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Case Report

Human corneal endothelial cell transplantation using nanocomposite gel sheet in bullous keratopathy

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Abstract: Transplantation of *in vitro* expanded human corneal endothelial precursors (HCEP) cells using a nanocomposite (D25-NC) gel sheet as supporting material in bovine's cornea has been earlier reported. Herein we report the transplantation of HCEP cells derived from a cadaver donor cornea to three patients using the NC gel sheet. In three patients with bullous keratopathy, one after cataract surgery, one after trauma and another in the corneal graft, earlier performed for congenital corneal dystrophy, not amenable to medical management HCEP cells isolated from a human cadaver donor cornea *in vitro* expanded using a thermoreversible gelation polymer (TGP) for 26 days were divided into three equal portions and 1.6×10^5 HCEP cells were injected on to the endothelium of the affected eye in each patient using the D25-NC gel sheet as a supporting material. The sheets were removed after three days. The bullae in the cornea disappeared by the 3rd-11th post-operative day in all the three patients. Visual acuity improved from Perception of light (PL)+/Projection of rays (PR)+ to Hand movements (HM)+ in one of the patients by post-operative day 3 which was maintained at 18 months follow-up. At 18 months follow-up, in another patient the visual acuity had improved from HM+ to 6/60 while in the third patient, visual acuity remained HM+ as it was prior to HCEP transplantation. There were no adverse effects during the follow-up in any of the patients.

Keywords: Corneal dystrophy, cell transplantation, nanocomposites, hydrogel

Introduction

Cornea is the transparent front portion of the eye, which transmits the images into the eye for visual perception. The cornea has three main layers, the outer epithelium, central stroma and the inner endothelium. Functions of the corneal endothelium include transport of nutrients from the aqueous humour to the stroma, as there are no blood vessels in the cornea, it maintains corneal transparency by maintaining stromal hydration, and it functions to remove water by osmosis from the stroma to the aqueous humour.

Bullous Keratopathy is a pathological condition affecting the cornea. Specifically, small vesicles, or bullae, are formed in the cornea due to endothelial dysfunction, which in turn affects other components of the cornea such as stroma. As a result, the corneal endothelial cell quantity and functional capability are hallmarks of optimal health of the cornea. Corneal transplantation is an effective approach to restore vision for Bullous Keratopathy. However, there is global donor cornea shortage [1]. Cell based therapies are a potential solution to this problem. Corneal endothelial cell density is an important factor used to adjudge the usefulness of the cornea for transplantation after it is obtained from a deceased donor. We previously demonstrated that it is possible to transport the highly sensitive corneal endothelial layer obtained from donor corneas (discarded due to lower endothelial cell density), which retain some of the corneal endothelial cells, over long distances without the need for cold chain preservation. By using a novel nanopolymer cocktail, it is possible to use viable corneal endothelial cells isolated from transported corneal

endothelial tissues [2] in cell based therapies. Further, human corneal endothelial precursor (HCEP) cell transplantation has already been reported in animal models of bullous keratopathy [3-5]. The major hurdle in clinical translation is the need to fix eyeballs, 24-36 hours facing down, without any movement to facilitate the gravity-assisted settling of the cells onto the endothelium [4]. Though animal derived Collagen sheet [6], gelatin [7], Descemet's membranes [8] etc., have been used to facilitate clinical transplantation, they have not come to routine application for want of technical simplicity or other hurdles like risk of biological contamination [9].

To overcome these hurdles, we have earlier reported [10] the successful transplantation of HCEP cells using a nanocomposite (D25-NC) gel sheet as supporting material in bovine's cornea. Herein, we report the transplantation of *in vitro* expanded HCEP cells derived from a human cadaver donor cornea to three human patients using the D25-NC gel sheet.

Case report

The study was approved by the Ethical committee of the Light eye hospital, Dharmapuri, India and was performed following all international and/or national guidelines/regulations available at the time of the study in accordance with the 1964 Helsinki Declaration. Three patients with bullous keratopathy, one after cataract surgery, one after trauma and another in the corneal graft, earlier performed for congenital corneal dystrophy, not amenable to medical management were considered for the study. Case selection was done on the basis of "less risk" and with very poor visual prognosis as our priority was to study the outcome of the procedure on the corneal edema and bullae.

Patient characteristics

Patient I: A 65 year old female patient presented with the complaints of watering, pain and irritation in the right eye. Observations revealed that this was a case of pseudophakic bullous keratopathy in the right eye with a history of intra-ocular lens implantation surgery six months earlier. The right eye was treated with hyperosmotic agents both topically and systemically but it did not respond to this medical line of management. Slit lamp examination showed the involvement of the entire cornea including the periphery. The bullae formation was subepithelial and recurrent in nature and hence the right eye needed an intervention to control the progressive bullous formation. There were few large bullae at the centre and many small bullae at the periphery. The stroma was cloudy with a visual acuity of Hand Movements (HM)+ in the right eye. The right eye was selected for the HCEP cells transplantation.

Patient II: A 45 year old male patient presented with the complaints of defective vision and irritation in the right eye. There was no pain or watering. History revealed an injury due to a trauma a year ago following which he was treated in one of the local eye clinics. For one year he did not have useful vision in the right eye. Thus this was a case of post-traumatic bullous keratopathy. The central cornea had epithelial and sub-epithelial bullae and the periphery was relatively free from bullae. This patient would otherwise be advised for penetrating keratoplasty (PKP) because the central cornea was compromised with the visual acuity of Perception of light (PL)+/Projection of rays (PR)+ in all quadrants. The visibility of the iris through the peripheral cornea thus gave a good prognosis for this patient if PKP was done. The right eye was selected for HCEP cells transplantation.

Patient III: A 40 year old male patient with congenital corneal dystrophy who had undergone a penetrating keratoplasty in the right eye presented with a visual acuity of HM+ in the right eye. On examination his right eye with the grafted cornea showed a few long standing central corneal bullae in the sub epithelial zone and anterior stroma with no epithelial eruptions. The left eye showed a well formed near total corneal opacity involving all the layers of the cornea. The right eye needed an intervention to provide clarity at the central cornea because the PKP done on the right eye showed signs of long standing graft rejection with endothelial decompensation. Though the patient was employed and was able to read Braille, he wanted to have a better clarity in vision. Hence the right eye was selected for HCEP cells transplantation.

Methodology

Informed consent was obtained from all the three patients after explaining the nature of this procedure. The HCEP cells were obtained from a deceased donor who was an Indian male

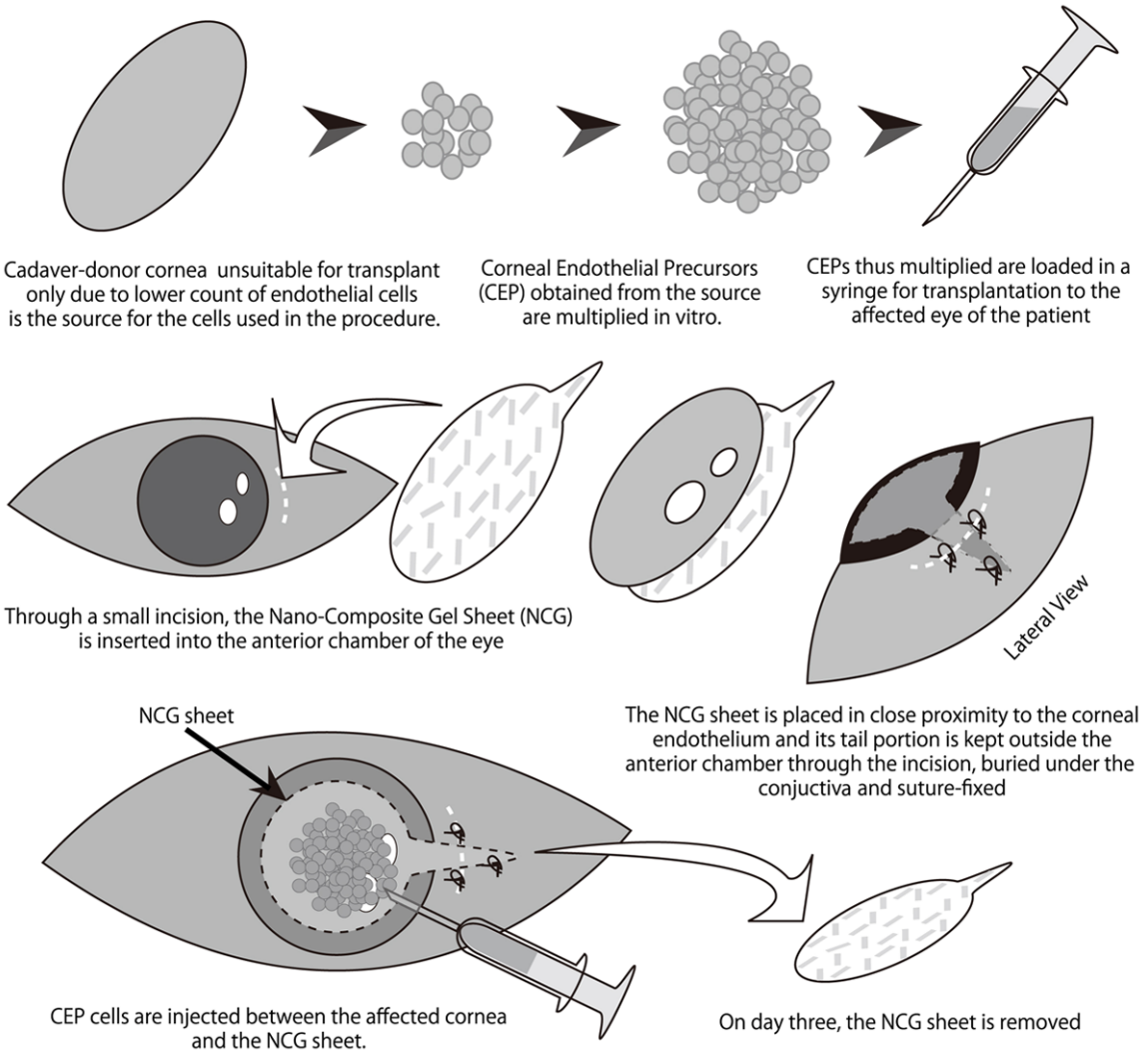

Figure 1. Schematic illustration of the various steps of clinical procedure performed in the study.

aged 30 years after informed consent from the guardian. The cornea was screened for infections like HIV, HBV, CMV and was found to be negative. The corneal endothelial cell layer and Descemet's membrane were dissected with fine forceps by an experienced ophthalmologist at the Light Eye hospital. The dissected corneal endothelial tissue was transported using a thermoreversible gelation polymer (TGP) based transportation methodology reported by Rao *et al* [2] without cool preservation to the laboratory located 300 km from the hospital taking a duration of 12 hours. In the laboratory, the tissue processing was done in a Class 10000 clean environment. Nearly 6×10^4 HCEP cells were isolated from the corneal endothelial tissue and *in vitro* expanded for 26 days using the sphere forming assay in a thermoreversible gelation polymer (TGP) hydrogel as reported earlier [9]. After expansion, 5×10^5 HCEP cells obtained were divided into three equal portions of nearly 1.6×10^5 cells in each portion. The D25-NC gel sheets [10, 11] (provided by M/s GN Corporation Co. Ltd., Japan) were cut into circular shapes with extension arms and perforations as described earlier [10]. Briefly, the peripheral margins of the sheets were trimmed to give a circular shape at the inferior end. The superior ends were trimmed in such a way that the central parts alone were left intact purposed to serve as handles and the sides were removed in a curved manner. The portion of the NC gel sheets closer to the handles were perforated with the 18 G needle in multiple places so as to allow the flow of aqueous humour through and through. The

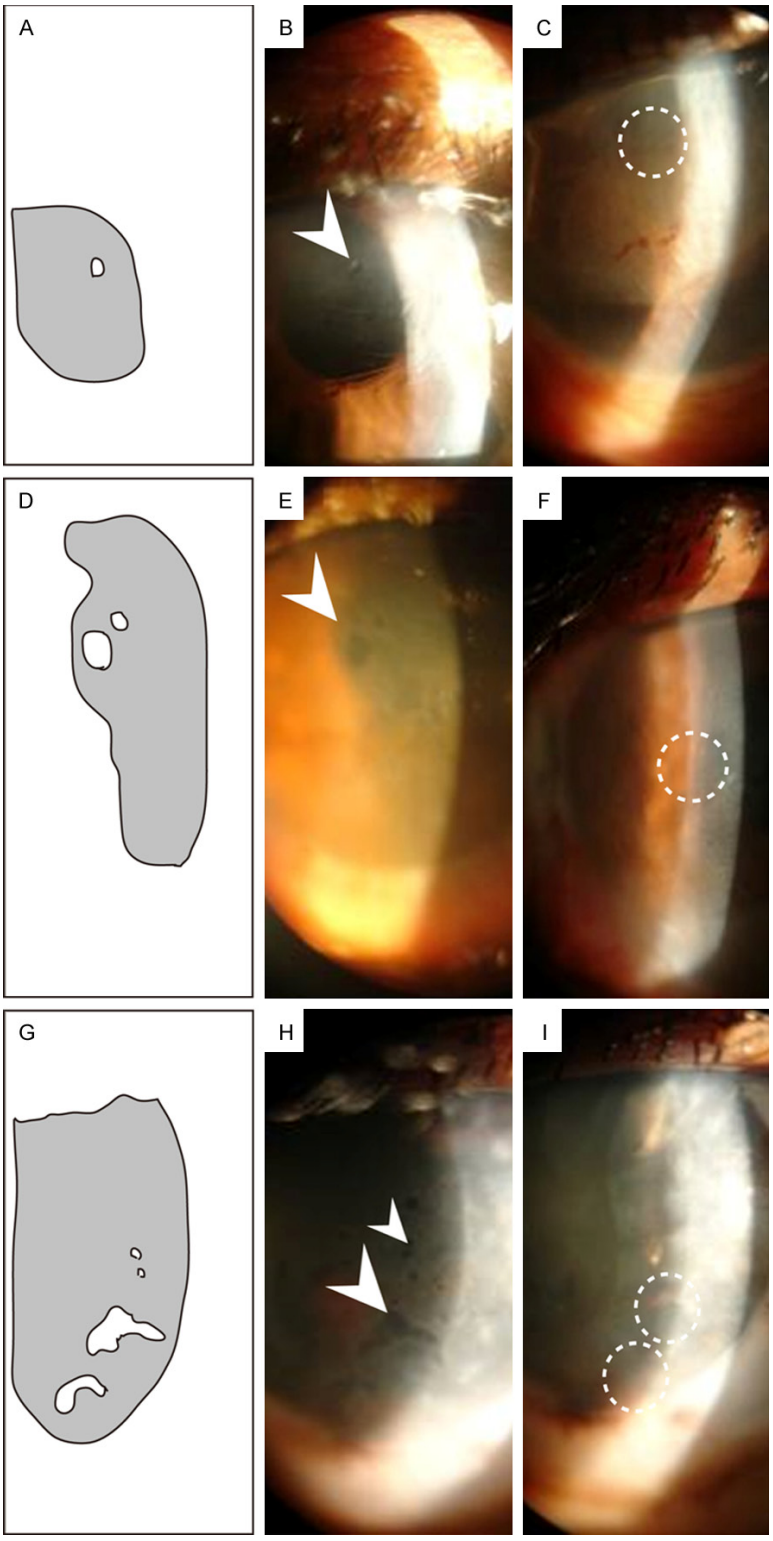

Figure 2. Pre and Post HCEP cell transplantation pictures of the three patients (Pt); (B) (Pt I), (E) (Pt II), (H) (Pt III) show the pre-transplantation slit lamp images of the eyes in which white arrow(s) point the bullae. (C) (Pt I), (F) (Pt II), (I) (Pt III) show the respective patients' post HCEP transplantation images with the corresponding regions where the bullae have disappeared as shown encircled. Corresponding diagrams (A, D, G) to the left show the shape of the bullae for easy identification.

schematic illustration of the various steps of the clinical procedure has been presented in **Figure 1**.

The HCEP cells were prepared to the dilution of 25000 cells/0.25 ml of saline. The cells for transplantation were screened for microbiological contamination and for endotoxin levels before transplantation to the patients. There was no microbiological contamination detected and endotoxin levels were below permissible limits. The cells were transplanted to the patients based on an earlier described protocol [10]. The patients' eyes indicated for HCEP transplantation were anaesthetised with a peribulbar injection with Xylocaine (lidocaine HCl) and Sensorcaine (bupivacaine HCl injections)® mixture. In the indicated eye of each patient, two side ports were made with a lancet blade one at 1 o'clock position and the other one at the 11 o'clock position. After preparations, the incision was made at the limbus at 12 o'clock position. A straight incision of approximately 10 mm size was made and viscoelastic substance (Appavisc, Appasamy Ocular Devices Pvt. Ltd., India) was injected into the anterior chamber to keep it formed. The inferior circular portion of the NC gel sheets, trimmed and shaped earlier was inserted through the incision so as to make the inferior margins of the sheet to rest within the inferior angle. The curvature was such that the circular inferior portion of the NC gel sheets occupy the anterior chamber's angles all through, between 3 o'clock position to 9 o'clock positions. The in-

Table 1. Observations on the reduction of the bullae over time after HCEP cell transplantation

S.No	Timeline	Patient I	Patient II	Patient III
1	Baseline Characteristics	Pseudophakic Bullous Keratopathy	Aphakic Bullous Keratopathy	Post keratoplasty Bullous Keratopathy
2	Total number of cells injected	50,000 cells in 0.25 ml	50,000 cells in 0.25 ml	50,000 cells in 0.25 ml
3	Duration of Follow-up	18 months	18 months	18 months
4	Immediate post-transplantation follow-up	Wound intact/Scaffold in position	Wound intact/Scaffold in position	Wound intact/Scaffold in position
5	Day I post-transplant follow-up	Striate keratitis+/Bullae present	Bullae present/no sign of rejection or reaction	Striate keratits++/Bullae not significant
6	Observation after NC Gel sheet removal (Day 3 post-transplant)	Striae clearing/Bullae reduced/no visible deposits on the scaffold	Bullae reduced/no visible deposits on the scaffold	Striate keratitis+/Bullae present/ no visible deposits on the scaffold
7	Day 7 post-transplant follow-up	No striae/Bullae+	Bullae insignificant	No striae/Bullae+
8	Day 11 post-transplant follow-up	No striae/Bullae+	Cornea clearing well	No striae/Bullae+
9	1 month post-transplant follow-up	Bullae+	No Bullous keratopathy	Bullae+
10	3 months post-transplant follow-up	No striae/Bullae+	No Bullous keratopathy	No striae/Bullae+
11	6 months post-transplant follow-up	No Bullous Keratopathy	No Bullous keratopathy	No Bullous Keratopathy
12	12 months post-transplant follow-up	No Bullous Keratopathy	No Bullous keratopathy	No Bullous Keratopathy
13	18 months post-transplant follow-up	No Bullous Keratopathy	No Bullous keratopathy	No Bullous Keratopathy

sertion was done by holding the handle-like projection cut at the superior aspect. Once the NC gel sheets were kept in position, 1.6×10^5 HCEP cells were loaded with 23 G needle into a 2 ml syringe and infused into the anterior chamber through the 11 o'clock position port in between the recipient endothelium and the NC gel sheets in each of the patient. The wounds were overlapped with conjunctivae along with the protruding portion of the handle of the NC gel sheets. The visco-elastic substance was not removed from the anterior chamber. Though a transient rise of Intraocular Pressure (IOP) was expected, the visco-elastic substance was left in the anterior chamber purposely to allow time for the infused endothelial cells to get attached to the recipients' endothelial cell layers. Immediately after the procedure the patients were kept in primary straight head position. All the three patients were kept under observation and examined under slit lamp at regular intervals in the post-operative period. The findings were documented. After three days the patients were posted for the removal of the NC gel sheets. The removal of the NC gel sheets was done under topical anaesthesia. The sheets were sent for microscopic examination.

Results

In the patient I, on post-operative Day 3, the central striae worsened while the lower half of the cornea was clearer. Lens glow which was seen in post-operative day 2 was absent. The patient had an exudative membrane at the pupillary area and hence the lens reflex could not be visualized properly on post-operative Day 3. After removal of the NC gel sheet and correction of the membranes, the lens reflex could be visualized. The bullae disappeared around the 11th day after HCEP cell transplant. Visual acuity improved from positive hand movements (HM) to 6/60 in 18 months. However the Iris pigments were seen deposited on the corneal endothelium. **Figure 2** shows the pre- and post HCEP cell transplantation images of the cornea of the three patients. In patient II, around the 3rd day after HCEP cell transplant the cornea was drastically clear and there was no evidence of the bullae. Vision had improved from PL+/PR+ to HM+. The status quo was maintained on post-transplant Day 11 and also during the follow-up after 18 months. In patient III, on the post-operative Day 3 the cornea was much clearer and bullae were totally absent. Mild striae were observed. On Day 11, the striae had come down and the cornea continued to be clear with no bullae. Follow-up after 18 months in the patient III revealed that there were few bullae but not in superficial zone. They appeared to be chronic bullae entrapped between the fibrous stroma. There was no evidence of new bullae. The transparency appeared to be the same. No worsening was observed and patient was asymptomatic. The visual acuity of HM+ present before the HCEP transplantation was maintained throughout the follow-up. **Table 1** gives the details of the reduction of bullae over time in the three patients after HCEP transplantation.

There were no adverse effects in any of the patients after HCEP transplantation throughout the follow-up period. Microscopic observation of all the three D25-NC gel sheets used in the study, removed three days after cell transplantation in each patient, showed no HCEP cells attached to them.

Discussion

The corneal endothelium composed of a single layer of regularly arranged hexagonal and pentagonal cells serves to maintain corneal transparency by regulating hydration while maintaining the diffusion of nutrients from the aqueous humour to the avascular cornea. Bullous keratopathy is a condition in which there is stromal edema along with epithelial and sub-epithelial blisters called bullae, caused due to endothelial cell loss or cell dysfunction secondary to several etiologies including surgical trauma especially intraocular lens implantation, inflammation or diseases like Fuch's Dystrophy [12]. The definitive treatment for bullous keratopathy is PKP (corneal transplantation procedure) which has been in practice since 1905. In corneal transplantation, one donor cornea is used to replace the defective endothelium in one recipient cornea [13]. However there is global donor cornea shortage [13]. *In vitro* expanded cell based approach for corneal endothelial diseases is a considerable option [13]. Yokoo *et al* [14] identified that the human corneal endothelium has progenitor cells that when isolated from a donor cornea and expanded *in vitro*, can be used to treat several eyes. Our animal study earlier proved the engraftment of transplanted HCEP cells onto a bovine's eye cornea [10]. This study proves that clinical transplantation of *in vitro* expanded HCEP cells using NC gel sheets is feasible. With the earlier reports proving that even from donor corneas unsuitable for transplantation precursors cells could be retrieved [2], this method of one-eye derived HCEP cells to treat multiple eyes without the risk of biological contamination, is worth a serious consideration. Also in patient III, since HCEP transplantation has helped to treat the bullae that developed over a corneal graft, this approach could probably be useful in patients with corneal graft failure. Larger studies are recommended to validate the outcome to make this a routine procedure.

Conclusion

In vitro expansion and transplantation of HCEP cells is safe and improves the visual acuity along with alleviating symptoms in patients with bullous keratopathy. After appropriate validation, this methodology can be employed for using corneal endothelial cells from one donor cornea to treat more than one patient with corneal endothelial diseases.

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Disclosure of conflict of interest

Authors Abraham and Haraguchi are applicants to a patent on usage of NC gel sheet. Author Parikumar is a stake holder in the Light eye hospital, Dharmapuri, India.

Abbreviations

HCEP, Human corneal endothelial precursor; NC, Nanocomposite; TGP, Thermoreversible gelation polymer; HM, Hand movements; PL, Perception of light; PR, Projection of rays; PKP, Penetrating keratoplasty; IOP, Intra-ocular pressure.

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