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Ice-free cryopreservation of natural and bioengineered tissues

, ffective improved tissue banking methods for natural and engineered tissues, complex vascularized allotransplants and organs are desperately needed for transplantation. Banking of living cellular tissues using current tissue banking practices employing conventional cryopreservation by freezing is not feasible due to the well-documented damage caused by ice formation. An alternative ice-free cryopreservation approach is vitrification. Formation of ice is prevented by the presence of high concentrations of cryoprotectants with preservation of extracellular matrix components and optional preservation of cells. Ice-free vitrification works for a variety of natural and engineered tissues, using a formulation consisting of DMSO, formamide and propylene glycol, known as VS55, but have been unsuccessful at sample volumes over a few mLs. The major constraints for scale-up of cryopreservation by ice-free vitrification have been avoidance of ice nucleation during warming and mechanical forces generated by glasses at low temperatures. In this presentation, I will focus on strategies for avoidance of ice nucleation. Our first successful strategy for large tissue samples was an 83% formulation based upon the same cryoprotectants, known as VS83. This formulation can be used to retain viable chondrocytes in large osteochondral grafts or for non-viable cardiovascular grafts with retention of extracellular matrix integrity, depending upon the way in which the formulation is added and removed before and after vitrification. Non-viable cardiovascular grafts with intact matrix have been a major research focus for the last 10 years and both in vitro and in vivo results demonstrated significantly reduced immunogenicity in heart valves, including reduced memory T-cell proliferation and most recently modulation of TGF-B1 from latent to active form among other statistically significant effects. We have been successful in scaling up the viable preservation of large tissue samples using either nano warming, inductive heating of iron nanoparticles, or convection warming using improved ice-free vitrification formulations.

Biography

Kelvin Brockbank, CEO, and Founder of Tissue Testing Technologies LLC, is a Research Professor of Bioengineering at Clemson University and Adjunct Professor of Regenerative Medicine and Cell Biology at the Medical University of South Carolina. His research interests include cell, tissue and organ cryopreservation for test systems and transplantation and manufacturing methods for cell-based bioengineered therapy products. His work has led to the establishment of two successful publicly traded low-temperature technology platform companies, CryoLife Inc., and Lifeline Scientific. He has over 500 publications and presentations at national and international conferences including more than 30 patents related to hypothermic, frozen and vitrified biomaterial preservation.

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