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AMNIOTIC MEMBRANE: NEW EMERGING RAY OF HOPE IN DENTAL FIELD

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ABSTRACT

Amniotic membrane is the inner most lining of the human placenta that is normally discarded afterparturition. The membrane has numerous growth factors like proteins and stem cell reserves that help in accelerating wound healing with regeneration of the lost tissues. In recent years, it has beenused in regenerative medicine because the amniotic-derived stem cells exhibit cellular plasticity, angiogenic, cytoprotective, antitumoral potential and the ability to generate induced pluripotent stem cells. The clinical application of amniotic membrane maintains the structural and anatomicalconfiguration of regenerated tissues, but also contributes to the enhancement of healing through reduction of post-operative scarring and subsequent loss of function and providing a rich source of stem cells. Thus, this review unfolds the inherent structure, properties, mechanisms, applications and how these membranes different from others for potential regeneration especially in the field of oral and periodontal surgeries.

Keywords; Amniotic membrane, Chorion membrane, Regeneration, Repair, stem cell, growth factor.

INTRODUCTION

Wound healing is a complex phenomenon that requires the coordinated interplay of many extracellular matrix proteins, growth factors, and cells. With manifold developments in our understanding on periodontal regeneration, biologic and materials sciences complete regeneration still is an unrealistic situation in many clinical situations due to the complexity of the biological events, factors, and cells involved in regenerative process in the periodontium. Recently, the fetalderived mesenchymal stem cells (MSC) from the placenta or other gestational tissues like the amniotic fluid, umbilical cord are novel materials with rich stem cell reserves. [1]

HISTORY[2]

The use of placental tissue for the treatment of wounds first done by Davis in 1910. He used thesefetal membranes as skin substitutes for the treatment of open wounds. In 1913 Sabella and Stern described its use for burnt and ulcerated skin surfaces. In 1965, Dino et al. demonstrated for the first time that amniotic membrane could be separated, sterilized and safely used. They evaluated the accelerative effect of the membrane on epithelialization and the reduction in pain in the wound.In 1940, De Röth first reported use of fetal membranes in the ocular surface. He used fresh amnionand chorion as a biological dressing material for management of conjunctival defects. Lawson in 1985 first, studied the use of amniotic membrane along

with pectoralis major muscle for oral cavityreconstruction. He concluded that placement of amnion over the deep aspect of the muscle that isexposed to the oral cavity resulted in a more rapid development of mucosa. Tinti and Vincenzi in1990 used the principles of guided tissue regeneration (GTR) to obtain coverage of the denuded root surface along with regeneration of the entire attachment apparatus.

WHERE TO FIND AMNIOTIC MEMBRANE IN BODY [3,1]

Human fertilization begins when a male gamete or sperm fuses with the female gamete or oocyte,generating a unique, and diploid chromosome aggregation called the zygote. The zygote undergoesmitotic divisions to increase the number of embryonic cells and the newly formed morula migratesinto the uterus and begins to fill with fluid to form a blastocyst. The telomere (DNA structure thatprotects chromosomal integrity by forming guanine-rich caps) length, a measure of the biologic age of a cell, and telomerase (an enzyme that maintains telomere length) have also been shown topeak at the blastocyst stage, possibly setting the biologic age of all fetal-derived cells. The blastocyst divides into two parts: an outer layer, the trophoblast, which will form the embryonic part of the placenta, and a group of centrally located blastomeres, the embryoblast, which will develop into an embryo (Watson, 1992). Blastocyst implantation in the uterine endometrium coincides with the development of a small space in the embryoblast that will form the amniotic cavity. Morphologic changes in the embryoblast result in the formation of the embryonic disc, a bilaminar plate formed by two layers: the epiblast, which constitutes the floor of the amniotic cavity, and the hypoblast, which together with the exocoelomic membrane forms the primitive yolk sac[3]. It is about 10-15 micrometer thick which consists of two fetal membranes; the inner amniotic membrane and the outer chorion. The AM encases the amniotic fluid and fetus, and is highly flexible because of which it is easily be separated from the chorion. AM has two types of cells with different embryological origins: amnion epithelial cells derived from embryonic ectoderm and amnion mesenchymal cells from embryonic mesoderm[1] as shown in Fig 1[3].

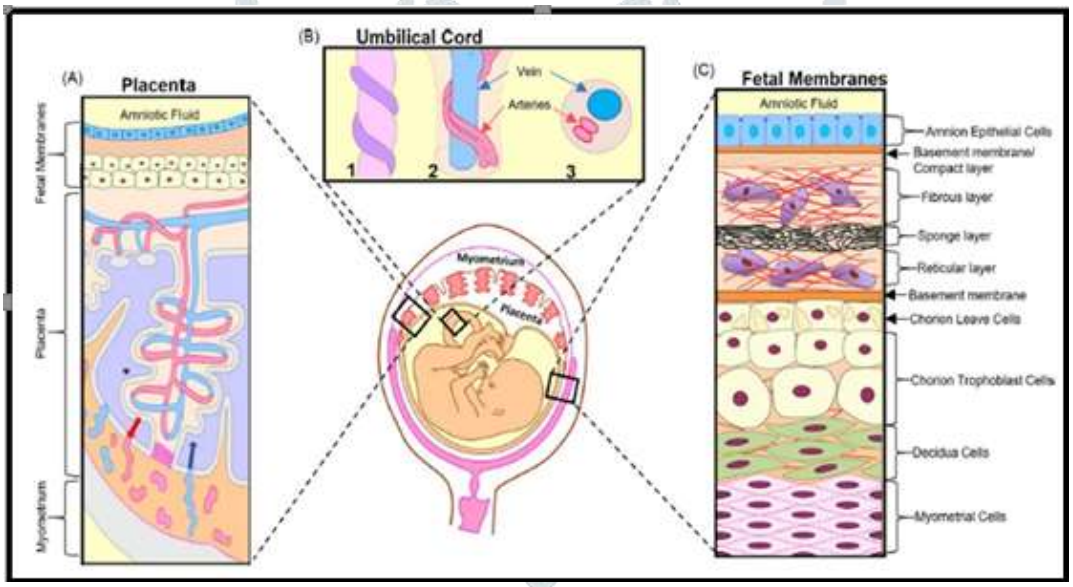


Fig 1 -Intrauterine cavity from outside to inside: maternal cavity, uterus/ myometrium, placenta, fetal membranes,amniotic fluid & fetus.

A) **Represent the placenta**, site of nutrients & oxygen exchange for the growing fetus. The placenta is attached to the maternal side by myometrium & the fetal side through the fetal membranes. The black asterisk identifies tertiary chorionic villi, while the res & blue arrows represent arteries & veins respectively.

B) **Represent the umbilical cord** on its external view: 1) internal view 2) &3) cross section

C) **Description starts** from the innermost layer **(Amnion)** & ends at the uterus.

Amnion epithelial cells (blue) are connected the first layer of the ECM called the basement membrane (orange) / compact layer (orange strips). The fibroblast (top red), spongy (black) and reticular layer (bottom red) follow, containing stromal cells (purple). The chorion (beige) is connected to the ECM through a pseudo -basement membrane (orange). The chorion is made up of 2 types of cells: 1) chorion leaves cells

that contain vacuoles (dark yellow circles) & 2) the chorion trophoblast cells. The chorion interfaces with the decidua (green), connecting the fetal tothe maternal compartments of the uterus.[3]

BASIC STRUCTURE OF AMNIOTIC MEMBRANE[1]

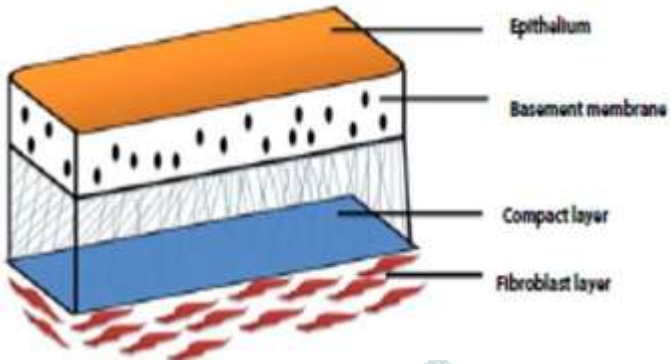


Fig 2- structure of amniotic membrane

Were,

Epithelium composed of single layer of flat cuboidal columnar cells, Basement membrane composed of collagen types III, IV and V, Fibronectin, Laminin and Nidogen, Compact layer composed of collagen types I, III, IV, V, VI and Fibronectin and Fibroblast layer composed of collagen types I, III and VI, Fibronectin, Laminin and Nidogen.

The amniotic epithelial cell layer is a single layer of flat, cuboidal and columnar cells that are indirect contact with the amniotic fluid. It is from this layer that amniotic MSC (AMSC) are isolated and stored to be used for regenerating tissues. There are no nerves, muscles, or lymphatics in the amniotic membrane. These HAE cells and human amniotic mesenchymal cells(HAM cells) express pluripotency and are potent stem cells reservoirs. The amniotic mesoderm layer consists of macrophages and fibroblast-like mesenchymal cells. The basement membrane of the amnion is very similar to the basement membrane found in the other parts of the body likethe conjunctiva or gingiva as shown in Fig 2.[1]

MECHANISM OF ACTION OF AMNIOTIC MEMBRANE[1]

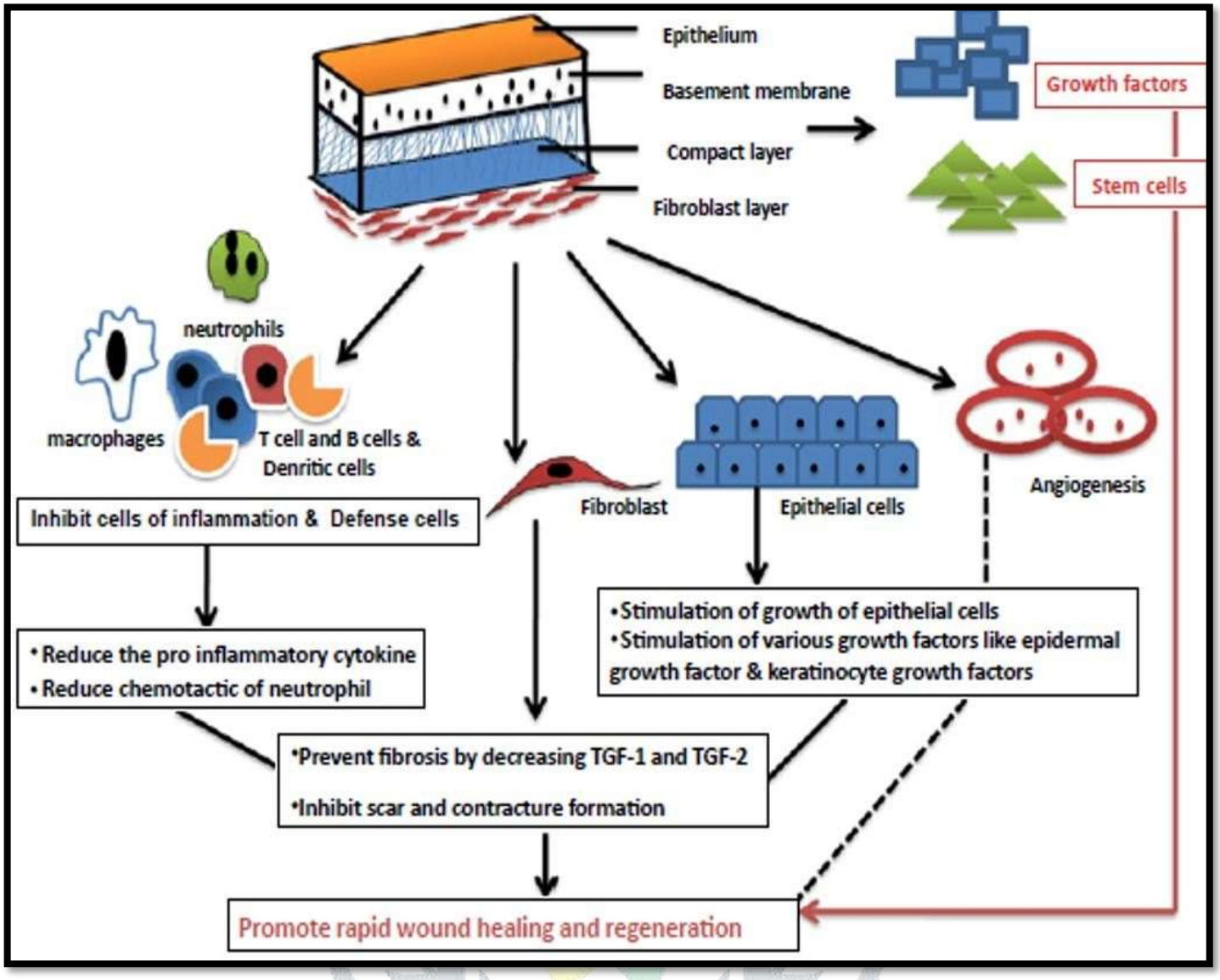


Fig 3- mechanism of action of amniotic membrane

The mechanisms involved in accelerated wound healing by amnion membrane can be divided asfollows:

1. Immunomodulative and Immune privilege
2. Anti-microbial (broad spectrum effect against bacteria, fungi, protozoa and viruses)
3. Reduction of pain
4. Anti-scarring and anti-Inflammatory
5. Tissue reparative activities with enhanced bone remodeling, osteogenesis and chondrogenesis
6. Speed fibrogenesis and angiogenesis
7. Increased extracellular matrix deposition
8. Potent source of mesenchymal stem cells[1]

PREPARATION OF AMNIOTIC MEMBRANE[1`]

AM can be used either alone with only amniotic epithelium (intact AM) or without it (denuded AM). In denuded AM only the cellular components are removed leaving the structural componentslike the basement membrane intact. A detergent-based protocol with sodium dodecyl sulphate (SDS) is used to remove the amniotic epithelium from AM while maintaining the histoarchitectureof the matrix. The amniotic cells were isolated by sequential trypsin and collagenase digestion thatremove the epithelial layer of cells and digested the stromal cells respectively. The amnion is thenwashed in phosphate-buffered saline (PBS) and then cut into pieces in PBS containing 0.03% hyaluronidase and 0.025% deoxyribonuclease I. The resulting minced amnion is digested with 0.2% trypsin and incubated at 37°C with constant stirring at 100 rpm for 30 min. The mixture is then poured to separate the dispersed amnion epithelial cells from the tissue pieces. The dispersedepithelial cells are collected by centrifugation and suspended in DMEM supplemented with heat-inactivated 10% Fetal Calf Saline and 1% antibiotic-antifungal solution. It is then seeded into culture dishes at a concentration of 3.0×104 cells /cm2 and incubated at 37°C under 5% carbon dioxide in air. Trypsinization of the amnion is repeated several times until no more HAE cells wereobtained. The dispersed mesenchymal cells of amnion are collected by filtration through gauze and centrifugation Special processing and sterilization is recommended to ensure consistent qualityand preservation of the properties of AM.

Various methods have been tried to preserve the AM include: hypothermic storage at 4°C, freezedrying through liquid nitrogen at -196°F, γ-sterilization, glycerol preservation and cryopreservation. The media and storage temperature used for the preservation process affects theviability of cells and growth factors in the AM. Sterilization with γ-rays has no significant effect on growth factor content in the human AM. While storage of AM in glycerol at 4°C will result inimmediate cell death, cooling will preserve the membrane for an indefinite time and make it bacteriologically pure and immunologically inert. Cryopreservation with dimethylsulphoxide (DMSO) at -80°C is an important modality for preservation of these tissues as it keeps the viable for a longer period of time but causes loss of some angiogenic factors and cell rupture.

To overcome these problems with cryopreservation, freeze dried - irradiated (Lyophilized) is the one the most commonly used preservation technique that preserves the original size and shape with minimum cell rupture. The membrane is first freeze dried at -60°C under vacuum (atmospheric pressure 102 mm of Hg) for 48 hours and then irradiated with 2.5 mega Rads (25 KGray) in a batch type cobalt-60 irradiator. The far-infrared rays and microwaves are also used forsterilization of amniotic membrane which is known as the Hyper-dry-amnion. During the drying process, the temperature inside the hyper drying device should not exceed 35°C as high temperatures on the surface that can reach 60°C can decrease the degradation of tissue-protein. Compared to cryopreserved amnion, which can be preserved for less than 3 months at 80°C, "Hyper-dry amnion" can be preserved at room temperature indefinitely until the packet is cut open.It is easily cut to the desired size and shape just before application. The freeze-dried membrane can be readied for use by soaking in normal saline for 1 minute.

Glutaraldehyde fixation method to fix the AM provides better stability and properties. Thisrequires neither antibiotics nor the use of special storage .[1]

Fig 4 - human fetal

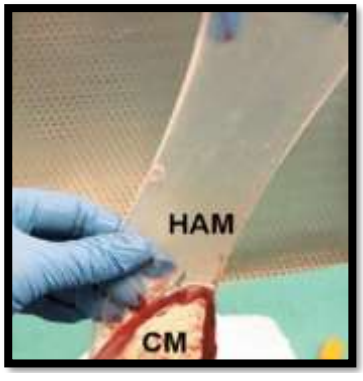


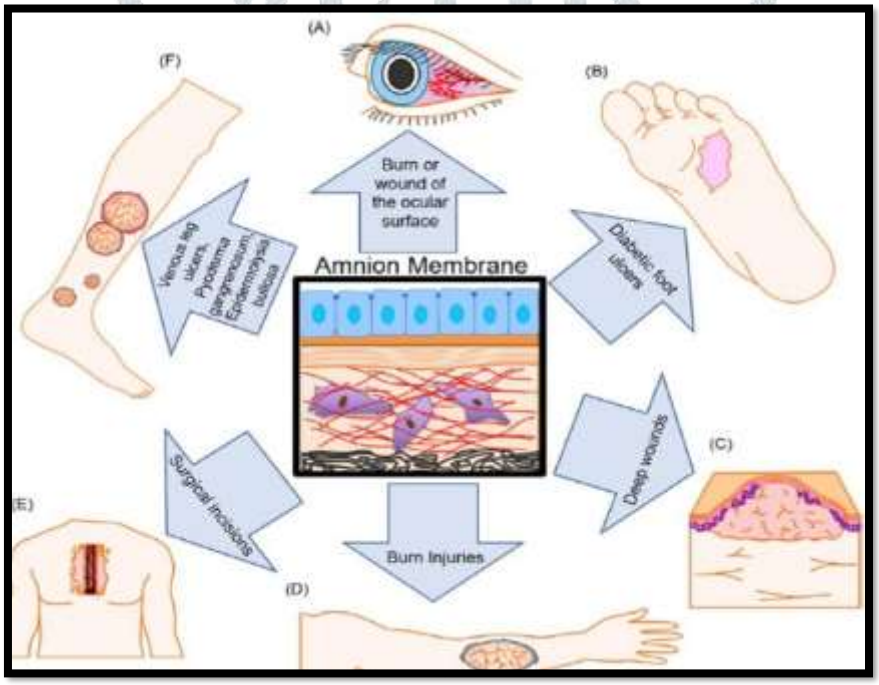
Fig 5- HAM is a thin translucent layer attached to the

PROPERTIES OF AMNIOTIC MEMBRANE [2]

1) Immunomodulatory
2) Antimicrobial
3) Reduction of Pain
4) Anti-Scarring
5) Anti-Inflammatory
6) Revascularization
7) Cellular Plasticity
8) Angiogenic
9) Cytoprotective
10) Anti-Tumoral Potential
11) Ability to generate induced pluripotent stem cells
12) Biocompatibility
13) Mechanical Properties
14) Good cell Adhesion

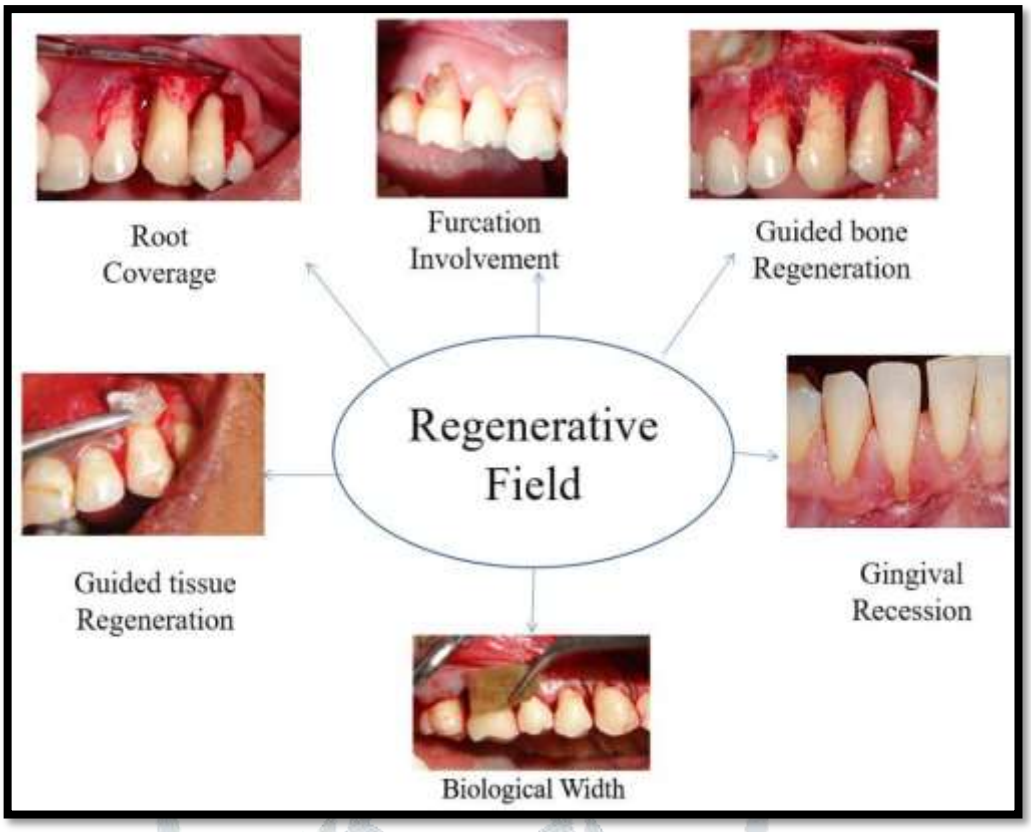
USES OF MEMBRANE

1- Medical field



2- Dental field[4]

a) Regenerative field



b) Non-healing ulcer

c) Manage wounds in the oral cavity such as-

i) Tongue

ii) Buccal Mucosa

iii)Vestibule

iv)Palatal Mucosa

v) Floor of the mouth

d) Rapid healing of ulcer

i) Herpes Simplex Virus (HSV)

ii) Varicella Zoster Virus

iii)Erythema Multiforme Major 9Stevens-Johnson syndrome)

iv)Cervical Necrotizing Fasciitis

e) Carrier for local delivery of various drugs such as

i) Antibiotic Netilmicin (NTM)

ii) Antiviral drugs such as acyclovir (ACV)

iii)Trifluridine (TFU)

f) Esthetic results in terms of texture and color match without postoperative discomfort and adverse reactions.

g) Regenerative procedures for endodontic surgery.

CONCLUSION

The mesenchymal stem cells derived from human amniotic membrane has given us a ray of hopeas its clinical application maintains the structural, anatomical configuration of regenerated tissues& improve post operative healing efficacy of the tissue.

REFERENCE

1) Amniotic Membrane: A Novel Material for Regeneration and Repair Aditi Chopra and Betsy S Thomas Department of Periodontology, Manipal College of Dental Sciences, Manipal University, Karnataka, India.

2) Human Amniotic Membrane: Hope in Periodontal Regeneration International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064 Index Copernicus Value (2013):6.14 | Impact Factor (2015): 6.391.

3) Characteristics, Properties, and Functionality of Fetal Membranes: An Overlooked Area inthe Field of Parturition Laura Martin*, São Paulo State University (UNESP), São Paulo, Brazil; The University of Texas Medical Branch at Galveston, Galveston, TX, United States.

4) Mohan R, Bajaj A, Gundappa M. Human amnion membrane: potential applications in oral and periodontal field. Journal of International Society of Preventive & Community Dentistry. 2017 Jan;7(1):15.

5) Leal-Marin S, Kern T, Hofmann N, Pogozhykh O, Framme C, Börgel M, Figueiredo C, Glasmacher B, Gryshkov O. Human Amniotic Membrane: A review on tissue engineering,application, and storage. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2021 Aug;109(8):1198-215.