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Use of Nonpreserved Human Amniotic Membrane for the Reconstruction of the Ocular Surface

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Purpose. To describe the use of nonpreserved human amniotic membrane (NP-AMT) as an alternative to preserved human amniotic membrane (AMT) for the reconstruction of the ocular surface in several diseases. **Methods.** NP-AMT was used in the treatment of five patients with the following diseases: noninvasive conjunctival squamous cell carcinoma, corneal persistent epithelial defect, severe alkali burn, near total limbal deficiency secondary to multiple surgeries, and ocular cicatricial pemphigoid. In some cases, a limbal autograft or allograft was employed simultaneously, sutured on top of the NP-AMT. All sutures were made with 10-0 Nylon and were removed at two weeks. **Results.** Ocular surface was satisfactorily reconstructed, eyes were quiet, and patients were comfortable despite prolonged deepithelialization in some cases. There was a case of a limbal autograft ischemia—in the burned patient—that caused partial corneal conjunctivalization. Initially, the NP-AMT looks thickened but thins around the fifth day and looks similar to AMT. **Conclusion.** Results using NP-AMT are similar to those of AMT. It is a good alternative and it is easily obtained in places were AMT is not available or is too expensive to procure.

Key Words: Amniotic—Membrane—Persistent epithelial defect—Conjunctival squamous cell carcinoma—Ocular surface disease—Nonpreserved—Limbal autograft.

The amniotic membrane is the innermost of the fetal membranes; it has an avascular stromal matrix, a thick and continuous basement membrane with a full complement of collagen type IV and V and laminin, and an epithelial monolayer.[1–3] It is avascular and antiangiogenic,[4] does not express histocompatibility antigens,[5,6] and has antibacterial[7,8] and antiadhesiveness[9,10] properties. It favors epithelial cell migration,[11] reinforces adhesion of basal epithelial cells,[12,13] diminishes their apoptosis,[14,15] and promotes their differentiation.[16–18]

De Rötth reported its use in ophthalmology for the first time in 1940, in symblepharon surgery, with partial success. [19] Then it was almost forgotten until 1995 when Tseng et al. began publishing their laboratory and clinical experience.[20] It has been used for persistent corneal epithelial defects,[21] leaking filtering blebs,[22] pterygium surgery,[23,24] symblepharon correction,[25] wide bulbar conjunctiva resections,[25] as a conjunctival flap substitute, and for the reconstruction of the diffusely compromised ocular surface with or without limbal conjunctival graft[26–28] with good results except in some cases.[23]

However, all of these new publications use preserved amnios (P-AMT), which is not available in all countries, is expensive, and therefore poses special difficulties for developing countries. Even more, controversy exists regarding the biochemical vitality of the epithelial monolayer after the chemical process and the freezing and unfreezing cycles. We have been using NP-AMT for more than a year, with similar results to those published by other authors who are using P-AMT.

PATIENTS AND METHODS

NP-AMT is obtained from a woman undergoing an elective cesarean who has given consent. She has previously—one week before—been tested seronegative for hepatitis B and C virus, syphilis, and HIV. Under sterile conditions the placenta, with all of its attached membranes, is profusely irrigated with saline solution to remove as much blood as possible. Then, a section of the membranes is obtained with scissors and the amnios is separated by blunt dissection from the chorion. This amniotic membrane is once again profusely irrigated and is aseptically stored in a vial with saline solution. No antibiotics or other substances are used for its storage. It is kept in the refrigerator without freezing and is used within 24 hours.

Ours is a heterogeneous group of patients whose demographic and clinical characteristics can be seen on Table 1.

We placed the NP-AMT epithelial side up in all cases, trimmed to cover the specific defect created on the ocular surface, used interrupted Nylon 10-0 sutures (Alcon Surgical Inc, Fort Worth, TX, U.S.A.) in all our patients, and at the end of surgery we gave the patient Cefazolin 100 mg plus Dexamethasone 4 mg subconjunctivally and Maxitrol ointment (Neomycin sulfate, Polymixin B Sulfate, and Dexamethasone; Alcon Laboratories Inc, Fort Worth, TX, U.S.A.). After surgery, all patients received artificial tears hourly while awake and Maxitrol (Alcon Laboratories Inc, Fort Worth, TX, U.S.A.) eyedrops three times a day, and then were tapered off in three weeks. Sutures were removed at two weeks.

RESULTS

The patients' cases evolved very favorably. All of them have had quiet eyes since the first postoperative day, notwithstanding

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TABLE 1. *Demographic and clinical data*

Patient, age, sex	Underlying cause, eye	Surgery	Outcome	Complications	Follow-up (mo)
1, 68, M	Noninvasive Conj. Squamous cell carcinoma, LE	Resection + 300° NP-AMT + 100° contralateral limbal autograft	Success. Epithelialization in 3 wk	None	3
2, 63, F	PED secondary to OCP, LE	Deepithelialization + 25 mm^2 NP-AMT sutured over PED	Success. Epithelialization in 3 wk	None	9
3, 31, M	Bilateral alkali burn, more severe RE	360° peritomy + 360° NP-AMT donut shaped + 90° contralateral limbal autograft	Partial success. Incipient inferior corneal conjunctivalization at last visit	Inferior limbal autograft ischemia, with incomplete epithelialization	3
4, 66, F	Near-total limbal deficiency secondary to multiple surgeries, LE	Superior and inferior 140° NP-AMT each. Concomitant 50° contralateral limbal autograft over each NP-AMT graft	Success	None	6
5, 55, F	OCP	Corneal deconjunctivalization + 360° donut shaped NP-AMT + 2 limbal allografts 100° each	Success	None	5

LE, left eye; RE, right eye.

some very large surgeries. There were no cases of NP-AMT loss, necrosis, or infection.

In patient one (Fig. 1), epithelialization was complete at six weeks. There has been no recurrence of the tumor.

In patient two (Fig. 2), there was a gradual replacement of the NP-AMT, with epithelialization being complete at three weeks. There has been no recurrence of the defect ever since. She is now under systemic chemotherapy for her ocular cicatricial pemphigoid (OCP).

Patient three began to epithelialize very early and rapidly, but developed ischemia on the inferior limbal autograft, which delayed the epithelialization more than three months. However, he did not develop any corneal edema, photophobia, or infection. At three months there was incipient conjunctivalization of the inferotemporal limbus; unfortunately the patient did not return for follow up. His eye was incredibly quiet, he was comfortable, and his last visit uncorrected visual acuity was 20/30.

Patient four had an adequate lateral growth of the limbal autograft on the NP-AMT, developing a healthy limbus, and three months later received an uneventful penetrating keratoplasty.

Patient five has been taking Oral Cyclosporine A (Novartis, S.A., Basilea, Switzerland) since the limbal allograft was made on the NP-AMT. She has a healthy limbus, with no signs of allograft rejection and is pending a penetrating keratoplasty.

DISCUSSION

Management of patients with severe ocular surface disease has always been a problem for the ophthalmologist. Ocular surface reconstruction techniques have advanced considerably during the last years, moving away from bare sclera techniques, trough free conjunctival autograft, oral and nasal mucosal grafts, and—the more potent and physiologic weapon—the limbal autograft first proposed by Dr. José I. Barraquer.[29] However, there are cases that cannot be solved with the mentioned techniques, and their prognosis is dismal. It is in these complicated cases where the amniotic membrane transplantation has proven to be helpful.

The amniotic membrane has an avascular stromal matrix and a thick basement membrane composed of collagen type IV and V and laminin.[1–3] The basement membrane reinforces the adhesion of the basal epithelial cells,[12,13] facilitates epithelial migration,[11] and prevents epithelial and fibroblast apoptosis.[14,15] All of these properties explain its usefulness in the reconstruction of the ocular surface, without ever being vascularized or conjunctivalized, but by serving as a scaffold for the host's epithelium. Also, thanks to its property of not expressing human leucocyte antigens, it is well tolerated and does not cause any rejection reaction in the host.[5,6]

The only difference we noted when using NP-AMT is that it is initially thicker than the P-AMT—which is no disadvantage during

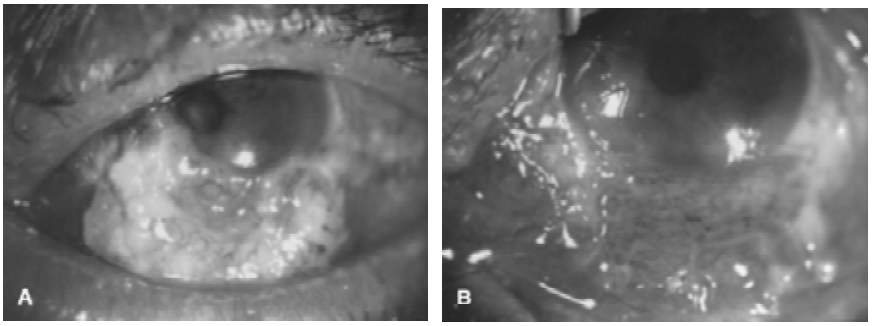

FIG. 1. Case 1. **(A)** Preoperative picture. Large Conjunctival mass encompassing the limbus from 2 to 11 clockwise. **(B)** Two weeks postoperative.

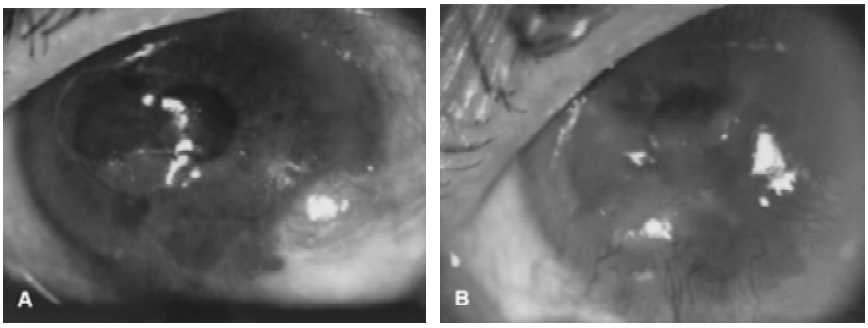

FIG. 2. Case 2. **(A)** Preoperative Picture, after six weeks of medical treatment. Note persistent epithelial defect of approximately 20 mm^2. **(B)** Four weeks postoperative. Epithelial defect is healed and stable; NP-AMT, not visible anymore.

surgery because eases its manipulation and orientation—but after approximately five days it appears to be identical. We have not had a single case of infection, necrosis, or NP-AMT graft loss. We do not think infection is a serious risk when using NP-AMT considering the aseptic manipulation of the tissue at all times and its antibacterial properties.[8] We think testing donors one week before the elective cesarean section for HIV, hepatitis, and syphilis is safe enough to almost eliminate the risk of infection by these agents; however, the possibility exists—albeit small—that the donor might get infected during that last week, and recipients must be informed about this.

It is worth noting how quiet these eyes looked since the first postoperative day, notwithstanding that some of these corneas persisted deepithelialized for several weeks without edema, loss of visual acuity, or even photophobia.

The vitality and lateral expansion of the limbal grafts over the amniotic membrane is very satisfactory. We have seen very small grafts able to populate 360° of a limbus without difficulties. This may reduce the need for limbal allografts in the future and their inconvenient need for lifetime immunosuppression.

Our results are similar to those published by other authors who use P-AMT. Procurement costs are minimal, availability is enormous, and it is very safe.

In the future, it will be interesting to compare NP-AMT with P-AMT because the hypothesis exists that the former might be more useful for acutely inflamed cases, since it does not go trough a process of preservation and freezing/unfreezing which may be deleterious for the biochemical function—cytokines in particular—of the epithelium.

We think the use of nonpresevered human amniotic membrane is a safe and sound technique for the reconstruction of the ocular surface, and may be advantageous over the preserved one in acutely inflamed eyes.

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