Outcomes of the First in Vivo Study of Osteochondral Allografts After Cryopreservation by Vitrification With Nanowarming in a Porcine Model

Kelvin GM. Brockbank, PhD - Tissue Testing Technologies LLC; Shangping Wang, PhD - Clemson University; Mary-Katherine Lynch, MD - Medical University of South Carolina; Jonathan Goodloe, MD - Medical University of South Carolina; Peng Chen, PhD - Medical University of South Carolina; Pengling Ren, PhD - Clemson University; Glenn Hepfer, PhD - Clemson University; Elizabeth Greene, LATG - Tissue Testing Technologies LLC; Lia Campbell, PhD - Tissue Testing Technologies LLC; Harris Slone, MD - Medical University of South Carolina; William Pullen, MD - Medical Univeristy of South Carolina; Yongren Wu, PhD - Clemson University; Hai Yao, PhD - Clemson University; Kristi Helke, DVM, PhD - Medical University of South Carolina

BACKGROUND: The limited supply and short storage time of fresh cartilage osteochondral allografts present challenges in clinical practice. Vitrification is a promising technique for tissue cryopreservation, however it has limited application to large specimens due to ice crystal formation during rewarming. Our previous study has shown that nanowarming improves the viability of vitrified grafts, particularly in the superficial layer, by reducing rewarming-induced damage (Cryobiology 109 (2022) S133). Building on these findings, we present the first in vivo experiences with nanowarmed articular cartilage specimens in a 4-month transplantation study.

OBJECTIVE: Determine whether our new cryopreservation method will result in repair of full thickness osteochondral defects.

METHODS: Osteochondral specimens from the trochlea groove of mature Yorkshire pigs were harvested and divided into fresh (control) and vitrified nanowarmed groups. After implantation in Hanford miniature pigs, the knees with implants were analyzed by imaging (µCT and MRI), macroscopic scoring and histopathology.

RESULTS: The in vivo pig model was successfully developed, with fresh grafts integrating well. The 4-month vitrified grafts showed partial bone loss, connective tissue and cartilage formation at the implant site, while fresh grafts displayed similar changes at earlier timepoints (1-3 months), suggesting that there is potential for normalization of the vitrified grafts if they had been maintained beyond 4 months. The cartilage of the vitrified grafts was thickened in all cases. Please see Figure 1 for representative fresh versus vitrified imaging. Location of vitrified grafts impacted results, with significant differences observed ($p=0.0046$) in proximal and distal placement (Figure 2). However, similar changes were noted in both fresh and vitrified samples. This study demonstrates the successful development of a pig model for assessment of future optimization of vitrification and nanowarming protocols. The vitrified grafts regenerated more slowly than fresh, possibly due to lower cell viability in the middle and deep zones of osteochondral plugs or residual cryoprotectants.

CONCLUSIONS: The study highlights the potential of nanowarming to improve the viability of vitrified grafts, while emphasizing the importance of graft location in future studies. Vitrified grafts in the distal

position were significantly better than in the proximal position similar to fresh implants. Further studies are in progress to improve outcomes in the proximal position.

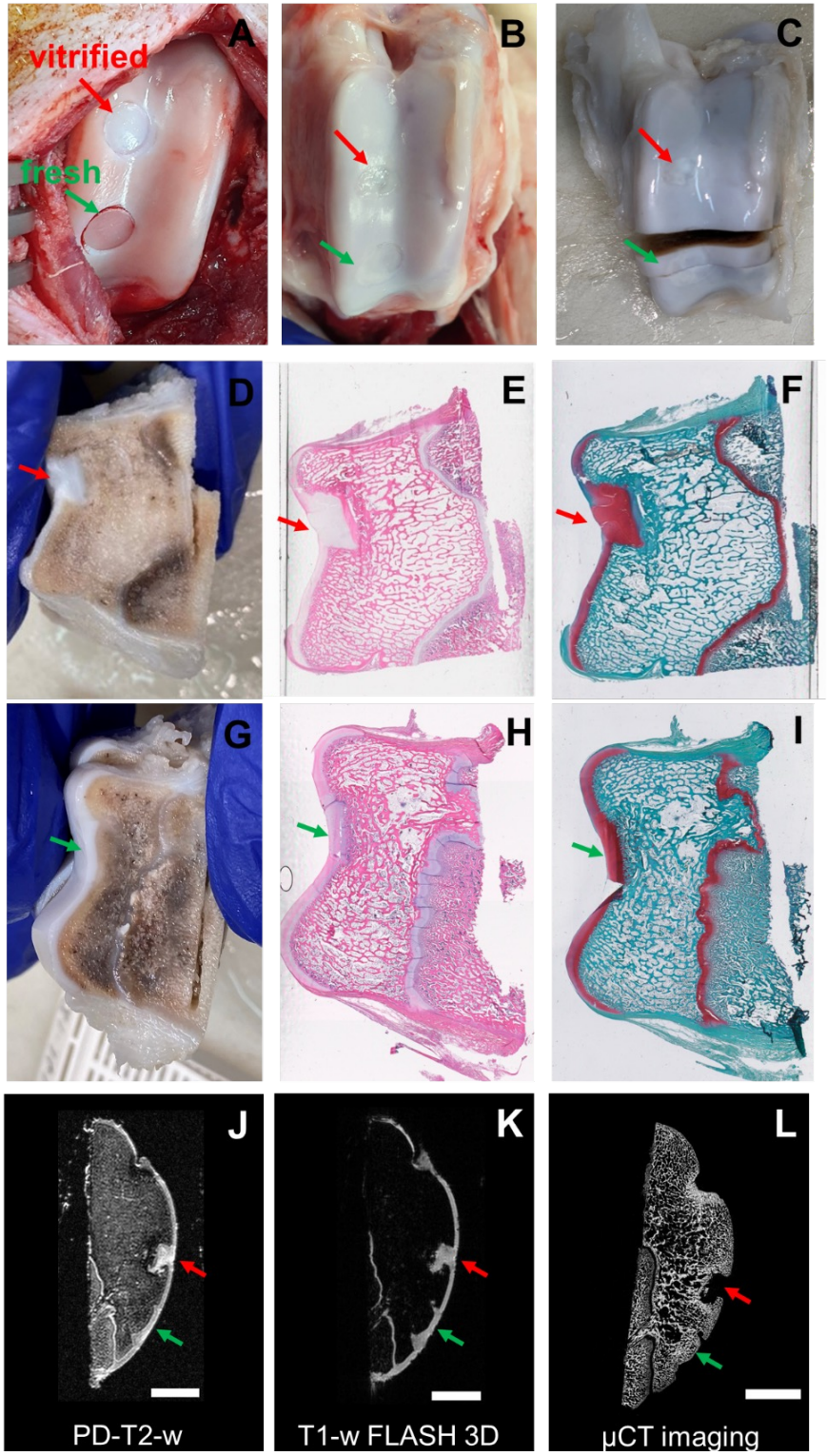

Figure 1: Representative gross, histological and radiological images of explants after 4-months. (A-C) Image of fresh (green arrow) and vitrified (red arrow) implants at closure (A), after 4-months of implantation (B), and after fixation in 10% formalin (C). (D-F) Unstained transverse section of the vitrified explant (D), an H&E stained image (E), and a Safranin O stained image (F) of the vitrified explant. (G-I) Transverse section of the fresh explant shown in (G), an H&E stained image (H), and a Safranin O stained image (I). (J-L) Sagittal sections of the same explants shown in radiological images including a PD-T2-w sequency MR image (J), a T1-w FLASH 3D sequency MR image (K), and a µCT image (L). H&E staining was used to assess overall tissue morphology, and Safranin O staining was used to evaluate proteoglycan content. Cartilage healing was assessed using MR images, whereas bone tissue healing was evaluated using µCT images. Scale bars: 1 cm in (J-L).

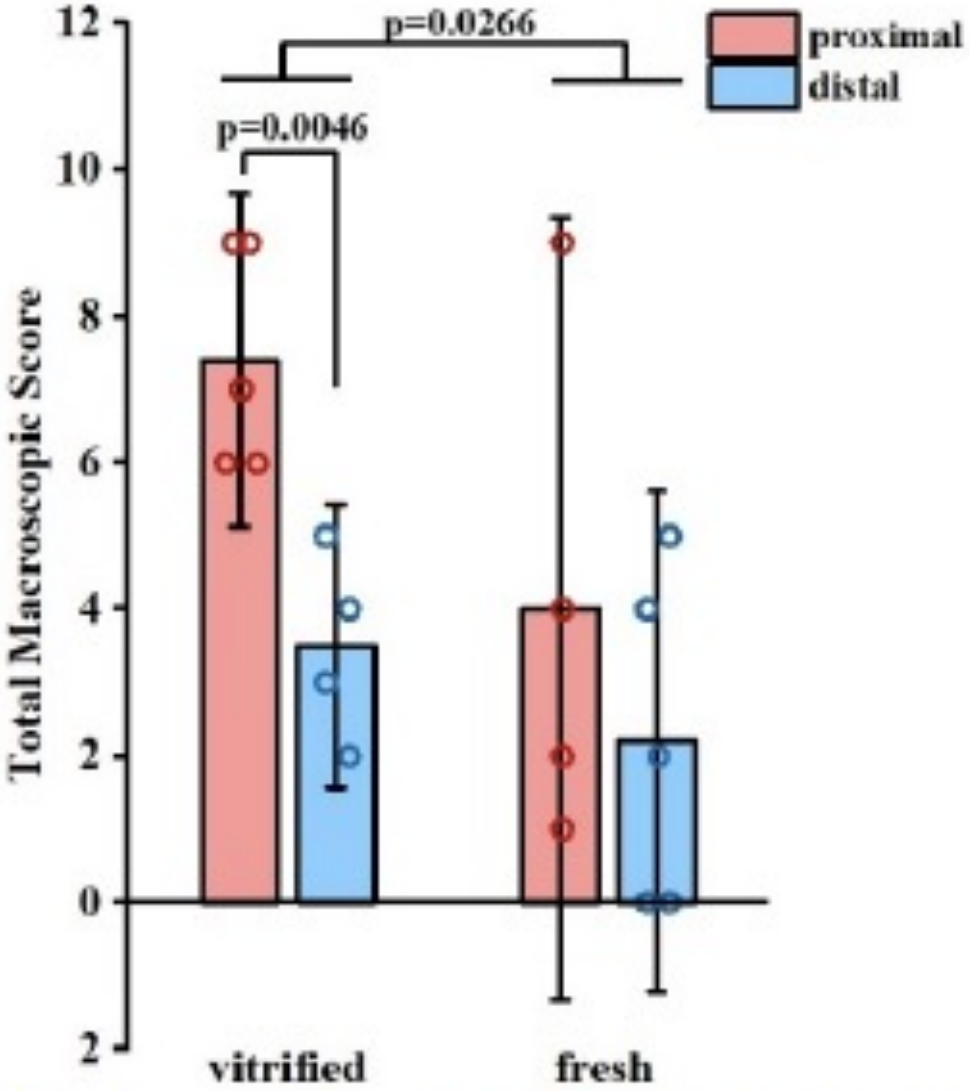

Figure 2: Total macroscopic scores of vitrified and fresh groups in proximal and distal placement. Data presented as mean ± SD.

Conclusions

- Fresh OCA grafts integrated well after 4 months. However, earlier 1-3 month explants were similar to the nanowarmed grafts at 4 months (not shown).

- Nanowarmed OCA grafts led to partial bone loss and connective tissue formation at the implant site after 4 months. The differences observed between fresh and nanowarmed suggests that the nanowarmed samples were taking longer to adapt to the recipient implant site.

- Location of vitrified grafts impacted macroscopic scores, with a statistically significant difference observed ($p=0.0046$) in proximal versus distal placement. There was no significant difference between the vitrified distal grafts and either the proximal or distal fresh grafts.

- The protocol used for OCA vitrification and nanowarming is being addressed for optimization in future transplant studies.