

Physical and Biological Characterization of the Gamma-Irradiated Human Cornea

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Purpose: To compare the physical and biological characteristics of commercial gamma-irradiated corneas with those of fresh human corneas and to determine suitability for transplantation.

Methods: The physical properties of gamma-irradiated and fresh corneas were evaluated with respect to light transmittance, hydration (swelling ratio), elastic modulus (compressive modulus by the indentation method), matrix organization (differential scanning calorimetry), and morphology (light and transmission electron microscopy). The biological properties of the gamma-irradiated cornea, including residual cell content and cellular biocompatibility, were evaluated by quantifying DNA content and measuring the proliferation rate of human corneal epithelial cells, respectively.

Results: The hydration, light transmittance, elastic modulus, and proliferation rate of human corneal epithelial cells were not significantly different between fresh and gamma-irradiated corneas. However, differences were observed in tissue morphology, DNA content, and thermal properties. The density of collagen fibrils of the gamma-irradiated corneal sample (160.6 ± 33.2 fibrils/ μm^2) was significantly lower than that of the fresh corneal sample (310.0 ± 44.7 fibrils/ μm^2). Additionally, in the gamma-irradiated corneas, cell fragments—but not viable cells—were observed, supported by lower DNA content of the gamma-irradiated cornea (1.0 ± 0.1 $\mu\text{g}/\text{mg}$) than in fresh corneas (1.9 $\mu\text{g}/\text{mg}$). Moreover, the denaturation temperature of gamma-irradiated corneas (61.8 ± 1.1 °C) was significantly lower than that of fresh corneas (66.1 ± 1.9 °C).

Conclusions: Despite structural changes due to irradiation, the physical and biological properties of the gamma-irradiated cornea remain similar to the fresh cornea. These factors, combined with a decreased risk of rejection and longer shelf life, make the gamma-irradiated tissue a viable and clinically desired option in various ophthalmic procedures.

Key Words: gamma irradiation, human cornea, corneal substitute, corneal transplantation, extracellular matrix

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Corneal disease is the fourth leading cause of blindness worldwide.¹ Many of these cases could be cured by allogeneic corneal transplantation, one of the most common and successful tissue transplantation procedures.² However, there are obstacles to corneal transplantation, including insufficient quality and quantity of donors, a short viable time frame between donation and transplantation, contamination by bacteria and fungi during donor preparation, and the possibility of disease transmission.^{2–4} In addition, conventional corneal transplantation has a high failure rate in high-risk populations because of immune rejection or mechanical graft failure.³

The gamma-irradiated human cornea (VisionGraft; Tissue Banks International, Baltimore, MD) may help to alleviate such problems. Donor corneas unsuitable for transplantation, typically because of low endothelium quality, become commercial gamma-irradiated corneas through cryogenic treatment and approximately 17 to 23 kGy of gamma irradiation from a cobalt-60 source. Those processes extend shelf life, expanding the pool of potential donor corneas. Another benefit of using the gamma-irradiated cornea is the reduction of the disease transmission risk and microbial contamination during donor preparation. Third, the gamma-irradiated cornea can potentially be transplanted in populations at high risk of immune rejection when using conventional human corneas.^{2,5,6} Although the gamma-irradiated cornea cannot be used for full-thickness corneal transplantation, it can be used for lamellar keratoplasty (which does not require viable endothelial cells) and for corneal patch grafts.⁶ In addition to applications in corneal surgery, this tissue can be used to cover glaucoma tube shunts and act as the skirt of the keratoprosthesis.² To accommodate the surgeon's preferences, these corneas can also be provided in various shapes and sizes with full- or partial-thickness stromas.

Gamma irradiation of donor corneas, however, may alter the structure of the corneal extracellular matrix (ECM) and change its physical and biological properties, which would in turn affect corneal physiology and limit its surgical utility. The effect of gamma irradiation has been studied on various tissues. Although there were no significant changes after applying a low dose (2–10 kGy) of gamma irradiation in the Achilles tendon,⁷ most studies have indicated that gamma

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irradiation induces substantial physical and/or biological alterations with structural changes.^{8–12} The structural alterations include intercollagen and intracollagen cross-linking, collagen molecular fragmentation or degradation by polypeptide chain scission, and collagen fibril reorganization.^{12–15} Because collagen is the main component of corneal proteins,¹⁶ gamma irradiation could be expected to biologically and physically alter the corneal ECM. In addition, altered physical and biological properties influence corneal wound repair and surgical manipulation such as handling and suturing the donor tissue.

Although the gamma-irradiated cornea has been used successfully in clinical situations and its popularity is rising, there is limited information about its optical properties and structure, especially compared with fresh donor corneas.^{17,18} Therefore, the purpose of this study was to characterize physical and biological properties of the commercial gamma-irradiated cornea and to determine whether it is a viable surgical material.

METHODS

Corneas

Gamma-irradiated human corneas were donated by Tissue Banks International (Baltimore, MD). Human corneas were handled according to the Declaration of Helsinki, and the study was approved by Johns Hopkins Medicine Institutional Review Boards. A total of 28 fresh and 25 gamma-irradiated corneas (within the expiration date) were used in this experiment. The average age of donors was 52.9 ± 15.8 years (range, 18–74 years), the average days of storage was 8.8 ± 4.4 days (range, 1–13 days), and the average death to preservation time was 12.5 ± 5.1 hours (range, 3–22.7 hours). As a negative control, to emulate a degrading tissue matrix, 6 gamma-irradiated corneas that were more than 2 years past the manufacturer's expiration date (outdated gamma-irradiated corneas) were evaluated for the characteristics of light transmittance, compressive modulus, and hydration. The term "gamma-irradiated cornea" indicates a cornea within its expiration date. Fresh corneas were preserved in Optisol GS (Bausch & Lomb, Rochester, NY) at 2 to 8°C, and gamma-irradiated corneas were stored in hyperosmolar albumin-based media at room temperature. All samples for the ensuing tests were prepared by removing the scleral rim and epithelium using conventional ophthalmic scissors and a #15 blade before performing experiments.

Light Transmittance

Five full-sized, fresh, and gamma-irradiated corneas and 3 full-sized outdated gamma-irradiated corneas were cut with a 10-mm trephine. Each cornea was washed 3 times using balanced buffer solution (BSS) and placed on a 48-well microplate. Using a multimode microplate reader (Synergy 2; BioTek, Seattle, WA), the blank-corrected light absorbance of each cornea was measured in 10-nm increments over the full visible spectrum (400–700 nm). The light transmittance was

derived by the Beer–Lambert law with the equation, transmittance (%) = $e^{-\text{absorbance}} \times 100$.

Compressive Modulus

Immediately after measuring light transmittance, the same corneas were used for measuring compressive modulus. Compression was applied to the full-sized sample from 0% to 10% thickness of the cornea using an ElectroForce 3200 testing instrument (Bose, Eden Prairie, MN) with a 250-g load cell at room temperature. The modulus was calculated using a computer program (MATLAB; MathWorks, Natick, MA) to find the slope of the best-fit curve with a linear region of stress versus strain plot, as performed previously.¹⁹

Hydration

The swelling ratio (wet mass/dry mass) was determined as follows: 5 half-sized, fresh, and gamma-irradiated corneas and 3 half-sized, outdated gamma-irradiated corneas were immersed in a sealed conical tube containing 10 mL of BSS and incubated at 37°C for 24 hours. After 24 hours, the swollen corneas were weighed after removing excess BSS using a filter paper and a digital balance (XS 105; Mettler Toledo, Columbus, OH). The samples were then lyophilized for 72 hours and weighed to obtain the dry mass.

Thermal Properties

The thermal properties of each cornea, collected from both the central and peripheral areas, were characterized using differential scanning calorimetry (DSC 8000; PerkinElmer, Norwalk, CT) as described previously.²⁰ Using a 4-mm trephine, samples approximately 20 mg in mass were collected from 5 full-sized, fresh, and gamma-irradiated corneas. One sample was taken from the central cornea, and 4 buttons were excised from the peripheral region. The specimens were sealed in a 30- μ L aluminum pan to prevent evaporation of the water content; an empty sealed pan was used as a reference. At a rate of 5°C/min, the samples were cooled from room temperature to -30°C under nitrogen flow and then heated to 90°C. A set of cooling and heating was repeated. The thermograms were analyzed and the denaturation temperature was determined using Pyris series software (PerkinElmer). Denaturation temperatures of each cornea were analyzed regardless of the location to determine whether there were any differences between fresh and gamma-irradiated corneas. The geometric effect on the denaturation temperature was then evaluated by comparing the central and peripheral regions of each cornea.

Structural Assessment

Five half-sized, fresh, and gamma-irradiated corneas were used to assess the corneal structure. After removing central and peripheral portions of the cornea using 1.5- and 4-mm trephines, each specimen was collected for microstructural assessment using a 3-mm trephine. Other portions of the cornea were used for evaluating the corneal macrostructure.

Both fresh and gamma-irradiated corneas were dehydrated, embedded in paraffin and stained with hematoxylin and eosin (H&E) using standard techniques. For transmission electron microscopy (TEM), the central part of corneas was fixed using a solution of 3% paraformaldehyde, 1.5% glutaraldehyde, 5 mM MgCl₂, 5 mM CaCl₂, 2.5% sucrose, and 0.1% tannic acid in 0.1 M sodium cacodylate buffer at pH 7.2 overnight in a cold room. After a phosphate-buffered saline (PBS) rinse, the samples were stained with 1% osmium tetroxide on ice for 1 hour, rinsed in deionized water, and stained en block overnight in Kellenberger uranyl acetate. After dehydration with a graded series (75%, 95%, and 100%) of chilled ethanol solutions, the samples were embedded in Epon resin (Eponate 12; Ted Pella, Redding, CA) at 60°C for 60 to 96 hours. The embedded samples were sectioned using an ultramicrotome (Ultracut UCT; Leica, Wien, Austria), collected on grids, and stained with uranyl acetate. The samples were examined and electronic digital images were taken using a TEM (Philips 420; FEI Co, Hillsboro, OR) system at 80 kV. Images were taken at depths between 20% and 80% of full corneal thickness. After images were converted to TIF files, 10 TEM images in each cornea were magnified approximately 100,000-fold. Five areas were randomly chosen in each image, and areas were defined as 1 × 1 μm². The collagen fibrils within the square were manually counted by masked observers, and the numbers of collagen fibrils were presented per square micrometers, as demonstrated previously.²¹

DNA Content

The DNA content of each cornea was determined using Hoechst 33258 dye (Molecular Probes, Eugene, OR) as described previously.²² Briefly, lyophilized corneas from the hydration test were digested in 125 μg/mL papain solution (Worthington, Lakewood, NJ) for 16 hours at 60°C, and the fluorescent intensity of a mixture of 30-μL papain digestion and 1 μg/mL of Hoechst dye in tris-NaCl-ethylenediaminetetraacetic acid (TNE) buffer was measured using a fluorometer (DyNA Quant 200; Hoefer, Holliston, MA). The DNA content was calculated from a standard curve generated using calf thymus DNA.

Human Corneal Epithelial Cell Proliferation Test

Fresh human corneas not used in the characterization were treated with 1.2 U/mL of dispase II (Roche Diagnostics, Mannheim, Germany) in the EpiLife medium (Invitrogen/Life Technologies, Grand Island, NY) at 4°C for 16 hours. Under a dissection microscope, epithelial sheets were collected and incubated in the 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) solution at 37°C for 30 minutes. After vigorous pipetting and neutralizing trypsin-EDTA, primary cells were cryopreserved at -120°C for preparation of the proliferation assay. To evaluate the proliferation of human corneal epithelial cells (hCECs) on corneas and tissue culture plates (TCPs), a 48-well microplate was prepared. Each of 16 TCP wells was either covered by an approximately 100-μm thick fresh or gamma-irradiated cornea or left untreated. hCECs were thawed in a 37°C water bath and subcultured in the

EpiLife medium with the human cornea growth supplement (Invitrogen/Life Technologies) and 2.5 ng/mL epithelial growth factor (Upstate Biotechnology, Lake Placid, NY) at a density of 5 × 10⁴ cells per square centimeter until confluence. After detaching confluent hCECs using 0.05% trypsin-EDTA, the cells were seeded on the microplate with a density of 1.0 × 10⁴ per well. At 0, 5, 14, and 21 days after seeding cells, the number of hCECs was measured in 4 wells of each of 3 groups using the cell-counting kit-8 CCK-8 (Dojindo Molecular Technology, Inc, Rockville, MD) following manufacturer instructions. The absorbance, correlated with the number of cells, was measured using a multimode microplate reader (Synergy 2; BioTek) at 450 nm.

Statistical Analysis

Data are displayed as mean ± SD. Results were analyzed by the Mann-Whitney test, Student *t* test, or Spearman rank correlation, depending on the size and characteristics of samples. Statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc, Chicago, IL). Statistical significance was established at *P* < 0.05.

RESULTS

Light Transmittance, Compressive Modulus and Hydration

Transparency—the main characteristic of a cornea—elasticity (compressive modulus), and cross-linking density (swelling) were measured in 3 different corneas: fresh, gamma-irradiated, and outdated gamma-irradiated. There was no significant difference between gamma-irradiated corneas and fresh corneas in transparency, elastic modulus, or cross-linking density (Fig. 1).

To quantify transparency, we measured the transmittance of visible wavelength. Although the transmittance of visible light through the gamma-irradiated cornea was slightly greater than that of the fresh corneas, the difference was not statistically significant. However, the light transmittance of the gamma-irradiated cornea was significantly greater than that of the outdated gamma-irradiated cornea from 400 to 540 nm (Fig. 1A). The compressive modulus was measured by indentation. There was no difference in the elasticity between fresh corneas (24.4 ± 6.4 kPa) and gamma-irradiated corneas (25.1 ± 5.8 kPa, *P* = 0.452). However, the elastic modulus of outdated gamma-irradiated corneas (7.5 ± 2.5 kPa) was significantly lower than that of gamma-irradiated corneas (*P* = 0.036; Fig. 1B).

Hydration (swelling ratio; wet weight/dry weight) was measured to assess the cross-linking density of the corneas. Wet masses of fresh, gamma-irradiated, and outdated gamma-irradiated corneas were 97.6 ± 6.6 mg, 96.2 ± 10.9 mg, and 108.7 ± 3.6 mg, respectively. After lyophilization, the dry masses of fresh, gamma-irradiated, and outdated gamma-irradiated corneas were measured as 14.0 ± 1.2 mg, 14.3 ± 1.8 mg, and 14.4 ± 0.7 mg, respectively. The dry masses between the different corneas were statistically similar (*P* > 0.75). There was no difference in the swelling ratio between

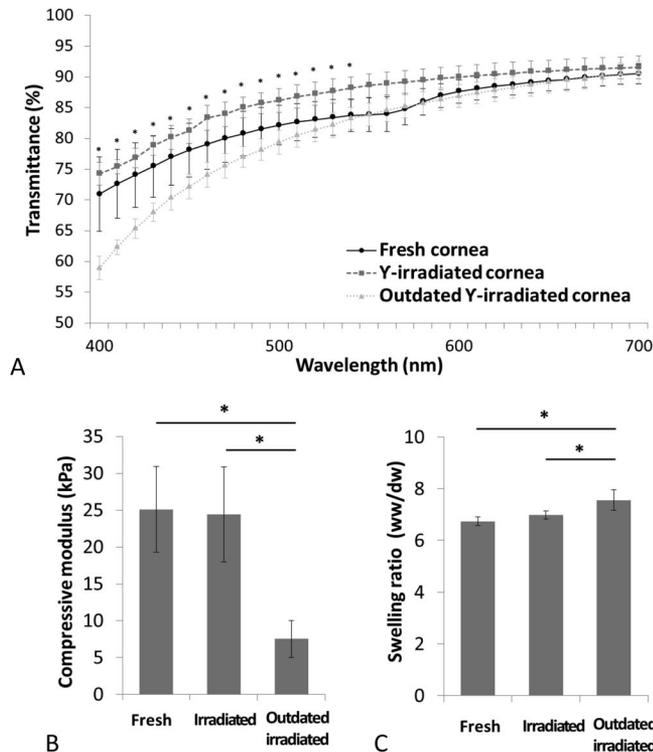


FIGURE 1. Light transmission, compressive modulus, and hydration of fresh gamma-irradiated corneas, and outdated gamma-irradiated corneas. A, Transmittance of visible wavelengths every 10 nm from 400 to 700 nm, (B) elastic modulus, and (C) swelling ratio [ratio of wet weight (ww) to dry weight (dw)] were determined for fresh ($n = 5$), gamma-irradiated ($n = 5$), and outdated gamma-irradiated ($n = 3$) corneas. $*P < 0.05$ versus outdated corneas unless otherwise noted, Mann-Whitney test. Data in A to C are mean \pm SD.

gamma-irradiated corneas (7.0 ± 0.2) and fresh corneas (6.7 ± 0.2 , $P = 0.096$; Fig. 1C). Outdated gamma-irradiated corneas, however, had a significantly larger swelling ratio (7.6 ± 0.4) than that of gamma-irradiated corneas (Fig. 1C), indicating less cross-linking density ($P = 0.036$).

Thermal Properties

The thermal properties, which represent matrix organization, differed between gamma-irradiated and fresh corneas. The denaturation temperature of whole gamma-irradiated corneas (61.8 ± 1.1 °C) was significantly lower than that of fresh corneas (66.1 ± 1.9 °C, $P = 0.004$; Figs. 2A, C). This difference varied based on the region, with similar denaturation temperatures in central regions ($P = 0.413$), but significantly lower in the peripheral region of gamma-irradiated corneas ($P = 0.029$; Fig. 2B). Within gamma-irradiated corneas, the denaturation temperature of the central region (64.2 ± 1.3 °C) was significantly higher ($P = 0.01$) than that of the peripheral regions (61.3 ± 1.5 °C). The denaturation temperatures of central (66.5 ± 3.0 °C) and peripheral (64.7 ± 2.8 °C) regions of fresh corneas were similar ($P = 0.149$; Fig. 2B).

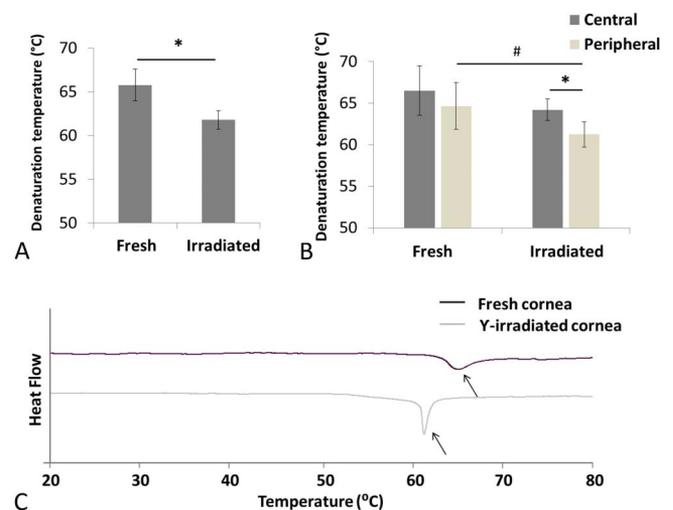


FIGURE 2. Thermal properties of fresh and gamma-irradiated corneas. A and B, Denaturation temperature across the whole area (A) and in the central and peripheral areas of corneas (B), Data in A and B are mean \pm SD ($n = 5$). C, Representative differential scanning calorimetry thermograms for fresh and gamma-irradiated corneas. Black arrows indicate denaturation curves in the thermogram. Statistical significance was established by the 1-tailed Student t test (*) or Spearman rank correlation (#) at $P < 0.05$.

Macrostructures and Microstructures of Corneas

H&E staining and TEM of the mid-peripheral region were used to measure the structure of gamma-irradiated corneas. Many keratocytes were found in the stromal layer of fresh corneas, whereas minimal cellular debris was observed in the stromal layer of gamma-irradiated corneas (Fig. 3). In the TEM images, the gamma-irradiated cornea demonstrated lower collagen fibril density than did the fresh cornea (Fig. 4A). Although intact cellular structures could not be detected, some cellular fragments were observed in the gamma-irradiated corneal stromal sample (Fig. 4B). In a defined $1\text{-}\mu\text{m}^2$ area, the density of collagen fibrils in the gamma-irradiated corneal stromal sample (160.6 ± 33.2 fibrils/ μm^2) was significantly lower than that in the fresh corneal stromal sample (310.0 ± 44.7 fibrils/ μm^2 , $P = 0.001$; Fig. 4C). Qualitatively, we observed thinner collagen fibrils in the gamma-irradiated corneas than in the fresh corneas. Additionally, the density of collagen fibrils in gamma-irradiated corneal stromal samples ranged from 80 to 240 fibrils/ μm^2 , whereas the fibril density in the fresh corneal stromal samples ranged from 260 to 400 fibrils per square micrometer (Fig. 4D).

DNA Content of the Corneas and hCECs Proliferation Test

Regarding biological properties, the residual cell content (amount of DNA) was decreased after gamma irradiation, with gamma-irradiated corneas (1.0 ± 0.1 μg DNA/mg-dry tissue) containing significantly ($P = 0.004$) less DNA than

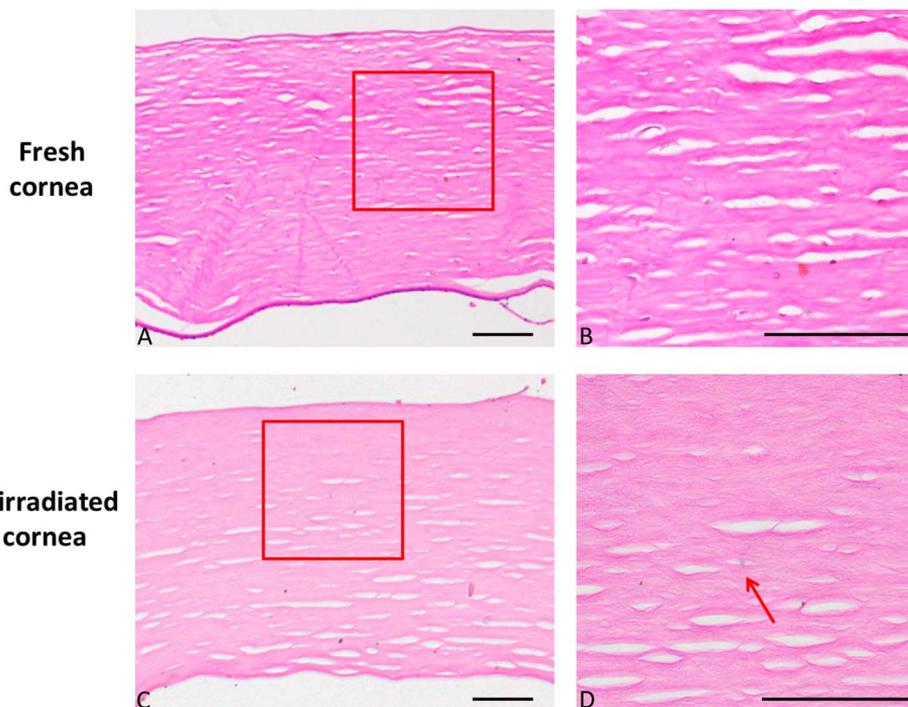


FIGURE 3. Histology of fresh and gamma-irradiated corneas. H&E staining of the mid-peripheral region revealed structure, keratocytes, and debris in gamma-irradiated corneas. Keratocytes are seen in blue in the fresh cornea. Cell-like debris (red arrow) was found in the gamma-irradiated cornea. Scale bars, 100 μm .

fresh corneas ($1.9 \pm 0.2 \mu\text{g}/\text{mg}$) as shown in the morphological evaluation (Fig. 5A). However, seeded cells did not respond differently to the gamma-irradiated and fresh corneas. There were no significant differences between proliferation of hCECs on the fresh and gamma-irradiated corneas at any time point over the course of 21 days (Fig. 5B). In general, cell proliferation increased over the course of 14 days and was more abundant when compared with the TCP. Between 14 and 21 days, the proliferation of hCECs in both corneas decreased, whereas proliferation did not change on the TCP (Fig. 5B).

DISCUSSION

Characterization of a biological material is important in establishing suitability for clinical application. Despite the availability of the gamma-irradiated donor human corneas to ophthalmic surgeons, many fundamental properties have not been carefully evaluated. In this study, we characterized several physical and biological properties, including the swelling ratio, light transmittance, elastic modulus, denaturation temperature, collagen fibril density, DNA content, and corneal epithelial cell proliferation.

The corneal ECM is an optically clear hydrogel comprised of primarily collagen and proteoglycans.²³ It is known that the amount of cross-linking (cross-linking density) governs the physical properties of a hydrogel,²⁴ as demonstrated by many riboflavin-UVA cross-linking studies of corneal tissue. When the cross-linking density of the corneal ECM is increased, light transmittance—one of the main properties of the cornea—increases,²⁵ as do the degree of material stiffness^{26,27} and brittleness,²⁸ which limit surgical handling while decreasing the swelling ratio.²⁵ Thus, the cross-

linking density, which affects the physical properties of the corneal ECM, must be balanced to ensure optimal qualities for a corneal replacement tissue. We demonstrated that the gamma-irradiated cornea shared similar hydration, light transmittance, and elastic modulus as the fresh cornea, indicating that gamma-irradiated corneas have the ideal cross-linking density. In addition, these shared properties suggest that 17- to 23-kGy irradiation does not affect certain functional qualities of the human cornea. Therefore, they could potentially share functional aspects of the fresh cornea, including surgical handling of the tissue and quality of vision. An example is lamellar keratoplasty in which stromal and epithelial (but not endothelial) corneal tissues can be replaced by a gamma-irradiated cornea.

Nevertheless, the denaturation temperature and collagen fibril density were significantly lower in the gamma-irradiated corneas than in fresh corneas. Thermal analysis provides information about the hierarchical organization of tissue,¹⁰ including corneal tissue²⁹ and its substitutable biomaterial.²⁰ The difference in the denaturation temperature in our study indicates that matrix organization of the cornea was altered by 17- to 23-kGy gamma irradiation. Although there is little information as to how thermal properties of the ECM affect corneal function, the matrix alteration by this amount of irradiation may not be great enough to change the cross-linking density of the cornea. Thermal analysis also showed that the denaturation temperature in the peripheral cornea is lower than that in the central cornea. This finding may be related to the fact that the structure of the corneal ECM differs depending on the location within the cornea. For example, the central cornea has a thinner stromal layer,³⁰ higher collagen density,³¹ thinner collagen diameter,³² and higher hydration³³ than those of the peripheral cornea. These

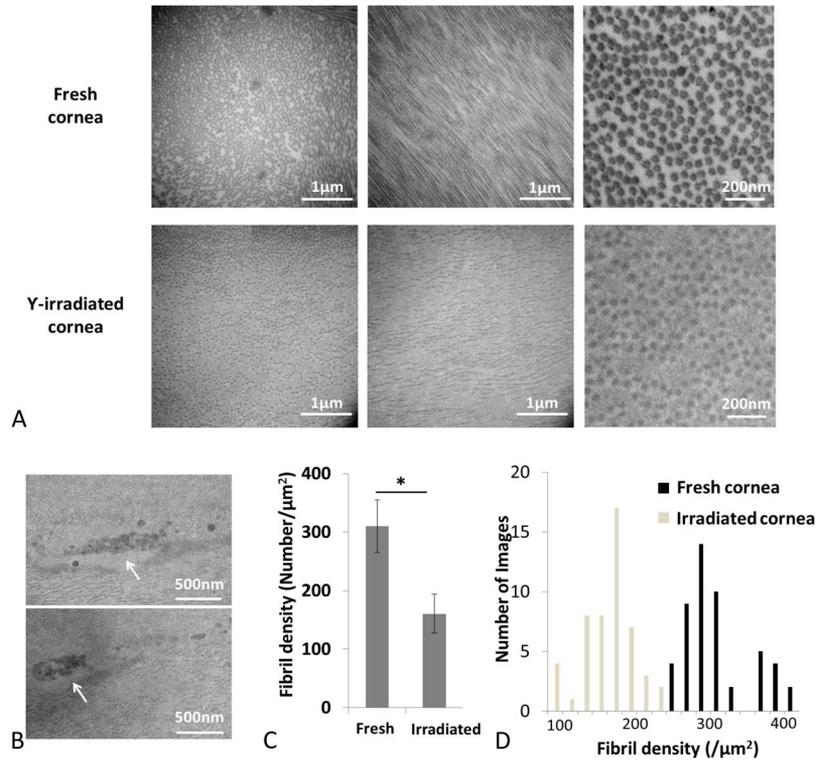


FIGURE 4. Transmission electron micrographs of fresh and gamma-irradiated human corneas in the mid-peripheral region. A, TEM images of fresh and gamma-irradiated corneas, (B) cell fragments (indicated by white arrows) in gamma-irradiated corneas. C and D, Collagen fibrils and fibril density quantified in 50 randomly chosen 1- μm^2 areas. * $P < 0.05$ by the 1-tailed Student t test. Data are mean \pm SD (n = 50).

regional differences in the ECM may influence the regional effect of gamma irradiation. Future studies could examine how irradiation may be performed to account for these localized differences.

Collagen density of corneas varies depending on the region and depth of the cornea, tending to be higher in the central and posterior regions than in the peripheral and mid-cornea.^{31,34} Owing to this variation, we performed microstructural assessments in a specific location—the mid-peripheral region at the mid-stromal depth of the cornea. In our study, the human mid-peripheral and mid-stromal corneal density (310.0 ± 44.7 fibrils/ μm^2) was lower than that

reported for the rabbit central and posterior-stromal corneas (396 ± 21 fibrils/ μm^2),²¹ although others reported that the human cornea has higher collagen fibril density than that of rabbit.³⁴ Furthermore, collagen fibril density of the irradiated cornea was significantly lower than that of the fresh cornea. However, a previous study reported that collagen density was decreased in the superficial area but not significantly different in the other areas of gamma-irradiated human corneas¹⁷ Owing to the irradiation dose-dependent structural changes in the various studies mentioned above, we suggest that the low fibril density of irradiated corneas is caused by the application of 17- to 23-kGy gamma irradiation, which may be different from other studies.

We demonstrated that light transmittance of gamma-irradiated corneas was not significantly different from that of fresh corneas. However, a recent study indicated that transmittance was improved in irradiated corneas compared with fresh corneas at certain wavelengths.¹⁸ We believe that this discrepancy is due to differences in the sample size, the methods for transmittance measurement, and statistical analysis between the present and previous studies. However, both studies clearly demonstrated similar light transmission properties of fresh and gamma-irradiated corneas. Corneal transparency increases with small-diameter collagen fibrils and low interfibrillar spacing.¹⁷ Because the collagen fibril density of gamma-irradiated corneas was lower than that of fresh corneas in our study, but transparency was unchanged, we posit that the small diameter of collagen fibril in the gamma-irradiated cornea increases transparency, which compensates for decreased corneal transparency by increased interfibrillar spacing.

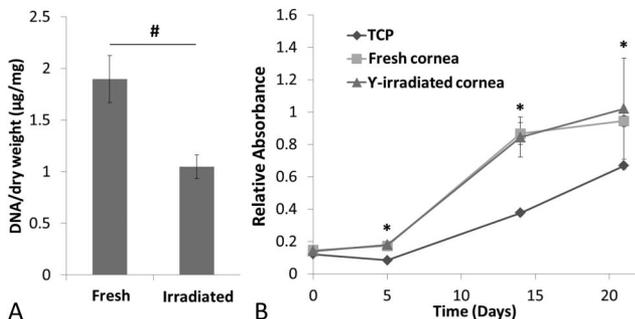


FIGURE 5. Biological characterization of fresh and gamma-irradiated corneas. A, DNA content in fresh and gamma-irradiated corneas relative to dry weight. B, Proliferation rate of hCECs over 21 days after seeding on fresh and gamma-irradiated corneas and on the TCP. * $P < 0.05$ compared with TCP unless otherwise noted, Mann-Whitney test. Data are mean \pm SD (n = 5).

Because the ECM strongly influences cell physiology, cell-based studies are essential to characterize the biological properties of the gamma-irradiated cornea.³⁵ Corneal epithelialization is the first step of corneal healing.³⁶ By measuring the rate of CEC proliferation, one can estimate the potential of a biomaterial for corneal reconstruction. In our study, the proliferation rate of hCECs was not significantly changed between fresh and irradiated corneas, indicating that the materials are equally compatible, although in vivo studies would be necessary to confirm. However, a previous study reported that the proliferation of rabbit CECs correlated with increased collagen-coating density on a synthetic material.³⁵ The discrepancy in proliferation between these 2 studies may be attributed to the differences in supporting materials and cell sources. Our study was conducted using fresh and gamma-irradiated human corneas and hCECs, which we believe to be clinically relevant.

Although hCEC proliferation was similar in fresh and irradiated corneas, gamma irradiation seemed to reduce the number of keratocytes in the stroma and DNA content. A previous penetrating keratoplasty study on mice similarly showed the potential of gamma irradiation to decrease the risk of immune rejection by damaging donor keratocytes.⁵ DNA content of both donor tissue and contaminating pathogens are known to activate immune response,³⁷ and allogeneic DNA may contribute to immune rejection by activating the major histocompatibility-I class molecule.^{38,39} Thus, by damaging both keratocytes and DNA, irradiating the transplant material could reduce immune rejection.

Fresh human corneal tissue prepared for tissue donation should be used within 14 days when kept in a preserved solution between 2 to 8°C in a refrigerator.⁴⁰ One of the advantages of gamma irradiation is that the procedure extends the shelf life up to 2 years without complex storage requirements. Nevertheless, outdated gamma-irradiated corneas had a lower swelling ratio (lower cross-linking density) than that of normal gamma-irradiated corneas, suggesting a finite shelf life for gamma-irradiated corneas as they undergo structural alterations with time. The decrease in the light transmittance and elastic modulus over time support this theory. Strategies to maintain cross-linking density in the gamma-irradiated cornea should be investigated to maximize the shelf life.

In conclusion, we have characterized the mechanical and biological properties of gamma-irradiated corneas and compared them with the properties of fresh human corneas. Gamma-irradiated corneas and fresh corneas have similar physical and biological properties, which imply that the 17- to 23-kGy of gamma irradiation does not affect surgical handling, healing process by epithelial cells, and tissue clarity. However, it is notable that the density of fibrils and the thermal stability of gamma-irradiated corneas are less than those of fresh corneas. Because of the similar biological and mechanical properties between irradiated and fresh corneas, longer shelf life of gamma-irradiated corneas compared with that of fresh donor tissue, and decreased risk of rejection, gamma-irradiated corneas may be a suitable tissue substitute in various types of ophthalmic surgeries.

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