Characterization of a Placental Extracellular Matrix Particulate for Wound Management

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INTRODUCTION

Collagen-based grafts are widely used for the management of both chronic and acute wounds. The majority of these products are derived from xenogeneic sources; whereas, placental extracellular matrix (PECM*) is a particulate product composed of human placental disc tissue. The intended use for PECM is to replace or supplement damaged or inadequate integumental tissue; therefore, processing techniques were developed with the specific intent of preserving the inherent composition and properties of the extracellular matrix. This study characterized PECM to evaluate the structure, handling characteristics and extracellular matrix components. Key structural proteins were identified and handling properties were evaluated. Additionally, *ex vivo* and *in vivo* models were used to demonstrate PECM supported cellular infiltration.

MATERIALS AND METHODS

Immunohistochemistry: PECM was hydrated and paraffin embedded. Immunohistochemistry was performed with antibodies against type 1 collagen, type 4 collagen, laminin, elastin, and fibronectin (Premier Laboratories). Images were acquired using Leica Microscope and 10X objective.

Scanning Electron Microscopy (SEM): SEM was performed at Particle Technology Labs using a JEOL NeoScope II. PECM was prepared for surface imaging by mounting an aluminum stub using a carbon tab followed by gold sputter coating.

Handling Properties: PECM (250 mg) was hydrated with varying amounts of saline (0.5-1 mL, 1.5-2 mL, or 2.5-3 mL). Qualitative assessment of the consistency was evaluated as well as surface area coverage when spread to 1 mm thickness.

***Ex vivo* skin model**: Biopsy punches were used to created a defect in human skin *ex vivo*. Each defect (4mm in diameter) was filled with hydrated PECM. Filled skin biopsy punches were cultured at 37°C, 5% CO_2 for 60 days followed by fixation in 4% paraformaldehyde for 24 hours. Each cultured biopsy was bisected and paraffin embedded. H&E staining was performed on paraffin embedded sections.

***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. The implant sites were harvested *en bloc* with >10 mm tissue margins to include epidermis, dermis, muscle, and other surrounding soft tissues. Samples were fixed in 10% NBF for at least 12-24 hours, then transferred into 70% ethanol. Histopathology assessment was performed on H&E sections.

RESULTS

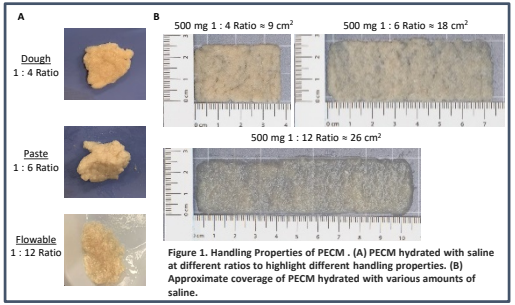

Figure 1. Handling Properties of PECM . (A) PECM hydrated with saline at different ratios to highlight different handling properties. (B) Approximate coverage of PECM hydrated with various amounts of saline.

RESULTS

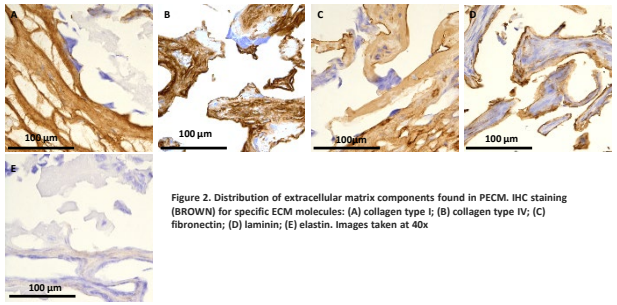

Figure 2. Distribution of extracellular matrix components found in PECM. IHC staining (BROWN) for specific ECM molecules: (A) collagen type I; (B) collagen type IV; (C) fibronectin; (D) laminin; (E) elastin. Images taken at 40x

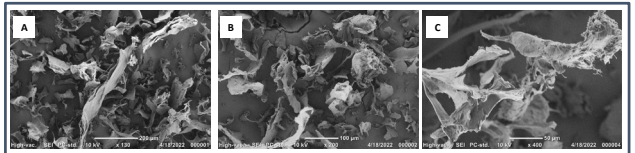

Figure 3. Scanning electron microscopy images of PECM. Visualization of single PECM particles at (A) 130x, (B) 200x, and (C) 400x magnification.

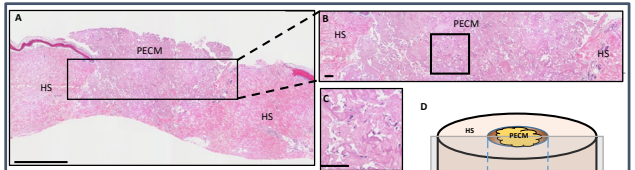

Figure 4. *Ex vivo* assessment of cellular infiltration. (A-C) Representative H&E images at increasing magnification demonstrating cellular infiltration into PECM from human skin (HS). Scale bar represents 100 µm. (D) Schematic demonstrating the HS biopsy defect was filled with hydrated PECM and allowed to culture for 60 days.

RESULTS

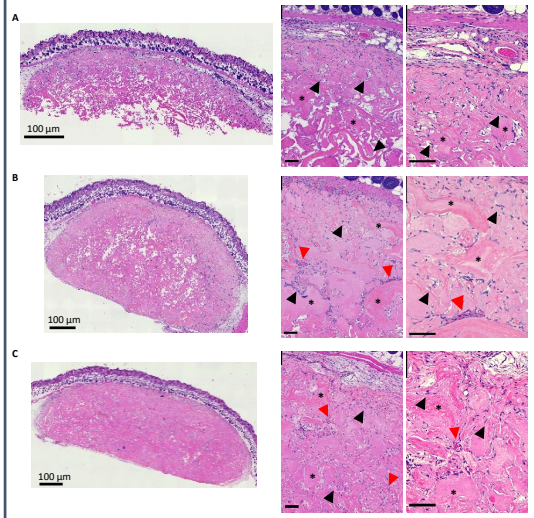

Figure 5. *In vivo* cellular response to PECM. The cellular response to PECM was assessed after (A) 1 week, (B) 2 weeks, and (C) 4 weeks of subcutaneous implantation in the nude mouse. PECM particles are visible at all time points (asterisks). Increased host cell infiltration observed over time (BLACK arrowheads) and associated with neocollagen formation and early neovascularization (RED arrowhead).

CONCLUSION

PECM is a human placental extracellular matrix particulate intended for the replacement or supplementation of damaged or inadequate integumental tissue. The structural matrix components of PECM provide a scaffold that restores site-appropriate and functional tissue by providing an environment conducive for cellular integration and remodeling. PECM is conducive for use in large wounds or irregular geometries. The dry particulate may be applied directly or, with the addition of saline, a paste can conform to the intricacies of the wound bed.

ACKNOWLEDGEMENTS

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

All authors are employees of MIMEDX Group, Inc.