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# Letter to the Editor

## Amniotic membrane transplantation in a perforated corneal graft: clinical and histopathological findings

Mario Nubile, Paolo Carpineto, Tatiana Liberali, Paola Barile, Manuela Lanzini and Leonardo Mastropasqua

Department of Medicine and Ageing Science, Ophthalmic Clinic, University of Chieti-Pescara, Chieti-Pescara, Italy

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Editor,

Amniotic membrane transplantation (AMT) has been successfully reported in patients with corneal perforations (Prabhasawat et al. 2001; Solomon et al. 2002) in which a prompt intervention to seal the defect and restore the anterior chamber is the main strategy for preserving ocular integrity and preventing infections. Amniotic membrane transplantation is thought to take effect by promoting epithelialization and reducing inflammation and by the ability of the amniotic membrane (AM) to act as a tissue substrate replacement. Theoretically, the successful outcome of multi-layer AMT using AM filling placed inside the stromal defect relies on the integration and epithelialization of the amniotic graft, which allow it to act as a substitute for collagen and to supply the stromal layers (Solomon et al. 2002). We describe the unusual clinical and histopathological findings in a case of corneal perforation after penetrating keratoplasty (PK) treated with multi-layer AMT.

A 47-year-old patient diagnosed with acute corneal graft perforation, which occurred 4 years after PK for post-herpetic leucoma, presented with massive stromal melting with epithelial defect in the central and inferior cornea and a descemetocele with a single perforation, 1 mm in size, athalamia and aqueous leakage (Fig. 1A). Cultures from corneal scraping ruled out microbial infection. On a clinical basis the diagnosis of necrotizing stromal HSV (herpes simplex virus) keratitis was presumed, in agreement with previously published criteria (Heiligenhaus et al. 2003). On the same day, cryopreserved multi-layer AMT was performed to seal the perforation and protect the exposed corneal stroma from progressive melting, according to a previously described technique (Nubile et al. 2007). The base of the ulcer was cleaned off the necrotic tissue, and properly sized small pieces of AM arranged in two layers with the epithelial side up, left unsutured, were used to cover the bottom of the perforation site and the stromal melting area, respectively. One additional AM layer was used as a graft to cover the de-epithelialized area and was sutured to the peripheral healthy cornea. The following day, slit-lamp examination revealed that the internal pieces of AM were displaced superiorly to the perforation site, whereas the external sutured AM graft was adherent (Fig. 1B). However, the cessation of the aqueous leakage and the formation of the anterior chamber ensured the therapeutic success of the surgery. Ultrasound biomicroscopic examination (UBM) performed 1 week after AMT showed a thin layer of amniotic tissue adherent to the corneal surface covering and sealing the perforation (Fig. 1C). Over the following 2 weeks, a significant reduction in inflammation and a stable, deep anterior chamber were noted. Three weeks after the primary AMT surgery, an elective PK was performed without any intraoperative complications. The removed corneal button was histologically evaluated by light microscopy. Healing and the clinical outcome up to 1 year after the graft were uneventful (Fig. 1D).

Histology revealed that the AM tissues were partially dissolved, but a thin layer, composed of the basement AM and a few residual amniotic stromal fibrils was found to be adherent to the corneal surface, acting as a 'bridge' of single-layer amniotic graft

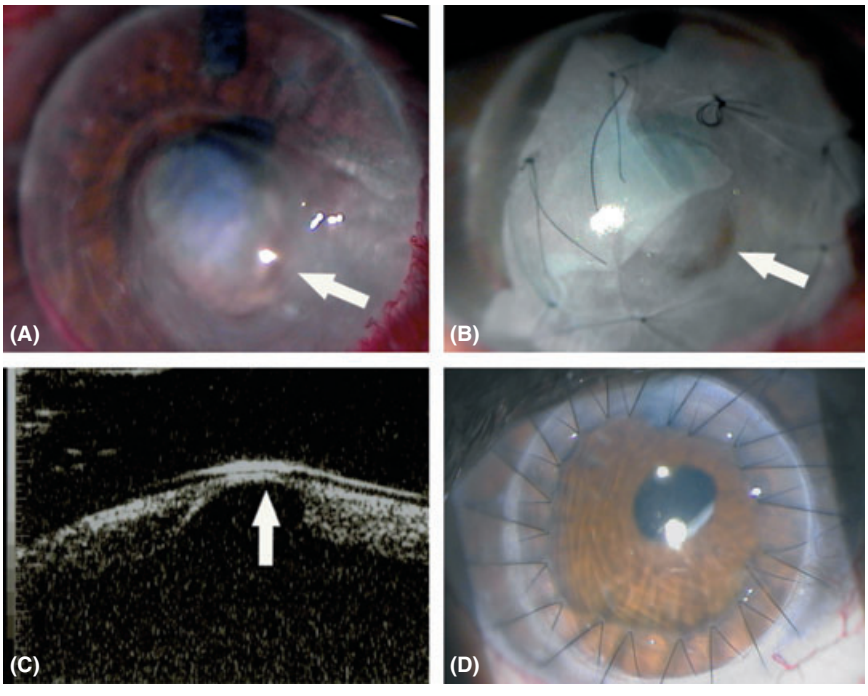


**Fig. 1.** (A) Slit-lamp photograph of the patient's right eye affected by necrotizing stromal HSV keratitis. The anterior chamber was flat, the corneal graft was massively infiltrated, and a large descemetocele with a perforated area (arrow) was present. (B) One day after multi-layer amniotic membrane transplantation, biomicroscopy showed that the internal amniotic layers were dislocated towards the centre of the cornea, but the external amniotic membrane was adherent to the corneal surface and effective in sealing the perforation (arrow), as evidenced by the restoration of the anterior chamber. (C) Ultrasound biomicroscopy performed 1 week after surgery showed a 1-mm, full-thickness perforation covered by a thin superficial bridge of tissue (arrow). The contact lens profile on the corneal surface is visible. (D) Slit-lamp photograph of the same eye 6 months after penetrating keratoplasty showing normal healing.

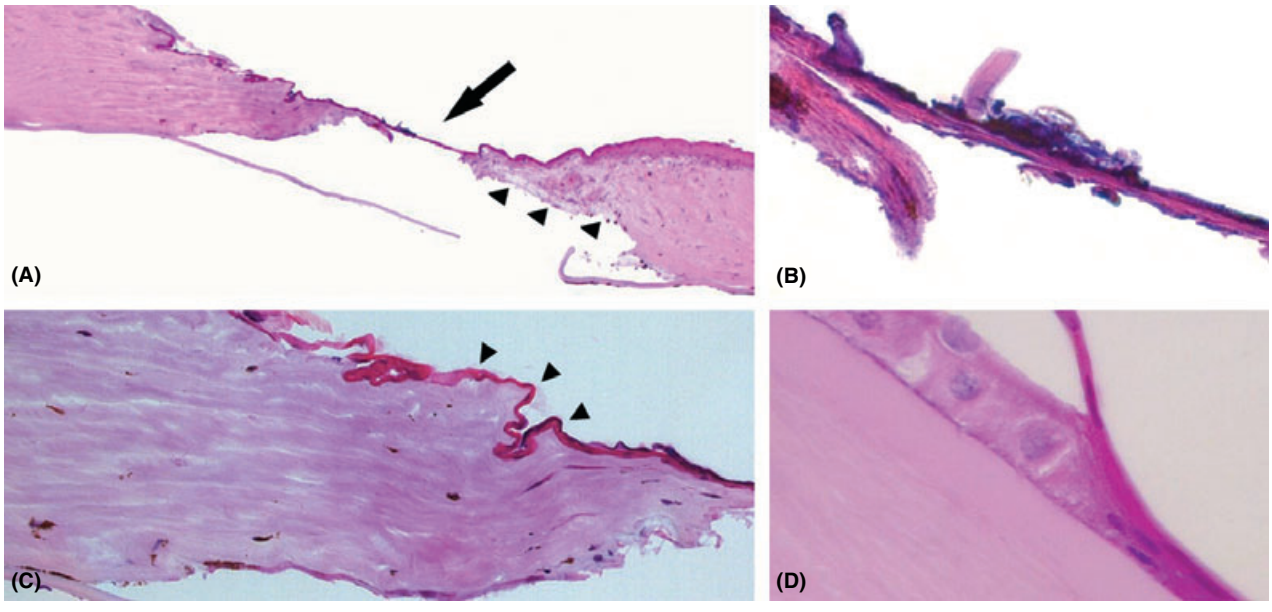


**Fig. 2.** Microphotographs of the removed corneal button after amniotic membrane transplantation at the time of penetrating keratoplasty (haematoxylin and eosin stains on frozen sections). (A) Panoramic view: the perforated corneal tissue is covered and sealed by a thin bridging layer of amniotic tissue found adherent to the corneal surface (arrow). Regenerating corneal epithelium was observed only adjacent to the inferior margin of the perforation (right side of the image). Few inflammatory cells infiltrating the corneal stroma were visible at that side (arrowheads). Descemet's membrane and endothelium were detached in the pathological area. (Original magnification × 50.) (B) Microphotograph showing the thin layer of mostly degraded amniotic tissue that covered the stromal defect. The amniotic epithelium was absent and few panoptic cells were visible. (Original magnification × 400.) (C) Superior margin of the perforation. A thin layer of amniotic tissue was found tightly adherent to the corneal surface, which was devoid of epithelium and Bowman's membrane. (Original magnification × 200.) (D) Microphotograph showing an immature regenerating single-layer corneal epithelium, migrating under the residual amniotic tissue. (Original magnification × 400).

tissue and sealing the perforation site (Fig. 2A, B). This AM bridge extended peripherally to the perforation, attached to the corneal surface which was devoid of corneal epithelium at the superior and lateral margin of the perforated area (Fig. 2C). Conversely, a regenerating epithelium, migrating under the AM, was found in the perilimbal cornea, inferiorly to the perforation (Fig. 2D).

This case report deserves particular interest because it shows that although the inner amniotic grafts failed to integrate into the stromal defect, the outer AM graft alone was capable of sealing the cornea and thus supporting the formation of a stable anterior chamber, despite the large size of the perforation. This finding was confirmed by UBM, which is not affected by the opacity of the membranes. The AMT outcome appeared stable as long as 3 weeks after surgery, when an elective PK was performed. Histology of the corneal button confirmed the absence of integration of the AM internal layers into the stromal defect, but nevertheless revealed that the outer AM single-layer graft was adherent to the corneal surface and effectively sealed the perforation. Surprisingly, this AM layer was only a few microns thick and was composed mainly of the AM basement membrane and minimally of AM stromal fibrils, indicating that the amniotic epithelium and stroma were almost entirely degraded at the time of PK. Bowman's membrane was absent throughout the majority of the cornea surrounding the stromal defect, and thus the AM adhered directly to the corneal stroma.

On the basis of these findings, and according to a previous histopathological study investigating the integration patterns of AM into the human cornea (Resch et al. 2006), we believe we observed a superficial localization (disintegration pattern) of the outer AMT. In such an event, the interaction between the amniotic and corneal tissue is not as strong as in 'intrastromal' and 'subepithelial' integration patterns of the AM, which provide a more stable tectonic support in a perforated eye. However, this case report demonstrates that a thin layer of mostly degraded AM, arranged into a superficial localization, can still achieve the sealing of a corneal perforation and restore the anterior chamber, while exerting favourable effects on tissue inflammation.

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*Correspondence:*
Mario Nubile MD
Via Valleparo, 8
66010 Torrevecchia T (CH)
Italy
Tel: +39 0871 358410
Fax: +39 0871 358794
Email: m.nubile@unich.it