

FREEZE DRIED STEM-CELLS

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Storage in a dry state and at room temperature is probably the safest way to keep biological samples for the long term. The storage is simple, no maintenance required and low cost. Storage requirements are simply to close samples under vacuum, and keep it protected from light, oxygen and irradiation.

In a recent publication we have shown that freeze-dried granulosa cells that were stored for 3 years at room temperature preserved their DNA integrity and were capable to produce normal embryos after nuclear transfer. We have then continued on improving the viability and functionality of dried cells after rehydration by adding an antioxidant to the lyophilization solution and by changing the freezing and drying parameters. This was done on mononuclear cells (MNC) derived from human UCB units.

A freezing solution named IMT-2 was added prior to freezing. Freezing was done using the MTG-1314 freezing device. We tested the viability, number of CD34+-presenting cells and ability of the rehydrated hematopoietic stem cells to differentiate into different blood cells in culture before freezing and after freeze thawing and freeze-drying.

The viability of the MNCs after freeze-drying and rehydration with pure water was 88%-91%. The total number of CD34+-presenting cells and the number of colonies did not change significantly when evaluated before freezing, after freeze-thawing and after freeze-drying ($5.4 \cdot 10^4 \pm 4.7$, $3.49 \cdot 10^4 \pm 6$ and $6.31 \cdot 10^4 \pm 12.27$ cells, respectively, and 31 ± 25.15 , 47 ± 45.8 and 23.44 ± 13.3 colonies, respectively).

Additionally, we have performed freeze thawing and freeze drying experiments with MNC derived from mice bone marrow which were transfused into lethally irradiated mice. We have seen increased survival following injection of freeze-dried and rehydrated cells. The freeze thawing experiments were done on blood from male mice that were injected into irradiated female mice. One month after the injection of the frozen thawed cells blood was taken from the female mice and PCR was performed showing the presence of Y chromosome. These preliminary findings suggest that the cells were capable to incorporate into the bone marrow and to form new white blood cells.

In summary, we have developed a freeze-drying technique for maintaining the viability and functionality of cells. Using this technique hematopoietic stem cells have survived complete desiccation while maintaining their clonogenicity capabilities upon rehydration.