RESEARCH

Comparison of Electrolyte Composition in Four Eye Bank Media During Corneal Preservation

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ABSTRACT

PURPOSE: To compare four different eye bank media for electrolyte composition and changes to concentrations during corneal preservation. Potential implications of variations in electrolyte composition will be discussed.

METHODS: Twenty-four human donor corneas were preserved in one of four types of media: Optisol GS (Optisol) at 4°C, Minimum Essential Medium (MEM) containing 2% Fetal Calf Serum (FCS) (MEM2) at 32°C, MEM with 8% FCS (MEM8) at 32°C, or Stem Alpha (STA) serum-free organ culture medium at 31°C. Cornea-free control media for each group were also stored. Samples were drawn at 0, 3, 7, 14, 21, and 28 days of storage and analyzed for K^+, Na^+, Ca^{2+}, Cl^-, glucose, and lactate concentrations using a blood gas analyzer. Media were not changed during the storage period.

RESULTS: Mean concentrations of K^+ at day 0 were 3.58, 4.56, 5.70, and 4.22 mmol/L for Optisol, MEM2, MEM8, and STA culture media, respectively. Concentrations of Na^+ were 174.1, 126.6, 136.9, and 129.8 mmol/L; Ca^{2+} 0.70, 1.33, 1.34, and 1.13 mmol/L; and Cl^- 103.3, 101.4, 113.6, and 97.8 mmol/L. Comparisons of the concentrations at day 0 against day 28 revealed no statistically significant differences, with the exception of Ca^{2+} concentrations in MEM2, STA, and Optisol.

CONCLUSIONS: Concentrations of electrolytes in various eye bank media differ. Concentrations vary little through the preservation period. Some electrolyte concentrations are not within the physiological range when compared to concentrations in aqueous humor or tear fluid. Optimizing electrolyte composition of eye bank media may be beneficial for the quality of donor tissue.

KEYWORDS: corneal preservation medium, endothelium, specular microscopy

Donor corneas, used in full-thickness or lamellar corneal transplantation, are preserved at different temperatures and in various eye bank media, according to routines independently established by eye bank associations or by individual eye banks themselves.

Hypothermic corneal storage is the method favoured in the US, with most departments using Optisol GS (Optisol) as the storage medium, maintained between 4°C to 8°C. The recommended maximum duration for hypothermic storage is 14 days.

The preservation technique used by most cornea bank members of the European Eye Bank Association (EEBA) is that of culturing in a Minimal Essential Medium (MEM)-based culture medium (including a varying percentage of fetal calf serum [FCS], usually either 2% [MEM2] or 8% [MEM8]) and maintaining the temperature close to that of physiological, about 30°C to 37°C.[1] Corneas preserved by this technique usually have a storage time of up to 4 weeks, though research has shown that longer periods are possible,[2,3] with transplanted corneas stored up to 7 weeks showing similar results to corneas stored under standard conditions.[4] Due to concern regarding the risk of Creutzfeldt-Jakob disease transmission inherent to serum-containing media, serum-free culture media have been receiving increased attention (eg, Eurosol,[5] Endothelial Serum Free Media[6] [SFM].

The electrolyte concentrations of tear and aqueous humor have been shown to be dramatically different from one another[7-12] (Table 1). During the storage of a donor cornea, however, the epithelium, stromal keratocytes, and endothelium are maintained in one compartment and exposed to the same microenvironment. Extracellular electrolyte composition is

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Table 1. Physiological values and electrolyte concentrations of various fluids

	Na^+	K^+	Cl^-	Ca^{2+}	Glucose	Lactate	pH
Aqueous[7]	162.9	2.2-3.9	131.6	1.8	2.7-3.7	2.5	7.38
Tear[8-12]	144.4-146	16.2-24.1	128-144.9	0.57	0.206	1-5	6.5-7.6
Plasma[7]	135-145	3.6-5.4	98-108	1.15-1.29	5-10	1-1.5	7.35-7.45

All electrolyte values are in mmol/L.

important to cellular function. Any electrolyte concentration abnormalities may interfere with important intra- and intercellular processes, leading to cellular compromise.

Each of these storage media is proprietary and as such their exact composition is kept confidential. Møller-Pedersen et al[13] compared 8 different test media and showed that SFM with 2% FCS maintained a superior level of post-storage viability, while MEM8 yielded better results that MEM2. Furthermore, Stoiber et al[5] found that Eurosol was comparable to MEM2 over a 30-day period. Additionally, in a small study, Frueh and Böhnke[14] showed that Optisol and MEM, supplemented by an unstipulated percentage of FCS, yielded similar graft results 2 years following transplantation. Despite these findings, the exact compositions of these media have not previously been examined. The identification of composition variation, coupled with previous knowledge, could identify the factors necessary to improve endothelial survival and thus clinical outcome.

The aim of this study is to compare the initial electrolyte, glucose, and lactate compositions of the 4 different media commonly employed in corneal preservation and to identify any variation in the concentrations throughout donor corneal storage. These results will be compared with reports on concentrations found in plasma, aqueous humor, and tear fluid. Finally, the potential effects of variations in the electrolyte composition of the storage media will be discussed.

METHODS

Consent and ethical approval was obtained from the Regional Committees for Medical and Health Research Ethics, Norway.

Donor Corneas

Following consent, investigators obtained a total of 12 cadaveric pairs of donor corneas by the excision of a corneoscleral disc and prepared them according to EEBA guidelines.[15] The average age at death was 72.5 years. The average time from death to removal of the tissue was 45 hours.

Media Preparation

Storage media was either purchased directly from the manufacturer or prepared at the Norwegian Eye Bank and Centre for Eye Research, Oslo University Hospital.

(1) Optisol GS (Optisol) (Bausch & Lomb, Rochester, New York, US) is a chondroitin sulfate-based, commercial cold storage media. The 20 mL of media were contained within a 30-mL glass bottle (prepackaged) with the lids exchanged for multiple layers of wax paper to allow for puncturing of the lid without breaking the seal. The corneas rested at the base during storage.

(2) Stem Alpha (STA) (Stem Alpha, Saint-Genis-l'Argentière, France) is an animal product-free commercial cultivation media tailored to corneal cultivation. Whilst being a 3-stage product (transport, storage, and deswelling), only the second phase was employed (storage). The 50 mL of media were contained within a 75-mL plastic bottle (prepackaged) with the lids exchanged for multiple layers of wax paper to allow for puncturing of the lid. The corneas rested at the base during storage.

(3) Minimal Essential Medium-based culture medium supplemented with 2% FCS (MEM2) (as used in most European eye banks) is buffered and contains standard concentrations of antibiotics and antifungal agents (Table 2). This media is produced locally (Norwegian Eye Bank) following EEBA guidelines. The 50 mL of media were contained in 75-mL glass bottles with self-sealing rubber stoppers. Corneas were suspended in the media from 6-0 prolene sutures.

(4) Minimal Essential Medium-based culture medium supplemented with 8% FCS (MEM8) is the standard medium at the Norwegian Eye Bank. MEM8 is

Table 2. MEM-based Culture Medium, Norwegian Eye Bank

Contents		Producer; Product Code	Weight or Volume
Minimal Essential Medium (MEM)		VWR; Art nr 61100-0053	9.53 g
HEPES buffer		Sigma; H4034	12.5 mL
Sodium Bicarbonate 7.5%		Sigma; S8761	29.3 mL
Milli Q water			873.95 mL
Fetal Calf Serum (Heat-inactivated)	2%		20 ml with 60 mL sterile water
	8%		80 mL
Amphotericin B (250 µg/mL)		Sigma Aldrich; A2942	2 mL
Gentamicin (40 mg/mL)		Sigma Aldrich; G1397	1.25 mL
Vancomycin (100 mg/mL)		Alpharma ApS (now Pfizer); 0069-2599	1 mL
Total			1 L*

*Mixed and sterile filtered. Media were divided into 50-mL aliquots and placed in sterile containers.

also buffered and contains standard concentrations of antibiotics and antifungal agents (Table 2). Both the medium and corneas were otherwise prepared as for MEM2.

Cultivation samples for MEM-containing media were placed in an incubator at 32°C as specified by the EEBA guidelines. STA samples were placed in an incubator at 31°C. Optisol hypothermic storage samples were placed in a refrigerator at 4°C, following the guidelines set out by the Eye Bank Association of America (EBAA). Six donor corneas were placed in each of the media with 6 cornea-free controls run in parallel.

To provide consistency amongst all of the media, the same preservation time (28 days) was used, despite the fact that the recommended maximum storage time for Optisol is shorter. The media were investigated after 0, 3, 7, 14, 21, and 28 days (D0, D3, D7, D14, D21, and D28) of tissue preservation. At each time point, 200 µL of fluid were removed from the media using a 22-gauge needle and 1-mL syringe and immediately placed into storage at -30°C. Each sample was obtained from within 1 cm of the cornea.

No changing or replenishing of the media occurred. This procedure was done for standardization purposes, as the practice varies from eye bank to eye bank.

The samples were later thawed at room temperature for a minimum of 2 hours and mixed to avoid any concentration variation within the syringe. Samples were then analyzed using the ABL718 blood gas analyzer (Radiometer, Copenhagen, Denmark).[16] Ninety-five µL of each thawed sample were used. This amount was used to assess concentrations of sodium, potassium, calcium, chloride, glucose, and lactate.

The pH was measured on day 0 samples using a PHM210 Standard pH Meter (Radiometer, Copenhagen, Denmark).

RESULTS

Results of the media comparisons are presented in Table 3.

Statistics

Results are expressed as mean (± standard deviation). Statistical differences between two time points were calculated using a T-Test (2 tail, simple, independent samples). A significance level of 5% was used. Error bars in figures represent the 95% confidence interval (Student's T distribution).

Appearance

During the 28 days of culture and storage, there was a gradual, but marked colour change from pink to orange in the serum-containing culture media, indicating a reduction in pH (Fig. 1). A similar, though less marked colour change was noted for STA. No colour change was noted in the Optisol.

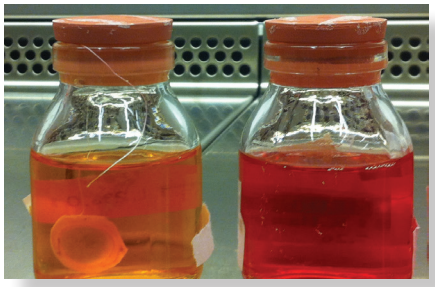


Fig. 1. pH-related colour comparison between Minimal Essential Medium with 2% Fetal Calf Serum (left) and its control at day 28 (right).

Potassium

There was no statistically significant difference in potassium concentrations between the various culture media and their respective controls over time (Fig. 2).

Sodium

The concentrations of sodium were fairly constant throughout the storage period with no statistical differences found between the individual media and their respective controls at any time point (Fig 3).

Optisol had a significantly higher sodium concentration than all other media, and values were statistically significant at all time points.

Table 3. Comparison of day 0 electrolyte concentrations with those of day 28 concentrations for all media used

Electrolyte	Medium	Day 0 (mmol/L)	Day 28 (mmol/L)	% Change	P value
K^+	MEM2	5.11 (0.33)	5.08 (0.36)	-0.49	0.898
	MEM8	6.01 (0.37)	6.00 (0.40)	-0.14	0.970
	Opt	3.58 (0.04)	3.56 (0.05)	-0.55	0.420
	STA	4.21 (0.10)	4.05 (0.36)	-3.75	0.665
Na^+	MEM2	136 (10.5)	135.2 (8.4)	-0.61	0.871
	MEM8	140.5 (9.3)	142.5 (9.5)	1.42	0.712
	Opt	174.1 (2.2)	173.9 (1.5)	-0.13	0.794
	STA	129.8 (2.4)	124.3 (10.3)	-4.19	0.254
Ca^{2+}	MEM2	1.44 (0.08)	1.34 (0.08)	-7.28	0.029*
	MEM8	1.41 (0.07)	1.38 (0.09)	-2.24	0.497
	Opt	0.70 (0.02)	0.67 (0.02)	-3.57	0.024*
	STA	1.13 (0.02)	1.02 (0.06)	-9.81	0.005*
Cl^-	MEM2	116.2 (10.4)	113.8 (8.7)	-2.01	0.645
	MEM8	121.3 (10.9)	119.8 (8.7)	-1.17	0.782
	Opt	103.3 (1.7)	103.9 (1.1)	0.53	0.389
	STA	97.8 (1.8)	94.5 (8.8)	-3.34	0.406

*Statistically significant difference

Abbreviations:

- MEM2, Minimal essential medium with 2% fetal calf serum supplement;
- MEM8, Minimal essential medium with 8% fetal calf serum supplement;
- STA, Stem Alpha (serum-free corneal culture medium);
- Opt, Optisol GS

Calcium

Ionized calcium levels were measured. There was a decrease in the control sample for MEM8 (cMEM8) that was significantly lower than MEM8 at day 3 (MEM8: 1.44 [0.06] mmol/L, cMEM8: 1.30 [0.06] mmol/L, P=0.002). Otherwise, all controls were not significantly different from their study samples (Fig 4).

Chloride

There were two instances in which study media were significantly different from the controls (MEM2, day 21, and MEM8, day 7) (Fig. 5).

Glucose

Cultivation samples showed reductions in the glucose concentrations that were all statistically significant from both their control samples and from their commencement concentrations (Fig. 6): MEM2: 5.00 (0.4) mmol/L to 0.3 (0.4) mmol/L; MEM8: 5.2 (0.4) mmol/L to 0.2 (0.2) mmol/L; STA: 21.5 (0.6) mmol/L to 15.3 (1.9) mmol/L. Optisol showed a minimal reduction over the time period (4.7 [0.1] mmol/L to 4.3 [0.24] mmol/L); however, that difference was also statistically significant (P<0.01).

Lactate

Lactate levels rose in all media throughout the period in relation to their controls, and all differences were significant (Fig. 6).

pH

The pH at day 0 was shown to be statistically significantly lower in STA (7.35 [0.03] mmol/L) than in the other three media (MEM2: 7.50 [0.02] mmol/L; MEM8: 7.50 [0.02] mmol/L; Optisol: 7.46 [0.08] mmol/L).

DISCUSSION

Changes in Media Composition during Preservation

There was little variation in the electrolyte composition throughout the culture period in any of the media. Concentrations of potassium, sodium, and chloride showed no significant changes during the culture period. Calcium concentrations did, however, manifest reduction in MEM 2, STA, and Optisol, with significant decreases in concentrations. In contrast, MEM8 showed no significant change.

Fig. 2. Potassium

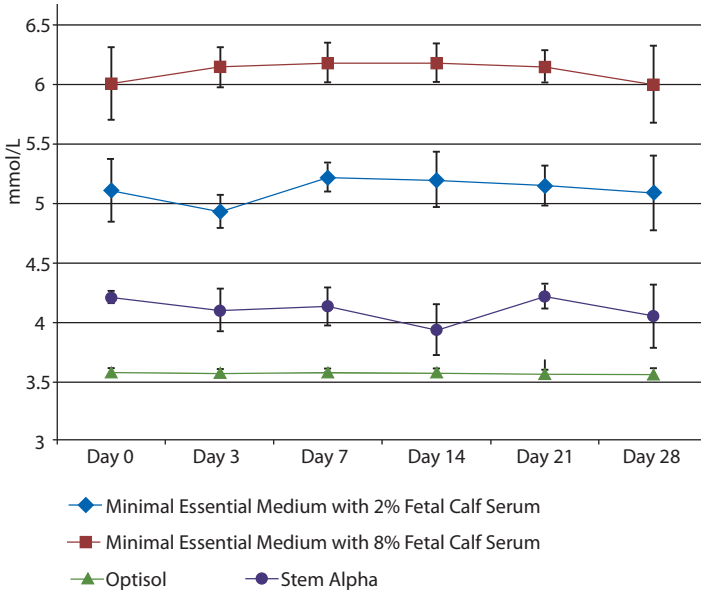


Fig. 2. Potassium concentration in the four media throughout the preservation period. Error bars in all figures represent 95% confidence interval.

Fig. 3. Sodium

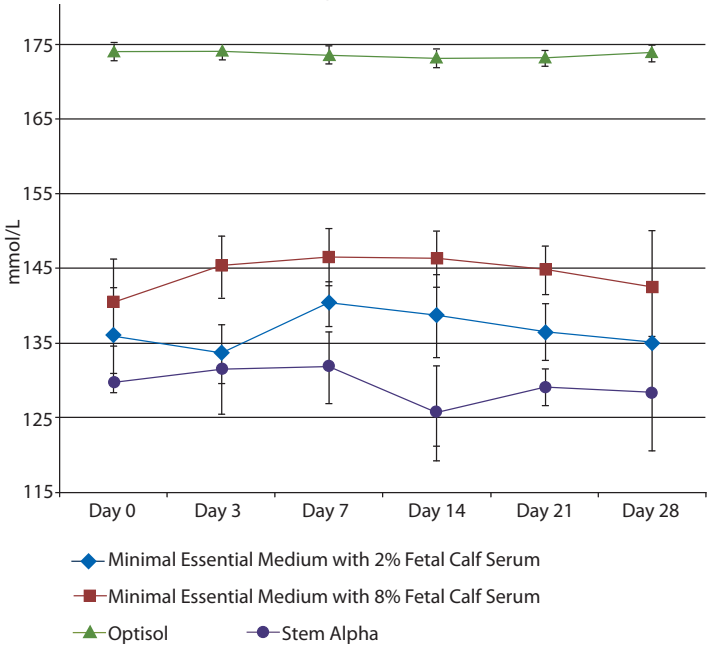


Fig. 3. Sodium concentration in the four media throughout the preservation period.

Fig. 4. Calcium

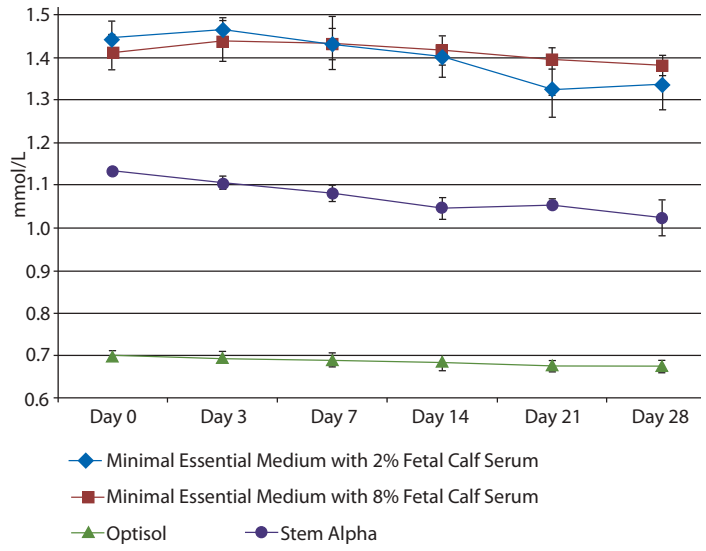


Fig. 4. Calcium concentration in the four media throughout the preservation period.

Fig. 5. Chloride

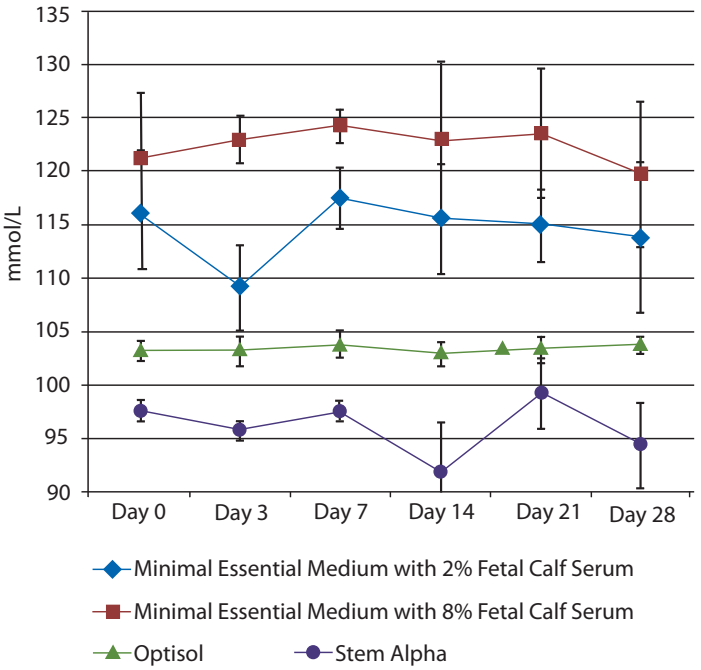
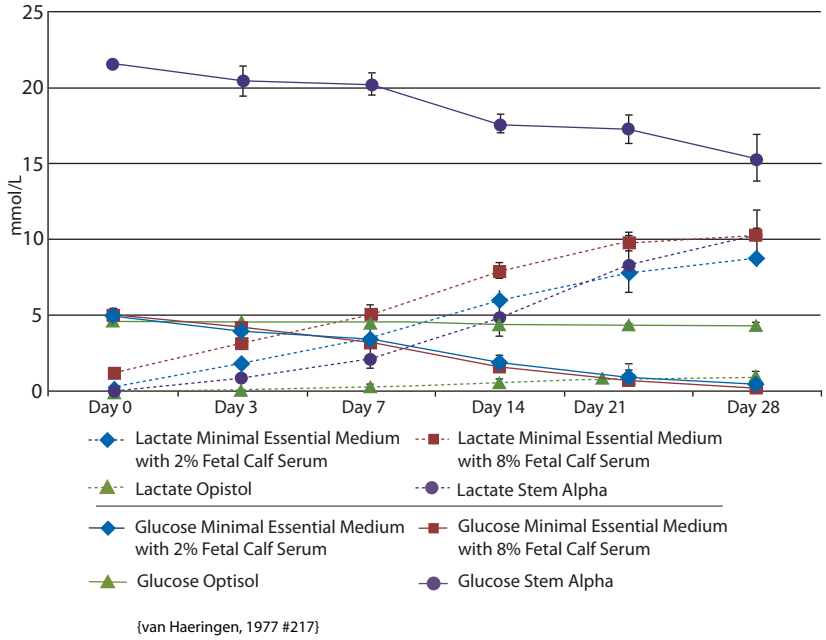


Fig. 5. Chloride concentration in the four media throughout the preservation period.

Fig. 6. Glucose and Lactate



{van Haeringen, 1977 #217}

Fig. 6. Glucose and lactate concentration in the four media throughout the preservation period.

As a method of validation, the results for glucose consumption and lactate production can be compared with the results from previous studies. Whilst studying donor corneal metabolism in 8% FCS-supplemented MEM-based medium, Hjortdal et al[17] showed a drop in glucose from 5.1 mmol/L to 0.25 mmol/L over a 28-day period. Similarly, our MEM8 samples showed a reduction from 5.15 mmol/L to 0.22 mmol/L over the 28-day period. Likewise, our findings were similar to Hjortdal et al with regards to lactate concentration.

Our findings indicate that no modification occurs to the electrolyte composition of the media during cultivation. With that said, there was little to no variation in the Optisol electrolyte composition from its control throughout storage, whilst there was a degree of non-statistically significant variation in concentration in the culture media when compared with their respective controls. Given that metabolism is minimized in Optisol hypothermic storage, this finding could suggest that in organ culture, active redistribution of electrolytes occurs between the intra- and extracellular compartments. Any changes being instigated by corneal metabolism or cellular death with the release of intracellular contents would likely be insignificant when compared to the overall volume of electrolyte and therefore are not reflected in our results. Furthermore, any potential concentration alterations could be lower than the sensitivity of the instrumentation.

Previous studies have shown the reduction in pH throughout the cultivation period, results confirmed by the colour change of our culture media (Fig. 1).

Given that our results do not show any significant changes in electrolyte composition, with the exception of calcium, the initial composition is likely the only variable that needs to be considered when designing a tailor-made corneal culture medium.

Variation in Media Composition

Initial electrolyte composition of the different media varied, both from each other and from aqueous humor and tear composition (Tables 1 and 3). Optisol was developed specifically with corneal storage in mind. STA was also developed specifically for corneal preservation, though as a culture medium, not as a storage medium. Whilst they both vary from both aqueous and tear fluids, they also vary from one another, with Optisol having a relatively high sodium concentration and low calcium and potassium levels. MEM was developed as a general culture medium for cell lines and its electrolyte levels are based on those of serum concentrations. This composition is minimally modified for use with corneal cultivation and as such the electrolyte concentrations resemble those of plasma. They showed significant differences when compared with Optisol and STA, with higher levels of potassium, calcium, and chloride.

When comparing the MEM-based media and Optisol, the initial pH varied little. However, STA is relatively acidic in contrast to other media and significantly so when compared to these media. Corneal intracellular pH has been shown to be maintained despite acidification by up to 0.6 units.[18] Furthermore, a reduction in pH (within the range of pH 6.7 – 7.4) does not limit corneal viability, despite a potential increase in metabolic stress.[13] Thus, the relatively low initial pH of STA may be of little clinical significance.

MEM-derived culture media showed significant variation in their electrolyte composition as well as lactate concentration. The only variation in their production was the addition of FCS in one of two concentrations (Table 2). The electrolyte concentrations of the calf serum differs from that of MEM.[19] As such, adding differing volumes of FCS to MEM to give either 2% or 8% concentrations will alter the electrolyte

concentration of the final product. Similarly, lactate concentrations are likely affected (Fig. 6). Interestingly, the calcium concentration was not overly affected by the increase in FCS concentration. As calcium is significantly protein bound, one possible explanation is that the increased protein load provided by the extra FCS acts as a buffer to maintain a more steady calcium concentration.

General Considerations

With each of the corneal layers being important in the clinical outcome of corneal transplant,[6,20,21] the goal of a tailor-made corneal preservation medium should be to maximize the survival of all corneal layers. The cornea, however, is preserved as a single entity, not as isolated layers. Thus the ideal culture medium may vary from that of physiological fluids.

For their part, endothelial cells must be preserved due to their inability to replicate *in vivo*.[6] However, studies have also shown that, given the right circumstances, mostly related to reduced calcium levels, endothelium can divide *in vitro*.[22-24] This phenomenon is of further interest as calcium concentration was shown to decrease during the preservation period in 3 of the 4 media and as such was the only electrolyte whose concentration changed significantly. Further investigation into individual electrolytes could identify media concentrations that are beneficial to the quality of the preserved cornea by stabilizing or indeed increasing the endothelial cell count.

There is ongoing research into the *in vitro* development and cultivation of corneal endothelial[25] and epithelial monolayers.[26] These cell cultures would place a different set of requirements on a tailored culture medium.

Given that previous studies have shown variation in the outcomes of corneas preserved in different media[24] and that the media employed are proprietary, the identification of differences in media composition is an important first step in isolating which individual factors affect corneal preservation. The optimization of the electrolyte composition of a corneal culture medium could lead to improved quality of donor corneal tissue following preservation and thereby improve the clinical outcome.

In conclusion, the concentrations of electrolytes in various eye bank media differ; however, the concentrations vary little through the preservation period. Furthermore, some electrolyte concentrations are not within the physiological range for the aqueous humor or tear fluid. Individual and specific modifications of electrolyte concentrations may lead to improved quality of cultured tissue.

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