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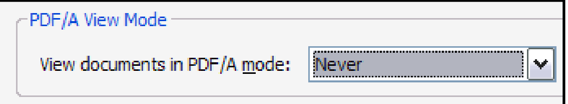

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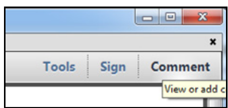

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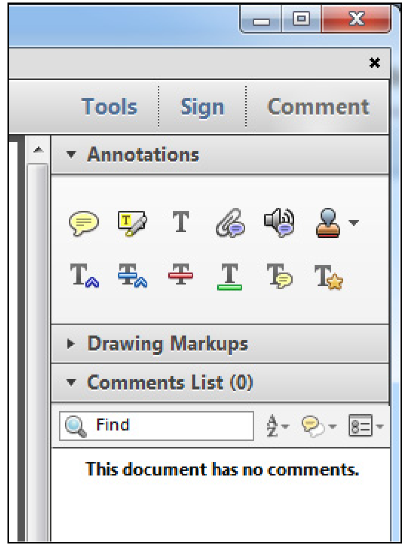

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Immunohistochemical study of corneal inflammation after femtosecond laser clear corneal incisions or manual surgery

Lisa Toto, MD, PhD, Claudia Curcio, PhD, Alessandra Mastropasqua, MD, Peter A. Mattei, MD, PhD, Erminia D'Ugo, MD, Chiara De Nicola, MD, Leonardo Mastropasqua, MD

PURPOSE: To use immunohistochemical staining to evaluate corneal inflammation and apoptosis induced after femtosecond laser incisions or manual incisions.

SETTING: Ophthalmology Clinic, University G. d'Annunzio, Chieti, Italy.

DESIGN: Experimental study.

METHODS: Ninety human cadaver corneas were cut manually or with the femtosecond laser at different energies and analyzed by immunohistochemistry after 5 minutes or 4 hours. The corneas were divided into 5 groups: untreated (Group 1), cut manually (Group 2), and treated with the femtosecond laser with increasing energies (Groups 3 to 5; 3.0 μJ, 6.0 μJ, and 15.0 μJ, respectively).

RESULTS: At 5 minutes, increased expression of interleukin (IL)-18 was observed in the femtosecond laser groups compared with the manual group ($P < .01$). Interferon gamma (IFNγ) positivity was significantly higher in Groups 4 and 5 than in Group 2 and between Groups 3 and 4 ($P < .05$). The terminal uridine deoxynucleotidyl nick end-labeling (TUNEL) positivity increased with higher energy (Group 2 versus Group 4 and Group 2 versus Group 5; $P < .05$). After 4 hours, IFNγ positivity was higher in Group 5 than in Group 2 ($P = .0021$) and between Group 5 and Groups 3 and 4 ($P < .05$). No sign of IL-18 positivity was found after 4 hours in any sample. Group 5 showed significant higher TUNEL positivity than all other groups ($P < .0001$).

CONCLUSION: The femtosecond laser technique at high energies induced a higher corneal inflammatory response and a higher corneal cell apoptosis than the manual technique.

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After damage to the cornea, different cell types (stromal keratocytes, endothelial cells, and basement membranes) show a rapid response by releasing cytokines.[1] This response induces epithelial regeneration, keratocyte proliferation,[2,3] migration,[4] differentiation into fibroblasts and myofibroblasts,[3] deposition of abnormal extracellular matrix (ECM) proteins, and recruitment of macrophages and other immune cells into the cornea.[1,5,6] Corneal wound healing is a complex process involving cell death, migration, proliferation, differentiation, and ECM remodeling.[7,8] Interleukin (IL)-4 and IL-13, both Th2-type cytokines that are preferentially involved in the disruption of the epithelial barrier of the ocular surface,[A] do not elicit a high level of epithelial cytokine secretion. These chemokines are involved in selective lymphocyte/leukocyte recruitment. The production of various cytokines, including IL-1, IL-6, IL-8, IL-18, interferon gamma (IFNγ), and transforming growth factor beta (TGFβ), has also been seen in cultured corneal cells in vitro.[9–13] Interferon γ is a pleotropic cytokine that is involved in a variety of immune functions, including the recruitment and polarization of naïve CD4 cells, which once differentiated, produce IFNγ.[14] Corneal epithelial cells are a potent source of IL-18, which might play an important role in initiating IFNγ-mediated inflammatory responses in the cornea.[13] Increased bioactive corneal IL-18 production can be induced by a number of

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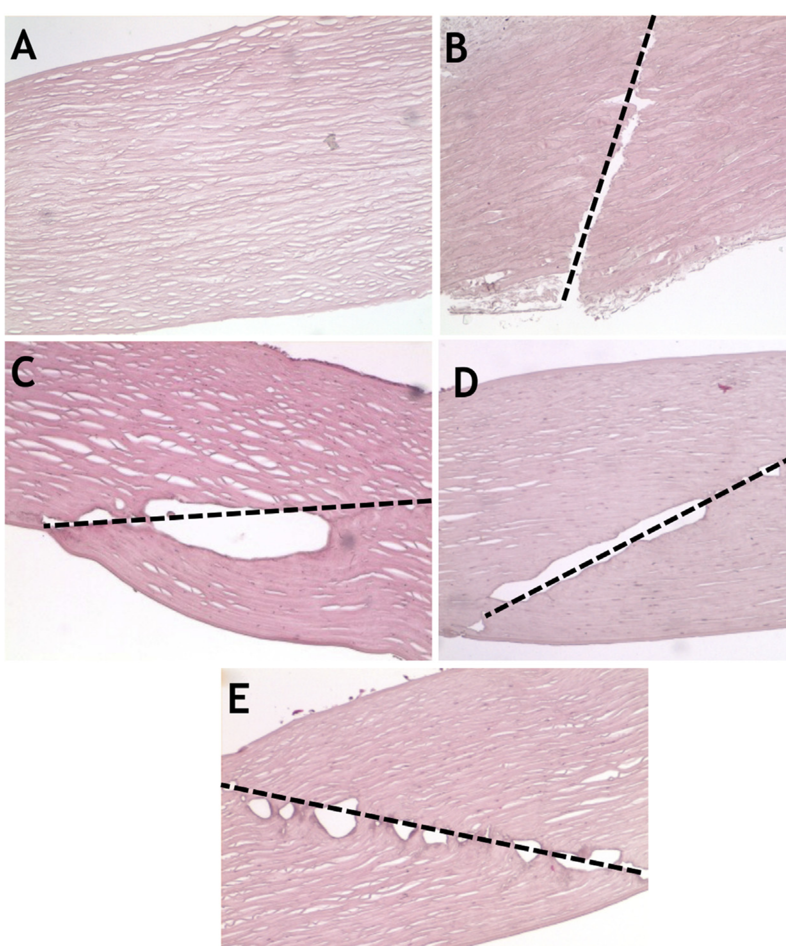

Figure 1. Hematoxylin–eosin staining of corneas cut with a manual (*B*: Group 2) or femtosecond laser technique at energy settings of 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15 μJ (*E*: Group 5). An untreated cornea is shown as the control (*A*: Group 1). The dashed lines indicate the cuts within the tissues (original magnification ×10).

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proinflammatory agents and might play an important role in initiating IFNγ-mediated inflammatory responses in the cornea.[13]

Apoptosis is a controlled form of cell death accompanied by characteristic ultrastructural changes that occur during tissue development, homeostatic responses to infection, and wound healing.[15,16] Keratocyte apoptosis was previously noted to be the first observable stromal response after epithelial injury.[17]

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Several authors have studied the inflammatory and cell death responses to corneal injuries related to different surgical corneal procedures, in particular after procedures using the latest energy sources in ophthalmic surgery, such as excimer lasers and, more recently, femtosecond lasers.[18,19] Interleukin-6 and tumor necrosis factor alpha (TNFα) were found to increase in human tear fluid during the first postoperative days after excimer laser phototherapeutic keratectomy (PRK).[18,19] In addition, Prada et al.[20] found increased gene expression of both IL-6 and TNFα after excimer laser ablation in Wistar rats. Leonardi et al.[21] found increased IL-12 expression in the tear film of patients after laser in situ keratomileusis (LASIK) and increased levels of IL-6, IL-8, and monocyte chemotactic protein-1 in human corneal fibroblast cultures after using a corneal excimer laser.

Keratocyte apoptosis is hypothesized to be an initiating event in the wound-healing response after a traditional excimer laser PRK, in which the epithelium is scraped before surface ablation.[22] Furthermore, the clinical differences in regression and haze in patients

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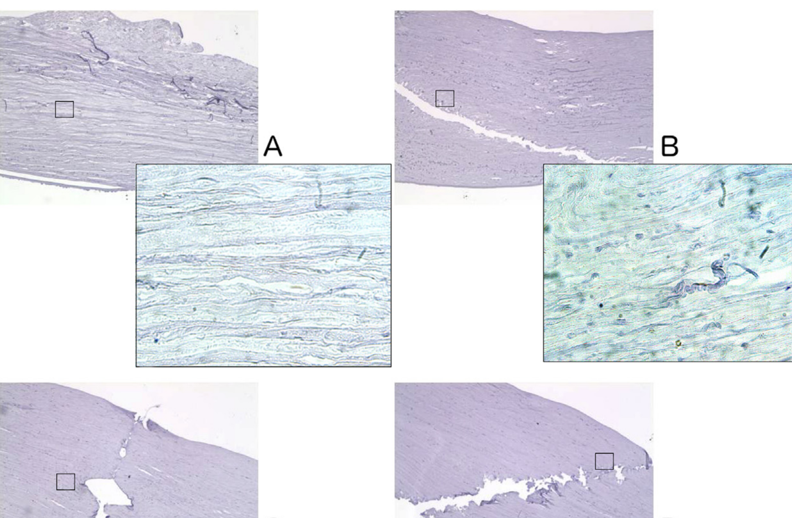

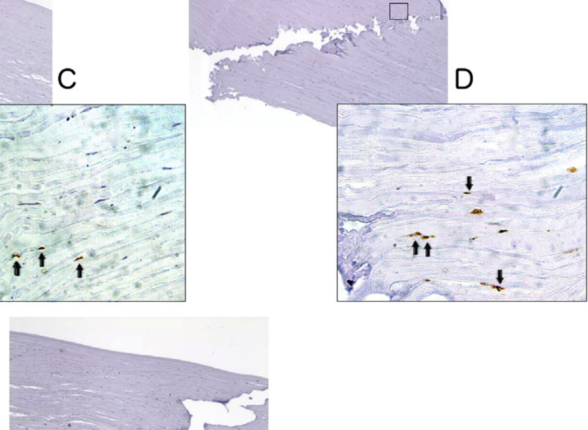

Figure 2. Interleukin-18 expression after manual surgery (*B*: Group 2) and use of the femtosecond laser technique at 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15 μJ (*E*: Group 5) energy settings evaluated via immunohistochemistry early (5 minutes) after surgery. Interleukin-18 expression in an untreated cornea (*A*: Group 1). A higher number of IL-18-positive cells (some *arrows* in the inserts showing positive cells characterized by brown staining) were observed in the corneas treated with femtosecond laser surgery at all of the analyzed settings. A scarce number of cells were detected in the other groups. Note cavities produced by cavitation bubbles at cut margins in the sites of femtosecond laser–tissue interaction. The images are representative of a single experiment. Each section was counterstained with hematoxylin (original magnification ×10, inserts ×63).

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treated with PRK and LASIK were attributed to diminished keratocyte apoptosis and an attenuated wound-healing response after LASIK.[22]

The use of femtosecond laser energy in corneal refractive surgery has been associated with greater inflammation and apoptosis compared to manual procedures, particularly when higher energy settings are used for the laser. A greater amount of inflammation and apoptosis was found in femtosecond LASIK at higher energy settings than in LASIK performed with a microkeratome.[23,24]

Figure 3. Computer-quantification of IL-18 staining in the immediate postoperative period in 3 fields ×200 for each sample. The data are expressed as the mean ± SEM, and the Mann-Whitney test was used to evaluate between-group differences of the mean levels of the marker. Statistically significant *P* values between different treatments are shown.

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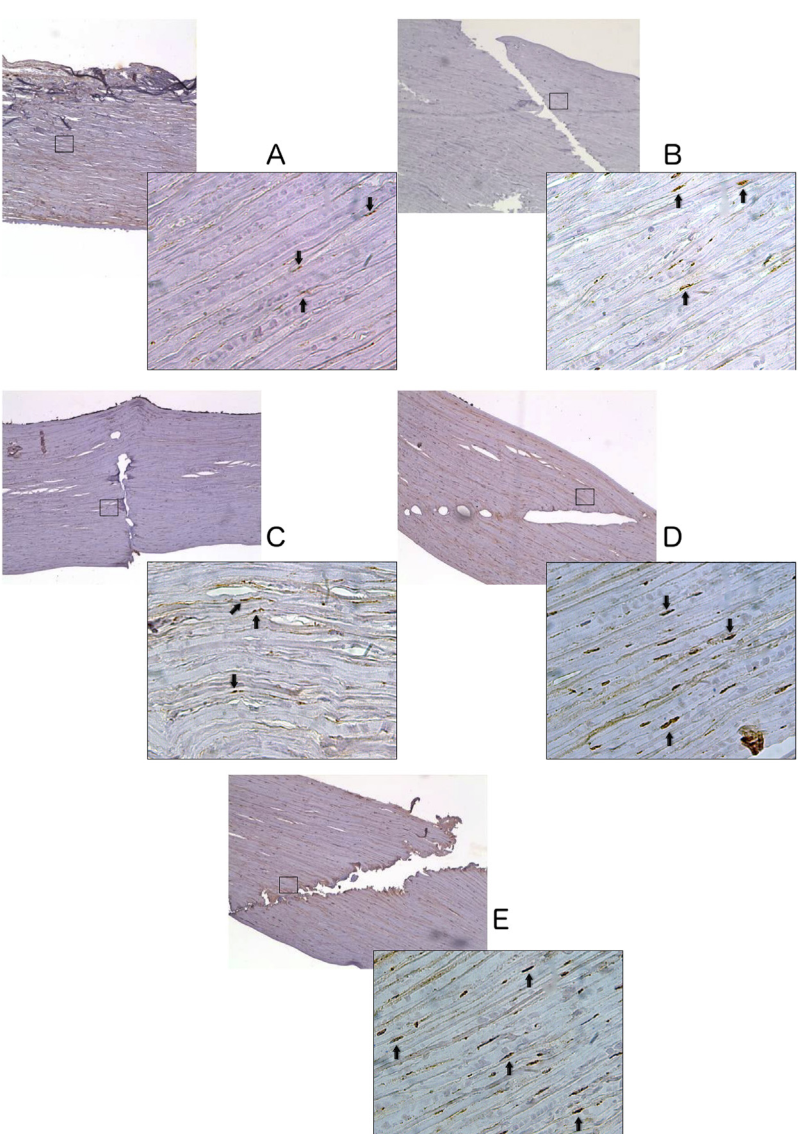

Figure 4. Assessment of IFNγ-positive cells after manual surgery (*B*: Group 2) and the femtosecond laser technique at 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15 μJ (*E*: Group 5) energy settings evaluated via immunohistochemistry early (5 minutes) after surgery. Interferon gamma expression in an untreated cornea (*A*: Group 1). A significant number of positive cells (*arrows* in the inserts showing positive cells characterized by brown staining) were found in the sample treated with the femtosecond laser at 6 μJ and 15 μJ energy compared with the manual technique. Note cavities produced by cavitation bubbles at cut margins in the sites of femtosecond laser–tissue interaction. The images are representative of a single experiment. Each section was counterstained with hematoxylin (original magnification ×10, inserts ×63).

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Clear corneal incisions (CCIs) are 1 of the 4 incisions (along with capsulotomy, lens fragmentation, arcuate keratotomy) performed with the femtosecond laser during femtosecond laser–assisted cataract surgery. Recently, Mayer et al.[25] studied monocytes and the dendritic cell response in human corneal buttons after CCIs were created with a femtosecond laser cataract surgery platform using 7 mJ laser pulse energy and found increased cell death compared with the manual technique. The safety and efficacy of femtosecond laser–assisted cataract surgery in performing corneal incisions were evaluated, and femtosecond laser CCIs showed better results than manual CCIs in terms of architectural stability and reproducibility.[26]

The aim of our study was to use immunohistochemistry in ex vivo human corneas to evaluate corneal inflammation and apoptosis induced after femtosecond laser CCIs at different energy settings or after manual surgery. Interleukin-18 and IFNγ expression were used to assess corneal inflammation, and the terminal uridine deoxynucleotidyl nick end-labeling (TUNEL) assay was performed to evaluate apoptosis in the early and late postoperative timepoints.

MATERIALS AND METHODS

This study adhered to the tenets of the Declaration of Helsinki. The protocol was approved by the university's Institutional Review Board.

Sample Features and Surgical Procedure

Ninety human eye-bank corneal buttons with scleral rims that were not suitable for transplantation (mean age 62.5 years ± 10.9 years [SD]; range 52 to 80 years) were included in the study. The mean time from death to

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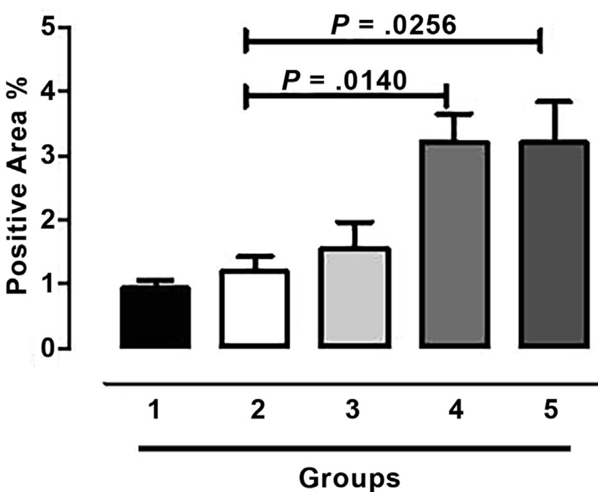

Figure 5. Computer-assisted quantification of IFNγ staining in the immediate postoperative period in 3 fields ×200 for each sample. The data are expressed as the mean ± SEM, and the Mann-Whitney test was used to evaluate between-group differences of the mean level of marker expression.

enucleation was 8 hours (range 4 to 10 hours). The mean storage time (Eusol-C, Alchimia Srl) between eye-bank procedures and fixation was 27 hours (range 20 to 48 hours). No evidence of disease, desiccation, or damage was noted.

The corneas were divided into 5 groups treated with femtosecond laser energy (Lensx platform, Alcon Laboratories, Inc.) or manually. Group 1 consisted of untreated corneas used as controls. A single-plane corneal incision was created at the periphery of the other corneal buttons. In the manual group (Group 2), a 2.75 mm disposable keratome knife was used. In the other 3 groups, a 2.7 mm single-plane CCI was created with a femtosecond laser with a spot separation of 3 μm and a layer separation of 3 μm (Groups 3 to 5, treated with 3.0 μJ, 6.0 μJ, and 15.0 μJ, respectively).

Fifty corneas were analyzed 5 minutes after surgery to assess the early response (10 specimens in each group), and 40 corneas were analyzed after 4 hours of permanence in organ culture (8 specimens in each group).

Sample Preparation for Immunohistochemical Staining in the Early-Response Assessment

Five minutes after treatment, samples were fixed in 4% formalin (Bio-Optica). Then, the samples were cut with a microtome (Leica Microsystems GmbH). The slices were stained with hematoxylin–eosin, and those containing cuts were collected for immunohistochemical staining.

Sample Preparation for Organ Culture in the Late-Response Assessment

The organ culture medium was prepared as previously described.[25] Briefly, the organ culture medium contained 25 mL Roswell Park Memorial Institute 1640 medium (with 2.0 g/L sodium bicarbonate, without phenol red and without L-glutamine) supplemented with 100 U/mL penicillin G, 100 g/mL streptomycin, 0.25 g/mL amphotericin B (Gibco/Life Technologies Corp.), 25 mM N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid buffer (Sigma-Aldrich Co.), and 5% fetal calf serum (Gibco/Life Technologies Corp.). Samples were fixed and cut as described in the previous paragraph.

Immunohistochemistry

All formalin-fixed paraffin-embedded serial tissue sections were cut into 4 μm thick slices, and peroxidase activity was inhibited by immersing the slides in a hydrogen peroxide 3.0% aqueous solution for 5 minutes. Each sample was stained with the following antibodies: IL-18 (1:500, order number ab137664, Abcam plc.), IFNγ (1:75, order number ab25101, Abcam plc.), and Apo BrdU in situ DNA fragmentation assay kit (TUNEL), according to the manufacturer's instructions (code K403-50, Biovision, Inc.). The tissue samples were deparaffinized and pretreated by microwave antigen retrieval using buffer containing ethylenediaminetetraacetic acid pH 9 (required for IFNγ) or citrate pH 6 (required for IL-18). For all of these antigens, the Envision system (Dako) was used before diaminobenzidine tetrahydrochloride (Dako) incubation. A negative control was performed for each antigen using the specific isotype control. All slides were stained for the same antigen together with the same antigen retrieval buffer, if required, and antibody dilution.

Image and Statistical Analysis

The Photoshop program (Adobe Systems, Inc.) was used to evaluate the total and positive pixels for cytoplasmic staining (IL-18 and IFNγ) as previously described[27,28] and previously implemented.[29] Briefly, an automated segmentation (color selection with tolerance determined on a per image basis) was used to automatically select cells. False-positive cells and background staining were manually deselected before measurements. Distances were measured in pixels and converted to microns using a conversion factor determined for each image during acquisition. For nuclear staining (TUNEL), the same software was used to view the images and manually count the keratocytes to determine the number of positive cells per area.[28] A nonparametric statistical test (Mann-Whitney) was used to evaluate the between-group differences in the mean levels of the marker expression for the selected antigens (Graphpad Prism 5 software, Graphpad Software, Inc.). Probabilities of less than 0.05 ($P < .05$) were considered statistically significant.

RESULTS

Hematoxylin–eosin staining was performed to detect samples that contained cuts (Figure 1).

Early Inflammatory and Apoptotic Response

Interleukin-18 expression was analyzed to evaluate the proinflammatory effects induced by cutting with the manual or femtosecond laser technique at different energy settings (Figure 2). There was a higher number of IL-18 positive cells in the cut region in all femtosecond laser samples (Groups 3, 4, and 5) than in the samples cut with the manual technique (Group 2) (Group 2 versus Groups 3 and 5, $P = .0012$; Group 2 versus Group 4, $P = .0051$), suggesting that treatment with a femtosecond laser modulates this inflammatory

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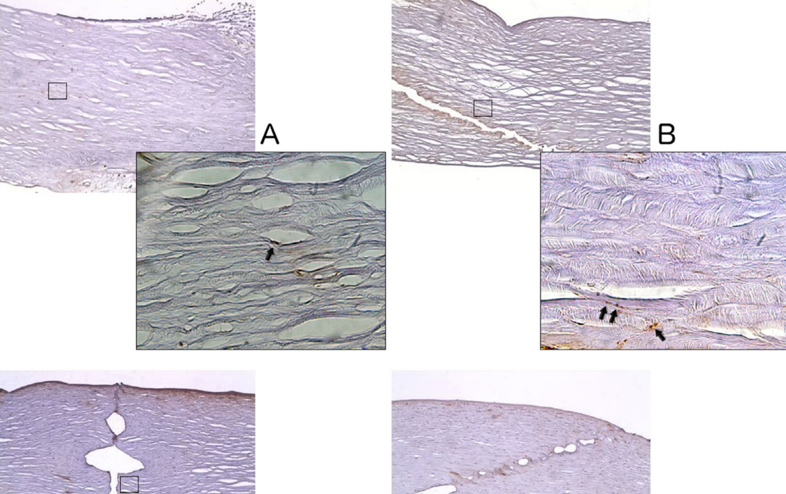

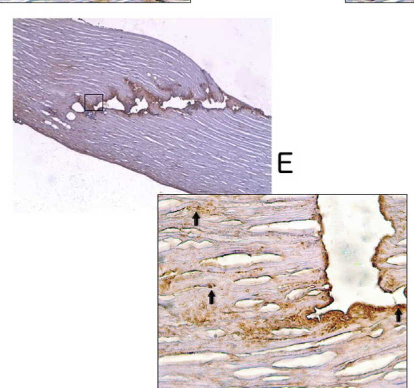

Figure 6. Assessment of TUNEL-positive cells (*arrows* Q2 in the inserts showing positive cells characterized by brown staining) after manual surgery (*B*: Group 2) and the femtosecond laser technique at 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15 μJ (*E*: Group 5) energy settings evaluated via immunohistochemistry early (5 minutes) after surgery. The TUNEL-positive cells in an untreated cornea (*A*: Group 1). The highest number of TUNEL-positive cells were observed in the femtosecond laser group at 15 μJ energy. Note cavities produced by cavitation bubbles at cut margins in the sites of femtosecond laser–tissue interaction. The images are representative of a single experiment. Each section was counterstained with hematoxylin (original magnification ×10, inserts ×63).

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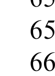
cytokine (Figure 3). In particular, IL-18 positivity increased from a mean value of 0.015 ± 0.021 in Group 2 to 0.085 ± 0.021 in Group 5 (Figure 3). Moreover, there were significant differences between control corneas and Groups 2, 3, and 5 ($P = .0031$) and Group 4 ($P = .0226$) (Figure 3).

The degree of inflammation evaluated by IFNγ staining (Figure 4) showed a high number of IFNγ-positive cells in the groups treated with femtosecond laser surgery compared with manual surgery. In particular, statistical significance was achieved comparing Group 2 with Group 4 ($P = .0140$) and with Group 5 ($P = .0256$) (Figure 5). In addition, a significant increase of IFNγ expression was observed between Groups 3 and 4 ($P = .0289$) (Figure 5) and between control corneas and high energy laser settings (Group 1 versus Group 4, $P = .0012$; Group 1 versus Group 5, $P = .0003$) (Figure 5).

The apoptotic cells, evaluated via the TUNEL assay in the immediate postoperative period, were distributed within the stroma near the cuts of the treated corneas (Figure 6). The number of positive cells was similar between Groups 2 and 3 in contrast to the significant increase observed in Group 4 ($P = .0221$) and Group 5 ($P = .0012$) compared with the manual technique (Figure 7). A significant increase in TUNEL expression was seen in Group 5 compared with Group 4 ($P = .0111$) (Figure 7) and between control tissues and treated tissues (Group 1 versus Group 2, $P = .0007$; Group 1 versus Group 3, $P = .0080$; Group 1 versus Group 4, $P = .0003$; Group 1 versus Group 5, $P = .0003$) (Figure 7).



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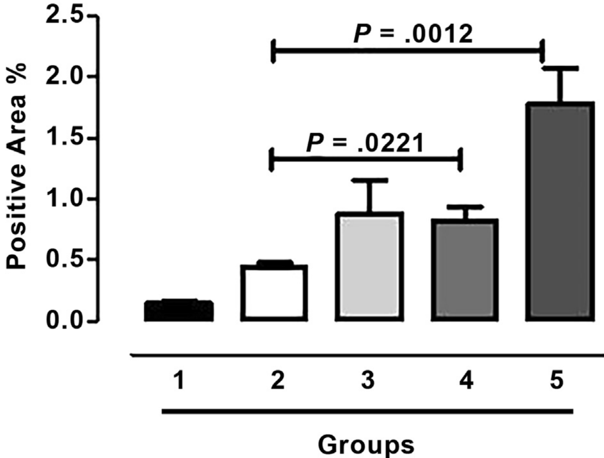

Figure 7. Statistical analysis of the data obtained from the different samples. The TUNEL-positive cells in the immediate postoperative period were identified by counting the number of cells showing an intense immune-labeling in 3 ×200 fields from each sample. The data are expressed as the mean ± SEM. The Mann-Whitney test was used to evaluate between-group differences of the mean levels of marker expression.

Late Inflammatory and Apoptotic Response

Interferon γ-positive cells were also found after 4 hours (Figure 8). A significantly higher number of IFNγ-positive cells in the cut region of group 5 of the femtosecond laser sample were observed compared with the manual group (Group 2) (Group 5 versus Groups 2 and 5, $P = .0021$) (Figure 9). Significant differences were also observed between Group 5 and Group 3 and 4 ($P = .0156$ and $P = .0255$, respectively) (Figure 9). No signs of IL-18 positivity were found after 4 hours in all samples of all groups (Figure 10). The apoptotic cells, evaluated via the TUNEL assay in the late postoperative period, were distributed within the stroma near the cuts of the treated corneas (Figure 11). Group 5 showed a significant increase compared with all other groups ($P < .0001$) (Figure 12).

DISCUSSION

The aim of current ocular surgical procedures is rapid tissue recovery without surgical sequelae. Several authors have evaluated the corneal tissue response after corneal surgical procedures in the context of corneal refractive or cataract surgery.[18–25] After a corneal injury, such as the injuries that occur with surgical corneal procedures, a change in the expression and localization of key cytokines and receptors were documented.[30] These modifications are considered to be important in wound healing and homeostasis in the cornea.[30] Stimulated keratocytes can produce several chemokines that have the potential to initiate severe corneal inflammation, which in turn can lead to corneal haze and other unsatisfactory sequelae.

The femtosecond laser is the vanguard in corneal refractive surgery and cataract surgery. It permits the automatization of various steps while optimizing the results in terms of predictability and repeatability. However, some authors recently evaluated tissue damage, showing tissue inflammation related to energy produced by the femtosecond laser.[25] Some studies compared the levels of inflammation and apoptosis between manual microkeratome and femtosecond laser after LASIK surgery, highlighting greater inflammation after the use of the femtosecond laser, in particular with higher energy settings, compared with the use of the microkeratome.[24] Kim et al.[23] found greater inflammatory cell infiltration in rabbit corneas after flap creation with the femtosecond laser than when a

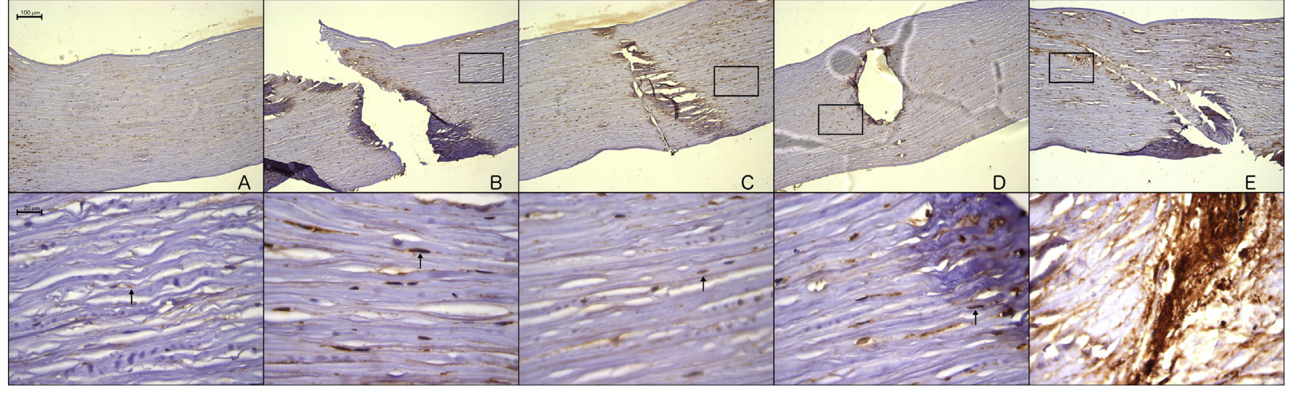

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Figure 8. Assessment of IFNγ-positive cells after manual surgery (*B*: Group 2) and the femtosecond laser technique at 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15 μJ (*E*: Group 5) energy settings evaluated via immunohistochemistry 4 hours after surgery. Interferon gamma expression in an untreated cornea (*A*: Group 1). A significant number of positive cells were found in the sample treated with the femtosecond laser at 15 μJ energy compared with all other groups. Note cavities produced by cavitation bubbles at cut margins in the sites of femtosecond laser–tissue interaction. The images are representative of a single experiment. Each section was counterstained with hematoxylin (original magnification ×10, inserts ×63).

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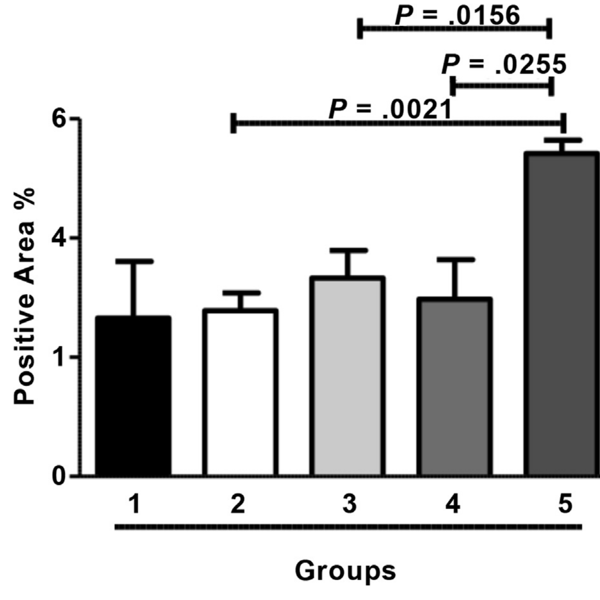

Figure 9. Computer-assisted quantification of IFNγ staining 4 hours after surgery in 3 fields ×200 for each sample. The data are expressed as the mean ± SEM and the Mann-Whitney test was used to evaluate between-group differences of the mean levels of marker expression.

mechanical microkeratome was used. Netto et al.[24] reported that LASIK flaps created with a femtosecond laser at a higher energy setting resulted in more keratocyte cell death, more severe cell inflammation, and more proliferation than flaps created using a microkeratome or a femtosecond laser at lower energy settings.

Recently, Mayer et al.[25] studied corneal inflammation and apoptosis ex vivo in human cadaver corneas after corneal incisions were made with the femtosecond laser platform for cataract surgery at a fixed energy level of 7 μJ compared to manual incisions. The authors observed no differences in the inflammatory response compared with the manual procedure. Conversely, higher apoptosis was present in corneas treated with the femtosecond laser than in corneas treated with manual incisions. Corneal inflammation was assessed by the presence of monocytes and antigen presenting cells along the cutting edge.[25] The authors hypothesized that the higher levels of cell death in the femtosecond laser–incised corneas were attributable to a direct thermal-induced energy-related apoptotic effect associated with the femtosecond laser energy.

The purpose of our study was to evaluate the possible differences in inflammation and apoptosis after manual and femtosecond laser–created corneal incisions at different energy settings in early and late inflammation and apoptotic response. Interleukin-18 and IFNγ immunohistochemical staining was used to assess corneal inflammation induced after surgery. During the early inflammatory response (5 minutes after surgery), we observed a lower expression of both IL-18 and IFNγ in samples treated with the manual technique than in those treated with femtosecond laser surgery. Interleukin-18 expression was evident in Groups 3, 4, and 5, suggesting the induction of an inflammatory response after treatment with femtosecond laser at all energy settings. In contrast, IFNγ positivity increased with femtosecond laser energy, especially after 6 μJ (Group 4) compared with the manual technique, confirming the induction of an inflammatory response due to femtosecond laser use. The late inflammatory response (4 hours after surgery) was characterized by a significant increase of IFNγ positivity in Group 5 compared with all other groups, while IL-18 positivity was not observed in any group.

Interleukin-18 has a controversial role in the pathogenesis of different diseases, with increased levels of

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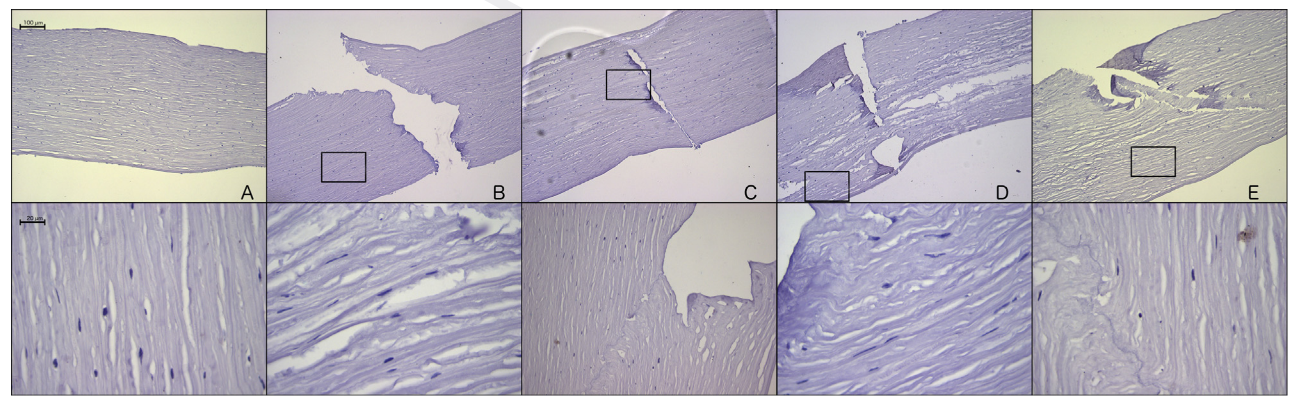

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Figure 10. Interleukin-18 expression after manual surgery (*B*: Group 2) and use of the femtosecond laser technique at 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15μJ (*E*: Group 5) energy settings evaluated via immunohistochemistry 4 hours after surgery. Interleukin-18 expression in an untreated cornea (*A*: Group 1). No IL-18 positivity was detected in any of the treated group. Note cavities produced by cavitation bubbles at cut margins in the sites of femtosecond laser-tissue interaction. The images are representative of a single experiment. Each section was counterstained with hematoxylin (original magnification ×10, inserts ×63).

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Figure 11. Assessment of TUNEL-positive cells (*arrows* in the inserts showing positive cells characterized by brown staining) after manual surgery (*B*: Group 2) and the femtosecond laser technique at 3 μJ (*C*: Group 3), 6 μJ (*D*: Group 4), and 15 μJ (*E*: Group 5) energy settings evaluated via immunohistochemistry 4 hours after surgery. The TUNEL-positive cells in an untreated cornea (*A*: Group 1). A statistically significant higher number of TUNEL-positive cells were observed in the femtosecond laser group at 15 μJ energy compared with all other groups. Note cavities produced by cavitation bubbles at cut margins in the sites of femtosecond laser–tissue interaction. The images are representative of a single experiment. Each section was counterstained with hematoxylin (original magnification ×10, inserts ×63).

IL-18 being reported in inflammatory and autoimmune diseases.[31–35] Its functional activities include promoting cytokine release, in particular TNFα, GM-CSF, IFNγ, and Fas-FasL-mediated cytotoxicity.[36] In our study, a significant increase in IL-18 and IFNγ expression was observed a few minutes after manual or femtosecond laser incisions, while only IFNγ was detectable 4 hours after incision creation. These results suggest that IL-18 activity was present only in the early phase of inflammatory response to corneal injury

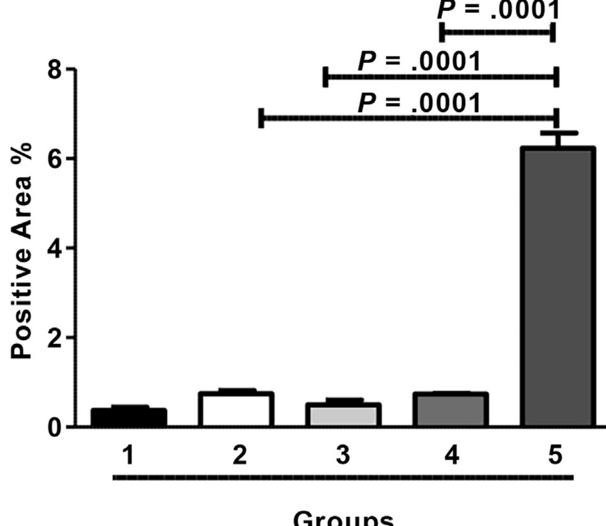

Figure 12. Statistical analysis of the data obtained from the different samples. The TUNEL-positive cells 4 hours after surgery were identified by counting the number of cells showing an intense immune-labeling in 3 ×200 fields from each sample. The data are expressed as the mean ± SEM. The Mann-Whitney test was used to evaluate between-group differences of the mean levels of marker expression.

and subsequently the signal was switched off and only IFNγ was still present.

The TUNEL assay was used to analyze apoptosis induced by the different types of surgery. Keratocyte apoptosis was previously noted to be the first observable stromal response after epithelial injury.[17] Early keratocytes apoptosis was detected using TUNEL assay after a few minutes (at 15 minutes); however, it was reported to be more prominent later, in particular after 4 hours,[37] and appeared to continue for a period extending for at least 1 week after the injury (epithelial scrape, epithelial scrape followed by PRK, and microkeratome cut).[38,39] However, apoptosis detected with the TUNEL assay appeared to diminish after 72 hours.[37]

In our study of human tissues, keratocytes apoptosis in the immediate postoperative period (after 5 minutes) and in the late period (after 4 hours) showed the highest number of TUNEL-positive cells in Group 5 compared with other femtosecond laser–treated tissues and compared with the manual group.

Our results suggest that the use of the femtosecond laser induced more proinflammatory agents and a significantly higher degree of keratocyte apoptosis than the manual technique, in particular when higher energy settings were used. These results partially agree with the results published by Mayer et al.[25] Both studies highlighted differences in apoptosis between laser surgery and manual surgery. In contrast, in our study a difference in inflammation cytokine response was also observed between the 2 types of surgery.

In our study, the use of the high-energy settings likely permitted the detection of differences between the 2 techniques. We hypothesize that the differences in inflammation and apoptosis between laser surgery and manual surgery may might be related to the

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thermal damage of the corneal tissue induced by laser energy, which increased when higher energy settings were used. The results in our study suggest that the use of low energy settings could reduce the inflammatory and apoptotic insults, thus promoting minimum tissue damage comparable to the manual procedure.

The main limitations of this study were that the in vitro analysis of the inflammatory and apoptosis corneal responses did not exactly follow the in vivo processes and that a relatively low number of the sclerocorneal rings were evaluated. Studies with larger samples that use a wider range of energy settings are required to determine the best setting for surgical optimization.

WHAT WAS KNOWN

- The use of femtosecond laser energy in corneal refractive surgery such as LASIK was associated with greater inflammation and apoptosis, in particular at high energy settings, compared with manual procedures.
- Corneal inflammation and apoptosis was also studied after CCI obtained with a femtosecond laser cataract surgery system at fixed low energy settings and did not show differences in corneal inflammation and apoptosis compared with the manual technique.

WHAT THIS PAPER ADDS

- The femtosecond laser–created CCIs at high energy settings induced a greater amount of IL-18, IFNγ, and TUNEL than the manual technique.
- A better understanding of the inflammatory cytokines and apoptosis induced by femtosecond laser would be useful for establishing an effective protocol with standardized parameters to optimize morphologic and functional results of cataract surgery.

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