Human Placental Extracellular Matrix Particulate Supports Fibroblast Cellular Activities: Therapeutic Potential for Wound Applications

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INTRODUCTION

Complex wounds arise from cellular insufficiencies that prevent progression through the healing cascade and often require a multifaceted treatment approach, including advanced wound care products.[1] Collagen dressings are intended to facilitate cellular integration by providing a substrate for ingrowth and remodeling.[2,3] However, most dressings are composed solely of purified collagen. Whereas, Placental Extracellular Matrix (PECM*) maintains the natural structure and composition which contains several key collagen types and other native ECM components. PECM particulate is a novel allograft, derived from PURION processed human placental tissue. This study sought to evaluate the effect of PECM on fibroblast activity both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Immunohistochemistry: Immunohistochemistry was performed on terminally sterilized PECM with antibodies against type I collagen, type IV collagen, laminin, elastin, and fibronectin (Premier Laboratories). Images were acquired using Leica Microscope and 10X objective.

Collagen Assessment: Total collagen of terminally sterilized PECM was quantified using the QuickZyme Total Collagen Assay.

Proteomics Characterization: High pressure liquid chromatography and tandem mass spectrometry assessed the extracellular matrix protein composition of terminally sterilized PECM (Creative Proteomics). Identified proteins were annotated to further identify which are known constituents of the human matrisome (matrisomeproject.mit.edu).

***In vitro* activity:** The following groups were generated for *in vitro* testing, PECM extract (PECM-EX), conditioned media (CM) from PCM-treated human dermal fibroblasts (HDF) (PECM/HDF-CM) and HDFs only (HDF-CM). PECM was rehydrated at a ratio of 10 mg/mL in Dulbecco's Modified Eagle Medium (DMEM) containing 0.5% Fetal Bovine Serum (FBS) for 37°C for 72 hours (PECM-EX). CM samples were created by culturing HDFs in the presence of PECM (PECM/HDF-CM) for 48 hours at 37°C. CM from HDFs cultured with out PECM (HDF-CM) were used as a control. A scratch wound assay was used to determine HDF cell migration by live cell imaging for 120 hours with automated image processing to determine % Wound Confluence at each time point (S3 IncuCyte, Sartorius). Cellular attachment and proliferation were assessed by coating non-tissue culture plates with extract or CM followed by UV crosslinking and Calcein AM staining. HDF attachment was determined after 24 hours and proliferation after 4 days. Fibroblast-derived regulatory factors were measured by multiplex ELISA assay.

***In vivo* mouse model:** Female and male NU/J athymic nude mice were implanted with 50 mg PECM into a 1 cm x 1 cm surgical pocket. Mice were euthanized at 1, 2, and 4 weeks post implantation. Samples were fixed in 10% neutral buffered formalin for at least 12-24 hours, then transferred into 70% ethanol. Samples were paraffin-embedded and sections stained for Hematoxylin and Eosin (H&E). H&E slides were reviewed and scored by an independent histopathologist at StageBio.

Immunofluorescence: Immunofluorescence was performed on formalin-fixed paraffin-embedded tissue sections. Briefly, sections were deparaffinized, subjected to antigen retrieval followed by blocking in Serum-Free Protein Block (Agilent Dako) for 1 hour at room temperature. Incubation with primary antibodies was carried out overnight at 4°C followed by incubation with fluorescently labeled secondary antibodies. Images were acquired on a Leica microscope fitted with 40X objective using Leica Application Suite Advance Fluorescence software and the THUNDER Imager (Leica Microsystems).

RESULTS

PECM composed of abundant human collagen and a large array of other extracellular matrix and ECM-associated proteins

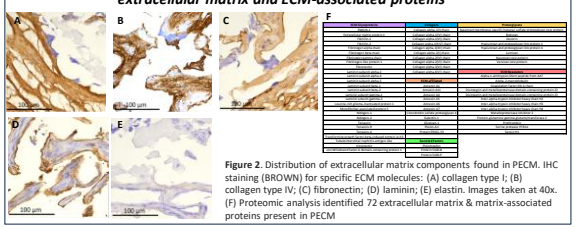

Figure 2. Distribution of extracellular matrix components found in PECM. IHC staining (BROWN) for specific ECM molecules: (A) collagen type I; (B) collagen type IV; (C) fibronectin; (D) laminin; (E) elastin. Images taken at 40x. (F) Proteomic analysis identified 72 extracellular matrix & matrix-associated proteins present in PECM

RESULTS

PECM promotes cellular activity in vitro

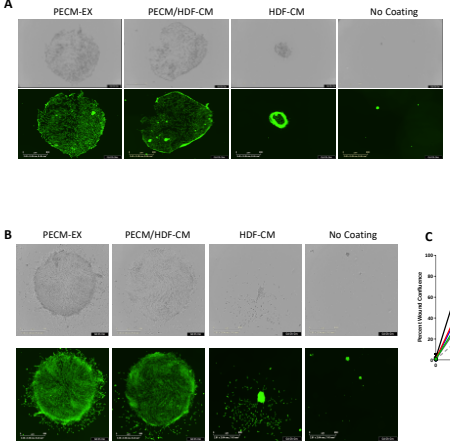

Figure 2. PECM effect on in vitro bioactivity. Effect of PECM-EX, PECM/HDF-CM or HDF-CM coating of low attachment plates on HDF attachment after 24 hours (A) and proliferation after 4 days (B). Cell viability was determined with Calcein AM staining. (C) Percent Wound Confluence (% cell confluence within the initial scratch area) plotted for each treatment over 5 days.

PECM alters the cellular secretome

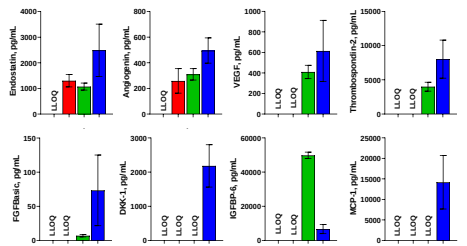

Figure 3. PECM alters the secretome of HDFs *in vitro*. Multiplex ELISA was used to detect HDF derived regulatory factors. LLOQ – lower limit of quantification

RESULTS

Host cells infiltrate particulate PECM implant in vivo, promoting collagen deposition and neovascularization

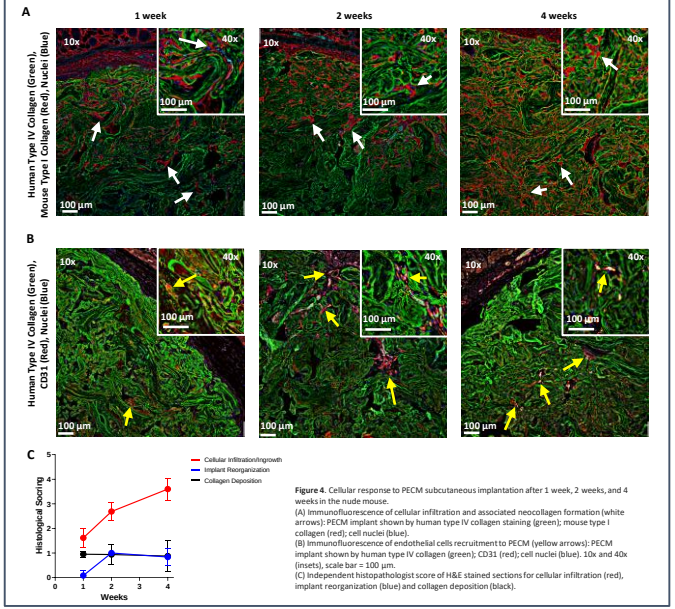

Figure 4. Cellular response to PECM subcutaneous implantation after 1 week, 2 weeks, and 4 weeks in the nude mouse.
(A) Immunofluorescence of cellular infiltration and associated neocollagen formation (white arrows): PECM implant shown by human type IV collagen staining (green); mouse type I collagen (red); cell nuclei (blue).
(B) Immunofluorescence of endothelial cells recruitment to PECM (yellow arrows): PECM implant shown by human type IV collagen (green); CD31 (red); cell nuclei (blue). 10x and 40x (insets), scale bar = 100 µm.
(C) Independent histopathologist score of H&E stained sections for cellular infiltration (red), implant reorganization (blue) and collagen deposition (black).

CONCLUSION

PECM is a PURION processed human placental tissue particulate intended for the replacement or supplementation of damaged or inadequate integumental tissue. *In vitro* data demonstrate that PECM promotes fibroblast activity. Furthermore, *in vivo*, the scaffold is permissive to infiltration by host cells, remodeling via deposition of neocollagen into implant voids, and endothelial cell recruitment, suggestive of neovascularization. These key features highlight the potential utility of PECM particulate to support the healing cascade and facilitate tissue repair in the management of large, complex wounds.

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