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*Case Report*

# Human corneal endothelial cell transplantation with nanocomposite gel sheet preserves corneal stability in post-corneal transplant bullous keratopathy: a 16-year follow-up

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**Abstract:** Post-corneal transplantation endothelial decompensation and subsequent bullous keratopathy often result in unfavorable clinical outcomes regardless of the treatment strategy employed. In this report, we present the outcomes of a patient managed with in vitro expanded human corneal endothelial cell (HCEC) transplantation facilitated by a nanocomposite gel (NC gel) sheet over 16 years. A 40-year-old male patient who presented with signs of graft failure after penetrating keratoplasty underwent HCEC transplantation. Additionally, HCECs were obtained from a deceased donor, cultured in vitro, and transplanted onto an NC gel sheet as a temporary scaffold to support the transplanted cells until engraftment. At the 16-year follow-up, the cornea had remained stable and did not exhibit active disease manifestations. Notably, no new bullae were formed, and the epithelial surface appeared smooth without signs of active fluid transport abnormalities. Although a slight reduction in corneal thickness was observed, the disease-free region at the time of the intervention remained transparent. HCEC transplantation with NC gel sheets is a promising, minimally invasive approach for achieving long-term corneal stability in cases of bullous keratopathy following corneal graft failure. Importantly, this technique circumvents the need for complex procedures and utilizes corneal endothelial precursors derived from donor corneas discarded for lack of sufficient endothelial cells. After in vitro culture, these cells were successfully transplanted in three patients, proving that one donated eye can be useful in treating three eyes of three patients. This technique addresses the donor cornea shortage concerns and makes our concept “an-eye-for-eyes”, a reality.

**Keywords:** Human corneal endothelial cell (HCEC) transplantation, bullous keratopathy, nanocomposite gel sheet, cornea, corneal stability

## Introduction

In a previous publication, we documented the successful transplantation of human corneal endothelial cells (HCEC) in three patients with bullous keratopathy [1]. In the current report, we present observations from a 16-year follow-up of one of these patients and provide insights for the effective management of similar cases in the future.

HCECs play a pivotal role in maintaining corneal transparency by regulating stromal hydration through a combination of ionic pumps and tight junction barriers [2]. Corneal endothelial dystrophies and bullous keratopathy can severely compromise HCEC function, leading to corneal decompensation when the cell density falls below a critical threshold of 500-700 cells/mm$^2$, necessitating intervention [3]. Although endothelial keratoplasty offers notable safety

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and efficacy, the scarcity of donor corneas in many regions underscores the need for alternative approaches [3].

Current clinical trials have explored diverse strategies, including (i) Endothelial self-repair: Excision of dysfunctional central endothelium in patients with healthy peripheral cells to stimulate natural regeneration; (ii) Transplantation of cultured HCECs [3-5]; (iii) Investigations into induced pluripotent stem cells, endothelial stem cell regeneration, gene therapy, and other biological and pharmacological approaches.

Among these approaches, we opted for transplantation of allogeneic HCECs, which were isolated from a single cadaveric donor cornea and transplanted into the eyes of three patients. This "eye-for-eyes" concept demonstrates the potential to alleviate donor cornea shortages by maximizing the use of available tissue resources [6].

In our previous report [1], we documented a patient with congenital corneal dystrophy who underwent penetrating keratoplasty (PKP) in the right eye and subsequently developed symptoms of graft failure with multiple bullae jeopardizing vision. Post-corneal transplant failure is influenced by various factors including donor and recipient characteristics. Parameters such as the primary indication for transplantation, previous corneal surgeries, neovascularization, prior graft rejection, and the type of transplantation procedure are known to affect graft outcomes [7]. Moreover, donor attributes such as endothelial cell density (ECD) affect graft rejection and failure. Even though factors such as ethnicity, lens status, and graft procurement/preservation techniques also play a role, recipient factors such as diabetes carry significant weight. Of particular importance is ECD, which naturally declines following PKP, leading to endothelial dysfunction even in the absence of overt graft rejection [8]. Therefore, minimizing postoperative endothelial cell loss is crucial for prolonging graft survival and maintaining optimal long-term visual outcomes after endothelial keratoplasty.

Endothelial dysfunction, characterized by enlarged endothelial cells, diminishes the available surface area for fluid pump sites and increases resistance to corneal fluid flow [8]. This delicate equilibrium is not solely governed by ECD, suggesting that the minimum ECD necessary to preserve corneal thickness and graft clarity may vary. Histopathological examination of grafts with endothelial failure and low ECD revealed unstable, stressed, and vulnerable endothelial cells, predisposing them to bullous keratopathy, a condition caused by widespread loss or dysfunction of the corneal endothelium. The compromised endothelial function leads to the inability to maintain corneal deturgescence. Clinically, bullous keratopathy progresses through distinct stages: initial stromal edema with Descemet's membrane folds, followed by intracellular epithelial edema (hydropic degeneration), and ultimately, separation of the epithelium from the Bowman layer. The aforementioned small separations, termed microcysts, can coalesce into large blisters known as bullae [9]. Advanced cases may exhibit changes in the secondary epithelial basement membrane, loss of stromal keratocytes, or pannus formation. In summary, corneal endothelial decompensation precipitates corneal edema, which progresses to bullous keratopathy in advanced stages.

Strategies for preventing graft rejection include early detection and prompt initiation of steroid therapy. In severe cases of endothelial graft rejection with an early presentation, intravenous steroids may be beneficial. However, topical, intracameral, or intravitreal steroids constitute the mainstay of treatment for all forms of graft rejection [10]. Supportive therapy is crucial and may encompass prophylactic administration of antibiotics, cycloplegics, topical lubricants, and antiglaucoma medications based on the presenting symptoms, signs, and intraocular pressure (IOP) [11]. Additionally, mitigating the risk factors for rejection and selecting grafts with high ECD are preventive measures that can be adopted. When left untreated or managed inadequately, this condition becomes a chronic concern, potentially resulting in pain and jeopardizing the existing vision, with the added risk of total vision loss. At times, this chronic condition may not respond to high doses of steroids and could culminate in corneal dehiscence and melting, necessitating repeat PKP or conjunctivoplasty and, in extreme cases, enucleation of the affected eye.

To address these challenges and combat endothelial cell loss following graft failure, we inves-

tigated the potential of HCEC transplantation as a novel therapeutic approach.

## Case report

A 40-year-old male with a history of congenital corneal dystrophy underwent PKP in the right eye and presented with graft failure [1]. Visual acuity was limited to hand motions (HM) in the affected eye. Examination revealed central corneal bullae within the grafted cornea, primarily located in the subepithelial zone and the anterior stroma, indicating endothelial dysfunction (**Figure 1A**). Notably, no active epithelial defects were noted.

Human corneal endothelial precursor (HCEP) cells were obtained from a deceased donor (30-year-old male) and transported using a thermo-reversible gelation polymer (TGP)-based method [12] to a laboratory 300 km away within 12 hours under sterile conditions. Approximately 6×10^4 HCEPs were isolated and expanded in vitro for 26 days using a sphere-forming assay in TGP hydrogel, yielding 5×10^5 HCEPs [12]. Subsequently, 1.6×10^5 HCEPs were prepared for transplantation to each of the three patients, after confirming sterility and acceptable endotoxin levels. The transplantation procedure, as described in detail in our previous studies [1, 13], involved the use of a pre-shaped nanocomposite gel sheet (NCG) to support the cells during transplantation, facilitating HCEP attachment and removal after 3 days [1].

The utilization of NCG sheets for cell support during transplantation was inspired by the successful application of HCEP cell transplantation in animal models of bullous keratopathy, as reported in previous studies [13]. However, a significant challenge in translating this approach into clinical practice arises from the need to immobilize the eyeballs in a face-down position for a few hours without movement to facilitate gravity-assisted cell adhesion to the endothelium [14, 15]. Although attempts have been made to use animal-derived collagen sheets, gelatin, and Descemet's membranes as supporting materials for clinical transplantation, these methods have not gained widespread adoption due to technical complexities or concerns regarding biological contamination. To address these limitations, we employed an NGC (D25) as a supporting material for cell transplantation [13], which sustained the cells until engraftment without the need for an eye-down position and was easily removable after 3 days.

On postoperative day 3, significant corneal clearing was observed with complete resolution of the bullae. However, mild corneal striae were observed. By day 11, the striae had diminished, and corneal clarity persisted without bullae (**Figure 1B**, **1C**). At the 18-month follow-up, a few chronic intrastromal bullae were identified, but no new bulla formation was evident. Corneal transparency remained unchanged, and no decline in visual acuity or symptoms was reported [5]. The patient underwent continuous follow-up over 16 years to monitor the corneal status and address any potential further deterioration, such as wound dehiscence, leading to corneal melting. Visual acuity was regularly assessed using slit-lamp examinations, and medical advice and medications were provided as necessary.

The 16-year follow-up examination revealed a smooth epithelial surface with leukocomatous stromal opacities and well-formed fibrous cavities (**Figure 1D**, **1E**). No signs of active fluid transport deformities were observed. Corneal thickness exhibited a slight reduction, as noted during slit-lamp examination. Notably, the region of the cornea devoid of preexisting endothelial defects has remained transparent for the past 16 years, with no new bulla formation (**Figure 1F**). Although an area of neovascularization extended into the corneal rim, it did not encroach on the donor cornea (**Figure 1G**). Furthermore, the other areas remained free of neovascularization. Corneal thickness measurement using pachymetry is not feasible.

The absence of complications and sequelae associated with bullous keratopathy during the extended follow-up period suggests the potential of HCEP transplantation as a viable alternative to invasive procedures for the treatment of corneal endothelial diseases, particularly graft failure. This approach may contribute to the long-term corneal stability.

## Discussion and conclusion

This report presents a 16-year case study highlighting the potential of NCG-delivered HCEPs to achieve long-term corneal preservation and

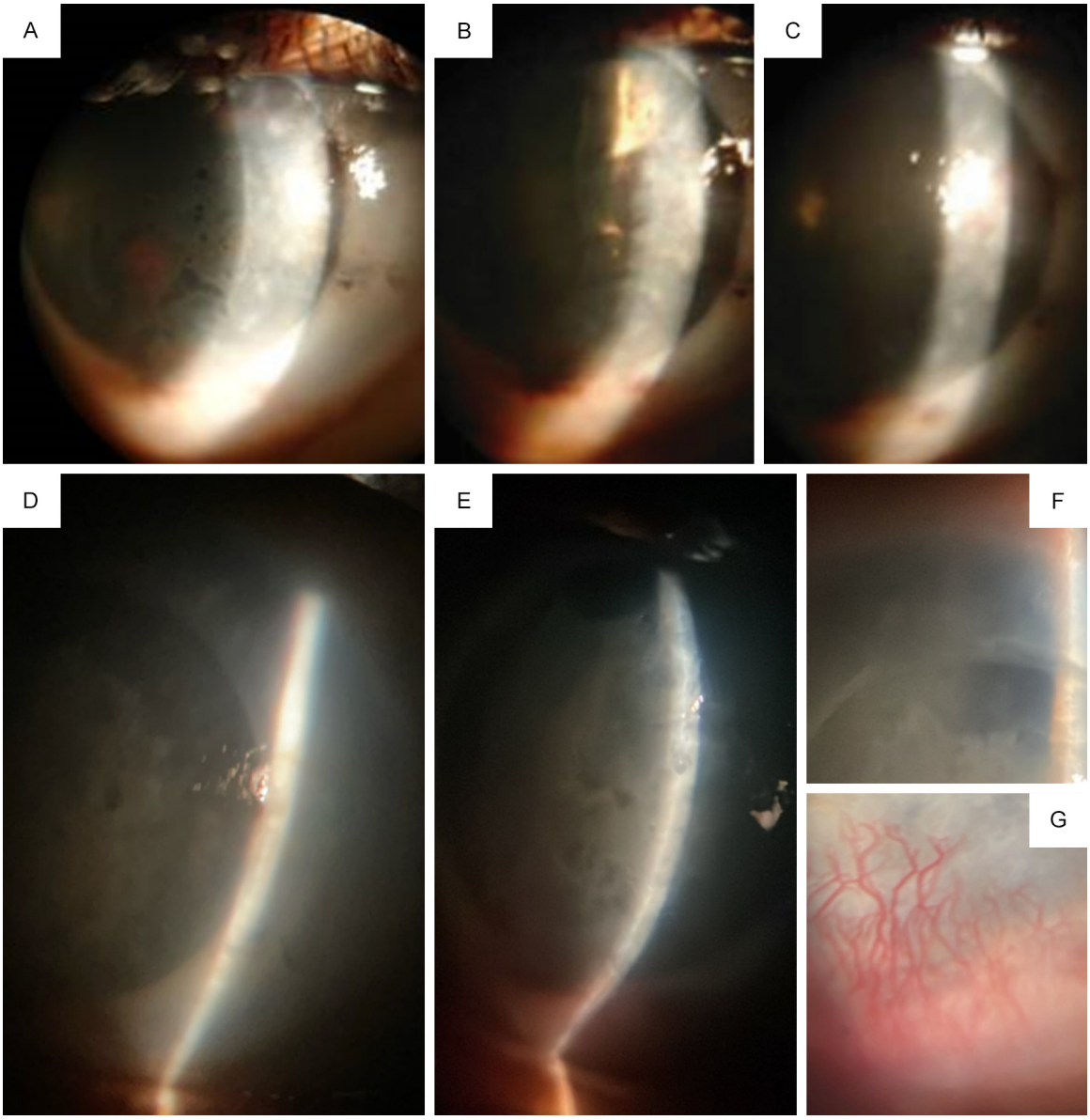


**Figure 1.** Human corneal endothelial cell (HCEC) transplantation and 16-year outcomes. A. Preoperative cornea demonstrates bullae due to endothelial cell deficiency. B. Cornea following HCEC transplantation (1-day post-surgery). C. Cornea 11 days post-transplantation, displaying resolved bullae. D, E. Sixteen-year follow-up of the HCEC-transplanted cornea, demonstrating maintained corneal clarity and stability. F. Region of the cornea at the 12 o'clock position that is clear without bullae preoperatively remains clear after 16 years. G. Neovascularization is observed only at the 6 o'clock position of the sclero-corneal junction.

stability in bullous keratopathy. Bullous keratopathy stems from corneal endothelial decompensation, a condition where the endothelium loses its ability to maintain corneal deturgescence [16]. This dysfunction can be triggered by various insults, including intraocular surgery, nonsurgical trauma, uncontrolled glaucoma, and Fuchs' endothelial dystrophy. When the visual potential remains viable, corneal transplantation is the preferred approach to alleviate pain, restore vision, and strengthen ocular surface defense. However, graft rejection or subsequent intraocular surgeries within the transplanted cornea can lead to bullous keratopathy, which was a problem in our case. The compromised endothelial function leads to a cascade of detrimental effects including increased stromal hydration. Loss of endotheli-

al pumping function results in progressive stromal edema. Keratocyte loss may further exacerbate this process. Epithelial basement membrane breakdown involves the weakening or rupture of the Bowman layer and epithelial basement membrane, potentially resulting in the loss of glycosaminoglycans from the stroma. Intraepithelial edema and poor adhesion occur when increased stromal hydration translates to intraepithelial edema, compromising epithelial adhesion and promoting recurrent or persistent erosion [16]. This vulnerability potentially contributes to the development of infectious keratitis and ulcers. For cases with limited visual potential or corneal graft failure, where repeat corneal transplantation is not suitable, management focuses on alleviating pain and promoting surface healing. Therapeutic options include bandage contact lenses, anterior stromal punctures, annular keratotomy, epikeratoplasty, excimer laser phototherapeutic keratectomy, and conjunctival flap procedures. Therefore, HCEP transplantation is considered a viable alternative. In addition, despite signs of clinical wound healing, the graft-host junction in graft failure may exhibit vulnerability to dehiscence owing to its inherent weakness [17]. Our HCEP transplantation technique demonstrates potential dual benefits such as maintaining corneal stability and preventing corneal neovascularization [18]. In cases where repeat corneal transplants are possible, our approach has the potential to obviate the need for more invasive surgical interventions such as PKP, Descemet's stripping endothelial keratoplasty, Descemet's stripping automated endothelial keratoplasty, and Descemet's membrane endothelial keratoplasty, all of which require a donor cornea with healthy HCECs [19]. This becomes particularly pertinent when bullous keratopathy persists beyond 12 months, leading to corneal stromal fibrosis and vascularization, which adversely affects outcomes [20].

Importantly, our procedure utilizes corneas deemed unsuitable for keratoplasty owing to their low HCEC count. By repurposing these discarded tissues from a single donor, we've successfully treated three patients. This approach stands as a promising solution for addressing the global shortage of donor corneas. Furthermore, by employing synthetic polymer-based corneal storage and transportation methods, the establishment of an allogeneic Corneal Endothelial Stem Cell Bank [21] becomes feasible, enabling the preservation of discarded, infection-free corneal tissues for future use and potentially treating a large patient population. Although conjunctival flap procedures may be necessary for corneal stabilization [22] in cases where keratoplasty is contraindicated, our minimally invasive approach of combining the cells with the NCG sheet as a supporting scaffold demonstrates efficacy with ease for the physician and comfort for the patient without having to be in an eye-down position. This approach resolves bullous keratopathy and maintains corneal stability for an extended period of 16 years.

Our technique also avoids the use of animal-derived proteins or chemicals, including Rho-associated protein kinase inhibitors [15], extracellular matrix proteins [23], and platelet-rich plasma treatment of cells [24]. Additionally, the procedure eliminates the need for complex HCEC transplantation procedures such as prolonged eye-down positioning [19] or magnetic cell delivery [25]. Consequently, this technique offers a potentially safe and versatile strategy for maintaining corneal endothelial cell function and stability.

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## Disclosure of conflict of interest

Authors Abraham and Haraguchi are inventors to a patent on usage of NC gel sheet. Author Abraham is a shareholder in GN Corporation Co. Ltd., Japan, an applicant to a patent on the NC Gel sheet. Author Parikumar is a stake holder in the Light eye hospital, Dharmapuri, India and The Light & Light Ventures Limited, Croydon, United Kingdom.

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