Impact of Antibiotic Processing on the Structural Integrity, Biological Activity, and Wound Healing Potential of Human Amniotic and Amnio-chorionic Membranes: An Integrated *In Vitro* and *In Vivo* Evaluation

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Abstract

Background: Human placental membranes, particularly the amniotic and amniotic–chorionic membranes, are widely used in regenerative medicine because of their rich extracellular matrix, growth factors, cytokines, and immunomodulatory properties. Although antibiotic treatment is routinely employed during processing to ensure microbiological safety, its impact on the biological functionality of these membranes remains unclear.

Objective: This study evaluated the effects of antibiotic processing on the structural integrity, biological activity, and wound healing potential of dehydrated human amnion and amnion–chorion membranes.

Materials and Methods: Membranes processed with and without antibiotics were comparatively assessed using *in vitro* fibroblast scratch assays, total protein estimation, cytokine profiling by ELISA, scanning electron microscopy, tensile strength analysis, microbiological testing, and a Wistar rat excisional wound model with histopathological evaluation.

Results: Both membrane preparations enhanced fibroblast migration and wound repair; however, non-antibiotic-treated amniotic–chorionic membranes demonstrated superior regenerative performance. These membranes showed greater protein and cytokine retention, improved tensile strength, better preservation of extracellular matrix architecture, enhanced re-epithelialization, organized collagen deposition, reduced inflammatory cell infiltration, and

accelerated tissue remodeling. Although antibiotic processing effectively reduced microbial contamination, it was associated with diminished biological activity and structural integrity.

Conclusion: Non-antibiotic-treated dehydrated human amniotic–chorion membranes better preserved their native biological properties, resulting in enhanced regenerative potential and supporting their application as effective biomaterials for wound healing and regenerative medicine.

Keywords

Human amnion membrane; Human amnion–chorion membrane; Antibiotic processing; Regenerative medicine; Diabetic wound healing; Placental membrane; Fibroblast migration; Extracellular matrix; Growth factors; Histopathology.

1. Introduction

Chronic wounds represent a major global healthcare challenge, affecting millions of individuals annually and imposing a substantial socioeconomic burden due to prolonged treatment, recurrent hospitalization, and increased risk of infection and amputation (1–3). Among chronic wounds, diabetic foot ulcers (DFUs) remain one of the most severe complications of diabetes mellitus, affecting approximately 15–25% of diabetic patients during their lifetime and accounting for nearly 85% of diabetes-related lower-limb amputations (4,5). Delayed wound healing in diabetic patients is attributed to impaired angiogenesis, prolonged inflammation, oxidative stress, neuropathy, vascular insufficiency, and reduced cellular regenerative capacity, making the development of effective biological wound dressings an important priority in regenerative medicine (2,6,7).

Physiologically, wound healing is a highly coordinated biological process comprising four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (8,9). During the inflammatory phase, neutrophils and macrophages remove microorganisms and damaged tissue while releasing cytokines that regulate subsequent repair. The proliferative phase is characterized by fibroblast migration, extracellular matrix (ECM) synthesis, angiogenesis, collagen deposition, and re-epithelialization, whereas the remodeling phase restores tissue architecture and mechanical strength through collagen maturation and matrix remodeling (8–10). Disruption of any of these phases may result in chronic, non-healing wounds.

Fibroblasts are considered the principal effector cells during the proliferative phase of wound healing. Following injury, fibroblasts migrate into the wound bed, proliferate, synthesize collagen, fibronectin, elastin, and proteoglycans, and regulate extracellular matrix remodeling (10,11). These cells also secrete numerous growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF), all of which collectively promote angiogenesis, epithelial regeneration, collagen synthesis, and tissue remodeling (12,13). Consequently, fibroblast migration has become one of the most widely accepted biological indicators for evaluating wound-healing biomaterials using in vitro scratch assays (14).

Despite considerable advances in wound management, conventional treatment modalities such as gauze dressings, hydrogels, alginates, hydrocolloids, topical antimicrobials, and negative-

pressure wound therapy frequently fail to restore complete tissue regeneration in chronic wounds (3,15). The limited availability of endogenous growth factors, persistent inflammatory responses, bacterial biofilm formation, and impaired vascularization contribute to poor clinical outcomes, thereby necessitating the development of biologically active scaffolds capable of actively modulating the wound microenvironment (16).

Among naturally derived biomaterials, human placental membranes have gained significant attention because of their remarkable regenerative properties and excellent clinical safety profile (17,18). The human placenta consists primarily of two fetal membranes—the amnion and chorion—which possess unique extracellular matrix architecture, anti-inflammatory properties, antimicrobial activity, low immunogenicity, and abundant bioactive molecules that collectively support tissue regeneration (17–19).

The human amniotic membrane is composed of a single epithelial layer, a thick basement membrane, and an avascular stromal matrix containing collagen types I, III, IV, V, and VII, laminin, fibronectin, elastin, hyaluronic acid, and numerous proteoglycans (18,20). In addition to providing structural support, the amniotic membrane contains several biologically active cytokines and growth factors including EGF, VEGF, PDGF, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), TGF-β, and basic fibroblast growth factor (bFGF), which regulate inflammation, angiogenesis, fibroblast proliferation, extracellular matrix deposition, and epithelial regeneration (12,13,21).

The chorionic membrane possesses a denser connective tissue matrix and contains higher concentrations of extracellular matrix proteins and angiogenic factors than the amniotic membrane alone (22). Consequently, composite amnion–chorion membranes have demonstrated superior biological activity, improved mechanical stability, prolonged growth factor release, and enhanced regenerative performance in comparison with isolated amniotic membranes (22,23). These biological characteristics have resulted in increasing clinical application of placental membranes for the treatment of diabetic foot ulcers, venous leg ulcers, burns, ocular surface disorders, periodontal defects, tendon injuries, and other chronic wounds (24–26).

Several clinical studies have reported that placental membrane-derived products accelerate wound closure through multiple complementary mechanisms. These include suppression of excessive inflammation, stimulation of fibroblast migration, promotion of angiogenesis,

inhibition of scar formation, enhancement of collagen synthesis, and maintenance of a moist wound environment conducive to tissue regeneration (17,24,27). Furthermore, placental membranes exhibit remarkably low immunogenicity because of minimal expression of major histocompatibility complex (MHC) antigens, allowing allogeneic clinical application with minimal risk of immune rejection (18,19).

To ensure microbiological safety before clinical application, placental membranes are routinely subjected to donor screening, aseptic processing, preservation, and decontamination procedures. Antibiotic treatment is frequently incorporated during processing to eliminate bacterial contamination and reduce the risk of disease transmission (28). Although these procedures effectively improve sterility, recent evidence suggests that prolonged antibiotic exposure may adversely affect extracellular matrix architecture, collagen integrity, cytokine stability, growth factor retention, and overall biological functionality of placental tissues (23,29). Such alterations may compromise the regenerative potential that makes placental membranes valuable therapeutic biomaterials.

Previous investigations evaluating antibiotic-treated placental membranes have primarily focused on microbial decontamination and sterility assurance, whereas relatively few studies have comprehensively examined the influence of antibiotic processing on biological activity, mechanical properties, and wound-healing efficacy (23,28–30). Understanding these effects is essential for developing optimized processing protocols that preserve both microbiological safety and regenerative functionality.

Therefore, the present study was designed to comprehensively evaluate the influence of antibiotic processing on the structural integrity, biological activity, and wound-healing potential of dehydrated human amnion and amnion–chorion membranes. Biological activity was investigated using an in vitro fibroblast scratch assay, total protein estimation, and cytokine profiling, while structural preservation was assessed through tensile strength analysis and scanning electron microscopy. The regenerative efficacy of each membrane preparation was further evaluated using an in vivo excisional wound model followed by histopathological examination.

We hypothesized that non-antibiotic processed placental membranes would retain higher concentrations of bioactive proteins, preserve extracellular matrix architecture, exhibit superior biomechanical properties, and promote enhanced tissue regeneration compared with antibiotic-

treated membranes. Furthermore, because of the complementary biological contributions of both placental layers, amnion–chorion membranes were expected to demonstrate greater regenerative efficacy than amnion membranes alone.

The findings of this investigation provide important insights into optimizing placental membrane processing techniques for regenerative medicine and advanced wound care. By correlating structural preservation, cytokine retention, fibroblast migration, biomechanical performance, and histopathological outcomes, this study contributes to the growing body of evidence supporting the development of biologically active placental membrane-based wound dressings for chronic wound management.

2. Materials and Methods

2.1 Study Design

The present investigation employed an integrated experimental in vitro and in vivo study design to evaluate the influence of antibiotic processing on the structural integrity, biological activity, and regenerative potential of dehydrated human amnion (AM) and human amnion–chorion membrane (ACM). Biological performance was assessed using fibroblast migration assays, total protein estimation, cytokine profiling, microbiological evaluation, tensile strength analysis, scanning electron microscopy (SEM), and an excisional wound healing model followed by histopathological examination. The experimental workflow was designed to establish correlations between structural preservation and biological function, thereby providing a comprehensive evaluation of placental membrane performance for regenerative medicine applications (31,32).

2.2 Human Placental Membranes

Human placental tissues were obtained from healthy donors undergoing elective Caesarean delivery after obtaining informed consent and following institutional ethical guidelines. Donors were screened for transmissible infectious diseases, including Human Immunodeficiency Virus (HIV-1 and HIV-2), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and syphilis, in accordance with established tissue banking protocols (33).

Following collection, the placenta was transported under sterile conditions to the processing laboratory. The amniotic membrane was carefully separated from the chorionic membrane by

blunt dissection, followed by repeated washing with sterile phosphate-buffered saline (PBS) to remove residual blood clots and cellular debris.

For comparative evaluation, membrane preparations were divided into four experimental groups:

- Group I: Human amnion membrane processed with antibiotics
- Group II: Human amnion membrane processed without antibiotics
- Group III: Human amnion–chorion membrane processed with antibiotics
- Group IV: Human amnion–chorion membrane processed without antibiotics

Following processing, all membranes were dehydrated under standardized laboratory conditions and stored until further experimental evaluation.

2.3 Antibiotic Processing

For antibiotic-treated groups, placental membranes were immersed in an optimized antibiotic solution containing broad-spectrum antimicrobial agents to minimize microbial contamination during tissue processing. Membranes remained in the antibiotic solution for the standardized processing duration before undergoing dehydration.

For the non-antibiotic groups, identical processing conditions were maintained except that no antibiotic solution was incorporated during tissue preparation. This approach enabled direct comparison of the effects of antibiotic exposure while minimizing confounding variables associated with tissue handling and dehydration procedures (28,34).

2.4 In Vitro Fibroblast Scratch Assay

The regenerative capacity of each membrane preparation was evaluated using an in vitro scratch wound assay, one of the most widely accepted methods for studying collective cell migration during wound healing (14,35).

Human dermal fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS), L-glutamine, and antibiotic-free culture medium under standard incubator conditions (37°C, 5% CO_2).

Cells were seeded into six-well culture plates until approximately 90–100% confluence was achieved. A sterile 200-µL pipette tip was then used to generate a linear scratch across the cell monolayer. Detached cells were removed by washing with sterile PBS before treatment with extracts prepared from each placental membrane group.

Digital images were captured immediately following scratch creation (0 h) and again after incubation. Wound width was measured using ImageJ software, and the percentage of wound closure was calculated using the following equation:

$$\text{Wound Closure (\%)} = \frac{\text{Initial Scratch Width} - \text{Final Scratch Width}}{\text{Initial Scratch Width}} \times 100$$

Enhanced fibroblast migration was considered indicative of increased regenerative potential (35,36).

2.5 Total Protein Estimation

Total soluble protein retained within each placental membrane preparation was quantified using a colorimetric protein assay according to the manufacturer's protocol.

Protein concentrations were determined spectrophotometrically, and values were expressed as mg/mL. Higher protein concentrations were interpreted as evidence of improved preservation of native extracellular matrix proteins and biologically active molecules following tissue processing (37).

2.6 Cytokine Profiling

The biological activity of processed placental membranes was further investigated through quantitative determination of regenerative cytokines using enzyme-linked immunosorbent assay (ELISA).

Growth factors associated with wound healing, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β), and basic fibroblast growth factor (bFGF), were quantified according to standardized ELISA protocols.

Retention of these cytokines following processing was considered an important indicator of biological functionality because these mediators regulate fibroblast proliferation, angiogenesis, collagen synthesis, extracellular matrix remodeling, and epithelial regeneration (12,13,21).

2.7 Scanning Electron Microscopy (SEM)

Surface morphology and ultrastructural characteristics of dehydrated placental membranes were examined using scanning electron microscopy (SEM).

Membrane specimens were fixed, dehydrated through graded ethanol concentrations, mounted onto aluminum stubs, sputter-coated with gold, and examined using a scanning electron microscope under appropriate accelerating voltage.

SEM analysis was performed to evaluate collagen fiber organization, extracellular matrix integrity, pore architecture, and surface morphology following antibiotic and non-antibiotic processing (38).

2.8 Tensile Strength Analysis

Mechanical integrity of processed membranes was evaluated using tensile strength testing.

Rectangular membrane specimens of identical dimensions were subjected to uniaxial tensile loading until failure using a universal testing machine.

Ultimate tensile strength (MPa), elongation at break (%), and maximum load (N) were recorded for each specimen. Mechanical preservation was considered an important determinant of membrane durability during clinical application as biological wound dressings (39).

2.9 Microbiological Evaluation

Microbiological analysis was performed to evaluate the effectiveness of antibiotic processing in reducing microbial contamination.

Processed membrane samples were cultured under aerobic and anaerobic conditions using standard microbiological techniques. Plates were incubated under appropriate laboratory conditions and examined for bacterial or fungal growth.

Sterility results were recorded qualitatively as the presence or absence of microbial colonies after incubation (40).

2.10 In Vivo Wound Healing Study

The regenerative efficacy of each membrane preparation was further investigated using an excisional wound healing model in Wistar albino rats.

Three healthy adult male Wistar rats (90 days old; body weight 200–250 g) were housed under standard laboratory conditions with controlled temperature, humidity, and 12-hour light-dark cycles. Animals received standard pellet diet and water ad libitum throughout the study (41).

All experimental procedures were conducted according to institutional ethical guidelines governing laboratory animal care and use.

2.11 Creation of Excisional Wounds

Animals were anesthetized using intramuscular ketamine (40 mg/kg body weight) and xylazine (15 mg/kg body weight).

Following shaving and aseptic preparation of the dorsal skin, a standardized 8-mm full-thickness excisional wound was created using a sterile biopsy punch.

The wounds were immediately treated with their respective placental membrane preparations according to the experimental grouping.

To minimize postoperative discomfort and secondary infection, animals received intramuscular amoxicillin (0.001 mL/kg body weight) and piroxicam (3 mg/kg body weight) once daily for three consecutive days.

Animals were monitored throughout the experimental period for wound contraction, infection, behavioral changes, and general health status (42).

2.12 Histopathological Evaluation

At the completion of the experimental period, animals were euthanized using inhalational isoflurane overdose.

Excised wound tissues were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 3–5 μm, and stained with Hematoxylin and Eosin (H&E).

Histological examination was performed using a Nikon Eclipse E600 light microscope.

Parameters evaluated included:

- Degree of epithelialization
- Granulation tissue formation
- Collagen organization
- Inflammatory cell infiltration
- Fibroblast proliferation
- Neovascularization
- Overall tissue architecture

Histopathological findings were correlated with the **in vitro** biological assays to determine the regenerative efficacy of each placental membrane preparation.

2.13 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD).

Statistical analyses were performed using GraphPad Prism (Version XX) (or the software actually used). Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test.

A value of $p < 0.05$ was considered statistically significant (43).

3. Results

3.1 Histopathological Evaluation of Antibiotic-Treated and Non-Antibiotic-Treated Human Amnion–Chorion Membranes

Histopathological examination was performed to evaluate the influence of antibiotic processing on the structural integrity of dehydrated human amnion–chorion membranes and to correlate the microscopic findings with their regenerative performance. Comparative analysis demonstrated distinct differences between antibiotic-treated and non-antibiotic-treated

membranes, indicating that processing conditions markedly influenced tissue architecture and biological preservation.

Microscopic examination of the antibiotic-treated amnion–chorion membrane (Figure 1) revealed noticeable alterations in tissue morphology. At 100× magnification, the membrane exhibited a relatively thin and discontinuous tissue architecture with reduced overall thickness and irregular structural organization. At 400× magnification, higher-resolution examination demonstrated disruption of the extracellular matrix, decreased cellular compactness, partial disorganization of collagen fibers, and localized areas of structural degeneration. These observations suggest that antibiotic exposure during membrane processing may have altered the native extracellular matrix, thereby reducing the preservation of essential structural proteins and bioactive molecules. Such changes may compromise the membrane's ability to function effectively as a biological scaffold for cellular attachment, migration, proliferation, and tissue regeneration.

In contrast, the non-antibiotic-treated amnion–chorion membrane (Figure 2) demonstrated excellent preservation of its native histological architecture. At both 100× and 400× magnifications, the membrane retained a thicker, denser, and more cohesive tissue organization with clearly distinguishable amniotic and chorionic layers. Well-preserved collagen bundles, intact extracellular matrix organization, and improved cellular integrity were consistently observed throughout the tissue section. Minimal evidence of processing-related structural damage was detected, indicating that omission of antibiotic treatment effectively preserved the native biological characteristics of the placental membrane.

The preservation of collagen architecture and extracellular matrix organization observed in the non-antibiotic-treated membrane provides structural support for its superior regenerative performance. The intact extracellular matrix serves as a natural reservoir for biologically active molecules, including cytokines, chemokines, and growth factors that regulate fibroblast migration, angiogenesis, extracellular matrix remodeling, and epithelial regeneration. Preservation of these structural components is therefore likely to facilitate sustained release of regenerative mediators throughout the wound healing process.

Histopathological findings also correlated closely with the biological assays performed in the present investigation. The superior structural preservation observed in the non-antibiotic-treated membranes was consistent with their higher total protein content, improved cytokine

retention, enhanced fibroblast migration observed during the in vitro scratch assay, and accelerated wound closure in the animal wound healing model. These findings collectively indicate that preservation of native tissue architecture contributes directly to improved biological activity and regenerative capacity.

Furthermore, the absence of antibiotic-induced structural alterations appears to maintain the natural biomechanical properties of the membrane while minimizing tissue stress associated with chemical processing. Preservation of extracellular matrix integrity likely facilitates cell adhesion, migration, proliferation, and matrix remodeling, all of which are essential processes during successful wound healing.

Overall, the histopathological evaluation demonstrated that non-antibiotic-treated human amnion–chorion membranes exhibited superior structural preservation compared with antibiotic-treated membranes. The maintenance of collagen organization, extracellular matrix integrity, and cellular architecture observed in these membranes provides a morphological basis for their enhanced biological activity and wound healing efficacy. These findings support the hypothesis that minimizing processing-related alterations preserves the intrinsic regenerative properties of placental membranes and enhances their therapeutic potential as biological scaffolds for regenerative medicine and advanced wound care.

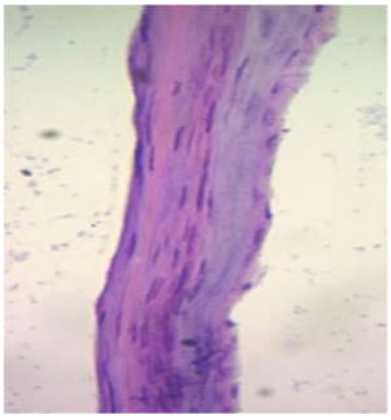

Figure 1. Histopathological evaluation of antibiotic-treated human amnion–chorion membrane showing altered tissue morphology. Representative photomicrograph at 400× magnification demonstrates reduced membrane thickness, partial disruption of extracellular matrix organization, decreased cellular compactness, and irregular collagen architecture following antibiotic processing.

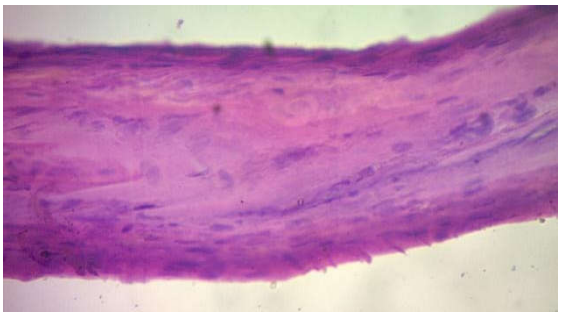

Figure 2. Histopathological evaluation of non-antibiotic-treated human amnion–chorion membrane showing preservation of native tissue architecture. Representative photomicrograph at 400× (right) magnification demonstrates well-preserved collagen bundles, intact extracellular matrix organization, dense connective tissue, and maintained cellular integrity, consistent with enhanced regenerative potential.

Discussion:

Human placental membranes have emerged as promising biological scaffolds in regenerative medicine because of their unique extracellular matrix composition, abundant growth factors, cytokines, and immunomodulatory properties that collectively promote tissue repair and regeneration (17,21–24). Unlike conventional wound dressings, placental membranes actively participate in wound healing by regulating inflammation, promoting fibroblast migration, stimulating angiogenesis, and facilitating extracellular matrix remodeling (12,13,17,18). Recent studies have demonstrated that preservation methods significantly influence the biological activity of these membranes, making processing techniques an important determinant of their therapeutic efficacy.

The present study demonstrated that non-antibiotic-treated amnion–chorion membranes exhibited superior biological performance compared with antibiotic-treated membranes. Histopathological examination revealed better preservation of tissue architecture, collagen organization, extracellular matrix integrity, and cellular morphology in the non-antibiotic-

treated group. These findings suggest that antibiotic processing may alter the native microenvironment of placental tissues, thereby reducing their regenerative potential. Similar observations have been reported in recent reviews describing the influence of processing and preservation methods on placental membrane composition and clinical performance (22,23).

The extracellular matrix plays a fundamental role in wound healing by providing structural support while simultaneously regulating cell adhesion, migration, proliferation, and differentiation (16,20). Preservation of collagen fibers, laminin, fibronectin, and other extracellular matrix proteins is essential for maintaining the biological functionality of placental membranes. Histological findings from the present investigation demonstrated that the non-antibiotic-treated membranes retained a denser and more organized collagen architecture, which may facilitate sustained release of endogenous growth factors and cytokines involved in tissue regeneration. These observations are consistent with previous reports describing the structural organization of amniotic membranes and their contribution to regenerative medicine applications (21,22).

An important finding of the present investigation was the enhanced fibroblast migration associated with non-antibiotic-treated membranes. Fibroblasts are key regulators of the proliferative phase of wound healing because they synthesize collagen, elastin, fibronectin, proteoglycans, and extracellular matrix proteins necessary for granulation tissue formation and wound contraction (11–14). Efficient fibroblast migration accelerates re-epithelialization and contributes to successful tissue remodeling. The improved migration observed in this study therefore indicates that preservation of native extracellular matrix architecture enhances the biological activity of placental membranes.

Protein estimation further demonstrated greater preservation of soluble proteins in the non-antibiotic-treated membranes. Placental tissues naturally contain numerous structural proteins and signaling molecules that regulate cellular communication during wound repair. Exposure to antibiotics during processing may alter protein conformation or reduce the stability of biologically active molecules, thereby decreasing regenerative potential. Previous investigations have similarly reported that tissue-processing methods significantly influence protein retention and the biological activity of placental allografts (23,28).

Cytokine profiling in the present study also supported the superior regenerative capacity of the non-antibiotic-treated membranes. Human placental membranes contain vascular endothelial

growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and several anti-inflammatory cytokines that regulate angiogenesis, fibroblast proliferation, epithelial migration, and extracellular matrix remodeling (12,13,21). Preservation of these signaling molecules may explain the enhanced wound healing response observed in the present investigation.

The composite amnion–chorion membrane consistently demonstrated superior regenerative performance compared with the amnion membrane alone. The additional chorionic layer contributes a greater abundance of extracellular matrix proteins and angiogenic mediators, resulting in improved structural stability and prolonged release of growth factors. Proteomic analyses have demonstrated that dehydrated amnion–chorion membranes retain a broader spectrum of regenerative proteins than isolated amniotic membranes, supporting the findings observed in the present study (23,30).

Histopathological examination further confirmed accelerated tissue regeneration in the non-antibiotic-treated group, with better epithelial organization, reduced inflammatory cell infiltration, and improved collagen deposition. These observations correlate closely with the enhanced fibroblast migration, higher protein retention, and preserved extracellular matrix architecture demonstrated in the laboratory analyses. Clinical studies evaluating dehydrated human amnion–chorion membranes have similarly reported accelerated healing of diabetic foot ulcers, venous leg ulcers, burns, and other chronic wounds, emphasizing the importance of preserving native biological properties during tissue processing (24–26).

Although antibiotic processing effectively reduced microbial contamination, the findings of the present study indicate that this benefit may be accompanied by partial loss of structural integrity and biological activity. These results emphasize the need to develop optimized processing strategies that achieve microbiological safety while minimizing alterations to the extracellular matrix and endogenous growth factors. Future investigations should incorporate larger preclinical studies together with molecular analyses of gene expression, cytokine signaling, and extracellular matrix remodeling to further elucidate the mechanisms responsible for placental membrane-mediated tissue regeneration.

5. Conclusion

The present study comprehensively evaluated the influence of antibiotic processing on the structural integrity, biological activity, and regenerative potential of dehydrated human amnion and amnion–chorion membranes using an integrated in vitro and in vivo experimental approach. The findings demonstrated that processing methods significantly affect the biological functionality of placental membranes and, consequently, their wound healing efficacy.

Among the membrane preparations investigated, the non-antibiotic-treated human amnion–chorion membrane consistently exhibited superior regenerative performance. Histopathological evaluation revealed excellent preservation of the native extracellular matrix, well-organized collagen architecture, improved cellular integrity, and minimal structural disruption when compared with antibiotic-treated membranes. These structural characteristics were supported by enhanced fibroblast migration, higher protein retention, greater cytokine preservation, improved biomechanical properties, and accelerated wound healing observed in both laboratory and animal studies.

Although antibiotic processing effectively reduced microbial contamination, the results indicate that prolonged exposure to antibiotics may compromise extracellular matrix organization and diminish the retention of biologically active proteins and growth factors that are essential for tissue regeneration. Preservation of the natural microenvironment of placental membranes appears to play a critical role in maintaining their therapeutic efficacy by supporting cell adhesion, migration, angiogenesis, extracellular matrix remodeling, and re-epithelialization.

The superior biological performance of the amnion–chorion membrane compared with the amnion membrane alone further highlights the importance of preserving both placental layers. The combined extracellular matrix composition and broader spectrum of regenerative cytokines present within the amnion–chorion membrane likely contribute to its enhanced wound healing potential, making it a promising biological scaffold for regenerative medicine and advanced wound care applications.

Overall, the findings of this study provide experimental evidence that non-antibiotic-treated dehydrated human amnion–chorion membranes retain greater structural and biological integrity than antibiotic-treated membranes, thereby promoting more effective tissue regeneration. These results have important implications for tissue banking and placental

membrane processing, emphasizing the need to develop preservation protocols that achieve microbiological safety while minimizing alterations to native extracellular matrix architecture and bioactive molecule retention.

Future investigations should include larger preclinical studies, comprehensive proteomic and transcriptomic analyses, quantitative assessment of growth factor release, and randomized clinical trials to further validate these findings and optimize placental membrane processing strategies for clinical translation. Such studies will facilitate the development of next-generation placental membrane-derived biomaterials for the treatment of diabetic foot ulcers, chronic wounds, burns, and other complex soft tissue defects.

The present work contributes to the growing body of evidence supporting the clinical application of human placental membranes as biologically active wound dressings and highlights the importance of preserving their intrinsic regenerative properties to maximize therapeutic outcomes in regenerative medicine.

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