A novel approach for storage of vascular cells at -20°C using a cryopreservation medium that minimizes ice recrystallization

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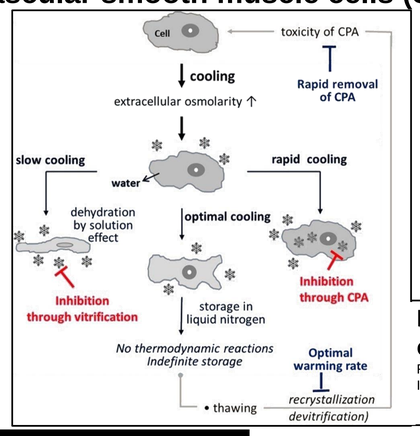
INTRODUCTION

Cryopreservation of blood vessels and bioengineered vascular grafts has great potential to facilitate bypass and other reconstructive surgeries. However, currently available cryopreservation methods are limited by a requirement for liquid nitrogen freezing, high concentrations of toxic cryoprotective agents (CPA), and impractical thawing protocols. The objective of this study was to determine if C80EZ®, a novel cryoprotective solution containing high concentrations of compact polysaccharide molecules that minimize ice recrystallization at non-cryogenic temperatures, can be used in conjunction with dimethyl sulfoxide (DMSO) to efficiently cryopreserve vascular smooth muscle cells (SMCs) at -20°C.



Physical events and cryoinjury of cells during freezing and thawing

From Jang TH, et al. Cryopreservation and its clinical applications. Integr Med Res. 2017 Mar;6(1):12-18.

METHODS

Human coronary artery SMCs were suspended in the following media and frozen at -20°C:

a) Fetal bovine serum with 5% DMSO (FBS/DMSO)
b) Dulbecco's modified Eagle medium with 5% DMSO (DMEM/DMSO)
c) 45% C80EZ, 50% DMEM, and 5% DMSO (C80EZ/DMEM/DMSO)

Upon thawing, cytotoxicity was assessed by measurement of lactate dehydrogenase (LDH) leakage. Cell viability was determined by plating cryopreserved cells and measuring cell proliferation over the following 96 hours.

RESULTS

- After 10 days of cryopreservation, SMCs suspended in C80EZ/DMEM/DMSO showed significantly less cytotoxicity compared to those suspended in DMEM/DMSO or FBS/DMSO, as reflected by a 3-fold increase in LDH leakage in the latter two groups compared to C80EZ/DMEM/DMSO; n=3/group; P<0.001.
- Furthermore, cells treated with C80EZ/DMEM/DMSO showed significantly greater proliferation (29 ± 0.5 x10^3 viable cells at 96 hours) compared to cells treated with DMEM/DMSO (2.6 ± 1.2 x10^3) or FBS/DMSO (5.1 ± 2.5 x10^3); n=3/group; P<0.001.

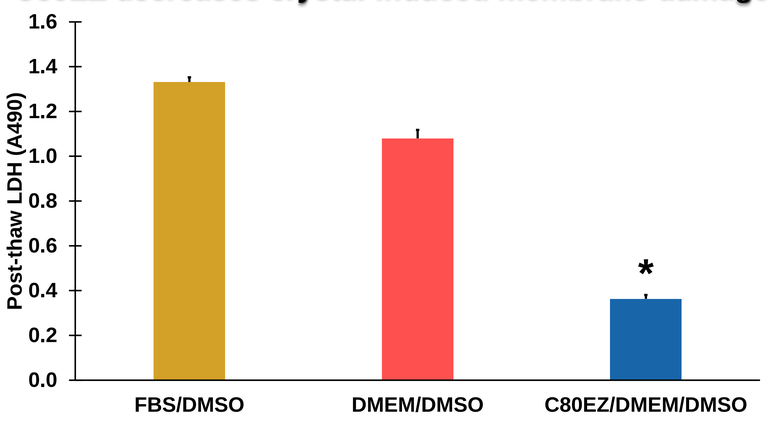


Figure 1. VSMC were incubated in their respective media at -20°C for 10 days. Upon thawing, cells incubated in C80EZ/DMEM/DMSO had less LDH leakage, reflecting less membrane damage caused by ice recrystallization. n=3; *P < 0.01 compared to other groups.

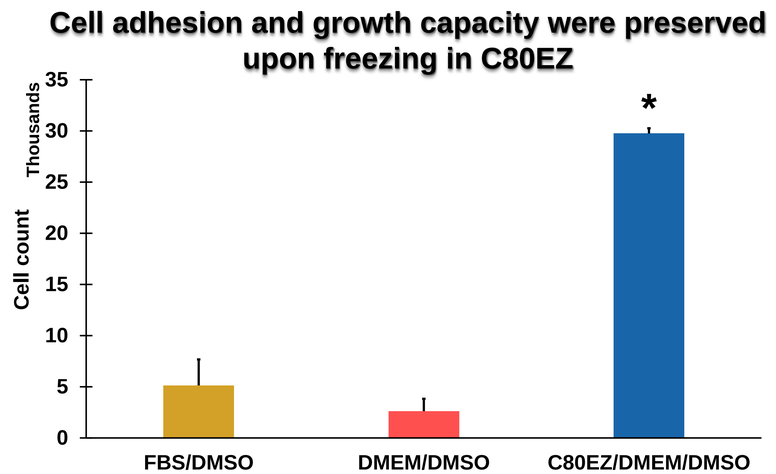


Figure 2. VSMC previously frozen in their respective media at -20°C were seeded and allowed to grow for 96 hours. Cells frozen in C80EZ/DMEM/DMSO had the highest plate attachment and subsequent growth capacity, reflecting intact cell membrane, channels and surface proteins. n=3; *P < 0.01 compared to other groups.

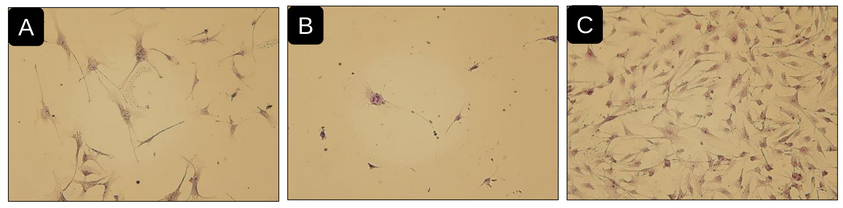


Figure 3. Cells previously frozen in different media were thawed, seeded and allowed to grow for 96 hours. Those that have been frozen in C80EZ/DMEM/DMSO (C) showed higher adhesion and proliferation capacity with an intact morphology compared to FBS/DMSO and DMEM/DMSO (A and B respectively). Cells are stained with Diff-quick (a variant of a Romanowski stain) and imaged at 10x magnification.

CONCLUSION

These results suggest that SMCs can be cryopreserved at -20°C in C80EZ/DMEM/DMSO.
This technology brings three main improvements to traditional cryopreservation techniques: decreasing the exposure of frozen samples to toxic CPA, bypassing the need for animal-derived fluids such as FBS, and not requiring special or hazardous equipment. Thus, it appears that this solution has the potential to markedly expand the utility of cryopreservation for storing vascular cells under readily available laboratory conditions, and may be suited to a number of research and clinical applications.