Advanced Cryopreservation of Viable Large Cartilage Tissues

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INTRODUCTION: Chondral defects are among the most common joint diseases worldwide. Because cartilage is avascular, its intrinsic capacity for self-repair is severely limited; without timely intervention, defects can progress and lead to severe joint disorders. Osteochondral allograft transplantation (OCA) has significantly improved outcomes and quality of life for patients with cartilage injuries, and more than 600,000 cartilage-related procedures are performed annually in the United States. However, the scarcity of donor tissue and the limited preservation of fresh OCAs (about 28 days at 4 °C) remain major obstacles, leading to frequent tissue loss and complicating screening, logistics, and surgical coordination. Vitrification offers a promising strategy to extend OCA preservation by preventing ice crystal–induced damage during cooling and warming through the use of high concentration cryoprotectants (CPAs). Despite this potential, two critical technical challenges remain: ensuring sufficient CPA penetration and achieving rapid and uniform warming, particularly for large tissue volumes, due to the avascular nature of articular cartilage. To address these barriers, we applied our previously developed microcompuational tomography (μCT)-based simulation approach to predict optimal CPA loading protocols by analyzing diffusion dynamics. In parallel, we employed an advanced radiofrequency electromagnetic (RF-EM) heating system, developed by the TDA team, to investigate the effects of vitrification on viability, structure, and functional properties on articular cartilage.

METHODS: Two sample volumes of porcine articular cartilage were studied: 3 mL discs and 10 mL pieces. Optimized loading protocols of VS55 solutions (55% of CPAs including dimethylsulfide oxide, propylene glycol, and formamide) were developed by modeling diffusion dynamics using a μCT-based simulation approach. Following those optimal protocols, vitrified samples were then rewarmed by either convection warming or the RF-EM warming system. Cell viability was assessed on transverse and sagittal sections using fluorescence live/dead staining and alamarBlue assays. Histological analysis of extracellular matrix (ECM) structure and composition was performed with hematoxylin and eosin (H&E), Safranin O, and Sirius Red staining, while biomechanical properties were determined by microindentation testing.

RESULTS SECTION: A μCT-based model was used to determine CPA diffusivities and predict concentration profiles across cartilage samples **(Fig. 1)**. For small cartilage discs (3 mL), the standard 2hr loading protocol achieved approximately 75% CPA penetration in the central region. However, when the tissue volume increased to cartilage pieces (10 mL), penetration at the center was only 41.1% and 57.2% for the 2hr and 3hr standard protocols, respectively. In contrast, with the newly developed 3hr+ loading protocol, VS55 penetration increased to 86.9%, and therefore this protocol was adopted for subsequent vitrification experiments of 10 mL cartilage pieces. Cell viability and metabolic assays demonstrated that for 3 mL cartilage discs treated with VS55, convection warming led to a significant reduction in viability due to ice crystal formation in the central regions caused by non-uniform heating (**Fig. 2A-C**). In contrast, RF-EM warming preserved both viability and metabolism at levels comparable to fresh samples, even with reduced CPA concentrations. With the increase in tissue volume to 10 mL, the 2hr standard loading protocol resulted in a marked reduction in viability. For 3hr+ loading protocol, live/dead assays first confirmed that the protocol did not exhibit notable cytotoxicity. Further, when combined with RF-EM warming, viable cells were preserved throughout the entire tissue thickness, achieving ~90% overall viability, which was significantly higher than 3hr+ convection warming or the 2hr loading protocol **(Fig. 2D-F).** Moreover, even in the central regions, the 3hr+ RF-EM group recovered ~70% metabolic activity after incubation—sufficient for clinical transplantation—while showing only minimal alterations in ECM structure and biomechanical strength relative to fresh tissues.

DISCUSSION: These findings provide strong evidence that 1) The optimized 3hr+ CPA loading protocol significantly improves CPA diffusion compared to the 2hr standard protocol, thereby enhancing the efficiency of RF-EM warming in larger tissue volumes; 2) The RF-EM warming system achieves faster and more uniform rewarming than conventional convection warming, exceeding the critical warming rate for VS55 solutions (~50 °C/min); 3) Compared to convection warming, RF-EM rewarming preserved over 90% cell viability, higher metabolic activity, and intact ECM composition in vitrified articular cartilage pieces; 4) This technology represents a major step toward the vitrification and rewarming of clinically relevant osteochondral allografts (OCAs), and establishes a clear path for future studies to scale up to larger OCAs with bone and other avascular tissues.

SIGNIFICANCE/CLINICAL RELEVANCE: The newly developed RF-EM rewarming system and the vitrification preservation method offer a promising strategy to alleviate the shortage of osteochondral grafts by extending preservation, increasing donor availability, and improving transplantation outcomes.

ACKNOWLEDGEMENTS: Funding for this project was provided by DHA W81XWH-21-C-0035, and NIH P20GM121342 (SC TRIMH COBRE), R01DE021134, and T32DE017551. This work was also supported in part by Cell & Molecular Imaging Shared Resource, Hollings Cancer Center, Medical University of South Carolina (P30CA138313).

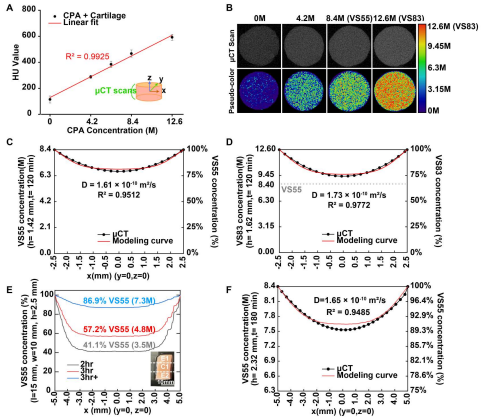

Fig 1. (A) The calibration curve for cartilage specimens equilibrated in different CPA concentration solutions; (B) Pseudocolor μCT images of cartilage tissues equilibrated in different concentrations of CPA solutions; (C) Concentration profiles of the VS55-loaded discs on sagittal sections; (D) Concentration profiles of the VS83-loaded discs on sagittal sections; (E) VS55 distribution after loading larger pieces of varying loading protocols at 0°C; (F) Concentration profiles of the VS55-loaded larger pieces on sagittal sections.

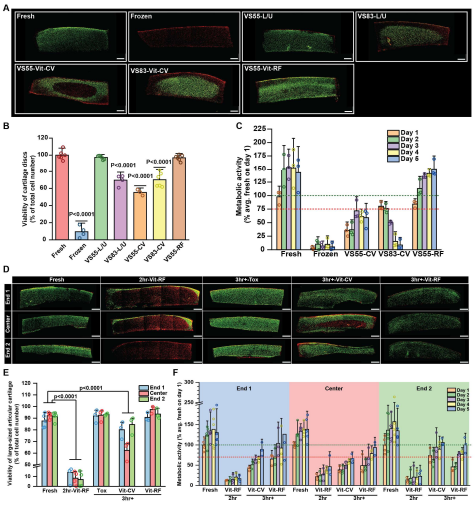

Fig 2. (A) Live/dead images of cartilage discs; (B) quantitative analysis of live/dead images; (C) metabolic activity measured by the alamarBlue assay. (D) Live/dead images of cartilage pieces; (E) quantitative analysis of live/dead images; (F) metabolic activity measured by the alamarBlue assay.