Corneal wound healing process following eximer laser photorefractive keratectomy and femtosecond laser assisted cataract surgery measured with modern diagnostic methods

Ph.D. Thesis

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INTRODUCTION

Among clinical anomalies affecting corneal transparency we studied postoperative corneal haze following photorefractive keratectomy and femtosecond laser assisted cataract surgery versus conventional phacoemulsification.

Superficial corneal haze following photorefractive keratectomy

During photorefractive keratectomy (PRK) corneal thinning is achieved with excimer laser photoablation following epithelium removal, performed in a 6 mm diameter central area.
Area of photoablation (treatment area) is in the center of the cornea when myopic procedure is performed, to achieve the decrease of the central corneal power. Paracentral, ring shaped photoablation is performed during hyperopic treatment, to achieve increase of the central corneal power. Transitional superficial scar (haze) formation following PRK can be observed with slit lamp examination in the area of ablation – in the center of the cornea following myopoic surgery, and in a paracentral ring shaped pattern following hyperopic treatment - mainly in the first three postoperative months. This scar formation is mainly the result of keratocyte repopulation, and new abnormally structured extracellular matrix deposition. Staging of corneal haze is based on the slit lamp findings of Hanna in 1992. The main severity stages are:

Grade 0.5: haze can be observed only with extensive examination, doesn’t affect visual acuity.

Grade 1.0: mild haze can be observed with broad tangential illumination, doesn't affect visual acuity.

Grade 2.0: can be observed with narrow slit illumination.

Grade 3.0: can be observed with direct focal illumination.

Grade 4.0: can be observed without a slit lamp.

Clinical importance of haze following PRK that the increase of the number of anterior stromal cells and collagen lamellae results in light scattering that can lead to subjective visual complains and stromal thickening can lead to refractive regression. The incidence of clinically significant corneal haze that can affect visual acuity is about 3%, and

persistent haze can be observed in about 5% of cases 1 year after the procedure. Staging of corneal scars according to Hanna is a subjective, observer dependent method; moreover documentation of haze is possible only with slit lamp photography, where retrospective evaluation can be questionable due to the quality of photographs. Nevertheless number of grade 3 and grade 4 hazes according to Hanna is gradually diminished thanks to the newly developed flying spot technology and the modern, personalized postoperative treatment protocol.
Scheimpflug principle is named after Theodore Scheimpflug, and means that subject, lens and image planes are not parallel, but have an intersection. This is the principle of reaching maximal depth of field with minimal image distortion and to achieve sharp imaging of object plane. Pentacam (Oculus-Pentacam GmbH, Wetzlar, Germany) contains a rotating Scheimpflug camera that creates a Scheimpflug image, where lens density can be objectively measured. We used this densitometry program to measure the density of corneal haze. On the Scheimpflug image there is a position marker line, which is automatically inserted on the apex of the cornea. With modifying the positions of the marker line corneal density (in units from 0-100) of the marked section of the cornea can be read from a densitogram on the right side of the image. The program measures objectively back scattered light from the corneal tissue.

Corneal oedema following femtosecond laser assisted cataract surgery

Femtosecond laser was introduced to clinical practice in 2001, mainly for corneal flap creation during refractive surgery. In 2008 we had the possibility at Semmelweis University to use the technique first in the world to create capsulotomy, lens fragmentation and corneal wounds before phacoemulsification. Femtosecond laser assisted lens fragmentation can separate the lens nucleus to any number of fragments, without the use of phaco energy. Phaco energy can be decrease with 43%, and phaco time with 51% with this technique, this can result in a heat protection of the cornea. Loss of corneal transparency develops when cornea has an excessed hydration from the anterior chamber following phacoemulsification. Endothelial cells play the most important role to prevent corneal oedema. Endothelial cells do not regenerate in vivo, and number of cells from birth decreases with age, about 0.3-0.6% yearly. This number reach 6.3-22.9% following phacoemulsification. Clinical importance of corneal oedema following phacoemulsification is that visual acuity decreases, and under 1000 cells/mm^2 irreversible corneal decompensation can occur. This process can have an especially great importance when surgery is performed on an eye with endothelial cell dystrophy. Scheimpflug imaging gives the possibility to objectively follow-up corneal oedma, with creating corneal thickness map and measuring 3-5-7 mm and total corneal volume. Modern method to examine endothelial cells is non-contact specular microscopy that can measure endothelial cell count, average cell area, polimegathism (variability of cell size) and pleomorphism (variability of cell shape) of endothelial cells without touching the surface of the eye, with an automated image focusing mechanism. Suzuki reported a non-invasive method to evaluate endothelial cell function, the volume stress index (VSI) in 2007. VSI is a parameter that corresponds with the postoperative increase of corneal volume, affecting each endothelial cell. VSI shows not a dynamic change of endothelial cell function, but shows the relationship between the development of corneal edema, and the individual endothelial cell count.

AIMS

Superficial corneal haze following photorefractive keratectomy
or aims were:

To apply the Pentacam Scheimpflug camera to objectively measure subepithelial corneal opacities following PRK, and to analyze applicability.

To analyse the correlation between corneal haze following myopic PRK according to Hanna's scale and according to the Scheimpflug densitometry measurement.

To support with the use of Pentacam Scheimpflug measured corneal densitometry the observation - published previously with other methods of other authors - that development of corneal haze following PRK relates strongly to the preoperative refraction and photo ablation depth.

To support with the use of Pentacam Scheimpflug measured corneal densitometry the observation - published previously with other methods of other authors - that corneal haze following PRK can be related to the postoperative refractive regression.

To analyse the relationship between the densities of peripheral normal corneas and patients' age with the use of Pentacam Scheimpflug densitometry.

Corneal oedema following femtosecond laser assisted cataract surgery

or aims were:

To examine the change in early postoperative central corneal thickness (CCT) and volume stress index (VSI) following femtosecond laser assisted cataract surgery, and to compare the results with the CCT and VSI following conventional phacoemulsification.

To analyse the morphological and numerical changes of central corneal endothelial cells following femtosecond laser assisted phacoemulsification compared to conventional phacoemulsification.

METHODS

Superficial corneal haze following photorefractive keratectomy

For statistical analysis first we graded post PRK corneal haze according to Hanna, than we performed a measurement with the Scheimpflug camera (Oculus-Pentacam GmbH, Wetzlar, Germany). Corneal density was measured on the Scheimpflug image, in the center of the cornea, than 1-2-3-4 and 4.5 mm right and left from the center. Means of right and left measured values were used for statistical analysis.

a.) Cross section study

79 eyes of 140 patients were included in the cross section study. Mean time of the control examinations was 4.5+/-3.8 months postoperatively. Two main groups were created: the myopic group contained 101 eyes of 56 patients and the hyperopic group contained 39 eyes of 23 patients. Both groups were divided to 3-3 different subgroups, according to the slit lamp findings: myopic or hyperopic subgroups of patient's pre operatively, with clear corneas, myopic or hyperopic subgroups of patient's post PRK with

clear corneas, and myopic or hyperopic subgroups of patient's post PRK with various grades of subepithelial haze according to Hanna.

b.) Patient follow-up

90 eyes of 46 patients treated with myopic PRK were included in the follow-up study. Preoperative mean spherical equivalent was -4.75+/-3.75D. Two patient groups were created according to the depth of photoablation: patients treated with less than 100 μm, and patients treated with more than 100 μm ablation depth. Scheimpflug corneal density and slit lamp based grading of corneal density according to Hanna were determined before and 1-3 and 6 months after surgery.

Corneal oedema following femtosecond laser assisted cataract surgery

We performed two different studies, and in both studies 2-2 patient groups were created.

In the "femtolaser" group patients were operated with femtosecond laser assisted phacoemulsification. In the "phaco" group patients were operated with conventional phacoemulsification. In both groups intraoperative phaco time and energy, effective phaco time, and Scheimpflug measured nucleus density were recorded. Biometry (anterior chamber depth, lens thickness, axial length) were performed with a non contact laser interferometer (Lenstar LS900, Haag-Streit AG, Switzerland).

a.) Measurement of changes in central corneal thickness, endothelial cell count and volume stress index

Both the „femtolaser" and „phaco" patient groups contained 38 eyes of 38 patients. Central corneal thickness, volume and endothelial cell count were measured, and VSI was calculated preoperatively and in the early postoperative period (1 day, 1 week and 1 month postoperatively).

b.) Measurement of the morphology of central corneal endothelial cells

Both the „femtolaser” and „phaco” patient groups contained 20 eyes of 20 patients.
During the 3 months follow-up we analysed changes in corneal endothelial cell count and morphology (average cell area, polimegethism and pleomorphism) compared to the preoperative values.

Statistical analysis was performed with the Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA) in all cases. Normality of the data was checked with Shapiro-Wilk W test, and the following statistical tests were used:

- ANOVA Kruskal-Wallis
- ANOVA repeated measures
- Multivariate regression analysis
- Spearman rank correlation
- Student’s t-test

Significance level was $p<0.05$ in all cases.

RESULTS

Superficial corneal haze following photorefractive keratectomy

a.) Cross section study

There wasn’t statistically significant difference between the subgroups regarding age, preoperative spherical equivalent, cylindrical correction and gender distribution neither in the myopic nor in the hyperopic patient groups ($p>0,05$).
Regarding the myopic group: central corneal density was significantly higher in the “myopic post PRK with haze” group (46.2+/-17.2) than in the myopic “post PRK with clear cornea” (29.9+/-8.7) and myopic “pre operative clear cornea” (25.1+/-2.4) groups ($p<0.05$). Maximum density value was measured in the corneal apex. Outside the treatment zone there was no statistically significant difference between the three myopic subgroups regarding mean corneal density values.
Regarding the hyperopic group: corneal density in the area of haze was significantly higher in the hyperopic “post PRK with haze” group (50.2+/-25.8) than in the hyperopic “post PRK with clear cornea” (22.9+/-3.1) and hyperopic “pre operative clear cornea”

(22.8+/-3.2) groups ($p<0.05$). Maximum density value was measured in the 6 mm diameter ring around the center of the cornea in the hyperopic “post PRK with haze” group. Outside the treatment zone there was no statistically significant difference between the three hyperopic subgroups regarding mean corneal density values.

There was a statistically significant decreasing tendency of mean density values in the myopic “post PRK with haze” group from the apex of the cornea to the 3 mm periphery from the apex (Spearman rank: $p<0.001$, r=-0.59).

There was also a statistically significant decreasing tendency of mean density values in the myopic “post PRK with haze” group from the 3mm periphery to the 4.5 mm periphery (Spearman rank: $p<0.05$, r=-0.21).

There was a statistically significant increasing tendency of mean density values in the hyperopic “post PRK with haze” group from the apex of the cornea to the 3 mm periphery from the apex (Spearman rank: $p<0.003$, r=0.4).

There was a statistically significant decreasing tendency of mean density values in the hyperopic “post PRK with haze” group from the 3 mm periphery to the 4.5 mm periphery (Spearman rank: $p<0.003$, r=-0 .47). Cornea density values at the periphery of the cornea, 4.5 mm from the apex were showing a statistically significant positive correlation with patients age in both the myopic and hyperopic groups (Spearman rank: $p<0.05$, r=0.62 in the myopic “pre operative clear cornea”; $p<0.05$, r=0.58 in the hyperopic “pre operative clear cornea” group).

b.) Patient follow-up

There was no statistically significant difference between the two patient groups regarding age and gender distribution. Mean treated diopter was significantly greater in the group treated with the greater photo ablation depth (spherical equivalent, SE=-7.0+/-4.25D) compared the the group treated with the smaller photo ablation depth (SE=-3.25+/-2.13D). Scheimpflug measured corneal density showed its peak value at 1 month postoperatively, than central corneal density decreased gradually to its preoperative value during the 6 months follow-up. Central corneal density of the group treated with more than 100 μm was significantly higher in the first (59.7+/-17.9) and third (46.3+/-12.8) postoperative months than in the group treated with less than 100 μm one (51.1+/-14.6) and three months (41.4+/-13.1) postoperatively. There was no statistically significant difference between the two group regaring preoperative and 6 months postoperative density values (more than 100 μm preoperative = 36.1+/-3.5, less than 100 μm preoperative = 35.1+/-3.7; more than 100 μm postoperative = 36.8+/-6.2, less than 100 μm postoperative = 35.7+/-5.0).

There was a statistically significant correlation between the measured corneal density and the photo ablation depth ($r=0.34$; $p<0.05$) and the corneal density and the preoperative spherical equivalent ($r=-0.36$; $p<0.05$) at 1 month postoperatively.

Scheimpflug measured corneal density showed a statistically significant positive correlation with the slit lamp grade of corneal opacities according to Hanna ($r=0.69$; $p<0.05$). There was a statistically significant negative correlation between the 6 months postoperative refraction corresponding with refractive regression and the Scheimpflug measured corneal density ($r=-0.36$; $p<0.05$).

Corneal oedema following femtosecond laser assisted cataract surgery

a.) Measurement of changes in central corneal thickness, endothelial cell count and volume stress index

There was no statistically significant difference between the groups operated with femtosecond laser assisted phacoemulsification and conventional phacoemulsification regarding gender distribution (18:28; 10:23), age (65.81+/-12.42; 66.93+/-10.99 years), axial length (24.1+/-3.0; 23.9+/-2.9 mm), manifest refraction (-1.0+/-4.7; -1.0+/-5.5D), chamber depth (2.57+/-0.39; 2.62+/-0.45 mm), lens thickness (4.5+/-0.5; 4.4+/-0.5 mm), Scheimpflug measured lens density (2.32+/-0.97; 2.13+/-1.22), endothelial cell count (2861+/-215; 2841+/-215/mm^2) and intraocular pressure (16.0+/-3.2; 15.6+/-2.9 mmHg). Phaco energy was significantly lower in the femtolaser group (14.7+/-8.3%) than in the phaco group (20.4+/-12.6%). Central corneal thickness increased significantly in both groups 1 day postoperatively, but central corneal thickness in the femtolaser group (580+/-42 μm) was significantly lower than in the phaco group (607+/-91 μm). Volume stress index was also significantly lower in the femtolaser group $3.0+/-2.3 \times 10^{-5}$ than in the phaco group ($5.3+/-6.0 \times 10^{-5}$) 1 day postoperatively. Multivariate regression analysis showed a statistically significant influence of preoperative endothelial cell count, Scheimpflug measured lens nucleus density, corneal thickness and patient group on the postoperative corneal thickness. Anterior chamber depth and effective phaco time did not have a statistically significant effect on the postoperative central corneal thickness.

b.) Measurement of the morphology of central corneal endothelial cells

There was no statistically significant difference between the femtolaser and phaco groups regarding male:female ratio (16:4; 13:7), lens nucleus density (2.4+/-1.1; 2.2+/-1.0) axial length (23.5+/-1.67; 24.0+/-3.2 mm) and anterior chamber depth (3.2+/-0.4; 2.6+/-0.6 mm).

There was a significant difference between the two groups regarding diagrams showing endothelial cell count decrease during the postoperative first 3 month: endothelial cell loss was significant in the phaco group, but it was not significant in the femtolaser group. Endothelial cell loss was 3.13+/-3.8% in the femtolaser group and 8.5+/-9.9% in the phaco group ($p<0.05$) 3 months postoperatively.

Increase of average cell area was significant in the phaco group (from 352.6+/-29 to 386.1+/-58.7 μm^2; p=0.01) and was not significant (from 358.3+/-266.5 to 366.5+/-25.6 μm^2; p=0.07) during the 3 months follow-up.

Hexagonality of endothelial cells decreased significantly from its preoperative value in the phaco group (from 46.2+/-7 to 41.6+/-7.2%; p=0.01), but not significantly in the femtolaser group (from 45.5+/-5 to 44.7+/-9%; p= 0.054) during the 3 months follow-up.

Diagrams showing the change in polimegethism were running parallel in the two groups, change in the variability of cell size was not significant in none of the groups.

CONCLUSIONS, NEW RESULTS

Superficial corneal haze following photorefractive keratectomy

1. We applied first in the literature the Pentacam Scheimpflug camera to objectively measure subepithelial corneal opacities following PRK with a cross sectional study and also with a 6 months patient follow-up.

2. We described first in the literature that corneal haze following myopic PRK according to Hanna's scale shows a statistically significant positive correlation with the Scheimpflug measured corneal density.

3. We supported first in the literature with the use of Pentacam Scheimpflug measured corneal densitometry the observation - published previously with other methods of other authors - that development of corneal haze following PRK relates strongly to the preoperative refraction and photo ablation depth, with the use of Pentacam Scheimpflug measured corneal densitometry.

4. We supported first in the literature with the use of Pentacam Scheimpflug measured corneal densitometry the observation - published previously with other methods of other authors - that corneal haze following PRK can be related to the postoperative refractive regression.

5. We published first in the literature the relationship between the densities of peripheral normal corneas and patients' age with the use of Pentacam Scheimpflug densitometry.

Corneal oedema following femtosecond laser assisted cataract surgery

6. We examined first in the literature the change in early postoperative central corneal thickness (CCT) and volume stress index (VSI) following femtosecond laser assisted cataract surgery, and we described, that CCT and VSI is significantly

less 1 day after femtosecond laser assisted phacoemulsification compared to conventional phacoemulsification.

7. We examined first in the literature the morphological and numerical changes of central corneal endothelial cells following femtosecond laser assisted phacoemulsification and we described, that endothelial cell count decrease and average cell area increase is less remarkable following femtosecond laser assisted phacoemulsification compared to conventional phacoemulsification, 3 months after surgery.

8. We described that 3 months diagrams showing pleomorphism and polymegathism are similar in the two groups. While hexagonality of endothelial cells decreased significantly following conventional phacoemulsification, this change was not significant following femtosecond laser assisted phacoemulsification.

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