CRYOPRESERVATION OF IN VITRO LIVER MODEL USING HEPARG CELLS FOR TOXICOLOGICAL STUDIES.

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Introduction

Animal experimentation raises ethical and scientific concerns, with in vitro models offering an alternative. However, constructing these models faces challenges such as limited biomass availability and delayed establishment. Among the different biological sources available, immortalized human cell lines are commonly used. Regarding liver engineering, HepaRG cells are valuable for studying drug metabolism, toxicity, and liver diseases due to their physiological relevance. Relatively expensive, this cell type requires prolonged differentiation, and exhibit batch variability.

Cryopreservation of living systems aims to maintain the viability and functionality of these systems while storing them over long periods [1]. Controlled-rate cryopreservation allows the sample to cool at a cell-specific controlled rate to minimize cellular damage caused by the formation of intracellular ice formation [2], and excessive dehydration. This method depends on several parameters, such as the utilization of a specific cryopreservation agent (permeating or non-permeating cryoprotective agents (CPAs) as well as single or combined CPA solutions), CPA loading duration, temperature ramp, addition of chemical factors [3], or the use of protective biomaterials [4]. The optimization of each of these parameters depends on the cell type as well as the organization of the cells.

The aim of this project was to use controlled rate freezing technologies in order to overcome technical obstacles related to inter-batch viability and create a ready to use *in vitro* liver model for toxicological studies.

Methods

HepaRG cell line was encapsulated with alginate serving as a cryoprotectant at a concentration of 1.E6 cells/mL of alginate. The samples were divided into two groups : one group undergoing conventional cryopreservation consisting of addition of CPA agent, 15 minutes of equilibration time at 4°C prior to 24H at -80°C into cell freezing container and transfer into nitrogen tank for long storage. The second group underwent controlled-rate freezing, following the same protocol as the first group, except using CryoMed™, allowing the sample to cool at a rate of -0.3°C/min until reaching -80°C. Five different CPA agents were used in order to compare the effects of CPA on cell activity. Metabolic assays were conducted on the samples prior to cryopreservation and after thawing.

Results and discussion

Results of cell activity on encapsulated HepaRG reported comparing global cell activity (Figure 1) as well as CYP activity depending on the cryopreservation technique and the CPA used.

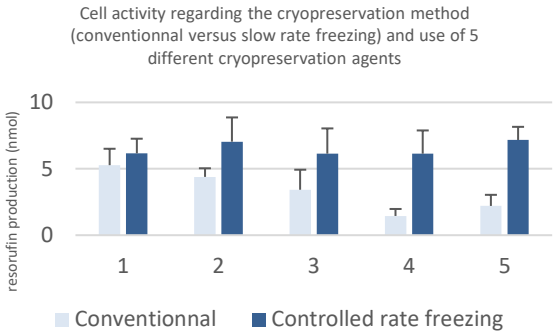

Figure 2: Mesured resorufin production witnessing cell activity by PrestoBlue™ Assay. Two conditions were tested, conventional cryopreservation (light blue), and controlled rate freezing (dark blue).

Conclusion

Samples undergoing controlled rate freezing presents better cell metabolism compared to conventional cryopreservation regardless of the choice of CPA used.

The choice of CPA used thus has an impact on different metabolism (data not shown). Depending on the metabolism of interest, the choice of CPA used is also a parameter to optimize.

This study will be extended to other cell line (IPSC-derived liver cells) and cell types (stellate cells, LSECs) as well as several cooling rates will be tested in order to optimize the best cryopreservation program for each cell type.

References

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