ELSEVIER

LABORATORY INVESTIGATIONS

Comparison of Stromal Remodeling and Keratocyte Response After Corneal Incision and Photorefractive Keratectomy

Toshiro Tanaka

Department of Ophthalmology,
Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan

Purpose: We investigated the keratocyte response and stromal remodeling after corneal incision and photorefractive keratectomy to understand the histophysiological and immunohistochemical differences between these two types of surgery.

Methods: Corneal incision or photorefractive keratectomy was performed in rabbits or rats. Then we chronologically observed the histological changes and the changes in the localization of extracellular matrix proteins.

Results: In both types of surgery, the keratocyte population at the damaged stroma became sparse, and the cells began undergoing apoptosis immediately after the surgical procedure. Subsequently, activated keratocytes migrated into the acellular zone, and the cells formed multiple layers at the resurfaced subepithelial regions. We observed deposition of amorphous substances between keratocytes that had migrated, and stromal remodeling appeared to start. Three months after surgery, the corneal structure had recovered to a near-normal condition at the corneal incision. After photorefractive keratectomy, however, extracellular matrix proteins were strongly immunoreactive at the subepithelial regions.

Conclusions: These results suggest that the stromal wound-healing processes are similar after corneal incision and photorefractive keratectomy. A corneal incision may induce a transient keratocyte response during stromal remodeling, but photorefractive keratectomy may induce a sustained keratocyte response. **Jpn J Ophthalmol 2000;44:579–590** © 2000 Japanese Ophthalmological Society

Key Words: Corneal incision, extracellular matrix, keratocytes, photorefractive keratectomy, wound healing.

Introduction

Radial keratotomy (RK) and other types of refractive surgery that require an excimer laser are now performed worldwide. Refractive surgery differs from conventional ophthalmic surgery because it is performed on translucent corneas with good visual acuity after correction. This surgery is unique in aiming for good visual acuity without correction as well as securing good visual acuity with correction.

The history of refractive surgery began with anterior-posterior RK by Sato in 1952.[1] Although unfavorable complications from this surgery developed many years later, the fundamental concept that relaxation of peripheral corneal tension induces flattening of the central cornea has been passed on to modern anterior RK. To save the endothelium, Fyodorov developed anterior RK in 1972.[2] In the 1990s, photorefractive keratectomy (PRK) using an excimer laser was developed to solve the problems of weakness of the postoperative cornea and inaccuracy of the surgery.[3–5] Later, to resolve problems of

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Correspondence and reprint requests to: Teruo NISHIDA, MD, DSc, Department of phthalmology, Yamaguchi University School of Medicine, 1-1-1, Minami-Kogushi, Ube-shi, Yamaguchi-ken 755-8505, Japan

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extension of the correcting power and postoperative haze, PRK was changed to laser-assisted in situ keratomileusis (LASIK), which is a combination of automated lamellar keratoplasty and excimer laser surgery.[6–8] Although there have been significant advances in surgical techniques in refractive surgery, the differences in the cornea's response to the various types of surgical invasion these techniques represent are not yet sufficiently understood.

Refractive surgery can be broadly classified into procedures in which the tension of the cornea is changed by incision (RK) and astigmatic keratotomy (AK), and procedures in which the shape of the cornea is trimmed (PRK, LASIK). In incision-type refractive surgery, such as AK and RK, allowable postoperative results on a relative scale can be obtained because of the development of the nomogram, but there are still unsolved problems, such as the difference in efficacy as a result of the patient's age, and the occurrence of hypermetropia long after the operation, which was clarified by the PERK study.[9,10] In contrast, PRK is a simpler procedure and can correct myopia with greater accuracy, but there are problems, such as the recurrence of myopia and subepithelial clouding, referred to as "haze," which results from irradiation of the center of the cornea.[9,11] These problems appear to be attributable to the physiological response of the cornea to surgical invasion and the wound-healing process.

The morphological changes in the cornea and the changes in localization of the extracellular matrix resulting from the two types of surgical invasions, corneal incision and photorefractive keratectomy, have been reported in previous studies.[12–24] In the past, however, time-course changes in corneal wound healing after each surgical invasion were not observed, and the difference in corneal response to different techniques was not examined comparatively.

The principal component of the cornea is stroma, which comprises more than 90% of the total thickness, and plays a very important role in corneal transparency and refractive power. Therefore, a better understanding of the response of the stroma to surgical invasion—namely, the healing process after corneal injury—is most important in determining safe and effective postoperative treatment, and will help to resolve the problems that occur after refractive surgery.

In the present study, we focused on stromal remodeling and keratocyte response to compare how the cornea responds, morphologically and immunohistologically, to corneal incision and photorefractive keratectomy, which are the basic techniques used in refractive surgery.

Materials and Methods

Corneal Incision and Photorefractive Keratectomy

Japanese white rabbits (30 males, 1.5 kg; KBT Oriental, Tosu City, Saga) were used in the histological examinations (light microscopy and electron microscopy) and detection of apoptosis. Ketamine hydrochloride (Ketalar®50; Sankyo) and xylazine hydrochloride (Seractar® 2% injection, Bayer) were administered to the rabbits to induce general anesthesia. Local anesthesia was induced by dropping 4% lidocaine into the rabbits' eyes. After the rabbits were anesthetized, a corneal incision (a straight incision 6 mm long and 250 μm deep through the center of cornea) was made with a diamond knife (Katena, Denville, NJ, USA) in both eyes. As simulated photorefractive keratectomy, mechanical ablation of the epithelium in a circle 6 mm in diameter in the center of the cornea, followed by irradiation with an excimer laser was performed in both eyes. An EC-5000 (Nidek, Gamagori) was used to generate the excimer laser. The irradiation conditions were 30 Hz and 78 scans at PRK mode. These conditions correspond to the dose for correcting human myopia of 3 diopters.

Rats (16 male Wistar rats, 250 g; Seac-Yoshitomi, Chikugami-gun, Fukuoka) were used in the immunohistological examinations. A straight incision 3 mm long and 150 μm deep was made in the center of the cornea in both eyes with a Katena diamond knife. After mechanical ablation of the corneal epithelium in a circle 3 mm in diameter, the site was irradiated in both eyes with an excimer laser (PTK mode, 30 Hz, 83 scans), corresponding to a depth of 50 μm in the human cornea.

After each type of simulated surgery, no medical treatment, such as antibiotic eyedrops, was applied. However, no serious corneal disorders, such as infection and ulceration, were observed in any of the eyes examined. One or two animals (2 or 4 eyes) from each experimental group were euthanized by injection of an excess amount of pentobarbital sodium (Nembutal®; Dainippon Pharmaceutical, Osaka) into an ear vein at defined intervals, and the enucleated eyes were examined histologically and immunohistologically.

The committee for animal research of the Yamaguchi University School of Medicine reviewed the present study. Care and treatment of animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Detection of Apoptosis

Japanese white rabbits that underwent corneal incision or excimer laser irradiation were euthanized 1

hour after surgery, the eyeballs were enucleated, embedded in OCT compound (Miles, Elkhart, IN, USA) and frozen in dry ice-acetone. Frozen sections 6 μm thick were prepared with a microtome cryostat (HM 505N; Zeiss, Oberkochen, Germany). After TUNEL-staining with the Apoptosis Detection System (Promega, Madison, WI, USA), sections were mounted using a mixture of phosphate-buffered saline (PBS) and nonfluorescing glycerine (1:2), and examined with a fluorescence microscope (Axioskop 50; Zeiss) under dark contrast. Micrographs were taken using Fujichrome ISO-400 film.

Histological Examination

Japanese white rabbits that underwent corneal incision or excimer laser irradiation were euthanized at 3 or 6 hours, 1 day, 1 or 2 weeks, or 1 or 3 months after surgery. Immediately after sacrifice, the animals were fixed with a perfusion of 2% glutaraldehyde through the aorta. Eyeballs were enucleated, and dually fixed with 2% glutaraldehyde and 2% osmium tetroxide. After dehydration by a series of acetone solutions, the tissue was embedded in Epon resin, thin-sectioned, stained with toluidine blue, and examined with a light microscope (Axioskop 50; Zeiss). Ultra-thin sections were also prepared, and stained with 2% uranyl acetate and lead citrate. The ultrastructure was examined with an electron microscope (JOEL-200X; Nihon Denshi, Tokyo).

Immunohistological Examination

Changes in localization of the extracellular matrix, laminin, type VI collagen, and fibronectin were examined immunohistologically.

Antibodies. Rabbit anti-human fibronectin antibody (LSL, Tokyo), rabbit anti-mouse laminin antibody (LSL), and rabbit anti-mouse type-IV collagen antibody (LSL) were used as primary antibodies. Normal rabbit serum (Cappel, Aurora, OH, USA) was used as a negative control. Fluorescein isothiocyanate-labeled goat anti-rabbit IgG antibody (Cappel) was used as a secondary antibody. Each antibody was diluted 1,000-fold with PBS containing 1% bovine serum albumin Fraction-V (BSA; Intergen, Purchase, NY, USA).

Immunohistochemistry for extracellular matrix proteins. The 16 rats that underwent corneal incision or excimer laser irradiation were sacrificed 1 day, 1 or 2 weeks, or 1 or 3 months later, and eyeballs were enucleated, embedded in OCT compound (Miles) and frozen in dry ice-acetone. Frozen sections 6 μm thick were prepared with a microtome cryostat (HM 505N; Zeiss). Sections were washed with PBS and incubated with 1% BSA in PBS for 1 hour (at room temperature) in order to block nonspecific reactions. The sections were then treated with a primary antibody or the negative control serum at room temperature for 1 hour, washed with PBS, and treated with the secondary antibody at room temperature for 1 hour. After another PBS wash, the sections were mounted using a mixture of PBS and nonfluorescing glycerine (1:2), and examined with a fluorescence microscope (Axioskop 50; Zeiss) under dark contrast. Micrographs were taken using Fujichrome ISO-400 film.

Results

Morphological Observations

Early stage after surgery. Soon after the corneal incision (within 6 hours after surgery), we observed through a light microscope that the incision site was open, forming a V-shape (Figure 1A). In addition, broken or irregularly stained degenerated keratocytes were found in the stroma around the incision site (Figure 1B). Using electron microscopy, we detected condensed nuclear chromatin (Figure 1C), and apoptotic bodies broken into fine pieces and surrounded by membrane (Figure 1D). After TUNEL-staining of the corneal tissue, a few TUNEL-positive cells were observed in the normal cornea, but many were found around the wound of the incised cornea (Figure 1E).

Light-microscopic observation, after excimer laser irradiation had exposed the stromal surface (Figure 1F), showed that the cells of the resected stromal surface had degenerated (Figure 1G). Electron microscopy showed condensation of the nuclear chromatin (Figure 1H)and apoptotic bodies (Figure 1I) in degenerated keratocytes. TUNEL-staining revealed many TUNEL-positive cells in the area irradiated with the excimer laser (Figure 1J).

These findings demonstrate that, after corneal incision or irradiation with an excimer laser, keratocyte death resulting from apoptosis occurred around the injury site, and an acellular zone developed in the early stage after surgery.

One week after surgery. One week after corneal incision, the incision site was resurfaced with epithelial cells, and many spindle-shaped keratocytes were found under the hyperplastic epithelium (Figure 2A). Electron microscopy also showed a gathering of spindle-shaped keratocytes in a layer just beneath the epithelium (Figure 2B). Because few intercellular collagen fibers were found, these keratocytes

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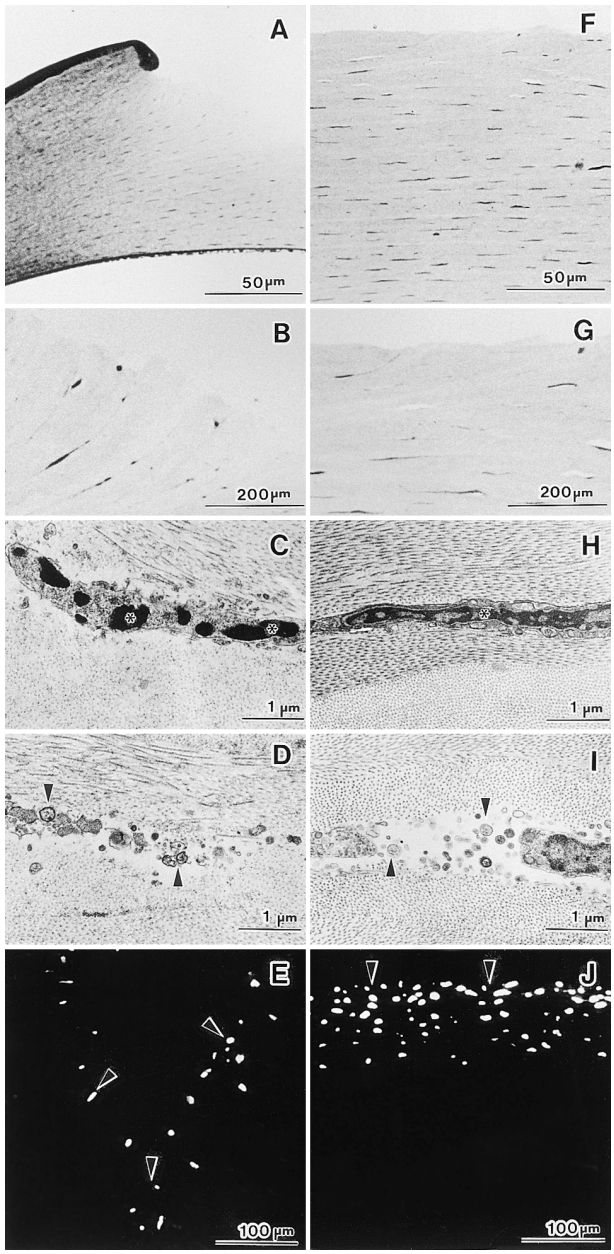

Figure 1. Histological changes in early stage after corneal incision (**A**–**E**) or excimer laser irradiation (**F**–**J**). Within 6 hours after corneal incision, injury site formed V-shape (**A**); after excimer laser irradiation, stroma was exposed (**F**). After both types of surgery, keratocytes at injury site had degenerated (**B**,**G**); there was condensation of nuclear chromatin (*,**C**,**H**). Apoptotic bodies (arrowheads, **D**,**I**) were found in degenerated cells, and TUNEL-positive cells (arrowheads) were found in wounded area (**E**,**J**).

were thought to be migrating under the resurfaced epithelial cell layer. In addition, these cells had many rough-surfaced endoplasmic reticula and seemed to be activated keratocytes (Figure 2C).

One week after excimer laser irradiation, the irradiation site was completely resurfaced with corneal epithelial cells. In the region around the irradiation site that was an acellular zone in the early stage after the operation, we found many keratocytes with thick processes. This finding suggests that activated keratocytes migrated from the surrounding area into the acellular zone generated by laser irradiation (Figure 2D). However, no cell components were found under the epithelium in the center of the irradiated area (Figure 2E), but some of the apoptotic bodies did not disperse but remained, forming a spindle-shaped mass (Figure 2F).

These findings suggest that 1 week after corneal incision, the inflammatory period of the early stage had already passed, and that the second stage, characterized by hyperplasia of the epithelial cells and synthesis of a new matrix by subepithelial gathering of activated keratocytes, had begun. This second stage, therefore, is the cell-activation stage in the wound-healing process. By contrast, after excimer laser irradiation, although resurfacing of the epithelium had

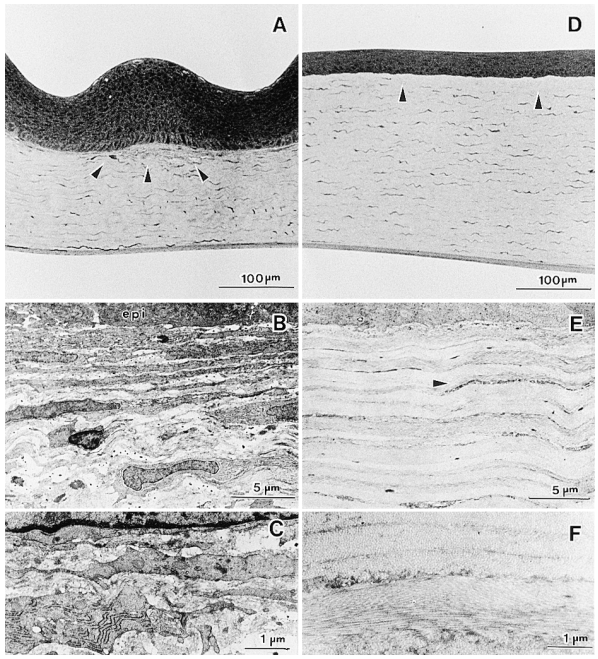

Figure 2. Histological changes 1 week after corneal incision or excimer laser irradiation. A: One week after corneal incision, epithelium was hyperplastic, and many keratocytes (arrowheads) were found under epithelium. (**B**,**C**) Keratocytes gathered just under epithelium (epi), and activated keratocytes were also found. (**D**) One week after excimer laser irradiation, keratocytes (arrowheads) started to infiltrate into irradiation site. (**E**,**F**) Electron microscopic observation detected no cell components under epithelium, but remnants of apoptotic bodies (arrowhead) were found.

been completed, an acellular zone remained in the stroma, and active keratocytes from the surrounding area had begun to migrate. Therefore, this stage appeared to be the transitional period between the inflammatory stage and the cell-activation stage.

Two weeks after surgery. Two weeks after corneal incision, the epithelium of the incised region began to thin, and epithelial cell differentiation had progressed. As was the case 1 week after incision, many keratocytes were observed under the epithelium at the incision site (Figure 3A). Electron microscopy revealed the formation of a layered structure by subepithelial keratocytes (Figure 3B). An amorphous extracellular matrix was found in the keratocyte intercellular spaces (Figure 3C).

Two weeks after excimer laser irradiation, the resurfaced epithelium at the irradiation site showed a multiple-layered structure, suggesting progress in differentiation. Many keratocytes were found under the epithelium (Figure 3D). Electron microscopy revealed that subepithelial keratocytes were gathering and forming a layer by keeping a small distance between collagen fibers of the remaining stromal surface and the epithelium (Figure 3E). An amorphous extracellular matrix was found in the keratocyte intercellular space (Figure 3F).

Thus, from 1 to 2 weeks after corneal incision, extracellular matrix components were synthesized by keratocytes that gathered under the epithelium, and amorphous substances accumulated in the intercellular space. Therefore, this period was considered the remodeling stage in the wound-healing process. By contrast, after excimer laser irradiation, activated keratocytes gradually migrated and synthesis of matrix began simultaneously. Therefore, this period was considered a transitional stage between cell activation and remodeling.

Three months after surgery. Three months after corneal incision, the epithelium became thinner but was still hyperplastic compared with normal corneal epithelium. In the subepithelial stroma, the intercellular distance between keratocytes increased and became almost the same as that in the normal cornea (Figure 4A). Electron microscopy revealed an irregular epithelial basement membrane structure. The stroma just under the epithelium contained almost no collagen fibers, but amorphous substances were found (Figures 4B,C). In the deep zone of the stroma, collagen fibers were observed in a relatively regular arrangement around keratocytes, but highly electron-dense amorphous substances were spread among the collagen fibers (Figure 4D).

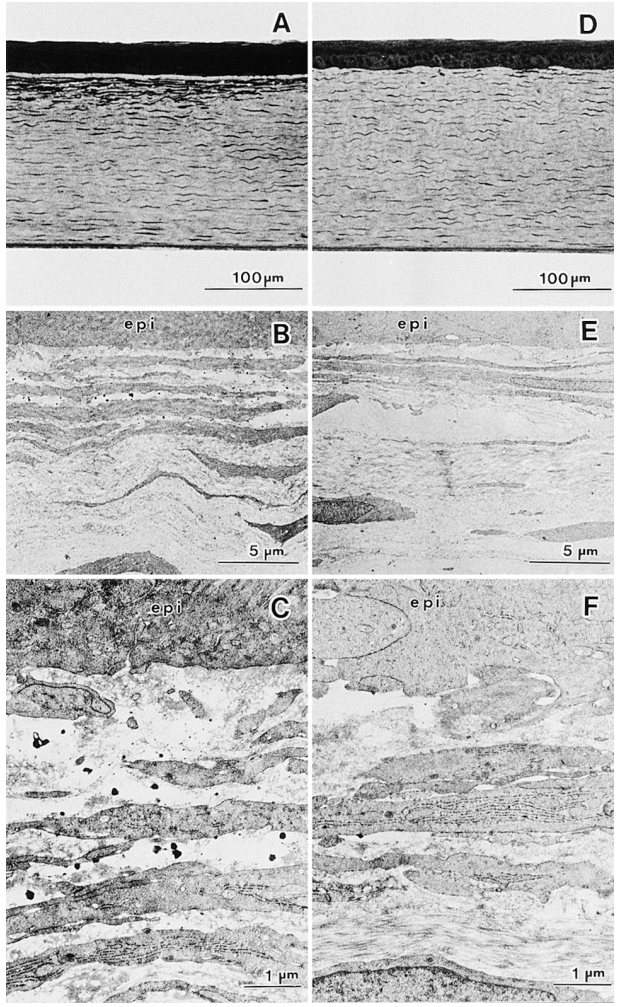

Figure 3. Histological changes 2 weeks after corneal incision or excimer laser irradiation. Two weeks after corneal incision, many keratocytes were found under epithelium (**A**). Keratocytes gathered under epithelium (epi), forming layer with spaces between keratocytes (**B**), and amorphous extracellular matrix was found in intercellular space (**C**). Two weeks after excimer laser irradiation, keratocytes had gathered under epithelium (**D**). Keratocytes were localized just under epithelium (epi) (**E**), and amorphous extracellular matrix was seen in intercellular space (**F**).

Three months after excimer laser irradiation, the layered structure of the epithelium was almost normalized, and the intercellular distance between the keratocytes in the stroma was normal (Figure 4E). Electron microscopy revealed a relatively smooth basement membrane structure under the epithelium. In the shallow zone of the reconstructed stroma just under the epithelium, an extremely electron-dense substance was found among collagen fibers in a relatively regular arrangement (Figures 4F–H).

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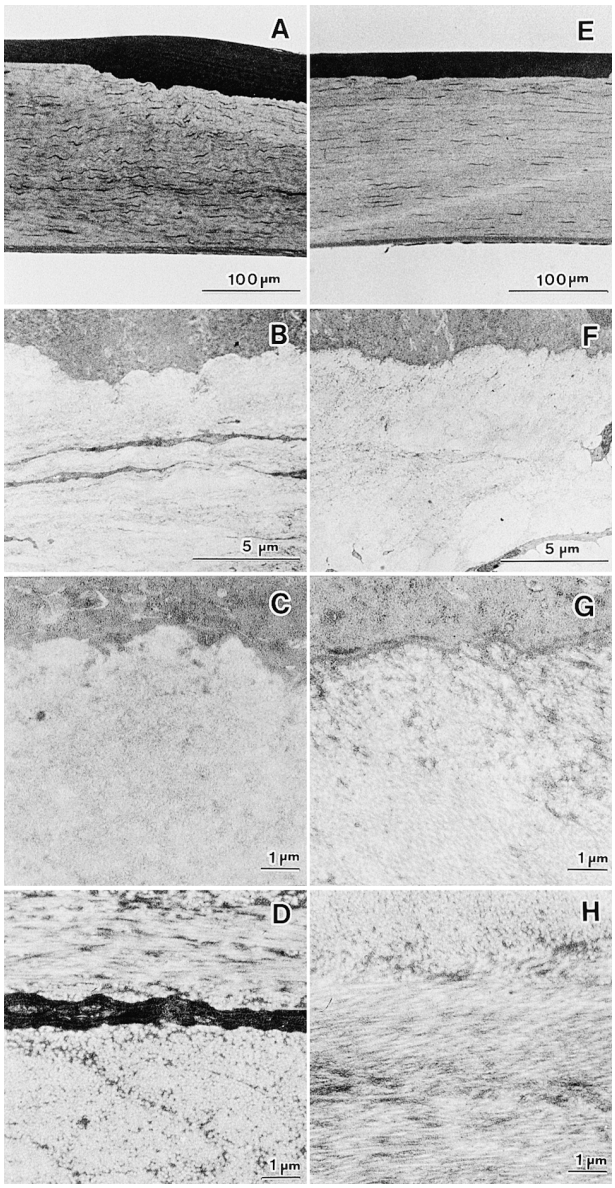

Figure 4. Histological changes 3 months after corneal incision or excimer laser irradiation. Three months after corneal incision, epithelium was still hyperplastic, but keratocyte density was almost normal (**A**). Although amorphous matrix was found in reconstructed region (**B**,**C**), arrangement of collagen fiber layers was seen in deep stromal zone (**D**). Three months after excimer laser irradiation, epithelium was almost normal, and keratocyte density was also normal (**E**). Reconstructed stroma consisted of amorphous extracellular matrix (**F**,**G**), including collagen fibers (**H**).

As shown above, the morphologically normal corneal structure seems to have been restored at 3 months after corneal incision. However, the boundary between the epithelial cell layer and the stroma was not yet smooth. By contrast, after excimer laser irradiation, collagen fibers in a nearly regular arrangement were found in the subepithelial stroma, suggesting considerable progress in tissue remodeling.

Immunohistological Examinations

Normal cornea. In the cornea of normal rats, fibronectin occurs along collagen fibers in the epithelial basement membrane, keratocytes and stroma, and it is also found in Descemet's membrane (Figure 5A). The fluorescence of laminin occurs in the epithelial basement membrane and Descemet's mem-

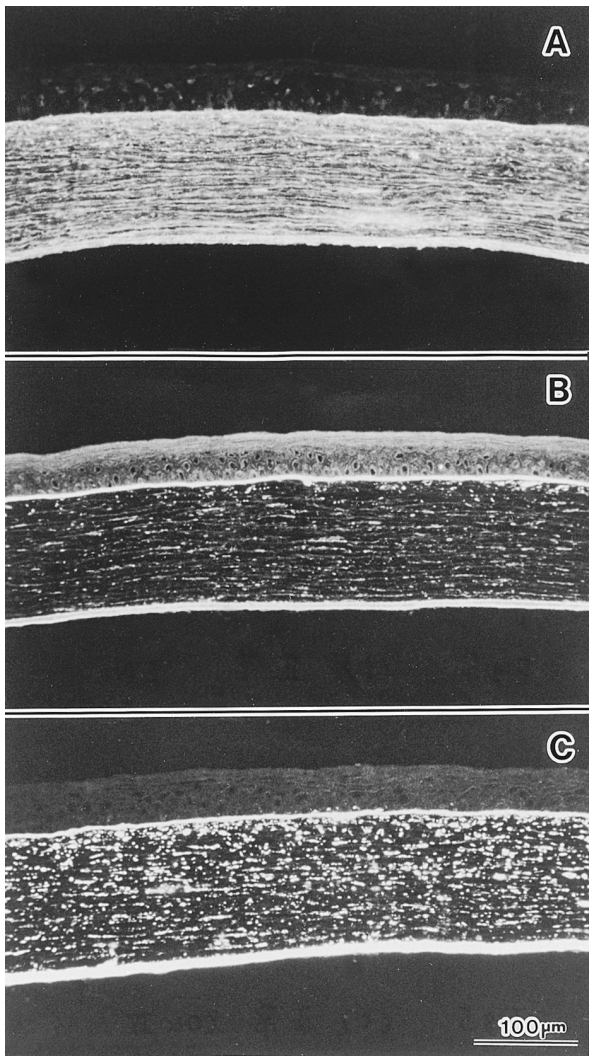

Figure 5. Localization of extracellular matrix components in cornea of normal rats. Fibronectin was found in epithelial basement membrane, stroma and Descemet's membrane (**A**). Laminin was found in epithelial basement membrane and Descemet's membrane (**B**). Type IV collagen was found in epithelial basement membrane and Descemet's membrane, and also as spots in stroma (**C**).

brane and as thin lines in the stroma (Figure 5B). The fluorescence of type IV collagen was also observed in the epithelial basement membrane and Descemet's membrane and as spots in the stroma (Figure 5C).

Wounded cornea. *Fibronectin.* One day after the incision, strong fluorescence of fibronectin was observed at the incision site (Figure 6A). One day after excimer laser irradiation, strong fluorescence of fibronectin was observed in the irradiated area (Figure 6C). Therefore, we demonstrated that with both techniques, fibronectin was expressed as a temporary basement membrane for resurfacing the denuded area with epithelial cells in the stromal surface of the injury site 1 day after surgery.

Three months after corneal incision, localization of fibronectin was almost the same as that in the normal cornea, but moderately strong fluorescence was found only under the incised epithelium (Figure 6B). However, 3 months after excimer laser irradiation, strong fibronectin fluorescence was observed under the epithelium (Figure 6D). This finding suggests active fibronectin synthesis by keratocytes in the reconstructed region.

Laminin. One day after incision, a slight fluorescence of laminin was observed in the boundary between the stroma and the epithelium that was resurfaced with epithelial cells, but not on the surface of the incision (Figure 7A). One day after irradiation with an excimer laser, no fluorescence of laminin was found on the irradiated surface (Figure 7C). Therefore, no matter which technique was used, laminin disappeared from the injured area of the epithelial basement membrane 1 day after either surgical dissection or resection of the basement membrane.

Three months after corneal incision, fluorescence of laminin in the subepithelial stroma of the incision site had decreased considerably after an initial increase (Figure 7B). In contrast, 3 months after excimer laser irradiation, localization of laminin was observed as strong fluorescence under the epithelium (this is similar to the result with fibronectin) (Figure 7D). This result suggests active laminin synthesis by migrating corneal epithelial cells and the presence of keratocytes in the reconstructed stroma.

Type IV collagen. One day after incision, no fluorescence of type IV collagen was found at the incision site (Figure 8A). One day after excimer laser ir-

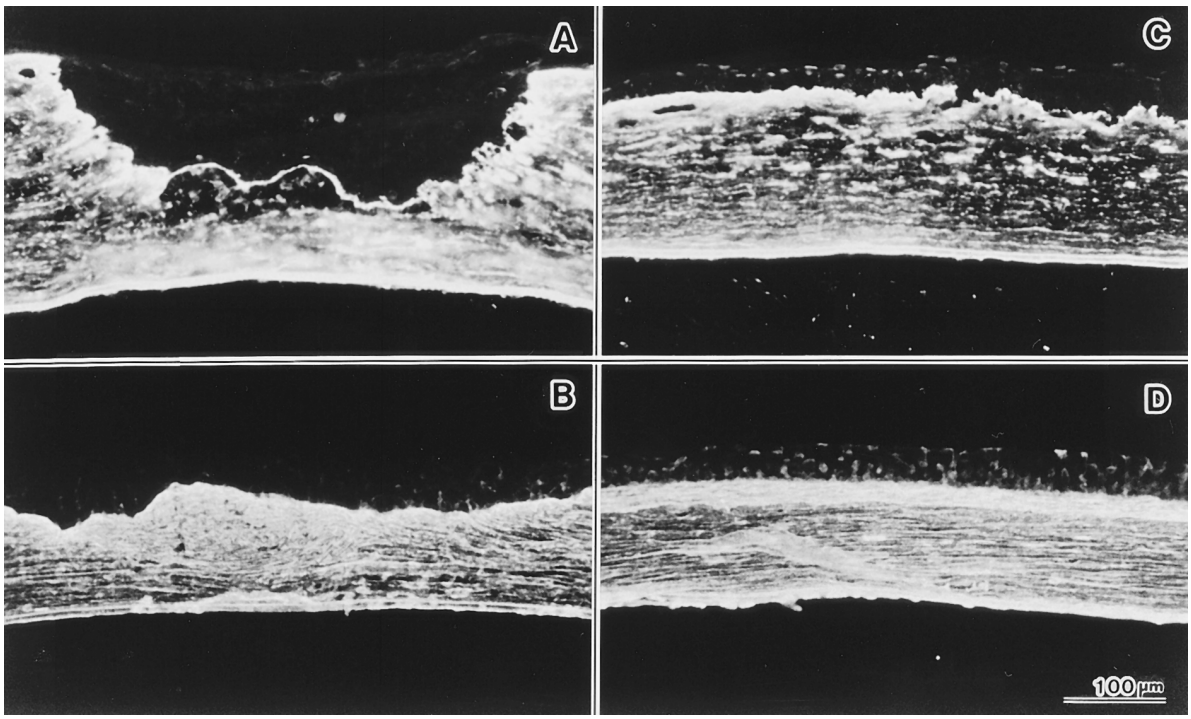

Figure 6. Localization of fibronectin after corneal incision or excimer laser irradiation. One day after incision, intense fluorescence was found at incision site (**A**). One day after irradiation, intense fluorescence was observed in shallow zone of stroma (**C**). Three months after corneal incision, moderately intense fluorescence was found in subepithelial region (**B**). Three months after irradiation, intense fluorescence was observed under epithelium (**D**).

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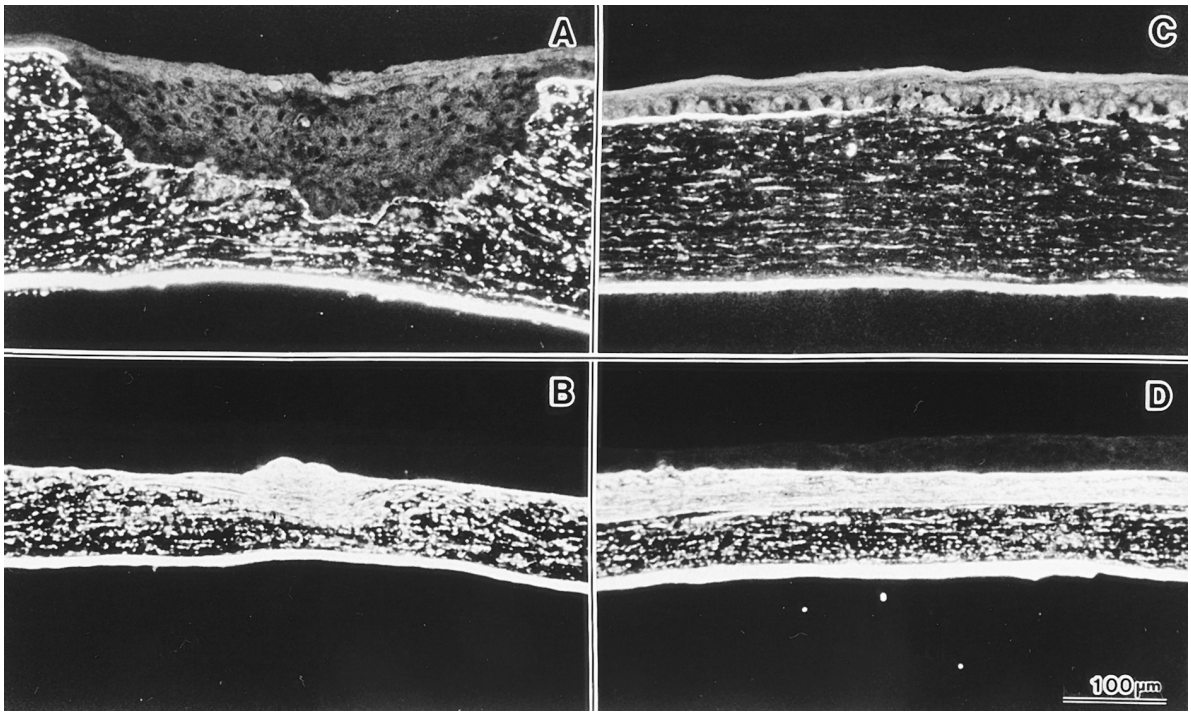

Figure 7. Localization of laminin after corneal incision or excimer laser irradiation. One day after incision, no fluorescence was found at incision site (**A**). One day after irradiation, no fluorescence was observed at irradiation site (**C**). Three months after corneal incision, slight fluorescence was found in subepithelial region (**B**). Three months after irradiation, intense fluorescence was observed under epithelium (**D**).

radiation, no fluorescence of type IV collagen was seen in the irradiated area (Figure 8C). Therefore, as was the case with laminin, type IV collagen disappeared from the injured area of the epithelial basement membrane within 1 day after the basement membrane had been dissected and resected, whether the surgery was performed by irradiation or incision.

As was the case with laminin, 3 months after corneal incision, fluorescence of type IV collagen in the subepithelial stroma of the incision site had decreased considerably after an initial increase (Figure 8B). In contrast, 3 months after excimer laser irradiation, localization of type IV collagen was observed as strong fluorescence under the epithelium (Figure 8D), suggesting active synthesis of type IV collagen by keratocytes in the reconstructed region.

Discussion

In the present study, the keratocyte responses to corneal incision and to photorefractive keratectomy were examined morphologically, and changes in localization of the extracellular matrix in the remodeled stroma were also examined immunohistologically by comparing the results of using these two surgical techniques. Although the quality of the wound-healing process in the cornea did not differ, the time of the response or the degree of the response of the cornea to surgical invasion was clearly different for the two techniques. That is, after corneal incision, the injury site was repaired relatively rapidly whereas photorefractive keratectomy resulted in protracted keratocyte activation and accumulation of matrix substances.

Using histophysiological and immunohistochemical techniques, many studies have investigated the morphological changes in the cornea and the changes in localization of the extracellular matrix proteins after corneal incision and photorefractive keratectomy.[12–24] The results of each surgical invasion agreed with our present study. However, as far as we know, this is the first report in the literature to investigate the time-course of changes in corneal wound healing after each surgical invasion and to compare the difference in corneal response to different techniques. In the present study, we simultaneously compared the corneal response to two types of surgical invasion, corneal incision and photorefractive keratectomy, and demonstrated a great difference in the corneal response to these different

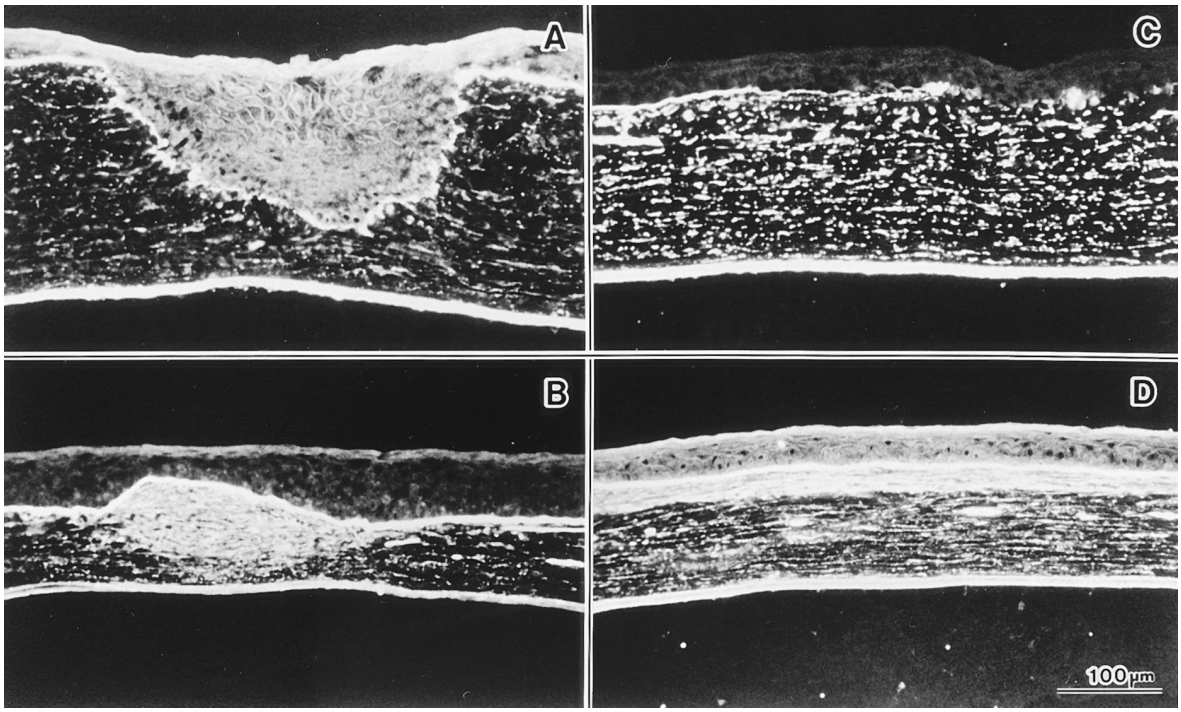

Figure 8. Localization of type IV collagen after corneal incision or excimer laser irradiation. One day after incision, no fluorescence was found at incision site (**A**). One day after irradiation, no fluorescence was observed at irradiation site (**C**). Three months after corneal incision, slight fluorescence was found in subepithelial region (**B**). Three months after irradiation, intense fluorescence was observed under epithelium (**D**).

techniques of refractive surgery that have the same goal. As several incisions are performed in RK or AK clinically, we cannot make a definite comparison of the morphological and immunohistochemical results of our two types of simulated surgery. Further detailed studies are needed to compare corneal responses after surgery.

The time-course observations of the wound-healing process in the corneal stroma after corneal incision and photorefractive keratotomy are shown in Figure 9. In the present study, in both techniques, keratocytes around the injured section died as a result of apoptosis, and an acellular zone was generated. Later, treatment by the two techniques resulted in similar corneal healing responses: the inflammation stage, consisting of the inflammatory cell infiltration; the cell-activation stage, consisting of gathering of activated keratocytes under the regenerating epithelium; and the remodeling stage, consisting of reconstruction with an extracellular matrix produced by migrating keratocytes. However, the timing or the degree of corneal response to these two types of surgical invasion was clearly different. With corneal incision, reaction in the inflammation stage was strong, but the remodeling stage progressed relatively rapidly, accumulation of abnormal matrix substance decreased gradually, and a recovery fairly close to the normal structure was observed after 3 months. With photorefractive keratectomy, however, inflammation was relatively mild, but transition from the cell-activation stage to the remodeling stage was delayed, and accumulation of abnormal matrix substance lasted for a long time. This extension of the remodeling stage agreed relatively well with keratocyte dynamics, and we considered that this indicated continuous keratocyte activity. As late as 6 months after photorefractive keratectomy, accumulation of extracellular matrix in the shallow layer of stroma was found (data not shown). Anderson et al[25] and Latvala et al[26] reported that fibronectin and type IV collagen were found in the shallow stromal layer more than 1 year after surgery. This continued tissue remodeling seems to be related to subepithelial clouding or haze, which is a problem after photorefractive keratectomy. In addition, not only synthesis of extracellular matrix after photorefractive keratectomy but also postoperative metabolic control of matrix substances, such as MMPs/TIMPs and the PA/plasmin system seem to be related to the prognosis after surgery.[27–30] Although one possible explanation for the corneal haze and refractive regression that occur clinically after photorefractive keratectomy may

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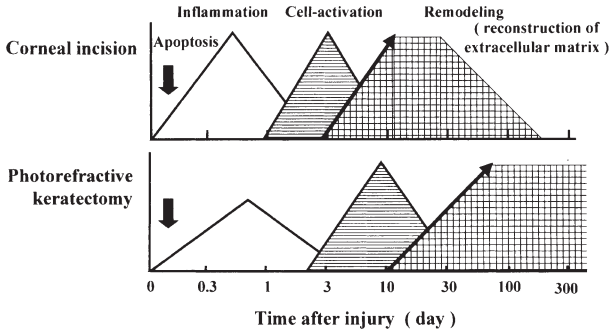

Figure 9. Time-course of wound-healing process of corneal stroma after corneal incision and photorefractive keratectomy.

be the continued tissue remodeling by activated keratocytes, further investigations are needed to resolve the postoperative problems.

Cell infiltration into the injury site in the inflammation period of the early wound-healing stage is considered to be migration of inflammatory cells, such as polymorphonuclear leukocytes derived from blood vessels of the sclera and conjunctiva, via tears or the corneal limbus into the stroma.[31,32] In a corneal incision, the injury site is opened to form a V-shape, and tear-derived inflammatory cells can easily infiltrate into the stroma of the injury site; the response in the inflammation stage seems to be strong after corneal incision. By contrast, in photorefractive keratectomy, a wide area of the stromal layer is irradiated after ablation of the epithelium, creating a large acellular zone after surgery. Therefore, gathering of activated keratocytes in the surrounding area is delayed, and transition to the cell-activation stage appears delayed. Activated keratocytes gather, extracellular matrix is actively synthesized, and the remodeling stage appears to continue. In a wide-area mechanical corneal incision, unlike in photorefractive keratectomy, a similar continuous remodeling stage was observed.[33] Moreover, it has been reported that, after photorefractive keratectomy, activated keratocytes produce and secrete various cytokines (IL-1, IL-6, TNF-α), growth factors (HGF, KGF, TGF-β, PDGF) and extracellular matrix components (FN, TN, SPARC).34-41 These humoral factors may induce continuous tissue remodeling by affecting corneal epithelial cells and keratocytes.

It is well known that an acellular zone forms in the stromal layer of the injury site shortly after invasion of the cornea.[31,42–44] Recent studies demonstrated involvement of apoptosis in developing this type of acellular zone. Some studies have suggested that apoptosis is induced in keratocytes around the injury site shortly after injury, regardless of the type of corneal invasion, and an acellular zone is formed This induction of apoptosis may act as a signal for initiation of wound healing .[40,45–47] In the present study, condensation of nuclear chromatin and formation of apoptotic bodies were found in keratocytes around the injury site shortly after both corneal incision and photorefractive keratectomy. Positive TUNEL-staining in the present study demonstrated that cells died as a result of induction of apoptosis and development of an acellular zone. As to the mechanism of apoptosis induction in response to injury, involvement of IL-1 and the Fas-Fas ligand system have been reported,[48–50] but have not been fully elucidated.

Refractive surgery is a technique that aims to improve visual acuity without correction by surgically altering translucent corneas. Therefore, maintenance of translucency in the pupillary area is absolutely required. At the same time, to obtain a better result, both an accurate monogram and control of the correction effect are required in each case. Therefore, more accurate correction should be achieved if postoperative wound healing is controlled. To control postoperative wound healing, clarification of the mechanism of wound healing in the cornea and its pharmacological control are vital.

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