

Ambient Temperature Viable Amnion Processed Via Novel Lyopreservation Method Retains Properties of Fresh Tissue

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Cryopreservation is currently the only method for long-term storage of living cells and tissues. Commercial viable cryopreserved placental membranes show positive outcomes in clinical trials for management of different types of wounds. However, cryopreservation requires specialized ultra-low temperature storage equipment that limits widespread use of cellular therapies. To address this limitation, a novel lyopreservation technology has been developed that allows for ambient storage of living cells and tissues. This method can be applied to many different cell and tissue types, including placenta, skin, bone and cartilage. Here, fresh human viable amniotic membrane (hVAM) was processed using the novel lyopreservation method. The structure, cell viability and wound-relevant properties of lyopreserved hVAM (hVLAM) were then investigated *in vitro* and *in vivo* in a diabetic mouse wound model. We found that both visual appearance and microscopic tissue structure, as well as the number of viable cells of hVLAM after rehydration, were comparable to both fresh and cryopreserved tissues. At the molecular level, hVLAM inhibited secretion of pro-inflammatory tumor necrosis factor- α by activated immune cells, and released more vascular endothelial growth factor in a hypoxic environment. These data demonstrate that hVLAM retains anti-inflammatory and pro-angiogenic properties of fresh placental tissue.

Application of hVLAM to wounds in diabetic mice resulted in accelerated wound closure that was associated with increased vascularization, granulation tissue formation, and restoration of the basement membrane in the wound. Taken together, our data demonstrate that hVLAM retains endogenous viable cells, as well as structural and functional properties of fresh amnion. This novel lyopreservation technique eliminates the need for low temperature storage, a limiting factor in the use of cryopreserved viable tissues, making these products accessible for widespread use.