Antibiotic Processing Attenuates Retention of Wound-Healing Cytokines in Human Amniotic and Amnio-chorionic Membranes: A Comparative ELISA-Based Study

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Abstract

Background: Human placental membranes are a rich reservoir of bioactive cytokines and growth factors that orchestrate the sequential phases of wound healing and tissue regeneration. Because antibiotic exposure during membrane processing can alter protein conformation and reduce cytokine bioavailability, its influence on the regenerative potential of these allografts warrants systematic evaluation.

Methods: This study quantified six wound-healing-associated cytokines — transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor-A (VEGF-A), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor isoforms AA and BB (PDGF-AA, PDGF-BB) — in dehydrated human amnion (AM) and amnion–chorion (ACM) membranes processed with and without antibiotic treatment. Following tissue lysis and homogenization, cytokine concentrations were determined by sandwich enzyme-linked immunosorbent assay (ELISA).

Results: Across all six cytokines, membranes processed without antibiotics retained significantly higher concentrations than their antibiotic-treated counterparts, and amnion–chorion membranes consistently exceeded amnion alone, yielding an overall hierarchy of Amnion–Chorion (no antibiotics) > Amnion–Chorion (with antibiotics) > Amnion (no antibiotics) > Amnion (with antibiotics).

Conclusion: Antibiotic exposure during processing measurably diminishes cytokine retention in placental membrane allografts, whereas the amnion–chorion composite preserves a greater

regenerative cytokine reservoir than amnion alone. These findings support antibiotic-free processing protocols and favor amnion–chorion over amnion-only grafts for applications in wound care and regenerative medicine.

Keywords: *Human amnion; Amnion–chorion membrane; Antibiotic processing; Wound-healing cytokines; ELISA; Regenerative medicine*

1 Introduction

1.1 The Burden of Chronic, Non-Healing Wounds

Chronic, non-healing wounds remain a persistent and growing clinical burden, particularly among patients with diabetes, burn injuries, and pressure ulcers (Choi et al., 2012). Population-based estimates indicate that chronic wounds affect between roughly 1.5 and 2.2 individuals per 1,000 in the general population, with the majority attributable to chronic leg ulcers (Martinengo et al., 2019). The clinical and economic toll of these wounds is substantial: beyond prolonged healing times and diminished quality of life, chronic wounds increase the risk of infection, hospitalization, and lower-limb amputation, while consuming a disproportionate share of healthcare expenditure in many national health systems (Graves et al., 2022).

Successful wound repair is a tightly orchestrated developmental process that depends on the coordinated action of multiple growth factors and cytokines governing inflammation, neovascularization, fibroblast migration, and reepithelialization (Mrugala et al., 2015; Galvez et al., 2025). When this cytokine signalling network is disrupted — as occurs in ischemic, diabetic, or chronically inflamed tissue — wounds stall in the inflammatory phase and fail to progress to proliferation and remodeling. This mechanistic gap has motivated substantial interest in biological dressings capable of directly replenishing the growth factor milieu of the wound bed.

Chronic wounds arise from a heterogeneous range of underlying aetiologies, including venous insufficiency, arterial disease, diabetic neuropathy, and prolonged pressure, yet they share a common endpoint: failure to progress through the normal, time-limited sequence of haemostasis, inflammation, proliferation, and remodeling. Standard-of-care management — debridement, offloading, compression, and moist wound dressings — remains inadequate for a substantial proportion of patients, which has driven interest in adjunctive biological therapies capable of directly supplementing the wound bed with the growth factors that non-healing tissue fails to produce or sustain on its own.

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1.2 Human Placental Membranes as Biological Wound Dressings

Human placental tissues, including amnion (AM) and amnion–chorion membranes (ACM), have attracted considerable interest as biological wound dressings owing to their regenerative, anti-inflammatory, and immunomodulatory properties (Choi et al., 2012). Anatomically, the amnion is an avascular, three-layered structure consisting of a single epithelial layer, an underlying basement membrane, and a stroma comprising compact, fibroblast, and spongy sublayers; the chorion, separated from the amnion by a gelatinous interstitial matrix, contributes additional trophoblastic and mesenchymal tissue (Dadkhah Tehrani et al., 2021). Amniotic epithelial and mesenchymal cells synthesize and store a broad repertoire of growth factors within this extracellular matrix, making the intact membrane a naturally concentrated biological reservoir rather than a mechanism that must synthesize new factors after grafting (Hu et al., 2023).

These membranes are naturally enriched in cytokines central to tissue repair, including transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF). Clinically, dehydrated human amnion/chorion membrane (HACM) grafts have been associated with an average 72% reduction in wound area within three weeks of application and complete wound closure in most patients within an average of 64 days. Systematic reviews and meta-analyses further indicate that HACM-based products outperform standard-of-care management for diabetic foot ulcers, a wound type historically associated with non-healing rates of 20–40% (Mrugala et al., 2015; Haugh et al., 2017).

Placental extracellular matrix (ECM) has additionally been shown to promote end-to-end wound closure with regrowth of hair follicles and reduced scarring in rat models, effects attributed to bioactive agents retained within the matrix (Choi et al., 2012). Fresh and cryopreserved homogenized amnion exhibits broad-spectrum antimicrobial activity against uropathogens, including resistant strains, that compares favorably with conventional antibiotic treatment (Ramuta

et al., 2020), while meta-analytic evidence indicates that amnion–chorion allografts accelerate healing of diabetic foot ulcers relative to standard-care controls (Brantley & Verla, 2015).

1.3 Cytokine and Growth Factor Mediators of Wound Repair

The cytokines assayed in this study each occupy a distinct, partially overlapping niche within the wound-healing cascade. TGF-β1 has among the broadest spectra of activity of any wound-healing mediator, regulating fibroblast recruitment, extracellular matrix deposition, and immune cell function across the inflammatory, proliferative, and remodeling phases (Barrientos et al., 2008). VEGF-A is the principal driver of wound angiogenesis: released by activated platelets, macrophages, and hypoxic keratinocytes, it restores microcirculatory perfusion and oxygen delivery to the healing tissue, and its expression is itself amplified by TGF-β1, EGF, bFGF, and PDGF-BB acting as upstream paracrine stimuli (Barrientos et al., 2008). EGF promotes keratinocyte proliferation and migration during reepithelialization, while bFGF stimulates fibroblast proliferation, angiogenesis, and turnover of the extracellular matrix. PDGF, in its AA and BB isoforms, is chemotactic for fibroblasts and monocytes and is a principal regulator of granulation tissue formation (Barrientos et al., 2008).

Because these factors act in a coordinated, dose-dependent, and temporally sequenced manner, the therapeutic value of a placental allograft is unlikely to depend on any single cytokine in isolation; rather, it reflects the aggregate concentration and balance of the full growth factor complement retained after processing. This provides the rationale for quantifying multiple cytokines in parallel, as undertaken in the present study, rather than relying on a single biomarker of regenerative potential.

This interdependence is well illustrated by the relationship between TGF-β1 and PDGF signalling in granulation tissue formation: PDGF isoforms recruit and activate fibroblasts, while TGF-β1 subsequently drives their differentiation and matrix-producing activity, such that deficiency in either factor alone can blunt the granulation response even when the other is present at adequate concentration. Likewise, VEGF-A-driven angiogenesis is of limited benefit to a healing wound unless accompanied by sufficient EGF- and bFGF-driven cellular proliferation to populate the newly perfused tissue. A processing method that uniformly depresses the full cytokine complement, as antibiotic exposure appeared to do in the present study, may therefore have a

compounding rather than merely additive effect on the regenerative competence of the finished allograft.

1.4 Antibiotic Processing of Placental Allografts: Rationale and Risk

The manufacturing of placental allografts commonly incorporates antibiotic treatment to minimize the risk of bacterial contamination, a step that may compromise protein integrity and reduce cytokine retention (Roy et al., 2022). Antibiotic incorporation during membrane processing reduces the likelihood of bacterial contamination, and the dense ultrastructural architecture of the placental stroma allows the tissue to retain antibiotics at relatively high concentrations, effectively functioning as a sustained local drug-delivery vehicle. Retained antibiotic, however, can concurrently disrupt the membrane's intrinsic antimicrobial factors and compromise the structural integrity of its constituent cytokines and proteins if the tissue is not thoroughly rinsed after processing, as illustrated by gentamicin-laden cryopreserved amnion (Mrugala et al., 2015; Ramuta et al., 2020).

Gentamicin and related aminoglycosides are highly polycationic molecules that act principally by binding the bacterial 30S ribosomal subunit, but their positive charge also promotes non-specific ionic interactions with negatively charged proteins and extracellular matrix components in host tissue (Chaves & Tadi, 2023). Such interactions, together with the osmotic and chemical stress of prolonged antibiotic-bath immersion during processing, provide a plausible mechanistic basis for the reduced cytokine yields observed in antibiotic-treated membranes, although the precise molecular pathway in placental tissue remains to be fully characterized.

From a manufacturing standpoint, most placental membrane allografts intended for wound care are regulated in the United States as human cells, tissues, and cellular and tissue-based products (HCT/Ps) solely under Section 361 of the Public Health Service Act, provided that processing satisfies the criteria of minimal manipulation and homologous use (U.S. Food and Drug Administration, 2020). Antibiotic incubation is typically framed by manufacturers as a sterility-assurance step compatible with minimal manipulation; however, any processing step that measurably degrades the tissue's native growth factor content raises the question of whether antibiotic exposure inadvertently compromises the biological — as opposed to merely microbiological — quality of the finished allograft.

1.5 Study Rationale and Objectives

Sandwich ELISA offers a sensitive and specific means of quantifying cytokine concentrations in biological samples. Despite widespread clinical use of both antibiotic-treated and antibiotic-free placental membrane allografts, direct, side-by-side quantitative comparisons of their cytokine content are limited. The present study was therefore designed to quantitatively compare the levels of six key wound-healing cytokines — TGF-β1, VEGF-A, EGF, bFGF, PDGF-AA, and PDGF-BB — in amnion and amnion–chorion membranes processed with and without antibiotics, in order to clarify how antibiotic exposure during processing influences the regenerative potential of these allografts and to inform evidence-based processing protocols for tissue banks and clinicians.

2 Materials and Methods

2.1 Study Design and Membrane Groups

This study employed a comparative, laboratory-based design encompassing four membrane groups defined by tissue type and processing condition: (i) amnion without antibiotic treatment, (ii) amnion with antibiotic treatment, (iii) amnion–chorion without antibiotic treatment, and (iv) amnion–chorion with antibiotic treatment. Each group was processed and analyzed under identical laboratory conditions so that observed differences in cytokine concentration could be attributed to tissue composition (amnion versus amnion–chorion) and processing condition (with versus without antibiotics) rather than to procedural variability.

2.2 Tissue Source and Regulatory Considerations

Amnion and amnion–chorion membranes were obtained and processed in accordance with standard tissue-banking practice for allografts intended for wound-care applications. Consistent with the regulatory framework governing human placental membrane allografts, such tissues are typically processed under criteria of minimal manipulation and homologous use, which constrain permissible processing steps — including sterilization and preservation procedures — to those that do not alter the tissue's fundamental characteristics or intended function (U.S. Food and Drug Administration, 2020).

Placental tissue intended for allograft processing is conventionally recovered following elective caesarean delivery from consenting donors who have undergone maternal serological screening, in keeping with standard tissue-bank donor-eligibility requirements for communicable disease

risk. Membranes are typically rinsed to remove blood and debris prior to the processing steps evaluated in this study, after which they are divided into antibiotic-treated and antibiotic-free processing streams so that the two conditions can be compared using tissue of common origin and comparable initial handling.

2.3 Preparation of Membrane Lysates

A 1.5× concentrated lysis buffer was prepared containing 75 mM Tris-HCl, 225 mM NaCl, 7.5 mM EDTA, and 1% Triton X-100, supplemented with 1× protease inhibitors for sample preparation (Moreno et al., 2024). Amnion and amnion–chorion membrane specimens (30 mg each) were minced into small fragments and incubated for 24 hours at 2–8 °C in 3 mL of lysis buffer containing protease inhibitors (10 mg/mL). Following incubation, the tissue was homogenized using a tissue homogenizer under ice-cold conditions and subsequently centrifuged at 10,000g. The resulting supernatant was collected, and 100 µL from each sample was transferred directly into ELISA well plates for analysis (Koob et al., 2014; Zhang et al., 2022). The use of a protease-inhibitor-supplemented buffer and continuous cold-chain handling throughout lysis and homogenization was intended to minimize ex vivo proteolytic degradation of the target cytokines, so that measured concentrations would reflect the cytokine content retained through membrane processing rather than artefactual loss during sample preparation.

2.4 Quantification of Cytokines by ELISA

Samples and standards (100 µL) were added to the wells and incubated for 80 minutes at 37 °C. After three washes with 1× wash buffer, wells were incubated with biotinylated detection antibody for 50 minutes at 37 °C, washed again, and then incubated with streptavidin–horseradish peroxidase (HRP) for a further 50 minutes. TMB substrate solution was subsequently added and allowed to develop for 20 minutes in the dark before the reaction was stopped; absorbance was read immediately at 450 nm (Elk Biotech, n.d.). This sandwich ELISA format, in which the target cytokine is captured between an immobilized capture antibody and a biotinylated detection antibody, was selected for its high specificity and sensitivity relative to competitive immunoassay formats, and was applied identically across all four membrane groups for each of the six cytokines assayed.

2.5 Data Handling and Presentation

Absorbance values obtained at 450 nm were interpreted against the standard curve generated for each cytokine assay, and cytokine concentrations were compared descriptively across the four membrane groups (amnion and amnion–chorion, each with and without antibiotic treatment) for each of the six cytokines. Results are presented in graphical form (Figs. 2–6) to allow direct visual comparison of cytokine concentration across processing conditions and membrane types, and are summarized qualitatively in Section 3 and Table 1.

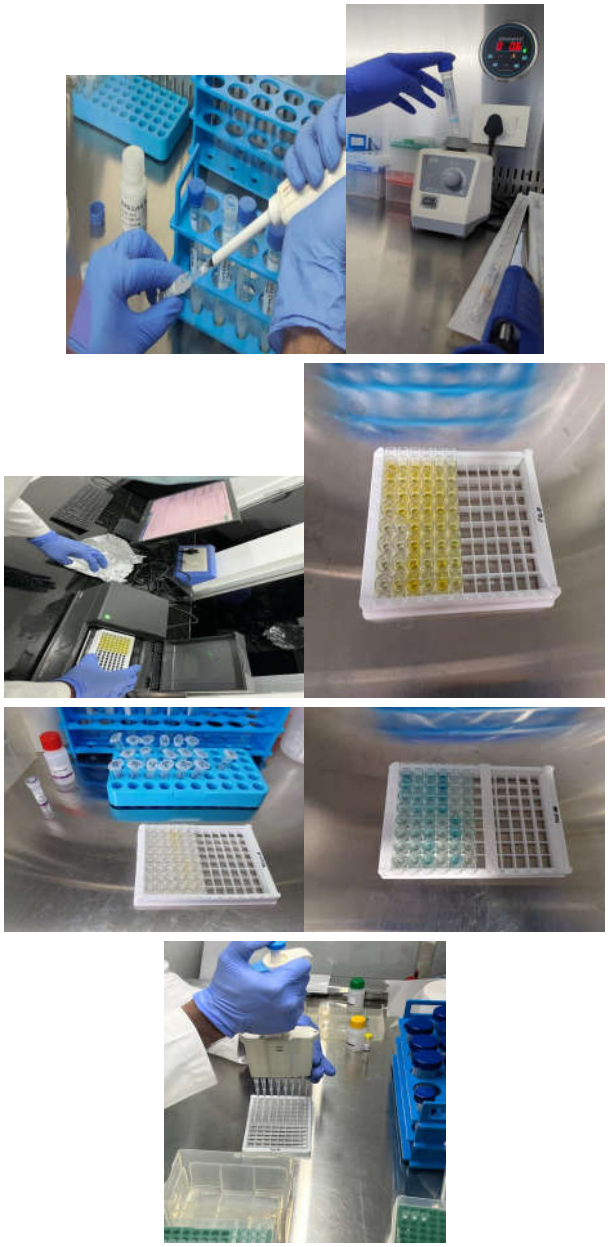

Fig. 1 Preparation and processing of amnion and amnion–chorion membranes, with and without antibiotic treatment, for ELISA analysis

3 Results

Quantification by ELISA revealed distinct cytokine expression profiles between amnion and amnion–chorion membranes processed with or without antibiotics. Findings for each cytokine are presented below and summarized in Table 1 and Figures 2–6.

3.1 TGF-β1

TGF-β1 concentrations were significantly higher in membranes processed without antibiotics than in antibiotic-treated membranes, with the highest concentrations observed in amnion–chorion preparations. Given the broad regulatory role of TGF-β1 in fibroblast recruitment and matrix deposition, this pattern suggests that antibiotic-free amnion–chorion allografts may offer the greatest capacity to support the proliferative phase of wound repair.

3.2 VEGF-A

VEGF-A, the most prominent angiogenic factor assessed, was likewise significantly elevated in amnion–chorion membranes relative to amnion alone, an effect most pronounced in the absence of antibiotic treatment. Because VEGF-A expression is itself amplified by several of the other cytokines measured in this study, the parallel elevation of TGF-β1, EGF, bFGF, and PDGF-BB in the same antibiotic-free amnion–chorion group is consistent with a coordinated, rather than isolated, preservation of the angiogenic growth factor network.

3.3 EGF

EGF concentrations were higher in non-antibiotic-treated membranes, consistent with a greater capacity to support epithelial cell regeneration. As EGF acts principally on keratinocytes during reepithelialization, its relative preservation in antibiotic-free membranes may be particularly relevant to wound closure rate.

3.4 bFGF

bFGF levels were also higher in amnion–chorion membranes, supporting a greater potential for fibroblast proliferation and extracellular matrix turnover in this tissue type. This finding parallels the pattern observed for TGF-β1 and VEGF-A, reinforcing the overall impression that the amnion–chorion composite retains a broader and more concentrated growth factor reservoir than amnion alone.

3.5 PDGF-AA and PDGF-BB

PDGF-AA and PDGF-BB concentrations followed the same pattern, with higher levels in non-antibiotic-treated membranes and the greatest cytokine accumulation observed in amnion–chorion preparations processed without antibiotics. As both PDGF isoforms are principal drivers of granulation tissue formation, their relative preservation may have particular relevance for wounds in which granulation tissue deficits are a rate-limiting step in closure.

3.6 Overall Cytokine Hierarchy

Taken together, cytokine concentrations across all groups followed a consistent hierarchy: Amnion–Chorion (no antibiotics) > Amnion–Chorion (with antibiotics) > Amnion (no antibiotics) > Amnion (with antibiotics). This pattern held across all six cytokines assayed, indicating that both tissue composition (amnion–chorion versus amnion) and processing condition (with versus without antibiotics) exert consistent, additive effects on cytokine retention. Table 1 summarizes the principal biological role of each cytokine alongside its observed retention pattern.

Table 1 Summary of assayed cytokines, their principal role in wound healing, and their observed retention pattern across the four membrane processing groups

Cytokine	Principal biological role in wound repair	Observed retention pattern across processing groups
TGF-β1	Fibroblast recruitment, extracellular matrix synthesis, and immune modulation	Highest in ACM without antibiotics; lowest in AM with antibiotics
VEGF-A	Principal angiogenic stimulus; restores tissue perfusion	Markedly elevated in ACM relative to AM, particularly without antibiotics
EGF	Keratinocyte proliferation and reepithelialization	Higher in non-antibiotic-treated membranes of both types
bFGF	Fibroblast proliferation and ECM turnover	Higher in amnion–chorion than amnion alone
PDGF-AA	Chemotaxis and mesenchymal cell proliferation	Higher in non-antibiotic-treated membranes
PDGF-BB	Granulation tissue formation and matrix remodeling	Greatest accumulation in ACM without antibiotics

As illustrated in Figures 2 through 6, the separation between antibiotic-treated and antibiotic-free groups was visually consistent across cytokines, as was the separation between amnion and amnion–chorion membranes within each antibiotic condition. This concordance across six independently assayed cytokines strengthens the inference that the observed differences reflect a genuine effect of processing condition and tissue composition rather than assay-specific variability.

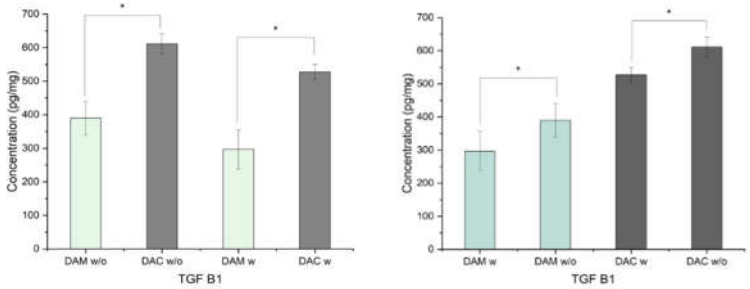

Fig. 2 TGF-β1 concentrations (left: amnion–chorion, with and without antibiotics; right: amnion, with and without antibiotics)

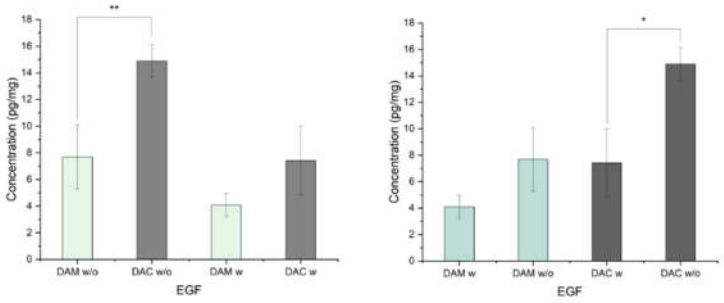

Fig. 3 EGF concentrations (left: amnion–chorion, with and without antibiotics; right: amnion, with and without antibiotics)

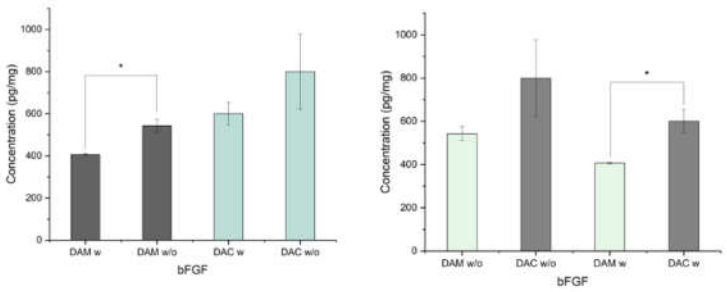

Fig. 4 bFGF concentrations (left: amnion–chorion, with and without antibiotics; right: amnion, with and without antibiotics)

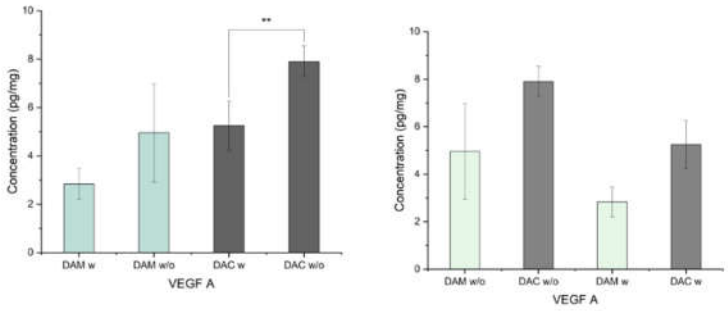

Fig. 5 VEGF-A concentrations (left: amnion–chorion, with and without antibiotics; right: amnion, with and without antibiotics)

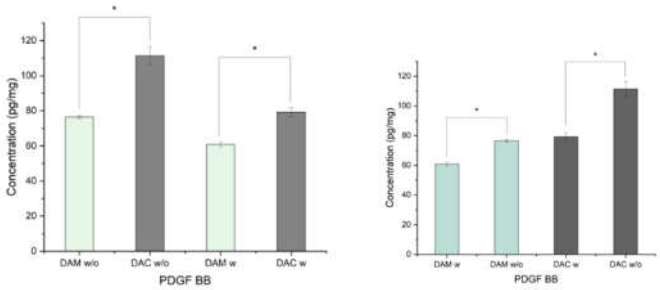

Fig. 6 PDGF-BB concentrations (left: amnion–chorion, with and without antibiotics; right: amnion, with and without antibiotics)

4 Discussion

Amnion–chorion membranes also consistently contained a greater abundance of cytokines than amnion alone, an observation likely attributable to the additional stromal and trophoblastic material contributed by the chorion layer during processing (Choi et al., 2012; Galvez et al., 2025). Because the chorion is a thicker, more cellular structure than the amnion and is separated from it only by a loose interstitial matrix, retaining the chorion effectively increases the total volume of growth-factor-producing and growth-factor-storing tissue per unit allograft, consistent with reviews describing the amniotic membrane's layered architecture as a reservoir for a broad complement of bioactive factors (Dadkhah Tehrani et al., 2021; Hu et al., 2023). This structural rationale offers a plausible explanation for why the amnion–chorion composite outperformed amnion alone irrespective of antibiotic exposure.

This pattern of cytokine loss aligns closely with the comparatively poorer outcomes reported for antibiotic-treated membranes in scratch assays, protein quantification studies, histopathological evaluations, and in vivo wound-healing models (Hofmann et al., 2023). Taken alongside the

established clinical benefits of HACM allografts for chronic wounds, including diabetic foot ulcers with historically high non-healing rates (Haugh et al., 2017), the present cytokine data provide a mechanistic underpinning for why processing choices at the tissue-bank level might translate into measurable differences in clinical healing trajectories, even though this study did not itself assess clinical wound outcomes.

From a manufacturing perspective, these findings are relevant to tissue banks operating within the minimal-manipulation and homologous-use framework that governs most placental membrane allografts (U.S. Food and Drug Administration, 2020). Because antibiotic incubation is typically adopted as a sterility-assurance measure rather than a therapeutically motivated step, tissue processors may wish to weigh the microbiological benefit of antibiotic exposure against its apparent cost to the biological potency of the finished allograft, and to consider alternative or adjunctive sterility strategies — such as more rigorous aseptic harvest and rinse protocols — that limit prolonged antibiotic–tissue contact.

This study's principal strength lies in its systematic, side-by-side comparison of four clinically relevant membrane processing conditions using a consistent ELISA methodology across six mechanistically distinct cytokines. Several limitations should nonetheless be acknowledged. First, this was an in vitro biochemical comparison; while cytokine retention is a plausible surrogate for regenerative potential, it was not directly correlated within this study to clinical wound-healing outcomes. Second, the specific antibiotic agent(s) and rinse protocol used during membrane processing were not varied systematically, so the generalizability of these findings to other antibiotic regimens used across different tissue banks remains to be established. Third, as with many single-center laboratory comparisons of biological allografts, donor-to-donor variability in placental tissue composition is a recognized source of biological variability that larger, multi-donor studies are better powered to characterize.

Future studies should aim to correlate cytokine retention directly with functional bioactivity assays (for example, fibroblast scratch-migration or endothelial tube-formation assays) and, ultimately, with clinical wound-healing endpoints in prospective cohorts. Comparative evaluation of alternative sterilization and preservation strategies — including irradiation-based and antibiotic-free antimicrobial protocols — would help clarify whether the cytokine losses observed here are

specific to antibiotic exposure or reflect a broader trade-off between sterility assurance and biological potency inherent to placental membrane processing more generally.

For clinicians selecting among commercially available placental membrane allografts, these findings suggest that processing history — specifically, whether a product was manufactured with or without antibiotic exposure — may be as relevant to expected regenerative performance as the choice between amnion-only and amnion–chorion tissue types. Product labelling and manufacturer documentation for placental allografts do not always make processing conditions readily apparent to end users, and greater transparency regarding antibiotic use during manufacturing could help clinicians match allograft selection to the specific regenerative demands of a given wound. For tissue banks, the cytokine hierarchy identified here offers a concrete, biochemically grounded target against which alternative sterilization strategies can be benchmarked as the field continues to refine placental membrane processing protocols.

5 Conclusion

This comparative ELISA-based analysis demonstrates that antibiotic treatment during the processing of human amnion and amnion–chorion membranes significantly reduces the retention of key wound-healing cytokines, including TGF-β1, VEGF-A, EGF, bFGF, PDGF-AA, and PDGF-BB. Amnion–chorion membranes processed without antibiotics retained the highest cytokine levels overall, underscoring both the added regenerative value of the chorion layer and the importance of antibiotic-free processing protocols in preserving allograft bioactivity. These findings support the continued optimization of placental membrane processing methods to maximize their therapeutic potential in wound care and regenerative medicine, and highlight the need for further studies correlating cytokine retention with functional clinical outcomes.

References

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. Wound Repair and Regeneration, 16(5), 585–601. https://doi.org/10.1111/j.1524-475X.2008.00410.x

Brantley, J. N., & Verla, T. D. (2015). Use of placental membranes for the treatment of chronic diabetic foot ulcers. Advances in Wound Care, 4(9), 545–559. https://doi.org/10.1089/wound.2015.0634

Chaves, B. J., & Tadi, P. (2023). Gentamicin. In StatPearls. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557550/

Choi, J. S., Kim, J. D., Yoon, H. S., & Cho, Y. W. (2012). Full-thickness skin wound healing using human placenta-derived extracellular matrix containing bioactive molecules. Tissue Engineering Part A, 19(3–4), 329–339. https://doi.org/10.1089/ten.tea.2011.0738

Dadkhah Tehrani, F., Firouzeh, A., Shabani, I., & Shabani, A. (2021). A review on modifications of amniotic membrane for biomedical applications. Frontiers in Bioengineering and Biotechnology, 8, 606982. https://doi.org/10.3389/fbioe.2020.606982

Elk Biotech. (n.d.). Biotin ELISA Kit. https://www.elkbiotech.com/upload/file/ELISA/ELK10918-1.pdf

Galvez, P., Omar, N. A., Siadous, R., Durand, M., Comperat, L., Lafarge, X., Gindraux, F., Sentilhes, L., Fricain, J., L'Heureux, N., & Fenelon, M. (2025). In vitro and in vivo assessment of a new acellular human amnion/chorion membrane device for guided bone regeneration. Scientific Reports, 15(1), 5483. https://doi.org/10.1038/s41598-025-88814-7

Graves, N., Phillips, C. J., & Harding, K. (2022). A narrative review of the epidemiology and economics of chronic wounds. British Journal of Dermatology, 187(2), 141–148. https://doi.org/10.1111/bjd.20692

Haugh, A. M., Witt, J. G., Hauch, A., Darden, M., Parker, G., Ellsworth, W. A., & Buell, J. F. (2017). Amnion membrane in diabetic foot wounds: A meta-analysis. Plastic & Reconstructive Surgery Global Open, 5(4), e1302. https://doi.org/10.1097/gox.0000000000001302

Hofmann, N., Rennekampff, H., Salz, A. K., & Börgel, M. (2023). Preparation of human amniotic membrane for transplantation in different application areas. Frontiers in Transplantation, 2, 1152068. https://doi.org/10.3389/frtra.2023.1152068

Hu, Z., Luo, Y., Ni, R., Hu, Y., Yang, F., Du, T., & Zhu, Y. (2023). Biological importance of human amniotic membrane in tissue engineering and regenerative medicine. Materials Today Bio, 22, 100790. https://doi.org/10.1016/j.mtbio.2023.100790

Ingraldi, A. L., Audet, R. G., & Tabor, A. J. (2023). The preparation and clinical efficacy of amnion-derived membranes: A review. Journal of Functional Biomaterials, 14(10), 531. https://doi.org/10.3390/jfb14100531

Koob, T. J., Lim, J. J., Zabek, N., & Massee, M. (2014). Cytokines in single layer amnion allografts compared to multilayer amnion/chorion allografts for wound healing. Journal

of Biomedical Materials Research Part B: Applied Biomaterials, 103(5), 1133–1140. https://doi.org/10.1002/jbm.b.33265

Martinengo, L., Olsson, M., Bajpai, R., Soljak, M., Upton, Z., Schmidtchen, A., Car, J., & Järbrink, K. (2019). Prevalence of chronic wounds in the general population: Systematic review and meta-analysis of observational studies. Annals of Epidemiology, 29, 8–15. https://doi.org/10.1016/j.annepidem.2018.10.005

Moreno, S., Massee, M., Campbell, S., Bara, H., Koob, T. J., & Harper, J. R. (2024). PURION® processed human amnion chorion membrane allografts retain material and biological properties supportive of soft tissue repair. Journal of Biomaterials Applications, 39(1), 24–39. https://doi.org/10.1177/08853282241246034

Mrugala, A., Sui, A., Plummer, M., Altman, I., Papineau, E., Frandsen, D., Hill, D., & Ennis, W. J. (2015). Amniotic membrane is a potential regenerative option for chronic non-healing wounds: A report of five cases receiving dehydrated human amnion/chorion membrane allograft. International Wound Journal, 13(4), 485–492. https://doi.org/10.1111/iwj.12458

Ramuta, T. Ž., Erjavec, M. S., & Kreft, M. E. (2020). Amniotic membrane preparation crucially affects its broad-spectrum activity against uropathogenic bacteria. Frontiers in Microbiology, 11, 469. https://doi.org/10.3389/fmicb.2020.00469

Roy, A., Mantay, M., Brannan, C., & Griffiths, S. (2022). Placental tissues as biomaterials in regenerative medicine. BioMed Research International, 2022(1), 6751456. https://doi.org/10.1155/2022/6751456

Sabol, T. J., Tran, G. S., Matuszewski, J., & Weston, W. W. (2022). Standardized reporting of amnion and amnion/chorion allograft data for wound care. Health Science Reports, 5(5), e794. https://doi.org/10.1002/hsr2.794

U.S. Food and Drug Administration. (2020). Regulatory considerations for human cells, tissues, and cellular and tissue-based products: Minimal manipulation and homologous use — Guidance for industry and FDA staff. U.S. Department of Health and Human Services.

Zhang, S., Gao, L., Wang, P., Ma, Y., Wang, X., Wen, J., Cheng, Y., Liu, C., Zhang, C., Liu, C., Yan, Y., & Zhao, C. (2022). A minimally manipulated preservation and virus inactivation method for amnion/chorion. Frontiers in Bioengineering and Biotechnology, 10, 952498. https://doi.org/10.3389/fbioe.2022.952498