

## Tissue preservation techniques

*Manasi Mahesh Chavan*

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Ever since American embryologist R. G. Harrison successfully grew cells as in vitro culture in the first decade of the 20th century, cell culture became widely popular in the medical and research field. Scientists around the world have made contributions to improving in vitro cell culture. As studies and treatment based on in vitro cell culture broadened and intensified, preservation of cell cultures for long-term studies and treatments became essential. Some of the materials that require preservation include seeds, embryos, ovaries, blood, sperms, cell lines, cell cultures, animal tissues, extracellular matrix, plant tissue samples and other organs that need to be preserved alive and in a functional state. There are many ways to preserve the live cells and tissues while maintaining their functionality; including dehydration, formalin-fixed paraffin embedding (FFPE), freeze-drying also called lyophilisation, vitrification and cryopreservation. Dehydration is a technique where all the water content is removed from the tissue by immersing it in concentrated alcohol. In FFPE, the tissue is fixed using formalin, followed by immersion in paraffin oil. Another method is freeze-drying, also called lyophilisation, in which the tissue is dehydrated and then stored in very cold conditions at  $-30^{\circ}\text{C}$  to  $-50^{\circ}\text{C}$ . Alternatively, vitrification is a very rapid freezing method that was developed to avoid crystal formation while freezing the sample specimen. The vitrified tissue acquires a glass-like appearance.

However, the most advanced and efficient technique is the cryopreservation method, in which living tissues are preserved in liquid nitrogen and carbon dioxide at  $-80^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$ . At such a low temperature, the biochemical activities of cells will slow down or stop. Cryopreservation techniques use chemical reagents to replace water present in the cells, these reagents are called cryoprotectants. Additionally, slow freezing causes removal of the water content from the cell which results in dehydration and extracellular crystal formation. Whereas, fast freezing causes intracellular crystallisation which eventually leads to cell death by rupture of the cell membrane. Hence, it is necessary to optimise and maintain the rate and speed of freezing for every specific sample. Nevertheless, all these methods possess their own limitations such as some of the methods can store specimens only for a short period, chemical reagents used in these procedures are mostly cytotoxic and can cause harm to the preserved tissues, etc. Furthermore, in the recovery of long-term preserved cells, significant cell damage may occur during the thawing procedure. Moreover, crystallisation is a major drawback of all the methods involving subzero temperature. However, scientists are working constantly on improving these techniques to overcome their drawbacks, especially cryopreservation, as it is the most efficient technique for long-term tissue preservation; thus it is gaining more research interest, to overcome its limitations with regards to avoiding crystal formation and decreasing the toxicity of the cryoprotectants.

*Keywords: Cell culture, Tissue preservation, Dehydration, Lyophilisation, Vitrification, Cryopreservation*

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### Citation:

Manasi Mahesh Chavan. Tissue preservation techniques. The Torch. 2021. 2(49). Available from:

<https://www.styvalley.com/pub/magazines/torch/read/tissue-preservation-techniques>.