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Simultaneous Transplantation of Limbal Stem Cells May Reduce Recurrences of Granular Dystrophy After Corneal Transplantation

2 Long-Term Case Reports

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Abstract: To present 2 cases with long-term relapse-free intervals only after limbo-keratoplasty but not after conventional penetrating keratoplasty in granular dystrophy.

Retrospective review of the patient charts and photographs taken during long-term follow-up of 2 cases with granular dystrophy, in which 1 eye received penetrating keratoplasty and the fellow eye received penetrating limbo-keratoplasty.

In the first patient, 1 eye showed extensive recurrence of granular deposits 17 years after penetrating keratoplasty was performed while in the second eye two-thirds of the corneal transplant adjacent to the transplanted limbal area remained clear 12 years after the limbo-corneal transplant. In the second patient, 1 eye showed no signs of recurrence 5 years after limbo-keratoplasty, whereas a recurrence of granular corneal deposits occurred 18 months after surgery in the fellow eye.

These cases show that the simultaneous transplantation of healthy donor limbus when performing penetrating keratoplasty may prolong recurrence in granular corneal dystrophy. Although we were unable to prove it on the molecular level, these clinical courses may support the hypothesis that a limbal transplant helps prevent a recurrence.

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Abbreviations: GCD = Granular corneal dystrophy, IC3D = The International Committee for Classification of Corneal Dystrophies.

INTRODUCTION

The autosomal dominant disorder granular corneal dystrophy (GCD) presents with the typical granular appearance of electron-dense, rod-shaped, trapezoid, and fenestrated bodies within the stroma of the central cornea. Characteristically, there are areas of clear corneal stroma in between the deposits and adjacent to the limbus. Two phenotypes exist. An early-onset form, the superficial variant, begins in childhood with confluent subepithelial and superficial stromal changes, frequent recurrent erosive attacks, and early visual loss. Genetically, these cases are homozygote for specific mutations on the BigH3/TGFB1 gene.[1–3] A late-onset and milder form is characterized by multiple, crumb-like stromal opacities, slow progression, fewer erosive attacks, less visual disturbance, and less need for therapeutic intervention. This form is caused by the p.Arg555Trp missense mutation, the most common mutation in GCD.[4] Penetrating or lamellar keratoplasty often is the only vision-improving therapeutic option in the advanced stages. Recurrences in the transplant, however, are inevitable and usually become a functional problem within the first 4 years after surgery.[5] Recurrences are first detected subepithelially and then accumulate in the central anterior stroma.[5–7] Mechanical abrasion in the very early stages or laser ablation has no lasting effects and often results in ''aggressive'' and more severe recurrences of the deposits[8] with no additional beneficial effect of topical mitomycin C treatment.[9,10]

Whether granular dystrophy is to be classified as a purely epithelial dystrophy, as Witschel and Sundmacher have long postulated,[11] or the keratocytes also contain dystrophic genes, as Lisch holds,[12] remains an open question. The International Committee for Classification of Corneal Dystrophies (IC3D) classification of corneal dystrophies considers granular dystrophy a stromal dystrophy.[13] However, there are indications that the corneal epithelium and the limbus may play a role in the etiology of the granular deposits in the cornea as well.[14–17]

In 1996, Sundmacher et al described a new therapeutic option for preventing conjunctivalization of the cornea in patients with limbal stem cell insufficiency. This consisted of an eccentrically trephined corneal graft, in which around one third of the circumference contains limbal tissue. With this technique, the simultaneous transplantation of clear corneal and limbal tissue is possible.[18,19] Later, this technique was shown to prevent recurrence in GCD.[19] His group further provided favorable long-term results of up to 9 years following penetrating limbo-keratoplasty in 2004.[20] The problem is that statistical evidence of the superiority of limbo-keratoplasty could not be shown due to the low number of patients who required the intervention. In addition, the effectiveness of limbal transplantation depends on the long-term survival of the transplanted stem cells in the host. This survival, however, is the crucial factor, which up until now has not been sufficiently granted or manipulated in the long run.[21–23] To learn more about recurrences in GCD, it may be helpful to study single patients in whom transplanted limbal stem cells seem to have survived for

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many years. This could provide at least some clarification of the potential of this technique in specific corneal diseases.

The 2 patients presented may be good examples from whom to gain more insights in the pathophysiology of corneal granular dystrophy and limbo-keratoplasty. Both cases provide a long postsurgical follow-up, and both had conventional keratoplasty done on one eye while the fellow eye was treated with limbo-keratoplasty. Thus, these cases provide the unique situation of an intraindividual control eye to study the long-term outcome after both techniques in GCD.

CASES

Written informed consent for publication was obtained from both patients.

Patient 1

A female patient, born in 1941, suffering from progressive vision decline due to corneal depositions of GCD, presented to our department 17 years ago and required surgery to improve her vision. At the time of the surgical intervention, vision was 20/100 in both eyes. We performed penetrating keratoplasty on the right eye, which at that time was state of the art. Best-corrected visual acuity following the intervention was 20/20 with spectacles. Due to the recurrence of granular deposits in the corneal transplant with vision decline, corneal abrasions were performed 2, 3, 6, and 9 years later following the intervention.

In the left eye, we performed penetrating limbo-keratoplasty with a 2-mismatch transplant 12 years ago. We attempted not only to restore a transiently clear corneal stroma but also to provide healthy donor limbal stem cells, which may serve as a permanent source for a nondiseased corneal epithelium. Best-corrected visual acuity following the intervention was 20/20 with spectacles. Immunosuppression with cyclosporin A was administered over a period of 6 months after the intervention to reduce rejection risk. No subsequent surgical intervention was performed except cataract extraction with the implantation of an intracapsular posterior chamber lens in both eyes.

The patient then presented to our department 17 and 12 years after the surgical interventions in the right and left eye, respectively. She complained about additional vision loss to 20/200 in her right eye. A slit-lamp examination revealed extensive intracorneal deposits of granular material in the whole corneal transplant and at its borders. Additionally, iron deposits in the anterior stroma were present paracentrally (Figure 1A).

In the left eye that received the corneolimbal transplant, visual acuity remained 20/25 with spectacle correction. The upper two-thirds of the corneal transplant, adjacent to the transplanted limbus, were clear, while the lower third of the transplant showed the typical stromal deposits of the recurrence of granular dystrophy (Figure 1B). Accordingly, the optical axis was clear allowing good vision in this eye. The limbal tissue of the transplant had gained access to the conjunctival vascularization, a phenomenon often seen after central limbo-keratoplasty and probably necessary for the permanent survival and function of limbal stem cells (Figure 1B).

Patient 2

In a male patient, born in 1931, the right eye was treated with a conventional penetrating keratoplasty in 1979, and the left eye in 1980. When the patient presented in 2007, both eyes showed a recurrence of intracorneal deposits of GCD with visual acuity of 20/50 in the right eye (Figure 2A). For resurgery, we performed limbo-keratoplasty on the right eye with a 2-mismatch transplant and a cyclosporin A eluting silicone matrix implanted subconjunctivally (Figure 2B). Vision improved to 20/30, and no recurrence of the dystrophy in the graft has been observed up to 5 years after surgery. Unfortunately, 2 years after the limbo-keratoplasty in this eye, vitreoretinal surgery became necessary for a macular hole, the effect of which was a persistent decrease in vision to 20/200 despite a clear limbo-corneal graft (Figure 2C).

The only way to improve the patient's visual function, therefore, was to perform a regraft on the left eye, too, since 31 years after the conventional corneal graft his vision had declined to 20/200, mainly due to recurrences of the dystrophy (Figure 3A). However, this eye also had an epiretinal membrane of the macula that contribute to visual loss to some extent. Although we recommended limbo-keratoplasty for this eye, the patient preferred conventional penetrating keratoplasty, which we performed in 2011. Sixteen months after surgery, a recurrence of the dystrophy occurred in this graft (Figure 3B). Best-corrected vision remained 20/200 with the macular area showing a slight increase in the epiretinal membrane.

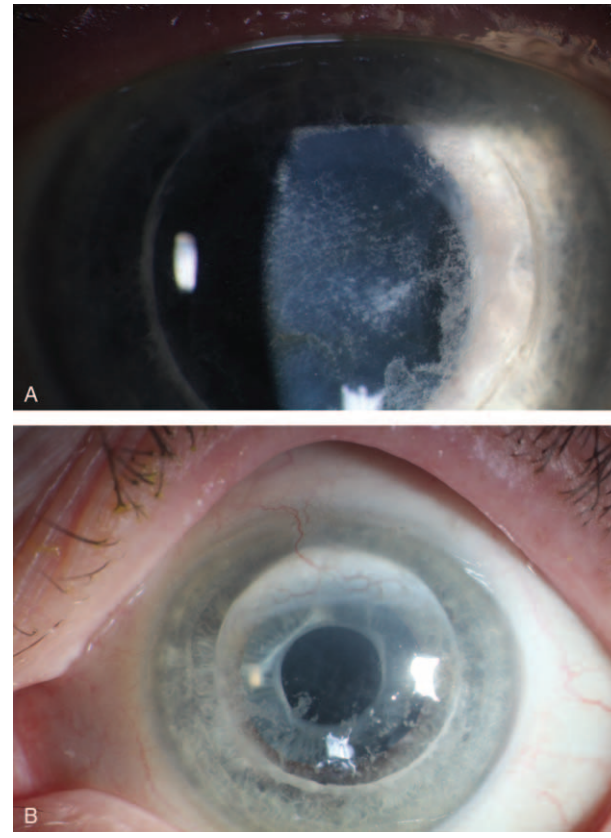

FIGURE 1. (A) Right eye of patient one 17 years after conventional penetrating keratoplasty. Subepithelial deposits with corneal opacification typical of corneal granular dystrophy have reoccurred. (B) Left eye of patient one 12 years after penetrating limbo-keratoplasty. In the superior part of the corneal transplant, the limbal transplant with access to the vascular system can be seen. Remarkably, the superior two-thirds of the corneal transplant remained clear while in the inferior one third of the corneal transplant, opposite the transplanted stem cells, subepithelial deposit formation as seen in granular dystrophy reoccurred.

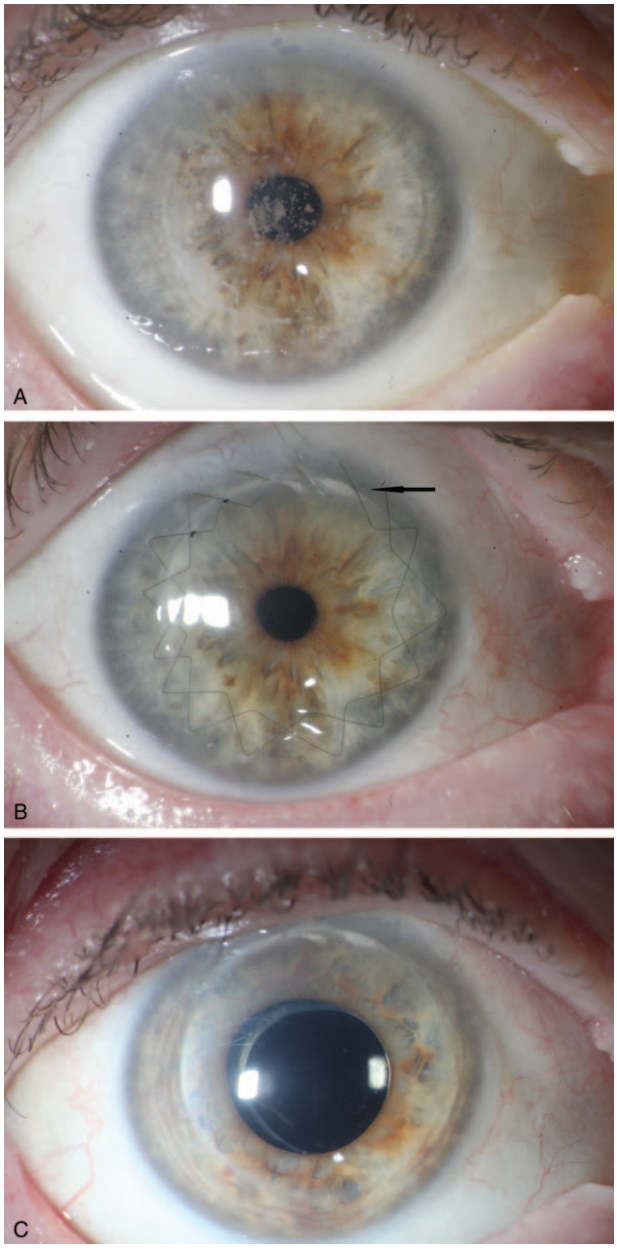

FIGURE 2. (A) Right eye of patient 2 before limbo-keratoplasty. Corneal transplant with the recurrence of intracorneal deposits of granular corneal dystrophy. (B) Right eye of patient 2 directly after limbo-keratoplasty. Clear corneal transplant with the sutures in place. The limbal area is at 12 o'clock (arrow). (C) Right eye of patient two 5 years after limbo-keratoplasty. Clear corneal transplant with no signs of subepithelial deposits.

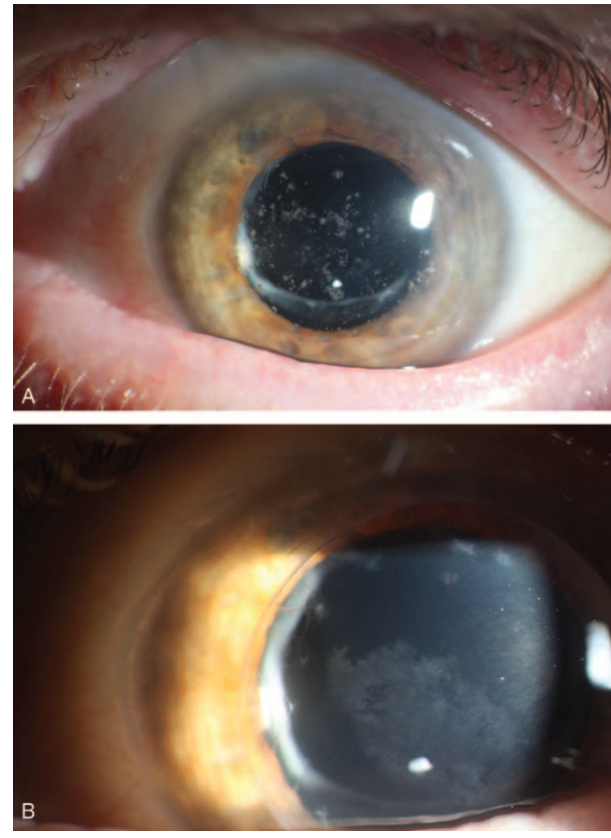

FIGURE 3. (A) Left eye of patient 2 before the second conventional penetrating keratoplasty. Corneal transplant with the recurrence of intracorneal deposits of granular corneal dystrophy. (B) Left eye of patient two 16 months after the second conventional penetrating keratoplasty. Subepithelial deposits with corneal opacification can already be seen.

DISCUSSION

The patients show how different the outcome can be after conventional penetrating keratoplasty and limbo-keratoplasty in GCD. In both cases, a clear visual axis was preserved only after limbo-keratoplasty in the long term (12 and 5 years, respectively). Since each surgical technique was performed in 1 eye of the patient, the difference in outcome may be attributed to whether limbal tissue was simultaneously transplanted. One explanation for this phenomenon may be that the repopulation of the graft surface by the host's epithelium could be prevented by the simultaneous transplantation of healthy limbal stem cells repopulating the donor disc so that the functionally important parts remain free from dystrophic deposits.

Two to 3 decades ago, granular dystrophy uniformly figured in the teaching books as a pure stromal dystrophy. The actual classification of corneal dystrophies (ICD-3) classifies it as purely stromal corneal dystrophy. The first discussion of a putative role for the dystrophic corneal epithelium apart from the general opinion that the host's keratocytes contribute to the deposits during the natural course of the disease was provided by Witschel und Sundmacher.[11] A putative role for the stromal keratocytes in recurrence should result in the full thickness occurrence of deposits, which is a very rare event since deposits in recurrence are usually located in the anterior stroma. To our knowledge, there is only 1 published case of deep stromal deposits in a recurrence after deep anterior lamellar keratoplasty.[24] Moreover, it may be observed that in this case a thin stromal lamella remained during preparation of the deep anterior lamella and that this remaining stroma may have contributed to the recurrence and not invading keratocytes from the border of the graft. A limitation of the presented cases is our inability to provide full evidence for the survival of the donor

limbal stem cells due to the patients' refusal to undergo a biopsy of the graft. However, both cases show an effect of the limbal tissue for preventing a recurrence in the graft. In addition to the effect of the limbal epithelial stem cells, a role for the limbal stromal mesenchymal cells in this context may be also possible. Limbal stromal mesenchymal cells have complex features and may directly or indirectly influence the outcome. Therefore, it cannot be ruled out that the limbal stromal mesenchymal cells, as well as the limbal epithelial stem cells, may contribute to the positive long-term effect seen after limbo-keratoplasty in our cases. Recently, mesenchymal stem cells have been discussed as a promising source of corneal healing, immunosuppression, and prevention of rejection in animal transplant models.[25,26] The potential role of these cells particularly in terms of preventing the recurrence of granular dystrophy in corneal grafts should be investigated further.

Although we believe we have found a principal way to avoid recurrences of granular dystrophy in the graft after keratoplasty, the practical achievements are far from being rewarding in every case. The reason, of course, is that we still do not have sufficient means of reliably inducing tolerance in a host, which is necessary for the long-term survival of homozygote donor cells or tissues, especially if these require ''proximity'' to host vessels, as seems to be the case with limbal stem cells.[21] This can be seen in our patients, who show a vascularization near the grafted limbus. This vascularization increases the risk of graft rejection. Seeking help from HLA matching and postoperative immune modulation, as we have in our patients, may have contributed to the favorable outcome. We do not claim, however, that these adjuvant measures worked for certain. It cannot be ruled out that the favorable outcomes presented here were mainly a chance event of ''lucky'' immunologic circumstances. It is clear that further substantial progress in this field will be achieved only if we succeed at better understanding and manipulating the phenomenon of immunologic tolerance in these cases.

Left with the choice of passively awaiting granular recurrences in the corneal graft after conventional perforating keratoplasty and the alternative of trying to avoid this outcome at least in some cases by simultaneously transplanting limbal tissue, we vote for the latter. With limbo-keratoplasty, we have developed a technically simple method that offers a chance for a good prognosis after keratoplasty for at least some patients with granular dystrophy who require keratoplasty.

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