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Preservation of donor corneal epithelium in McCarey-Kaufman medium

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Purpose: To evaluate the role of McCarey-Kaufman (MK) medium in maintaining the integrity of donor corneal epithelium. **Methods:** Nineteen corneal buttons were harvested and stored in MK media at 2°C–8°C for four days. Serial photographs were done every day till the 3rd day, and images were then analyzed with ImageJ software (LOCI, University of Wisconsin, USA). The area of exposure and epithelial defect (ED) was calculated every day for each corneal button. **Results:** The average age of the donors was 56.5 ± 22.7 years and mean time from death to preservation of the corneal buttons was 7.7 ± 3.1 hours. The average corneal area was 145.6 ± 18.8 mm^2. The total mean area of exposure was 3.6 ± 4.8, 7.2 ± 9.2, and 9.0 ± 11.9 mm^2, and ED was 1.7 ± 4.6, 2.8 ± 5.3, and 3.3 ± 5.9 mm^2 on days 1, 2, and 3, respectively. The percentage of increase in the area of exposure and ED in MK media was 3.71% and 1.1% from day 1 to day 3, respectively. Six out of 19 corneal buttons (31.57%) were utilized for keratoplasties, of which two were utilized in house and four were distributed outside. Of the two utilized corneas, none had epithelial defect on postoperative day 1. Rest 13 corneas were either used for training and research purposes, stored in glycerol media, or discarded. **Conclusion:** Since the percentage change in area of exposure/ED is not much at the end of day 3, corneas stored in MK media can be safely used even after three days of storage. Hence, MK medium serves as an excellent medium in maintaining the integrity of donor corneal epithelium.

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Prior to the advent of storage media, whole globes were stored in ice chests at 4°C.[1] Tissues could be safely kept in moist chambers till 48 hours. The whole donor eye was kept in a sterile jar. McCarey-Kaufman (MK) medium was introduced in 1974, which allowed the storage of donor corneas for 3–4 days at 4°C. It revolutionized the corneal transplantation by converting it from emergency to elective and scheduled procedure. Also, it made the transportation and distribution of donor cornea to faraway places feasible.[2] Since then, many storage media have been described, which extended the storage until 14 days. Organ culture allows effective storage of up to four weeks.[3] In developing countries, where the magnitude of cornea blindness exceeds the available donor corneas, MK media acts as a reliable media for short-term storage.[4] Also it is easy and simple to manufacture. It consists of tissue culture medium (TC 199), 5% dextran, HEPES buffer (N-hydroxyethyl piperazine-N-ethane-sulphonic acid), and an antibiotic like gentamycin.[5]

The function of any storage media is to maintain the integrity of corneal tissue. Although maintaining the viability of donor endothelium is of utmost importance, integrity of donor epithelium is also essential.[6] The donor epithelium is eventually replaced by the host epithelium through the process of mitosis, migration, and hemi-desmosomal attachment.[7] Since these changes takes place eventually, epithelial defects (ED) in the donor cornea can lead to persistent epithelial defects (PED). It might cause complications such as infections, perforations, and graft failures. The role of intact epithelium becomes more pertinent in the cases of ocular surface diseases and host corneas with diminished corneal sensations.[8] Therefore, this article evaluates the efficacy of MK media in preserving the epithelium of donor cornea.

Methods

Human corneas were harvested by an eye bank in a tertiary eye care hospital in eastern India. The study is exempted from the Ethics Committee review. The harvested corneal buttons with 2–3 mm of scleral rim were stored in MK media at the site of retrieval. On reaching the eye bank, they were thoroughly evaluated[9] and preserved in MK media at 2°C –8°C for four days. Most of the corneas were utilized or distributed, and only the corneal buttons not yet utilized or distributed for three days were included in the study. The corneal epithelium was viewed within the vial using slit-lamp biomicroscope (SL 800, Carl Zeiss Meditec, Inc., Germany). Serial photographs (10×, SL Imaging Solution, Carl Zeiss Meditec, Inc., Germany) were done at days 1, 2, and 3.

The images were then analyzed by a single observer using ImageJ software (LOCI, University of Wisconsin, USA) and the

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Table 1: Data of donor corneal buttons and their utilization

Corneal Button Number	Age of Donors (Years)	Time of Death to Preservation (Hours)	Cause of Death	Utilization of Corneal Buttons
1, 2‡	35	11.5	Cardiac Arrest	Distributed, Distributed
3, 4‡	73	5	Cardiac Arrest	Training/Research, Training/Research
5,6‡	76	8	Road Traffic Accident	Discarded, Discarded
7, 8‡	15	4.5	Suicide by Hanging	Keratoplasty, Training/Research
9, 10	50	3	Road Traffic Accident	Training/Research, Training/Research
11, 12	69	10.16	Cardiac Arrest	Distributed, Distributed
13, 14	80	4.5	Chronic Renal Failure	Preserved in Glycerol, Preserved in Glycerol
15, 16	28	8.67	Suicide by Poisoning	Training/Research, Keratoplasty
17, 18	78	11	Chronic Renal Failure	Preserved in Glycerol, Preserved in Glycerol
19*	65	14.33	Cardiac Arrest	Preserved in Glycerol

*All corneal buttons were paired except number 19‡ This represents the corneal buttons harvested from refrigerated bodies

Table 2: Day-wise percentage area of exposure and epithelial defect of donor corneal epithelium

	Percentage of Exposure	Percentage of Epithelial Defect
Day 1	2.47%	1.16%
Day 2	4.94%	1.92%
Day 3	6.18%	2.26%
Total increase from day 1 to day 3	3.71%	1.10%

area of ED and exposure was calculated for each corneal button, daily. Before analyzing, the software was calibrated each time using a constant calibration picture. In the software, the various areas were then marked, and the arithmetic sum was done to calculate the total area of exposure and ED for a given corneal button. The total surface area of anterior corneal surface was also calculated. Exposure was defined as the loss of superficial layers, and ED was defined as the loss of all the layers of donor corneal epithelium. The corneal buttons were either used on the 4th day or were transferred to Cornisol (Aurolab, Madurai, India) media for further usage. Fig. 1 depicts the calculation of exposure and ED in the two corneal buttons.

Statistical analysis was done, and values were calculated in mean, standard deviation, and *P* value using MedCalc v. 20.013 statistical software. A $P < 0.05$ was considered significant.

Results

Nineteen corneal buttons were kept in MK-media and were serially imaged for three consecutive days. Four pairs of corneal buttons were harvested from refrigerated bodies (Group I) and rest 11 were procured from the bodies lying in the normal room temperature (Group II). Table 1 describes the data of 19 corneal buttons and their utilization status, of which nine corneal buttons were paired. The average age of donors was 56.5 ± 22.7 (range: 15–80) years and the mean time of death to preservation (DTP) of the corneal buttons was 7.7 ± 3.1 hours. DTP was 7.25 ± 2.8 and 8.09 ± 3.6 hours in Group I and Group II, respectively. The average corneal area was 145.6 ± 18.8 mm^2.

On evaluation, three donor corneas had no exposure on day 1. However, all nineteen buttons developed exposure from day 2 onwards. Thirteen donor buttons did not have any ED on day 1, of which three developed ED on day 2 and one on day 3. Therefore, ten donor corneal buttons had ED and all had exposure on day 3. The mean total area of exposure was 3.56 ± 4.83 (median: 2.15), 7.19 ± 9.23 (median: 3.14), and 9.03 ± 11.97 (median: 4.96) mm^2 on days 1, 2, and 3, respectively. The increase in total area of exposure from day 1 to day 3 was non-significant ($P = 0.07$). The mean total area of ED was 1.7 ± 4.6, 2.8 ± 5.3, and 3.3 ± 5.9 mm^2 on respective days. The increase in total area of ED from day 1 to day 3 was non-significant ($P = 0.35$). The percentage area of the exposure ([surface area of exposure/average surface area of cornea)] ×100), and percentage area of ED ([surface area of ED/average surface area of cornea] ×100) for days 1, 2, and 3 are described in Table 2. Subgroup analysis for ED and exposure was done for Group I and Group II and is described in Table 3. It was observed that ED and exposure was non-significant ($P < 0.05$) for all three days in both groups.

Out of the 19 corneal buttons, 4 were distributed to other centers, 2 were utilized in the institute for optical keratoplasty, 2 were discarded (Venereal Disease Research Laboratory test positive), 6 were utilized for training and research purposes, and 5 were preserved in glycerol for future use. No epithelial defects were seen on day 1 postoperatively in the two patients who were operated at our institute.

Discussion

With a prevalence of 4.9 million cases of bilateral corneal blindness worldwide, corneal transplantation remains the definitive treatment for visual rehabilitation.[10] In a developing country like India, the rate of procurement and utilization is disproportionately less than a developed country. Various factors affect the utilization rate, among which storage media plays a major role. The most commonly used media in India is MK media, as opposed to organ culture or Optisol-GS (Bausch and Lomb, Rochester, New York, USA) media in developed countries.[11]

In 1994, Ramayamma International Eye Bank, India with the aid of International Federation of Eye and Tissue Bank Baltimore, USA had undertaken the initiative of creating a facility for production of MK media for India and its neighboring countries.[12] It had allowed the storage and transportation of donor corneal tissues feasible up to 72–96 hours in a developing country like India as opposed

Table 3: Day-wise exposure and epithelial defect of two groups

	Epithelial Defect (Mean±SD) in mm²			Exposure (Mean±SD) in mm²		
	Group I	Group II	*P*	Group I	Group II	*P*
Day 1	0.23±0.39	2.80±5.81	0.23	3.74±3.03	3.43±5.80	0.89
Day 2	2.34±3.86	3.15±6.09	0.74	10.10±11.77	5.07±5.98	0.24
Day 3	3.32±5.62	3.32±6.09	1.00	12.71±16.21	6.36±6.28	0.25

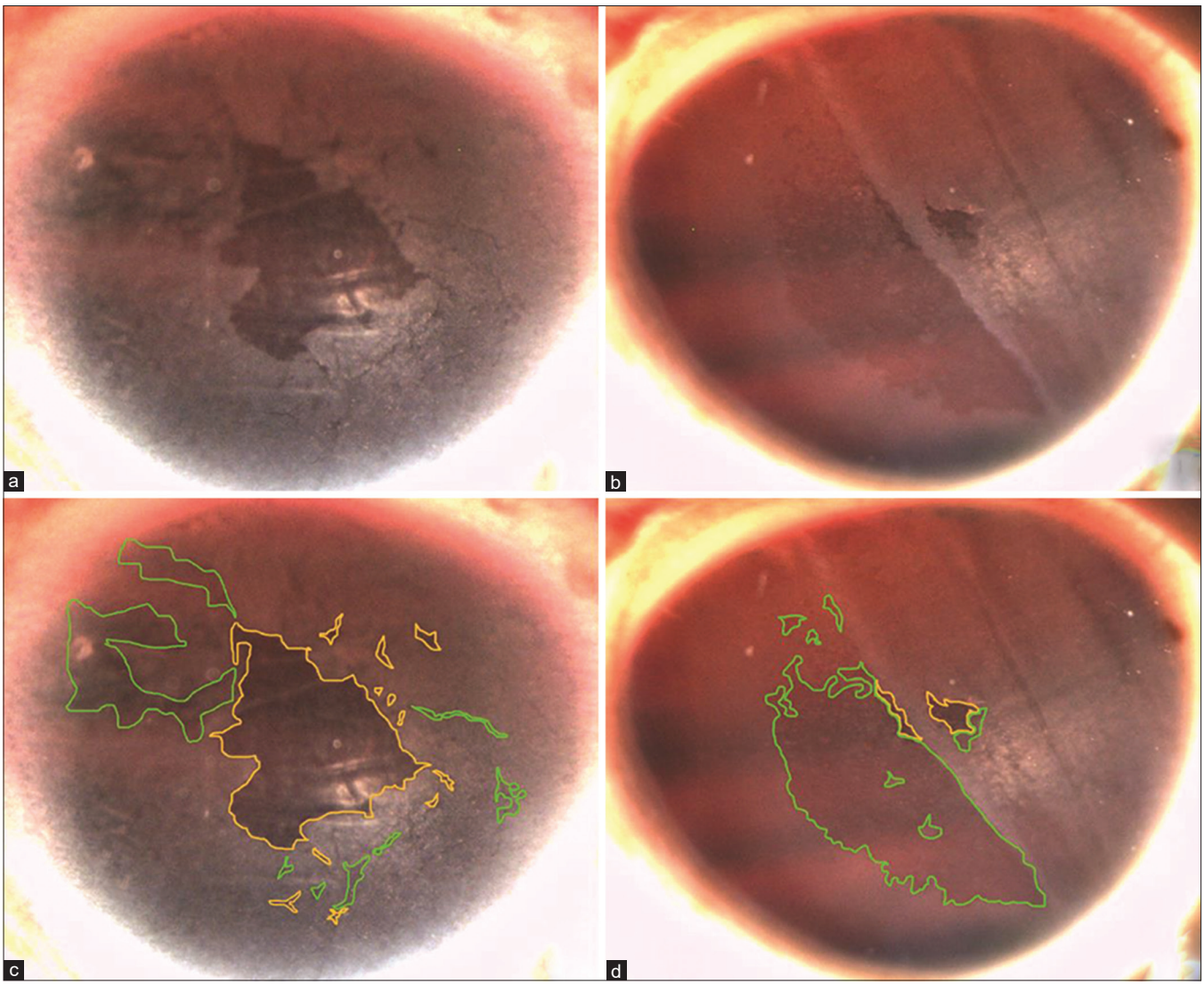

Figure 1: (a and b) Depicts the donor corneal buttons photographed in diffuse illumination of slit-lamp biomicroscope in the vial; (c and d) Green depicts the exposure and yellow border depicts the epithelial defect. Their respective areas were then calculated on ImageJ software

to 24-hour storage offered by moist chambers. Though the endothelial cell counts are slightly lower in the donor tissue transferred from MK media to other intermediate storage media as compared to those tissues which are stored in the intermediate media from the beginning,[13] relative early utilization of donor corneas (vast deficit between number of tissues harvested and demand)[4] and cheap manufacturing of MK media[11,14] has made it the practice pattern in our eye bank to store corneal tissue in MK media for four days. Donor corneas which are non-utilized on the 3rd day are transferred to other intermediate storage media (Cornisol in our case).

Various studies have evaluated the efficacy of MK media for maintaining endothelial integrity,[15,16] but its role in maintaining the integrity of epithelium of donor cornea has often been overlooked. ED in donor cornea may lead to PED and can subsequently cause infections, scarring, perforations, or graft failures. Pre-existing ED can delay the recovery in patients especially those with prior ocular surface disorders.[17–19] To minimize the damage while direct handling of tissues,[20] donor corneal epithelium was examined in the vials itself. Using the diffuse illumination of slit-lamp biomicroscope, serial photographs were done for three consecutive days. The

area of exposure and ED was calculated using the ImageJ software. This image processing software is developed by the National Institutes of Health and serves as an excellent platform for analysis for both non-programmers and professional developers.[21,22] In our study, it was observed that 13 out of 19 corneal tissues (68.4%) stored in MK media had no ED on the 1st day, but by the end of the 3rd day, only 9 out of 19 (47.4%) did not have ED. Exposure is the precursor of ED and all corneal buttons (100%) had exposure by day 3. The percentage increase of area of exposure and ED in MK media was 3.71% and 1.1% from day 1 to day 3, respectively.

Prior studies on the effect of MK media on the integrity of donor epithelium are lacking, but similar studies exist for other media. Greenbaum[8] *et al*. have observed that there was no significant differences between Optisol-GS and Dexsol (Chiron Ophthamics, Irvine, CA, USA) for the magnitude of epithelial loss. Similar to our study, it was noticed by them that the epithelial loss correlated with the duration of storage. Soni *et al*.[23] conducted a similar study and observed that there was no significant difference between Optisol-GS and Life 4°C (Numedis, Inc., Minneapolis, USA) media for preserving the donor epithelium. They also observed that most cornea developed ED within the 14-day observation period. For ED and exposure, subgroup analysis was also done (tissue harvested from refrigerated cadaver vs non-refrigerated cadaver), which was found to be non-significant in our study. However, the subgroup sample size was small and needs further evaluation.

This affirms that MK media acts as a good storage media for the donor corneal epithelium. Since the percentage area of ED is not much at the end of the 3rd day of preservation, corneas stored in the MK media can be used for various surface disorders or keratoplasty in a host with decreased corneal sensations. Apart from the storage media, the integrity of donor corneal epithelium may also depend upon the death-to-preservation time, exposure to the environment after death, and mechanical movement of the tissues stored in the media. Therefore, to add to this study, a study on the various factors affecting the health of donor epithelium before its placement in MK media needs to be studied. Also, a comparative study between MK medium and other storage media to further substantiate the efficacy of MK medium in maintaining the integrity of donor corneal epithelium as any other medium is obligatory. Further, to ensure repeatability of readings, storage-cum-viewing (where tissue can be fixed) can be used. This will ensure lesser variations in the angulations while viewing and analyzing the tissue.

Conclusion

MK medium serves as an excellent medium in maintaining the integrity of donor corneal epithelium. Corneas stored in MK media can be safely used even after three days of storage.

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Conflicts of interest

There are no conflicts of interest.

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