The Lure of Placental Membranes: Chemotaxis and Haptotaxis

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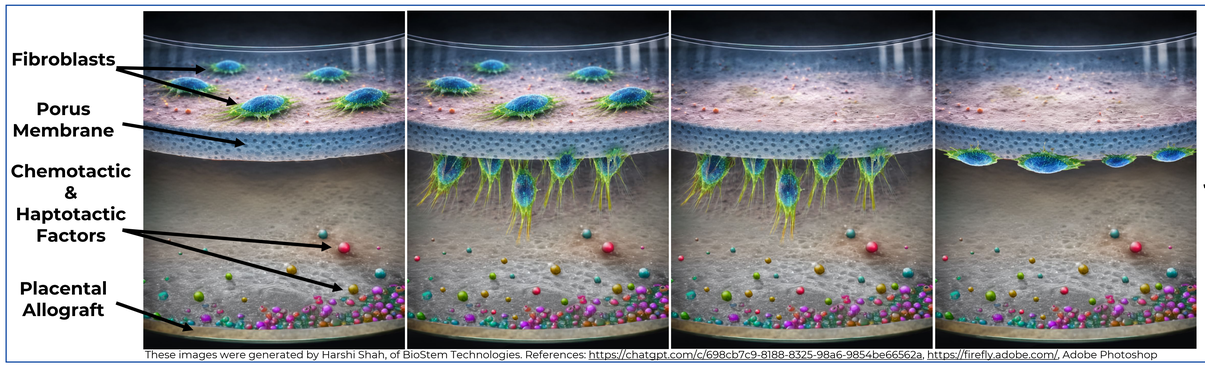
Abstract

Placental membranes are known to contain numerous structural proteins, growth factors and cytokines. However, the way these tissues are processed can affect the concentration of these beneficial factors. Some factors within placental membranes affect fibroblast migration. The Boyden Chamber assay is used to detect and measure the migration of cells due to a cytokine gradient, in this case, sourced from the extracellular matrix (ECM) of the membrane. Movement along these gradients are mechanisms called chemotaxis and haptotaxis. We hypothesize that a retention-focused tissue processing method (RE-AC/RE-AM) will promote local haptotaxis and chemotaxis of fibroblasts. We further quantify chemotactic and haptotactic factors released into the local environment from these tissues.

Methods

The Boyden Chamber assay, or cell migration assay, is conducted using chambers inserted into wells of a cell culture plate. The chamber has a polycarbonate membrane (8um pore size) that is suspended in the media. Amnion/chorion, amnion, or amnion/amnion are placed at the bottom of the well and covered with media. The polycarbonate membrane is seeded with fibroblasts and placed into the well. Migration of the fibroblasts through the membrane is measured. Separately, potential growth factors responsible for chemotaxis and haptotaxis are quantified by ELISA using eluate from tissue samples incubated for 72 hours.



These images were generated by Harshi Shah, of BioStem Technologies. References: https://chatgpt.com/c/698cb7c9-8188-8325-98a6-9854be66562a, https://firefly.adobe.com/, Adobe Photoshop

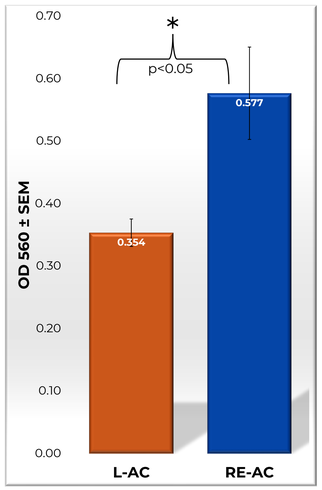
Local Chemotaxis
Fibroblasts are migrating along a gradient formed by factors within the cell culture media, which are concentrated near the placental allografts

Local Haptotaxis
Fibroblasts are migrating along a gradient formed by factors within the extracellular matrix of the placental allografts

Results

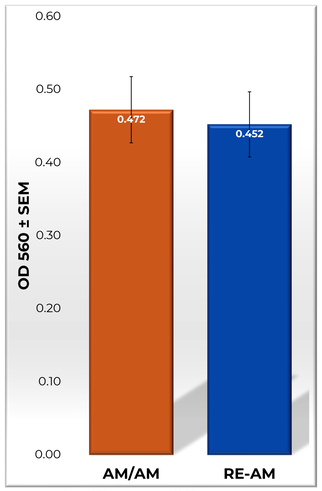
Migration

Amnion/Chorion



RE-AC showed significantly higher fibroblast migration compared to leading Amnion/Chorion on the market product (L-AC), n=5. *significance defined as p<0.05.

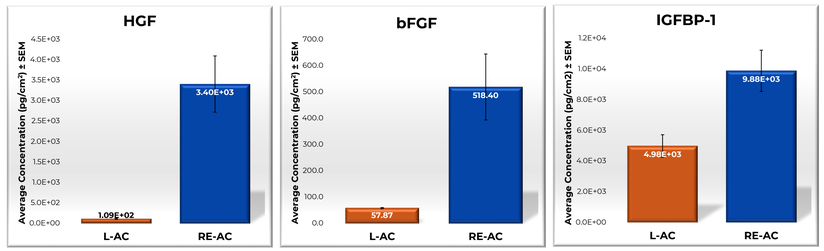
Amnion



No significant difference was found between **dual-layer** amnion (AM/AM) and **single-layer** RE-AM, n=5. This data suggests that **single-layer RE-AM** has **equivalent** fibroblast migration to a **dual-layer** product.

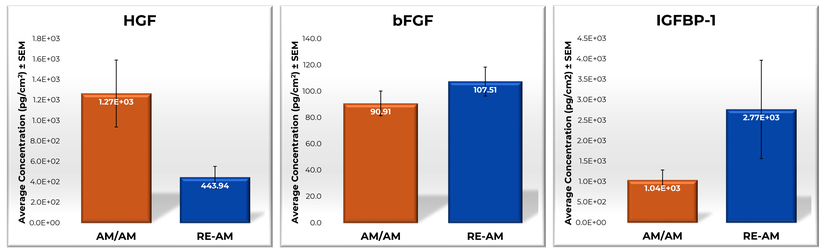
Chemotactic Factors

Amnion/Chorion



RE-AC showed higher concentrations of the chemotactic factors HGF, bFGF, and IGFBP-1, when compared to the leading Amnion/Chorion competitor (L-AC), n=5. This corresponds to the fibroblast migration data, supporting the correlation of higher factor concertation and higher fibroblast migration.

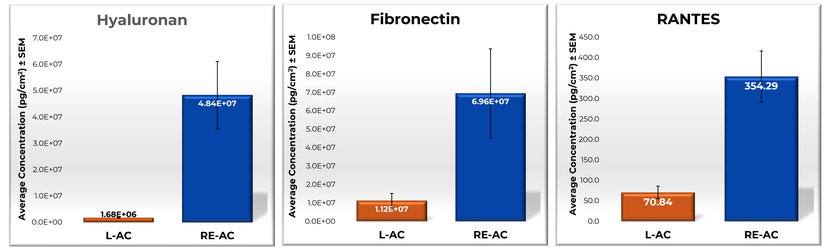
Amnion



Single layer RE-AM showed higher concentrations of the chemotactic factors bFGF and IGFBP-1, excluding HGF, when compared to the **dual layer** Amnion/Amnion competitor (AM/AM), n=5. The lower concentration of HGF may be attributed to the RE-AM having only one layer of amniotic membrane within the product as opposed to two. This corresponds to the fibroblast migration data, supporting the performance the **single layer** product in the migration assay.

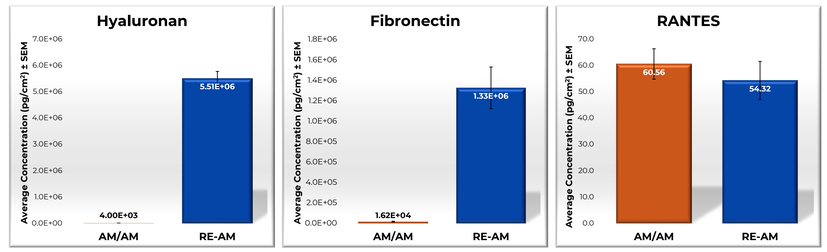
Haptotactic Factors

Amnion/Chorion



RE-AC showed higher concentrations of the haptotactic factors Hyaluronan, Fibronectin, and RANTES, when compared to the leading Amnion/Chorion competitor (L-AC), n=5. This corresponds to the fibroblast migration data, supporting the correlation of higher factor concertation and higher total migration.

Amnion



Single layer RE-AM showed higher concentrations of the haptotactic factors Hyaluronan and Fibronectin, excluding RANTES, when compared to the **dual layer** Amnion/Amnion competitor (AM/AM), n=5. The lower concentration of RANTES may be attributed to the RE-AM having only one layer of amniotic membrane within the product as opposed to two. This corresponds to the fibroblast migration data, supporting the performance of the **single layer** product in the migration assay.

Discussion

Fibroblast migration towards placental membranes by chemotaxis and haptotaxis was found to be favorable to retention-processed membranes. The RE-AC showed significantly higher migration and **single layer** RE-AM showed to be comparable to the **dual layer** AM/AM. The local chemotactic and haptotactic properties of each membrane were robustly determined and quantified using both migration assays and growth factor analysis. The concentrations of growth factors within the membranes support the migration data, further fortifying the results. Considered together, the higher concentrations of factors and the fibroblast migration towards these membranes, indicates that retention-based processed membranes are a promising option as a treatment modality for wound covering.

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