INTRODUCTION

Given the worldwide shortage of donor corneas needed for corneal transplantation, there is a need to store viable tissue for longer periods of time [1]. The successful outcome of corneal transplantation surgery depends to a large degree on the presence of a viable corneal endothelium [1]. Refrigeration at 4 °C is the most commonly applied method to store donor corneas, but typically cannot preserve corneas useful for transplantation for more than 1-2 weeks [2]. Cryopreservation of corneas, i.e. freezing the tissue, is currently not in clinical use due to cell damage from ice crystal formation during the freezing process [3]. We hypothesize that cryopreservation of corneal tissue in cryoprotectant media, result in clinically acceptable damage to the cornea. The goal of this project was therefore, to assess the viability of corneas after cryostorage in cryoprotective media as a means of extending the storage time of donor corneas.

METHODS

18 porcine corneas were obtained post mortem and stained with the nucleic acid stain 4',6-diamidino-2-phenylindole and fluorescently labeled phalloidin, a filamentous actin binding molecule, to determine changes in the corneas' structure. Three-dimensional images of the corneal tissue were acquired after two weeks of cryopreservation or refrigeration, respectively, using confocal microscopy. Area of cells and extracellular structures were determined using morphometry and image analysis software. Quality of structure was measured using morphometry of cytoskeletal structures as surrogate markers and the scale below (Figure 1) [4]

Figure 1: Grading of corneal endothelium viability

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RESULTS

Figure 2: Corneal structure and quality is well preserved after cryopreservation.

- [A] Representative sections of the corneal endothelium of the control that was fixed immediately is shown as a representative section of a corneal endothelium. [B] Representative section of the corneal endothelium of the corneas preserved using the current standard technique (refrigeration at 4 °C for two weeks) shows a very disorganized and degenerated endothelium compared to the fixed control. [C] Corneas cryopreserved in 5% DMSO at -80 °C lasting for two weeks showed an endothelium that closely resembled the healthy fixed control endothelium, indicating relative preservation of the original endothelium structure

Figure 3: Quantitative analysis of structure and quality of corneal endothelium.

- [A] Fixed Control 4°C Control
- [B] 10% DMSO 5% DMSO
- [C] Grade 1
- [D] Grade 2
- [E] Grade 3

Figure 3: Quantitative analysis of structure and quality of corneal endothelium.

- [A] Present area of phalloidin-labeled tissue comparing the structure of endothelium in the fixed control and in refrigerated and cryopreserved corneas after two weeks of storage. The refrigerated control showed a significant decline, while cryopreserved corneas were not significantly different from the immediately fixed control (one-way ANOVA, p = 0.137). [B] Grading of corneal endothelium viability using phalloidin cytoskeleton staining grade was performed to measure the quality of the endothelium. 4°C control and DMEM control showed significant differences from the fixed control while 5% DMSO and 10% DMSO did not (one-way ANOVA, p = 0.005). Bar represent mean ± SEM, * p-values of <0.05, ** p < 0.01, and *** p < 0.001 are indicated by * *, **, and ***, respectively, as determined by unpaired Student’s t-test to compare the control that was fixed immediately.

CONCLUSIONS

- Our results confirm that current refrigeration-based techniques used to preserve donor corneas do not allow storage for longer than two weeks without significant structural deterioration of the tissue.
- Cryopreservation maintained the quality and structure of the corneal endothelium significantly better than refrigeration at 4 °C.
- Our data indicate that cryopreservation of cornea tissue in cryoprotectant media containing DMSO represents a potentially clinically relevant method to extend the storage period addressing unmet clinical needs.
- Further research is needed to assess the structure and quality recovery of cryopreserved corneas after transplantation in a live recipient.

REFERENCES

4. Tharasanit D, Mintz D, Deligiozova D, Koulen P. Cryopreservation of corneal endothelium with 10% DMSO containing cryoprotectant media shows no significant difference to controls that had been fixed immediately only with respect to amount and quality of cell structure.

SUMMARY

- Corneas that were cryopreserved showed a well preserved endothelial structure when compared to the fixed control (Figure 2).
- After two weeks, corneas cryopreserved at -80 °C in 10% DMSO containing cryoprotectant media showed no significant difference to controls that had been fixed immediately with respect to amount and quality of cell structure.
- Corneas stored at 4 °C for the same time period, however, showed a significant decline in amount and quality of cell structure (p = 0.014 and p < 0.001, respectively).
- Corneas cryopreserved at -80 °C in 5% DMSO containing cryoprotectant media showed no significant difference to the controls that had been fixed immediately only with respect to amount of cell structure.