Tissue Engineering

Biopreservation

Advances in tissue engineering, cell transplantation, and genetic technology have created intense interest in the use of living cells as therapeutic tools. Several engineered products are already available to replace organs either temporarily or permanently or to augment organ function. Recent progress in stem cell biology has further fueled the excitement by providing a potentially unlimited source of cells for reparative medicine.

As these new therapies approach clinical utility, certain fundamental and practical issues must be addressed to allow translation of these technologies from bench to bedside. In particular, storage of living cells and complex tissue constructs has received insufficient attention.

In the body, natural processes preserve the physiologic functions of cells, tissues, and organs. When cells are damaged, or as they age and die, biological events ensure their repair or replacement. Unfortunately, when cells are removed from the body, changes in the external environment can result not only in cell damage but also in inhibition or elimination of the natural repair and replacement processes.

The goal of biopreservation is to preserve the viability and activity of cells, tissues, and organs held outside their native environment for extended periods of time. Biopreservation can be categorized into four areas based on the techniques used for stabilization: tissue culture, hypothermic storage, cryopreservation, and desiccation.

"Tissue Engineering" is edited by Jeffrey R. Morgan of the Division of Biology and Medicine, Brown University, and Martin L. Yarmush of the Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School.

Tissue culture is the process of maintaining the viability of a cell population or tissue by providing the necessary components and conditions required for normal cell growth and division ex vivo. The technique has been widely used in the biotechnology industry to maintain the activity of bacteria and yeast used in the production of pharmaceutical agents and in research labs to maintain cultures of mammalian cells.

Recent efforts have attempted to extend the tissue culture approach to clinically important cells and tissues. Ex vivo expansion of stem cells and long-term storage of skin, corneas, and islet cells are examples of successful applications of tissue culture. Unfortunately, extended tissue culture is an expensive preservation strategy that is highly susceptible to contamination and genetic drift of the cell populations being preserved.

When a cell is cooled below normal physiologic temperatures, the rates of its biochemical reactions decrease. Exploiting this effect of temperature, placement of cells, tissues, and organs in hypothermic environments has been used to minimize storage effects. Hypothermic conditions are those in which the temperature is lower than normal physiologic temperature but higher than the freezing point of the storage solution.

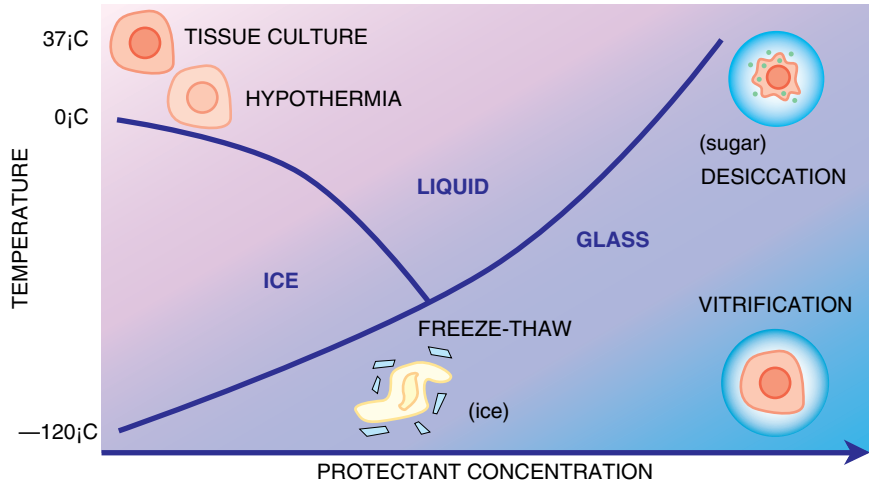
Extensive studies on the effects of hypothermia on cell physiology and biochemistry have identified some of the critical storage requirements for cells and tissues. One achievement has been extended storage times for platelets, erythrocytes, corneas, and split-thickness skin. While cellular metabolism is slowed during hypothermic storage, it is not completely suppressed, and accumulating cell damage and cell death eventually result in a decrease in the biological activity of the system. Because of the limited shelf-life of biological products that are stored at hypothermic temperatures, this technique is not currently a viable solution for long-term storage of engineered cells and tissues.

Cryopreservation is a process whereby biological function is maintained by freezing and storage at ultralow subzero temperatures. Below –80°C, all metabolic and biochemical reactions cease, and the cell is held in stasis. When cells are cooled below the freezing point of their storage solution, ice forms in the extracellular space, resulting in the concentration of extracellular solutes and osmotic shrinkage of the cells.

Cell damage can result from both rapid cooling (due to intracellular freezing) and slow cooling (due to exposure to higher concentrations of solutes). The successful cryopreservation of a wide variety of cell types has been achieved with the development of novel cryoprotective agents that minimize both kinds of damage.

There are two approaches to cryopreservation: slow freeze-thaw in the presence of 1 to 2 M concentrations of cryoprotectants, and vitrification, which is rapid cooling using much higher concentrations of cryoprotectants (in the range of 6 to 8 M). Once a cell or tissue has been cryopreserved successfully, it can be stored indefinitely.

Although cryopreservation has been used extensively for the long-term storage of various clinically important cells and tissues, areas for improvement exist. First, high



Cells and tissues can be preserved in different physical states by manipulating the temperature and/or protectant concentration.

concentrations of chemical cryoprotectants can adversely affect transplant patients, necessitating costly post-thaw removal. Second, there are a number of cell types from different species that have limited viability after cryopreservation, including hepatocytes, granulocytes, and oocytes.

Moreover, the increased complexity of tissues compared to isolated cells limits the ability to use traditional techniques to preserve cell viability at low temperatures. Finally, the cryopreservation process itself is costly and requires highly trained technicians and specialized equipment for processing and storage, making it logistically prohibitive for routine use in large-scale or remote operations.

In natural systems, desiccation is used as a strategy to preserve biological activity through times of extreme environmental stress. Termed *anhydrobiosis*, the ability to survive in a dry state for extended times has been identified in plant seeds, bacteria, yeast, brine shrimp, fungi and fungal spores, and cysts of certain crustaceans.

Studies of these organisms has revealed a series of complex molecular and physiologic adaptations that permit survival despite water loss exceeding 99%. Central to this work has been the discovery that large amounts of mono- and disaccharides can act to protect biological structures during dehydration. They do this by forming a stable glass matrix or by binding to sites previously stabilized by water.

By incorporating sugars into the storage medium, freeze-drying has been used successfully for the dehydration and storage of pharmaceutical agents, bacteria, yeast, and liposomes. Current efforts are focusing on the desiccation of mammalian cells.

For sugars to be maximally effective at protecting against the damaging effects of dehydration, they need to be present both intra- and extracellularly. Because mammalian cells do not naturally synthesize the polysaccharides that function in this process, nor is the plasma membrane permeable to them, getting the sugars into the cells is the main impediment. Techniques so far suggested and tried are (1) expression of sucrose and trehalose synthase genes, (2) a metal-actuated switchable membrane pore, (3) thermal and osmotic shock, and (4) microinjection.

It also appears that a certain minimum concentration of intracellular sugars is needed to confer protection. Based on that knowledge, mammalian cells have been dried to low residual moisture levels and have regained significant viability upon immediate rehydration. Whether this method will allow stable storage in the dried state is now being investigated.

Because the role of intracellular sugars in long-term cell and tissue storage is still uncertain, other strategies are also being examined. For example, another protective mechanism used by natural systems that undergo seasonal exposure to environmental stresses is a hypometabolic state called *diapause*. In this dormant state, organisms are remarkably resistant to anoxia and dehydration. A possibility is to metabolically and genetically engineer mammalian cells to mimic the behavior of diapausing organisms and thus to enhance the dehydration tolerance of these cells.

Although new, desiccation promises to overcome some of the problems presented by other preservation methods. The low concentrations of intracellular sugars required for protection and the reduced toxicity of these natural protectants would eliminate the costly removal of toxic chemical cryoprotectants before transplantation. Dehydrated cells also could be stored in ambient conditions, reducing the high cost of maintaining large inventories at subzero temperatures and simplifying distribution.

The need for effective preservation in cell and tissue engineering will be the impetus for more efforts toward advancing the development of cryopreservation and dry storage technologies. The remaining challenges are formidable, but significant progress has been achieved through interdisciplinary research, with encouraging prospects for the future of biopreservation.

JASON P. ACKER
MEHMET TONER

Department of Surgery,
Massachusetts General Hospital and
Harvard Medical School
Boston