesis by illustrating that the central epithelium (up to 5 mm diameter) gains in thickness after surgery, while the peripheral epithelium (7-9 mm diameter) becomes thinner.

Furthermore, the correlation between postoperative epithelial thickness changes and curvature gradient in different regions after different surgeries were evaluated for the first time in this study. The changes from pos1w to pos6m after surgery were selected for analysis to avoid the effect of early postoperative epithelial healing response. However, the expected correlation between curvature gradient and epithelial thickness changes could be identified only in the paracentral region after tPRK. This weak compliance with the curvature gradient hypothesis may be attributed to a number of factors. Firstly, the hypothesis was derived using data of a population with high preoperative myopia (mean SE: -9.4±2.4D) and high postoperative curvature gradient (> 5 D/mm)[39], which could result in more postoperative corneal epithelial remodeling than in the present study (SE: -5.63±1.73D). Secondly, the curvature changes at 12 months postoperatively were used in Vinciguerra's study, and this longer follow-up period may reveal more epithelial changes than those identified in the 6-month period adopted in our study. Thirdly, the corneal curvature gradient was analyzed separately over the 3 regions (circular, paracentral, midperipheral) that constitute the 7 mm central region, over which regions the change in curvature gradient could vary, possibly weakening the correlation with epithelial thickness change.

There are several limitations to this study. Firstly, this study sample sizes were relatively small so that the variability in terms of lenticules diameters, ablation diameters or aberration free ablation pattern cannot be ruled out. Secondly, this study only considered a certain range of myopic correction, and therefore its findings may not be applicable to corrections outside this range. Thirdly,

while epithelial remodeling seems to have stabilized by, and beyond, pos3m in FS-LASIK and SMILE, a stable epithelial thickness may not have been reached in tPRK. Further studies with larger sample sizes and longer follow-up are being planned by our group. Next, all the tPRK patients of our study experienced complete epithelialization by day three. Since this is different from normal clinical practice, in which the epithelium of some patients takes longer than three days to heal, there is potential for our study to involve selection bias. Finally, this study did not analyze the effect of gender on epithelial remodeling, but it is acknowledged that different gender ratios could have had a potential impact on the postoperative outcome.

In conclusion, we evaluated corneal epithelial remodeling across a 9mm diameter after FS-LASIK, SMILE and tPRK in a 6-month follow-up period and identified differences in the remodeling profile in the three surgical methods. In the early stages after operation, epithelial thickening was lowest after SMILE and most unstable after tPRK. However, a similar epithelial thickness profile was reached at pos6m in the three surgery groups. While epithelial remodeling after FS-LASIK and SMILE stabilized by, and beyond, pos3m, epithelial remodeling may not have reached stability by pos6m after tPRK.

Figure Captions

Figure 1 Corneal areas used in thickness analysis. T-temporal; S-superior; N-nasal; I-inferior. 2: paracentral areas（2–5 mm diameter）; 5: midperipheral areas (5–7 mm diameter); 7: peripheral areas (7–9 mm diameter)

Figure 2 Curvature gradient map.

Figure 3 Epithelial thickness (mean ± standard deviation) in the cornea's 25 areas – depicted in Figure 1 – at different follow-up stages after (a) FS-LASIK, (b) SMILE and (c) tPRK. Gray vertical lines are used to separate the four corneal regions.

Figure 4 Mean epithelial thickness in FS-LASIK, SMILE and tPRK in the cornea's 25 areas – depicted in Figure1 (a) before surgery, and after surgery by (b) 1 or 3 days, (c) 1 week, (d) 1 month, (e) 3 months, and (f) 6 months. Gray vertical lines are used to separate the four corneal regions. Gray double horizontal lines are used to separate different follow-up time points.

Table Captions

Table 1 Preoperative information and surgery parameters for all participants

Table 2 Mean and standard deviation of changes in epithelial thickness relative to the pre stage in μm in corneas undergoing FS-LASIK, SMILE and tPRK.

Table 3 Curvature gradient (mean ± standard deviation) in D/mm after FS-LASIK, SMILE and tPRK

Table S1 Required sample size and statistical power calculated based on comparison between the three surgery groups

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Tables

Table 1 Preoperative information and surgery parameters for all participants

Characteristic	FS-LASIK	SMILE	tPRK	P
patient/eyes	23	22	31	-
Gender (male:female)	11:12	7:15	7:24	0.147
Age, mean±SD (years)	27.52±6.53	27.77±5.53	27.87±5.51	0.976
BCVA, mean±SD, LogMAR	-0.01±0.03	-0.01±0.03	-0.01±0.03	0.871
SE, mean±SD (D)	-5.58±1.78	-5.32±1.57	-5.63±1.73	0.786
Optical zone diameter, mean±SD (mm)	6.60±0.33	6.67±0.25	6.63±0.36	0.797
Stromal ablation depth, mean±SD (μm)	95.04±19.66	112.00±16.68	95.14±21.43	0.005

SD = standard deviation; D = diopters; SE = spherical equivalent; BCVA = best corrected visual acuity; P values from ANOVA. For the stromal ablation depth, the differences between FS-LASIK and SMILE, and between SMILE and tPRK were statistically significant (P=0.015, 0.009 by Bonferroni test), while the difference between FS-LASIK and tPRK was not significant (P=1.000 by Bonferroni test).

Table 2 Mean and standard deviation of changes in epithelial thickness relative to the pre stage in μm in corneas undergoing FS-LASIK, SMILE and tPRK

Follow-up stage	Region	FS-LASIK	SMILE	tPRK	FS-LASIK vs SMILE (P value)	SMILE vs tPRK (P value)	tPRK vs FS-LASIK (P value)
pre	Central	53.59±3.78	53.33±3.39	52.51±3.37	1	1	0.789
	Paracentral	53.55±3.12	53.32±3.38	52.44±3.46	1	1	0.696
	Midperipheral	53.27±2.55	52.93±3.36	52.24±3.70	1	1	0.774
	Peripheral	51.34±2.34	50.95±3.38	50.47±3.64	1	1	0.979
pos1-3d changes	Central	5.37±9.08*	1.72±1.51*	-4.47±8.17*	0.40	0.026	0
	Paracentral	6.63±8.80*	1.94±1.46*	-5.95±7.42*	0.124	0.001	0
	Midperipheral	3.20±8.45	1.58±1.92*	-12.52±10.00*	1	0	0
	Peripheral	3.53±8.28	-1.57±1.79*	-13.92±11.03*	0.212	0	0
pos1w changes	Central	2.54±3.92*	1.32±3.02*	-2.69±5.42*	1	0.006	0
	Paracentral	3.82±3.62*	1.46±2.65*	-3.16±4.90*	0.163	0	0
	Midperipheral	0.13±2.80	0.34±2.89	-6.79±5.55*	1	0	0
	Peripheral	-0.12±3.59	-2.83±2.92*	-6.89±5.45*	0.124	0.004	0
pos1m changes	Central	2.27±3.51*	2.23±2.63*	-4.74±5.00*	1	0	0
	Paracentral	3.31±3.39*	2.27±2.57*	-4.15±4.52*	1	0	0
	Midperipheral	-1.35±2.70	0.74±2.82	-6.88±4.41*	0.154	0	0

	Peripheral	-2.22±3.03*	-2.53±2.71*	-6.08±3.77*	1	0.001	0
	Central	4.44±4.41*	4.67±2.72*	-1.31±4.22	1	0	0
pos3m	Paracentral	4.60±3.74*	4.33±2.29*	-0.82±4.55	1	0	0
changes	Midperipheral	-1.10±3.73	2.36±2.46*	-3.42±4.35*	0.007	0	0.076
	Peripheral	-2.24±3.82*	-1.08±2.34*	-2.60±3.76*	0.792	0.347	1
	Central	4.73±3.85*	4.30±2.41*	2.42±5.14*	1	0.644	0.268
pos6m	Paracentral	5.09±2.21*	4.03±2.19*	3.06±5.27*	1	1	0.326
changes	Midperipheral	-0.59±2.00	1.54±2.66	-0.41±5.10	0.465	0.47	1
	Peripheral	-2.04±1.60*	-1.56±2.28*	-0.76±4.30	1	1	0.654

Note: * significant difference against preoperative thickness values with $P < 0.05$

Table 3 Curvature gradient (mean ± standard deviation) in D/mm after FS-LASIK, SMILE and tPRK

Follow-up stage	Region	Curvature gradient			
		FS-LASIK	**SMILE**	**tPRK**	**P value**
preoperative	Central	-0.45±0.26	-0.32±0.29	-0.42±0.42	0.487
	Paracentral	-0.54±0.45	-0.69±0.72	-0.89±0.48	0.106
	Midperipheral	-2.62±0.64	-2.86±1.21	-2.61±1.13	0.705
1-3 days	Central	-0.71±1.32	-0.78±0.29	-1.75±0.97	0.015
	Paracentral	3.74±2.91	1.80±1.33	4.81±3.01	0.013
	Midperipheral	4.99±2.46	4.96±2.38	1.28±2.61	0.001
1 week	Central	-0.44±0.81	-0.49±0.42	-0.72±1.11	0.593
	Paracentral	4.94±2.66	2.24±1.49	4.69±2.75	<0.001
	Midperipheral	4.46±1.65	4.92±2.57	3.64±2.03	0.138
1 month	Central	-0.09±0.56	-0.56±0.65	-0.65±0.92	0.044
	Paracentral	4.19±1.98	2.38±2.10	4.45±2.96	0.013
	Midperipheral	3.29±1.50	4.91±2.75	3.79±2.09	0.059
3 months	Central	-0.08±0.65	-0.44±0.51	-0.57±0.60	0.031
	Paracentral	4.00±2.37	2.61±1.85	3.95±2.38	0.010
	Midperipheral	3.14±1.23	4.54±2.70	4.08±2.06	0.054
6 months	Central	-0.73±0.56	-0.61±0.54	-0.31±0.66	0.172
	Paracentral	3.85±1.80	2.31±1.72	3.72±2.61	0.248
	Midperipheral	3.11±1.10	4.16±2.04	3.57±1.68	0.386

Table S1 Required sample size and statistical power calculated based on comparison between the three surgery groups

Follow-up stage	Region	Comparison between three groups	
		Required sample size in each group	Achieved statistical power
Pos1-3d vs Pre	Central	15	0.97
	Paracentral	10	1.00
	Midperipheral	9	1.00
	Peripheral	9	1.00
Pos1w vs Pre	Central	16	0.96
	Paracentral	10	1.00
	Midperipheral	9	1.00
	Peripheral	12	0.99
Pos1m vs Pre	Central	9	1.00
	Paracentral	8	1.00
	Midperipheral	8	1.00
	Peripheral	15	0.97
Pos3m vs Pre	Central	11	1.00
	Paracentral	11	1.00
	Midperipheral	12	0.99
	Peripheral	96	0.27

Figure 4

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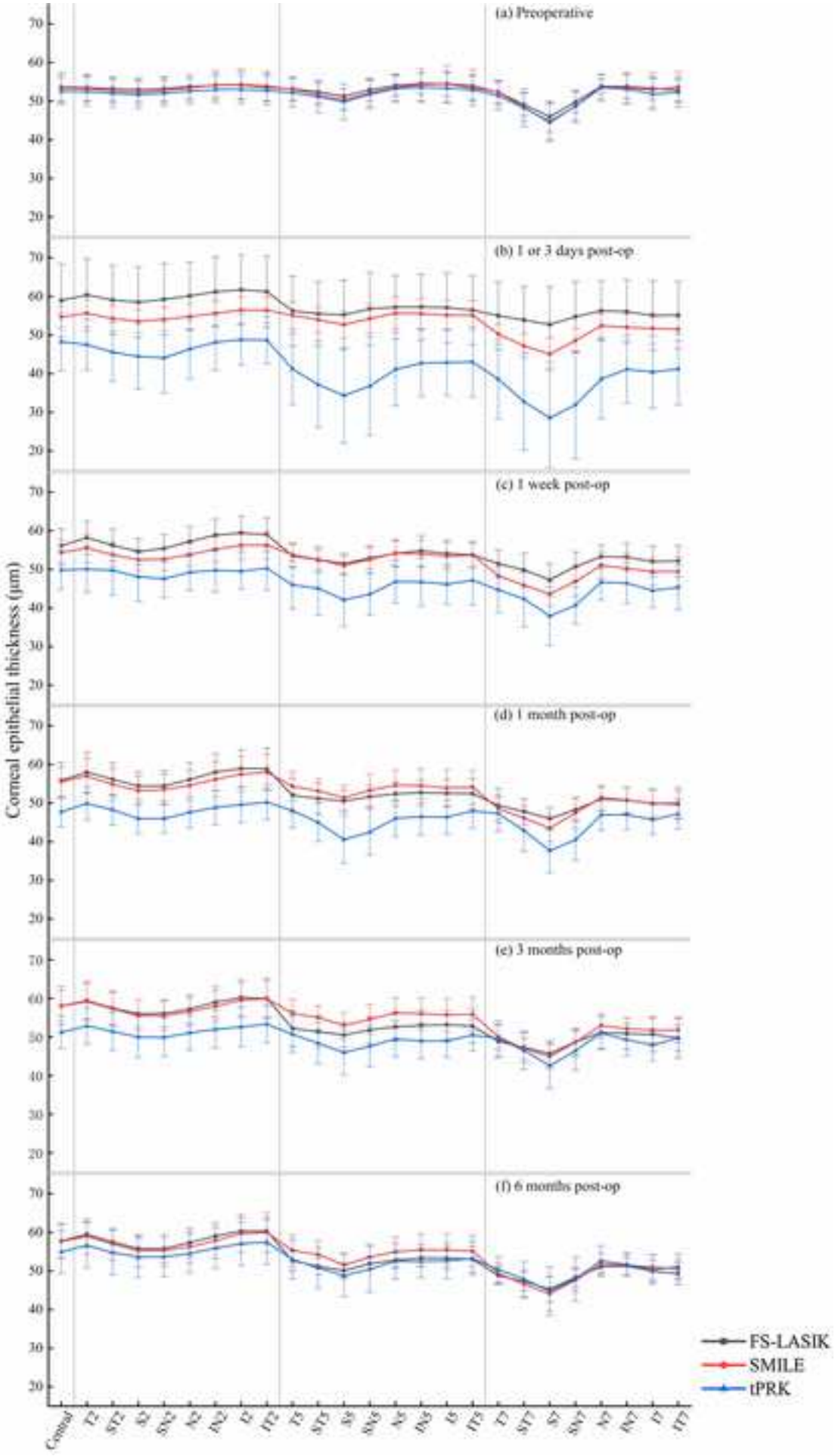

Figure 2

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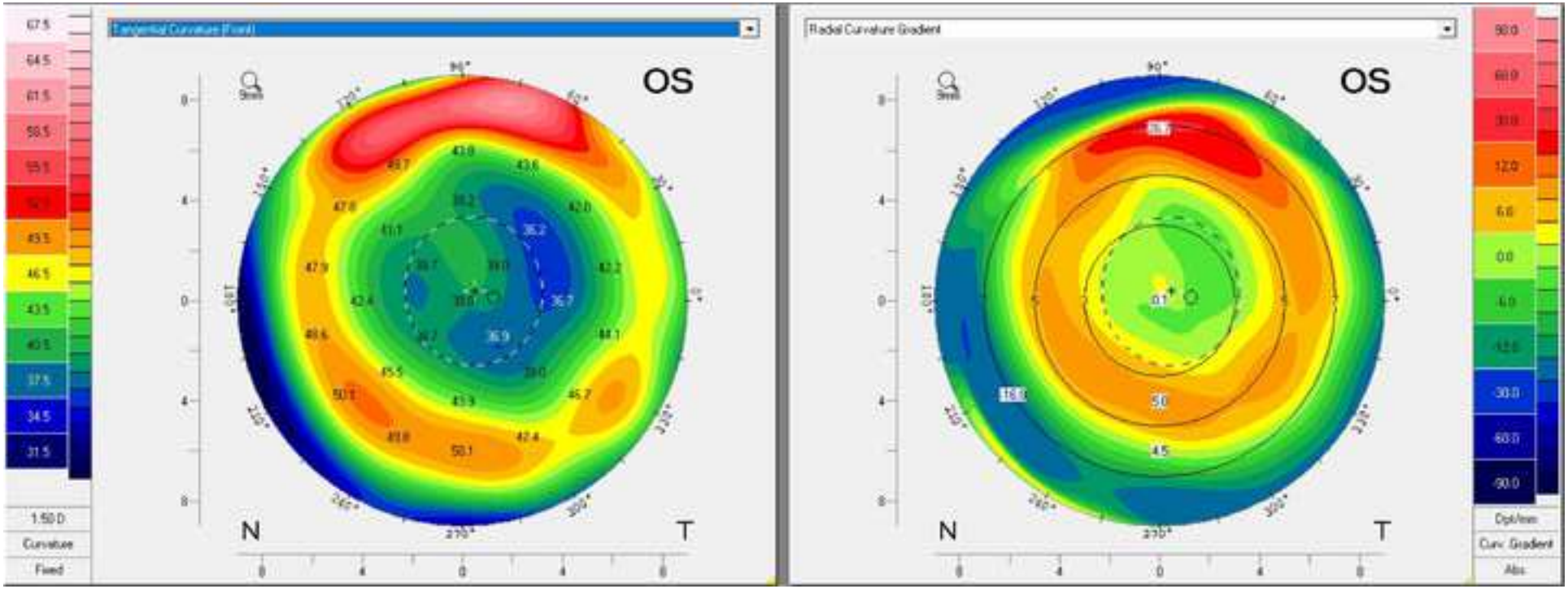

Figure 3

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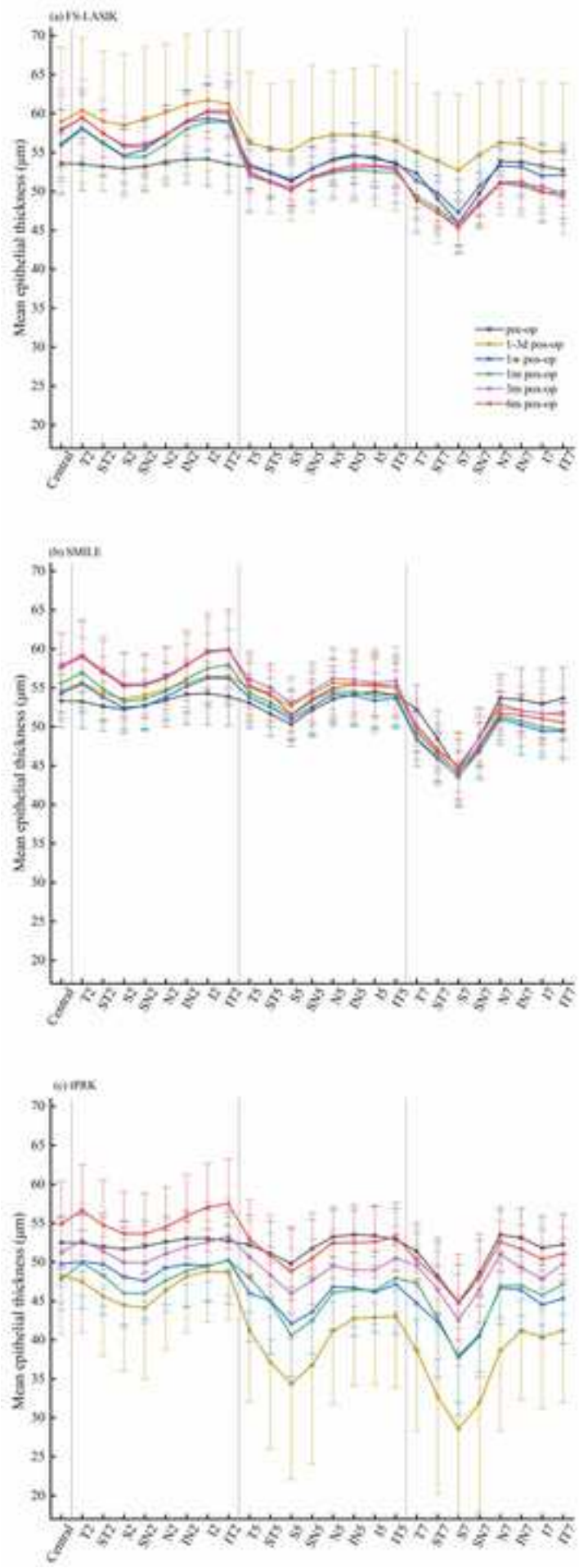

Figure 1