



Uses of amniotic membrane in ophthalmic practice

Eassy

***Submitted For Partial Fulfillment of Master Degree in
Ophthalmology***

By

Marwa Salah Mohamed Ibrahim

M.B.B.CH

Supervised by

Prof. Dr. Ahmed Ibrahim Abo El Naga

Professor of Ophthalmology

Faculty of Medicine - Ain Shams University

Prof. Dr. Ahmed Atef Zaki

Professor of Ophthalmology

Research institute of ophthalmology

Dr. Tarek Mohamed Abd El Aziz

Lecture of Ophthalmology

Faculty of Medicine - Ain Shams University

Ain Shams University

Cairo, Egypt

2015

Acknowledgements

*First of all, I want to **THANK GOD** for supporting me and guiding me throughout my life.*

*I would like to express my profound gratitude to Professor **Doctor/ Ahmad Ibrahim Abo El Naga**, Professor of Ophthalmology, Ain Shams University, for his most valuable advises and support all through the whole work and for dedicating much of his precious time to accomplish this work,*

*I'm also grateful for **Prof. Dr. Ahmad Atef Zaki**, Professor of Ophthalmology, Research Institute Of Ophthalmology, for his valuable support, supervision and guidance throughout this work,*

*I am also grateful to lecturer **Doctor/ Tarek Mohammad Abdel Aziz**, Lecturer of Ophthalmology, Ain Shams University, for his considerable help, and knowledge he offered me throughout this work*



استخدامات الغشاء الأمنيوسى فى طب العيون

رسالة

توطئة للحصول على درجة الماجستير في طب وجراحة العيون

مقدمة من

مروة صلاح محمد إبراهيم
بكالوريوس الطب والجراحة
كلية الطب جامعة ٦ أكتوبر

تحت إشراف

الأستاذ الدكتور / احمد إبراهيم ابوالنجا
استاذ طب وجراحة العيون
كلية الطب - جامعة عين شمس

الأستاذ الدكتور / احمد عاطف زكى
استاذ طب وجراحة العيون
معهد بحوث امراض العيون

الدكتور / طارق محمد عبد العزيز
مدرس طب وجراحة العيون
كلية الطب - جامعة عين شمس

جامعة عين شمس
القاهرة - مصر

٢٠١٥

LIST OF THE CONTENTS

List of Figures	<u>I</u>
List of Abbreviations.....	<u>IV</u>
Chapter 1: Anatomy of amniotic membrane	<u>1</u>
Chapter 2: pathophysiology of amniotic membrane	<u>5</u>
Chapter 3: Properties of amniotic membrane.....	<u>8</u>
Chapter 4: Preparation and preservation of amniotic membrane.....	<u>13</u>
Chapter 5 : Uses of amniotic membrane	<u>17</u>
Chapter 6 : Surgical techniques.....	<u>49</u>
Summary	<u>66</u>
References	<u>68</u>
Arabic Summary	-

LIST OF FIGURES

Fig. No.	Title	Page No.
(1.1)	Embryonic membranes and placenta	2
(1.2)	Schematic presentation of the structure of the fetal membrane at term	3
(5.1)	amniotic membrane transplantation in a patient with neurotrophic Corneal ulcer	20
(5.2)	Combined technique: AM graft covered by AM patch in neurotrophic ulcer	20
(5.3)	AM as a graft in bullous keratopathy	21
(5.4)	Pre- and postoperative slit lamp photographs of a patient with band keratopathy who underwent combined EDTA chelation, PTK, and permanent AMT	23
(5.5)	Corneal perforation in different stages	26
(5.6)	AM used to cover bare sclera after excision of primary pterygium	29
(5.7)	Chronic cicatricial complications of SJS/TEN	30
(5.8)	Autologous transplantation of limbal epithelium cultivated ex vivo	33
(5.9)	Surgical excision of a corneal-displaced recurrent conjunctival melanoma	36
(5.10)	Eyes with total LSCD caused by an acid burn , Stevens-Johnson syndrome,and alkali burn	37
(5.11)	AM as a patch in total limbal stem cell deficiency	39

Fig. No.	Title	Page No.
(5.12)	Surgical technique of AM in strabismus surgery	40
(5.13)	Amniotic membrane buffer technique	44
(6.1)	HAM, oriented with the stromal side in contact with the nitrocellulose filter paper and epithelial side facing up	50
(6.2)	The stromal side of an amniotic membrane	50
(6.3)	Schematic, representation of preserved frozen and freeze-dried HAM	52
(6.4)	ProKera contains a piece of cryopreserved AM	53
(6.5)	AM used as an inlay graft	55
(6.6)	Corneal thinning, Corneal thinning, post multilayered AMT, Multilayered AMT healing stage, Multilayered AMT after healing with cataract	55
(6.7)	AM used as an overlay patch	56
(6.8)	Single layer inlay covered by a larger “patch” or “onlay” and ,multi-layer inlay	57
(6.9)	Diagrammatic representation of a small, peripheral and large, central PED. Visual axis and the central cornea is left exposed in small PED, whereas it is covered by HAMT in the larger PED	59
(6.10)	Corneal ulcer with perforation	60
(6.11)	A multilayered, blanket-fold of HAM is used to manage a large descemetocoele surgically	60
(6.12)	Conjunctival defect is sealed with an AM surface graft using interrupted Vicryl sutures	61

Fig. No.	Title	Page No.
(6.13)	HAMT over the entire ocular surface including the cornea	62
(6.14)	Surgical steps in performing AM strip grafting of the eyelid margins	63
(6.15)	HAMT on the external skin surfaces of the upper and lower eyelids	64

LIST OF ABBREVIATIONS

5-FU	5 –flurouracil
AGV	ahmad glaucoma valve
AM	amniotic membrane
AMG	amniotic membrane graft
AMP	amniotic membrane patch
AMT	amniotic membrane transplantation
AS	absorbable suture
B FGF	basic fibroblast growth factor
DMSO	dimethyle sulfoxide
EDTA	ethylene –diamine tetra-acetic acid
EGF	epidermal growth factor
ELISA	enzyme linked immunoabsorbed assay
FDA	food and drug administration
GDD	glaucoma drainage device
HAM	human amniotic membrane
HGF	hepatocyte growth factor
HIV	human immunodeficiency virus
HLA	human leucocytic antigen
IL	interleukin

KGF	keratinocyte growth factor
MMC	mitomycin C
m-RNA	messenger –ribonucleic acid
NAS	non absorbable sutures
NGF	nerve growth factor
OCP	ocular cicatricial pemphigoid
PAA	para acetic acid
PCR	polymerase chain reaction
PTK	photo refractive keratectomy
RA	receptor antagonist
SJS	steven Johnson syndrome
TEN	toxic epidermal necrosis
TGF	transforming growth factor
TNF	tumour necrosis factor

Anatomy and Histology of the Amniotic Membrane

Amniotic membranes (AM) develop from extra-embryonic tissue and consist of a fetal component (the chorionic plate) and a maternal component (the deciduas). These two parts are held together by the chorionic villi and connect the cytotrophoblastic shell of the chorionic sac to the decidua basalis. (*Parry S and Strauss JF.1998*)

The fetal component, which includes the amniotic and chorionic fetal membranes, separates the fetus from the endometrium. The amniochorionic membrane forms the outer limits of the sac that encloses the fetus, while the innermost layer of the sac is the AM. The AM consists of an epithelial monolayer, a thick basement membrane, and an avascular stroma. (*Parry S and Strauss JF.1998*)

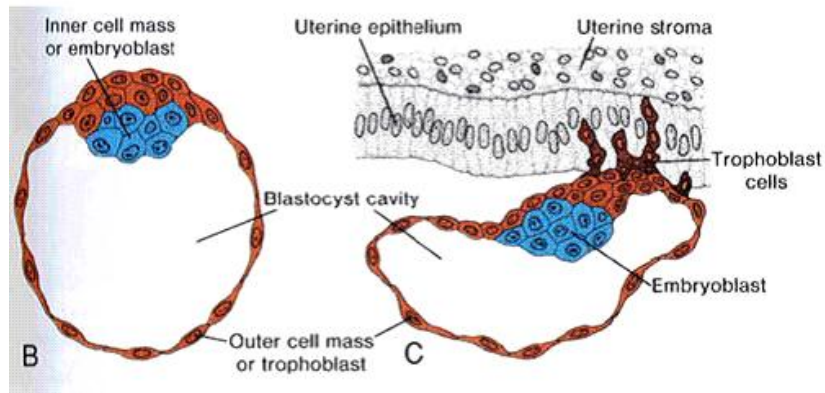


Fig. (1.1): Embryonic membranes and placenta are derived from the outer cell mass and the fetus is derived from the inner cell mass of the blastocyst. (*Rafii et al.2007*)

The AM, which is normally 0.02 mm to 0.05 mm in thickness, lines the amniotic cavity and has its inner apical surface bathed by the amniotic fluid, whereas the outer basal surface is in direct proximity to the chorion. Unlike the chorion, amnion is devoid of any vasculature. (**Bourne GL. 1960**)

The AM consists of five layers from within outward:

- a.** A single layer of highly metabolically active, columnar to cuboidal epithelium.
- b.** A thin basement membrane.
- c.** A compact layer made of reticular fibers virtually devoid of cells.
- d.** A loose network of reticulum containing fibroblasts, called the fibroblast layer.
- e.** A spongy layer of wavy bundles of reticulum bathed in mucin, which forms the interface with the chorion. (**Bourne, 1960**)

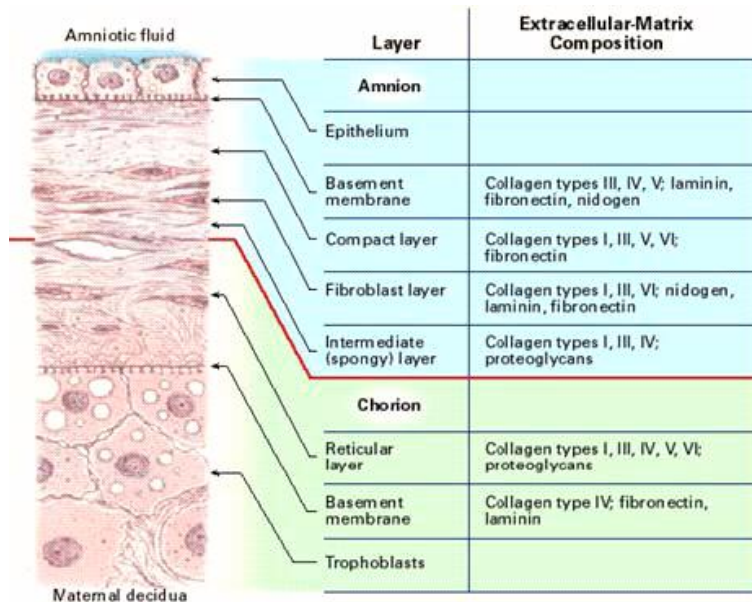


Fig. (1.2): Schematic presentation of the structure of the fetal membrane at term. The extracellular matrix components of each layer are shown. (*Parry S and Strauss JF. 1998*)

It was documented that matrix amniotic basal lamina contains large quantities of proteoglycans rich in heparin sulphate, where amnion contains a large amount of collagen, hyaluronan and predominantly smaller proteoglycans such as biglycan and decorin, with decorin being more prominent of the two, and is located in close connection with the collagen fibrils. (*Meinert M et al.2001*)

Collagen types I, III, IV, V and VII, laminin and fibronectin, have been identified in amniotic basement membrane and stromal amnion. Similarities between the lamini-1, laminin-5, fibronectin and type VII collagen components of the basement

membranes of conjunctiva, cornea and AM have been demonstrated. (*Fukuda K et al.1999*)

Furthermore, the α -subchain components of collagen IV have been shown to be similar between AM and conjunctiva but different between AM and cornea. (*Fukuda K et al.1999*)

Pathophysiology of AM

Some components of the membrane that are relevant in the context of its mechanism of action, and that help understand its limitations and indication.

Enzymes: Important amongst these are enzymes involved in prostaglandin synthesis such as phospholipases, prostaglandin synthase and cyclo-oxygenase as the presence of prostaglandins in the membrane would promote inflammation, whereas, the presence of prostaglandin inactivating enzyme would suppress inflammation (*Smieja et al., 1993*)

Secretory leukocyte protease inhibitor: a potent inhibitor of human the AM. Its concentrations can be up regulated by exposing amniotic cells to interleukins(IL)-1a, IL-1b, and tumour necrosis factor (TNF α), whereas, the presence of secretory leukocyte protease inhibitor would suppress inflammation. (*Zhang et al., 2001*).

Cytokines: IL-6 and -8 are the predominant cytokines associated with amnion cells. Expression of these cytokines was increased in the presence of IL-1b, TNF α and bacterial lipopolysaccharide. IL-10 and IL-1RA (receptor antagonist), both anti-inflammatory cytokines, have been also shown in amnion epithelial and mesenchymal cells. (*Keelan et al., 1997*)

The presence of anti-inflammatory cytokines such IL-1Ra and IL- 10 would suppress inflammation but the presence of IL-6 and IL-8 would promote inflammation. In eyes that are inflamed due to injury, other proinflammatory cytokines such as IL-1 α , IL-1 β and TNF α could also promote both pro- and anti inflammatory cytokines and enzymes. (*Hao et al., 2000*)

Growth Factors: Studies on human amniotic membrane have revealed the presence of EGF, TGF α , KGF, HGF, bFGF, TGF-b1, and - b2 by RT-PCR for the mRNA and by ELISA for the protein products. (*Koizumi et al., 2000*)

TGF-b3 and growth factor receptors KGFR and HGFR were also detected by RT-PCR, with a higher level of various growth factors was found in AM with epithelium than without epithelium, indicating an epithelial origin for these growth factors, the presence of various growth factors like EGF would support epithelial growth and TGF would support wound healing. (*Koizumi et al., 2000*)

However, TGF would itself promote scar tissue formation and be contradictory to the ‘anti-adhesive or scar suppressing’ action proposed for the membrane in preventing corneal and conjunctival cicatrisation (*Koizumi et al., 2000*)