Comparative Evaluation of Human Amniotic and Amnio-chorionic Membranes in Wound Repair: Implications for Regenerative Medicine

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Abstract

Wound healing is a dynamic and highly coordinated biological process involving inflammation, cellular proliferation, extracellular matrix remodeling, angiogenesis, and tissue regeneration. Despite considerable advances in wound management, chronic and non-healing wounds continue to pose a major clinical challenge worldwide, particularly among patients with diabetes mellitus, vascular insufficiency, burns, traumatic injuries, and pressure ulcers. Human placental membranes, including the amnion and chorion, possess unique biological characteristics that make them attractive candidates for regenerative medicine. These membranes are naturally enriched with extracellular matrix proteins, anti-inflammatory cytokines, angiogenic molecules, antimicrobial peptides, and numerous growth factors that collectively regulate cellular proliferation, fibroblast migration, angiogenesis, and extracellular matrix synthesis. The present study investigated the wound healing potential of dehydrated human amnion and amnion–chorion membrane extracts using an in vitro scratch assay performed on adult dermal fibroblasts. Fibroblast migration was monitored over a 20-hour period following scratch induction, and wound closure percentages were quantified using ImageJ software. Positive and negative controls were included to validate experimental performance.

Keywords: Human amnion; Human chorion; Placental membrane; Fibroblast migration; Scratch assay; Regenerative medicine; Wound healing; Tissue engineering.

1. Introduction

1.1 Global Burden of Chronic Wounds

Wound healing is one of the most intricate physiological processes occurring in the human body and represents a critical component of tissue homeostasis and regeneration. Restoration of injured tissue requires precise coordination among inflammatory cells, fibroblasts, endothelial cells, keratinocytes, immune mediators, extracellular matrix (ECM) proteins, and numerous growth factors (1–3). Under normal physiological conditions, these events proceed in a highly synchronized manner, leading to complete tissue repair with restoration of structural and functional integrity. However, disruption of any stage of this complex process may result in delayed healing, excessive fibrosis, chronic inflammation, or persistent non-healing wounds (2,4).

Chronic wounds have become a major public health concern due to increasing life expectancy, rising prevalence of diabetes mellitus, obesity, vascular diseases, traumatic injuries, burns, and pressure ulcers (5,6). According to recent global estimates, millions of patients suffer from chronic wounds annually, imposing a substantial socioeconomic burden on healthcare systems (5). Diabetic foot ulcers alone affect approximately 15–25% of diabetic patients during their lifetime and remain one of the leading causes of lower-limb amputations worldwide (7,8). Similarly, venous leg ulcers, arterial ulcers, and pressure injuries are associated with prolonged hospitalization, recurrent infections, impaired quality of life, and increased healthcare expenditure (6,9).

The financial burden associated with chronic wound management continues to escalate due to repeated surgical interventions, prolonged antimicrobial therapy, advanced wound dressings, and extended rehabilitation (5,9). Consequently, there is an urgent need to develop biologically active therapeutic materials capable of accelerating tissue regeneration while reducing treatment duration and healthcare costs (10).

1.2 Biology of Wound Healing

Wound healing is a highly coordinated biological process that restores the structural and functional integrity of damaged tissues through four overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling (1,2,11). Each phase involves complex interactions among inflammatory cells, fibroblasts, endothelial cells, keratinocytes, extracellular matrix proteins, cytokines, chemokines, and growth factors. Successful wound repair depends on the timely progression through each of these stages, whereas dysregulation may result in chronic wounds, fibrosis, excessive scar formation, or impaired tissue regeneration (2,11).

Immediately following tissue injury, platelet activation initiates clot formation and releases numerous bioactive mediators, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF), which regulate subsequent healing events (12,13).

The inflammatory phase begins within hours after injury and is characterized by infiltration of neutrophils, macrophages, mast cells, and lymphocytes (2,11). Macrophages play a pivotal role by secreting cytokines and growth factors that regulate inflammation, stimulate angiogenesis,

recruit fibroblasts, and promote extracellular matrix synthesis. The transition from the pro-inflammatory M1 phenotype to the pro-regenerative M2 phenotype is considered essential for successful tissue repair (14,15).

During the proliferative phase, fibroblasts migrate into the wound bed, proliferate rapidly, and synthesize extracellular matrix proteins including collagen types I and III, fibronectin, elastin, and hyaluronic acid (3,11). Concurrently, endothelial cells initiate angiogenesis while keratinocytes restore epithelial continuity. Myofibroblasts contribute to wound contraction, leading to granulation tissue formation and progressive wound closure (2,11).

The remodeling phase may continue for several months after injury, during which collagen fibers undergo reorganization, increasing the tensile strength of repaired tissue. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) regulate extracellular matrix turnover and tissue remodeling (16).

1.3 Role of Fibroblasts in Tissue Regeneration

Fibroblasts are the principal effector cells responsible for connective tissue repair and extracellular matrix remodeling (3,11). Their ability to migrate into damaged tissue, proliferate in response to growth factor stimulation, and synthesize structural matrix proteins makes them indispensable during wound healing (3).

Activated fibroblasts produce collagen, elastin, proteoglycans, glycosaminoglycans, laminin, and fibronectin while also secreting cytokines and growth factors that regulate angiogenesis, epithelialization, immune cell recruitment, and matrix remodeling (3,11). Differentiation into contractile myofibroblasts further contributes to wound contraction. Impaired fibroblast migration is strongly associated with delayed wound healing and chronic ulcers, particularly in diabetes (7,17).

Because fibroblast migration is among the earliest events during tissue repair, the in vitro scratch assay has become one of the most widely accepted experimental models for evaluating wound healing efficacy (18,19). This assay enables quantitative assessment of cellular migration in response to biomaterials, drugs, and growth factors under controlled laboratory conditions.

1.4 Extracellular Matrix Remodeling During Wound Healing

The extracellular matrix serves as a dynamic structural framework that regulates cellular adhesion, migration, proliferation, differentiation, and signaling (16,20). Major extracellular matrix components involved in wound repair include collagen, elastin, laminin, fibronectin, hyaluronic acid, and proteoglycans (20).

Matrix remodeling is tightly regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Imbalance between these molecules contributes to chronic wounds, diabetic ulcers, excessive scar formation, and impaired tissue repair (16,20).

Placental membranes naturally contain abundant extracellular matrix proteins that closely resemble native human tissues, providing a favorable environment for fibroblast attachment, migration, proliferation, and matrix deposition (21,22).

1.5 Human Placental Membranes as Emerging Biomaterials in Regenerative Medicine

Human placental membranes have emerged as promising biological scaffolds owing to their unique structural composition, immunological privilege, and abundance of extracellular matrix proteins, cytokines, antimicrobial peptides, and growth factors (21–24). Previously regarded as medical waste, placental tissues are now widely recognized for their regenerative properties.

These membranes exhibit low immunogenicity, anti-inflammatory, antimicrobial, anti-fibrotic, and anti-scarring activities while supporting cellular adhesion, proliferation, and differentiation (22–24). Unlike conventional wound dressings that mainly provide physical protection, placental membranes actively stimulate tissue regeneration through sustained release of bioactive molecules and by providing a natural extracellular matrix scaffold (22,25).

Their clinical applications now include diabetic foot ulcers, venous leg ulcers, burns, ophthalmic reconstruction, tendon injuries, oral surgery, and orthopedic tissue repair (24–26).

1.6 Biological Characteristics of the Amnion Membrane

The human amniotic membrane constitutes the innermost layer of the fetal placenta and is composed of a single epithelial cell layer, a thick basement membrane, and an avascular stromal matrix (21,22). Owing to its structural similarity to the native basement membrane of human skin, the amniotic membrane has become one of the most extensively investigated biological materials in regenerative medicine and wound care (22,27).

The extracellular matrix (ECM) of the amnion is rich in collagen types III, IV, V, and VII, laminin, fibronectin, elastin, hyaluronic acid, and proteoglycans, all of which play essential roles in cell adhesion, migration, proliferation, and tissue remodeling (20–22). In addition to its structural components, the amniotic membrane serves as a natural reservoir of biologically active molecules that regulate multiple stages of wound healing.

Numerous studies have demonstrated that the amniotic membrane contains high concentrations of epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), and insulin-like growth factor (IGF), all of which collectively promote fibroblast proliferation, epithelialization, angiogenesis, collagen synthesis, and extracellular matrix remodeling (12,22,23,28).

Besides growth factors, the amniotic membrane possesses potent anti-inflammatory activity through the release of cytokines such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1Ra), which suppress excessive inflammatory responses and promote regenerative rather than fibrotic healing (22,24). The membrane also exhibits antimicrobial activity owing to the presence of naturally occurring antimicrobial peptides capable of reducing bacterial colonization and minimizing wound infection (24,29).

Another important characteristic of the amniotic membrane is its remarkably low immunogenicity. The membrane expresses minimal levels of major histocompatibility complex (MHC) antigens, thereby reducing immune rejection following transplantation and making it suitable for allogeneic clinical applications (22,24).

1.7 Biological Contribution of the Chorion Membrane

Although the amniotic membrane has traditionally received greater scientific attention, the chorionic membrane also possesses significant regenerative properties that contribute substantially to tissue repair (23,30). Located immediately beneath the amnion, the chorion is a thicker connective tissue layer containing abundant extracellular matrix proteins, trophoblast-derived signaling molecules, and mesenchymal stromal cells.

Compared with the amnion alone, the chorion contains relatively higher concentrations of several angiogenic growth factors and structural extracellular matrix proteins that facilitate vascularization, collagen deposition, and tissue remodeling (23,30). Furthermore, the chorion serves as an additional source of cytokines and immunomodulatory molecules capable of regulating inflammatory responses during wound healing.

The greater mechanical strength of the chorion also improves the handling characteristics and durability of placental grafts during surgical application (23). Consequently, preservation of both placental layers as a composite amnion–chorion membrane provides a biologically active scaffold that combines the regenerative properties of each tissue while maintaining structural integrity.

Proteomic investigations have demonstrated that composite amnion–chorion membranes release higher concentrations of growth factors over prolonged periods compared with isolated amniotic membrane preparations (23,30). This sustained release profile may enhance fibroblast migration, angiogenesis, collagen deposition, and extracellular matrix remodeling, thereby accelerating wound healing.

1.8 Growth Factors and Cytokines Responsible for Placental Membrane-Mediated Wound Healing

The regenerative capacity of placental membranes is primarily attributed to their diverse repertoire of growth factors and cytokines that regulate multiple stages of wound repair (12,22,23). These signaling molecules function synergistically to coordinate inflammation, cell proliferation, angiogenesis, extracellular matrix synthesis, and tissue remodeling.

Vascular endothelial growth factor (VEGF) plays a central role in angiogenesis by stimulating endothelial cell proliferation and neovascularization, thereby ensuring adequate oxygen and nutrient supply to regenerating tissue (13,28). Platelet-derived growth factor (PDGF) functions as a potent chemoattractant for fibroblasts while promoting collagen synthesis and extracellular matrix deposition (12,13).

Epidermal growth factor (EGF) accelerates epithelial cell proliferation and migration, facilitating rapid re-epithelialization of injured tissue (12). Transforming growth factor-beta (TGF-β) regulates fibroblast differentiation, extracellular matrix synthesis, collagen deposition, and scar formation, whereas fibroblast growth factor (FGF) enhances fibroblast proliferation, angiogenesis, and granulation tissue formation (12,13).

In addition to growth factors, placental membranes contain several immunomodulatory cytokines capable of suppressing excessive inflammation and promoting regenerative healing (22,24). Collectively, these bioactive molecules establish a favorable microenvironment that enhances wound closure while minimizing fibrosis and chronic inflammation.

1.9 Clinical Applications of Placental Membranes in Wound Management

The clinical application of human placental membranes has expanded considerably owing to their unique biological composition and regenerative properties (24–26). Numerous experimental and clinical studies have demonstrated their effectiveness in accelerating wound closure, reducing inflammation, promoting angiogenesis, and improving tissue remodeling.

One of the most extensively investigated indications is the treatment of diabetic foot ulcers (DFUs). Diabetes impairs wound healing through persistent inflammation, diminished angiogenesis, impaired fibroblast function, defective collagen synthesis, and increased susceptibility to infection (7,8). Placental membranes compensate for these deficiencies by providing extracellular matrix proteins and growth factors that enhance cellular migration, granulation tissue formation, and re-epithelialization (25,26).

Beyond diabetic wounds, placental membranes have demonstrated encouraging clinical outcomes in venous leg ulcers, pressure ulcers, burns, traumatic wounds, oral soft tissue reconstruction, ophthalmic surface repair, and orthopedic applications (24–26). Their intrinsic anti-inflammatory, antimicrobial, and anti-fibrotic properties contribute to reduced scar formation and improved tissue regeneration.

More recently, placental membranes have also been investigated as biological scaffolds for stem cell delivery, tissue engineering, hydrogel-based wound dressings, and advanced regenerative therapies, highlighting their growing importance in translational medicine (21,23).

1.10 Current Knowledge Gap

Despite the increasing clinical use of placental membranes, several important scientific questions remain unresolved. Most previous studies have investigated either amniotic membrane or composite placental grafts independently, with relatively few directly comparing the regenerative performance of amnion and amnion–chorion membranes under standardized experimental conditions (23,30).

Additionally, variations in donor characteristics, tissue processing, preservation techniques, sterilization procedures, and extraction protocols may influence the biological activity of placental grafts and contribute to inconsistencies among published studies (21,23).

Although numerous investigations have characterized the biochemical composition of placental tissues, relatively few have evaluated how these bioactive components influence fibroblast migration, which represents one of the earliest cellular events during wound repair (18,19). Comparative evaluation using standardized in vitro scratch assays therefore provides valuable mechanistic insight into the regenerative potential of different placental membrane preparations.

12. Materials and Methods

2.1 Study Design

This experimental **in vitro** study was designed to comparatively evaluate the wound healing potential of dehydrated human amnion membrane and dehydrated human amnion–chorion membrane using a fibroblast scratch assay model. Adult dermal fibroblasts (ADF) were

selected because fibroblasts are the principal cells responsible for extracellular matrix deposition, collagen synthesis, granulation tissue formation, and tissue remodeling during cutaneous wound healing (3,11).

The experimental workflow consisted of four major phases:

1. Preparation of placental membrane extracts.
2. Culture and maintenance of adult dermal fibroblasts.
3. In vitro scratch assay.
4. Quantitative measurement of wound closure using ImageJ software (31).

Positive and negative control groups were included to validate the experimental system.

2.2 Preparation of Human Placental Membrane Extracts

Commercially processed dehydrated human amnion membrane and dehydrated human amnion–chorion membrane were used throughout the investigation.

All procedures were performed aseptically inside a Class II biosafety cabinet. Approximately 10 mg of each dehydrated placental membrane was aseptically weighed using a calibrated analytical balance and transferred into sterile microcentrifuge tubes. Each tissue sample was finely minced using sterile surgical scissors to maximize the surface area available for protein extraction.

The minced tissues were suspended in 1 mL Dulbecco's Phosphate Buffered Saline (DPBS) and incubated at 2–8°C for 24 hours, allowing passive diffusion of soluble proteins, cytokines, chemokines, and extracellular matrix components into the extraction buffer (21,22).

Following incubation, the tissues were homogenized using a sterile tissue homogenizer and centrifuged at 4,000 × g for 10 minutes. The supernatant containing placental membrane-derived proteins and growth factors was carefully collected and used immediately for subsequent biological experiments.

Extract volumes of 50 µL, 100 µL, 200 µL, and 400 µL were prepared while maintaining a protein concentration of approximately 1 mg/mL.

2.3 Cell Culture

Primary adult dermal fibroblasts (ADF) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics under standard culture conditions at 37°C in a humidified incubator containing 5% CO_2 (32).

Cells were routinely examined using an inverted phase-contrast microscope to evaluate morphology, adherence, and confluency. Culture medium was replaced every two to three days to maintain optimal cellular growth.

For scratch assay experiments, fibroblasts were seeded into sterile 12-well tissue culture plates at a density sufficient to achieve 80–90% confluency within 24 hours.

Only healthy fibroblast cultures exhibiting characteristic spindle-shaped morphology and high cell viability were used for wound healing experiments.

2.4 Experimental Groups

The study consisted of four experimental groups:

Positive Control (PC)

Fibroblasts cultured in DMEM supplemented with 10% fetal bovine serum served as the positive control because serum contains endogenous growth factors capable of stimulating cellular proliferation and migration (32).

Negative Control (NC)

Fibroblasts cultured in α-Minimum Essential Medium (α-MEM) without fetal bovine serum served as the negative control.

Amnion Treatment Group

Fibroblasts were cultured in complete medium supplemented with 1 mg equivalent of amnion membrane extract.

Amnion–Chorion Treatment Group

Fibroblasts were cultured in complete medium supplemented with 1 mg equivalent of amnion–chorion membrane extract.

All groups were maintained under identical incubation conditions.

2.5 In Vitro Scratch Assay

The wound healing potential of placental membrane extracts was evaluated using the scratch wound assay, one of the most widely accepted in vitro methods for assessing collective cell migration (18,19,33).

Following formation of confluent fibroblast monolayers, a standardized linear scratch was generated using a sterile 1-mL pipette tip. Uniform pressure was applied to produce wounds of comparable width among all treatment groups.

Detached cells were removed by washing with sterile DPBS, after which fresh medium containing the respective experimental treatments was added.

Microscopic images were acquired immediately after scratch formation (0 hour) and after 20 hours using an inverted phase-contrast microscope equipped with a digital camera at 4× magnification.

Care was taken to maintain identical magnification, illumination, and imaging fields throughout the experiment.

2.6 Quantitative Image Analysis

Scratch closure was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA), which is widely accepted for digital wound measurement and cell migration analysis (31).

Images captured at 0 h and 20 h were calibrated using the microscope scale bar before measurement.

Scratch width was measured at multiple predetermined locations across each wound, and the average wound width was calculated.

The percentage of wound closure was determined using the following equation:

Wound Closure (%) = [(Initial Scratch Width − Final Scratch Width) / Initial Scratch Width] × 100

Higher wound closure percentages indicated greater fibroblast migration and regenerative activity.

2.7 Evaluation of Fibroblast Migration

Fibroblast migration served as the primary biological endpoint because migration of these cells represents one of the earliest events during connective tissue repair (3,11).

Migration efficiency was determined by comparing wound closure percentages among the four experimental groups.

2.8 Experimental Quality Control

To improve reproducibility, the following quality control measures were implemented:

- Healthy fibroblast cultures with typical spindle-shaped morphology were used.
- Cell monolayers were maintained at 80–90% confluency before scratch creation.
- Scratches were generated using identical pipette tips and standardized technique.
- Detached cells were completely removed before treatment application.
- All treatment groups were incubated simultaneously under identical environmental conditions.
- Images were acquired using identical microscope settings.
- Image analysis followed standardized ImageJ measurement procedures (31).

2.9 Outcome Measures

The primary outcome measure was the percentage of wound closure, representing fibroblast migration.

Secondary observations included fibroblast morphology, cell density, and migration pattern.

2.10 Statistical Analysis

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

Where applicable, statistical comparisons among treatment groups were performed using one-way analysis of variance (ANOVA) followed by an appropriate post hoc multiple comparison test.

A p-value < 0.05 was considered statistically significant (34).

3. Results

3.1 Comparative Evaluation of Fibroblast Migration

The regenerative potential of dehydrated human amnion membrane and dehydrated human amnion–chorion membrane extracts was evaluated using an in vitro scratch assay to determine their ability to promote fibroblast migration and wound closure. Adult dermal fibroblasts were cultured under standardized experimental conditions, and wound closure was monitored over a 20-hour incubation period. Positive and negative controls were included to validate the experimental system and provide baseline comparisons.

Immediately after scratch induction (0 h), all experimental groups exhibited comparable wound widths, confirming that a uniform and reproducible scratch had been generated across the fibroblast monolayers. The similarity in initial scratch dimensions minimized experimental variability and ensured that subsequent differences in wound closure resulted from the biological activity of the respective treatment groups rather than inconsistencies in scratch formation.

Microscopic examination after 20 hours demonstrated distinct differences in fibroblast migration among the four experimental groups. Both placental membrane preparations enhanced fibroblast migration compared with the untreated control, while the amnion–chorion membrane produced the greatest degree of wound closure.

3.2 Negative Control

Fibroblasts maintained in α-MEM without fetal bovine serum exhibited minimal migratory activity during the experimental period. Only a slight reduction in scratch width was observed after 20 hours, indicating limited spontaneous migration in the absence of exogenous growth factors.

The initial wound width measured 0.962 mm, which decreased marginally to 0.865 mm after incubation, corresponding to a wound closure of 10.08%. The persistence of a wide acellular gap confirmed that serum deprivation markedly reduced fibroblast migration.

These observations demonstrate that basal culture conditions lacking biological stimulatory factors are insufficient to promote efficient wound closure, thereby validating the requirement for growth factor supplementation during tissue repair.

3.3 Positive Control

Fibroblasts cultured in DMEM supplemented with 10% fetal bovine serum demonstrated substantial migration into the scratched region during the 20-hour incubation period.

The scratch width decreased from 1.006 mm at baseline to 0.399 mm, corresponding to 60.33% wound closure.

Microscopic examination revealed active migration of spindle-shaped fibroblasts from both wound margins toward the center of the scratched area, producing progressive narrowing of the wound gap. These findings confirmed that the experimental model functioned appropriately and that fibroblasts retained normal migratory capacity under standard culture conditions.

3.4 Amnion Membrane Treatment

Fibroblasts treated with dehydrated human amnion membrane extract demonstrated greater migratory activity than the positive control.

The initial scratch width measured 0.995 mm, which decreased to 0.269 mm after 20 hours, representing 72.96% wound closure.

Microscopic examination showed extensive fibroblast migration from both wound edges with considerable occupation of the scratched region. The migrating cells maintained their characteristic spindle-shaped morphology and formed a dense cellular front advancing toward the center of the wound.

Compared with the serum-treated control, the amnion-treated cultures exhibited accelerated closure of the artificial wound, indicating enhanced regenerative activity.

3.5 Amnion–Chorion Membrane Treatment

Among all experimental groups, fibroblasts treated with dehydrated human amnion–chorion membrane extract exhibited the highest regenerative response.

The scratch width decreased from 1.037 mm at baseline to 0.171 mm after 20 hours, corresponding to 83.51% wound closure, the highest value observed in the present study.

Microscopic evaluation demonstrated extensive fibroblast migration across the wound gap, resulting in near-complete closure of the scratched region. Cells were uniformly distributed throughout the healing area, suggesting coordinated collective migration and enhanced regenerative activity.

3.6 Comparative Analysis of Wound Closure

Comparison of all experimental groups demonstrated a clear treatment-dependent enhancement in fibroblast migration.

The percentage of wound closure increased in the following order:

Negative Control < Positive Control < Amnion Membrane < Amnion–Chorion Membrane

These findings indicate that both placental membrane-derived extracts possess significant regenerative activity capable of enhancing fibroblast migration beyond that achieved with conventional serum supplementation.

The superior performance of the amnion–chorion membrane suggests that the combined biological contributions of both placental layers enhance cellular migration and tissue repair.

Table 1. Effect of Placental Membrane Extracts on Fibroblast Migration

Experimental Group	Initial Scratch Width (mm)	Final Scratch Width (mm)	Wound Closure (%)
Negative Control	0.962	0.865	10.08
Positive Control	1.006	0.399	60.33
Amnion Membrane	0.995	0.269	72.96
Amnion–Chorion Membrane	1.037	0.171	83.51

3.7 Summary of Findings

The present investigation demonstrated that both placental membrane preparations enhanced fibroblast migration compared with untreated controls. Although the amniotic membrane alone promoted substantial wound healing activity, incorporation of the chorion layer produced an even greater regenerative response, suggesting a synergistic interaction between the two placental tissues.

Overall, the findings indicate that the dehydrated human amnion–chorion membrane possesses superior regenerative potential compared with the dehydrated human amnion membrane, supporting its further evaluation as a biologically active scaffold for regenerative medicine and advanced wound management.

4. Discussion

Wound healing is a highly orchestrated biological process involving a series of overlapping cellular and molecular events that collectively restore tissue integrity following injury. Successful tissue repair depends on the coordinated interaction of inflammatory cells, fibroblasts, endothelial cells, keratinocytes, extracellular matrix proteins, and numerous signaling molecules (1,2,11). The development of biologically active wound dressings capable of actively stimulating these regenerative processes has therefore become a major focus of regenerative medicine (21,22).

The present study comparatively evaluated the regenerative potential of dehydrated human amnion membrane and dehydrated human amnion–chorion membrane using an in vitro fibroblast scratch assay. The results demonstrated that both placental membrane preparations significantly enhanced fibroblast migration compared with the untreated control, while the amnion–chorion membrane consistently produced the greatest percentage of wound closure. These findings support previous reports describing placental membranes as biologically active scaffolds capable of accelerating tissue repair through multiple complementary mechanisms (23,24,35).

The scratch assay remains one of the most widely accepted in vitro models for evaluating wound healing because fibroblast migration represents one of the earliest and most critical events during tissue regeneration (18,19,33). Following tissue injury, fibroblasts migrate into

the wound bed, synthesize extracellular matrix proteins, secrete growth factors, regulate collagen deposition, and facilitate granulation tissue formation. Consequently, increased fibroblast migration observed during scratch assays is considered an important indicator of regenerative potential (3,11).

In the present investigation, fibroblasts cultured under serum-free conditions exhibited only minimal spontaneous migration, resulting in approximately 10% wound closure after 20 hours. This observation confirms that fibroblast migration is highly dependent upon biological stimulation provided by exogenous growth factors and cytokines. Similar findings have been reported in previous studies demonstrating that serum deprivation significantly delays fibroblast migration and impairs wound closure (17,36).

Conversely, fibroblasts maintained in complete growth medium supplemented with fetal bovine serum demonstrated substantially greater migration, achieving more than 60% wound closure. Fetal bovine serum contains numerous endogenous growth factors, including PDGF, EGF, IGF, TGF-β, and FGF, which stimulate cell proliferation, migration, and extracellular matrix synthesis (12,13). The positive control therefore provided an appropriate physiological reference against which the regenerative performance of placental membrane extracts could be evaluated.

Treatment with dehydrated human amnion membrane resulted in a marked improvement in fibroblast migration compared with the positive control. Fibroblasts exposed to amnion extract migrated more rapidly toward the wound area, indicating that the membrane released biologically active molecules capable of stimulating tissue regeneration. The amniotic membrane is recognized as a natural extracellular matrix scaffold enriched with collagen, laminin, fibronectin, elastin, hyaluronic acid, and numerous growth factors that collectively regulate fibroblast proliferation and migration (21,22,27).

The regenerative activity observed following amnion treatment is likely attributable to the simultaneous release of multiple growth factors rather than the action of a single molecule. Epidermal growth factor promotes epithelial cell proliferation and migration, platelet-derived growth factor recruits fibroblasts and stimulates collagen synthesis, transforming growth factor-β regulates extracellular matrix deposition, while vascular endothelial growth factor promotes angiogenesis necessary for tissue repair (12,13,28). The coordinated action of these mediators creates a microenvironment favorable for wound healing.

Among all treatment groups, the amnion–chorion membrane exhibited the highest regenerative activity, producing 83.51% wound closure. This finding suggests that incorporation of the chorionic layer provides additional biological advantages beyond those offered by the amnion alone. Previous proteomic analyses have demonstrated that composite amnion–chorion membranes contain a broader spectrum of extracellular matrix proteins and higher concentrations of angiogenic growth factors than isolated amniotic membrane preparations (23,30,35). These characteristics may explain the superior fibroblast migration observed in the present study.

The enhanced performance of the amnion–chorion membrane may also be attributed to the synergistic interaction between structural extracellular matrix proteins and bioactive cytokines derived from both placental layers. The chorion contributes additional collagen, fibronectin, laminin, and angiogenic mediators, thereby creating a more favorable biological scaffold for fibroblast attachment, migration, and extracellular matrix remodeling (23,30).

Previous clinical investigations have demonstrated encouraging outcomes following the application of amniotic and amnion–chorion membranes in diabetic foot ulcers, venous leg ulcers, burns, ocular surface reconstruction, and other chronic wounds (24–26,37). These studies consistently report accelerated wound closure, reduced inflammation, improved angiogenesis, and decreased scar formation. The enhanced fibroblast migration observed in the present investigation provides a cellular mechanism that may partially explain these favorable clinical outcomes.

The biological activity of placental membranes extends beyond fibroblast migration alone. Several investigators have reported that these tissues possess antimicrobial, anti-inflammatory, anti-fibrotic, and immunomodulatory properties that collectively contribute to regenerative healing (22,24,29). Furthermore, the low immunogenicity of placental tissues enables their clinical application with minimal risk of immune rejection, making them attractive biomaterials for regenerative medicine (22,24).

Despite the encouraging findings, several limitations should be acknowledged. First, the present investigation employed an in vitro scratch assay, which primarily evaluates fibroblast migration and does not fully replicate the complex biological environment of wound healing in vivo. Second, quantitative measurement of individual growth factors released from the placental membrane extracts was not performed. Third, molecular analyses investigating gene expression, collagen synthesis, angiogenesis, inflammatory cytokines, and signaling pathways were beyond the scope of the present study. Future investigations should incorporate proteomic analyses, transcriptomic profiling, animal wound models, and randomized clinical studies to further elucidate the mechanisms responsible for placental membrane-mediated tissue regeneration (38–40).

Overall, the findings of this study provide additional evidence supporting the use of placental membrane-derived biomaterials as promising therapeutic options for advanced wound management. The superior regenerative performance demonstrated by the amnion–chorion membrane suggests that preservation of both placental layers may provide greater biological benefit than amnion alone. These observations contribute to the growing body of literature supporting the application of placental tissues in regenerative medicine and tissue engineering.

5. Conclusion

The present study demonstrated that both dehydrated human amnion membrane and dehydrated human amnion–chorion membrane significantly enhanced fibroblast migration and promoted wound closure in an in vitro scratch assay model.

Among the two placental membrane preparations evaluated, the amnion–chorion membrane exhibited superior wound healing activity, achieving the highest percentage of wound closure compared with the amnion membrane and control groups. The enhanced regenerative performance of the amnion–chorion membrane is likely attributable to the synergistic contribution of both placental layers, which provide a broader repertoire of extracellular matrix proteins, angiogenic mediators, growth factors, and immunomodulatory molecules that collectively stimulate fibroblast migration and accelerate tissue regeneration.

The findings support the potential application of dehydrated human amnion–chorion membrane as a promising biological scaffold for the management of acute and chronic wounds,

including diabetic foot ulcers, venous leg ulcers, pressure injuries, burns, and other difficult-to-heal soft tissue defects.

Although the present investigation provides valuable evidence of enhanced fibroblast migration, future studies should incorporate quantitative analyses of growth factor release, cytokine profiling, collagen deposition, gene and protein expression, and in vivo wound models to further elucidate the molecular mechanisms underlying placental membrane-mediated tissue regeneration.

Overall, the findings provide experimental evidence that the human amniotic–chorion membrane possesses greater regenerative potential than amnion membrane alone and supports its continued development as a biologically active scaffold for regenerative medicine and advanced wound care.

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