A safe, cheap and effective method for slow-freezing human stem cells

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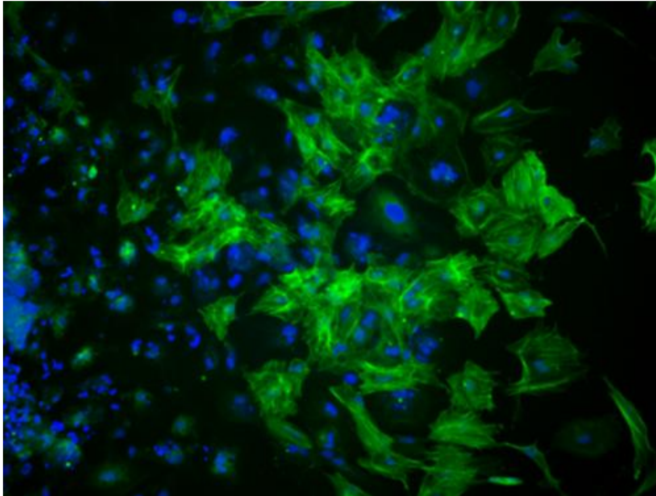

Figure 1: Fluorescence microscopy image showing the differentiation of healthy thawed stem cells into distinct germ layers. Green shows labeled mesodermal proteins; blue shows cell nuclei. Credit: K. Imaizumi et al.

Human pluripotent stem cells (hPSCs) show great potential and versatility in regenerative medicine and new therapeutic approaches to fight disease. Patient-specific, individualized treatments using stem cells have even been generated for a number of diseases. Although further research into hPSCs is needed in order to harness their full potential, preserving the stem cells and storing them in the large numbers required for research has proved difficult.

Teruo Akuta and colleagues at the RIKEN Center for Developmental Biology, together with scientists from the Foundation for Biomedical Research and Innovation, have now developed a cost-effective, efficient and reliable slow-freezing method for preserving hPSCs in large numbers with a high survival rate.

Vitrification, which involves the use of cryoprotectants to chill cells to low temperatures without freezing, and conventional slow-freezing techniques are currently used for the cryopreservation of hPSCs. "Vitrification using liquid nitrogen is a highly skilled task," notes Akuta, "and is not suitable for stock freezing of hPSCs. Slow-freezing methods, on the other hand, typically have poor post-thaw recovery and low yields. We needed to find an easier, more efficient and robust method to preserve hPSCs."

A key problem in the cryopreservation of hPSCs is 'cell clumping', by which cells stick together during the freezing process and do not survive because the cryoprotectant is unable to penetrate the cell clumps. Akuta and his team hypothesized that loose cell adhesion could be encouraged through the use of freezing solutions containing cell detachment reagents.

The researchers modified a safe, cheap and readily available freezing agent called CP-1 to optimize it for use with hPSCs. They tested five different combinations of CP-1 and cell detachment reagents, avoiding expensive or animal-based products.

The most successful formula consisted of a mix of hydroxyethyl starch, a natural cryoprotectant from plants; dimethyl sulfoxide and ethylene glycol, used to prevent the formation of ice crystals inside cells; and a cell detachment agent called Pronase/EDTA, used for the first time in cryopreservation.

After freezing using the new freezing agent, CP-5E, and a conventional freezer at –80 °C, followed by rapid thawing in a simple water bath, Akuta's team found that over 80% of the harvested hPSCs retained their ability to differentiate into different mature cell types (Fig. 1).

"We believe this is in part due to the loose connections between cells created by the Pronase solution, but the exact mechanisms need

verification," says Akuta. "We hope to mass-produce CP-5E in the near future for use in research and clinical applications."

More information: Imaizumi, K., Nishishita, N., Muramatsu, M., Yamamoto, T., Takenaka, C., Kawamata, S., Kobayashi, K., Nishikawa, S. & Akuta, T. "A simple and highly effective method for slow-freezing human pluripotent stem cells using dimethyl sulfoxide, hydroxyethyl starch and ethylene glycol." *PLoS ONE* 9, e88696 (2014). dx.doi.org/10.1371/journal.pone.0088696

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