THE ROLE OF EXTRACELLULAR MATRIX IN
CORNEAL WOUND HEALING

PH.D. THESIS

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INTRODUCTION

Regular organisation of the extracellular matrix (ECM) is essential in the transparency and refractive index of the cornea. Corneal wound healing is different from other tissues of the human body because of its unique avascular and bradytrop structure. The main goal of corneal wound healing is the preservation of transparency. Relative dehydration of the cornea is necessary in transparency, which is provided by the intact epithelium, imbibation pressure of the extracellular matrix and the active endothelial pump.

In transparent corneas, water content of the cornea is binded to the ECM. Keratocytes ensure the relative steady state of the ECM composition, thus transparency. Main components of the ECM are the collagen fibres, and the equal interfibrillar distance is determined by proteoglycans. Proteoglycans are composed of glucosaminoglicans (GAG) attached to polypeptide chains. GAGs consists of disacharide monomers. Among disacharide monomers, in the cornea the most important is keratan sulfate (KS). Besides collagens and proteoglycans, ECM contains lipids as well. Composition of the ECM can change during degenerative disorders and as the result of surgical corneal intervention on the cornea.

In degenerative disorders, deposits occur in the cornea leading to the loss of corneal transparency. The most frequent corneal degeneration in the age over 60 years is arcus lipoides (also called corneal arcus, arcus senilis or gerontoxon), when lipid content increases, extracellular lipid deposits (cholesterine, phospholipid, triglyceride) accumulate. The changes of ECM and deposits modify corneal wound healing, which can be observed after cataract surgery.

After injury or refractive surgery (photorefractive keratectomy – PRK, Laser in situ keratomileusis – LASIK) transient loss of integrity in intact cornea can be seen, which is partly the consequence of extracellular – and in some cases clinically detectable –abnormalities. Biochemical changes of the ECM results in the decrease of dehydration, the water content, thus thickness of the cornea increases, and transparency diminishes.
OBJECTIVES

Aim of our work was to investigate the role of extracellular matrix in the healing of iatrogenic wounds, in healthy corneas and corneas with degenerative disorders after iatrogenic corneal. Clinical diagnostic methods on human eyes and histopathological methods on animal model were planned.

1. The impact of arcus lipoides on corneal wound healing after cataract extraction

In corneal degeneration, such as arcus lipoides extracellular lipid deposits accumulate in the cornea. Our aim was to analyze the – clinically detectable – effects of lipid deposition on the corneal wound healing.

- To follow up keratometric astigmatism after extracapsular cataract extraction through a 9.0 mm long sclerocorneal incision in a rare, unilateral case of corneal arcus associated with ptosis.
- Based on the results of the above mentioned case report. A prospective clinical study was planned to examine the role of ECM changes due to arcus lipoides in corneal topography parameters after corneal incision phacoemulsification.

2. Ultrasound pachymetric evaluation of corneal edema in LASIK (human clinical study)

Early post-LASIK visual acuity is determined by transient corneal edema, which is parallel with the increase of the central corneal thickness.

- To investigate the dynamics of corneal edema development.
- Applying intraoperative measurement of the corneal thickness the assessment of corneal edema was planned.
- To examine the changes of corneal thickness after LASIK during wound healing period. When does stromal bed and flap edema start and how long does it persist?
3. Spatial distribution of GAG and KS after PRK (animal model)

Excimer laser photoablation of the corneal surface results in the desintegration of the ECM at the expense of transparency and visual acuity. Our purpose was the examination of spatial distribution of GAG and KS (main components of the corneal ECM) in the following aspects:

- How does the distribution of GAG and KS change in the rabbit cornea after PRK?
- Which layers of the corneal stroma are involved in the changes?
- Are GAG and KS alterations in correlation with epithelial regeneration, inflammation or apoptosis and proliferation of the keratocytes?
MATERIALS AND METHODS

1. The impact of arcus lipoides on corneal wound healing after cataract extraction

Effects of extracellular lipid deposition on the corneal surface and wound healing was examined in a case report and a prospective clinical study.

Case report. A case of unilateral arcus lipoides associated with unilateral ptosis was followed up by TMS-2 (Tomey, Erlangen, Németszág) corneal topography: simulated keratometry (SimK) values were measured after extracapsular cataract extraction and suture removal. Possible pathomechanical correlation between ptosis and corneal arcus was evaluated.

Prospective clinical cornea topographic study. On forty eyes of 40 patients uncomplicated phakoemulsification was performed through a 3.2 mm clear corneal incision at 12 o’clock position. Corneal wound was enlarged to 4.0 mm, then a posterior chamber lens (PCL) was implanted in the capsular bag. Eyes were classified according to the circumferential extension of corneal arcus: Group 0. (n=6, age: 56.3 +/- 4.8 year) no arcus lipoides, Group 1. (n=18, age: 70.1 +/- 12.0 year) arcus lipoides ≤180°, Group 2. (n=16, age: 71.9 +/- 7.4 year) arcus lipoides >180°. Corneal topography (TMS-2, Tomey, Erlangen, Germany) was carried out on the preoperative day, postoperative days 1 and 10, and at month 1 and 3. Main outcome measures: keratometric cylinder (Dcyl), surface regularity index (SRI), surface asymmetry index (SAI), potential visual acuity (PVA). Data were statistically analyzed applying the Wilcoxon and Mann-Whitney non-parametric tests (p<0.05) using the SPSS (version 10.0 for Windows; SPSS Inc., Chicago, USA) software.
2. Ultrasound pachymetric evaluation of corneal edema in LASIK (human clinical study)

Corneal thickness represents corneal edema. By ultrasonic corneal thickness measurements the hydration changes during and after LASIK were evaluated. On twenty-one myopic (28.9+/−8.6 year; -8.40+/−2.40 D) and nine hyperopic (32.7+/−10.7 year; +5.06+/−0.87 D) eyes LASIK was performed combined with ultrasound pachymetry (Humphrey Model 850 San Leandro, CA, USA). Corneal LASIK-flap was created with the Moria CB Microkeratome (Moria SA, Antony, France) set to 130 μm. Photoablation of thickness depending on the refractive error was performed applying the Zeiss Meditec MEL 70_G-Scan excimer laser (Asclepion-Meditec GmbH, Jena, Germany), then flap was replaced.

Ultrasound pachymetry was carried out: preoperatively, after flap creation, after photoablation, after replacing the flap (5 minutes), on days 1 and 5 and at month 1 and 6. Data of three measurements were evaluated.

**Directly measurable parameters:**

- preoperative central corneal thickness (CCT)
- stromal bed thickness (CST) after flap creation
- CST after photoablation
- CCT after flap replacement
- postoperative CCT (day 1, day 5, month 1, month 6)

**Calculated parameters:** Calculated by subtraction pachymetry from directly measurable parameters:

- calculated flap thickness = preoperative CCT − CST after flap creation
- calculated photoablation thickness = CST after flap creation − CST after photoablation
- predicted CCT = preoperative CCT − predicted photoablation depth
- Edema = measured CCT − predicted CCT at all timepoints.

**Statistics.** Statistical analysis with the SPSS (version 10.0 for Windows; SPSS Inc., Chicago, USA) software was performed using analysis of variances (ANOVA), dependent sample t-test and Pearson correlation analysis (p<0.05).
3. Spatial distribution of GAG and KS after PRK (animal model)

A rabbit modell was used for histopathological detection of the distribution alterations in keratan sulfate in the corneal ECM. Myopic PRK (-6.0 D) was performed on the right eyes of 37 pigmented rabbits. After manual deepithelisation an area of 6.0 mm diameter and centrally 82 µm thick tissue was photoablated applying the Aesculap Meditec MEL 70G-Scan flying-spot argon-fluoride excimer laser. Cornea was examined in 8 groups (4-5 rabbits per group) according to the time between PRK and enucleation: 4 hours, day 1, 4, 7, 14, 28, 56 (2 months), 112 (3 months) and 208 (7 months). Left eyes served as a control group. Corneas were examined by the following methods:

- **Histology**: hematoxylin-eosine, PAS, trichrom, alcian-blue (GAG detection)
- **Immunohistochemistry**:
  - monoclonal anti KS-antibody (Seikagaku Corporation, Tokio, Japan) to detect KS
  - DAPI (diamino phenylindrol dihydro chloride, Vector Laboratories Inc., Burlingame, CA, USA) nucleus staining.
  - PCNA (proliferating cell nuclear antigen, DAKO, Glostrup, Denmark) for proliferating epithelium
  - TUNEL-test (terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick-end labelling; Apoptag, Q-Biogene, Strasbourg, France) for keratocyte apoptosis.
  - Ki67 (DAKO, Glostrup, Denmark) for proliferating keratocytes
- **Measurements**: Distances were measured by the ocular micrometer of the light and fluorescent microscope:
  - Epithelium thickness.
  - Central stromal thickness (CST).
  - Anterior and posterior stromal KS-positive layers
  - Depth of infiltration (in percentage of the CST in the anterior stroma).
- **Calculations**:
  - Keratocyte density (500x1000µm area),
  - Apoptotic keratocyte density (high power field)
  - Proliferating keratocyte density (high power field)
  - Inflammatory cell density (high power field)
- **Statistics**: Statistica 6.0 program (Statsoft Inc, Tulsa, Okla) application of independent samples t-test (p<0.05).
RESULTS

1. The impact of arcus lipoides on corneal wound healing after cataract extraction

After 9.0 mm corneoscleral incision, 12 D corneal astigmatism developed, later decreased to 10 D, after suture removal further decreased to 1.8 D in 2.5 years. Delayed normalisation of corneal astigmatism and modified wound healing was observed in corneal arcus. Potential role of congenital ptosis is due to local hyperthermy, increased circulation and vascular permeability leading to excessive lipid deposition in the corneal stroma.

In our prospective study after clear corneal incision, corneal astigmatism in group 2 was higher at day 1 (3.1+/-1.9D) at day 10 (2.7+/-.21D) than preoperatively (1.8+/-1.9D; all cases p<0.05, Wilcoxon-test) and from control values (preop.: 1.3+/-0.7D; 1 nap: 1.2+/-0.4D; 10 nap:1.1+/-021D; all relations p<0.05, Mann-Whitney-test). SRI, SAI and PVA values were not markedly influenced by arcus lipoides. The 4.0 mm superior clear corneal cataract incision is a safe method for cataract surgery in arcus lipoides. Such a procedure does not appear to induce more corneal astigmatism, surface irregularity, or surface asymmetry in eyes with arcus lipoides as compared to normal corneas. The wound healing at 3 months was independent of the grade of arcus lipoides in respect of corneal topographic results, although the stabilization of the corneal surface did show a slower tendency. In higher grade corneal arcus an incision larger than 4.0 mm is not favourable.

2. Ultrasound pachymetric evaluation of corneal edema in LASIK (human clinical study)

Significant difference between predicted and calculated photoablation depth was found in myopic eyes (dependent sample t-test p=0.018), but linear correlation (Pearson, R=0.725, p=0.001). Thickness difference can be explained by corneal edema, which appeared to be 55.4+/-48.1 µm after photoablation (dependent sample t-test p<0.01). After flap replacement edema comprised 98.3+/-49.7 µm (dependent sample t-test p<0.01), than reduced to 29.8+/-30.4 µm at day 1 (dependent sample t-test p=0.02). Significant (dependent sample t-test p=0.19) thickness difference (16.7+/-17.7 µm) disappeared only 5 days after LASIK. In hyperopic eyes, no such correlation could be identified due to the geometry of photoablation.
Ultrasound pachymetric results revealed that LASIK performed on healthy corneas leads to the accumulation of water in the extracellular matrix. Edema appears already intraoperatively: at photoablation and after flap replacement, and disappears only at postoperative day 5. Presence of intraoperative edema – especially in cases with prolonged or complicated flap creation – can worsen predictability of photoablation and thus might lead to undercorrection of myopia.

3. Spatial distribution of GAG and KS after PRK (animal model)

In control corneas homogenous GAG distribution by alcian-blue staining was found in the stroma. Accumulated GAG was detected in the anterior third of the stroma on postoperative days 7 to 28. No significant difference in GAG distribution was found later in the follow up period.

Two characteristic patterns of KS-staining could be differentiated in the corneal stroma: a. lamellar and b. granular, irregular. In controls only lamellar pattern was detected, mainly in the posterior stroma. Three phases of changes in KS distribution were described:

**Phase 1. [4 hours – 14 days (2 weeks)]:** In the first phase only in the anterior corneal layers were involved: lamellar structure was desintegrated in the early postoperative period, granular, irregular, foam-like KS-staining appeared. Thickness of the anterior KS-positive stroma layer peaked at day 1 (200±/-35 µm).

**Phase 2. [14 days (2 weeks) – 56 days (2 months)]:** In the second phase, both anterior and posterior stroma were affected. In the anterior stroma irregular KS was gradually replaced by lamellar pattern of KS-staining. From day 7, thickness of posterior, lamellar pattern KS-positive layers increased, and appeared the thickest at postoperative day 28 (100±28 µm).

**Phase 3. [56 days (2 months) - 208 nap (7 months)]:** In the last phase, no significant change was noted in the anterior stroma, lamellar KS, described in phase 2 was still detectable until day 112, but absent at the end of follow up (208 days). In the posterior stroma however, the KS-positive stroma thickness did not differ from control (independent sample t-test, p=0.654) at day 112 (month 3).

Proliferating (PCNA positive) epithelial cells were found 4 to 14 days in the central cornea. Density of keratocytes dropped 4 hours and 1 day (22+/−18) after PRK, on day 7 (58+/−32) was not different from controls (62+/−20), then on day 14 (82+/−40) and day 28
(75+/-24) was greater than in controls. On postoperative days 1 to 14 keratocyte apoptosis (1-5-2-5 cell / 5 high power field) and proliferating (Ki67-positive) keratocytes (4-6-7-6 cell / 5 high power field) were simultaneously observable.

Inflammatory cells appeared 4 hours after PRK on the anterior surface of the corneal stroma, later invaded anterior stroma reaching the maximum of 30 cells / high power field at day 4. Cellular inflammatory reaction lasted until day 14.

Our results pointed to the fact, that excimer laser surface ablation induces extracellular changes in the posterior stroma as well. Large amount of degraded KS accumulate in the early postoperative period in the anterior stroma, which later transforms into lamellar pattern parallel with the activation of keratocytes. In the posterior stroma, after apoptosis and proliferation of keratocytes, pattern of KS remained lamellar, but accumulated.
CONCLUSION AND CLINICAL RELEVATIONS

1. Clear corneal cataract incision is a safe method for phakoemulsification in arcus lipoides in context of corneal surface, but delayed stabilization of corneal topographic parameters (SAI, SRI és PVA) can be expected. In higher grade corneal arcus an incision larger than 4.0 mm is not favourable because of the risk of high surgically induced corneal astigmatism.

2. By the application of ultrasound pachymetry in LASIK, corneal flap and stromal bed edema can be detected already intraoperatively and persists until postoperative day 5. Intraoperative edema can lead to the decrease of photoablation depth, thus undercorrection of the myopic refractive error.

3. Keratan sulfate distribution was described after PRK in the rabbit cornea. We have demonstrated, that excimer laser surface ablation results changes in the deeper stromal layers.

4. In the anterior stroma 4 hours after PRK irregular staining of keratan sulfate appears, which transforms into lamellar staining from day 14, later disappears. In the posterior stroma the lamellar keratan sulfate staining – which is characteristic to controls – peaks at postoperative month 1 in the rabbit.

5. Apoptosis and proliferation of the keratocytes have been demonstrated in the postoperative days 1-14., simultaneously with the inflammatory reaction. Changes of the extracellular matrix in the rabbit starts earlier and normalisation lasts longer than the cellular reactions.
PUBLICATIONS

Articles in relation with the thesis


Other articles


4. Popper M, Resch M, Süveges I: Results of LASIK with Topography Supported Customized Ablation (TOSCA) on eyes with a high degree of myopia and irregular astigmatism previously treated with perforating keratoplasty. Ophthalmologica Hung. 2003;140:21-25.


Citable abstracts in relation with the thesis


Other citable abstracts


Presentations in relation with the thesis


Other presentations

3. „Videorefraktiva” – Comments on 4 liver refractive operations (MSZT 2003. Budapest)