



## Deep eutectic solvents for cryopreservation

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Cryopreservation has had huge benefits for the world at large, including preservation of blood and stem cells, and assisted reproductive technologies. (Fuller, Paynter, & Watson, 2004) However, there are many cell types that cannot be stored using current cryopreservation methods, and no organs. (Hunt, 2011; Mazur, 1970; Sputtek & Sputtek, 2004) In fact, 60% of all donated hearts and lungs are discarded due to inadequate storage methods, and this waste could be overcome with cryopreservation. (Manuchehrabadi et al., 2017)

The main limitation in cryopreservation is the ongoing reliance on predominantly just two cryoprotective agents (CPAs), both of which are toxic: dimethylsulfoxide (DMSO) and glycerol. (Lovelock & Bishop, 1959; Polge, Smith, & Parkes, 1949) The toxicity of existing CPAs means that cells must be frozen immediately after addition of the CPA. These CPAs are inappropriate for tissues and organs because there is insufficient time to penetrate to deeper cell layers, leaving them vulnerable to freezing damage. (Fuller et al., 2004) Thus there is a need for different, non-toxic CPAs with tuneable properties. (Raju, Bryant, Wilkinson, & Bryant, 2021)

Deep eutectic solvents (DESs) are highly tuneable solvents, many of which are non-toxic. To date, only a very few studies have examined the cryoprotective applications of DESs, but these have shown comparable viability of cells stored using DESs compared to those stored using DMSO. (Jesus, Meneses, Duarte, & Paiva, 2021) We have characterised a number of DESs for their thermal properties and interactions with mammalian cells, including toxicity and permeability. One DES was then carried forward and tested for its cryoprotective effect on four distinct mammalian cell lines. It was just as effective, and in some cases more effective, than DMSO at protecting the cells during cryopreservation.

These results provide new avenues of cryopreservation for cell types which cannot be preserved with existing CPAs. This in turn has wide-ranging benefits, especially in the biomedical field.

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