Changing the Organ Preservation Game

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Published in:
Transplantation

DOI:
10.1097/TP.0000000000003076

Changing the Organ Preservation Game

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Every medical specialty has its own big dreams. In organ transplantation, we dream about unlimited organ supply by engineering bio-artificial organs, perhaps xenotransplantation and banking organs. Certainly, storing donor organs for prolonged periods of time could significantly change the field, enabling optimal donor-to-recipient matching, a worldwide organ exchange, and elective surgeries.[1] In stem cell transplantation, cryopreservation-based banking allows the distribution of viable cells around the globe, allowing transplant procedures to be planned well in advance. Freezing a solid organ, however, is a very different ball game. When cooling down to subzero temperatures, it becomes crucial to prevent the formation of disruptive ice crystals that mechanically compromise cellular viability. One or more "cryoprotectant" agents, which block intracellular and extracellular water crystallization, need to be added to the preservation solution facilitating cell integrity. Although this approach had been proven feasible for cryopreservation of single cell suspensions and very small tissues, larger tissue volumes such as whole organs do not easily allow enough distribution and intracellular uptake of essential cryoprotectants. In addition, homogeneous rewarming of whole organs after deep subzero cryopreservation is nearly impossible. During rewarming, the slightest uneven temperature gradient occurring in a deep frozen organ may cause it to literally break apart.[2] Given these and other challenges, only a few groups have been motivated to actively pursue the development of a whole organ cryopreservation protocol. Consequently, the area of research has seen very little real progress to date.

The group of Korkut Uygun at the Center for Engineering in Medicine at Harvard is among the brave minority pushing forward to tackle important issues that are holding back any breakthrough in the whole organ cryopreservation field. They prefer to call their approach "supercooling." The justification for this cartoon hero-worthy name lies in one of the main differences from cellular cryopreservation: supercooling is done at temperatures that are only a few degrees below zero Celsius. In addition, perhaps even more importantly, their protocol does not rely on vitrification. Vitrification is the formation of solid, amorphous (glass-like) water, which occurs when crystallization is prevented either by very careful freezing or adding cryoprotectants.[3] In itself, vitrification is a tissue-friendly way to cryopreserve whole organs. As the entire organ still ends up frozen solid, a homogeneous rewarming process after vitrified cryopreservation has thus far not been possible for human-sized organs.

In earlier publications, the authors had introduced their supercooling protocol for rodent livers and demonstrated that successful posttransplant survival could be obtained.[4,5] In their current work, they have slightly adapted their methodology and taken the next step: supercooling of *human* livers.[6] In short, 5 discarded human livers were statically cold stored after procurement and then subjected to a 3-hour period of subnormothermic machine perfusion at 21°C. Next, the machine perfusion temperature was gradually reduced to 4°C. During this phase, the perfusion solution was further supplemented with cryoprotectant agents to prevent intracellular ice crystal formation and suppress the freezing point of the perfusion medium to below −4°C. Following this machine perfusion-driven preconditioning phase, perfusion was discontinued and the organ was cooled to −4°C and kept "supercooled" at that temperature for 20 hours. Subsequently, machine perfusion was restarted and cryoprotectants were washed out at 4°C, followed by 3 hours of 21°C machine perfusion during which organ viability markers were measured. Three livers also underwent a 2-hour period of whole blood normothermic reperfusion at 37°C, simulating transplant conditions. The authors reported no major differences between liver viability markers during subnormothermic machine perfusion before and after supercooling. Moreover, they reported that the 3 livers that underwent whole blood reperfusion appeared viable with a good energy charge, normal bile and urea production, and good lactate clearance. Histologies showed preserved lobular architecture with patches of reversible hepatocellular injury comparable to the structural changes that had already been present before supercooling. With this recent Nature Biotechnology publication, de Vries et al are the first to report potentially successful 20-hour supercooling of human livers to −4°C.

How might this paper change the organ preservation game?

The focus of organ preservation-related research has gradually shifted from static cold storage, via cold machine perfusion, to (sub)normothermic perfusion.[7,8] With this

Received 25 November 2019.

Accepted 25 November 2019.

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The authors declare no funding or conflicts of interest.

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ISSN: 0041-1337/20/1045-895

DOI: 10.1097/TP.0000000000003076

<zdoi;10.1097/TP.0000000000003076>

trend during the recent 10 years, the future appears to be predominantly warm. In addition, there are good reasons for this development, since marginal-quality organs may require objective ex vivo viability assessment with opportunities for active organ reconditioning before transplantation. Given the recent dominance of research into normothermic preservation, going back to the cold and even passing the 0°C boundary is refreshing from a scientific point of view. In addition, the group from Boston has also demonstrated another supercool trick: in 1 protocol, they have elegantly combined every possible organ preservation technique available: static cold storage, hypothermic machine perfusion, subnormothermic machine perfusion, and normothermic machine perfusion, all facilitating their novel approach of supercooling. In addition, the unique methodology that these investigators have developed for subzero organ preservation represents a significant advancement. Avoiding vitrification and "keeping things fluid" might be the key to preserving tissue integrity during cryopreservation and rewarming. However, it remains to be determined whether the associated higher subzero temperatures are good enough for long-term organ storage. Real organ banking would of course require more than just 20 hours of storage. Nevertheless, adding only a few hours to current maximum organ preservation times would already be a significant gain.

It needs to be recognized, however, that this study represents just a first step, as the discarded livers have not been transplanted after supercooling. Although viability assessment was performed ex vivo, this approach may not reflect the complexity of clinical transplantation. Thus, it remains to be seen whether the protocol really preserves organ viability to a level mandatory for transplantation. Nevertheless, if favorable posttransplant function and survival can indeed be demonstrated after supercooling, this approach may eventually revolutionize the way we preserve, exchange, and transplant organs. To find out whether supercooling is feasible, large animal transplantation studies would be a logical next step for these investigators. In addition, hopefully, other research groups will now be motivated to join this unique effort to change the organ preservation field in a somewhat unexpected direction. The game is on!

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