Human Trabecular Meshwork Organ Culture: Morphology and Glycosaminoglycan Synthesis

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Human corneoscleral explants were maintained for several weeks in defined, serum-free media. Trabecular cell vitality, as judged by vital stain exclusion, is high for at least one month. Trabecular ultrastructure, as compared to that of fresh eyes, first shows minor cellular and extracellular matrix degradation after 3 weeks in culture. The biosynthetic profiles of trabecular glycosaminoglycans (GAGs) change significantly by 3 weeks in culture. Eyes that are stored at 5°C for up to 48 hr postmortem exhibit changes in trabecular ultrastructure and in GAG profiles; both characteristics return to normal by 7 days in culture. The incorporation pattern of 35S-sulfate and 3H-glucosamine into the GAGs of the trabecular meshwork (TM) is distinct from corneal or scleral incorporation. The relative incorporation of 3H-glucosamine into trabecular GAGs, as determined by sequential enzymatic degradation, is: 22.3% hyaluronic acid (HA), 27.9% chondroitin sulfate (CS), 21.3% dermatan sulfate (DS), 5.9% keratan sulfate (KS), 17.7% heparan sulfate (HS) and 4.9% unidentified material. The relative incorporation of 35S-sulfate into trabecular GAGs is: 0% HA, 32.9% CS, 34.8% DS, 7.7% KS, 13.8% HS and 11.1% into unidentified material. This profile is in good agreement with the profile that was previously obtained for human and nonhuman primate meshworks prior to culture. We conclude that corneoscleral explant organ culture is a useful tool for extracellular matrix studies within a time window from 7 to at least 14 days in culture. Invest Ophthalmol Vis Sci 29:90–100, 1988

Glycosaminoglycans (GAGs), which are the major carbohydrate portion of proteoglycans, may play an important role in maintaining intraocular pressure by acting as the aqueous humor outflow resistance barrier.1–9 In direct biochemical studies, hyaluronic acid (HA), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS) and heparan sulfate (HS) have been identified in the human trabecular meshwork.1–3,7–10 In histochemical studies with Alcian Blue and Ruthenium Red, staining that is removed by GAG-degrading enzymes has been identified in several regions of the meshwork.6,11,12 Fibronectin, laminin, heparan sulfate proteoglycan and types I, III and IV collagen have been identified in the TM by immunohistochemical methods.13–15 Enzymatic perfusion studies have been used to implicate GAGs in the maintenance of resistance to aqueous humor outflow.4,5,16,17

Materials and Methods

Materials

Human eyes, which were enucleated within 3 hr postmortem (mean time 1.7 hr) and stored at 5°C, were obtained from local eye banks and used within
48 hr postmortem. The average donor was between 50 and 75 years of age, although some were younger or older. A total of 102 eyes were used for the biochemical analysis and 12 for the morphologic studies. The paired eye of each of the latter 12 was included in the former group. In all of the studies reported herein, eyes from donors with any history of major eye surgery or disease, diabetes, liver failure, sepsis, immune or neurological disease, major head wounds or drowning were excluded. These studies use human tissue designated "exempt" under category number five by the Public Health Service (PHS). The protocol has been accepted by both the PHS and the Oregon Health Sciences University Human Subjects Committee. L-glutamine, 100X Antibiotic-antimycotic mixture, Dulbecco's modified Eagle medium with added L-glutamine and glucose, Dulbecco's phosphate buffered saline and 0.4% Trypan blue stain in normal saline were obtained from GIBCO (Santa Clara, CA). GAG degrading enzymes (Miles Laboratories, Elkhart, IN), cellulose acetate electrophoresis supplies and standards were as described earlier. 3 Radioactive precursors: D-[6-3H(N)]-glucosamine hydrochloride (~20 Ci/mmol) and carrier-free 35S-sulfate (~43 Ci/mmol) and Atomite liquid scintillation solution were purchased from New England Nuclear (Boston, MA). Epon 812 and acacodylic acid (sodium salt) were purchased from Ernest F. Fullam (Schenectady, NY); EM grade glutaraldehyde was purchased in 10 ml sealed vials from Stevens Metallurgical Corp. (New York, NY); paraformaldehyde was purchased from Mallinkrodt (St. Louis, MO); osmium tetroxide was purchased from Polysciences (Warrington, PA); Alcian Blue 8GX was purchased from Sigma Chemical (St. Louis, MO); Bio-Gel P-6DG desalting gel and columns were purchased from Bio-Rad Labs (Richmond, CA). All other chemicals were the best Reagent Grade available.

**Explant Organ Culture**

Explants were removed by a scleral incision 2 mm posterior to the limbus and lifted with a gentle teasing-away of the iris and ciliary body. Associated iris root was removed with tweezers without disruption of the TM or scleral spur. This complete, undivided corneoscleral explant was used for culture. Using aseptic technique, the explants were rinsed in 10 ml of Dulbecco's phosphate buffered saline and incubated, concave side up, for 30 min at room temperature in 10 ml of Dulbecco's modified Eagle medium (DMEM) with IX added Antibiotic-antimycotic mixture (final incubation concentrations: 100 units/ml Penicillin G, 100 μg/ml Streptomycin sulfate, 0.25 μg/ml Fungizone) in Falcon 60 × 15 mm culture dishes. Explants were then transferred to 10 ml of fresh DMEM containing 1X antibiotic-antimycotic and 0.292 mg/ml additional L-glutamine in Falcon 60 × 15 mm culture dishes and incubated, concave side up, with gentle shaking at 37°C under a humidified 5% to 95%, carbon dioxide to air atmosphere. Cultures were changed to fresh media every 2 or 3 days and were allowed to stabilize for 7 days prior to use, except as indicated.

**Microscopy/Morphology/Vitality**

Small wedges, 2 mm at the circumference, were cut from corneoscleral explants with a double-edged razor blade and fixed for 4 hr in 2.5% glutaraldehyde, 2% paraformaldehyde, 5 mM calcium chloride, 130 mM sucrose and 100 mM sodium cacodylate (pH 7.4). The wedges were then washed three times in this buffer without aldehydes, post-fixed for 1 hr in 1% osmium tetroxide in the same buffer, washed in the same buffer, dehydrated through graded alcohols to propylene oxide, embedded in Epon and sectioned on an LKB (Bromma, Sweden) III microtome with a glass or diamond knife for light or transmission electron microscopy. Sections were viewed and photographed on a Zeiss (Oberkochen, West Germany) Photomicroscope or a Siemens (Hamburg, West Germany) Elmiskop-101 TEM. Parallel wedges were vital stained with 0.4% trypan blue in saline for 30 min at room temperature and processed for light microscopy. Several eyes were received within 1 to 4 hr postmortem (designated as "fresh" in the text) and immediately fixed for electron microscopy.

**Radiolabel Incorporation**

Explants, stabilized for 1 week in culture except as indicated, were changed to fresh media containing 100 μCi of 35S-sulfate and 75 μCi of 3H-glucosamine per dish and incubated under standard culture conditions for the times indicated. Media and label were changed every 48 hr. A standard labeling time of 48 hr was used where no time is indicated. At the designated times, explants were removed from culture and the TM, sclera and cornea separated under a dissecting scope as described previously.

**GAG Isolation and Analysis**

The methods for isolation, partial purification, cellulose acetate electrophoresis (CAE) and sequential enzymatic degradation of the GAGs have been described in detail previously. 3 Briefly, tissue lipids were extracted with chloroform/methanol; explant proteins were digested with papain; proteins were precipitated with trichloroacetic acid (TCA); small molecules, TCA and free radiolabeled were removed by
molecular sieve chromatography on Bio-Gel P-6DG columns; and the GAGs were collected by precipitation from an ethanol/potassium acetate solution.

For electrophoretic studies, these semi-purified GAGs were dissolved in 5 μl of water and 0.25 μl aliquots were electrophoresed on cellulose acetate in a Beckman (Fullerton, CA) Microzone apparatus for 2 hr at 4 mamps using 0.3 M cadmium acetate buffer (pH 4.1). The cellulose acetate membranes were stained with Alcian Blue for visualization of the GAGs. Each lane of the membrane was cut into 20 slices (1 mm wide); the slices and both ends of each lane were counted in Atomlite in a Packard Tri-carb (4000 Series, Downers Grove, IL) liquid scintillation counter. The amounts of each isotope were determined using a program that corrects to disintegrations per minute (DPM) in external standard mode with automatic gain compensation; corrections are based upon dual-label quench curves using Packard 3H-toluene and 14C-toluene quench standards or 

35S-sulfate quenched with chloroform. Isotope counting separations allowed less than 5% uncorrected cross-channel spill. DPM values for 35S-sulfate were corrected for radioactive decay.

For sequential enzymatic degradation studies, the enzyme sequence to degrade each respective GAG (given below in parenthesis after the enzyme name) was as detailed previously, 3 ie Streptomyces hyaluronidase (HA), chondroitinase AC (CS), chondroitinase ABC (DS), keratanase (KS) and heparitinase (HS). The remaining unidentified material which was resistant to all enzymatic degradation is termed “REM.” Instead of applying the respective degraded GAG samples to CAE as previously reported, the supernatant from each alcohol precipitation step was dried in a scintillation vial, dissolved in 100 μl of water and then counted in 10 ml of Atomlite. Culture medium from some experiments was digested with papain and then treated identically to the tissue samples. The number of eyes used in individual experiments is indicated in the text or figure captions.

Results

Morphology of Explant Organ Cultures

Tissue wedges were evaluated for trypan blue exclusion and light microscopic morphology after 0, 1, 3, 5, 7, 10, 14, 21 and 28 days in culture. Tissue morphology and vital staining, as judged by light microscopy, indicate that the explants regain apparently normal morphology and essentially 100% viability within 3 to 5 days in culture and maintain this for at least 1 month (data not shown). Light micrographs of explants after 7, 28 and 49 days in culture are shown in Figure 1. At this level of resolution, it is difficult to detect significant changes until at least 4 weeks. By 7 weeks (Fig. 1c), deterioration is quite apparent.

The transmission electron micrographs shown in Figure 2 are of the TM of fresh eyes that were obtained and placed in fixative within 2 hr postmortem. Figure 3 is an electron micrograph of the TM of an eye that was enucleated 3 hr and fixed 43 hr postmortem. The trabecular morphology in this micrograph is typical of the worst eyes that we accept for culture. Compared to fresh eyes or 7-day cultured explants, the TMs of these eyes have a large number of electron-lucent vesicles (arrow) throughout the cytoplasm and exhibit occasional loss of beam integrity near the cellular surface (*). Figure 4a and b shows two electron micrographs of typical TMs after 1 week in culture. The morphology is quite similar to that of fresh specimens (Fig. 2).

Figure 4c and d contains electron micrographs of the TM of explants which had been in culture for 14 days (c) and 21 days (d). Occasionally, a few cells (less than 5%) can be found at 21 days which have begun to show the beginnings of possible cytoplasmic changes (see Fig. 5 for more extreme examples). By 28 days (Fig. 5a, b), however, appreciable beam disintegration (*) is observed and more cells can be found which exhibit nuclear changes (arrows) or loss of cytoplasmic integrity (just to left of arrow in a). The proportion of damaged cells is still low at 28 days (between 5% and 20%). By 49 days in culture (Fig. 5c), cellular degradation is very advanced. The cells have released most of their cytoplasmic contents, the membranes are compromised (arrow) and the beams are disorganized (*). Vesicles are quite apparent by this time as is an increased amount of amorphous material in the spaces between the beams. The nuclear changes beginning to appear at 28 days (arrows 5a, b) are becoming pronounced (double arrows 5c).

Cellulose Acetate Electrophoresis of GAGs

In Figure 6, a comparison of the electrophoretic profiles of radiolabel incorporation into trabecular, corneal and scleral GAGs, after a 48 hr incubation with label under standard culture conditions, is presented. Figure 6a shows the CAE profile of 3H-glucosamine and 35S-sulfate incorporation into trabecular GAGs. Figure 6b shows the 35S-sulfate incorporation into the corneal and scleral GAGs. These profiles are the averages of three separate experiments, and are typical of many others that we have conducted. In these experiments, the GAGs from the three tissues of one explant were run in parallel lanes on the same acetate strips and therefore reflect identical electrophoretic conditions.
Fig. 1. Light micrographs of corneoscleral explants. After culture for 7 (a), 28 (b) and 49 (c) days, thick sections were cut from fixed and embedded wedges, stained with 0.1% toluidine blue in 0.1% sodium borate and photographed. Schlemm's canal is seen near the center with the trabecular meshwork in the upper right and the sclera in the lower left of each photomicrograph. Magnifications: (a) ×400, (b) ×250, (c) ×210.

Fig. 2. Transmission electron micrographs of fresh human TM. Micrograph (a) of a “fresh” human eye, fixed within 3 hr postmortem, showing trabecular cells and adjacent beam from the deep corneoscleral region near the juxtaglomerular area at a magnification of ×12,667. Note pigment granules (P), the extracellular matrix beams (*), the plasma membrane integrity (arrow), the close association of the beams with lamina lucida and lamina densa of the basement membrane at the cell surface and the lack of open beams without cells between them and the open trabecular spaces (S). Lower magnification (×6933) micrograph (b) showing beams (*), villous cytoplasmic projections into the spaces (S) between trabecular cells and beams and the typical trabecular nucleus with the nuclear membrane and closely-associated electron dense heterochromatin (arrow).
Incorporation Time Courses

In Figure 7, the results from time courses for the total incorporation of 3H-glucosamine into the GAGs of the TM, cornea and sclera are presented. Similar curves are obtained for 35S-sulfate incorporation (data not shown). In the TM, the incorporation of both labels is at steady state within 2 days. This is not the case for either the cornea or the sclera, both of which show increasing incorporation for at least 7 days. The CAE profiles for the time courses of 35S-sulfate and 3H-glucosamine incorporation into the TM show that the relative distribution into the respective peaks is constant over the times studied (data not shown).

Sequential Enzymatic Analysis of the GAGs of the TM

Sequential enzymatic degradation of the GAGs from the TMs, corneas and scleras of explants resulted in the relative incorporation profiles presented in Figures 8 and 9, respectively. For comparison purposes, we have included in Figure 8 (solid bars) the relative amounts of Alcian blue staining determined in our previous studies with uncultured human trabecular GAGs.3 The explants were preequilibrated in culture for 7 days and incubated for 48 hr with 35S-sulfate and 3H-glucosamine. The GAGs were extracted and analyzed enzymatically as outlined in the Methods section. To check for incomplete degradation in the individual steps, several pools of three to five TMs were analyzed without increasing the enzyme incubation times or enzyme concentrations; no significant differences were observed. The means of the percentages of incorporation into each GAG have relatively small standard errors (SEM). Somewhat larger SEM values (approaching 10% of the total amount for each GAG) were observed when the raw DPMs were averaged. Most of this variability is due to differences in the total incorporation of label, rather than from the biochemical analysis. When replicate analyses of pooled samples are conducted, the variations are quite small.

These GAG profiles are distinctive for each radiolabel and for each tissue. The relative incorporation of 3H-glucosamine into the trabecular GAGs is: 22.3% HA, 27.9% CS, 21.3% DS, 5.9% KS, 17.7% HS and 4.9% unidentified material. The relative incorporation of 35S-sulfate into trabecular GAGs is: 0% HA, 32.9% CS, 34.8% DS, 7.7% KS, 13.8% HS and 11.1% unidentified material.

Effects of In Vitro Incubation Times Upon GAG Profiles

Based upon the morphological studies (Figs. 1–5), it appears that the TMs of explants that have been in culture for between 7 and 21 days resemble closely the TMs of fresh eyes. To determine a range of in vitro incubation times that produces a profile of trabecular GAGs resembling that determined earlier for noncultured eyes,3 explants were cultured for several time intervals prior to the initiation of the standard 48 hr incubation with radiolabels; GAGs were then analyzed as before. This study used standard explant conditions and eyes that were obtained within 48 hr and were enucleated within 3 hr postmortem. The exception to this was the group of eyes which are designated “fresh.” In this group, labeling in culture was begun within 4 hr postmortem. The results for 3H-glucosamine incorporation into the respective GAGs are presented in Figure 10. When the GAG profiles which result from the different preequilibration times are analyzed statistically, four obvious groups emerge. These groups are: (1) Explants from...
Fig. 4. Electron micrographs of explants after 7 to 21 days in culture. After explants were in culture for 7 days, they were processed for TEM and typical micrographs are presented showing (a) trabecular cells and beams from the juxtacanalicular region and at lower magnification (b) cells and beams of the TM from the corneoscleral region. Micrographs are also presented of the cells and beams in the deep corneoscleral region of an explant that had been in culture for 14 days (c) and in the central corneoscleral region of an explant that had been in culture for 21 days (d). The magnifications are ×9600, ×7867, ×8000 and ×11,333, respectively. Note the prominent Golgi (arrow in a), phagocytic and/or secretory vesicles (arrow in d), mitochondria (arrow in c), rough endoplasmic reticulum (arrow in b), “curly” collagen (in a) and the close association of the plasma membranes with each other and with the adjacent beams.

fresh eyes; (2) explants from 0 to 5 days in culture; (3) explants from 7 to 14 days in culture; and (4) explants from 21 and 28 days in culture. Because of the resemblance between the trabecular GAG profiles that we previously obtained for pools of eyes prior to culture and the profiles obtained (Fig. 10), statistical analysis is presented as a comparison of groups 2 and 4 above to the combined groups 1 and 3 of fresh and 7 to 14 days in culture. The (*) indicates significance at level of $P < 0.005$. 
As can be seen in Figure 10, several of the GAGs are produced in significantly different amounts, when compared to the fresh and 7 to 14 day culture groups. HA is produced in higher amounts from 0 to 5 days in vitro, and in lower amounts from 21 to 28 days in vitro. The other GAGs are different either in the early, or in the late groups, but not in both. No simple overall pattern for GAG production as a function of culture preequilibration time is apparent. The results from the analysis of 35S-sulfate incorporation into the respective GAGs obtained in this study (data not shown) are similar, although not identical and the changes are slightly less pronounced. No general patterns are apparent here either.

Other Observations and Controls

To test for an effect of the antibiotics in the media upon the GAG profile, pairs of eyes from the same donor were cultured, one with and one without the addition of the antibiotic/antimycotic solution to the media, for 7 days and then labeled for 48 hr. No significant differences in GAG profiles were observed (data not shown) within four respective pairs of explants.

Individual variation (standard error of the mean < 10%) in the total radiolabel incorporated into trabecular GAGs in the cultures is much larger than the observed differences between paired eyes from the same donor. Retrospective regression analysis indi-
Fig. 6. Cellulose acetate electrophoretic profiles of radiolabel incorporation into GAGs of the TM, cornea and sclera after standard 7-day preequilibration followed by 48 hr of incubation with labels. Panel A shows CAE profiles of 3H-glucosamine (solid line) and 35S-sulfate (dashed line) incorporation into the GAGs of the TM. Panel B shows the CAE profiles of 35S-sulfate incorporation into the cornea (solid lines) and the sclera (dashed lines). Electrophoresis was in the direction of the arrow (negative-to-positive) and values are averages of three experiments using separate single explants. Individual experiments used the three tissues from the same explant and were run on the same CAE strip.

cates no significant effect of donor age on individual GAG production. However, it should be mentioned that in this analysis, the proportion of donors which were under 50 years of age was small (9 of 41 total) and more data would be required to draw a meaningful conclusion on the effect of donor age.

As might be expected, reduced culture pH has drastic effects upon GAG profiles and morphology, therefore media with a colored pH-indicator are advantageous. Infrequently, yeast infections can occur in cultures, nearly always in pairs of eyes suggesting the donor as the source of infection. In these cases the complete explants are quickly permeated (within 12 hr) by these microorganisms. Infections are very easily detectable by media color changes (acidification turns the phenol red indicator yellow), a yeasty odor and by very noticeable increases in media turbidity. Careful attention to the media and microscopic observation of media aliquots are recommended to safeguard against this infrequent problem.

Analysis of the radiolabeled macromolecules in the culture medium shows that less than 10% of the label incorporation into GAGs is secreted without being

Fig. 7. Time courses for the total incorporation of 3H-glucosamine into the GAGs of the TM, cornea and sclera after various periods of continuous radiolabel exposure. Explants were preequilibrated for 7 days prior to initiation of time courses. Media were changed every 48 hr and fresh label added. Values are means in which three complete explants were used for each time point.

Fig. 8. Trabecular GAG incorporation profiles obtained by sequential enzymatic analysis. Explants were preequilibrated for 7 days, incubated with radiolabels for 48 hr and then the individual GAGs were analyzed. Relative incorporation values for 3H-glucosamine (3H, open boxes) and 35S-sulfate (35S, hatched boxes) are compared with Alcian Blue staining values (AB, solid boxes) reported previously for pools of noncultured eyes. Incorporation values are means with vertical lines showing the SEM for 18 samples analyzed in five separate experiments; 12 were analyzed individually and the remaining six samples were analyzed as pools of tissue from three explants each.
integrated into the extracellular matrix of the explant. No individual GAG predominates the labeled material released into the media by the explants.

Discussion

From these studies, we conclude that serum-free corneoscleral explant organ culture provides a satisfactory system for studies of the extracellular matrix of the trabecular meshwork. With very fresh eyes, these studies can be initiated immediately. However, if the eyes have been stored for more than a few hours postmortem, then a 1 week preequilibration period in culture is required prior to initiation of experiments. Sometime between 2 and 3 weeks in culture detrimental changes begin to occur and results become questionable. The supportive evidence for these conclusions is summarized below.

The ultrastructure of trabecular meshworks, which have been in explant organ culture for a period of from 7 to 21 days, is quite similar to that of "fresh" eyes and to that reported previously by other investigators.\(^\text{20,28-30}\) Comparison of our ultrastructural results with those of "activated" trabecular cells in culture\(^\text{2,22,26,31}\) leads us to conclude that our TM cells are not "activated." Micrographs of TMs from explants that have been in culture between 7 and 21 days do not show the migration from the beams, changes in the heterochromatin and nuclear structure, increased numbers of mitochondria, overly prominent Golgi complex, increased rough endoplasmic reticulum with enlarged cisterns, or generally enlarged cells that are characteristic of this "activation." Cellular migration is normally observed from meshwork explants which have been exposed to serum or growth factors, to phagocytic loads or to proteinases. In addition, migration of cells from the beams is observed from TMs which have been separated from the cornea and sclera before culture.\(^\text{2,20,22,25,26,29}\) From our studies, we cannot differentiate between these two potential contributing factors.

The trabecular GAG profile that is synthesized by fresh explants or by explants from eyes stored for up to 48 hr and then cultured for 1 week prior to initiation of experiments is very similar to the GAG profile
reported previously for the TMs of noncultured human eyes.\textsuperscript{3} A strict comparison is not possible here, since Alcian Blue staining is not quantitatively identical to either 3H-glucosamine or 35S-sulfate incorporation into the individual GAGs. However, the profiles should be, and are, sufficiently similar for us to conclude that within the time window from 7 to at least 14 days or when “fresh” eyes are used the corneoscleral explant culture system provides a very good approximation of the in vivo state of the TM.

This conclusion has recently been verified by the use of a new microanalytic method to determine the GAG content of individual human TMs which had not been cultured.\textsuperscript{32–34} In one of these studies,\textsuperscript{33} human trabecular GAG microanalysis results were compared directly with our published values\textsuperscript{3} and very good agreement was observed.

The incorporation profile of the trabecular GAGs is also clearly distinct from that of the cornea or the sclera in our culture system. The time courses for total radiolabel incorporation are also quite different; the maximal incorporation of both labels into the TM, which probably reflects a steady-state balance between GAG synthesis and turnover, occurs long before the maximum is reached in the other tissues of the explant. This probably means that the extracellular matrix of the TM is more dynamic than that of the adjacent tissues, although other explanations are possible. Electrophoretic migration profiles of the GAGs of these three tissues are also quite different. These three lines of evidence and the additional fact that very little labeled macromolecular material is found free in the medium argue strongly against the possibility of the cornea or sclera contributing to our determination of the trabecular GAG profile.

One difficulty of all culture studies is establishing the extent to which the behavior of the cells in culture reflects the in vivo state. The relatively undisturbed state of corneoscleral explants, the relatively avascular nature of the TM and the exclusion of serum or growth factors favor a close resemblance between cultured and in vivo trabecular behavior. Observations similar to ours have been reported for the corneal GAGs in a very similar corneoscleral explant organ culture system.\textsuperscript{24,35} We do observe minor corneal opacity and hydration in the explant cultures, suggesting that our conditions may be less than perfect for some types of long-term studies of the cornea. We also observe differences between scleral CS levels in our culture (Fig. 9b) and our previous analysis.\textsuperscript{3} We do not assume that the sclera is maintained optimally in this culture system, since there are no serum or growth factors in the media and the sclera is a vascular tissue. We do contend, from our results, that the other tissues in the explant are not affecting the TM as regards its morphology or its GAGs. Clearly, since we have not evaluated other aspects of trabecular behavior, we cannot comment beyond this limited contention. It is also important to mention that careful interpretation will be required in evaluations of the effects of drugs or natural modulators upon the TM in these corneoscleral explants, since trabecular changes could be indirect as a consequence of changes in the adjacent tissues.

Trabecular cell culture is one step further removed from the in vivo state and early studies with dividing trabecular cells\textsuperscript{20} found very high HA levels synthesized and secreted into the media. A GAG profile very similar to ours has recently been reported for densely-confluent trabecular cell cultures.\textsuperscript{27} This latter study reported the same, very high hyaluronic acid synthesis (75–91% of the total incorporation into GAGs) that has been reported for most trabecular cell cultures, while the cells were in the proliferative phase.\textsuperscript{7,16–20,22,27} However, it is only after 2–3 weeks in cell culture, or when dense confluence is reached, that the profile of trabecular GAGs changes; the production of HA drops to around 10% while the production of other GAGs, particularly HS, increases.\textsuperscript{7,20,22,27} We have recently compared the proteoglycans produced by our explant organ culture with those produced in trabecular cell culture\textsuperscript{36–38} and found the similarities to be more striking than the differences. This has also recently been verified.\textsuperscript{39}

We conclude then that either cell culture or corneoscleral explant organ culture will reproduce the intact GAG profile faithfully, if treated appropriately, and that both systems have considerable potential, albeit somewhat different ones, for future studies.

\textbf{Key words:} aqueous outflow pathway, glycosaminoglycans, human corneoscleral explant organ culture, trabecular extracellular matrix

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\section*{References}


