

**Characterization and Comparison of Cryopreserved Umbilical Cord Products**

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**ABSTRACT**

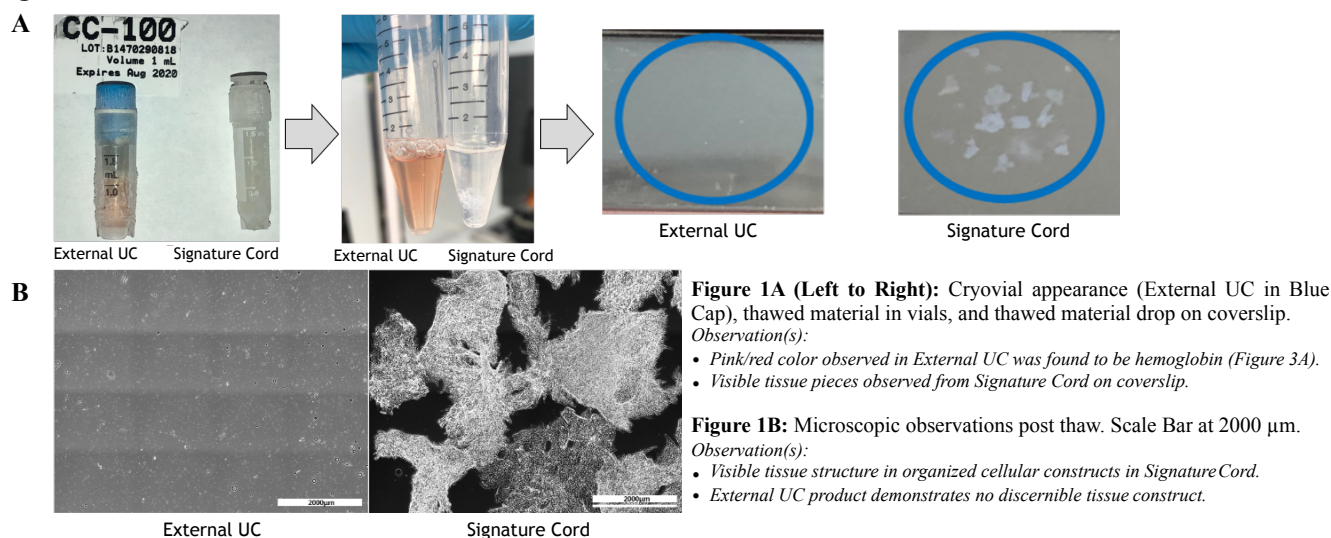
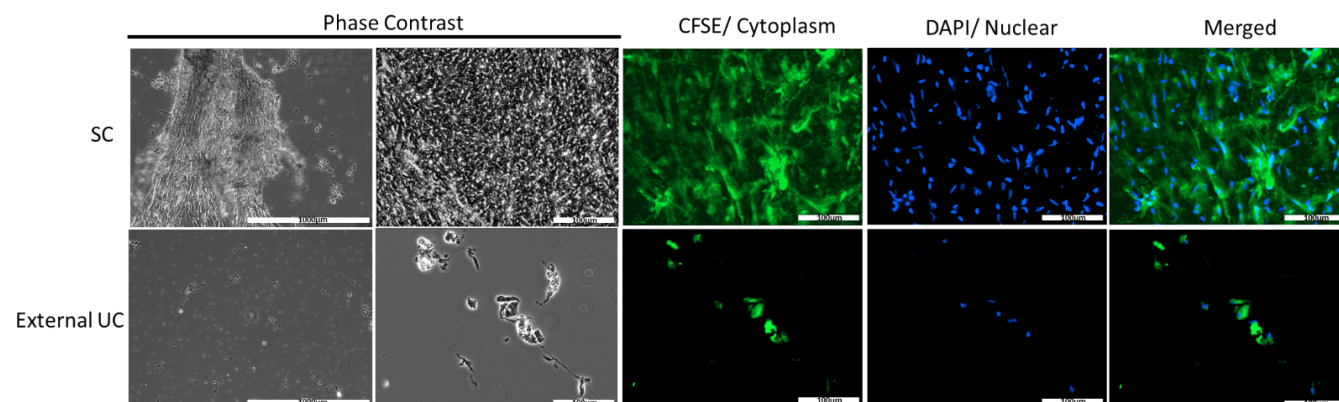
Umbilical cord (UC) is a placental organ that provides nutrients from mother to fetus. UC is structurally soft and spongy, allowing it to mechanically buffer physical impacts to the fetus [1, 2]. The UC contains Wharton's jelly (WJ), which is rich in collagen and hyaluronic acid. The UC has unique immune invasive properties which enables the tissue to evade host rejection and makes it a valuable tool for tissue therapy [3]. Moreover, UC tissue is well known to have anti-inflammatory properties and have been shown to promote healing in various orthopedic indications [4]. This study characterizes and defines the viability of the cellular and molecular components of cryopreserved Signature Cord and an external UC product.

**METHODOLOGY**

Minimally manipulated UC/WJ tissue products manufactured by Signature Biologics and an external (Non Signature Biologics) UC product, manufactured in the USA, were used in this study. For viable cell localization, fluorescence microscopy using CFSE (metabolically active cytoplasm) and Hoechst 33342 (nucleus) were used. Homogenates of all the products were used to quantify hyaluronic acid, prostaglandin E2 (PGE2) and human hemoglobin using enzyme-linked immunosorbent assay (ELISA). A panel of 440 cytokines were quantified using Quantibody® Human Cytokine Antibody Array 440 (RayBiotech) in homogenates of the products.

**RESULTS**

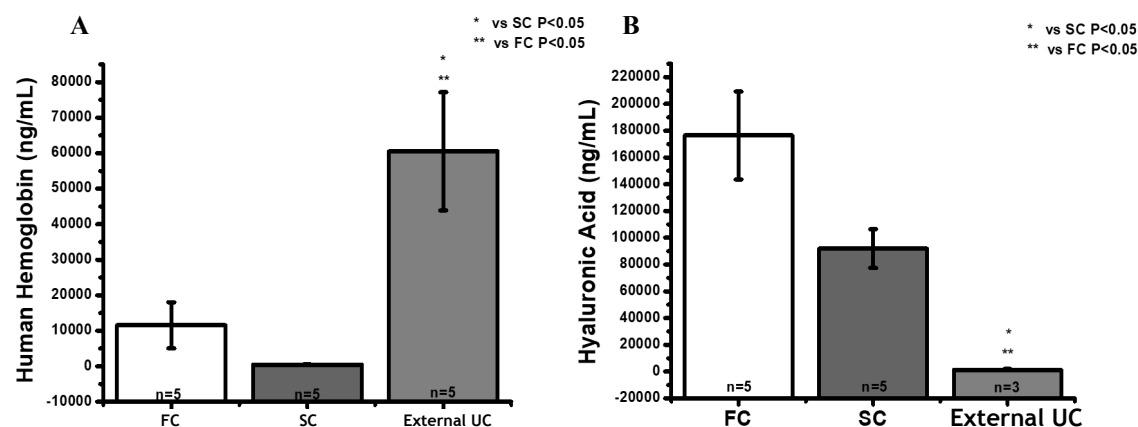
CSFE and DAPI staining showed a high amount of viable cells within Signature Biologics' products compared to the external UC product. PGE2 abundance in Signature Biologics' products were significantly different to external UC but not to fresh tissue. We found significantly higher levels of human hemoglobin in external UC products compared to Signature Biologics' products and fresh umbilical cord. Significantly low levels of hyaluronic acid were found in external UC product compared to Signature Biologics and fresh tissue. 440 Quantibody Analysis showed a high presence of angiogenic, tissue regeneration, and anti-inflammatory factors in Signature Biologics' products compared to external UC product.

**Figure 1 - Characteristics Post Thaw****Figure 2 - Assessment of Cryopreserved Tissue Cellular Viability Post Thaw**

**Figure 2. (Left to Right):** Phase Contrast images of thawed products, Scales Bar at 1000 µm and 100 µm. Cytoplasm stained by CFSE. Cell nucleus stained by DAPI. Merged CSFE and DAPI images to show intact cytoplasm containing cell nucleus.

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**Figure 3 - Molecular Components of Placental-derived Products**



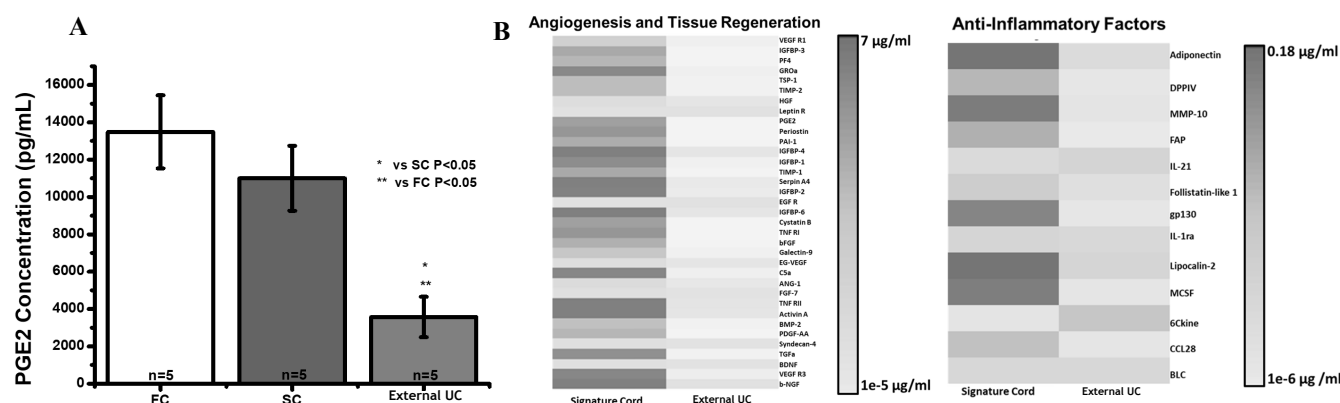
**Figure 3A:** Quantitative results for the presence of hemoglobin in Fresh Umbilical Cord (FC), SC product and External UC product.

Observation(s): Presence of blood in External UC sample, notably higher than fresh umbilical cord tissue (FC) control.

**Figure 3B:** Hyaluronic acid (HA) quantification in FC, SC and External UC tissues. HA is non-sulphated GAG [2-4]. HA provides viscosity through its water content, even at low concentrations [5-6].

Observation(s): External UC product hyaluronic acid levels were statistically different from the FC tissue.

**Figure 4 - Characterization of Molecular Components of Placental-derived Products**



**Figure 4A:** Quantitative results for the presence of PGE2 in Fresh Umbilical Cord (FC), SC product and External UC product.

Observation(s): External UC product PGE2 concentrations were statistically different from the FC tissue.

**Figure 4B:** 440 Quantibody cytokine analysis of SC and External UC. Conducted by RayBiotech, Inc. (Peachtree Corners, GA). Selectively grouped into i) Angiogenesis and Tissue Regeneration, and ii) Anti-Inflammatory Factors.

Observation(s): Heat maps of the cytokine abundance show a strong representation of key cytokines from SC product..

## REFERENCES

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