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REVIEW OF LITERATURE

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Ever since man first learnt to make fire, while on the one hand it has served the humanity tremendously, it is considered man's worst enemy for causing burns. The difficulties in the treatment of burns have been lamented by all writers for centuries. The information regarding burns is found in different literature.

Papyrus (1500 BC) had been using boiled cow dung topically. Indian literature shows that Sushutra used mixture of butter with red ochre or the bark of a fig tree. He also recommended debridement of severe burns with loose skin and flesh.

Adams (1939) reports that Hippocrates applied warm mixture over the burn and avoided separation by simple cleanliness. Paulus aegineta (AD 625-690) recommended application of moderate detergent materials which were not definitely heating or cooling.

Razes (AD 880-923) had been using white ointment composed of white lead oil of roses and wax. Apart from excision of contracted scars described by Celsius, surgery had no place in the treatment of burns with Greek and Romans. Volesco de Tarenta of Montpellier (1490) described method to avoid syxnderely in the burnt hands.

William Cloves (1544-1604) stands out in history as the first surgeon since the middle ages to use the physical signs of burns 'where the skin was burnt off, and the parts were made raw and painful' to indicate his local treatment.

Hildamus in his book De combustionibus (1607) insisted that the classification of burns should be a guide for
treatment, classifying burns into three degree by external appearance; redness and blistering; withering and skin without charring; and eschar formation and charring. He warned against cooling burnt skin which would harden the tissues. In very deep burns, he made incisions to let the moisture escape, as otherwise gangrene and infection would supervene. So he was also the first to perform 'escharotomy'.

Wiseatt (1676) observed that an organ which is burnt superficially is far more painful than the deep. He too classified burns into three degrees. Haister (1743) suggested a new classification into four degrees, including a time factor as a further diagnostic aid.

Sir James Earle (1799) described several cases of burns which he had treated with ice as an antidote. He suggested that application of cold to a burnt limb alleviates pain, and if immediately applied, prevents the formation of blisters and limits the change to an area of erythema. Trusti (1816) was the first person to carry out controlled experiments on dogs, which were sealed and treated with cold water.

Kentish (1797) justified his mode of treatment with oil of terpine, which aims to 'diminish the increased action of system'. He had classified burns into two categories.

The Baltimore Medical and philosophical Lyceum (1811) described carded cotton dressings, indicating the important principle of dressings a burn 'to give the most perfect protection and comfort'.

Boyer (1814) classified burns into three degrees: Erythema: Blistering leading to superficial ulcers; and eschar. Dupuytren divided burns into six degrees: First erythema or superficial phlogosis which blanches on pressure. Second cutaneous inflammation, with the loss of
Specifics can be advantageous in addressing burn wounds. Third the destruction of a portion of the papillary body. Fourth the disorganization of the whole dermis to a subcutaneous cellular tissues. Fifth the formation of eschars of all the carbonization of the whole thickness of the burnt part.

Hobra (1861) treated burn patients in warm water baths. Believing that Dupuytren's last degrees of burns were of academic interest only, he returned to three degree classification (Hobrae 1866) erythema with swelling and pain; blistering with petichial hemorrhages; eschar and devoid of sensations.

The 19th century presented two basic important principles for the local treatment of burns—skin grafting (Pollock 1871) and open treatment (Copeland Alabama 1887, Stocker 1894 and Reid 1898). However Bouisson had used open treatment about 400 years back, Lusgarten (1871) suggested and Wilms (1901) carried out excision of burnt tissue for the first time but he never grafted the excised area. Juszkovic (1968) revived the idea of excision of burnt tissue and immediate skin grafting thus making a footnote to the contemporary principle of early tangential excision and early grafting.

At the termination of 19th century, the combat was to avoid infection and within the 20th century pathophysiology elucidated causes and indicated methods of systemic treatment of burns, counteracting shock. Neiss (1890) and Tomassoli (1897) introduced the systemic treatment of shock by intravenous saline infusions in severely burnt patients. Brown (1896) and Snee (1905) followed the same suit. But this treatment
became in fourth decade of this century, a routine practice after the remarkable work of Davidson (1926), Underhill (1930) and Blalock (1931). Previously people had been using alcoholic drinks and opium for correcting shock in these patients.

This was the turning point, and since the mid 20th century, an increased understanding of the metabolic, nutritional, immunological and wound healing process have been recognised improving treatment and comfort of burn patients.

Oppenheimer (1906) advocated the use of pector acid therapy and Davidson (1925) Tannic acid in treatment of burns. As a consequence, the popularity of the 'exposure' method rapidly declined and only in 1949 was this treatment revived by Wallace.

Little progress had been made from the ancient time in the local treatment of burns. Man's main concern had been to reduce pain. To achieve this various medicaments from pigeon dung (Aegineta 1535) to enzymatic sloughing agents and antibacterial agents have been used.

Work of Leitbug, Reiss and Artz (1953) indicated septicaemia as primary cause of death in burns and staphylococci as the major organism. With the availability of antibacterial agents against gram positive organism, Pseudomonas emerged as the major organism responsible for sepsis and death. This led other workers to find out antibacterial agents that would penetrate scar. 5% Ag Nog (Moyer), Mefenide (Moncrief), Silver sulphadiazine (Foxe Jr & others), cerium nitrate (Williams & N. Nonofo, Sonn & Tandan) are the topical agents which minimize bacterial counts over the wound.

**Burn Wound Coverings**

Thermal injury results in striking anatomic, metabolic and physiologic disturbances which prejudice survival of burn
patients. In major degree of burns, the patient is exposed to death from shock or toxæmia due to absorption of poisons from the injured surface or from loss of skin covering or from exhaustion due to the long continued fight for recovery or due to their combined effects.

Autogenous skin grafting is the best covering material, but it has its own limitation in terms of limited supply, unfitness of the already shocked patient for skin grafting. To overcome this problem, various biological and synthetic covering materials, either for short period till the healing of the wound or permanent in place of lost skin, have been suggested by different workers.

Biological Dressings:

Homografts

Pollock (1871), Girder (1881) and Shade (1881) were the pioneers in this field. Ivanova (1890) suggested the advantageous use of foetal skin over the adult because the infantile tissue possessed more "energetic vitality".

Dago (1952) introduced the use of Postmortem allografts as temporary biological dressings. The use of fresh Postmortem allografts have added measurably to the successful treatment of burns. As temporary biological coverings, they decrease fluid and protein loss, diminish infection and prepare granulating surface for the application of autogenous grafts.

Brown (1952) used allografts as emergency dressings for burn. He stated that the skin may be removed even days after death if the cadaver has been placed in cold storage.

Bade (1959) and Norries (1960) observed that the homografts have organizational and debride-mental effects on healing wound.
Healed epidermis shows alteration in the architecture and the dermis contains aedematous connective tissue in 2nd degree burns where homografts are not used. When the homografts are used, the healed epidermis shows normal architecture with recognizable basal layer and normal collagen bundles in the dermis (Miller 1967).

James O’Neill Jr (1967) used temporary homografts coverage over open wounds including 2nd and 3rd degree burns. Such coverage was of distinct benefit following eschar separation in burn injury. Shamas et al (1978) reported the same results.

Allografts skin, besides being satisfactory, biological dressings have their own limitations. Cadavers suitable for skin donations are limited in number. Buxton (1970) has estimated 6 physician hours and hospital cost of $225 per patient needed to use cadaver homografts.

**Xenografts**

The use of Heterologous tissue as a temporary dressings for full thickness skin defects was largely a result of the difficulty of obtaining adequate amount of homografts. Brown, Burleson and Lewis have shown that the adherence of allografts and xenografts is similar. Heterograft provides a readily available, easily stored and sterilized dressings in contrast to homografts. The only xenograft in common use is pig skin. Variable results have been reported with porcine xenograft coverage of donor sites and partial thickness burns ranging from early re-epithelialization to conversion of full thickness skin loss. Salisbury (1973) has reported that incorporation of xenograft tissue on healing donor sites occurs in 35 percent of cases. There appears to be no significant difference in the effectiveness of fresh
The most striking advantage with the procine xenograft is that of immediate and lasting pain relief. Xenograft has most of the properties of the ideal skin substitute. A viable xenograft is antigenic but the dead is not. The major problem is the propensity to digestion by wound collagenase and subsequent infection.

**Collagen Sheets**

Collagen, fibrous protein is present in many animal tissue like skin, muscle and bone. Its structure and immunologic chemistry are well characterized and antigenicity can be altered. It also possesses a haemostatic effect; when implanted in pure from over a living animal tissue, no antigenic reaction is seen. Air borne infection is minimised and fluid loss is prevented. Thus it is ideal raw material to be used in burns. It is derived from serous and sub serous layers of freshly slaughtered cattle intestine and are commercially available in 10 cm x 15 cm size packed in cylindrical glass tubes containing ethylene oxide which acts as sterilizing agent.

Sinha (1972), Shankar (1975) and Gupta et al (1976) used collagen sheets as primary cover material in the management of burns. Elhans et al (1978) used sheets as biological dressings in 22 patients and reported its role in prevention of infection and in increasing the rate of healing. Jain et al (1976) reported the similar findings.

**Synthetic Materials**

The problem associated with biological materials provided an impetus to search for synthetic material with ideal properties for a skin Prosthesis. Earlies research work was a sulfonamide film (Pickrell, 1942). Many of these materials
adhere by intrapment of coagulum in the interstices of the material. Silicon polymer membrane is the best material available because it is elastic, durable and the water vapour transfer characteristics can be controlled by varying the thickness. Kornbrey et al (1972) have used thin silicon membrane bonded to cotton gauze for temporary skin substitution but it lacks elasticity and creates non uniform pattern of adherence. Other materials are modified polyvinyl chloride or similar plastics which provides more elasticity and water vapour transfer characteristics. (James et al, 1975; Lamhey et al, 1977; Towsend, 1977). The material is deep but the greatest disadvantage is lack of adherence to wound itself. These materials seem to have great promise as a temporary skin substitute for short time applications.

**Amniotic Membrane**

The quest for a cheap, painless and easily available biological dressing having most of the properties of the ideal skin substitute led people to use amniotic membrane.

Amniotic membrane is the inner of the two fetal membranes having two surface. Its outer surface is separated from the deciduous of the maternal uterus by chorion. The inner surface is in contact with the content of the amniotic sac i.e. the fluid and the fetal body.

A section of amnion under a light microscope shows 5 different layers:

1. **Epithelium**: This is a single layer of non ciliated cuboidal cells having a role in the exchange of fluids and electrolytes between amniotic sac and mother.

2. **Basement membrane**: This is a narrow band of reticular tissue at the base of epithelial layer adherent to it.
3. **Compact layer**: It is a dense acellular layer deep to basement membrane. It is firmly adherent to the basement membrane and cannot be separated from it normally.

4. **Fibroblast layer**: It is composed of fibroblast net work present in mesh of reticulum. Fibroblast and hofbour cells (Macrophages) are normally present in this layer. It forms a considerable part of the thickness of amnion.

5. **Spongy layer**: It is composed of extra embryonic coelomic reticulum. It is capable of great distension. It contains mucous in its structure which enables the layer to alter its shape.

The normal thickness of the membrane is 1/50 to 1/2 m.m. which may increase to as much as 2-5 cm, due to change in the amount of the mucin and fluid within the spongy layer and the capability of loose connective tissue to great variation.

**The Chorion**: The chorion consists of four layers, these are from within outwards:

1) **Cellular layer**: thin layer consisting of an interlacing fibroblast net work.

2) **Reticular layer**: It forms the majority of thickness of the fibrous chorion and consists of a reticular net work, the fibres of which tend to be parallel. Nodes present on the fibres at those places where branching occurs.

3) **Pseudo basement membrane**: It is a layer of dense argyrophil connective tissue that is firmly adherent to the reticular layer above.

4) **Trophoblast**: It forms 2 to 10 layers of trophoblast cells in contact, on their deeper aspect, with material decidua. This layer contains obliterated chorionic villi.
Amnion does not have any blood supply at term as well as not at any stage of pregnancy.

Nerve Supply: Nerve supply have been described in the amnion but the findings have not been confirmed.

Lymphatic Vessels: Possibilities that amnion contains lymphatic vessels have been suggested by some workers.

Embryonic Development:

In the human embryo, development of amnion begins during the transformation of morula to blastocyst stage at the time of implantation, about 7-8 days after fertilization. There is separation from inner all mass of the germ disc at the periphery of the ectodermal layer of polyhedral cells. "Amniogenic cells", to form a slitlike cavity, with appearance of primary extra embryonic mesoderm. The amniotic mesenchyme is derived epithelium becomes separated from the primitive trophoblast. Amniotic mesenchyme is derived from the primary extra embryonic mesoderm of the trophoblast.

Finally the foetal membrane consists of an inner amniotic membrane consisting of a single layer of ectodermally derived amnion cells. Collagen rich mesenchyme of 5-8 cells in the thickness. The chorion consisting of compressed trophoblastic tissue of the chorion leave and mesenchymal tissue.

Immunology

Various studies have been carried out to observe the facts concerning this important aspect of the subject.

Amnion when implanted to its own newborn infant "takes" as a permanent graft. Neovascularisation has not been seen. Nourishment of the graft appears to be by simple diffusion.

When implanted subcutaneously as allograft, results were similar to autograft for first 14-17 days. Later on these grafts were transformed into hyalinised substances. Similar results
When the mesenchymal surface was placed towards the host "superior take up" or "fixation" was seen, while on placing amnion towards host, little fixation was noticed at the end of 72 hours. No neovascularization was observed in any case.

The allograft amnion membrane appeared viable histologically after 21 days. When it was placed in pelvic cavity after pelvic exenteration, granulation tissue and fibroblast tissue activity was markedly inhibited as compared to control cases.

When allograft amnion was implanted intra peritoneally in the experimental animal in whom the caecum was damaged and contaminated, prevention of adhesions and gradual disintegration of membrane without any host response occurred.

These experiments suggest that antigenicity of amnion is low and no violent host reaction noted yet.

When the chorion was placed over host tissue as autograft and allograft neovascularization and migration of host cells was observed. It provokes strong cellular and less antibody response. The tissue had an accelerated rejection phenomenon in 72 hrs, being rejected by 11th day. This rejection phenomenon can be delayed by high doses of progesterone.

**Clinical and experimental application**

In 1910, Davis, a senior medical student reported attempts at grafting pieces of the lining of the amniotic sac onto granulating wounds.

Sabella (1912) for the first time treated a burn patient, applying amniotic side of the membrane towards the wound because of its ectodermal origin and reported results as: Absence of infection, reduction in pain and rapid re epithelization. While Burger (1937) used amnion in construction of vagina; De Roth (1940) reported its successful use in conjunctival repair.
Chao et al. (1940) and Johnstone (1941) suggested the use of "amnio plastin", a preparation made by the immersion of amnion in alcohol for fixing, followed by drying in sheets and boiling in water for 20 minutes for sterilization. This was washed in normal saline before use. This fixed, dead amnion preparation was employed as a covering to prevent adhesions during operations on the brain. Later Pinkerton (1942) and others used it for mobilizing adherent tendon fixed by adhesions.

The credit for using living amnion as a homograft in burns goes to Kubani, a Hungarian. He also used sterile amnion to cover raw surface of the peritoneum after separating adhesions. His contemporary, Henson (1950) used amniotic membrane in the management of chronic skin ulcerations with smooth side of amnion facing wound. He observed that granulation tissue never raised above margins which occurred when plaster of paris was used to cover the wound.

Douglas (1952) used heterografts (human skin to chorio-allantoic membrane of chick and human amnion to chick amnion) and reported successful results. He further applied heterografts in experimental dogs using amnion and chorion over the wounds. Healing was quick and infection was less and he noticed that dressings separated readily from the surface, grafted with amnion, leaving a shiny, dry and pinkish surface. The chorion grafts were more opaque and more salmon pink coloured.

Douglas (1951) also did the first successful homografting in an extensively burnt patient.

Douglas and other (1954) transplanting homologous and heterologous chorionic membrane to the mouse concluded that homologous and heterologous grafts of foetal membrane are
tolerated as covering for open wounds as well as homologous grafts of skin and for two or three times the duration of the homografts of the skin. The transplants of foetal membrane served as viable transplants, capable of cellular division and epithelization. Their experiments indicated that chorionic grafts may be more useful as temporary coverage of wounds than are homplastic skin grafts.

Jullian (1956) using the amnion membrane over old infected flame burns successfully, suggested the amniotic membrane as dressing material for emergency measurement of trauma.

Hanson (1956) treated patients with intermittent claudication, grafting amniotic membrane and found favourable results. Similar results were reported by Rowling (1958) Unger-Hamilton (1958) and Hansen (1960).

Pigeons (1960) contention in using amniotic membrane was that it is similar to skin as it arises from ectoderm of the foetus.

Massee et al (1962) reported favourable results with amniotic membrane grafts in experimental dogs where he carried out pelvic exenteration. They showed that there were few adhesions and dense scar formation.

Dins (1965) in his study using various layers of foetal membrane on burnt patient, observed that following application of membrane, there was immediate relief in pain. However, he did not report any allergic reaction, crust formation was there over grafted areas which remained dry and uninfected until their pealing off (9th to 20th day) following grafting. He used amnion, amnion with chorion and chorion alone and did not find any difference after using these
Dino's next study (1966) was to find out the best preservative. He preserved the amniotic membrane in different sterile solutions at 4°C Temp. viz., 1. Normal saline, 2. Benzal bromine solution (1:1000 dilution), 3. Sodium hypochlorite (1:40 dilution) in normal saline solution, 4. Saline solution (500 cc) with 1 million units of crystalline penicillin and 1 gr. of streptomycin sulphate or Kanamycine. Amniotic membranes were preserved from the fresh stage to one month and were used in treatment. Bacteriological examinations of membranes were done on 1st, 3rd, 7th and 30th day. He labelled solution of sodium hypochloride 2. Solution of crystalline penicillin with streptomycin sulphate or Kanamycine sulphate as best.

Galask et al. (1970) observed the presence of several factors in the human amniotic membrane which are known to be antibacterial. They have clearly shown inhibition of number of bacterial genera by amniotic fluid, even by amniotic fluid supplemented by casein hydrolysate.

Trelford et al. (1970) confirmed the observation of Douglas that the mesenchymal side towards the host provided more consistent 'take' while using amnion as auto and allografts in experimental sheep.

Robson et al. (1973) studied the effect of human amniotic membranes on the bacterial population of infected rat burns. They concluded that compared to human skin, the amniotic membrane was more effective at decreasing the bacterial counts in the burn wounds. They sought specific antibacterial substance using invitro techniques with amniotic membrane homografts but no such substance was found, and proposed that the invitro antibacterial effect seen is due to achievement of a biologically closed wounds by the membrane, thus allowing
the host's own defence mechanism to deal with the bacterial population as did other biological dressings.

Robson et al (1973) treated 50 patients having open wounds with full thickness amniotic membrane. Over full thickness defects, the amniotic membranes were placed on the wound with the chorion against the granulating surface, changing them at every 48 hours. Before applying membrane and while changing the membrane, specimens were taken for bacterial analysis. In partial thickness wounds, membranes were applied with chorion facing the wound and in some, amnion facing the wound. They observed that amniotic membrane adhered to all wounds regardless of their depth. In all of the full thickness wound, the bacterial count decreased and the decrease was equal to allograft skin and superior to xenograft skin.

Colocho & others (1974) observed the effect of human amniotic membrane in clinical and experimental studies. Amniotic membranes were used to cover the split thickness donor sites and partial thickness burn wounds. In rats, open wounds were covered with amnion and in some, it was placed in sub-cutaneous pockets. None of the biopsy specimens showed vascularization of the amnion by the host. The membrane became disintegrated forming a dry eschar over the surface of burn that gradually became detached as reepithelialization proceeded beneath the membrane.

Bapat and Kothari (1974) successfully used living amniotic membrane grafts for the restoration of the floor of the mouth in the patients of advanced cancer of the tongue, following radical total glossectomy. They observed that the healing was rapid with induction of metaplasia in a
fore-night. Grafted area showed hardly any scarring. The floor remained flexible and pliable.

Trelford- Souder and other (1977) used the amniotic membrane to cover the raw area after pelvic exenteration and innumerated advantages as: Readily availability of low antigenic tissue, lack of intestinal complications, reduced fluid and protein loss, technically easy method, reduced hospitalization and reduced number of intra-abdominal adhesions.

Rose B (1979) used membrane as biological dressing's over burns, pointing out that amnion adheres more firmly than any other biological dressing. Agarwal V K (1982) reported similar results.