Human Trabecular Meshwork Cell Survival Is Dependent on Perfusion Rate

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Purpose. To determine whether suppression of flow may be detrimental to trabecular cell survival and to the morphologic characteristics of the trabecular meshwork.

Methods. The anterior segments of normal human eye bank eyes were placed in perfusion organ culture. The effect of various perfusion rates of culture medium, and of the constant flow and constant pressure methods of delivery of culture medium, were studied. Trabecular cell survival was determined by quantitation of cell nuclei in histologic sections and by morphologic observation.

Results. Trabecular meshworks with perfusion rates of 1 µl/minute and higher had significantly more trabecular cells than meshworks with lower perfusion rates. A significant loss of trabecular cells was found in meshworks cultured with the constant pressure technique when compared with fellow eyes cultured with the constant flow of medium. Those constant pressure cultured meshworks with surviving cells had higher flow rates than those with necrotic cells.

Conclusions. A minimum perfusion rate of 1 µl/minute is required for trabecular cell survival in perfusion organ culture. Constant pressure perfusion of medium is unsuccessful in maintaining trabecular cells in long-term culture if low perfusion rates occur. Constant flow appears to mimic the in vivo situation more closely.


Trabecular cells are dependent on the aqueous humor for nourishment because the trabecular meshwork has no intrinsic blood supply. The rate of aqueous flow is variable, ranging from peaks as high as 4.26 µl/minute during the day to rates as low as 1.08 µl/minute at night. Suppression of aqueous flow may occur with the use of β-adrenergic blocking agents, carbonic anhydrase inhibitors, and the α2 agonist apraclonidine. A decrease in aqueous flow through the trabecular meshwork has been reported to cause secondary changes within this tissue. Filtration surgery, which allows aqueous to bypass the meshwork, may cause the accumulation of electron-dense deposits in the juxtacanalicular tissue. A cyclodialysis cleft also may divert aqueous from the meshwork, and the acute elevation of intraocular pressure observed with closure of a cleft has been theorized to be caused by accumulation of debris in the meshwork while the cleft was functioning.

The current study was conducted to examine the effect of flow rates on the trabecular meshwork to determine whether a minimum for trabecular cell survival exists. Two commonly used techniques for the delivery of culture medium—constant flow using a microinfusion pump or constant pressure delivery using gravity as the driving force—were also compared. Results indicate that a decreased flow rate is detrimental to trabecular cells in perfusion organ culture, with a perfusion rate of approximately 1 µl/minute the lower limit for cell survival.

MATERIALS AND METHODS. Culture Technique. Tenets of the Declaration of Helsinki were followed. Seventy-six normal human eye bank eyes were studied. The average age of the donor eyes was 72 ± 12 years (±SD; range, 35 to 97 years). Six donors were younger than 60 years of age, and these eyes were distributed equally between the experimental groups. No eyes had glaucoma or uveitis, nor were they on topical medication. Two eyes had undergone cataract surgery; no anterior chamber lenses had been implanted. The culture technique was similar to that described previously. Eyes were bisected at the equator, and the iris, lens, and vitreous were removed. The anterior segment was clamped in a modified petri dish, and the eye was perfused with culture medium (Fig. 1). The culture period was either 7, 14, or 21 days. Culture medium was Dulbecco’s modified Eagle’s medium with the addition of a mixture of antibiotics: penicillin (10,000 U), streptomycin (10 mg), amphotericin B (25 µg) (Sigma, St. Louis, MO), and gentamicin (17 mg, Sigma) in 100 ml of medium. It was perfused at a predetermined flow rate (2.5 µl/minute) using a Harvard microinfusion pump (Harvard Bioscience, S. Natick, MA). Anterior segments were cultured at 37°C in a 5% CO₂ atmosphere.

FIGURE 1. Perfusion culture system. After bisection of the eye at the equator and removal of lens, iris, and vitreous, the eye is clamped in a modified petri dish. Culture medium flows into the eye through a cannula in the bottom of the dish and must exit through the trabecular meshwork.
At the end of the culture period, the anterior segments were perfused with 2% paraformaldehyde–1% glutaraldehyde fixative at 17 mm Hg. Wedges of limbal tissue from at least two quadrants 180° apart were processed for light and electron microscopy by dehydration in a graded series of ethanol and embedded in either Araldite resin or LR White resin. Staining of 1-μm thick araldite sections was performed using toluidine blue.

**Experiments. Perfusion Rates.** Perfusion rates of culture medium ranged from 0.5 to 10 μl/minute. Low perfusion rates were studied in 17 pairs of anterior segments (0.5 μl/minute, n = 5; 0.75 μl/minute, n = 5; 1 μl/minute, n = 7). Meshwork from the experimental eye received medium at the lower flow rate, whereas meshwork from the fellow eye served as a control and received medium at the standard perfusion rate of 2.5 μl/minute.

The normal physiologic aqueous flow rate, 2.5 μl/minute, was studied in 25 anterior segment cultures that served as controls for the other experiments in this study. High perfusion rates were studied in 18 anterior segments (unpaired; 5 μl/minute, n = 12; 10 μl/minute, n = 6).

**Media Delivery.** Anterior segments of 10 pairs of eyes were studied to compare constant flow and constant pressure delivery of medium. One anterior segment of the pair received medium at a constant perfusion rate using a microinfusion pump (2.5 μl/minute), whereas the fellow anterior segment received medium at a constant pressure of 15 mm Hg. The flow rate of the medium was measured every 24 hours during the culture period and was used to calculate a single mean flow for the entire culture period, expressed as microliters of medium per minute.

Four anterior segments from two pairs of eyes were cultured with the nonperfusion technique reported by Acott et al3 by placing the dissected anterior segments cornea-side down in a large dish filled with medium in a shaker-equipped incubator. Medium was changed every 48 hours, and anterior segments were cultured for 7 days.

**Assessment of Trabecular Meshwork. Cellularity.** The trabecular meshwork was considered to be the region extending from Descemet’s membrane to the end of Schlemm’s canal, and from Schlemm’s canal to the uveal meshwork. For counting purposes, each cell was assumed to have only one nucleus, and each nucleus thus represented one cell. Cells without nuclei were not counted.4-7 Nuclei were counted at 400X using a combination of photographic montages and direct counting from the microscope slide. All meshworks were assessed for cellularity, and any visible nucleus was counted, even if the cell appeared edematous or otherwise abnormal.

**Histologic Observation.** Morphologic characteristics of trabecular cells and the trabecular meshwork were examined with light microscopy. Transmission electron microscopy was performed on 35 eyes, including some from each group. Histologic observation provided an additional dimension to the evaluation of cell survival because quantitation of cell numbers alone does not give information on the condition of the cell and its presumed viability. Cells with edematous cytoplasm or nuclei, edematous or ruptured mitochondria, and cytoplasmic lipid vacuoles, and cells off the trabecular lamellae were considered to represent abnormal cells. Such cells would appear swollen and round and would lose their usual thin profile and horizontal position on the lamellae. These findings were combined to give an overall assessment of the meshwork, normal or abnormal, and are reported along with the cellularity values.

Culture of intact human eye tissue presents a challenge in distinguishing the results of an experimental manipulation from those postmortem changes that occur in eyes before culture. The expected survival rate in meshworks cultured under standard conditions is 78% (n = 482 cultures; includes published4,8,9 and unpublished data). Hence, even some control cultures would be anticipated to be unsuccessful in any experimental series. This necessitates the use of paired eyes, adequate numbers of cultures, and histologic evaluation to distinguish experimental changes from “donor failures.”

**Statistical Methods.** The values of the cellularity of each meshwork from an experimental group were combined to give a mean value for the group. Comparisons of cellularity between paired eyes were analyzed with a paired, two-tailed t-test; comparisons among unpaired eyes used the unpaired, two-tailed t-test. All meshworks were included in the cellularity assessment, even if they were considered abnormal by subjective criteria. Chi-square analysis was used to compare the numbers of normal and abnormal meshworks between groups.

The study was approved by the Institutional Review Board of the Mayo Clinic on the use of human tissue. Consent for use of all autopsy eyes was obtained from next of kin.

**RESULTS.** Of the 76 anterior segments cultured, three pairs had necrotic cells in both the experimental and control meshworks and were considered donor failures (one pair each in the 0.75 μl/minute group, 1 μl/minute group, and 5 μl/minute group). These were excluded from further analysis and do not appear in the figures; in total, 70 anterior segments were used to assess experimental results. Minor culture-associated changes were present in approximately 50% of meshworks, most commonly the scattered loss of trabecular cells and the accumulation of intracellular lipid vacuoles.4 These changes usually were limited to the uveal meshwork region.
Perfusion Rate. All perfusion rates were delivered by an adjustable microinfusion pump. No significant difference was noted between culture durations of 7, 14, or 21 days, and these data were combined.

High Perfusion Rate (5 and 10 μL/minute). The mean cellularity was similar in the two groups (57.5 ± 29.1 and 66.7 ± 14.5, respectively) and was decreased by approximately one third compared to the meshworks of the control group (P < 0.01, unpaired; Fig. 2). Histologic examination revealed scattered cell loss but relatively normal appearance to the remaining cells in 81% (15/16) of meshworks. The finding that the remaining cells appeared normal differs from the findings in the low perfusion groups, in which the remaining cells were abnormal. Cells maintained remaining cells appeared normal differs from the histologic appearances similar to those of the experimental eyes.

Normal Perfusion Rate (2.5 μL/minute). The mean cellularity of these meshworks was 94.1 ± 22.7 (Fig. 2). Eighty four percent (21/25) of the anterior segments had a normal appearance of the trabecular meshwork.

Low Perfusion Rates. At lower perfusion rates, cell survival appeared dependent on perfusion rate. With a perfusion rate of 1 μL/minute (approximately the average nocturnal aqueous flow rate), cellularity was similar to fellow control eyes (77.5 ± 28.7 versus 85.2 ± 23.5; Fig. 2). Subjective assessment revealed that meshworks remained normal. The anterior segments from fellow control eyes, perfused at the average physiologic aqueous flow rate of 2.5 μL/minute, showed that meshworks appeared intact in 3 of 6 eyes, with histologic appearances similar to those of the experimental eyes.

At 0.5 μL/minute, all anterior segments had necrosis of trabecular cells, with marked cell loss. The mean cell count was approximately one third of the fellow control eyes (39.6 ± 31.3 versus 101 ± 16, P = 0.01; Fig. 2). Cells that remained were swollen, rounded, and lifted off the trabecular beams. Cytoplasm was pale staining, cell boundaries were indistinct, intracellular lipid vacuoles were common, and nuclei were rounded. Cells in the anterior meshwork and juxtaocular region also were necrotic. Subjective histologic analysis revealed that 4 of 5 fellow control anterior segments appeared to have intact meshworks.

The perfusion rate of 0.75 μL/minute appeared to be in the transition zone for trabecular cell survival. The overall cellularity was decreased by approximately one half from fellow control eyes (40 ± 33.9 versus 79.8 ± 19.5, P = 0.025; Fig. 2). Subjective assessment revealed 2 of 4 anterior segments cultured at 0.75 μL/minute to have a normal appearance of the meshwork. Analyzing the cellularity of these two meshworks revealed a mean cell count of 64.5 ± 25.2, whereas the two meshworks with many necrotic cells had a mean cellularity of 15.5 ± 19.1. Subjective assessment of fellow control eyes revealed all four to have normal-appearing meshworks.

Media Delivery. Constant Flow Versus Constant Pressure. Cellularity was decreased significantly in anterior segments cultured with the constant pressure method of delivery of medium when compared with fellow eyes (35.6 ± 41.3 versus 101.8 ± 24.2, P = 0.002; Fig. 2). Only 3 of 10 meshworks were assessed subjectively as normal. These anterior segments all had flow rates of greater than 1 μL/minute. The mean cellularity of these three meshworks was 90 ± 22.5. Anterior segments with flow rates of 0.89 μL/minute and less had significant loss of meshwork cells and edematous rounding of many of the remaining cells, even if they were cultured for as briefly as 7 days (Fig. 3). The mean cellularity of these seven meshworks with flow rates below 1 μL/minute was 12.3 ± 16.5. Fellow control eyes, cultured with a constant flow of medium (2.5 μL/minute, as delivered by the microinfusion pump) had normal-appearing meshworks in 9 of 10 anterior segments (Fig. 4).

Nonperfusion Method. All four anterior segments had nearly normal-appearing meshworks, with preservation of trabecular cells and lamellae. Loss of cells in the uveal meshwork area was common. Schlemm's canal appeared wide, and the cells lining the canal remained intact. No giant vacuoles were seen.
FIGURE 3. Trabecular meshwork of a 61-year-old woman, cultured with constant pressure technique for 7 days. Mean flow rate for this eye was 0.89 ± 0.38 μl/minute, (range, 0.62 to 1.66 μl/minute) at a pressure of 15 mm Hg. Trabecular cells are necrotic (arrows), and Schlemm’s canal cells are absent. Magnification, ×630.

DISCUSSION. Trabecular cells require a minimum perfusion rate of approximately 1 μl/minute for long-term survival, as determined by the perfusion culture model. At lower flow rates, the exchange of nutrients appears inadequate for the maintenance of normal cell numbers and normal cell appearance. It is of interest that the flow rate of 1 μl/minute is similar to the minimum aqueous flow rate in vivo.1 Cellularity was reduced markedly in meshworks with flow rates below 1 μl/minute when compared with higher flow rates: 25.9 ± 27.6 versus 81.3 ± 27.7 (P < 0.001; combines cell counts from all experiments). Histologic evaluation of individual meshworks, which considers the morphologic characteristics of cells and the meshwork as well as cellularity data, also found decreased survival at low flow rates. Only 13% (2/16) of meshworks appeared normal with perfusion rates below 1 μl/minute, whereas 86% (43/50) with perfusion rates above this level were judged as appearing normal (P < 0.001; combines meshworks from both experiments). The cells that remained in the high perfusion rate meshworks appeared normal despite an overall loss of some cells, whereas the cells remaining in the low perfusion rate meshworks were markedly abnormal in appearance.

The interpretation of experimental results in a system using intact human eye tissue presents a challenge in distinguishing the results of an experimental manipulation from postmortem changes that may occur before culture. Cells may die before culture, or they may be so compromised that their reactions are altered. In addition, culture medium cannot duplicate all the growth factors and other components of aqueous humor. Because of this, even some control cultures would be anticipated to be unsuccessful in any experimental series. A survival rate of 78% has been found in other experiments in our laboratory (n = 482 cultures, includes published4,8,9 and unpublished data). This indicates that 1 in 4 control cultures would be expected to be nonsurviving, and it necessitates the use of paired eyes and of adequate numbers of cultures to distinguish experimental changes from donor failures. This expected loss probably accounts for the nonsurvival of some control meshworks in each experiment.

The use of fellow eyes in comparisons of meshwork cellularity and morphology provides the best experimental control possible. The difference in cellularity between fellow eyes is small, 12.8%, which gives statistical support to the logical idea of using the fellow eye as a control.6 The cellularity of the trabecular meshwork at age 70, the mean age of the eyes in the current study, is approximately 110 cells per histologic section.7 The cellularity of 94.1 ± 22.7, found in the cultured control meshworks in the current study, indicates a 15% cell loss in cultured meshworks, similar to the 20% to 40% loss reported previously.4

The finding that a minimal rate of flow of culture medium is necessary for trabecular cell survival may be the reason that meshwork cultures are rarely successful if attempted beyond 24 hours postmortem.4,5,8,10 Because aqueous flow has ceased in these eyes at the time of death, stagnant aqueous may result in an inadequate supply of nutrients. Lack of aqueous

FIGURE 4. Fellow eye to that in Figure 3, cultured with constant flow technique at 2.5 μl/minute for 7 days. Note preservation of trabecular cells (arrows) and Schlemm’s canal lining cells. Giant vacuoles are numerous (small arrows). Magnification, ×630.
turnover also allows metabolic waste and products released from stressed and dying cells to accumulate. Thus, the experimental use of human eyes older than 24 hours postmortem may be confounded by dying trabecular cells. Of interest, the four anterior segments cultured with the nonperfusion method had intact trabecular cells, consistent with the findings of Acott and colleagues. The use of a shaker base for the culture plates in this method probably created enough mixing of culture medium to expose the trabecular cells to an adequate supply of nutrients.

The constant pressure technique for delivery of culture medium appears unsatisfactory for long-term maintenance of trabecular cells. This is in agreement with the lack of trabecular cell survival beyond a few days, reported with the constant pressure culture technique. An explanation may be the scattered cell loss that occurs in meshwork culture, even when considered successful, as mentioned above. As trabecular cell loss occurs in culture, debris from necrotic cells travels downstream into the intertrabecular spaces and the juxtacanalicular tissue. This debris may cause temporary increases in outflow resistance, which would impede the flow of culture medium in a constant pressure-driven system. The constant flow technique, which can maintain cellular viability for up to 28 days, would tend to drive the debris through the meshwork, causing a temporary elevation of intraocular pressure. It is likely this debris accounts for the variable intraocular pressure curves noted in the constant flow perfusion culture system, which may vary 3 to 5 mm Hg over a 24-hour period. The constant flow technique, which mimics the pressure-independent formation of aqueous humor in vivo, appears preferable for the long-term survival of trabecular cells.

**Key Words**
organ culture, trabecular meshwork

**References**