# Minimally Manipulated Amniotic Membrane Highly Retains Cellular Structure and Molecular Components as in Native Tissue

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## ABSTRACT

Amniotic membrane (AM) is a placental organ that protects the fetus and keeps it isolated from the organs of the mother. Rich in collagen and hyaluronic acid, the AM is structurally elastic, soft and spongy, allowing it to mechanically buffer physical impacts to the fetus [1-3]. Another relevant property of AM is the unique immunosuppressive and angiogenic properties of enabling this tissue to evade host rejection and induce vessel regeneration, respectively, making it a valuable tool for tissue therapy [4-6]. Moreover, it is well known to have anti-inflammatory properties and has been shown to promote healing in various orthopedic indications [7]. This study characterizes and defines the viability of Amniotic Membrane cellular and molecular components observed in cryopreserved Signature Matrix.


## METHODOLOGY

Minimally manipulated AM tissue products manufactured by Signature Biologics and naïve/fresh AM were used in this study. Signature Matrix product used was selected at random from company Finished Goods inventory (average of 5±2 months in cryopreservation) and thawed according to manufacturer instructions. To observe viable cell localization, fluorescence microscopy using CSFE (metabolically active cytoplasm) and DAPI (nucleus) were utilized. Homogenates of all the products were used to quantify hyaluronic acid, prostaglandin E2 (PGE2) and human hemoglobin using enzyme-linked immunosorbent assay (ELISA).

## RESULTS

H&E and Collagen staining showed an abundance of cells and collagen, respectively, in Signature Matrix. CSFE and DAPI staining showed than in a single 2.5mm hexagon of AM ~$1.22x10^6$ of living cells reside. We found significantly higher levels of human hemoglobin in fresh AM (FAM) compared to Signature Matrix (SM). Significant differences in hyaluronic acid were not observed between groups. No differences in PGE2 abundance were found between Signature Matrix products and fresh amniotic membrane. We concluded that Signature Matrix did not present any structural and molecular differences compared to fresh/naïve or after cutting the amniotic membrane.

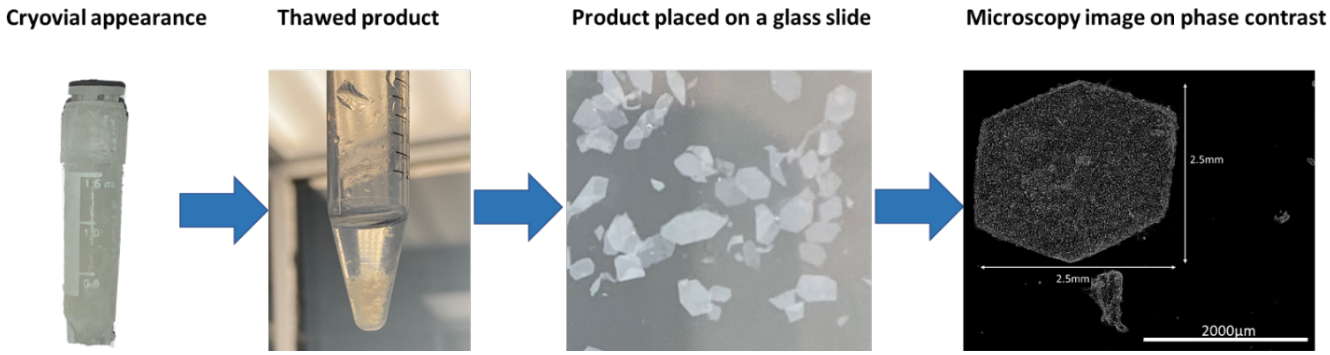


**Figure 1. General characteristics after thawing:** Visual appearance of the cryopreserved product from the cryovial to the microscope. 135mg of hexagonal-shaped pieces of amniotic membrane are intact.

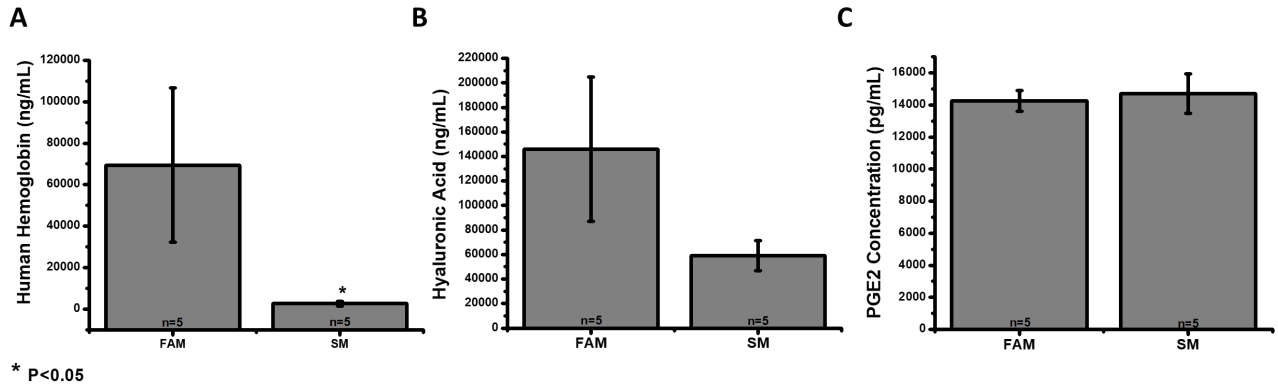


**Figure 2. Molecular components of Fresh Amniotic Membrane and Signature Matrix.** A) Quantitative results for the presence of hemoglobin in Fresh Amniotic Membrane (FAM) and Signature Matrix (FM). B) Hyaluronic acid quantification in FM tissue and SM products. C) Quantitative results for the presence of PGE2 in FAM and SM demonstrating that the levels of this molecule remain the same as in the native tissue.

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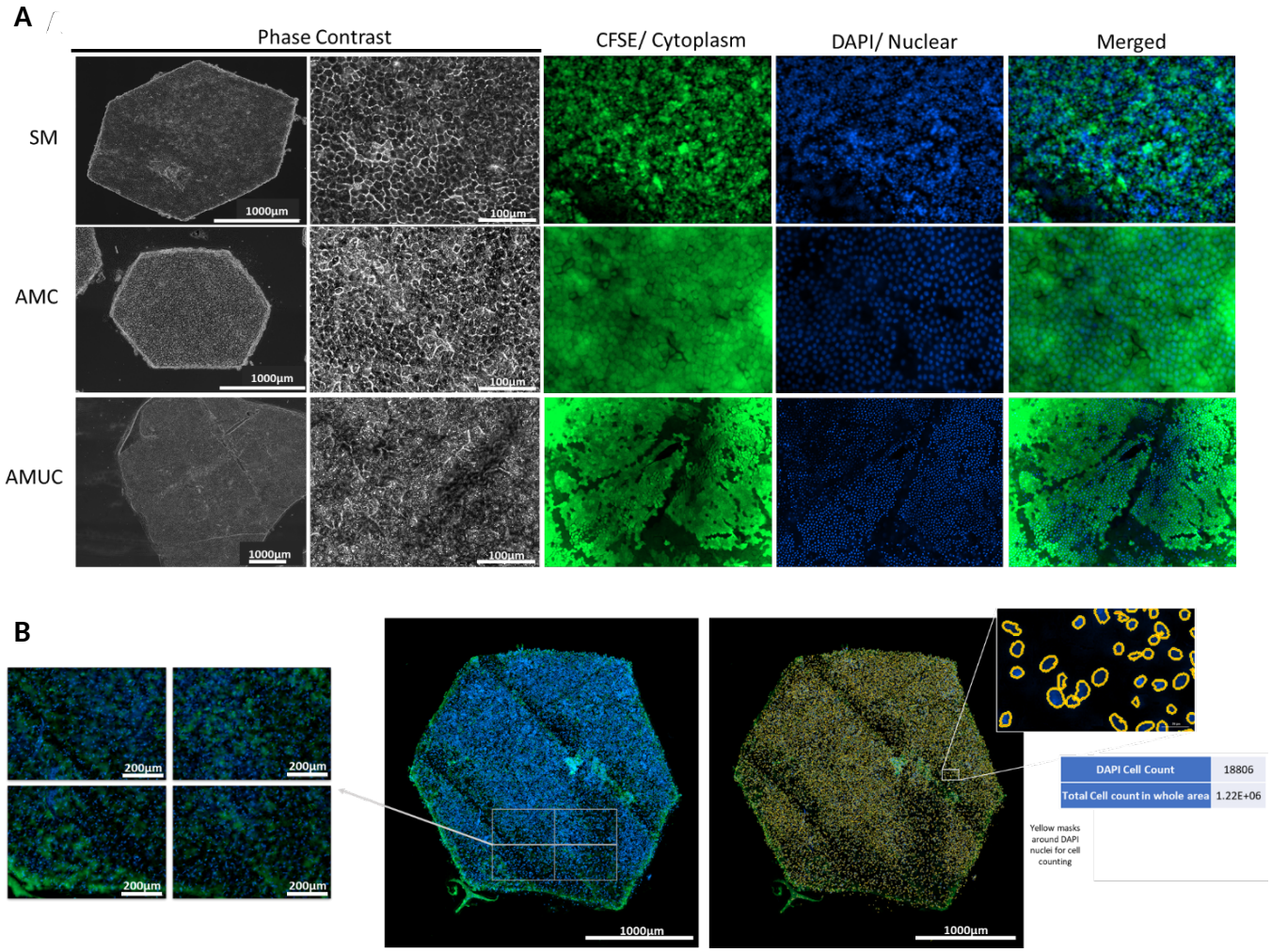


**Figure 3. Live staining of Amnion Membrane.** A) Images of post thaw Signature Matrix (SM), Amniotic Membrane after cut but not cryopreserved (AMC) and Amniotic Membrane Uncut/Native (AMUC). Left panel: Phase Contrast and Right Panel: CSFE staining for metabolically/living cell cytoplasm, DAPI for cell nucleus identification and merged mage of both fluorescent dyes showing the correlation of cytoplasm with the nucleus. B) (From Left to Right) Single SM hexagon, enhanced resolution with multi-image collage stitching, and DAPI Cell Count obtained using Gen5™ Software from BioTek®.

## REFERENCES


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