Corneal Function After Storage in Commercial Eye Bank Media

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Rabbit corneas were stored in commercially prepared media (K-Sol, M-K, CSM, or Dexsol) for 3, 6, 9, or 12 days. Corneas stored in each medium showed decreased poststorage deturgescence with increased storage time. This effect was most pronounced in corneas stored in K-Sol, followed by those in M-K, CSM, and Dexsol. Corneas stored in K-Sol also exhibited the fastest swelling rate when cultured in the presence of 20 μM ouabain, followed by those in M-K, Dexsol, and CSM. An estimate of the active ion transport capacity of each experimental group was made by determining the area between each group’s corneal thickness profiles in the presence and absence of ouabain. Corneas stored in M-K, K-Sol, or CSM retained approximately 70% of control activity after 3 days storage and 65% after 6 days storage. Corneas stored in Dexsol had 92% of control activity after 3 days of storage, 78% after 6 days, and 44% after 12 days.

Since 1975, the most common storage method for human corneas awaiting keratoplasty has been suspension in modified tissue culture medium at 4°C. McCarey and Kaufman1 initiated this protocol by formulating M-K medium (M-K) which contained 5% dextran (40,000 kD) to retard corneal swelling in M-199 tissue culture medium. Although preliminary evidence indicated that corneal viability could be maintained for up to 2 weeks,1 additional laboratory2-8 and clinical2-11 experience with M-K has limited its use in eye banking to a maximum storage time of 4 days.10,11 In 1985, Kaufman et al11,12 introduced a modification of M-K called K-Sol. The primary change from the original M-K formulation was the replacement of 5% dextran with 2.5% chondroitin sulfate which was column treated to remove small molecular weight components. Although successful grafts have been reported using rabbit12 and human11 corneas stored in K-Sol for as long as 2 weeks, most surgeons hesitate to use tissue stored in K-Sol more than 7 days. CSM medium was introduced more recently after some years of development.13 It differs from the previous media in its use of Minimum Essential Medium (MEM) as the tissue culture medium and lower content (1.35%) of chondroitin sulfate. The most recent medium to be marketed is Dexsol. Its composition is similar to that of CSM, with 1% dextran added to increase colloidal osmotic activity.

There have been many reports of laboratory and clinical observations of corneas stored in M-K and K-Sol, primarily using specular microscopy to observe endothelium and estimate cell densities. As valuable as this type of information is, there have been no in-depth studies on the functional capability of corneas after storage in K-Sol, CSM, or Dexsol and only limited information on corneas stored in M-K.1,4,14 It is generally accepted that corneal transparency is maintained in vivo by the function of energy-dependent ion transport process(es) located primarily in the endothelium which effectively move ions out of the cornea; water is therefore also removed osmotically. This deturgescence process maintains the collagen fibrils and glycosaminoglycan components of the corneal stroma in proper alignment to permit light transmission.15

A popular method to assess corneal function has been to observe a cornea’s ability to become thinner from a partially edematous state.16 Since corneas need to achieve and maintain a relatively deturgescent (thin) state after keratoplasty, it would seem that a test of a cornea’s ability to deturgesc itself in vitro after storage would be the most direct means of assessing tissue vitality. We therefore compared the deturgescence capabilities of rabbit corneas in vitro after storage in M-K, K-Sol, CSM, or Dexsol for up to 12 days, using a simplified deturgescence protocol which we described recently.17

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Materials and Methods

The data to be presented are the results of three identical experiments. The experimental protocol consisted of three phases: corneal processing and storage, corneal preincubation, and corneal culture.

Corneal Processing and Storage

For each experiment, 102 New Zealand white rabbits (2-3 kg) were decapitated, and their heads were immediately placed on ice by a nearby packing facility. The heads were maintained at 4°C until enucleation of the eyes within 4 hr post mortem. Each cornea was gently flushed with sterile 0.9% saline, and several drops of Neosporin (Burroughs Wellcome, Research Triangle Park, NC) were applied. The eyes were kept in a closed, sterile, moist chamber at 4°C for up to 4 hr more before corneal processing.

For each of the four storage medium, 48 corneas with intact epithelium were dissected along with 1 mm of surrounding sclera from the eyes of 24 rabbits using sterile technique. Each cornea was placed in an eyebank viewing chamber filled with the appropriate (coded) storage medium and stored at 4°C for up to 12 days.

Corneal Preincubation

In addition to the stored corneas, each experiment used six pairs of identically processed corneas to serve as "no storage" controls. Each control cornea was processed identically and immediately preincubated at 4°C in an eyebank viewing chamber filled with 25 ml of medium (CM) composed of Medium 199 supplemented with 30 mM NaHCO3, 5 mM HEPES buffer, and 50 µg/ml gentamicin. This medium has been shown to allow slow swelling of corneas when preincubated at 4°C, but promoted energy-dependent corneal deturgescence when cultured at physiologic temperatures.17 The CM medium was titrated to pH 7.50 and an osmolality of 305 ± 5 mOsm with NaOH and NaCl, respectively. Control corneas thickened during preincubation at 4°C from 350 to 550 µm in 40 hr.

At each storage time indicated, six randomly selected pairs of corneas from each storage medium group were removed from storage, placed in CM medium, and preincubated at 4°C until each group’s mean thickness increased to 550 ± 20 µm. Because of changes in corneal thickness during storage, the preincubation time needed to reach 550 µm decreased with increasing storage time; from approximately 24 hr after 3 days of storage to 2 hr after 12 days of storage.

Preliminary experiments showed that the endothelium of corneas stored for 12 days in any of the commercial media were viable by trypan blue extrusion criteria (not shown).

Corneal Culture

At the end of each preincubation period, 6 of the 12 corneas from each experimental group were cultured at 34°C in fresh CM. Their contralateral corneas were cultured in parallel with CM media with 20 µM ouabain added (OM), which has been shown to inhibit maximally corneal endothelial Na+/K+ ATPase and corneal deturgescence.20 Corneal thicknesses were measured optically every 1.5 hr for 6 hr with a specular microscope as described previously.17

Each point in Figures 1-4 indicates the mean of at least 16 corneas, calculated from three identical experiments. The standard error of the mean (when graphically larger than the symbol) is indicated by brackets. Statistical differences in corneal function, where determined to be significant, reflect results of the student t-test for unpaired samples with P < 0.01.

Corneal viewing chambers were obtained from Coopervision, Irvine, CA. K-Sol was purchased from Coopervision Cilco, Bellevue, WA, just before its removal from the market. Dexasol was purchased from Chiron, Irvine, CA. M-K and CSM were purchased from Sigma, St. Louis, MO. The institutions, investigators, or their families had no proprietary or other financial interest in any of these companies.

Results

Thickness Changes During Storage

Corneal thicknesses changed substantially during storage in commercial media. Corneas stored in M-K medium increased in thickness approximately 12 µm per day (Fig. 1). Corneas in K-Sol decreased in thickness during the first few days of storage but then exhibited the highest rate of swelling thereafter. CSM and Dexasol showed greater initial swelling, but their overall swelling for the entire storage period were essentially identical to those of K-Sol or M-K.

Because of the different rates of swelling exhibited by corneas in each medium during the storage and preincubation phases of the experiment, it was not practical to produce corneas of identical thickness at the start of culture. Since differences in swelling pressure may exist in corneas of unequal thickness,13 we did preliminary experiments to find the range of starting corneal thickness in which corneal swelling pressure would remain inconsequential. Figure 2 shows profiles of two subgroups of control corneas not stored in eyebank medium but simply preincubated to mean thicknesses of 570 µm or 530 µm. The data in
storage time (days)

Fig. 1. Corneal thickness changes during storage. Thickness measurements after 2 days storage in K-Sol (— O —), M-K (— A —), CSM (— △ —), or Dexsol (— □ —) were performed on six corneas per experimental group, randomly selected for temporary removal from 4°C storage (approximately 30 min). For other storage times, thicknesses were measured identically from corneas about to undergo preincubation in CM.

The left panel shows that the relative rates of deturgescence in CM and the rates of swelling in OM were essentially identical despite the difference in starting thickness. These observations can be more easily seen in the right panel, where the data are portrayed as a percent of each cornea’s starting thickness. Using this presentation, the two identically treated groups have essentially identical corneal-thickness profiles. Most of the subsequent data therefore uses this simpler graphic presentation.

Fig. 2. Corneal thickness changes during culture of control (no storage) corneas of different starting thicknesses. Left panel: six pairs of corneas were selected from a group of preincubated control corneas so that their mean starting thickness was 570 μm. One subgroup of six corneas was cultured at 34°C in CM (— ● —) while their contralateral corneas were incubated in OM (— O —). An analogous group of corneas with a mean thickness of 530 μm was identically cultured in CM (● · · · ) or OM (— △— ). Right panel: same data plotted at a percent of each cornea’s thickness at the initiation of culture (I₀).

A summary of the deturgescence capability of corneas cultured in CM after storage in eyebank media for up to 12 days is shown in Figure 3. After 3 days of storage, all corneas became significantly thinner than their starting thickness within 4.5 hr of culture. However, all stored corneas deturgesced significantly more slowly than control (no storage) corneas. Differences in deturgescence capability were more demonstrable after 6 days of storage. Corneas stored in K-Sol no longer deturgesced during culture in CM, becoming significantly thicker within 4.5 hr of culture. Corneas stored in M-K remained essentially at their starting value for 4.5 hr, then exhibited some deturgescence capability. Corneas stored in CSM or Dexsol for 6 days exhibited deturgescence ability which was not significantly different from their 3-day storage profiles. After 9 days of storage, corneas in K-Sol showed an increased rate of swelling; corneas in M-K became slightly thicker during culture, but the difference from their starting value was not significant after 4.5 hr of culture. The deturgescence profiles of corneas stored in Dexsol or CSM were essentially identical after 9 days of storage. Although deturgescence was not quite as great as after 6 days of storage, corneas stored in either media were significantly thinner than their starting value after 4.5 hr of culture.

Corneas stored in K-Sol for 12 days swelled more rapidly in culture than at earlier storage times. This precluded their thickness measurement at 6 hr of culture. Corneas stored in M-K for 12 days became significantly thicker within 4.5 hr of culture. Corneas stored in CSM or Dexsol for 12 days again showed essentially identical deturgescence profiles. After this
storage time, however, significant deturgescence was no longer exhibited within 4.5 hr of culture.

According to the "pump-leak" theory of corneal thickness equilibrium,\textsuperscript{15} a decrease in corneal deturgescence capacity could result from either a decrease in energy-dependent ion transport activity (pump), an increased permeability to water through the epithelial and/or endothelial barriers into the stroma (leak), or a combination of both factors. To assess the importance of the latter mechanism, identically stored, contralateral corneas were cultured in a parallel manner using ouabain, an inhibitor of Na\textsuperscript{+}/K\textsuperscript{+} ATPase, to prevent energy-dependent ion transport. Thus, only the corneas' propensity to imbibe water was expressed. Figure 4 summarizes this series of experiments. All corneas stored for 3 days showed increased swelling rates compared with control (no storage) corneas, but only those corneas stored in K-Sol were significantly thicker within 4.5 hr of culture. After 6, 9, and 12 days in storage, all corneas thickened at progressively faster rates than their 3-day stored counterparts. A consistent rank order of swelling was observed throughout, with corneas stored in K-Sol swelling the most, followed by those stored in M-K, Dexsol, and CSM. All corneas stored for 12 days thickened significantly more than control corneas except the group stored in CSM.

The thickness profiles of stored corneas during culture in medium with and without ouabain are expressed in Figure 5 for each eyebank medium. These data clearly portray the substantial effect that increased passive swelling has on the corneas' ability to deturgescence after storage. This point is made most strikingly by corneas stored for 6 days in K-Sol. This group of corneas no longer exhibited corneal deturgescence in CM. These corneas, however, were attempting to deturgescence in the face of a substantially increased passive swelling rate, as indicated by their group of contralateral corneas cultured in OM. The data in Figure 5 clearly show that measurement of corneal deturgescence in CM does not directly reflect a cornea's active ion transport (pump) capacity but only its net thinning after overcoming its passive swelling (leak) component.

To estimate the total active ion transport (pump) capacity of each group of corneas, the areas between

![Fig. 3. Corneal deturgescence during culture with CM following storage in eye bank media for 3, 6, 9, or 12 days. Control corneas (---) or corneas stored in K-Sol (---), M-K (• ∆ •), CSM (--- • ---), or Dexsol (--- □ ---) were cultured in CM at 34°C for 6 hr after their appropriate preincubation periods at 4°C to bring each groups mean corneal thicknesses to 550 ± 20 μm. Thicknesses are portrayed as a percent of their thickness at the start of culture (Co). The thicknesses of corneas stored in K-Sol for 12 days could not be measured after 6 hr of culture.](image1)

![Fig. 4. Corneal thickness changes during culture with OM following storage in eye bank media for 3, 6, 9, or 12 days. Control corneas (---) or corneas stored in K-Sol (---), M-K (• ∆ •), CSM (--- • ---), or Dexsol (--- □ ---) were cultured in OM at 34°C. All experimental groups were composed of contralateral corneas to those used in the experiments depicted by Figure 3. The thicknesses of corneas stored in K-Sol and in Dexsol for 12 days could not be measured after 6 hr of culture.](image2)
Fig. 5. Corneal thickness profiles when cultured with and without ouabain after storage in each eye bank media. The data are redrawn from Figures 3 and 4 to portray the changes in net deturgescence (— ● —), passive swelling (— ○ —), and active ion transport of corneas after storage.

The thickness profiles of corneas cultured in CM and OM were quantified and shown in Table 1. All corneas lost significant energy-dependent ion activity within 3 days of storage with respect to control (no storage) corneas. Corneas stored in K-Sol lost this activity the fastest, retaining 72% of the no-storage control activity after 3 days' storage and 25% after 12 days of storage. Corneas stored in M-K and in CSM retained somewhat more pump activity, especially after 9 days of storage. Corneas stored in Dexsol retained the most energy-dependent activity, exhibiting 92% of control after 3 days' storage and 44% of control after 12 days of storage.

Table 1. Active ion transport of stored corneas

<table>
<thead>
<tr>
<th>Storage time</th>
<th>K-Sol</th>
<th>M-K</th>
<th>CSM</th>
<th>Dексол</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>227 (72%)</td>
<td>221 (70%)</td>
<td>217 (69%)</td>
<td>290 (92%)</td>
</tr>
<tr>
<td>6 days</td>
<td>204 (65%)</td>
<td>192 (61%)</td>
<td>214 (68%)</td>
<td>246 (78%)</td>
</tr>
<tr>
<td>9 days</td>
<td>151 (48%)</td>
<td>191 (61%)</td>
<td>195 (62%)</td>
<td>201 (64%)</td>
</tr>
<tr>
<td>12 days</td>
<td>78 (25%)</td>
<td>116 (37%)</td>
<td>126 (40%)</td>
<td>137 (44%)</td>
</tr>
</tbody>
</table>

Active ion transport during corneal culture was estimated by calculating the area between the corneal thickness profiles cultured ± ouabain (Fig. 5) for the first 4.5 hr of culture.

K) exhibited much higher poststorage swelling rates than corneas stored in media formulated with MEM (CSM and Dexsol). To determine whether the tissue culture medium formulation affected corneal swelling patterns, laboratory-prepared storage media were made using a combination of 1.35% chondroitin sulfate and 1% dextran in both M-199 and MEM tissue culture media. Twenty-four corneas from 12 rabbits were stored in each medium for 9 days using the protocol described for storage in commercial media.

Corneal function after storage in these media is shown in Figure 6. There was essentially no difference in the deturgescence or swelling patterns exhibited by corneas stored in either medium, suggesting that functional differences observed in the experiments using commercial media were the result of the colloidal osmotic agents used or other additives, not the tissue culture media.

Discussion

Although it has been widely assumed that corneal function decreases with increasing corneal storage time, the mechanism(s) and rate of deterioration have remained largely a matter of speculation. Clearly, there are large changes in a cornea's ability to exhibit deturgescence (Fig. 3) after storage. The loss in detur-
Fig. 6. Corneal thickness profiles of corneas stored in laboratory-prepared media using M-199 or MEM. Twelve pairs of corneas were stored in media containing 1.35% chondroitin sulfate and 1% dextran in either M-199 (circles) or in MEM (triangles). After 9 days of storage at 4°C, paired corneas were preincubated and cultured in either CM (open symbols) or OM (closed symbols), as described previously. Data points indicate the mean ± standard error from a typical, reproducible experiment.

gescence ability was time dependent with any of the storage media used. Moreover, there were substantial differences among each commercial storage medium’s ability to retain deturgescence ability.

A substantial loss in deturgescence was most quickly seen in corneas stored in K-Sol. Within 6 days of storage in K-Sol, corneas could no longer maintain their starting thickness in culture, whereas at least some level of deturgescence was observed in corneas stored in M-K after 6 days, in CSM after 9 days, and in Dexsol even after 12 days of storage.

Applying the pump-leak hypothesis of corneal thickness homeostasis to these data, a cornea that is able to thin during culture expresses a pump rate which is faster than its leak rate; a cornea that becomes thicker exhibits a pump rate less than its leak rate. It is not possible to interpret from the experiments in Figure 3 alone whether a loss in deturgescence resulted from a decrease in pump ability or an increase in leakiness (or both) of the cornea. To differentiate between the two mechanisms, parallel cultures of identically stored corneas were tested with 20 μM ouabain in the medium to block the pump component (Fig. 4). These experiments indicate that the passive leak component changes substantially during storage in different commercial media. Corneas stored in K-Sol (with 2.5% chondroitin sulfate) increased their swelling characteristics the fastest at all storage times tested. Corneas stored in K-Sol for 6 days had a passive swelling rate almost threefold higher than control corneas. This change may account for the numerous clinical reports of prolonged postsurgical swelling using K-Sol-stored corneas. The swelling effect was somewhat lower in corneas stored in M-K (5% dextran) and still lower in corneas stored in Dexsol (1.35% chondroitin sulfate and 1% dextran). Corneas stored in CSM (1.35% chondroitin sulfate) only slightly increased their passive swelling rate throughout the 12-day storage period. Thus, there was an association between higher concentrations of each respective colloidal osmotic agent and increased post-storage tendency to swell during culture. This effect may be due to penetration of the osmotic agents into the corneal stroma during storage, which would then act to pull water osmotically into the cornea during poststorage culture. Both dextran21 and chondroitin sulfate22 have been reported to be taken up by tissues. Conversely, it is possible that alterations in corneal swelling patterns could arise from the removal of endogenous glycosaminoglycans from the corneal stroma during storage. Little is known concerning the rate and extent of glycosaminoglycan extraction during corneal storage, or whether different storage media affect the process.

Another plausible mechanism suggests that both 2.5% chondroitin sulfate and 5% dextran (or contaminants in these preparations) are toxic to the corneal endothelial and/or epithelial cell junctions, and the water permeability characteristics of these barriers are physically changed during storage. The experimental evidence in Figures 3–5 are consistent with any of the mechanisms. The data in Figure 1, however, do not favor the first mechanism, since relatively large amounts of colloid would need to penetrate the cornea to change the swelling rates of corneas during this storage period.

In addition to increased passive swelling characteristics, the cornea’s capacity to perform energy-dependent ion transport decreased with storage time for all the media tested. Dexsol was significantly superior to the other media in retaining energy-dependent ion transport at storage times through 6 days, exhibiting 92% and 78% of the control capacity after 3 and 6 days, respectively. After 9 and 12 days of storage, corneas stored in K-Sol did significantly worse than corneas in the other media. Since the control (no storage) corneas used in these experiments were actually 48 hr post mortem when cultured, the above percentages probably overestimate stored corneal performance with respect to in vivo deturgescence capacity.

It remains an open question whether the first 6 hr of culture accurately reflect a stored cornea’s long-term ability to regain pump capacity or barrier integrity. It
is feasible that corneas need more time to recover from the storage environment. We attempted to address this possibility in several experiments (not shown) by continuing to culture corneas which showed no deturgescence ability during the first 6 hr of culture for up to 16 hr. No indications of increasing deturgescence capacity were observed during these extended cultures.

The results of these experiments do not concur with findings of other studies using morphologic criteria to gauge corneal viability. In general, corneas appear "viable" by specular or endothelial microscopy for longer storage periods than seen in this study.1,11,23 In most of these studies, however, the corneas were not removed from the storage medium before observation or fixation. The transition of a cornea from its storage medium to a more physiologic environment may be more traumatic than generally assumed. Van Horn et al.2 noted a marked increase in trypan blue vital staining and cellular disruption of endothelium in stored cat corneas if the corneas were incubated for 2 hr at 35°C in fresh tissue culture medium before evaluation. The time course of endothelial cell death using this approach was similar to the decrease in active ion transport estimates made in the studies herein. Moreover, Hartmann and Rieck28 recently used a new vital staining technique to monitor viability of endothelium on pig corneas stored in M-K. The rate of decrease in viable cells with storage time was very close to the loss of active ion transport activity shown here. Measurement of corneal function after storage appears to be a direct and accurate means to evaluate storage methods and tissue quality.

Key words: cornea, storage, deturgescence, eyebanking, rabbit

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References