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Review

Advances and impact of human amniotic membrane and human amniotic-based materials in wound healing application

Parisa Heydari [a], Maryam Mojahedi [a], Pouya Javaherchi [a], Maede Sharifi [a], Anousheh Zargar Kharazi [b,c,*]

[a] Department of Biomaterials Nanotechnology and Tissue Engineering, School of Advanced Technology in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Department of Biomaterials Nanotechnology and Tissue Engineering, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[c] Applied Physiology Research Center, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

Wound healing is a complicated process, especially when surgical, traumatic, burn, or pathological injury occurs, which requires different kinds of dressing covers including hydrogels, hydrocolloids, alginates foams and films for treatment. The human amniotic membrane (hAM) is a biodegradable extracellular matrix with unique and tailorable physicochemical and biological properties, generated by the membrane itself or other cells that are located on the membrane surface. It is noted as a promising aid for wound healing and tissue regeneration due to the release of growth factors and cytokines, and its antibacterial and immunosuppressive properties. Moreover, hAM has optimal physical, biological, and mechanical properties, which makes it a much better option as a regenerative skin treatment than existing alternative materials. In addition, this layer has a structure with different layers and cells with different functions, which act as a regenerative geometry and reservoir of bioactive substances and cells for wound healing. In the present work, the structural and biological features of hAM are introduced as well as the application of this layer in different forms of composites to enhance wound healing. Future studies are recommended to detect possible further functionalization to enhance the hAM effectiveness on wound healing.

1. Introduction

Wounds can be caused by various factors such as surgery, trauma, burns, or pathologic disorders like diabetes [1]. These skin defects are generally categorized into two types: chronic wounds and acute wounds [2]. Acute wounds typically undergo a healing process that leads to long-term anatomical and functional integrity [3]. In contrast, chronic wounds struggle to maintain maximum anatomical and functional integrity [4]. A successful wound-healing process involves restoring the normal anatomical structure, function, and appearance of the skin after an injury. This can be achieved by following the natural wound-healing mechanism [5].

The processes of wound healing is an intricate biological process that requires the regulated interaction of several cellular and molecular activities [6–8]. Fig. 1 illustrates the wound healing process schematically characterized into three main phases: Hemostasis and Inflammation Proliferation, and Remodeling. Different cellular and molecular processes have crucial roles in successful wound healing and functional regeneration [4,9].

Medical dressings are crucial in healthcare as they play a significant role in the healing process. They protect wounds from external harm and create a favorable environment for tissue regeneration. Different types of wounds at various stages can benefit from the use of dressings, aiding in their healing. Dry dressings, like gauze, sterile absorbent cotton, and bandages, are commonly used in medical settings due to their cost-effectiveness. However, they have limited benefits in terms of wound healing and infection prevention, mainly providing physical protection. In clinical practice, healthcare professionals frequently employ dressings such as hydrogels, hydrocolloids, alginates, foams, and films. In recent years, new materials like hydrogel, nanofibers, and sponges have been developed and used to treat chronic wounds [10,11]. Effective dressings should possess biocompatibility, flexibility, and air permeability to enhance wound healing. They should also have excellent physical swelling and antibacterial properties, as chronic wounds are

* Corresponding author at: Biomaterials Nanotechnology and Tissue Engineering Faculty, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail address: a_zargar@med.mui.ac.ir (A.Z. Kharazi).

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susceptible to exudate penetration and severe bacterial infections. Furthermore, an ideal dressing should help maintain moisture balance, promote oxygen exchange, protect against proteases, encourage the production of beneficial factors, prevent infection, aid in the removal of dead tissue, and facilitate the growth of new tissue and skin. However, currently, no single dressing can fulfill all these requirements [11].

In recent years, amniotic membrane coverings have been used in medicine as a skin replacement. The use of amniotic membrane (AM) as a dressing for chronic wounds has been proven to be beneficial. AM has antimicrobial and immunomodulatory effects, and it secretes growth factors like β-TGF and EGF, which stimulate the migration of keratinocytes and promote wound healing [12].

Human amniotic membranes (hAM) are potential biomaterials for wound healing, described to their beneficial characteristics, minimal ethical issues, and demonstrated efficacy in various study. hAM facilitates chronic wound healing and is utilized in tissue engineering and surgical reconstruction. Table 1 provides an overview of several commercially available amniotic membrane-based products, highlighting their key properties.

The study begins with a brief description of the structure and composition of the human amniotic membrane, followed by an assessment of its antibacterial effects, angiogenesis, and re-epithelialization. The review aims to provide valuable insights and perspectives to encourage further advanced studies on the design of human amniotic membrane-based wound dressings, leading to advancements in wound treatment applications.

Table 1
Summary of commercial wound dressing based human amniotic membrane.

	Commercial samples	Description	Ref
Cryopreserved	Bio-Tissue AmnioGraft®	Enhancing collagen deposition Improving cell proliferation Enhancing anti-inflammatory	[13]
	GRAFIX® Membrane	Suitable flexibility and strength properties Absorbing and eliminating without secondary surgery Exhibiting immune response	[14]
	NuCel®	Improving regeneration Increasing wound healing process Maintaining cell viability	[15]
Lyophilized	Aminofix®	Anti-inflammation activity Anti-bacterial and reducing infection risk Promotion formation new tissue	[16]
	Bio-A®	Promotion wound closure Acceleration vascularization Minimizing scar formation	[17]
	Epifix®	Protective barrier Reducing transmission diseases Exhibiting natural antimicrobial properties	[18]

2. Architecture and biological attributes of the amniotic membrane for wound healing

The human amnion is the innermost layer of fetal membranes, forming a sac that holds amniotic fluid. This sac serves as a protective barrier for the growing baby [19]. The structure of the amnion includes a monolayer of epithelium, a dense basement membrane, a layer without blood vessels, and a stromal matrix with a thickness ranging from 0.02 to 0.5 mm. The epithelial layer consists of metabolically active cuboidal and columnar cells that are in direct contact with the amniotic fluid. The basement membrane is made up of various components of the extracellular matrix (ECM), such as collagen, elastin, laminin, fibronectin, and vitronectin. These are non-collagenous glycoproteins. Additionally, the basement membrane contains glycosaminoglycans like hyaluronic acid, natural inhibitors called matrix metalloproteinases (MMPs), and biologically active factors like cytokines and growth factors, which are essential for wound healing and tissue regeneration [20]. Fig. 2 illustrates the structure and components of the hAM. In general, the human amnion is a multi-layered tissue consisting of a single layer of epithelial cells, a deep basement membrane, a compact layer, a fibroblast layer, and a spongy layer adjacent to the chorion [21]. It typically lacks nerves, muscles, and lymphatics. The epithelial cell layer is composed of a monolayer of flat, cuboidal, and columnar cells that are in direct contact with the amniotic fluid [22]. The basement membrane of the amnion is similar to those found in other areas of the body, such as

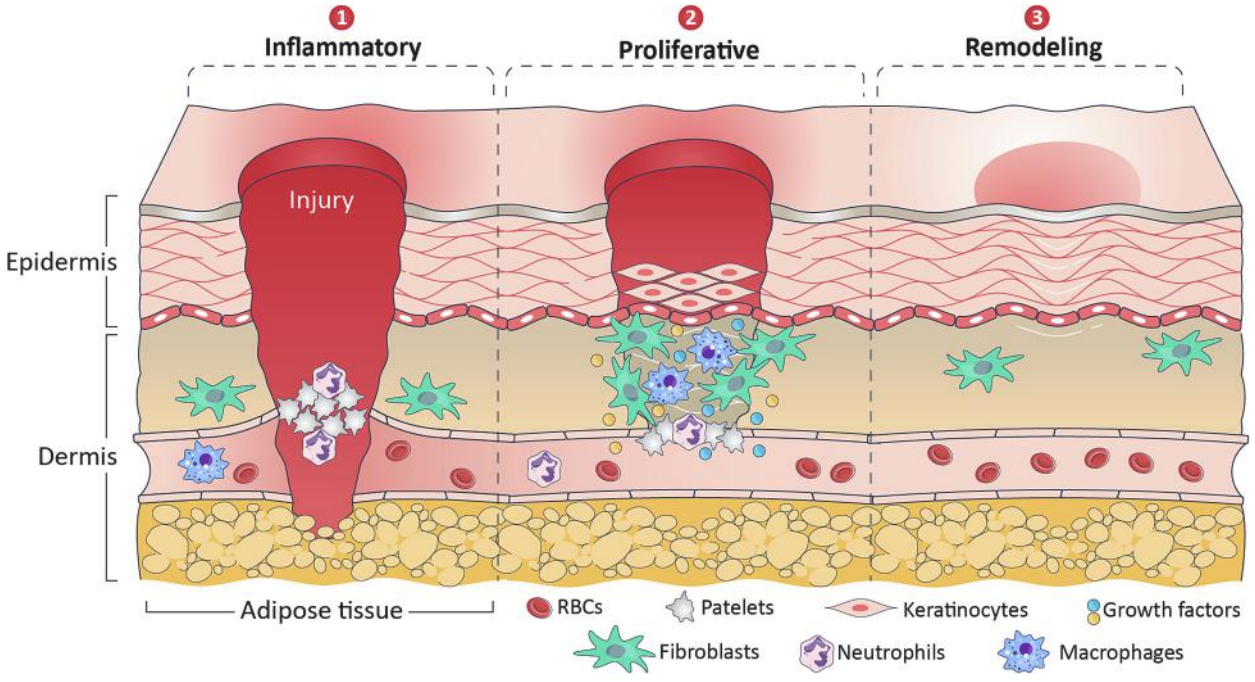

Fig. 1. The schematic of three time-dependent stages in wound healing processes containing 1) inflammatory, 2) proliferative, and 3) remodeling with various cells and biological agents involved in each step.

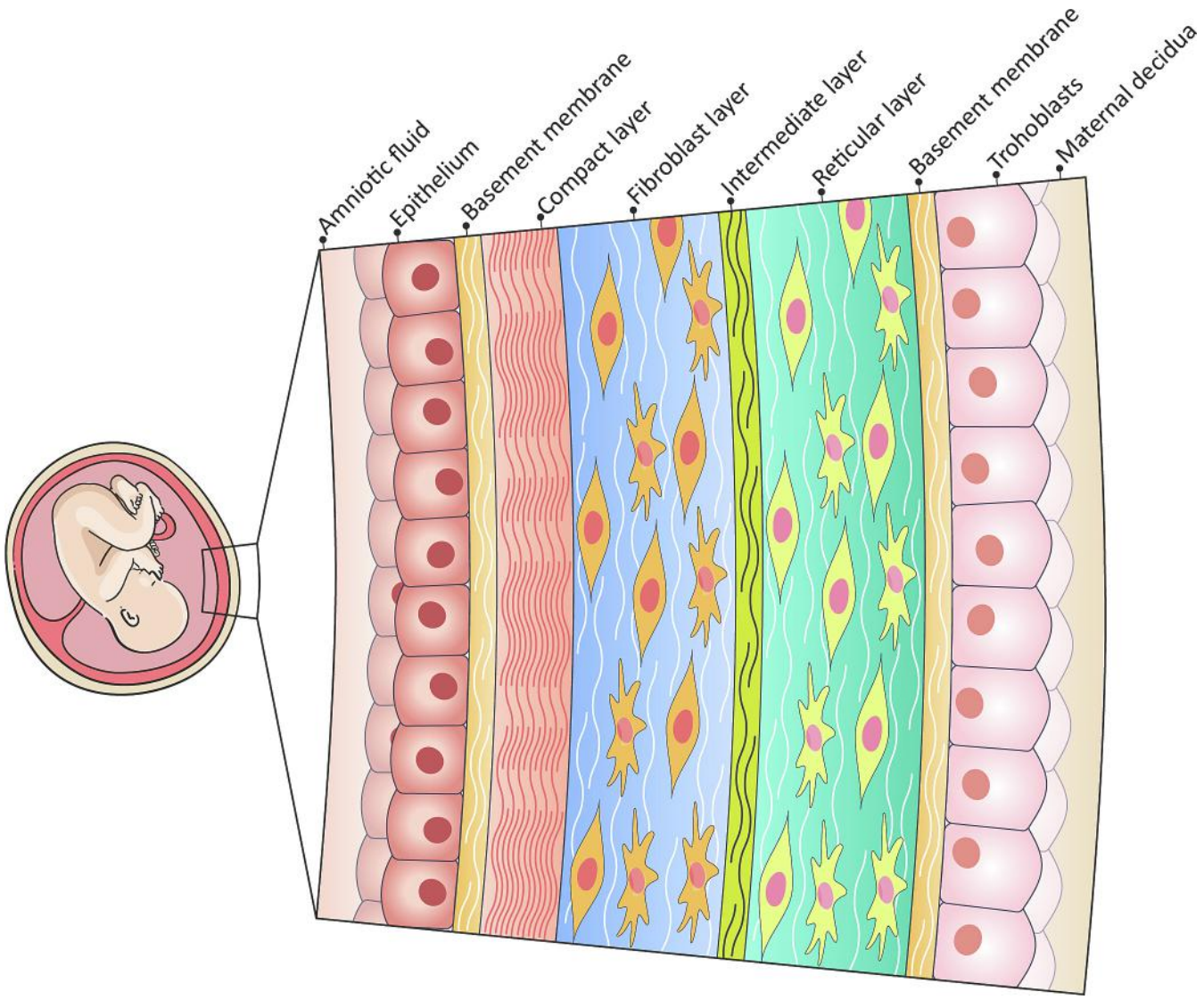

Fig. 2. The hAM anatomy may be shown by a schematic image consisting of an epithelial layer, a basement membrane, a compact layer, a fibroblast layer, and a spongy outer layer.

the conjunctiva and gingiva. The sponge layer contains hydrated proteoglycans and glycoproteins that form a non-fibrillar network alongside collagen [23]. During the 8th day of pregnancy, pluripotent cells give rise to amniotic epithelial cells [24]. Gastrulation, a crucial developmental process, may occur between days 15 and 17, during which pluripotent cells differentiate into three primary germ layers: ectoderm, mesoderm, and endoderm. This transformation is sometimes referred to as the "tipping point" [25]. Amniotic tissues consist of two types of cells with stem cell-like properties: human amniotic epithelial cells and human amniotic mesenchymal stromal cells [26]. The following sections provide a detailed examination of the biological composition, antibacterial characteristics, angiogenesis, and re-epithelialization of the hAM.

2.1. Biological agents in hAMs

Tumor necrosis factor (TNF), transforming growth factor beta-1 (TGF-β1), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatic growth factor (HGF), interleukin-4 (IL-4), IL-6, IL-8, IL-10, natural inhibitors of metalloproteases, angiogenin (ANG), dipeptidyl peptidase IV (DPPIV/CD26), serine protease inhibitor (serpin) E1 (also known as type 1 plasminogen activator inhibitor or PAI-1), insulin-like growth factors (IGF), and their binding proteins (IGFBPs) are various growth factors, cytokines, and signaling molecules found in the structure of the human amniotic membrane (hAM) [19,27–32]. For example, EGF and KGF enhance the proliferation and migration of epithelial cells, playing a crucial role in wound healing. bFGF has similar effects on keratinocytes, promoting the development of significant granular tissue. TGF-β, a versatile cytokine present in the hAM, regulates cell proliferation, migration, differentiation, and remodeling of the extracellular matrix [19]. TGF-β1 exhibits high migratory activity against fibroblasts and macrophages, promoting granulation [31].

However, when used in allogeneic transplantation, hAM has low immunogenicity due to its low antigenicity (specifically, Human Leukocyte Antigen (HLA)-DR DR negative, CD59 positive) and weakly positive expression of Major Histocompatibility Complex (MHC) Class I, along with the absence of MHC Class II on human AM. As a result, it presents minimal immunological challenges. Additionally, the hyperdry method used in HD-AM ensures that there are no living cells, significantly reducing the likelihood of causing any immunological complications [29,31,33,34]. Fig. 3 illustrates the presence of all cytokines, growth factors, and other biologically active components in the amniotic membrane.

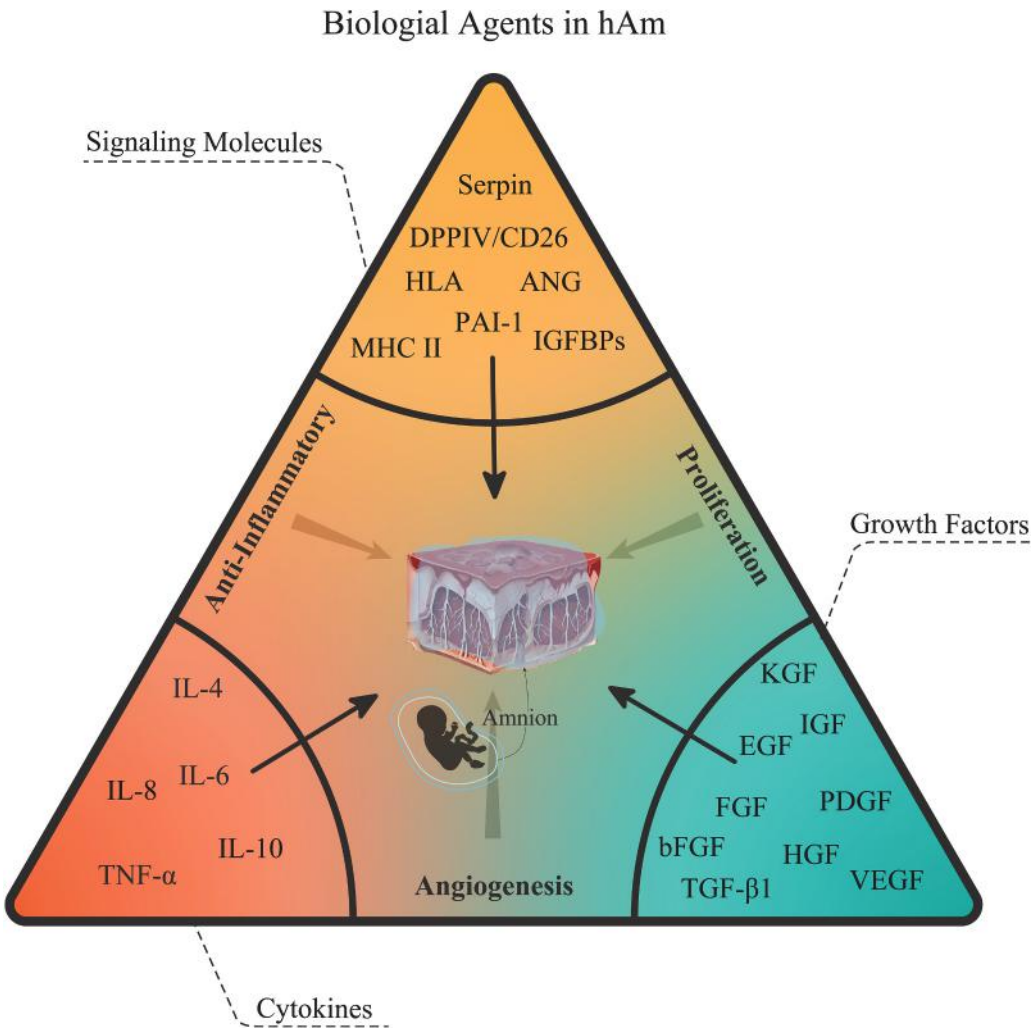

Fig. 3. Schematic illustrating specific biological agents in hAM for promoting angiogenesis, anti-inflammation and cell proliferation applications.

2.2. The anti-bacterial effect of hAM

With the increasing development of bacterial resistance, researchers are constantly searching for effective natural treatments [35]. Another notable characteristic of this membrane is its ability to fight against bacteria. It has a significant antimicrobial impact on various bacterial and fungal infections [36]. This makes it beneficial for healing skin and tissues that are prone to infections [37,38]. The antibacterial properties of the amniotic membrane also help prevent fetal ascending infections [39]. Maternal bacterial infections were found to be the cause of preterm labor and fetal and neonatal diseases The amniotic membrane's natural antimicrobial compounds are innate components of the immune system that provide protection against Gram-negative and Gram-positive bacteria, as well as fungal and viral infections [40,41].

The antibacterial properties of the amniotic membrane are demonstrated through a complex combination of physical and biological processes [42]. Due to its compact multilayered composition of epithelial cells, basement membrane, and stromal layers, it serves as a physical barrier that successfully prevents bacterial infiltration and colonization of the underlying tissue [43,44]. Moreover, the membrane includes several kinds of antibacterial constituents, including as antimicrobial peptides, growth factors, and cytokines [45,46]. These components synergistically interact to disturb microbial membranes, facilitate wound healing, regulate immunological responses, and inhibit inflammation, all of which contribute to the antibacterial effectiveness of the membrane.

Furthermore, in addition to its direct antibacterial properties, the amniotic membrane also shows promise in preventing the development of biofilms. Biofilms, which are formations of bacterial populations enclosed in a protective matrix, provide a substantial obstacle to traditional antibiotics [47]. Inhibition of bacterial attachment to wound surfaces by the amniotic membrane can impede the development of biofilms. Moreover, its capacity to expedite the process of wound healing decreases the duration during which bacteria may form biofilms, therefore augmenting its antibacterial characteristics significantly [45].

Dallal et al. [48] demonstrated the inhibitory effect of amniotic membrane on common bacterial strains, including *E. coli* (ATCC25922), *S. enterica* (BAA-708), and *P. aeruginosa* (ATCC27853). However, the standard strains of *K. pneumoniae* (ATCC7881) and *E. faecalis* (ATCC29212) showed resistance to the antibacterial properties of the amniotic membrane. Kjaergaard et al. [49] examined the antibacterial effect of amniotic and chorionic membranes on strains of Streptococcus group A, Streptococcus Group B, *S. aureus*, *S. saprophyticus*, and *E. faecalis* and reported positive results in terms of growth inhibition and inhibition zone diameter in Streptococcus Group A, *S. aureus*, and *S. saprophyticus*. Elafin (a skin-dependent antileukoproteinase) and secretory leukocyte protease inhibitor (SLPI) are two antimicrobial peptides found in whey acidic peptide (WAP) [50,51].

2.3. The angiogenesis and re-epithelialization of hAM

The human amniotic membrane (hAM) possesses a secondary set of

peptides that contribute to innate immunity. These peptides can be classified into two primary groups: α-defensin and β-defensin [52]. Neutrophils typically contain α-defensins, while paneth cells of the small intestine and other epithelial tissues have β-defensins. Additionally, the hAM is composed of natural substances such as lactoferrin and interleukin-1 receptor blockers, which help minimize inflammation and bacterial infection [41]. Moreover, the amniotic membrane contains angiogenic factors such as vascular endothelial growth factor and basic fibroblast growth factor, which promote neovascularization and revascularization [30]. hAM application provides several benefits over skin transplantation. The use of hAM in clinical treatment has been reported for over a century, with donated placenta being the most commonly used source for wound healing [53]. The hAM can be used either denuded (without the amniotic epithelium) or intact (without de-epithelialization) [54]. While fresh hAM was previously used in therapeutic settings, it is now considered impractical [55]. Due to its biochemical and physiological cues that mimic the natural microcellular milieu, the hAM is regarded as one of the most efficient functional biomaterials. Basic hAM degrades rapidly, which limits its use for repairing extensive or full-thickness skin defects, as it may break down before complete tissue healing occurs. Therefore, further research and development are necessary to fully explore the potential of hAM in the field of tissue engineering [30]. Given its unique structure and biological properties, including various bioactive substances, the hAM is an ideal biological dressing [56].

Re-epithelialization is the process of resurfacing a skin wound with fresh epithelium from the wound borders to the center of excisional damage. This generates a continuous rebuilding of a differentiated epidermis [57]. The mechanisms by which the amniotic membrane induces re-epithelialization are complex. Amniotic membrane has been shown to promote wound healing by accelerating keratinocyte migration from the wound edge and promoting differentiation, resulting in the formation of intact epithelium [58]. It has been hypothesized that the stimulatory effects on epithelialization from the wound bed and/or wound edge are mediated by progenitor cells and growth factors released by the amniotic membrane. Maintaining the integrity of the stromal matrix and basement membrane is also important for the healing efficacy of the amniotic membrane. Preservation of these structures promotes fast re-epithelialization. However, it remains unclear which cell type in the amniotic membrane is responsible for its positive effects on chronic wound epithelialization [59].

In normal wound healing, keratinocytes migrate, proliferate, and differentiate to maintain the epidermal barrier structure. Keratinocyte migration is one of the earliest and most crucial steps in the wound-healing process. Keratinocytes at wound edges undergo morphological changes in response to growth factors (TNF-α), cytokines (IL-1), and extracellular matrix, resulting in protrusive adhesions and cytoskeleton rearrangements. In a paracrine manner, IL-1 and TNF trigger dermal fibroblasts to produce FGF-7, which promotes epithelialization and initiates the inflammatory cascade [60–62].

Recent research has shown that the human amniotic membrane (hAM) has a successful effect on chronic wounds, particularly in the process of re-epithelialization. Previous studies have focused on the impact of hAM on wound healing using cell models. When hAM is present, it triggers the activation of the mitogen-activated protein (MAP) kinase and c-Jun N-terminal kinase (JNK) pathways in keratinocytes, resulting in an increased synthesis of c-Jun. Transforming growth factor-beta (TGF-β) is an essential modulator of wound healing, playing a pivotal function in the regeneration of epithelial cells and the restoration of tissues [63]. Although the accumulation of TGF-β is crucial for the process of wound healing and reaches its highest point during the inflammatory phase, its continued presence in chronic wounds can lead to severe scarring and inhibited tissue regeneration. The human amniotic membrane (hAM) exhibits a subtle equilibrium in regulating TGF-β signaling, which enhances its therapeutic effectiveness [59].

Through epidermal growth factor (EGF) signaling, hAM precisely modulates the TGF-β pathway. This fine-tuning includes the simultaneous increase and decrease of TGF-β signaling, therefore maintaining the preservation of the necessary levels for the ideal potential wound healing. The upregulation of TGF-β can stimulates cell proliferation, migration, and the deposition of extracellular matrix. Nevertheless, total inhibition of TGF-β signaling did not increase hAM-induced migration, indicating the need for a carefully managed homeostasis. These findings indicate that hAM successfully regulates TGF-β signaling, facilitating a prompt and regulated reaction, prevents excessive formation of scar tissue, and promotes appropriate regeneration of tissues [64,65]. In terms of migratory machinery, hAM affects the dynamics of focal adhesions, resulting in a faster remodeling process with a high turnover rate. This is evident in the activation of proteins like Paxillin by hAM [59]. Furthermore, preserving the integrity of the basement membrane and stromal matrix has been found to boost the healing effectiveness of hAM, which is crucial in permitting fast re-epithelialization. Furthermore, hAM releases progenitor cells and growth factors that stimulate epithelialization from the wound bed and/or tip. It is also crucial to preserve the cohesiveness of the stromal matrix and basement membrane, as it enhances the healing effectiveness of the basement and allows for faster re-epithelialization [66]. The diverse cell types present in hAM contribute to its ability to heal wounds, and there is no specific hAM cell type responsible for its beneficial effects on chronic wound epithelialization [67].

Research suggests that a conditioned medium consisting of human amniotic epithelial cells (hAECs) can mimic some of the effects of hAM on keratinocytes, but the extracellular matrix (ECM) of the hAM basal membrane (BM) plays a role in preserving or altering the capabilities and features of amniotic cells [68]. The structure of the BM is similar to that of hAM [54] and further investigation is needed to understand how these proteins contribute to creating an optimal environment for hAECs [69]. It is important to consider the structure of the BM to ensure the proper condition of hAECs and maximize their therapeutic potential. Additionally, more research is needed to determine the importance of hAECs and human amniotic mesenchymal stromal cells (hAMSCs) in wound healing.

2.4. The anti-fibrotic activity of hAM

The amniotic membrane has several important functions in wound healing and skin tissue engineering. It has anti-fibrotic properties that aid in anti-scarring regeneration and overall wound healing. The anti-fibrotic properties of hAM are due to its composition, which includes collagen, extracellular matrix proteins, and physiologically active cells that produce growth factors, cytokines, and chemokines. It also contains anti-fibrotic cytokines, growth factors, and extracellular proteins that enhance its therapeutic capacity. These components help reduce fibrosis and scarring, promoting more effective regenerative repair [70].

Interestingly, the amniotic membrane's ability to inhibit fibrosis may be connected to its immune regulatory capacity. Regulatory T cells are important in maintaining immune system balance and can regulate the healing of skin wounds and decrease the formation of fibrous tissue [71]. Additionally, it inhibits the activation of fibroblasts by reducing the production of transforming growth factor beta (TGF-β), which in turn has an anti-fibrotic effect, and decreases protease activity by releasing tissue inhibitors of TIMPs [43].

TGF-β is predominantly recognized for its function as a critical mediator of fibrosis, in which it facilitates the activation and proliferation of fibroblasts [72]. The antifibrotic effects of hAM are achieved by suppressing the TGF-β1 signaling pathway, which is essential for the development of fibrosis. hAM prevents the transformation of fibroblasts into myofibroblasts by reducing TGF-β1 expression, thereby reducing the accumulation of collagen in the tissue [73]. TGF-β is a critical activator of fibroblasts and other cell types, including macrophages, epithelial cells, and vascular cells, which in turn regulates fibrosis [74]. Furthermore, hAM enhances its antifibrotic efficacy by reducing

proinflammatory cytokines, including TNF-α and IL-6 [73]. Furthermore, the membrane induces apoptosis or reverses the phenotype of activated myofibroblasts, thereby slowing the progression of fibrosis. hAM is a prospective therapeutic strategy for the treatment of fibrosis due to its combined anti-inflammatory and antiscarring effects [73,75].

To summarize, the amniotic membrane has a wide range of functions in wound healing and skin tissue engineering, particularly in preventing fibrosis. The process incorporates the inherent characteristics of AM, including its elevated levels of matrix and growth factors, as well as its capacity to modulate immune responses, minimizing excessive scarring and facilitating regenerative healing. AM possesses these attributes, which make it a promising choice for the development of novel therapies with the goal of enhancing wound healing while minimizing fibrotic outcomes [20,71].

2.5. The regulate of immune function of hAM

The AM is known for its remarkable immunomodulatory capabilities, particularly in regulating interleukin levels crucial for managing inflammatory responses. It reduces pro-inflammatory cytokines like IL-1α and IL-1β while increasing the anti-inflammatory cytokine IL-1RA, thus creating an anti-inflammatory environment. These attributes highlight AM's therapeutic potential in reducing inflammation and improving inflammatory conditions, especially in ocular applications [76,77].

hAM and its derivatives possess potent immunomodulatory properties, contributing to their therapeutic potential in treating chronic inflammation and promoting transplant tolerance. This unique characteristic stems from the release of growth factors, anti-inflammatory cytokines (e.g., IL-10), and immunomodulatory proteins like HLA-G. AM exhibits a multifaceted anti-inflammatory effect, suppressing pro-inflammatory cytokines, decreasing oxidative burst, inhibiting chemotaxis, and enhancing phagocytic function. Its ability to attract and trap inflammatory cells further underscores its anti-inflammatory nature, likely mediated by both cytokine and mechanical mechanisms [32]. Furthermore, AM's immune-privileged status, attributed to the presence of immunoregulatory factors such as HLA-G and Fas ligand, contributes to its low immunogenicity [76]. Decellularized AM (dHAM) retains these essential anti-inflammatory properties while also promoting tissue regeneration, making it a valuable tool in regenerative medicine and tissue engineering for treating burns and wounds [32,78]. Integrating AM within collagen scaffolds significantly enhances its immunomodulatory effects, promoting optimal homeostatic balance by either suppressing or stimulating the immune system as needed. This synergy not only reduces inflammation but also facilitates a more organized arrangement of fibroblasts and collagen fibers during the early stages of healing. Notably, AM distinctly reduces the expression of key inflammatory response genes such as TNF-α, collagen I, and MMP-3, crucial for tendon healing [78].

AM has several complex processes that modulate the immune system, contributing to wound healing and skin tissue engineering. AM is recognized for the presence of physiologically active cells, such as stem cells, that generate various growth factors, cytokines, and anti-inflammatory compounds that help in the process of wound healing [20,79].

Remarkably, the immunomodulatory role of AM is not just ascribed to its cellular constituents but also to its matrix and structural characteristics. The collagen and extracellular matrix present in AM contribute to tissue remodeling and serve as signals for tissue morphogenesis, which is crucial for skin regeneration [20,79]. The preparation of the membrane and its healing processes in skin regeneration involve the use of AM, which facilitates the production of growth factors and proteins by fibroblasts induced by AM [20].

The immune response may be modulated by these components of AM, which are essential for the regeneration and healing of injured skin. The inclusion of extracellular matrix proteins, such as collagen, lamanins, and fibronectins, in the amniotic membrane also facilitates cell attachment and proliferation. These proteins act as a scaffold for tissue engineering purposes [79].

In Fig. 4, a schematic illustrates the specific characteristics of hAM that promote wound healing. These characteristics include antibacterial and antiviral properties, anti-fibrotic activity, the ability to regulate immune function, and improved angiogenesis at the wound site.

3. Composite-based human amniotic membrane

Recent research has contributed to the development of biocomposites based on AM through the addition of polymers, fibrin glue, Ham extract, hydrogel, and other materials. However, AM is sometimes used in composite form solely to enhance the biological properties of another substance. As mentioned earlier, AM's unique characteristics have led researchers to explore its application in various fields. In certain cases, additional biological or non-biological materials are combined with AM to further improve its qualities and functionality. Secondary agents, such as particles, gel, biological adhesives, polymers, or electrospun layers, can be utilized. Table 1 provides an overview of the research conducted on AM-based composites, which are categorized into three subgroups: coated AM, powdered AM-based composites, hAM extract, and Hydrogel-based hAM (Fig. 5).

3.1. Powder amniotic membrane

The powdered amniotic membrane is a potential wound dressing material derived from the innermost layer of the placenta, known as amniotic membrane (Table 2). This powder maintains the characteristics of the original amniotic membrane and offers several advantages for wound treatment. Amniotic membrane powder is produced through freeze-drying or spray-drying the amniotic membrane. This process preserves the extracellular matrix (ECM) components, growth factors, and cytokines naturally present in the tissue [81,95].

The production techniques for Powder amniotic membrane encompass cryogenic grinding, freeze-drying, enzyme-assisted decellularization, and chemical processing, each imparting distinct advantages and constraints. By subjecting the membrane to low temperatures, cryogenic grinding efficiently maintains its biological components, leading to the production of a highly biocompatible powder that is abundant in growth factors and extracellular matrix proteins. Furthermore, freeze-drying generates a porous powder that improves biocompatibility and facilitates cell infiltration, rendering it well-suited for tissue scaffolding. In sensitive applications, enzyme-assisted decellularization selectively targets cellular components while preserving the extracellular matrix, therefore minimizing the possibility of immunogenicity. However, chemical processing is a more economical and scalable approach; yet, it may undermine the biocompatibility of the membrane because of the use of abrasive chemicals. Hence, the selection of the manufacturing technique should be in accordance with the planned uses and specified characteristics of the powder amniotic membrane. The Fig. 6 provides a summary of various powder amniotic membrane manufacturing methods and their properties.

For instance, Rodella et al. [81] conducted a study to assess the therapeutic effects of amniotic membrane powder on a patient with a diabetic ulcer. Their findings demonstrated that the application of amniotic membrane powder reduced both the pain and size of the chronic diabetic ulcer. Laboratory experiments validated that this effect could be attributed to angiogenesis and stimulation of the epithelium. Powdered amniotic membrane shows great potential as a wound dressing material due to its various biological features that actively promote the healing process. It possesses anti-inflammatory, antibacterial, pro-angiogenic, and re-epithelialization capabilities, making it a promising therapeutic option for different types of wounds. Further research is needed to improve the application and distribution techniques of this powder and to fully comprehend its therapeutic capabilities [81,96].

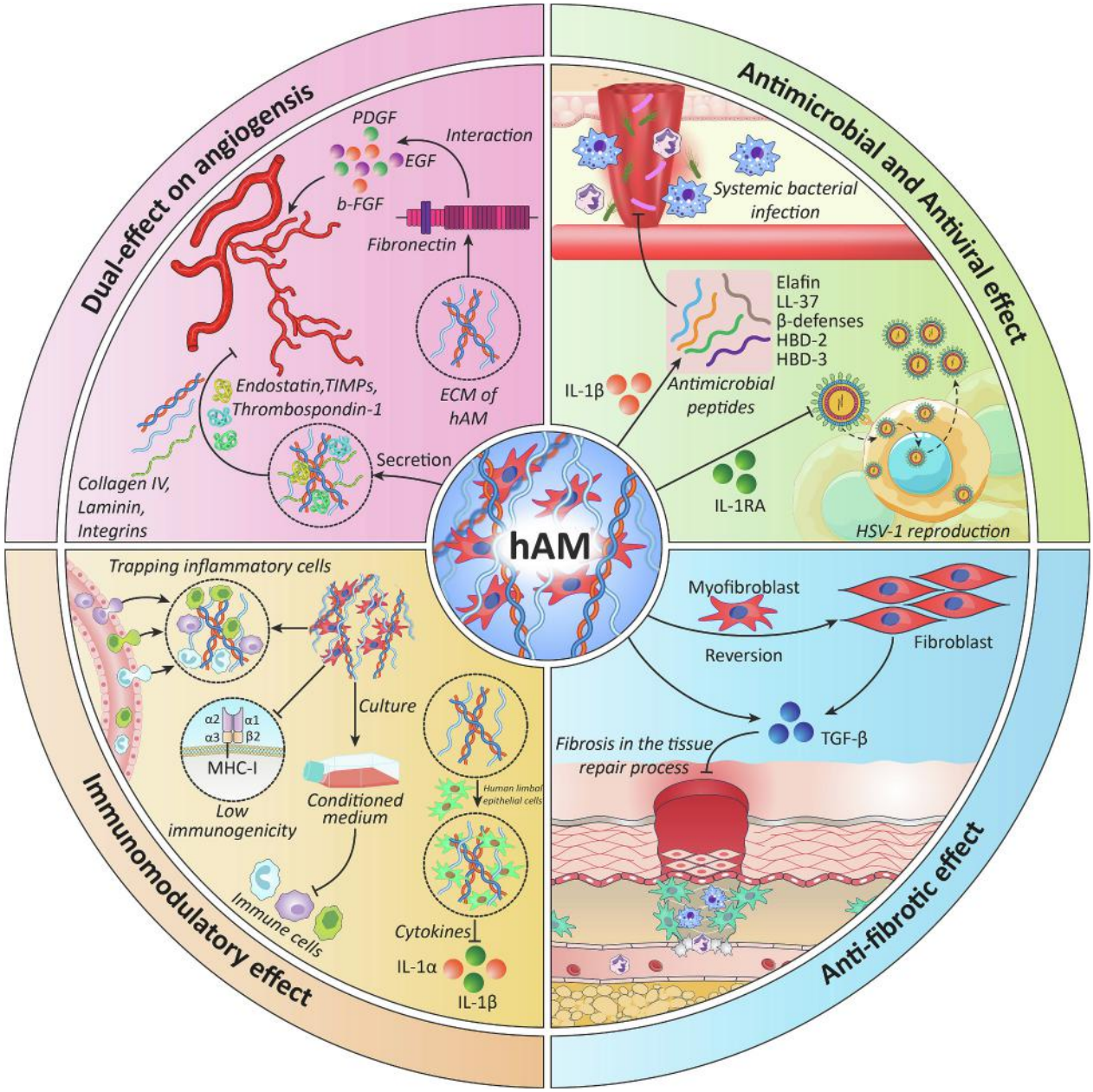

Fig. 4. Schematic illustrating specific characteristics of hAM for the purpose of promoting wound healing. The characteristics encompass antibacterial and antiviral properties, anti-fibrotic activity, the capacity to regulate immune function and improved angiogenesis in wound bed.

3.2. Coated amniotic membrane

One viable method to increase the therapeutic effectiveness of AM is to apply various biomaterials as coatings [56].AM has been coated with a variety of materials, including:

- Collagen: Coating AM with collagen serves as a supporting structure for the development and movement of cells, facilitating the process of re-epithelialization and tissue regeneration [97].
- Fibrin: Coating AM with fibrin imitates the natural wound healing environment, promoting the production of blood clots and tissue regeneration [82].
- Silk: Coating AM with silk can improve mechanical and structural behavior, as well as enhance biodegradability and biocompatibility for wound healing applications [86].
- Nanoparticles: Adding nanoparticles, such as silver nanoparticles, to AM coatings can improve their ability to kill germs and accelerate wound healing [98].
- Growth factors: Coating AM with growth factors delivers concentrated doses of bioactive molecules directly to the wound site, promoting tissue regeneration [83].

For instance, Malekabadi et al. [86] evaluated the effects of silk nanofiber-coated AM in a rabbit model and demonstrated that by adding hAM reduced the development of hypertrophic scars due to decrease collagen deposition and increasing MMP1 expression and deposition via hAM. On the other word, the application of coated amniotic membranes shows great potential as a therapeutic approach to improve the healing process of skin wounds. Coated AM combines the regenerative qualities of AM with the advantages of different coating materials, resulting in enhanced cell adhesion, angiogenesis, inflammatory management, and antibacterial protection. Further studies are needed to improve the

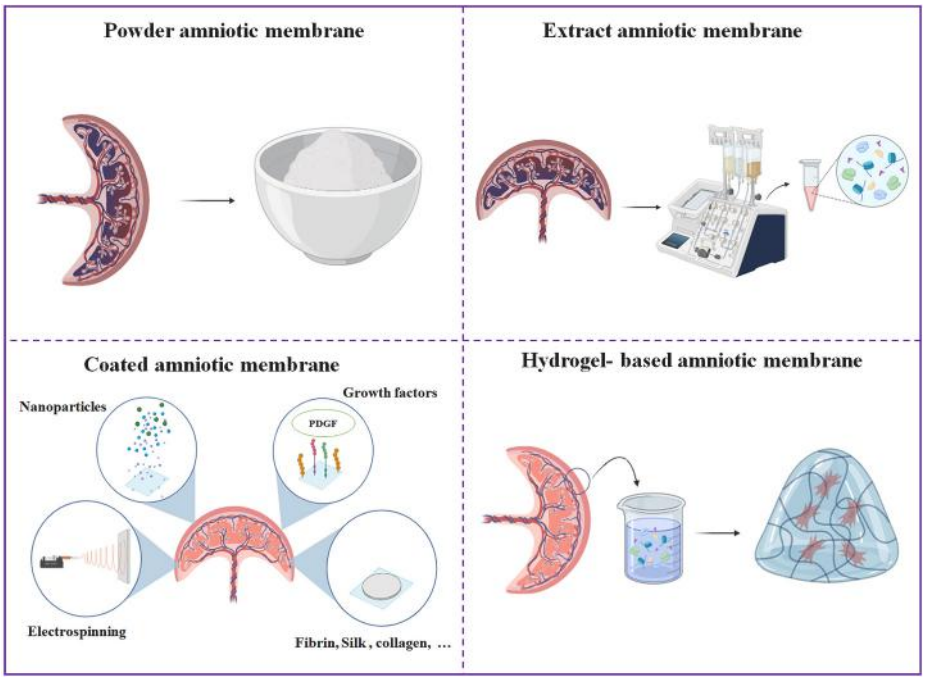

Fig. 5. The schematic of various composite based amniotic membrane such as powdered AM-based composites, coated AM, amniotic membrane extract, hydrogel based on amniotic membrane.

composition and application techniques of coatings, as well as to assess the long-term effectiveness of coated antimicrobial agents in a wider range of wound types [56].

3.3. Amniotic membrane extract (AME)

Amniotic membrane extract (AME) shows great promise as an antibiotic for promoting wound healing. It contains a variety of bioactive substances that stimulate cell growth, promote the formation of new blood vessels, regulate inflammation, and provide antimicrobial defense. Further research is needed to improve the formulations and administration techniques of AME and to assess its long-term effectiveness on a wider range of wound types [87,99].

Fig. 7 illustrates the various extraction methods, including aqueous extraction, organic solvent extraction, enzymatic extraction, and ultrasonic extraction. The extraction process for amniotic membrane across these four methods generally involves several key steps: sourcing and preparing the amniotic membrane, implementing the extraction methods, optimizing the extraction process, followed by purification and concentration. Finally, the extracts are sterilized and stored, typically at −80 °C for long-term storage or at 4 °C for short-term preservation [99,100].

Furthermore, pulverization is a much easier process that produces over twice as much extractable HGF when performed up to three times. Comparing different storage conditions, it was found that HGF is resistant to repeated freeze-thaw cycles. The researchers also discovered that while storage temperature does not have a noticeable impact on HGF levels, preserving AME at −170 °C causes the least amount of HGF to decrease after six days. However, at −170 °C, this factor becomes unstable when stored for a longer period. Lastly, they demonstrated that there is no noticeable change in HGF and protein levels when the AME is sterilized using a 0.2-μm filter. The results obtained show that non-sterile conditions can be used to prepare AME [101].

Amniotic membrane extract (AME) and chorionic membrane extract (CME) have been shown by Mukesh et al. [85] to inhibit the growth of *S. pneumoniae* in both planktonic and biofilm states. Additionally, these extracts have strong biofilm-eradicating properties. The antimicrobial and anti-biofilm activities of AME/CME are attributed to several antimicrobial proteins and peptides present in the AM/CM. The AME/CME membranes overcome the limitations of complete membranes as they can be easily preserved, handled, and sterilized through filtration. They can also be used in combination with antibiotics. Despite the potential risk of transmitting infectious diseases associated with allogeneic tissue, pathogen transmission can potentially be prevented through filter sterilization and donor screening for communicable illnesses [102].

The effects of deferoxamine and AME on angiogenesis in wound healing were investigated by Farzan et al. [87]. The results indicated that amniotic membrane extract enhanced the angiogenic markers, particularly new vessel numbers, and CD31+, compared with the control group. Deferoxamine also significantly increased new vessel numbers and Von Willebrand factor (vWF) compared with the control group. The combined group showed an increase in angiogenic factors, although this difference was not statistically significant.

Laranjeira et al. [88] demonstrated that hAME can prevent the development of the inflammatory response by directly interfering with T cells' ability to proliferate in response to mitogenic activation, secrete IL-2, and produce Th1/Th17-related cytokines (TNFα, IFNγ, IL-17). This is in contrast to inhibiting the production of pro-inflammatory cytokines by monocytes and mDC, which can also be achieved by inhibiting their interaction with T cells through the expression of chemokines. By preventing the expression of proteins with crucial cytotoxic functions like TNFα, granzyme B, and perforin in CD8+ T cells and γδ T cells, hAME further impairs the effector arm of the Th1/Th17 immune response. Furthermore, hAME's ability to block the production of IL-9 by T cells could provide important information regarding allergies.

Litwiniuk et al. [89] evaluated the influence of AME on the proliferation of keratinocytes (HaCaT), fibroblasts (Wi-38), and endothelial cell lines (HECa-10) using a colorimetric tetrazolium salt reduction. Significant amounts of TGF-β, TIMP-1, and EGF were identified in all of the amnion samples. All of the evaluated amnion extracts stimulated the proliferation of HaCaT and Wi-38 cells, with keratinocytes being more stimulated than fibroblasts in samples derived from the cervical part of the amniotic membrane generated from cesarean sections. However, all of the investigated extracts decreased the proliferation of the HECa-10

Table 2
Summary of recent studies on the effect of composite based human amniotic membrane for wound healing application.

	Substrates	Type of wound	Description	Ref
Powder amniotic membrane	hAM powders	Full thickness porcine	The study showed a notable and fast healing of wounds, mostly due to the growth of new epithelial cells rather than the wound closing by contraction. This resulted in the production of a fully developed outer layer of skin and underlying tissue that had a comparable composition to healthy skin.	[80]
	hAM powders	Chronic diabetic ulcer	The lesion showed signs of re-epithelization, therefore confirming that AM powder preserves proteins associated with anti-inflammatory and angiogenesis processes.	[81]
	hAM powders with *Aloe vera* extract	Skin burn wound	Wounds treated with AM+AV showed a thicker regenerated outer layer of skin, a higher number of blood vessels, and a bigger number of rapidly dividing skin cells within the outer layer.	[29]
Coated amniotic membrane	Coated hAM with poly-[Lactide-*co*-Glycolide-co-Caprolactone] containing silver nanoparticles	Chronic wound	The biological membranous matrix is reinforced by AM to enhance mechanical strength, hence preventing wound contraction.	[82]
	Coated hAM with FGF and EGF growth factors	Burn wound	Rapid increase in wound healing rate, growth of new skin cells, accumulation of collagen, and development of new blood vessels during the initial week.	[83]
	Coated hAM with silver	Burn wound	Deposition of silver particles on AM results in the formation of an antibacterial barrier with controlled release of moisture vapor and a high absorption capacity.	[84]
	Coated hAM with sodium alginate and silk fibroin biopolymers	Chronic wounds	Improved adhesion and proliferation of Mesenchymal Stem Cells in prepared scaffolds and increased water wettability for biomedical application.	[85]
	Coated hAM with silk nanofibers	Chronic wound	Reduced the development of hypertrophic scars following damage in vivo by decreasing collagen deposition and increased MMP1 expression and deposition.	[86]
Amniotic membrane extract (AME)	AME	Various wounds	Improved the angiogenic markers CD31+, new vessel numbers, and Von Willebrand factor (vWF).	[87]
	hAME	Allergic wounds	By inhibiting the inflammatory response and suppressing the generation of IL-9 by T cells, valuable insights into allergies might be obtained.	[88]
	AME	Various skin wounds	Indicated that biological dressings made with AME might effectively support wound healing by promoting the growth of fibroblast and HaCat cells.	[89]
Hydrogel based on amniotic membrane	Chitosan/PVP hydrogel containing hAMs	Burn wound model	demonstrated that AME-Gel improves the process of epidermal and dermal regeneration by promoting the formation of granulation tissue, stimulating the proliferation of fibroblasts, and enhancing the development of blood vessels	[90]
	Decellularized human amniotic membrane (dHAM)/ methacrylate (MA)	Chronic wounds	Promoted cell proliferation, migration, angiogenesis, and effectively reduces inflammation.	[91]
	Hyaluronic acid/freeze-dried conditioned medium obtained from the amnion	Diabetic wounds	High elasticity, superior biocompatibility, controlling the polarization of macrophages and stimulating the formation of new blood vessels.	[92]
	Silk fibroin (SF) fibers/alginate hydrogel - contained amniotic fluid (AF).	Critical wounds	Improvement in cellular proliferation, spreading, and collagen production	[93]
	Polylactic acid (PLA)/thermosensitive hydrogel of chitosan and β-glycerophosphate (β-GP) with a layer of human-am decellularized tissue	Various wounds	Enhanced cell adhesion, proliferation and accelerated wound healing process.	[94]

cell line, unlike HaCaT and Wi-38 cells. The results demonstrated that biological dressings made from the amniotic membrane, particularly the placental section, could provide appropriate assistance for wound healing applications.

3.4. Hydrogel based on amniotic membrane

Amniotic membrane hydrogels are widely recognized as highly effective biomaterials for promoting wound healing. These hydrogels imitate the natural extracellular matrix of the amniotic membrane, creating a moist environment that enhances cell development and migration. They also transport growth factors and other bioactive substances. AM-based hydrogels have distinctive characteristics such as biocompatibility, biodegradability, and the ability to stimulate angiogenesis and minimize inflammation. These qualities make them suitable for use in wound healing applications [30,103]. In the following, we will discuss recent studies on types of hydrogels containing hAM for healing skin wounds.

Zhang et al. [30] first grafted dHAM with methacrylic anhydride (MA) and integrated dHAMMA with methacrylated gelatin (GelMA) to fabricate a skin hydrogel. GelMA dHAMMA was also found to have good physical and chemical properties, as well as a porous structure of a bicomponent polymer network. GelMA-dHAMMA has been shown to stimulate fibroblast proliferation and α-smooth muscle actin (α-SMA) expression in vitro. In addition, in vivo investigations have indicated that GelMA-dHAMMA can accelerate tissue healing, stimulate wound collagen deposition and angiogenesis, and effectively mimic the extracellular microenvironment.

Tehrani et al. [94] reported the development and description of an oxygen-generating wound dressing based on AM, given the significance of oxygen in wound healing. This construct included polylactic acid (PLA) microparticles containing H2O2 encapsulated in a thermosensitive hydrogel of chitosan and β-glycerophosphate (β-GP), coated with a layer of human-am decellularized tissue. Based on MTT results, the hydrogel/dAM extract was non-toxic after 7 days, and the resultant composite enhanced cell adhesion and proliferation. This sample showed less than 5 % hemolysis and the least amount of blood cell adhesion. The results indicate the essential biological, chemical, and physical properties of the suggested structure as an active wound dressing.

The printability and stability of hydrogels after three-dimensional (3D) bioprinting are limited by their weak mechanical durability and inferior rheological behavior. Therefore, to improve the characteristics of hydrogels derived from decellularized amniotic membranes (dAM), it

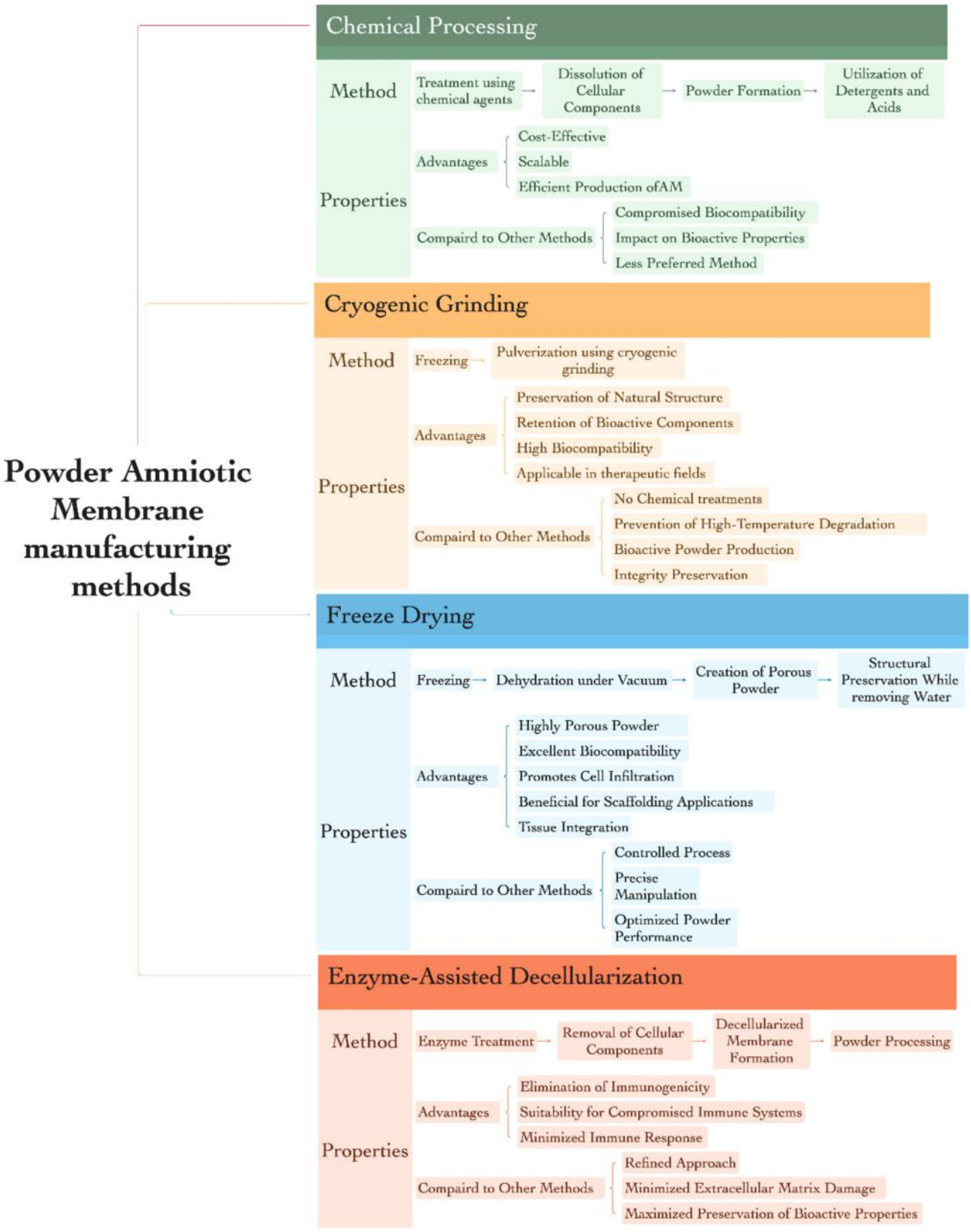

Fig. 6. Diagram illustrating the different methods for producing amniotic membrane powder, along with the advantages, disadvantages, and characteristics of the powder obtained from each method.

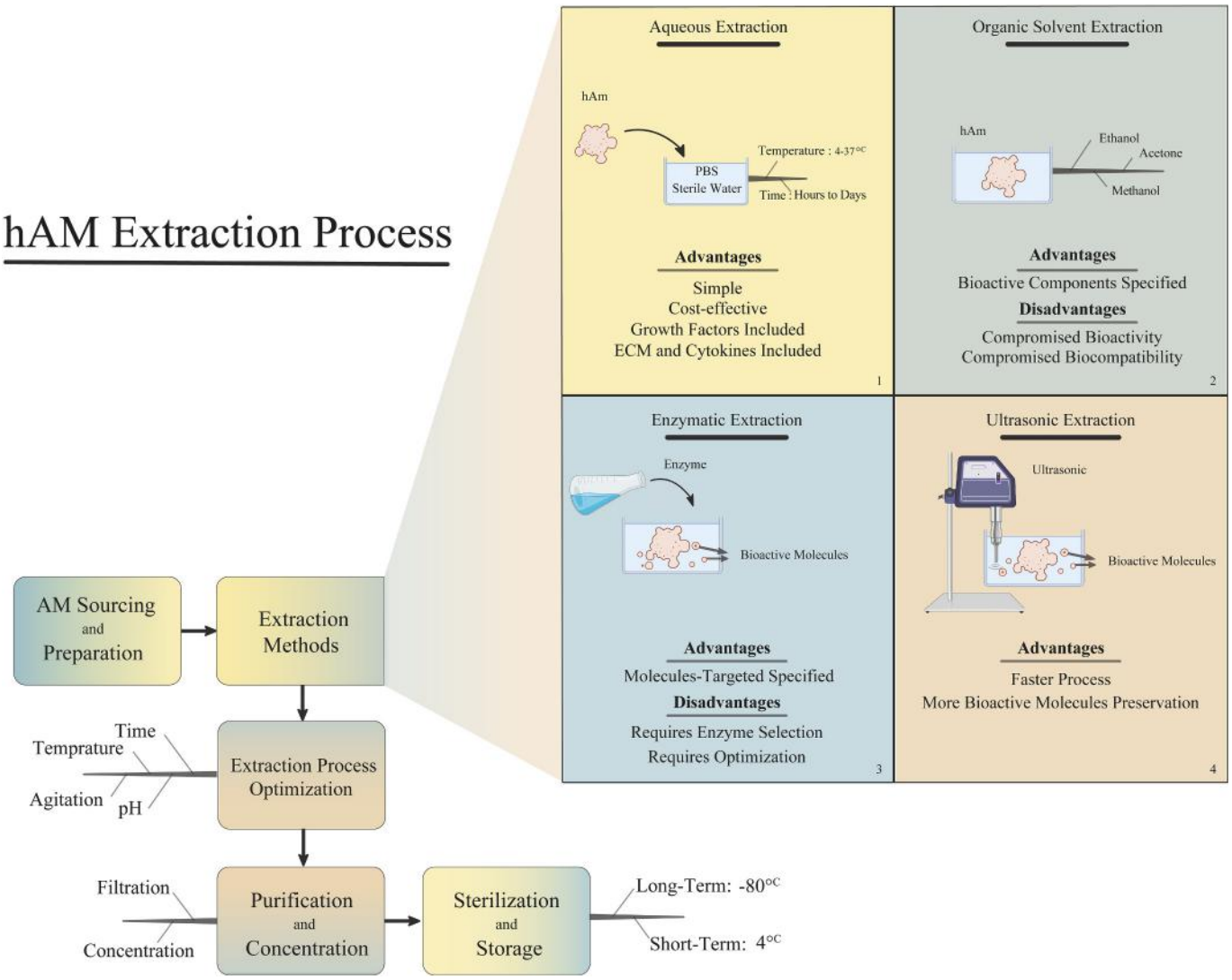

Fig. 7. Diagram illustrating various methods for preparing amniotic membrane extracts (such as aqueous extraction, organic solvent extraction, enzymatic extraction, and ultrasonic extraction), detailing the extraction processes, the advantages and disadvantages of each method, and the key parameters influencing the extraction efficiency.

is necessary to incorporate biocompatible materials through chemical or physical modification. Kafili et al. [103] present an approach that involves enhancing dAM-derived hydrogels (DAMHs) with Laponite nanoplatelets and sodium alginate. The rheological behavior of the DAMHs was evaluated using these Laponite nanoplatelets.

Another study evaluated a novel cell-free hydrogel based on collagen, human amnion, and sodium carboxymethyl cellulose for second-degree burns wound healing application. The results demonstrated that the hydrogel containing a combination of amnion and collagen showed significantly faster wound healing and completed re-epithelialization at wound site [96].

Kafili et al. [104] propose a method for modifying the surface of two-dimensional nanosilicates (laponite) to alter the characteristics of decellularized amniotic membrane-derived hydrogels (DAMHs) by acting as a rheological modifier. The results indicate that the hydrogel enhances cell viability and adhesion. This study's findings have the potential to contribute to the development of injectable DAMHs with improved properties for tissue engineering (TE).

Murphy et al. [80] conducted an investigation using a full-thickness porcine skin wound model to assess the effectiveness of amnion hydrogel and a less-processed amnion product made from freeze-dried amnion membrane powder in promoting wound healing. The results showed that the application of amnion hydrogel and amnion powder resulted in significant and rapid wound healing, primarily through the development of new epithelial cells rather than closure through contraction. Another study describes a new product obtained by solubilizing amnion blended with hyaluronic acid (HA) hydrogel carrier (HA-AM). This process is straightforward. The researchers demonstrated that HA-AM significantly improves the wound closure process by promoting the development of new epithelial cells and inhibiting lesion shrinkage [105].

Momeni et al. [90] investigated the potential of developing a novel chitosan gel dressing derived from amniotic tissue as an effective substrate for wound healing. The study evaluated the wound healing effectiveness and scar prevention effects of a chitosan/polyvinylpyrrolidone (PVP) gel containing human amniotic membrane extract (AME-Gel) in a rat burn model. The results demonstrated that AME-Gel enhances epidermal and dermal regeneration by promoting granulation tissue formation, stimulating fibroblast proliferation, enhancing blood vessel development, and facilitating collagen synthesis.

Chen et al. [91] used decellularized human amniotic membrane (dHAM) as a bioactive extracellular matrix (ECM) and modified it by grafting methacrylate (MA) for engineering applications, resulting in the development of photosensitive dECMMA. This material promotes cell

proliferation, migration, angiogenesis, and effectively reduces inflammation.

Ghalei et al. [93] initially used electrospinning to produce silk fibroin (SF) fibers. These fibers were then combined with an alginate hydrogel containing amniotic fluid (AF). By adjusting the ratio of alginate to AF, different dressings were created. In vitro release profiles of AF revealed that increasing the concentration of alginate decreased the amount of AF released. Fibroblast culture on the dressings showed that as AF increased, cellular proliferation, spreading, and collagen production improved. Overall, these results offer a unique bioactive dressing with significant potential for promoting healing in critical wounds.

Rahman et al. [29] developed a novel gel by combining amnion with *Aloe vera* extract to enhance burn wound healing and eliminate grafting limitations. Observations indicated that the AM/AV gel improved the adhesion and proliferation of HaCaT and HFF1 cells. In vitro, scratch experiments showed that the combination of AM and AV effectively enhanced wound healing by promoting HaCaT cell migration. Skin irritation experiments confirmed the suitability of the gels, as there was no erythema or edema. The results also indicated that a gel containing a blend of amnion and *Aloe vera* extract is an effective therapeutic solution for burn wound healing.

Phan et al. [106] developed sponges using amnion hydrogel for wound treatment. These sponges were manufactured through a combination of crosslinking and freeze-drying processes. The results suggest that the sponge containing AM holds promise as a wound dressing.

Zhang et al. [92] created a hydrogel containing hyaluronic acid modified with methacrylic anhydride and N-(2-aminoethyl)-4-[4-(hydroxymethyl)-2-methoxy-5-nitrophenoxy]-butanamide (NB) groups. This hydrogel was used to encapsulate freeze-dried conditioned medium obtained from the amnion (AM-CM). Additionally, the hydrogel exhibited excellent mechanical characteristics, high elasticity, superior biocompatibility, and sustained release of AM-CM. Subsequent in vitro and in vivo investigations showed that the hydrogel effectively accelerated the healing process of diabetic wounds by controlling macrophage polarization and promoting new blood vessel formation. The hydrogel developed with AM-CM release holds great promise for treating chronic wounds in clinical settings.

4. Conclusion

The human amniotic membrane (hAM) and hAM-based materials have shown great promise in the field of wound healing. This is mainly due to their antibacterial properties, ability to regulate the immune response, and ability to release growth factors. The unique composition and biological characteristics of hAM make it an intriguing option for regenerative skin treatments, as it can improve the wound healing process in various ways. However, further research is needed to explore potential innovations that can enhance the therapeutic effectiveness of the human amniotic membrane (hAM) in wound healing.

CRediT authorship contribution statement

Parisa Heydari: Writing – review & editing, Writing – original draft, Visualization, Investigation. **Maryam Mojahedi:** Writing – original draft, Methodology. **Pouya Javaherchi:** Writing – original draft, Conceptualization. **Maede Sharifi:** Writing – original draft. **Anousheh Zargar Kharazi:** Writing – review & editing, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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