Biomechanical Characterization of Human Amniotic Membrane Preparations for Ocular Surface Reconstruction

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Key Words
Amniotic membrane · Biomechanical properties · Transplant · Ocular surface reconstruction

Abstract

\textbf{Purpose:} To investigate the tensile and elastic properties of both commercially available and experimental human amniotic membrane preparations. \textbf{Method:} Nine preparations of human amniotic membrane were studied. The four dry preparations were untreated (nonirradiated, \(n = 20\)), and gamma (\(n = 25\)), low-dose (AmbioDry\textsuperscript{\textregistered}, Okto Ophtho Inc., Costa Mesa, Calif., USA, \(n = 20\)) and high-dose (\(n = 20\)) electron beam sterilized. The same dry membranes were moistened with balanced salt solution (\(n = 20, 34, 20 \text{ and } 20\), respectively). The ninth group consisted of thawed medium-frozen amniotic membrane (AmmioGraft\textsuperscript{\textregistered}, Bio-Tissue Inc., Miami, Fla., USA, \(n = 20\)). The membranes were cut into thin strips, loaded on a gram range load sensor, and stretched incrementally to the point of rupture. The modulus of elasticity, displacement until rupture and maximum tolerated stress were recorded and compared. \textbf{Results:} The dry preparations exhibited higher moduli of elasticity when compared with the moist samples, with the low-dose electron beam-irradiated samples having the greatest mean modulus of elasticity overall and maintaining a high modulus of elasticity as a moist sample (\(p < 0.05\)). Moist nonirradiated preparations and thawed medium-frozen preparations stretched the farthest before rupture and experienced the greatest mean stresses at the point of rupture. While 3 of 4 membranes had greater stretch when moistened as compared to their dry counterparts, there was no difference in the membrane stiffness between dry and moistened low-dose electron beam-irradiated samples (\(p > 0.8\)). \textbf{Conclusions:} Low-dose electron beam-irradiated amnion appeared to maintain desirable elastic characteristics in transition from a dry to rehydrated state and may thus provide an easy-to-manipulate transplant tissue for ocular surface reconstruction. Moist nonirradiated and thawed medium-frozen tissues, however, may provide surgical advantages as they required greater forces to rupture.

Introduction

Ocular surgeons are frequently faced with the challenge of reconstructing surface defects in either the conjunctiva or the cornea. Defects in the corneal epithelium requiring surgical reconstruction may result, for example, from ulcers [1–7], bullous [8, 9] or band [10] keratopathy, and thermal and chemical burns [11, 12]. Large defects in the conjunctiva carry the risk of scarring that can disfigure the conjunctiva and even restrict ocular motility. Conjunctival defects may result from neoplasms removal [13, 14], symblepharon removal [15], pterygium surgery.
[16, 17], and scar revisions. Reconstruction of the ocular surface has been attempted with conjunctival autografts [18] and other mucosa [19, 20]. Currently, there has been a resurgence in the use of amniotic membrane grafts in the treatment of ocular surface defects.

The first use of what were then described as ‘fetal membranes’ appeared in the literature in 1940 by de Roth [21] who reported the use of the tissue to repair conjunctival defects. He described using the tissue on a few select patients in whom the transplanted tissue shrank in size and eventually disappeared completely after 10 weeks, leaving behind the healed conjunctiva. Subsequently, Sorsby and Symmons [22] reported the transplantation of amniotic membrane to the ocular surface in the treatment of caustic burns. Concurrently, a case report by Lavery [23] described ‘human amnioplastin’ grafted to the ocular surface, again to treat a caustic burn from lime. For the next 50 years, very few references documenting the application of amnion to cover corneal and conjunctival defects [40–42, and Battle JF, et al. Ophthalmology 1993;100: Abstract 107] were found in the scientific literature on the topic of ocular amniotic membrane transplantation. In 1995, the first formal studies of amniotic membrane transplantation in an animal model were reported by Kim and Tseng [24, 25]. They reported that amniotic membranes grafted onto a damaged rabbit cornea denuded of all epithelial cells encouraged successful restoration of a cornea-like epithelium.

Recently, amniotic membrane transplantation has been used in the repair of many types of epithelial [26–31] and conjunctival [32] defects. Transplantation of the membrane has also been combined experimentally with limbal epithelial stem cell grafts [31, 33]. Although the benefits of amniotic membrane transplantation have been well demonstrated, working with the fragile amniotic membrane often presents a technical challenge for the surgeon.

The biomechanical properties of different amniotic membrane preparations have been little studied and compared [34, 43]. In this study, we present a biomechanical evaluation of the tensile and elastic properties of commercially available and experimental amniotic membrane preparations.

Methods

Nine preparations of amniotic membrane were examined. These included four dehydrated amniotic membranes, the same four preparations saline-moistened, and a thawed medium-frozen amnion (table 1).

### Table 1. Amniotic membrane preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Group</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonirradiated</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Irradiated gamma (20–40 kGy)</td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td>low-dose E-beam (18–20 kGy)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>high-dose E-beam (30–40 kGy)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Medium-frozen (thawed)</td>
<td>not available</td>
<td>20</td>
</tr>
</tbody>
</table>

1 AmbioDry (Otto Ophtho Inc.).
2 AmnioGraft (Bio-Tissue Inc.).

The dehydrated tissues were processed by isolating the amnion from chorion followed by cleansing and osmotic destruction of cells with a saline solution to reduce the tissue to its nonliving components. Using hypotonic saline solution, the tissue was washed further to remove the surface columnar and embedded cellular components within the amniotic membrane. The tissues were dehydrated under low heat and air vacuum during which time an elevated grid pattern was dried within the graft structure.

The first dry tissue group (nonirradiated, n = 20) underwent no additional processing beyond these steps. The second study group was sterilized using 25–40 kGy of gamma irradiation (gamma-irradiated, n = 25). The third and fourth study group membranes were sterilized using low-dose (18–20 kGy, n = 20; AmbioDry®, Otto Ophtho Inc., Costa Mesa, Calif., USA) and high-dose (30–40 kGy, n = 20) electron beam (E-beam) sterilization cycles. Another four sample groups consisted of rehydrated membranes from each of the four dry acellular preparations (n = 20, 34, 20, and 20, respectively). These four sample groups were hydrated using a balanced salt solution (Alcon, Fort Worth, Tex., USA) for 10 min before testing. The ninth group used in the study was a commercially available preparation thawed from frozen culture medium (AmnioGraft®, Bio-Tissue Inc., Miami, Fla., USA, n = 20). Dry preparations of this medium-frozen membrane are not available.

The examiner was blinded to the membrane preparations being examined with the exception of the thawed medium-frozen preparation which was visually different from the other preparations and thus unable to be blinded. Number-coded samples represented lot numbers from different amnion donors. Two to three different lot samples were represented in each preparation group.

Samples of each of the nine amniotic membrane preparations were cut into thin (1–2 mm × 4–8 mm) strips. Width and thickness of each strip was measured with a digital (YT202, Geneva Gage Inc., Albany, Oreg., USA) and metric micrometer (Geneva Gage Inc.) and the cross-sectional area of each individual piece was calculated.

Each strip was loaded into a two-axis adjustable tensiometer connected to a gram range load sensor (GS20 Precision Gram Sensor, Transducer Techniques, Temecula, Calif., USA) (fig. 1A) by securing the aluminum end plates to each end of the piece of membrane (fig. 1B). Each secured membrane was preconditioned twice by increasing the membrane length in a stepwise fashion in incre-


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Fig. 1. Measurement apparatus. A Gram range load sensor (white arrow) connected to voltmeter (black arrow). Strips of amnion placed between clamps of the load sensor are stretched incrementally. Voltmeter readings (mV) are converted to measurements of force (mN). The digital micrometer (black arrowhead) used to measure the length and width of test strips is shown. B A strip of dry amnion (*) is seen here positioned between the aluminum end plates of the load sensor.

Characterization of Amniotic Membrane

[Text continues...]
Results

Of the 213 individual amniotic membranes tested, 199 generated meaningful data. The results of 14 strips were discarded because of damage to the tissue during handling and insertion into the tensile testing equipment or during early preconditioning. The moduli of elasticity for all nine membrane preparations are compared in figure 2A. All of the dry membranes exhibited higher moduli of elasticity when compared to the five moist preparations.

There was very little difference between the elasticity of the dry samples tested, with a significant difference only between the means of low-dose E-beam (mean modulus of elasticity, 86.08 ± 33.70 N/mm²) and high-dose E-beam (66.45 ± 26.05 N/mm²; p < 0.05) (fig. 2A, table 2). The modulus of elasticity was significantly decreased for all moistened samples, with gamma-irradiated samples having the lowest mean value (11.78 ± 5.80 N/mm²).

Fig. 2. Mean values (± SD) of the biomechanical properties of amniotic membrane preparations. A Modulus of elasticity (or stress tolerated per unit strain) is shown and compared for all nine preparations of membrane. B Maximum stress tolerated before membrane rupture. C Change in length before rupture.

Table 2. Summary of p values for biomechanical measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Sample preparation</th>
<th>Comparison</th>
<th>Samples compared</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOE</td>
<td>dry moist</td>
<td>higher MOE higher MOE after rehydration</td>
<td>low-dose E-beam vs. high-dose E-beam</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>low-dose E-beam vs. all rehydrated samples</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maximum stress at rupture</td>
<td>dry moist</td>
<td>rupture at lowest stress highest level of stress tolerated lowest level of stress tolerated lower stress at rupture</td>
<td>high-dose E-beam vs. all dry preparations</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nonirradiated and medium-frozen vs. all others</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>gamma-irradiated vs. all others</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>moist samples vs. dry counterparts</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Strain</td>
<td>moist</td>
<td>greater stretch before rupture least stretch before rupture</td>
<td>nonirradiated and medium-frozen vs. all others</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>low-dose E-beam vs. all others</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

MOE = Modulus of elasticity.
Table 3. Amniotic membrane thickness

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Strip thickness, μm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dry</td>
<td>moist</td>
</tr>
<tr>
<td>Nonirradiated</td>
<td>19.25 ± 1.18</td>
<td>20.25 ± 1.60</td>
</tr>
<tr>
<td>Irradiated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gamma</td>
<td>21.30 ± 3.69</td>
<td>19.71 ± 2.02</td>
</tr>
<tr>
<td>low-dose E-beam</td>
<td>18.50 ± 2.05</td>
<td>20.00 ± 1.99</td>
</tr>
<tr>
<td>high-dose E-beam</td>
<td>20.14 ± 2.36</td>
<td>19.50 ± 1.54</td>
</tr>
<tr>
<td>Medium-frozen (thawed)²</td>
<td>not available</td>
<td>32.13 ± 5.21³</td>
</tr>
</tbody>
</table>

Figures are mean ± SD.
1 AmbioDry (Oktopho, Inc.).
² AmnioGraft (Bio-Tissue, Inc.).
³ p < 0.0001 vs. all other preparations.

followed by the thawed medium-frozen membranes (20.04 ± 8.88 N/mm²), the moist nonirradiated preparations (21.65 ± 6.82 N/mm²), and high-dose E-beam (25.23 ± 11.44 N/mm²). Low-dose E-beam preparations maintained a relatively high mean modulus of elasticity after rehydration (64.26 ± 22.03 N/mm²), significantly higher than any other moist preparation (p < 0.0001). Of note, rehydrated gamma-irradiated preparations had a significantly lower mean modulus of elasticity than any other preparation tested (11.78 ± 5.80 N/mm², p < 0.0001).

The maximum stress at the point of rupture that was tolerated by each type of membrane was measured and compared. The means of the maximum stress (N/mm²) endured by each membrane preparation are presented in figure 2B. There was no significant difference between the means of the dry nonirradiated, gamma-irradiated, and low-dose E-beam-irradiated samples. Of the dry samples, only the high-dose E-beam-irradiated preparations ruptured at a significantly lower level of stress with a mean of 9.39 ± 3.05 N/mm² (p < 0.01). Of the rehydrated preparations tested, both the moist nonirradiated and medium-frozen tissue samples demonstrated significantly higher tissue stress levels at the point of rupture as compared to all other moist samples (11.82 ± 2.34 and 12.10 ± 4.02 N/mm², respectively; p < 0.01) (table 2). Both the low- and high-dose E-beam preparations tolerated the next highest levels of stress (8.57 ± 2.26 and 9.30 ± 2.59 N/mm², respectively) with no statistical difference between these two rehydrated preparations (p > 0.25). The gamma-irradiated membrane exhibited by far the lowest mean tissue stress at rupture of 5.46 ± 1.44 N/mm² (p < 0.0001). With the exception of the high-dose E-beam preparation, all of the moist samples presented lower stress at the point of rupture when compared to their dry counterparts (p < 0.01 for all comparisons). In the high-dose E-beam samples, there was no change in mean stress at rupture between the dry and moist forms (p > 0.9).

The change in length from L₀ to the point of rupture (strain) was tested for each individual membrane and compared between membrane preparations (fig. 2C, table 3). Of all the moist membranes examined, the nonirradiated and thawed medium-frozen preparations stretched substantially further before rupture with means of 1.46 ± 0.15 mm and 1.48 ± 0.29 mm, respectively, compared to other membrane preparations (p < 0.0001). Low-dose E-beam-irradiated samples demonstrated significantly less mean displacement (0.62 ± 0.17 mm) when compared to the other moist preparations (p < 0.0001). The amount of stretch exhibited by the rehydrated low-dose E-beam-irradiated samples was, in fact, unchanged from the dry form of the same membrane (0.61 ± 0.20 mm, p > 0.8). Alternatively, all other membranes that could be examined in both the dry and rehydrated state demonstrated significantly increased stretch when moistened versus the dry samples of the same membrane (fig. 2C).

The cross-sectional area used to compare the values of maximum tolerated stress per unit area was determined for each membrane sample individually. Comparisons of strip dimensions by group were also performed (table 3). The thawed medium-frozen membrane preparations are significantly thicker than each of the acellular dehydrated groups, in either the moist or dry state. Of note, there was virtually no increase in membrane thickness after rehydration of the dry acellular preparations. Mean membrane thickness of all dry acellular preparations combined (n = 85) was 19.88 ± 2.75 μm, while combined membrane thickness of rehydrated acellular preparations (n = 94) was 19.84 ± 1.83 μm (p > 0.9).

Discussion

Persistent ocular surface defects, both as a consequence of disease as well as iatrogenic, are a common problem in ophthalmology. Amniotic membrane grafting offers an effective treatment option for aiding in the closure of both corneal epithelial and conjunctival defects. Preserved amniotic graft tissues are either rehydrated from the dry state [43–45], or thawed from the frozen state [1–7, 9, 10, 12–17, 24–33, 37–39]. We examined several preserved preparations of amniotic membranes
that are either currently available or in development and measured and compared the biomechanical properties of each. While in this study both dry and rehydrated tissues were examined, it should be noted that all tissues used in surgery will be manipulated and sutured on the ocular surface in the rehydrated state. Thus, it is important to be aware of the response of the membrane to physical stress and manipulation in the moistened state. Such data may allow the surgeon to reliably anticipate how the tissue will be handled during surgery.

All dry preparations examined in this study exhibited a higher modulus of elasticity than the moist preparations of membrane. Of the four acellular preparations that could be compared in both the dry and rehydrated state, the modulus of elasticity for two of these membranes, nonirradiated and high-dose E-beam-irradiated, decreased significantly in the moistened state to levels that were statistically equivalent to the thawed medium-frozen preparation. Of note, the rehydrated low-dose E-beam-irradiated samples maintained a substantially higher modulus of elasticity as compared to all other moist samples tested. The modulus of elasticity of the moist low-dose E-beam tissue was actually near that of the same preparation in the dry form. This may indicate that the elastic properties of the tissue or the ‘feel’ of its elasticity in the hands of the surgeon would be very similar to the dry tissue before rehydration. This characteristic of the tissue is further demonstrated in the change in length prior to rupture. In the case of the low-dose E-beam-irradiated sample group, there was no significant difference between dry and moist tissue displacement.

The moist gamma-irradiated preparation exhibited the lowest modulus of elasticity by a substantial margin. It may be that the gamma-irradiation process affects the collagen cross-linking and thus the properties of the tissue. Recent studies in amnion treated with gamma irradiation indicate this may be the case secondary to scission of collagen chains with increasing levels of irradiation [36]. This may also explain why the rehydrated gamma-irradiated samples ruptured under a much lower level of stress than the other moist membranes. Gamma-irradiated samples may not be as desirable for use in human amniotic membrane transplantation surgery because of the weakening effect that the gamma irradiation seems to have on the tissue.

Two moist membranes, the nonirradiated and thawed medium-frozen preparations, were able to withstand the greatest tissue stretch prior to rupture. The same preparations tolerated higher maximum stress before rupture than the other three rehydrated sample groups. Clinically, this may be advantageous as the membranes may stretch further to adjust the size of an amnion patch during fixation to the ocular surface. Of course, this could also present a challenge in the operating room if a precut patch of amnion does not maintain predictable dimensions. However, some stretch beyond the borders of the defect is easily remedied and would likely be tolerable.

Currently, only two of the membranes examined in this study are available commercially, the low-dose E-beam-irradiated tissue (AmbioDry, Okto Optho, Inc.) and the medium-frozen (AmnioGraft, Bio-Tissue, Inc.) preparations. Direct discussion and comparison of the biomechanical properties of these two preparations is warranted. In comparing the moist state of each membrane, the greatest difference is apparent in the modulus of elasticity. Since modulus of elasticity represents the slope of the stress-strain curve, a tissue that demonstrates a higher modulus of elasticity will stretch less for a given level of force exerted on the tissue. AmbioDry demonstrates a modulus of elasticity that is more than three times that of AmnioGraft. Regarding displacement of the tissue, AmnioGraft strips stretched more than twice the length of the AmbioDry samples. AmnioGraft also endured a greater force per unit area before rupture than did the AmbioDry samples, requiring more than 40% greater force to break. The ocular surface surgeon will need to decide on the continuum of stiffness versus pliability which membrane is most desirable to use.

Biomechanical characteristics of amniotic membrane preparations are only some of the tissue qualities to consider in evaluating amnion preparations. When choosing between available preparations, ophthalmologists must consider a variety of factors. Some surgeons may prefer to store, handle, measure, and cut tissue to size while in a room temperature dry state, while others prefer the medium-frozen technology. Additional factors to consider include the steps of amnion preparation itself. When processing amniotic membrane, it is desirable to preserve the demonstrated ability of amnion to reduce inflammatory response, encourage proliferation of neighboring native cell populations and serve as a substrate for epithelial cell migration and expansion. Therefore, side-by-side comparisons of available amniotic membrane preparations are needed to study the growth, proliferation and support abilities of the tissues in addition to the biomechanical properties.

The biomechanical properties of amniotic membrane grafts are important to consider in predicting intra- and
postoperative surgical behavior. The membrane preparations studied have displayed a wide range of tensile behavior upon stretching. Further work to correlate the measured properties with perceived material behavior during surgical manipulation will need to be performed.

In addition to the medium-frozen (AmnioGraft) and dehydrated acellular (AmbioDry) preparations available in the United States, fresh amnion is being studied and transplanted to the ocular surface in other countries [8, 37–39]. Therefore, we also recommend that future studies include evaluation of the biomechanical properties of fresh amniotic membranes and their comparison to the membrane preparations studied here.

Acknowledgements

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References


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