Maintaining Tissue Characteristics of Lyophilized and Terminally Sterilized Full Thickness Amniotic Membrane

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INTRODUCTION

Placental tissues including amniotic membranes, are widely used in the treatment of diabetic foot ulcers, venous leg ulcers, and other chronic wounds. Based on scientific studies, their healing potential is related to the scaffold provided by the extracellular matrix and action of regulatory proteins on cells. Our study was aimed to maintain the innate structural and biological properties of native placental tissue by evaluating tissue function after processing.

METHODS

The full thickness amniotic membrane (FT-AM) was processed and preserved using proprietary lyophilization and sterilization methods. Hematoxylin-Eosin (H&E) staining was used to verify maintenance of the native structure. Tissue inhibitor of matrix metalloproteinases-1 and basic fibroblast growth factor were used as example regulatory proteins implicating tissue regeneration; both were measured by enzyme-linked immunosorbent assay. Cell proliferation and chemotaxis assays using human adult dermal fibroblast (HDFa), and an anti-inflammatory assay using THP-1 human monocytic cells were utilized to confirm the tissue's biological function after processing.

RESULTS

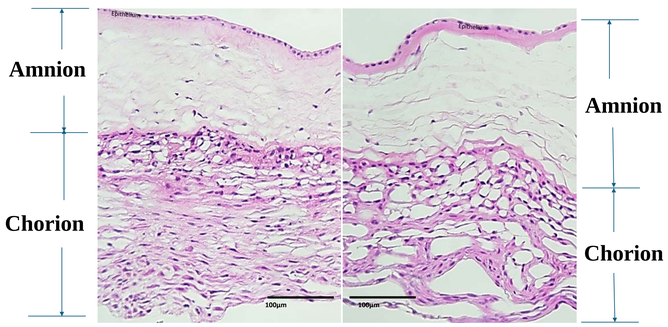

Figure 1. H&E histology of tissue prior to processing (left), and post processing and rehydration in saline (right, FT-AM). Histology shows preservation of the different layers of Amnion-Chorion.

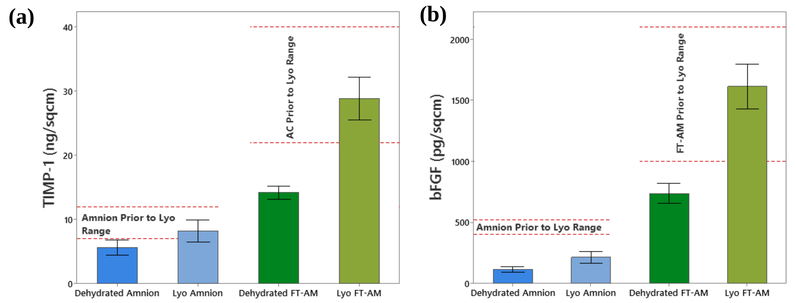

Figure 2. Regulatory protein levels for amnion alone and full thickness amnion-chorion (FT-AM) for LifeLink proprietary lyophilization/sterilization process compared to traditional dehydration / sterilization. Gamma sterilization was performed for all tissues. TIMP-1 (a) and βFGF (b) are example regulatory proteins implicated in wound healing and tissue regeneration measured with ELISA. Lyophilization is preferable to dehydration for regulatory protein preservation and amnion-chorion has significantly higher regulatory protein levels.

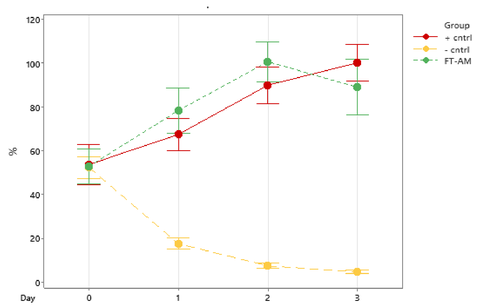

Figure 3. Cell proliferation assay using HDFa cells for FT-AM conditioned cell culture media without serum. Positive control (+ cntrl) was media including serum to promote cell proliferation. Negative control, (- cntrl) was media without serum. In vitro data show that FT-AM upregulates cell proliferation to levels similar to the positive control for cell proliferation.

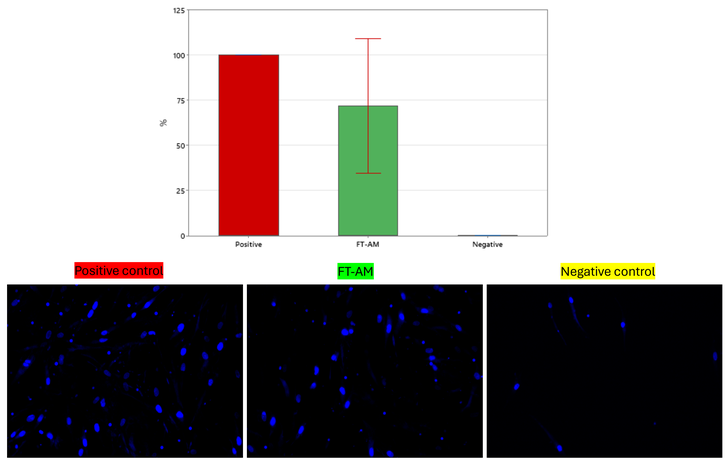

Figure 4. Chemotaxis assay using HDFa cells for FT-AM conditioned cell culture media without serum. Cells were seeded in the upper membrane, and test groups were added to the lower compartment. Positive control was media including serum, and negative control was serum-free cell culture media on the lower compartment. In-vitro data show that FT-AM has chemo-attractants to promote cell migration from serum-free media to FT-AM conditioned media to a level close to the positive control.

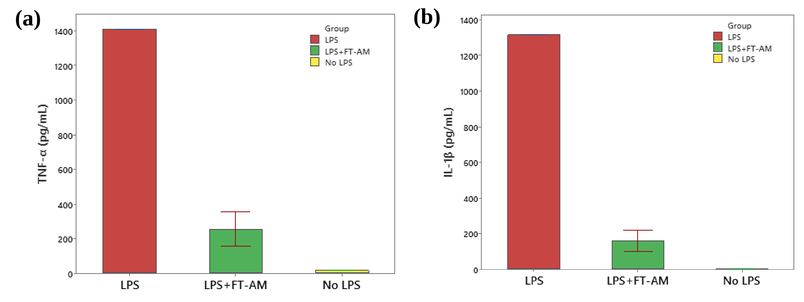

Figure 5. Anti-inflammatory assay *in-vitro* using THP-1 human monocytic cell line. TNF-α and IL-1 β are signaling molecules indicating an inflammatory response when secreted by THP-1 monocytes. Lipopolysaccharide (LPS) is a major component of gram-negative bacteria cell walls and can cause an acute inflammatory response. The level of TNF-α (a) and the IL-1β (b) in monocytes cultured with LPS (LPS), without LPS (No LPS) and with LPS and FT-AM conditioned media (LPS+FT-AM) are shown in Figure 5. FT-AM attenuates the inflammatory response *in vitro* as indicated by down regulation of TNF-a and IL-1β for cells cultured in the presence of the LPS inflammatory molecule with FT-AM conditioned media compared to LPS in media without the conditioning.

H&E results confirmed preservation of different layers of native membrane's structure. Lyophilization was preferable to the traditional dehydration methods for regulatory protein preservation. FT-AC maintained levels of both tested regulatory proteins, at similar levels to the native tissues. HDFa proliferation was increased when cells were exposed to the media conditioned with FT-AC. THP-1 macrophage inflammatory response in the presence of lipopolysaccharide (LPS) in media was attenuated when cells were exposed to LPS in the FT-AC conditioned media. Chemo-attractants in FT-AC promoted cell invasion and migration to FT-AC conditioned media from serum-free media.

DISCUSSION

FT-AC maintained physical structures, regulatory proteins, and biological properties that are known to aid external or internal tissue defects (e.g., wounds) by providing an extracellular matrix and promoting cell proliferation and migration while reducing inflammation. These outcomes indicate FT-AC can be used to enhance the healing cascade in external or internal tissue defects.

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