Cryopreservation Maintains the Structural Integrity and Biochemical Properties of Fresh Amniotic Membrane and Umbilical Cord Tissues

Ek Kia Tan\textsuperscript{1}, Julie O’Connell\textsuperscript{2}, Scheffer C.G. Tseng\textsuperscript{1,3}

\textsuperscript{1}TissueTech, Inc., Miami, FL
\textsuperscript{2}Amniox Medical, Inc., Atlanta, GA
\textsuperscript{3}Ocular Surface Center, Miami, FL
Disclosure

Tan EK – Employee of TissueTech Inc.
O’Connell J – Employee of Amniox Medical Inc.
Tseng SC – Employee of TissueTech Inc.
Background

- Cryopreserved amniotic membrane (AM) and umbilical cord (UC) tissues have been used clinically for decreasing post-operative inflammation, pain, and adhesion following soft-tissue and lower extremity reconstructive procedures.
- AM and UC tissues contain an abundance of the anti-inflammatory mediator, hyaluronic acid.
- HC-HA/PTX3, a unique matrix proteoglycan, has been identified as a key mediator of the anti-inflammatory and anti-scarring properties of amnion tissues.
- The purpose of this study was to examine the effect the cryopreservation process may have on amnion tissue structural and biochemical properties that may affect the anti-inflammatory and anti-scarring properties of these tissues.
Materials & Methods

• **Materials:**
  • Cryopreserved amniotic membrane (AM; CLARIX™ 100) and cryopreserved umbilical cord (UC; CLARIX™ 1K, Amniox Medical Inc., Atlanta, GA) were directly compared to fresh AM and UC tissue obtained from elective Cesarean section.

• **Methods:**
  • **Histology:** Fresh and cryopreserved AM and UC samples were stained for hematoxylin and eosin, Masson’s trichrome, and Safranin-O.
  • **Histochemistry:** Tissue matrix hyaluronic acid (HA) content was measured using HA binding protein (HABP) fluorescent histochemistry.
  • **Macrophage functional assessment:** RAW264.7 macrophage proliferation and cell death was assessed in cultures on cryopreserved AM tissues.
  • **TGF-β1 promoter activation:** TGF-β1 promoter activity was examined in human corneal fibroblasts cultured in the presence of fresh and cryopreserved AM extracts.
Comparison of fresh and cryopreserved AM and UC tissues show that the cryopreservation process does not significantly alter tissue structure (A-D), collagen (E-H), or glycosaminoglycan content (I-L).
Histochemistry with HA binding protein revealed that the distribution of HA was similar in both fresh and cryopreserved AM and UC tissues.

Cryopreserved AM and UC tissues contained significantly higher ratios of total HA/Protein content compared to fresh samples.
Results

- High molecular weight HA is retained in the loading well in both fresh and cryopreserved AM and UC tissues (A) but is lost with treatment with HAase (B)
- Overall, **UC contains a higher amount of HA compared to AM**

- The high molecular weight HC-HA complex, shown to exert anti-inflammatory and anti-scarring effects, was retained in the loading well for both fresh and cryopreserved AM and UC tissues
Results

I

• Live/Dead staining shows viable (green) and dead (red) RAW264.7 macrophages after 48h of culture on fresh and cryopreserved AM (I – panels A-D)

• Proliferation of RAW264.7 macrophages was significantly inhibited in response to both fresh and cryopreserved AM extracts (II)

II

![Graph showing proliferation](chart.png)
Results

- Human corneal fibroblasts were cultured in the presence of increasing concentrations of fresh and cryopreserved AM extracts.
- Both fresh and cryopreserved AM extracts dose-dependently decreased TGF-β1 promoter activity.
- These results suggest that cryopreservation preserves the anti-scarring activity of AM tissues.
Discussion

• Cryopreservation does not significantly alter the structural and biochemical composition of amniotic membrane and umbilical cord tissues when compared to fresh tissues

• Both cryopreserved AM and UC tissues contain high molecular weight hyaluronic acid and the HC-HA complex that has been demonstrated to impart the anti-inflammatory and anti-scarring properties of amnion tissues

• Cryopreserved AM tissue extract inhibited RAW264.7 macrophage proliferation and human corneal fibroblast TGF-β1 promoter activity to the same extent as fresh AM tissue
Conclusions

• Collectively, these results indicate that cryopreservation of AM and UC effectively preserves the structural, biochemical, anti-inflammatory, and anti-scarring functional properties of these tissues.
References


