Evaluation of Dehydrated Human Umbilical Cord Biological Properties for Wound Care and Soft Tissue Healing

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Background

- Although these fetal membranes have been successfully used for the treatment of chronic wounds, the umbilical cord has garnered increasing attention as an allograft for soft tissue healing[1]
- The umbilical cord transports oxygen and nutrients from the placenta to the fetus and is composed of amnion epithelium, two arteries, one vein, and Wharton's jelly[2]
- Preliminary studies have shown that a cryopreserved umbilical cord product has been used as a graft to treat complex foot ulcers and can promote re-epithelialization in a murine corneal abrasion model[3]
- It has yet to be determined whether these properties and biological signals also exist in a dehydrated form of the umbilical cord matrix as well
- In this study, donated umbilical cords were processed using a proprietary gentle cleansing process[†], dehydrated under controlled conditions, and terminally sterilized

Purpose

The aim of this study was to characterize PURION® PLUS-processed dehydrated human umbilical cord* (dHUC) and identify its biological properties relevant to wound healing.

Methods

Extracellular Matrix and Regulatory Protein Composition

- Embedded dHUC was cryosectioned, stained for collagen type I, hyaluronic acid (HA) and DAPI
- HA, laminin, and fibronectin were quantified using single factor ELISA (Table 3)
- Soluble regulatory protein characterization was measured using a multiplex ELISA Quantibody® and performed by RayBiotech

References
1. Koob TJ, Lim JJ, Massee M, Zabek N, Denoziere G. Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration. Journal of Biomedical Materials Research Part B. 2014;102:1353-62.
2. Spurway J, Logan P, Pak S. The development, structure and blood flow within the umbilical cord with particular reference to the venous system. Australasian Journal of Ultrasound in Medicine. 2012;15:97-102.
3. Caputo WJ, Vaquero C, Monterosa A, Monterosa P, Johnson E, Beggs D, et al. A retrospective study of cryopreserved umbilical cord as an adjunctive therapy to promote the healing of chronic, complex foot ulcers with underlying osteomyelitis. Wound Repair Regen. 2016;24:885-93.

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*§ Both authors contributed equally to this work; † PURION® PLUS process; * EpiCord® umbilical cord allograft; EpiCord® and PURION® PLUS are registered trademarks of MiMedx Group, Inc., Marietta, GA.*

Methods

dHUC Extracts

- dHUC was minced, and allowed to extract overnight at 4°C into basal media
- Tissue residue was removed by centrifugation, and extracts were sterile filtered

Migration

- Human dermal fibroblasts (HDFs) were plated at 10,000 cells/well in a 96-well plate and allowed to adhere overnight
- A cell-free lane was created in each well using a WoundMaker tool (Essen)
- Cells were treated with positive and negative control media, or dHUC extract at 20, 10, 5, or 1 mg/mL (Table 1)
- Percent wound confluence was determined using an image processing algorithm to differentiate HDF cells and cellular debris from background to avoid the influence of user bias during image analysis

Table 1. Media formulations

	Migration	Vessel Formation
Positive Control	Cell specific media WITH serum supplement	Cell specific media + 20ng/mL vascular endothelial growth factor (VEGF)
Negative Control	Cell specific media WITHOUT serum supplement	Cell specific media + 20ng/mL VEGF + 1000µM suramin Cell specific media only (Vehicle Control)
dHUC Extract	dHUC extracted overnight into basal media (without serum)	dHUC extracted overnight into basal media (without serum)

Endothelial Cell Vessel Formation

- Adipose-derived stem cells (ADSCs) and human endothelial colony forming cells (ECFCs) provided by the Angiogenesis StemKit (Essen) were co-cultured in a 96-well plate
- Cells were treated with positive or negative control media, or dHUC extracts at 10, 5 or 1mg/mL (Table 1)
- Tube formation, measured as network area (mm^2/mm^2) and network length (mm/mm^2), by the ECFCs was quantified using the Angiogenesis module (Essen)

In vivo Biocompatibility and Bioresorption

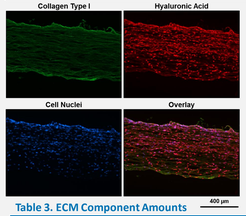
- 5mm x 5mm dHUC grafts were implanted subcutaneously in 22 normal Sprague-Dawley rats (T3 Laboratories, Atlanta, GA)
- Tissue samples were harvested en bloc from the implant site 24 hours, 7, 14, 22, 42, and 97 days after implantation
- Tissues were fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) and Movat's Pentachrome Stain (Alizee Pathology, Thurmont, MD)
- Images were scored for inflammation and residual material by an independent, board certified histopathologist (Table 2)

Table 2. Histological Scoring

Score	Inflammation	Residual material
0	none	None present.
1	Rare, 1-5/hpf.	Extensive bioresorption with less than one third of original amount of test or control material remaining.
2	Mild, 5-10/hpf.	Moderate bioresorption with between one-third and two-thirds of original amount of test or control material remaining.
3	Moderate, heavy infiltrate.	Mild bioresorption with approximately two-thirds of original amount of test or control material remaining.
4	Severe, packed.	Little to no bioresorption of residual test or control material.

Results

dHUC Contains ECM Components and Regulatory Proteins



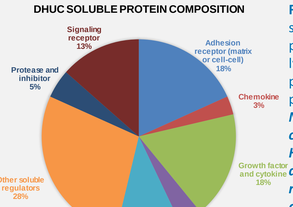
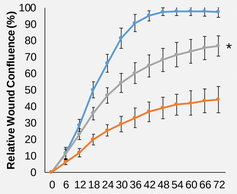


Table 3. ECM Component Amounts

ECM Component	Amount in dHUC
Fibronectin	444.6 ± 176.8 ng/mg
Laminin	183.0 ± 48.3 pg/mg
Hyaluronic Acid	676.5 ± 54.9 µg/mg

Figure 1. Immunofluoresence staining of dHUC grafts (left), protein composition of dHUC lysates reported as percent protein concentration of total protein amount detected (right). ***Native umbilical cord ECM components, such as collagen I, HA, fibronectin and laminin were detected in dHUC tissue. 241 regulatory proteins were detected, with many having functions that play a role in tissue remodeling and homeostasis.***

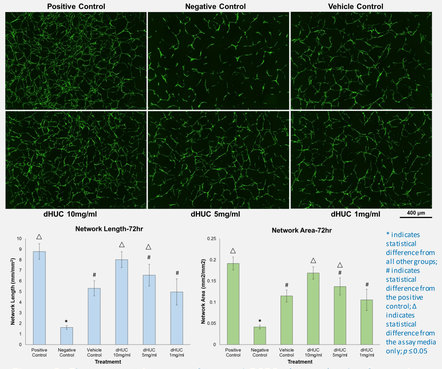
dHUC Stimulates Human Dermal Fibroblast Migration



* indicates a statistically significant difference from positive and negative controls at 72 hours, $p \leq 0.05$.

Figure 2. Migration of HDFs over 72 hours in response to 20mg/mL dHUC treatment. ***dHUC treatment at 20mg/mL promotes HDF migration greater than the negative control.***

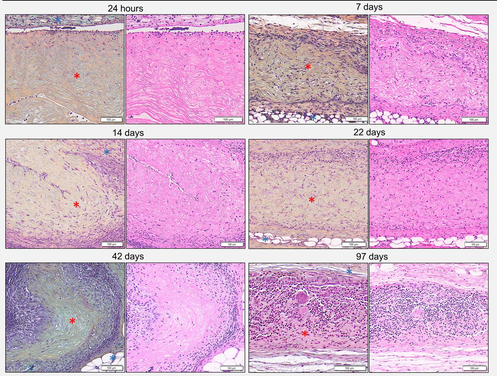
dHUC Promotes Vessel Formation



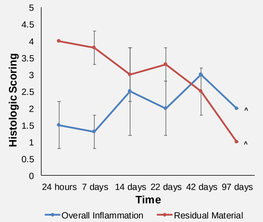
* indicates statistical difference from all other groups; # indicates statistical difference from the positive control; Δ indicates statistical difference from the assay media only; $p \leq 0.05$

Figure 3. Fluorescent images of tagged ECFCs (green) tube formation after 72 hours (top), angiogenic response measured by average network length and network area in response to dHUC extracts (bottom). ***dHUC treatment promotes an angiogenic response in ECFCs in a dose dependent manner.***

dHUC is Resorbed Without Fibrous Encapsulation



* denotes implant; * denotes native tissue



^ indicates scores that are only based on one sample because material could not be located in other animals.

Figure 4. Movat's Pentachrome and H&E staining of dHUC allografts in a rat subcutaneous implant model at 24 hours, 7 days, 14 days, 22 days, 42 days, and 97 days (left), histological scoring for overall inflammation and residual implant material over 97 days (right).

dHUC tissue does not elicit an adverse effect and is actively resorbed when implanted. Moderate inflammation was observed but declined after 97 days as the implant was degraded, as seen by the lack of detection in three animals at that timepoint.

Conclusions

dHUC is a promising therapy for the treatment of acute and chronic wounds due to its vast regulatory protein content, ECM components and ability to promote cell responses such as proliferation, migration and angiogenesis. Additionally, dHUC does not elicit an adverse effect and is actively resorbed when implanted in a rat model. These findings demonstrate that dHUC has biological properties that can promote cell activity involved not only in wound closure, but also other general healing responses necessary for soft tissue regeneration.