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# Sinasol versus Optisol-GS for cold preservation of human cornea: a prospective ex vivo and clinical study

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**Abstract** To compare ex vivo results of donor corneas maintained in Sinasol with those stored in Optisol-GS and reporting clinical outcomes of grafted Sinasol-versus Optisol-GS-stored corneas. In phase I, paired donor corneas were maintained in Sinasol or Optisol-GS. Afterward, the corneas were subjected to slit-lamp biomicroscopic and specular microscopic examinations on days 1 and 7, and then to trypan blue staining on day 7. The same examinations were performed on the corneas that were kept in Sinasol or Optisol-GS for 14 days. In phase II, the post-operative reports of 72 consecutive corneal transplantations were recorded using Sinasol- or Optisol-GS-preserved corneas. In phase I, 128 corneas from 64 donors and 59 corneas from 33 donors were investigated for 7 and 14 days, respectively. The EC indices were comparable between the groups at the measurement periods. The EC losses over 7 and 14 days were 3.7% and 19.9% in Sinasol against 4.6% and 20.8% in Optisol-GS. Although fair quality corneas were more common in Optisol-GS group after 7 ($P = 0.04$) and 14 days ($P = 0.034$), changes of stromal edema, Descemet's fold, and other quality ratings during 14 days were not different between the groups. In phase II, all the transplanted corneas were postoperatively clear with no adverse reactions. The overall results indicate that Sinasol is a safe, effective, and affordable intermediate cold storage medium for preservation of corneas.

**Keywords** Sinasol · Optisol-GS · Donor corneas · Corneal transplantations

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## Introduction

Donor corneal endothelial integrity is considered as a fundamental factor for a successful penetrating and/or endothelial keratoplasty; therefore, a proper storage system is necessitated to maintain the corneal endothelial cells (ECs) viability and also to minimize post-mortem cell lysis during the storage (Feizi 2016;

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Wilson and Bourne 1989). The history of corneal preservation backs to 1911 when the donated corneas were stored in a hemolyzed blood serum at 5–7 °C (Patel 2017). Since then, 3 types of corneal storage media including short—(moist chamber for 1 day at 4 °C and McCarey–Kaufman for 2–4 days at 4 °C) (Wilson and Bourne 1989; Patel 2017), intermediate—(K-sol, Dexol, Optisol-GS, Procell, Eusol-C, Chen, Life4C, and Cornisol, all for 14 days at 4 °C, and Cornea Cold® for ≤ 21 days at 4 °C) (Wilson and Bourne 1989; Patel 2017; Jeng 2006; Kanavi et al 2015; Nelson et al 2000; Pham et al 2013; Basak and Prajna 2016; Parekh et al 2014), and long-term (European organ culture for 120 days at 37 °C, Eurosol for 28 days at 31 °C, and cryopreservation for unlimited period at − 80 °C) (Patel 2017) maintenance media have been introduced. In contrast to the organ culture (warm storage) media that are popular in the European eye banks, hypothermic storage media, especially Optisol-GS, are known as the most preferred maintenance option in the United states and Asian eye banks including the Central Eye Bank of Iran (Wilson and Bourne 1989; Kanavi et al 2015; Møller-Pedersen et al 2001; Parekh et al 2015).

Sinasol (made by Sinadarou Labs Company, Tehran, Iran, ordered by the Central Eye Bank of Iran) is a recently manufactured intermediate-term cold storage medium (unpublished data), which aimed to achieve self-sufficiency, national production, cost-effectiveness, and accessibility for Iran and other countries. In this regard, it is notable that the components of Sinasol and Optisol-GS are almost similar except in the lack of accessory components (Table 1) (Basak and Prajna 2016; Parekh et al 2014). The Medical Equipment Department of the Iran Ministry of Health and Medical Education approved Sinasol for cold maintenance of the donated corneal tissues up to 14 days. Given that, except the in-house data from the Sinadarou Labs Company, there was no published data on comparing the Sinasol with Optisol-GS, so this study was designed to perform this in two phases. In the 1st phase, a prospective randomized ex vivo study was conducted to compare these two media and also to determine any superiority of one medium over another in the preservation of donated corneas in terms of qualitative and quantitative indices over 7 and 14 days of storage. In the second phase, the results of graft clarity and the rates of post-operative adverse reactions that were reported to the Central Eye Bank of Iran after transplantation of Sinasol- and Optisol-GS-preserved corneas, were investigated.

## Materials and methods

The prospective ex vivo and the clinical protocols were reviewed and approved by the Institutional Review Board of the Central Eye Bank of Iran and the ethics committee of the Ophthalmic Research Center affiliated with the Shahid Beheshti University of Medical Sciences, Tehran-Iran (ethical approval committee code number # IR.SBMU.ORC.REC.1395.14). For the second phase of the study, the signed informed consent was obtained from the cornea recipients enrolled in the study in terms of the principles of the Declaration of Helsinki.

### Phase I (prospective randomized ex vivo investigations)

#### *Donated corneas*

Corneas from donors with death to preservation time less than 30 h, a minimum of ‘‘an apparent good cornea rating’’ on slit-lamp biomicroscopy (Kanavi et al 2015), and not suitable for surgery due to a reactive serologic result were enrolled in this phase of the study at the Central Eye Bank of Iran. Corneas with apparent fair endothelial quality or from donors aged < 3 and over > 65 years were excluded. In this prospective single blind comparative study, a total of 128 corneas from 64 donors were investigated for 7 days; one cornea from each donor was arbitrarily preserved in Optisol-GS (Bausch & Lomb, Inc., Rochester, NY, USA) and the mate cornea was stored in Sinasol (Sinadarou Labs Company, Tehran, Iran) at 4 °C. To obtain a single masked study, the commercial labels of the media were covered and then re-labeled as medium#1 and medium#2 for Optisol-GS and Sinasol, respectively. Afterward, all the corneas were subjected to slit-lamp biomicroscopic and specular microscopic examinations on days 1 and 7. On day 7, the viability of the ECs was assessed by trypan blue staining. Apart from these corneas, 59 corneas from 33 donors were also stored in Sinasol or Optisol-GS for 14 days, which were then subjected to slit-lamp biomicroscopic and specular microscopic examinations, as well as trypan blue staining.

**Table 1** Compositions of Sinasol, Optisol-GS, Cornisol, and Cornea Cold®

| Composition | Optisol-GS[8,9] | Sinasol | Cornisol[8] | Cornea Cold®[9] |
|---|---|---|---|---|
| Basic Components | A mixture of TC-199 and MEM-Earle media | MEM | MEM | MEM |
| Buffer | HEPES | HEPES | HEPES | HEPES |
| Antibiotics | Gentamicin | Gentamicin | Gentamicin | Present |
| | Streptomycin | Penicillin–Streptomycin | Streptomycin | |
| Others | Sodium bicarbonate | Sodium bicarbonate | Sodium bicarbonate | Sodium bicarbonate |
| | Chondroitin sulfate | Chondroitin sulfate | Chondroitin sulfate | Dextran-T500 |
| | Dextran-T40 | Dextran T70 | Dextran-T40 | Pyruvate |
| | Pyruvate | | Nonessential aminoacids | Nonessential aminoacids |
| | Nonessential aminoacids | | Human recombinant insulin | Vitamins |
| | 2-mercaptoethanol | | | *L*-glutamine |
| | Ascorbic acid | | | |
| | Vitamins | | | |
| | ATP precursors | | | |
| | *L*-glutamine | | | |

*Slit-lamp biomicroscopy*

The qualitative indices on slit-lamp biomicroscopy, including corneal stromal edema and Descemet's folding, were graded by an expert eye bank technician (T.Ch), which were then confirmed by an ophthalmologist/eye bank specialist (MRK). The grading protocol for the tissues followed the same protocol recommended by the Eye Bank Association of America and the European Eye Bank Association (EBAA 2013; EEBA 2020). Briefly, the stromal edema and Descemet's folding were separately graded as none, mild, moderate, and severe.

*Specular microscopy*

At the Central Eye Bank of Iran, each pair of corneas was examined by an eye bank technician (T.Ch) using a specular microscope (KeratoAnalyzer EKA-10; Konan Medical Inc., Hyogo, Japan) in similar time periods (days 1 and 7) and the obtained results were rechecked by the ophthalmologist/eye bank specialist (MRK). Before performing specular microscopy, the corneal tissues were thawed for 30 min, and four specular microscopic images were taken from four different parts of the corneal endothelium at the central and paracentral regions per each cornea. Moreover, the center mode of cell counting was used to quantify EC density (ECD), percentage of hexagonality, percentage of polymegethism, and mean EC area (MECA). Based on the obtained specular microscopic images, the EC vacuolation was graded as minimal (1–2), few (3–7), moderate (> 8), and numerous (when half of the field was vacuolated). Finally, considering the specular microscopic data, the overall corneal endothelial rating was determined as fair, good, very good, and excellent (Feizi et al 2014).

*Trypan blue staining for detection of dead ECs or denuded Descemet's membrane*

On days 7 and 14, all corneal tissues preserved in Sinasol and Optisol-GS were subjected to staining with trypan blue, as it was described earlier (Sperling 1986; Pels and Rijneveld 2009). Briefly, after removing the cornea from the corresponding storage medium, rinsing with PBS, and placing the endothelial side on a sterile Petri dish, one to two drops of 0.4% trypan blue solution were put on the endothelial side and remained there for 45 s. Subsequently, the scleral rim of the cornea was grabbed with a forceps and after shaking off the dye, the cornea was rinsed with BSS to remove the remaining trypan blue. Afterward, the endothelium was inspected using light microscopy

(BX41, Olympus, Tokyo, Japan) and photographed from 2 to 3 different areas with a digital camera (DP12 Microscope Camera, Olympus, Tokyo, Japan). This investigation was performed for each pair of corneas almost at the same time period. Moreover, the corresponding photographs were reviewed in terms of the presence of the blue stained nuclei of membrane-damaged cells and denuded Descemet's membrane. Also, the corresponding area was quantified using Image J software (National Institutes of Health, Bethesda, Md., USA) via the pathway of setting the scale, splitting channels, adjusting the image threshold, and analyzing the particles.

Phase II (post-transplantation investigation)

The eligibility criteria for the donated corneas applied in this phase of study were corneas from donors with death to preservation time less than 30 h, a minimum of ''an apparent good cornea rating'' on slit-lamp biomicroscopy (Kanavi et al 2015), and having nonreactive serologic results for hepatitis B virus, hepatitis C virus, human immune deficiency virus 1&2, human T cell lymphoma virus 1&2, and syphilis. After preserving the corneas in Sinasol or Optisol-GS, they were subjected to slit-lamp biomicroscopic and specular microscopic examinations, and while preserving at 4 °C, they were transplanted after a 3-day preservation. In a prospective single-masked study, the corneas preserved in Optisol-GS or Sinasol were randomly allocated to the patients that had been assigned for a penetrating keratoplasty in three university-based referral ophthalmic centers. No limitation, in terms of sex, age, or indication for keratoplasty, was considered when allocating the corneas. Post-transplantation data in terms of graft clarity and the rates of post-operative adverse reactions that were reported to the Central Eye Bank of Iran, were investigated.

Statistical analyses

To describe the data, we used frequency (percent), mean ± standard deviation, median, and range. T-test was also implemented to assess the differences between the groups at the measurement time periods in terms of quantitative indices. In this regard, for the qualitative indices, Chi-square and fisher exact tests were used to analyze the differences between the studied groups. Moreover, T-test was also used to evaluate the changes of the quantitative indices over the study period between the groups. All the statistical analyses were performed by SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). *P* value of less than 0.05 was considered as statistically significant.

## Results

Phase I: ex vivo results of corneas stored in Optisol-GS versus those kept in Sinasol

A total of 64 pairs with donors' mean age of 44.3 ± 11.6 years old (range of 18–60 years) were enrolled in the phase I of the study. All pairs were harvested as whole globe enucleation and of phakic status. Sixty-four corneas were maintained in Optisol-GS and the 64 mate corneas in Sinasol. Regarding the slit-lamp biomicroscopic examinations (Table 2), the grading of stromal cloudiness was not statistically different between these two groups on days 1 and 7 ($P > 0.999$). Although the rate of moderate to severe Descemet's folding was higher in the corneas stored in Sinasol compared to those kept in Optisol-GS on day 1 ($P = 0.04$), it showed a significantly lower rate after 7-days storage in comparison with the Optisol-GS group ($P = 0.001$). The changes of stromal edema were also significant for both groups over the 7-day period ($P = 0.015$ for Sinasol and $P = 0.013$ for Optisol-GS); however, there was no significant difference between these two media ($P = 0.92$). Unlike the significant changes of Descemet's folding in Optisol-GS group ($P < 0.001$), no remarkable change was observed in the Sinasol group over the 7-day storage ($P = 0.292$). Accordingly, the changes of Descemet's folding were not significantly different between the two groups ($P = 0.17$).

The statistical comparisons of EC indices of the donated corneas preserved in Optisol-GS and Sinasol were illustrated in Table 3. The ECD, coefficient of variation, percentage of hexagonality, MECA, EC vacuolation, and mortality were compared between the groups at the measurement periods. Notably, the EC loss over 7 days was 3.7% in the Sinasol group and 4.6% in the Optisol-GS group ($P = 0.31$). A decreasing pattern was also observed for ECD in both groups

**Table 2** Statistical comparison of qualitative indices of the donated corneas preserved in Optisol-GS and Sinasol for 7 days

| | Day 1 | | *P*-between | Day7 | | *P*-between | *P*-within Sinasol (1–7) | *P*-within Optisol-GS (1–7) | *P*-between (1–7) |
|---|---|---|---|---|---|---|---|---|---|
| | Sinasol | Optisol-GS | | Sinasol | Optisol-GS | | | | |
| *Stromal edema* | | | | | | | | | |
| No-Mild | 63 (98.4%) | 64 (100.0%) | >0.999 | 50 (78.1%) | 50 (78.1%) | >0.999 | | | |
| Moderate-Severe | 1 (1.6%) | 0 (0.0%) | | 14 (21.9%) | 14 (21.9%) | | 0.015 | 0.013 | 0.92 |
| Total | 64 (100.0%) | 64 (100.0%) | | 64 (100.0%) | 64 (100.0%) | | | | |
| Descemet's folding | | | | | | | | | |
| No-Mild | 49 (76.6%) | 61 (95.3%) | 0.004 | 47 (73.4%) | 27 (42.2%) | 0.001 | | | |
| Moderate-Severe | 15 (23.4%) | 3 (4.7%) | | 17 (26.6%) | 37 (57.8%) | | 0.292 | <0.001 | 0.17 |
| Total | 64 (100.0%) | 64 (100.0%) | | 64 (100.0%) | 64 (100.0%) | | | | |

over a 7-day period; however, this trend was not statistically significant within ($P = 0.203$ for Optisol GS and $P = 0.568$ for Sinasol) and between the groups ($P = 0.824$). Unlike EC hexagonality that exhibited a downward slope, the EC polymegathism, MECA, and EC vacuolation showed upward trends from day 1 to day 7 in both groups. In addition, the change in the hexagonality trend was statistically significant per each group ($P < 0.001$); however, it did not show a significant intergroup difference ($P = 0.22$). The EC polymegathism, MECA, and EC vacuolation exhibited no significant difference between the groups ($P = 0.703$, $P = 0.598$, and $P = 0.74$, respectively). Unlike EC polymegathism and MECA, there was a significant change in terms of EC vacuolation in each group over the 7-day storage period ($P = 0.003$ and $P = 0.002$ for Sinasol and Optisol-GS, respectively).

After 7 days, although there was no significant difference in the good and very good overall endothelial rates between the two groups ($P = 0.277$ and $P = 0.376$, respectively), the fair quality corneas were more common in the Optisol-GS than the Sinasol group ($P = 0.04$). Notably, none of the corneas were rated as excellent on day 7. With respect to the overall cornea rating, except the changes of cornea rating to fair quality ($P = 0.009$), there was no significant change for other quality ratings between the two groups from day 1 to day 7. In the Sinasol group, all quality ratings except the "fair" quality, showed remarkable changes over the 7-days storage (Table 3).

The mean area of trypan blue stained nuclei of membrane-damaged ECs along with denuded Descemet's membrane in the corneas kept for 7 days in Sinasol, was 135,392.6 ± 168,392.8 $\mu m^2$ (range of 9257–541,336.5 $\mu m^2$) against 154,587.7 ± 189,873.6 $\mu m^2$ (range of 4997.5–535,614.5 $\mu m^2$) for the corneas stored in Optisol-GS ($P = 0.771$) (Fig. 1).

## Phase I: ex vivo results of corneas stored in Sinasol and Optisol-GS for 14 days

Thirty-one corneas from 19 sero-positive donors aged between 16 and 65 years old (44.7 ± 13.4) were preserved in Sinasol for 14 days. Twenty-eight corneas from 14 sero-converted donors aged between 18 and 59 years old (36.8 ± 14.3) were also stored in Optisol-GS for 2 weeks. After a 14-days storage, more than half of the corneas in both groups were graded as "moderate" or "severe" for stromal edema, and all the preserved corneas demonstrated moderate to severe Descemet's folding with no significant difference between the two groups (Table 4). Over the 2-week storage, significant changes of stromal edema and Descemet's folding were observed in each group ($P < 0.001$).

EC indices of the corneas preserved in Sinasol were comparable with those in Optisol-GS after 2 weeks (Table 5), and the EC loss over 14 days in Optisol-GS group was similar to that in Sinasol group (20.8% vs. 19.9%, $P = 0.861$). The changes in the trends of EC hexagonality, polymegathism, and MECA from day 1 to day 14 were statistically significant per each group ($P < 0.001$); however, they did not show a significant intergroup difference. Unlike EC polymegathism and

**Table 3** Statistical comparison of quantitative indices of the donated corneas preserved in Optisol-GS and Sinasol for 7 days

| | Day 1 | | *P*-between | Day 7 | | *P*-between | *P*-within Sinasol (1–7) | *P*-within Optisol-GS (1–7) | *P*-between (1–7) |
|---|---|---|---|---|---|---|---|---|---|
| | Sinasol | Optisol-GS | | Sinasol | Optisol-GS | | | | |
| ECD (cell/mm$^2$) | 2829 ± 423 | 2946 ± 457 | 0.135 | 2723 ± 419 | 2835 ± 493 | 0.18 | 0.568 | 0.203 | 0.824 |
| | 2778[a] (2028–4000) | 2849[a] (2020–4149) | | 2721[a] (1272–3690) | 2786[a] (1508–4291) | | | | |
| Hexagonality (%) | 54 ± 10 | 55 ± 11 | 0.613 | 48 ± 11 | 46 ± 11 | 0.36 | <0.001 | <0.001 | 0.22 |
| | 56[a] (30 to 75) | 57[a] (28 to 76) | | 48[a] (23 to 70) | 48[a] (25 to 65) | | | | |
| Polymegathism (%) | 38 ± 6 | 38 ± 9 | 0.896 | 41 ± 7 | 40 ± 9 | 0.519 | 0.086 | 0.119 | 0.703 |
| | 38[a] (28–54) | 37[a] (25–71) | | 40[a] (29–62) | 39[a] (26–75) | | | | |
| MECA (μm$^2$) | 344 ± 56 | 338 ± 56 | 0.761 | 361 ± 76 | 353 ± 80 | 0.413 | 0.638 | 0.233 | 0.598 |
| | 334[a] (238–493) | 328[a] (242–495) | | 359[a] (271–786) | 351[a] (370–663) | | | | |
| *Vacuolation* | | | | | | | | | |
| Minimal to Few | 55 (85.9%) | 60 (93.8%) | 0.241 | 32 (50.0%) | 34 (53.1%) | 0.86 | 0.003 | 0.002 | 0.74 |
| Moderate to Numerus | 9 (14.1%) | 4 (6.3%) | | 32 (50.0%) | 30 (46.9%) | | | | |
| *Quality* | | | | | | | | | |
| Fair | 0 (0.0%) | 0 (0.0%) | – | 3 (4.7%) | 14 (21.9%) | 0.004 | 0.19 | 0.013 | 0.009 |
| Good | 15 (23.4%) | 13 (20.3%) | 0.699 | 28 (43.8%) | 22 (34.4%) | 0.277 | 0.015 | 0.034 | 0.351 |
| Very good | 41 (64.1%) | 37 (57.8%) | 0.469 | 33 (51.6%) | 28 (43.8%) | 0.376 | 0.043 | 0.034 | 0.795 |
| Excellent | 8 (12.5%) | 14 (21.9%) | 0.16 | 0 (0.0%) | 0 (0.0%) | – | 0.043 | 0.013 | 0.164 |

*ECD* endothelial cell density, *MECA* mean endothelial cell area

[a]Mode

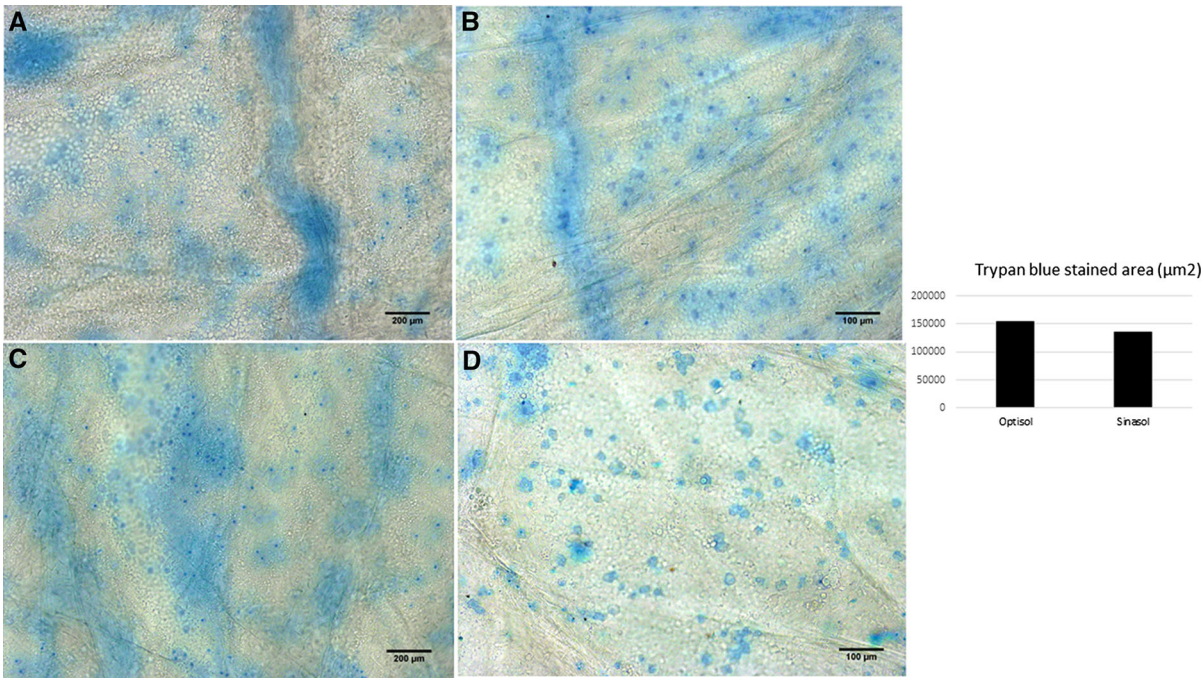


**Fig. 1** Representative microphotographs of trypan-blue stained corneal endothelium after 7 days storage in Optisol-GS and Sinasol. Note the blue stained nuclei of membrane-damaged endothelial cells along with denuded Descemet's membrane in the corneas kept in Optisol-GS (**a**, **b**) against Sinasol (**c**, **d**), and no significant difference between the groups in the representative graph

**Table 4** Statistical comparison of qualitative indices of the donated corneas preserved in Sinasol and Optisol-GS for 14 days

| | Day 1 | | *P*-between | Day14 | | *P*-between | *P*-within Sinasol (1–14) | *P*-within Optisol-GS (1–14) | *P*-between (1–14) |
|---|---|---|---|---|---|---|---|---|---|
| | Sinasol | Optisol-GS | | Sinasol | Optisol-GS | | | | |
| *Stromal edema* | | | | | | | | | |
| No-mild | 30 (96.8%) | 28 (100.0%) | >0.999 | 10 (32.3%) | 12 (42.9%) | 0.488 | <0.001 | <0.001 | 0.563 |
| Moderate-severe | 1 (3.2%) | 0 (0.0%) | | 21 (67.7%) | 16 (57.1%) | | | | |
| Total | 31 (100.0%) | 28 (100.0%) | | 31 (100.0%) | 28 (100.0%) | | | | |
| *Descemet's folding* | | | | | | | | | |
| No-mild | 23 (74.2%) | 27 (96.4%) | 0.038 | 0 (0.0%) | 0 (0.0%) | >0.999 | <0.001 | <0.001 | 0.041 |
| Moderate-severe | 8 (25.8%) | 1 (3.6%) | | 31 (100.0%) | 28 (100.0%) | | | | |
| Total | 31 (100.0%) | 28 (100.0%) | | 31 (100.0%) | 28 (100.0%) | | | | |

MECA that showed upward slopes from day 1 to day 14, the EC hexagonality exhibited a decreasing trend in both groups. "Moderate" or "numerous" EC vacuolation was also observed in almost all corneas in both groups after a 14-day storage ($P < 0.949$) and demonstrating significant changes in comparison to those in day 1 in each group ($P < 0.001$) but the corresponding changes of trend over the 2-week period were not different between the groups ($P > 0.999$) (Table 5).

After 14 days, the majority of the corneas kept in Sinasol and all the corneas kept in Optisol-GS

**Table 5** Statistical comparison of quantitative indices of the donated corneas preserved in Sinasol and Optisol-GS for 14 days

| | Day 1 | | *P*-between | Day 14 | | *P*-between | *P*-within Sinasol (1–14) | *P*-within Optisol-GS (1–14) | *P*-between (1–14) |
|---|---|---|---|---|---|---|---|---|---|
| | Sinasol | Optisol-GS | | Sinasol | Optisol-GS | | | | |
| ECD (cell/mm$^2$) | 2804 ± 423 | 2880 ± 383 | 0.782 | 2245 ± 589 | 2282 ± 699 | 0.851 | <0.001 | <0.001 | 0.861 |
| | 2315[a] (2028–3623) | 2712[a] (2513–3650) | | 2421[a] (526–3390) | 2265[a] (743–3305) | | | | |
| Hexagonality (%) | 55 ± 9 | 54 ± 10 | 0.849 | 39 ± 10 | 41 ± 10 | 0.430 | <0.001 | 0.001 | 0.548 |
| | 56[a] (33–75) | 54[a] (33–77) | | 45[a] (20–59) | 42[a] (25–66) | | | | |
| Polymegathism (%) | 38 ± 7 | 38 ± 8 | 0.933 | 47 ± 12 | 48 ± 9 | 0.599 | 0.001 | 0.003 | 0.730 |
| | 31[a] (28–54) | 38[a] (25–54) | | 37[a] (28–69) | 47[a] (30 to 63) | | | | |
| MECA (μm$^2$) | 352 ± 62 | 354 ± 42 | 0.889 | 459 ± 132 | 460 ± 82 | 0.792 | <0.001 | <0.001 | 0.280 |
| | 432[a] (238–493) | 332[a] (274–398) | | 413[a] (295–856) | 438[a] (227–604) | | | | |
| *Vacuolation* | | | | | | | | | |
| Minimal to few | 27 (87.1%) | 24 (85.7%) | > 0.999 | 0 (0.0%) | 1 (3.6%) | 0.949 | <0.001 | <0.001 | >0.999 |
| Moderate to numerus | 4 (12.9%) | 4 (14.3%) | | 31 (100.0%) | 27 (96.4%) | | | | |
| *Quality* | | | | | | | | | |
| Fair | 0 (0.0%) | 0 (0.0%) | – | 12 (38.7%) | 15 (53.6%) | 0.377 | <0.001 | <0.001 | 0.034 |
| Good | 8 (25.8%) | 3 (10.7%) | 0.248 | 15 (48.4%) | 13 (46.4%) | >0.999 | 0.113 | 0.006 | 0.269 |
| Very good | 19 (61.3%) | 17 (60.7%) | > 0.999 | 4 (12.9%) | 0 (0.0%) | 0.05 | <0.001 | <0.001 | 0.344 |
| Excellent | 4 (12.9%) | 8 (28.6%) | 0.242 | 0 (0.0%) | 0 (0.0%) | – | 0.112 | 0.004 | 0.144 |

*ECD* endothelial cell density, *MECA* mean endothelial cell area

[a]Mode

demonstrated fair or good overall endothelial rating. Except the changes of cornea rating to fair quality ($P = 0.034$), there was no significant change for other quality ratings between the two groups from day 1 to day 14. Although a very good overall endothelial rate was observed in 12.9% after the 14-day storage period in Sinasol, it was not statistically different from that in Optisol-GS ($P = 0.05$). None of the corneas were rated as excellent on day 14; however, 61.3% of the corneas in the Sinasol group revealed a good or very good endothelial quality rating after 2 weeks. In the Sinasol group, except the significant change in "fair" and "very good" quality ratings over the 14-day storage ($P < 0.001$), no remarkable change was observed in other quality ratings (Table 5).

The mean area of trypan blue stained nuclei of membrane-damaged ECs along with denuded Descemet's membrane in the corneas kept in Sinasol (498,664.8 ± 514,602.5 $\mu m^2$, ranged from 105,963 to 1,827,007 $\mu m^2$) for 14 days was comparable with that in Optisol-GS (566,741.7 ± 479,398.4 $\mu m^2$, range of 172,637–1,531,880 $\mu m^2$) ($P = 0.668$) (Fig. 2).

Phase II (post-transplantation results)

Between August 2018 and November 2019, a total of 72 corneas from 36 donors aged from 20 to 55 years old (30.4 ± 7.9) were preserved in Sinasol or Optisol-GS, and then transplanted after a 3-day preservation in 72 recipients. Thirty-six patients received corneas stored in Sinasol and the other 36 received corneas preserved in Optisol-GS. Characteristics of the donated corneas and the recipients of the corresponding corneas were illustrated in Table 6. No significant difference was noted between the two groups in terms of mean ECD of the donated corneas (3151 ± 304 cell/$mm^2$, ranged from 2604 to 3906 versus 3153 ± 297 cell/$mm^2$, ranged from 2618 to 3953; $P = 0.972$) and the overall quality rating. The cornea recipients in the Sinasol group were not significantly different from those in the Optisol-GS group in terms of age, sex, and indications for keratoplasty. Postoperative reports to the eye bank disclosed clarity of the grafted corneas in all cases of both groups and no evidence of adverse reactions was reported up to 22 months after surgery.

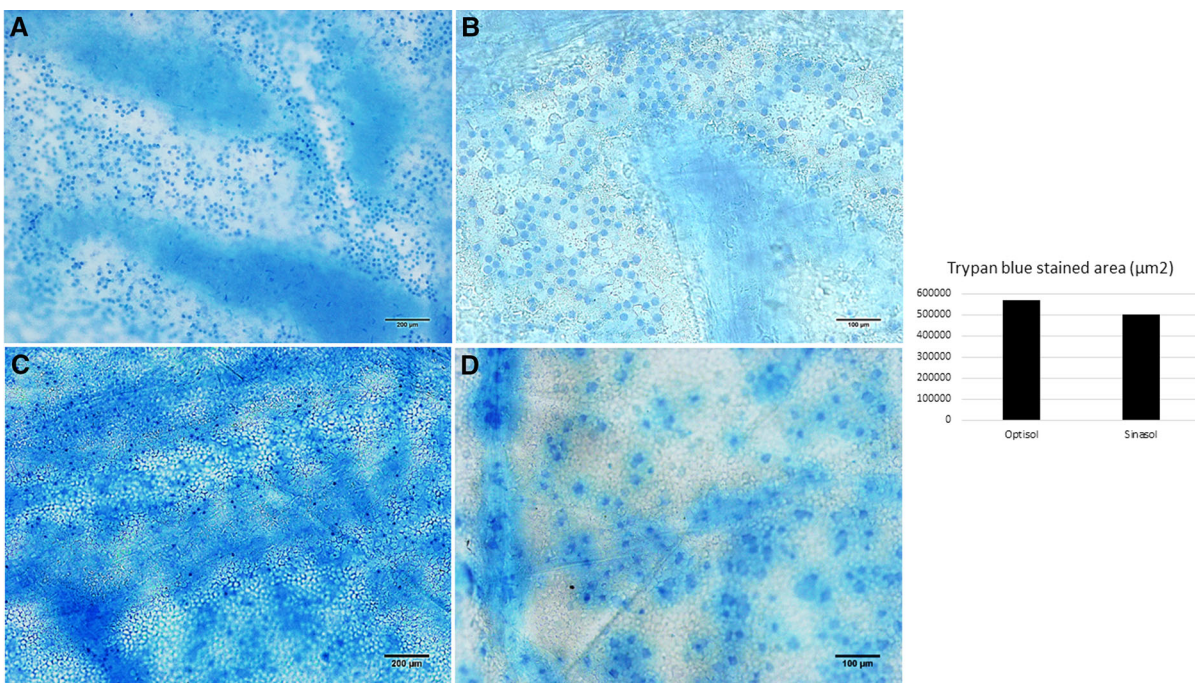


**Fig. 2** Representative microphotographs of trypan-blue stained corneal endothelium after 14 days storage in Optisol-GS and Sinasol. The blue stained nuclei of membrane-damaged endothelial cells along with denuded Descemet's membrane in the corneas kept in Optisol-GS are illustrated in images **a**, **b**, and those of the corneas stored in Sinasol are shown in images **c**, **d**, demonstrating no significant difference between the groups in the illustrative graph

**Table 6** Donor corneal tissue and recipient criteria in the Sinasol and Optisol-GS groups

| Donor corneas/recipients | Sinasol group (n = 36) | Optisol-GS group (n = 36) | *P* value |
|---|---|---|---|
| Donor corneal ECD (cell/mm$^2$) | 3151 ± 304 | 3153 ± 297 | 0.972 |
| *Donor corneal overall rating* (n, %) | | | |
| Excellent | 1 (12.8%) | 1 (2.8%) | 0.315 |
| Very good | 35 (97.2%) | 34 (94.4%) | |
| Good | 0.0% | 1 (2.8%) | |
| Age (year) | 45.7 ± 14.4 | 37.1 ± 20.5 | 0.061 |
| *Sex* | | | |
| Male | 26 (72.2%) | 25 (69.4%) | 0.755 |
| Female | 10 (27.8%) | 11 (30.6%) | |
| *Indications for keratoplasty* | | | |
| *Keratoconus* | 14 (38.9%) | 13 (36.1%) | 0.282 |
| Failed previous grafts | 10 (27.8%) | 11 (30.6%) | |
| Pseudophakic bullous keratopathy | 1 (2.8%) | 6 (16.7%) | |
| Corneal stromal dystrophies | 6 (16.7%) | 4 (11.1%) | |
| Corneal scars and opacities | 3 (8.3%) | 2 (5.5%) | |
| Miscellaneous | 2 (5.5%) | 0 (0.0%) | |
| *Post-operative graft clarity* | | | |
| Yes | 36 (100%) | 36 (100%) | >0.999 |
| No | 0 (0.0%) | 0 (0.0%) | |
| Post-operative adverse reaction | | | |
| Yes | 0 (0.0%) | 0 (0.0%) | >0.999 |
| No | 36 (100%) | 36 (100%) | |

*ECD* endothelial cell density

## Discussion

The first phase of this study, which was a randomized controlled investigation with a paired design of the inclusion criteria and blind assessment of the results, demonstrated no superiority in terms of EC density, polymegathism, hexagonality, MECA, EC vacuolation, and mortality between Optisol-GS and Sinasol in the preservation of corneas after 7 and 14 days. However, Sinasol showed superiority over Optisol-GS in terms of lower rates of moderate to severe Descemet's folding after 7 days as well as lower rates of fair quality endothelial rating after the 7- and 14-day storages. Additionally, more than half of the corneas that were kept in Sinasol for up to 14 days, revealed a good or very good endothelial quality rating. The clinical outcomes of the transplanted Sinasol-stored corneas in the second phase of our study were similar to the grafted Optisol-GS-preserved corneas and revealed graft clarity in all the patients as well as lack of adverse reactions up to 22 months post-operatively. The results of the current study were all indicative of the effectiveness and safety of Sinasol for the preservation of the donated corneas.

The EC losses at 7 and 14 days in Sinasol were comparable with those in Optisol-GS. While there are no published data on Sinasol, the EC loss over 7-day in the Optisol-GS group (4.6%) was consistent with our previous findings (2.9%) (Kanavi et al 2015) and much lower than the findings reported by Basak and Prajna (9.4%) (Basak and Prajna 2016) and Parekh et al. (8.0%) (Parekh et al 2014) during 7 days. In the current study, although the EC loss in Optisol-GS during 14 days (20.8%) was slightly higher than the reported values by Basak and Prajna (15.7%) (Basak and Prajna 2016) and Parekh et al. (16.04%) (Parekh et al 2014), the difference was not significant. The decreasing changes of ECD in the groups of the current study, similar to the study by Basak and Prajna

(2016), were not statistically significant between the investigated maintenance media over the 7- and 14-day storages.

In the current study, except a significant decreasing trend in EC hexagonality and an increasing trend in EC vacuolation over the 7 and 14 days in each group, no remarkable difference was observed between the two media in term of changes in EC hexagonality, polymegathism, MECA, and EC vacuolation. While there were no published information regarding Sinasol, the EC hexagonality in the Optisol-GS group was comparable with our previous finding during 7 days (46 ± 11 vs. 50 ± 12) (Kanavi et al 2015) as well as the results of Basak and Prajna's study at 7 (46 ± 11 vs. 47 ± 5) and 14 days (41 ± 10 vs. 43 ± 5) (Basak and Prajna 2016). The polymegathism at 7 days were also consistent with the similar studies (40 ± 9 vs. 39 ± 9 and 40 ± 9 vs. 44 ± 6, respectively) (Kanavi et al 2015; Basak and Prajna 2016). The polymegathism in Optisol-GS at 14 days (48 ± 9) is also comparable with the reported value in the Basak and Prajna's study (45 ± 5) (Basak and Prajna 2016).

The changes of stromal edema in both groups were significant along with an increase in the maintenance time of the donated corneas from day 1 to day 7 and from day 1 to day 14. The changes of Descemet's folding were also significant along with an increase in the maintenance time of the donated corneas from day 1 to day 7 in Optisol-GS group and from day 1 to day 14 in both groups. The corresponding changes were in line with the results of the study by Basak and Prajna (2016).

Our study demonstrated no significant difference in the mean area of dead ECs and denuded Descemet's membrane between the two storage media after 7- and 14-day periods. Unlike Parekh et al. (2014) who used a manual method for counting viable ECs in trypan blue stained tissues, in this study, we used ImageJ software for the quantification of the blue-stained areas of interest to have a lower error rate and be apart from the subjectivity inherent to manual counting. In the current study, the methodology used was similar to that of reported by Jardine et al. (2014), in which a plug-in feature obtained from the Fiji imaging software was used to count the ECs loss in trypan blue stained Descemet's membrane endothelial keratoplasty tissues.

By extending the storage time of the corneas to 14 days in Sinasol, more than half of the corneas still had a good to very good overall endothelial rating. However, similar to the optisol-GS group, the majority of the corneas displayed moderate to severe degrees of stromal edema and Descemet's folding. Moreover, the changes in the ECD, hexagonality, polymegathism, EC vacuolation, and MECA over 2 weeks were significant. Furthermore, the EC loss after a 14-day period was 19.9%, and the mean area of dead ECs and denuded Descemet's membrane of the Sinasol-stored corneas for 14 days was approximately 3.7 times more than that of the corneas stored for 7 days (498,665 $\mu m^2$ vs. 135,393 $\mu m^2$). Nevertheless, given the similar rates of EC loss after 14 days that were observed in corneas stored in Optisol-GS in the current study (20.8%) as well as those preserved in Cornisol (17.4%) and Optisol-GS (15.7%) in the Basak and Prajna's study (2016), Sinasol can be used as an effective medium for preserving the corneas for a time period up to 14 days.

Sinasol contains dextran T70 that have physical and chemical properties similar to the Dextran T40 in Optisol-GS and Cornisol (Basak and Prajna 2016). Moreover, it induces more colloid osmotic pressure and less shear viscosity in comparison to the same concentration of dextran T500 (de Belder 2003). Correspondingly, the latter is one of the main components of Cornea Cold® (Table 1) (Parekh et al 2014). Besides, dextran T70, similar to albumin, was demonstrated to be retained in the intravascular spaces (de Belder 2003); therefore, its presence in Sinasol can induce very similar maintenance conditions to the biological conditions needed for the body tissues.

Development of a proper, available, and affordable cold storage medium has been a long-standing goal in eye banks of developing countries that have a short interval between cornea preservation and transplantation (Kanavi et al 2015; Basak and Prajna 2016). In this regard, Sinasol and Cornisol are considered as perfect examples of the development of effective intermediate cold storage media for preserving donated corneas in Iran and India (Basak and Prajna 2016), which can not only be beneficial for their corresponding eye bank communities, but also for eye bank communities in other developing countries. These new products can inspire competitions, especially in terms of costs of the storage media. The final cost of Sinasol production is less than half of the cost of the Optisol-GS. It is noteworthy that Sinasol, unlike Cornisol and Optisol-GS, lacks amino acids and adenosine triphosphate precursors. Moreover, unlike

Cornisol (Table 1), human recombinant insulin, L-glutamine, and vitamins are not present in Sinasol. Accordingly, these may make Sinasol as a more cost-effective medium compared to the Optisol-GS.

In addition to the random assignment and paired design of the inclusion criteria in the phase I, the presence of post-operative clinical outcomes was another strength of the current study. However, the ex vivo nature of the phase I and lack of data on corneal thicknesses are the weakness points of this study. Additionally, our clinical phase was an open label study that investigated post-transplantation clarity of the corneas kept for 3 days in Sinasol. Further study with longer storage time and paired design in Sinasol versus Optisol-GS should be conducted to investigate the graft success and ECs loss for at least 6–12 months. Generally, the results of the phases I and II of this study ensure that Sinasol is a safe, effective, and cost–benefit intermediate cold storage medium for the preservation of the corneas. It is as effective as Optisol-GS for maintaining the corneal tissues and even superior to Optisol-GS in terms of a lower rate of fair endothelial ratings and being more affordable.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The ex vivo and the clinical protocols were reviewed and approved by the Institutional Review Board of the Central Eye Bank of Iran and the ethics committee of the Ophthalmic Research Center affiliated with the Shahid Beheshti University of Medical Sciences, Tehran-Iran. Additionally, signed informed consent was obtained from the cornea recipients enrolled in the second phase of the study in terms of the principles of the Declaration of Helsinki.

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