time, successful recovery of a rat kidney from the vitrified state using nanowarming, is shown. First, kidneys are perfused via the renal artery with CPA and silica-coated iron oxide nanoparticles (sIONPs). After cooling at −40 °C min−1 in a controlled rate freezer, microcomputed tomography (μCT) imaging is used to verify the distribution of the sIONPs and the vitrified state of the kidneys. By applying a radiofrequency field to excite the distributed sIONPs, the vitrified kidneys are nanowarmed at a mean rate of 63.7 °C/min. Experiments and modeling show the avoidance of both ice crystallization and cracking during these processes. Histology and confocal imaging show that nanowarmed kidneys are dramatically better than convective rewarming controls. This work suggests that kidney nanowarming holds tremendous promise for transplantation.

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Conflict of Interest: The following patents are published—"Cryopreservative compositions and methods" U.S. Patent Application 14/775998 (Bischof, J.C., Etheridge, M.L., and Choi, J., University of Minnesota, 2016), "Mesoporous silica-coated nanoparticles" U.S. Patent 10493098 (Haynes, C.L., Hurley, K.R., and Egger, S.M., University of Minnesota, 2019), "Cobalt-iron nanowires for remote heating using an alternating magnetic field." U.S. Patent Application 16/852850 (Shore, D.E., Gao, Z., Tabakovic, I., Bischof, J., and Stadler, B.J.H., University of Minnesota, 2020), "System and Method for cryopreservation of tissues." International Publication Number WO2020/150 529 A1 (Lee, C.Y., Bischof, J.C., Finger, E.B., Sharma, A., the University of North Carolina at Charlotte, University of Minnesota, 2020, this is a provisional patent). All other authors declare that they have no competing interests.

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VITRIFICATION AND REWARMING OF MAGNETIC NANOPARTICLE-LOADED RAT HEARTS

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Heart transplantation is lifesaving treatment of choice for patients with end-stage heart failure. However, the scarcity of donor organs is the biggest impediment due to shorter ischemic time of the heart (4-6 hours). Therefore, developing an advanced storage method to extend the current static cold storage time (<6 hours) is crucial. We propose heart cryopreservation by vitrification-cryogenic storage in a glass-like state. In order to vitrify successfully, we perfused the rat heart with cryoprotective agent (CPA) that inhibits ice during cooling. We developed "nanowarming" wherein and silica coated iron oxide nanoparticles (sIONPs) are loaded throughout the organ vasculature, and radiofrequency (RF) coil is used to rewarm the organ fast and uniformly. We optimized cannulation methods to load CPA and sIONPs into the heart and studied distribution of CPA and sIONPs via μCT imaging and ICP-OES. The CPA and sIONP loaded hearts were then vitrified in a control-rate freezer and were rewarmed by either convective warming or nanowarming. Both experimental and modelling data showed the convective warming causes larger temperature difference compared to the temperature difference in nanowarmed hearts. The huge thermal stress resulted in cracks in the convectively warmed hearts. Further, nanowarmed hearts were shown to be largely equivalent in tissue integrity and morphology to sIONP and CPA loaded and unloaded hearts: they retained some electrical activity and were clearly superior to convective controls. This study demonstrates that a whole rat heart can be physically vitrified and rewarmed and that biological outcomes can be expected to improve by reducing or eliminating CPA toxicity during loading and unloading.

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ADVANCING ORGAN CRYOPRESERVATION THROUGH SCALABLE POLYMER COATING OF IRON OXIDE NANOPARTICLES

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Using radiofrequency excited iron oxide nanoparticles (IONPs) dispersed in cryoprotective agents (CPAs), we have successfully rewarmed vitrified, cryopreserved (-150oC) biological samples. Previous IONPs, our silica coated sIONP, have limited Fe saturation concentration in CPA. We present a small molecule phosphonate linker (PLink) IONP coating method that is CPA stable, improves saturation concentration, and inexpensive for scale-up (> 1g).

PLink contains a phosphonate "anchor" for high irreversible IONP core binding and a carboxyl "handle" for ligand attachment. PLink displaces initial coatings on commercial IONPs (Ferrotec Inc.): EMG-1200 (hydrophobic) and EMG-308 (hydrophilic) and allows attachment of polymers of interest, through a simple ligand exchange. PLink-polyethylene glycol (PEG) increased colloidal stability, decreasing aggregation, in water and CPAs from minutes (uncoated) to up to 14 days. Heating properties of EMG-1200 in water, measured at 360 kHz and 20 kA/m, increased from 20 to 150 W/g Fe by increasing PLink-PEG5000 as aggregation decreased. Additionally, PLink-PEG did not decrease the 400 W/g Fe heating of water stable EMG308, indicating PLink preserves the core. Further, we successfully nanowarmed cryopreserved HDF cells in VS55 (common CPA) using both 308-PEG5000 and 1200-PEG5000, viability comparable to fresh. The concentrations of IONP in VS55 reached 25 mg Fe/mL of 308-PEG5000 and 60 mg Fe/mL of 1200-PEG5000, which is above our previous sIONP at 10 mg Fe/mL. The heating rate reached 200 °C/min, 2.5 times faster than our sIONPs tests. PLink coated IONPs have been scaled to over 10 g synthesis and used to nanowarm rat kidneys at and above these rates.

The PLink coating allows for facile, inexpensive, and scalable synthesis of PEG-functionalized IONPs for, as needed for human scale organ cryopreservation. In future experiments, PLink IONPs will be tested at higher Fe concentration in various CPAs, maximizing the heating rates with EMG308 IONPs and translating nanowarming to transplantation.

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COMPUTATIONAL MODELLING AND OPTIMISATION OF SLOW COOLING PROFILES FOR THE CRYOPRESERVATION OF CELLS IN SUSPENSION

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The cryopreservation of biological materials is a highly complex process, as it involves numerous factors such as the cooling and thawing procedures, the administration of cryoprotective agents (CPAs), as well as the type and composition of cells. While theoretical work has yielded a better understanding of the processes occurring during cryopreservation, the design of cryopreservation protocols and their parameters is currently based on