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Assessing the resistance of sheep fibroblasts to increasing concentration of Trehalose

Y. Ressaissi, M. Czernik, P. Toschi, P. Loi*

Faculty of Veterinary Medicine, University of Teramo, Via Balzarini 1, 64100 Teramo, Italy

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Introduction

Trehalose

Good glass-forming properties

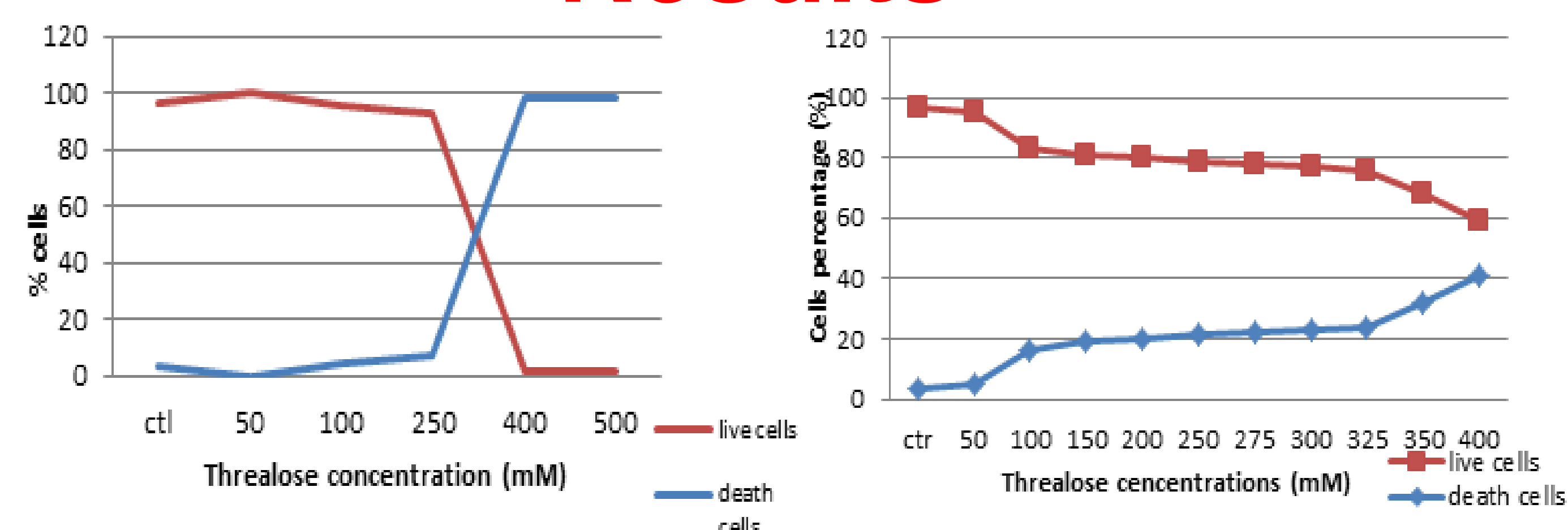
Replace hydrogen bonds of water with biomolecules upon dehydration

Stabilize biomolecules

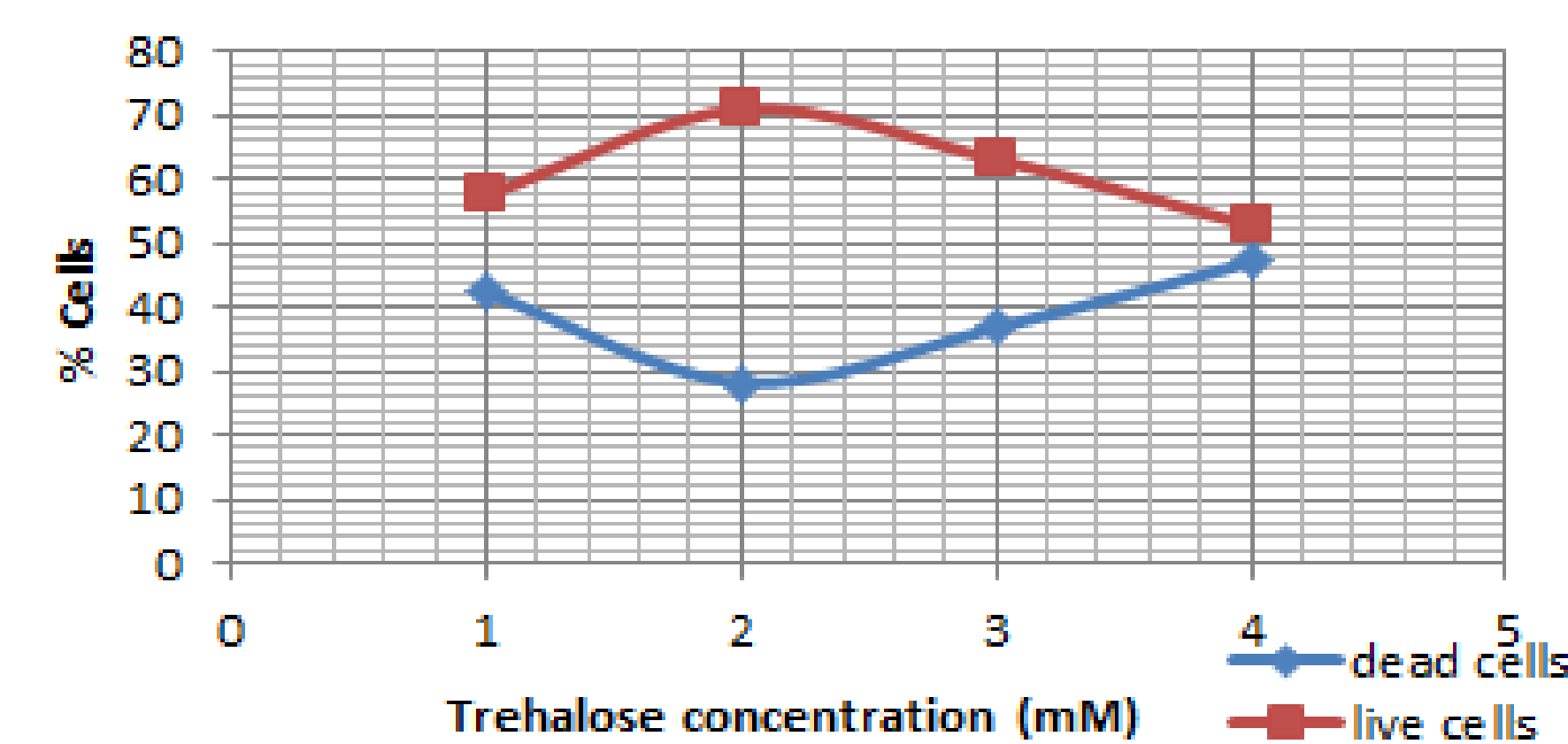
A main specific protectant used in dry preservation of biological materials

Assessing the resistance of sheep fibroblasts to Trehalose in perspective of use as lyoprotectant in freeze drying

Results



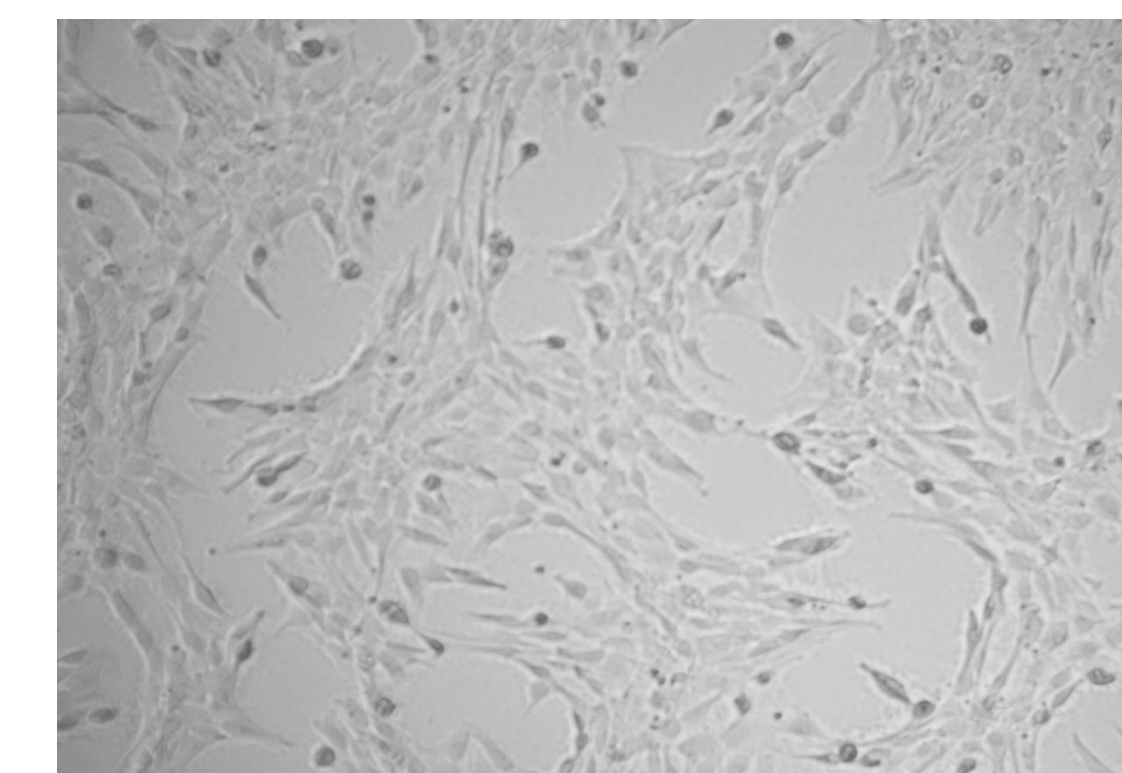
Percentage of survival cells after 24h within Trehalose concentrations



Percentage of survival cells within Trehalose after 1h



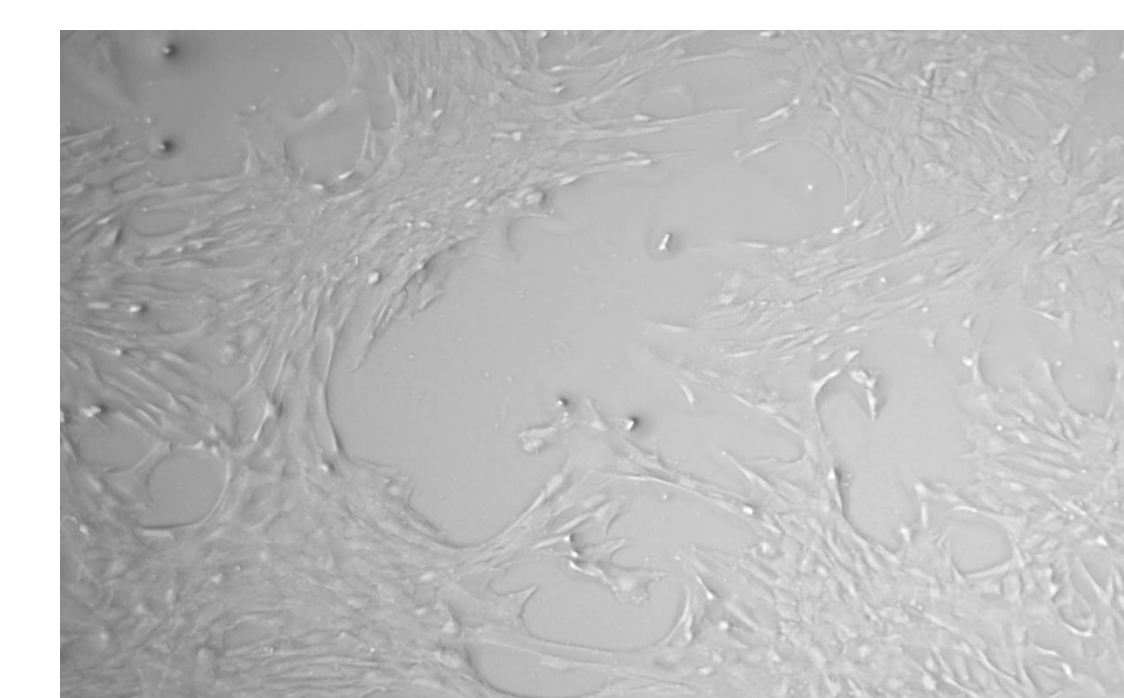
Cells shape after 3h with MEM



Cells shape after 3h with 50mM Trehalose



Cells shape after 2h with 100mM Trehalose



Cells shape after 3h with 200mM Trehalose

- Negative correlation between the percentage of live cells and the sugar concentration
- Death of the cells after 2h and 3h within each concentration

Method

24h

- Sheep fibroblast incubation in Minimum Essential Medium (MEM) with 50mM, 100mM, 250mM, 400mM and 500mM of Trehalose under 38.50°C in incubator
- Coloration with Trypan blue
- Live/dead cell counting after 24h

24h

- Sheep fibroblast incubation in Minimum Essential Medium (MEM) with 50mM, 100mM, 150mM, 200mM, 250mM, 275mM, 300mM, 325mM, 350mM and 400mM under 38.50°C in incubator
- Coloration with Trypan blue
- Live/dead cell counting after 24h

3h

- Sheep fibroblast incubation in Minimum Essential Medium (MEM) with 50mM, 100mM and 200mM which osmolarities are 376, 462 and 711mOsm/kg in a stove under 37°C, 12% humidity and without gas
- Coloration with Trypan blue
- Live/dead cell counting after each hour

Reference

1.Miao, Z.; Harriette, O.; Bulat, S.; Judith, B.; Harald, S; Willem, F.W.. *Scientific reports* 2017, 7.

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*To whom correspondence should be addressed: ploi@unite.it

Conclusions

We have found that the concentration of trehalose compatible with the maintenance of cell viability ranges from 50mM To 200mM. Trehalose treated cells undergo morphological changes (shrinking), that was however reversible upon further culture of the cells. These data prepare the ground for next studies aiming to induce trehalose import into the cell through the transient expression of the trehalose carrier. Trehalose-loaded cells will be submitted to controlled drying.