Corneal cryopreservation: and endothelial cell viability and histomorphological study of cornea

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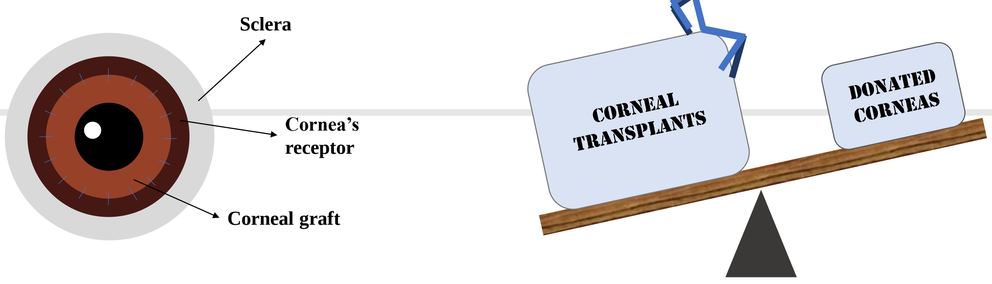
INTRODUCTION

Corneal transplant (keratoplasty) is one of the most frequent transplant in the world. Nevertheless, there is an imbalance between the number of people who need a keratoplasty and the number of donated corneas. Cryopreservation could be one of the solutions to increase the corneal availability, as it would allow to storage them during an unlimited time. Cryopreserved corneas, then, must maintain their histomorphological structure and their endothelial cell viability.



OBJECTIVE

Preliminary evaluation of four cryopreservation protocols, attending to endothelial cellularity, endothelial cell viability, and to the histology of whole corneas.

METHODS

12 corneas were cryopreserved using the protocols showed in Table 1: Protocol 1: slow cryopreservation (SC) with albumin and DMSO in 199 media; Protocol 2: SC with DMSO in 199 media; Protocol 3: fast cryopreservation (FC) with VS55 media, and Protocol 4: FC with DP6 media. An assay with LIVE/DEAD imaging kit and Hoechst was realised to evaluate cellularity and endothelial cell viability. A Masson trichrome staining was preformed to the histological evaluation.

RESULTS

Endothelial cellularity was huge in all corneas (in descending order, Protocols 4, 3, 1 and 2). Regarding cell viability, corneas which were cryopreserved with Protocol 1 showed the high viable cells rate (66,79%) (Figure 1 a), while, with the other three protocols there were non-viable cells (Figures 1 b, c and d). Cryoinjuries were observed in endothelium detachment in corneas cryopreserved with Protocols 2, 3 and 4 (Figure 2 c). Moreover, corneas cryopreserved with Protocols 1, 3 and 4 showed holes among collagen fibers of stroma (Figure 2 c). In all cryopreservation groups, epithelium was thinner (Figure 2 a), and some cells were lost or showed a serrated epithelium (Figure 2 b) that would be able to have been created by extracellular ice.

CONCLUSIONS

Cryopreservation with any of the four protocols allow a high endothelial cellularity after thawing. Fast cryopreservation offers the best result in cellularity. Despite of all, endothelial cell viability is affected by fast cryopreservation while slow cryopreservation retains some cell viability. Endothelium, epithelium and stroma are three sensitive corneal layers that suffer cryoinjuries, in special, when the fast cryopreservation is carried out.

Table 1. Cryopreservation type and cryoprotectant agents used in each of the four cryopreservation protocols. The number of corneal cryopreservation is n=3. DMSO: dimethyl sulfoxide, PrOH: 1,2-propanediol.

	Cryopreservation Type	Cryoprotectant Agent
Protocol 1 (n=3)	Slow Cryopreservation (SC)	Albumin DMSO
Protocol 2 (n=3)	Slow Cryopreservation (SC)	DMSO
Protocol 3 (n=3)	Fast Cryopreservation (vitrification; FC)	PrOH DMSO Formamide
Protocol 4 (n=3)	Fast Cryopreservation (vitrification; FC)	PrOH DMSO

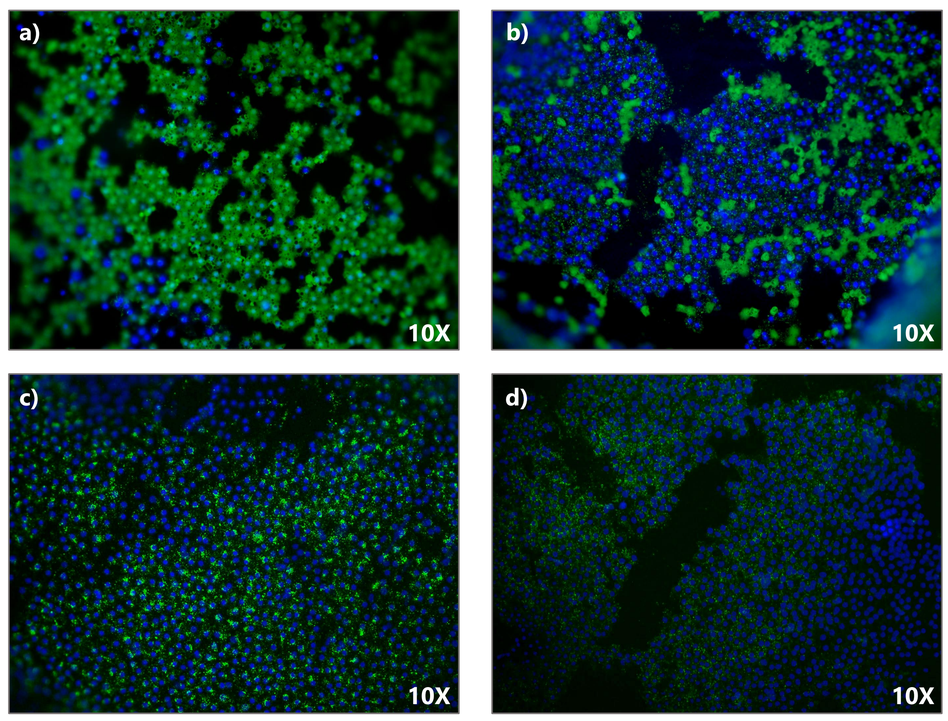


Figure 1. Cell viability assay on endothelia of cryopreserved corneas with Hoechst and the LIVE/DEAD imaging kit (green, calcein): a), Protocol 1; b), Protocol 2; c), Protocol 3, y d) Protocol 4.

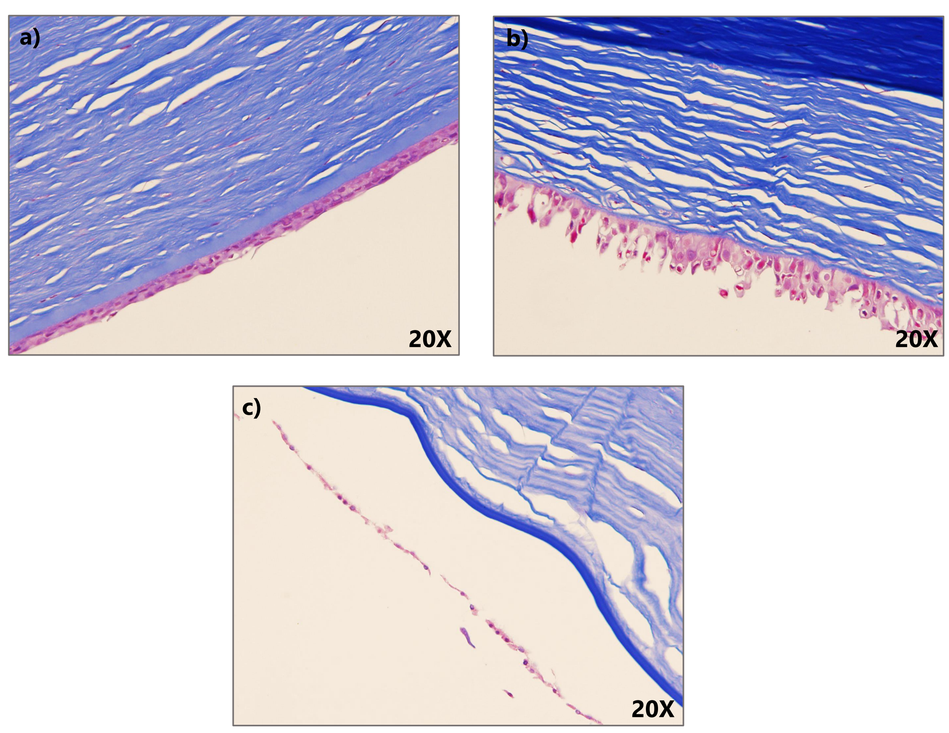


Figure 2. Potential cryoinjuris. a) epithelial thinning (Protocol 1), b) serrated epithelium (Protocol 1); c) huge holes in stroma and endothelial separation (Protocol 4).

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