Reference Note

Bambanker™ hRM - the best cryopreservation medium to freeze corneal endothelial cells

Product	Bambanker™ hRM
Distributor	NIPPON Genetics EUROPE GmbH
Manufacturer	GC LYMPHOTEC Inc.

Reference

This reference note is based on the following publication:

Feasibility of a cryopreservation of cultured human corneal endothelial cells

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Department of Biomedical Engineering, Faculty of Life and Medical Sciences, Doshisha University, Kyotonabe, Japan, 2019

Summary

Corneal endothelial (CE) cells are specialized cells responsible for maintaining corneal transparency by regulating water flux across the cornea. Damage to these cells can result in corneal swelling and vision loss. Currently, corneal transplantation is the only treatment available for CE dysfunction. However, the limited availability of corneal tissue has increased the demand for alternative therapies, such as the use of cultured CE cells. Cryopreservation is a potential approach to preserve cultured CE cells for future use. This study aimed to investigate the feasibility of different cryopreserving media for human CE cells. Bambanker™ hRM exhibited better cell viability, cell growth and cell density than other cryopreservation media.

Method

Human CE cells (HCEC) were cultured and cryopreserved using a slow-freezing method. The cells were frozen at -80 °C and stored in liquid nitrogen (-196 °C) for additional 13 days. After cryopreservation, HCECs were incubated at 37˚C in a water bath for 1–2 minutes, centrifuged and resuspended in a culture medium. The viability and morphology of the cells were assessed before and after cryopreservation. The following cryopreservation reagents were used in the screening experiments:

- Cellbanker 2 (Nippon Genyaku Kogyou Co., Ltd, Ltd, Fukushima, Japan)
- Bambanker™ (Nippon Genetics Co., Ltd, Tokyo, Japan)
- KM Banker (Cosmo Bio Co., Ltd, Tokyo, Japan)
- Stem-Cell-banker (Nippon Genyaku Kogyou Co., Ltd)
- Bambanker™ hRM (Nippon Genetics Co., Ltd, Tokyo, Japan)
- ReproCryo DMSO Free RM (ReproCELL Inc., Kanagawa, Japan)
- Opti-MEM + 10% v/v dimethyl sulfoxide (DMSO; Nacalai tesque) + 10% v/v FBS was used as a control cryopreservation reagent

Results

HCECs were screened for cell viability, growth speed and cell density after cryopreservation.
For non-preserved control cells, the percentage of viable cells was 89.2 %, whereas it was 75 % for cells preserved with Opti-MEM + 10 % DMSO + 10 % FBS medium. Cells preserved with Bambanker™ hRM showed the highest cell viability with 89.4 % (Figure 1).

HCECs were cultured for 28 days after cryopreservation with the various agents. Cells preserved with Bambanker™ hRM and Bambanker™ grew faster than cells preserved with other cryopreservation media (Figure 2).

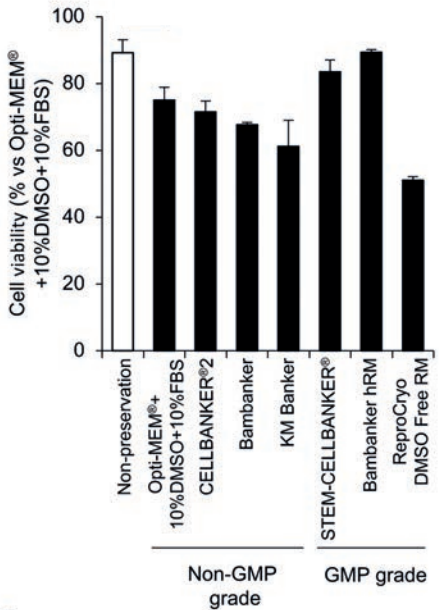

Figure 1: Percentage of viable HCECs for different cryopreservation media, compared to non-preserved cells. Bambanker™ hRM significantly increased the percentage of viable cells to 89.4 %.

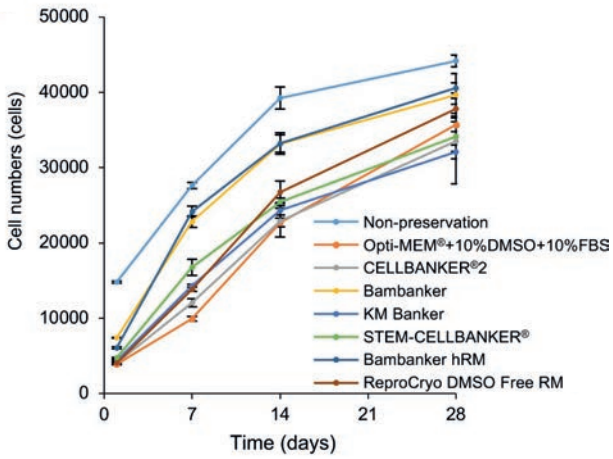

Figure 2: HCECs were grown for 28 days after cryopreservation. Cells that were cryopreserved in Bambanker™ hRM or Bambanker™ grew faster than cells that were cryopreserved in other media.

HCEC cells cryopreserved in Bambanker™ hRM maintained a cell density of 96.6 %, compared to non-cryopreserved control cells. HCEC cells preserved in Opti-MEM + 10 % DMSO + 10 % FBS medium only showed a cell density of 76.2 % (Figure 3).

Bambanker™ hRM was also tested for cryopreservation of HCEC cells at conditions typically used for clinical cell-based therapy (passage 3-5, cell density >2000 cells/mm²). The cell density and viability did not significantly decrease after cryopreservation, compared to non-preserved control cells (Figure 4).

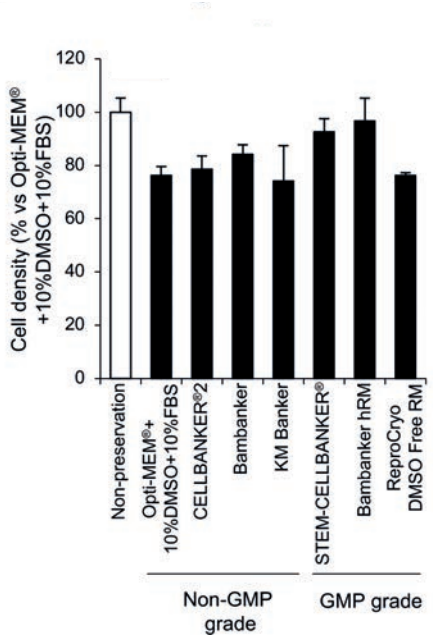

Figure 3: HCECs were grown for 28 days after cryopreservation. Cells cryopreserved with Bambanker™ hRM showed the highest cell density with 96.6 % of the non-preserved control cells.

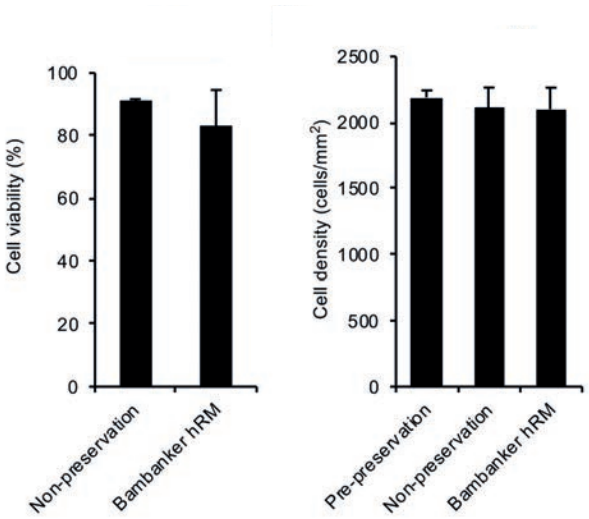

Figure 4: Cell viability and cell density of clinically used HCECs, cryopreserved with Bambanker™ hRM, showed simial values compared to non-preserved control cells.

Furthermore, cells cryopreserved with Bambanker™ hRM grew in a similar cobblestone-like sheet structure (Figure 5). Immunofluorescence staining showed a comparable expression of typical cell markers (Figure 6).

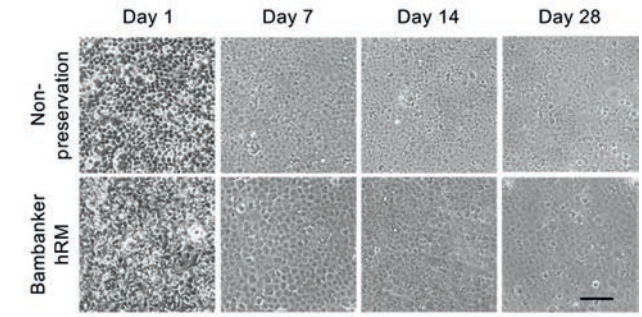

Figure 5: Phase contrast images of HCECs cryopreserved with Bambanker™ hRM. The cells show similar cell growth, shape and structure to the non-preserved control. Scale bar: 200 µm.

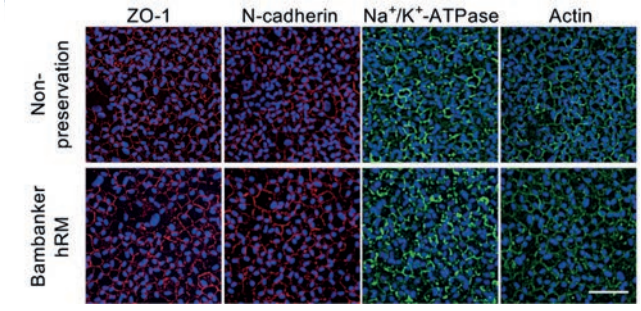

Figure 6: Cell marker immunofluorescence stainings of HCECs cryopreserved with Bambanker™ hRM, compared to non-preserved cells. The cells show a similar expression of ZO-1 (tight junctions), N-cadherin (adherence junctions), Na^+/K^+-ATPase (pump function) and Actin. Scale bar: 200 µm.

Conclusion

The study demonstrated the feasibility of cryopreservation of cultured human corneal endothelial cells using Bambanker™ hRM as a cryoprotectant. Bambanker™ hRM exhibited better cell viability, morphology preservation, and cellular function than other cryopreservation media and can also be used for cryopreservation of clinical-grade HCECs.

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