Know What's in Your Membranes: Quantification of Factors in Placental Allografts[3]

Taylor J Sabol, MS[1,2], Jason V Matuszewski, BS[1], Wendy W Weston, PhD[1,2]

[1] BioStem Technologies, Inc., [2] CTBS

Abstract

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. While using the digested, pre-processed membrane to release all proteins may give 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 should be 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. Analysis of dehydrated, sterilized amnion and amnion/chorion revealed elution of factors that are conducive to wound healing.

Methods

A Standardized Process

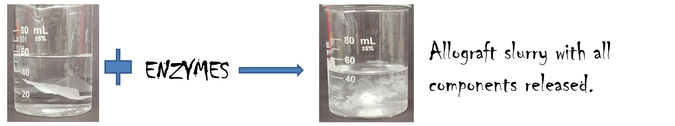
- Donated human placentas were acquired and processed in accordance with FDA Good Tissue Practices and AATB standards.
- AM or AC was isolated from the placenta and BioRETain® processed, resulting in sterile AM[i] or AC[ii].
- **The final sterilized products were used for all tests.**
- 8 mm biopsy punches were taken from tissue.
- Punches were placed in 500 uL DPBS$^{Ca\text{-}Mg\text{-}}$ at 37° C for 72 hours, rocking.
- The supernatant was collected, clarified by centrifugation and stored at -80° C until use.
- Custom 5-plex cytokine panels and ELISA assays were performed.
- Results were plotted using a 5-parameter logistic standard curve.
- The average pg/ml of growth factor was multiplied by the milliliters of eluate= total GF content in the eluate .
- The total GF content in the eluate was divided by 0.503 (8mm punches have an area of 0.503 cm^2) to reveal the pg of growth factor in 1 cm^2 of tissue.

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

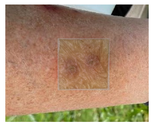
This can be utilized for *any tissue, volume of eluate* used and *any sample punch size*.

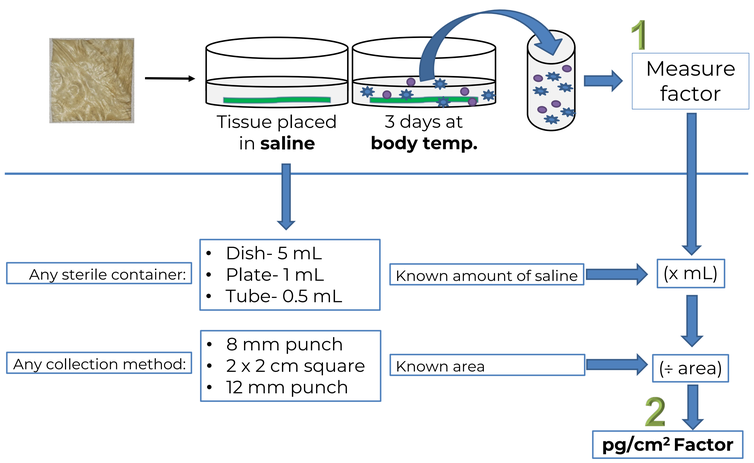
[i] Vendaje® [ii] Vendaje AC®

How most tissue is processed to get data?



Does this really represent what will happen on the patient?



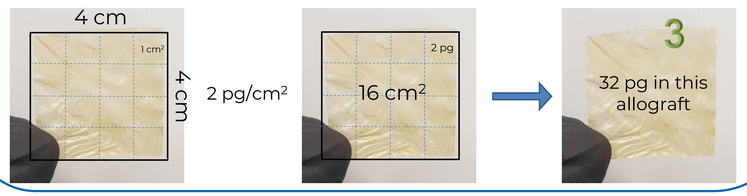


I have a 4 x 4 cm allograft:

How much "Factor" is in this?

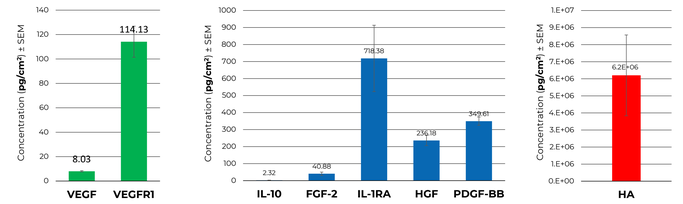
Reported in pg/**mg** ➡ Weigh it in the surgical room?
Reported in pg/**mL** ➡ Determine its volume?
Reported as pg/**cm²** ➡ Amount available in 1 cm² of intact membrane

For example: your assay and calculations give 2 pg/cm²

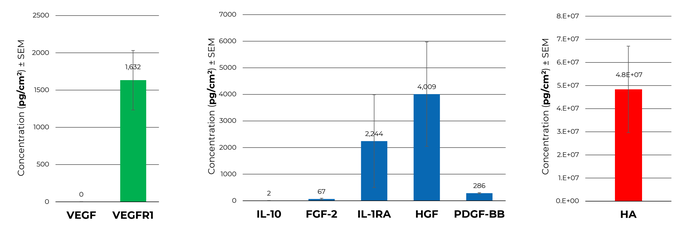


Results

Amniotic membrane



Amnion/Chorion



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 can be reported as a function of available per square centimeter of tissue.

Discussion

We highlight the need for standardized data collection and reporting in the field of dry membrane allografts. The quantification of factors released 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] Collection and Quantification of Elements per SQ CM of Tissue. Patent pending 63/391,031