ROKEPIE®

HIBERNATION TECHNOLOGY FOR CELLS

Protection of Adipose Tissue-derived Stem Cells during 4°C storage

When working with human Adipose Tissue-derived Stem Cells (hADSC) preservation of the cells for a short period is often needed. Storing or transporting hADSC at hypothermic conditions is easier than at cryothermic conditions, however preventing hypothermal-induced cell damage and maintaining cell viability and functionality is always a challenge. ROKEPIE® now offers the ideal solution to address these issues.

Maintaining both stem cell viability and functionality

ROKEPIE® can protect hADSCs from hypothermal damage and maintain both its viability and functionality. This was assessed by experiments with hADSC on cell viability, adhesion and proliferation conducted by the University of Groningen. As multipotent stem cells hADSCs play a crucial role in the suppression of inflammation, apoptosis and promote angiogenisis by the secretion of several growth factors [2, 3]. And are located in white adipose tissue as pericytes or periadventitial cells [1]. These cells show a great potential in regenerative medicine by using ADSCs or ADSC-derived tissue engineered constructs in the treatment of several diseases. Cell and tissue engineering can benefit from easy and non-damaging short-term preservation of ADSCs in order to conduct effective quality control.

Used methods

Cells were cultured in DMEM supplemented with 10% FBS, 1% Penicilin/Streptomycin and 2mM L-glutamine. The hADSCs were cultured both with and without ROKEPIE® (1:10 or 1:100) and stored under hypothermic conditions (4°C) for 24 hours. The caps of the flasks were shut airtight to prevent a damaging pH increase of the cell culture media. Subsequently, the cells were re-warmed for two hours under standard cell culture conditions in a humidified incubator (37 C°, 5% CO_2) with an open cap to facilitate gas exchange. ADSC viability was assessed by flow cytometry with an Apoptosis & Necrosis Kit (Promokine, #PK-CA707-30018).

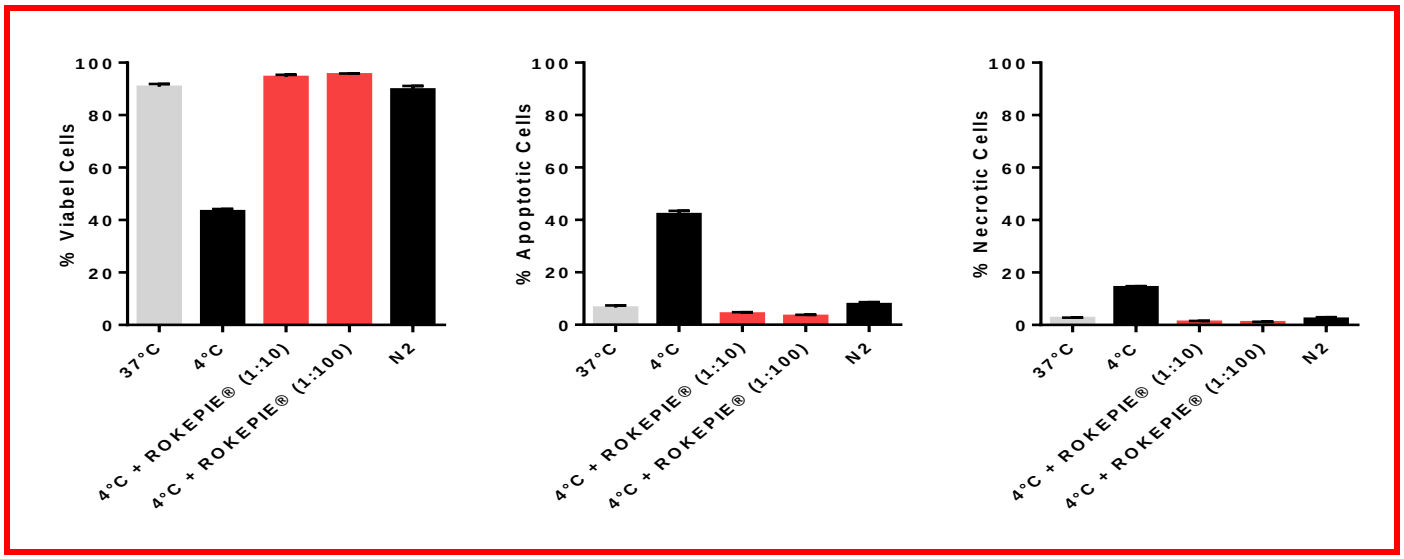

Fig. 1. The viability of hADSCs after 24 hours of hypothermal storage. ROKEPIE® protects cells from hypothermia-induced cell death. The N_2 group represents the cells that were stored on cryopreservation.

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The cell adhesion was investigated by dissociating the cells, staining the ADSCs with a fluorescent label and allowing the cells to adhere for 15 up to 240 minutes in 24-wells plates. Following the adherence phase, fluorescence was measured with a Varioskan spectrofuorometer. Automated fluorescence microscopy software TissueFax was used to asses proliferation.

Results

By adding ROKEPIE® the cell viability is maintained equal to the control group at 37 °C. Hypothermia-induced cell death was mainly the result of apoptosis and not necrosis, however ROKEPIE® inhibits both responses substantially with respectively 40% and 13% (fig. 1). The difference in cell viability between storage with ROKEPIE® and cryopreservation is minimal, however ROKEPIE® is easier to use for short-term storage and without toxic additives.

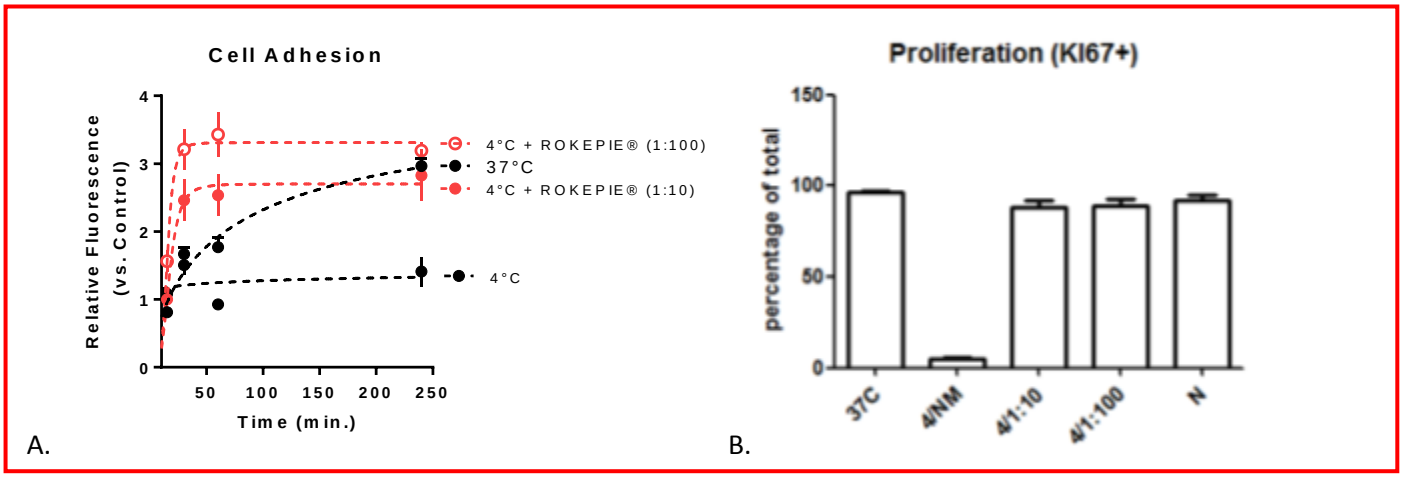

Fig. 2. (A) The cell adhesion of hADSC after hypothermal storage. (B) The proliferation of hADSC after hypothermic storage. ROKEPIE® addition resulted in faster adherence and the maintenance of a proliferative capacity. Abbreviations: 4/NM: cells hypothermically stored without ROKEPIE®, N: cells stored with cryopreservation. KI67: cellular marker for proliferation [4].

The cells that are supplemented with ROKEPIE® adhered faster to the tissue culture plastic than the control cells, indicating that the expression and function of adhesion receptors was not altered (fig. 2a). ADSCs hypothermically stored with ROKEPIE® had proliferation rates similar to the control cells that were cultured on 37°C (fig. 2b). Storage without ROKEPIE® impaired the proliferative capacity of hADSCs significantly to almost 0%.

Conclusion

By adding ROKEPIE® to preserve cells, the cell viability, adhesive and proliferative properties will be maintained at levels equal to 37 °C control cells. These results indicate that ROKEPIE® maintains cell functionality. This needs to be further investigated for each application individually, as the differentiation potential of ADSCs is considered a key feature for regenerative medicine applications. The cell viability experiments show minimal difference between ROKEPIE® and cryogenic storage, however ROKEPIE® is easier to use and therefore a better solution for short-term storage.

References:

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