

Thesis

Clinical applications of amniotic membranes in wound healing

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Wien, März 03, 2025

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Zusammenfassung

Hintergrund: Die Amnionmembran ist ein dünnes, aber äußerst strapazierfähiges Biomaterial, das verschiedene Zytokine, Wachstumsfaktoren und Signalmoleküle enthält und möglicherweise eine entscheidende Rolle in der fetalen Entwicklung spielt. Aufgrund der komplexen Natur der Wundheilung wurden Produkte aus menschlichem Amnion entwickelt, um die Geweberegeneration und den Wundverschluss zu unterstützen. Die signifikanten Mengen an bioaktiven Molekülen, wie Zytokinen und Wachstumsfaktoren in Amnionmembranen, zusammen mit ihren schmerzlindernden Effekten, antibakteriellen Eigenschaften, der Reduktion von Entzündungen und der Fähigkeit, als biologische Barriere zu fungieren, machen sie besonders vorteilhaft zur Beschleunigung des Wundheilungsprozesses. Diese Arbeit zielt darauf ab, einen umfassenden Überblick über die erfolgreichen Anwendungen und die Wundheilungseigenschaften von Amnionmembranen zu bieten, unterstützt durch klinische Beispiele. Sie wird auch zukünftige Möglichkeiten und Einschränkungen im Zusammenhang mit dieser Technik diskutieren.

Methoden: Diese Studie ist eine Literaturübersicht. Die Literatursuche umfasst hauptsächlich Studien aus PubMed, Google Scholar, ScienceDirect und anderen Literaturdatenbanken. Darüber hinaus werden medizinische Lehrbücher verwendet, um das Verständnis des aktuellen Wissensstands zu diesem Thema zu vertiefen.

Ergebnisse: Die Amnionmembran ist eine äußerst effektive Behandlung zur Wundheilung und bietet Vorteile gegenüber anderen Methoden, nicht nur aufgrund ihrer einzigartigen Eigenschaften, sondern auch weil sie reichlich vorhanden, leicht zugänglich und sicher für verschiedene Patient:innen, einschließlich solcher mit Begleiterkrankungen, ist. Amnionmembranen können in einer Vielzahl von Anwendungen eingesetzt werden, wie z. B. zur Behandlung chronischer oder traumatischer Wunden, und können mit anderen Materialien kombiniert werden, um ihre Wirksamkeit zu verbessern. Allerdings hat die Amnionmembran auch einige Nachteile, wie eine schnelle Zersetzung und unterschiedliche Eigenschaften, die die Handhabung erschweren. In einigen Regionen hemmt sie die Blutbildung, während sie in anderen diese fördert, und bestimmte Bereiche können an funktionalen Zellen fehlen.

Schlussfolgerung: Nach der Untersuchung zahlreicher Fälle und Studien ist es offensichtlich, dass Amnion ein bemerkenswert bedeutendes biologisches Gewebe ist.

Seine vielfältigen und einzigartigen Eigenschaften machen es besonders vorteilhaft für medizinische Anwendungen. Durch das Verständnis, wie man seine Anwendung verbessern und effektivere Methoden zur Verarbeitung und Lagerung entwickeln kann, können wir erhebliche Vorteile erzielen. Darüber hinaus machen die regenerativen Stammzell-Eigenschaften von Amnionmembranen sie in der regenerativen Medizin äußerst wertvoll, und ihre Nutzung kann auch in verschiedenen medizinischen Bereichen verbessert werden. Letztendlich verdienen Amnionmembranen innerhalb der Medizin größere Anerkennung, und weitere Forschungen zu diesem Gewebe könnten es uns ermöglichen, nahezu alle Arten von Wunden in der Zukunft zu behandeln, was zu einer Verringerung des Leidens der Patient:innen und einer geringeren Belastung des öffentlichen Gesundheitssystems aufgrund der Wundversorgung führen könnte, insbesondere bei chronischen oder nicht heilenden Wunden.

Abstract

Introduction: The amniotic membrane is a thin yet highly durable biomaterial that contains various cytokines, growth factors, and signaling molecules, potentially playing a crucial role in fetal development. Due to the complex nature of wound healing, human amnion products have been developed to aid in tissue regeneration and wound closure. The significant levels of bioactive molecules, such as cytokines and growth factors in amniotic membranes, along with their pain-reducing effects, antibacterial properties, inflammation reduction, and ability to act as a biological barrier, make them particularly advantageous for expediting the wound healing process. This work aims to offer a comprehensive overview of the successful applications and wound healing properties of amniotic membranes, supported by clinical examples. It will also discuss future opportunities and limitations associated with this technique.

Methods: This study is a literature review. The literature search primarily includes studies from PubMed, Google Scholar, ScienceDirect, and other literature databases. Additionally, medical textbooks are used to enhance the understanding of the current state of knowledge on this topic.

Results: The amniotic membrane is a highly effective treatment for wound healing, offering advantages over other methods, not only because of unique qualities but also that it is abundant, easily accessible, and safe for diverse patients, including those with comorbidities. Amniotic membranes can be utilized in a variety of applications, such as treating chronic or traumatic wounds, and can be combined with other materials to improve its efficacy. However, the amniotic membrane has also some drawbacks, such as rapid decomposition and varying properties that complicate handling. Some regions inhibit blood formation, while others promote it, and certain areas may lack functional cells.

Conclusions: After examining numerous cases and studies, it is evident that amnion is a remarkably significant biological tissue. Its diverse and distinctive characteristics make it particularly advantageous for medical uses. By understanding how to improve its application and creating more effective methods for processing and storage, we can achieve substantial benefits. Moreover, the regenerative stem cell properties of amnion membranes make them highly valuable in regenerative medicine and their utilization can

also be improved across different medical fields. Ultimately, amnion membrane warrant greater acknowledgment within the medical community, and further research on this tissue may enable us to treat nearly all types of wounds in the future, resulting in reduced patient suffering and decreased liability on the public health system due to wound care, particularly for chronic or non-healing wounds.

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List of Abbreviations

AE	Amniotic epithelium
AECs	Amniotic epithelial cells
AF	Amniotic fluid
AM	Amniotic membrane
AMs	Amniotic membranes
AMCs	Amniotic mesenchymal cells
AME	Amniotic membrane extract
AMSCs-CM	AMSCs- conditioned medium
Bcl	B-cell lymphoma
BDNF	Brain-derived neurotrophic factor
BM	Basement membrane
CD	Cluster of differentiation
CFC	Chordin-like factor
COX	Cyclooxygenase
CL	Compact layer
CNTF	Ciliary neurotrophic factor
DKK1/3	Dickkopf-related proteins 1 and 3
DPPA	Developmental pluripotency associated
dAM	Decellularized amnion
ECM	Extracellular matrix
EGF	Epidermal growth factor
FDA	Food and drug administration
FGF	Fibroblast growth factor

FL	Fibroblast layer
GAGs	Glycosaminoglycans
GCTM	Glycophorin C transmembrane
HA	Hyaluronic acid
hASCs	Human amniotic stem cells
HBD	Human-beta-defensin
HGF	Hepatocyte growth factor
HLA	Human leukocyte antigen
ICAM	Intercellular adhesion molecule
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
KGF	Keratinocyte growth factor
KLF	Kruppel-like factor
miRNA	Micro RNAs
MMPs	Matrix metalloproteinases
MPO	Neutrophil myeloperoxidase
MRSA	Methicillin-resistant staphylococcus aureus
M-CSFR	Macrophage colony-stimulating factor receptor
NK	Natural killer cells
NT	Neurotrophin
OCT	Octamer-binding transcription factor
PA	Placental amnion

PAECs	Placental amniotic epithelial cells
PAMCs	Placenta amniotic mesenchymal cells
PAX	Paired box gene
PDGF	Platelet-derived growth factor
PEDF	Pigment epithelial - dependent factor
PD-L	Programmed cell death ligands
PECAM	Platelet endothelial cell adhesion molecule
PROM	Prominin
PTX3	Pentraxin 3
PGE 2	Prostaglandin E2
PTGS2	Prostaglandin-endoperoxide synthase
RA	Reflected amnion
RAECs	Reflected amniotic epithelial cells
RAMCs	Reflected amniotic mesenchymal stromal cells
REX	RNA export factor
ROS	Reactive oxygen species
SL	Spongy layer
SLPI	Secretory leukocyte proteinase inhibitor
SOX	SRY (Sex Determining Region Y)-box
SSEA	Stage-specific embryonic antigen
STRO	STEM cell antigen-like protein
TERT	Telomerase reverse transcriptase
TGF	Transforming growth factor
TIMP	Tissue inhibitors of metalloproteases

TRA	Trophoblast antigen
TSP	Thrombospondin
UCA	Umbilical or cord amnion
VEGF	Vascular endothelial growth factor
WH	Wound healing

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1. Introduction

From individual cells to complex organisms such as humans, all living beings own an outer sheath (Reinke and Sorg, 2024) that shields them from the external world's potentially harmful influences (Sorg *et al.*, 2016). In humans, the skin serves as this protective shield while also carrying out numerous vital functions that contribute to maintaining the body's homeostasis and ensuring overall integrity. Injuries to the skin and as a result, the formation of wounds represent a primary threat to its integrity, highlighting the critical importance of wound healing for preserving the body's physiological functions. The process of wound healing in the skin is a dynamic and elaborate mechanism that involves the systematic cooperation of various cell types, molecules, cytokines, hormones, and signaling mediators (Sorg *et al.*, 2016). There are two foundational methods for tissue defect closure: regeneration (*restitutio ad integrum*) and repair. Besides, the wound healing process is often divided into four distinct phases: Hemostasis, Inflammation, Proliferation, and Remodeling. Understanding the type of wound, evaluating the extent of tissue damage, and recognizing their impact on overall health is crucial and significantly influence wound healing, at the same time the trauma mechanism, the distinct appearance, and the presence of complicating factors are important determinants in the individualized examination of a wound. These variables determine the particular healing mechanism and phases through which a wound heals over time. There are three main types of wounds—traumatic, iatrogenic, and chronic—each of which can be categorized further into subtypes (Lippert, 2012).

Throughout the rich history of medicine, evidence exists that even prehistoric civilizations acknowledged the importance of wound treatment and developed multiple methods to take care of wounds and inhibit infections. Early wound management techniques, including the use of compression for hemostasis, were described in ancient Egyptian papyrus scrolls (3200–300 BC), as well as Hippocrates underscored the significance of draining pus ('Ubi pus, ibi evacua'), and Galen established the concepts of primary and secondary intention healing. Nevertheless, over time a prominent part of this therapeutic method was slowly neglected and eventually forgotten (Reinke and Sorg, 2024). The exploration for effective wound treatment techniques, despite this, proceeded, and ultimately gave rise to the finding of the amniotic membrane's potential in wound healing, a discipline with a long-established background that traces back over a century. The first implantation of the Amniotic membrane in medicine took place at the beginning of the 1900s when Davis in

the year 1910 published his attempt at using the lining of the amniotic sac as a skin graft, inspired by the idea of a fourth-year medical student. Even though he did not get favorable outcomes, Davis stated that it was “well worth a trial” and he was also convinced about the potential of this material, which he mentioned could be a breakthrough in medicine, in case an effective technique is developed for the usage of AM. This particular research awoke the interest of many other scientists (m/w/d) and led light to further exploration of AM use in medicine as well as its wound healing properties. Two separate researchers (m/w/d) from France and Germany in 1913 discovered the potential of AMs as a possible alternative for healing skin wounds through skin grafting, presenting successful results comparable to those obtained with autologous skin grafts. In 1940 Dr. De Roth implemented the use of AM in the field of ophthalmology for the first time. The delivered results from using both chorion and amnion together to treat ocular burns along with conjunctival defects were successful. (Moy, 2019). In that particular year, Chao and Humphreys experimented with applying AM in severe head injury patients with dural perforation to reconstruct the dura, this research outcome was also favorable. These two versatile uses of AM in different surgical disciplines highlighted the diverse and noteworthy roles this biological material can play in medical practice and pointed out the future perspective of AM utilization for tissue repair one more time. Furthermore, in 1986, AM was incorporated into vulvovaginoplasty for vaginal reconstruction as an effective substitute for split-thickness skin grafts (Elkhenany et al., 2022).

Despite promising results, the limitations in storage and preservation techniques restricted the widespread implantation of AM. To tackle these challenges, the cryopreservation of AMs at -80°C to sustain their biological and physical properties was proposed by Kim and Tseng. In a study, these cryopreserved amniotic membranes were used to reconstruct ulcerated corneas in 11 patients and showed a success rate of more than 90%, which demonstrated the effectiveness of this method. In 1997 the lyophilized (freeze-dried and sterile) AMs were used for the first time by Güler and Ercan. They used it in mandibular vestibuloplasty and demonstrated relevant angiogenic effects this biological material possesses (Elkhenany et al., 2022).

Wound care, especially the management of chronic wounds from a financial viewpoint is a massive burden to healthcare, most importantly, however, also a great burden to patients, who often suffer because of the limited success of conventional treatments. In comparison, Amniotic membrane usage offers multiple advantages in economic as well as clinical

settings. The placenta, from which the AM is derived, is typically discarded after delivery as a biological waste. For that reason, readily available AM is cost-effective, and abundant, and also acquiring it is non-invasive, as well as safe. Thus, the above-mentioned utilization of AM can contribute to reducing healthcare costs while providing a potentially effective therapeutic option (Mamede and Botelho, 2015).

1.1 Anatomy, structure, and composition of the AM

Historically, in the context of culture, superstitious beliefs were deeply tied to the human amniotic sac. When a baby was born with an intact amniotic sac, which was called a “caul”, was believed to grant life-long happiness and protection, particularly against drowning as a protective charm, but also bestow mystical powers, such as spirit sight. These mystical associations with the amniotic sac were so widespread, that in the 19th century, it was even preserved and traded, especially among sailors to shield against maritime dangers. These beliefs show the long-standing cultural relevance of the amniotic membrane, which predates today’s knowledge and use in medicine, and underlines future perspectives (Fairbairn, Randolph and Redmond, 2014).

Understanding the anatomy and structure of the AM in medicine is essential for its application. AM’s unique structural features, including a rich extracellular matrix and abundance of growth factors, play an important role in stimulating tissue regeneration and suppressing inflammatory responses. These are really important qualities, particularly for regenerative medicine, which enables the effective application of its properties in various therapeutic interventions. By grasping the AM’s inherent capacities, we can optimize its use in clinical practice, ultimately can achieve significant success and improve outcomes in various areas of medicine (Mamede and Botelho, 2015).

The amniotic membrane is a remarkable tissue unique to pregnancy, playing a major role in fetal development. Together with the chorion and decidua, it builds the fetal membranes—a complex, dynamic unit with both anatomical and physiological significance. The amnion is the innermost and translucent membrane, originates from the embryoblast, and plays a dual role: secreting amniotic fluid while maintaining direct contact with it to support nutrient transfer to its cellular components. In contrast, the chorion is a thicker and more opaque intermediate membrane and is derived from the cytotrophoblast. The decidua, or outer membrane, originates from the uterine decidua

capsularis, which envelops the implantation site. These membranes have multiple critical functions: anatomically, they serve as a barrier that separates the fetal and maternal environments while simultaneously facilitating communication and nutrient exchange between the two systems (Mamede and Botelho, 2015).

1.1.1 Embryonic development

The exact mechanisms, of how the human amnion and amniotic cavity form, remain poorly understood. However, research on primate embryos suggests that the amniotic cavity emerges in the dorsal region of the embryonic node at the onset of implantation or immediately right after it. A distinct population of cells known as mesodermal cells emerges during subsequent stages of embryological development. These cells, together with the amniotic epithelium-ectoderm, contribute to the formation of the extraembryonic membrane known as the amnion. The extraembryonic mesenchyme derives from hypoblast cells and gives rise to the fibroblast layer of the amnion. This mesenchyme proliferates and then extends to envelop the amniotic epithelium, eventually differentiating into the amniotic mesoderm. The amniotic mesoderm by 7 and 10 weeks of gestation integrates with the chorionic mesoderm, beginning at the umbilical cord insertion point on the chorionic plate. Despite this connection, the amnion and chorion remain loosely attached, allowing the two membranes to glide smoothly relative to one another. (Mamede and Botelho, 2015).

This theory states that the amniotic cavity arises from the embryonic node and the amniotic membrane originates from embryoblast-derived material. So the amniotic epithelium develops prior to the process of gastrulation, the stage during which the three primary embryonic germ layers develop from the epiblast, and therefore it has pluripotent stem cells of the epiblast that persist within the amnion throughout pregnancy, even until term (Mamede and Botelho, 2015).

As the amniotic cavity expands the amnion fuses with the chorion and as a result, the inner surface of the chorion borders the outer layer of the amnion. At the same time, the outer layer of the chorion, together with the trophoblastic villi that extend from its surface, establishes contact with the decidua. The decidua capsularis lies above the chorion laeve and, as the chorionic sac enlarges to fill the endometrial cavity, it fuses with the parietal decidua of the uterine wall (Mamede and Botelho, 2015).

By the end of the third month of gestation the amnion and chorion laeve, which is the early form of the chorion, are clearly distinct from one another by the presence of the chorionic cavity. As the fetus grows, the amniotic sac enlarges and accumulates fluid, and following this, the amnion gradually attaches to the innermost layer of the chorion, which results in the eventual disappearance of the chorionic cavity. Even though there is a close connection between amniotic and chorionic membranes, they do not undergo fusion and maintain their physiological separation. As a result, despite the fact that during the process of placenta expulsion following the delivery of the baby, the placenta is ripped apart and in most cases requires reconstruction to regain its physiological structure, the amniotic and chorionic membranes can easily be detached from one another with just peeling (Roy et al., 2022).

1.1.2 Anatomy and structure

The placental membrane (PM) is composed of the amnion facing the fetus, the chorion facing the maternal side, and the intermediate layer between and has been reported in the literature that at term to range in thickness from 0.02 mm to 0.6 mm, covering an average surface area of roughly 1600 cm². The PM undergoes ongoing remodeling of its extracellular matrix during pregnancy to preserve its structural integrity while supporting the development of the growing fetus. Each layer of the PM exhibits specific compositional characteristics that are tailored to fulfill its particular role in maintaining the overall function of the placental barrier (Roy et al., 2022).

The typical thickness of AM is between 35-60 μm and it is a delicate, thin, avascular membrane, which by visual inspection after the delivery of the placenta looks transparent and has a subtle blue reflection. The nourishment of the membrane occurs in multiple ways, via diffusion from mesenchymal-epithelial cells located between the amnion and chorion, amniotic fluid, fetal vessels on the surface, and glands of the decidua (Mamede and Botelho, 2015).

Development of the AM begins by forming a single layer of cuboidal ectodermal cells derived from the extraembryonic ectoderm and bordered by a mesothelium-like layer of extraembryonic mesoderm origin, which is nonvascular and nerve-free. As development progresses, additional intermediate layers start to gradually form within the membrane (Mamede and Botelho, 2015).

Ultimately, the AM consists of three distinct layers. The single epithelial layer is the innermost layer, which directly interacts with the amniotic fluid and is supported by the intermediate layer, called the basement membrane. The third layer of the membrane is the collagen-rich stromal layer and is further composed of three sublayers: the compact layer, the fibroblastic layer, and the outermost spongy layer (Fénelon *et al.*, 2021).

Furthermore, the AM consists of three distinct layers. The single epithelial layer is the innermost layer, which directly interacts with amniotic fluid (AF) and is supported by the intermediate layer, called the basement membrane. The third layer of the membrane is the collagen-rich stromal layer and is further composed of three sublayers: the compact layer, the fibroblastic layer, and the outermost spongy layer (Fénelon *et al.*, 2021).

In addition to the histological classification, AM can also be divided into three subregions based on anatomy: Placental amnion (PA) - the part over the placenta, Reflected amnion (RA) - which covers the uterine wall and creates the amniotic sac, Umbilical or Cord amnion (UCA) - envelops the umbilical cord. Findings imply that in application in regenerative medicine, these subregions may show diverse outcomes, and they can even be further classified into different Zones (Weidinger *et al.*, 2021), considering the weight of the fetus and gravity, but also other, with the growing fetus connected factors existed, these classifications are not surprising (Mamede and Botelho, 2015).

Throughout the whole pregnancy period, from the first day of AM development even until birth, the cellular configuration and morphology of AM continuously change. Of course, it is not unexpected, given that the Fetus constantly develops and grows, therefore AM also has to continuously change and reform, in order to withstand the increasing mechanical and metabolic load (Dua *et al.*, 2004).

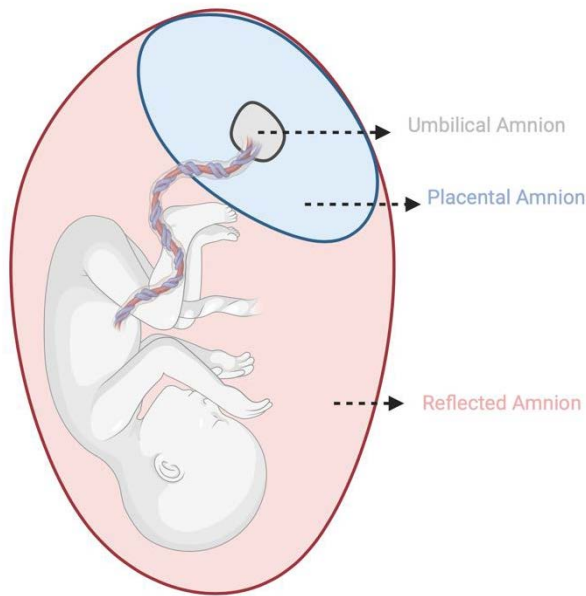


Figure 1: Subregions of the amniotic membrane

Created in <https://BioRender.com> (Weidinger et al., 2021)

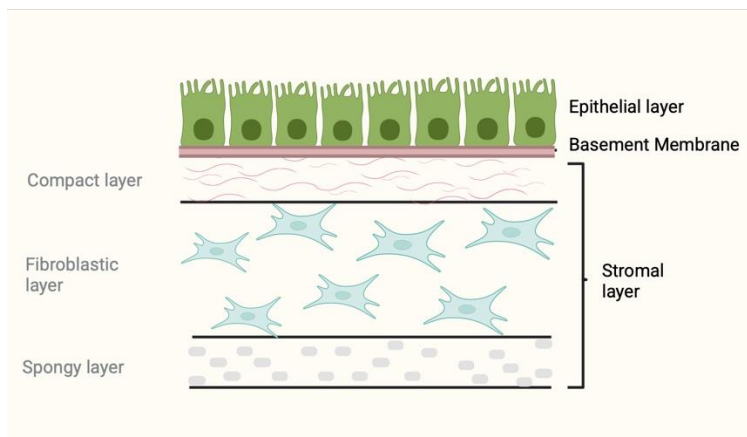


Figure 2: Layers of the amniotic membrane

Created in <https://BioRender.com> (Schmiedova et al., 2021)

Epithelial layer

The epithelial layer is the cellular layer of the AM that faces the fetus and accordingly is in direct interaction with the AF. Across all regions of this layer, human amniotic epithelial cells (AECs) are distributed and these epithelial cells depending on the region can be divided into two subtypes: human reflected amniotic epithelial cells (RAECs) and human placental amniotic epithelial cells (PAECs) (Silini *et al.*, 2020).

The AM epithelium is composed of a single sheet of epithelial cells that possess a polygonal morphology (Mamede and Botelho, 2015). These cells have a columnar shape, where the amnion overlaps the placenta, and is called Placental Amnion, but are cuboidal or flattened in regions, that are distant from the placenta, to be more accurate in the reflected amnion subregion. However, the epithelial cells of the umbilical amnion do not have one layer, but rather three or four layers (Weidinger *et al.*, 2021).

While the basal surface of the epithelial cells possesses podocytic invaginations, that anchor to the underlying basal membrane, which are more prominent in PA than in RA (Weidinger *et al.*, 2021), and are supported by tonofilaments, over the apical surface, face the fetus, they have microvilli and their density fluctuates throughout the whole course of the pregnancy. The main function of these microvilli is uptake, but also production of the fluid and hydrophilic substances. Furthermore, within the stromal connective tissue of the chorionic villi of the human amniotic sac wall, the Hofbauer cells resembling large macrophage-like cells were identified (Mamede and Botelho, 2015).

The nucleus of the epithelial cells has an irregular shape with several notches along the surface of the membrane, whereas the nucleolus is large and uniform, demonstrating active nucleolar function (Mamede and Botelho, 2015). The nuclei of the epithelial cells also show regional differences. In the RA subregion epithelial cells have centrally located nuclei and in PA nuclei are positioned at the apex of the cells, in comparison UCA possess perinuclear halos in lower layer cells, but cells of the upper layer own pyknotic nuclei, or they do not even have nuclei (Weidinger *et al.*, 2021).

The cytoplasmic components of the amniotic cells, rough endoplasmic reticulum (Mamede and Botelho, 2015), Golgi apparatus (DUA, 1999), glycogen deposits, and lipid granules, using Electron microscopy were found (Mamede and Botelho, 2015). In the final weeks of pregnancy, lipid inclusions become increasingly prominent, due to a believed phenomenon linked to the uptake of surfactants that are produced by the fetal lungs, which is also present in the AF near term (Mamede and Botelho, 2015). The quantity of existing Lipid droplets is higher in RA and the periphery of the PA (Weidinger *et al.*, 2021).

Based on studies, AECs demonstrate stem cell characteristic surface markers, they possess the multipotent properties of the undifferentiated epiblast, and pluripotency-associated transcription factors, thereby they are able to transform into the three fundamental germ

layers (Fénelon *et al.*, 2021). These Markers and Factors are stage-specific embryonic antigens 3 and 4 (SSEA-3 and SSEA-4), TRA-1-60, TRA-1-81, Oct-4 (Mamede and Botelho, 2015), and Nanog. They give these epithelial cells the ability to convert or more precisely, to differentiate into a variety of cell types, including fat, bone, nerve, muscle, liver, bone marrow, endothelial, kidney, heart muscle, and retinal cells (Ingraldi *et al.*, 2023).

Another crucial role of AECs is to regulate cellular processes such as proliferation, cellular mechanisms governing molecular movement and excretory role, producing a variety of growth factors and cytokines that support tissue development as well as repair (Gholipourmalekabadi *et al.*, 2020), such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), transforming growth factors (TGF- α/β 1-3), and platelet-derived growth factor (Hu *et al.*, 2023). Furthermore, AECs also release integral components of the basement membrane, which are collagen types III and IV, as well as non-collagenous glycoproteins, including laminins, nidogen, fibronectin, and vitronectin.

It is no surprise that, throughout the entire gestational period the amniotic epithelium needs a significant amount of energy, in order to be able to maintain the previously mentioned functions. Taking into consideration that AE doesn't possess any blood vessels, their oxygen supply relies on other sources, which are chorionic fluid, AF, and the circulation of the fetus, nevertheless, the main energy source is anaerobic ATP production. On the apex of the epithelial cells GLUT1 and GLUT3 (Glucose transporter proteins 1 and 3) are present, facilitating glucose uptake (Dua *et al.*, 2004).

Basement membrane

The middle layer of the AM is the structurally very compact Basement Membrane. It has structural similarities to human skin and is among the most tightly packed tissues, therefore BM owns properties of high resistance to tension and architectural robustness. These characteristics are extremely important to AM to be able to adapt to the growing fetus during the entire gestation, particularly regarding overlying epithelium, which consists of cells that continuously multiply and evolve.

The BM's two main components are lamina densa and lamina lucida (Ingraldi *et al.*, 2023) and it consists of key structural proteins, including collagen types I, II, III, and IV, as well as laminins 1 and 5, and fibronectin. Abundant in hyaluronic acid and other vital extracellular matrix (ECM) components, this membrane forms a crucial interface between the epithelial layer and the underlying connective tissue.

These extracellular matrix proteins facilitate cell attachment, molecular communication, and engagement with receptors on the plasma membrane (Chopra and Thomas, 2013). Laminins play a vital role in the development, migration, and morphology of cells, controlling gene expression (Mamede and Botelho, 2015), as well as preserving phenotypical characteristics and viability of tissue by engaging with proteins on the cell surface like integrins and dystroglycans, particularly the isoforms 2, 4, 5, 6, 7, 10, and 11 (Chopra and Thomas, 2013). In maintaining cell attachment fibronectins are very crucial, while collagens ensure the resilience and robustness of the BM (Mamede and Botelho, 2015).

Stroma

The outermost layer, referred to as the mesenchymal or stromal layer is also the thickest one between layers. Structurally, the stroma is divided into three distinct sub-layers, from innermost to outermost: the compact, fibroblastic, and spongy layers (Doudi *et al.*, 2022).

- Compact layer

As the innermost layer of the stroma, the compact layer (CL) is cell-free, located directly underneath the basement membrane, and consists of Collagen types I, III, V, and VI. These collagens are produced by mesenchymal cells, that are found in the underlying fibroblast layer and they give not only CL but also AM its mechanical strength (Mamede and Botelho, 2015). While collagen types I and III are the most abundant and possess structural endurance properties, that organize into parallel bundles (Sarvari *et al.*, 2022), collagen types V and VI are mainly responsible for adhesion, as well as binding with the BM, and as an outcome, AM has a better tensile strength (Mamede and Botelho, 2015).

- Fibroblastic layer

The fibroblastic layer is the finest between the layers of the AM and contains mesenchymal cells (AMCs) as well as a few macrophages surrounded by a loose network of reticulum (Mamede and Botelho, 2015). These mesenchymal cells depending on the region are divided into two morphologically identical subtypes: reflected amniotic membrane mesenchymal stromal cells (RAMC) and placental amniotic membrane mesenchymal stromal cells (PAMC). AMSCs also have pluripotent properties, due to existing markers that are: SSEA-3 and SSEA-4, however, they do not own TRA-1-60 and TRA-1-81 markers (Silini *et al.*, 2020).

- Spongy (mesenchymal) Layer

This layer has many names: spongy, mesenchymal, intermediate, or, as some authors call it, conjunctive layer, and represents the contact site between the AM and the Chorion. The mesenchymal layer is composed of collagens, particularly type III, as well as proteoglycans and glycoproteins, which gives this layer its spongy-like morphology. The connection to the underlying CM is not tight so that AM moves and slides over the chorion, therefore they can also be easily peeled apart (Mamede and Botelho, 2015).

The spongy layer also shows morphological variations based on amnion subregions, particularly in collagen number and alignment. Collagens are more abundant in PA than in RA, but also in distal parts of the RA further away from the placenta, they become far less than the parts of the RA that are in closer proximity to the placenta. Collagens are arranged in a parallel configuration proximally from the placenta, but distally in a perpendicular configuration (Weidinger *et al.*, 2021).

Extracellular matrix

Overall, the extracellular matrix of the amnion has many different components that give the AM its most important structural properties. Furthermore, these properties are also, one of the characteristics of the AM, which makes it attractive to wound repair and regenerative medicine (Roy *et al.*, 2022).

Glycoproteins are mostly contributing to the architectural robustness of the AM. While fibronectin connects with collagen, proteoglycan, and cells of the amnion as well as chorion, laminin adheres to the proteins of the surrounding layers. Proteoglycans help the connection of the growth factors within amnion, but they are also responsible for the structural cohesion and strength of the AM (Roy *et al.*, 2022).

It is apparent that the AM also needs elements, which make it elastic and play a padding role against the forces. Elastin fibers and Glycosaminoglycans, especially Hyaluronic acid, are the primary components for this role. Elastin fibers are great for stretch resistance and pull strength. Hyaluronic acid on the other hand provides AM with flexibility and reduces friction between layers, it also decreases immunological response. Data has shown that an abundance of HA correlates with collagen existence, particularly collagen types IV and V, therefore BM and mesenchymal layer are rich in HA (Roy *et al.*, 2022).

1.2 Physiological and biological properties of AM

After understanding the anatomy and histology of the AM, the second most important things to know are their biological and chemical features, so to say to know what makes them attractive for medicine. However, the findings of different researchers (m/w/d), determine what markers, genes, factors, etc. the AM and their cells possess and they depend on the cultivation, isolation, preparation, culture, and other such methods (Mamede and Botelho, 2015).

The Amniotic membrane has numerous functions, some of them found in vitro, but some of them found in vivo studies. As we know, AM possesses AECs, AMCs, as well as, extracellular matrix components, which explain its vast number of useful properties, on the other hand, it also produces many different kinds of cytokines, growth factors, protease inhibitors, etc, that give AM other important features and controls normal growth of the fetus during pregnancy (Grzywocz *et al.*, 2016).

Next, we will examine the molecules possessed by amnion for each characteristic (Mamede and Botelho, 2015).

Anti-inflammatory

In general, PA exhibits more tendency toward inflammation, because it possesses a high amount of pro-inflammatory molecules, for example, C-X-C motif chemokine 6, and prostaglandin-endoperoxide synthase 2 (PTGS2) or cyclooxygenase 2 (COX2), compared to other subregions of the AM (Weidinger *et al.*, 2021).

- Serpins (serine proteinase inhibitor)
Secretory Leukocyte Proteinase Inhibitor; Elafin (Elkhenany *et al.*, 2022)
- Cytokines
Transforming Growth Factor-beta (TGF- β) plays an anti-inflammatory role and found in RA more (Weidinger *et al.*, 2021).
Anti-inflammatory interleukins: IL-10 inhibits pro-inflammatory cytokines IL-1 β , IL-8 (Mamede and Botelho, 2015), IL-6, TNF- α (Elkhenany *et al.*, 2022), interferon- γ , IL-2, IL-3 and GM-CSF (granulocyte-macrophage colony-stimulating

factor) at the same time promotes viability, replication and immunoglobulin synthesis of B-cells (Fairbairn, Randolph and Redmond, 2014).

- Soluble factors

Prostaglandin E2 (PGE2) reduces T-cell division and synthesis of the cytokines, that cause inflammation (Mamede and Botelho, 2015); Hepatocyte growth factor (HGF); Indoleamine 2, 3 dioxygenase (IDO) (Gholipourmalekabadi et al., 2020).

- Exosomes

These are ultrasmall vesicles, produced by AECs and AMCs, and filled with a variety of molecules, that have many different properties. One of the functions is to reduce inflammation by suppressing neutrophil myeloperoxidase (MPO), boosting phagocytosis in Macrophages, and downregulating T cells, that are stimulated by CD3 and CD28 (Elkhenany et al., 2022). MPO enzyme is a reactive oxygen species (ROS) found in Neutrophils, leukocytes that get primarily activated when an infection exists, and they eliminate pathogens by hypochlorous acid (HOCl), which is derived from hydrogen peroxide (H₂O₂) (Nauseef, 2015). In Macrophages they support phagocytosis by switching polarisation from M1, pro-inflammatory, to M2, anti-inflammatory, condition (Elkhenany et al., 2022; Xia *et al.*, 2023).

- Protease inhibitors

α 1-anti-trypsin; inter- α -trypsin inhibitor; IL-1 inhibitors (IL-1RA) (Elkhenany et al., 2022)

- Hyaluronic acid

HA supports the immune system through CD44, It also builds the HC*HA complex with the inter- α -inhibitor's heavy chain (HC), which is also against inflammation and fibrosis (Mamede and Botelho, 2015).

- Migration-inhibitory factor (MIF)

It is produced by AECs and suppresses the function of Macrophages and NK cells (Mamede and Botelho, 2015).

Anti-bacterial

The research has shown that they prevent gram-positive, but at the same time, gram-negative bacteria (Elkhenany et al., 2022), and they are effective especially against MRSA (Munoz-Torres et al., 2023).

- Peptides
The AM produces these peptides against bacteria: elafin; HBD-2 (human-beta-defensin); HBD-3; Cathelicidin LL-37 (Elkhenany et al., 2022).
- Intercellular adhesion molecule-1 (ICAM-1)
Through TNF- α and IL-1 β AECs exhibits ICAM-1. This molecule gathers leukocytes, macrophages, and granulocytes in the inflammation zone (Fairbairn, Randolph, and Redmond, 2014).
- Lysozyme (Elkhenany et al., 2022);
- Lactoferrin (Elkhenany et al., 2022)
- Secretory leukocyte proteinase inhibitor (SLPI) (Gholipourmalekabadi et al., 2020)
- Immunoglobulins
Particularly IgA plays an important role (Munoz-Torres et al., 2023)
- Fas ligand
Through controlling cell death mechanisms, they exhibit anti-bacterial function (Munoz-Torres et al., 2023)

Anti-fibrotic/Anri-scarring agents

- Matrix Metalloproteinases (MMPs) -2/9
It secreted by AECs (Hu et al., 2023) and breaks down excessive collagen accumulation (Tency *et al.*, 2012).
- Tissue Inhibitors of Metalloproteinases (TIMPs)

Produced by AECs, inhibits enzymatic degradation (Fénelon et al., 2021) and controls MMPs function (Tency *et al.*, 2012).

- Exosomes
Exosomes of the AECs carry molecules, that prevent fibrosis, such as growth factors EGF (Epidermal Growth Factor), FGF (Fibroblast Growth Factor), PDGF (Elkhenany et al., 2022), KGF, and HGF, that regulate fibroblast activity (Gholipourmalekabadi *et al.*, 2020), MiRNAs function via influencing TGF- β (Elkhenany et al., 2022), which stimulates fibroblast differentiation (Frangogianni, 2020).
- Hyaluronic acid
Via reducing the effect of SMAD and also non-SMAD signaling hyaluronic acid hinders TGF- β , which is a cytokine and one of the functions is to catalyze fibrosis while supporting immune response (Elkhenany et al., 2022). HC*HA complex also has an anti-scarring function (Mamede and Botelho, 2015).
- Proteins
IGFBP-3 (Insulin-like Growth Factor Binding Protein-3) as well as DKK1/3 (Dickkopf-related proteins 1 and 3) are produced by AMCs and in research in mice, they reduced fibrosis of the liver (Hu et al., 2023).

Pro-angiogenic properties

PA promotes angiogenesis more compared to RA, because of more angiogenin and Olfactomedin-like protein 3 expression, additionally, RA in the cervix is even less pro-angiogenic than other parts of the RA (Weidinger et al., 2021).

- Growth factors
AMCs produce PDGF, VEGF (Elkhenany et al., 2022), HGF, EGF, and FGF-2, which promote the formation of new blood vessels (Hu et al., 2023).

- **Proteins**
 Angiogenin is produced by AMCs and through endothelial cells it facilitates angiogenesis (Elkhenany et al., 2022; Lyons *et al.*, 2016).
 Angiopoietin-1 and the antiapoptotic protein AKT-1 generated from AMCs have angiogenesis-promoting roles (Hu et al., 2023). Olfactomedin-like protein 3 stimulates migration and replication of vascular endothelial cells (Weidinger et al., 2021).

Anti-angiogenic properties

- **Tissue inhibitors of metalloproteinases (TIMP-1, 2, 3, and 4)**
 These are regulators of Matrix Metalloproteinases (MMPs), which play a role in neovascularization, particularly in tumor development, hence through these the AM also participates in the prevention of tumor growth (Elkhenany et al., 2022; Quintero-Fabián *et al.*, 2019).
- **IL-1Ra (Interleukin-1 receptor antagonist)**
 They are produced by AECs and suppresses specifically IL-1, through this it hinders IL-1 α , that have an angiogenesis function, in tumor tissue as well (Elkhenany et al., 2022; Gong *et al.*, 2018)
- **Thrombospondin-1 (TSP-1)**
 It is a glycoprotein with many functions. It shows anti-angiogenic properties by attaching to the CD36 receptor on microvascular endothelial cells and hindering VEGF signal transduction (Elkhenany et al., 2022; Chu, Ramakrishnan, and Silverstein, 2013)
- **Endostatin and Collagen XVIII**
 Endostatin emerges from the breakdown of Collagen XVIII, which is a part of ECM and expressed by cells of AM (Elkhenany et al., 2022; Wenzel *et al.*, 2024). Collagen XVIII possesses anti-angiogenic qualities, accordingly endostatin as well (Elkhenany et al., 2022).

- Pigment epithelial- dependent factor (PEDF) (Munoz-Torres et al., 2023)

Immunomodulation

Although the AM is immunologically active, it shows no immune response or host rejection in transplantation (Roy *et al.*, 2022).

- Surface Markers

The AM has reduced immune response, due to the downregulation of CD86, CD40, CD80, HLA-Ia, and HLA-DR, therefore inhibiting T-cell multiplication (Elkhenany et al., 2022).

Another non-classical major histocompatibility complex (MHC) is HLA-G and it suppresses the activity of the natural killer (NK) and T cells by binding to the receptors ILT 2/4 and KIR2DL4 on the cells (Hu et al., 2023).

On the other hand, the AM does not possess HLA-II and exhibits reduced or non-HLA-I expression, which plays a role in immune activation (Fénelon et al., 2021). The majority of the AM cells possess HLA-I, while a subset of AM fibroblasts exhibits HLA-II (Chopra and Thomas, 2013).

- Programmed cell Death Ligands (PD-L)

These are immune checkpoint molecules and by connecting to PD-1 receptors of the T-cells, they prevent T-cell function (Hu et al., 2023; Gutic *et al.*, 2023). AECs produce these proteins, however, AMCs possess them (Hu et al., 2023).

- Surfactant Protein D and A

RA exhibits mostly Surfactant Protein D expression and PA mostly Surfactant Protein A, they are responsible for modulating the immune response (Weidinger et al., 2021).

- Proteins

Pentraxin 3 (PTX3) (Elkhenany et al., 2022), Fas Ligand (Hu et al., 2023).

Anti-apoptotic factors

- AMCs - conditioned medium (AMCs-CM)

They are produced by AMCs and suppress heat-stress-related apoptosis in epidermal keratinocytes and fibroblasts of the dermis in humans (Hu et al., 2023).

- Exosomes
AECs synthesized exosomes hinder apoptosis and stimulate cell survival (Hu et al., 2023).
- Bcl-2 (B-cell lymphoma 2)
As an anti-apoptotic protein modulates cell survival while hindering apoptosis (Elkhenany et al., 2022).

Regenerative and reparative properties

- Growth factors
FGF-6, PDGF- AB, macrophage colony-stimulating factor receptor (M-CSFR), VEGFR3, neurotrophin-4 (NT-4), insulin-like growth factor binding protein 4 (IGFBP-4), IGFBP-6 (Elkhenany et al., 2022), EGF, IGF-1 (Hu et al., 2023), keratinocyte growth factor and hepatocyte growth factor (Weidinger et al., 2021).
- Neuroprotective factors
Brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and forskolin are produced by AECs, and in pregnancy, their function is in the development of the nervous system of the embryo, so they influence tissue repair and regeneration (Hu et al., 2023).
- MMP-2/9
Through ECM-remodeling, they boost regeneration (Hu et al., 2023).
- Monocyte chemoattractant protein (MCP)-1
Synthesized by AECs in a low oxygen environment (Hu et al., 2023).
- AMCs-conditioned medium (AMCs-CM)

IL-8 together with EGF and IGF-1, secreted by AMCs-CM, speeds up the closure of the wound, thereby showing regenerative and tissue repair properties (Hu et al., 2023).

- ECM components

Laminin, fibronectin, HA, collagens, proteoglycans, etc. promote regeneration and tissue repair (Gholipourmalekabadi *et al.*, 2020). β IG-H3 is also a part of the ECM components and plays a role in cell migration and tissue healing (Mamede and Botelho, 2015).

- Cyclic AMP response element binding (CREB) protein

It is very crucial for the differentiation, as well as, proliferation of cells, and the AM exhibition of CREB protein on the periphery is more than in other areas (Weidinger et al., 2021).

- PTHrP (parathyroid hormone-related protein)

PTHrP is produced by AECs and its expression in placental amnion is more than reflected amnion. It induces cell differentiation, migration, and tissue repair. Particularly, it is very important in cartilage formation and bone repair, so that PTHrP promotes chondrogenesis, as well as reduces hypertrophy of the chondrocytes. It also promotes Keratocyte differentiation (Weidinger et al., 2021).

- microRNAs

It is expressed by AM, and they are found in PA more than in RA. They transform into smooth muscle cells (Weidinger et al., 2021).

- Integrin α 6/ β 4

One of the important molecules in BM, they play a role in the attachment of AECs to the BM, therefore on the wound they secure stem cell connection to the wound surface (Chopra and Thomas, 2013).

Non-tumorigenic

The other great property of the AMSCs is that, in vivo grafting they do not cause teratomas (Elkhenany et al., 2022).

- TIMP-1, 2, 3, and 4
As mentioned above, downregulating MMPs, as a result, hinders neovascularisation in tumor tissue and inhibits cancer growth (Elkhenany et al., 2022; Quintero-Fabián *et al.*, 2019)
- Proteins
IGFBP-3 and DKK1/3 reduce the multiplication of the cells, at the same time it stimulates the tumor cell death (Hu et al., 2023).

Stem cell qualities

Before talking about the stem cell qualities of AM, first of all, one should understand what stem cells are and which properties make a cell so-called stem cells (Mamede and Botelho, 2015).

The undifferentiated cells that can transform, so to say give rise to various cell types of the body, as well as, are able to restore and renew themselves are called stem cells. On the other hand, these cells also possess specific intracellular and extracellular stem cell markers, which is very helpful in identifying them. There are different types of stem cells based on their potency: totipotent, pluripotent, multipotent, oligopotent, and unipotent. Totipotent stem cells can develop and produce the whole organism, The Zygote is a very good example of this, which is the result of a fertilized egg and a starting point of the organism's creation. Pluripotency is when stem cells are able to give rise to any cells of the organism excluding extraembryonic cells, for instance, Embryonic stem cells. Multipotent stem cells, however, can only differentiate into the cells of a particular cell lineage, like neural stem cells, which transform into only the cells of the nervous system. Oligopotent stem cells can specialize into a few cell types, for example, myeloid stem cells. Unipotent stem cells on the other hand just specialize into one cell type and can proliferate many types (Zakrzewski et al., 2019).

As we mentioned before, the origin of the AECs is ectodermal epiblasts, which is why AECs possess pluripotency markers, however, the origin of the AMCs is extraembryonic mesoderm, so that is the reason for their multipotency (Mamede and Botelho, 2015). However, not all of the AECs and AMCs express the same markers, it also depends on the embryonic development period, as well as, from which part of the AM these cells are obtained. As an example, approximately 50% of the AECs exhibit SSEA-4, but 1-3% possess NANOG (Hu et al., 2023). On the other hand, in vivo studies of AECs and AMCs have not displayed any tumor-building activity of these cells, despite stem cell properties (Elkhenany et al., 2022).

- AECs
 - Embryonic markers
SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 (Canciello *et al.*, 2017), REX-1, FGF-4, GCTM-2, CFC-1, DPPA-3, PROM-1 and PAX-6 (Mamede and Botelho, 2015).
A subset of AECs display these embryonic markers: Oct4, Sox2, Nanog, Tert (Canciello *et al.*, 2017).
 - Mesenchymal and hematopoietic cell markers
CD 10, CD 13, CD 29, CD 44, CD 73, CD 90, CD 105, CD 166, and STRO-1 (Mamede and Botelho, 2015).
 - Epithelial markers
CD 324, CD 326, CD 73 (Weidinger *et al.*, 2021), cytokeratins, and vimentin (Chopra and Thomas, 2013)
 - Epidermal Markers: CA125 (Chopra and Thomas, 2013)

Undifferentiated hAECs also express neural, hepatic, pulmonary, cardiomyogenic, and pancreatic markers (Mamede and Botelho, 2015).

- AMCs
 - Embryonic markers
SSEA-3, SSEA-4 (Silini *et al.*, 2020), OCT-4, REX-1 and SOX-2.
A subset of cells expresses: OCT-3/4 and KLF-4 (Mamede and Botelho, 2015).
 - Mesenchymal stem cell markers

CD 13, CD27^{low}, CD 29, CD 44, CD 49e, CD 54, CD 73, CD 90, CD 105, CD 117 (weak), CD 166, and STRO-1 (Mamede and Botelho, 2015).

Undifferentiated hAMCs also express the neural, hepatic, cardiomyogenic, and pancreatic markers, as well as, PECAM-1/CD31 (platelet/endothelial cell adhesion molecule), vWF (von Willebrand Factor), VEGF, VEGFR-2 (vascular endothelial growth factor receptor 2), basic FGF and angiopoietin-1 (Mamede and Botelho, 2015).

1.3 Research aim and question

Research aim

The primary aim of this research is to systematically investigate the clinical applications and efficacy of amniotic membranes in wound healing. This study seeks to evaluate their benefits and limitations, as well as to explore potential future directions for their use in various medical contexts.

Research question:

What are the clinical applications of amniotic membranes in wound healing, and how do they compare to traditional wound care methods in terms of efficacy, safety, and patient outcomes?

Sub-questions:

1. What specific types of wounds demonstrate the greatest benefit from amniotic membrane treatment?
2. In what ways do the biological properties of amniotic membranes contribute to their effectiveness in promoting wound healing?
3. What challenges and limitations are associated with the implementation of amniotic membranes in clinical practice?
4. How do patient demographics and comorbidities influence the outcomes of amniotic membrane treatments?

2. Materials and methods

This literature-based thesis delved into the role of amniotic membrane in skin wound healing, exploring its advantages, disadvantages, challenges, and future perspectives. The methodology involved systematic search, as well as, selection techniques to identify, analyze, and synthesize relevant academic research.

Electronic databases such as PubMed, Google Scholar, ScienceDirect, Wiley Online Library, BioMed Central (BMC), World Medical Association (WMA), Frontiers, Springer Science and Business Media LLC, Karger, Oxford academic, Taylor&Francis, MDPI (Multidisciplinary Digital Publishing Institute), Scopus, Nature portfolio, Web of Science and websites like WHO, Human Tissue Authority, European Commission were utilized.

Keywords like “amniotic membrane,” “wound healing,” “biological dressings,” “tissue regeneration,” and “wound management” were used, and boolean operators such as AND and OR were applied in order to sharpen search results, as well as secure thorough search.

The research’s inclusion criteria were publications mostly in English that investigated the application of AM in wound healing, particularly skin wound healing while exclusion criteria excluded studies unrelated to amniotic membrane in wound healing or articles without available full text.

Sixty studies meeting the criteria were selected for detailed analysis based on examination of titles and abstracts for relevance.

The findings and conclusions from the literature review are presented and discussed in subsequent chapters.

3. Results, discussion and conclusion

In this chapter, we will explore the applications and role of the amniotic membrane in skin wound healing and regeneration through conducted research, studies, and case reports, to determine its efficacy and advantages/disadvantages as a treatment.

3.1 Amnion membrane and wound healing

First, we will delve into the physiological process of wound healing and also identify where and how this process can fail in order to understand the role of the AM in it. The next step involves reviewing research and studies that have used amnion in the WH, specifically in skin lesions, determining their success or failure, and analyzing the factors contributing to these outcomes. Subsequently, we will summarize the findings and assess whether further research in this area would be beneficial.

3.1.1 Understanding wounds and their healing process

Any injury of the tissue that results in the separation of the biological material from one another or even the loss of a part of the tissue is described as a wound. Damage to the skin, with or without inner organ trauma, is called an open or external wound, however, injuries to any visceral organ, when the skin remains intact, are characterized as closed or internal wounds. Restoration of these defects is identified as wound healing. Two mechanisms take place after the initial wound-healing phases depending on the tissue. One of them is Regeneration, the process, that totally restores the prior form and function of injured tissue, this is also referred to as *Restitutio ad integrum*, for example, when only the epidermis of the skin or a mucosa of the intestinal tract is injured. Another method is reparation, where a new structure replaces the old one, but this doesn't restore prior function, resulting in scar tissue. The majority of complex wounds are examples of this (Lippert, 2012).

Wound types

Firstly, wounds are classified into 3 broad categories based on their causes: traumatic, iatrogenic, and chronic, but certainly there are other categories as well, based on depth, the form of the edges, how clean they are, the severity of the damage, etc (Lippert, 2012).

Traumatic wounds are the result of external forces and they make the most of all the accidental injuries. We can divide traumatic wounds into further categories, such as (Lippert, 2012):

- Mechanical - here, the shape of the wound edges is crucial. It reveals the cause of the wound and vice versa, the cause determines the shape, for example, knife, axe, or car accident wounds have distinct shapes, which is essential for forensic medicine and certainly also for the treatment (Lippert, 2012).
- Chemical - burns from acids and alkalis (Lippert, 2012).
- Thermic - burns, scalds, electrical injuries, frostbites (Lippert, 2012).
- Radiation-induced (Lippert, 2012).

Iatrogenic wounds arise from medical procedures that are carried out for diagnostic or treatment purposes, which include surgical treatments as well (Cheng *et al.*, 2019).

Wounds that have delayed recovery even under therapy and do not show signs of getting better even after 4 weeks are called chronic wounds. They are mostly a result of underlying chronic diseases, such as diabetes, venous insufficiency, chronic occlusive arterial disease, etc (Lippert, 2012).

Wound healing

Wound healing is a dynamic process, in which the catabolic and anabolic processes can take place simultaneously. It is physiologically divided into 4 stages, which overlap one another, and the exact differentiation of the phases is not possible. These 4 phases are (Lippert, 2012):

- Haemostasis

This phase is the very first step of wound healing, which begins immediately right after the injury. The initial reaction in this phase is vasoconstriction to stop the blood loss, afterward

with the help of leukocytes, blood platelets together and fibrin temporary wound closure happens, which protects the wound from external influences (Schultz et al., 2011).

- Inflammation

After the temporary closure of the wound blood vessels dilate and let immune cells migrate to the wound area, which leads to local inflammatory response. Particularly neutrophils and macrophages start cleansing, they get rid of cell debris and kill microorganisms. These processes set the stage for the formation of new tissue (Schultz et al., 2011).

- Proliferation

As it is clear from the name in this stage, the building of the new tissue formation begins, and therefore above all angioblasts, fibroblasts, and keratinocytes proliferate. Angioblasts build new blood vessels and fibroblasts produce collagen, proteoglycan, etc, which result in building granulation tissue. Subsequently, wound contraction and epithelialization start (Schultz et al., 2011).

- Remodeling

In the last stage of wound healing the previously formed granulation tissue turns into scar tissue and the wound area becomes stronger. However, the injured tissue never gains its prior strength (Schultz et al., 2011).

Types of wound closure

On the other hand, the classification of wound closure into primary, secondary, or tertiary depends on the wound's size, depth, edges, overall severity etc (Chhabra *et al.*, 2016).

Primary Intention	Clean uninfected wounds that have minimal tissue deficit and well-defined edges can be closed using methods such as sutures or staples, low risk of scarring
Secondary Intention	Wounds with a significant tissue deficit that have poorly defined edges tend to heal by forming granulation tissue, high risk of scarring

Tertiary Intention	Complex wounds that heal slowly, initially left open to granulate, then closed with primary intention or with grafts, substitutes, etc.
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Table 1: Types of wound closure (Chhabra *et al.*, 2016)

Disruptors of wound healing

All processes that alter or delay the physiological course of wound healing are counted as disturbance factors. While normal healing processes get through all the WH phases in the right order and end up gaining anatomical and functional integrity of the tissue, wounds with impaired healing do not complete those stages and consequently lead to anatomical or functional anomalies, in the worst case can even have life-threatening results. Clearly, WH is not a simple process, on the contrary, many known and unknown factors influence it and the most important factors are the size of the wound, what type of wound it is, and of course the location in the body. As a result, those numerous general and local issues can hinder WH, therefore every wound needs both professional and personalized treatment (Lippert, 2012).

General disruptive factors are those endogenous systemic elements that interfere with the organism's homeostasis and balance. They impair the healing with their overall effect on the organism. Here are some of those factors (Lippert, 2012):

- Age - even though aging is an unavoidable part of life, we people are still trying to slow it down in one way or another. In wound healing aging also has negative effects for multiple reasons. One of the significant reasons is the high prevalence of polymorbidity in older populations, such as diabetes, hypertension, cardiovascular disease, etc. Other reasons are reduction in cell proliferation, new capillary formation, wound contraction, reaction to mitogenic stimuli, fibroblasts, and slowing down of the epithelization (Lippert, 2012).
- Underlying and accompanying diseases - diabetes mellitus, hyperbilirubinemia, liver cirrhosis, malignant disease, anemia, uremia, connective tissue diseases, deficiency of coagulation factors, etc (Lippert, 2012).

- Nutritional status - such as malnutrition, cachexia, low-protein diet, and obesity. Malnutrition is a complex of protein, carbohydrate, fatty acid, vitamins, as well as, trace elements like iron, zinc, copper, etc. deficiencies, which all are important for WH. Patients with obesity frequently experience impaired wound healing. This is primarily caused by reduced blood circulation in adipose tissue, accompanied by comorbidities, thus creating conditions favorable for infection (Lippert, 2012).
- Immune status - the immune system has an immense role in wound healing, especially in the first phase, so processes that impair the immune system, for example, infectious and immune deficiency disease, also impair the WH process (Lippert, 2012).
- Medications - every pharmaceutical product has an adverse effect, so drugs that inhibit DNA synthesis, mitosis, and cellular defense mechanisms like cytostatics and immunosuppressants influence WH negatively, however with careful indication and balanced dosing, the effects are minimal (Lippert, 2012).
- Circulatory disorders - a good blood supply is a prerequisite for the undisturbed healing of wounds. So ischemia, as well as venous and lymphatic drainage issues lead to slower or even non-healing of wounds (Lippert, 2012).

There are also local disruptive factors that have an immense influence on the WH as well. Depending on the localization, morphology, formation mechanism, size, contamination, foreign bodies, local therapeutics, and etc. the wound can heal faster or slower. For example, wounds in areas of good blood supply heal more effectively than those in poorly supplied organs or anal wounds are at risk of infection due to their location, so they need special types of treatments (Lippert, 2012).

In the majority of cases, it has been reported that wound healing is unable to progress beyond the inflammation or proliferative phase, as a result, acute wounds can transition into chronic wounds. For instance, the examination of wound discharge in leg ulcers has revealed a high concentration of mediators and cytokines that promote inflammation, like TNF- α , IL-1 β , and TGF- β 1, which can block the progress of the WH and lead to unsuccessful epithelialization (Castellanos *et al.*, 2017).

Wound healing complications

WH disorders are a consequence of disruptive factors and manifest in diverse wound complications categorized by their morphological features. Main wound complications are these (Lippert, 2012):

- Seromas - are in the wound collected serous fluids, such as lymph or serum, that have built up in the cavity, mainly because of open lymph channels or incised lymph nodes. Clinically swelling is present at the wound site, however, there's no visible redness or pain (Lippert, 2012).
- Wound hematomas - are areas within a wound that are filled with blood and often arise from insufficient bleeding control, slipped ligatures of the vessels, incomplete drainage of the wound cavity, or premature displacement of the drainage tube. The presence of a hematoma increases the risk of infection, as blood serves as a conducive environment for pathogens to thrive. Therefore, larger hematomas should be surgically removed (Lippert, 2012).
- Wound margin necrosis and dehiscence - inadequate suturing technique, trauma to the skin and soft tissue, inadequate incision, and insufficient blood supply can result in wound margin necrosis. The necrosis area initially appears pale or cyanotic cool, which then gradually turns brown and eventually necrotizes, however, on the opposite side of the wound at first adhesions appear, which after a few days leads to wound dehiscence (Lippert, 2012).
- Wound infections - are characterized by the classic signs of infection: redness (Rubor), swelling (Tumor), localized warmth (Calor), pain (Dolor), and loss of function (Functio laesa). Moreover, typical symptoms including fever and elevated white blood cell count are possible, and the specific presentation depends on the causative bacteria (Lippert, 2012).
- Hypertrophic scarring and keloids - hypertrophic scars and keloids have many differences. First of all, they have different clinical, as well as, histomorphological appearances. On the other hand, although hypertrophic scars extend beyond the level of the skin, they do not spread to other areas and remain confined to the area of the healed wound, as well as, within a few months or years they regress. On the

contrary, keloids go beyond the boundaries of the healed wound, do not show any tendency to regress, and can also develop spontaneously (Lippert, 2012).

These are the result of the excess granulation tissue. However up until now the causes for the formation of the keloids remain unclear, we just only know of some predisposing factors. One factor can be the disorder of the melanocyte-stimulating hormone metabolism, so that also explains why dark-skinned people are 10 times more prone to develop keloids. In one study they have also found genetic predisposition on chromosomes 2q23 and 7p11. Furthermore, hypertrophic scarring is anticipated in wounds subjected to significant vertical tension aligned perpendicular to the skin's tension lines. Molecularbiologically it is believed that cytokines and some growth factors have a role in excess healing. On one hand, Lymphocytes linger in overgrown wound tissue, contributing to excess granulation via heightened blood vessel density. On the other hand, another reason could be the TGF- β -induced increase in collagen production, which also overstimulates fibroblasts to synthesize excessive collagen. Hypertrophic scar tissue samples reveal fibroblasts with an inadequate amount of collagen, which showed that proline hydroxylase activity, a marker of collagen synthesis, was 6 times higher in hypertrophic scars than in healthy skin and 20 times higher in keloid scars (Lippert, 2012).

- Malignant degeneration - Scar-related carcinomas are rare, arising from persistent irritation such as unstable scars, chronic fistulas, ulcers, and radiation-induced skin damage (Lippert, 2012).

3.1.2 Application of AM in wound healing

Despite facing some challenges, amniotic membrane possesses nearly all the necessary properties required for a wound care material, graft, or dressing (Arasteh et al., 2019). In wound healing, the amnion can be utilized in numerous ways, such as a dissolving transplant, covering, extract, gel, or liquid (Antoinette Bruwer, 2024).

The properties required for the wound dressings are as follows: they need to create a protective layer to shield the wound from physical, chemical, and microbial pathogens, should maintain the moisture balance and retain hydration, while also absorbing any excess

fluid from wound drainage, should provide an optimal environment for healing. Furthermore, the application and removal of these dressings should be simple and convenient, causing no damage and at the same time must be biocompatible, affordable, safe, and free from allergies or irritations. AM possesses nearly all of the properties mentioned above, and these requirements can be obtained or enhanced through various processing techniques or by adding other substances (Arasteh et al., 2019).

Numerous studies have been conducted on amniotic membrane's relation to wound healing, which has been known for almost more than a century, and in recent decades, the number of studies has increased. In this chapter, we will try to highlight some of them, even if not all of them (Silini et al., 2020).

In a mouse model of full-thickness skin wounds, Arasteh et al. compared the effects of bilayer silk-decellularized amniotic membrane scaffolds, AM treatment alone, and untreated controls within silicon splints to simulate human wound healing. The findings indicate that while the AM performed better than the untreated wound site in terms of epidermal, dermal, and subcutaneous tissue repair, the bilayer Silk - AM scaffold showed superior results in every aspect from the formation of a relatively complete keratinizing epidermal, dermal, and subcutaneous tissue to the improved accessory skin appendages that exhibited normal structure, dimensions, and form. Moreover, the bilayer scaffold demonstrated reduced inflammatory response, an abundance of differentiated fibroblasts, a high amount of glycosaminoglycans, and orderly arranged collagen strands in the dermal layer, unlike the AM-treated wound site. There was no significant difference in the expression of type I collagen and markers of new capillary growth (CD31 and VEGF) between the AM and bilayer scaffold-treated sites. In conclusion, the various beneficial effects of the AM further confirmed, and simultaneously showed the effectiveness of tissue engineering in the WH (Arasteh et al., 2019).

In one study, the regenerative property of AM was examined by separating the epidermis from the skin, leaving only the dermis, and then AECs culture, along with fibroblasts, was applied. The outcome was the emergence of the epidermis on the surface of the dermis and not only did it resemble physiological skin in terms of morphology, but histological examination also revealed similarities in molecular structures such as cells, their interactions, and the existence of ECM.

Several comparable studies examined the reepithelialization characteristics of amnion. In one such study, researchers cultivated AECs and AMCs on a fibrin-based air-liquid interface cell culture to replicate the biological milieu of normal skin. Following 3 weeks of observations, they noted the formation of epidermal keratinocytes from AECs. The development of multiple layers of keratinocyte epidermis layers is attributed to the embryonic epidermal transformation of the AECs, as evidenced by the lack of keratinocyte epidermal biomarkers in the newly formed layers. On the other hand, in the absence of AMCs, only a single cell layer forms, indicating that the support and nourishment provided by AMCs enable amniotic epithelial cells to transform into keratinocytes, which aids in the formation of the epidermis (Farhadhosseinabadi et al., 2018).

In one study they have used AM in the treatment of omphalocele and gastroschisis or in the deep extensive tissue loss after a high-impact injury, aggressive infection, or surgical tumor removal as a short-term biological wound cover to shield the open wound against external physical, chemical, and microbiological threats (Fairbairn, Randolph and Redmond, 2014).

In another study, they used AM as a replacement for the Bogota bag. The results were not only better but working with this biological material, whether applying it to the affected area or suturing it, was much more functional and easier. However, these findings still require further research to obtain more solid and applicable conclusions (Fairbairn, Randolph, and Redmond, 2014; Tekin *et al.*, 2007).

Chronic wounds

Chronic wounds pose a significant challenge within the healthcare system, requiring specialized care for healing. Despite regular treatments, these wounds often remain stuck in the inflammation phase and fail to heal. This can be attributed to pre-existing illnesses in patients, such as diabetes mellitus, circulatory issues, cachexia, and obesity. As a result, patients experience reduced overall well-being, continuous pain, discomfort, and a sense of hopelessness. In severe cases, non-healing and infected wounds may lead to limb or body part amputation, or even endanger the patient's life due to possible sepsis. Furthermore, this wounds also has a negative economic impact on the healthcare system. Therefore, finding an effective treatment alternative would be a significant step towards a better future in medicine in multiple aspects (Antoinette Bruwer, 2024).

In these types of wounds, AM is significantly helpful, specifically due to its proangiogenic, anti-inflammatory, anti-microbial, and immune modulation effects. Other beneficial effects of AM for WH are epidermal regeneration, epithelial cell migration (Dadkhah Tehrani et al., 2021), and pain-relieving properties (Rodríguez-Valiente *et al.*, 2024).

In the developing world, the prevalence of diabetes is steadily increasing, posing significant challenges for both patients and the healthcare system. One of the major issues with diabetes is foot ulcers, which are not only slow to heal but also prone to high recurrence due to diabetes-associated problems in organisms such as peripheral neuropathy, abnormal blood sugar levels, and micro- and macroangiopathy, which consequently reduces the life quality of the patient and in extreme cases can even lead to amputations (Zelen, Serena and Fetterolf, 2013).

Zelen et al. compared treatments with dehydrated amnion membrane allografts versus conventional wound therapy and the differences were massive. Despite applying dehydrated amnion membrane grafts just once every two weeks, in addition to conventional treatment, healing was not only drastically faster but also better than the therapy just with routine wound management (Zelen et al., 2013).

In a study by Zelen, Serena, and Fetterolf they followed up after 9 to 12 months with the patients who had diabetic foot ulcers and healed successfully after the therapy with the dHACM to determine the recurrence rate of the ulcers. The study's results were pleasing, showing a 94.4 % success rate with only one recurrence among 18 patients (Zelen, Serena, and Fetterolf, 2013).

During the research experiment with mice, the researchers focused on stimulating slow-healing wounds, therefore they specifically chose to fixate the wounds because it is known that healing in mice primarily occurs through contraction and this approach allowed them to create a model of slow-healing wounds in mice that closely resembled the same process in humans. To investigate the effects of AM on chronic wounds, both viable and devitalized AM were used and the aim was not only to prove the WH effect of AMs on such wounds but also to compare the differences between these two types of amnion membrane. The results showed that both viable and devitalized AM enhanced the concentration of mitotic active cells in the epidermis and dermis, promoted angiogenesis, and ultimately accelerated the process of granulation. However, it was observed that viable

AM had a profound effect on accelerating epithelization than devitalized AM (Dolivo et al., 2022).

One method that still requires further development is amniotic membrane extract (AME), which possesses nearly all the WH properties of cryopreserved amnion membranes and has been shown to enhance healing speed in chronic wounds by promoting vascular regeneration. Additionally, they also exhibit anti-inflammatory, anti-scarring, and anti-apoptosis properties, that are inherent to amniotic membranes due to the presence of growth factors, chemokine, protease inhibitors, HC*HA complex, and inhibition of T-cell replication. In the study on leg ulcers, researchers found out the mechanism by which AM promotes healing. They uncovered that AME use does not trigger fibroblastic cells in ulcer tissue but only in normal healthy tissues, which is believed to arise from a disrupted signaling pathway in fibroblastic cells in ulcers and leads to healing, while also modulating inflammation (Dadkhah Tehrani et al., 2021).

Rodríguez-Valiente et al. conducted a study on the treatment of non-healing leg wounds in 5 patients. The patients were aged between 61 to 74 with various comorbidities and the wounds on their legs had not healed for 6 weeks. The results showed that 4 patients achieved full recovery and epithelialization within 4 to 6 weeks of AM treatment. However, one patient only achieved 40% epithelialization after 14 months of treatment, and the reasons for this incomplete healing are not fully understood. Another positive outcome of the treatment was a reduction in pain sensation with each application (Rodríguez-Valiente et al., 2024).

Burns

As one of the most widespread injuries in the world, burn wounds are not that simple to be overlooked. Besides being aesthetically unappealing, these issues can lead to severe and potentially fatal health problems like bacterial infections, sepsis, hypovolemic shock, or compartment syndrome, as well as functional losses, reduced quality of life, or impaired physiological functions. Therefore, it is crucial to develop better treatments for these wounds to save lives and enhance quality of life (Shpichka *et al.*, 2019).

In a Malaysian university hospital study, 33 patients, that with superficial second-degree burns on their faces were treated with dried AM. Out of the total participants, only 8 were

female, the rest were male and the average age of the patients was 16.5 years old. The burns were caused by scald, fire, or electric exposure. The results indicated that 28 of the burn patients healed after a single application of dried AM, while the remaining patients required two applications. The treatment duration was between 2 to 14 days. In the post-treatment follow-up, only in 3 patients skin discoloration observed, 2 had darkening and 1 had lightening of the skin and additionally, one patient developed hypertrophic scar tissue. Overall, the outcomes of the study were deemed satisfactory (Bujang-Safawi *et al.*, 2010).

A case study involving a 41-year-old man who suffered from severe electrical burns of both second and third degrees on various body parts at work was treated with the amniotic membrane on his burned chest and abdominal regions one week after the incident.

Throughout the treatment, the patient experienced no complications and his symptoms improved. Upon a 10-month follow-up, researchers noted full skin restoration with hyperpigmentation but no scarring. In addition to providing pain relief and promoting faster healing, the amnion tissue proved advantageous due to its elasticity and ease of application on uneven surfaces. On the other hand, the clear nature of the tissue allowed for visibility of the wound bed, eliminating the need to remove the applied AM during site examinations (Campos Gómez *et al.*, 2024).

In another study, although AM was not directly used as a graft, it was applied over the dermal graft to secure it on the wound surface without the need for stitches or staples. The study involved a total of 54 patients, with an equal distribution of male and female participants and treatments were administered on 108 extremities, divided into amnion and control groups. As is clear from the name in amnion group they applied amnion over the graft and in control group graft were stapled to the skin. A follow-up was conducted after 6 months by a specialist who was unaware of the treatment allocation and the results were evaluated using the Vancouver scar scale. The findings indicated significant improvements in AM applied burn wounds, showing better outcomes in terms of itching and hypertrophic scar formation, particularly in the amnion group, no severe itching or scar formation was reported (Johari *et al.*, 2017).

Surgical wounds

In a case study, a 74-year-old female patient with vulvar cancer experienced a reopening of the wound site after the surgical removal of the cancer tissue and reconstruction of the

surgical site with a flap. Two months after the surgery, it was decided to use cryopreserved AM for treatment upon the occurrence of wound dehiscence. Ten days after the cryopreserved AM was placed on the wound site, there was a remarkable improvement in the healing process, which was not observed previously. Unfortunately, the patient passed away shortly after due to other health-related issues, leaving the long-term outcome of the therapy unknown. Nevertheless, this case study serves as a crucial step toward further research on the treatment of oncological patients who undergo aggressive oncological surgery (Restaino et al., 2022).

Traumatic wounds

In one study, a wound caused by a dog bite was treated with the AM after conventional wound therapy had been administered for six weeks with little progress. The patient was an 87-year-old female suffering from dementia and various other health issues, which complicated her treatment. After just one application of AM, the improvement was remarkable. Following this, treatments were continued once a week for the next six weeks. By the end of the eight weeks of AM treatment, total tissue repair was observed. In comparison, during the first six weeks of conventional therapy right after the incident, healing was only at 20%. However, with the AM treatment over the subsequent eight weeks, healing reached 100% (Antoinette Bruwer, 2024).

A study reported on a 64-year-old male who lost a forefoot due to a workplace accident. During surgery because of skin disruption due to trauma, there was insufficient tissue to cover the exposed bone, so AM was used as a covering. Within four days of applying the AM, granulation tissue began to form over the grafted bone. By the end of four weeks, the entire bone was enclosed without scar tissue. The treatment with AM not only accelerated the healing process but also helped reduce the patient's pain, and discomfort and minimized the risk of infections or complications (Antoinette Bruwer, 2024).

Another study investigated the AM's role in severe traumatic wounds larger than 10 x10 centimeters in sixty patients and they were randomly assigned to two equal groups, one treated with AM, the other with traditional wound covering. The evaluation of the wounds was conducted on the 7th and 14th days using the Bates-Jensen Score. The results showed significant advancement in healing of with AM-treated wounds, particularly in terms of

infection, tissue swelling, and pain sensation. However, there were no significant differences in the full coverage of the wounds (Choudhary *et al.*, 2023).

Scar formation

The development of scars can indicate a failed wound-healing process, characterized by excessive stimulation and process regulation problems that lead to increased collagen buildup. These scars not only pose visual concerns but also impact functionality and can lead to psychological stress. Currently, there is no definitive treatment for hypertrophic scars or keloids and the success of therapy depends on various factors such as the patient's age, overall health, lifestyle, smoking habits, as well as the size, depth, and location of the scar. Predominantly forming before age 30, they are thought to be influenced by hormones and high collagen levels (Hassan *et al.*).

To study the impact of amnion on keloids, researchers at Alexandria University's dentistry faculty conducted a study involving 10 patients aged between 6 and 15 with keloids on their face or neck. The scar area was prepared with a combination of polyvinylpyrrolidone and iodine, followed by the application of AM directly onto the wound, with the inner side in contact with the skin. The AM was sutured, and a second layer was added as a covering. Follow-ups conducted one week and three months post-treatment using the Vancouver Scar Scale and revealed significant improvements, with scores decreasing from the 6-10 range to 4-8 intervals. These findings hold promise for more effective scar treatments in the future (Hassan *et al.*).

Skin hyperpigmentation

In some individuals, because of excessive production as well as clustering of melanin, comes to hyperpigmentation and other skin issues like melasma, lentigines, and sun spots. While the specific reason is unclear, we do know about some contributing factors and genetic predisposition. Although anti-melanogenic products are available, their limited effectiveness and side effects are problematic. The favorable effects of the AMCs such as dermal wound repair, boosting recovery, cell reproduction, and suppressing programmed cell death are already observed. However, in the study by Wang *et al.*, they reported effects of hASCs on hyperpigmentation as well. They have documented that, microRNAs 181a-5p

and 199a that are released from hASCs through exosomes inhibit melanogenesis, simultaneously promote melanosome destruction through self-digestion and consequently, reduce skin hyperpigmentation (Wang et al., 2021).

Sex-based differences in AM-associated wound healing

Research indicates that there are notable differences in wound healing properties between men and women. The high levels of estrogen in women enhance the wound recovery process by promoting the movement of keratinocytes, stimulating fibroblast function, and facilitating the formation of new blood vessels. Furthermore, women tend to exhibit stronger innate and acquired immune responses, which contribute to more rapid wound healing. In men, testosterone may have the opposite effect, hindering the healing process by suppressing inflammatory responses, which could result in prolonged inflammation, slower recovery, and an increased chance of developing fibrous tissue. Moreover, a more structured collagen configuration and thus less scarring is typically observed in women. In opposition, males commonly exhibit more distinct scars and are subject to an elevated chance of fibrosis stemming from ongoing inflammation (Gilliver *et al.*, 2008).

Nevertheless, further research is needed in these areas, especially considering the influence of age-related hormonal fluctuations (Ashcroft, 2004). It is also important to note that no studies have investigated the influence of amniotic membranes on wound healing across genders.

3.1.3 Differences between AM and other wound healing strategies

Several wound therapy methods exist and the optimal choice depends on the wound type, the patient's overall health, comorbidities, and lifestyle. Currently, negative pressure wound therapy, via vacuum-assisted closure, is highly effective for managing complex, large wounds such as surgical wounds, slow-healing wounds, and diabetic ulcers. Other therapy options include hyperbaric oxygen therapy in air-pressure special chambers, platelet-rich plasma therapy derived from the patient's blood, electrical stimulation, or low-level laser therapy and etc. However, it should be noted that these alternative therapies are not very effective. On the one hand, they tend to be quite expensive and not easily accessible, for instance, hyperbaric oxygen therapy is only available in specific large

hospitals or medical centers. On the other hand, these therapies come with various complications and contraindications. Therefore, it is crucial to carefully select and closely monitor patients and indications throughout the entire treatment process (Antoinette Bruwer, 2024).). There are also various other therapy options available, such as silicone-based therapy, corticosteroid application topical or injections, treatments with laser, radiotherapy, and others, but they have not been delivering satisfactory results.

There are primarily two types of skin substitutes: biological and synthetic. Each category has its own set of positive and negative aspects. Non-biological materials carry a minimal risk of pathogen transfer, are easier to manage and especially they can be customized to meet the specific needs of the patient. Additionally, substances can be incorporated into them and they can be easily combined with other biological materials as well. However, due to their differences from natural skin, particularly the absence of ECM, they may not always be suitable for use. On the other hand, the main advantage of biological materials is that they possess ECM, which allows for better compatibility with the physiological skin and facilitates the regeneration of the epithelial layer (Farhadhosseinabadi *et al.*, 2018)

Autologous split-thickness skin grafts remain the gold standard treatment for deep dermal wounds and burns. While this treatment does yield acceptable therapeutic results, it is important to note that there are also certain limitations associated with this procedure. One issue is that the results are not entirely satisfactory (Murphy *et al.*, 2019). Another one is, that while autologous skin grafts heal the burned site, they form another wound while generating the autograft. Therefore these newly formed wounds also have to be treated and have to heal properly, otherwise, they can be an additional burden for the patient by being painful, getting infected, forming scar tissue, etc (Ahuja *et al.*, 2020). A further issue is the insufficient skin surface area in some patients (Murphy *et al.*, 2019), particularly in children, which hinders the possibility of autografting (Ahuja *et al.*, 2020).

Conversely, skin allografts from other individuals fail because recipient immune systems reject them, even with immunosuppressants, as prolonged use is often intolerable (Murphy *et al.*, 2019).

There are also other various grafts, biologic grafts like porcine, cadaveric skin (Ahuja *et al.*, 2020), ECM derived from sources like the submucosa of the small intestine, the matrix of the urinary bladder, or acellular liver scaffolds (Murphy *et al.*, 2019), synthetic materials such as hydrocolloids, hydrogels and silicon, and biosynthetics as an example Integra,

Apligraf, or Dermagraft, and all of them have different failing reasons. For example, Grafts from cadaveric skin pose a risk of high pathogen spread and skin pigmentation mismatch, Integra yields better results compared to other techniques but lacks the epidermis, requiring the use of skin autografts for patients and Apligraf carries a high infection risk and must be utilized within 15 days (Ahuja *et al.*, 2020). Moreover, scaffolds that are created with tissue engineering techniques even though some of them can outperform allografts and autografts, almost all of them are costly, not only in manufacturing but also in storage and applications (Murphy *et al.*, 2019).

The amniotic membrane, when compared to other treatment methods is more advantageous in WH. We are already familiar with the various characteristics of the amniotic membrane and understand how some of these properties work. The amnion is a biological tissue that contains a wide range of bioactive molecules, as well as pluripotent and multipotent stem cells (Elkhenany *et al.*, 2022).

First of all, AM is plentiful and easily accessible, as well as the process of acquiring it is safe and harmless. It can be used for almost any type of wound, whether acute, simple, chronic, or severely traumatic, without concern for the size of the wound due to the abundance of AM tissue available. On the other hand, it can be almost applied to patients of all demographics without complications, even in cases of comorbid patients, since there is no complication observed. Furthermore, amniotic membrane processing offers diverse techniques, enabling versatile applications and combinations with other materials, tissues, or molecules, thus enhancing AM's suitability. When considering all these factors, the amniotic membrane emerges as a more advantageous treatment method compared to others (Elkhenany *et al.*, 2022).

3.1.4. Challenges with AM

Despite all the great properties of the amnion, some drawbacks need to be discussed. The main weakness of the amniotic membrane is first of all the high decomposition ability by enzymes, which in some cases can even be favorable, but because of this problem, AM needs to be applied multiple times to get the needed therapeutic effect (Farhadhosseinabadi *et al.*, 2018). Another one is low and fluctuating physical strength, depending on from which location of the amnion it is obtained, for example, placental amnion is mechanically more robust than reflected amnion (Weidinger *et al.*, 2021).

Therefore AM needs to be handled delicately, and suturing it can be quite challenging, especially in certain procedures. Based on the part of the amnion, it also shows different properties, for instance, AECs mostly reduce new blood formation, but AMCs promote it or placental AM has some different not only structural but also biochemical qualities than other parts of the amnion, so each part has the same but also unique individual qualities. One more example would be a zone of extremely altered morphology that lacks intact AECs and AMCs (Farhadhosseinabadi et al., 2018).

To overcome the challenges associated with AM and achieve better results, several approaches can be considered. These include cross-linking or creating AM-based composites using either synthetic or biological materials, molecules or substances. Additionally, a straightforward and effective method is to thoroughly examine the physical and biological properties of the amnion tissue before using them for their intended purpose. This careful evaluation can help avoid potential limitations. However, it's important to note that there may also be unexpected challenges with AM that arise depending on how and where it is utilized, and these drawbacks cannot always be anticipated (Farhadhosseinabadi et al., 2018).

One more issue is that, although amnion is plentiful and easily obtained, preparing, storing, maintaining viability, and testing it for pathogens still requires additional procedures. Particularly, the short lifespan of AM required developing storing methods, that in some cases can be costly. That's why AMs cost-effectiveness is still questionable (Antoinette Bruwer, 2024).

3.2 Preparation and clinical application of the AM

We have already mentioned various important biological, as well as, mechanical properties of AM. However, it is important to note that, on the one hand, harvesting, processing, and preservation techniques, on the other hand, choosing the right application method for the right clinical problem has an immense influence on the effectiveness of these properties. Moreover, there are still many missing pieces of knowledge about AM that yet need to be explored (Ingraldi, Audet, and Tabor, 2023). Moreover, all of the processes, from the beginning until the point of application, must follow the regulations and protocols of the respective country. In the European Union, they are regulated by Directive 2004/23/EC protocols and in the USA guidelines of the FDA, which are essential not only for the safety of the recipient but also from a public health point of view (Mamede and Botelho, 2015).

3.2.1 Methods of harvesting and processing

First and foremost, it is apparent that the right donor must be found. For the intended purpose the potential donors` medical and personal backgrounds are checked to be sure that the donor is healthy. Women with health issues and also backgrounds of various intimate encounters, as well as, substance abuse are not eligible. The next move is to inform the patient about all the procedures, processes, and applications of the tissue and then, attain the consent. Afterward in the last trimester, serological tests take place, which will also be performed again half a year after the birth, to prevent misleading negative results and ensure the full safety of the tissue (Mamede and Botelho, 2015). Consequently, the human amnion membrane can be harvested (Ingraldi, Audet, and Tabor, 2023).

Clinically applied AMs are mostly from electively scheduled cesarean section placenta because harvesting takes place in a sanitized environment. However, during physiological vaginal childbirth, on the one hand, vaginal contamination of the amnion membrane through physiological vaginal flora occurs, on the other hand, mechanical properties of the amnion change, such as reduced strength and elasticity, accompanied by epithelial to mesenchymal transition (EMT), which enabling as expected the normal child delivery happen (Ingraldi, Audet and Tabor, 2023).

The next step is the transportation of the placenta to the biological tissue storage facility, which in the USA must follow the FDA regulations outlined in 21 CFR Part 1271. The

storage container for transport has to display proper information about the donor's data and the placenta is then placed in a contamination-free solution for transport, for example, isotonic sodium chloride solution, in the optional presence of antibiotic agent depending on the guideline. The container must be held within the temperature range of +2–8 °C until further handling (Ingraldi, Audet, and Tabor, 2023).

To secure the safety of the recipient the donor must be tested for a wide spectrum of pathogens, such as human immunodeficiency virus I/II (HIV), human T-cell lymphotropic virus (HTLV), human hepatitis virus B (HBV) and C (HCV), as well as, syphilis, in some cases also for cytomegalovirus, toxoplasmosis, tuberculosis, Creutzfeldt-Jakob disease and other serious health conditions, furthermore, the biological material must be also examined via a 7-day bioburden test (Ingraldi, Audet and Tabor, 2023).

For further preparation, the amnion/chorion membranes are separated from the placenta and then rinsed with a solution, such as phosphate-buffered saline, that does not change the pH of the tissue, together with antibiotics to clean the material. Afterward, amnion and chorion membranes can be separated (Ingraldi, Audet, and Tabor, 2023). All of these processes must take place in a sterile environment (Munoz-Torres et al., 2023), such as a cleanroom, biosafety cabinet, laminar flow cabinet (Ingraldi, Audet and Tabor, 2023), etc, with sterilized equipment (Munoz-Torres et al., 2023).

The subsequent step in processing depends on the application purpose and tissue regulation rules. There are already numerous methods for preparation and every one of those can alter the structural, physical, and physiological characteristics of the AM, so for this reason the right method for the intended use must be chosen (Ingraldi, Audet, and Tabor, 2023).

Decellularized

As the name clearly indicates, this method aims to eliminate all cells of AM through different techniques, and as a consequence, AM consists of only ECM and other existing molecules, such as growth factors, proteins, peptides, etc. Removal of these cells evidently clears the amnion from the properties that cells give to it and leads to low immune response in allografts, as well as, AM becomes thinner. Therefore AM prepared with this technique is in ocular surface reconstruction particularly preferred (Ingraldi, Audet, and Tabor, 2023). Decellularization can take place with enzymes, like sodium dodecyl sulfate

(SDS), Triton X-100, ethylenediaminetetraacetic acid (EDTA), urea, or trypsin, mechanically like rubbing, as well as, a combination of the different techniques (Ingraldi, Audet and Tabor, 2023).

Sterilization

This process is one of the important steps in the preparation of the AM, to ensure the safety of the patient and to avoid the spread of transmissible infections. These are actively used decontamination techniques: ethylene oxide, glutaraldehyde, formaldehyde, ionizing irradiation, high-dose electron beam irradiation, and supercritical CO₂, but gamma (γ) irradiation and peracetic acid (PAA) are more frequently used ones. Sterilization can also alter the structural and biological features of the AM, so it is vital to use the proper and suitable dose. For example, for gamma irradiation the ideal dose is 25 kGy, in which the tissue is sterilized, however, no damage is done to its cellular, biological, or morphological components. Additionally, this dose is also accurate according to ISO standard (ISO 11137), which has a Sterility Assurance Level (SAL) of 10^{-6} . The gamma irradiation dose higher than that destroys not only the molecular components of AM, but also its layers. Standard decontamination compound PAA on the other hand successfully eradicates pathogens and does not act toxic if remnants are left on the tissue, while not destroying AM's morphology, which can be rather difficult to achieve with gamma irradiation. These decontamination methods can also be combined (Ingraldi, Audet, and Tabor, 2023).

Cross-linking

We already know about different vital and unique properties of AM and how AM products are used and also researched in medicine. However, there is another problem with the application of AM, particularly as a tissue graft, which is the liability of the prepared graft's membrane to be harmed by collagens of the recipient on the applied site and consequently destroying AM before even being able to treat the applied site. It creates other difficulties with itself, such as reapplying the graft regularly, slowed treatment, etc. Crosslinking methods are intentionally developed to solve this important problem and difficulties. The most frequently used agents for crosslinking with AM are Glutaraldehyde (GA), and 1-ethyl-3(3-dimethyl aminopropyl) carbodiimide (EDC) and in a study using

0.1% glutaraldehyde (GA)-crosslinked amniotic membrane (AM) demonstrated enhanced physical robustness as well as, a slower breakdown rate of the grafted membrane and led to prolonged viability of the graft up to 3 month, which was prior to crosslinking just a week. As with other methods, here also the agent, mainly GA, must be entirely washed out, otherwise, it can lead to toxication (Ingraldi, Audet, and Tabor, 2023).

AM-based composites

AM is also utilized with various materials to eliminate undesirable characteristics, enhance desired functions, improve outcomes, and address the challenges of the AM, and vice versa, creating materials that are structurally and functionally better for the intended use. Different substances exist to create these superior materials by combining AM with biological and non-biological substances (Dadkhah Tehrani *et al.*, 2021).

Silks obtained from spiders or Lepidoptera worms and consisting of fibrous proteins are used to create a bilayer scaffold with the AM. They get through an electrospinning process, which generates pores, thus increasing surface area and creating an ECM-like surface-to-volume ratio. Afterward, this created a fibroin nanofibrous layer handled by air plasma to enhance its water solubility features and at the same time eliminate pathogens from the constructs. Next, the layer is placed on top of the decellularized AM (Arasteh *et al.*, 2019), resulting in a bilayer scaffold that enhances mechanical strength and slows down the decomposition of the AM (Dadkhah Tehrani *et al.*, 2021).

A combination of dAM with clavainin A improves AM's fungal-inhibiting properties, physical stability, and biological compatibility, consequently making it more suitable for some ophthalmological treatments (Dadkhah Tehrani *et al.*, 2021).

In some cases, the single layer of the AM is not sufficient, and more layers must be used. Attaching these layers to each other through stitches is not that easy, so in some studies, they used fibrin glue for these challenges. Fibrin glue added AM is stronger, and there is no need for stitches, as it glues not only AM layers one to another but also to the applied surface, for example, this method can be very beneficial in ocular surgeries (Dadkhah Tehrani *et al.*, 2021).

In cardiac-related treatments, dAM is combined with human cardiac ECM hydrogel to enhance the regenerative properties of AM (Dadkhah Tehrani *et al.*, 2021).

In abdominal surgeries, a polypropylene mesh is used to reinforce the closed hernia sac, preventing the formation of a secondary sac in the future. This mesh strengthens the sac, but can also cause issues such as abdominal adhesions. However, when an AM-prepared polypropylene mesh is used, it significantly reduces the likelihood of the adhesion forming (Dadkhah Tehrani *et al.*, 2021).

There are a vast number of AM combinations that are being used or developed for different medical specialties and various issues, proving a good deal of applicability of the AM (Dadkhah Tehrani *et al.*, 2021).

3.2.2 Preservation techniques

In some instances the AM is still used fresh, just a few hours after the dissection from the chorion (Munoz-Torres *et al.*, 2023), however, it is not easy to obtain fresh AM and utilize it immediately within the specified timeframe (Ingraldi, Audet and Tabor, 2023), moreover, it also brings with itself safety issues, since the tests can take a while even up to 6 months (Mamede and Botelho, 2015). Therefore some preservation techniques have been found, and proven to be very useful. Preservation techniques are apparently also highly dependent on the application purpose of the AM. Each of these preservation methods can alter every possible quality of the tissue, for instance, in one study they demonstrated that, through cryopreservation, AM becomes thicker due to fluid absorption, whereas in lyophilization the AM first gets thinner as a result of dehydration, but right after fluid restoration it gains its normal density. The differences are even macroscopically visible (Ingraldi, Audet and Tabor, 2023).

Fresh

There are still ongoing discussions about, whether fresh amnion membrane has advantages over AM, which are with other methods preserved and they defend that in fresh AM all the properties remain intact and functional, which is not the case with the AM after processing. As mentioned above, fresh AM has some limitations, the most important one being is a carrier of various dangerous infections, which can lead to public health disasters. Additionally, contrary to those beliefs, it has been proven that fresh amnion in fact provides no benefits over the processed and preserved AM. The AM can be temporarily

stored at below 0°C. For longer periods of time to the extent of 6 Weeks it should be slightly processed. Firstly AM must be washed with 0.025 % sodium hypochlorite solution and then it can be preserved at 4°C in one of these solutions: 20 % glycerine, silver nitrate, or sterile saline. Moreover, for preservation of more than a year, it must be held in 85 % glycerol solution at 4°C (Mamede and Botelho, 2015).

Cryopreservation

This is a vital technique, that keeps the viable cells stored at a hypothermic state for a long time without damaging the cells and keeping important molecules as well as cell structures intact (Jaiswal and Vagga, 2022). This method is used more frequently, due to being secure and effective (Ingraldi, Audet and Tabor, 2023). It is performed mostly either with dimethyl sulfoxide (DMSO) and 1% human serum albumin in saline solution or DMEM and glycerol at the ratio of 1:1, and then the temperature will start to decrease slowly up to the point of -80°C (Munoz-Torres et al., 2023). In one study they found that there is no difference between fresh AM and cryopreserved AM with DMEM and the glycerol 1:1 ratio in terms of stretching capacity and histology (Ingraldi, Audet and Tabor, 2023). The cryopreserved AM can be preserved for an extended period of months and according to reports even for as long as 2 years (Mamede and Botelho, 2015).

Undoubtedly this method also has some drawbacks. On the one hand, some important molecules get removed unintentionally, on the other hand before utilizing the AM it has to be washed off to get rid of the cryopreservative layer, however in some instances the layer is not removed entirely and it results in being toxic. One another problem with cryopreservation is that, in order to keep the tissue intact until utilization cryopreserved refrigeration compartments are needed, which are very large and expensive, which makes the availability difficult (Ingraldi, Audet and Tabor, 2023).

Lyophilization/dry freezing

With this method, all the fluid that the AM possesses gets evaporated, which lets the tissue be preserved for an extended period of time more easily, as well as, transportation becomes also easier. Firstly AM is distributed over a nitrocellulose sheet, then divided into pieces, the next step is treatment with trehalose solution, afterward replacing the tissue in a freezer

at -80°C and the last step is lyophilization. Apparently, this process also changes the properties of the AM, which is the case with every method. Although, in cryopreservation, the important molecules, such as proteins, growth factors, and HA, are retained better, the lyophilization process does not change the antibacterial properties of AM and keeps its structural form better. This is mostly due to incubation in a trehalose solution, which exists in nature and helps organisms regain prior structure and properties after long-term dehydration (Ingraldi, Audet and Tabor, 2023). In the AM trehalose prohibits the forming of ice crystals during the freezing period, which damages the cells, instead, it secures the cell membrane and other components of the AM by forming a matrix and it has been found that lyophilized AM without trehalose treatment is (Mamede and Botelho, 2015). Additionally, other benefits of lyophilization are that its storage and transportation are easier, as well as, low cost, which is not the case with cryopreservation (Mamede and Botelho, 2015).

Controlled dehydration

This method also uses water depletion and tissue drying, however, the temperature is more regulated, and here different techniques are used, such as thermal treatment or cold storage at $4-8^{\circ}\text{C}$ (Ingraldi, Audet and Tabor, 2023).

Air-drying

At room temperature in a sterile workbench, for example laminar flow chamber, AM are prepared and then left to air dry for a determined timeframe. Many chose to sterilize the AM as the last step, in order to guarantee, that the tissue is contamination-free. This dehydration technique is less complex, more efficient, and inexpensive than all other methods. Moreover, with this method prepared AM seals the used surface against microbiomes, such as Bacillus, Escherichia coli, Pseudomonas, Citrobacter, Flavimonas, and Staphylococcus, therefore, can be effectively used in wound dressing (Ingraldi, Audet and Tabor, 2023).

Other methods

Using chemicals or physical techniques to preserve AM on one hand reduces immune rejection because of the eradication of the vital membrane components, on the other hand, it can also change and ruin the AM structure (Munoz-Torres et al., 2023).

Sterilization methods	Boiling, Autoclave, Peracetic acid, Ethylene oxide, Glutaraldehyde, Formaldehyde, Ionizing irradiation, High-dose electron beam irradiation, Supercritical CO ₂ , Gamma irradiation, Peracetic acid
Preparation methods	Fresh, Cryopreservation, Lyophilization/dry freezing, Controlled dehydration, Air-drying, Amnio-M sponge, AM-extract, Gel form
Methods for developing AM-based composites	Cross-linking with: γ -ray and electron beam Glutaraldehyde irradiation, Carbodiimide, Photo cross-linking UV irradiation Hybridization with: Atelocollagen, HA, Aloe vera gel, Gelatin Methacryloyl, Nano-fibrous Fibroin, POC polymer Cell integration: Dental pulp derived cells, TGF- β 3 BMSCs, Corneal stromal cell, ASCs Other combinations: Tissue glue, Amino acids, Ca and Ph, Cefazolin, Moxifloxacin

Tabel 2: Overview of AM processing (Elkhenany et al., 2022)

3.2.3 Other clinical uses of AM

Other than wound healing, there are many different clinical applications of the AM in various medical fields (Munoz-Torres et al., 2023).

Ophthalmology

AM utilization in ophthalmology can be the most studied clinical application of the amnion. The defects of the ocular surface by burns, pterygium, conjunctival, or limbal tumors, corneal ulcers, as well as, immune-mediated eye disease, such as Sjögren's syndrome, Mooren's ulcer, peripheral ulcerative keratitis, rheumatoid arthritis, atypical hemolytic uremic syndrome, etc have been cured with amnion. They have mostly used cryopreserved AM, but also frozen or fresh AM, and outcomes were positive.

Ophthalmologically AM demonstrated a low immune response that inhibits tissue rejection, which is very crucial in ocular transplantation use. Other ocular benefits are antimicrobial characteristics, minimized complications, decreased fibrosis, and scar tissue formation. Therapy of pterygium with AM graft additionally illustrated a low incidence of recurrence (Munoz-Torres et al., 2023). Moreover, the additional properties that are very important for ophthalmology and distinguish it from other available graft materials are that AM is fine, partially translucent, resilient, malleable and also weight-efficient (Ingraldi, Audet and Tabor, 2023).

Gynecology

From the early 20th century the use of AM in gynecology, precisely in the reconstruction of the vagina was researched. Since then multiple applications of the AM for different indications have been found, such as patients with vaginal agenesis, vaginal ulcers caused by vaginal mesh, and utero-vesical fistula repair. Amnion compared to other therapeutic methods shows better outcomes and has fewer complications, for example, the rate of intrauterine adhesions is low, tissue rejection, pain, and vaginal dryness are reduced, and better menstrual flow and reproductivity are noticed (Munoz-Torres et al., 2023).

Orthopedic surgery/traumatology

The musculoskeletal system also benefits from AM's many properties, such as the AM utilization results in better tendon recovery, bone fracture recovery, and bone regeneration, as well as because of anti-inflammatory, anti-fibrosis, anti-microbial, anti-tumorigenic and other qualities of AM, overall utilization results in good outcomes. As an example, in carpal tunnel release surgery, the application of frozen AM reduced post-operative

symptoms, and complications, additionally induced repair of the median nerve, which can atrophy because of the compression for a longer time. Another interesting use of AM is in a tissue-engineered bone graft (TEBG), that benefits from ECM's and stem cells' osteogenic and pro-angiogenic qualities (Munoz-Torres et al., 2023).

Gastrointestinal surgery

In gastrointestinal surgery, the AM has many applications. In one study on the rat liver with cirrhosis and fibrosis, they microscopically confirmed, that applying fresh AM reduced the fibrosis. The AM was used in surgical lesions of many abdominal organs and the results of the different studies all confirmed these findings, that AM decreases the inflammatory response, boosts recovery and regeneration, slows septic reaction, as well as, reduces complications. It is also affirmed, that AM can be used to close the open abdomen temporarily as a Bogota bag, because of its pathogen-inhibiting properties (Munoz-Torres et al., 2023).

Neurosurgery

As we know any surgical intervention, regardless of how minor that process seems, in the nervous system is very critical and can impair the patient, causing them to have a reduced life quality. So any effective and promising discovery in neurosurgery is very valuable. Many studies used AM in dura matter repair and had positive outcomes. They utilized fresh as well as dried AM as a substitute and found out that, in the predominant number of instances AM blended with the dura mater so that it faded and patched the injury area, furthermore stopping cerebrospinal fluid (CSF) outflow. Other applications of AMs are after neurosurgeries, for example, laminectomy or neurorrhaphy, that showed AM reduces inflammation, fibrosis, and adhesion creation, moreover induces better, as well as, faster healing, on top of those qualities it also regenerates nervous tissue. Undoubtedly these results and applications have to be researched more to come to better conclusions and to find better application methods for AM (Munoz-Torres et al., 2023).

Oral cavity surgeries

It is evident by means of studies, that disease of the oral cavity also profits from amnion's valuable qualities. We can see the use of cryopreserved AM following dental implant procedure or lyophilized AM after removal of premalignant growth, which exhibits favorable results, such as reduced pain, regeneration of the injury site through building new epithelial cells, and better recovery. In one study hyper dry AM utilization is compared to vestibuloplasty in the therapy of prosthesis preparation, as excision of widespread mucosal abnormalities and applied AM depicted greater outcomes, while vestibuloplasty led to bone protrusion (Munoz-Torres et al., 2023).

Urology

Here also AM demonstrates effectiveness. In robotic-assisted laparoscopic prostatectomy (RRP) amnion/chorion membrane, on the one hand, has been used to secure the neurovascular bundle (NVB), on the other hand, utilization led to a faster return of normal bladder and erectile function. Additionally, frozen AM is used to repair severe ureteral wall damage and has positive outcomes (Munoz-Torres et al., 2023).

3.2.4 Ethical consideration and safety

Since the amniotic membrane is a biological tissue, that once was a part of the human body, there are many important points to be considered. First of all, as it is a human-derived tissue, at every step of the way it must be handled with respect and care. Then there are ethical concerns, and legal and protective principles, which must be taken into account and worked on even before the tissue is obtained (Mamede and Botelho, 2015).

Every country has its own regulations for human-derived tissues, which helps with the above-mentioned issues and more, additionally, there are international and regional legislations (Mamede and Botelho, 2015). In Europe, it is Directive 2004/23/EC, which was created in 2004 by the European Parliament and the Council of the European Union, furthermore over the years new Commission Directives were also added (European Commission, n.d.). In the UK, more particularly in England, Wales, and Northern Ireland, they follow The Human Tissue Act 2004 and related guidelines (Human Tissue Authority, n.d.). In the States, biological tissue has to fulfill the requirements of the FDA section 21

CFR 1271 and also must be sterilized according to the guidelines of the American Association of Tissue Banks (AATB) (Ingraldi, Audet and Tabor, 2023). Globally the International Organization for Standardization (ISO) (International Organization for Standardization, n.d.), the World Health Organization (WHO) (World Health Organization, 2010), and the Declaration of Helsinki provide many standards and guidelines for human-derived tissues (WMA, 2013).

On the market, there are many products, that are made from AM and for these products to be safe, they have to be approved by at least one of those organizations. These approvals guarantee, that these products are clear of infections or any harmful agents and also have been prepared according to the criteria (Munoz-Torres et al., 2023).

There are also laws regarding the data protection, that have to be upheld and also identities of neither the donor nor the recipient never be revealed (Mamede and Botelho, 2015).

Informed consent

Informed consent of the donor is a crucial requirement, that has to be obtained a certain period prior to the birth and only from the donor itself. It has to be clear, thorough, as well as, easily comprehensible. The donors have the right to withdraw their consent at any time without penalty and they are not obliged to give any explanation. The donors have to know these informations before signing the consent (Mamede and Botelho, 2015):

- how the tissue will be acquired
- what amount and type of material will be collected
- designated use of the tissue
- data privacy
- preservation and storage information, how long the tissue will be kept

(Mamede and Botelho, 2015).

3.3 Summary and outlook

It is undeniable that the placenta plays a crucial role in gestation, carrying out a wide range of essential functions from the beginning of pregnancy until the child is born and the amnion membrane, as a vital component of the placental tissue, holds a variety of unique qualities. It contains a numerous of bioactive molecules, structural elements, as well as pluripotent and multipotent stem cells, giving it valuable properties that are yet to be fully explored. These distinctive characteristics make the AM highly suitable for clinical applications (Dadkhah Tehrani et al., 2021).

The potential of the amnion membrane was discovered at the beginning of the last century and since then, research has been conducted to investigate its effects, leading to the development of many methods and techniques to enhance this biological tissue for clinical use. In recent decades, advancements in technology and medicine have increased interest in this valuable tissue, resulting in significant improvements in the implementation of AM and the conduction of numerous new studies. The properties of the amnion membrane allow for a wide range of medical applications, from being used as a patch in neurosurgery to its application in the reconstruction of the female reproductive system, many successful cases have been reported. (Elkhenany et al., 2022).

Amnion has proven to be particularly effective in wound healing. It not only accelerates the healing process but also reduces inflammation, prevents infections and tumor formation, and promotes skin regeneration through epithelialization, which is crucial for wound closure. Additionally, it enhances blood supply through endothelialization, making it especially beneficial for deeper or chronic wounds, also minimizes scar formation and can even reduce existing scars when treated with it. Moreover, its effectiveness in chronic wound healing is especially significant, as these wounds pose a challenge not only for the patients who suffer from them but also for the healthcare system, which causes substantial costs due to the prolonged nature of these cases and the lack of clear pathways to complete healing (Dadkhah Tehrani et al., 2021).

However, amnion has several limitations as well, such as a short viability period and weak mechanical strength. While some of these issues are being addressed through various processing methods that enhance its desired qualities, it still remains more applicable compared to alternative treatment approaches. Its use in clinical settings results in a low

risk of complications, and so far, it has not caused any damage. Additionally, the processing techniques for amnion are still simpler than those of other treatment modalities (Elkhenany et al., 2022).

Future outlook

After examining numerous cases and studies, it is evident that amnion is a remarkably significant biological tissue with extensive implications for the field of medicine.

It possesses an array of diverse and distinctive characteristics, that make it particularly advantageous for medical use and a powerful tool in the realm of wound healing (Elkhenany et al., 2022).

To fully harness the potential of AMs, it is crucial to focus on improving their application through the development of more effective methods for processing and storage. By advancing these techniques, we can ensure that the therapeutic properties of the amnion are preserved and optimized for clinical use and enhanced processing methods will not only increase the viability of the membrane once it is harvested, but they will also facilitate its widespread adoption in medical settings, thereby maximizing the benefits for patients in need (Elkhenany et al., 2022).

Moreover, the regenerative stem cell properties of amnion membranes make them highly valuable in regenerative medicine and allow for the promotion of cellular repair and regeneration, making AM a promising candidate for treating a wide range of conditions beyond wound healing (Elkhenany et al., 2022).

Ultimately, amnion membrane warrant greater acknowledgment within the medical community, and further research on this extraordinary tissue will develop innovative treatment protocols for nearly all types of wounds, resulting in reduced suffering associated with chronic or non-healing wounds, moreover with improved wound management stemming from the use of amniotic membranes, the strain on the public health system may significantly lessen, leading to improved resource allocation and a better quality of life for patients. (Elkhenany et al., 2022).

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