Establishment of A Pig Model for Preclinical Evaluation of Vitrified Meniscal Allograft Transplantation

Ge Pan[1], Haiyang Ma[1], Kristi Helke[2], William Michael Pullen[2], Harris Slone[2], Meenakshi Mukherji[1], Daniel Eldridge[2], Peng Chen[1], Dustin Mueller[1,2], Shuchun Sun[1], Yongren Wu[1,2], Tong Ye[1], Hai Yao[1,2], Shangping Wang[1,2]
[1]Clemson University, Clemson, SC, [2]Medical University of South Carolina, Charleston, SC
pan4@g.clemson.edu

Disclosures: Ge Pan (N), Haiyang Ma (N), Kristi Helke (N), William Michael Pullen (N), Harris Slone (N), Meenakshi Mukherji (N), Daniel Eldridge (N), Peng Chen (N), Dustin Mueller (N), Shuchun Sun (N), Yongren Wu (N), Tong Ye (N), Hai Yao (N), Shangping Wang (N)

INTRODUCTION: Meniscus injuries affect millions of people each year, yet the intrinsic healing capacity of the meniscus is severely limited due to its avascular nature. The standard treatment, meniscectomy, requires partial or total removal of the tissue and substantially increases the long-term risk of osteoarthritis. Meniscal allograft transplantation (MAT) offers a more advanced option and has shown promising long-term outcomes by restoring joint function. However, despite the high demand for meniscus replacement among hundreds of thousands of patients, only about 2,000 procedures are performed annually in the United States, primarily because of the limited availability of suitable donor grafts. Vitrification has emerged as a promising approach to address this limitation by enabling long-term preservation of viable tissues at cryogenic temperatures. Unlike conventional freezing, vitrification prevents ice crystal formation through the use of concentrated cryoprotectants (CPAs). Previous work demonstrated that an optimized 3-hour VS55 loading protocol, developed using a microcomputational tomography (μCT)-based simulation approach, preserved more than 70% cell viability in intact menisci harvested from 6-week-old miniature pigs. However, clinical translation requires scaling up to menisci from 6-month-old pigs, which are developmentally and anatomically comparable to those of 6-7-year-old humans, where higher tissue density and complexity hinder CPA penetration and increase toxicity risks. In this study, we established an optimized VS55 loading protocol that balances CPA diffusion and cytotoxicity, enabling efficient penetration and long-term storage (1 year) of large meniscal grafts. To evaluate functional outcomes, we conducted a 4-month transplantation study in a pig model, comparing vitrified meniscal allografts with fresh allografts using both in vivo and ex vivo assessments.

METHODS: To ensure sufficient CPA penetration into larger tissues, computational modeling was used to optimize loading protocols. To assess cell viability of vitrified menisci, live/dead staining and AlamarBlue were performed on fresh, standard 3-hour vitrification (3hr), and optimized 3-hour vitrification (3hr+) specimens. Histological analysis of the structure and composition of the extracellular matrix (ECM) was performed with hematoxylin-eosin (H&E), safranin O, and picrosirius red staining. For MAT animal studies, 12 pigs (6 males and 6 females) were assigned to Sham (n=2), autograft (n=2), fresh allograft (n=4) and vitrified allograft (n=4) transplantations. A 12-mm-wide meniscal segment was transplanted into the right hind limb of each animal. Postoperative outcomes, including swelling, redness, wear, and pain, were monitored for four months. Blood samples were collected five times during this period to assess systemic inflammation and infection. MRI scans were performed at two and four months to assess meniscal regeneration and joint pathology. At the four-month endpoint, explanted implants underwent gross inspection, joint function testing, cell viability assessment, and histological analysis.

RESULTS SECTION: For the in vitro assessment, the standard 3hr loading group exhibited extensive cell death throughout the tissue. In contrast, the optimized 3hr+ loading protocol did not adversely affect cell viability during CPA loading and unloading process and successfully maintained cell viability above 80% for up to 11 months of storage following vitrification (**Fig 1**). Vitrified menisci were then transplanted into pigs and compared with autografts and fresh allografts. MRI and gross inspection of the sham group confirmed that the surgical procedures including incision and closure did not adversely affect knee joints with intact menisci and articular cartilage. In all graft transplantation groups, substantial tissue growth was observed within 4 months, and the grafts integrated with the surrounding native tissue (**Fig 2**). At the four-month endpoint, we found no significant differences in meniscal cell viability among the vitrified allografts, fresh allografts, and autografts, with all groups maintaining viability above 90%. Furthermore, analysis of cartilage from the femur and tibia demonstrated comparable outcomes cross groups, with cell survival consistently exceeding 85%.

DISCUSSION: These findings offer compelling evidence that 1) the enhanced 3hr+ loading protocol improved CPA penetration from 46–61% to 84–92% in larger whole menisci, which achieved cell viability above 80% throughout the tissue after vitrification and rewarming; 2) the sham operation verified that surgical procedure itself did not cause additional effects on the tissues; 3) Vitrified meniscal segments (12 mm wide, harvested from the central region) demonstrated integration with native tissue comparable to that of autografts and fresh allografts over the 4-month post-operative period.

SIGNIFICANCE/CLINICAL RELEVANCE: This pilot animal study provides critical evidence that vitrification can preserve cellularly, structurally and functionally competent menisci at clinically relevant scales, offering a potential pathway to expand the availability of donor grafts and advance meniscal transplantation therapy.

ACKNOWLEDGEMENTS: Funding for this project was provided by the Musculoskeletal Transplant Foundation (MTF) Biologics Extramural Research Grant and National Institutes of Health: 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).

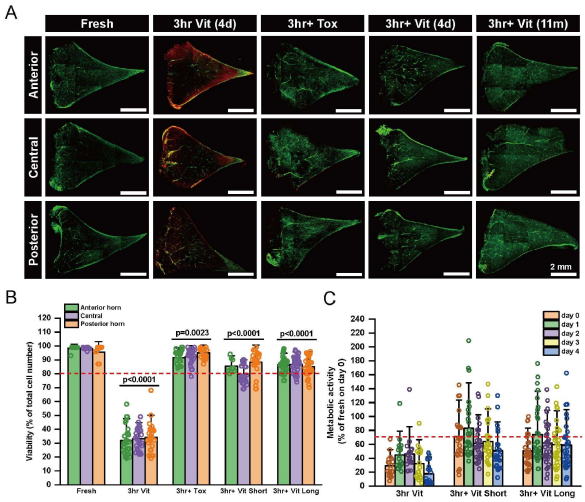

Fig 1. (A) Live/dead images of entire menisci from different groups; (B) quantitative analysis of cell viability across different regions; (C) metabolic activity measured by alamarBlue assay.

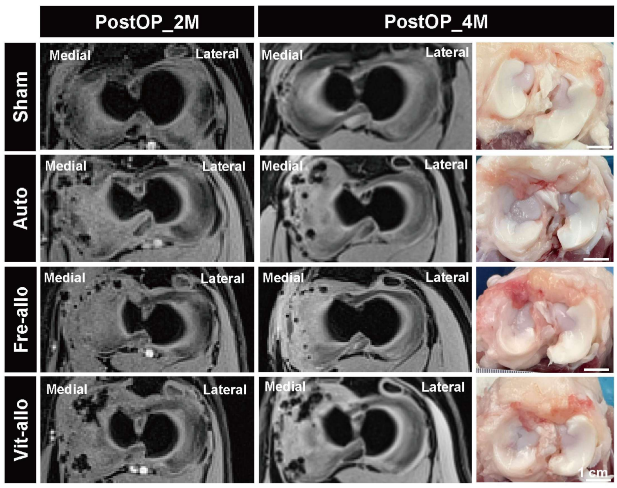

Fig 2. MRI images obtained at 2- and 4-months post-operation, with gross inspection at the endpoint.