Epithelization of alloplants applied in eyelid and conjunctiva repair


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Purpose. To develop allografts providing an optimum bed for regeneration of conjunctiva epithelium.

Methods. Selection and processing of allografts were carried out under the Alloplant technology (E.R.Muldashev et al., 1978-1981). Two types of alloplant were selected for study purposes: the first - for eyelid repair, prepared from allogenic derma, and the second - for conjunctiva repair, prepared from allogenic fascia. The control conjunctiva repair was performed with a fresh allogenic fascia. The epithelization process was studied experimentally (72 rabbits) and clinically (46 patients). Histologic and biomicroscopic methods were used.

Results. The used biologic materials are collagen-proteoglycan complexes with high content of heparansulphate. The surface of the alloplant is coated by a structurally and functionally adequate epithelium soon after implantation (during 2 weeks). Two types of alloplant epithelization were observed: marginal growth and migration of epithelial cells onto free surface of the biomaterial. For fresh allografts is characteristic atypical epithelium.

Conclusion. The biologic material "Alloplant" for eyelid and conjunctiva repair due to its high content of heparansulphate creates favorable conditions for epithelium regeneration.

Human lamellar tendon graft in corneal surgery

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ABSTRACT

Background: Due to the lack of donor corneas or unsuitable conditions for keratoplasty because of preexisting conditions such as vascularization, infections, and multiple rejections, scientists have searched for new synthetic and biological materials that can temporarily or permanently substitute for corneal tissue.

Purpose: The purpose of this article is to show our personal experiences with the use of commercially available donor tendon tissue which has been chemically treated and was developed by Muldashev.

Methods: Ten eyes unsuitable for a corneal allograft underwent lamellar keratoplasty using tendon allografts. Due to differing pathologies various surgical procedures were used. The following period ranged from 6 to 18 months.
Results: Transparent or semitransparent corneas resulted in seven of the ten eyes. In the three remaining eyes, conditions were improved for future penetrating keratoplasty because there was a significant decrease in corneal vascularization. The donor tendon was not rejected in any case.


Therapeutic keratoplasty is a surgical procedure in which damaged corneal tissue is removed and replaced by autograft, isograft, or allograft donor tissue. In some cases or special situations, synthetic material or a xenograft can be used.

In some countries such as United States, where approximately 40,000 corneal transplants are performed yearly, and in tropical countries such as Brazil, where corneal diseases are the cause (18.1%) of blindness, the supply of available donor material is not keeping up with the demand. In some countries, this low supply is due to social-cultural and religious reasons, and the spread of diseases through viruses such as AIDS.

Continuous high demand for donor corneas has led to the development of alternative materials and tissues for use in corneal surgery.

MATERIALS

Two tissues were used on the patients in this study. One, "Homotransplant for Layer-by-Layer Keratoplasty", as the Russian named it (US Patent 4,348,374, May 24, 1983), is derived from donor tendon tissue. This material was developed by Professor Ernest R. Muldashev, from the University of Ufa, Russia, who did experimental studies with over 10 years. This author has been successfully using this tissue in lamellar keratoplasties on humans in the former Soviet Union and many other countries including Brazil. The second material used, "Homotransplant for Conjunctivoplasty", as the Russians call it, comes from the retroperitoneal fasciae of donor tendon tissue (US Patent 4,383,338, May 17, 1983). This tissue was only used on one patient.

The material was removed in pieces from the tendon of the musculus extensor digitorum, the musculus biceps brachi, the musculus palmaris longus in sections that measured 1.0 x 1.0 x 5 cm. The material was deep-frozen with the aid of ethyl-chloride and cut with a microtome between 50 to 300 mkm thick, with a diameter of 7 to 12 mm and irregular in form. Materials were kept in ampoules with 70% solution of ethyl-alcohol. After gamma sterilization, and testing for HIV, the material was stored at 00 to 300 C, for up to a period of 5 years.

PATIENTS

Ten eyes (one eye per patient), three male and seven female, varying in age from 6 to 80 years, and presenting different corneal pathologies, were submitted to typical keratoplasty using a trephine, 3.5 to 8 mm in diameter, and atypical or "free hand" technique, where the graft was cut according to the shape of the corneal receptor (Table 1). The thickness of all the grafts used was 100 mkm the depth of the receptor cornea varied according to the pathology, averaging 0.35 mm.

Table 1 shows preoperative diagnosis that all eye had corneal vascularization and loss of transparency from previous infections with a poor prognosis for penetrating keratoplasty.

Patients experienced photophobia, pain, and lacrimation. The follow up varied from 6 to 18 months.

SURGICAL TECHNIQUE

Eight of the ten eyes underwent peribulbar anesthesia (lidocaine 2% and bupivacaine 5%, 50:50). Patients II and VII (Table 1) had general anesthesia because of their age. The operations were performed using an operation microscope.

The purpose of the following procedures was to remove diseased epithelial, subepithelial, and corneal stroma. The surgeon must be cautious not to cause accidental perforations during surgery.

The first step of the operation was to prepare the recipient bed so that it was slightly deeper than the graft thickness and slightly smaller than the area of the graft. The procedure was done with either a disposable Hessburg-Barron trephine, Storz E 3094, or E 3100 keratoplasty trephine. The next step was to choose the predetermined graft size for "Homotransplant for Layer-by-Layer
Table 1. Clinical and Surgical Data in 10 Eyes Receiving a Lamellar Tendon Allograft

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (Yr)</th>
<th>Sex</th>
<th>Pathology</th>
<th>Type of Lamellar Tendon Graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>38</td>
<td>F</td>
<td>Pellucid marginal degeneration</td>
<td>7 x 5 mm atypical</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>M</td>
<td>Leukoma after recurrent herpes simplex</td>
<td>Central atypical 8 x 5 mm</td>
</tr>
<tr>
<td>III</td>
<td>80</td>
<td>F</td>
<td>Suppurative keratitis, peripheral vascularization</td>
<td>Typical 3.5 mm</td>
</tr>
<tr>
<td>IV</td>
<td>19</td>
<td>M</td>
<td>Leukoma after recurrent herpes simplex with vascularization</td>
<td>Central atypical 7.5 mm</td>
</tr>
<tr>
<td>V</td>
<td>43</td>
<td>F</td>
<td>Infectious ulcer, caused by contact lenses</td>
<td>Central 3 mm</td>
</tr>
<tr>
<td>VI</td>
<td>60</td>
<td>F</td>
<td>Unspecific degeneration, infection, deep vascularization</td>
<td>Ring-shaped 8 mm 3.5 central hole</td>
</tr>
<tr>
<td>VII</td>
<td>9</td>
<td>F</td>
<td>Chemical burn, deep vascularization, conjunctiva damage</td>
<td>Typical central 8 mm (first step)</td>
</tr>
<tr>
<td>VIII</td>
<td>32</td>
<td>M</td>
<td>Keratoconus with multiple rejections after penetrating keratoplasty, deep vascularization, edema</td>
<td>Typical 8 mm (first step)</td>
</tr>
<tr>
<td>IX</td>
<td>43</td>
<td>F</td>
<td>Central ulcerative keratitis followed by multiple rejections after penetrating keratoplasty, deep vascularization</td>
<td>Typical 8 mm (first step)</td>
</tr>
<tr>
<td>X</td>
<td>71</td>
<td>F</td>
<td>Neurotrophic keratitis, ulcerative keratitis, vascularization</td>
<td>Atypical 7 x 6 mm irregular</td>
</tr>
</tbody>
</table>

Keratoplasty”, remove it from the ampoule,, and immerse it in a 9% saline solution. The last step was to correctly place the selected implant and suture it with interrupted or continuous running suture 10-0 nylon that extended approximately 5 mm into the graft and longer in the recipient,, especially when the cornea was weak. All suture knots were trimmed and buried on the side of the recipient cornea. In the typical tendon allograft, it may be necessary to perform a radial incision with a scissors, to remove a wrinkle in the graft since the cornea is curved and the implant is plane.

For peripheral grafts or grafts that were irregular in form and thickness, the opaque corneal tissue was excised with a gem blade knife. The graft was cut with a scissors, according to the shape of the corneal defect (patients I, II, and X; Table 1).

Patient VII (Table 1) was the only case in which damaged conjunctiva was removed and approximately 4.5 mm of the corneal limbus was replaced by "Homotransplant for Conjunctiviplasty" of the same size and form (annular), sutured in the episclera and in the remaining conjunctiva, with interrupted 10-0 nylon suture, followed by lamellar keratoplasty as with the previous patients.

During the postoperative period, corticosteroid-free antibiotic drops were applied five times a day combined with pressure patching until reepithelization was completed.

Sutures were removed either when they became loose or approximately 15 days after the operation, when the graft was completely joined to the corneal tissue.

RESULTS

The overall results of the use of this material were excellent (Table 2). There was a decrease in symptoms in all patients. Vascularization was greatly decreased. Infections were eliminated (patients III, IV, VI, and X).

No rejection of the tendon allograft was observed, and epithelization was complete in all eyes between the third and fourth postoperative days. Patients presenting severe vascularization had faster absorption and a less transparent regenerated cornea.
Table 2. Visual Acuity Data

<table>
<thead>
<tr>
<th>Patients</th>
<th>Spectacle Corrected Preoperative Visual Acuity</th>
<th>Postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Refraction (D)* First Step</td>
<td>Visual Acuity</td>
</tr>
<tr>
<td>I</td>
<td>20/100 - 2.00 - 1.25x75</td>
<td>20/20</td>
</tr>
<tr>
<td>II</td>
<td>20/100 + 2.50</td>
<td>20/50</td>
</tr>
<tr>
<td>III</td>
<td>hand movements 0.00</td>
<td>20/200 cataract</td>
</tr>
<tr>
<td>IV</td>
<td>counts fingers + 2.00</td>
<td>20/30</td>
</tr>
<tr>
<td>V</td>
<td>counts fingers + 4.00</td>
<td>20/25</td>
</tr>
<tr>
<td>VI</td>
<td>counts fingers + 4.00-1.50180</td>
<td>20/60</td>
</tr>
<tr>
<td>VII</td>
<td>light perception NA</td>
<td>Hand movements</td>
</tr>
<tr>
<td>VIII</td>
<td>light perception NA</td>
<td>Hand movements 0.50-3.00x30 =20/20 after penetrating keratoplasty</td>
</tr>
<tr>
<td>IX</td>
<td>light perception NA</td>
<td>Counts fingers</td>
</tr>
<tr>
<td>X</td>
<td>counts fingers NA</td>
<td>20/80 cataract</td>
</tr>
</tbody>
</table>

* irregular astigmatism; NA - not applicable

Complications were rare when the surgeon was experienced in the lamellar graft surgical technique. However, epithelial growth in the interface can occur, when sutures are loose or when the surface of the graft is above the surface of the recipient cornea, resulting in the loss of the graft.

Even if there is very intense, deep vascularization such as after a caustic burn (case VII), or multiple rejections (cases VIII and IX), the graft can still be absorbed within 2 to 3 months. However, a less transparent cornea will result. We suggest that in these eyes a tendon allograft be performed again 6 months following the surgery, to further decrease vascularization; therefore allowing for a future penetrating graft with fresh donor cornea.

One disadvantage of the tendon allograft with this tissue is that the graft is visibly opaque right after the surgery. However, the graft becomes transparent within a few months of surgery.

In some eyes, irregular astigmatism can result from the surgery. Correction can be obtained with the use of hard contact lenses (cases II, VI). We did not perform keratoplasty.

DISCUSSION

During the 18 moths that the tendon allografts were performed, it was confirmed that once the absorption of the tissue began, the corneal tissue itself formed, little by little where areas of the graft were already surrounded by regenerated tissue. This tendon tissue has been chemically treated with polysaccharide substances according to patent procedures (Russian patent No. 940768, 1978, Muldashev ER, Nigmatullin RT, Salikhov AV). The chemical composition of the tissue inhibits the formation of proliferative fibroblasts, thus allowing the keratoblasts to produce transparent collagen without the proliferation of scar tissue. The transplant tissue is not only naturally absorbed by the body but it serves as “scaffolding”, for corneal regeneration (Muldashev E, 1992, personal communication).

As expected, Bowman’s layer did not regenerate. The epithelium and stroma did regenerate.

In patient I, an extremely thin protrusion was observed 2 mm from the inferior limbus, similar to pellucid marginal degeneration. But, within 2 months of surgery, the thickness satisfactory improved.

Overall, the results were considered excellent when compared to those of other therapeutic methods.
Three eyes (VII, VIII, and IX) had transparent tendon grafts but poor visual results because of this material is undeniable. In the present series, the symptomatology decreased immediately after surgery in all patients. It is believed that complications occur only when the material is used in unsuitable cases and pathologies or when this procedure is performed by someone with relative inexperience in using the surgical lamellar technique.

The tendon allograft does not substitute for a corneal allograft, but it is a therapeutic alternative for eyes that need therapeutic, tectonic, or cosmetic grafts.

This technique has numerous advantages:

1. Neither corticosteroid not antiinflammatory drugs are necessary.
2. There is no rejection of the graft tissue.
3. Vascularization is prevented.
4. The tendon tissue is not infected by bacteria or viruses.
5. Absorption of the graft tissue occurs within 1 to 6 months and is replaced by clearer "corneal" tissue.
6. The graft tissue is easy to store.
7. There is no need to depend on donor corneas.

Currently, there is little published literature concerning cornea graft alternatives. Therefore, we suggest the continued cautious use of this material, to see if it is possible to achieve results comparable to the ones presented here.

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**ALLOPLANT LAMELLAR KERATOPLASTY**

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**Purpose.** To develop a new transplant for lamellar keratoplasty as an alternative to allogenic cornea.

**Methods.** Histological (35 rabbits), immunological and clinical study. The typical lamellar keratoplasty technique was used. 312 patients were operated on for the following diagnoses: herpes keratitis, after-burn corneal spots, corneal ulcerations, pterygium, dystrophic corneal lesions. The patients were followed up for 1-5 years.

**Results.** Tendon tissues with high content of keratan sulphate can readily take root after lamellar keratoplasty, epithelialization of the surface thereof is complete in 3-5 days. Then the transplant is gradually replaced by corneal tissue. The replacement of the transplant by transparent corneal tissue is directly dependent on the transparency of the deeper layers: opaque replacement occurs when the tissue bed is opaque, transparent replacement - when the tissue bed is transparent. Clinical studies have shown transparent replacement in 36.2%, nearly transparent - in 26.9%, semi-transparent - in 27.0%, opaque - in 9.9%. The best results have been achieved in treating herpes keratitis, corneal ulcerations and various kinds of dystrophy.

**Conclusions.** The properties such as: no immune response, long-term shelf-life (up to 5 years) and easy modeling allow it to recommend Alloplant for wide clinical application.