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Corneal reinforcement using an acellular dermal matrix for an analysis of biocompatibility, mechanical properties, and transparency

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ABSTRACT

The aim of this study was to analyze the viability of using an acellular dermal matrix (ADM) as a reinforcement material for peripheral corneal thinning disease. The complete removal of cell components was confirmed by hematoxylin and eosin (H&E) and 4′,6-diamidino-2-phenylindole (DAPI) staining. Transmission electron microscopy determined that the stromal structure was well preserved. Uniaxial tests revealed that the ADM had strong mechanical properties. After being implanted into rabbit cornea the ADM showed no sign of rejection and even achieved good transparency 24 weeks post-surgery. H&E staining demonstrated that keratocytes grew in the ADM and the ADM–cornea interface became blurry. Picrosirius red staining revealed great changes of collagen in the ADM. Uniaxial testing of the reinforced cornea showed better mechanical strength than the normal rabbit cornea, but this did not exhibit statistical significance. These results suggest that ADM is a worthy candidate for future exploration as a reinforcement material for peripheral corneal thinning problems.

1. Introduction

The cornea, the most important part of the outer ocular tunic, is a well-differentiated connective tissue mostly composed of extracellular matrix [1]. It serves as a physical barrier between the eyeball and the external environment, as well as being the primary refractive element in the optical pathway [2]. Most characteristics of the cornea, including its physical strength, stability of shape, and transparency, are largely attributed to the ultrastructure of collagen fibrils in the corneal stroma, which represents the largest portion, more than 70%, of the cornea's dry weight [3]. The normal human corneal stroma is rich in type I collagen, and contains relatively large proportions of type V and VI collagens [4,5]. Type III collagen is present in low proportions but increases during wound healing, inflammation, and several pathological conditions [1]. The remarkable uniformity of fibril diameter and regularity of interfibrillar spacing are major determinants of corneal transparency [6,7]. The collagen fibril direction and orientation are closely related to corneal tensile strength, which provides protection against external trauma and maintains the corneal shape and curvature [8].

Progressive stromal thinning is the hallmark of several corneal ectatic disorders, such as keratoconus with central/para-central corneal thinning, as well as pellucid marginal degeneration and Terrien's marginal degeneration with peripheral corneal thinning [9,10]. A localized or extensively thinned stroma results in bulging of the corneal surface and leads to high against the rule astigmatism. The poorer mechanical properties puts the thinning cornea at risk of rupture and perforation. Treatment consists of contact lenses in the early stages and keratoplasty with human donor material for severe cases to restore vision. However, sometimes the grafts are large and close to the limbus because of the location of the thinning, making surgery technically more difficult and the graft more prone to rejection [11,12]. Other factors that limit the worldwide use of corneal transplantation include the shortage of donor tissue as well as the risk of infectious disease transmission [3,13].

There has been major progress in corneal tissue engineering over the past few years. The idea of scaffolding in tissue engineering has been the inspiration for an acellular collagenous frame that could be used for corneal reinforcement. Such a scaffold must demonstrate several critical features for potential utility in vivo, including mechanical integrity, biocompatibility, slow biodegradation, and transparency.

An acellular dermal matrix (ADM) is a commercially available tissue graft derived from donated human skin [14,15]. ADM has

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been widely used since 1992 for various reconstructive surgeries, such as in cases of severe burns, recurrent hernias, abdominal wounds, and oculoplastic applications [16–23]. Clinical experience has suggested that its use in regenerative medicine can potentially offer a lower infection rate and fewer adhesions, while maintaining sufficient tensile strength. ADM contains intact collagen fibers, elastin filaments, and glycosaminoglycans, but lacks cellular components after mechanical and chemical processing. Absence of the keratin and epidermal layers reduces the antigenicity and provides a hypocellular material suitable for grafting. The collagen structure of ADM shows a good ability to induce correct native cell ingrowth when applied to the skin. Additionally, the collagenous composition of the dermis is similar to that of the cornea, mainly type I with a small amount of type III, but the dermal fibers are much stronger and thicker [24–26]. Taking all these properties together, we hypothesized that ADM might be an ideal corneal reinforcement material, with good mechanical properties and a native cell-friendly environment. Thus, we assessed (1) the biocompatibility of ADM with the cornea, (2) the mechanical properties of an ADM-reinforced cornea, and (3) corneal transparency after ADM implantation.

2. Materials and methods

2.1. Materials and animals

Commercially available ADM was tailored into rectangular pieces, half of which were implanted into rabbit corneas, while the rest were used for histological analysis and mechanical property testing.

Two pieces of normal eyelid skin were obtained from two patients who came to our department for palpebral surgery and gave informed consent to the experimental use of the removed skin. Structure analysis of the eyelid skin samples were used for comparison with ADM.

Fifteen New Zealand white rabbits (Center for Experimental Animals of Peking University Health Science Center, Beijing, China), weighing 1.5–2.0 kg, were used for animal corneal implantation. All animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. All animal experiments were approved by the Medical Ethics Committee of Peking University Third Hospital.

2.2. Implantation and clinical observation

ADMs dehydrated in sterile glycerol were implanted into the right corneas of 15 New Zealand white rabbits to evaluate scaffold biocompatibility and transparency. Under general anesthesia, 3×6 mm corneal stromal pockets were made in the temporal peripheral area in the superior–inferior direction at a depth of half the corneal thickness. The ADMs were then implanted into the pockets with a pair of tweezers. No suture was applied to the incision. The non-operated, contralateral eye was used as a positive control. Sub-conjunctival injection of gentamicin was used on the operation day. Eye drops of tobramycin and dexamethasone were used over the first post-operative week. Follow-up clinical examinations included slit-lamp examination to assess corneal optical clarity, neovascularization, and signs of rejection.

2.3. Histology, electron microscopy, and immunofluorescent staining

ADMs, eyelid skin, operated corneas at different time points (post-operative and 1, 12, and 24 weeks, $n = 2$, respectively), and non-operated normal corneas were fixed in 4% formaldehyde, and embedded in paraffin, from which 4 mm sections were cut. Some of the specimens were stained with hematoxylin and eosin (H&E) and viewed under a light microscope. Others were stained with picrosirius red and observed under a polarizing microscope.

ADMs for transmission electron microscopy (TEM) were cut into 1×1 mm pieces, both transversely and vertically. The samples were treated using routine procedures [27], and were viewed with a transmission electron microscope (JEM-1400, JEOL, Japan).

4′,6-Diamidino-2-phenylindole (DAPI) staining was performed on frozen sections of ADMs, eyelid skin, and non-operated normal cornea using a previously reported method [28]. The samples were examined under a fluorescence microscope.

2.4. Mechanical properties

The maximum load, tensile strength, elastic modulus, and elongation at break of the ADMs, non-operated normal corneas, and reinforced corneas at post-operative week 24 ($n = 5$, respectively) were measured according to a previously reported method [29], using an Instron 3365 tester equipped with Origin 7.0 software. The specimens were kept wet using phosphate-buffered saline (PBS) and cut into 5×10 mm rectangular strips. Corneal strips were cut in the superior–inferior direction near the limbus. The cross-head speed was 10 mm min^{-1}. Statistical analysis was performed using an independent *t*-test for ADMs and normal corneas and a one-way analysis of variance (ANOVA) for ADMs, normal corneas, and reinforced corneas. The statistical significance was set at $p < 0.05$.

3. Results

3.1. Characterization of the ADM

3.1.1. Microscopy and histology

The ADMs were translucent on gross observation and became totally transparent after being soaked in 100% sterile glycerol for 10 min (Fig. 1A), which implied that it had good optical potential. No visible cellular components were observed in the ADM on H&E or DAPI staining (Fig. 1B). Eyelid skin and rabbit cornea were used as control groups, the H&E and DAPI staining of which showed normal cell distributions. The organization of collagen fibrils in the ADM was very compact, similar to the structure of the upper papillary dermis in the skin, and different from the deeper loose reticular layer. The collagen fibrillar spacing of ADM was rather narrow compared with that of the cornea. Additionally, TEM images (Fig. 1C) of longitudinal ADM sections demonstrated a regular arrangement of the collagen fibrils with no cells among them, while the transverse section showed good uniformity in terms of fibril diameter, as well as in interfibrillar spacing.

3.1.2. Mechanical properties

The maximum load of the ADM was 57.98 ± 22.36 N, higher than that of the rabbit cornea (14.19 ± 3.43 N, $n = 5$, $P = 0.003$) (Fig. 2A).The tensile strength of the ADM was 16.97 ± 6.66 MPa, which is much stronger than that of the normal rabbit cornea (3.83 ± 0.91 MPa, $n = 5$, $P = 0.002$). Finally, the elastic modulus of the ADM was 32.23 ± 14.43 MPa, which is also stronger than that of normal rabbit corneas (11.57 ± 3.05 MPa, $n = 5$, $P = 0.014$) (Fig. 2B and D). The elongation at break of ADM was 86.39 ± 17.04%, similar to that of native rabbit corneas (78.11 ± 26.11%, $n = 5$, $P = 0.572$) (Fig. 2C).