Novel Sutureless Keratoplasty with a Chemically Defined Bioadhesive

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Purpose. The purpose of this study was to evaluate sutureless keratoplasty using a chemically-defined bioadhesive (CDB) made from food or medical additives.

Methods. Sutureless automated lamellar therapeutic keratoplasty (ALTK) using a CDB was performed on three rabbit eyes. Allogenic lamellar graft was transplanted onto the recipient bed using either suture fixation or a sutureless technique using the CDB. Slit-lamp examination was performed at selected intervals to evaluate the grade of epithelialization and the corneal clarity. The rabbits were killed at 90 days after operation and the eyes processed for histology, electron microscopic examination, and immunohistochemistry for cytokeratins and cell junction-related proteins.

Results. Sutureless keratoplasty was successfully performed with appropriate handling time before the CDB gelatinized. All the glued grafts were rapidly epithelialized within 7 days, and thereafter remained clear and attached for 90 days. Histologic and ultrastructural findings on the sutureless group showed the normal feature of stromal and epithelial cells and the grafts to be closely adhered with no inflammatory or scarring changes on the interface. Immunohistochemistry of the epithelial cells on the sutureless group revealed a similar expression pattern to the control group.

Conclusions. These results demonstrate that sutureless keratoplasty using the CDB is easy to perform, with reliable attachment and no fear of toxic effects or disease transmissions. The authors expect the CDB to become a major choice for corneal treatment, especially in lamellar keratoplasty, posterior keratoplasty, and amniotic membrane transplantation on corneas. (*Invest Ophthalmol Vis Sci.* 2009;50:2679–2685) DOI: 10.1167/iovs.08-2944

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Penetrating keratoplasty (PK) is a commonly performed corneal transplantation procedure for treating corneal opacities or lesions, such as corneal dystrophies, scarring after corneal infection, bullous keratopathy, and keratoconus. Recently, a variety of keratoplasties have been developed with an aim toward targeting replacement of corneal tissue to retain maximal unaffected tissue to minimize surgical damage and immunologic response. These corneal surgeries typically need sutures for the secure attachment of the donor corneal graft to the recipient corneal bed. However, the use of suturing as the bonding method is the source of many complications. Sutures prolong operative time and incite additional inflammation and vascularization,[1] act as a nidus for infection,[2] and can induce unexpected astigmatism.[3] If a tissue adhesive could be used safely to attach the graft, it might shorten the procedure time, avoid the complications related to sutures, and reduce astigmatism compared with the suture method.

There has been a prolonged search for a bioadhesive that can be successfully used in corneal surgery. One of the early uses of bioadhesives in corneal surgery was in the management of corneal perforations using cyanoacrylate derivatives in the 1960s.[4] Cyanoacrylate derivatives are compounds with very high tensile strength and rapid polymerization. However, they can induce an inflammatory foreign body reaction, including neovascularization and tissue necrosis,[5,6] because they are synthetic and nonbiodegradable. Other disadvantages have also been reported related to their inflexibility, inability to be reabsorbed, and lack of transparency.[7] Thus, the cyanoacrylate derivatives are far from ideal adhesives for use on corneas. Fibrin adhesives have also been used in ophthalmology to treat corneal thinning and perforations,[5,8] ocular surface disorders,[9,10] and glaucoma.[11] Although they have a lower tensile strength and slower polymerization than cyanoacrylates, being biological and biodegradable, they may induce minimal inflammation. Most recently, fibrin-based adhesives have been used for corneas to perform sutureless lamellar keratoplasty[12,13] and "top hat" PK.[14] These methods using fibrin glue achieved successful outcomes; however, fibrin glue carries a small risk of infection from a contaminated donor pool. Although the risk is extremely low, patients should be informed of the risk before surgery.

Hence, many attempts have been made for developing new, less toxic, synthetic adhesives for corneas. Ideally, corneal adhesives should have adequate tensile strength to maintain corneal integrity, allow for sufficient working time before becoming totally hardened, not cause inflammation, be clear to permit vision, and eventually disappear to permit healing. If a nonbiological bioadhesive that fills these requirements can be successfully applied for the cornea, corneal surgeries will become very simple and easy to perform.

Materials and Methods

Preparation of Chemically Defined Bioadhesive (CDB)

The CDB was prepared as previously described.[15] The solution of 14 w/w% aldehyded dextran (molecular mass: 75 KDa) with aldehyde

introduced at the ratio of 0.43 per sugar unit and 7 w/w% ε-poly(L-lysine) (molecular mass: 4 KDa) containing 1.4 w/w% acetic anhydride was filled into each cylinder of a double-syringed container after filtration through a 0.2-μm pore size filter. The mixing tip was attached before use. Gel formation time was approximately 19 seconds at the temperature of 37°C, counted by the same method as in the previous report.[16] The CDB in this report was self-degradable at around 7 days at the temperature of 37°C in vitro.

Sutureless Keratoplasty Using CDB

As a model of sutureless keratoplasty, automated lamellar therapeutic keratoplasty (ALTK) was selected. All experiments in this study were performed in accordance with the Committee for Animal Research at Kyoto Prefectural University of Medicine and according to the ARVO statement on the Use of Animals in Ophthalmic and Vision Research. The rabbits were anesthetized with ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (5 mg/kg) injected intramuscularly. The ALTK was performed on two rabbits simultaneously by exchanging the extracted grafts between the two eyes of each rabbit. The corneal epithelium was mechanically scraped (Fig. 1A). A microkeratome (MK-2000L; Nidek, Aichi, Japan) was used to create a nasally hinged corneal flap approximately 120 micrometers thick in each eye (Fig. 1B). Hinges were cut with surgical scissors to make the free-flap donor corneal button. The corneal buttons extracted from each corneal surface were exchanged to be placed onto the ocular surface of the eye of the other rabbit. For the sutureless ALTK, the excessive fluid on the ocular surface was soaked up with a surgical sponge and subsequently the CDB was applied from the tip of the double-barreled syringe to the recipient bed (Fig. 1C). Immediately after the application, the allograft was transferred onto the graft-removed area in the proper position (Fig. 1D). The CDB extruded from the graft edge was sufficiently removed with a surgical sponge (Fig. 1E). For the sutured ALTK as a control, the allograft was sutured in place with eight interrupted 10-0 nylon sutures. Operations were performed on three rabbits for each group. A bandage soft contact lens was postoperatively placed on each eye, and left in place for 7 days. The operated eye was patched with a topical steroid/antibiotic ointment, and a topical steroid/antibiotic ointment was applied once daily for a week.

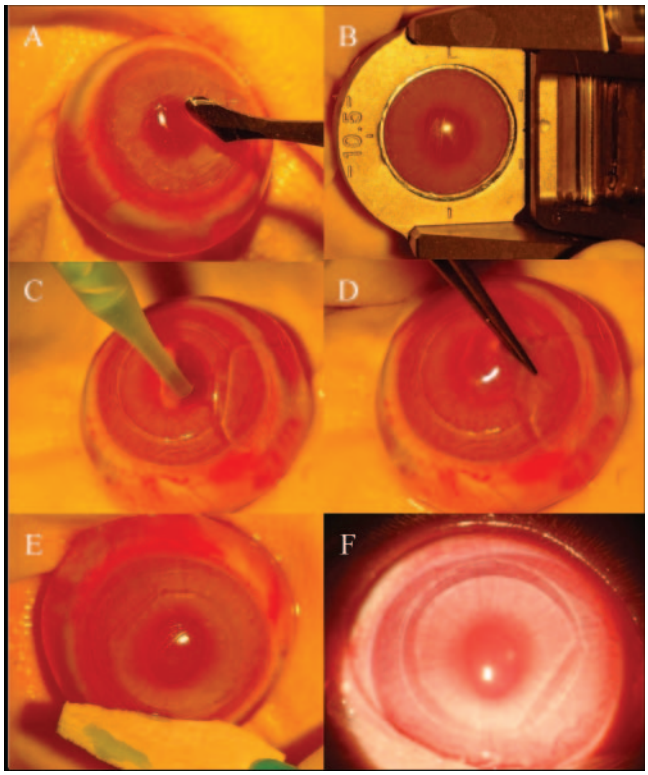

FIGURE 1. Surgical steps of sutureless ALTK using the CDB. The corneal epithelium was mechanically scraped with a blade (**A**). A microkeratome was used to create a nasally hinged corneal flap (**B**) and then the hinges were cut with surgical scissors to make the free-flap donor corneal button. The corneal buttons extracted from each corneal surface were exchanged to be placed onto the ocular surface of the eye of the other rabbit. The excessive fluid on the ocular surface was soaked up with a sponge and subsequently the CDB was applied from the tip of the double-barreled syringe to the entire area of the recipient corneal bed (**C**). Immediately after the application, the allograft was transferred onto the recipient corneal bed in the proper position (**D**). The CDB extruded from the graft edge was sufficiently removed with a sponge (**E**). The recipient eye at the end of transplantation (**F**).

Slit Lamp Microscopy and Histology

Slit lamp examination and photography were carried out at 0 (a few minutes), 2, 7, 14, and 90 days after transplantation to evaluate the attachment of the graft and corneal transparency and the status of epithelialization. At 90 days, the rabbit was euthanatized by the phleboclysis of 1 mL pentobarbital sodium, and the tissue was embedded in OCT compound (Tissue-Tek; Miles Inc., Elkhart, IN) and frozen with liquid nitrogen. Cryostat sections were stained with hematoxylin and eosin. To investigate the further biological effect of CDB on cells and surrounding tissues, collected tissues were ultrastructurally, and immunohistochemically examined on the 90-day samples.

Electron Microscopic Examination

The graft-host interface and the corneal epithelial cells migrated on the graft were examined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Samples were fixed in 2.5% glutaraldehyde in a phosphate buffered saline (PBS) buffer, dissected into small pieces, and washed three times in PBS for 15 minutes. Samples were post-fixed in 2% osmium tetroxide for 2 hours and washed again in PBS before being passed through a graded ethanol series. For TEM preparation, specimens were then transferred to propylene oxide twice for 20 minutes each time. They were placed in a solution containing 50% propylene oxide and 50% resin (Araldite; Agar Scientific Ltd., Stansted, UK) overnight, after which they were transferred to 100% resin and infiltrated overnight under agitation. The samples were embedded in molds containing fresh resin and polymerized at 60°C for 24 to 36 hours. Ultrathin sections (50- to 70-nm thick) were cut on a microtome (Reichert Ultracut E; Reichert-Jung, Nussloch, Germany) collected on naked copper grids and counterstained for 1 hour each with 1% vanadyl sulfate and phosphotungstic acid, and then for 15 minutes with Reynolds' lead citrate before examination on an transmission electron microscope (JEOL JEM 1010; JEOL UK Ltd., Welwyn Garden City, UK). For SEM preparation, samples were subsequently transferred to hexamethyldisilazane (HMDS) for 20 minutes and then air dried. They were then mounted on aluminum stubs and sputter coated with gold using a sputter coater (Edwards S150A; Edwards Ltd., Crawley, UK). Samples were then examined on a scanning electron microscope (JEOL JSM 5600; JEOL UK Ltd.).

Immunohistochemistry

Immunohistochemical studies were performed on both groups of the samples at 90 days after operation using our previously described method.[17] Briefly, semi-thin (8 μm) cryostat sections were obtained from unfixed tissue embedded in compound (Tissue-Tek OCT; Sakura Finetek, Torrance, CA). After fixing with cold acetone for 10 minutes, the sections were incubated with 1% bovine serum albumin for 30 minutes. Subsequently, the sections were incubated at room temperature (RT) for 1 hour with the primary antibody (Table 1) and washed three times in PBS containing 0.15% Triton X-100 phosphate buffered saline with tween (PBST, Wako, Osaka, Japan) for 10 minutes. In the negative controls, the primary antibody was replaced with the appropriate nonimmune IgG. The sections were then incubated at RT for 1 hour with appropriate secondary antibodies (Alexa Fluor 488 conju-

TABLE 1. Primary Antibodies and Sources Used in this Study

Specificity	Immunized	Dilution	Sources	Catalog Number
Cytokeratin 3	Mouse, mAb	×50	Progen	61807
Cytokeratin 4	Mouse, mAb	×200	Novocastra	NCL-CK4
Cytokeratin 12	Mouse, mAb	×200	Transgenic	KR074
Cytokeratin 13	Mouse, mAb	×200	Novocastra	NCL-CK13
ZO-1	Mouse, mAb	×200	Zymed	33-9100
Desmoplakin	Mouse, mAb	×1	Progen	651109
Collagen type VII	Mouse, mAb	×100	Chemicon	MAB1345
Integrin α6	Mouse, mAb	×200	Chemicon	CBL458

gated anti-mouse IgG antibody; Molecular Probes Inc., Eugene, OR). After several washings with PBS, the sections were coverslipped using anti-fading mounting medium containing propidium iodide (Vectashield; Vector, Burlingame, CA) and examined by confocal microscopy (TCS-SP2; Leica, Tokyo, Japan).

RESULTS

Sutureless Keratoplasty Using CDB

Sutureless keratoplasty using the CDB was successfully performed (Fig. 1). The appropriate amount of the CDB mixture was properly applied and spread over the entire area of the recipient corneal bed. Sufficient handling time was allowed to transfer the graft onto the recipient corneal bed and remove the extruded CDB with a sponge before the CDB was totally gelatinized. Once the CDB had gelatinized, the gel did not disintegrate even when a balanced saline solution (BSS) was poured onto the ocular surface.

Slit Lamp Microscopy

A few minutes after the sutureless ALTK, the graft had been securely attached without loosening or dislocation (Fig. 2A1). Fluorescein staining revealed that the total corneal epithelial defect that had covered nearly the entire cornea (except for the limbus) just after the operation had gradually diminished in size by postoperative day 2 and disappeared completely within 7 days (Figs. 2A2, 2A3). The slight corneal edema that was observed a few minutes after the operation was increased at 2 days under the epithelial defect, and gradually decreased during the time course, especially after total epithelialization (Figs. 2A2–2A5). The graft was fixed without loss or dislocation until the epithelialization and the epithelialized surface remained intact even at 90 days. Slit lamp microscopy showed that all the lamellar keratoplasty grafts and the recipient corneas remained clear, without excessive inflammatory changes. There was no scarring or epithelial ingrowth observed within the interface during the follow-up time (Figs. 2A1–2A5). No significant difference was detected between the sutureless and suture models as to corneal clarity, epithelialization, and inflammatory change (Figs. 2A1–2A5, 2B1–2B5). Both models exhibited no major complication, but suture-related discharges were prominent in the suture model (Figs. 2B3, 2B4). Sutures were removed at 30 days since the total epithelialization had been achieved.

Histologic and Morphologic Examination

Histologic examinations of the suture and sutureless models showed that the transplanted graft adapted securely to the recipient corneal bed without large gaps throughout the interface, and on the edge without any epithelial-cell ingrowth into the interface. Both models of the donor and host corneal stroma looked healthy with no apparent scarring or inflammatory change (Figs. 3A–D). Electron microscopic examination of the interface showed that the CDB in some regions appeared to have completely disappeared while minimal amount of the CDB was still visible in other regions (Fig. 4A). The stroma adjacent to the interface and the keratocytes nearby appeared normal on the sutureless model (Fig. 4B).

H-E staining of both samples showed that the regenerated epithelium had completely covered the graft surface and were well stratified and differentiated. There were no significant differences between the suture and sutureless models in regard to inflammatory reactions (Figs. 3A–D). SEM and TEM examination of the regenerated corneal epithelial cells on the graft showed them to be completely normal, with numerous desmosomal cell junctions between neighboring cells and frequent hemi-desmosomal contacts between the basal cells and the basement membrane (Figs. 5A–D). This finding was observed in whole corneal epithelium from the center to the periphery.

Characterization of the Corneal Epithelial Cells on the Lamellar Graft

The characterization of the migrated corneal epithelial cells on both groups of samples was compared at 90 days by immunohistochemistry for cytokeratins (CK; types 3, 4, 12, and 13) and cell junction-related proteins (ZO-1, desmoplakin, collagen VII, and integrin α6; Fig. 6). There was no significant difference between suture and sutureless models in the staining pattern of all the above-listed antibodies. The staining pattern was uniform from the center to the periphery of the epithelium on all the slides that we investigated.

DISCUSSION

In this study, we demonstrated that our CDB can be successfully applied to ALTK as a model of sutureless keratoplasty. The CDB maintained the bond between the graft and the recipient bed until the graft was re-epithelialized, and the totally epithelialized ocular surface lasted for at least 90 days without any additional inflammatory reactions or residual toxic effects being observed. Compared with fibrin glue, the main advantage of the CDB is the theoretical safety. In addition, several suitable properties associated with CDB is that it is stronger, easier to handle, and more flexible than fibrin glue.

Suture enables secure attachment between the graft and the recipient cornea. However, suture itself inflicts trauma to corneal tissues that can cause inflammation and neovascularization.[1] Moreover, high levels of astigmatism are frequently produced by corneal sutures.[3] The postoperative course of a keratoplasty may also be complicated by a cascade of suture-related problems such as suture loosening, sterile infiltrates, secondary infections, corneal ulcerations, wound dehiscence with spontaneous wound leakage, and allograft rejection.[2,18] Concerns about these problems last until the sutures are removed, and early suture removal may lead to wound dehiscence if the wound healing is incomplete. The suturing

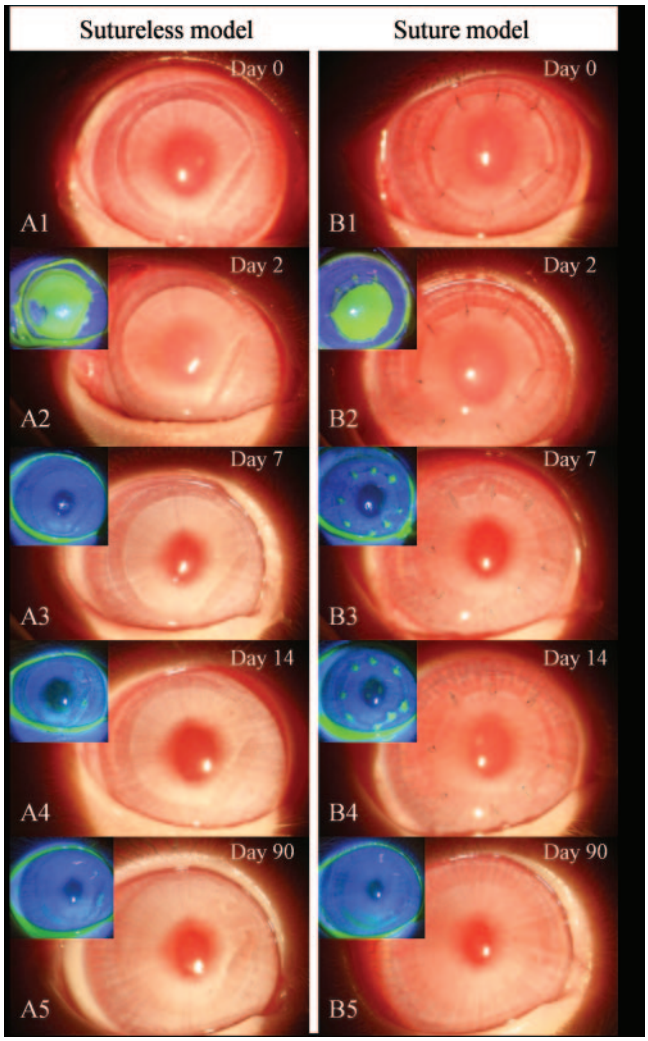

FIGURE 2. Representative slit lamp photographs of rabbit eyes taken 0 (a few minutes), 2, 7, 14, and 90 days after sutureless ALTK (**A1–A5**) and sutured ALTK (**B1–B5**). A few minutes after the sutureless ALTK, the graft was securely attached without loosening or dislocation (**A1**). Fluorescein staining of both models revealed that the total corneal epithelial defect but limbus just after the operations was gradually diminished in size at 2 days, eventually disappeared within 7 days (**A2**, **A3**, **B2**, **B3**). The slight corneal edema which was observed a few minutes after both operations was increased at 2 days under the epithelial defect, and gradually decreased during the time course, especially after total epithelialization (**A2–A5**, **B2–B5**). The graft was fixed without loss or dislocation until the epithelialization, and the epithelialized surface remained intact even at 90 days after both operations. Sutures on the suture model were removed at 30 days since the total epithelialization had been achieved. Slit lamp microscopy showed that all the lamellar keratoplasty grafts and the recipient corneas remained clear, without excessive inflammatory changes. There was no scarring or epithelial ingrowth observed within the interface of both groups during the follow-up time (**A1–A5**, **B1–B5**). No significant difference was detected between the sutureless and suture models as to corneal clarity, epithelialization, or inflammatory change (**A1–A5**, **B1–B5**). Both models exhibited no major complication, but suture-related discharges were prominent in the suture model (**B3**, **B4**).

method often entails prolonged surgical time and surgical skill, which is one of the hurdles for surgeons performing keratoplasties. However, the sutureless technique allows surgeons to perform keratoplasties more simply and quickly, with easier post-surgical management than the suturing method. Duarte et al.[13] recently showed that sutureless lamellar keratoplasty using fibrin glue could be easier and faster to perform than the suturing technique, and that it could provide additional support to prevent against dislodgement of the graft where the donor graft is edematous. Although there are some problems associated with the use of fibrin glue, sutureless keratoplasty itself has been reported to be a satisfactory procedure.

Regarding fibrin adhesives, several disadvantages make the fibrin glue less ideal for use and increase the need for developing new adhesives. First, the increasing concern about the transmission of disease has raised questions about using fibrin adhesives. Some viruses, such as parvovirus B19 (HPV B19), are particularly difficult to totally remove or inactivate, and transmission of HPV B19 has been reported after the use of commercially available fibrin glue in thoracic surgery.[19] There is a potential possibility to develop severe conditions such as transient aplastic crisis in patients with shortened red cell survival or fetal death after intrauterine infection. Prions or other unknown pathogens can also be included in commercially available human plasma, thus causing some diseases. Although the risks of contracting these diseases are extremely low, patients should be informed of these risks before surgery. Second, fibrin glue has relatively low strength and is also known to rapidly disintegrate when exposed to the tear film,[20] thus increasing the risk of postoperative graft loss. Third, mixing and preparing the ingredients of fibrin glue, which must be carried out in the operating room, requires a substantial amount of time and is somewhat complicated. The components of commercially available fibrin glues must be warmed in a water bath for approximately 20 minutes or more, and subsequently the four components must be mixed separately into two solutions, usually to be placed into the double-barreled mixing system syringes or to be applied separately.

Instead of fibrin adhesives, synthetic adhesives have been developed such as a biodendrimer,[21] a hydrogel adhesive,[22] a chondroitin sulfate aldehyde adhesive,[23] and a photopolymerized sealant composed of hyaluronic acid[24] for sealing corneal

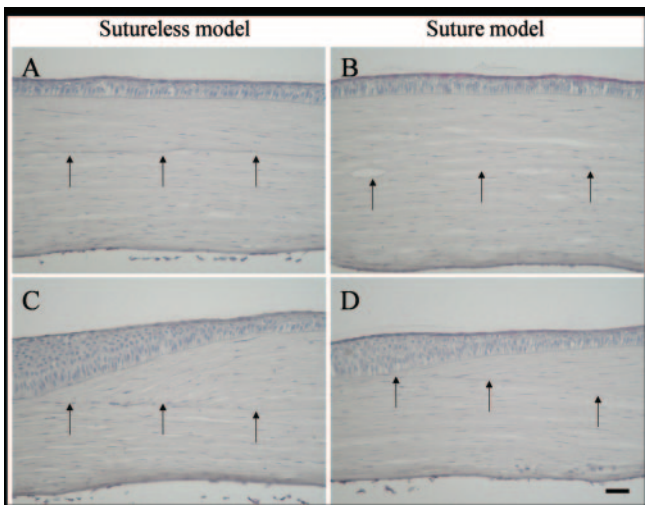

FIGURE 3. Histologic examinations of the 90-day samples of the sutureless (**A**, **C**) and suture models (**B**, **D**). The transplanted graft adapted securely to the recipient corneal bed without large gaps throughout the interface (**A–D**) and on the edge without any epithelial-cell ingrowth into the interface (**C**, **D**). Both models of the donor and host corneal stroma looked healthy with no apparent scarring or inflammatory change (**A–D**). The epithelial cells of both samples had completely covered the graft surface and were well stratified and differentiated (**A–D**). There were no significant differences between the suture and sutureless models in regard to inflammatory reactions. Scale bar: 50 μm. *Arrows*: the interface between the donor and host corneas.

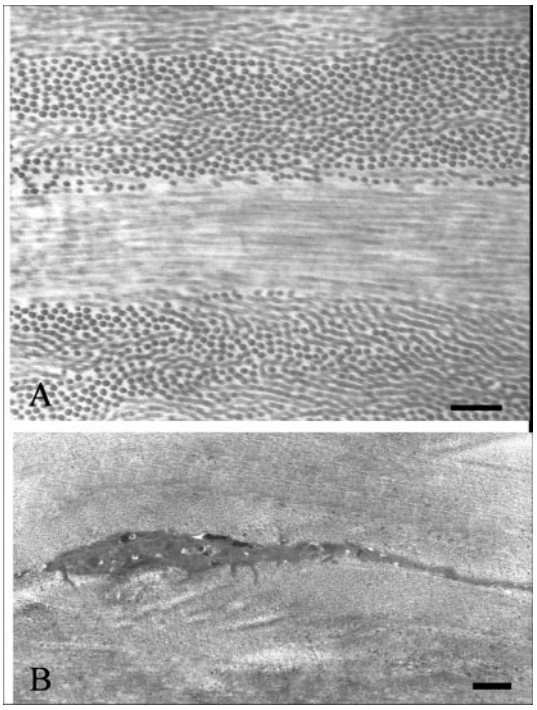

FIGURE 4. Electron microscopic examination on the corneas proximal to the interface between the host and donor corneas of the sutureless model. The interface in some regions showed close adherence between the graft and the recipient corneal bed without gaps or debris. The layer of CDB was almost undetectable and appeared to have completely disappeared (**A**). The stroma proximal to the interface between the host and donor cornea and the keratocytes nearby appear normal (**B**). Scale bar: (**A**) 1 μm, (**B**) 2 μm.

incisions. Although these adhesives can effectively close the corneal incision, they remain largely uninvestigated in regards to toxicity, biocompatibility, and biodegradability, and are not optimal for other ophthalmic surgical procedures.

Our CDB in this report is a synthetic adhesive that is made from dextran and ε-poly-(L-lysine), two kinds of food or medical additives, as starting materials. Dextran is a commercially available polysaccharide generally used as a plasma volume expander to reduce blood viscosity administered by intravenous infusion. It is also used as a thickening agent for foods. Cytotoxicity testing of aldehyded dextran using the mouse established cell line L929 has shown that the IC_{50} measurement (sample concentration in culture medium which suppresses the cell viability down to 50%) was 1000 times greater than those of formaldehyde and glutaraldehyde (1.7 μg/mL and 3.9 μg/mL, respectively), suggesting that aldehyded dextran has quite low toxicity. E-poly-(L-lysine) is commercially used as a food preservative with an antimicrobial effect against yeast, fungi, Gram-positive bacteria, and Gram-negative bacteria.[25] The mechanism of the inhibitory effect of ε-poly-(L-lysine) on microbial growth is the electrostatic adsorption to the cell surface of microorganisms on the basis of its poly-cationic property.[26] Although its influence on tissue is not fully understood, it was found to be nontoxic at high levels in acute animal studies and was not mutagenic in bacterial reversion assays to be categorized into "Generally Recognized As Safe" (GRAS) by the US Food and Drug Administration (FDA).[25]

Attention was focused on the bacteriostatic effect of not only one component of CDB, poly-L-lysine, but also the CDB mixture itself. Although this effect has not been fully investigated in vivo, our in vitro preliminary experiment has shown the bacteriostatic effect of the CDB against *Staphylococcus aureus* and *methicillin resistant Staphylococcus aureus* (MRSA) through colony forming assay (data not shown). If the CDB has bacteriostatic effect in vivo, that would be an additional advantage of the bioadhesive, especially for infectious tissue. Another advantage of the CDB is that its preparation is very simple and easy compared to that of fibrin glue. As soon as a mixing tip is attached, the CDB is ready to use. Moreover, the CDB in this report has proven to be sufficiently strong, safe, easy to use, and suitable for performing the lamellar attachment on corneas as previously shown for amniotic membrane transplantation on sclera.[15] Furthermore, its properties, such as being self-degradable, transparent, more flexible than fibrin glue, and possibly bacteriostatic, are especially ideal for corneal applications. To the best of our knowledge, this is the first attempt to apply this kind of chemically defined bioadhesive for corneas.

We successfully performed the sutureless keratoplasty with this adhesive on rabbit cornea, and we found that the manipulation time is sufficient to place the graft in the appropriate position. In situations where a surgeon may require additional time to manipulate the graft, they can choose from a variety of CDB compositions beforehand to adjust the appropriate gel formation time to the required handling time since the gelatinization time can be altered by aldehyde introduction in the dextran.[16] Thus, the CDB can be made suitable for every surgeon to perform keratoplasties. Although sutureless keratoplasty has been performed successfully, preliminary experiments have shown that surgeons should pay attention to minor

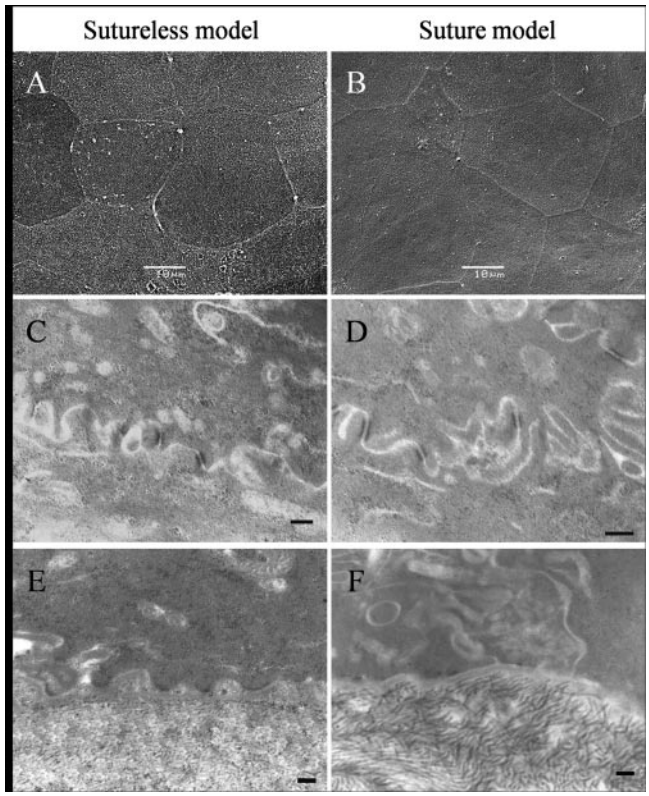

FIGURE 5. TEM and SEM micrographs of the migrated corneal epithelial cells on the sutureless model (**A**, **C**, **E**) and the suture model (**B**, **D**, **F**). The SEM micrograph of both models shows that on the surface, irregular polygonal cells look healthy with distinct junctions (**A**, **B**). In the middle layer of both models, the TEM micrograph shows numerous desmosomal cell junctions between neighboring cells (**C**, **D**). The TEM micrograph of both models shows that the basal epithelium is well attached to the basal lamina with numerous hemi-desmosomes (**E**, **F**). Scale bar: (**A**, **B**) 10 μm, (**C**–**F**) 200 nm.

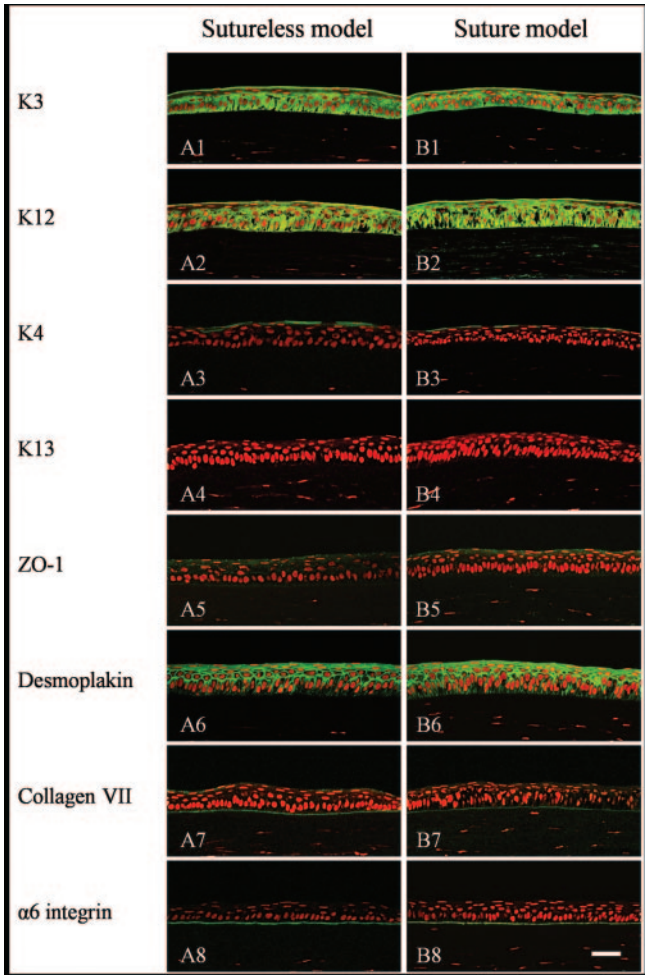

FIGURE 6. Representative immunohistochemical staining of CKs 3 (**A1**, **B1**), 12 (**A2**, **B2**), 4 (**A3**, **B3**), and 13 (**A4**, **B4**), and cell junction-related proteins ZO-1 (**A5**, **B5**), desmoplakin (**A6**, **B6**), collagen type VII (**A7**, **B7**), and integrin α6 (**A8**, **B8**) on the sutureless model (**A1–A8**) and sutured model (**B1–B8**). CKs 3 and 12, which are specific for corneal epithelium, were expressed in the whole layer of the epithelial cells on both groups (**A1–A2, B1–B2**). CKs 4 and 13, which are specific for conjunctival epithelial cells, were generally unexpressed on both corneal epithelial cells. The expression pattern of CK 4 on both groups is similar to normal with only the superficial expression (**A3**, **B3**). The expression pattern of cell junction-related proteins was also similar to normal on both models with only surface expression of cell junctions in ZO-1 (**A5**, **B5**), whole epithelial layer of cell membrane expression between adjacent cells in desmoplakin (**A6**, **B6**), and linear expression correspondent to the basement membrane in collagen VII (**A7**, **B7**) and integrin α6 (**A8**, **B8**). Scale bar: 50 μm.

technical difficulties associated with the application. First, the CDB does not act as basement membrane nor does it facilitate the epithelialization on the surface of it. Therefore, the excess CDB extruded from the interface should be removed as much as possible. Second, the excess fluid on the ocular surface tends to reduce the concentration of the CDB, resulting in prolonged gelation time and lower adhesive strength. Therefore, the interface should be dried with a sponge before application of the CDB. Paying strict attention to these technical matters significantly improves the success rate of sutureless keratoplasty.

For the application of bioadhesives, the main requirement is its bonding strength. Slit lamp microscopy, histology, and electron microscopy have all shown that the CDB provides sufficiently strong corneal lamellar adhesion. The firm attachment of the graft not only prevents its loss or dislocation, but can also inhibit epithelial-cell ingrowth into the interface, although control models also made good adhesion without epithelial-cell ingrowth.

It is important for the good prognosis and outcomes after sutureless keratoplasties that the adhesive is nontoxic and thus has little influence on the surrounding tissues. Histologic and ultrastructural findings around the interface revealed no apparent inflammatory cells or scarring and showed healthy keratocytes nearby, even though a minimal amount of CDB still remained at the interface. The toxic effect of adhesives can lead to enhanced postoperative inflammation on the cornea, thus causing slower epithelialization that increases the difficulty of postoperative management. However, the CDB used in this report facilitated the epithelialization after the sutureless ALTK as rapidly as that after ALTK with sutures. The corneal epithelial cells that migrated on the graft also appeared similar to normal on the histologic and electron microscopic examinations, even in the presence of CDB nearby. Moreover, they expressed CKs 3 and 12, which are specific for corneal epithelium, indicating that the CDB did not affect normal differentiation of the corneal epithelial cells. Expression of cell junction–related proteins was also similar to that observed in the controls, indicating that the corneal epithelial cells on the graft had formed efficient cell junctions. Collectively, these results suggest that the CDB has very little negative influence on surrounding tissues and is sufficiently safe for corneas.

For corneal application, especially for the lamellar technique, it is of critical importance to retain corneal transparency for good visual outcomes. Sutureless ALTK resulted in a high-enough degree of corneal clarity as to not affect visual acuity. Moreover, the flexibility of adhesives is required especially on the application for flexible tissues. The CDB in this report retained a sufficient amount of flexibility to be compatible with surrounding tissues.

Our investigations have shown that the CDB used in this study is sufficiently safe and useful for attaching corneas, especially when used for sutureless keratoplasty. Based on our results, we expect that this CDB will become a highly chosen adhesive for use in a variety of sutureless operations on the cornea, including anterior and posterior lamellar keratoplasty, amniotic membrane transplantation on corneas, and cultivated corneal or oral mucosal epithelium transplantation. This CDB has the potential for supplemental usage in femtosecond laser–assisted PK, yet the adhesive will weaken if it comes into contact with aqueous humor. Moreover, we expect that this CDB will be used to support corneal attachment and promote corneal stability after LASIK enhancement because insufficient corneal attachment can lead to a minor problem such as epithelial ingrowth under the LASIK flap.[27] We are currently in the process of investigating a variety of applications.

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