

Chapter 3.2

Prospective clinical evaluation of McCarey-Kaufman and organ culture cornea preservation media; 14- year follow-up

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3.2

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Abstract

Purpose

To compare the outcome of corneal grafts preserved in McCarey-Kaufman (MK) medium versus organ culture after penetrating keratoplasties.

Methods

Paired corneas were stored in McCarey-Kaufman medium for 2-44 hours (mean 21 hours) and in organ culture (OC) for 144-240 hours (mean 192 hours). Penetrating keratoplasties were performed by 2 surgeons in 2 groups of patients with keratoconus, matched for age. Each pair was transplanted by the same surgeon using the same technique. Visual acuity, central corneal thickness, and central endothelial cell density were assessed at 166 ± 7.8 months postoperatively.

Results

Nine pairs of patients were recovered for a long term follow-up. The mean endothelial cell densities for the MK and OC groups were 611 ± 155 and 683 ± 168 cells/mm² respectively, which were not significantly different. A first rapid cell loss rate of 2.07 % and 2.52% per month and a second slow of 0.78 % and 0.69 % per month were observed in the respective groups. Individual values of best corrected visual acuity were all the same (value 1.00) for both groups. Corneal thicknesses were, respectively, 571 ± 52 and 540 ± 35 μ m, and were significantly different ($p= 0.013$).

Conclusions

After 14 years of penetrating keratoplasties performed with corneas stored in MK versus OC, no significant differences were observed in visual acuity, endothelial cell density and cell loss. The observed thinner grafts after OC compared to MK could not be explained.

Key words

corneal preservation - keratoconus - penetrating keratoplasty - organ culture - endothelial cell density.

Introduction

Corneal storage may be classified as short- and intermediate-term hypothermic storage at 4°C and as long-term storage based on organ culture (OC) at 30.5 - 37°C. McCarey-Kaufman (MK) storage allows a storage time of 72 hours. It has been succeeded by solutions, such as Optisol GS and others, which allow a storage time exceeding that of the MK medium.¹⁻⁴ OC allows 4-5 weeks of storage⁵⁻⁹ and yields excellent preservation of endothelial viability.¹⁰ Good results of penetrating keratoplasties with corneas in OC stored have been reported as well.^{11,12} The extended storage time allowed by OC makes it possible to schedule surgery, to minimize waste of donor tissue, to facilitate tissue typing and matching, to check the medium for microbiological infections and to perform a second endothelial cell evaluation. Since 1982, OC has become the storage method of choice in the Netherlands.¹³

In 1990, we started a prospective study to compare the clinical functionality of corneal grafts stored in MK or OC. The paired corneal grafts, originating from the same donor, were transplanted only in patients with keratoconus. Under these equal conditions, the 2 preservation methods could be compared and evaluated adequately. Inspections 13.9 ± 5.2 months postoperatively (1 year postoperatively) revealed no significant difference between the 2 groups in visual acuity, central corneal thickness, and central endothelial cell density (ECD).¹⁴

At 166 ± 7.8 months postoperatively (14 years postoperatively), 9 pairs of patients could be traced for follow-up. To our knowledge, this study is the only prospective, randomized study performed on a group of patients with the same diagnosis, comparing OC with MK, with a long-term follow-up. The long-term influence of the graft preservation method on corneal grafts and their exponential endothelial cell loss after keratoplasty are reported in this study.

Materials and Methods

Fourteen paired human corneas, 1 stored in MK medium and the other in OC, were transplanted between 1986 and 1988 in the Rotterdam Eye Hospital. All consecutive patients with keratoconus on the waiting list for penetrating keratoplasty were selected for the study. After the preservation procedures, described elsewhere,¹⁴ the paired corneas were transplanted into 2 age-matched recipients. All transplantations were performed by 2 surgeons (WHB, GvR), who used the same operative technique. Each pair was transplanted by the same surgeon. Donor buttons were punched from the endothelial side with a trephine 0.25 mm larger than the one used for the recipient. Each pair of recipients received a donor button with the same diameter. The suturing method used was a combination of interrupted (nylon 10.0) and running (nylon 11.0).

Fourteen years later, 9 of the 14 pairs of patients could be recovered for follow-up. The five excluded pairs included 1 because of a herpetic keratitis and graft rejection,¹⁴ one because of a cataract extraction and 3 as a consequence of address changes.

Data adjusted for the remaining 9 pairs were as follows: donor age varied between 63 and 81 years (mean 72.2 years). The interval between donor death and cornea preservation varied from 4 to 17.5 hours (mean 10.1 hours). The time from enucleation to preservation was 1 hour or less. Storage time varied from 2 to 44 hours (mean 21 hours) for MK and 144 to 240 hours (mean 192 hours) for OC. Recipient ages ranged from 19 to 49 years (mean 36.2 years) in the MK group and 22 to 44 years (mean 34 years) in the OC group.

Pre-operative ECD was only determined in the grafts stored in OC. Those donor buttons were evaluated and stored by the Cornea Bank Amsterdam, while the donor buttons in MK were stored in the Rotterdam Eye Hospital until surgery. The endothelium of the MK donor corneas was not evaluated because this was not a part of the standard procedure at that time.¹⁴ The pre-operative cell density in paired corneas was considered to be the same.¹⁵ In both groups, endothelial cell countings were performed 13.9 ± 5.2 months and 166 ± 7.8 months postoperatively.

At the 14 years follow-up examination, central corneal thickness was measured by ultrasonic pachymetry (3 central measurements per cornea). All pachymetry measurements were performed in the afternoon between 1 and 5 pm. Best corrected visual acuities were determined with spectacle or contact lens correction. The paired patients were measured in the same manner, either with contact lenses, or with spectacle correction. Visual acuity was determined using a Snellen chart and expressed in decimals. For statistical analysis, those data were converted to a logarithmic scale (logMar). The central ECD was assessed by taking 3 consecutive images of the central cornea by one observer with the Topcon SP-2000P. It was interactively analyzed with corrected Image Net software.¹⁶ The postoperative endothelial cell losses were compared with those predicted by the biexponential model of Armitage et al.¹⁷ The first period was defined as the period from the pre operative to the 1-year postoperative follow-up moment. The second period was the period from the 1- to the 14 year postoperative follow-up moment. Cell loss rates were calculated using the formula $N_t = N_0 e^{-rt}$, where N_0 is cell density at the start of a follow-up interval, N_t is cell density at the end of the interval of length t and r is the decay (loss) rate per unit time.

The half time for endothelial cell loss was calculated as the ratio of $0.693 [-\ln(0.5)]$ to the average decay rate r .^{18,19} Differences between the two paired storage media and the two periods were statistically analyzed using the Wilcoxon matched pairs signed-rank test (cell density results) or the paired t-test (corneal thickness). Ninety-five percent confidence intervals (CI) were calculated parametrically using the t distribution. A 2-tailed probability of 5% or less was considered statistically significant.

Results

Table 1 - Overview of post operative results after 1 and 14 years

Pair no	Storage	pre-op	1 year postoperatively				14 years postoperatively			
		Cell count	Months	Cell count	VA	Pachymetry	Months	Cell count	VA	Pachymetry
1	MK	-	23	1780	1.0	537	176	574	1.0	539
	OC	2600	23	1900	0.5 (A)	533	176	796	0.25 (T)	534
2	MK	-	22	2053	0.8	586	-	-	-	-
	OC	3000	22	1660	1.2	550	-	-	-	-
3	MK	-	23	2050	1.0	-	176	741	0.25 (C)	486
	OC	2600	23	1950	1.0	-	176	967	0.8	478
4	MK	-	24	1800	1.2	537			-	-
	OC	2700	24	1380	0.3 (A)	525			-	-
5	MK	-	17	2320	1.0	500	174	406	1.0	617
	OC	2700	17	1980	1.2	615	174	518	1.0	579
6	MK	-	23	2120	1.0	-	-	-	-	-
	OC	2900	23	1650	1.0	635	-	-	-	-
7	MK	-	14	1825	0.9	556	167	450	1.0	625
	OC	2500	15	1440	1.0	592	167	743	1.0	543
8	MK	-	14	2060	1.2	640	157	557	1.0	601
	OC	-	14	1460	0.8	665	165	398	1.0	531
9	MK	-	13	2200	0.9	546	165	818	1.0	539
	OC	2700	13	2300	1.2	581	165	754	1.0	531
10	MK	-	11	1660	0.6 (A)	516	164	459	0.4 (C)	558
	OC	-	11	2040	1.0	-	163	692	0.25 (C)	535
11	MK	-	7	1940	1.0	482	158	755	1.0	532
	OC	2600	7	2050	1.0	520	159	565	1.0	527
13	MK	-	8	2130	1.0	483	-	-	-	-
	OC	2700	8	1700	0.7	497	-	-	-	-
14	MK	-	11	1620	1.0	613	153	740	1.0	641
	OC	2400	11	2100	0.9	580	155	710	1.0	603

A = amblyopia; C = after cataract extraction; T= after trauma

MK = McCarey-Kaufman medium; OC = organ culture; VA = visual acuity

A complete listing of the collected data is set out in Table 1. The preoperative ECD and the mean ECD for both groups at 1 year postoperatively and 14 years postoperatively are set out in Table 2.

Table 2 - Endothelial cell density (cells/mm²)*

	MK		OC		Wilcoxon matched pairs signed- rank test	n
	Cell density	Cell loss (%) per month	Cell density	Cell loss (%) per month		
pre-op	2673 ± 168 †		2673 ± 168			11 ‡
1 yr #	1966 ± 214	2.07 ± 1.06	1816 ± 288	2.52 ± 1.40	P = 0.20	13 §
14 yrs **	611 ± 155	0.69 ± 0.18	683 ± 168	0.78 ± 0.18	P = 0.25	9 ¶

MK= McCarey-Kaufman medium; OC= organ culture

* Values are expressed as mean ± SD

† pre operative cell densities were considered to be the same in paired corneas.

‡ pre operative cell densities for patient 8 and 10 were missing.

§ one patient, who received an MK preserved cornea had a herpetic keratitis at six months and rejection of the graft, which failed. This patient and his matched subject were excluded from further study.

¶ one patient had a cataract extraction, so he and his fellow were excluded, three pairs were not traceable.

endothelial cell loss in the first period = pre-op to 1 year postoperative

** endothelial cell loss in the second period= 1 year postoperative to 14 years postoperative

The evaluation of the endothelial cell decay in the first period revealed rapid mean decay rates of 2.07% (95% CI: 1.36 to 2.78) cell loss per month in the grafts stored in MK and 2.52% (95% CI: 1.57 to 3.46) in the grafts stored in OC; those values were not significantly different (P = 0.24). The mean decay rates in endothelial density in the second period were slower and were 0.78% (95% CI: 0.64 to 0.92) per month for the MK and 0.69% (95% CI: 0.56 to 0.82) per month for the OC group; those values were not significantly different (P = 0.36). The mean difference MK - OC was 0.09 percent point (95% CI: -0.09 to +0.26). The half times for the first and second period of decrease in cell density were 33.5 months (95% CI: 24.9 to 51.0) and 88.9 months (95% CI: 75.3 to 108.3) for MK storage and 27.5 months (95% CI: 20.0 to 44.1) and 100.5 months (95% CI: 84.5 to 123.8) for OC storage.

Mean differences in loss rate between the first and second period could be estimated in 7 pairs and were significant for either storage: -1.39% points/month (95% CI: -2.67 to -0.11; P = 0.031) for MK and -1.33% points/month (95% CI: -2.35 to -0.31; P = 0.016) for OC.

The data points of endothelial cell loss were superimposed on the plot of endothelial cell loss predicted by Armitage et al.¹⁷ (figure 1)

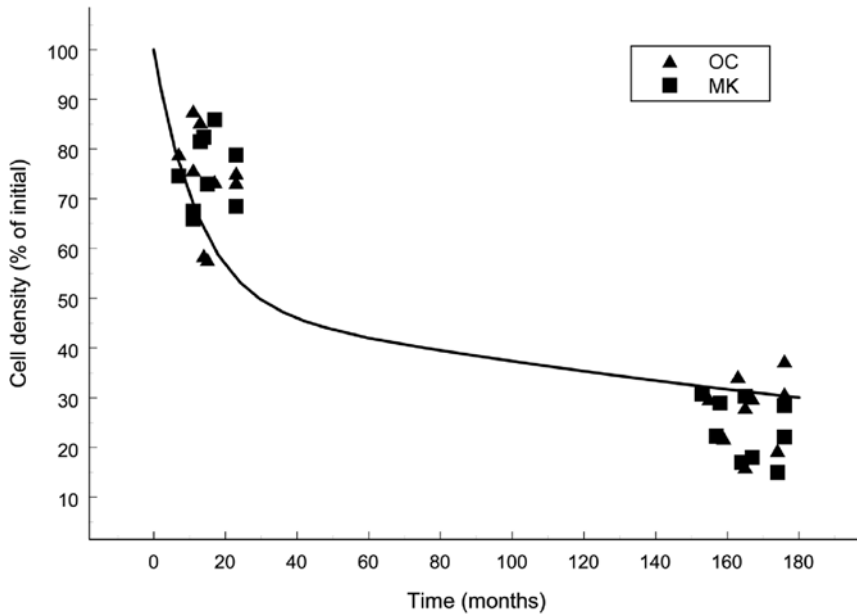


Figure 1

Cell density as percentage of initial density versus time after keratoplasty for grafts stored in OC and McCarey-Kaufman medium. Data points were superimposed on the plot of predicted endothelial cell loss by Armitage and Bourne.

Crude analyses on the cell density values without taking account of the varying follow-up times yielded essentially the same results in terms of significance.

Visual acuity could be compared in 6 of the 9 pairs of patients with a prolonged post-keratoplasty follow-up (table 3). One patient was excluded because of amblyopia, 2 other pairs were excluded because of cataract. All 6 pairs had the same best corrected visual acuity (value 1.00) under MK and OC storage.

Corneal thickness measurements after 14 years in all 9 pairs were 571 ± 52 microns and 540 ± 35 μm respectively for the MK and OC groups with a mean difference MK - OC of 31 μm (95 % CI: 9 to 53; $P=0.013$). No significant correlation was found between ECD and central corneal thickness.

Discussion

This randomized, prospective study of paired corneas showed that, up to 14 years after penetrating keratoplasty, no significant difference was observed for visual acuity and ECD for corneas stored in MK and OC. Pachymetry revealed that corneas stored in OC were significantly thinner than those stored in MK.

Few studies which compared OC with MK reported variable results. Bourne et al found a decrease in endothelial cell survival in transplanted corneas preserved in 3 modifications of OC as compared with grafts stored in MK.^{20,21} However, this was a nonrandomized study with a short follow-up time of 2 months. Also, the OC method used in that study was different from the one used in Europe.

A retrospective study by the same authors comparing the 2 media in a non randomized way revealed no difference in the endothelial cell loss after 2 months.²² Our group also found no difference between MK and OC for a 1- to 2-year follow-up period.¹⁴

Optisol has, in many cases, replaced MK medium. Frueh and Bohnke²³ reported no significant difference in clinical outcome between OC and Optisol. They included patients with different indications for grafting, whereas in our study only patients with keratoconus were selected. The storage time in their study was comparable with ours.

Table 3 - Post-operative visual acuity (decimals)*

Time in years	Visual acuity						Number of pairs
	MK			OC			
1	0.98	±	0.10	1.0	±	0.20	N = 10 †
14	1.0	±	0.0	1.0	±	0.0	N = 6 ‡

MK, McCarey-Kaufman medium; OC, organ culture.

* Values are expressed as mean ± SD

† One patient, who received an MK preserved cornea at 6 months had a herpetic keratitis and rejection of the graft, which failed. This patient and his matched subject were excluded from further study.

Three patients and so the pairs were excluded because of amblyopia.

‡ One patient is excluded because of amblyopia. Three pair of patients were not traceable. Four patients had cataract causing three pairs to be excluded.

Thuret et al²⁴ reported a higher endothelial cell count in corneas stored up to 12 days than in those stored for more than 21 days. Therefore we cannot exclude that longer storage times may result in a different clinical outcome, but corneas showing significant cell loss during prolonged storage are routinely excluded before grafting.⁹

We observed a high endothelial decay rate for MK and OC in the first period of 2.07% and 2.52% per month, compared to 0.78% and 0.69% per month in the second period. These differences are significant which means that a first-order decay model, as described by Redmond et al²⁵ is not likely.

Armitage et al proposed a biexponential decay model for endothelial cell loss after penetrating keratoplasty and cataract surgery.^{17,26} Our data did not allow testing this bi-exponential decay model in a conclusive manner, because there were not sufficient data. However, the rapid and slow component in the loss of ECD in our data fitted well in their graph.

The difference in corneal thickness between the 2 groups was not apparent at 1 year after keratoplasty. After 14 years the mean corneal thickness of the corneas stored in MK tended to be different to those of the corneas stored in OC. A significant correlation between ECD and corneal thickness was not observed. The presence of some cell counts that reach the cornea decompensation level may have interfered with the results.

The main limitation of our study is the relatively small number of corneas (14 pairs) and the fact that only 9 pairs could be re-evaluated after 14 years. The power of the analyses is likely to be low given the small sample size, which means that a true difference between the groups may have been missed. However, the mean difference in ECD between the groups was not large even 14 years after transplantation. Even if this difference was statistically significant, its clinical significance is questionable.

In the longer run, failure rates may provide an indication about the different pachymetry findings in favor of OC in this study. Selection of one system over another should be based on expertise and logistics of cornea banks, desirability of tissue typing and other factors specific for each setting.

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