Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine

By

Unaiza Ali Khan

(Registration No: 364029)

Department of Biomedical Engineering & Sciences

School of Mechanical and Manufacturing Engineering (SMME)

National University of Sciences & Technology (NUST)

Islamabad, Pakistan.

(2024)

Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine

By

Unaiza Ali Khan

(Registration No: 364029)

A thesis submitted to the National University of Sciences and Technology, Islamabad,

In partial fulfillment of the requirements for the degree of

MS Biomedical Sciences

Supervisor: Dr. Asim Waris

Co Supervisor: Dr. Aftab Ahmed Chattha

School of Mechanical and Manufacturing Engineering (SMME)

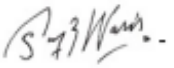
National University of Sciences & Technology (NUST)

Islamabad, Pakistan.

(2024)

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS/MPhil thesis written by **Regn No. 00000364029 Unaiza Ali Khan** of **School of Mechanical & Manufacturing Engineering (SMME)** has been vetted by undersigned, found complete in all respects as per NUST Statues/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis titled. **Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine**

Signature: 

Name (Supervisor): Muhammad Asim Waris

Date: 19 - Feb - 2024

Signature (HOD): 

Date: 19 - Feb - 2024

Signature (DEAN): 

Date: 19 - Feb - 2024

Form TH-4

National University of Sciences & Technology (NUST)

MASTER'S THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: Unaiza Ali Khan (00000364029)
Titled: Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine be accepted in partial fulfillment of the requirements for the award of MS in Biomedical Sciences degree.

Examination Committee Members

1. Name: Adeeb Shehzad Signature:

2. Name: Muhammad Asim Waris Signature:

Supervisor: Muhammad Asim Waris Signature:

Date: 19 - Feb - 2024

Head of Department

19 - Feb - 2024
Date

COUNTERSINGED

19 - Feb - 2024
Date

Dean/Principal

CERTIFICATE OF APPROVAL

This is to certify that the research work presented in this thesis, entitled "Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine" was conducted by Ms. Unaiza Ali Khan under the supervision of Dr.Asim Waris and Dr.Aftab Ahmed Chattha.
No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the department of Biomedical Engineering & Sciences, School of Mechanical and Manufacturing Engineering (SMME), in partial fulfillment of the requirements for the degree of Master of Science in Field of Biomedical Sciences.
Department of Biomedical Engineering & Sciences, National University of Sciences and Technology, Islamabad.

Student Name: Unaiza Ali Khan Signature: _____

Supervisor Name: Dr.Asim Waris Signature: _____

Name of Dean/HOD: Dr.Asim Waris Signature: _____

AUTHOR'S DECLARATION

I hereby declare that the research work titled "Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine" presented in the following thesis is my original work and has not been submitted previously by me for taking any degree from National University of Sciences and Technology, Islamabad or anywhere else in the country/ world.

At any time if my statement is found to be incorrect even after I graduate, the university has the right to withdraw my MS degree.

Name of Student: Unaiza Ali Khan

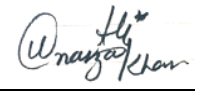
Date: 19 February, 2024

PLAGIARISM UNDERTAKING

I solemnly declare that research work presented in the thesis titled "Human Placental-Derived Extracellular Matrix Sheets as Scaffolds for Cell Growth in Cornea Transplantation: A Promising Approach in Regenerative medicine" is solely my research work with no significant contribution from any other person. Small contribution/ help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and National University of Sciences and Technology (NUST), Islamabad towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS degree, the University reserves the rights to withdraw/revoke my MS degree and that HEC and NUST, Islamabad has the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized thesis.

Student Signature: 

Name: Unaiza Ali Khan

To my wonderful parents and brothers, this thesis is dedicated to you. Your love and support mean everything to me. Thank you for always believing in me, cheering me on, and being there through thick and thin. Your sacrifices have paved the way for my academic journey, and this achievement is as much yours as it is mine. I am grateful for the values and strength you've given me.

ACKNOWLEGMENTS

First and foremost, I want to thank to the Almighty Allah for helping me to complete my research work successfully. I'm grateful for the blessings received throughout this journey.

I would like to express my special thanks of gratitude to my supervisor ***Dr. Asim Waris***, *HOD Department of Biomedical Engineering & Sciences (SMME, NUST).*

I extend my heartfelt thanks to my Co-supervisor ***Dr. Aftab Ahmad Chattha***, *founder of Chattha Bio Care and National Academy of Young Scientists (NAYS),* whose guidance, expertise, and unwavering support have been instrumental in shaping this research and give the golden opportunity to do this wonderful research project.

I would also like to appreciative my family and specially my elder brother ***Zulqarnain Haider*** for their constant encouragement and understanding during the ups and downs of this academic endeavor. Thanks to my mother for their exceptional dedication to managing household chores, creating a comfortable space for us all.

I would also acknowledge my friends specially Nadia Khan for their moral support throughout this project.

Lastly, I would like to acknowledge NUST research funding department for their financial support, which made this research possible.

Each of you has played a crucial role in this journey, and I am sincerely thankful for your support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

Page No:

LIST OF FIGURES

Page No:

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

AMSCs	Amniotic Membrane Scaffolds
AM	Amniotic Membrane
AMD	Age Related Macular Degeneration
BAK	Benzalkonium chloride
CHAM	Cryopreservation Human Amniotic Membrane
CPAs	Cryo-protectants
CECs	Corneal Endothelial Cells
DHAM	Dry Human Amniotic Membrane
DM	Decemets Membrane
ECM	Extracellular Matrix
E coli	Escherichia coli
FTIR	Fourier Transform Infrared Spectroscopy
GAGs	Glycosaminoglycan’s
GBD	Global burden of diseases
hAM	Human Amniotic Membrane
HIV	Human immunodeficiency virus
H &E	Hematoxylin and Eosin staining
HA	Hyaluronic Acid
IL	Interleukin

PBS	Phosphate Buffer Saline
PM	Placental Membrane
RD	Retinal Detachment
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SDS	Sodium dodecyl sulfate
TE	Tissue Engineering
TNF-β	Tumor Necrosis Factor-β
TIMP	Tissue Inhibition of Metalloproteinase
UV	Ultraviolet
UTM	Ultimate Tensile Strength
YLDs	Years Lived with Disability

ABSTRACT

Eye is a sensory organ designed for human vision. Its intricate components work together to make the process of sight possible. The cornea is a critical part of the eye responsible for clear vision, and corneal diseases or injuries can lead to visual impairment or blindness. However, the limited availability of suitable donor tissue poses a significant challenge. There is a significant influence on the quality of life when the visual acuity is reduced. In terms of the overall prevalence of blindness and visual impairment Pakistan ranks third position, following the India and Bangladesh across all age groups, totaling 21.78 million. Placenta-derived extracellular matrix (ECM) sheets have become an effective therapeutic approach due to their rich composition of bioactive molecules, growth factors, and supportive microenvironment for tissue regeneration. The unique composition of placental-derived ECM sheets can provide a favorable microenvironment for the growth of corneal cell and promote the regeneration of corneal tissue. In this study amniotic membrane sheets, have been prepared by decellularizing placental tissue and different characterization techniques have been used for a thorough examination of the human amniotic membrane. Scanning Electron Microscopy (SEM) reveals intricate surface features, while Hematoxylin and Eosin (H&E) staining provides insights into tissue architecture. Fourier Transform Infrared Spectroscopy (FTIR) offers a detailed examination of biochemical composition. Microbial activity testing provides valuable information of the membrane's antimicrobial properties. A p-value < 0.05 in the ANOVA analysis indicated a significant difference in antimicrobial activity among the three bacterial strains. The characterization approaches utilized in this study contribute to a better

knowledge of the biological characteristics of the human amniotic membrane, paving the path for advances in regenerative medicine and tissue engineering. In this study a human placental-derived extracellular matrix (ECM) sheets have been used to investigate the integration potential of the ECM sheets with host corneal tissue. The positive outcome was associated with a noticeable reduction in size of corneal defect due to the application of amniotic membrane transplant. The use of AM proved to be essential in reducing notable subjective symptoms like pain, as well as clinical signs such as redness and the size of corneal ulcers.

Key words: Amniotic membrane, Characterization techniques, corneal epithelial defect, Extracellular matrix.

CHAPTER 1: INTRODUCTION

The eye functions as a sensory organ, primarily designed to facilitate human vision. Its intricate components work in harmony, collectively contributing to the complex process of sight. By absorbing light from the surrounding environment, the eye transmits visual data to the brain for intricate processing. . (*Eyes: Structure, Function, and Disease*, n.d.). The front of the eye constitutes the visible portion, whereas the remaining structures are located within the eye socket, commonly referred to as the orbit. (*Eyes: Structure, Function, and Disease*, n.d.).

A histological understanding of the layers of the eye is essential for comprehending the pathophysiology of diseases and gaining insight into specific therapeutic approaches. External components of the eye include features like eyelashes, lids, muscles, accessory glands, and the conjunctiva. Internally, the eye is arranged into three concentric layers: the outermost layer consisting of the sclera and cornea, the middle layer comprising the uvea (iris, ciliary body, choroid), and the innermost layer encompassing the lens, vitreous, and retina. (Pradeep et al., 2023).

The cornea is made up of several layers including the epithelium, stroma, Bowman’s layer, descement’s membrane and endothelium (Sridhar, 2018). The epithelium acts as a protective barrier and absorbs oxygen and nutrients. Beneath it lies Bowman's layer, a transparent tissue prone to scarring when injured. The stroma, constituting the majority of the cornea, is primarily composed of water and collagen, providing strength and elasticity. Descemet's membrane, positioned under the stroma, act as a defensive barrier and repairs itself after damage. The endothelium, which is the innermost layer, maintains corneal clarity by pumping excess fluid out of the stroma. Injury to the endothelium can result in corneal edema and potential blindness, where transplantation stands as the sole viable treatment option (*What Is The Cornea & How To Treat Corneal Issues*, n.d.).

A wide range of conditions falls under the umbrella of eye diseases and corneal disorders, affecting the overall health and functionality of the eye. The global prevalence

of these conditions varies based on the specific disorder and geographical location. Refractive errors, including myopia, hyperopia, and astigmatism, impact billions of people globally, and there is a growing prevalence of myopia, notably observed in East and Southeast Asia. (Holden et al., 2016).

A decline in visual acuity significantly influences the quality of life, particularly affecting individuals dealing with various chronic health conditions. (SJ Park et al., 2015), (Y. Park et al., 2015). This issue has evolved into a prominent global health concern.

Within the South Asian region, Pakistan holds the third position, following India and Bangladesh, in terms of the overall prevalence of blindness and vision impairment across all age groups, totaling 21.78 million. The percentage change in the overall burden of vision loss in Pakistan between 1990 and 2017 was 67%, ranking it as the third least increased among South Asian countries, with Sri Lanka at 58% and Nepal at 64%. Notably, prevalent eye disorders such as presbyopia, retinal detachment (RD), and cataract persist as predominant issues across all South Asian nations, including Pakistan (Hassan et al., 2019).

The placenta functions as a sophisticated organ facilitating physiological interaction between the mother and the fetus. It has an abundance of bioactive molecules and extracellular matrix (ECM) (Wildman, 2011). The placental membrane comprising three main layers, this includes amnion facing the fetus, chorion facing the mother, and an intermediary layer positioned between them (Mamede et al., 2012), (Niknejad et al., 2008).

Amniotic membrane (AM) is conventionally believed to be 0.02-0.05 mm thick and is generally considered to have three layers. These layers are made up of a stromal layer that contains the extracellular matrix (ECM) and a single layer of epithelium that is supported by a basement membrane. The stromal layer consists of an acellular compact layer and a fibroblast layer containing a limited cell population (Malhotra & Jain, 2014).

The Placental membrane (PM) is comprised of a sophisticated network of components including collagens (type I, III, IV, V, VI), laminin, fibronectin, hyaluronic acid (HA), vitronectin, elastin, and proteoglycans. All of these components work together to provide the mechanical strength, elasticity, flexibility, and required stiffness to maintain

the barrier's integrity in the uterine environment (Niknejad et al., 2008), (Meinert et al., 2001), (Lei et al., 2017), (Cooper et al., 2005), (Mohan et al., 2017). Every layer of PM has a distinct composition tailored to fulfill its specific function (Roy & Griffiths, 2020).

In the 16th century, China documented the medical use of the human placenta (Medicine & 1979, n.d.), Although the amniotic membrane was first used in medicine in the early 20th century, Since 1910, The human amniotic membrane (hAM) has been used for medicinal purposes, notably skin transplantation ((1866-1922) & 1913, n.d.), (Association & 1913, n.d.).

In 1986, the Amnio-M emerged as a highly efficient alternative to split skin grafts for reconstructing the vagina in vulvovaginoplasty (MORTON & DEWHURST, 1986). Since 1995, there has been a notable increase in reported instances of using human amniotic membrane in tissue engineering (TE), particularly in areas such as dermatology, plastic surgery, skin transplantation, and as a natural dressing for promoting ophthalmic healing (Niknejad et al., 2008), (Sripriya & Kumar, 2016), (Jirsova & Jones, 2017).

In 2005, the initial application involved the use of suspension eye drops (AMEED®) for the treatment of corneal ulcers, presenting a less invasive alternative to the suturing of the Amnio-M graft (Bonci et al., 2005).

The diverse applications of the amniotic membrane arise from its unique biological features, which include: (a) its ability to generate anti-inflammatory factors like hyaluronic acid, leading to anti-inflammatory effects; (b) the capacity to suppress pro-inflammatory cytokines; (c) Compounds like β-defensins and elafin are said to exhibit antibacterial activity (Mamede & Botelho, 2015); (d) The down-regulation of TGF-β and its receptor expression leads to anti-fibrotic effects (Niknejad et al., 2008); (e) low antigenicity (Gholipourmalekabadi et al., 2016); and (f) Epithelial cells produce substances that limit the migration of macrophages and natural killer cells, preventing maternal immunological responses (Mamede & Botelho, 2015).

The mechanical characteristics of the human amniotic membrane (hAM), including as elasticity, stiffness, and tensile strength, are closely linked to the composition of the

placenta. The configuration of collagen fibrils within extracellular matrix (ECM) plays a role in determining tensile strength, and elastic deformation is impacted by the presence of elastin fibers, laminin, hyaluronic acid, and glycosaminoglycan (Friel et al., 2017).

The amniotic membrane have versatile applications in various medical fields, notably in treating skin burns and preventing tissue adhesion during surgeries involving the head, neck, abdomen, larynx, and genitourinary tract (A. Mamede et al., 2012). Its diverse uses include serving as a surgical dressing for burns, aiding in oral cavity and bladder reconstruction, and contributing to procedures like tympanoplasty, arthroplasty, repair of omphaloceles, as well as the prevention of adhesions in pelvic and abdominal surgeries (Schmiedova et al., 2021).

The several applications of amniotic membrane stem from its distinctive characteristics, particularly its antimicrobial properties. These features make it well-suited for applications in post-surgery scenarios, including wound healing, burn injuries, oral injuries, and ophthalmology. (Trelford & Trelford-Sauder, 1979), (Dadkhah Tehrani et al., 2021). The amniotic membrane is biocompatible, meaning it can interact with living tissue without causing toxic, injurious, carcinogenic, or immunological responses. Its ability to endure inflammation and respond appropriately to host reactions enhances its importance. Additionally, its biodegradability makes it a suitable option for use as a scaffold (Niknejad et al., 2008b).

Several methods have been developed for preserving the amniotic membrane (AM). The aim of these preservation techniques is to retain its characteristics as closely as possible to native tissue, providing a readily available and on-demand therapeutic option. (Sharma et al., 2023). The preservation techniques of amniotic membrane are (a) Cryopreservation (b) Freeze-drying/Lyophilization (c) heat or air preservation (d) Utilizing low-temperature vacuum evaporation (e) irradiation and (f) Vitrification (Sharma et al., 2023), (Leal-Marin et al., 2021).

Managing chronic wounds is a substantial and complex health problem for individuals and medical professionals. Utilizing the grafts in a range of sizes and forms not

only minimizes material wastage but also improves cost-effectiveness (Schmiedova et al., 2021). Amnion-M's complex makeup makes it an excellent natural bio-scaffold for a range of therapeutic applications. Amniotic membrane scaffolds (AMSCs) possess unique qualities, rendering them suitable for a broad spectrum of tissue repair scenarios (Walkden, 2020), (Dua et al., 2004), (Adinolfi et al., 1982).

The amniotic membrane market was estimated to be worth USD 2.26 billion globally as of 2017, and is anticipated to reach USD 5.81 billion by 2025, as reported by Grand View Research, Inc. The majority of organizations engaged in the research, development, or production of amniotic membranes are situated in the United States (Schmiedova et al., 2021)

1.1. Aims and Objectives

- To explore the potential of ECM sheets derived from placenta in facilitating corneal cell attachment, proliferation, and differentiation.
- To contribute to the development of regenerative medicine approaches in the field of cornea transplantation.
- To offer support for the potential clinical use of ECM sheets derived from placenta in enhancing the outcomes of corneal transplantation.
- To generate data that can guide future research and clinical trials in utilizing placental-derived ECM as a viable option for cornea transplantation.
- To address the limitations of donor tissue availability and offer a sustainable solution for meeting the growing demand for cornea transplantation.
- To explore the optimal methods of preparation, preservation, and sterilization of placenta-derived ECM sheets to ensure their bioactivity and safety.

CHAPTER 2: LITERATURE REVIEW

2.1. The Eye

Eyes are a key sensory organ, and it serve as integral components of the visual system, offering a living organisms the capability to perceive and analyze visual information. They facilitate various photo response functions beyond just vision and are adept at detecting light, converting it into electro-chemical impulses within neurons (*Eyes: How They Work, Anatomy & Common Conditions*, n.d.).

2.1.1. Function of Eye

The primary purpose of the eyes is to facilitate human vision. All the intricate components of the eye function harmonious, collectively contributing to the process of seeing. They absorb light from the surroundings, transmitting visual data to the brain for intricate processing.

- Light traverses the cornea, a gracefully arched structure that skillfully bends the incoming light, aiding the eye in achieving focus.
- The iris acts as a selective gatekeeper, allowing a specific amount of this light to enter through the pupil.
- Light passes through the lens, Collaborating with the cornea, the lens actively focuses and directs the light onto retina situated at the back of the eye.
- Retina transforms the light signal into electrical impulses.
- Acting as a messenger, the optic nerve carries these impulses to the brain, where the signals undergo processing, leading to the formation of the image (*Eyes: Structure, Function, and Disease*, n.d.)

2.1.2. Structure of Eye

The visible portion of the eye is its front, while the remaining structures are situated within the eye socket, also known as the orbit. Muscles linked to the eyeball facilitate its movement in alignment with the individual's gaze (Eyes: Structure, Function, and Disease, n.d.).

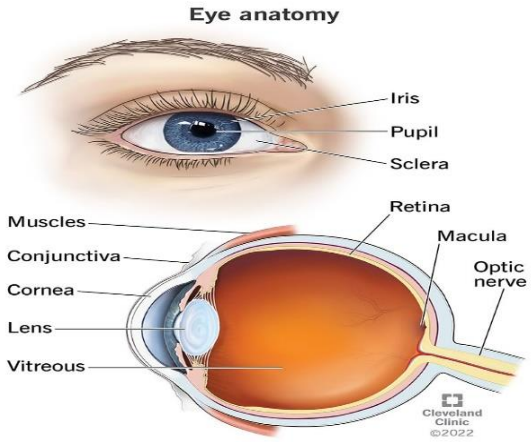


Figure 2. 1: Anatomy of Eye. Adapted from (https://my.clevelandclinic.org/health/body/21823-eyes) [Accessed on: 7 Dec 2023].

The eye comprises three primary types of tissues i.e., refractive tissue, light sensitive tissue and supporting tissues.

i. Refractive Tissues

Refractive tissues work to concentrate incoming light onto light-sensitive tissues, ensuring the formation of a crisp and clear image. When these tissues are improperly shaped, misaligned, or damaged, it can result in blurry vision. The refracting tissues include:

- Pupil (This is the dark spot in the center of the colored part of the eye)
- Iris
- Lens
- Ciliary muscles

- Cornea
- Vitreous and aqueous fluid

ii. Light Sensitive Tissues

- The retina
- Optic nerve

iii. Supporting Tissues

There are many support tissues in the eye, three of these are;

- The sclera
- The conjunctiva
- The uvea. (The uvea is the middle layer of the eyeball (*Eyes: Structure, Function, and Disease*, n.d.).

A histological understanding of the eye's layers is crucial for comprehending the pathophysiology of diseases and grasping specific therapeutic methods. Generally, when viewed anatomically, the eye can be perceived as a succession of tissue layers that overlap.

External elements of the eye encompass features such as eyelashes, lids, muscles, accessory glands, and the conjunctiva. Internally, the eye is structured with three concentric layers of tissue (Pradeep et al., 2023).

Outermost Layer: Sclera and Cornea

- **Sclera (the white part of eye)**

Commonly referred to as the "whites of the eyes," this part is fibrous and provides structural support to the eyeball, ensuring its proper shape. Connected to muscles, it enables versatile movement, allowing the eye to shift in nearly any direction (Sclera: Definition, Anatomy & Function, n.d.).

- **Cornea (transparent front layer of the eye)**

The outer covering or protective layer of the eyeball comprises cornea and sclera, serving as a primary function of safeguarding the internal eye structures. The cornea, being

a transparent avascular tissue, functions as a critical structural barrier, protecting the eye from harmful infections (DelMonte et al., n.d.).

Cornea comprising both cellular and acellular elements, the cornea features epithelial cells, keratocytes, and endothelial cells as its cellular components. The acellular components encompass collagen and glycosaminoglycans. Originating from the epidermal ectoderm, the epithelial cells, while the keratocytes and endothelial cells originate from the neural crest (Sridhar, 2018).

2.1.3. Structure of Cornea

The corneal tissue is arranged into five basic layers, each of which has a specific and essential function.

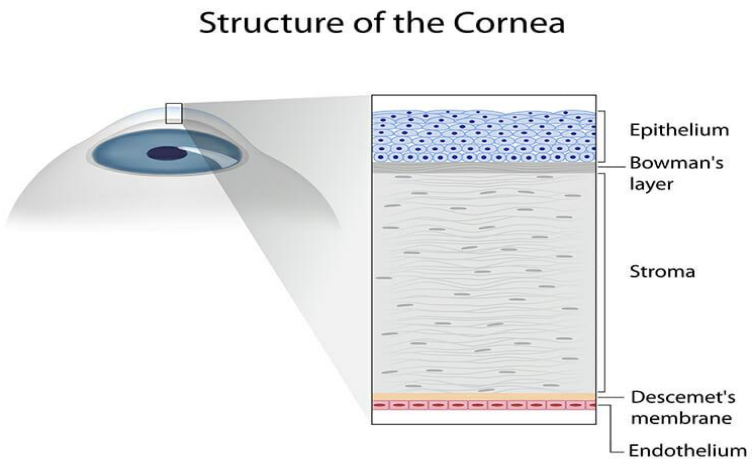


Figure 2. 2: Structure of the cornea. Adapted from (https://wilmingtoneye.com/specialties/cornea/structure-of-the-cornea/) [Accessed on: 7 Dec 2023].

- **The corneal Epithelium**

The outermost layer of cornea, known as epithelium, constitutes approximately 10% of the tissue's thickness. Its major role is to keep external elements like dust, water, and germs from entering the eye and other corneal layers. Additionally, the epithelium contributes to maintaining a smooth surface that takes up oxygen and nutrients from tears,

distributing them across the cornea. Notably, the epithelium is rich in numerous small nerve endings, heightening the cornea's sensitivity to pain when subjected to rubbing or scratching. The basement membrane is the fundamental part of the epithelium where cells attach and arrange themselves.

- **Bowman’s Layer**

Positioned just beneath the epithelium's basement membrane, there exists a transparent tissue sheet known as Bowman's layer. This layer consist of resilient, layered protein fibers called collagen. In the event of an injury, Bowman's layer has the capacity to develop a scar during the healing process. Vision loss may occur if these scars are sizable and situated in central areas.

- **Stroma** (**also substantia propri)**

Stroma is below the bowman’s layer constituting approximately 90% of the cornea's thickness. This layer is predominantly composed of water (78%) and collagen (16%), devoid of any blood vessels. The presence of collagen imparts strength, elasticity, and structure to the cornea.

- **Descemet’s Membrane (also posterior limiting membrane)**

Descemet's membrane is a thin but strong tissue layer that acts as a protective barrier against infections and injuries. Descemet's membrane is made up of collagen fibers that differ from those found in the stroma. It is formed by the underlying endothelial cells. Notably, Descemet's membrane demonstrates a remarkable capacity for regeneration following injury.

- **Corneal Endothelium**

The endothelium, an incredibly thin inner layer of the cornea, is responsible for preserving corneal clarity by actively pushing excess fluid from the stroma. This prevents stromal swelling, haziness, and opacity. A delicate balance is normally maintained between fluid entering and leaving the cornea. However, if endothelial cells are damaged due to

disease or trauma, they are irreversibly lost. Retinal edema and blindness may result from excessive loss of these cells, for which corneal transplantation is the only available therapy. (What Is The Cornea & How To Treat Corneal Issues, n.d.).

Middle Layer: Uvea (Iris, Ciliary Body, Choroid)

- **Iris:**

The term "iris" refers to the pigmented area of the eye that contains muscles that regulate the pupil's size and the quantity of light that reaches the retina (*Eyes: Structure, Function, and Disease*, n.d.).

- **Ciliary Body:**

Ciliary body Comprising the ciliary muscle and the ciliary epithelium, this structure forms a muscular ring connected to the lens (Pradeep et al., 2023). Through contraction or relaxation, it induces changes in the lens shape, a mechanism referred to as accommodation (Eyes: Structure, Function, and Disease, n.d.).

- **Choroid**

Choroid consisting of dense network of blood vessels that provide essential nourishment to eye structures, embedded within a loose connective tissue framework. The choriocapillary layer, situated in the innermost section of the choroid, serves the crucial function of supplying the retina with blood (Pradeep et al., 2023).

Innermost layer: Lens, Vitreous, And Retina

- **Lens**

Lens is a transparent and convex structure. Once passing through the pupil, light proceeds to the lens, the lens possesses the ability to alter its shape, aiding the eye in precisely focusing light onto the retina. However, as the lens ages, it tends to become less flexible and more rigid, leading to increased difficulty in focusing (*Eyes: Structure, Function, and Disease*, n.d.).

- **Vitreous and aqueous fluid**

Two fluids circulate within the eyes, contributing to their structure and providing essential nutrients. Vitreous fluid, located at the back of the eye, is dense and gel-like, constituting the majority of the eye's mass. In contrast, aqueous fluid, which is more watery, circulates through the front of the eye (Eyes: Structure, Function, and Disease, n.d.).

- **Retina**

The retina, positioned as the innermost layer of the eye, houses millions of light-sensitive photoreceptor cells responsible for detecting light and transforming it into electrical signals sent to the brain for interpretation. These cells, equipped with opsins sensitive to light, come in two main types known as "rods" and "cones."

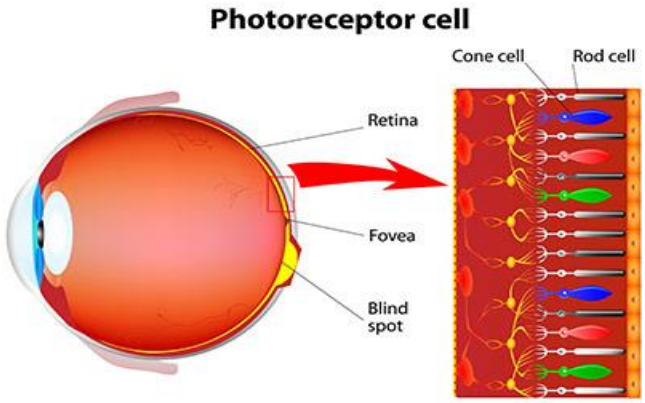


Figure 2. 3: Anatomy of photoreceptor cells. Adapted from (https://www.aao.org/eye-health/anatomy/photoreceptors) [Accessed On: 7 Dec 2023]

Cones, concentrated in the macula at the retina's center, number around 6 million and play a crucial role in typical light conditions and color differentiation. The fovea, a high-concentration area within the macula, specifically houses cones, enabling sharp vision and color perception.

Cones are categorized by the colors they are sensitive to, including red, green, and blue, with red and green cones predominating in the foveae center and blue cones situated toward the periphery. In contrast, rods, concentrated around the retinal edges, contribute to black and white vision, excel in low-light conditions, and facilitate night vision. Each eye possesses approximately 125 million rods (*Eyes: Structure, Function, and Disease*, n.d.).

2.1.4. Eye diseases

Eye diseases and corneal disorders encompass a broad spectrum of conditions that can impact the well-being and functionality of the eye. The following are several prevalent eye ailments, each accompanied by concise descriptions:

1. Keratoconus

Keratoconus is an advancing corneal condition determine by the progressive thinning of the cornea, result in a conical shape. Although relatively uncommon, this eye disorder can lead to substantial distortions in vision (*What Is Keratoconus? - American Academy of Ophthalmology*, n.d.).

2. Keratitis

Keratitis is an inflammation impacting the cornea, commonly triggered by factors like infections, injuries, or complications associated with contact lens usage (Keratitis - Symptoms and Causes - Mayo Clinic, n.d.). Common symptoms include photophobia, sensitivity to light, redness, impaired vision, and feeling like something alien is in the eye (What Is a Corneal Ulcer (Keratitis)? - American Academy of Ophthalmology, n.d.).

3. Eye Injuries

Injuries to the eyes may occur due to accidents, exposure to foreign objects, or blunt trauma, leading to symptoms like eye pain, redness, tearing, and blurred vision. These injuries vary in severity, ranging from minor scratches to more severe trauma, emphasizing the importance of seeking immediate medical attention in all instances (*Eye Injury: Symptoms, Treatment, Causes*, n.d.).

4. Eye Allergies (Allergic Conjunctivitis):

Allergic conjunctivitis manifests as an allergic response impacting the conjunctiva, resulting in symptoms such as itching, redness, tearing, and swollen eyelids. Commonly, this condition is triggered by allergens like pollen, pet dander, or dust mites (*Eye Allergies | Causes, Symptoms & Treatment | ACAAI Public Website*, n.d.).

5. Dry Eye Syndrome:

Dry eye syndrome emerges when the eyes fail to generate an adequate quantity of tears or produce tears of insufficient quality, resulting in symptoms like dryness, burning, itching, redness, and blurred vision. Factors contributing to dry eye syndrome encompass aging, environmental conditions, medications, or underlying health issues (*Dry Eye | AOA*, n.d.).

6. Glaucoma

Glaucoma refers to a collection of eye diseases that harm the optic nerve, potentially resulting in vision loss. Often termed the 'silent thief of sight,' glaucoma can advance without manifesting noticeable symptoms (*What Is Glaucoma? Symptoms, Causes, Diagnosis, Treatment - American Academy of Ophthalmology*, n.d.).

7. Cataract

Cataract is a common age-related eye condition identified by the clouding of the eye's natural lens, representing a significant contributor to global vision impairment and blindness (*Blindness and Vision Impairment*, n.d.). The onset of cataracts is gradual, often impacting older individuals, although it can occur across all age groups. Usual symptoms involve blurry vision, sensitivity to glare, challenges in night vision, and diminished perception of colors (*Cataracts - Symptoms and Causes - Mayo Clinic*, n.d.).

8. Presbyopia:

Presbyopia represents a frequent age-related vision issue that typically manifests around the age of 40 due to the natural aging process. This condition arises as the eye's lens

loses flexibility, resulting in challenges when attempting to focus on close objects. Common symptoms encompass difficulties in reading small print, the necessity to hold reading material at arm's length, and eye strain (*Presbyopia | AOA*, n.d.).

9. Corneal Dystrophies:

Corneal dystrophies refer to a set of inherited conditions marked by the buildup of abnormal material in the cornea, the transparent front surface of the eye. These dystrophies have the potential to impact different layers of the cornea, potentially causing vision disturbances, discomfort, and, in some instances, significant visual impairment.

There are more than 20 types of corneal dystrophies, each presenting unique clinical features and genetic patterns (*What Are Corneal Dystrophies? - American Academy of Ophthalmology*, n.d.).

10. Corneal Ulcer

A corneal ulcer, also known as keratitis, is a painful condition with potential threats to vision, characterized by an open sore or erosion on the clear front surface of the eye. It can arise from various factors such as infections, injuries, or complications related to contact lens usage (*Keratitis - Symptoms and Causes - Mayo Clinic*, n.d.).Typical symptoms include eye pain, redness, tearing, blurred vision, photophobia (sensitivity to light), and a feeling of having a foreign body in the eye (*What Is a Corneal Ulcer (Keratitis)? - American Academy of Ophthalmology*, n.d.).

Diagnosis involves a thorough eye examination utilizing a slit lamp to assess the severity of the ulcer and determine its root cause usage (*Keratitis - Symptoms and Causes - Mayo Clinic*, n.d.). Treatment approaches vary depending on the specific cause, potentially including antibiotics for bacterial keratitis, antifungal medications for fungal keratitis, antiviral drugs for viral keratitis, and pain management through anti-inflammatory medications. Seeking immediate medical attention is crucial to prevent complications and safeguard vision (*What Is a Corneal Ulcer (Keratitis)? - American Academy of Ophthalmology*, n.d.).

2.2. Global Prevalence of eye disease

The occurrence of eye diseases globally varies depending on the specific condition and geographical region. Refractive errors, encompassing myopia, hyperopia, and astigmatism, affect billions of individuals worldwide, with an increasing prevalence of myopia, particularly noted in East and Southeast Asia (Holden et al., 2016). Cataracts, a prominent cause of vision impairment, are widespread, notably in low- and middle-income countries (Pascolini & Mariotti, 2012). Glaucoma, a significant concern affecting millions, stands as a second leading cause of irreversible blindness(Quigley & Broman, 2006).

Age-related macular degeneration (AMD) is common in developed countries with aging populations, and the incidence of diabetic retinopathy is growing due to the worldwide diabetes epidemic (Wong et al., 2014),(Yau et al., 2012). Trachoma remains a substantial cause of blindness in areas with limited access to healthcare (Pascolini & Mariotti, 2012).Moreover, uncorrected refractive errors impose a significant burden, impacting a substantial portion of the global population (Holden et al., 2016). It's crucial to acknowledge that the prevalence rates can fluctuate based on geographical, socioeconomic, and demographic factors, as noted in these referenced studies.

The most recent global assessment and projection on blindness, as well as distance and near vision impairment among individuals aged 50 years and above, indicate that approximately 43.3 million individuals worldwide experienced blindness, while 295 million people had moderate to severe vision impairment in the year 2020 (Bourne et al., 2021).

2.2.1. Prevalence of eye disease in Pakistan

The study utilized the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD 2017) to evaluate the prevalence and Years Lived with Disability (YLDs) associated with vision loss. In 2017, among Pakistan's population of 207.7 million, an estimated 1.12 million individuals were affected by blindness, 1.09 million experienced severe vision loss, and 6.79 million had moderate vision loss. Notably, presbyopia emerged as the most

common ocular condition, impacting around 12.64 million individuals, with 61% being female (Hassan et al., 2019).

In comparison to 1990, the all-age YLDs count for blindness and vision impairment in 2017 showed a 55% increase. This positions it as the tenth highest increment among major causes of health loss, alongside factors such as dietary iron deficiency, headache disorders, and low back pain, within the Pakistani context (Hassan et al., 2019).

The comprehensive prevalence of total blindness and vision impairment across all age groups exhibited a consistent increase from 1990 to 2017, and projections indicate a continued rise in 2025. Furthermore, the prevalence ratio of females to males is anticipated to increase in 2025. In the context of South Asian countries, Pakistan holds the third position, following India and Bangladesh, in the all-age prevalence of blindness and vision impairment, totaling 21.78 million cases (Hassan et al., 2019).

Age-specific vision loss burden with leading causes in South Asian countries in year 2017.

Countries	*Age-specific Vision Loss Burden (Prevalence %)*					*All-Age Leading Causes (%)*			*Rank**
	1–4 years	**5–14 years**	**15–49 years**	**50–69 years**	**70+ years**	**Presbyopia**	**RD**	**Cataract**	
Afghanistan	3	10	53	23	11	69	18	5	2
Bangladesh	1	4	36	39	20	65	18	12	3
Bhutan	1	4	45	35	15	77	14	5	8
India	1	3	38	41	17	71	15	10	1
Maldives	1	4	44	33	18	65	19	10	6
Nepal	1	4	36	41	18	62	20	10	5
Pakistan	**2**	**7**	**43**	**34**	**14**	**58**	**22**	**12**	**4**
Sri Lanka	1	3	27	45	24	63	19	12	7

RD = Refraction Disorders

*Rank in terms of age-standardized Years Lived with Disability (YLDs) rate per 100K.

Figure 2. 4: Age-specific vision loss burden with leading causes in South Asian countries in year 2017. Adapted from (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0216492) [Accessed On: 8 Dec 2023]

2.2.2. Primary contributors of health loss in Pakistan from 1990 to 2017

In Pakistan, the health loss burden due to blindness and vision impairment increased significantly between 1990 and 2017. The all-age Years Lived with Disability (YLDs) count for this category observed a 55% increase, ranking it as the tenth highest among various causes of health loss. On the other hand, among the many causes of health loss, the age-standardized YLDs rate for blindness and vision impairment decreased by 21% in 2017, ranking it as the fourth largest decline. Without significant interventions, this trend is expected to persist steadily, with further increases anticipated by 2025 (Hassan et al., 2019).

2.3. Human placenta

The placenta serves as a sophisticated organ that enables the physiological exchange between the fetus and the mother. It contains a highly abundant reservoir of extracellular matrix (ECM) and bioactive molecules (Wildman, 2011).The placenta is a vascular organ that has a specific lifespan and forms exclusively during pregnancy. It attaches to the uterine wall and connects to the fetus through the umbilical cord, providing essential support for fetal growth and development (Schuette *et al.*, 2017).

The term "placenta" originates from the Greek word "plakoenta," signifying a flat cake, and it exhibits a flat, round, or oval shape. The outer and inner membranes are referred to as the chorion and the amnion, respectively(Pan *et al*., 2017),(Longo & Reynolds, 2010).

2.3.1. Anatomy and Development

The placental membrane (PM) envelops the developing fetus, creating the fetal compartment that contains amniotic fluid during gestation. This membrane consists of three main layers: the amnion, which is in contact with the fetus; the chorion, facing the mother; and an intermediary layer positioned between them (A. C. Mamede et al., 2012), (Niknejad et al., 2008a).

The amnion, comprised of epithelium, basement membrane, compact layer, and fibroblast layer, surrounds both the fetus and the amniotic fluid, providing a protective cushion throughout gestation (Verbruggen et al., 2017).

On the side facing the mother, the chorion interacts with the maternal decidua and consists of three layers: the reticular layer, a basement membrane, and a trophoblast layer (A. C. Mamede et al., 2012), (Bryant-Greenwood, 1998), (Hieber et al., 1997). The chorion serves as a protective barrier, creating a separation between the fetal environment and the maternal immune system (Hong et al., 2013), (Kim et al., 2008).

As pregnancy progresses, the amnion and chorion develop independently after the blastocyst implants, usually around day 14, marking the formation of the placental disc (Eslani et al., n.d.), (*Sadler: Langman's Medical Embryology - Google Scholar*, n.d.). The blastocyst's wall transforms into what we call the chorion. By day 10 post-conception, you can start seeing the amnion, emerging from special cells known as amniogenic cells in the inner trophoblast layer of the embryo (Eslani et al., n.d.).

The placental membrane (PM) undergoes continuous growth to accommodate the developing fetus. After the third month of gestation, differentiation occurs between the amnion and the chorion leave, the precursor to the chorion, marked by the formation of the chorionic cavity. As the amniotic sac fills with fluid and expands, the amnion attaches to the inner surface of the chorion, causing the disappearance of the chorionic cavity. Despite their intimate connection, the amniotic and chorionic layers of the PM do not physically merge and maintain distinguishable characteristics under microscopic examination (Eslani et al., n.d.).

When approaching the delivery period, research suggests that the intrinsic thickness of the PM can vary between 0.02 mm and 0.6 mm, with the average surface area estimated to be around 1600 cm2 (S. Gupta et al., n.d.), (T. V. Rao & Chandrasekharam, 1981b).

2.3.2. Physiology

The placental membrane (PM) plays a crucial role as a protective barrier, shielding the developing fetus from the surrounding maternal environment and handling the stretching and pressure caused by the fetus's growth and movements (Bryant-Greenwood, 1998), (Favaron et al., 2015). Beyond its defensive duties, the PM is a hub of metabolic activity, serving as both an immune barrier against infections and a shield preventing the mother's immune system from rejecting the fetus (Togarrati et al., 2019).

Throughout the entire pregnancy journey, the PM goes through continuous matrix remodeling, ensuring its barrier remains intact and capable of accommodating the expanding fetus (Lei, Priddy, Lim, Massee, et al., 2017). The composition of each layer in the placental membrane (PM) undergoes slight variations to fulfill the overall function of the membrane. Within the amnion, the basement membrane is a compact tissue layer that serves as a selectively permeable barrier, facilitating the exchange of nutrients (A. C. Mamede et al., 2012), (Keene et al., 1987).

To enhance the strength of the membrane, the compact layer emerges as the most fibrous among the layers of the amnion. This fibrous nature is attributed to a significant collagen content secreted by mesenchymal cells in the fibroblast layer (A. C. Mamede et al., 2012), (Niknejad et al., 2008a), (Strauss, 2013). The fibroblast layer plays a crucial role in tensile strength and provides a scaffold for cell-to-cell interactions (Rousselle et al., 1997), (Smith & Ockleford, 1994).

Simultaneously, the intermediate layer functions as a separator between the amnion and the chorion, providing mechanical support through a non-fibrillar network primarily composed of proteoglycans, glycosaminoglycans (GAGs), and type III collagen (A. C. Mamede et al., 2012), (Meinert et al., 2001), (Roy & Griffiths, 2020).

The reticular layer functions as a supportive framework for the chorion, characterized by a mesh of fibers and embedded mesenchymal cells, resembling the fibroblast layer in the amnion (Uchide et al., 2012). Positioned between the reticular layer and the trophoblasts, the basement membrane enhances the structural integrity of the

chorion by providing cellular scaffolding for the trophoblast layer. This contribution to the tissue's immune privilege reinforces the chorion's resilience (Hong et al., 2013), (Koob et al., 2014), (Roy et al., 2022).

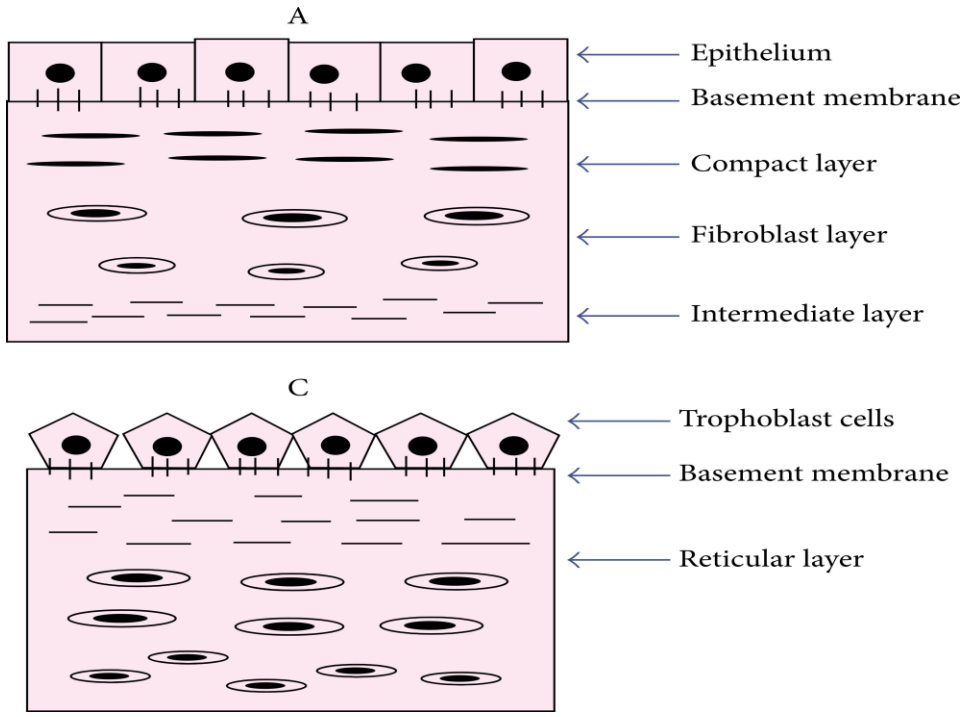


Figure 2. 5: Histological architecture of amnion (A) and chorion (C) membranes depicted as line diagram. Adapted from (https://www.researchgate.net/figure/Line-diagrammatic-representation-of-histological-architecture-of-amnion-A-and-chorion_fig2_286035236) [Accessed on: 15 Nov 2023].

The outermost layer of the chorion, known as the trophoblast layer, is made up of trophoblasts, myofibroblasts, and macrophages (Hong et al., 2013). The overall structure of the placental membrane (PM) is designed to support resident cells while maintaining a delicate balance between tensile strength and elasticity. This balance is crucial for the regular turnover of the extracellular matrix (ECM), facilitated by endogenous cytokines within the PM (Lei, Priddy, Lim, & Koob, 2017).

2.3.3. Structural composition

Comprising a network of collagens (types I, III, IV, V, and VI), laminin, fibronectin, hyaluronic acid (HA), vitronectin, elastin, and proteoglycans, the PM provides mechanical strength, elasticity, flexibility, and suitable stiffness to uphold barrier integrity in the uterine environment (Niknejad et al., 2008a), (Meinert et al., 2001), (Lei, Priddy, Lim, & Koob, 2017), (Cooper et al., 2005), (Mohan et al., 2017). Each layer of the PM possesses a unique composition tailored to fulfill its specific function (Roy & Griffiths, 2020).

2.3.4 Morphology

The postpartum placenta adopts a disc-shaped morphology, typically measuring 16–20 cm in diameter and averaging a weight of 500 g. The main cell types found in the placenta are trophoblast cells, mesenchymal cells, and endothelial cells of vessels. Ethical considerations arise when using "early placenta" from the first two trimesters of pregnancy, leading most researchers to concentrate on the third-trimester placenta, often termed the "mature placenta," which occurs between 38 and 40 weeks of pregnancy (Yoshizawa, 2013), (Van Griensven et al., 2015).

2.4. The Historical Background of Amniotic Membrane Transplant

Historically, the placenta has been acknowledged as a form of traditional folk medicine not only in China but also in various regions across the globe, because for its rich nutrients and bioactive components (Pan et al., 2017). During the 16th century, China documented the medical utilization of the human placenta (Medicine & 1979, n.d.). The Modern medical use of amniotic membrane began in the early 20th century. The efficacy of placental membrane in skin grafting was initially reported by Davis in 1910, highlighting its superior performance compared to cadaveric tissue (J & 1910, n.d.).

Since 1910, the use of human amniotic membrane (hAM) has been applied for therapeutic purposes, particularly in skin transplantation ((1866-1922) & 1913, n.d.),

(Association & 1913, n.d.). In 1940, de Röth pioneered its application in ophthalmology to repair symblepharon and conjunctival defects (Rötth De, 1940). Furthermore, in the latter half of the 20th century, hAM became a pioneering biomaterial for creating tissue-engineered constructs, fostering cell migration, and facilitating the growth of new tissue (Lim, 2017), (Niknejad et al., 2008a).

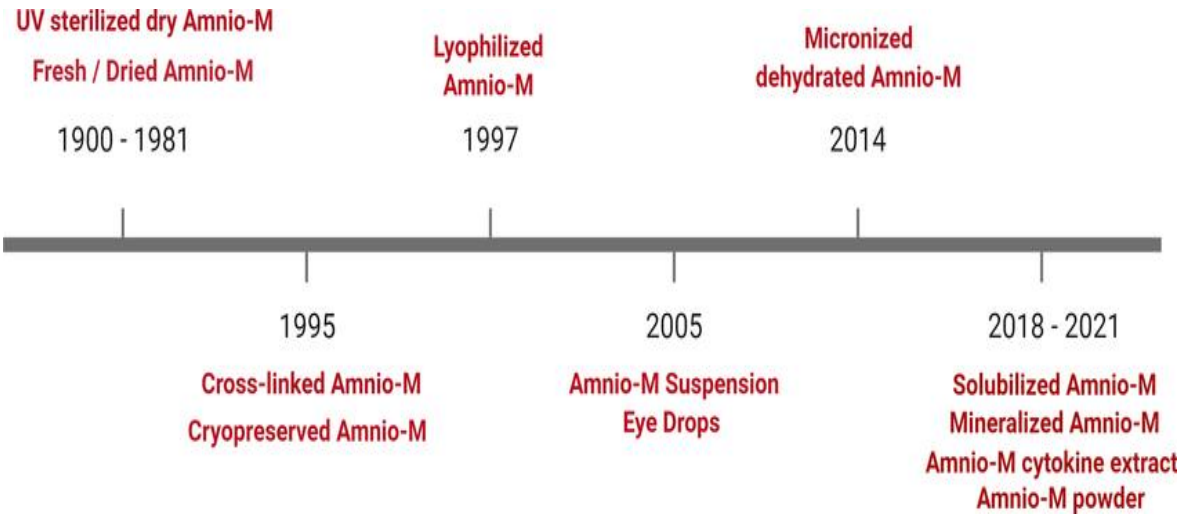


Figure 2. 6: The history of Amnio-M transformations and technological advancement. Adapted from (https://www.researchgate.net/figure/History-of-Amnio-M-modifications-and-technological-enhancement_fig1_357732139) [Accessed On: 15 Nov 2023].

Starting from 1995, there has been a noticeable surge in reports on the utilization of (hAM) in tissue engineering (TE), especially in dermatology, plastic surgery, skin transplantation, and as a biological dressing for ophthalmic healing (Niknejad et al., 2008a), (Sripriya & Kumar, 2016), (Jirsova & Jones, 2017). Moreover, hAM is gaining acknowledgment in the cardiac field for its exceptional properties as a scaffold for blood vessels and as a substitute for pericardial tissue (P. H. Lee et al., 2012), (Francisco et al., 2016).

In 1986, the Amnio-M emerged as a highly effective alternative to split skin grafts for reconstructing the vagina in vulvovaginoplasty (MORTON & DEWHURST, 1986). Recognizing the importance of preserving its biological and physical qualities, Kim and

Tseng proposed cryopreserving the Amnio-M at -80 °C (Jae Chan Kim & Tseng, 1995).Utilizing the cryopreserved Amnio-M for reconstructing ulcerated corneas in 11 patients resulted in a success rate exceeding 90% (S. H. Lee & Tseng, 1997).

In 1997, Güler and Ercan pioneered the examination of lyophilized (freeze-dried and sterile) Amnio-M in mandibular vestibuloplasty, noting significant angiogenic effects (Güler et al., 1997). To enhance ease of application, commercial versions of Amnio-M in suspension form have recently become available. Initially utilized in 2005 as suspension eye drops (AMEED®), it provided a less invasive alternative to suturing the Amnio-M graft for treating corneal ulcers (Bonci et al., 2005).

In the early 1990s, ophthalmologists started to explore alternative applications once again. With the development and improvement of various preservation methods, enhanced storage, and distribution techniques, accessibility to the tissue significantly improved (Walkden, 2020a).

2.5. General Structure of Amniotic Membrane

The amnion is structured into five layers, typically with a thickness ranging from 20–500µm (T. Rao et al., n.d.). It includes an epithelial monolayer supported by a basement membrane and an extracellular matrix (ECM) stromal layer, consisting of an acellular compact layer and a fibroblast layer with a sparse cell population. The innermost layer, known as the spongy layer, acts as the interface between the fibroblastic layer of the amnion and the reticular layer of the chorion (McLaren et al., 1999), (Wu & Hui, 2006).

The structural proteins found in the amnion's extracellular matrix (ECM) and basement membrane, including laminin, fibronectin, and collagens, create a scaffold that facilitates cellular interactions, promoting epithelial regeneration (Scheffer C.G. Tseng et al., 2004), (Hopkinson et al., 2008). At full term, there is a single layer of amniotic cells firmly attached to a mesenchymal layer, typically ranging from six to eight cells in thickness (Bourne, 1960), (Danforth & Hull, 1958). Notably, it lacks vascularity and does not have a direct blood supply (Dua et al., 2004), (Trelford & Trelford-Sauder, 1979), (Matthews et al., n.d.).

Since it lacks its own blood supply, the amnion receives nutrition and oxygen from the nearby chorionic fluid, amniotic fluid, and blood vessels on the fetal surface. Its main source of energy comes from anaerobic glycolysis pathways (Dua et al., 2004).

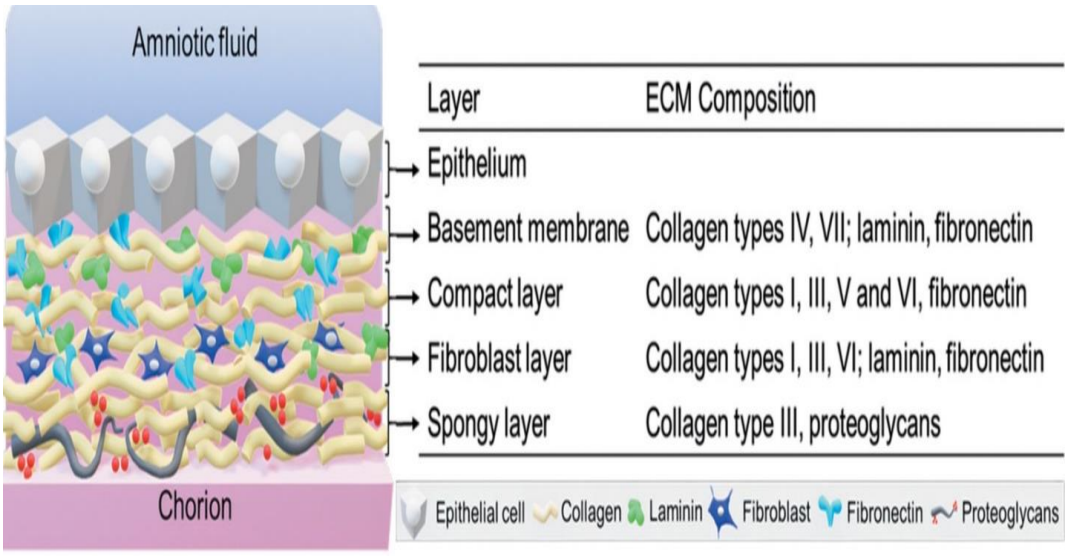


Figure 2. 7: Human placental membranes: (a) diagram of the structure of hAM and the content of extracellular matrix for each layer.Adapted from (https://onlinelibrary.wiley.com/doi/pdf/10.1002/jbm.b.34782) [Accessed on: 15 Nov 2023].

The fetal membranes comprise two layers: the outer chorion, which contains blood vessels and is in contact with the uterine wall, and the inner avascular amnion, situated beneath the chorion and in contact with the amniotic fluid. The amniotic membrane (AM) is typically 0.02-0.05 mm thick and is traditionally considered to consist of three layers (Malhotra & Jain, 2014a).

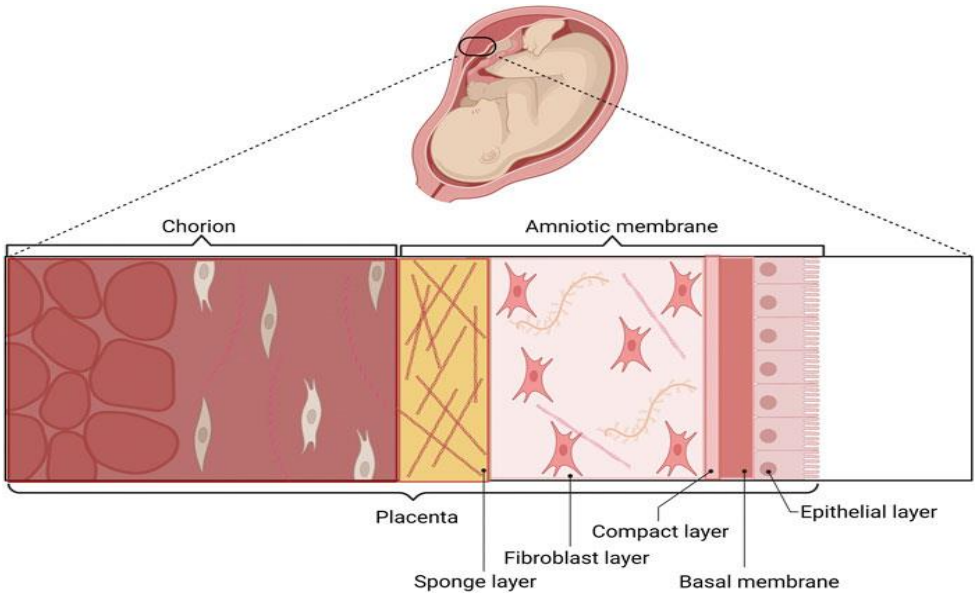


Figure 2. 8: Components of the placenta and AM. Adapted from (https://www.frontiersin.org/articles/10.3389/fbioe.2022.1067480/ful) [Accessed On: 15 Nov 2023]

- **Epithelium**

The epithelium is made up of a single layer of dynamic cuboidal cells with microvilli on the top surface (Malhotra & Jain, 2014a).

- **Basement membrane**

The basement membrane includes type IV, V, and VII collagens (also present in conjunctival and corneal basement membranes), as well as fibronectin and laminin. Known for its significant thickness, it ranks as one of the thickest membranes in the human body and exhibits resistance to contemporary cryopreservation methods (Malhotra & Jain, 2014a).

- **Stroma**

Structured into three interconnected yet distinct layers, the amniotic membrane exhibits a well-defined organization. The inner compact layer, which directly interfaces with the basement membrane, enhances the membrane's tensile strength. The middle

fibroblast layer is substantial, featuring a loose fibroblast network, and together with the outermost spongy layer, it constitutes the overall stroma structure (Malhotra & Jain, 2014a).

The stroma is composed of three layers: compact, fibroblast, and spongy. The compact layer stands out as the strongest, capable of withstanding inflammation and edema. The fibroblast layer, characterized by its thickness, comprises fibroblasts embedded in reticular tissue, occasionally showcasing phagocytic abilities. The outermost layer is spongy and gelatinous, composed of reticulin bundles within mucin. This layer has the potential to become edematous, enabling the amnion to smoothly glide over the securely anchored chorion (Malhotra & Jain, 2014b). Remarkably, a single placenta can supply up to 40 grafts of amniotic membrane for ophthalmic applications(Blood & Services, n.d.).

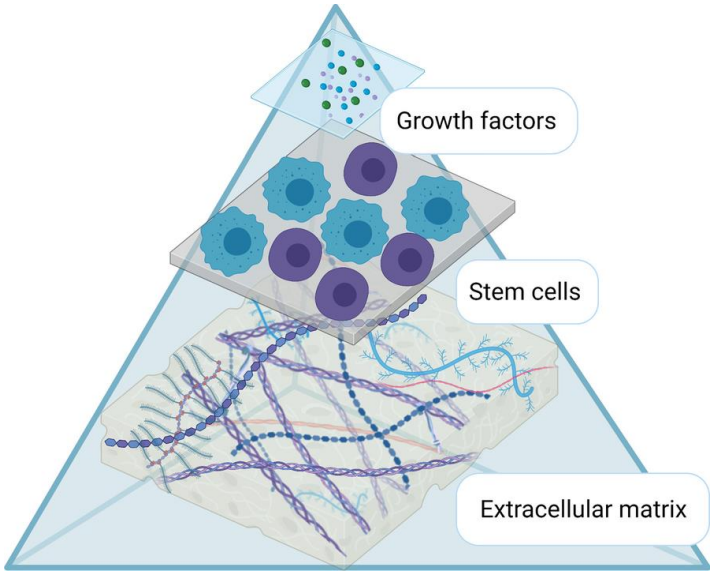


Figure 2. 9: The components of the Amnio-M meets the standards of the "tissue engineering pyramid". Adapted from (https://www.researchgate.net/figure/The-components-of-the-Amnio-M-fulfil-the-requirements-of-the-tissue-engineering-pyramid_fig2_357732139) [Accessed On: 15 Nov 2023].

The incorporation of cytokines and growth factors has broadened the utilization of Amnio-M across different clinical scenarios within the field of regenerative medicine. This encompasses its function as a three-dimensional (3D) scaffold for tissue engineering and

in the realm of drug delivery. The diverse applications of Amnio-M have not only progressed medical treatments but have also played a pivotal role in deepening our insights into stem cell biology. The distinctive properties of Amnio-M position it as an optimal platform for creating favorable conditions for cell culture (Elkhenany et al., 2022).

2.5.1 Amniotic membrane transplantation advantages

The intricate composition of Amnion-M makes it a valuable natural bio-scaffold for various clinical purposes. Amniotic membrane scaffolds (AMSCs) exhibit distinct qualities that make them applicable in a wide range of tissue repair scenarios. While the precise mechanism of action of AM hasn't been fully quantified, it is understood to result from a combination of the tissue's barrier function, providing structural advantages, and the presence of bioactive proteins within its structure (Walkden, 2020a), (Dua et al., 2004), (Adinolfi et al., 1982a). These properties work together synergistically, delivering a consistent advantage when utilized as a tissue transplant (Sharma et al., 2023).

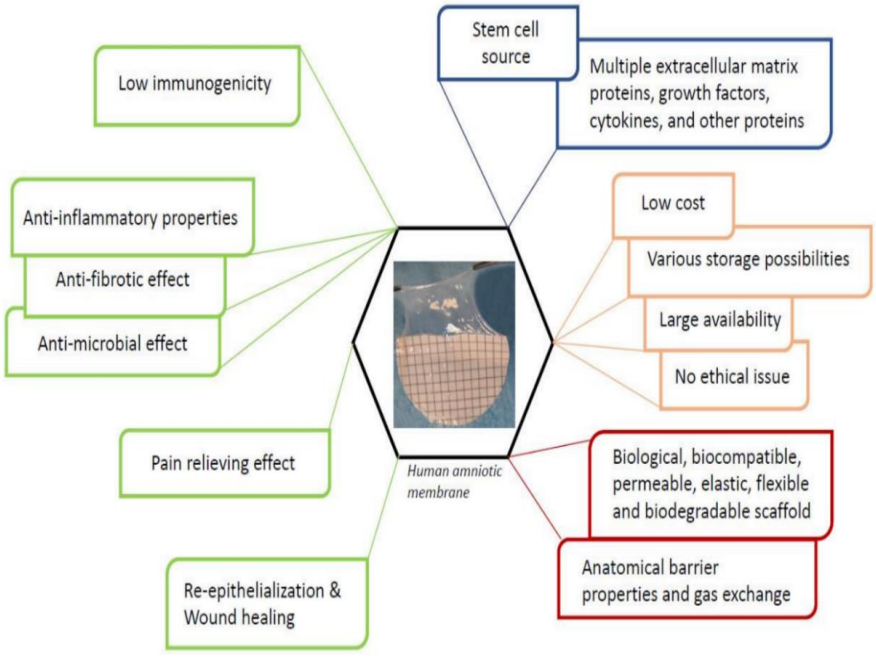


Figure 2. 10: The properties of the human amniotic membrane make it a perfect scaffold for tissue engineering. Adapted from (https://www.researchgate.net/figure/Human-amniotic-membrane-properties-as-an-ideal-scaffold-for-tissue-engineering_fig1_351856896) [Accessed On: 15 Nov 2023].

2.5.2. Biological properties

- **Anti-inflammatory**

Clinically, the anti-inflammatory effects of AM are evident through a noticeable reduction in redness. Numerous experiments have been conducted to decipher the precise mechanisms underlying these effects, starting with initial trials on preclinical animal models. In the year 2000, AM demonstrated its ability to trap and prevent the infiltration of polymorph-nuclear cells in a rabbit model of photorefractive keratectomy (Park et al., n.d.), (Woo et al., n.d.).

Subsequent to this, in 2001, the application of AM was found to facilitate the reduction of lymphocyte and macrophage infiltration in the corneal stroma of a murine model with HSV-1 necrotizing keratitis (Heiligenhaus et al., n.d.). These early experiments involved using AM as a dressing, suggesting the potential for AM to function as a physical cover and barrier against inflammatory cell infiltration (Tighe et al., 2010).

The anti-inflammatory impact of AM is credited to its ability to hinder the expression of pro-inflammatory cytokines originating from the damaged ocular surface. These cytokines encompass interleukin (IL) 1a, IL-2, IL-8, interferon-γ, tumor necrosis factor-β, basic fibroblast growth factor, and platelet-derived growth factors (Solomon et al., 2001). In addition to the chemical mediation of anti-inflammatory effects, Shimmura et al. have also demonstrated a mechanical influence, revealing that inflammatory cells become entrapped in the matrix of the AM and undergo apoptosis (Malhotra & Jain, 2014a).

- **Anti-Microbial properties**

A review of the literature unveils varying opinions regarding the antimicrobial characteristics of AM. Studies indicate that the application of AM in treating burn patients is associated with reduced bacterial counts and effective infection control (T. V. Rao & Chandrasekharam, 1981a), (Robson et al., n.d.).The antibacterial properties extend to both gram-positive cocci, such as streptococci and *Staphylococcus aureus,* and gram-negative

bacilli, including *Escherichia coli* and *Pseudomonas aeruginosa* (Kjaergaard, Hein, et al., n.d.), (Kjaergaard, Helmig, et al., n.d.).

The ability of AM to exert antibacterial effects is linked to the presence of various antimicrobial factors in the amniotic fluid. These factors include bactricidin, beta-lysine, lysozyme, transferrin, and 7S immunoglobulin (Immunology & 1962, n.d.), (Galask et al., n.d.). On the contrary, some researchers argue that AM may not inherently contain chemical antimicrobial substances. Instead, they propose that its effectiveness lies in serving as a robust physical barrier against infection due to its capacity to closely adhere to the underlying surface (Kjaergaard, Helmig, et al., n.d.), (Inge et al., n.d.).

The amnion actively releases elafin (peptidase inhibitor 3) and secretory leukocyte proteinase inhibitor, both serving as essential components of the innate immune system with antimicrobial properties (Sangwan & Basu, 2011). Furthermore, the amnion is recognized for its antiviral characteristics, credited to the existence of cystatin E, an analog of a cysteine proteinase inhibitor (Sangwan & Basu, 2011).

- **Promotion of Epithelization**

The amniotic membrane plays a pivotal role in facilitating the migration of epithelial cells (S. C.G. Tseng et al., 1997), improving basal cell adhesion (Shimazaki et al., 1998), nurturing epithelial differentiation (Guo & Grinnell, 1989), preventing epithelial apoptosis (Boudreau et al., 1995), and fostering wound healing through effective epithelialization (Surgery et al., n.d.). Moreover, it releases various growth factors that encourage epithelialization (Koizumi et al., 2000) and supports the expansion and maintenance of epithelial progenitor cells in vivo (Grueterich & Tseng, 2002).

The amniotic membrane can produce endothelin-1 and parathyroid hormone-related protein as well. Serving as a secure and suitable bed, its basement membrane facilitates the growth of epithelial cells, while its excellent permeability ensures sufficient oxygenation a feature not found in many synthetic materials. Consequently, the amniotic membrane emerges as an optimal tissue for promoting the growth, migration, and differentiation of epithelial cells (Tosi et al., 2005), (Kurpakus-Wheater, 2001).

- **Lack of Immunogenicity**

The risk of acute rejection following amniotic membrane transplantation is minimized as amniotic epithelial cells lack HLA-A, HLA-B, HLA-D, and HLA-DR antigens, expressing HLA-G on their surfaces (Hori et al., 2006). Immunological elements, including interferon-γ, have been identified in the amniotic membrane, indicating that viable epithelial cells may induce immunologic reactions. Research has shown that fresh amniotic membrane transplantation is associated with a mild inflammatory response, possibly linked to the expression of HLA I antigens by viable epithelial cells (Akle et al., 1981).

The immunogenicity of cryopreserved amniotic membrane is lower compared to fresh amniotic membrane due to the loss of epithelial cells during cryopreservation (Adinolfi et al., 1982b). Amniotic membrane exhibits the ability to suppress T lymphocytes in allografted limbus cells, suggesting its immunosuppressive properties, which contribute to the potential success of grafting (Ueta et al., 2002). The use of placental membrane materials as tissue grafts is associated with a minimal risk of immune rejection, leading to their recognition as possessing an "immune privilege" (Streilein, 1995), (Whitsett et al., n.d.).

- **Cell differentiation property**

The fetal placental tissues exhibit the capability to differentiate into various cell lineages. Specifically, the chorion, allantois, and yolk sac comprise members of the hematopoietic lineage, while the chorion and amnion contain members of the mesenchymal lineage. Cells originating from the chorion serve as valuable reservoirs for both hematopoietic and mesenchymal lineage cells, owing to their possession of these distinct properties. It is believed that the amniotic membrane can sustain the potential for pluripotent stem cells, allowing for diverse cell differentiations (A. Gupta et al., 2015).

- **Anti-Scarring**

Fibroblasts with anti-scarring properties play a key role in the proliferation phase of wound healing. Their main responsibility is to generate collagen, which offers structural support and helps reduce the size of the wound. Nevertheless, the collagen and glycosaminoglycan's produced during the initial stages of repair exhibit an uneven arrangement of fibrils and composition, potentially contributing to the development of opacity and scarring.

In the intricate process of wound healing, inflammatory cells play a vital role in collaboration with fibroblasts. These cells express mediators that stimulate various actions in fibroblasts, including migration, proliferation, extracellular matrix production, and differentiation into myofibroblasts the primary cells responsible for wound contraction (Tighe et al., 2010).

Following this, fibroblasts, neutrophils, and macrophages release proteases that contribute to collagen remodeling, ultimately replacing granulation tissue with a fibrotic scar. To prevent scarring effectively, it becomes crucial to curb excessive inflammation, regulate collagen formation by fibroblasts, and inhibit the differentiation of fibroblasts into myofibroblasts. The anti-scarring properties of birth tissue have been substantiated through numerous clinical and preclinical studies (Tighe et al., 2010).

- **Anti-Fibrotic Factors**

Amnio-M demonstrated an anti-fibrotic impact by suppressing the expression of TGF-β3 and its receptor. This action fosters the process of wound healing rather than contributing to scar formation. Notably, TGF-β3 serves as an antagonist to TGF-β1 and TGF-β2. These latter factors typically stimulate the synthesis of the extracellular matrix (ECM), elevate collagen deposition in the wound area, and encourage the formation of scars (Gilbert et al., 2016).

- **Anti-angiogenic properties**

The amniotic membrane possesses anti-angiogenic properties due to the release of potent chemicals that hinder angiogenesis, including thrombospondin-1, endostatin, and all four tissue inhibitors of metalloproteinase (TIMP-1, 2, 3, and 4) (Hao et al., n.d.). While generally beneficial, it is essential to carefully consider and strike a balance between the anti-angiogenic effect of the amniotic membrane and its other potential advantages when using it in cases of limbal stem cell deficiency linked to limbal ischemia, such as in ocular surface injuries from chemical exposure (Malhotra & Jain, 2014a).

2.5.3. Mechanical properties

The mechanical properties of the human amniotic membrane (hAM), such as elasticity, stiffness, and tensile strength, are closely linked to the composition of the placenta. The organization of collagen fibrils in the extracellular matrix (ECM) plays a role in determining tensile strength, and the presence of elastin fibers, laminin, hyaluronic acid, and glycosaminoglycan influences elastic deformation (Friel et al., 2017).

Research indicates that the shear modulus of the amniotic membrane falls within the range of 100 to 400 Pa, with variations in measurements linked to the state of the hAM being examined. Interestingly, decellularized hAM exhibits a higher shear modulus compared to the native form. This disparity is attributed to the denudation process, which results in membrane dehydration and a subsequent reduction in thickness. Moreover, studies have demonstrated a correlation between the elastic modulus and increasing hAM thickness, revealing a decrease in elastic modulus with thicker hAM specimens (Chen et al., n.d.), (Benson-Martin et al., n.d.).

2.6. Methods of Amniotic Membrane preservations

In order to facilitate convenient access to tissue transplantation, various preservation methods for amniotic membrane (AM) have been devised. These methods ensure that the therapeutic material can undergo thorough testing for infectious diseases, be transported, and stored effectively before application. The goal of AM preservation is to maintain its

characteristics as closely as possible to native tissue, enabling an on-demand and easily accessible therapeutic option (Sharma et al., 2023).

- **Cryopreservation**

Cryopreservation stands out as the most widely employed technique for preserving amniotic membrane (AM). The AM is carefully divided into either small (2cm by 2cm) or larger (3cm by 3cm) pieces, then positioned on nitrocellulose paper with the stromal side facing down. Following this, the AM undergoes storage in a solution containing 50% glycerol and is frozen at temperatures as low as -80 degrees (Blood & Services, n.d.).

CHAM, or cryopreserved amniotic membrane, can be safely maintained for a period of 48 hours and requires storage in a freezer maintaining temperatures below -40 degrees. After this designated timeframe, obtaining a license from the Human Tissues Authority (HTA) becomes necessary. When ready for use, CHAM is thawed for 10 minutes at room temperature and must be promptly utilized (Malhotra & Jain, 2014b), (Blood & Services, n.d.).

Certain studies in the literature propose that the freezing of tissues might induce the formation of ice crystals, posing a potential risk to the integrity of cells. Additionally, the thawing process before application could lead to the loss of crucial soluble proteins essential for the wound healing process (Malhotra & Jain, 2014b), (Allen et al., 2013).

- **Freeze-Drying/Lyophilization**

Freeze-drying encompasses cooling the amnion to -80°C, succeeded by a sublimation process to remove water from the tissue. Gamma irradiation is then employed for tissue sterilization, ensuring its safety. While this method can cause ice crystal damage to the tissue, unlike to cryopreservation, it offers the advantage of not encountering logistical challenges associated with cold chain storage. Furthermore, unlike cryopreserved products, there's no need for thawing before utilization (Rodríguez-Ares et al., 2009).

Lyophilization is a technique involving the removal of water through sublimation, preventing chemical reactions that could negatively impact tissue integrity (Rodríguez-

Ares et al., 2009). The lyophilized tissue, as a result, can be stored at room temperature for extended durations, addressing the primary drawbacks associated with cryopreservation and facilitating convenient transportation (Walkden, 2020a).

On comparing cryopreserved and lyophilized samples, scientists observed a consistent presence of type IV collagen in the basement membranes for both preservation methods. Notably, there were no significant variations in growth factors and total protein content between the two methods, except for specific fibroblast growth factors, which exhibited higher levels in cryopreserved samples (Rodríguez-Ares et al., 2009).

- **Preservation through air or heat drying**

In the preservation process, heat or air is employed to eliminate residual moisture from processed tissue, ensuring its protection (Walkden, 2020a). This method effectively prevents tissue damage that could occur if subjected to freezing. Studies have observed that tissues preserved through heat or air drying are notably thin, typically measuring around 20–30 microns (Versen-Höynck et al., 2004).However, the adoption of this technique is relatively infrequent due to challenges associated with standardizing the procedure (Sharma et al., 2023).

- **Dehydration and low temperature vacuum evaporation**

Utilizing low-temperature vacuum evaporation methods involves applying a sugar protectant, such as trehalose, to effectively preserve and stabilize the tissue structure, cellular membrane, and proteins (Allen et al., 2013), (Riau et al., 2010). The careful removal of residual water occurs through controlled low temperatures and specified vacuums, resulting in a dried product. In this preservation process, sugar protectants replace intracellular water, safeguarding the cellular matrix of the amniotic membranes (AMs) and preventing damage (Allen et al., 2013), (Wolkers et al., 2002).

Tissues preserved through this method can be shipped and stored without encountering logistical issues, making them readily available worldwide, even in war zones (Walkden, 2020b). Certain authors propose that this technique offers a more effective

substrate when compared to traditional cryopreserved amniotic membrane (AM) (Allen et al., 2013), although conflicting reports exist (Cooke et al., 2014). Furthermore, this product exhibits stability, enabling its global transport for application in clinical and military sectors (Schrimpf, 1954).

- **Irradiation method**

In the preservation processes of most tissue preparations, it is common to treat them with an antibiotic and antimycotic solution to eliminate any remaining bacteria and fungi. Some tissue preparations undergo additional sterilization through methods such as irradiation, like gamma sterilization (Nakamura et al., 2004), or the use of fluids, such as carbon dioxide in a supercritical state (Wehmeyer et al., 2015). However, there are reports suggesting that terminal sterilization methods may compromise the integrity of the tissue (Versen-Höynck et al., 2004), (Riau et al., 2010). A recent publication by (Marsit et al., 2019) highlights an antibiotic-based, aseptic decontamination technique suitable for dried amniotic membrane (AM).

- **Vitrification**

Vitrification is a technique wherein liquid nitrogen is directly introduced into the aqueous phase, leading it to transform directly into a glass phase. This method has an advantage over slow freezing since it creates a vitreous, or glassy, condition throughout the vitrification process (El-Danasouri & Selman, 2005). But to get this vitreous condition, large cryo-protectant concentrations are required, usually between 40% and 60% (w/v).

Using higher concentrations of penetrating cryo-protectants (CPAs) in this approach, which consist of DMSO, ethylene glycols, and glycerol, presents a potential risk of increased toxicity. This heightened toxicity may result in potential genetic and epigenetic changes in cells, including processes such as DNA methylation and post-translational histone modifications (Anamika Chatterjee et al., 2016), (A. Chatterjee et al., 2017). Additionally, successful execution of this method demands precise manipulation skills, and there is a notable risk of contamination (Jang et al., 2017).

Primarily utilized for preserving germ plasm, encompassing reproductive cells like oocytes and sperm, vitrification has also found application in preserving epithelial cells in the human amniotic membrane (hAM) and mesenchymal stem cells derived from the hAM (Leal-Marin et al., 2021).

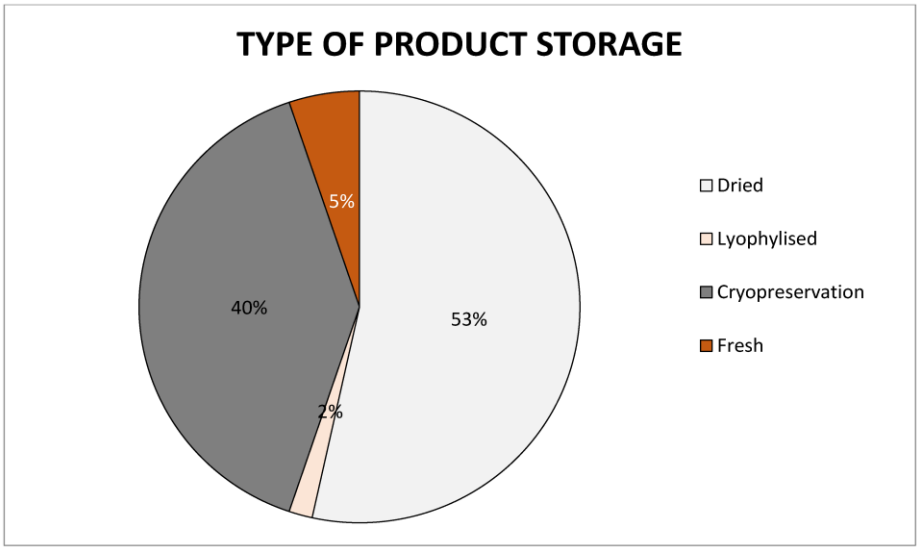


Figure 2. 11: An overview of available amniotic membrane preservation methods. Adapted from (https://www.mdpi.com/2077-0375/11/12/941) [Accessed On: 15 Nov 2023].

2.7 Amniotic Membrane Applications in Clinical Practice

The amniotic membrane serves as both a temporary and permanent graft in transplantation. When used temporarily, it is sutured as a bandage or patch to promote healing of the host tissue, dissolving once epithelialization is complete within 2 to 6 weeks. As a permanent graft, it is sutured to fill tissue defects, facilitating the integration of host cells for a robust connection with the surrounding tissue, especially in procedures involving the cornea or conjunctiva (A. Gupta et al., 2015).

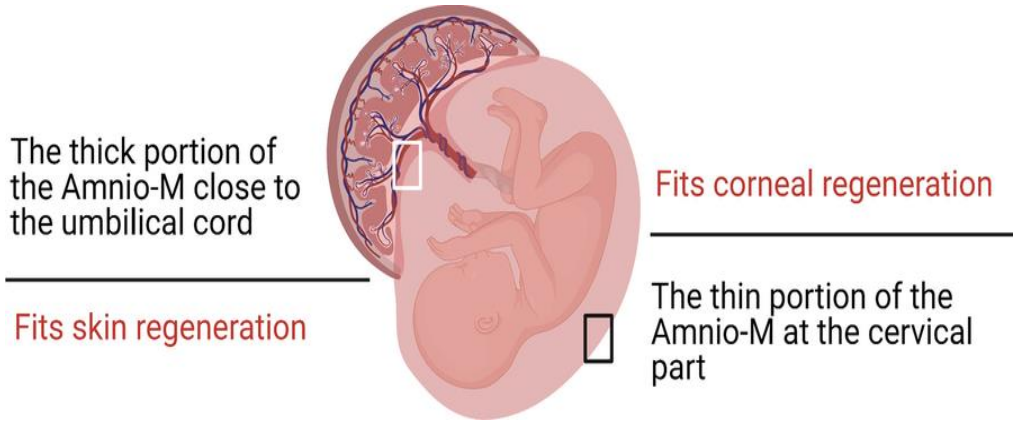


Figure 2. 12: Amnio-M site selection based on thickness to suit different clinical applications. Adapted from (https://www.researchgate.net/figure/Site-selection-of-the-Amnio-M-based-on-its-thickness-to-fit-various-clinical-applications_fig5_357732139) [Accessed On: 15 Nov 2023].

The versatile applications of the amniotic membrane extend across various medical fields, particularly in treating skin burns and preventing tissue adhesion during surgeries involving the head, neck, abdomen, larynx, and genitourinary tract (A. Mamede et al., 2012). It is utilized in diverse medical procedures, serving as a surgical dressing for burns, aiding in oral cavity and bladder reconstruction, and contributing to interventions like tympanoplasty, arthroplasty, repair of omphaloceles, and the prevention of adhesions in pelvic and abdominal surgeries (Schmiedova et al., 2021).

The varied applications of the amniotic membrane stem from its distinctive characteristics, particularly its antimicrobial properties. These qualities render it suitable for various post-surgery applications, encompassing wound healing, burn injuries, dental injuries, and ophthalmology. Given the prevalence of bacterial infection and biofilm growth in these areas, the amniotic membrane emerges as a valuable option (Trelford & Trelford-Sauder, 1979), (Dadkhah Tehrani et al., 2021).

The amniotic membrane possesses the crucial quality of biocompatibility, signifying its ability to interact with living tissue without eliciting toxic, injurious, carcinogenic, or immunological responses. Its capacity to withstand inflammation and appropriately respond to host reactions adds to its significance. Equally important is its biodegradability, rendering it a suitable choice for utilization as a scaffold (Niknejad et al., 2008b).

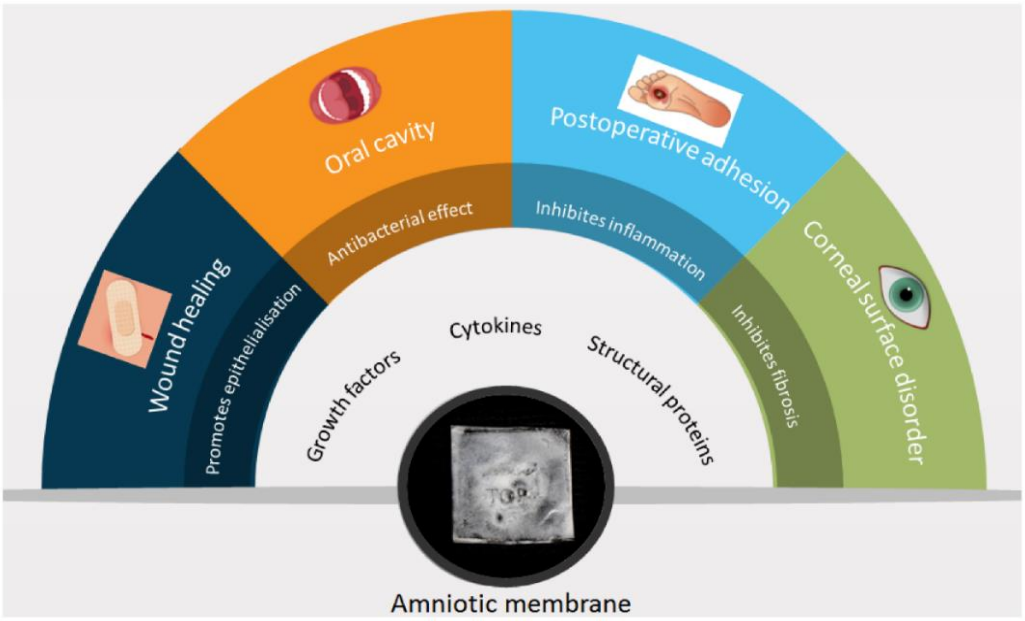


Figure 2. 13: Diagrammatic representation of the applications, properties, and components of amniotic membrane. Adapted from (https://www.mdpi.com/2077-0375/11/12/941) [Accessed On: 15 Nov 2023].

The utilization of amniotic membranes in treating corneal burns and other epithelial defects in ophthalmology is widespread, resulting in the creation of various commercially available products (Fairbairn et al., 2014). Furthermore, the amniotic membrane functions as an alternative reservoir of stem cells, and several studies suggest their potential use in tissue repairs, spanning areas such as corneal tissue, spinal cord injuries, brain infarction, and conditions like Parkinson's disease (Díaz-Prado et al., 2011).

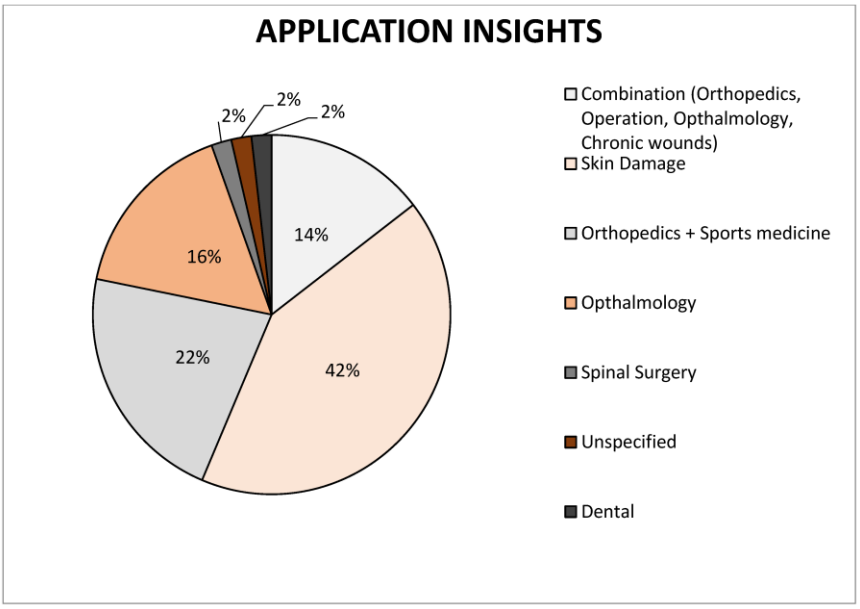


Figure 2. 14: A commercial representation of the various applications of amniotic membranes. Adapted from (https://www.mdpi.com/2077-0375/11/12/941) [Accessed On: 15 Nov 2023]

2.8. Amniotic membrane in world market

Long-term wound care presents a serious and difficult health risk to individuals as well as medical professionals. Grafts in a range of sizes and forms are readily available, which reduces material waste and enhances cost-effectiveness. Features like ease of handling and a longer shelf life without requiring a lot of preparation all contribute to the reduction of the administrative and clinical time that is usually involved in using advanced wound care products (Schmiedova et al., 2021).

Any advanced therapy or product used in wound care must be evaluated for cost-effectiveness by taking into account a number of factors, such as the rate at which the wound heals completely and the speed at which it closes. Despite the potential expenses associated with advanced wound care products, overall costs can be mitigated by shorter treatment durations, lower complication rates, reduced hospitalizations, and a decreased likelihood of amputations (Fetterolf et al., n.d.), (Zelen et al., 2014). To treat chronic wounds, Snyder et al. found 76 commercially accessible skin replacements.

The majority of these replacements are made from donated human dermis, animal tissue, or human placental membrane; none of them contain any cells. The anticipated expenses associated with treating diabetic foot ulcers varied from $6.2 billion to $18.7 billion, encompassing both non-infected and infected wound scenarios. Projections indicated a range of $0.7 billion to $1.5 billion for venous leg ulcers and $3.9 billion to $22 billion for pressure ulcers (Snyder et al., 2020), (*MiMedx Provides Update Regarding Timing of Restatement*, n.d.), (Dulemba & Mirzakhani, 2016).

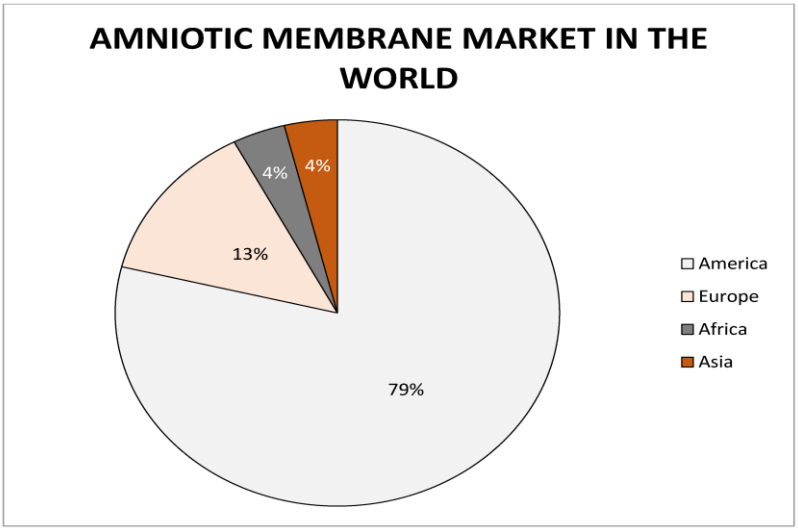


Figure 2. 15: A diagram of the global amniotic membrane market. Adapted from (https://www.mdpi.com/2077-0375/11/12/94). [Accessed On: 15 Nov 2023].

The success of treatments involving placental derivatives is exemplified by the distribution of over one million medicinal products by MiMedx (Kesting et al., 2008). Other key players in this field include Amnio Technology LLC, Celularity Inc., Human Regenerative Technologies LLC, Katena Products Inc., Integra Life Sciences Corporation, Skye Biologics Inc., and Amniox Medical Inc. (Schmiedova et al., 2021).

As of 2017, the global amniotic membrane market was valued at USD 2.26 billion, projected to reach USD 5.81 billion by 2025, according to Grand View Research, Inc. The majority of firms working in amniotic membrane research, development, or manufacture are situated in the United States (Schmiedova et al., 2021).

2.9. Techniques for causing injury to the corneal endothelium

- **Transcorneal cryo-injury**

The transcorneal cryo-injury method has been widely employed to induce dysfunction in corneal endothelial cells (CEC) (Maumenee & Kornblueth, 1948), (Khodadoust et al., n.d.). Although it is a relatively non-invasive and easily applicable technique, the resulting CEC wound and subsequent healing behavior depend on factors such as temperature, probe size, and application duration, necessitating careful standardization (Han et al., n.d.), (Chi & Kelman, 1966).

Typically, liquid nitrogen is utilized to cool the probe to a range of −80 to −196 ℃. It's crucial to note that the probe's temperature increases during the initial 3–5 seconds after application, underscoring the importance of immediate cooling before each use (Chi & Kelman, 1966). The application should result in the creation of an ice ball in the anterior chamber if the temperature and time are handled properly. Corneal epithelial defects normally recover completely after 5-7 days of damage (Okumura et al., 2011), (D. Van Horn et al., n.d.), (D. L. Van Horn & Hyndiuk, 1975), (Chi & Kelman, 1966).

- **Mechanical injury**

Mechanical damage is caused by scraping and removing the endothelium with an instrument in the anterior chamber. Various methods have been employed to scrape corneal endothelial cells (CECs) while maintaining Descemet's Membrane (DM), such as making linear or circular incisions with a dull spatula or needle or using specially designed tools to remove CECs (Tuft et al., n.d.), (Mills & Donn, 1960), (Huang et al., n.d.), (Ichijima et al., 1993), (Solomon et al., 1997), (Landshman et al., 1989).

- **Chemical injury**

In contrast to mechanical and cryoinjury methods, the chemical injury approach was initially introduced in 1977 during animal corneal transplant experiments. This method aimed to eliminate all host cells, induce persistent corneal edema, and prevent corneal

neovascularization (Maurice & Perlman, 1977). To mitigate collateral tissue damage and potential consequences like anterior uveitis, secondary glaucoma, and corneal neovascularization, physiological saline is irrigated into the anterior chamber following the injection of the chemical agent (Maurice & Perlman, 1977), (Britton et al., 1976).

While various pharmacologic agents have been used for anterior chamber injection to induce corneal endothelial cell (CEC) cytotoxicity, benzalkonium chloride (BAK) stands out as the most frequently employed agent in experimental animals, leading to bullous keratopathy. Chemical injury via intracameral injection is expected to cause more extensive CEC damage compared to cryoinjury or mechanical debridement (Zhang et al., 2017), (Maurice & Perlman, 1977), (Segarra et al., 2018).

- **Laser injury**

The Neodymium-doped yttrium aluminum garnet (Nd: YAG) laser functions through photo disruption, creating micro-explosions within tissues. This laser technology enables healthcare professionals to target intraocular tissues without the necessity of entering the anterior chamber. Typical applications of the Nd: YAG laser involve peripheral iridotomy and capsulotomy, used to address acute angle-closure glaucoma and posterior capsular opacification following cataract surgery, respectively.(Zhang et al., 2017), (Seitz & Langenbucher, 2000), (Dragnea et al., 2020). These operations are typically regarded as safe, with few related problems like corneal ulcers and uveitis (Zhang et al., 2017).

- **UV irradiation**

The prolonged exposure of corneal endothelial cells (CECs) to light, especially UV radiation, and their high oxygen demand during active pump operation make them particularly susceptible to oxidative stress (Jurkunas et al., 2010). Researchers have subjected animal corneas to UV irradiation to simulate a prooxidative environment that damages nuclear and mitochondrial DNA and causes corneal endothelial dysfunction (Sangwan Park et al., 2021).

The extent of damage induced by UV irradiation generally relies on factors such as the wavelength, light intensity, and the absorption spectrum specific to each tissue (Ivanov et al., 2018). Experimenting with UV irradiation, like the cryoinjury model, damages the corneal epithelium, a process that generally heals within one week (Liu et al., 2020).

2.10. Surgical methods of Amniotic membrane Transplantation and its clinical application

The common practice involves placing the amniotic membrane (AM) onto nitrocellulose filter paper, positioning it with the epithelial side facing upward, and having the stromal side in direct contact with the paper. The stromal surface is identifiable by the presence of vitreous-like strands, which can be lifted using a sponge or delicate forceps. Confirmation of the stromal surface may require this procedure at multiple points, depending on the specific indication for its usage (Malhotra & Jain, 2014).

The positioning of the amniotic membrane (AM) is crucial and depends on the desired outcome. Placing the AM with the epithelial side up (where the epithelium faces the host's lids and the stromal side is directed towards the host's cornea) facilitates the growth of corneal and conjunctival epithelial cells, promoting the re-epithelialization of the host's epithelium. Conversely, positioning the AM with the epithelial side down (facing the host's cornea and the stromal side towards the host's lids) utilizes the stromal matrix to help reduce inflammation (Malhotra & Jain, 2014), (Meller et al., 2011). The epithelial side of the AM is characterized by a smooth and shiny surface, whereas the stromal side is sticky and gelatinous. Typically, it is supplied on nitrocellulose filter paper, with the epithelial side facing up and the stromal side facing down (Blood & Services, n.d.).

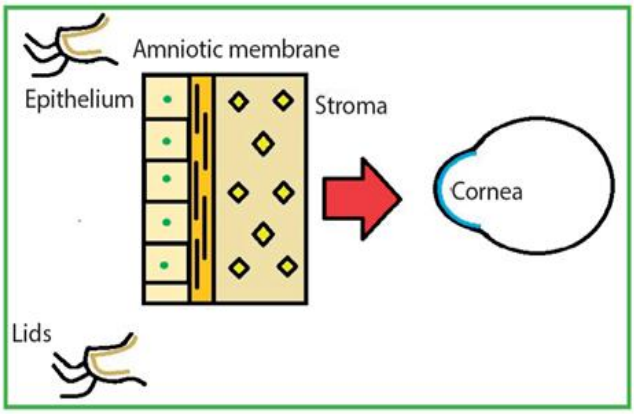


Figure 2. 16: Epithelial side UP amniotic membrane. Adapted from (https://www.eyenews.uk.com/education/trainees/post/understanding-amniotic-membrane-grafts) [Accessed On: 8 Dec 2023]

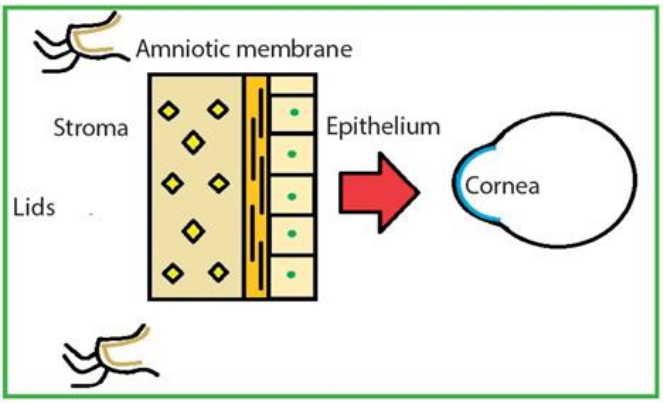


Figure 2. 17: Epithelial side DOWN amniotic membrane. Adapted from (https://www.eyenews.uk.com/education/trainees/post/understanding-amniotic-membrane-grafts) [Accessed On: 8 Dec 2023]

- **Graft or inlay Transplantation**

In this technique, the amniotic membrane (AM) serves as a substrate or scaffold to support the proliferation of epithelial cells. The AM is placed with the epithelial/basement membrane side facing upward and is customized to match the size of the underlying epithelial or stromal defect. Typically, non-absorbable 10-0 nylon sutures are used to secure the AM to the cornea. The decision to orient the epithelial/basement membrane side up is rooted in the superior substrate provided by the amnion's basement membrane. This

substrate promotes the growth of progenitor epithelial cells by prolonging their lifespan, preserving clonogenicity, and preventing apoptosis (Grueterich et al., n.d.).

This procedure can be employed as a single-layer graft inlay, utilizing a single layer of AM, or as a multilayer graft inlay, incorporating multiple layers of AM into the ulcer base without the need for sutures, depending on the depth of the underlying defect. In the latter case, a ring-shaped area surrounding the corneal ulcer is depithelialized, and a superficial graft is sutured to the ulcer's edge. The objective of this multilayer graft is for the epithelium to develop over the top layer (Seitz et al., n.d.). Another term for this method is the layered or fill-in method. Layering can be achieved by cutting the AM into multiple pieces and stacking them or by using a larger piece of AM that is folded (blanket fold) upon itself repeatedly (Malhotra & Jain, 2014).

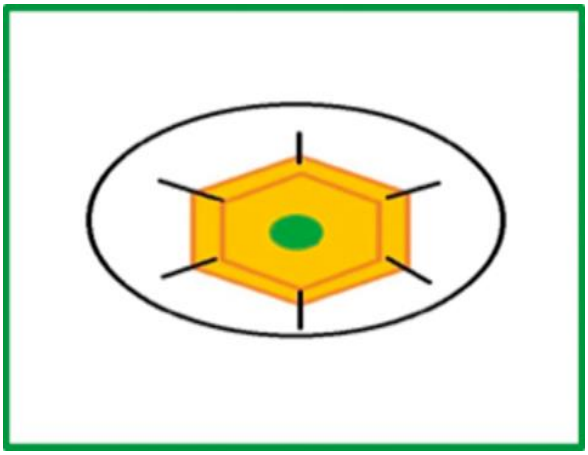


Figure 2. 18: Diagram of amniotic membrane graft. The corneal defect (green circle) is covered by the amniotic membrane (orange hexagon) held with interrupted corneal sutures. Adapted from (https://www.eyenews.uk.com/education/trainees/post/understanding-amniotic-membrane-grafts) [Accessed On: 8 Dec 2023].

- **Patch or overlay Transplantation**

An onlay graft, commonly referred to as a patch application of amniotic membrane (AM), serves as a temporary biological bandage, acting as a protective barrier to shield the wound from external damage or pressure exerted by the eyelids (Walkden, 2020). Patch

transplantation is typically employed in cases involving superficial damage or severe inflammatory disorders (Sharma et al., 2023).

When using the patch or overlay (temporary basement membrane) amniotic membrane (AM), it can be positioned with the epithelial side facing up or down. It is securely placed over the host's epithelium to the conjunctiva or episclera using 10-0 vicryl or adhered in place with fibrin. In this manner, the AM functions as a 'natural' patch and usually undergoes breakdown within one to two weeks (Malhotra & Jain, 2014), (Elhassan, 2019).

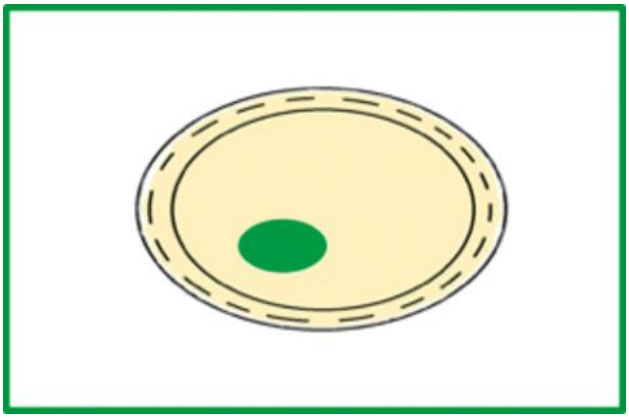


Figure 2. 19: Diagram of amniotic membrane patch. The corneal defect (green circle) is covered with amniotic membrane patch (cream circle) sutured in place with vicryl in a purse-string fashion in the peri-limbal region. Adapted from (https://www.eyenews.uk.com/education/trainees/post/understanding-amniotic-membrane-grafts) [Accessed On: 8 Dec 2023].

- **Combinatorial (inlay and overlay) Transplantation**

The sandwich amniotic membrane (AM) transplantation technique is also known as the combined technique (Jirsova & Jones, 2017).Combinatorial transplantation involves incorporating both inlay and onlay transplantation methodologies. This approach combines the structural support provided by the graft with the protective function of the patch, ensuring the integrity of the overall procedure. Both transplants contribute to the delivery of anti-inflammatory and pro-epithelialization factors (Jirsova & Jones, 2017).

When the graft is positioned with the epithelial side up and the patch with the epithelial side down, the anticipated outcome is for the epithelium to develop between the two layers (Dua et al., 2004). The integrated transplantation, combining both inlay and overlay techniques, supports the growth of the epithelium beneath the patch and above the graft over the course of several weeks (Malhotra & Jain, 2014), (Elhassan, 2019).

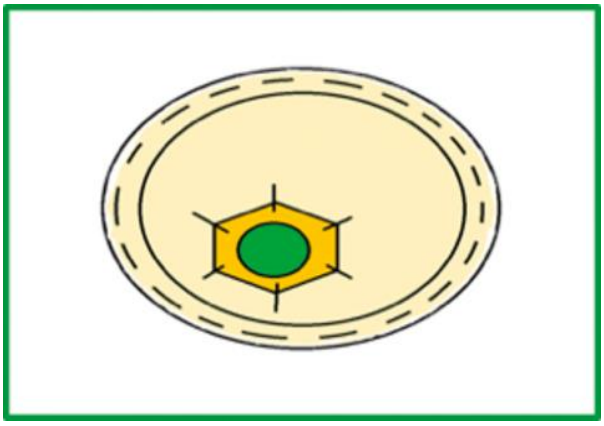


Figure 2. 20: Diagram of combined AM. The corneal defect (green circle) is filled with AM graft (orange hexagon) and then covered with AM patch (cream circle). Adapted from (https://www.eyenews.uk.com/education/trainees/post/understanding-amniotic-membrane-grafts) [Accessed On: 8 Dec 2023].

CHAPTER 3: MATERIALS AND METHODS

Table 3. 1: Materials used for Amniotic membrane preparation

Materials			
1.	Human placenta	**2.**	Ciprofloxacin injection
3.	Fine tissue roll	**4.**	Examination gloves pack
5.	Yellow pipette tips	**6.**	10cc syringes
7.	Test tube	**8.**	Ethanol solution 2.5L
9.	Distilled water	**10.**	Para tulle (bandage)
11.	Aluminum foil	**12.**	Disposable petri plate
13.	Sterile saline	**14.**	Conical flask
15.	Sodium dodecyl sulfate (SDS)	**16.**	Streptomycin
17.	Cutter	**18.**	Nitrocellulose membrane

3.1. Human Amniotic Membrane (HAM) Preparation

3.1.1. Collection of human placenta

The human amniotic membrane (hAM) is derived from the human placenta and collected by healthcare personnel after a cesarean birth with the donors' informed consent. Every maternal donor undergoes prior serological screening to ensure they test negative for the infectious diseases such as human immune deficiency virus (HIV), hepatitis B and

C viruses, and syphilis. Placentas from vaginal deliveries are excluded from use due to the risk of potential bacterial contamination from the vaginal environment (Klama-Baryła et al., 2020).

3.1.2. Transportation

Following collection, the placenta was carefully preserved in a sterile container and quickly delivered to the laboratory for processing. To maintain optimal conditions, it is advisable to minimize the transport time, ideally within a maximum recommended duration of 24 hours (Leal-Marin et al., 2021).

3.1.3. Preparation

The human placenta undergoes multiple washes using distilled water to eliminate blood components. Subsequently, in a controlled lamellar flow environment, the placenta undergoes an additional wash with sterile saline to ensure the removal of any lingering blood clots.

The placenta was put in a sterile container, and the amnion was carefully removed from chorion using a gentle and sterile blunt dissection. Extra cellular matric sheet (EMC) suspension was bathed in a ‘cocktail’ of antibiotics containing 1 ml (penicillin, streptomycin and ciprofloxacin) and sterile saline for 24 hours and washed with sodium dodecyl Sulfate (SDS) (Elhassan, 2019).

3.1.4. Decellularization of human amniotic membrane

Pour the 15g of 0.5% SDS in 3L of distilled water to prepared solution for decellularization. The extracellular matrix (ECM) suspension underwent a treatment process involving buffered 0.5% sodium dodecyl sulfate (SDS) diluted 1:1 , along with an antibiotic cocktail. This treated ECM suspension was then placed in a gently shaking incubator at 50 rpm and rinsed with distilled water twice a day for a total of 4 days at room temperature. This rinsing process continued until any remaining SDS was completely removed. The rinsing is repeated until a clean solution is obtained.

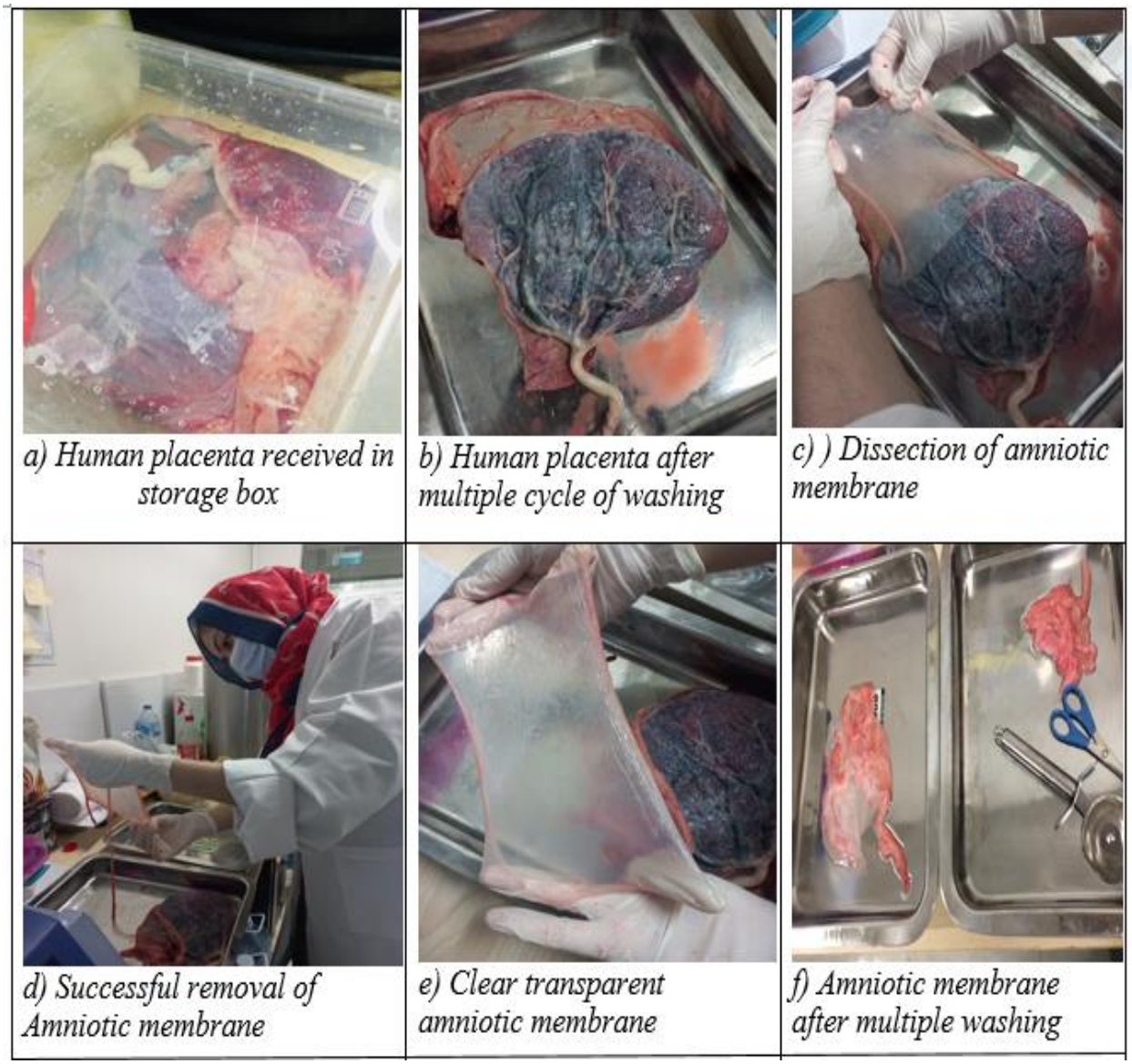


Figure 3. 1: Steps of human amniotic membrane preparations

Following that, the suspension underwent a 10-minute treatment at 37°C with a combination of 0.2% DNase (2000 U; Sigma) and 200mg/ml RNase (Sigma) (Choi *et al*., 2013). The amnion was then delicately put on a glass plate, and any excess blood and mucus were carefully removed. For the purification of placental tissues, mechanical techniques were employed to effectively eliminate blood clots and other potential contaminants.

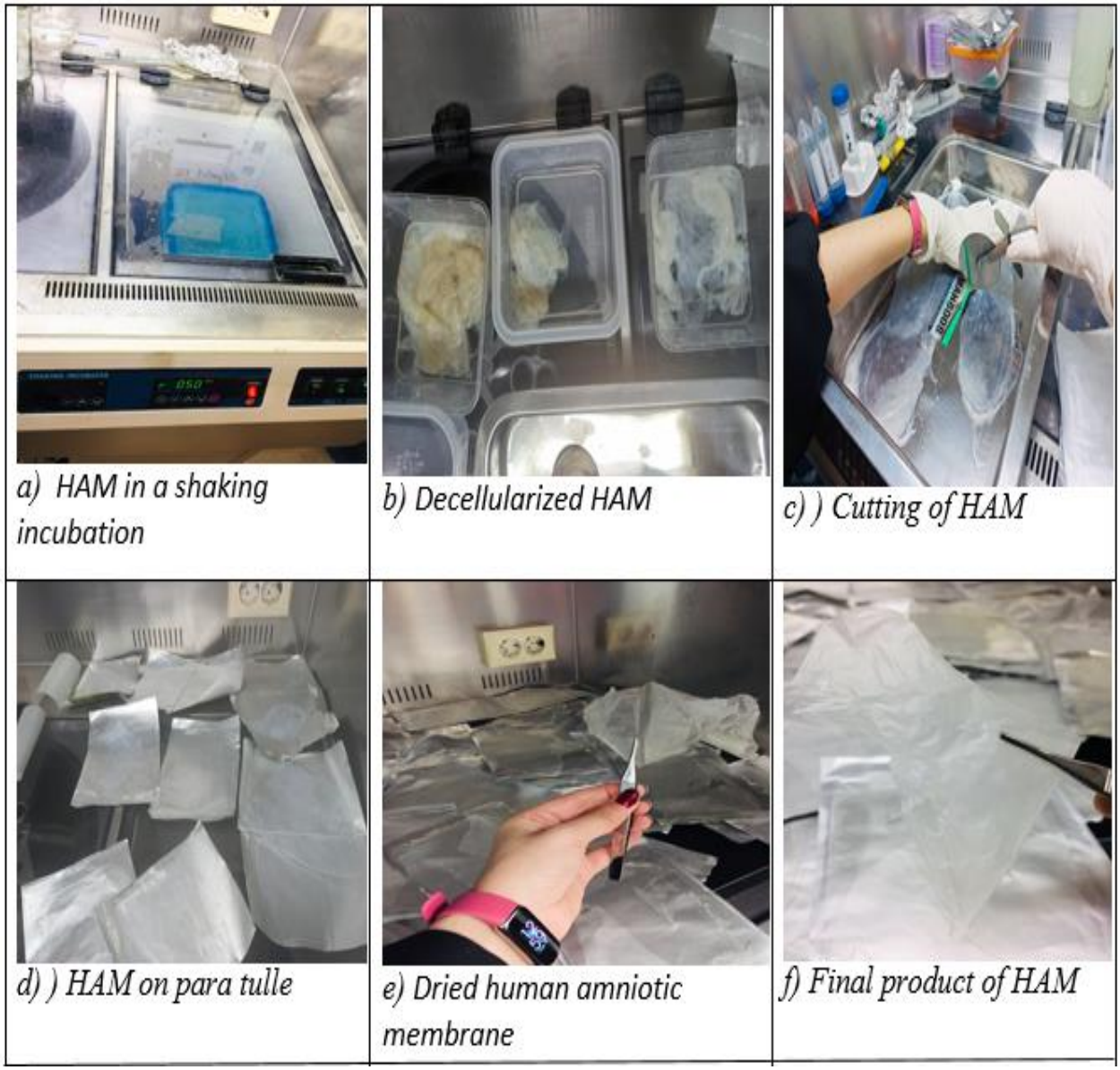


Figure 3. 2: Decellularization process of human amniotic membrane

After removing the media from the decelluarized hAM, it was transfer to container containing ethanol solution. The membranes, once separated, are cut into various sizes and carefully positioned on nitrocellulose paper strips, ensuring that the epithelial side faces upward. Amniotic membrane can be preserved through cryopreservation (CHAM) or in a dry de-epithelialization form (DHAM) (Blood & Services, n.d.).

Mesenchymal stem cells, fibroblasts, and an epithelial cell layer make up the human amniotic membrane (HAM). Decellularization is a technique used to remove cells and cell debris from the amniotic membrane without damaging the extracellular structural proteins to minimize the possibility of immunogenic reactions.

Human amniotic membrane (hAM), which has not been decellularized, functions well as a scaffolding material. Few investigations have documented a mild immunogenic response resulting in tissue inflammation in the treated area (Woong Lee & Young Park Eun Ah Kim Il Han Yun, 2014), (*Glaucoma Filtration Surgery Using Amniotic Membrane Transplantation - PubMed*, n.d.).

3.1.5. Dry human amniotic membrane (DHAM)

Sterilization of the amniotic membrane (AM) was achieved by air vacuum and gentle heating. It has a two to five year shelf life and is suitable for room-temperature storage. However, it's crucial to remember that an HTA license is necessary for storage beyond 48 hours. DHAM is usually hydrated before use by using sterile saline (Blood & Services, n.d.), (Malhotra & Jain, 2014). According to published research, AMs that have been air-dried are stable and may be kept in a different environments without losing their therapeutic effectiveness. (Naeem, 2018).

CHAPTERR 4: RESULTS AND DISSCUSSION

4.1. Antimicrobial activity of human amniotic membrane

4.1.1. Media preparation

Prepared 200ml growth media by dissolving 7.4g of nutrient agar in conical flask. Autoclave the media and petri dishes at 121°C for 20 minutes. The purpose of autoclaving the media is to ensured that it is free from any living microorganisms, including bacteria, fungi, and their spores.

4.1.2. Amniotic membrane preparation

Dry decellularized human Amniotic membrane is cut into 3*3 pieces and exposed to UV radiation for 10 to 15 minutes.

4.1.3. Strain selection

Three different bacterial strains have been used to check the antimicrobial activity of human amniotic membrane i.e.

i. *Bacillus subtilis*
ii. *Escherichia coli (E coli)*
iii. *Staphylococcus aureus*

4.1.4. Plate's preparations

In the laminar flow hood pour the media in petri plates and allow it to solidify. With the help of micro pipette cultivate the microorganisms in a suitable growth medium. Sterilized glass rod was used to spread the bacteria strains on the growth media.

With the help of tweezers placed the amniotic membrane patch in the center of petri plates. Incubate the plates at the optimal temperature for 24 hours. Measure the diameter of the clear zone (inhibition zone) around each patch after incubation.

4.1.5. Results and Discussion

Only Amniotic Membrane

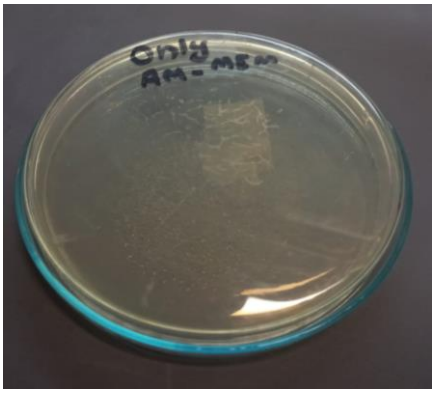


Figure 4. 1: AM membrane on nutrient agar

Amniotic membrane is placed alone on the growth media to check whether it is free from pathogen or not. Results after incubation shows that Amniotic membrane is sterilized and don't contain any pathogen.

i. Bacillus subtilis

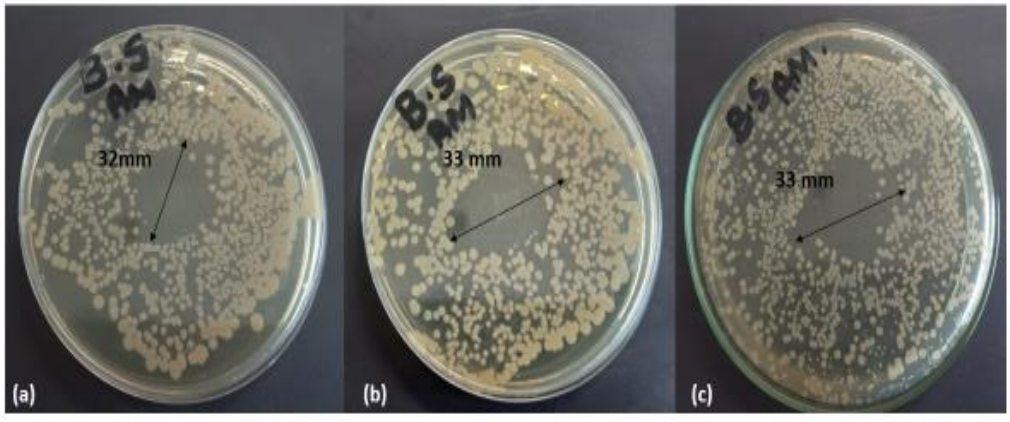


Figure 4. 2: Human amniotic membrane microbial activity against *Bacillus subtilis*

Bacillus subtilis a common gram-positive bacterium. The antimicrobial activity of dry human amniotic membrane patches against *Bacillus subtilis* shows a significant zone of inhibition. Figure (a) illustrates a 32 mm zone of inhibition (b) 33 mm zone of inhibition and (c) 33 mm zone of inhibition.

The average mean of the zone of inhibition for *Bacillus subtilis* is 32 mm and standard error of 0.333.

ii. Escherichia coli (E coli)

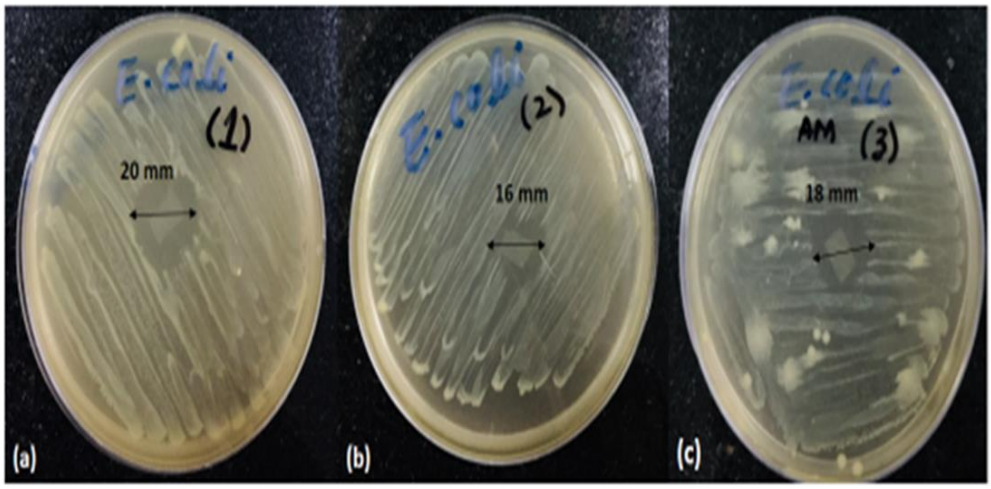


Figure 4. 3: Human amniotic membrane microbial activity against Escherichia coli

The antimicrobial activity of human amniotic membrane was investigated against the *Escherichia coli***.** *Escherichia coli* is a gram-negative bacterium. The zone of inhibition for each plates was calculated after incubation for 24 hours. The figure (a) 20 mm zone of inhibition (b) 16 mm zone inhibition (c) 19 mm zone of inhibition. Results are presented as mean and standard error. Average mean = 18 mm standard error of 1.154.

iii. Staphylococcus aureus

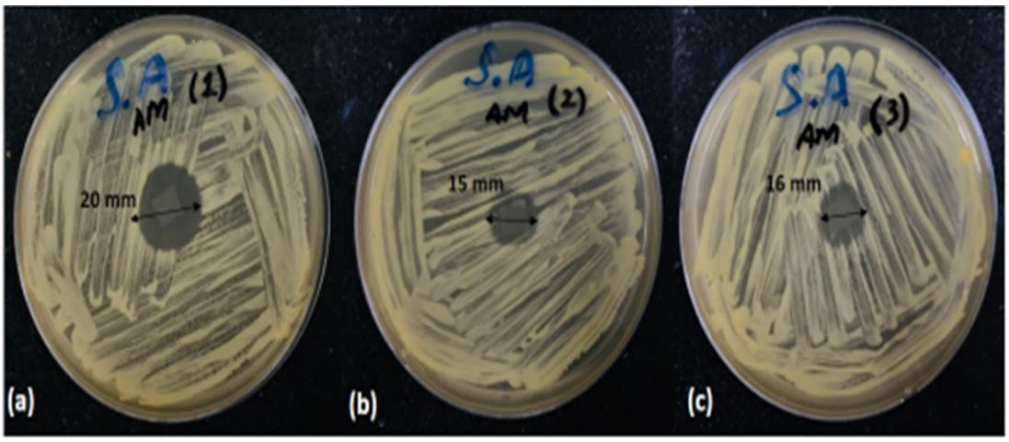


Figure 4. 4: Human amniotic membrane microbial activity against *Staphylococcus aureus*

Staphylococcus aureus a common gram-positive bacterium. The antimicrobial activity of human Amniotic membrane against *Staphylococcus aureus* was investigated and it shows a clear circular zone of inhibition around the human amniotic membrane patches. The figure (a) 20 mm in a diameter area of inhibition (b) 15 mm area of inhibition and (c) 16 mm area of inhibition. The mean result of *Staphylococcus aureus* is 17 mm with the standard error of 1.527.

4.1.6. Conclusion

In conclusion, our study demonstrates the significant antimicrobial activity of human amniotic membrane against *Bacillus subtilis, Staphylococcus aureus*, and *Escherichia coli.* .Following a 24-hour incubation period, no colonies were discovered beneath the human amniotic membrane patches. All three strains of bacteria shows significant circular zone of inhibition. Our antimicrobial study is focus on a three bacterial strains. Future research should explore the broader antimicrobial activity of human amniotic membrane against various gram-negative and gram-positive microorganisms.

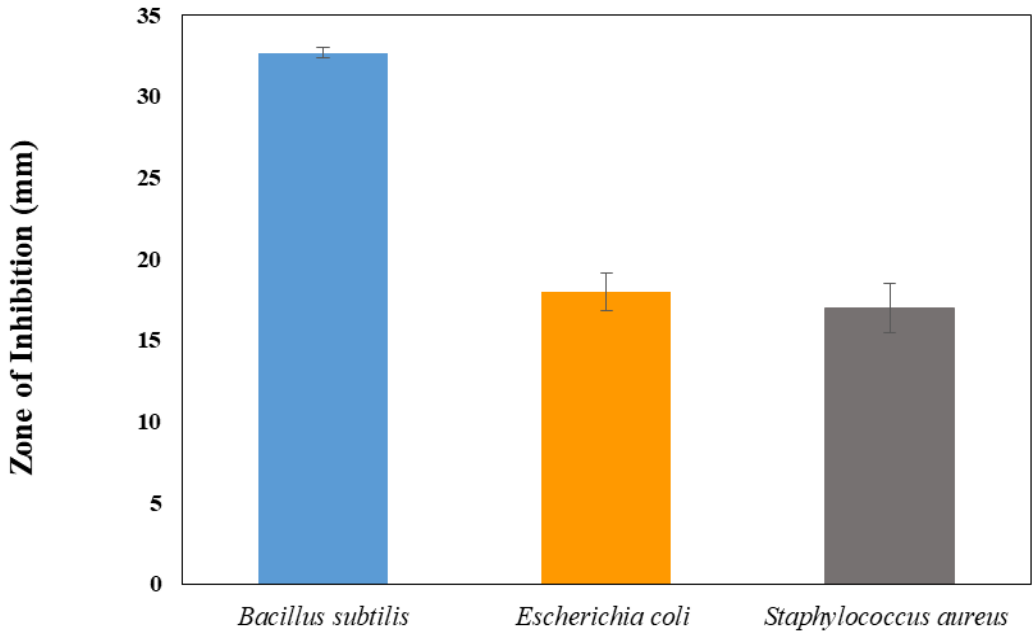


Figure 4. 5: Antimicrobial activity of three different bacterial strains

The one-way analysis of variance (ANOVA) revealed a statistically significant difference in the antimicrobial activity among the three bacterial strains. The p-value was 0.000103 which is ($p<0.05$) this indicates that there are likely variations in the antimicrobial activity of the tested strains.

Table 4.1: Result of One-way ANOVA for Antimicrobial activity

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	461.5556	2	230.7778	61.08824	0.000103	5.143253
Within Groups	22.66667	6	3.777778			
Total	484.2222	8				

4.2. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is useful tool in tissue engineering research, which uses the beam of electron to produce the detailed image of the samples. SEM image provide the significant information about the surface morphology, cross sectional view, composition and properties of the samples.

SEM samples must be completely dry. Biological samples of any kind such as cells, tissues, and membranes need to be chemically fixed in order to preserve its properties and stabilized their structure (Al Shehadat *et al.,* 2018).

4.2.1. Methodology

The samples were fixed in 2.5% glutaraldehyde for two hours at 4 degrees Celsius before undergoing scanning electron microscopy. The specimens were then thoroughly cleaned with PBS. For 10 minutes, graded ethanol solutions of 30%, 50%, 70%, 90%, and 100% each were used to dehydrate human amniotic membranes. In 100% ethanol, the dehydration procedure was repeated twice. (Shehadat and colleagues, 2018).

The specimens were then dried and mounted on suitable microscopic slides. Afterward, a thin layer of gold was applied via sputter coating, and a scanning electron microscopy (JEOL-JSM-6490LA model) was used to observe the samples. The coated samples were visualized at a 20 kV electron beam.

4.2.2. Results and Discussion

4.2.3. Human amniotic membrane (hAM) Surface view

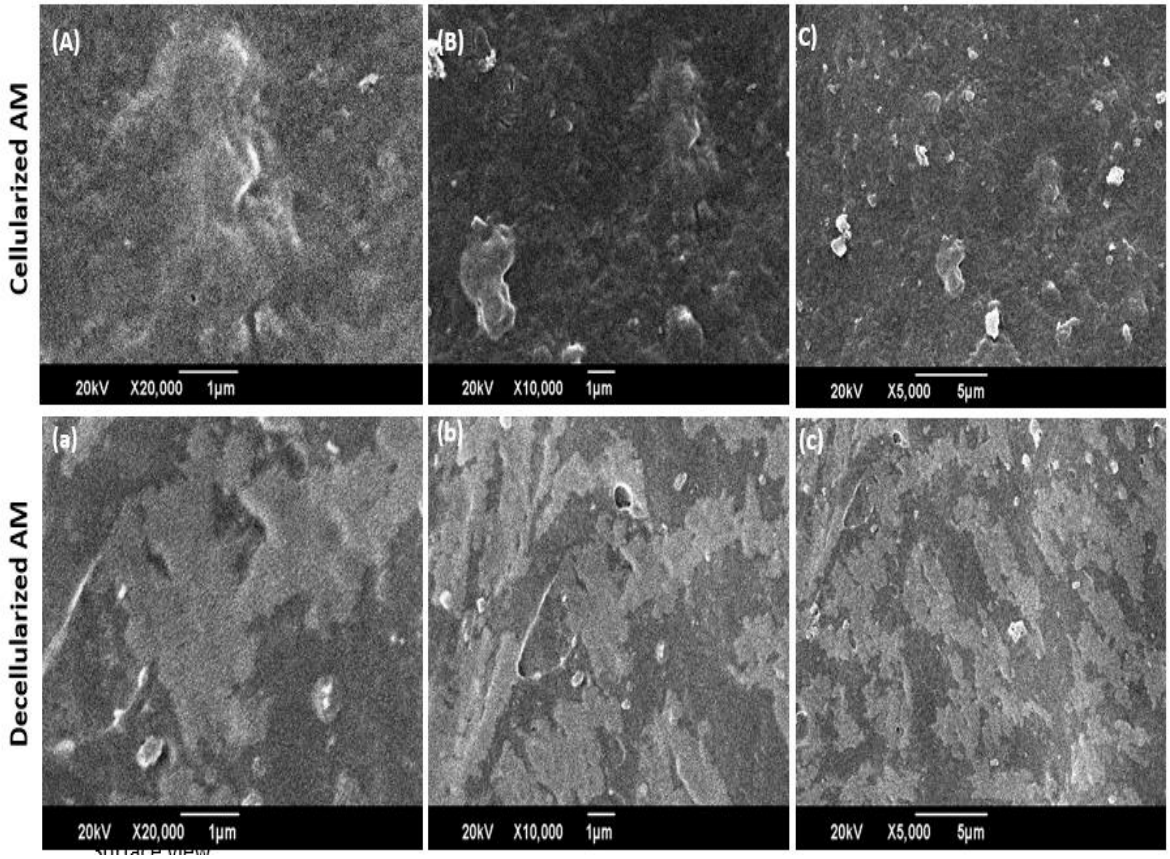


Figure 4. 6: Representations of the human amniotic membrane obtained by scanning electron microscopy at different magnifications **(A, B, & C)** surface view of the unprocessed or cellularized human amniotic membrane and **(a, b, & c)** surface view of processed or decellularized human amniotic membrane.

The human amniotic membrane, both cellularized and decellularized, has been evaluated using scanning electron microscopy (SEM). Image (A) cellularized hAM and (a) decellularized hAM at the X20, 000 magnification. Image (B) cellularized hAM and (b) decellularized hAM at the X10, 000 magnification. Image (C) cellularized hAM and (c) decellularized hAM at the X 5,000 magnification operation at a 20kv electron beam voltage.

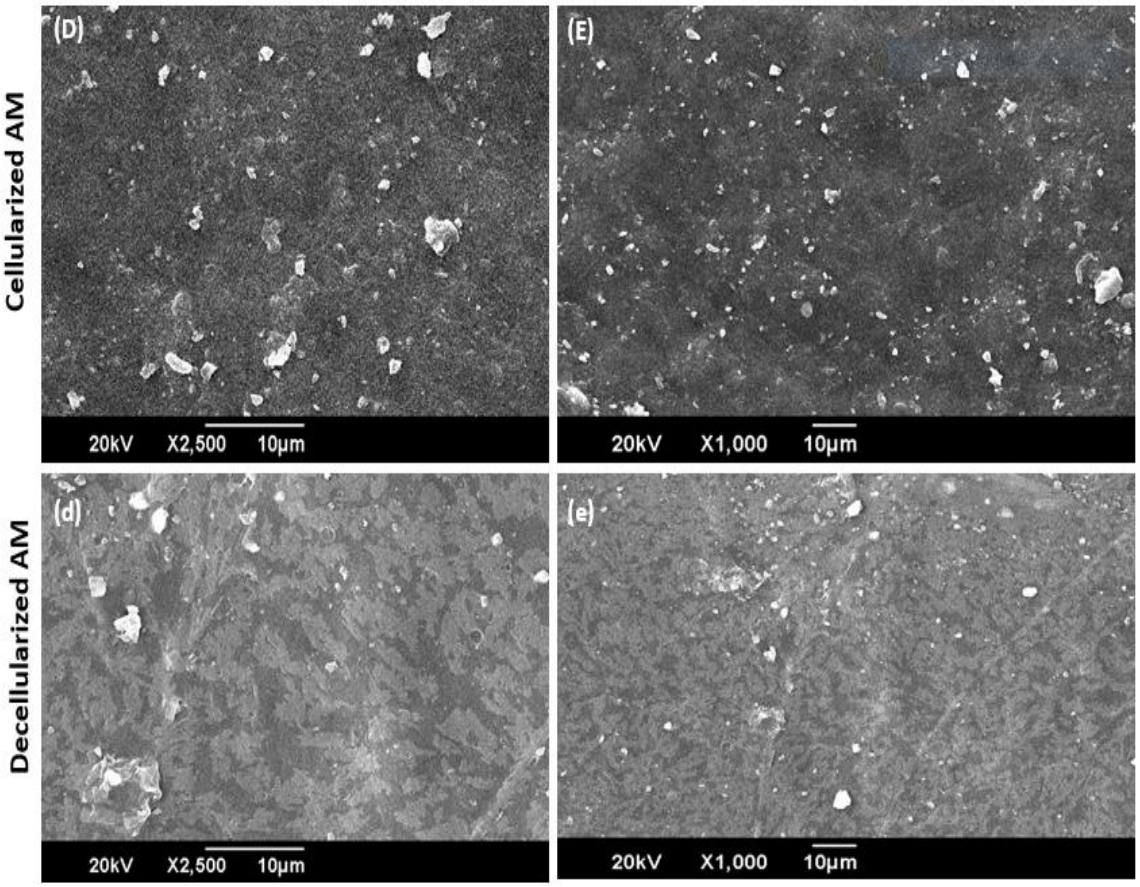


Figure 4. 7: A scanning electron microscopic image of human amniotic membrane **(D, E)** surface or top view of the unprocessed or cellualrized human amniotic membrane and **(d, e)** surface or top view of processed or decellularized human amniotic membrane.

SEM Image (D) cellularized hAM and (d) decellularized hAM at the X 25, 00 magnification. Image (E) cellularized hAM and (e) decellularized hAM at the X 1,000 magnification operation at a 20kv electron beam voltage.

SEM of human amniotic membrane at magnifications of X20000, X10000, X5000, X2500, and X1000 operating at a 20kv electron beam. Results of the scanning electron microscopy shows that cellularized or unprocessed hAM has epithelial cells present on the surface with intact extracellular matrix while the decelluarized hAM shows the absence of epithial cell with intact extra cellular matrix. The presence of white spots on the surface shows the cells on epithelial side of hAM.

4.2.4. Human amniotic membrane (hAM) Cross-Sectional view

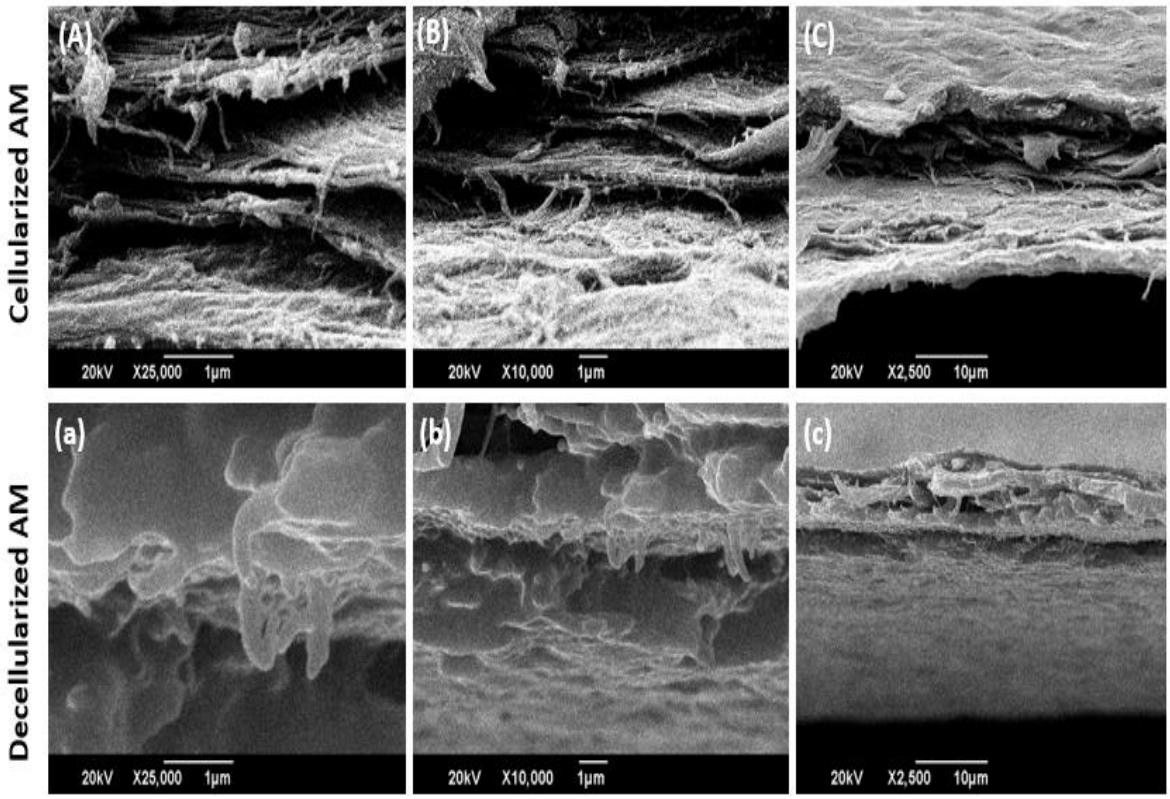


Figure 4. 8: A cross-sectional scanning electron microscope (SEM) image of human amniotic membrane **(A, B, C)** cross-section view of the unprocessed or cellualrized human amniotic membrane and **(a, b, c)** cross section view of processed or decellularized human amniotic membrane.

SEM cross sectional Image (A) cellularized hAM and (a) decellularized hAM at the X25, 000 magnification. Image (B) cellularized hAM and (b) decellularized hAM at the X10, 000 magnification. Image (C) cellularized hAM and (c) decellularized hAM at the X 25, 00 magnification operation at a 20kv electron beam voltage.

Cross sectional view of human amniotic membrane at the magnification of X25000, X10000, X2500 shows that the cellularized human amniotic membrane consists of amniotic epithelial cells and a smooth basement membrane. The decellularized human amniotic membrane, on the other hand, has the extracellular matrix intact but lacks epithelial cells.

4.3. Hematoxylin and Eosin (H & E) Staining

Hematoxylin and Eosin (H&E) staining is widely used staining technique in biomedical research. It is used to visualized and differentiate the tissue and cell components under the microscope.

Human amniotic membranes that had been cellularized and decellularized were stored with 10% (v/v) neutral buffered formalin, and they were then subsequently dehydrated using by graded series of ethanol. After dehydration hAM was embedded in paraffin wax and Hematoxylin & Eosins stain were applied to specimen for histological examination (Sripriya & Kumar, 2016)**.**

Pixel pro software were used to analyze the H&E staining.

4.3.1. Results and discussion

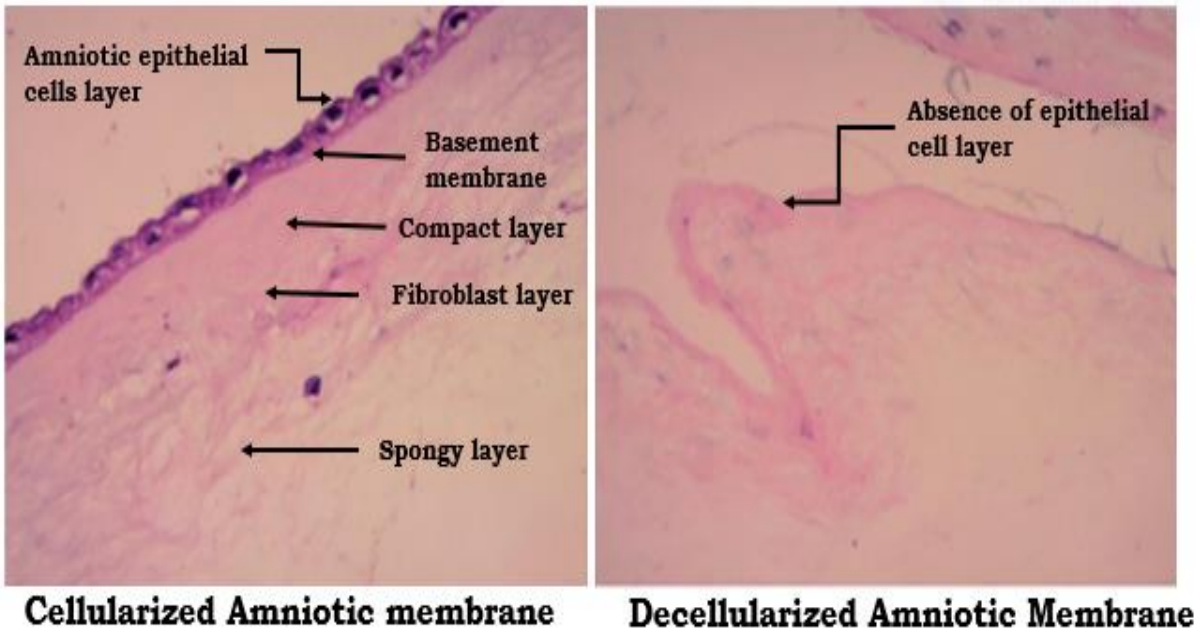


Figure 4. 9: H&E staining of cellularized and decellularized human amniotic membrane

H&E Image (A, B, C) are the cellularized human amniotic membrane while (a, b, c) are decellularized human amniotic membrane. A light microscopic picture of the human amniotic membrane that has been cellularized and stained with H&E reveals that the membrane is made up of columnar epithelial cells that are attached to a thick basement membrane.

Nuclei stained purple also verifies the existence of epithelial cells on the cellularized human amniotic membrane. Decellularized human amniotic membranes lack epithelial cells, and the absence of a nuclei structure indicates effective decellularization.

4.4. Fourier transform infrared spectroscopy (FTIR)

FTIR is infrared spectroscopic technique widely used in material sciences and chemistry to analyze the substance and its components. FTIR additionally offers valuable insights into the molecular structure and chemical composition of the specimen.The magnitude of peaks in the spectrum serves as a direct indicator of the quantity of material present. In modern software algorithms, FTIR has shown to be a highly effective tool for quantitative analysis (Ganzoury *et al.*, 2015).

4.4.1. Results and discussion

FTIR spectra of human amniotic membrane both cellularized and decellularized were examined to understand the presence of molecular structural component of amniotic membrane. The spectra were obtained within the 400–4000 cm-1 range. Origin 7.5 software had been used to plot the FTIR spectrum.

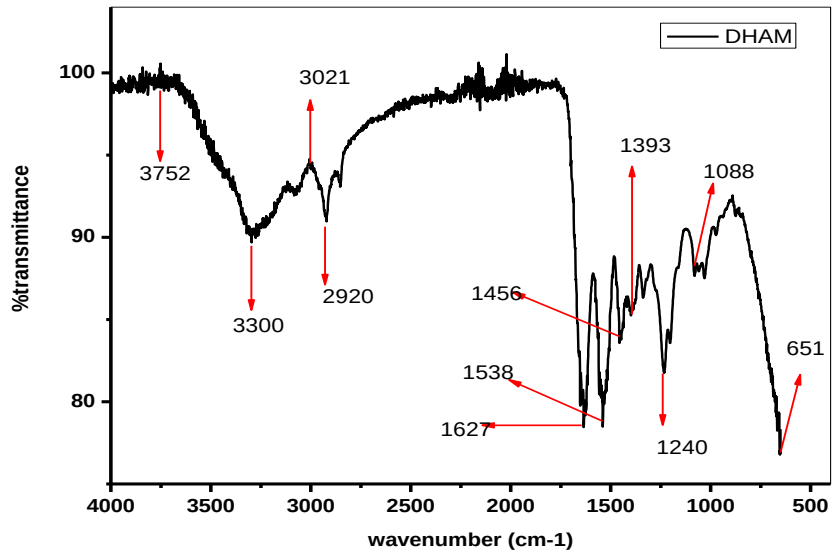


Figure 4. 10: FTIR spectra of the decellularized human amniotic membrane (DHAM)

The FTIR spectrum of the decellularized human amniotic membrane shows different absorbance band at the frequency of 3752 cm-1, 3300 cm-1, 3021 cm-1, 2920 cm-1, 1627 cm-1, 1538 cm-1, 1456 cm-1, 1393 cm-1, 1240 cm-1, 1088 cm-1, and 651 cm-1 respectively.

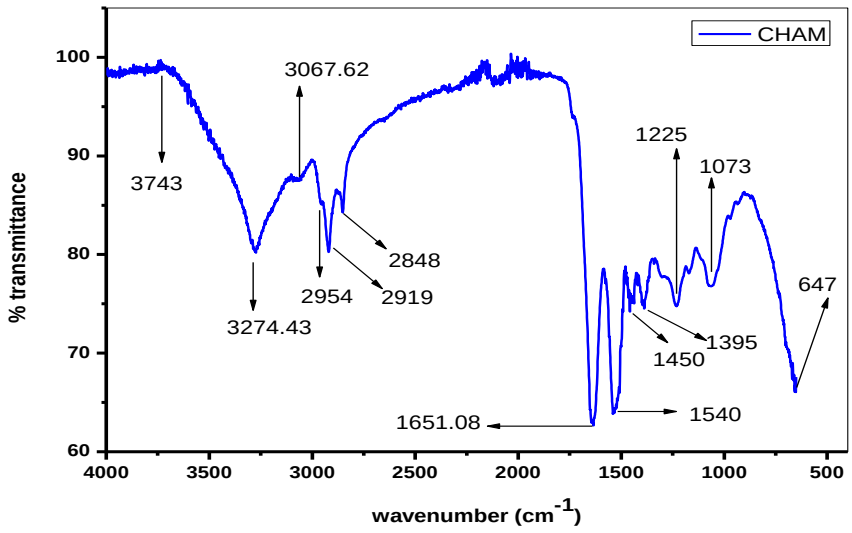


Figure 4. 11: FTIR spectra of cellularized human amniotic membrane (CHAM)

The FTIR spectra of cellularized human amniotic membrane (CHAM) having different characteristic absorption band at the frequency of 3743 cm-1, 3274 cm-1, 3067 cm-1, 2954 cm-1, 2919 cm-1, 2848 cm-1, 1651 cm-1, 1540 cm-1, 1450 cm-1, 1395 cm-1, 1225 cm-1, 1073 cm-1, and 647 cm-1 respectively.

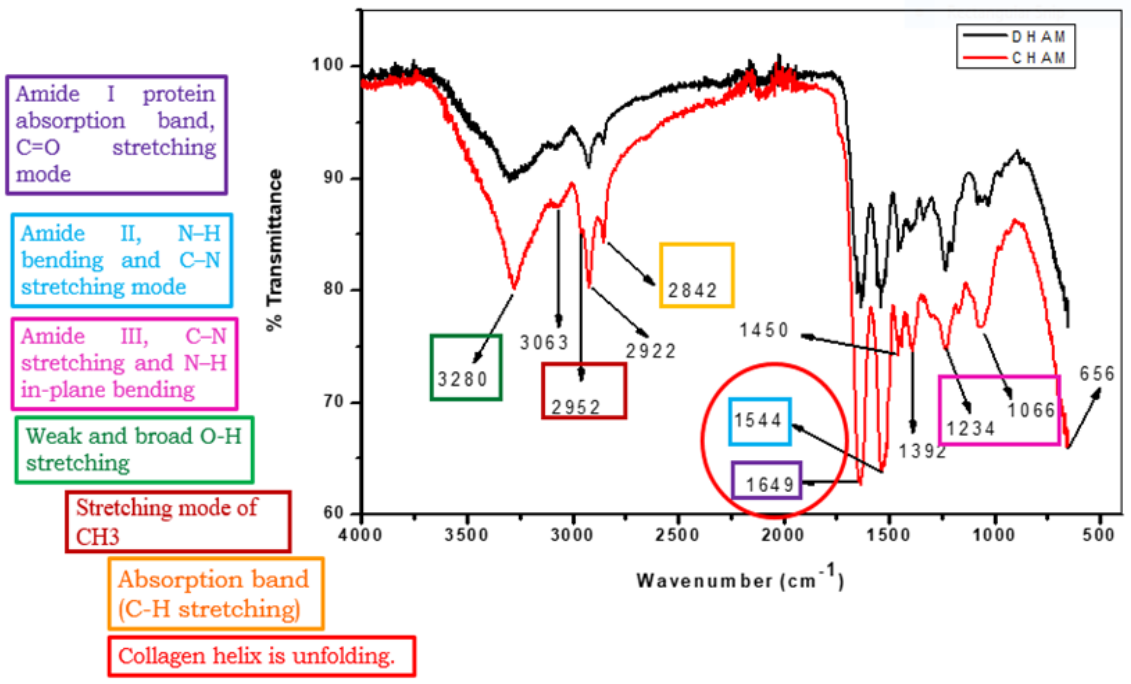


Figure 4. 12: FTIR spectra of both cellularized and decellularized human amniotic membrane.

The FTIR spectra of both cellularized and decellularized human amniotic membrane exhibit peak shifts, indicating the variations in the matrix's nature. The FTIR examination of the human amniotic membrane identified unique peaks associated with different functional groups present in the tissue.

The absorption band at 3280 cm-1 represents the weak and broad O-H stretching, while the peak at 2960 cm-1 is associated with the CH3 asymmetric stretching mode. Additional peaks reveal various absorption bands in the range of 2842 cm-1 (C-H stretching), 1649 cm-1 (Amide I protein absorption band, C=O stretching mode), 1544 cm-1 (Amide II, N-H bending mode, and C-N stretching mode), 1234 cm-1, and 1066 cm-1 (Amide III, C-N stretching, and N-H in-plane bending).This peak region appears notably

broader in cellularized human amniotic membrane, attributed to the presence of cellular components.

Table 4.2: A Comparison of intensities of all major peaks of Amniotic Membrane

Peak Wavenumber (cm-1)	Corresponding Peak Functional Group	Cellularized Amniotic Membrane (CHAM)	Decellularized Amniotic Membrane (DHAM)
1600–1640 cm-1	Amide I (Absorption band, C=O stretching mode)	1651 cm-1	1627 cm-1
1510–1560 cm-1	Amide II (N–H bending and C–N stretching mode)	1540 cm-1	1538 cm-1
1210–1300 cm-1and 1070–1080 cm-1	Amide III (C–N stretching and N–H in-plane bending)	1225 cm-1, 1073 cm-1	1240 cm-1, 1088 cm-1
1398, 640–650 cm-1	Amide IV (carboxylate ion and C=O planar deformation vibration)	1392 cm-1, 656 cm-1	1393 cm-1, 651 cm-1
3280 cm-1	Weak and broad O-H stretching	3274 cm-1	3300 cm-1
2842 cm-1	C-H stretching (Absorption band)	2848 cm-1	2920 cm-1
1550 to 1530 cm-1	Collagen helix is unfolding.	1544 cm-1	1538 cm-1
2960 cm-1	Stretching mode of CH3.	2954 cm-1	2957 cm-1

Specifically, the carboxylate ion and the C=O planar deformation vibration of amide IV are linked to the peaks in the 640–650 cm-1 region. A clear change in the collagen structure happens when the signal at 1550 shifts to 1530 cm-1. This shift shows that the collagen helix is unfolding. It also means that the gaps between the signals at 1650 and 1550 cm-1 are getting bigger. However, in the DHAM spectrum, which represents the processed membrane, there are no noticeable changes in the collagen signals. This indicates that collagen molecules in the treated membrane maintain their natural state (Grdadolnik & Maréchal, 2001), (*Download Infrared and Raman Characteristic Group Frequencies: Tables and Charts*, n.d.),.

4.5. Animal Model protocol

Table 4.3: Materials used for animal preparation

Materials			
1.	Rabbits	**2.**	Anesthesia (Ketamine)
3.	Amniotic membranes	**4.**	sterile gauzes
5.	Sodium hydroxide 10%	**6.**	Syringe
7.	Fluorescein Sodium Ophthalmic Strips	**8.**	moxifloxacin 0.5%
9.	micro sponge	**10.**	scissors
11.	Ciprofloxacin 0.35%	**12.**	needle holders
13.	Filter paper	**14.**	sterile gauzes
15.	Surgical kit	**16.**	Surgical gloves
17.	Surgical sutures 10-0 nylon	**18.**	Wood's lamp
19.	0.9% sterile saline solution	**20.**	Electric magnifying glass

4.5.1. Animal Selection

Four healthy adult female white New Zealand rabbits, with weights ranging from 2.0 to 2.5 kg, were obtained from the National Institutes of Health (NIH) to established animal model. The surgical procedures were conducted on the left eyes.

- **Treatment with Amniotic membrane plus Antibiotic**

Treatment of rabbit with decellularzied human placental derived amniotic membrane along with moxifloxacine antibiotic.

- **Standard or Control group**

  Treatment of rabbit with antibiotic moxifloxacine eye drops.

4.5.2. Recipient Rabbit Preparation

- Anesthetize the rabbits using appropriate anesthesia protocols to minimize pain and discomfort.
- Corneal defect induced experimentally by using chemical material Sodium Hydroxide (10 %) to destroy the corneal epithelial.
- A micro sponge was used to debride the weakly adhering epithelium from the base of an epithelial defect or stromal ulcer.
- After application of imbedded filter paper with sodium hydroxide 10 % on rabbit eye wait for fifth day before transplantation of amniotic membrane.

4.5.3. Amniotic Membrane Transplantation Procedure

- Anesthetize the rabbits using appropriate anesthesia protocols to minimize pain and discomfort.
- Rabbits were administered intramuscular ketamine hydrochloride at 1 mg/kg along with local anesthesia using atropine sulfate.
- The positioning of the rabbits involved placing their treated eyes at the forefront of the operating table, securing the mandible and forehead on respective supports.
- The cornea of the eyes undergoing inspection was observed by adjusting the position of an indicator light. Following the examination, the recorded images and videos were saved.
- Cleaning of the mandibular and forehead supports was carried out using alcohol.

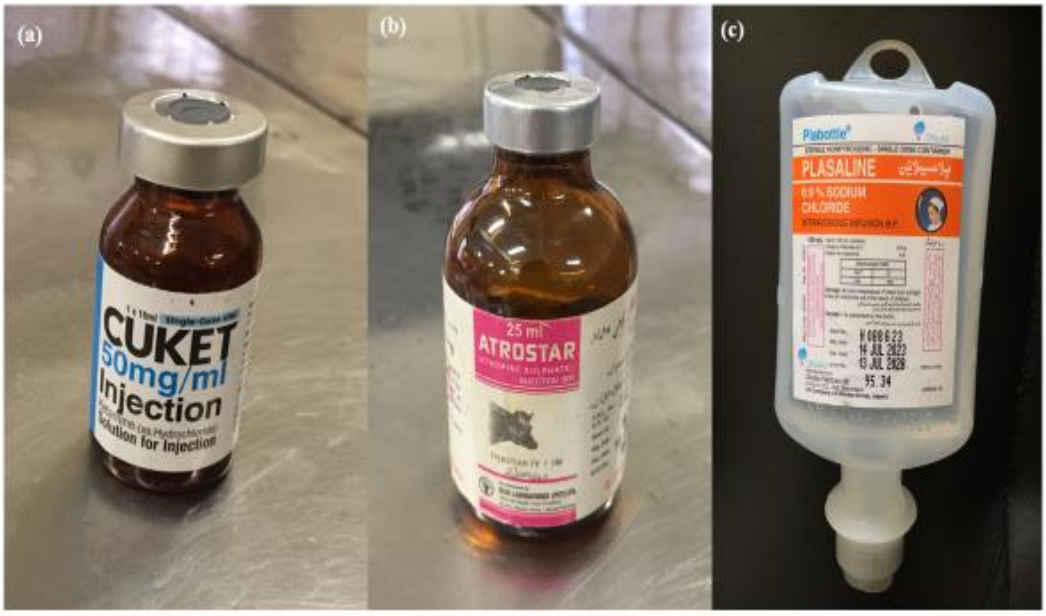


Figure 4. 13: Diagrammatic representation of anesthesia (a) anesthesia ketamine injection (b) local anesthesia atropine sulphate and (c) 0.9% normal saline

- The amniotic membrane was gently removed from nitrocellulose filter paper
- A circular cut was made on the amnion to ensure the appropriate size, and it was then placed on the lesion with the epithelial side facing up.
- The whole surface of the cornea and the surrounding conjunctiva were carefully covered with the amniotic membrane.
- After that, the amniotic membrane was subsequently secured to the perilimbal conjunctiva using interrupted 10 - 0 nylon sutures.
- This procedure was done by using electric magnifying glass.

4.5.4. Postoperative Care

The ocular surface is rinsed daily with normal saline. Moxifloxacine % eye drops applied four times a day. The amniotic membrane dissolved over the period of two weeks

Figure 4. 14: Diagrammatic representation of surgical instruments (a) ophthalmic surgical skit and (b) nylon 10-0 wegosutures.

4.5.5. Clinical observations

The evaluation of the surgical outcome was based on the following parameters.

- **Visual observation**

The surgical result was evaluated using subjective symptoms, specifically pain, on the 7th day after the operation, at the end of the second week (day 15), and at the conclusion of the month (day 30)

- **Measuring Scale**

Objective signs (hyperemia and size of the corneal ulcer) with the help of photo documentation.

4.5.6. Results

After applying a 10% sodium hydroxide solution to chemically induce a corneal defect, on the first day, the eye showed signs of redness and inflammation in the conjunctiva (hyperemia), accompanied by excessive tearing. The symptoms of pain gradually faded away by the end of the first week.

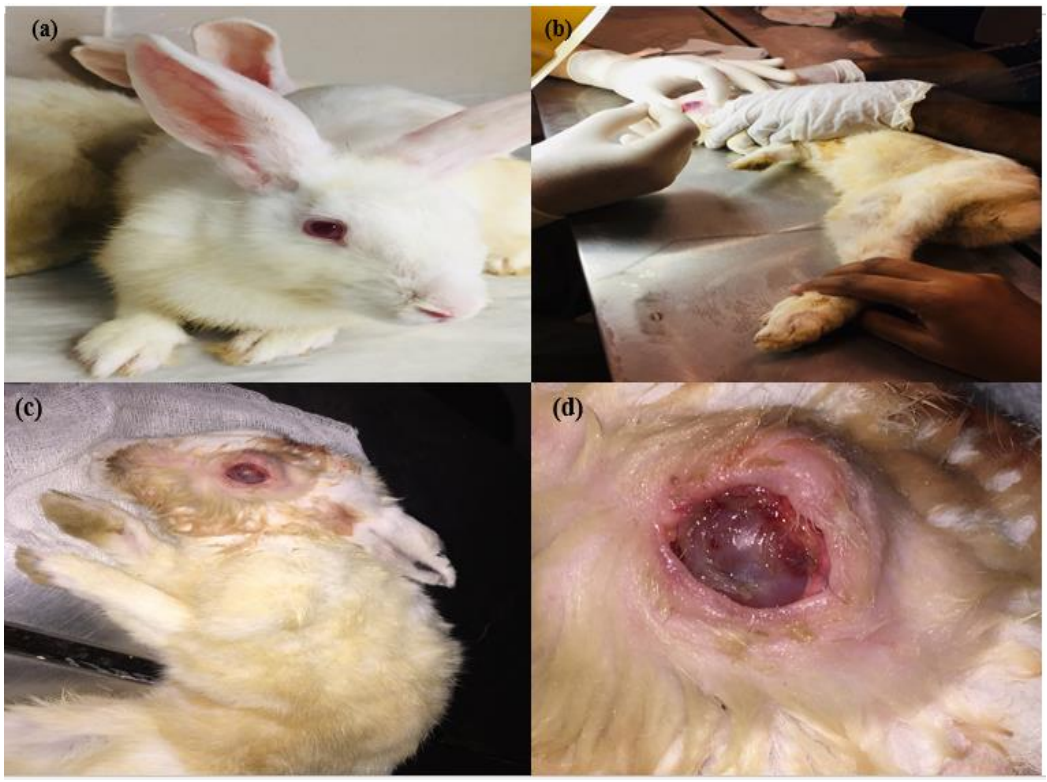


Figure 4. 15: Diagrammatic representation of AM transplantation (a) normal eye of rabbit before surgery and (b, c, d) transplantation of Amniotic Membrane graft with sutures.

It was also observed that both the congestion and pain completely disappeared after two weeks. This positive outcome was associated with a noticeable reduction in the size of the corneal defect due to the application of amniotic membrane transplant as it clearly seen in Figure 4.16.

We can clearly see that using the amniotic membrane results in a reduction in redness and the size of the ulcers over time, especially noticeable on the last day of the second week. The external eye images taken from electric magnifying glass revealed that the cornea looked normal and transparent after the amniotic membrane transplantation in rabbit eyes. There were no signs of significant swelling or immune rejection.

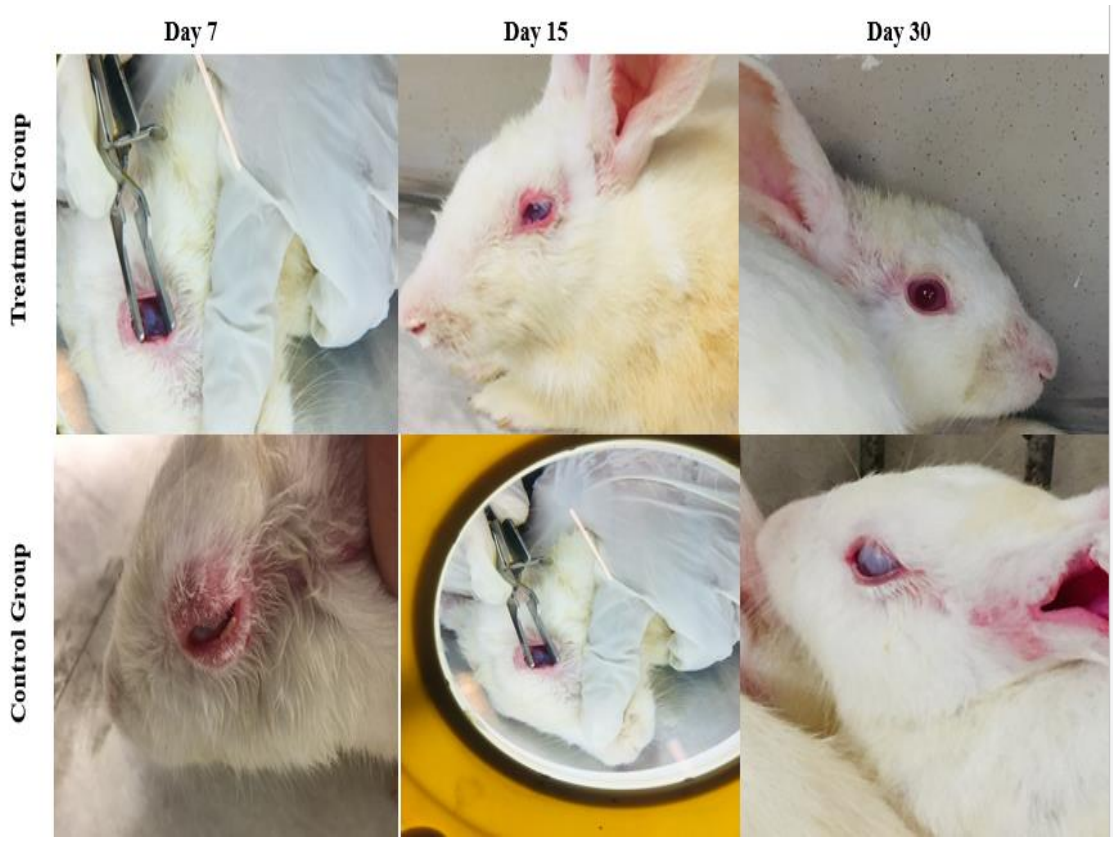


Figure 4. 16: The progression of cornea healing with and without a human placental-derived ECM sheet, imaged at days 7, 15, and 30.

4.5.7. Discussion

The goal of this study was to evaluate the application of extracellular matrix (ECM) sheets derived from human placenta as scaffolds in enhancing the recovery of ocular surface damage in rabbit corneas. In our investigation, we conducted a comparative analysis between amniotic membrane transplant (AMT) and a control group treated solely with moxifloxacin antibiotics for repairing corneal defects in a rabbit model.

The results demonstrated the effectiveness of amniotic membrane transplant in fostering the healing process of corneal defects. Our clinical findings indicate that applying the AM has a beneficial impact on the recovery of injured corneas. The use of AM proved to be essential in reducing notable subjective symptoms like pain, as well as clinical signs such as redness and the size of corneal ulcers.

The amniotic membrane was characterized by using SEM, FTIR, H & E staining and antimicrobial activity. SEM and H&E staining revealed the presence of epithelial cells in cellularized hAM, contrasting with the absence of epithelial cells in decellularized hAM. FTIR analysis showed a peak shift in processed hAM, indicating ECM variations without changes in collagen signals, this implied that collagen molecules within the treated membrane preserve their inherent or natural conformation. The p-value < 0.05 from the ANOVA analysis signifies a significant difference in antimicrobial activity among the three bacterial strains.

The amniotic membrane is essential for facilitating the migration of epithelial cells (S. C.G. Tseng et al., 1997) and facilitating effective wound healing through the epithelialization (Surgery et al., n.d.).

The intricate composition of Amnion-M makes it a valuable natural scaffold for various clinical application. Amniotic membrane scaffolds (AMSCs) have distinct characteristics that make them well-suited for a wide range of situations where tissue repair is needed (Walkden, 2020), (Dua et al., 2004), (Adinolfi et al., 1982).

The human amniotic membranes were used in individuals experiencing different conditions affecting the eye surface, such as defects in the corneal epithelium (Prabhasawat et al., 2001; Letko et al., 2001), ulcers (Lakimento et al., 2013), or perforations (Chen et al., 2006). When it comes to the healing of the corneal epithelium, success rates ranged from 19% to 91% (Nubile et al., 2011).

This study shows that amniotic membrane transplant significantly reduces clinical signs, particularly redness and pain, as well as the size of the corneal defect. This aligns

with the findings of Yana Manolova et al. in 2016, where the application of AM led to a notable decrease in symptoms across all categories.

The amniotic membrane produces numerous anti-inflammatory proteins, and studies have illustrated its capacity to diminish inflammation in the eyes. This is achieved by preventing the infiltration of white blood cells and limiting the activity of enzymes called proteases (Li 2003; Zhou et al. 2003; Zhen & Xie 2006).

Utilizing the amniotic membrane as tissue grafts carries a minimal risk of provoking an immune rejection response. This has led to the acknowledgment that these materials hold a special status known as "immune privilege." (Streilein, 1995), (Whitsett et al., n.d.).

CHAPTER 5: CONCLUSION AND FUTURE RECOMMENDATION

Our findings suggest that incorporating the human amniotic membrane as a scaffold in corneal transplantation proves effective in enhancing corneal healing, while concurrently reducing signs of inflammation and redness in a rabbit model. The findings of this study underscore the promising role of human placental-derived ECM sheets as scaffolds for cell growth in cornea transplantation, offering a potential alternative to conventional donor tissue transplantation.

The results of this research emphasize on the potential using of extracellular matrix sheets derived from human placenta for supporting cell growth in cornea transplantation, providing a possible substitute for the traditional transplantation of donor tissues.

The future use of amniotic membrane (AM) in cornea transplant procedures shows promise, with ongoing research aiming to establish its safety and efficacy. Clinical trials are crucial for refining processing techniques, determining optimal patient criteria, and ensuring consistent application across different surgical settings. Combining AM with innovative approaches like regenerative medicine or advanced biomaterials holds potential for improving outcomes in corneal transplantation. Long-term follow-up studies will be vital for assessing the durability of therapeutic effects and monitoring potential complications, contributing valuable data to refine best practices. Efforts to enhance cost-effectiveness, regulatory approval, and global accessibility are essential for the widespread integration of AM into routine cornea transplant procedures. Educating healthcare professionals and patients about the advantages and limitations of AM in corneal transplantation is key for successful adoption in clinical practice.

In conclusion, future recommendations for AM in cornea transplant involve a comprehensive approach focusing on research, standardization, combination therapies, long-term monitoring, cost-effectiveness, and global accessibility to advance both scientific understanding and practical implementation in ophthalmology.

REFERENCES

[1] (1866-1922), N. S.-M. R., & 1913, undefined. (n.d.). Use of the fetal membranes in skin grafting. *Search.Proquest.ComN SABKLLAMedical Record (1866-1922), 1913•search.Proquest.Com*. Retrieved November 14, 2023, from https://search.proquest.com/openview/6b9eab5a92d512b049940e7168bff38e/1?pq-origsite=gscholar&cbl=40146

[2] Adinolfi, M., Akle, C. A., McColl, I., Fensom, A. H., Tansley, L., Connolly, P., Hsi, B. L., Faulk, W. P., Travers, P., & Bodmer, W. F. (1982a). Expression of HLA antigens, β2-microglobulin and enzymes by human amniotic epithelial cells. *Nature 1982 295:5847*, *295*(5847), 325–327. https://doi.org/10.1038/295325a0

[3] Adinolfi, M., Akle, C. A., McColl, I., Fensom, A. H., Tansley, L., Connolly, P., Hsi, B. L., Faulk, W. P., Travers, P., & Bodmer, W. F. (1982b). Expression of HLA antigens, β2-microglobulin and enzymes by human amniotic epithelial cells. *Nature*, *295*(5847), 325–327. https://doi.org/10.1038/295325A0

[4] Akle, C. A., Welsh, K. I., Adinolfi, M., Leibowitz, S., & Mccoll, I. (1981). IMMUNOGENICITY OF HUMAN AMNIOTIC EPITHELIAL CELLS AFTER TRANSPLANTATION INTO VOLUNTEERS. *The Lancet*, *318*(8254), 1003–1005. https://doi.org/10.1016/S0140-6736(81)91212-5

[5] Allen, C. L., Clare, G., Stewart, E. A., Branch, M. J., McIntosh, O. D., Dadhwal, M., Dua, H. S., & Hopkinson, A. (2013). Augmented dried versus cryopreserved amniotic membrane as an ocular surface dressing. *PLoS ONE*, *8*(10). https://doi.org/10.1371/JOURNAL.PONE.0078441

[6] Association, M. S.-J. of the A. M., & 1913, undefined. (n.d.). The grafting of preserved amniotic membrane to burned and ulcerated surfaces, substituing skin grafts: a preliminary report. *Jamanetwork.Com*. Retrieved November 14, 2023, from https://jamanetwork.com/journals/jama/article-abstract/214644

[7] Benson-Martin, J., Zammaretti, P., … G. B.-E. journal of, & 2006, undefined. (n.d.). The Young's modulus of fetal preterm and term amniotic membranes. *Elsevier*. Retrieved November 15, 2023, from

https://www.sciencedirect.com/science/article/pii/S0301211506000121

[8] Blood, N. H. S., & Services, T. (n.d.). *Amniotic Membrane Frozen Small*.

[9] Bonci, P., Bonci, P., & Lia, A. (2005). Suspension made with amniotic membrane: Clinical trial. *European Journal of Ophthalmology*, *15*(4), 441–445. https://doi.org/10.1177/112067210501500403

[10] Boudreau, N., Sympson, C. J., Werb, Z., & Bissell, M. J. (1995). Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. *Science*, *267*(5199), 891–893. https://doi.org/10.1126/SCIENCE.7531366

[11] Bourne, G. L. (1960). The microscopic anatomy of the human amnion and chorion. *American Journal of Obstetrics and Gynecology*, *79*(6), 1070–1073. https://doi.org/10.1016/0002-9378(60)90512-3

[12] Bryant-Greenwood, G. D. (1998). The extracellular matrix of the human fetal membranes: Structure and function. *Placenta*, *19*(1), 1–11. https://doi.org/10.1016/S0143-4004(98)90092-3

[13] Chatterjee, A., Saha, D., Niemann, H., Gryshkov, O., Glasmacher, B., & Hofmann, N. (2017). Effects of cryopreservation on the epigenetic profile of cells. *Cryobiology*, *74*, 1–7. https://doi.org/10.1016/J.CRYOBIOL.2016.12.002

[14] Chatterjee, Anamika, Saha, D., Glasmacher, B., & Hofmann, N. (2016). Chilling without regrets. *EMBO Reports*, *17*(3), 292–295. https://doi.org/10.15252/EMBR.201642069

[15] Chen, B., Jones, R., Mi, S., Foster, J., Matter, S. A.-S., & 2012, undefined. (n.d.). The mechanical properties of amniotic membrane influence its effect as a biomaterial for ocular surface repair. *Pubs.Rsc.OrgB Chen, RR Jones, S Mi, J Foster, SG Alcock, IW Hamley, CJ ConnonSoft Matter, 2012•pubs.Rsc.Org*. https://doi.org/10.1039/C2SM26175H

[16] Cooke, M., Tan, E. K., Mandrycky, C., He, H., O'Connell, J., & Tseng, S. C. G. (2014). Comparison of cryopreserved amniotic membrane and umbilical cord tissue with dehydrated amniotic membrane/chorion tissue. *Journal of Wound Care*, *23*(10), 465–476. https://doi.org/10.12968/JOWC.2014.23.10.465

[17] Cooper, L. J., Kinoshita, S., German, M., Koizumi, N., Nakamura, T., & Fullwood, N. J. (2005). An investigation into the composition of amniotic membrane used for ocular

surface reconstruction. *Cornea*, *24*(6), 722–729. https://doi.org/10.1097/01.ICO.0000154237.49112.29

[18] Dadkhah Tehrani, F., Firouzeh, A., Shabani, I., & Shabani, A. (2021). A Review on Modifications of Amniotic Membrane for Biomedical Applications. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/FBIOE.2020.606982/FULL

[19] Danforth, D. N., & Hull, R. W. (1958). The microscopic anatomy of the fetal membranes with particular reference to the detailed structure of the amnion. *American Journal of Obstetrics and Gynecology*, *75*(3), 536–550. https://doi.org/10.1016/0002-9378(58)90610-0

[20] Díaz-Prado, S., Muiños-López, E., Hermida-Gómez, T., Cicione, C., Rendal-Vázquez, M. E., Fuentes-Boquete, I., de Toro, F. J., & Blanco, F. J. (2011). Human amniotic membrane as an alternative source of stem cells for regenerative medicine. *Differentiation*, *81*(3), 162–171. https://doi.org/10.1016/J.DIFF.2011.01.005

[21] Dua, H. S., Gomes, J. A. P., King, A. J., & Maharajan, V. S. (2004). The amniotic membrane in ophthalmology. *Survey of Ophthalmology*, *49*(1), 51–77. https://doi.org/10.1016/J.SURVOPHTHAL.2003.10.004

[22] Dulemba, J., & Mirzakhani, P. (2016). Evaluation of Dehydrated Human Amnion/Chorion Membrane as an Adhesion Barrier in Women Undergoing Robotic Laparoscopy. *Gynecology & Obstetrics*, *06*(10). https://doi.org/10.4172/2161-0932.1000405

[23] El-Danasouri, I., & Selman, H. (2005). Vitrification versus conventional cryopreservation technique. *Middle East Fertility Society Journal*, *10*(3), 205–206.

[24] Elkhenany, H., Derby, A. El, Elkodous, M. A., Salah, R. A., Lotfy, A., & Badri, N. El. (2022). *Applications of the amniotic membrane in tissue engineering and regeneration : the hundred - year challenge*. *0*, 1–19. https://doi.org/10.1186/s13287-021-02684-0

[25] Eslani, M., Baradaran-Rafii, A., … A. C.-A. journal of, & 2019, undefined. (n.d.). Amniotic membrane transplantation in acute severe ocular chemical injury: a randomized clinical trial. *Elsevier*. Retrieved November 14, 2023, from https://www.sciencedirect.com/science/article/pii/S0002939418306251

[26] Fairbairn, N. G., Randolph, M. A., & Redmond, R. W. (2014). The clinical applications of human amnion in plastic surgery. *Journal of Plastic, Reconstructive and*

Aesthetic Surgery, *67*(5), 662–675. https://doi.org/10.1016/J.BJPS.2014.01.031

[27] Favaron, P. O., Carvalho, R. C., Borghesi, J., Anunciação, A. R. A., & Miglino, M. A. (2015). The Amniotic Membrane: Development and Potential Applications – A Review. *Reproduction in Domestic Animals*, *50*(6), 881–892. https://doi.org/10.1111/RDA.12633

[28] Fetterolf, D., (Langhorne, N. I.-… care, Pa.), undefined, & 2014, undefined. (n.d.). An evaluation of healing metrics associated with commonly used advanced wound care products for the treatment of chronic diabetic foot ulcers. *Pubmed.Ncbi.Nlm.Nih.Gov*. Retrieved November 15, 2023, from https://pubmed.ncbi.nlm.nih.gov/25199368/

[29] Francisco, J. C., Correa Cunha, R., Cardoso, M. A., Baggio Simeoni, R., Mogharbel, B. F., Picharski, G. L., Silva Moreira Dziedzic, D., Guarita-Souza, L. C., & Carvalho, K. A. T. (2016). Decellularized Amniotic Membrane Scaffold as a Pericardial Substitute: An In Vivo Study. *Transplantation Proceedings*, *48*(8), 2845–2849. https://doi.org/10.1016/J.TRANSPROCEED.2016.07.026

[30] Friel, N. A., de Girolamo, L., Gomoll, A. H., Mowry, K. C., Vines, J. B., & Farr, J. (2017). Amniotic Fluid, Cells, and Membrane Application. *Operative Techniques in Sports Medicine*, *25*(1), 20–24. https://doi.org/10.1053/J.OTSM.2016.12.004

[31] Galask, R., gynecology, I. S.-A. journal of obstetrics and, & 1970, undefined. (n.d.). Antimicrobial factors in amniotic fluid. *Elsevier*. Retrieved November 15, 2023, from https://www.sciencedirect.com/science/article/pii/0002937870901262

[32] Gilbert, R. W. D., Vickaryous, M. K., & Viloria-Petit, A. M. (2016). Signalling by Transforming Growth Factor Beta Isoforms in Wound Healing and Tissue Regeneration. *Journal of Developmental Biology*, *4*(2). https://doi.org/10.3390/JDB4020021

[33] Grueterich, M., & Tseng, S. C. G. (2002). Human limbal progenitor cells expanded on intact amniotic membrane ex vivo. *Archives of Ophthalmology*, *120*(6), 783–790. https://doi.org/10.1001/ARCHOPHT.120.6.783

[34] Güler, R., Ercan, M. T., Ulutunçel, N., Devrim, H., & Uran, N. (1997). Measurement of blood flow by the 133Xe clearance technique to grafts of amnion used in vestibuloplasty. *The British Journal of Oral & Maxillofacial Surgery*, *35*(4), 280–283. https://doi.org/10.1016/S0266-4356(97)90048-6

[35] Guo, M., & Grinnell, F. (1989). Basement Membrane and Human Epidermal Differentiation In Vitro. *Journal of Investigative Dermatology*, *93*(3), 372–378.

https://doi.org/10.1111/1523-1747.EP12280275

[36] Gupta, A., Kedige, S. D., & Jain, K. (2015). Amnion and chorion membranes: Potential stem cell reservoir with wide applications in periodontics. *International Journal of Biomaterials, 2015*. https://doi.org/10.1155/2015/274082

[37] Gupta, S., IJSR, R. G.-I. J. S. R., & 2014, undefined. (n.d.). Placental tissues-from reproductive to regenerative biology. *Researchgate.NetS Gupta, R GuptaInt. J. Sci. Res. IJSR, 2014•researchgate.Net*. Retrieved November 14, 2023, from https://www.researchgate.net/profile/Swati-Gupta-38/publication/276077143_Placental_Tissues-From_Reproductive_to_Regenerative_Biology/links/57ea366908aeb34bc091f979/Placental-Tissues-From-Reproductive-to-Regenerative-Biology.pdf

[38] Hao, Y., Ma, D., Hwang, D., Kim, W., Cornea, F. Z.-, & 2000, undefined. (n.d.). Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Journals.Lww.ComY Hao, DHK Ma, DG Hwang, WS Kim, F ZhangCornea, 2000•journals.Lww.Com*. Retrieved November 15, 2023, from https://journals.lww.com/corneajrnl/fulltext/2000/05000/identification_of_antiangiogenic_and.18.aspx

[39] Heiligenhaus, A., Bauer, D., science, D. M.-… & visual, & 2001, undefined. (n.d.). Improvement of HSV-1 necrotizing keratitis with amniotic membrane transplantation. *Tvst.Arvojournals.OrgA Heiligenhaus, D Bauer, D Meller, KP Steuhl, SCG TsengInvestigative Ophthalmology & Visual Science, 2001•tvst.Arvojournals.Org*. Retrieved November 15, 2023, from https://tvst.arvojournals.org/article.aspx?articleid=2122851

[40] Hieber, A. D., Corcino, D., Motosue, J., Sandberg, L. B., Roos, P. J., Yu, S. Y., Csiszar, K., Kagan, H. M., Boyd, C. D., & Bryant-Greenwood, G. D. (1997). Detection of elastin in the human fetal membranes: Proposed molecular basis for elasticity. *Placenta, 18*(4), 301–312. https://doi.org/10.1016/S0143-4004(97)80065-3

[41] Hong, J. S., Romero, R., Kusanovic, J. P., Kim, J. S., Lee, J., Jin, M., El Azzamy, H., Lee, D. C., Topping, V., Ahn, S., Jacques, S., Qureshi, F., Chaiworapongsa, T., Hassan, S. S., Korzeniewski, S. J., Than, N. G., & Kim, C. J. (2013). "Trophoblast islands of the chorionic connective tissue" (TICCT): A novel placental histologic feature. *Placenta,*

34(4), 360–368. https://doi.org/10.1016/J.PLACENTA.2013.01.012

[42] Hopkinson, A., Shanmuganathan, V. A., Gray, T., Yeung, A. M., Lowe, J., James, D. K., & Dua, H. S. (2008). Optimization of amniotic membrane (AM) denuding for tissue engineering. *Tissue Engineering - Part C: Methods*, *14*(4), 371–381. https://doi.org/10.1089/TEN.TEC.2008.0315

[43] Hori, J., Wang, M., Kamiya, K., Takahashi, H., & Sakuragawa, N. (2006). Immunological Characteristics of Amniotic Epithelium. *Cornea*, *25*(Supplement 1), S53–S58. https://doi.org/10.1097/01.ICO.0000247214.31757.5C

[44] Immunology, J. G.-T. J. of, & 1962, undefined. (n.d.). A bactericidin for Bacillus subtilis in pregnancy. *Journals.Aai.OrgJP GusdonThe Journal of Immunology, 1962•journals.Aai.Org*. Retrieved November 15, 2023, from https://journals.aai.org/jimmunol/article-abstract/88/4/494/67558

[45] Inge, E., Talmi, Y., Sigler, L., Finkelstein, Y., Placenta, Y. Z.-, & 1991, undefined. (n.d.). Antibacterial properties of human amniotic membranes. *Elsevier*. Retrieved November 15, 2023, from https://www.sciencedirect.com/science/article/pii/014340049190010D

[46] J, J. D.-J. H. M., & 1910, undefined. (n.d.). Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. *Books.Google.ComJW DavisJohns Hopkins Med J, 1910•books.Google.Com*. Retrieved November 14, 2023, from https://books.google.com/books?hl=en&lr=&id=yqFXAAAAMAAJ&oi=fnd&pg=PA307&ots=EtC5xlqfjN&sig=0MtFZHJ9QOqBTybIIwkrei7UVFw

[47] Jae Chan Kim, & Tseng, S. C. G. (1995). Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea*, *14*(5), 473–484. https://doi.org/10.1097/00003226-199509000-00006

[48] Jang, T. H., Park, S. C., Yang, J. H., Kim, J. Y., Seok, J. H., Park, U. S., Choi, C. W., Lee, S. R., & Han, J. (2017). Cryopreservation and its clinical applications. *Integrative Medicine Research*, *6*(1), 12–18. https://doi.org/10.1016/J.IMR.2016.12.001

[49] Jirsova, K., & Jones, G. L. A. (2017). Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting—a review. *Cell and Tissue Banking*, *18*(2), 193–204. https://doi.org/10.1007/S10561-017-9618-5/METRICS

[50] Keene, D. R., Sakai, L. Y., Lunstrum, G. P., Morris, N. P., & Burgeson, R. E.

(1987). Type VII collagen forms an extended network of anchoring fibrils. *Journal of Cell Biology*, *104*(3), 611–621. https://doi.org/10.1083/JCB.104.3.611

[51] Kesting, M. R., Wolff, K.-D., Hohlweg-Majert, B., & Steinstraesser, L. (2008). The role of allogenic amniotic membrane in burn treatment. *Academic.Oup.ComMR Kesting, KD Wolff, B Hohlweg-Majert, L SteinstraesserJournal of Burn Care & Research, 2008•academic.Oup.Com*, *29*(6), 907–916. https://doi.org/10.1097/BCR.0b013e31818b9e40

[52] Kim, S. S., Romero, R., Kim, J. S., Abbas, A., Espinoza, J., Kusanovic, J. P., Hassan, S., Yoon, B. H., & Kim, C. J. (2008). Coexpression of myofibroblast and macrophage markers: novel evidence for an in vivo plasticity of chorioamniotic mesodermal cells of the human placenta. *Laboratory Investigation*, *88*(4), 365–374. https://doi.org/10.1038/LABINVEST.3700749

[53] Kjaergaard, N., Hein, M., Hyttel, L., … R. H.-E. J. of, & 2001, undefined. (n.d.). Antibacterial properties of human amnion and chorion in vitro. *Elsevier*. Retrieved November 15, 2023, from https://www.sciencedirect.com/science/article/pii/S0301211500003456

[54] Kjaergaard, N., Helmig, R., … H. S.-E. J. of, & 1999, undefined. (n.d.). Chorioamniotic membranes constitute a competent barrier to group b streptococcus in vitro. *Elsevier*. Retrieved November 15, 2023, from https://www.sciencedirect.com/science/article/pii/S0301211599000093

[55] Koizumi, N., Inatomi, T., Sotozono, C., Fullwood, N. J., Quantock, A. J., & Kinoshita, S. (2000). Growth factor mRNA and protein in preserved human amniotic membrane. *Current Eye Research*, *20*(3), 173–177. https://doi.org/10.1076/0271-3683(200003)2031-9FT173

[56] Koob, T. J., Lim, J. J., Massee, M., Zabek, N., & Denozière, G. (2014). Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *102*(6), 1353–1362. https://doi.org/10.1002/JBM.B.33141

[57] Kurpakus-Wheater, M. (2001). Laminin-5 is a component of preserved amniotic membrane. *Current Eye Research*, *22*(5), 353–357. https://doi.org/10.1076/CEYR.22.5.353.5494

[58] Leal-Marin, S., Kern, T., Hofmann, N., Pogozhykh, O., Framme, C., Börgel, M., Figueiredo, C., Glasmacher, B., & Gryshkov, O. (2021). Human Amniotic Membrane: A review on tissue engineering, application, and storage. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *109*(8), 1198–1215. https://doi.org/10.1002/jbm.b.34782

[59] Lee, P. H., Tsai, S. H., Kuo, L., Hwang, C. Y., Kuo, C. Y., Yang, V. C., & Chen, J. K. (2012). A prototype tissue engineered blood vessel using amniotic membrane as scaffold. *Acta Biomaterialia*, *8*(9), 3342–3348. https://doi.org/10.1016/J.ACTBIO.2012.05.012

[60] Lee, S. H., & Tseng, S. C. G. (1997). Amniotic membrane transplantation for persistent epithelial defects with ulceration. *American Journal of Ophthalmology*, *123*(3), 303–312. https://doi.org/10.1016/S0002-9394(14)70125-4

[61] Lei, J., Priddy, L. B., Lim, J. J., & Koob, T. J. (2017). Dehydrated Human Amnion/Chorion Membrane (dHACM) Allografts as a Therapy for Orthopedic Tissue Repair. *Techniques in Orthopaedics*, *32*(3), 149–157. https://doi.org/10.1097/BTO.0000000000000229

[62] Lei, J., Priddy, L. B., Lim, J. J., Massee, M., & Koob, T. J. (2017). Identification of Extracellular Matrix Components and Biological Factors in Micronized Dehydrated Human Amnion/Chorion Membrane. *Https://Home.Liebertpub.Com/Wound*, *6*(2), 43–53. https://doi.org/10.1089/WOUND.2016.0699

[63] Lim, R. (2017). Concise Review: Fetal Membranes in Regenerative Medicine: New Tricks from an Old Dog? *Stem Cells Translational Medicine*, *6*(9), 1767–1776. https://doi.org/10.1002/SCTM.16-0447

[64] Longo, L. D., & Reynolds, L. P. (2010). Some historical aspects of understanding placental development, structure and function. *The International Journal of Developmental Biology*, *54*(2–3), 237–255. https://doi.org/10.1387/IJDB.082774LL

[65] Malhotra, C., & Jain, A. K. (2014a). Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. *World Journal of Transplantation*, *4*(2), 111. https://doi.org/10.5500/WJT.V4.I2.111

[66] Malhotra, C., & Jain, A. K. (2014b). *World Journal of Transplantation © 2014*. *4*(2), 111–122. https://doi.org/10.5500/wjt.v4.i2.111

[67] Mamede, A. C., Carvalho, M. J., Abrantes, A. M., Laranjo, M., Maia, C. J., & Botelho, M. F. (2012). Amniotic membrane: From structure and functions to clinical applications. *Cell and Tissue Research*, *349*(2), 447–458. https://doi.org/10.1007/S00441-012-1424-6/METRICS

[68] Mamede, A., Carvalho, M., … A. A.-C. and tissue, & 2012, undefined. (2012). Amniotic membrane: from structure and functions to clinical applications. *SpringerAC Mamede, MJ Carvalho, AM Abrantes, M Laranjo, CJ Maia, MF BotelhoCell and Tissue Research, 2012•Springer*, *349*(2), 447–458. https://doi.org/10.1007/s00441-012-1424-6

[69] Marsit, N. M., Sidney, L. E., Britchford, E. R., McIntosh, O. D., Allen, C. L., Ashraf, W., Bayston, R., & Hopkinson, A. (2019). Validation and assessment of an antibiotic-based, aseptic decontamination manufacturing protocol for therapeutic, vacuum-dried human amniotic membrane. *Scientific Reports*, *9*(1). https://doi.org/10.1038/S41598-019-49314-7

[70] Matthews, R., annual, W. F.-… and gynecology, & 1982, undefined. (n.d.). A review of the role of amniotic membranes in surgical practice. *Pubmed.Ncbi.Nlm.Nih.Gov*. Retrieved November 15, 2023, from https://pubmed.ncbi.nlm.nih.gov/7050796/

[71] McLaren, J., Malak, T. M., & Bell, S. C. (1999). Structural characteristics of term human fetal membranes prior to labour: Identification of an area of altered morphology overlying the cervix. *Human Reproduction*, *14*(1), 237–241. https://doi.org/10.1093/HUMREP/14.1.237

[72] Medicine, W. O.-B. of the N. Y. A. of, & 1979, undefined. (n.d.). Notes on placentophagy. *Ncbi.Nlm.Nih.GovWB OberBulletin of the New York Academy of Medicine, 1979•ncbi.Nlm.Nih.Gov*. Retrieved November 14, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1807646/

[73] Meinert, M., Eriksen, G. V., Petersen, A. C., Helmig, R. B., Laurent, C., Uldbjerg, N., & Malmström, A. (2001). Proteoglycans and hyaluronan in human fetal membranes. *American Journal of Obstetrics and Gynecology*, *184*(4), 679–685. https://doi.org/10.1067/mob.2001.110294

[74] *MiMedx Provides Update Regarding Timing of Restatement*. (n.d.). Retrieved November 15, 2023, from https://www.prnewswire.com/news-releases/mimedx-provides-update-regarding-timing-of-restatement-300974332.html

[75] Mohan, R., Bajaj, A., & Gundappa, M. (2017). Human amnion membrane: Potential applications in oral and periodontal field. *Journal of International Society of Preventive and Community Dentistry*, *7*(1), 15–21. https://doi.org/10.4103/JISPCD.JISPCD_359_16

[76] MORTON, K. E., & DEWHURST, C. J. (1986). Human amnion in the treatment of vaginal malformations. *British Journal of Obstetrics and Gynaecology*, *93*(1), 50–54. https://doi.org/10.1111/J.1471-0528.1986.TB07813.X

[77] Nakamura, T., Yoshitani, M., Rigby, H., Fullwood, N. J., Ito, W., Inatomi, T., Sotozono, C., Nakamura, T., Shimizu, Y., & Kinoshita, S. (2004). Sterilized, Freeze-Dried Amniotic Membrane: A Useful Substrate for Ocular Surface Reconstruction. *Investigative Ophthalmology and Visual Science*, *45*(1), 93–99. https://doi.org/10.1167/IOVS.03-0752

[78] Niknejad, H., Peirovi, H., Jorjani, M., Ahmadiani, A., Ghanavi, J., & Seifalian, A. M. (2008a). Properties of the amniotic membrane for potential use in tissue engineering. *European Cells and Materials*, *15*, 88–99. https://doi.org/10.22203/ecm.v015a07

[79] Niknejad, H., Peirovi, H., Jorjani, M., Ahmadiani, A., Ghanavi, J., & Seifalian, A. M. (2008b). Properties of the amniotic membrane for potential use in tissue engineering. *European Cells and Materials*, *15*, 88–99. https://doi.org/10.22203/ECM.V015A07

[80] Pan, S. Y., Chan, M. K. S., Wong, M. B. F., Klokol, D., & Chernykh, V. (2017). Placental therapy: An insight to their biological and therapeutic properties. *Journal of Medicine and Therapeutics J Med Therap*, *1*(3), 1–6. https://doi.org/10.15761/JMT.1000118

[81] Park, W., visual, S. T.-I. ophthalmology &, & 2000, undefined. (n.d.). Modulation of acute inflammation and keratocyte death by suturing, blood, and amniotic membrane in PRK. *Arvojournals.OrgWC Park, SCG TsengInvestigative Ophthalmology & Visual Science, 2000•arvojournals.Org*. Retrieved November 15, 2023, from https://arvojournals.org/article.aspx?articleid=2123423

[82] Rao, T., Surgery, V. C.-A. of, & 1981, undefined. (n.d.). Use of dry human and bovine amnion as a biological dressing. *Jamanetwork.Com*. Retrieved November 15, 2023, from https://jamanetwork.com/journals/jamasurgery/article-abstract/587665

[83] Rao, T. V., & Chandrasekharam, V. (1981a). Use of dry human and bovine amnion as a biological dressing. *Archives of Surgery (Chicago, Ill. : 1960)*, *116*(7), 891–896.

https://doi.org/10.1001/ARCHSURG.1981.01380190029007

[84] Rao, T. V., & Chandrasekharam, V. (1981b). Use of Dry Human and Bovine Amnion as a Biological Dressing. *Archives of Surgery*, *116*(7), 891–896. https://doi.org/10.1001/ARCHSURG.1981.01380190029007

[85] Riau, A. K., Beuerman, R. W., Lim, L. S., & Mehta, J. S. (2010). Preservation, sterilization and de-epithelialization of human amniotic membrane for use in ocular surface reconstruction. *Biomaterials*, *31*(2), 216–225. https://doi.org/10.1016/J.BIOMATERIALS.2009.09.034

[86] Robson, M., surgery, T. K.-A. of, & 1973, undefined. (n.d.). The effect of human amniotic membranes on the bacteria population of infected rat burns. *Ncbi.Nlm.Nih.GovMC Robson, TJ KrizekAnnals of Surgery, 1973•ncbi.Nlm.Nih.Gov*. Retrieved November 15, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1355552/

[87] Rodríguez-Ares, M., … M. L.-A., & 2009, undefined. (2009). Effects of lyophilization on human amniotic membrane. *Wiley Online LibraryMT Rodríguez-Ares, MJ López-Valladares, R Touriño, B Vieites, F Gude, MT SilvaActa Ophthalmologica, 2009•Wiley Online Library*, *87*(4), 396–403. https://doi.org/10.1111/j.1755-3768.2008.01261.x

[88] Rötth De, A. (1940). Plastic repair of conjunctival defects with fetal membranes. *Archives of Ophthalmology*, *23*, 522–525.

[89] Rousselle, P., Keene, D. R., Ruggiero, F., Champliaud, M. F., Van Rest, M. Der, & Burgeson, R. E. (1997). Laminin 5 Binds the NC-1 Domain of Type VII Collagen. *Journal of Cell Biology*, *138*(3), 719–728. https://doi.org/10.1083/JCB.138.3.719

[90] Roy, A., & Griffiths, S. (2020). Intermediate layer contribution in placental membrane allografts. *Journal of Tissue Engineering and Regenerative Medicine*, *14*(8), 1126–1135. https://doi.org/10.1002/TERM.3086

[91] Roy, A., Mantay, M., Brannan, C., & Griffiths, S. (2022). *Placental Tissues as Biomaterials in Regenerative Medicine*. https://doi.org/10.1155/2022/6751456

[92] *Sadler: Langman's medical embryology - Google Scholar*. (n.d.). Retrieved November 14, 2023, from https://scholar.google.com/scholar_lookup?title=Langman's+medical+embryology&auth

or=T.+W.+Sadler&author=J.+Langman&

[93] Sangwan, V. S., & Basu, S. (2011). Antimicrobial properties of amniotic membrane. *British Journal of Ophthalmology*, *95*(1), 1–2. https://doi.org/10.1136/BJO.2010.184259

[94] Schmiedova, I., Dembickaja, A., Kiselakova, L., Nowakova, B., & Slama, P. (2021). Using of amniotic membrane derivatives for the treatment of chronic wounds. *Membranes*, *11*(12). https://doi.org/10.3390/membranes11120941

[95] Schrimpf, W. J. (1954). IX Repair of tympanic membrane perforations with human amniotic membrane: Report of fifty-three cases. *Annals of Otology, Rhinology ... Laryngology*, *63*(1), 101–115. https://doi.org/10.1177/000348945406300109

[96] Schuette, S. A., Brown, K. M., Cuthbert, D. A., Coyle, C. W., Wisner, K. L., Hoffman, M. C., Yang, A., Ciolino, J. D., Newmark, R. L., & Clark, C. T. (2017). Perspectives from Patients and Healthcare Providers on the Practice of Maternal Placentophagy. *Https://Home.Liebertpub.Com/Acm*, *23*(1), 60–67. https://doi.org/10.1089/ACM.2016.0147

[97] Sharma, R., Nappi, V., & Empeslidis, T. (2023). The developments in amniotic membrane transplantation in glaucoma and vitreoretinal procedures. *International Ophthalmology*, *43*(5), 1771–1783. https://doi.org/10.1007/s10792-022-02570-5

[98] Shimazaki, J., Shinozaki, N., of, K. T.-B. J., & 1998, undefined. (1998). Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. *Bjo.Bmj.ComJ Shimazaki, N Shinozaki, K TsubotaBritish Journal of Ophthalmology, 1998•bjo.Bmj.Com*, *82*(3), 235–240. https://doi.org/10.1136/bjo.82.3.235

[99] Smith, J., & Ockleford, C. D. (1994). Laser scanning confocal examination and comparison of nidogen (entactin) with laminin in term human amniochorion. *Placenta*, *15*(1), 95–106. https://doi.org/10.1016/S0143-4004(05)80240-1

[100] Snyder, D., Sullivan, N., Margolis, D., & Schoelles, K. (2020). *Skin substitutes for treating chronic wounds*. https://europepmc.org/article/nbk/nbk554220

[101] Solomon, A., Rosenblatt, M., Monroy, D., Ji, Z., Pflugfelder, S. C., & Tseng, S. C. G. (2001). Suppression of interleukin 1alpha and interleukin 1beta in human limbal epithelial cells cultured on the amniotic membrane stromal matrix. *The British Journal of*

Ophthalmology, *85*(4), 444–449. https://doi.org/10.1136/BJO.85.4.444

[102] Sripriya, R., & Kumar, R. (2016). Denudation of human amniotic membrane by a novel process and its characterisations for biomedical applications. *Progress in Biomaterials*, *5*(3–4), 161–172. https://doi.org/10.1007/S40204-016-0053-7/FIGURES/6

[103] Strauss, J. F. (2013). Extracellular Matrix Dynamics and Fetal Membrane Rupture. *Reproductive Sciences 2013 20:2*, *20*(2), 140–153. https://doi.org/10.1177/1933719111424454

[104] Streilein, J. W. (1995). Unraveling immune privilege. *Science*, *270*(5239), 1158–1159. https://doi.org/10.1126/SCIENCE.270.5239.1158

[105] Surgery, M. R.-, Obstetrics, G. &, & 1973, undefined. (n.d.). Membranes as a Temporaary Wound Dressing. *Cir.Nii.Ac.Jp*. Retrieved November 15, 2023, from https://cir.nii.ac.jp/crid/1572824499207768320

[106] Tighe, S., Mead, O. G., Lee, A., & Tseng, S. C. G. (2010). *Basic science review of birth tissue uses in ophthalmology*. https://doi.org/10.4103/tjo.tjo_4_20

[107] Togarrati, P. P., Dinglasan, N., Yee, E., Heitman, J. W., Jackman, R. P., Geisberg, M., Norris, P. J., Bárcena, A., & Muench, M. O. (2019). Potential of Membranes Surrounding the Fetus as Immunoprotective Cell-Carriers for Allogeneic Transplantations. *Transplantation Direct*, *5*(6). https://doi.org/10.1097/TXD.0000000000000901

[108] Tosi, G., … M. M.-J. of cellular, & 2005, undefined. (2005). Amniotic membrane transplantation in ocular surface disorders. *Wiley Online LibraryGM Tosi, M Massaro-Giordano, A Caporossi, P TotiJournal of Cellular Physiology, 2005•Wiley Online Library*, *202*(3), 849–851. https://doi.org/10.1002/jcp.20181

[109] Trelford, J. D., & Trelford-Sauder, M. (1979). The amnion in surgery, past and present. *American Journal of Obstetrics and Gynecology*, *134*(7), 833–845. https://doi.org/10.1016/0002-9378(79)90957-8

[110] Tseng, S. C.G., Prabhasawat, P., & Lee, S. H. (1997). Amniotic membrane transplantation for conjunctival surface reconstruction. *American Journal of Ophthalmology*, *124*(6), 765–774. https://doi.org/10.1016/S0002-9394(14)71693-9

[111] Tseng, Scheffer C.G., Espana, E. M., Kawakita, T., Di Pascuale, M. A., Li, W., He, H., Liu, T. S., Cho, T. H., Gao, Y. Y., Yeh, L. K., & Liu, C. Y. (2004). How does amniotic membrane work? *Ocular Surface*, *2*(3), 177–187. https://doi.org/10.1016/S1542-

0124(12)70059-9

[112] Uchide, N., Ohyama, K., Bessho, T., Takeichi, M., & Toyoda, H. (2012). Possible roles of proinflammatory and chemoattractive cytokines produced by human fetal membrane cells in the pathology of adverse pregnancy outcomes associated with influenza virus infection. *Mediators of Inflammation*, *2012*. https://doi.org/10.1155/2012/270670

[113] Ueta, M., Kweon, M. N., Sano, Y., Sotozono, C., Yamada, J., Koizumi, N., Kiyono, H., & Kinoshita, S. (2002). Immunosuppressive properties of human amniotic membrane for mixed lymphocyte reaction. *Clinical and Experimental Immunology*, *129*(3), 464–470. https://doi.org/10.1046/J.1365-2249.2002.01945.X

[114] Van Griensven, M., Hasirci, V. N., Wolbank, S., Parolini, O., Silini, A. R., Cargnoni, A., Magatti, M., & Pianta, S. (2015). *The long path of human placenta, and its derivatives, in regenerative medicine*. *3*. https://doi.org/10.3389/fbioe.2015.00162

[115] Verbruggen, S. W., Oyen, M. L., Phillips, A. T. M., & Nowlan, N. C. (2017). *Function and failure of the fetal membrane: Modelling the mechanics of the chorion and amnion*. https://doi.org/10.1371/journal.pone.0171588

[116] Versen-Höynck, F. von, Syring, C., … S. B.-C. and T., & 2004, undefined. (2004). The influence of different preservation and sterilisation steps on the histological properties of amnion allografts—light and scanning electron microscopic studies. *SpringerF von Versen-Höynck, C Syring, S Bachmann, DE MöllerCell and Tissue Banking, 2004•Springer*, *5*(1), 45–56. https://doi.org/10.1023/B:CATB.0000022276.47180.96

[117] Walkden, A. (2020a). Amniotic membrane transplantation in ophthalmology: An updated perspective. *Clinical Ophthalmology*, *14*, 2057–2072. https://doi.org/10.2147/OPTH.S208008

[118] Walkden, A. (2020b). Amniotic Membrane Transplantation in Ophthalmology: An Updated Perspective . *Clinical Ophthalmology*, *14*, 2057–2072. https://doi.org/10.2147/OPTH.S208008

[119] Wehmeyer, J., Natesan, S., C, R. C.-T. E. P., & 2015, undefined. (2015). Development of a sterile amniotic membrane tissue graft using supercritical carbon dioxide. *Liebertpub.ComJL Wehmeyer, S Natesan, RJ ChristyTissue Engineering Part C: Methods, 2015•liebertpub.Com*, *21*(7), 649–659. https://doi.org/10.1089/ten.tec.2014.0304

[120] Whitsett, C., Priest, J., … R. P.-A. J. of, & 1983, undefined. (n.d.). HLA typing of cultured amniotic fluid cells. *Academic.Oup.ComCF Whitsett, JH Priest, RE Priest, J MarionAmerican Journal of Clinical Pathology, 1983•academic.Oup.Com*. https://doi.org/10.1093/ajcp/79.2.186

[121] Wildman, D. E. (2011). Review: Toward an integrated evolutionary understanding of the mammalian placenta. *Placenta*, *32*(SUPPL. 2), S142–S145. https://doi.org/10.1016/J.PLACENTA.2011.01.005

[122] Wolkers, W. F., Tablin, F., & Crowe, J. H. (2002). From anhydrobiosis to freeze-drying of eukaryotic cells. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *131*(3), 535–543. https://doi.org/10.1016/S1095-6433(01)00505-0

[123] Woo, H.-M., Kim, M. S., Kweon, O.-K., Kim, D.-Y., Nam, T.-C., & Kim, J. H. (n.d.). Effects of amniotic membrane on epithelial wound healing and stromal remodelling after excimer laser keratectomy in rabbit cornea. *Bjo.Bmj.ComHM Woo, MS Kim, OK Kweon, DY Kim, TC Nam, JH KimBritish Journal of Ophthalmology, 2001•bjo.Bmj.Com*. https://doi.org/10.1136/bjo.85.3.345

[124] Wu, Z. Y., & Hui, G. Z. (2006). Materials for neuro-transplantation and the amnion. *Chinese Medical Journal*, *119*(16), 1323–1326. https://doi.org/10.1097/00029330-200608020-00001

[125] Yoshizawa, R. S. (2013). Review: Public perspectives on the utilization of human placentas in scientific research and medicine. *Placenta*, *34*(1), 9–13. https://doi.org/10.1016/J.PLACENTA.2012.10.014

[126] Zelen, C. M., Gould, L., Serena, T. E., Carter, M. J., Keller, J., Li, W. W., & Zelen, C. (2014). A prospective, randomised, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft. *Wiley Online LibraryCM Zelen, L Gould, TE Serena, MJ Carter, J Keller, WW LiInternational Wound Journal, 2015•Wiley Online Library*, *12*(6), 724–732. https://doi.org/10.1111/iwj.12395

[127] Gholipourmalekabadi, M., Sameni, M., Radenkovic, D., Mozafari, M., Mossahebi-Mohammadi, M., & Seifalian, A. (2016). Decellularized human amniotic membrane: How

viable is it as a delivery system for human adipose tissue-derived stromal cells? *Cell Proliferation*, *49*(1), 115–121. https://doi.org/10.1111/CPR.12240

[128] Mamede, A., & Botelho, M. (2015). *Amniotic membrane: origin, characterization and medical applications*. https://books.google.com/books?hl=en&lr=&id=X0kwCgAAQBAJ&oi=fnd&pg=PR5&ots=h_zAAOVokT&sig=M8YAiGhEUpiSsACFoqtPk7cUH2g

[129] Blood, N. H. S., & Services, T. (n.d.). *Amniotic Membrane Frozen Small*.

[130] Choi, J. S., Kim, J. D., Yoon, H. S., & Cho, Y. W. (2013). Full-thickness skin wound healing using human placenta-derived extracellular matrix containing bioactive molecules. *Tissue Engineering - Part A*, *19*(3–4), 329–339. https://doi.org/10.1089/ten.tea.2011.0738

[131] Elhassan, B. Y. S. (2019). *Understanding amniotic membrane grafts*. *25*(6).

[132] *Glaucoma filtration surgery using amniotic membrane transplantation - PubMed*. (n.d.). Retrieved November 13, 2023, from https://pubmed.ncbi.nlm.nih.gov/11431440/

[133] Klama-Baryła, A., Rojczyk, E., Kitala, D., Łabuś, W., Smętek, W., Wilemska-Kucharzewska, K., & Kucharzewski, M. (2020). Preparation of placental tissue transplants and their application in skin wound healing and chosen skin bullous diseases - Stevens-Johnson syndrome and toxic epidermal necrolysis treatment. *International Wound Journal*, *17*(2), 491–507. https://doi.org/10.1111/iwj.13305

[134] Leal-Marin, S., Kern, T., Hofmann, N., Pogozhykh, O., Framme, C., Börgel, M., Figueiredo, C., Glasmacher, B., & Gryshkov, O. (2021). Human Amniotic Membrane: A review on tissue engineering, application, and storage. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, *109*(8), 1198–1215. https://doi.org/10.1002/jbm.b.34782

[135] Malhotra, C., & Jain, A. K. (2014). Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. *World Journal of Transplantation*, *4*(2), 111. https://doi.org/10.5500/WJT.V4.I2.111

[136] Naeem, L. A. (2018). *Clinical Study To Evaluate the Effectiveness of Amniotic Membrane Transplantation on Corneal Epithelial Defect Clinical Study To Evaluate the Effectiveness of Amniotic Membrane Transplantation on Corneal*. *December*, 984–991. https://doi.org/10.22192/iajmr.2017.3.1.6

[137] Woong Lee, J., & Young Park Eun Ah Kim Il Han Yun, W. (2014). E-Mail Tissue Response to Implanted Ahmed Glaucoma Valve with Adjunctive Amniotic Membrane in Rabbit Eyes. *Ophthalmic Res*, *51*, 129–139. https://doi.org/10.1159/000357097

[138] *Blindness and vision impairment*. (n.d.). Retrieved December 8, 2023, from https://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment

[139] Blood, N. H. S., & Services, T. (n.d.). *Amniotic Membrane Frozen Small*.

[140] Bourne, R. R. A., Steinmetz, J. D., Flaxman, S., Briant, P. S., Taylor, H. R., Resnikoff, S., Casson, R. J., Abdoli, A., Abu-Gharbieh, E., Afshin, A., Ahmadieh, H., Akalu, Y., Alamneh, A. A., Alemayehu, W., Alfaar, A. S., Alipour, V., Anbesu, E. W., Androudi, S., Arabloo, J., … Vos, T. (2021). Trends in prevalence of blindness and distance and near vision impairment over 30 years: An analysis for the Global Burden of Disease Study. *The Lancet Global Health*, *9*(2), e130–e143. https://doi.org/10.1016/S2214-109X(20)30425-3

[141] Britton, B., Hervey, R., Kasten, K., Gregg, S., & McDonald, T. (1976). Intraocular irritation evaluation of benzalkonium chloride in rabbits. *Ophthalmic Surgery*, *7*(3), 46–55. https://doi.org/10.3928/1542-8877-19761001-08

[142] *Cataracts - Symptoms and causes - Mayo Clinic*. (n.d.). Retrieved December 8, 2023, from https://www.mayoclinic.org/diseases-conditions/cataracts/symptoms-causes/syc-20353790

[143] Chi, H. H., & Kelman, C. D. (1966). Effects of freezing on ocular tissues. I. Clinical and histologic study of corneal endothelium. *American Journal of Ophthalmology*, *61*(4), 630–641. https://doi.org/10.1016/0002-9394(66)91199-8

[144] DelMonte, D., Surgery, T. K.-J. of C. & R., & 2011, undefined. (n.d.). Anatomy and physiology of the cornea. *Elsevier*. Retrieved December 8, 2023, from https://www.sciencedirect.com/science/article/pii/S0886335010019243

[145] Dragnea, D. C., Dhubhghaill, S. N., Bourgonje, V. J. A., Dapena, I., Oellerich, S., Melles, G. R. J., & Lavy, I. (2020). In Vitro Evaluation of the Feasibility of Slit-Lamp Nd:YAG Laser Descemetorhexis. *Cornea*, *39*(2), 229. https://doi.org/10.1097/ICO.0000000000002109

[146] *Dry eye | AOA*. (n.d.). Retrieved December 8, 2023, from

https://www.aoa.org/healthy-eyes/eye-and-vision-conditions/dry-eye?sso=y

[147] Dua, H. S., Gomes, J. A. P., King, A. J., & Maharajan, V. S. (2004). The amniotic membrane in ophthalmology. *Survey of Ophthalmology*, *49*(1), 51–77. https://doi.org/10.1016/j.survophthal.2003.10.004

[148] Elhassan, B. Y. S. (2019). *Understanding amniotic membrane grafts*. *25*(6).

[149] *Eye Allergies | Causes, Symptoms & Treatment | ACAAI Public Website*. (n.d.). Retrieved December 8, 2023, from https://acaai.org/allergies/allergic-conditions/eye-allergy/

[150] *Eye Injury: Symptoms, Treatment, Causes*. (n.d.). Retrieved December 8, 2023, from https://my.clevelandclinic.org/health/diseases/16988-eye-injuries

[151] *Eyes: How They Work, Anatomy & Common Conditions*. (n.d.). Retrieved December 8, 2023, from https://my.clevelandclinic.org/health/body/21823-eyes

[152] *Eyes: Structure, function, and disease*. (n.d.). Retrieved December 8, 2023, from https://www.medicalnewstoday.com/articles/320608#anatomy-of-the-eye

[153] *Global Burden of Disease Study 2017*. (2017). file:///C:/Users/Harrison/AppData/Local/Mendeley Ltd./Mendeley Desktop/Downloaded/Unknown - 2017 - Global Burden of Disease Study 2017.pdf

[154] Grueterich, M., Ophthalmology, S. T.-A. of, & 2002, undefined. (n.d.). Human limbal progenitor cells expanded on intact amniotic membrane ex vivo. *Jamanetwork.Com*. Retrieved December 8, 2023, from https://jamanetwork.com/journals/jamaophthalmology/article-abstract/271046

[155] Han, S., Ang, H., Balehosur, D., … G. P.-M., & 2013, undefined. (n.d.). A mouse model of corneal endothelial decompensation using cryoinjury. *Ncbi.Nlm.Nih.GovSB Han, H Ang, D Balehosur, G Peh, SS Chaurasia, DTH Tan, JS MehtaMolecular Vision, 2013•ncbi.Nlm.Nih.Gov*. Retrieved December 8, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3675054/

[156] Hassan, B., Ahmed, R., Li, B., Noor, A., & ul Hassan, Z. (2019). A comprehensive study capturing vision loss burden in Pakistan (1990-2025): Findings from the Global Burden of Disease (GBD) 2017 study. *PLoS ONE*, *14*(5). https://doi.org/10.1371/JOURNAL.PONE.0216492

[157] Holden, B. A., Fricke, T. R., Wilson, D. A., Jong, M., Naidoo, K. S., Sankaridurg,

P., Wong, T. Y., Naduvilath, T. J., & Resnikoff, S. (2016). Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050. *Ophthalmology*, *123*(5), 1036–1042. https://doi.org/10.1016/J.OPHTHA.2016.01.006

[158] Horn, D. Van, Sendele, D., … S. S.-… ophthalmology & visual, & 1977, undefined. (n.d.). Regenerative capacity of the corneal endothelium in rabbit and cat. *CiteseerDL Van Horn, DD Sendele, S Seideman, PJ BucoInvestigative Ophthalmology & Visual Science, 1977•Citeseer*. Retrieved December 8, 2023, from https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=52101bb49cc72225c807e3f57ffb7c436114fa03

[159] Huang, P., … L. N.-… ophthalmology & visual, & 1989, undefined. (n.d.). The morphology and function of healing cat corneal endothelium. *Iovs.Arvojournals.OrgPT Huang, LR Nelson, WM BourneInvestigative Ophthalmology & Visual Science, 1989•iovs.Arvojournals.Org*. Retrieved December 8, 2023, from https://iovs.arvojournals.org/article.aspx?articleid=2178438

[160] Ichijima, H., Petroll, W. M., Jester, J. V., Barry, P. A., Andrews, P. M., Dai, M., & Cavanagh, H. D. (1993). In vivo confocal microscopic studies of endothelial wound healing in rabbit cornea. *Cornea*, *12*(5), 369–378. https://doi.org/10.1097/00003226-199309000-00001

[161] Ivanov, I. V, Mappes, T., Schaupp, | Patrick, Lappe, | Christian, & Wahl, | Siegfried. (2018). Ultraviolet radiation oxidative stress affects eye health. *Wiley Online LibraryIV Ivanov, T Mappes, P Schaupp, C Lappe, S WahlJournal of Biophotonics, 2018•Wiley Online Library*, *11*(7). https://doi.org/10.1002/jbio.201700377

[162] James, S., Abate, D., Abate, K., Abay, S., Lancet, C. A.-T., & 2018, undefined. (n.d.). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017. *Thelancet.ComSL James, D Abate, KH Abate, SM Abay, C Abbafati, N Abbasi, H Abbastabar, F Abd-AllahThe Lancet, 2018•thelancet.Com*. Retrieved December 8, 2023, from https://www.thelancet.com/article/S0140-6736(18)32279-7/abstract

[163] Jirsova, K., & Jones, G. L. A. (2017). Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review. *Cell and Tissue Banking*, *18*(2), 193–204. https://doi.org/10.1007/S10561-017-9618-5

[164] Jurkunas, U. V., Bitar, M. S., Funaki, T., & Azizi, B. (2010). Evidence of oxidative stress in the pathogenesis of fuchs endothelial corneal dystrophy. *American Journal of Pathology*, *177*(5), 2278–2289. https://doi.org/10.2353/AJPATH.2010.100279

[165] *Keratitis - Symptoms and causes - Mayo Clinic*. (n.d.). Retrieved December 8, 2023, from https://www.mayoclinic.org/diseases-conditions/keratitis/symptoms-causes/syc-20374110

[166] Khodadoust, A., & K. G.-I. O., & 1976, undefined. (n.d.). Physiological function of regenerating endothelium. *Iovs.Arvojournals.OrgAA Khodadoust, K GreenInvestigative Ophthalmology & Visual Science, 1976•iovs.Arvojournals.Org*. Retrieved December 8, 2023, from https://iovs.arvojournals.org/article.aspx?articleid=2158543

[167] Landshman, N., Solomon, A., & Belkin, M. (1989). Cell Division in the Healing of the Corneal Endothelium of Cats. *Archives of Ophthalmology*, *107*(12), 1804–1808. https://doi.org/10.1001/ARCHOPHT.1989.01070020886032

[168] Liu, C., Miyajima, T., Melangath, G., Miyai, T., Vasanth, S., Deshpande, N., Kumar, V., Tone, S. O., Gupta, R., Zhu, S., Vojnovic, D., Chen, Y., Rogan, E. G., Mondal, B., Zahid, M., & Jurkunas, U. V. (2020). Ultraviolet A light induces DNA damage and estrogen-DNA adducts in Fuchs endothelial corneal dystrophy causing females to be more affected. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(1), 573–583. https://doi.org/10.1073/PNAS.1912546116

[169] Malhotra, C., & Jain, A. K. (2014). Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. *World Journal of Transplantation*, *4*(2), 111. https://doi.org/10.5500/WJT.V4.I2.111

[170] Maumenee, A. E., & Kornblueth, W. (1948). Regeneration of the corneal stromal cells. I. Technique for destruction of corneal corpuscles by application of solidified (frozen) carbon dioxide. *American Journal of Ophthalmology*, *31*(6), 699–702. https://doi.org/10.1016/S0002-9394(48)91915-1

[171] Maurice, D., & Perlman, M. (1977). *Permanent destruction of the corneal endothelium in rabbits.* https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL7850098439

[172] Meller, D., Pauklin, M., Thomasen, H., Westekemper, H., & Steuhl, K.-P. (2011). Amniotic membrane transplantation in the human eye. *Deutsches Arzteblatt International*,

108(14). https://doi.org/10.3238/ARZTEBL.2011.0243

[173] Mills, N. L., & Donn, A. (1960). Incorporation of Tritium-Labeled Thymidine by Rabbit Corneal Endothelium. *Archives of Ophthalmology*, *64*(3), 443–446. https://doi.org/10.1001/ARCHOPHT.1960.01840010445019

[174] Okumura, N., Koizumi, N., Ueno, M., Sakamoto, Y., Takahashi, H., Hirata, K., Torii, R., Hamuro, J., & Kinoshita, S. (2011). Enhancement of corneal endothelium wound healing by Rho-associated kinase (ROCK) inhibitor eye drops. *British Journal of Ophthalmology*, *95*(7), 1006–1009. https://doi.org/10.1136/BJO.2010.194571

[175] Park, Sangwan, Leonard, B. C., Raghunathan, V. K., Kim, S., Li, J. Y., Mannis, M. J., Murphy, C. J., & Thomasy, S. M. (2021). Animal models of corneal endothelial dysfunction to facilitate development of novel therapies. *Annals of Translational Medicine*, *9*(15), 1271–1271. https://doi.org/10.21037/ATM-20-4389

[176] Park, SJ, Ahn, S., Woo, S., ophthalmology, K. P.-J., & 2015, undefined. (2015). Extent of exacerbation of chronic health conditions by visual impairment in terms of health-related quality of life. *Jamanetwork.Com*, *133*(11), 1267–1275. https://doi.org/10.1001/jamaophthalmol.2015.3055

[177] Park, Y., Shin, J. A., Yang, S. W., Yim, H. W., Kim, H. S., & Park, Y. H. (2015). The relationship between visual impairment and health-related quality of life in Korean adults: The Korea national health and nutrition examination survey (2008-2012). *PLoS ONE*, *10*(7). https://doi.org/10.1371/JOURNAL.PONE.0132779

[178] Pascolini, D., & Mariotti, S. P. (2012). Global estimates of visual impairment: 2010. *The British Journal of Ophthalmology*, *96*(5), 614–618. https://doi.org/10.1136/BJOPHTHALMOL-2011-300539

[179] Pradeep, T., Mehra, D., & Le, P. H. (2023). Histology, Eye. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK544343/

[180] *Presbyopia | AOA*. (n.d.). Retrieved December 8, 2023, from https://www.aoa.org/healthy-eyes/eye-and-vision-conditions/presbyopia?sso=y

[181] Quigley, H., & Broman, A. T. (2006). The number of people with glaucoma worldwide in 2010 and 2020. *The British Journal of Ophthalmology*, *90*(3), 262–267. https://doi.org/10.1136/BJO.2005.081224

[182] *Sclera: Definition, Anatomy & Function*. (n.d.). Retrieved December 8, 2023, from

https://my.clevelandclinic.org/health/body/22088-sclera

[183] Segarra, S., Leiva, M., Costa, D., Coyo, N., Sabés-Alsina, M., Ríos, J., & Peña, T. (2018). A dose-escalation ex vivo study on the effects of intracameral benzalkonium chloride in,. *BMC Veterinary Research*, *14*(1). https://doi.org/10.1186/S12917-018-1349-8

[184] Seitz, B., … M. R.-A. of, & 2006, undefined. (n.d.). Histopathology and ultrastructure of human corneas after amniotic membrane transplantation. *Jamanetwork.Com*. Retrieved December 8, 2023, from https://jamanetwork.com/journals/jamaophthalmology/article-abstract/418637

[185] Seitz, B., & Langenbucher, A. (2000). Lasers in ophthalmology. *Lancet*, *356*(9248). https://doi.org/10.1016/S0140-6736(00)92012-9

[186] Sharma, R., Nappi, V., & Empeslidis, T. (2023). The developments in amniotic membrane transplantation in glaucoma and vitreoretinal procedures. *International Ophthalmology*, *43*(5), 1771–1783. https://doi.org/10.1007/s10792-022-02570-5

[187] Solomon, A., Solberg, Y., Belkin, M., & Landshman, N. (1997). Effect of corticosteroids on healing of the corneal endothelium in cats. *Graefe's Archive for Clinical and Experimental Ophthalmology*, *235*(5), 325–329. https://doi.org/10.1007/BF01739643

[188] Sridhar, M. S. (2018). Anatomy of cornea and ocular surface. *Indian Journal of Ophthalmology*, *66*(2), 190–194. https://doi.org/10.4103/IJO.IJO_646_17

[189] Tuft, S., Williams, K., … D. C. & visual, & 1986, undefined. (n.d.). Endothelial repair in the rat cornea. *Iovs.Arvojournals.OrgSJ Tuft, KA Williams, DJ CosterInvestigative Ophthalmology & Visual Science, 1986•iovs.Arvojournals.Org*. Retrieved December 8, 2023, from https://iovs.arvojournals.org/article.aspx?articleid=2177572

[190] Van Horn, D. L., & Hyndiuk, R. A. (1975). Endothelial wound repair in primate cornea. *Experimental Eye Research*, *21*(2), 113–124. https://doi.org/10.1016/0014-4835(75)90076-7

[191] Walkden, A. (2020). Amniotic Membrane Transplantation in Ophthalmology: An Updated Perspective . *Clinical Ophthalmology*, *14*, 2057–2072. https://doi.org/10.2147/OPTH.S208008

[192] *What Are Corneal Dystrophies? - American Academy of Ophthalmology*. (n.d.).

Retrieved December 8, 2023, from https://www.aao.org/eye-health/diseases/corneal-dystrophies

[193] *What Is a Corneal Ulcer (Keratitis)? - American Academy of Ophthalmology*. (n.d.). Retrieved December 8, 2023, from https://www.aao.org/eye-health/diseases/corneal-ulcer

[194] *What Is Glaucoma? Symptoms, Causes, Diagnosis, Treatment - American Academy of Ophthalmology*. (n.d.). Retrieved December 8, 2023, from https://www.aao.org/eye-health/diseases/what-is-glaucoma

[195] *What Is Keratoconus? - American Academy of Ophthalmology*. (n.d.). Retrieved December 8, 2023, from https://www.aao.org/eye-health/diseases/what-is-keratoconus

[196] *What Is The Cornea & How To Treat Corneal Issues*. (n.d.). Retrieved December 8, 2023, from https://wilmingtoneye.com/specialties/cornea/structure-of-the-cornea/

[197] Wong, W. L., Su, X., Li, X., Cheung, C. M. G., Klein, R., Cheng, C. Y., & Wong, T. Y. (2014). Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet. Global Health*, *2*(2). https://doi.org/10.1016/S2214-109X(13)70145-1

[198] Yau, J. W. Y., Rogers, S. L., Kawasaki, R., Lamoureux, E. L., Kowalski, J. W., Bek, T., Chen, S. J., Dekker, J. M., Fletcher, A., Grauslund, J., Haffner, S., Hamman, R. F., Ikram, M. K., Kayama, T., Klein, B. E. K., Klein, R., Krishnaiah, S., Mayurasakorn, K., O'Hare, J. P., … Wong, T. Y. (2012). Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*, *35*(3), 556–564. https://doi.org/10.2337/DC11-1909

[199] Zhang, W., Hu, Y., Lu, L., Liu, Y., Yang, X., Sun, H., Ruan, J., Chen, J., Yao, Q., Yan, C., Gu, P., Fu, Y., Shao, C., & Fan, X. (2017). Rabbit Model of Corneal Endothelial Injury Established Using the Nd:YAG Laser. *Cornea*, *36*(10), 1274–1281. https://doi.org/10.1097/ICO.0000000000001324

[200] Al Shehadat, S., Gorduysus, M. O., Hamid, S. S. A., Abdullah, N. A., Samsudin, A. R., & Ahmad, A. (2018). Optimization of scanning electron microscope technique for amniotic membrane investigation: A preliminary study. *European Journal of Dentistry*, *12*(4), 574–578. https://doi.org/10.4103/EJD.EJD_401_17/ID/JR_20/BIB

[201] *Download Infrared and Raman Characteristic Group Frequencies: Tables and Charts*. (n.d.). Retrieved November 12, 2023, from https://infinity.wecabrio.com/470093072-infrared-and-raman-characteristic-group-frequencie.pdf

[202] Ganzoury, M. A., Allam, N. K., Nicolet, T., & All, C. (2015). Introduction to Fourier Transform Infrared Spectrometry. *Renewable and Sustainable Energy Reviews*, *50*, 1–8. https://doi.org/10.1016/j.rser.2015.05.073

[203] Grdadolnik, J., & Maréchal, Y. (2001). Bovine serum albumin observed by infrared spectrometry. I. Methodology, structural investigation, and water uptake. *Biopolymers - Biospectroscopy Section*, *62*(1), 40–53. https://doi.org/10.1002/1097-0282(2001)62:1<40::AID-BIP60>3.0.CO;2-C

[204] Sripriya, R., & Kumar, R. (2016). Denudation of human amniotic membrane by a novel process and its characterisations for biomedical applications. *Progress in Biomaterials*, *5*(3–4), 161–172. https://doi.org/10.1007/s40204-016-0053-7

[205] Li XD (2003): Treatment of severe keratoconjunctival chemical burns with pre-served amniotic membrane transplantation combined with limbal transplantation. Chin J Ocular Trauma Occupational Eye Dis 25: 660–661 (in Chinese).

[206] Zhen S & Xie HP (2006): Progression in application of amniotic membrane transplantation in ophthalmology. Rec Adv Ophthalmol 26: 71–73 (in Chinese).

[207] Zhou HJ, Lin SP, He GH, Lu B, Wang J, Mao W & Zhou YS (2003): A clinical observation of full-thickness lamellar keratoplasty with stem cells and cryopreserved amniotic membrane transplantation for eye chemical burn. Chin J Prac Ophthalmol 21: 739–741 (in Chinese).

[208] Prabhasawat, P., Tesavibul, S and Komosuradej, W. 2001. Single and multilayer amniotic membrane transplantation for persistent corneal epithelial defect with and without stromal thinning and perforation. Brazilian Journal of Ophthalmology, 85: 1455 – 1463.

[209] Letko, E., Stechschulte, S and Kenyopn, K. R. 2001. Amniotic membrane grafting for corneal epithelial stromal ulcers. Archives of Ophthalmology, 119: 659– 663.

[210] Lakimento, S. A., Buznyk, O. I and Rymgayllo Jankowska, B. 2013. Amniotic membrane transplantation in treatment of persistent corneal ulceration after severe chemical and thermal eye injuries. European Journal of Ophthalmology, 23: 496 – 503.

[211] Chen, H. C., Tan, H. Y., Hsiao, C. H., Huang, S., Lin, K. K and Ma, D. 2006. Amniotic membrane transplantation for persistent corneal ulcers and perforations in acute fungal keratitis. Cornea, 25: 564 – 572.

[212] , M., Dua, H. S and Lanzini, M. 2011. In vivo analysis of stromal integration of multilayer amniotic membrane transplantation in corneal ulcers. American Journal of Ophthalmology, 151: 809 – 822.

[213] Yana Manolova, Cvetan Popov and Christina Grupcheva. 2016. Amniotic membrane transplantation - Excellent biological scaffold for anterior ocular surface reconstruction, Department of Ophthalmology and Visual Science, Medical University of Varna Center for Translational Medicine and Cell Therapy - Varna, Bulgaria. Scripta Scientifica Medica, 48 (3): 72 - 79.