Amniotic Membrane in Surgery

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The first reported use of fetal membrane as skin substitute was by Davis in 1910. In 1913, Salbella presented the first clinical report of successful use of amniotic membrane in the treatment of burns and skin ulcerations. In 1940, DeRoth reported the use of amniotic membrane in the repair of conjunctival defects. From 1940 to 1965 a number of clinical trials of successful use of amniotic membrane for use in acute skin injuries appear in the literature. However no practical methods of preparation, sterilization and storage were suggested and this fact seems to have limited the use of this modality prior to 1965. In 1965 Dino et al demonstrated that amniotic membrane from routine deliveries could be sterilized and kept for six weeks at 4°C and safely used on acute second degree burns and on skin donor sites. This encouraging report stimulated great interest amongst clinicians and has resulted in numerous reports in the world literature documenting thousands of patients with successful healing of all kinds of skin lesions.

Amnion is a thin, tough, transparent membrane. It is about 10-15 micrometer thick. It is made up of two membranes, the inner amniotic membrane and the outer chorion. Chorionic side of the membrane is rougher and mucinous. Amnion can easily be separated from chorion leave and placenta as far as the umbilical cord. Once separated, the amnion is found to be smooth and shining and much tougher and more elastic and easier to clean than the thicker chorion, which does not strip from the placenta. The chorion, although thicker, is more easily torn because it is much less elastic.

It is not entirely clear whether the amniotic membrane is primarily nourished by the amniotic fluid or diffusion from the chorion, but the presence of significant AIP and glycogen in the amniotic fluid suggests that the latter is the main source of nourishment of the amniotic membrane.

Immunogenicity of Amniotic Membrane

Amniotic membrane has low or no antigenicity, a fact that might be related apparently to a distinct collagen present in the amniotic membrane. McIntyre and Faulk had isolated a glycoprotein from amnion and credited it to be responsible for suppressing any “foreign body” type reaction by acting on lymphocytes and preventing ymphoblastogenesis. Amniotic membrane when used as an allograft in peritoneal cavity or buried under skin has shown long term survival with no evidence of any immune reaction. Like wise, when used as xenograft from human to animals or cattle to humans no significant antigenicity is revealed. Robson, Samburg and Krizek preferred to use the unseparated amniochorionic membrane with the chorion placed against the wound in deep second degree skin injuries. Although vascularization and vigorous “rejection” develops from the mesenchymal side of the chorion, both the procedures seem to be less intense when the epithelial cells are placed on the wound. Walker has seen very little difference after 24 hours in the appearance of the membrane or of the wound whether amnion or chorion is placed next to it. The difference in immunology of amnion and chorion is related to the presence of
fragments of maternal decidua on the chorion. Animal experimentation suggests that the chorion alone can be responsible for the reaction. If the chorion is separated from the amnion and the mesenchymal side is applied to the host, a clear immune reaction may be observed.

**Functions of Amniotic Membrane**

When used as a biological dressing, amniotic membrane has been credited with the following functions:

**Vapour Barrier**

By acting as an effective vapor barrier it prevents the evaporation of fluids from burn wound, thereby reducing insensible loss and in turn the overall fluid requirement of the body. Furthermore, by preventing evaporation from the wound surface, the temperature regulation mechanism is not over-strained and the caloric requirement to maintain body temperature is also correspondingly reduced. Vapor barrier property of amniotic membrane has been attributed to a firm bond between it and the wound, composed mostly of fibrin and elastin. When compared to homograft skin and porcine graft skin, amnion causes a very minor reduction of evaporation. Salisbury, Carnes and Enterline showed that in both full thickness and partial thickness wounds, allograft was as effective as sheet porcine skin and five times as effective as meshed porcine skin or amnion.

**Bacteriostatic Function**

This function is said to be due to the presence of antibodies, possibly allantoin, a bactericidal product of purine metabolism and lysozyme, a bacteriolytic protein. Adherence of amnion to the burn wound by eliminating its exposed status may itself lower bacterial count in the wound. The close adherence of the membrane to the wound is said to be via a fibrin and elastin. Furthermore, the amniotic membrane has a high thrombin activity which allows a very rapid and efficient attachment to living dermis or granulating tissue. This close adherence allows restoration of lymphatic integrity, protects circulating phagocytes from exposure and allows removal of surface debris and bacteria. It has been shown that amniotic membrane takes onto a granulating wound. This initial neo-vascularization is held responsible for effective decrease in bacterial counts.

**Reduction of Pain**

This is a frequently observed and well recognized quality of amniotic membrane when used as a skin substitute. It appears to follow diminished inflammation and possibly better state of hydration of wound bed. Another explanation is that soft mucoid lining of amniotic membrane protects the exposed nerve endings from external irritant, the most important of which is the surrounding air.

**Enhanced Wound Healing**

Many workers have noted enhanced healing of wounds with the application of amniotic membrane. Robson, Krizek, Koss and Samburg in 1973 have subjectively noted rapidity of ingrowth of epithelium from the borders of the wound in full thickness defects and rate of re-epithelization of partial thickness burns appear to be increased by the use of amniotic membrane. Mechanism of healing by Amniotic Membrane

The most striking effect noted by Faulk et al using amniotic membrane on chronic leg ulcer was the development of new vessels which they thought was due to some
angiogenic factors acting on capillary endothelium.

Burgos\textsuperscript{38} confirmed the presence of angiogenic and mitogenic factors in amniotic membrane and held them responsible for producing healing in the wounds. Freeze dried (lyophilized) amniotic membrane was used by Unger\textsuperscript{39} on split skin graft donor sites who found it to be equivalent to an ordinary dressing and fail to notice any enhanced rate of healing.

**Preparation of Amniotic Membrane**

The difference in potential of stimulating neovascularization and re-epithelization of fresh and lyophilized membrane is still controversial.\textsuperscript{10} Klen and Skalska\textsuperscript{41} compared freeze dried amniotic membrane with freeze dried dermo-epidermal graft and concluded that chorion-amnion grafts were as effective as dermoepidermal grafts.

For clinical use the membrane can be prepared in the following forms: Fresh membrane as already described is obtained from the placenta at the time of delivery, either vaginal or caesarian section. Robson and Krizek\textsuperscript{12} rinsed the membrane in a 0.025\% solution of sodium hypochiorite and stored at 4°C in sterile solution containing penicillin. They showed that membranes remained sterile up to 6 weeks.

Dinno and associates\textsuperscript{28} performed cultures to study sterilization of amniotic membranes. Preservation with 1:40 dilution of sodium hypochlorite revealed no positive cultures until 30 days.

Similar results were obtained with aqueous penicillin 50,000 units and streptomycin 1 gm. In 400 ml of normal saline. When kanamycin sulfate, 1.0 gm. in 400 ml normal saline was used no positive cultures were found even at the end of 30 days.

**Dried Membrane**

Rao and Chandreskharam\textsuperscript{13} after cleaning and rinsing the membranes spread them on a plastic sheet and allowed to dry in the open air. He found it 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.\textsuperscript{42,43}

**Freeze Dried - Irradiated (Lyophilized)**

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.\textsuperscript{44,45} 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.\textsuperscript{46} The freeze dried membrane can be readied for use by soaking it in normal saline for 1 minute.

**Stabilized Amniotic Membrane**

The idea of gluteraldehyde fixation of amniotic membrane was popularized which has led to the development of stabilized amniotic membrane (SAM).\textsuperscript{47} Glutaraldehyde 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 interpositional graft in experimental animals.\textsuperscript{48}

**Storage of Amniotic Membrane**
Dino36 suggested the idea of human amnion bank which was seconded by Rao and Chandrasekhram also adding bovine amnions. Burgos and Faulk describe a method to keep the amniotic membrane in culture for 2-3 weeks 50-90% viability.

**Side of the Membrane towards Wound**

There is great controversy as to which side of the membrane, amniotic or chorionic, should be applied next to the wound. Trelford transplanted “trophoblastic tissue” (i.e. chorion) in sheep as an autograft and has shown that an immunological response occurs suggesting maternal decidua fragments may inadvertently be accompanying the chorion. He recommended that the mesenchymal side should be placed towards the wound for better survival. He had demonstrated that capillary and cellular invasion does not occur in the absence of chorion. Robson considered “vascular invasion” to be the sole criteria for a dressing to be labeled as physiological and recommends that chorionic surface must be applied to deep or second degree burns to have any benefit. If amnion and chorion are separated and the amnion’s mesenchymal side is applied to the host tissue, then vascularization and rejection phenomenon are not seen. Walker saw very little difference after 24-48 hours in the appearance of the membrane or of the burn wound whether amnion or chorion was applied.

**Method of Use**

Before the membrane is applied, the wound should be prepared as for any dressing or for skin grafting. Surgical scrub with antiseptic and minimal debridment are followed by moist compression until ooze has stopped and the wound surface is reasonably dry. This procedure is preferably done in a clean sterile dressing room, observing all aseptic measures. No local or general anesthesia is required.

Membrane is applied with rough (chorionic) surface next to the wound. Care is taken to ensure no trapping of air bubbles between membrane and wound by gentle pressing. Membrane is followed by a layer of anti-bacterial gauze (e.g. Soframycin tulle), some moist gauze, dry gauze, cotton and bandage. Dressing should be changed along with the membrane at least every 48 hours and preferably after every 24 hours. Dressing should be continued for 7-10 days or until wound appears clinically clean. Split skin grafting should be done after 7-10 days or when wound contains less than 105 organisms/gram of tissue.

**Use of Amniotic Membrane in other Surgical Disciplines**

Freeze dried irradiated membrane is also used as described above, but before application it is soaked in sterile saline for 1-2 minutes.

Apart from established use of amniotic membrane in acute superficial burn wounds and acute second-degree injuries, in particular facial burns, some of the other indications are as follows:

- Following facial derrnabrasion, vaginal reconstruction, replacing nasal mucosa,
- bladder wall reconstruction, Stevens-Johnson syndrome, non-healing leg ulcers, reconstruction of the floor of the mouth following total glossectomy, micro-vascular interpositional grafts, conjuctiva and comel defects, graft donor sites and radiation burns.

Advantages of Amniotic Membrane as a Biological Membrane It is readily available at no cost if fresh, sterilization, storage and application are simple, prevents fluids, protein and energy loss, combats infection, promotes healing and relieves pain.
Disadvantages
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.

Amniotic Membrane in a Developing Country
Considering the properties of amniotic membrane and the easy availability, low cost of procurement and cheap storage makes it appear to be a useful dressing material for burn wounds and other non-healing skin lesions in developing countries.

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