Creative technology advances tissue preservation

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Provenance: This is an invited Editorial commissioned by the Section Editor Dr. Mingzhu Gao (Department of Laboratory Medicine, Wuxi Second Hospital, Nanjing Medical University, Wuxi, China).

Comment on: Manuchehrabadi N, Gao Z, Zhang J, *et al.* Improved tissue cryopreservation using inductive heating of magnetic nanoparticles. Sci Transl Med 2017;9. pii: eaah4586.

Submitted Aug 22, 2017. Accepted for publication Sep 06, 2017.
doi: 10.21037/atm.2017.09.08
View this article at: http://dx.doi.org/10.21037/atm.2017.09.08

Technological advances within the last two decades vastly increased options to restore damaged or diseased tissues and organs with viable replacements. Techniques to harvest, generate and implant them continue to evolve. Specialized knowledge, expertise and facilities make it possible to restore tissue function previously only imagined within current memory. Unfortunately, there are constraints on the total distance between donor and the recipient. This is due to fixed windows of time for tissue survival between harvest and implantation. The maximum allowable time reduces the transport distance to somewhat local versus global. Mechanisms to improve tissue and organ preservation will precipitously increase the potential recipient pool and reduce loss of invaluable tissue donations.

From a historical perspective, cell cryopreservation had a strong early start in the field of theriogenology (1,2). Preservation of cells and oocytes for long distance travel was small scale and possible with dedicated shipping containers. Rapid cooling in the presence of high concentrations of cryoprotectants during the vitrification freezing process prevents formation of cytotoxic ice crystals. The technique is a proficient mechanism to preserve cells and small tissue samples for extended periods. Unfortunately, successfully revitalizing or warming the cells, including those surrounded by tissue, to a metabolically active state has not had comparable success.

Cell warming involves passage through temperatures from −160 to 37 ℃ including those for prime crystal formation , −15 to −60 ℃ (3). The highly permeable cryoprotectant dimethylsulfoxide (DMSO) included in the cryopreservation medium VS55 helps reduce crystallization by creating chemical barriers to molecular organization. However, cryoprotectants are not entirely effective, especially if the thawing process includes prolonged periods at favorable crystallization temperatures (4). Another vital point is that the warming process must allow time for fluid exchange between medium and cells to prevent irreversible membrane or organelle damage as well as consequences of "heat shock" (5). Hence, the thawing process is a delicate balance of efficiency (rate to target temperature) and efficacy (cell survival).

The authors, Manuchehrabadi *et al*, tested an innovative combination of technologies to facilitate rapid, large scale tissue warming post-vitrification in their manuscript "Improved tissue cryopreservation using inductive healing of magnetic nanoparticles (mNPs)" (6). Using a process of nanowarming, the authors confirmed rapid revitalization of frozen cells and tissue up to 50 mL, a volume that is a large step closer to tissue grafts than the standard 1 mL vitrified sample volumes. Viability outcomes were comparable to established convective warming and superior to slow thawing. Additionally, mechanical properties of porcine artery tissue were not detectably altered by the process. The creative combination of low-radiofrequency energy (RF)-alternating magnetic fields with biocompatible iron oxide nanoparticles to thaw vitrified cells and tissue was tested with a series of measures, and resulting outcomes support the feasibility of the novel technology.

The work was premised on previous work by several of the authors surrounding modification of commercially

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available mNPs and generation of heat by the particles within cryopreservation solutions during exposure to RF-alternating magnetic fields (7,8). When mNPs are exposed to an alternating current, relaxation of the magnetic field inside the material lags behind the external field. This causes magnetization reversal that generates a relatively large amount of heat from rotation of the magnetic moment within the particle, Neel relaxation, or rotation of the particle as a whole, Brownian relaxation. For the present study, nanoparticles were coated with a mesoporous silica shell followed by combined polyethylene glycol and trimethoxysilane (msIONPs) that effectively prevented particles from aggregating, increased size homogeneity and sustained particle solubility in full and half concentrations of VS55, the cryopreservation medium. The authors also created a radiofrequency inductive heating coil and system, 15-kW, 80-mL, 5-cm-diameter, 4-turn coil, that has a magnetic field strength 3-fold higher at the same frequency range than that of a smaller 1-kW, 1-mL, 1-cm-diameter, 2.5 turn coil system. The radiofrequency inductive heating coils and coated, mNPs were used for tissue testing in the study following optimization of tissue loading with VS55 medium and nanoparticles described below.

The cytotoxicity of the cryoprotectant in VS55, DMSO, is well documented (9,10). Effects are exacerbated with prolonged exposure (11). To optimize the time for tissue permeation and limit the exposure of metabolically active cells to cryoprotectant, the authors determined the rate of VS55 loading in porcine carotid artery tissue at 4°C with microcomputed tomography (12). They also used microcomputed tomography to assess sample solidification following vitrification which was successful at 15 °C/min with an annealing step just prior to the lowest temperatures. Nanowarming of the vitrified porcine artery tissue was effective at 130 °C/min. The rate exceeds the established critical warming rate (CWR) of VS55. The CWR is the maximum warming rate for a given solution that avoids crystallization. Hence, the preliminary work provided the proof of concept that nanowarming increased the CWR of VS55 to avoid sample crystallization over the course of rapid thawing.

Prior to use of a combination of msIONPs and VS55 on cells and tissues, the investigators used a one-dimensional conduction and thermal stress computer model to test the limits of convection and nanowarming. They used VS55 thermal properties within cylinders with continuous increases in radii from 0.5 to 2.5 cm. In terms of volume, 0.5 and 2.5 cm radii correspond to 1 and 80 mL, respectively. Using a thermal stress limit of 3.5 MPa, the critical yield stress of the material, convective cooling was efficacious at radii of up to about 1.16 cm, convective warming up to about 0.76 cm and nanowarming up to 2.50 cm. Failure of convective cooling was considered ice ball formation and warming failure was confirmed by devitrification or crystallization. Hence, use of established values in a virtual model confirmed the potential advantages of nanowarming at volumes up to 80 mL.

Human dermal fibroblasts were used to test the impact of the cryopreservation medium alone or in combination with msIONPs on cell viability prior to vitrification and, subsequently, benefits of nanowarming versus slow convection warming. Based on Hoechst propidium iodide viability staining, there was little to no impact of VS55 loading alone or with nanoparticles. Viability following nanowarming of cells with both medium and nanoparticles was higher than medium alone and both were higher than cells after slow warming on ice or after they were maintained in VS55 on ice for the same period. Radiofrequency energy exposure was not found to impact cell viability. With cell loading, cooling and warming techniques established, the system was tested on porcine carotid artery tissues at 1 and 50 mL volumes. Again, the cryopreservation medium and nanoparticles were not found to affect cell viability based on alamarBlue assays® [resazurin reduction to resorufin during cellular respiration (13)]. The cell viability in 1 mL samples of nanowarmed tissue was not significantly different from fresh tissue and both had comparable cell viability to fast-convective warming. All had higher cell viability than the 1 mL slow-convective warmed samples. In 50 mL samples, cell viability in nanowarmed samples was comparable to that in nanowarmed and fast-convective warmed 1 mL samples and significantly higher than in 50 mL samples warmed with fast-convective heating and 1 mL samples warmed with slow-convective heating.

As the last steps in this sequential series of investigations, the ability to remove nanoparticles in porcine carotid artery tissue was confirmed with magnetic resonance imaging. Samples were evaluated after 4 and 24 hours of loading and following washout. The impact of nanowarming on tissue mechanical properties of 1 mL samples was compared to convective and slow warming and relative to fresh control samples. Reported findings included lower elastic modulus, a shorter toe region and longer initial length (diameter) in samples thawed by slow-warming.

The work described in the Manuchehrabadi manuscript

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is an initial step to advance tissue preservation. The authors anticipate that it will be possible to expand the technology to accommodate volumes as high as 1 L. It is also surmised that it will be possible to perfuse tissues with cryopreservation medium and nanoparticles using techniques similar to those currently applied to renal tissues. The study design and conclusions support attention to detail and conscientious consideration of safety and efficacy by the authors. As with all new technology, additional testing will be necessary for more general application of the discoveries.

The cryoprotectant, DMSO, in VS55 cryopreservation medium is a known carcinogen that can cause serious side effects when administered intravenously at relatively low concentrations in cell suspensions (14,15). Removal can be complicated and time consuming (16). Mechanisms to remove DMSO following vitrification will likely need to be developed and validated for distinct tissues.

The vitrification period used for the investigations in the manuscript was reported as 19 to 22 hours (overnight). This is consistent with a goal of extending the period of organ viability beyond a few hours or days. It will be of interest to assess the impact of different warming techniques on tissues stored for longer time periods as well. The ability to revitalize tissues after days or weeks of cryopreservation will allow more time for recipient identification and preparation. There is also a question of accessibility to radiofrequency inductive heating coils required for nanowarming. It is not yet clear if it will be possible to transport the coils with cryopreserved tissues or organs. Alternatively, it is possible that the technology will become standard at transplantation sites. Future work will likely address these possibilities.

The tissues used in the study were relatively thin and homogenous compared to complex organs like liver or kidney. Biomechanical assessments in the study showed no impact of nanowarming on porcine carotid artery tissue properties. Size and resistance to elongation is critical to normal vessel function. There are numerous potential mechanical parameters that are also germane to tissue structure and material properties that might be considered for future studies. Additionally, success of complex organ implantation is related to performance of the various tissue components. As such, proof that cells are not only viable, but that they also resume their specialized behavior within an organ following vitrification will benefit cryopreservation science immensely.

Overall, the work by Manuchehrabadi *et al.* to improve cell and tissue revitalization with inductive heating of mNPs represents a large contribution to the future of tissue and organ transplant preservation. As indicated above, new discoveries raise additional questions that help guide the path to implementation. Nonetheless, nanowarming has significant potential to shift the paradigm of tissue and organ transplants by eradicating time constraints between harvest to implantation. Based on the significant foundation on which this work is premised, there is no question that the authors are poised and ready to meet this challenge.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 1949;164:666.
2. Whittingham DG, Leibo SP, Mazur P. Survival of mouse embryos frozen to -196 degrees and -269 degrees C. Science 1972;178:411-4.
3. Gao D, Critser JK. Mechanisms of cryoinjury in living cells. ILAR J 2000;41:187-96.
4. Weng LD, Li WZ, Zuo JG, et al. Osmolality and unfrozen water content of aqueous solution of dimethyl sulfoxide. J Chem Eng Data 2011;56:3175-82.
5. Bakhach J. The cryopreservation of composite tissues: Principles and recent advancement on cryopreservation of different type of tissues. Organogenesis 2009;5:119-26.
6. Manuchehrabadi N, Gao Z, Zhang J, et al. Improved tissue cryopreservation using inductive heating of magnetic nanoparticles. Sci Transl Med 2017;9. pii: eaah4586.
7. Etheridge ML, Xu Y, Rott L, et al. RF heating of magnetic nanoparticles improves the thawing of cryopreserved biomaterials. Technology 2014;02:229-42.
8. Hurley KR, Ring HL, Etheridge M, et al. Predictable heating and positive MRI contrast from a mesoporous silica-coated iron oxide nanoparticle. Mol Pharm 2016;13:2172-83.
9. Liu Y, Xu X, Ma X, et al. Cryopreservation of human bone marrow-derived mesenchymal stem cells with reduced dimethylsulfoxide and well-defined freezing solutions.

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Biotechnol Prog 2010;26:1635-43.
10. Scheinkönig C, Kappicht S, Kolb HJ, et al. Adoption of long-term cultures to evaluate the cryoprotective potential of trehalose for freezing hematopoietic stem cells. Bone Marrow Transplant 2004;34:531-6.
11. Fuller BJ. Cryoprotectants: the essential antifreezes to protect life in the frozen state. Cryo Letters 2004;25:375-88.
12. Bischof JC, Mahr B, Choi JH, et al. Use of X-ray tomography to map crystalline and amorphous phases in frozen biomaterials. Ann Biomed Eng 2007;35:292-304.
13. Chen JL, Steele TW, Stuckey DC. Modeling and application of a rapid fluorescence-based assay for biotoxicity in anaerobic digestion. Environ. Sci. Technol 2015;49:13463-71.
14. Júnior AM, Arrais CA, Saboya R, et al. Neurotoxicity associated with dimethylsulfoxide-preserved hematopoietic progenitor cell infusion. Bone Marrow Transplant 2008;41:95-6.
15. Quimby JM, Webb TL, Habenicht LM, et al. Safety and efficacy of intravenous infusion of allogeneic cryopreserved mesenchymal stem cells for treatment of chronic kidney disease in cats: results of three sequential pilot studies. Stem Cell Res Ther 2013;4:48.
16. Thirumala S, Goebel WS, Woods EJ. Clinical grade adult stem cell banking. Organogenesis 2009;5:143-54.

Cite this article as: Lopez MJ. Creative technology advances tissue preservation. Ann Transl Med 2017;5(23):463. doi: 10.21037/atm.2017.09.08

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