PLACENTAL-DERIVED BIOMATERIALS – AN ANALYSIS OF NOVEL NON-DELAMINATED PLACENTAL MEMBRANE ALLOGRAFTS

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INTRODUCTION

Human placental membranes (PM) have been chosen as biological wound dressings for decades due to their unique structural and biochemical compositions. The interplay of the three distinctive layers of the PM, the amnion, intermediate layer (IL), and chorion, supports the PM's barrier features and helps maintain the biochemical environment to support a developing fetus.[1]

The IL, a critical portion of the PM, makes up approximately 40% of the native unprocessed membrane thickness.[2] This critical portion of the tissue houses many cytokines, growth factors, and other ECM components, such as hyaluronic acid, that may be important for wound healing. Historic processing techniques involve cleaning the PM tissue by separating the layers and removing the IL, yielding delaminated dual layer (DL) amnion/chorion allografts. Delamination of the layers risks the removal of native biochemical factors as well as key structural and functional proteins.[2]

A dehydrated complete placental membrane (dCHPM) allograft was developed using patented technology to retain key native PM structures and components for use as a wound covering or barrier membrane. Through this novel processing technique, dCHPM allografts are never delaminated and are preserved in both lyophilized and oven dehydrated formats,[3–5] providing flexibility in graft appearance and handling properties. This study investigates the structural compositions of commercially available lyophilized and oven dehydrated dCHPM allografts compared to a delaminated DL competitor allograft. The levels of key components were analyzed in dCHPM allografts, and the composition of lyophilized dCHPM allografts were compared to that of delaminated DL competitor allografts. This evaluation explores the favorable characteristics of non-delaminated full-thickness PM allografts for clinical care.

Figure 1. (A) Unprocessed human placental tissue (B) Histological section of native unprocessed PM stained with H&E, showing the amnion, intermediate layer, and chorion morphology.

Donated human PMs were recovered from full-term, healthy births under full consent of donors who underwent screening for infectious diseases. Tissues were dissected, cleaned and lyophilized or oven dehydrated, utilizing proprietary processes, to create dCHPM allografts for testing. Commercially available DL competitor allografts were obtained for comparison purposes. In all cases, a minimum of n=3 unique lots per test group was used.

LYOPHILIZED AND OVEN DEHYDRATED DCHPM

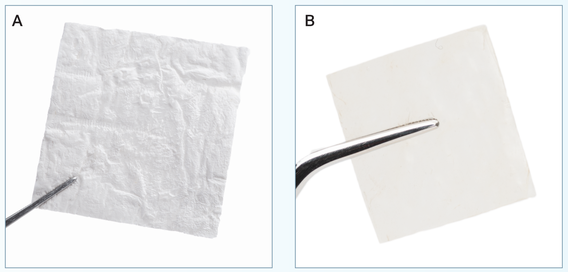

Figure 2. (A) Lyophilized and (B) oven dehydrated dCHPM allografts produced using patented technology that retains the natural structure of the native PM. Lyophilized dCHPM is opaque whereas oven dehydrated dCHPM is provided in a translucent format. While both dCHPM allografts retain the natural structure, they each have unique visual characteristics that have valuable indications for clinical applications.

STRUCTURE AND COMPOSITION OF dCHPM ALLOGRAFTS

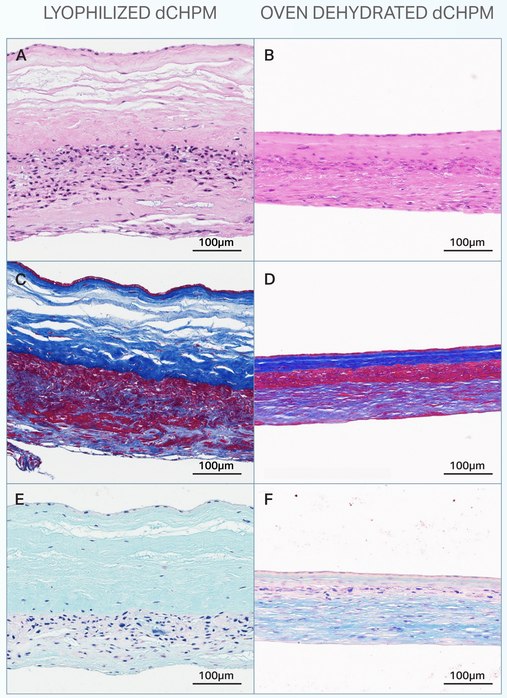

Figure 3 (Left). Extracellular matrix content of lyophilized and oven dehydrated dCHPM allografts. Histological cross-sections are shown demonstrating (A and B) cell nuclei and extracellular matrix content (H&E), (C and D) collagen (Masson's Trichrome), and (E and F) mucopolysaccharides (Alcian Blue).

Target Component	Lyophilized dCHPM	Oven Dehydrated dCHPM
TGFβ	+++	++++
PDGF-AA	+++++	++++
PDGF-BB	++++	+++
TGFα	++	+
bFGF	+++++	++++
EGF	+++	+++
IL-5	++	+
IL-10	+	+
VEGF	++	++
TIMP-1	+++++	+++++
TIMP-2	+++++	+++++
TIMP-4	++++	+++
Hyaluronic Acid	+++++	+++++

Figure 4. Lyophilized and oven dehydrated dCHPM allografts were analyzed for comparison of composition. Mean concentrations of each component were calculated (pg/cm^2), and concentration assignments were given. Results show that, on average, both dCHPM allografts have comparable target protein and HA concentration levels.

Concentration (pg/cm^2)	Concentration Assignments
<1	+
1<10	++
10<100	+++
100<9000	++++
>9000	+++++

EVALUATION OF dCHPM AND DL ALLOGRAFT COMPOSITION

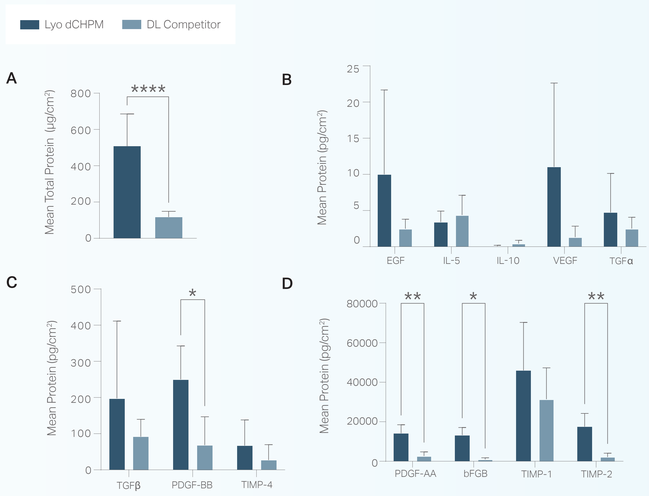

Figure 5. Lyophilized dCHPM and DL competitor allografts were tested for comparative analysis of (A) total protein and (B), (C), (D) target components including growth factors and enzyme inhibitors. Results demonstrate that, on average, lyophilized dCHPM tissue contains more total protein and specific target protein PDGF-AA, PDGF-BB, bFGF, and TIMP-2 than the DL competitor allograft. Significance defined as p<0.05 (*), p<0.01 (**), p<0.001 (***), p<0.0001 (****).

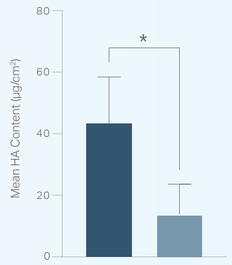

Figure 6. Lyophilized dCHPM and DL competitor allografts were tested for comparative analysis of hyaluronic acid content. Results demonstrate that, on average, lyophilized dCHPM contains significantly more HA than a delaminated DL competitor. Significance defined as p<0.05 (*).

THICKNESS COMPARISON OF dCHPM AND DL COMPETITOR ALLOGRAFTS

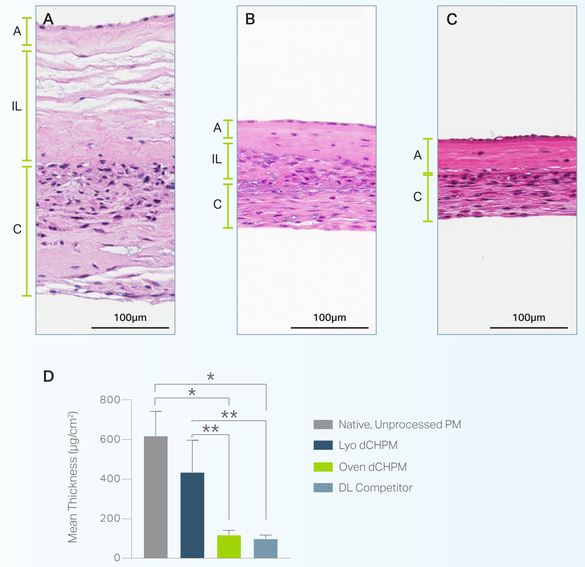

Figure 7. Histological sections of (A) lyophilized and (B) oven dehydrated dCHPM were compared to (C) a DL competitor for structural analysis and identification of membrane layers. The amnion, intermediate layer, and chorion are labelled as A, IL, and C, respectively, on the representative images of dCHPM allografts. The amnion and chorion are labelled as A and C on the DL competitor image. (D) An analysis of allograft thickness was also performed for both dCHPM allografts and compared to the DL competitor allograft; native unprocessed PM thickness is shown for thickness comparison purposes. Significance defined as p<0.05 (*), p<0.01 (**).

CONCLUSIONS

- Lyophilized and oven dehydrated dCHPM allografts demonstrated retention of collagens, glycosaminoglycans, and other structural components in all 3 layers (amnion, IL, and chorion).
- Lyophilized and oven dehydrated dCHPM allografts had comparable protein and HA concentrations; both contained target proteins that have clinical relevance in wound applications.[2]
- Lyophilized dCHPM allografts retain significantly more total protein, HA, and select cytokines, enzyme inhibitors, and growth factors than the DL competitor allograft.
- When compared to a delaminated DL competitor, lyophilized dCHPM and oven dehydrated dCHPM allografts were thicker. A clear separation of membrane layers and absence of the IL was shown in the DL allograft whereas the IL was distinguishable and visibly anchored to the amnion and chorion in the intact full-thickness dCHPM allografts. Additionally, on average, lyophilized dCHPM retained more than 70% of the native, unprocessed PM thickness.
- Further side-by-side comparisons of non-delaminated, intact lyophilized and oven dehydrated dCHPM allografts would provide insight into their unique potential for tissue engineering and clinical applications.

References: 1. Bryant-Greenwood, G. D. (1998). The extracellular matrix of the human fetal membranes: Structure and function. Placenta, 19(1), 1–11. https://doi.org/10.1016/s0143-4004(98)90092-3 2. Roy, A., & Griffiths, S. (2020). Intermediate layer contribution in placental membrane allografts. Journal of Tissue Engineering and Regenerative Medicine, 14(8), 1126–1135. https://doi.org/10.1002/term.3086 3. Daniel, J., Griffiths, S. & Berg, R. (2021) "Compositions derived from placenta and methods of producing the same." (U.S. Patent No. 11,116,871 B2). 4. Daniel, J., Griffiths, S. & Roy, Annelise. (2021) "Translucent, dehydrated placental tissue and methods of producing and using the same." (U.S. Patent No. 11,154,641 B2). 5. Daniel, J., Griffiths, S. & Berg, R. (2022) "Compositions derived from placenta and methods of producing the same." (U.S. Patent No. 11,413,372 B2). EDU23-003
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