Efficient Cryopreservation of Primary Chromaffin Cells Using A Novel Cryopreservation Medium

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Introduction

Adrenal chromaffin cells are a great model to study exocytosis and endocytosis events to explain various important mechanisms in neuroscience. In this study, we demonstrated and improved the efficiency using a novel and commercially available cryoprotective medium, C80EZ®, for cryopreservation of primary bovine chromaffin cells in both -80ºC deep freezers and liquid nitrogen dewars.

The two corresponding major functioning components are the compact polysaccharide (A) and glyconate antioxidant (B). Additional polymer and antioxidant molecules were added to the existing formula (AB) and tested their effects on the cell life and activity across different timelines.

Component Name	Identification and Purpose
A	Compact polysaccharide, the C80EZ® major component
B	Glyconate antioxidant, another C80EZ® component
C	Dextran, a regular polymer used for traditional cryopreservation protocols to maintain cell membrane integrity (Fisher Scientific, USA)
D	Lactobionate, a regular antioxidant (Sigma-Aldrich, USA)

Goal

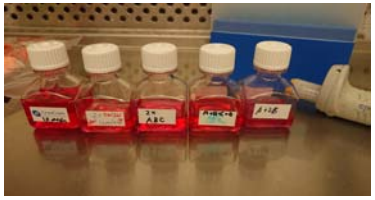
To be able to store chromaffin cells at -80°C for up to 6 months with minimum loss of cells and to avoid liquid nitrogen facilities to provide a more efficient storage technique, as well as to improve the outcome of traditional liquid nitrogen storage protocols of chromaffin cells.

Methods

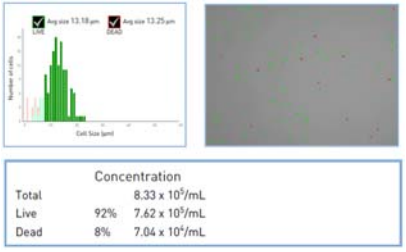
- In total, five different versions of the original formula were tested. The versions of the media were named as; AB, AC, ABC, ABCD and A2B, all of which were supplied with 5% dimethyl sulfoxide (DMSO) as the standard protocol of using C80EZ® products.
- Results were taken at two weeks, two months and six months. All results collected were compared to a control group which were frozen with regular chromaffin cell culture media, which contains 10% fetal bovine serum with 10% DMSO, following the same physical procedures.

Media Type	Composition
AB	[A]+[B]
AC	[A]+[C]
ABCD	[A]+[B]+[C]+[D]
A2B	[A]+ Double the concentration[B]

- Traditional procedures suggest 10% DMSO to be added to the media for cell survival. However, considering the toxic effect of DMSO on the cells, results of using 5% DMSO with the C80EZ® media were compared to those using10% DMSO with the C80EZ®.
- Cells are prepared with a version of C80EZ® media and contained in 1ml cryovials and frozen by using standard freezing boxes, CoolCell® (Fisher Scientific, USA) until the final temperature of -80°C is reached.



- For liquid nitrogen storage, the cryovials were kept at -80ºC overnight and then transferred to a liquid nitrogen dewar for storage.
- Cell counts were obtained by Countess II (ThermoFisher Scientific, USA) automated cell counter with using Trypan blue imaging assay.

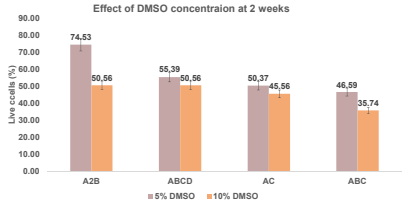


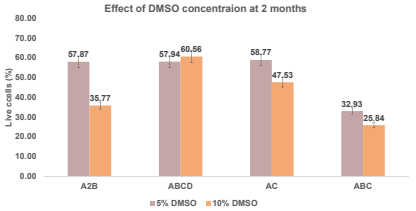
- Moreover, cell viability was also tested by measuring the electrochemical activity of cells. Cells were stimulated by a high concentration potassium solution to promote exocytosis and create amperometric spikes.

Results

DMSO concentration effect

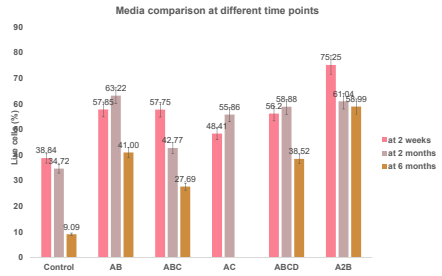
- The Impact of the concentrations of DMSO Comparing the results from using different concentration of DMSO in various freezing media tested, it was found that 5% DMSO in the freezing media yielded higher percentage of live cells. Therefore, all upcoming studies were done with using 5%DMSO in the freezing media.

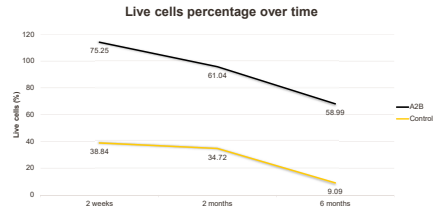




Time course studies

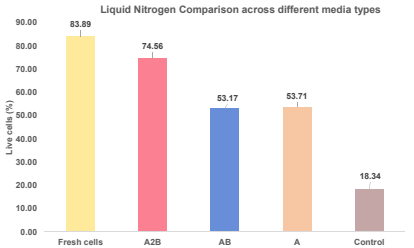
- Different versions were tested at 2 weeks and at 2 months and compared to the control group. Out of all the measurements, A2B yielded the highest rate of cell survival making
- The addition of Dextran or lactobionate didn't improve the survival efficiency.
- Comparison of the results of using A2B to those from the control group which lacks the C80EZ® media showed that using the C80EZ® medium is a much better option for freezing chromaffin cells.





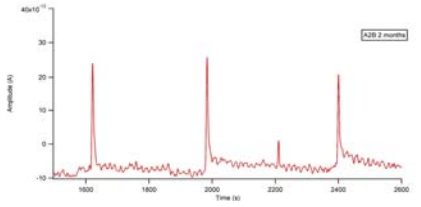
Liquid Nitrogen trials

- Studies showed that, out of all the groups, preserving chromaffin cells in A2B version of C80EZ® provided the significantly better results compare to the other two options.



Electrochemical Studies

- Amperometric spikes were detected by custom made devices at Gillis lab, after the cells were chemically stimulated by a high concentration potassium solution. Recorded spikes provide an evidence for the event of exocytosis and thus demonstrate that cells are still biologically functioning.



Conclusions

- The C80EZ® technology utilizes macromolecular crowding effects to significantly reduces the size of ice crystals during cooling and prevents recrystallization during storage and warming procedures.
- The results show that A2B version of C80EZ® media provides significantly higher chance of survival of bovine chromaffin cells than the other cryopreservation methods.
- Using 5% DMSO in the freezing media instead of the 10% DMSO, enhances the cell survival percentage after being frozen for months.