Use of a Hyperdried Cross-Linked Amniotic Membrane as Initial Therapy for Corneal Perforations

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Abstract

Purpose: To report the use of hyperdried cross-linked (HDCL) amniotic membrane (AM) patching with tissue adhesive as an initial therapy for corneal perforations.

Methods: Cryopreserved AM was cross-linked with 0.1% glutaraldehyde and then dried using far-infrared rays and microwaves (hyperdry method). Three eyes of three patients with corneal perforations of up to 3 mm in diameter were included in this study. They were treated with a single-layer patch of HDCL-AM applied with a tissue adhesive (2-octyl-cyanoacrylate). We also evaluated the resistance of HDCL-AM to collagenases during in vitro digestion testing.

Results: In all three cases, the corneal perforations were repaired within 28 days (range, 17–28 days). No recurrence occurred during the follow-up period (3–6 months). In the collagenase digestion testing, the HDCL-AM did not dissolve until 48 h, whereas the cryopreserved AM completely dissolved within 60 min.

Conclusions: Three cases of corneal perforations were successfully managed using HDCL-AM patching with tissue adhesive. The HDCL-AM was resistant to collagenases during in vitro digestion testing. The HDCL-AM was a useful substrate for corneal perforations. This simple surgical technique may be one of the initial therapeutic options for corneal perforations.

Keywords: corneal perforation, cross-linked, glutaraldehyde, hyperdried amniotic membrane

Introduction

Corneal perforations are emergent ocular conditions, resulting in vision-threatening bacterial endophthalmitis. Several techniques to repair corneal perforations using cryopreserved amniotic membrane (AM) have been reported.1–3 AM transplantation onto the ocular surface is performed using a graft or patch.4 When a graft is used, the grafted AM is incorporated into the ocular tissues, and when a patch is used, the patched AM gradually dissolves and falls off. We have often noted that the patched AM dissolves and falls off earlier than expected, thus thwarting a positive outcome. It has been reported that ocular collagenolytic enzymes play important roles in the pathogenesis of corneal ulceration and that those enzymes exist in the aqueous humor.5–8 We postulate that a patched cryopreserved AM may be dissolved by those enzymatic digestion processes. A crucial problem in conventional AM patching appears to be the limited durability of the AM against those ocular enzymes. Spoerl et al.9 reported that AM cross-linked by glutaraldehyde is a useful material for ocular surface reconstruction, and that the cross-linked AM is not dissolved by ocular enzymatic digestion for several months. Toda et al.10 recently reported on a new type of dried AM prepared by hyperdrying.
We recently reported on the efficacy of the HD-AM patch in combination with use of a tissue adhesive in treating corneal perforations or bleb leaks. In the present study, we developed a new HDCL-AM by combining the methods of Spoerl et al.9 and Toda et al.10 to improve both the durability and the convenience of AM in ophthalmic clinical applications. We applied this HDCL-AM patching to cases of corneal perforations and also evaluated the resistance of HDCL-AM to collagenase digestion by performing in vitro digestion testing.

Patients and Methods

Three eyes of three patients with corneal perforation were included in this study. All surgical procedures were performed at Toyama University Hospital between 1 January 2008 and 31 January 2009, after the patients were informed of the risks and alternative treatments. Written informed consent for the treatment was obtained from each patient before the surgical procedure.

Preparation of HDCL-AM

Human AM was obtained from seronegative donors undergoing cesarean sections at Toyama University Hospital. Informed consent for treatment and research was obtained from the donors. The AM was washed with sterile phosphate-buffered saline (PBS). According to the method described by Spoerl et al.,9 the AM was cross-linked with 0.1% glutaraldehyde solution. The cross-linked AMs were washed thoroughly with saline and dried with a hyperdrying device (Sakura, Nagano, Japan). During the drying process, the temperature within the hyperdrying device was controlled within the range of 5–35°C under continuous vacuum conditions. The HDCL-AM was cut into 5-cm squares, packaged, and then sterilized by 25 kGy of γ-ray irradiation (Fig. 1A, B).

Collagenase Digestion Testing

Eight-millimeter discs of cryopreserved AM, HD-AM, and HDCL-AM were prepared and incubated with a 0.1% collagenase A solution (Lot. 032-10534, 150 U/mg; Wako Pure Chemical Industries, Osaka, Japan) in PBS (pH 7.5) (Wako Pure Chemical Industries) at 35°C for 72 h under static conditions. After three washes in PBS, the discs were fixed in 4% formalin and embedded in paraffin. Four-micrometer-thick paraffin sections were stained with hematoxylin and eosin and observed by light microscopy (Olympus AX80; Olympus, Tokyo, Japan).

Surgical Procedure

All three patients received a single-layer patch of HDCL-AM applied using a tissue adhesive in the minor procedure room under topical anesthesia with 2% lidocaine. The HDCL-AM was trimmed to the appropriate size and shape before application. The tissue adhesive, 2-octyl cyanoacrylate, was applied to the epithelial side of the HDCL-AM. After the perforated site was dried with a cellulose sponge, forceps were used to place the HDCL-AM with the tissue adhesive was placed over the lesion. Topical 0.5% levofloxacin was instilled, and a hydrogel contact lens installed as a bandage.

Results

Collagenase Digestion Testing

The discs of both the cryopreserved AM and the HD-AM were almost completely dissolved within 60 min in the 0.1% collagenase A solution, but the morphologic appearance of the HDCL-AM remained unaltered for 48 h. On histologic sections of HDCL-AM exposed to collagenases, the layer structure of the HDCL-AM was maintained completely for 6 h, and the basal portion of the HDCL-AM was not dissolved until 72 h of digestion (Fig. 2). These findings are representative of those of the four discs (original magnification, ×400).

Clinical Outcome

Clinical data and outcomes in the three patients are summarized in Table 1. A single-layer HDCL-AM patch was used to treat each of the three patients, and reapplication of the AM patch during the follow-up was not needed. Management of the corneal perforations in all three cases proceeded as expected. The patient's final postoperative visual acuity was improved in cases 1 and 2. The healing times for repair of the corneal perforations were 17, 21, and 28 days in cases 1, 2, and 3, respectively. In case 1, the perforated site was repaired with faintly hazy tissue of normal thickness (Fig. 3A, B). In case 2, the perforated site was repaired with opaque tissue of normal thickness. In case 3, the perforated site was repaired with thin, almost clear tissue. In all three cases, corneal re-epithelialization was observed over the repaired areas.

Case Example: Case 3

A 76-year-old woman was referred to our clinic because of a corneal perforation in her right eye. She had undergone two failed trabeculectomies for secondary glaucoma due to uveitis in the right eye at another clinic over the past 15 years. Her right visual acuity had been no light perception for several years owing to optic atrophy. On examination, a large corneal perforation (3 mm in diameter) with aqueous leakage was found to have developed at the center of the cornea (Fig. 4A). The anterior chamber had disappeared, and a dense, whitish cataract was seen through the perforation site. The iris was not prolapsed. The patient immedi-