Standardized Quantification of Factors in Placental Membranes[4,5]

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Abstract

Although the favorable biological and mechanical properties of the amnion and chorion have been explored for regenerative medicine indications, published data from placental tissues in their final, useable form is lacking. During treatment with membrane product, the tissue is usually sterile, intact and laid on a wound or treatment area. The factors available to the treatment area from the product so applied need to be elucidated and presented in a relatable form. Current reporting for eluted growth factor results are not informative with respect to the factors available from the area of tissue, as applied, and may differ among techniques. We report here a method of quantifying these factors such that any membrane may be characterized, and data generated relates to the product in hand. Furthermore, use of this method facilitates evaluation of process improvements by providing a standardized methodology.

Introduction

A variety of membrane products have been developed for applications ranging from cosmetic to invasive surgery. Various preparations focus on exploiting the available growth factors and adding strength to AM and AC. These include lamination, cryopreservation, dehydration and freeze drying. Each processing and preservation method results in the optimization of different properties of the membrane.

Although there is a vast array of information collected on amnion and amnion/chorion membranes, the studies often utilize enzymatically digested, macerated and/or centrifuged extracts. Although, using the digested, pre-processed membrane to release all proteins gives us a clear look at the potential of the membrane, it does not allow us to determine the factors available to a patient. For treatment with membrane product, the tissue is usually sterile, intact and laid on a wound or treatment area. The factors present as available to the treatment area from product, as applied, should be the quantified and reported in a standardized manner.

To address this, we characterized dehydrated sterilized AM and AC from the perspective of factors eluted from a specific area (per square centimeter) of finished product, as would be eluted to patient tissue. This information adds to the ever-expanding understanding of why dehydrated placental membranes are efficacious and presents a quantitative aspect to reporting that is applicable to both use and comparison. Furthermore, this method of characterization may be applied to any membrane size or composition.

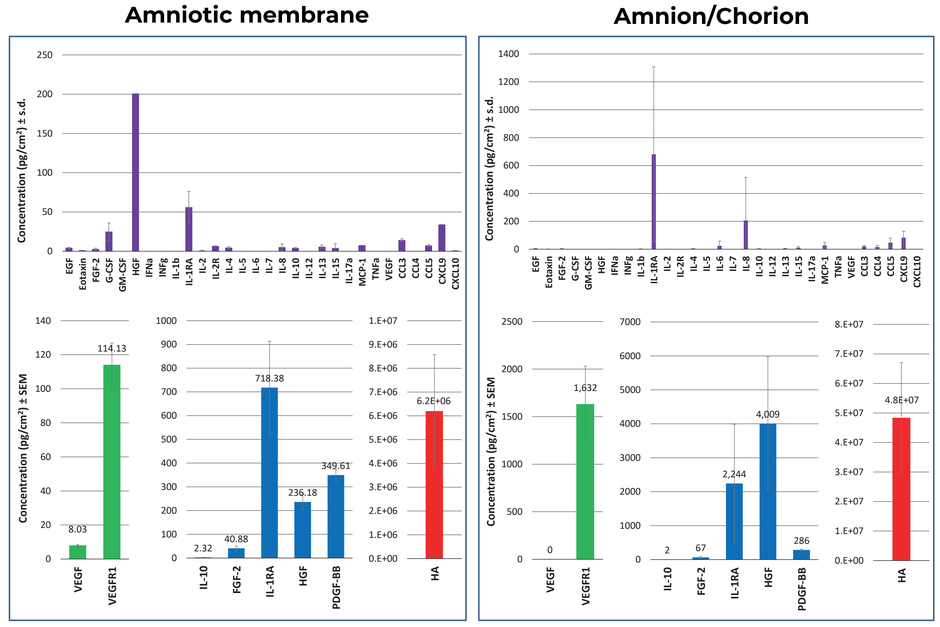
Methods

- Donated human placentas were acquired from accredited after planned cesarean sections with informed consent and processing completed in accordance with FDA Good Tissue Practices AATB standards.
- AM or AC was isolated from the placenta and BioRetain® processed[3].
- Processed AM (Vendaje®) or dehydrated AC (Vendaje AC®) were e-beam sterilized.
- **The final sterilized products were used for all tests.**
- 8 mm biopsy punches were placed in DPBS at 37^0 C.
- The supernatant was collected and stored at -80^0 C until use.
- A 30-plex cytokine assay was performed.
- A custom 5-plex panel was performed.
- Multiplex panels used 5-parameter logistic standard curves.
- ELISA kits were performed.
- ELISAs were calculated from a 4-parameter logistic standard curve.
- The average pg/ml of growth factor was multiplied by the milliliters of eluate.
- The 8mm punches have an area of 0.503 cm^2, hence, total GF content in the eluate was divided by 0.503 to reveal the pg of growth factor in 1 cm^2 of tissue.

$$\frac{GF\left(\frac{pg}{ml}\right)*ml}{0.503\ sqcm} = pg\ of\ growth\ factor\ per\ cm^2.$$

- This can be utilized for *any volume of eluate* used and *any sample punch size*.

Results



Analysis of membrane supernatants show that dehydrated, sterilized amnion and amnion/chorion elute factors that are conducive to wound healing, which are available to recipient tissues. Importantly, these measurable factors eluted from dehydrated, sterilized membranes can be reported as a function of available factors per square centimeter of tissue.

Discussion/Applications

We highlight the need for standardized data collection and reporting in the field of dry membrane allografts. The characterizations of **release of factors from** final membranes to recipient under physiologic conditions is critical for comparison of products across graft sizes and types, when optimizing processing methods, and for clinical studies. By reporting the data per cm^2, the user may apply the data to larger or smaller grafts and understand the eluate-able concentration available to the patient from the membrane that is in their possession.

It is not known how much of these factors are taken up by recipient tissues nor their activity at the wound bed, hence the functional effects must still be investigated.

[3]Placental Processing for Retention of Factors. Patent pending 63/391,032

[4]Collection and Quantification of Elements per SQ CM of Tissue. Patent pending 63/391,031

[5]Sabol TJ, Tran GS, Matuszewski J, Weston WW. Standardized reporting of amnion and amnion/chorion allograft data for wound care. Health Sci Rep. 2022;5(5):e794. PMID: 36032519.