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SUCCESSFUL TRANSPLANT OF KIDNEYS AFTER VITRIFICATION AND NANOWARMING

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Organ banking would remove the influence of time from organ transplantation. A promising approach to achieve this is vitrification wherein viable storage is theoretically indefinite. While vitrification of rabbit kidneys was first successfully demonstrated decades ago, the ability to reproducibly rewarm whole organs while preserving viability and function has proven elusive due to ice formation (i.e., devitrification) and/or cracks during rewarming due to insufficient and/or non-uniform rewarming. To overcome these limitations, we first applied an engineering-based transport model to optimize CPA delivery and achieve reproducible vitrification of VMP-perfused rat kidneys. Next, we used “nanowarming”, employing alternating magnetic fields to remotely couple to and heat iron oxide nanoparticles that are perfused in CPA throughout the organ vasculature prior to cooling to achieve fast and uniform rewarming of vitrified kidneys. After unloading, nanowarmed kidneys were assessed by normothermic perfusion and transplantation. Nanowarmed kidneys (n=4) produced urine and functioned comparable to 24-hour cold-stored or VMP-only treated organs when tested by ex vivo normothermic perfusion. Nanowarmed kidneys (n=5) were transplanted in nephrectomized recipients, and all demonstrated rapid reperfusion and urine production. There was some early dysfunction lasting 2-3 weeks (peak Cr 12.6 mg/dL), but renal function normalized (Cr 0.4 to 0.8 mg/dL) by day 21. All measures of renal function were normal at the end of follow-up (day 30), including serum Cr, protein, and electrolytes; calculated glomerular filtration rate; and absence of proteinuria. Histologic and TEM appearance was comparable to fresh control kidney transplants. Using a rat kidney model, we demonstrate the first reproducibly successful vitrification, long-term storage (up to 100 days), rewarming, and transplantation of organs that restore full renal function and solely sustain the life of nephrectomized transplant recipients. Importantly, since this approach provides uniform rewarming, it is scalable to larger organs and provides a viable pathway to clinical organ banking.

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Conflict of Interest: The authors declare the following intellectual property related to this work: “Cryopreservative compositions and methods” Pending U.S. Patent Applications 14/775,998 and 17/579,369 (M.L.E and J.C.B). All other authors declare no competing interest.

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OPTIMIZATION OF REWARMING PROCESS FOR CRYOPRESERVED ORGANS USING A SINGLE-MODE ELECTROMAGNETIC CAVITY: A NUMERICAL STUDY

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Cryopreservation of large-scale biospecimens, tissues or organs is critical for advancing biomedical research and clinical applications, such as organ transplantation. However, currently used warming methods may result in low rewarming rate and temperature non-uniformity in the cryopreserved organs, which can cause lethal ice-recrystallization/devitrification and thermal-stress induced fracture in organs. Electromagnetic heating offers a volumetric heating approach to achieve optimal rapid and uniform warming. In this study, a biophysical model and computer programs were developed to investigate the temperature changes in multiple types of tissues and organs during the warming processes inside a single-mode electromagnetic resonant (SMER) cavity. The model was first validated by comparing the temperature profiles between experimental data and numerical simulation results. They agreed well with each other. The validated model and computer simulation was then applied to study key factors and characteristics of organ rewarming processes, including: (1) temperature profiles and rewarming rates in the cryopreserved organs; (2) temperature gradient and thermal stress inside organs; (3) optimization of the cavity design to fit organ variations in various organ biophysical properties and dimensions. From this study, the SMER performance to generate rapid and uniform rewarming in large-scale tissues and organs were simulated and investigated, which provides guidance for the future cavity development and the elimination of the harmful thermal stress. In addition, the findings from this study revealed valuable insights on the cavity design to accommodate different size of organs and its further optimization to ensure the rapid and uniform rewarming under different cryopreservation conditions.

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VITRIFICATION AND ULTRA-RAPID WARMING OF MOUSE SKIN

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Around 1.1 million burn injuries in the US require medical attention annually. The definitive treatment for severe burns is skin grafting, but many patients are too sick on initial presentation to undergo autologous grafting. Instead, cryopreserved human allogeneic skin grafts are used for coverage to prevent infection and allow for patient resuscitation. Traditional skin cryopreservation uses slow cooling with 10-20% Me_2SO or glycerol as cryoprotective agents (CPAs) but results in poor cell viability, which is vital for the neovascularization and recovery of wounds. Injury results from ice formation that disrupts cells and the macroscopic skin architecture. Therefore, we developed an alternative skin cryopreservation technique using ice-free vitrification paired with rapid rewarming (VR) using metal form radiofrequency (RF) heating. This study tested VR of full-thickness tail skin grafts in a mouse model. CPA (DP6) was loaded stepwise using a protocol developed by predictive diffusion modeling and verified by micro-CT and empiric testing of viability using AlamarBlue. DP6-loaded skin grafts (~8x6mm) were vitrified and stored at -150°C until needed and rewarmed using our ultra-rapid metal form approach in an RF coil. CPA was unloaded (stepwise), and skin grafts were tested directly for viability or transplanted onto the back of syngeneic recipients. The viability of CPA-only controls was 100% (N=8), VR skin was 100% (N=7), and slow warming was 81% (N=4). The 30-day transplant result shows that the VR skin grafts demonstrated high-level engraftment (consistent with revascularization) and no necrosis (N=10) and were comparable to the control transplant group (N=10). In contrast, the injury control (N=2) and convectively warmed skin grafts (N=4) had reduced viability after rewarming and appeared pale and necrotic after transplant. These positive results show that vitrification can improve the viability, reliability, and function of donor skin for transplant to address burn therapy and other regenerative medicine applications.

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METRICS OF CORAL MICROFRAGMENT VIABILITY

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Coral reefs are being degraded at unprecedented rates and decisive intervention actions are urgently needed to help them. One such intervention in aid of reefs is coral cryopreservation. Although the cryopreservation of coral sperm and larvae has been achieved, preservation of coral fragments including both its tissue and skeleton, has not. The goal of this paper was to understand and assess the physiological stressors that might underlie coral fragment cryopreservation and the long-term consequences of these physiological exposures to continued growth. Therefore, we assessed small fragments (~0.5 x0.5 mm^2) from the Hawaiian coral, *Porites compressa*, examining: 1) the sensitivity of the fragments and their algal symbionts to chilling temperatures; 2) the sensitivity of the coral to complex cryoprotectants; 3) methods to safely remove the algal symbionts from the coral fragment for cryopreservation, given the two symbiotic partners may require different cryopreservation protocols; 4) continued growth over time of coral fragments once returned to running seawater after treatment exposures; and, 5) assessment of health and viability of microfragments after treatments examining the distribution of green fluorescent protein and fluorescent symbionts. Technological advances in cryo-technology promise to support successful coral fragment cryopreservation soon, and its success could help secure much of the genetic and biodiversity of reefs in the next decade.
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CRYOPRESERVATION OF DIVERSE SYMBIODINIACEAE SPECIES: FATTY ACID PROFILES, GROWTH AND PHOTOSYNTHETIC PERFORMANCE

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Symbiodiniaceae are a diverse group of dinoflagellates, which can be both free-living and/or associated with a variety of protists and other invertebrate hosts. Maintenance of isolated strains in culture is labour-intensive and expensive. Cryopreservation provides an excellent avenue for their long-term storage. Different cultured isolates from six Symbiodiniaceae genera were cryopreserved using either rapid freezing or controlled rate freezing techniques using dimethyl sulfoxide (Me_2SO) as the cryoprotectant agent (CPA). Symbiodiniaceae isolates from four genera had high cell survival (> 40%) after thawing. The isolates that failed to cryopreserve had very low survival rates (< 15%). These isolates were cryopreserved using [a] a lower final 10% Me_2SO concentration, and [b] grown in media with increased salinity before cryopreservation experiments. For the two isolates that had low survival rates when using a lower concentration of Me_2SO, increased salinity treatments were trialled before freezing. There were high cell viabilities in *Symbiodinium pilosum* cultures after treatment with increased salinities of 44 parts per thousand (ppt) and 54 ppt culture medium. *Fugacium* sp. cryopreserved after salinity treatments of 54 ppt and 64 ppt. Fatty acid (FA) analyses after the salt pre-treatment experiment showed changes in FA production. FAs production increased in response to high salinity treatments as compared to normal salinity (34 ppt). After thawing and allowing the cultures to recover in their normal salinity media, we investigated the photosynthetic performance and growth rates of an endosymbiotic and free-living isolate using Pulse Amplitude Modulated (PAM) fluorometry. The results showed that cryopreservation does not affect their photosynthetic ability nor growth rates. Molecular investigations are underway to explore whether genes involved in enhancing FA production and overall cryopreservation success can be identified.
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THE LAST OF US: SURVIVAL OF CRYOPRESERVED SALAMANDER SPERM DEPENDS ON CRYODILUENT

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Gamete survival through the cryopreservation process can be impacted by a range of factors including diluents and cryoprotectants. This research aimed to test six different cryodiluent treatments and their effects on several sperm parameters in tiger salamanders (n=11). The diluent HAM's F-10 nutrient mix (HAM's), diluted HAM's, or 10% Holtfreter's was added to milt samples to bring the concentration to 1x10^6 sperm/mL. Then, the cryoprotectant dimethylformamide (DMFA) or dimethylsulfoxide (Me_2SO) was mixed 1:1 with the sperm samples and cryopreserved in 200μL straws using liquid nitrogen. A total of 87 straws (1-4 straws/treatment/male) were thawed in a 40°C water bath for 5 seconds and assessed for relative total motility and viability. A subset of straws (n=41) was used for *in-vitro* fertilization with fresh eggs from female tiger salamanders (n=8). We found that both cryoprotectant and diluent had a significant effect on post-thaw relative motility, where Me_2SO was better ($p<0.01$) than DMFA (38.7±4.5% vs. 29.6±5.0%) and HAM's was better ($p<0.01$) than diluted HAM's and 10% Holtfreter's (45.4±6.4% vs. 34.1±4.0% or 23.0±4.0%). Viability was significantly higher ($p<0.05$) in samples frozen with Me_2SO compared to DMFA (32.8±3.9% vs. 27.6±4.1%), while samples with diluted HAM's had higher ($p<0.01$) viability compared to 10% Holtfreter's (34.3±3.9% vs. 26.9±3.5%). DMFA resulted in higher ($p<0.05$) embryo cleavage rates than Me_2SO regardless of diluent (29.8±3.2% vs. 5.7±1.7%). The combination of diluted HAM's and DMFA resulted in the highest ($p<0.05$) embryo cleavage rates (40.1±5.9%) overall. These findings support the use of HAM's as a diluent for cryopreserving salamander sperm, however further work is needed into methodologies for washing salamander sperm cryopreserved with Me_2SO as the chemical appears toxic during the fertilization process. Biobanking cryopreserved sperm is a critical component to genetic management of declining amphibian species. By increasing fertilization capabilities of frozen gametes, both captive and wild populations can avert extinction.
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