Teacher Background

Cryobiology

Note: The Teacher Background Section is meant to provide information for the teacher about the topic and is tied very closely to the PowerPoint slide show. For greater understanding, the teacher may want to play the slide show as he/she reads the background section. For the students, the slide show can be used in its entirety or can be edited as necessary for a given class.

<u>What is cryopreservation</u>?

Cryopreservation is defined as the use of very low temperatures (usually the temperature of liquid nitrogen, -196^{o}C) to preserve structurally intact living cells and tissues. At this temperature, almost all biological activities, including the ones that lead to cell death, are stopped and can be restored upon warming. The goal of cryopreservation is to preserve the integrity of the living cell by avoiding ice formation inside the cell.

<u>How are cells protected from damage as they are cryopreserved</u>?

Since water expands when it freezes (forming ice), it can cause the container it is in to expand and potentially burst, such as a pipes or hoses full of water do during freezing temperatures. Because it is estimated that 60% of the human adult male body and 55% of human adult female body are made of water, freezing tissues can cause damage to them.

There are two theories about the cause of damage to cells as they freeze; both are related to the amount of water in living tissue. One is that freezing living tissue causes ice to form inside of cells which damages fragile membranes of organelles in the cell and can potentially rupture cell membranes causing cell death. The second is that damage comes from the secondary effect caused by the concentrating of solutes left in solution as the water freezes. In the latter case, if cryoprotectant compounds could be added to the cells to increase the total concentration of all solutes in the system, the amount of ice that could form at any temperature would be reduced. Ideally, these cryoprotectants should be able to diffuse or osmose into and out of the cell and have low toxicity. Cryoprotectants that have these properties, such as glycerol, dimethyl sulfoxide, ethylene glycol, propylene glycol, ethanediol, propanediol, and sugars, have been used to keep the intracellular (within the cell) ice formation to a minimum. It is generally agreed that intracellular ice formation is damaging and extracellular (outside the cell) ice formation is harmless. However, damage has been found to be caused by the mechanical stresses of extracellular ice formation in densely packed cells within the channels where they are sequestered, such as in complex multicellular systems. (1, 2)

<u>Does cryopreservation occur in nature</u>?

Long before scientists figured out how to achieve cryopreservation, it was discovered in nature. Survival strategies relying on cryopreservation exist in bacteria, fungi, plants, insects and other animals during harsh winters. The common wood frog, *Rana sylvatica*, uses sugar as a cryoprotectant and arctic insects use low molecular weight non-protein antifreeze-type substances in their blood, such as glycerol and other sugar alcohols, to protect them from freezing to death during the winter.

The wood frog ranges from southern Ohio to Arctic Circle and can survive very cold winters. It was found that one touch of ice sets off signals in the frog which include dehydration of tissues, circulation of sugar in the bloodstream, and slowing of metabolism. The heartbeat slows and the frog can become so frozen that its heart stops beating and ice forms around the heart and inside the chambers of the heart. This state of arrested heart function can occur for many days and perhaps months. When the frog thaws, its heart contractions spontaneously resume, and the frog begins to move, and even to mate. (3)

Arctic insects live above the Arctic Circle and include a wide variety of species. Most are freeze-tolerant like the wood frogs and survive the winter in a frozen state with much of the water in their bodies converted to extracellular ice. It has been found that frozen insects use less energy and lose water more slowly than unfrozen insects at the same temperature. Most species of insects use low molecular weight cryoprotectants, such as glycerol, to protect tissues during freezing and thawing. In these small insects, water loss can become a problem and the insects can become dessication-resistant by controlling the behavior of water in cells and tissues through solute production (cryoprotectants) and ion transport. Like the wood frog, the insects also make *nucleators* which initiate freezing at relatively high subfreezing temperatures to prevent very rapid growth of ice crystals that form once the first crystal appears in cold supercooled fluids. When spring comes, the insects thaw and resume their normal activities. (4, 5)

<u>What other variables are important to consider in cryopreservation</u>?

Equally important to consider in the freezing process are the type of cell, the cooling rate, and the warming rate. Slow rate freezing avoids ice formation inside the cell by making water diffuse out of the cell; however, since ice still forms outside the cell, the process of vitrification was investigated. Vitrification is a super rapid rate freezing method which produces a glassy state that is defined as a viscosity which behaves more like a solid without the formation of crystallization. Vitrified liquids appear clear, like glass, which is what *vitrified* means in Latin. Pure water can be vitrified (no ice formation) if cooled at a rate of millions of degrees Celsius per second. This very rapid freezing process avoids overall ice formation inside and outside the cell. Slow rate freezing can be done as well with no ice crystals forming inside the cell but the results are not as close to being like fresh tissues as vitrified tissues are since ice crystals do form outside the cell.

Nitrogen goes from the gas phase to the liquid phase at -196^{o}C (-321^{o}F) and from the liquid phase to the solid phase at -210^{o}C (-346^{o}F). As a liquid, it is so cold that it freezes living tissue on contact causing the sensation and appearance of a bad burn. Vitrification using a cryoprotective agent, like glycerol, protects the cell, since ice crystals won't form inside or outside the cell when exposed to liquid nitrogen. The concentration of glycerol/water affects how much or how little ice can form when placed in liquid nitrogen. Glycerol forms hydrogen bonds with water thus interfering with the hydrogen bonding between water molecules which form as water cools as part of ice formation and keeps the solutes in proper concentrations to prevent ice formation. Since no ice forms in the cell, the cell will survive the freezing and thawing process. Work performed by Alison Y. Ting, PhD, at ONPRC found that a 53% concentration of glycerol/water solutions gives the best result for vitrification of ovarian tissue and ooctyes (eggs).

<u>How can cryopreservation be used for fertility preservation in male and female cancer patients</u>?

When a cancer patient is facing the use of chemotherapy and/or radiation treatment to help destroy cancer cells, preserving fertility is an important aspect of cancer treatment so that infertility will not be a permanent side-effect of the cancer treatment after the patient recovers from the cancer. Vitrification in liquid nitrogen is a way to preserve whole ovaries, ovarian tissue, oocytes (including social egg banking to postpone having children until later on one's career), and embryos in females, and in males, whole testicles, testicular tissue, sperm, and embryos for later use by the cancer survivor. Many of these methods are still experimental, however. At this time, freezing embryos and sperm, if possible, continue to be the best options for future live births, in monkeys and in humans.

<u>Does vitrification occur in everyday life</u>?

Vitrification occurs both inside and outside of the laboratory setting. When sucrose in solution is cooled slowly, the result is crystal sugar (or rock candy). However, when the sucrose solution is cooled rapidly, the result is syrupy and airy cotton candy which can be collected on a paper cone. Another example is old ice cream which becomes less appetizing because of the formation of large ice crystals as a result of devitrification. As the ice cream is unfrozen and refrozen over time, the water slowly freezes and forms ice crystals throughout the ice cream.

<u>Is cryopreservation useful in other ways</u>?

Dwindling numbers of many species are leading to endangerment and extinctions of a variety of living things. In some species, numbers have dwindled due to catastrophic events, as in the case of the cheetah. In others, increase in the human population and its overuse of resources have been key factors. This would include overharvesting some species for food, clothing, etc. and decreasing the amount of habitat available for their survival. Being able to successfully freeze the oocytes and sperm cells from endangered species might allow us to use them later to create more members of the species and perhaps provide greater genetic diversity.

Bibliography

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