AMNION MEMBRANE HARVEST THE BEST HEALING SUPPORT IN MEDICAL FIELDS

Mrs. Lissa J, Asst. Professor,

AMNIOTIC MEMBRANE

The amniotic membrane (or amnion) is the innermost layer of the placenta that lines the amniotic cavity. The membrane itself consists of a special combination of tissue layers, making amnion a unique membrane in the human body. The special composition of cellular components in this membrane provide specific functions which aid in the healing process.

Layers Of Human Amniotic Membrane:

The amniotic membrane is one of the fetal membranes covering the growing fetus. It’s the inner most of the fetal membranes and its tough, tenacious at term.

It has 5 layers

1. Innermost layer consists of uninterrupted single layer of cuboidal epithelial cells bathed with amniotic fluid
2. Second layer is basement membrane
3. Next layer a cellular compact layer composed of interstitial collagen I, III, V

Collagen III serves for tissue strength and helps for tissue integrity.

Collagen V of amniotic membrane shows same structure as the collagen fibrils of cornea and thus helps in maintaining transparency.
Amniotic membrane acts as a healthy basement membrane which helps normal epithelization in the injured part.

4. On outer side of compact layer, there is row of fibroblast like mesenchymal cells.

5. Outermost layer of amnion is relatively a cellular zona spongiosa contiguous with chorion laevae.

The amniotic membrane lacks smooth muscle, lymphatics, nerves and blood vessels

**History of amniotic membrane used in wound healing**

Live fetal membrane which included both amnion and chorion was first documented by De Rotth in 1940 for use in conjunctiva reconstruction. A very low success rate was reported. During the same year, Dr. Brown used rabbit peritoneum as a temporary patch for ocular surface burns which Dr. Sorsby later modified with human amniotic membrane in 1946 and 1947. In 1995 Dr. Kim and Tseng re-introduced the idea of amniotic membrane for ophthalmic use which has since become increasingly more popular.

**Advantage of using Amniotic Membrane In Wound Healing**

- It is easy to handle and Flexible
- It offers protection through presence of Lysozyme and interleukin.
- There is no immuno-rejection
- Exudation of wound also takes place very easily due to its porosity.
- Amniotic membrane is easy to apply without hurting patient especially in burns patient.
- It is easy to observe the progress of wound due to its transparency.
- Scar formation is very minimal after application of amniotic membrane.
- It is quite cumbersome in collection and preservation of amniotic membrane.
- **Pain reduction** – application of amnion to the burns surface can protect the exposed nerve ending from external irritants such as clothing and even the soft moving air
- Amnion has good adherence to wound surface and is able to maintain a moist environment.
- Good wound adherence-the bounding is established between the amnion and wound surface is biological and not a mechanical.
- The strong bond and adherence can also maintain the moisture level over the wound surface, which can accelerate re- epithelialization and protect the newly formed epithelium from trauma .the amnion will become dry and readily peel off once the wound is completely healed
- **Thin, light and transparent** – amnion membrane is thin and lightweight, which makes it practical to apply, these characteristic avoid bulky dressing .the transparent nature of the
membrane also allows visualization of the underlying wound bed without removing membrane. Therefore, any collection or wound surface is easily detectable.

- **Minimizing fluid loss**—The amnion is made of five layers: epithelium, basement membrane, connective tissue, fibroblast, and spongy layer. These structures resemble a skin epidermis, which is a natural vapor barrier. By acting like a vapor barrier, amnion can prevent excessive evaporation from the wound surface thus directly and markedly reducing the insensible water loss.

- **Anti bacterial properties**—Human amniotic membrane can provide a mechanical barrier to microbial invasion, and when used, has produced a lower rate of burns wound infection. It also has been demonstrated that the pH fall after amniotic membrane application and the temperature of the underlying wound bed rises. Both of this changes promotes phagocytosis and reduce the exudation of interstitial fluid.

- **No rejection phenomenon**—As human amniotic membrane is embryologically derived from fetal ectoderm, it carries the advantage of fetal skin allograft and there is no rejection phenomenon. The glycoprotein that is present in the trophoblast and amnio-chorion is very important in suppressing the detection of fetus as “foreign” to the mother by acting on the maternal lymphocytes and preventing rejection throughout the pregnancy.

**Who can Donate Amniotic Membrane?**

The AM grafts were prepared from placentas which were harvested during caesarean sections or from surrogate after full-term pregnancy in medically cleared donors.

Eligible donor mothers were accepted for the AM donation after a medical interview and after a written informed consent was obtained from them.

**Criteria used for selection and processing of donors membranes included:**

1) No history of jaundice, premature rupture of membranes, endometritis, malaria, toxemias and venereal disease;

2) Donors to be sero-negative for HIV, hepatitis B and C surface antigen and syphilis, toxoplasmosis, and cytomegalovirus;

3) Only membranes of caesarean sections were obtained;

4) Meconium stained membranes were rejected.
Those sero-negative for HIV, hepatitis B and syphilis were used. Placentas from women undergoing caesarean section were procured at the time of delivery and placed in the jar of saline.

If not procured at actual delivery, the placenta was kept in a sterile container in the main compartment of the theatre refrigerator and was transferred within two hours into the jar containing normal saline.

The placenta was then processed immediately for separation of amniotic membrane. If the placenta could not be processed immediately, it was kept in a refrigerator at 4°C and was processed within 24 hours.

After wearing sterile gloves, the placenta was taken out of the jar. The membrane stripped from the placenta. The placenta was cleaned of all blood clots with sterile phosphate buffer saline solution containing 1000 U/ml of penicillin, 20 µg/ml of streptomycin, 2.5 µg/ml of amphotericin B.

The amnion was separated from chorion by blunt dissection through potential spaces situated between these 2 tissues and flattened with epithelium/basement membrane surface up onto a nitrocellulose paper of pore.

The paper with the amniotic membrane was then cut into 3 x 4 cm disks and stored before transplantation in freezer in a sterile vial containing Dulbecco modified Eagle medium and glycerol at ratio 1:1. A piece of amniotic membrane was sent for culture and sensitivity. The membrane was used within 6 week. Part left at the end of this period was discarded.

Different Method of Preparation of Amniotic Membrane

**Fresh membrane:** Fresh membrane as is obtained from the placenta at the time of delivery, either vaginal or caesarian section. The membrane in a 0.025% solution of sodium hypochlorite and stored at 4°C in sterile solution containing penicillin. They showed that membranes remained sterile upto 6 weeks.

**Dried Membrane**

After cleaning and rinsing the membranes spread them on a plastic sheet and allowed to dry in the open air. It is found to be equally effective when compared with the fresh.

**Frozen Membrane**

Amniotic membrane is frozen by passing through liquid nitrogen at -196°F. Cooling
preserves the membrane for an indefinite time, produces bacteriologically pure and immunologically almost inert material.

**Freeze Dried – Irradiated**

In this process, membrane, after obtained from placenta is freeze dried at -60°C under vacuum (atmospheric pressure 102) for 48 hours. It is then irradiated with 2.5 mega rads (25 K Gray) in a batch type cobalt-60 irradiator. By the method of freeze drying there is sublimation of liquid moisture of membrane to gaseous state without having undergone the intermediate solid stage. This method helps the membrane to maintain its original size and shape with minimum cell rupture. The freeze dried membrane can be readied for use by soaking it in normal saline for 1 minute.

**Stabilized Amniotic Membrane**

The gluteraldehyde fixation of amniotic membrane was popularized which has led to the development of stabilized amniotic membrane (SAM). Gluteraldehyde treatment required neither the antibiotics nor the use of special storage techniques and renders the amnion sterile as well as non-immunogenic. Successful use of gluteraldehyde treated amnion (SAM) is employed as a microvascular inter-positional graft in experimental animals.

Commercially Available In Market

**Use of amniotic membrane**

1. For the treatment of burns wound
2. For the treatment of diabetic ulcer and pressure ulcer
3. For treatment in cosmetic medicine
4. For treatment of eye (corneal ulcer)
5. Preparation of synthetic mesh
6. Preparation of eye drops

**Preparation of synthetic mesh**

collagen part of amniotic membrane is separated and combined with synthetic bio-absorbable poly glycolic acid mesh in preparing mesh product.

**Preparation of eye drops**

Amniotic membrane extract contain all of cytokine Viz growth factor, receptors, molecules for wound healing, interleukin, fibroblast growth factor ect these extract are used for the preparation eye drops.
Treatment in cosmetic medicine

Dermoabrasion of face and in exposed body parts, application of amniotic membrane dressing showed a remarkable recovery with minimal scar. It is also used in moisturizing and treating dry skin.

Treatment of burns wound

Burn wound management is a complex phenomenon in which the main aim is to obtain physiological closure of wound in short time. Failure to do so will result in fluid loss through skin less burn wound. Amniotic membrane dressing is a widely used modality of care for burns wound management which showed the remarkable improvement in wound healing, control infection, decrease mortality rate and overall decrease in hospital stay.

Treatment of diabetic ulcer and pressure ulcer

The main complication of diabetic and pressure ulcer is infection and extension of suppurative process and tissue degeneration. Application of amniotic membrane in these is very effective in recovery of wound and preventing tissue degeneration. It is very effective in promoting epidermal growth factor and tissue regeneration.

For treatment of eye (corneal ulcer)

Amniotic membrane is used as a graft for healing corneal ulcer, especially ulcer due to infectious and inflammatory process like scleritis, keratitis etc.

Disadvantage of using AM

When the proper laboratory test is not performed before application of amniotic membrane it may leads to infection.

It is highly fragile and becomes firmly adherent to the wound. Attempts to remove it, even after soaking the area, can cause considerable bleeding and pain to the patient. Patients accepted the AMT.

Conclusions:

AMT becomes an attractive alternative for country like ours where there is shortage of tissues. Hence used either as a substrate or patch graft, to replace ocular surface. Lastly efficacy of AMT is better in ocular surface disorders and in burns wound.