Major Article

The effect of amniotic membrane grafting on healing and wound strength after strabismus surgery in a rabbit model

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BACKGROUND Amniotic membrane grafts (AMGs) are used, with mixed results, as a platform for ocular healing and to reduce pathologic scarring. This study evaluated wound tensile strength and histopathologic changes after strabismus surgery with AMGs in 20 New Zealand white rabbits.

METHODS All subjects underwent 4 mm inferior rectus hang-back recessions to both eyes. The right eyes served as controls. Ten left eyes (group 1) received processed dehydrated amniotic membrane allografts (Ambiodry2, IOP Inc, Costa Mesa, CA) and ten left eyes (group 2) received cryopreserved human amniotic membrane allografts (AmnioGraft, Bio-Tissue, Miami, FL) between the sclera and muscle insertion and between the muscle and repositioned conjunctiva. At postoperative month 1, tensile strengths of the muscle–globe and conjunctiva–globe attachments were measured, and histopathologic analysis of each eye was performed.

RESULTS In group 1 the mean tensile strength of the muscle–globe attachments was 441.4 ± 274.4 g; of the conjunctiva–globe attachments, 640.3 ± 266.4 g. In the control eyes, the comparable values were 365.8 ± 199.8 g and 595.2 ± 315.3 g, respectively ($P = 0.19$, $P = 0.13$). In group 2 the mean tensile strengths were 456 ± 297.5 g and 608.2 ± 306.7 g, compared with control values of 352.7 ± 114.8 g and 583.8 ± 347.1 g ($P = 0.43$, $P = 0.45$).

CONCLUSIONS There was no significant change in tensile strength of the muscle insertion using AMGs. In a rabbit model, AMGs do not reduce inflammation or improve scar formation 1 month after strabismus surgery. (J AAPOS 2017;■:1-5)

Postoperative scarring and adhesions can lead to unpredictable results both in complex, consecutive, and routine strabismus cases.[1,2] These adhesions may involve conjunctiva, Tenon's capsule, extraocular muscles, sclera, intramuscular septum, and orbital fat. Adhesions may have a profound influence on surgical outcomes and increase the complexity of subsequent surgeries. Previous studies have demonstrated the efficacy of 5-fluorouracil, mitomycin C, and other agents in reducing postoperative adhesions; however, routine use has been limited by potential side effects of these medications as well as inconsistent results.[1,3-8]

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In strabismus surgery, mixed results have been reported with the use of AMG to prevent subconjunctival and sub-Tenon's adhesions.[9-17] AMG is available in multiple preparations, including fresh, cryopreserved, and lyophilized. In this study, we evaluate the effect of both cryopreserved and processed dehydrated AMG on pathologic postoperative scarring through histopathologic analysis and measurement of the tensile strength of the muscle and overlying conjunctiva. Following strabismus surgery, the operated extraocular muscle forms a new insertion with adhesion to the underlying sclera, and the overlying conjunctiva forms a new adhesion attachment to the underlying sclera anterior to the recessed muscle. The force necessary to release those attachments was measured as a means to determine whether there was more or less pathologic scarring, based on the assumption that more tensile strength than normal would represent pathological scarring.

Materials and Methods

This study was approved by the Institutional Animal Committee for Use and Care at the University of Colorado and was performed in accordance with the ARVO statement for Use of Animals in Ophthalmic and Vision Research. Twenty New Zealand

white rabbits were randomized into two groups. Both eyes of all animals underwent inferior rectus recession using a hang-back technique. Briefly, following general anesthesia with 1%-2% inhaled isofluorane in oxygen and intramuscular injection of ketamine HCL (30 mg/kg) and xylazine (5 mg/kg), the ocular surface and periocular area was washed with 5% povidone solution. An inferior conjunctival peritomy was created using Westcott scissors and the insertion of the inferior rectus muscle was exposed. The muscle was isolated using a Jameson muscle hook, and intramuscular membranes were removed using blunt dissection. The inferior rectus muscle was secured using 6-0 polyglactin 910 suture placed approximately 1.0 mm posterior to the insertion, and the muscle was disinserted from the globe 0.5 mm anterior to the suture. In both groups, the left eye was the treatment eye. Prior to reattaching the muscle, in group 1 eyes dehydrated AMG (Ambiodry2, IOP Inc., Costa Mesa, CA) was bisected and a piece approximately 10 × 7.5 mm was placed between the sclera and the inferior rectus muscle, stroma side up, anteriorly up to the inferior rectus muscle stump (Figure 1). The muscle was reattached to the muscle stump using a hang-back technique such that the muscle was approximately 4.0 mm posterior to the original muscle stump. The second half of processed dehydrated AMG was then placed on top of the muscle, stroma side down. The conjunctiva was closed using 8-0 polyglactin 910 suture. The same procedure was performed on the right eye of all animals, but without placement of AMG. In group 2, the same procedure was performed using cryopreserved AMG (AmnioGraft, Bio-Tissue, Miami, FL) in the treatment eyes. Neomycin, polymyxin B, and dexamethasone ophthalmic ointment (Perrigo, Dublin, Ireland) were applied to both eyes of all animals after surgery and, in ophthalmic suspensions, twice daily for 5 days beginning on postoperative day 1. All animals were also administered the oral analgesic meloxicam (Boehringer Ingleheim, St. Joseph, MO) 0.3mg/kg 24 hours prior to surgery and once daily for 2 days following surgery.

One month postoperatively, the animals were euthanized. Small radial snip incisions were made through the conjunctiva on either side of the IRM insertion. A modified tensometer attached to a Green eye muscle hook was used to isolate the muscle. The force required to disinsert the muscle from the underlying sclera as well as break through the overlying conjunctiva were measured sequentially. Force was measured as a means to determine if there was more or less pathologic scarring, and the assumption was made that more tensile strength than normal would represent pathological scarring. Following tensile strength analysis, the animal was sacrificed by intravenous injection of 26% sodium pentobarbital (SleepAway, Zoetis, Florham Park, NJ). The orbital contents were then exenterated and preserved in Davidson's Fixative (70% ethanol, 10% glacial acetic acid, 2% formalin) for histopathologic analysis. Serial sections through the muscle stump were stained with hematoxylin-eosin for evaluation of inflammatory response, Masson trichrome for evaluation of fibrosis, and human placental collagen type 1 to evaluate remaining AMG. The grading guidelines are described in eTable 1; the histopathological results are given in Table 1. The grading system used was modified from that previously described by Kassem and colleagues.[10]

Statistical analysis of tensile strength was performed on GraphPad Prism software using analysis of variance analysis (ANOVA) with Tukey's post hoc. Graded histopathologic evaluation of fibrosis and inflammation were compared using two-tailed paired *t* test. During analysis, amniotic membrane treated eyes were compared only to controls within the same group in order to control for inter-animal variability. Differences were considered statistically significant if $P < 0.05$.

Results

Tensile Strength

In group 1 normal intra-animal variation of the tensile strength of muscle and conjunctiva measurements was noted (Figure 2). The mean tensile strength of the muscle attachment in processed dehydrated AMG-treated eyes was 441.1 ± 274.4 g; of the control eyes, 365.8 ± 199.8 g. The mean tensile strength of the overlying conjunctiva was 640.3 ± 266.4 g in the AMG-treated eyes; 595.2 ± 315.3 g, in the control eyes. There was no significant difference in the tensile strength of the muscle attachment ($P = 0.14$) or conjunctival adhesion ($P = 0.30$).

In group 2 intra-animal variation of the tensile strength measurements was again noted. Tensile strength measurements are shown in (Figure 2). The mean tensile strength of the muscle attachment in cryopreserved AMG-treated eyes was 456 ± 297.5 g; in control eyes, 352.7 ± 114.8 g. The tensile strength of the overlying conjunctiva was 608.2 ± 306.7 g in the AMG-treated eyes; 583.8 ± 347.1 g, in the control eyes. There was no significant difference in the tensile strength of the muscle attachment ($P = 0.43$) or conjunctival adhesion ($P = 0.45$).

Histopathology

Graded evaluation of fibrosis and inflammation for each animal in groups 1 and 2 as well as mean scores for each group are shown in Table 1. In group 1 there was no significant difference in inflammation or fibrosis compared to control eyes. There was a trend toward decreased incidence of muscle-scleral adhesion inflammation in the eyes treated with processed dehydrated AMG, but it did not rise to the level of statistical significance ($P = 0.07$). In group 2 there was significantly more scleral inflammation in eyes treated with cryopreserved amniotic membrane compared to control eyes ($P = 0.01$). There was no significant difference in all other evaluated parameters.

Discussion

The present study compared eyes treated with standard inferior rectus hang-back recession to fellow eyes treated using the same technique but with application of AMG. Both processed dehydrated AMG and cryopreserved AMG were used in order to discern whether there was a significant difference in inflammatory response or fibrosis based on histology and on tensile strength of the 28-day