Studies on ophthalmic surface laser surgery and corneal regeneration following excimer laser treatment

Ph.D. Thesis

A cornea felszíni-lézersebészet körülményeinek és a cornea regenerációjának tanulmányozása excimer-lézer kezelés után

Egyetemi doktori értekezés

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1. Background

The use of laser technology in the medical practice has developed rapidly. Excimer laser, for instance, has gained widespread applications in ophtlalmologic surgery worldwide and found its way into the ophtlalmologic practice in Hungary in the last decade. By now several thousands of corneal laser treatments are done in one year in Hungary alone for photorefractive (PRK) and therapeutic (PTK) purposes.

I had the privilege to be part of the first Hungarian study group, including laser physicists, Zsolt Bor, Gábor Szabó, Béla Rácz and Béla Hopp (Deptartment of Optics and Quantum Electronics) and ophthalmologist colleagues, Ildikó Süveges, Judit Mohay and Ágnes Füst (Department of Ophthalmology), which formed in 1991 at the University of Szeged. Our group played a leading role in Hungary in studying the physics and biological effects of the 193nm excimer laser in experimental corneal surgery in order to properly set up laser parameters for clinical applications.

My research experience gained in animal models could later be extended to the clinical practice when I spent nearly two years starting in 1993 at the Department of Opthalmology of the Westfalien Willhelms University in Münster. There, my scientific interest was focused on applied reasearch on a large number of patients in connection with the use of the 193nm excimer laser for therapeutic and refractive purposes.

Recently, in a joint project with the Department of Pathology, University of Szeged we have investigated the molecular basis of corneal wound healing in a rabbit model after excimer laser treatment, with particular attention to the direct cell-cell communication through gap junctions. In another cooperation with a research group from the USA we have been studying the circumstances for the clinical applications of a revolutionary new laser type, Nd-glass infrared femtosecond laser, which offers further improvements of the available ophthalmologic laser treatment protocols and chances for introducing new and more efficient treatment techniques.

2. Aims of the dissertation

1. To better understand the biophysical mechanisms of action of the 193nm excimer laser in order to more accurately set up laser parameters for clinical applications and therefore, optimise the efficiency, safety and reproducibility of the laser treatment.
2. To reveal how the changing physiology of the human cornea with age require corrections of the laser parameters in order to achieve the intended ablation rate and by doing so the neccessary myopic correction.
3. To analyse the molecular and cellular mechanisms involved in the corneal wound repair process following excimer laser ablation. To reval the possible role of direct cell-cell communication through gap junctions in normal corneal physiology and in the healing cornea in correlation with

other epithelial cell junctions, epithelial cell proliferation and epidermal growth factor receptor expression.

4. To establish the potential in ophthalmologic surgery of a revolutionary, ultrafast laser type, the Nd-glass femtosecond laser through *in vitro* studies, animal experiments and early clinical trials.
5. To utilise research results of points 1-4 for clinical applications and validate laser treatment protocols by determining their medical efficiency on a large number of patients undergone phototherapeutic or photorefractive laser surgery.

3. Summary

3.1. Studies on the biophysical effects of excimer laser in the cornea

Excimer lasers have been used for corneal surface surgery since 1988, however, the biophysical mechanism of photoablation of the cornea is still incompletely understood. In a team with laser physicists we analyzed and visually dissected the biophysical interaction between individual laser pulses and the corneal surface. A high-speed laser-based photographic arrangement was constructed, with a temporal resolution of <1nsec, which could also be converted to a shadowgraph setup. By utilizing these facilities we could detect the ejection of the ablation plume and the formation of shock waves in the air above the eye caused by individual laser shots. In addition, we could demonstrate, for the first time, high-amplitude surface waves propagated in the targeted corneal surface.

Our further studies were focused on the surgical use of the excimer laser. Laser parameters were tested on *ex vivo* pig eyes and the wound healing process was studied *in vivo* in rabbit eyes. Scanning electron microscopy proved the high spatial accuracy of the 193nm UV laser in pig corneas. In preclinical studies, we found positive correlation between the etching depth and the number of laser shots and between the etching rate and the logarithm of the energy density. Based on these results we could calculate the value of absorption coefficient and the range of the fluence applicable for safe corneal surgery. Further studies, by treating groups of *ex vivo* sheep and lamb corneas or a polymethyl-metacrylate (PMMA) test resin system, showed that: 1. with increased diameter of the ablation zone the ablation rate is significantly decreased, 2. there is no correlation between the repetition rate (up to 30Hz) and the ablation rate when energy density is constant, and 3. there is a positive correlation between the energy density and ablation rate at constant ablation diameter.

3.2. Molecular and morphological studies on the healing rabbit cornea after laser ablation

The visual performance of corneas following excimer laser photorefractive keratectomy is highly dependent on the rapid and co-ordinated rebuilding of its epithelium and stroma which is based on intimate interactions between the cells, matrix and soluble factors involved. There had been only few experimental reports studying corneal regeneration after excimer laser treatment and very limited attention focused on the role of direct cell-cell communication in wound healing. In a series of experiments we investigated the healing process in rabbits by devoting particular attention to gap

junction expression correlated with the rearrangement of other cell junctions, cell proliferation and epidermal growth factor receptor (EGFR) expression. We found two types of gap junctions, connexin43 (Cx43) and Cx26, the latter of which had not been described in the cornea before, appearing as early as in the migrating, highly proliferating epithelium which also expressed high level of EGFR. In untreated corneas Cx-s were localized to different regions of the same basal epithelial cells, Cx26 (β2) junctions were concentrated on the basolateral side while Cx43 (α1) types on the apical cell membranes. Since the two isotypes represent evolutionary different families of Cx-s (Cx43 is α1 and Cx26 is β2) they can not form heteromeric functional channels and they have selective permeabilities to molecules of ~<1kD. Therefore, they may represent alternative pathways for fine tuning direct cell-cell communication between basal epithelial cells.

During wound repair, the expression of both Cx-s were transiently upregulated by appearing in upper cell layers also, which suggested the involvement of direct cell-cell communication in corneal wound healing. Cell proliferation in corneal epithelium is facilitated by growth factor receptor activation including EGFR, particularly upon wound healing. Proliferating cell pool in both unwounded and wounded corneal epithelium had been thought to be restricted to the limbal basal cell region where EGFR expression is also elevated. In our experiments not only epithelial cells adjacent to the wound but those migrating towards and over the wounded area were found to show and excessive proliferation rate and high level of membrane-bound EGFR expression. Mitotic activity in the migrating and pharmacologically not influenced corneal epithelial cells is another important and novel finding in our experiments which is most probably the sign of the excessive demand for new epithelial cells not met by the proliferating limbal stock alone, due to the relatively large size of the wound. Our results showed that gap junctional communication is most probably involved in the regulation of wound repair, and the proliferative capacity of the basal corneal epithelium is highly flexible and it is not restricted to the peripherial areas when robust healing is required.

3.3. Clinical use of excimer laser for phototherapeutic and photorefractive purposes

To improve the efficiency of photorefractive keratectomy (PRK) we monitored the long-term refractive outcome at several hundreds of our treated patients. We found that in patients older than ~40 years the effective ablation rate proportionally increased with age, therefore, the intended correction calculated on the basis of the corneal refraction before treatment may cause overcorrection. As opposed to them, in the younger group up to ~30 years of age, the calculated intended correction may result in undercorrection. These observations may be explained by the decreasing hydration rate of the cornea with age, since there is a negative correlation between the ablation rate of the laser and the hydration of the corneal stroma. In cooperation with a physicist colleague, we elaborated nomogram based on a calculation equation which summarizes these correlations and assist in calculating the value

of necessary compensation. By using this we can effectively reduce the risk of over- and undercorrection.

We also observed, that larger ablation diameter caused less side-effect including halos, and regressions, however, deeper ablation due to the increased diameter may have a risk of haze. We used the excimer laser also for therapeutic purposes to treat superficial corneal diseases in 252 eyes, suffering from recurrent erosion, band keratopathy, map-dot-fingerprint-, crystalline dystrophy, amyloidosis, scars or pterygium. In comparison to PRK, PTK is not a standardized procedure and in many cases only the symptoms are treated. To reduce the risk of hyperopic effect, we modified our earlier surgical strategy for combining the possible largest ablation zone with the most superficial PTK. We also found diclofenac sodium to effectively reduce postoperative pain and the need for systemic analgesics after PTK and PRK without any delay in wound healing and without using a contact lens.

3.4. Animal studies and clinical trials with an ultrafast femtosecond laser

By now the laser-assisted in situ keratomileusis (LASIK), where flap creation preceeds excimer laser ablation of the corneal stroma, has become the most commonly performed refractive surgical technique.

Flap creation with a mechanical microkeratome is the source of most LASIK complications. To overcome this a high repetition-rate femtosecond laser with computer controlled scanning optical delivery system was developed. Femtosecond laser offers clear advantages for flap creation including the flexible adaptation of the size and depth of the flap, the lack of unwanted increase in intraocular pressure. All of these promise better reproducibility and postoperative flap stability, greater clinical safety and less pain and side effects. I have had the privilege to test this revolutinary new laser, for the firs time, in animal studies and later, when FDA approval was granted, in a clinical trial in non-sighted and partially sighted eyes. We could optimize the dissection and surface quality, the side cut and hinge architecture of the flaps produced by femtosecond laser, and succesfully used the Pulsion FS1 instrument for femtosecond laser keratomileusis, IntraLasik, intracorneal ring implantation and intrastromal corneal refractive surgery. The latter intervention is based on the speciality of the ultrafast femtosecond laser which allows to perform surgical cuts in the corneal stroma without any interference with the overlying cell layers.

Novel results in the thesis:

1. In cooperation with laser physicist colleagues we demostrated the biophysical effects of the 193 nm excimer laser pulse on the corneal surface including the surface and shock wave formation as well as the plume emission in the air right above the surface.

2. We found a positive correlation of the ablation rate with both the intended refraction correction and the patients' age. A nomogram based on a calculation equation was elaborated which assisted well in practice in determining the modified correction values and thus avoiding over- and undercorrections.
3. In animal experiments we detected connexin26 type gap junctions in the cornea which had not beed found by others before. It was localized in the basolateral membrane while Cx43, a known isotype, was concentrated on the apical membrane of the same cells. Since the two isotypes belong to evolutionary different families of Cx-s (Cx43 is $\alpha 1$ and Cx26 is $\beta 2$) with selective permeabilities, they may represent alternative pathways for direct cell-cell communication between basal epithelial cells.
4. During wound healing, we, for the first time, detected the transient upregulation of both Cx-s in the form of overlapping relocation which involved the upper cell layers also. This finding clearly suggested the involvement of direct cell-cell communication in corneal wound healing.
5. During wound healing we found evidence that the proliferative capacity of the basal corneal epithelium is highly flexible and it is not restricted to the peripherial areas, as it had been a common view by others, when robust healing is required. All the proliferating cells expressed high level of EGFR protein which might be involved in facilitating cell cycling.
6. We were among the first to test and adjust a new ultrafast femtosecond laser for corneal surgical applications: to create corneal flap for IntraLasik, removable stromal lenticule for Femtosecond Laser Keratomileusis; to make a tunel and entry cut for intracorneal ring implantations and for intrastromal refractive surgery. We found much less intra- and postoperative complications by these operations compared to commercial technics. Wound healing and the regaining of visual acuity were rapid without any pain.

List of full papers on which the thesis is based:

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22. **Ratkay-Traub I**, Ferincz I, Kiss K, Juhasz T, Kurtz R: The first clinical results with femtosecond laser microkeratome. (submitted to J. Refr Surg).

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3. **Ratkay I**, Füst Á, Mohay J, Süveges I, Szabó G, Rácz B, Hopp B, Bor Zs: Scanning electron microscopical study of corneal incisions induced by an excimer laser (II. Annual Congr. Summit International Laser Users, 4-6 Sept, 1992. Montreaux, Switzerland)
4. **Ratkay I**, Süveges I, Füst Á, Bor Zs: Corneal wound healing after photorefractive keratectomy (International Conference on Cornea, Eye Banking and External Diseases, Jerusalem, Israel, June 20-24, 1993. Abstract 157)
5. **Ratkay I**, Süveges I, Füst Á, Bor Zs, Szabó G: Corneal wound healing in rabbits eyes after 193 nm excimer laser ablation (ARVO, Sarasota, FL USA, May 1-6, 1994. Abstract 3488)
6. Förster W, Czernak A, Radda TM, Dangel P, Lunecke C, **Ratkay I**: Myopic PRK with different optical zones (ASCRS, San Diego, CA USA, Apr 1-5, 1995. Abst. 55)
7. **Ratkay I**, Förster W, Czernak A, Radda TM, Busse H: Myopic photorefractive keratectomy with different optical zones (II. Ametropia Kongresszus, Szeged, 1995. ápr. 29, Abstract 42)
8. **Ratkay I**, Förster W, Heide K, Gruchmann T, Grenzebach U, Gerding H: Dependence of the ablation rate on laser parameters in excimer laser surgery of the cornea (ARVO, Fort Lauderdale, FL USA, May 14-19, 1995. Abstract 4895)
9. **Ratkay I**, Förster W, Busse H, Kolozsvári L: Experience with excimer laser phototherapeutic keratectomy (2nd International Conference on Cornea, Eye Banking and External Diseases - Joint Meeting with the IFEB, Prague, Czeh Republic June 26-30, 1996. Abstract 221)
10. Förster W, Czernak A, **Ratkay I**: Stability of refraction in photorefractive keratectomy (PRK) with different optical zones: a brief report (Fifth International Congress on Laser Technology in Ophthalmology, Lugano, Switzerland June 26-29, 1996. Abstract 1087)
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12. **Ratkay I**, Dinnyés M, Ferincz I: Schwind Keratom Magyarországon (SHIOL, Seregélyes, 1997. 04. 18-19)
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14. **Ratkay-Traub I**, Ferincz I, Makarova T, Dinnyés M: Excimer Laser PRK for correction of rest Myopia after RK. (ESCRS 2nd Winter Refr Surg Meeting, Munich, Feb 13-15, 1998. Abst. 50)
15. **Ratkay I**, Ferincz I: Schwind Keratom II-vel nyert tapasztalataink myopia, astigmia és radiális keratotómia után végzett excimer lézeres kezelések kapcsán. (felkért elõadás - I. Magyar Excimer Lézer Szimpozium, Debrecen, 1998. március 6-7)
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17. **Ratkay I**: Radiális keratotómia után végzett excimer lézer kezelések. (SHIOL, Keszthely, 1998. 03. 28, továbbképzõ kurzus - felkért elõadás)
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20. **Ratkay Imola**: A piko- és femtosecundumos laserek szemészeti alkalmazásának lehetõségei. (SHIOL, Keszthely, 1999. 03. 19, továbbképzõ kurzus - felkért elõadás)
21. **Ratkay-Traub I**, Ferincz I, Kiss K, Marchi V, Gualano A, Marchi S, Bertini F, Juhasz T, Kurtz R: A femtosecond Laser Keratome: Preliminary Clinical Results (20th Anniversary World Summer Refr Surg Symposium of the ISRS, Miami, FL USA, July 16-18, 1999. Abstract 59)
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23. **Ratkay-Traub I**, Ferincz I, Kiss K, Kurtz R, Juhász T, Horvath C: Femtosecond lasik: preliminary clinical results (XXIII. International Congr of Ophthalmol Alpe Adria Association, Keszthely, 8-9 October 1999)
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27. **Ratkay-Traub I**: Clinical Experience with a Femtosecond Laser Microkeratome. (Invited Speaker and Moderator for the ISRS World Refr Surg Symp. Dallas, Tx, USA, Oct 19-21, 2000)
28. **Ratkay-Traub I**: The Evolution of the Pulsion FS Laser (Invited Speaker for IntraLase Corporation presents: IntraLasik™ The All Laser Solution™, Dallas Texas USA, 104th AAO, October 22, 2000)
29. **Ratkay-Traub I**: Early experience with the IntraLase Pulsion FS for Creation of Tunnels and Entry Cuts for INTACS (Invited Speaker for IntraLase Corp, 104th AAO, Dallas, Texas USA, October 22-25, 2000)

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9. **Ratkay I**, Grenzebach U, Busse H: Reconstruction of the tarsus function of the eyelids (XIth Congress of the European Society of Ophthalmology, Budapest, June 1-5, 1997. Abstract 955)
10. Gonda Gy, Pastajev N, **Ratkay I**, Makarova T: Nagyfokú myopia epiphakias korrigálása negativ lencsével. (SHIOL, Keszthely, 1998. 03. 26-28, Abstract 51)
11. **Ratkay I**: A LASIK eljárás indikációs területe, a műtét kivitelezése, várható eredmények, esetleges komplikációk. (SHIOL, Keszthely, 1999. 03. 19, továbbképzõ kurzus - felkért elõadás)

12. Hassan Z, **Ratkay I**, Csutak A, Berta A: Our first experiences and results with LASIK in Hungary (XVIIth Congress of the ESCRS, Vienna, 4th-8th September 1999. Abstract 131)
13. **Ratkay I**: Új laserek a kutatásban, fejlesztésben, helyük az orvosi gyakorlatban (MTA Biomedicinális Optikai Albiz, Magyar Orvosi Laser & Optikai Egyesület Symp, Budapest MTA Székház, 1999. 05. 8)
14. **Ratkay-Traub I**: Svyatoslav N. Fyodorov Tribute (Invited Memorandum for 2000 ISRS Annual Awards Dinner, Dallas, Texas, USA, October 20, 2000)

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