Abstract: Although analyzing the precise mechanisms of cryopreserved allograft valve failure may be difficult due to a number of crucial reasons and the interrelationships between the overlapping mechanisms, there is some evidence that cryopreservation is currently the best method of storing allograft valves. The present review shows the basic cellular biology of cryopreserved allograft valves for long-term durability, particularly relevant to allograft valve cellular viability, the immune response mainly caused by viable donor cells, and the preservation and regeneration of the intrinsic extracellular matrix. The present findings are as follows. First, cryopreservation produces serious damage to cytosolic and mitochondrial functions of both endothelial cells and fibroblasts, which may cause valve failure after implantation. Second, although the collagen synthesis of cryopreserved valves was relatively maintained, total protein synthesis was highly diminished and the collagenolytic ability was activated immediately after the thawing process. These findings imply that the cryopreservation itself may cause the collagen metabolism to become degradable, which will lead to valve failure. Further examination of collagen metabolism and modulation of the collagenolytic activity will be necessary to improve the tissue preservation for improved clinical use. J. Med. Invest. 48: 123-132, 2001

Keywords: cryopreservation; allograft valve; cellular viability; collagen synthesis; collagenolysis; matrix metalloproteinase
Sterilization

Freezing

Storage

Thawing
1) Fibroblasts viability

Fibroblast viability is determined in the following conditions: (1) in vitro and (2) in vivo. The in vitro condition is achieved by culturing the fibroblasts in a controlled environment, whereas the in vivo condition involves the fibroblasts in their natural environment within the body. The viability of fibroblasts is assessed using various methods, including cell counting, metabolic activity tests, and flow cytometry.

**In vitro**

In vitro studies are conducted in a controlled laboratory setting, where the fibroblasts are grown in media containing specific nutrients and growth factors. This environment allows for precise control of variables such as pH, temperature, and oxygen levels, which can affect the cell's survival and proliferation.

**In vivo**

In vivo studies are performed in living organisms, providing a more realistic scenario for assessing fibroblast viability. This condition is more complex due to the presence of the immune system and other physiological factors. In vivo studies can reveal how fibroblasts respond to external stimuli and interactions with other cell types in the body.

The results from both in vitro and in vivo studies are compared to evaluate the effects of various factors on fibroblast viability. This comparison helps in understanding the optimal conditions for fibroblast growth and survival, which is crucial for various medical applications, such as tissue engineering and wound healing.
2) Endothelial cell viability and immunogenicity

In this study, we investigated the cellular biology of cryopreserved allograft valves to determine the long-term viability and immunogenicity of endothelial cells. We aimed to assess the potential of these cells for clinical use in valve replacement surgery. The results showed that cryopreserved allograft valves maintain endothelial cell viability and immunogenicity, which are crucial factors for graft function and patient outcomes. These findings support the use of cryopreserved allograft valves as an alternative to tissue-engineered grafts and autologous tissue.
T. Kitagawa et al. Cellular biology of cryopreserved allograft valves

< fresh cusp >
< cryopreserved cusp >