

Comparison of cryopreserved amniotic membrane and umbilical cord tissue with dehydrated amniotic membrane/chorion tissue.

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Why This Study Is Relevant:

Compares processing methods of amniotic membrane and umbilical cord tissue

Compares the structural and cellular differences between cryopreserved amniotic membrane and dehydrated amniotic membrane/chorion

Compares the structural and cellular difference between cryopreserved umbilical cord and dehydrated amniotic membrane/chorion.

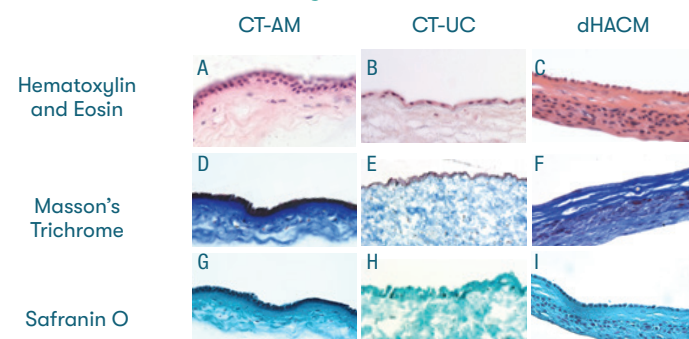
Use of amniotic membrane (AM) and umbilical cord (UC) tissues are well-known to help expedite regenerative healing in a wide range of medical specialties including ophthalmology, orthopedics, urology, wound care, and more.¹⁻⁵ However as with other allografts, the use of fresh tissue is often impractical and can pose a serious risk of disease transmission, therefore processing methods must be used to clean the tissue. While several processing methods exist, the most widely employed are cryopreservation and dehydration via heat drying. While cryopreservation has previously been shown to be comparable to fresh AM/UC (Tan, 2014), Cooke et al. investigated structural and biochemical differences of cryopreserved AM (Neox®/Clarix® 100; Amniox Medical, Inc., now BioTissue Surgical), cryopreserved UC (Neox®/Clarix® 1K; Amniox Medical, Inc., now BioTissue Surgical), and dehydrated amniotic membrane/chorion (dHACM; EpiFix/AmnioFix; MiMedx).

In brief, Hematoxylin and Eosin (H&E) staining revealed that the structural morphology of cryopreserved AM and UC (termed “CT-AM” and “CT-Thick” respectively in Cooke et al.), i.e., AM and UC processed by BioTissue’s CryoTek method closely resembles that of fresh tissue, while dHACM was dramatically compacted in comparison to cryopreserved AM. Both Masson’s Trichrome and Safranin O staining used to indicate collagen content and sulfated proteoglycans, respectively, corroborated these results. Tissue extracts showed CT-AM and CT-UC had approximately 3 and 50 times more HA, respectively, than dHACM. Furthermore, agarose gel electrophoresis confirmed that CT-UC showed similar high molecular weight (HMW HA) distributed as control (1000-6000 kDa), indicating UC preferentially contained HMW HA. In comparison, dHACM contained no HMW HA and exhibited a low molecular weight HA smear (500 kDa). Hence, dehydration compromises tissue structure while cryopreserved tissues retains structural similarities to fresh tissue.

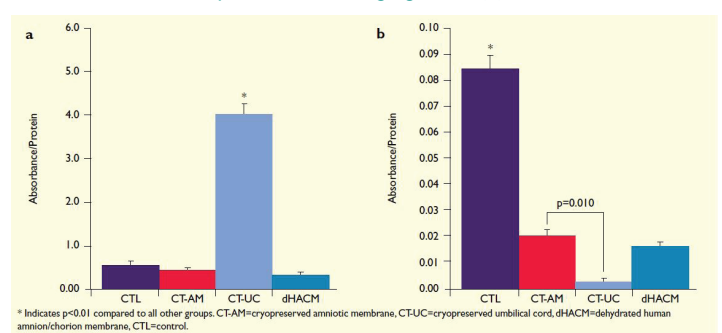
Since previous research suggests HC-HA/PTX3 as a key factor in the regenerative qualities found in these tissues, characterization of this unique complex was performed in cryopreserved and dehydrated tissue.⁶ Western Blot analysis of tissue extracts confirmed the HC-HA/PTX3 complex was present in CT-AM and CT-UC but not dHACM. In addition, the AM’s anti-inflammatory activity was shown to be present in CT-AM and CT-UC but absent in dHACM. Pro-inflammatory macrophages treated with cryopreserved tissue extracts exhibited significant inhibition of proliferation whereas macrophages treated with dHACM demonstrated no such inhibition. ELISA analysis further confirmed CT-UC dramatically increased IL-10 and decreased IL-12 expression compared to dHACM, indicative of a more anti-inflammatory macrophage phenotype.

In summary, the authors showed strong evidence suggesting the cryopreservation processing method of AM/UC tissues is superior to dehydration with respect to maintaining structural and biochemical properties of fresh tissue.

Histological staining of cryopreserved and dehydrated amniotic tissues. CT-AM (a, d, g), CT-UC (b, e, h) and dHACM (c, f, i). All images are 20x magnification.



Macrophage cytokine expression in response to tissue extracts. IL-10, an anti-inflammatory cytokine (a) and IL-12, a pro-inflammatory cytokine (b).



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