Effects of Cryopreservation on Structure and Quality of Corneal Tissue

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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 cryoprotectant media as a means of extending the storage time of donor corneas.

METHODS

Method of Preservation	T = 0	T = 2 weeks
Fixed Control at 4°C (n = 6)	Samples #1-6 fixed	
DMEM + 1% dextran + 2% chondroitin sulfate at 4°C (n = 4)	Samples #1-4 stored	Samples #1-4 fixed
DMEM + 1% dextran + 2% chondroitin sulfate + 10% Dimethyl Sulfoxide (DMSO) at -80°C (n =4)	Samples #1-4 stored	Samples #1-4 thawed, fixed
DMEM + 1% dextran + 2% chondroitin sulfate + 5% DMSO at -80°C (n = 4)	Samples #1-4 stored	Samples #1-4 thawed, fixed

Current standard preservation media Cryopreserved media with cryoprotectant media (DMSO)

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]

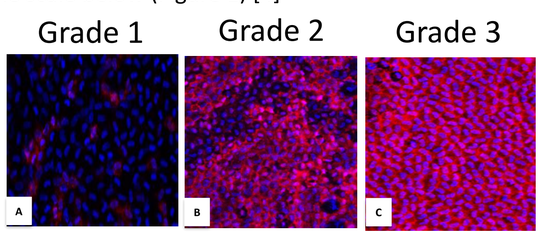

Figure 1: Grading of corneal endothelium viability

(A) Grade 1: large areas lack actin staining and/or cells; **(B)** Grade 2: less distinct cell borders with small clumps of actin in cytoplasm; **(C)** Grade 3: precise, sharp restriction of actin staining to cell borders, which is grade 3

RESULTS

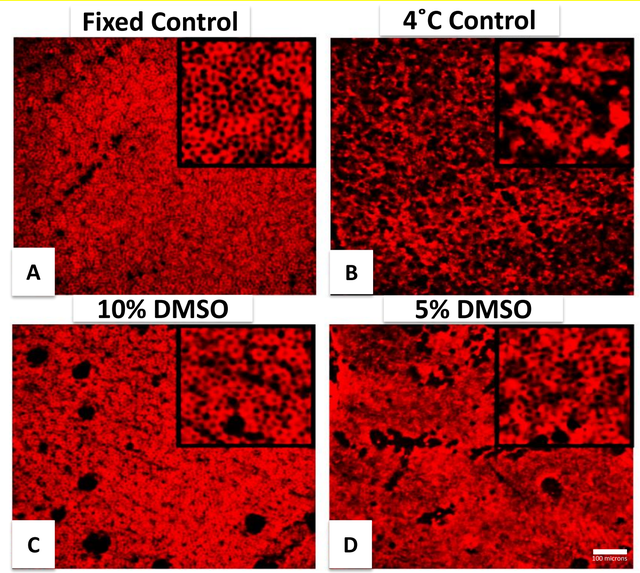

Figure 2: Corneal structure and quality is well preserved after cryopreservation.

(A) Representative section 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-D)** Corneas cryopreserved in 10% (C) and 5% DMSO (D) for two weeks showed an endothelium that closely resembled the healthy fixed control endothelium, indicating relative preservation of the original endothelium structure

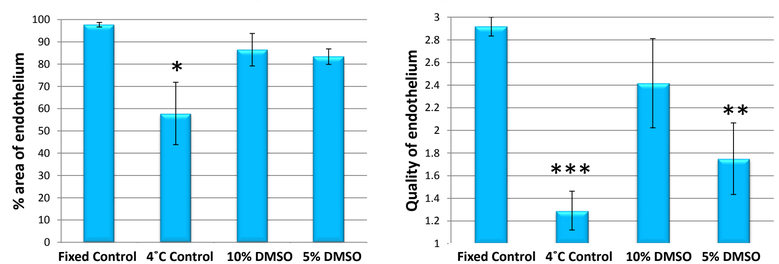

Figure 3: Quantitative analysis of structure and quality of corneal endothelium.

(A) Percent 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 = 0.027). **(B)** Grading of corneal endothelium viability using phalloidin cytochemistry staining grade was performed to measure the quality of the endothelial structure. 4˚C control and 5%DMSO showed significant difference from the fixed control while 10%DMSO did not (one-way ANOVA, p = 0.005). Bars represent mean ± SEM; p-values of < 0.05, < 0.01, and < 0.001 are indicated by *, **, and ***, respectively, as determined by unpaired Student's t test comparison to the control that was fixed immediately .

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.

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

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