# ABSTRACT

ČECHOVÁ, K. 2024. ***Effect of cryopreservation with low concentrations of dimethylsulfoxide on selected properties of keratinocytes*** [*Thesis*]

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Cryopreservation of cells is one of the main challenges of contemporary cryobiology, especially with regard to the progressively growing field of tissue engineering. The method of cryopreservation can significantly affect the quality of cells after thawing. For this reason, it is necessary to establish protocols that ensure a good physiological and adherent state of frozen cells, because their effective regeneration leads to high application efficiency in cell therapies. Successful cryopreservation requires careful consideration of several key factors, including the choice of cryoprotective agent (CPA) and its concentration.

The presented dissertation deals with the study of the effect of cryopreservation of cells with different low concentrations of the cryoprotective substance dimethylsulfoxide (DMSO). DMSO is known to be able to ameliorate freezing-related cell damage during slow cooling, but its apparent toxicity is still a matter of debate.

We investigated the cryopreservation of human keratinocytes using standard and lower doses of DMSO in the freezing mixture, which minimize its toxic effects but still retain cryoprotective effects. Human skin keratinocyte cells were frozen with (1.8 %; 2.2 %; 5 % and 10 % v/v) concentrations of DMSO and stored for a short time (for 4 days) at a temperature of − 80 °C. After thawing, we compared them with unfrozen cells. We focused on the viability and proliferation of cells after thawing and monitored the changes in biophysical characteristics that occur in cells due to cryopreservation. We analyzed changes in morphology and ultrastructure caused by freezing, monitored the effect of DMSO depending on its concentration, and thus considered procedures to reduce its toxic effects. We have shown that DMSO even in lower concentrations (≥ 2.2 %) can protect keratinocytes during their cryopreservation and short-term storage at low temperature. A DMSO concentration lower than 2.2 % may no longer have a sufficient cryoprotective effect, as differences in the structural and dynamic properties of the freezing mixture have been shown to cause cells to undergo apoptosis after thawing. These findings could have benefits in optimizing skin cell cryopreservation methods for both biotechnology and basic research.

**Key words**: keratinocytes, DMSO, cryopreservation, viability, proliferation morphology, arrangement of lipid bilayers, fluorescent probe DPH, scanning electron microscopy, transmission electron microscopy.