Conductive Keratoplasty: Histological Study of Human Corneas

TATIANA L. NAOUMIDI, MD, PHD, IOANNIS G. PALLIKARIS, MD, PHD, IRINI I. NAOUMIDI, PHD, AND NIKOLAOS I. ASTYRAKAKIS, DO

• PURPOSE: **To determine the morphologic changes in human corneas over time following radiofrequency-based conductive keratoplasty (CK) treatment.**
• DESIGN: **Prospective, observational case series.**
• METHODS: **In a single-center study six human corneas of six patients with localized peripheral keratoconus underwent CK treatment followed by penetrating keratoplasty. Three spots were applied in the periphery of each cornea (6 mm optical zone). Corneal buttons were examined with light and electron microscopy at different postoperative intervals up to 6 months post-CK.**
• RESULTS: **In samples assessed on day one post-CK, small areas of detachment between the basal layer of epithelial cells and Bowman's layer were observed. At 1 week after the CK procedure, the epithelium appeared almost normal. Endothelium and Descemet's membrane had no alterations. In all samples, thermally induced misconfiguration of collagen fibers, described as "crumpled" changes of collagen layers, was observed reaching 75% to 80% of the stromal depth. The area of alterations had a cylindrical shape with a diameter of 120 μm.**
• CONCLUSIONS: **The conductive keratoplasty procedure produced collagen "crumpling and splitting" changes in human corneas, which were observed during the follow-up of 6 months. Areas adjacent to treatment site were minimally damaged. (Am J Ophthalmol 2005;140:984–992. © 2005 by Elsevier Inc. All rights reserved.)**

MORE THAN A HUNDRED YEARS AGO, LANS DEMonstrated that localized heating of the cornea could change its curvature by inducing collagen shrinkage.[1] Gasset and Kaufman first performed thermokeratoplasty as a surgical attempt of flattening keratoconus through central contraction of stromal collagen in 1975, resulting, though, in delayed epithelial healing, corneal scars, recurrent erosions, and corneal neovascularization.[2] Similar studies by Keats and associates[3] reported on regression of corneal topographic changes several months after the treatment. As reported by Aquavella and associates,[4] this type of thermokeratoplasty caused stromal melting and perforation because of dissolution of stromal collagen at high temperatures in some cases. Thermal damage to basement membrane as well as frequent destruction of Bowman's layer during treatment resulted in persistent epithelial defects, recurrent erosions, and superficial stromal scarring.[4] A new modification, Los Alamos thermokeratoplasty proposed by Rowsey and Doss[5] involved heating of deep stromal collagen without raising the temperature of the epithelium or Bowman's membrane to destructive levels, but again the procedure was not able to provide a lasting effect.

In 1981, Fyodorov developed a technique with the use of a retractable hot nickel-chromium probe preset to penetrate the cornea at 95% of its depth and produce controlled thermal burns.[6] Nevertheless, regression of the initially promising results inevitably limited the technique's clinical utility. Attempts to correct hyperopia were also made by Peyman and associates[7] with carbon dioxide laser. The complications included superficial retraction of the corneal collagen and early regression of the refractive effect. Lack of stability, along with high complication rate of the techniques discussed above, gave rise to a development of other laser and radiofrequency thermal keratoplasty procedures: laser thermal keratoplasty[8–12] and conductive keratoplasty.[13–16,18,22]

In 1993, Mendez and his group started to work on conductive keratoplasty (CK), the procedure involving high frequency current brought in contact with collagen fibers of the cornea.[16] Dr Mendez believed that it was essential for the energy to be delivered directly to the stroma to achieve the desired amount of the effect with less energy and heating required.[16]

When attempting to "shrink" corneal collagen through heating, the temperature gap is very important. Temperatures of less than 30 to 50°C[17] minimally affect the biochemical properties of the cornea. When heated up to 55 to 58°C, human corneal collagen shrinks to one third of

Accepted for publication Jun 11, 2005.
From the University of Crete, School of Medicine, Vardinoyannion Eye Institute of Crete, Heraklion, Greece.
Inquiries to Tatiana L. Naoumidi, University of Crete, School of Medicine, Vardinoyannion Eye Institute of Crete, Voutes PO 1352, 71110 Heraklion Crete, Greece, fax: +30 2 810 210 334; e-mail: tnaoumidi@hotmail.com

0002-9394/05/$30.00
doi:10.1016/j.ajo.2005.06.027

TABLE. Conductive Keratoplasty: Histological Study of Human Corneas—Case Summary

Case No.	Gender, Age	Treated Eye	Follow-up (Time Between CK and PKP)	CK Spots Location
1	M 32	OD	24 hours	3 spots at 12, 1, 2 o'clock at 6 mm optical zone
2	M 33	OS	24 hours	3 spots at 1, 2, 3 o'clock at 6 mm optical zone
3	F 41	OS	3 days	3 spots at 9, 10, 11 o'clock at 6 mm optical zone
4	M 47	OD	7 days	3 spots at 12, 1, 2 o'clock at 6 mm optical zone
5	M 38	OD	3 months	3 spots at 10, 11, 12 o'clock at 6 mm optical zone
6	M 35	OS	6 months	3 spots at 1, 2, 3 o'clock at 6 mm optical zone

CK = Conductive keratoplasty; PKP = penetrating keratoplasty; M = male; F = female.

its length (30% to 50%). This leads to local flattening of the corneal surface. At higher temperatures, heat-sensitive intermolecular bonds between collagen fibers begin to dissolve, resulting in corneal necrosis, scarring, and permanent destruction of corneal tissue.[17]

With conductive keratoplasty, the tissue temperature rise is induced by electric impedance in the flow of energy through corneal tissue, causing collagen shrinkage when the temperature reaches 65°C (data on file, Refractec, Inc, Irvine, California, USA). CK uses the electrical conductive properties of the corneal tissue to propagate the energy through the stroma.[19] Tissue resistance to current flow generates localized heat, while the delivery probe remains cool, inserted approximately to the 80% of the cornea's depth (500 μm). The thermal effect proceeds from the bottom up as it finds the path of least resistance. As a result, CK-treated tissue is exposed to the same temperature at the tip of the probe deep in the stroma, as at the top of the probe on the corneal surface.[20] This process should result in denaturation and shrinkage of corneal collagen, which is self-limiting because resistance to the flow of the current increases with the increasing dehydration of collagen.[13] The histology of a pig eye showed a cylindrical footprint extending down to 80% of the midperipheral cornea following a CK in vitro treatment.[14,21]

In the present study, morphologic changes in human corneas induced by conductive keratoplasty procedure were examined up to 6 months following the treatment with the means of light and electron microscopy.

PATIENTS AND METHODS

IN THIS PROSPECTIVE CASE SERIES STUDY, SIX PATIENTS scheduled to undergo penetrating keratoplasty for keratoconus (regardless of the present study), received a CK treatment before PKP. The treatment was performed with a ViewPoint CK system (Refractec, Inc, Irvine, California, USA). All eyes were treated with the standardized setting of 350 kHz, 60% power for 0.6 seconds/spot. The study population consisted of one female and five male patients; ages 32 to 47 years. Informed consent was obtained from the entire patient group before surgery. The study was carried out with the approval from the Institutional Review Board.

The inclusion criterion was localized peripheral keratoconus. Eligible patients were examined preoperatively to obtain full medical history and to undergo a complete ophthalmic evaluation. The number of CK spots was three in all cases (at 6 mm optical zone). The CK spots were applied in the premarked areas with clear cornea, according to the slit-lamp and topography image, avoiding the areas compromised by the disease. All treatments were unilateral.

After the conductive keratoplasty procedure, the corneas of the subjects were obtained through penetrating keratoplasty (PKP) at different postoperative periods (Table) and evaluated histologically. The longest follow-up was 6 months.

Immediately after PKP, a triangular tissue piece containing the three CK spots was prepared in each eye. All samples were placed in glutaraldehyde 2.5% in 0.1 mol/l cacodylate buffer (pH 7.3) at 4°C, for at least 24 hours, and then postfixed in 1% osmium tetroxide in 0.1 mol/l cacodylate buffer (pH 7.3) at 4°C for 1 hour. After dehydration and embedding, samples were sectioned, stained, and examined using light microscopy. In the areas with mostly pronounced morphologic alterations, electron microscopy was performed. Standard techniques for the preparation and electron microscopy examination were used. All semi-thin sections were stained with modified trichrome stain[29]; all sections for electron microscopy were stained with uranyl acetate and lead citrate.

RESULTS

TWENTY-FOUR HOURS AFTER TREATMENT, THE HUMAN corneal epithelium in the CK-treated area had only minor morphologic alterations. The most marked observation was accumulation of fluid under the epithelial layer (Figure 1) in the upper part of Bowman's layer. The fluid accumulation was localized within the Bowman's layer, splitting it into two parts (bullous separation). The upper part con-

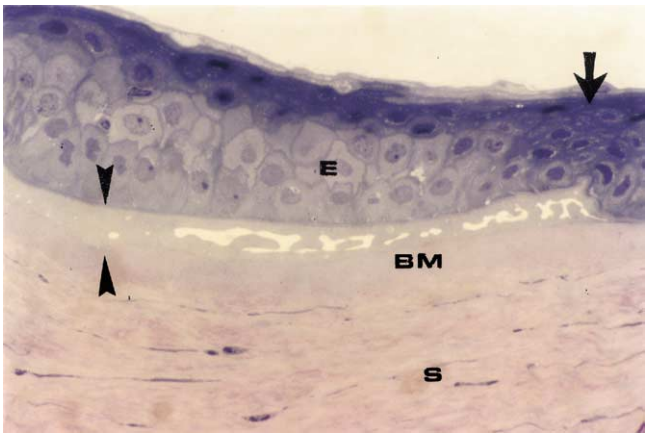


FIGURE 1. Twenty-four hours after the conductive keratoplasty (CK) treatment (human cornea): the upper part of CK-treated area. The zone of fluid accumulation (between arrowheads) is distinguished under the epithelium (E). It surrounds the tip's penetration site (thick arrow). S = corneal stroma; BM = Bowman's membrane. Light microscopy, × 500.

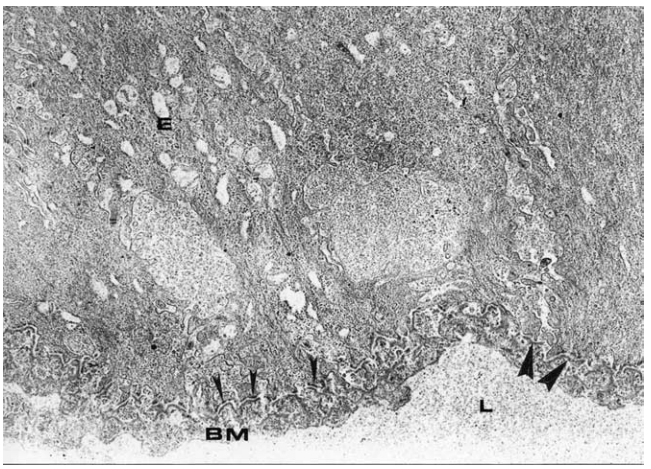


FIGURE 2. Twenty-four hours after the conductive keratoplasty (CK) treatment (human cornea): the basal part of epithelial layer (E), basement membrane (big arrowheads), and the upper part of the Bowman's membrane (BM). The liquid accumulation (L) surrounding the tip's entrance is seen beneath. The density and distribution of hemidesmosomes (small arrowheads) are not different from normal despite considerable folding of the basement membrane. Electron microscopy, × 5000.

tained the epithelium, the basement membrane, and the superficial layer of the Bowman's layer (approximately 1/10 of the total membrane's thickness), whereas the lower part included the rest of the 90% of the splitted Bowman's layer. Despite splitting, the total thickness of the Bowman's layer remained unaltered. The epithelium appeared slightly disruptured, most probably attributable to trauma caused by the tip's penetration. The cells, however, appeared viable. No signs of cell fragmentation or loss of intercellular contacts were observed. A moderate enlargement of the intercellular spaces in the area, an indication to slight edema, was also present (Figure 2).

In contrast to intact tissues, the lower epithelial border and the basement membrane within the zone of fluid accumulation, contained considerable number of microfolds. The structure of the basement membrane, as well as that of hemidesmosomes, was close to normal. The Bowman's layer in this area had slight alterations: disruption at the tip's penetration site, as well as relatively reduced fibril structural component when compared with intact tissue. The area of the disruption in the Bowman's layer had a size of 7 to 10 basal epithelial cells' diameters.

The area that was mostly affected by the treatment was corneal stroma. Smooth and parallel grating of intact collagen layers crumpled in the treated spot area. Besides the crumpled collagen layers, we also noted wavy appearance of keratocytes. Their wavy shape followed the altered architecture of the extracellular matrix (Figure 3).

The zone of crumpled collagen layers started right below the Bowman's membrane within the CK-treated area and extended through approximately 80% of corneal thickness. The degree of the crumpling changes did not vary considerably across the treatment zone; consequently its border with the intact collagen layers could be clearly distinguished. The area of alterations had a cylindrical shape with a diameter of 120 μm.

Transmission electron microscopy of CK-treated zones demonstrated that crumpling of collagen layers was associated with the appearance of diffuse-shaped electron-dense substance with chaotic microfibrillar structure (Figure 4A). At higher magnification, the electron microphotographs demonstrated that these microfibrillar aggregations were often mixed with collagen fibers, the appearance of which was typical for intact cornea. The diameter of these microfibrillar aggregations usually exceeded the typical diameter of intact collagen fibers by two to three times (Figure 4B).

The keratocytes within the corneal stroma in the CK-affected zone followed the architecture of the extracellular matrix. Other observed morphologic alterations of keratocytes included: cytoplasm fragmentation, vacuolization, and in some cases picnotic nuclear changes (Figure 4A). No inflammatory cells were present in any of the specimens. At this postop period, the Descemet's membrane beneath the treatment area typical for intact cornea thickness and structure, was continuous, without any folds, breaks, or cracks.

In the observed specimens, the morphologic appearance of the endothelium within the CK treatment zone was close to normal. Some endothelial cells had tendency towards vacuolization. Intracellular contacts as well as the connections between the endothelium and the Descemet's membrane were normal (Figure 5).

Three days postoperatively, the main difference in the histologic appearance of a CK-treated cornea from the 24

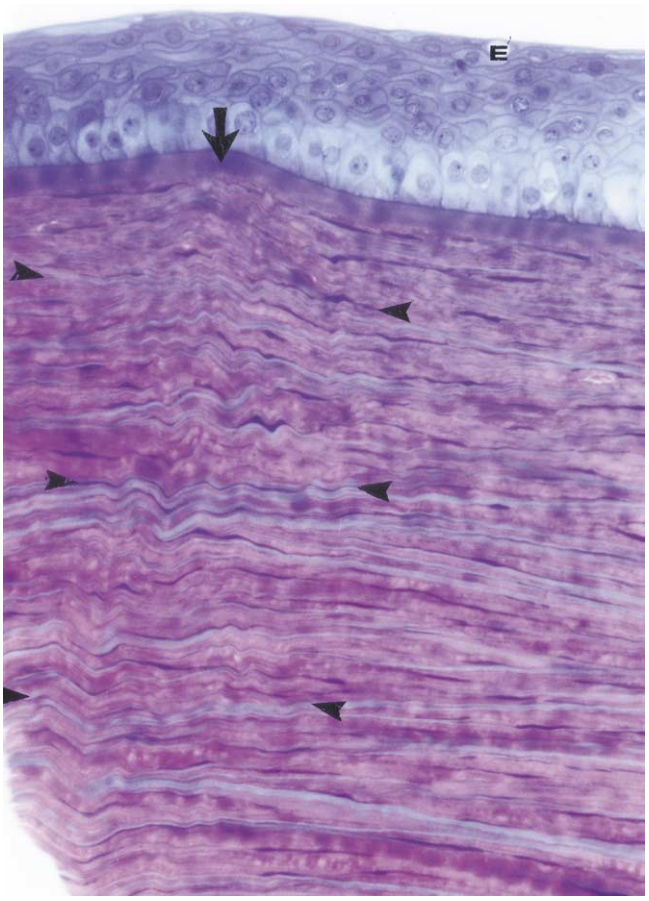


FIGURE 3. Twenty-four hours after the conductive keratoplasty (CK) treatment (human cornea): crumpled collagen layers (between arrowheads) within the central part of the CK-treated area (thick arrows). E = epithelium. Light microscopy, × 200.

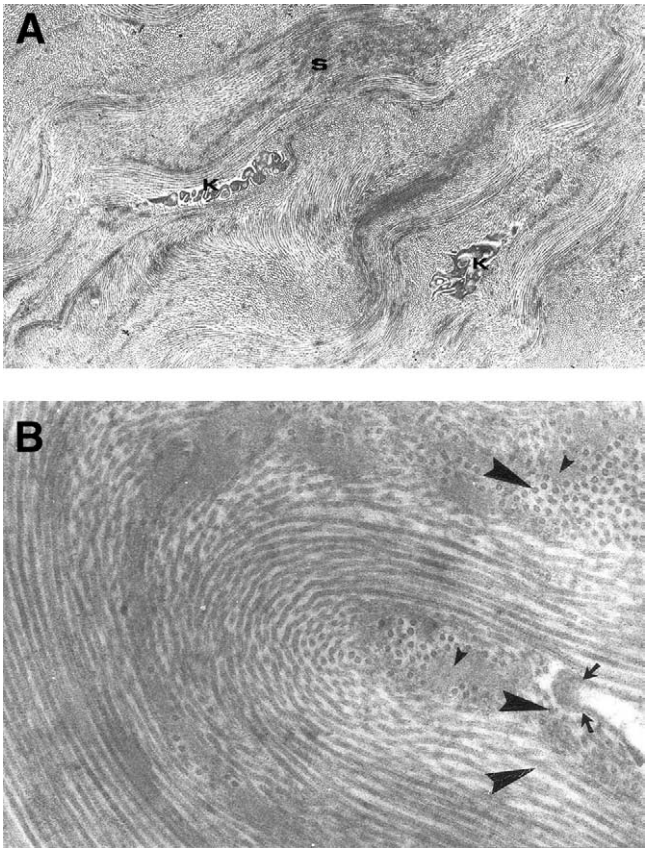


FIGURE 4. (A) Twenty-four hours after the conductive keratoplasty (CK) treatment (human cornea): crumpled collagen layers in the corneal stroma (S), typical for this postoperative interval. Keratocytes (K) have cytoplasm fragmentation, vacuolization and picnotic nuclear changes. Electron microscopy, × 3300. (B) Twenty-four hours after the conductive keratoplasty (CK) treatment (human cornea): collagen fibrils within the CK treatment zone. Besides the typical structure of stromal collagen (big arrowheads), microfibrillar aggregations (small arrowheads) can also be observed throughout the area of crumpled collagen layers. Small arrows point the transition of a single collagen fibril from its typical morphology into splitting. Electron microscopy, × 20,000.

hours' specimens was the condition of the epithelial layer at the tip's penetration site. By the third postoperative day, the integrity of the epithelial layer was restored. Analysis of the histologic specimens 3 days postoperatively did not reveal any evidence of liquid accumulation in the subepithelial space. The surface around the tip entrance was elevated above the corneal plane. Rupture of the Bowman's layer could often be observed in the center of the tip's penetration site (Figure 6).

Morphologic alterations of corneal stroma 3 days after the CK procedure were pronounced in the same degree as one day after the treatment: crumpling of collagen layers in the treatment area had the same morphologic characteristics as 24 hours postop. There was no sign of inflammatory reaction at the site of conductive keratoplasty application.

Three days after the treatment, the keratocytes within the CK-affected area were "sandwiched" between the crumpled collagen layers, thus the structure and the shape of keratocytes, determined by the crumpled collagen layers, was diverse. Some keratocytes within the stroma had signs of activation. Three days postoperatively, the Descemet's membrane and the endothelium beneath the treated area were intact and continuous. Seven days following the CK treatment, the only morphologic abnormality observed in the epithelium within the treated zone was an area of wing cells with picnotic changes of the nuclei. This finding could be caused by a thermal trauma of these cells, which were located in the basal epithelial layer at the time of a CK treatment.

The corneal layers of collagen remained crumpled within the entire treated area (Figure 7). Overall the thickness of the CK-treated stroma was greater than the one in the intact areas. The area of the crumpling changes extended to approximately 500 μm within the stroma at the CK application site.

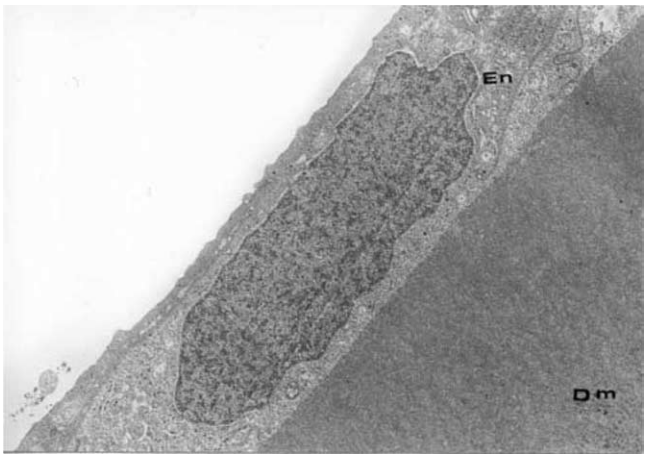


FIGURE 5. Twenty-four hours after the conductive keratoplasty (CK) treatment (human cornea): the posterior stroma, Descemet's membrane (Dm) and endothelial layer (En) right beneath the central part of the CK treatment zone. Note the continuous endothelial layer and the absence of endothelial edema or detachment from the Descemet's membrane. Electron microscopy, × 5000.

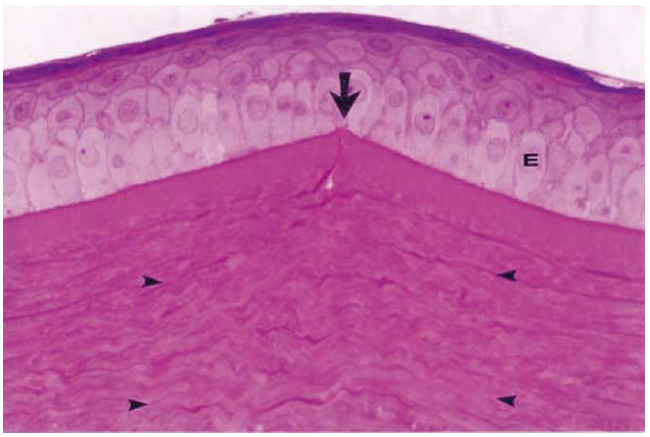


FIGURE 6. Three days after the conductive keratoplasty (CK) treatment (human cornea): the condition of the epithelium (E) within the tip's penetration site is similar to the intact one. The tip's penetration site can be recognized by the Bowman's membrane disruption (thick arrow). Elevation of the cornea and crumpling of collagen layers within the CK treatment zone (between arrowheads) are also noted. Light microscopy, × 160.

Six months after the procedure, only a slight increase in the thickness of epithelial layer was noted (Figure 8A). Alterations of the Bowman's layer were present: at two to three locations, normal structure of the Bowman's layer was completely missing and replaced by an irregular connective tissue with numerous cells that resembled activated fibroblasts. The sites with the most pronounced abnormalities of basal epithelial cells were their contacts with the irregular fibrous tissue that had replaced the Bowman's layer in the areas where it was missing (Figures 8B, 9).

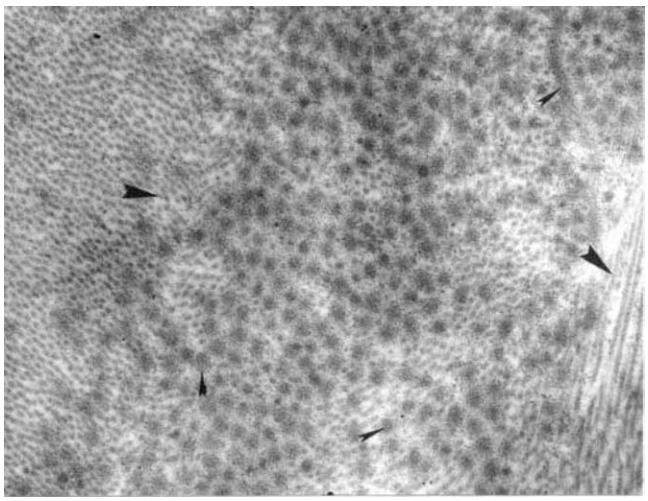


FIGURE 7. Seven days after the conductive keratoplasty (CK) treatment (human cornea): the corneal stroma remains crumpled throughout the treated area, microfibrillar aggregations (small arrowheads) are observed between normal collagen fibers (big arrowheads). Electron microscopy, × 16,000.

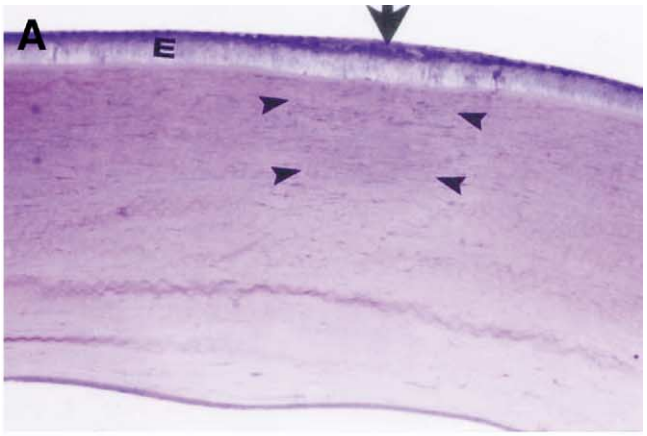
The stroma in the CK-treated zone did not appear swollen; moreover, a slight reduction of corneal thickness was noted in the gross specimen within the CK-affected area. A zone of loose fibrous connective tissue was observed within the stroma, not only in its superficial layer but also actually within the entire area of thermally altered collagen. A noticeably increased number of cells similar to activated fibroblasts were distributed over the entire CK-affected area.

Six months after the treatment, the Descemet's membrane in the treated areas did not differ from the intact one in the control areas. The morphology of the endothelial cells was typical for intact tissue, with only one exception, which was a slightly increased amount of vacuolized structures.

DISCUSSION

IN THE COURSE OF THIS STUDY, WE DETERMINED THE spectrum of histologic changes induced in human corneas by conductive keratoplasty treatment. We believe that when discussing these findings, it is sensible to describe and compare them to data obtained only from human studies following thermokeratoplasty procedures. According to our experience with post-CK rabbit and pig corneas, the animal findings differ greatly from the human ones, even if animal and human corneas were treated under the same experimental conditions. In this study, 24 hours after the treatment the most marked observation in the corneal epithelium was an accumulation of fluid under the epithelial layer in the upper part of Bowman's layer.

Guimaraes and her group (Guimaraes MR, Guimaraes RQ, Castro RD. Radio frequency to correct hyperopia and



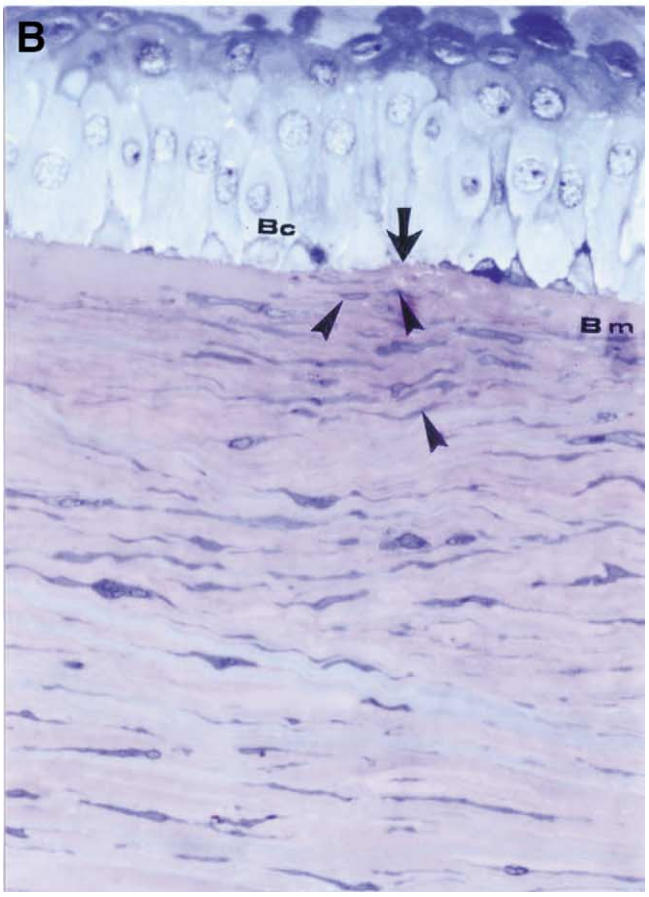


FIGURE 8. (A) Six months after the conductive keratoplasty (CK) treatment (human cornea): the foci of the Bowman's membrane as well as the affected corneal stroma, are substituted with fibrous connective tissue along with a big amount of cells resembling fibroblast cells (between small arrowheads). E = epithelial layer. The center of CK-treated area = thick arrows. Light microscopy, × 40. (B) Six months after the conductive keratoplasty (CK) treatment (human cornea) = larger magnification of Fig. 8 (fragment): the Bowman's membrane (Bm) around the tip's entrance (thick arrow) is substituted with loose fibrous tissue with numerous fibroblast-like cells (arrowheads). Basal epithelial cells (Bc) have edema and hyperpolarization. Light microscopy, × 500.

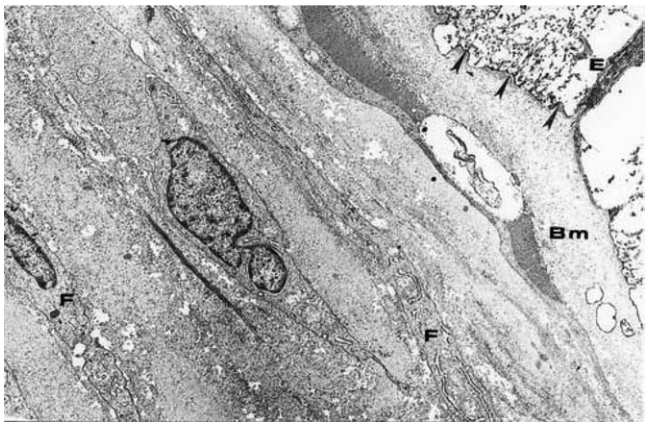


FIGURE 9. Six months after the conductive keratoplasty (CK) treatment (human cornea): edema of the basal cells of epithelial layer (E). The epithelial basal membrane is characterized by numerous microfolds (arrowheads). A large part of the Bowman's membrane (Bm), as well as the stromal collagen layers, are substituted with loose fibrous (scar type) tissue with a big amount of activated fibroblasts (F). Electron microscopy, × 3300.

astigmatism: short-term histopathology of six human corneas. Presented at the Symposium on Cataract, IOL, and Refractive Surgery. San Diego, California. 1995; best papers of sessions edition: 31 to 35) also described this type of bullous-like separation 24 hours following a CK treatment. Overall, their post-CK short-term study of human corneas reported on more destructive findings in the epithelium, than the ones we have observed. The Guimaraes group commented on a total absence of the epithelium at the site of the radio frequency spot. As a result of trauma, the epithelial cells at the site of the spot were necrotic, shrunken nuclei, and cell disruptions were present. In the present study, the epithelium appeared only slightly disruptured, with viable cells and without cell fragmentation or any loss of intercellular contacts. In the Guimaraes study, the Bowman's layer was reported to be intact in all cases with no ruptures, folds, or thinning areas. It was also noted that a mild attenuation of fibers at the site of heat application, suggesting a discrete shrinkage of fibers, was present. The authors did not observe any severe necrosis or inflammatory cells within the limits of the thermal burn following CK treatment.

Overall, laser thermal keratoplasty (LTK) studies comment on more destructive findings in the epithelium compared with the observations of the present study. A report on LTK treatment of human corneas by Koch and associates,[10] comments on epithelial sloughing and increased staining of the remaining epithelial cells, thinning of the Bowman's layer, and disruption of the linear structure of the basement membrane in the treated area at all energy densities. Koch and associates[10] also described loss of structural definition of the Bowman's layer at higher energy levels 24 hours following LTK treatment. Earlier

thermokeratoplasty studies, such as a human study by Arentsen and associates,[23] reported on epithelial thinning and necrosis along with focal absence of the Bowman's layer. Unlike CK studies, Arentsen and associates[23] pointed on evidence of acute inflammation in the subepithelial zone.[23] Aquavella and associates[24] also mentioned bullous keratopathy, thickening of the epithelial basement membrane. At later postoperative periods, loose fibrous connective tissue was reported to produce scarring without vascularization, recurrent erosions were also present.[24]

In the stroma of the cornea one day after the CK treatment, the observed crumpling changes of collagen layers were associated with the appearance of diffuse-shaped electron-dense substance with chaotic microfibrillar structure. These morphologic alterations of corneal stroma suggest that possible splitting of collagen fibrils took place because of the tissue heating in the CK treatment zone. According to thermal denaturation models proposed by Allain and associates[27] and our data, the structures affected by the CK procedure probably involve the heat-labile cross links that form the collagen network by interconnecting the collagen molecules. Most probably, other two types of heat-sensitive bonds are also affected by the CK procedure: the collagen triple-helix bonds (hydrogen bonds), forming the collagen molecules, and some peptide bonds forming the α-helices of the collagen molecule.[26–28] This issue needs clarification, especially if we take into consideration the fact that part of the collagen fibrils within the CK zone preserved their original structure, while the rest collagen fibers "split" to microfibrils.

Taking into account these arguments, the term "shrinkage" with relation to collagen fibers does not appear to be fully adequate when characterizing thermal damage of collagen. First, the electron microscopic analysis proved that after heat application, the volume occupied by a solitary collagen fiber increased. Second, the central areas of the CK-treated zone, where the considerable amount of collagen fibers underwent thermal "splitting," suffered a noticeable posterior corneal swelling within the first postop week. In most cases, a relevant elevation of superficial stroma around the tip entrance zone was also observed. It cannot be excluded that the volume of collagen fibers increased after the CK procedure.

Human corneal stroma 24 hours following a CK treatment, described by Guimaraes (Guimaraes MR and associates, Radio frequency to correct hyperopia and astigmatism: short-term histopathology of six human corneas. Presented at the Symposium on Cataract, IOL, and Refractive Surgery. San Diego, California, 1995) had "decreased or shrunken keratocyte population, edema between the stromal lamellae, leading to increased thickness of the area, and collagen disorganization. The surrounding stroma kept its staining properties with preservation of its nuclei and collagen structure." The authors did not report on inflammatory cells to be present within the limits of a thermal burn, although mild stromal edema was present (bullous separation, similar to the findings of the present study).

An early thermokeratoplasty study by Aquavella and associates[24] reported on severe damage to corneal stroma, including superficial stromal scarring, persistent inflammatory infiltrate, or even aseptic stromal necrosis following the treatment. Arentsen and associates[23] commented on marked edema of keratocytes through the whole thickness of the cornea, evaluated with transmission electron microscopy in their thermokeratoplasty study. Ariyasu and associates[24] have reported on stromal edema, contraction of stromal collagen, forming striae extending between adjacent treatment sites. The depth of the conical-shaped corneal opacities ranged from 25% of the corneal thickness to 75% of the corneal stroma in patients treated with a pulsed mode LTK therapy.[24] In an LTK study by Koch[10] and associates, keratocytes in the anterior stroma were reported to be injured, fragmented, and reduced in size and number. The degree and depth of these changes increased with increased energy densities. Stromal lamellae were disorganized in the anterior stroma at low energy densities: this effect extended to two-thirds depth at high energy densities. Corneal swelling was reported in the posterior region of the cornea.[10] Koch and associates also observed randomly distributed, electron-dense particulate matter and splitting of individual fibrils into subfibrillar structures.[10]

The condition of the endothelium beneath the treated zone is a very important safety feature for any refractive procedure. In the present study, the Descemet's membrane and the endothelium were reported to be very close to normal through the whole post-CK follow-up period. Guimaraes and her group (Guimaraes MR and associates, Radio frequency to correct hyperopia and astigmatism: short-term histopathology of six human corneas. Presented at the Symposium on Cataract, IOL, and Refractive Surgery. San Diego, California, 1995) reported on similar condition of the Descemet's membrane, but the endothelial cell number was attenuated though in two of the six cases.

In the LTK group, Ariyasu and associates[24] reported no endothelial damage in human corneas using specular microscopy immediately after the treatment. Most of the thermokeratoplasty studies, though comment on serious damage to the endothelium and the Descemet's membrane following the treatment. Aquavella,[5] Arentsen,[23] and Koch[10] described a zone of damaged endothelial cells and exposed Descemet's membrane beneath the zone of a laser treatment in human specimens, most probably related to a nonspecific reaction to thermal damage. The small zone of endothelial cell damage at the border of the coagulation demonstrated high temperature gradient from the center to the periphery of the coagulated tissue.[12] In the LTK study by Koch[10] and his colleagues, a dose-dependant potential for endothelial cell damage was reported, when the laser

beam approached the posterior cornea. A continuous endothelium was described only at the lowest energy density, whereas the endothelium and the Descemet's membrane were reported to be completely absent in the specimens with higher energy density.[10]

We believe that the slight increase in the thickness of epithelial layer noted in our specimens at 6 months postop, was caused instead by epithelial hyperplasia than by hyperpolarization of the basal epithelial cells. Several areas within the Bowman's layer were completely missing and replaced by an irregular connective tissue with numerous cells that resembled activated fibroblasts. We speculate that this replacement was responsible for local abnormalities of basal epithelial cells within the described areas.

In an early thermokeratoplasty study, Arentsen and associates[23] noted that although irregular epithelial regeneration was present in human specimens, the epithelial basement membrane was absent and hemidesmosomes were deficient in the treated areas at 2, 5, and 8 months following the treatment. Like we anticipated, the corneal stroma in the CK-treated zone was no longer swollen, moreover a slight reduction of corneal thickness was observed within the CK-affected area at 6 months postop.

A zone of loose fibrous connective tissue, which was observed within the stroma, extended through the entire area of thermally altered collagen. A noticeably increased number of cells similar to activated fibroblasts were distributed over the entire CK affected area. Unconditionally, this area of loose fibrous connective tissue may be characterized as scar tissue, although neither infiltration by lymphocytes nor vascular channels were observed in any of the specimens examined.

Arentsen and associates[23] reported the endothelial cells to be normal in human specimens, central stroma to be thinned with focal scar formation at 2, 5, and 8 months following the thermokeratoplasty treatment.[23] Unfortunately, there is a severe lack of human data with a substantial follow-up of thermokeratoplasty-treated corneas.

We believe that one more issue that needs to be mentioned here is the stability of the induced changes, which is always a hot topic with thermokeratoplasty procedures. The basic difference between the laser- and radiofrequency-based treatments lies in the mechanism of their action.

With laser thermal keratoplasty the thermal energy applied by the Holmium:YAG laser to the surface of the cornea is absorbed not only by the tears on the surface of the cornea but also by the surrounding tissue differentially along a thermal gradient through the depth of the treatment site (anterior cornea with a higher temperature). Additionally, leukomas at the treatment site may function as filters during the thermokeratoplasty procedure with the Holmium:YAG laser.[19] The above are backed up by the histologic findings in LTK-treated corneas, reported mostly in animal studies: the maximum volume of tissue alterations is observed in the upper corneal layers (epithelium, Bowman and superficial stroma) fading out towards the deeper parts of the cornea. The area of collagen alterations has a conical shape.[9,10,25] Induced by LTK morphologic changes (intense tissue damage associated with an inflammatory reaction, before the formation of new collagen tissue of the cornea) cause regression of the effect produced by holmium laser, as reported by Ayala and associates.[9] Koch and associates[10] demonstrated acute epithelial and stromal tissue changes following an LTK treatment, which in rabbit corneas stimulated a brisk wound healing response. According to the authors, these changes could contribute to postoperative regression of induced refractive correction after laser thermal keratoplasty treatment.[10]

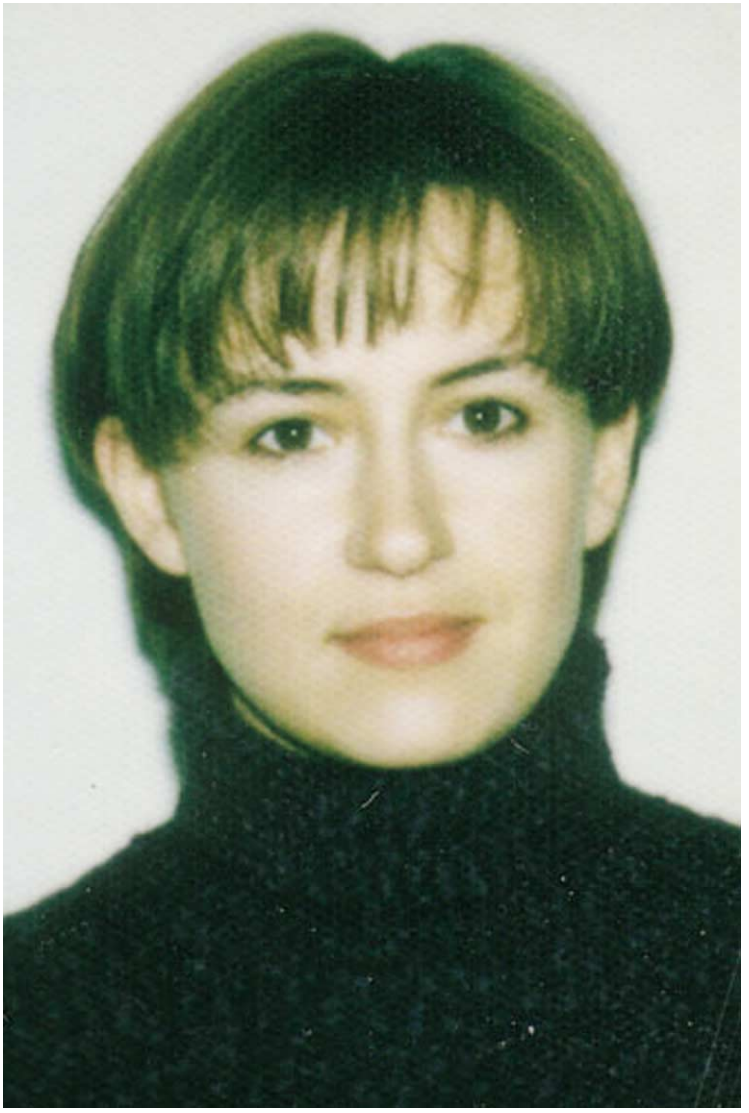
With conductive keratoplasty, the resistance to the current flow through the tissue generates the thermal energy, not the probe itself. As denaturation of the corneal stroma occurs, the impedance in the tissue rises, which functions as an autoregulatory mechanism.[19] As a result, a more homogenous and deep (approximately 500 μm) cylindrical thermal footprint is produced, as seen in the present and already reported CK histologic studies (Guimaraes MR and associates, Radio frequency to correct hyperopia and astigmatism: short-term histopathology of six human corneas. Presented at the Symposium on Cataract, IOL, and Refractive Surgery. San Diego, California, 1995). In the present study, we observed the CK-induced alterations in the corneal stroma to be present at 6 months postoperatively. Finally, the reported absence of inflammatory cells or severe necrosis along with minor epithelial damage is also thought to contribute to the stability of the achieved effect.

We believe that further investigation of the healing process along with more histologic and clinical data with a long-term follow-up will help enhance both the technical parameters of thermokeratoplasty procedures, and understand the impact of the procedure on human cornea in more detail.

REFERENCES

1. Lans LJ. Experimentelle Untersuchungen uber Entstehung von Astigmatismus durch nicht-perforierende Corneawunden. Graefes Arch Ophthalmol 1898;45:117–152.
2. Gasset AR, Kaufman HE. Thermokeratoplasty in the treatment of keratoconus. Am J Ophthalmol 1975;79:226–232.
3. Keates RH, Dingle J. Thermokeratoplasty for keratoconus. Ophthalmic Surg 1975;6:89–92.
4. Aquavella JV, Smith RS, Shaw EL. Alterations in corneal morphology following thermokeratoplasty. Am J Ophthalmol 1977;83:392–401.
5. Rowsey JJ, Doss JD. Preliminary report of Los Alamos keratoplasty techniques. Ophthalmology 1981;88:755–760.
6. Fyodorov SN, Ivashina AI, Aleksandrova OG, Bessarabov AN. Surgical correction of compound hypermetropic and

mixed astigmatism by sectoral thermal keratocoagulation. Implants Ophthalmol 1990;2:43–48.
7. Peyman GA, Larson B, Raichand M, Andrews AH. Modification of rabbit corneal curvature with the use of carbon dioxide laser burns. Ophthalmic Surg 1980;11:325–329.
8. Seiler T, Matallana M, Bende T. Laser thermokeratoplasty by means of a pulsed Ho: YAG laser for hyperopic correction. Refract Corneal Surg 1990;63:335–339.
9. Ayala Espinoza MJ, Alio JL, Ismail MM, Sanchez Castro P. Experimental corneal histological study after thermokeratoplasty with holmium laser. Arch Soc Esp Oftalmol 2000; 75:619–626.
10. Koch DD, Kohnen T, Anderson JA, et al. Histological changes and wound healing response following 10-pulse noncontact Holmium: YAG laser thermal keratoplasty. J Refract Surg 1996;12:621–634.
11. Moreira H, Campos M, Sawusch MP, Mc Donnell JM, Sand B, McDonell PJ. Holmium laser thermokeratoplasty. Ophthalmology 1993;100:752–761.
12. Wirbelauer C, Koop N, Tuengler A, et al. Corneal endothelial cell damage after experimental diode laser thermal keratoplasty. J Refract Surg 2000;16:323–329.
13. McDonald MB, Davidorf J, Maloney RK, Manche EE, Hersch P. Conductive keratoplasty for the correction of low to moderate hyperopia: 1-year results of the first 54 eyes. Ophthalmology 2002;109:637–649.
14. McDonald MB, Hersh PS, Manche EE, et al. Conductive keratoplasty for the correction of low to moderate hyperopia: U.S. clinical trial one-year results on 355 eyes. Ophthalmology 2002;109:1978–1989.
15. Pallikaris IG, Naoumidi TL, Panagopoulou SI, Alegakis AK, Astyrakakis NI. Conductive keratoplasty for low to moderate hyperopia: 1-year results. J Refract Surg 2003;19:496–506.
16. Mendez A, Mendez Noble A. Conductive keratoplasty for the correction of hyperopia. In: Sher NA, editor. Surgery for hyperopia and presbyopia. Philadelphia: Williams and Wilkins, 1997;163–171.
17. Sporl E, Genth U, Schmalfuss K, Seiler T. Thermomechanical behavior of the cornea. Ger J Ophthalmol 1996;5:322–327.
18. Stringer H, Parr J. Shrinkage temperature of eye collagen. Nature 1964;204:1307.
19. Haw WW, Manche EE. Conductive keratoplasty and laser thermal keratoplasty. Int Ophthalmol Clin 2002;42:99–106.
20. Huang B. Update on nonexcimer laser refractive surgery technique: conductive keratoplasty. Curr Opin Ophthalmol 2003;14:203–206.
21. Pearce J. Corneal reshaping by radio frequency current: numerical model studies. Proc SPIE 2001;4247.
22. Pallikaris IG, Naoumidi TL, Astyrakakis NI. Conductive keratoplasty to correct hyperopic astigmatism. J Refract Surg 2003;19:425–432.
23. Arentsen J, Rodriquez M, Laibson P. Histopathologic changes after thermokeratoplasty for keratoconus. Invest Ophthalmol Vis Science 1977;16:32–38.
24. Ariyasu R, Sand B, Menefee R, et al. Holmium laser thermal keratoplasty of 10 poorly sighted eyes. J Refract Surg 1995; 11:358–365.
25. Ren Q, Simon G, Parel J. Noncontact laser photothermal keratoplasty III: histological study in animal eyes. J Refract and Corneal Surg 1994;10:529–539.
26. Brinkmann R, Radt B, Flamm C, Kampmeier J, Koop N, Birngruber R. Influence of temperature and time on thermally induced forces in corneal collagen and the effect on laser thermokeratoplasty. J Cataract Refract Surg 2000;26: 744–754.
27. Allain JC, Le Lous M, Cohen Solal L, et al. Isometric tensions developed during the hydrothermal swelling of rat skin. Connect Tissue Res 1980;7:127–133.
28. Darnell J, Lodish H, Baltimore D. Molecular cell biology. New York, New York, Scientific American Books Inc, 1986.
29. Rock ME, Anderson JA, Binder PS. A modified trichrome stain for light microscopic examination of plastic-embedded corneal tissue. Cornea 1993;12:255–260.



Biosketch

Tatiana L. Naoumidi, MD, PhD, is a clinical researcher in the Vardinoyannion Eye Institute of Crete (VEIC), Greece. She is in charge of the Conductive Keratoplasty International Centre of Excellence at the University of Crete, School of Medicine. Her research interests include photodynamic therapy of glaucoma and thermokeratoplasty techniques.

Biosketch

Ioannis G. Pallikaris, MD, PhD, is Professor and Chair of Ophthalmology at the University of Crete, Greece, President of the University of Crete, Greece and the founder and director of the Vardinoyannion Eye Institute of Crete (VEIC). Prof. Pallikaris is an opinion leader in Refractive Surgery and is widely recognized as the inventor of LASIK. His research interests include refractive surgery and surface ablations in particular, as well as vitreoretinal surgery.