In Vitro Analysis and Comparison of Dehydrated Human Amniotic Membrane: Dual vs Tri-layer Membrane Constructions

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Introduction

Human placenta is the biomaterial of choice for a wide array of regenerative applications. Placenta membranes have been used as wound coverings for chronic wounds. Dehydrated wound coverings have an extended shelf life as compared to fresh or frozen products.

The material properties of dehydrated membranes are important to understand for handling capabilities of these types of products. We captured the properties of two types of dehydrated wound coverings: Artacent Wound® (dual layer, dehydrated human amniotic membrane) and Artacent AC® (tri-layer, dehydrated human amniotic and chorionic membrane).

In the current study, the properties of these products were assessed with the following techniques:

- HISTOLOGY WAS DONE TO VISUALIZE THE EXTRACELLULAR MATRIX (ECM) NANO STRUCTURE AND THE EXTENT OF DE-CELLULARIZATION OF THE SCAFFOLDS.
- SURFACE TOPOGRAPHY WAS MEASURED TO UNDERSTAND SURFACE CHARACTERISTIC IN AN ATTEMPT TO UNCOVER THE PRESENTATION OF CELL BINDING DOMAINS AVAILABLE FOR CELL INFILTRATION, MIGRATION AND SURFACE WETTABILITY.
- SCANNING ELECTRON MICROSCOPY WAS PERFORMED TO VISUALIZE SCAFFOLD MORPHOLOGY AND QUANTIFY FIBRIL DIMENSIONS.
- NANOINDENTATION WAS PERFORMED TO EVALUATE THE LOCAL MICRO STRUCTURE BIOMECHANICAL PROPERTIES OF THE SCAFFOLD.

Methods

1 **HISTOLOGY**
A 20 x 20 mm section of each tissue was prepared and sent to Histowiz Inc. for histological analysis. The samples were paraffin embedded and stained with Hematoxylin and Eosin (H&E). Cell nuclei appear purple and extracellular matrix is stained pink. Slides were scanned and thickness measurements were taken utilizing ImageJ software.

2 **SURFACE TOPOGRAPHY**
The ContourX-100 Optical Profilometer was used to measure the surface roughness of both wound coverings. It provides a high-throughput, highly repeatable etch depth, film thickness, step-height, and surface roughness measurement of the surface of the covering along which cells adhere and infiltrate the material.

3 **SCANNING ELECTRON MICROSCOPY**
Artacent Wound (W) and Artacent AC (AC) were tested under dry conditions. Samples were sputter coated with platinum (Pt) to achieve a 20 nm thickness. Samples then were mounted on an SEM sample holder in two orientations: flat and at 45° using double-sided carbon tape.

4 **NANO BIOMECHANICAL PROPERTIES NANOINDENTATION**
Artacent Wound (W) and Artacent AC (AC) samples were used under dry conditions and samples were mounted to sample holder for nanoindentation measurement.

Results

1 **HISTOLOGY**

Figure 1: Artacent product histology. Tissues were fixed, dehydrated, and embedded in paraffin wax. Sections were cut using a microtome following with Hematoxylin & Eosin (H&E) staining. Histology image of (A) Amniotic Membrane (B) Artacent Wound and (C) Artacent AC. Figure 2: Measured thickness of Artacent AC & Artacent Wound shows average value of 70.7 µm & 36.9 µm, respectively.

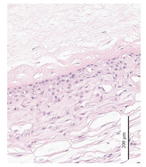
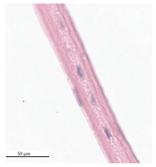
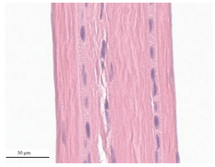


FIG1A. AMNIOTIC MEMBRANE



1B. ARTACENT WOUND



1C. ARTACENT AC

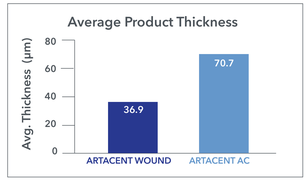


FIG. 2

2 **SURFACE TOPOGRAPHY**

Artacent AC provides a more textured surface when compared to Artacent Wound. Figure 3 and 4: Surface topography analysis of Artacent products. Roughness analysis of Artacent AC and Wound, shows Artacent AC has a more textured surface when compared to Artacent Wound.

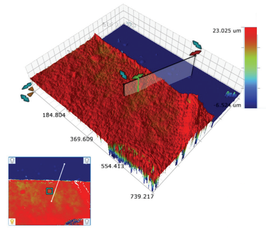


FIG. 3

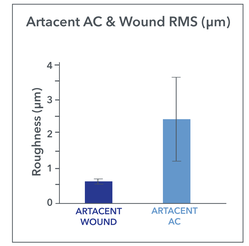


FIG. 4

3 **SCANNING ELECTRON MICROSCOPY (SEM)**

Figure 5: SEM images of Artacent AC and Artacent Wound product on (A&B) flat and (C&D) angled images. (A) SEM image of Artacent Wound with uniform human collagen fiber distribution with porosity between 50 nm-100 nm. (B) SEM image of Artacent AC showing human collagen fibers. The enlarged image shows fibrillated collagen fibers with 45.6 nm fibers. (C) The SEM image of Artacent Wound showing a thickness of 13.8 µm. (D) The SEM image of Artacent AC shows the three membrane layers in the product.

ARTACENT WOUND

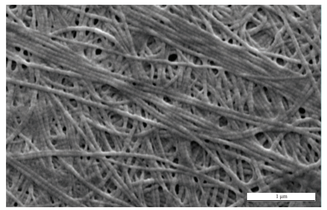


FIG. 5A.

ARTACENT AC

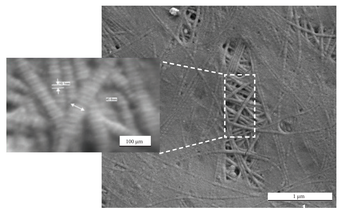


FIG. 5B.

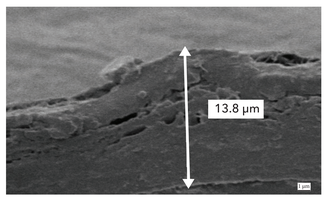


FIG. 5C.

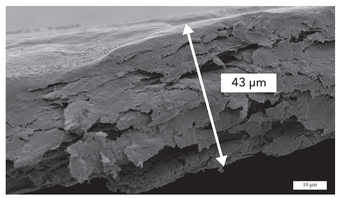


FIG. 5D.

4 **NANO BIOMECHANICAL PROPERTIES NANOINDENTATION**

Figure 6: Compression Young's Modulus of Artacent AC and Artacent Wound shows nano compression Young's Modulus of 407.2 MPa and 651.8 MPa, respectively, (P<0.0001).

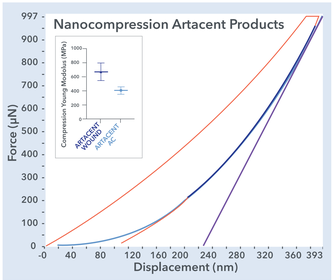


FIG. 6

Discussion

Wound covering design and processing impacts the material properties of the final product. Differences in the material properties of a bi-layered amniotic membrane and a tri-layered amnion-chorion-amnion membrane were measured.

Histologically, the dual layers of Artacent Wound and three layers of Artacent AC can be clearly identified. Artacent AC is twice as thick as Artacent Wound. Comparing surface topography of the two, Artacent AC is rougher when compared to Artacent Wound. Collagen fibers are visible on the surface of Artacent Wound in the SEM micrographs. Artacent AC micrographs illustrate a surface with regions of fibrillar and non-fibrillar extracellular matrix. Finally, nanoindentation analysis revealed a significant difference in the compressive Young's Modulus of the two products, which could translate into a difference in cellular attachment and migration.[1]

An understanding of the material properties of wound coverings plays an important role in the selection of an optimal wound care product. Both clinician preference and efficacy are important considerations in wound covering design.

Conclusions

- ARTACENT AC IS 2X THICKER THAN ARTACENT WOUND.
- ARTACENT AC IS 1.5 TIMES MORE COMPRESSIBLE WHEN COMPARED TO ARTACENT WOUND IN THE NANOSCALE.
- ARTACENT PROCESSING, AKA ARTACLEANSE®, IS GENTLE, AS EVIDENT FROM THE RETENTION OF PROTEIN MICRO AND MACRO STRUCTURES FROM HISTOLOGY AND SEM IMAGES, YET STRONG ENOUGH TO REMOVE CELL MEMBRANE AND OTHER POTENTIALLY INFLAMMATORY CUES.
- THE DIFFERENCE IN TEXTURE BETWEEN ARTACENT AC AND ARTACENT WOUND CAN IMPACT THE INFILTRATION OF CELLS, WETTABILITY OF THE SCAFFOLD, AND MIGRATION OF CELLS IN THE SCAFFOLD. ALL THESE VARIABLES PLAY A ROLE IN THE REGENERATION OF WOUNDS (1,2).
- THE ARTACEANSE PROCESS RESULTS IN A WOUND COVERING WITH FIBRILLATED COLLAGEN, SIMILAR TO THAT IN NATIVE TISSUE.

REFERENCES

1. Liu et al ACS Appl Mater Interfaces. 2019 Jul 3; 11 (26): 23558-23572.
2. Chung et al Biomaterials 2003, 24 (25), 4655-4661.

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