Matrix Composition of a Decellularized Human Placental Extracellular Matrix Particulate

Matthew Giedd, Naretsi Rosas, Lisa Godwin, Sarah Moreno, Michelle Massee, Heather Bara PhD, Thomas J Koob PhD, and John Harper PhD

SAWC Fall, October 2022

INTRODUCTION

Despite broadening usage of human placental tissues in the management of chronic and acute wounds, the placental disc remains an underutilized material. The placental disc is a vascular tissue rich in extracellular matrix components, but transplantation is complicated by the presence of maternal cells. The risk of adverse immunological responses to maternal elements necessitates complete decellularization prior to use. A proprietary processing technique has been developed to decellularize the placental disc yet maintain the native components of the matrix, yielding a product amenable to cellular infiltration. This study characterizes the impact of processing on the makeup and quality of a placental extracellular matrix (PECM*) particulate.

MATERIALS AND METHODS

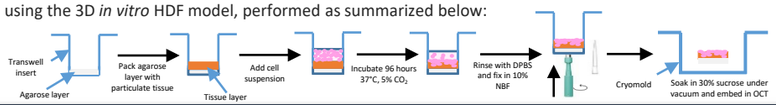
Histology: Decellularization was visualized with hematoxylin & eosin (H&E) staining of 5 µm sections of paraffin-embedded samples. Immunohistochemistry was performed by Premier Laboratories with antibodies against human type I collagen (Abcam, ab138492) and human type IV collagen (Dako, M0785). Images were acquired using a Leica DMB6 Microscope.

Hydroxyproline Assay: Total collagen was quantified pre- and post- decellularization using the QuickZyme Total Collagen Assay. Pre-decellularization samples were collected after initial rinsing of the disc to remove gross blood content. Post-decellularization samples were collected after completion of all rinsing steps.

Differential Scanning Calorimetry (DSC): The molecular integrity of placental disc collagen was assessed by DSC in samples taken both pre- and post-decellularization. Samples were heated in hermetic aluminum pans at 10°C/min from 15 - 85°C using a TA Instruments Discovery DSC 25.

Proteomics (HPLC MS/MS): High pressure liquid chromatography and tandem mass spectrometry assessed the ECM protein composition of PECM (Creative Proteomics). Raw data was analyzed using Label Free Quantification in MaxQuant. Processing of the analyzed data files was performed using Perseus. Identifications from <1 unique peptide and with MaxQuant scores <10 were filtered out. Identification of ECM proteins was performed by searching the Human Matrisome Project database (matrisomeproject.mit.edu).

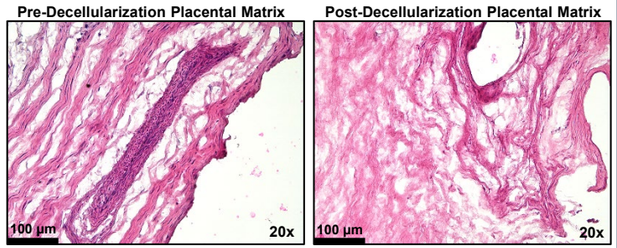
3D *in vitro* Human Dermal Fibroblast (HDF) Model: Cellular interaction with PECM was demonstrated using the 3D *in vitro* HDF model, performed as summarized below:



RESULTS

Effective decellularization of the placental matrix

Figure 1. Decellularization of placental matrix visualized by H&E staining. Placental disc before decellularization (left), and after (right). Placental cells are shown by dark purple staining and placental tissue by pink staining. 20x; scale = 100 µm.



RESULTS

Decellularized placental matrix is rich in collagen

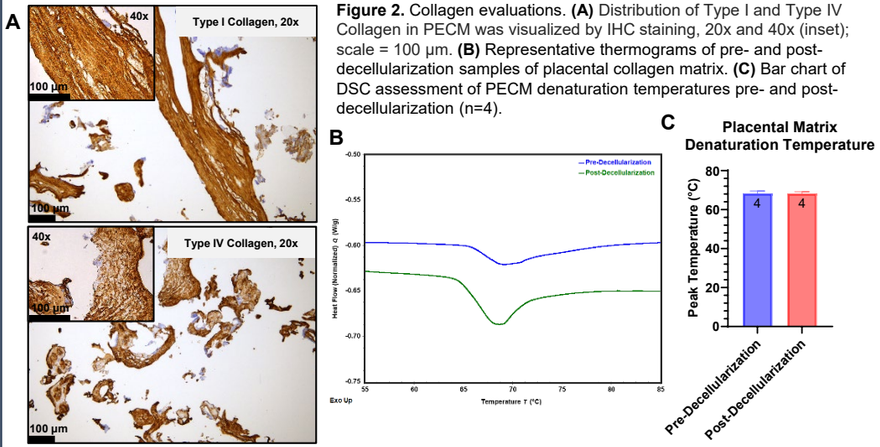
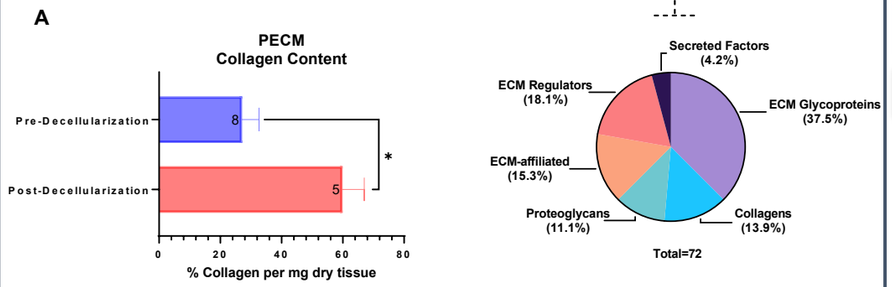


Figure 2. Collagen evaluations. **(A)** Distribution of Type I and Type IV Collagen in PECM was visualized by IHC staining, 20x and 40x (inset); scale = 100 µm. **(B)** Representative thermograms of pre- and post-decellularization samples of placental collagen matrix. **(C)** Bar chart of DSC assessment of PECM denaturation temperatures pre- and post-decellularization (n=4).

Collagen-rich PECM also contains a large array of additional extracellular matrix proteins

Figure 3. **(A)** Total collagen content evaluated pre-decellularization (n=8) and post-decellularization (n=5). * indicates p<0.0005. **(B)** Proteomic analysis by HPLC-MS/MS identified 1310 unique proteins present in PECM, 72 (5.5% of identified proteins) of which were further classified as extracellular matrix & matrix-associated proteins (left). ECM proteins were then further categorized (right). All percentages shown are of the total positive identifications across three PECM samples, not relative protein abundance.



RESULTS

Human dermal fibroblasts readily infiltrate into the decellularized PECM particulate

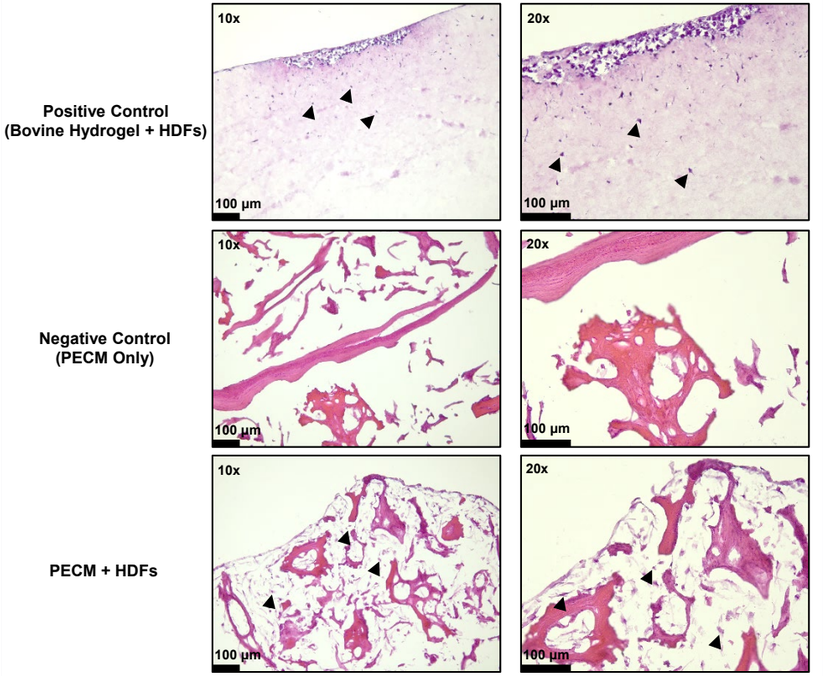


Figure 5. *In vitro* cellular infiltration into PECM. Positive control/PC, HDFs cultured on PureCol EZ gel (top row). Negative control/NC, PECM particulate only (middle row). HDFs cultured on PECM particulate (bottom row). Infiltrating cells are indicated by black arrows. Images on the left are 10x, and images on the right are 20x; scale = 100 µm.

CONCLUSION

PECM has been optimized to effectively decellularize the tissue while retaining elements of the functional placental extracellular matrix. Characterization reveals not only abundant collagen, distributed throughout the particulate matrix, but also a diverse array of proteins, including 72 proteins identified as components of the matrisome. Cellular compatibility of PECM is further demonstrated through the cellular infiltration of the product in an *in vitro* model. This initial characterization of the decellularized disc matrix, PECM particulate, supports its continued development for a variety of wound management applications.

*AXIOFILL ECM Particulate, MIMEDX Group Inc. Marietta, GA

All authors are employees of MIMEDX Group, Inc.