

ORGAN BANKING

Mechanical engineers have the know-how to push back the boundaries of cryopreservation of human tissues.

BY YOED RABIN AND JEDEDIAH LEWIS

Surgeons rush to make the best of a tragic situation, recovering organs from the victim of a tragedy so they can be readied for transplantation, saving a waiting patient's life. It's an extraordinarily delicate situation, and time is the enemy.

At present, organs can be maintained outside of the body for hours at most and often must be matched to recipients far from the site of donation. Surgical teams and waitlisted patients are on call around the clock to receive organs flown in by jet or helicopter. Those time constraints mean that opportunities to match donated organs with the best recipients are profoundly limited. Today in the United States, some two-thirds of hearts from eligible donors go untransplanted, and 20 percent of kidneys must be discarded.

In the United States, around 30,000 organs find recipients each year—a fraction of the need. Roughly 120,000 patients are currently awaiting an organ

transplantation, and the number of patients on official transplant waitlists is dwarfed by true potential of transplantation. Millions worldwide suffer from diseases for which transplantation is the gold-standard treatment. Some peer-reviewed articles suggest that organ transplantations could avert more than 30 percent of all U.S. deaths.

The inability to preserve human tissues for long periods outside the body impacts other areas of medicine as well. For example, research in areas such as tissue engineering and drug discovery is hampered by the limited shelf life of organs and tissue samples. The long-term preservation of ovaries and ovarian tissue—an approach that has succeeded experimentally—is another area where new technology could promote human well being.

For these reasons, the long-term preservation of organs and tissues is a major priority in biomedical engineer-





ing research. While slowing the natural degradation of biological materials can be accomplished by mild cooling to hypothermic conditions, only extremely low temperatures—far below the natural freezing point of biological solutions—can facilitate long-term storage, in a process generally known as cryopreservation (*cryo* means ice-cold in Greek). To develop the science and technology of cryopreservation, researchers are tapping expertise from core engineering areas such as heat and mass transfer, solid mechanics, materials science, nanotechnology, computer modeling, information technology, and microelectronics—with prominent leadership by mechanical engineers.

To further explore how engineers from various disciplines can offer a broader and improved set of tools to tackle preservation challenges, ASME and the Organ Preservation Alliance are co-organizing the Summit on Organ Banking through Converging Technologies in Boston, Mass., this August. (See sidebar on page 49.) In addition, the new Advanced Regenerative Manufacturing Institute, launched late last year with \$300 million of funding from the Department of Defense, will focus on logistical challenges in tissue engineering, including the extension of shelf-life for engineered tissue constructs.

The shortage of organs for transplantation is a public health crisis. The gap between the current state of the art and the technology needed for cryopreservation is one that an orchestrated effort between cryobiologists and mechanical engineers can bridge.

Preserved in glass

Time is a critical factor for organ transplants. As soon as tissues are removed from the body, they undergo natural degradation which causes their structure to disintegrate and their cells to die. The only way to stop the clock on cell death is

to trap the biological material in a solid-like state in cryogenic conditions.

Techniques for successful cryopreservation have been developed over the past five decades for several tissue types, with some dramatic advances being made in recent years. At present, however, successful applications are generally related to small specimens, ranging from micrometer-scale cell clusters such as stem cells to millimeter-scale tissues like corneas. In humans, researchers have been able to cryopreserve larger specimens only for tissues such as heart valves where the mechanical function, rather than the inherent biological functionality, is of higher priority.

It's important to note that cryopreservation is different, and more difficult, than simple freezing, since freezing involves the formation of ice crystals that can mutilate cell membranes and other important cell structures. Instead, effective cryopreservation of large structures requires trapping the biological material in an amorphous, glassy state in a process known as vitrification. While the concept of cryopreservation via vitrification is almost a century old, its application to large-size specimens is only now on the verge of becoming a practical reality.

In order to achieve vitrification, cryobiologists introduce glass-promoting solutions known as cryoprotective agents, or CPAs, into the tissue. CPAs exhibit an exponential increase of viscosity when the temperature decreases rapidly.

To achieve vitrification, tissues are permeated with CPAs and then quickly cooled to very low temperatures over a time period shorter than it takes for crystallization to occur. Below a threshold known as the glass transition temperature, the CPAs become so viscous that the specimen can be considered solid for all practical purposes, yet no ice crystals are present that might damage the cell structures. Rapid rewarming is also required when the material is recovered from cryogenic storage, to eliminate the



possibility of crystallization when the CPA regains fluid behavior while the temperature is still below the freezing point.

It shouldn't be a surprise that fundamental thermal engineering is paving the road for the design and optimization of cryopreservation protocols. One example of the contribution of thermal engineering is in the thermal characterization of new CPAs and biocompatible materials developed by biologists and chemists.

Engineers have found, for instance, that the thermal conductivity of a crystalline dimethyl sulfoxide—one of the most common ingredients in CPA cocktails—is up to an order of magnitude higher than that of its amorphous state. This means that it is much more difficult than originally anticipated to cool the center of a large organ, and to achieve the high cooling rates necessary for vitrification. It follows that without the detailed knowledge of the thermal conductivity, a thermal engineer would not be able to help the cryobiologist in correlating cryopreservation success with the thermal conditions that led to it.

Cryopreservation success is not only about temperatures and cooling rates, but more

The kidney on the right was cryopreserved in a glassy state, while the one on the left was frozen.

Photo: Gregory M. Fahy

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importantly about the thermal history that the specimen experiences. For example, two nearly identical tissue samples that were exposed to different thermal histories along the path to cryogenic storage may have radically different propensities to form crystals. That path dependency, along with the thermal properties and geometry of the tissue, the CPA cocktail used, and even the kind of container that holds the sample, adds virtually endless complexity to the analysis of experimental systems and computational models—it represents a major hurdle in developing guidelines to assist cryobiologists wishing to preserve tissues.

Cooling and stress

As researchers push the boundaries on the ability to cryopreserve bulky tissues and large organs, a new thermal challenge emerges. Rapid cooling can potentially give rise to dangerous thermomechanical stress driven by the tendency of the material to contract with temperature. That stress can surpass the strength of the material and cause structural damage or even fractures. Structural damage can be evident even in

milliliter-size specimens.

More gradual cooling is one possible solution, and researchers still can achieve vitrification in samples that are cooled slowly by increasing the concentration of CPAs. But that poses another danger: At high concentrations, CPAs are toxic, and the longer a metabolically active tissue is exposed to the chemicals, the greater the damage the CPAs cause.

As the cryopreservation process progresses, the toxicity of the CPA wanes with the decreasing temperature of the specimen. But with a decrease in temperature, there is a need for an increase in the CPA concentration to suppress the harmful effect of water crystallization.

Another approach is to increase in CPA concentration gradually, concurrent with the decrease in the temperature of the sample. However, CPA concentration is only one parameter, where the resistance to CPA perfusion into the tissue only increases with the increased viscosity as temperatures drop, further affecting CPA permeation through cell membranes and other barriers.

The three research goals to achieve cryopreservation—promoting vitrification, reducing toxicity, and averting structural damage—appear to be working against each other. Fortunately, mechanical engineers have the tools to potentially solve the trilemma, based on engineering analysis techniques and optimization methods.

One cutting-edge approach that lowers the barriers to solve the trilemma is to develop synthetic ice modulators, or SIMs, that impede the formation and growth of ice nuclei and crystals. Small quantities of SIMs could reduce the concentrations of other, more toxic ingredients in the CPA cocktail and enhance

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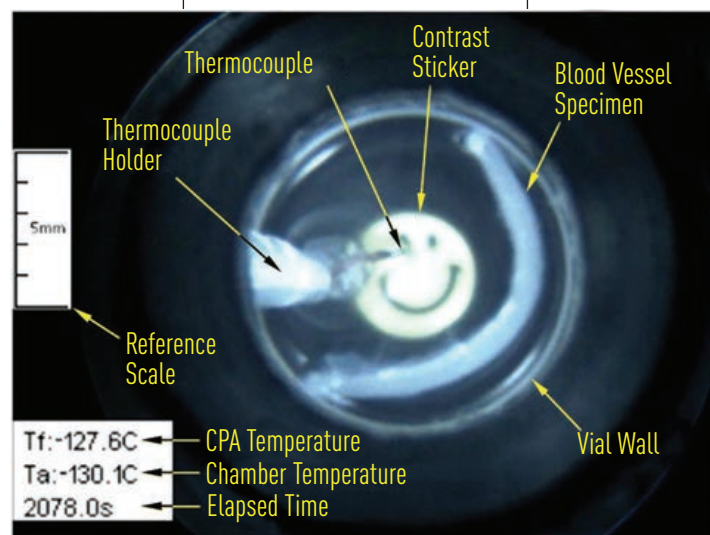
the stability of the vitrified tissues. Key mechanical engineering challenges with SIMs include measuring their physical properties, investigating their effect on the thermal history and the resulting mechanical stress, developing mathematical models to explain ice modulation, and exploring biotransport effects at the cellular level.

The competing needs for reducing toxicity, preventing ice formation and growth, and reducing mechanical stress complicate the process of recovering the specimen from cryogenic storage. The process of rewarming requires a very high rate of temperature change, which risks further mechanical stress. One concept for solving the trilemma during rewarming involves heating the tissue internally throughout the sample rather than from the outside in. While microwave heating—similar to food thawing—could create the desired internal warming, nano heaters can help organ recovery from cryogenic storage.

A very attractive concept called nanowarming is accomplished by infusing the tissue with magnetic nanoparticles prior to vitrification. Upon demand, the cryopreserved tissue is then subjected to an oscillating electromagnetic field in the radiofrequency range, which excites the nanoparticles and causes them to heat up. However, there are many engineering challenges remaining, ranging from the synthesis of the particles themselves to their uniform dispersion within the tissue to modeling and controlling

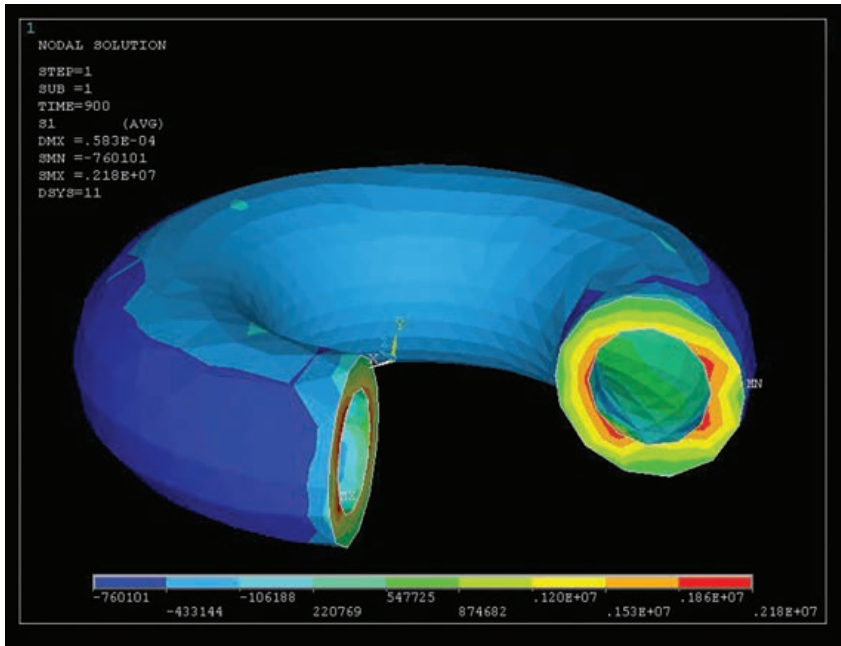
the process in order to meet the desired rewarming outcomes.

Clearly, mechanical stress and structural integrity play key roles in cryopreservation success, during both as the specimen



This image shows a successful vitrification of a blood vessel in controlled conditions.

Image: Yoed Rabin, Carnegie Mellon University.



cools and as it recovers from cryogenic storage. The cryobiology community has known for decades that it needed better solid-mechanics knowledge and tools to characterize structural damage suffered by cryopreserved samples and to devise means to prevent it. At present, however, the area is still relatively uncharted.

It is not just that the mechanical behavior of biological materials undergoing vitrification has not been fully understood, but also that only a small range of measurement instrumentation has been devised and a limited number of analysis techniques have been formulated. Preserving structural integrity represents an emerging engineering area in cryopreservation research, which becomes more critical with the increasing size of the organs to be preserved.

Interdisciplinary effort

Cryobiologists and medical researchers cannot do this alone. Considerably more engineering contributions are needed as part of an organized, interdisciplinary effort in order to make organ banking a practical reality.

A solid-mechanics model simulates a blood vessel undergoing vitrification.
Image: Yoed Rabin, Carnegie Mellon University.

Heating
 tissue
 internally
 throughout
 the sample
 can help organ
 recovery from
 cryogenic
 storage.

Engineers are needed to help foster the emergence of organ banking through work in areas beyond cryopreservation. It will take engineers to design ways to better protect biological material between recovery and transplantation. It will be engineers who develop the hardware needed at every step of the road between the donor and the recipient.

For example, engineers have been developing the tools undergirding the Internet of Things and Big Data that will enable the rise of a more deliberate approach to organ banking. From the donor to the recipient, Big Data tools will elevate the ability to monitor and control the history of the organ. That history includes organ preparations and handling, short-term organ storage during shipping, cryogenic storage in biobank facilities, organ reconditioning, and even the history of the organ after transplantation, as transplantation success is evaluated over an extended period of time. By incorporating Big Data tools, many parameters can be correlated with patient recovery, such as the quality of donor-recipient matching, specific clinical procedures, and the history of the organ.

Further into the future, those computation tools may serve a global infrastructure for organ banking. The high-volume data traffic that will fuel that global system calls for the development of smart, networked sensors with the ability of onboard compilation and local evaluation. Such sensors will, in essence, place cryopreserved organs into the Internet of Things, monitoring a wide array of critical data including temperature, stress, toxicity, and metabolic activity, without affecting the integrity of the organ, clinical procedures, and biocompatibility needs.

The engineering approach

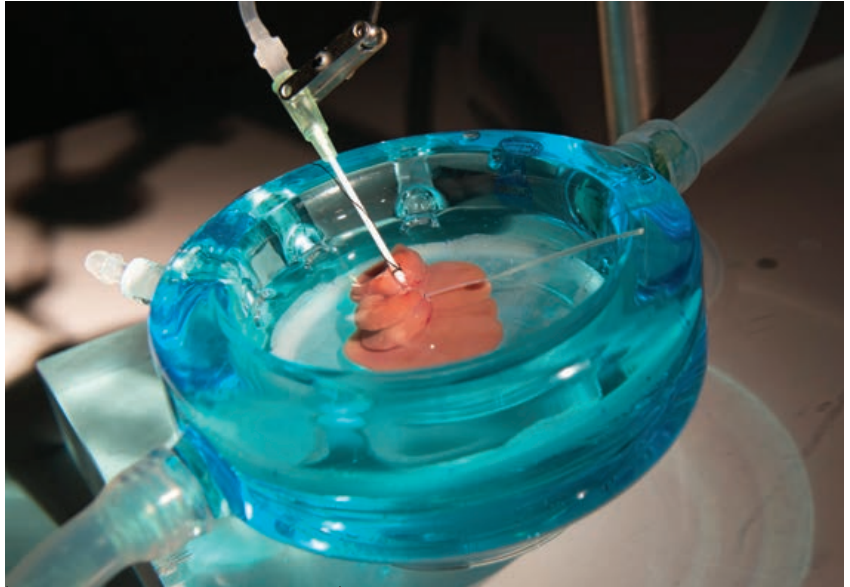
When it comes to developing lifesaving medical technology, we often resort first to biologists and medical researchers. Their approach to research is typically geared

toward hypothesis-driven and diagnostic studies. While the diagnostic approach may yield a quick response to burning questions, it is often prone to trial-and-error investigation and sometimes scattershot progress.

In a field such as cryopreservation that has real and urgent needs, it makes sense for there to be diversity in research cultures, where the engineering approach can make special contributions. The engineering approach can integrate predictive tools that are rooted in physical measurements, mathematical modeling, and computation power. By applying a mechanistic approach, complex tissues and cryopreservation processes can be understood by examining the workings of their individual parts and the manner in which they are coupled. The engineering approach requires a higher upfront investment, but it provides a high return in the long run, especially as computer modeling and simulation techniques can gradually replace a significant portion of physical experiments.

Cryopreservation of organs, critical native tissues, and engineered tissue constructs is the key means to meet one of the largest public health challenges of the 21st century: the shortage of organs and tissues for transplantation. But it is not a challenge that can be met by biologists and medical researchers alone. Engineers—especially mechanical engineers—have demonstrated their invaluable contribution towards making tissue and organ banking a reality, with an ever-expanding role, as medical progress occurs on the boundaries of life sciences and engineering technology. **ME**

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A nonfrozen rat liver supercooled to $-6\text{ }^{\circ}\text{C}$ sits in preservation solution within a perfusion system.

Image courtesy: Wally Reeves, Bote Bruinsma, Martin Yarmush, Korkut Uygun, Harvard University/Massachusetts General Hospital

Summit on Organ Banking

The upcoming Summit on Organ Banking through Converging Technologies (obs2017.org) is a venue for bringing diverse research approaches together. Held across multiple sites at Harvard Medical School and around Boston, the summit will bring together engineers and scientists with a broad area of research interests to explore ways to apply new platform technologies to the grand challenge of organ banking. Many of the researchers come from fields outside of cryopreservation but have built complementary engineering expertise that can be applied to make breakthroughs.

The summit is also suited for engineers who have little or no background in cryopreservation and biobanking, since it will provide a crash course on the topics and an array of potential collaborators eager to make cross-disciplinary strides in these emerging areas of research.

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