·Original article·

Assessment of stromal interface quality after femtosecond laser-assisted lamellar cuts in cat cornea

Tao Zhang[1], Shao-Wei Li[1,2], Jing-Liang He[1], Yan-Wei Kang[1], Shuai Shi[2], Wei Li[2]

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[1]Department of Ophthalmology, Aier School of Ophthalmology, Central South University, Changsha 410083, Hunan Province, China

[2]Department of Ophthalmology, Beijing Aier-Intech Eye Hospital, Beijing 100021, China

Correspondence to: Shao-Wei Li. Panjiayuan Plaza, No. 12 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China. shaoweili2005@vip.163.com

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飞秒激光辅助的猫角膜基质板层切割界面光滑度的研究

张　涛[1],李绍伟[1,2],何景良[1],康艳伟[1],石　帅[2],李　巍[2]

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(作者单位:[1]410083 湖南省长沙市中南大学爱尔眼科学院眼科;[2]100021 北京市北京爱尔英智眼科医院眼科)

作者简介:张涛,中南大学,博士研究生,主治医师,研究方向:角膜病、白内障

通讯作者:李绍伟,青岛大学,博士研究生,主任医师,研究方向:角膜病、白内障. shaoweili2005@vip.163.com

摘要

目的:评估飞秒激光 FS200 在不同模式下切割猫角膜基质板层界面的光滑度。

方法:新鲜的猫眼球 20 只,随机平均分为 4 组(A、B、C、D 组),分别在不同的模式下进行飞秒激光角膜基质板层切割:A 组为常规切割组:负压吸引环吸引、角膜完全压平,压平直径约为 13mm;B 组不使用负压吸引环、角膜完全压平,压平直径约 13mm;C 组负压吸引环吸引、角膜压平直径约为 8mm;D 组不使用负压吸引环、角膜压平直径约为 8mm。采用 5 分制的评分标准,对切割后的猫角膜基质界面的电子显微镜扫描照片进行评分,以评估切割后的角膜基质界面的光滑度。

结果:裂隙灯显微镜图片显示出四组角膜基质界面光滑度的结果,D 组最光滑,A 组最粗糙。30 倍扫描电镜照片的评分结果显示 D 组光滑度明显优于 A 组(P=0.007),D 组光滑度明显优于 B 组(P =0.007),D 组光滑度明显优于 C 组(P =0.016)。100 倍电镜照片评分结果显示 D 组光滑度明显优于 A 组(P =0.01),D 组光滑度明显优于 B 组(P =0.016)。其余各组间差异无统计学意义。

结论:在无负压环吸引且压平面积小的模式-对角膜组织的压缩变形较小的切割状态下,飞秒激光辅助的猫角膜基质后板层切割界面的光滑度可得以部分改善。

关键词:飞秒激光,角膜基质后板层界面,电子显微镜,猫角膜,压平锥

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Abstract

• AIM: To assess posterior corneal stromal interface (PCSI) quality after FS200 femtosecond laser (FSL) lamellar cuts were applied in different patterns in cats.

• METHODS: A total of 20 fresh cat eyeballs were randomly separated into 4 groups: Group A, the routine (control) group, cuts were made using a suction ring and complete corneal applanation within an approximate diameter of 13 mm; Group B, no suction ring was used, but complete corneal applanation was performed using an approximate diameter of 13 mm; Group C, a suction ring was used, and corneal applanation was performed using an approximate diameter of 8 mm; and Group D, no suction ring was used, and corneal applanation was performed using an approximate diameter of 8 mm. Scanning electron microscopy (SEM) images of the resulting PCSI were graded for ridges and roughness using a subjective 5-point grading scale.

• RESULTS: Photography performed using a slit lamp microscope showed that the best PCSI was achieved in Group D, and the worst group was Group A. SEM images (×30 magnification) indicated that the macroscopic interface quality was significantly different between Group D and Group A (P=0.007), between Group D and Group B (P=0.007), and between Group D and Group C (P=0.016). Other SEM images obtained at ×100 magnification indicated that the grades for the microscopic surface quality between Group D and Group A (P=0.01) and between Group D and Group B (P=0.016) were significantly different. The grades of the other groups were not significantly different.

• CONCLUSION: The quality of PCSI on the cat corneas can be partially improved if the deformation of the extruded corneal stroma is slight without using suction ring or an excessive corneal applanation scope by the applanation cone.

• KEYWORDS: femtosecond laser; posterior corneal stromal interface; scanning electron microscope; cat cornea; applanation cone

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INTRODUCTION

Lamellar keratoplasty has been accepted as a superior treatment option for anterior stromal disease with a low rejection rate[1] and low intraoperative risk[2]. But the corneal stromal interface that is achieved by manual dissection is not smooth enough[3]. Performing deep anterior lamellar keratoplasty (DALK) may produce a smooth interface and also presents more challenges. Meanwhile, that will result in a long learning curve[4] and there is the possibility that the corneal descemet membrane could be perforated[5-7]. Therefore, for middle-shallow corneal stromal disorders, femtosecond laser-assisted lamellar keratoplasty is considered to be more secure and reliable[8]. In 2008, Soong *et al*[9] reported a case series of lamellar keratoplasties that were performed using a femtosecond laser that showed the effectiveness and safety of femtosecond-assisted lamellar keratoplasty. Precisely controlling the depth and configuration of a femtosecond laser cut can create an excellent complex donor and host wound edge configuration[10-12]. However, the irregular PCSI that are made by femtosecond laser-assisted cuts remains a problem and is likely the result of excessively changing of the corneal curvature to achieve an applanation effect and the use of an inappropriate femtosecond laser setting[13-14] Some studies have indicated that adjustments of the femtosecond laser setting just can partly improve the quality of PCSI[15-16]. However, these approaches got only limited success.

According to our clinical experience, we found that modifying the femtosecond laser setting indeed had a limited effect on the improvement of PCSI quality, and the irregularity of the PCSI was attributed to the serious compressive deformation of the corneal posterior stroma that resulted from the limited expansion by the constraint of the suction ring when the applanation cone placed excessive pressure on the cornea. In accordance with the above-mentioned hypothesis, we designed and launched a laboratory research study and altered the scope of the corneal expansion and the corneal applanation to assess the PCSI quality following the application of femtosecond laser-assisted posterior corneal stromal cuts on cats and also to validate our hypothesis.

MATERIALS AND METHODS

Eye Preparation A total of 10 cats were procured from Beijing Jin Mu Yang Biotechnology Company. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Aier School of Ophthalmology of Central South University. The 20 freshly enucleated eyeballs of these cats were randomly distributed into 4 groups consisting of 5 eyes each: Group A, as the routine group, was cut with a suction ring and using complete corneal applanation with an approximately 13 mm diameter; Group B was cut without a suction ring and with complete corneal applanation with an approximately 13 mm diameter; Group C was cut with a suction ring and with corneal applanation with an approximately 8 mm diameter; and Group D was cut without a suction ring and with corneal applanation at an approximately 8 mm diameter. The eyes were used within 24h of each animal's death. Before femtosecond laser cuts were made, the central corneal thickness was measured using an ultrasound pachymeter (SP-3000, Tomey, Nagoya, Japan).

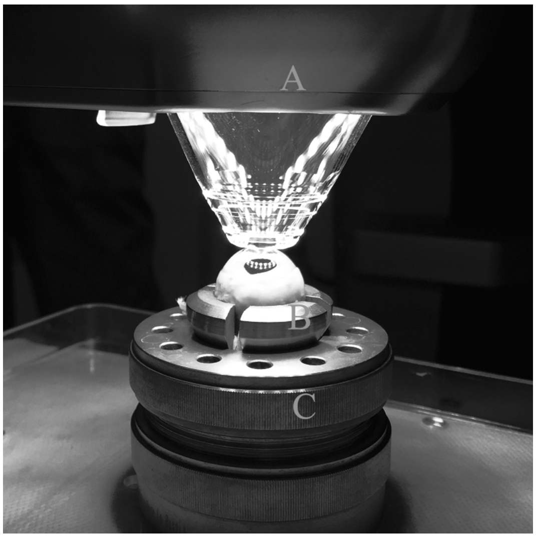

Figure 1 The new holder for cat eyeball and femtosecond laser applanation cone (A). At the top of holder, four arms (B) hold the eyeball to make its proper position and pressure. As the rotation of top rounding screw (C) of the the holder, the strength of arm of the holder will be adjusted in order to control the pressure of the eye ball.

Femtosecond Laser Settings and Deep Lamellar Cuts A FS200 femtosecond laser were used in all groups with the same settings. An anterior side cut was created at a 90 degree angle at a depth of 400 μm (energy, 2.4 μJ; spot separation and line separation, 4 μm), and anterior full lamellar cuts were made at the same depth (diameter, 7.5 mm; raster energy, 1.5 μJ; spot and line separation, 6 μm and 7 μm). The whole globe was then placed on a customized holder (Mediheica, Vostanya str, Kazan Russia, Figure 1) and pressurized to achieve intraocular pressure (approximately) by adjusting the tightness of the eyeball holder. The suction ring was tightly placed on the corneoscleral limbus to establish negative pressure. The pipeline of the suction ring was clamped in the groups without suction. We then adjusted the intraocular pressure in accord with the requirements of the emission of the femtosecond laser by adjusting the eyeball holder. Digital photographs were taken through using a slitlamp microscope after the lifting of the cap was performed for each eyeball. The corneal stromal surface quality of each eye was graded using SEM. The specimens used for the SEM procedure were fixed in 3% glutaraldehyde.

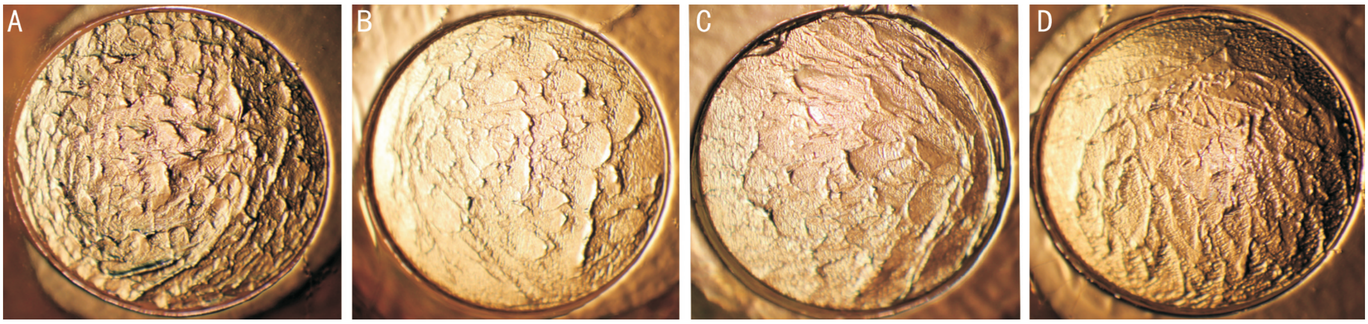

Figure 2 Slit lamp photographs of the corneal stromal surface The best PCSI was achieved in Group D, and worst PCSI was achieved in Group A. Gropu B or Group C was in the intermediate level.

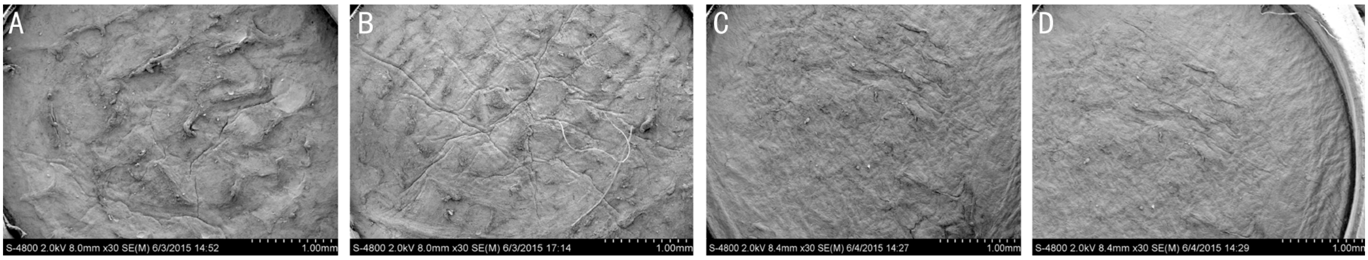

Figure 3 SEM images at ×30 magnification were used to evaluate the severity of the ridges Group A showed the maximum ridges. Group D showed the minimal ridges. The ridges in Group B or Group C was intermediate level compared to the other groups.

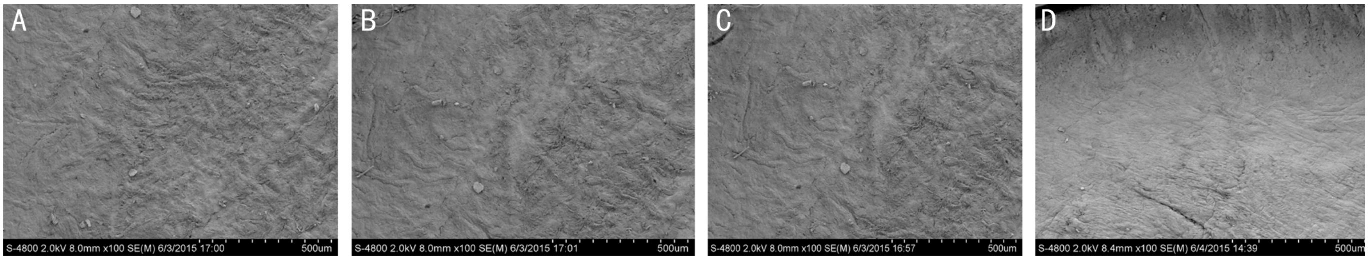

Figure 4 SEM images at ×100 magnification were used to evaluate microscopic roughness Group A showed the worst smoothness. Group D showed the best smoothness. The microscopic roughness in Group B or Group C was moderate level.

Scanning Electron Microscopy After fixation (see above) of the corneal cap and bed specimens, the corneoscleral specimens that were used in SEM were fixed in 2% glutaraldehyde in 0. 1 M phosphate-buffered solution and dehydrated in increasing concentrations of ethanol. They were dried using a t-butyl-alcohol freeze-drying method and coated with osmium. The specimens were viewed using a JSM-6320F scanning electron microscope (Jeol, Ltd, Tokyo, Japan). Four photographs were taken at different randomly selected areas of each sample.

Interface Quality Grading A subjective 5-point grading scale based on SEM results was used to compare the quality of the PCSI. A subjective 5-point integer score (1 = the smoothest samples among all; 2 = next smoothest; 3 = the median group; 4 = rough, but not the worst; 5 = the roughest samples) was used in this analysis. Ridges and roughness were the two indices that were used to assess the quality of the PCSI. SEM images obtained at ×30 magnification that reflected the macroscopic interface quality of the cornea were used to evaluate ridge grades. SEM images were taken at ×100 magnification of each quadrant of each specimen, and these images reflected the microscopic interface quality of the cornea. Mann-Whitney (non-parametric) statistical analysis and Kruskal-Wallis test were performed to compare different groups.

RESULTS

Slitlamp photographs were used to assess the quality of the PCSI immediately after laser cutting and cap lifting were performed. However, quantitative grading was not attempted because only gross ridges were observed in these images. The results showed that the best quality of the PCSI was achieved in Group D, and the worst one was achieved in Group A. Gropu B and Group C were in the intermidiate level. (Figure 2).

There was a statistically significant difference in the the quality of the posterior corneal stromal interface (PCSI) of the different groups at ×30 magnification ($P=0.004$) and ×100 magnification ($P=0.01$). SEM images (×30 magnification) indicated that the macroscopic quality of the PCSI was significantly different between Group D and Group A ($P=0.007$), between Group D and Group B ($P=0.007$), and between Group D and Group C ($P=0.016$) (Figure 3). Other SEM images obtained at ×100 magnification indicated that the grades for the microscopic surface quality between Group D and Group A ($P=0.01$) and between Group D and Group B($P=0.016$) were significantly different. The grades of the other groups were not significantly different (Figure 4).

DISCUSSION

Many studies have indicated that there were several reasons for the irregularity of the quality of the PCSI cut by the femtosecond laser. First, the anatomy of the compact anterior corneal stroma differs from the more loosely separated lamellar fibrils in the posterior corneal stroma. The anterior corneal lamellae have more bridging fibres that can increase shear strength, and these are relatively lacking in the posterior stroma[17]. Accordingly, comparing the anterior corneal stroma, the squeezing by the suction ring and and femtosecond laser cone may have more influence on the posterior corneal stroma. Peyrot *et al*[18] indicated that the percentage of scattered light is a function of corneal thickness, and they showed that scattering increased with increasing tissue thickness. Storing mild oedematous donor corneas in corneal storage medium results in the functional decline of corneal endothelial cells and the perturbation of the structural arrangement of corneal fibrils. These effects result in light scattering and optical aberrations when a laser beam is passed through the tissue and also affect the quality of the PCSI.

According to our laboratory results, SEM images acquired at ×30 and ×100 magnification both showed that the grade of Group D was significantly different from Group A ($P<0.05$). Comparing the processing factor of the two groups, the corneas in group D underwent the minimum compression and deformation of the posterior corneal stroma without suction and with small corneal applanation area, while the corneas in Group A underwent the maximum compression and deformation of the stroma. Meanwhile, the PCSI quality of Group B or Group C is better than Group A , which indicatied that eliminating either of the two processing factors, the PCSI quality will be elevated. Hence, we speculated that femtosecond laser setting was appropriate under excessive compression conditions and also can create a relative smooth PCSI. After the suction ring is released, the cornea will expand and the stromal bed surface will then become rough. Provided that there was no suction ring is used to limit the expansion of the cornea during applanation, the stroma can expand and will not be excessively compressed, resulting in less wrinkling. Therefore, the cut stromal surface will become relatively smooth under these circumstances.

Soong *et al*[9] postulated that the irregularities may result from microscopic circular wrinkles in the posterior stroma that may have been induced by the flattening action of the applanation cone. His viewpoint may also proved that reducing compression of the posterior corneal stroma will improve the quality.

Many specialists have investigated some methods for improving the quality of PCSI cut by the femtosecond laser. Rousseau *et al*[15] compared methods using single path and double path procedures with a femtosecond laser to apply posterior stromal lamellar cuts and verified that the two successive layered cuts made using the double path procedure can create a smoother stromal surface. After the first cut, the posterior stroma became thinner and more capable of bending. This minimised the swelling of the posterior stroma and contributed to stress collagen lamellae. This approach also supported our opinion that less compressive deformation will improve the interface quality of posterior stroma. Some studies have indicated that adjustments to the femtosecond laser setting can improve the quality of the corneal stromal interface instead of achieving a complete resolution[13,19-20]. Because the structure of posterior stroma has been altered by the excessive pressure, these procedures just changed the tiny improvement on the anterior corneal stromal interface. Therefore, we deduced that the excessive deformation of the posterior corneal stroma is induced by the limitation of corneal stromal expansion that results from the constraint of the suction ring and that the large applanation extent of the femtosecond laser cone is the main reason for achieving an unsatisfactory corneal stromal surface quality.

Our study demonstrates that reduction of limit of expansion by the suction ring and applying a smaller applanation area when using a femtosecond laser cone decreased the range of compression of the cornea possiblly will improve the quality of PCSI. We hope that the results of our research will provide theoretical guidance for updates to the use of femtosecond laser -assisted posterior lamellar keratoplasty.

REFERENCES

1 Reinhart WJ, Musch DC, Jacobs DS, Lee WB, Kaufman SC, Shtein RM. Deep anterior lamellar keratoplasty as an alternative to penetrating keratoplasty a report by the american academy of ophthalmology. *Ophthalmology* 2011;118(1):209-218

2 Kawashima M, Kawakita T, Shimmura S, Tsubota K, Shimazaki J. Characteristics of traumatic globe rupture after keratoplasty. *Ophthalmology* 2009;116(11):2072-2076

3 Cleary C, Song JC, Tang M, Li Y, Liu Y, Yiu S, Huang D. Dual laser - assisted lamellar anterior keratoplasty with top hat graft: a laboratory study. *Cornea* 2012;31(7):791-797

4 Shimmura S, Tsubota K. Deep anterior lamellar keratoplasty. *Curr Opin Ophthalmol* 2006;17(4):349-355

5 Den S, Shimmura S, Tsubota K, Shimazaki J. Impact of the descemet membrane perforation on surgical outcomes after deep lamellar keratoplasty. *Am J Ophthalmol* 2007;143(5):750-754

6 Leccisotti A. Descemet's membrane perforation during deep anterior lamellar keratoplasty: prognosis. *J Cataract Refract Surg* 2007;33(5):825-829

7 Rama P, Knutsson KA, Razzoli G, Matuska S, Viganò M, Paganoni G. Deep anterior lamellar keratoplasty using an original manual technique. *Br J Ophthalmol* 2013;97(1):23-27

8 Mosca L, Fasciani R, Tamburelli C, Buzzonetti L, Guccione L, Mandarà E, Balestrazzi E. Femtosecond laser - assisted lamellar keratoplasty: early results. *Cornea* 2008;27(6):668-672

9 Soong HK, Mian S, Abbasi O, Juhasz T. Femtosecond laser-assisted posterior lamellar keratoplasty: initial studies of surgical technique in eye bank eyes. *Ophthalmology* 2005;112(1):44-49

10 Mehta JS, Shilbayeh R, Por YM, Cajucom-Uy H, Beuerman RW, Tan DT. Femtosecond laser creation of donor cornea buttons for Descemet - stripping endothelial keratoplasty. *J Cataract Refract Surg* 2008; 34 (11):1970-1975

11 Sarayba MA, Ignacio TS, Binder PS, Tran DB. Comparative study of

stromal bed quality by using mechanical, IntraLase femtosecond laser 15- and 30-kHz microkeratomes. *Cornea* 2007;26(4):446-451

12 Farid M, Kim M, Steinert RF. Results of penetrating keratoplasty performed with a femtosecond laser zigzag incision initial report. *Ophthalmology* 2007;114(12):2208-2212

13 Serrao S, Buratto L, Lombardo G, De Santo MP, Ducoli P, Lombardo M. Optimal parameters to improve the interface quality of the flap bed in femtosecond laser-assisted laser *in situ* keratomileusis. *J Cataract Refract Surg* 2012;38(8):1453-1459

14 Phillips PM, Phillips LJ, Saad HA, Terry MA, Stolz DB, Stoeger C, Franks J, Davis-Boozer D. "Ultrathin" DSAEK tissue prepared with a low-pulse energy, high-frequency femtosecond laser. *Cornea* 2013;32(1):81-86

15 Rousseau A, Bensalem A, Garnier V, Savoldelli M, Saragoussi JJ, Renard G, Bourges JL. Interface quality of endothelial keratoplasty buttons obtained with optimised femtosecond laser settings. *Br J Ophthalmol* 2012;96(1):122-127

16 Serrao S, Lombardo M, De Santo MP, Lombardo G, Schiano Lomoriello D, Ducoli P, Stirpe M. Femtosecond laser photodisruptive effects on the posterior human corneal stroma investigated with atomic force microscopy. *Eur J Ophthalmol* 2012;22(Suppl 7):S89-S97

17 Bergmanson JP, Horne J, Doughty MJ, Garcia M, Gondo M. Assessment of the number of lamellae in the central region of the normal human corneal stroma at the resolution of the transmission electron microscope. *Eye Contact Lens* 2005;31(6):281-287

18 Peyrot DA, Aptel F, Crotti C, Deloison F, Lemaire S, Marciano T, Bancelin S, Alahyane F, Kowalczuk L, Savoldelli M, Legeais JM, Plamann K. Effect of incident light wavelength and corneal edema on light scattering and penetration: laboratory study of human corneas. *J Refract Surg* 2010;26(10):786-795

19 Monterosso C, Galan A, Böhm E, Zampini A, Parekh M, Caretti L. Effect of 60 kHz and 150 kHz Femtosecond Lasers on Corneal Stromal Bed Surfaces: A Comparative Study. *ISRN Ophthalmol* 2013; 2013: 971451

20 Lombardo M, De Santo MP, Lombardo G, Schiano Lomoriello D, Desiderio G, Ducoli P, Barberi R, Serrao S. Surface quality of femtosecond dissected posterior human corneal stroma investigated with atomic force microscopy. *Cornea* 2012;31(12):1369-1375