REVIEW OF LITERATURE
REVIEW OF LITERATURE

Even with the recent advances in medical sciences problem of burn management is still a threat to the life. These injuries either due to fire, radiation, electricity or chemical agents have grown more important socially, medically, and economically with every coming year due to change in the life style of mankind.

As far back as 1500 B.C. Papyrus had used cow-dung topically. Ancient Indian literature shows that Sushtra used mixture of butter with red achre or the bark of a fig tree. He also debrided severe burns with loose skin and flesh. Around 5th - 6th centuries, B.C. the Egyptians were treating burns by incineration and a mixture of gum, goat's hair and milk from a lady who has given birth to a son. Chinese and Japanese were using tinctures and extracts made from tea leaves around 430 B.C.

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

In Ancient Rome three methods were used
1. Celsius suggested mixture of honey and bran.
2. Fliny and Elder suggested exposure method.
3. Galen suggested local application of vinegar or wine over burn surface.

In 7th century A.D. Paulus of Aegina used the various emollient preparations. Rhazes (AD 580-923) had been using white ointment composed of white lead, oil of roses and wax. He also used ice cold water locally. 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 syndactyly in the burnt hands.

Ambrose Pare (1517-1590) suggested ointments for the treatment of burn wounds. Claws (1591) used 5 different complex preparations on the different parts of body area involved in burn. He 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.

Gaihelmus Fabricus Hildanus (1607), the father of German surgery, described 3 degrees of burn according to depth. L. Heister (1683-1758) classified the burns into four degrees according to depth and including time faster.

David cleghron (1792) used the Vinegar and Chalk poultices locally. Edward Kentish (1797) suggested the pressure dressings to relieve pain and to stop blisters.
Sir James Earle (1799) suggested the ice cold water and told that it acts as a good analgesic and prevents oedema formation. Syme (1827) suggested the use of dry cotton wool dressing with firm pressure.

Boyer (1814) classified burns into three degrees: erythema, blistering leading to superficial ulcers and eschar. Dupaytren (1932) classified the burn into 6 degrees according to depth of involved tissue.

1. Erythema or superficial philogosis which blanches in pressure.
2. Cutaneous inflammation, with the loss of epidermis and the development of vesicles filled with serum.
3. The destruction of a portion of the papillary body.
4. The disorganization of the whole dermis to a subcutaneous cellular tissue.
5. The formation of eschars of all the carbonization of the whole thickness of the burnt part.

He also described the 4 periods during the natural course of burn injury.
1. Period of irritation
2. Period of inflammation
3. Period of suppuration
4. Period of Exhaustion

He also described the gastrointestinal haemorrhage in the burn cases. Later Curling (1842) recognized gastric and duodenal ulcers as a cause of gastrointestinal haemorrhage in burn cases.
Passowant (1853) suggested the use of saline bath, Copeland (1877) suggested the exposure method. Edward Clark Davidson (1894-1933) used tannic acid on burn surface in 1925. He suggested that these agents decreases the fluid loss, relieve pain and produce a clean scar. Later on Meclure in 1944 found this agent to be hepatotoxic and as a cause of many death in burn patients. Therefore, its use abandoned since that time. Addridge (1933) suggested the use of gention violet as escharotic agent on burn wound for cleaner scar. 5% silver nitrate was also used.

In 1942 Allen and Koch of chicago suggested the use of petroleum gauze piece to apply over burn wound with strict immobilization and abandoned the use of pressure bandages.

Wallece of Edinburg (1949) reintroduced the exposure method for treatment of burn in England. Pulaki, Artz and Blocker also started this method in united state of America in 1950. Later on other surgeons also accepted the same remedy with view that development of crust and scar provides physiological covering to burn wound and in this way reduce the disadvantages of raw areas in burns.

Leidbug, Reiss and Artz (1953) pointed out that septicaemia was the primary cause of death in burns and many of the deaths were due to staphylooccci. As improved antibiotics against the gram positive organism become
available, pseudomonas sepsis became very common and was considered as primary cause of death. To reduce the growth of bacteria under the burn surface different agents like 5% Ag₂O₃ (Moyer) Nefemide or sulfamylon (Moncrief), Silver sulfadiazine (Fox C.L.Jr. Rappole B.J. Stanford W, (1969) cerium nitrate (William W. monoto, Som N. Tandon), Cerium nitrate and silver sulfadiazine (Fox C.L.Jr. 1975) were suggested. But these topical agents are effective merely in control of bacterial population of burn wound, not in sterilization.

**Burn Wound Coverings**

Effects of thermal injuries are due to immediate traumatic shock and loss of skin covering leading to anatomic, metabolic and physiological disturbances. Traumatic shock is not a significant contributing factor to mortality and morbidity due to burns now-a-days, because up to 80% of burnt area shock can be managed successfully. The main cause of morbidity and mortality is toxaemia due to absorption of poisons from the injured surface or from loss of skin covering leading to abnormal loss of heat and body constituents, and invasion of micro-organism. So the new concept of burn care is restoration of impaired barrier. Autogenous skin grafting is the best coverage material amongst all, suggested till this time, but it has its own limitations, in form of limited supply. Unfitness of the already shocked
patient for surgical procedure involved in skin grafting and the refusal of patients or his attendant on religious, sentimental or ethical ground. To overcome this problem, various biological and synthetic coverage materials wether for short period till the healing of wound or permanent in place of lost skin, has been suggested by different workers.

**Biological Dressings**

**Homografts**— About a century ago, Pollack (1871) applied the first homograft on a burn patients. In 1881 Girdner treated a lightening burn with the skin from a suicide victim. Skede used skin from amputation specimen as well as from cadavers with the limit of 24 hours. Ivanova in 1890 stressed the use of foetal skin as a homograft on burn surface because of its more energetic vitality.

In 1952 Dago of Italy used the post mortam allografts as a temporary biological dressings. He noted that cadaver skin is useful when obtained especially while it is still viable and where it is possible to preserve the tissue till the time of application. He proved skin viability by determining the tissue oxygen uptake in the warburg apparatus. The skin was preserved at 3°C in physiological solution. The oxygen consumption of cadaver skin was noted unaffected upto 16 hours after death.

Brown (1952) reported the use of allografts as emergency dressing for burns. He stated that skin may be removed even days after death if cadaver has been placed in cold storage.
Eade (1958) and Horries (1960) observed that the homografts have organizational and debridemental effects on healing wound. They pointed out that the count of bacteria on granulating infected wound decreases within 2 hours of homografts application.

In 1967, Miller et al studied the use of frequently changed skin homografts to promote healing in non healing infected ulcers. He observed that in 2nd degree burns if healing occurs without homografts, epidermis shows the alterations in the architecture and is disorganised and dermis contains oedematous connective tissue, while if healing occurs under the coverage of homografts, the epidermis shows normal architecture with recognizable basal layer and normal collagen bundles in the dermis.

Sharma et al (1978) used the preserved homografts in 25 cases out of which 15 were of the superficial burns. The period of normal survival of grafts over wound surface was 11 days if recipient and donor's blood groups was same. Where blood groups was not same, it was 13 days.

Allograft skin, even being satisfactory biological dressing, have their limitations. It has limited supply and personnel are required for procurement. Boxter (1970) has estimated that six physician hours and hospital cost of $225 per patient are needed to use cadaver allografts.
**Xenografts:**

Because of limitations in the use of homografts, xenografts came in use. Brown, Burleson and Tavis have shown that the adherence of allografts and xenografts is similar. Hetrografts provides a readily available easily stored and sterilized dressing in contrast to homografts. In 1960, canine skin has been used by Switzer et al. Porcine skin is the xenograft material of choice however, and Bromberg et al and Elliott and Hoehn have used pig skin. Variable results have been reported from early re-epithelialization to conversion of full thickness skin loss. Salisbury (1973) has reported some poor results, when they used this type of dressing on donor sites, with increased inflammation and delayed repair following treatment. Comparative experiments have shown no significant difference in the effectiveness of fresh compared with fresh frozen or frozen irradiated porcine skin.

The most striking advantage with the porcine 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 Sheet**

Collagen is a fibrous protein which is present in many animal tissues like skin, muscle and bone, when implanted in
living animals, tissue in pure form, it does not produce any antigenic reaction. Collagen sheets are derived from serous and subserous layers of freshly slaughtered cattle intestine. These are available in 4" x 6" size and packed in cylindrical glass tubes containing ethylene oxide which acts as sterilizing agent.

Sinha (1972), Shanker (1975) and Gupta et al (1976) used collagen sheets as primary cover material in management of burns. Gupta and Chaturvedi (1974) used it to cover donor areas. Thukral and Gupta (1976) have used collagen material in repair of hernia and to cover surgical defects. Alhans et al (1978) used sheets as biological dressing in 32 patients and reported its role in prevention of infection and in increasing the rate of healing. Jain et al (1976) reported the similar findings.

The effects of collagen sheet are, a) prevention of air born infection, b) minimising fluid loss, c) promoting formation of healthy and pink granulation tissue. But it is expensive material and is not available at every centre.

**Synthetic Materials**

Pickrell (1942) worked on sulfonamide film. Many of these materials adhere by intrapment of coagulum, in the interestices 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. Kornberg et al (1977) 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; Lamkey et al 1977; Townsend, 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**

It is most economic and freely available biological dressing, have most of properties of ideal skin substitute.

Amnion is the inner one of two foetal membranes. Its inner surface is in contact with contents of amniotic sac i.e. amniotic fluid and foetal body. Its outer surface is separated from decidua of maternal uterus by chorion.

It has following anatomical parts:

a- Placental amnion: Lines inner aspect of placenta
b- Reflected amnion: Lines rest of amnion
c- Dependent amnion: Overlies the internal os of cervix.

The amnion which is normally 0.02 to 0.5 mm in thickness, consists of five layers. These are from with-in outwards

(A) Epithelium (B) Basement Membrane (C) Compact layer
(D) Fibroblast layer and (E) Spongy layer.
(A) **The Epithelium**: This is the inner most layer in contact with the amniotic fluid. It consists of a single layer of cells which are usually cuboidal but may be columnar over the placenta or flattened to pavement cells on the reflected amnion. They normally contain a single nucleus and are densely adherent to the underlying basement membrane on their free, normally convex surface. They are surmounted by microvilli to form a brush border. The cells contain a number of vacuoles of varying size.

(B) **The Basement Membrane**: This is a thin layer composed of a network of reticular fibres and is well marked over both the placental and the reflected parts of the amnion. Short blunt processes from the bases of the epithelial cells inter digitate with similar process that arise from the basement membrane.

(C) **The Compact Layer**: This consists of a complex network of reticular fibres but devoid of cells. This layer which is probably the strongest of the amniotic layers is rarely thickened by oedema and it appears to resist, to some extent penetration by leukocytes.

(D) **The Fibroblast Layer**: This is the thickest layer of the amnion. It is composed of a loose fibroblast network embedded in a mass of reticulin. The cells occasionally show phagocytic activity.

(E) **The Spongy Layer**: The tissue of the extra embryonic celom is compressed between the amnion and the chorion to form the
spongy layer. These are wavy bundles of reticulin with branching fibres having triangular shaped nodes at the junctions. A few isolated fibroblasts are present in this layer. This layer frequently becomes oedematous and as such, accounts for the increase in thickness. It permits the amnion to slide upon the underlying chorion.

The amnion has no blood supply or lymphatic system. The nerve supply is still not confirmed.

**Embryologic Development:**

The 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 cell mass of the germ disk at the periphery of the ectodermal layer of polyhedral cells, "amniogenic cells" to form a slit like cavity with appearance of primary extra embryonic mesoderm. The amniotic epithelium becomes separated from the primitive trophoblast. Amniotic mesenchyme is derived from the primary extra embryonic mesoderm of the blastocyst.

**Immunology:**

Concerning this aspect following studies has been carried out when amnion was implanted to its own new born infants, It has been demonstrated that 'take' was as permanent graft. Amnion, not formed attachment to host tissue and mesenchyme cells, was kept towards host in those studies. Neovascularization was not occured.
Nourishment of graft appeared to be by simple diffusion.

When subcutaneous implantation was done of allograft amnion, results were same as autograft for first 14-17 days. Latter on these grafts were transformed into hyalinized substances. Only mild infiltration of round cells was observed by 20-30 days. When the amniotic membrane was used as biological dressing for surface defects as allografts and autografts same result has been observed. Superior 'take' or 'fixation' was noted when mesenchymal surface was placed towards host. When amnion was placed towards the host, little fixation was noticed at the end of 72 hours. No neovascularization was observed in any case.

When the allograft amniotic membrane was placed in pelvic cavity after pelvic extenteration, it was recovered at the end of 21 days and appeared viable histologically. Granulation tissue and fibroblastic tissue activities were markedly inhibited as compared to control cases.

When allograft amnion implantation was done, in intra peritoneal cavity, in the experimental animals, in whom the caecum was damaged and contaminated, prevention of adhesions add gradual disintegration of membrane without any host response were observed.

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

The chorion consists of four layers. These are from within outward: (F) Cellular layer (G) reticular layer (H) pseudo basement membrane and (I) trophoplast.

(F) Cellular layer: This is a thin layer consisting of an interlacing fibroblast network. It is frequently imperfect or completely absent from the chorion when examined at term.

(G) Reticular layer: This forms the majority of the thickness of the chorion and consists of a reticular network. The fibres of which tend to be parallel. Nodes are present on the fibres at those places where branching occurs. A few fibroblasts are present together with many macrophages.

(H) Pseudo basement membrane:

This forms a type of basement membrane for the trophoblast. It is a layer of dense connective tissue that is firmly adherent to the reticular layer above and which sends anchoring and branching fibres down into the trophoblast.

(I) Trophoblast: It consists of from two to ten layers of trophoblast cells in contact, on their deeper aspect, with maternal decidua. This layer contains the obliterated chorionic villi.

The chorion contain branches of umbilical vessel in the substances of its reticular layer but does not receive any capillary blood supply from them.
When the chorion was placed over host tissue as autograft, neovascularization and migration of host cells was observed, causing host versus graft rejection phenomenon. It provokes strong cellular and less antibody response. The tissue had an accelerated rejection phenomenon in 72 hours and being rejected by 11th day. This rejection phenomenon can be delayed by high dose progesterone.

Antigens have been demonstrated by a number of workers on trophoblastic cells. Recent studies shows that chorionic tissue may have 3 usual antigens, two from mature placenta and one relatively specific for chorion.

**Clinical and Experimental application**

John Staigu Davis, at John Hopkins University was first person to report the attempts of grafting pieces of lining of the amniotic sac on granulating wounds in 1910.

For the first time in 1913, Sabella reported the use of amniotic membrane on the raw surfaces caused by burn or ulceration. He applied the amniotic side of amniotic membrane towards the wound because of its ectodermal origin and observed reduced pain, rapid re-epithelization and absence of infection.

Brindeau (1935) and Burger (1937) used amnion for construction of artificial vagina. Burger successfully used the amnion also in repair of experimental dural defects in rabbits, dogs and cats. De Roth (1940) reported successful use of amnion in conjunctival repair.
Chao et al (1940) used the preparation of amniotic membrane known as "amnioplastin". The amnion is immersed in alcohol for fixsing, 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 used to prevent the adhesions following the craniotomy for head injury. He applied it over lacerated pia mater, dura mater and temporal muscle were stitched over it. There was no adhesions in any case. The amnioplastin gradually disappeared after 10 days. Mucoid material was present as remanent of membrane up to 20 days. There was slight evidence of mucoid material. On 30th day, there was no gross or microscopic evidence of mucoid material.

Histologically, after 10 days, there was no evidence of foreign body reaction or organized adhesions. The growth of fibroblasts beneath and above the membrane was only reaction to vanishing foreign body. Remains of amnioplastin was present as amorphus substance without cellular structure. After 30 days, dura and pia defect was completely filled and there was no evidence of adhesion bands even over injured brain. Pinkerton (1942) reported the use of amnioplastin to prevent the adhesions between flexor tendons and their sheaths.

Kubani (1941-43) a Hungarian used amnion in various conditions like burn, traumatic skin loss, in prevention of
intra abdominal adhesions. He also tried it for repair of enterocutaneous fistula successfully in one case.

In 1950 Henson reported the use of amniotic membrane in the management of chronic skin ulceration and kept the smooth side of amnion for contact with wound surface. He found the granulation tissue never raised above margins as compared with other management of wound like coverage of wound under plaster of Paris.

Douglas (1952) reported the use of homografts of foetal membrane as a biological dressing over burn wound surfaces. In 1954, he used the human membranes on chorio-allantoic membrane of chick. He also studied the use of amnion and chorion as surface covering. Healing was quick and infection was less and he noticed that dressing 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.

By tissue chamber technique he observed that plasmic and haemic circulation remained active growing in perimeter of membrane transplant.

Jullian A. Sterling (1956) reported the successful use of the amniotic membrane over old infected flame burns. He suggested the amniotic membrane as dressing material for emergency management of trauma.
Hensen (1960) reviewed 100 cases of peripheral vascular disease treated by amnion implantation to the fat of thigh deeply. The patient were kept in bed for four days and normally discharged on 6th day without symptoms. Similar results were reported by Rowling (1958) under Hamilton (1958).

In 1960, Pigeon reported the application of amniotic membrane in burn cases with full aseptic precautions and observed following effects:

(a) Immediate effects:

1. Reduction in pain
2. Antibiotics were used only in development of complication.
3. Dressing were generally found quite dry.
4. Healing of wound was rapid and complete.

(b) Delayed effects:

1. No discoloration
2. Minimal scar tissue formation

He also stated that amniotic membrane undergoes change similar to which occur in cornified cells.

Massee and colleagues (1962) reported the use of foetal membrane to replace the parietal peritoneum in experimental studies in dogs undergoing pelvic exenteration. Animals were killed after 59 days, there were very few adhesions and pelvic cavity was filled with dense scar tissue. Human trials were failed at that time.
Dino (1965) pointed out the use of amniotic membrane in burn cases. In favour of his study following points were suggested.

1. It is the homograft which most closely resembles the skin being a direct continuation of foetal integuments along the umbilical cord.

2. It can be easily available and have minimal contact with the maternal blood.

3. It is highly stretchable and can cover a wide surface.

4. It is fairly strong to be handled.

5. It is available with negligible cost.

Dino (1966) preserved the amniotic membrane in following types of solutions.

1. Sterile normal saline solution

2. Lensal koniom chloride (1:1000 dilution) in sterile saline solution.

3. Sodium hypochloride (1:40 dilution) in sterile saline solution.

4. Saline solution (400 cc) with 50,000 units of crystalline penicillin & 1 gm streptomycin sulphate.

5. Saline solution (400 cc) with 1 gm Kenamycin sulphate.

The preserved grafts were kept in refrigerator at 4°C temperature. Bacteriological studies at regular interval were done to test efficacy of used preservative. Amniotic membrane
were preserved from fresh stage to one month and used in the treatment. From the bacteriological studies of preserved membrane which was done on 1st, 3rd, 7th, 14th and 30th day, he concluded that

(1) Solution of sodium hypochloride;
(2) solution of crystalline penicillin and streptomycin;
(3) kanamycin sulphate; were found to be best. On histological examination, cellular necrosis was seen from 2nd day after preservation. However according to kirschbain and hernaudaz (1963) cellular elements of amnion survived even after 45-60 days. Grossly the membrane remained intact and appeared thickened.

Galask et al (1970) observed the several antibacterial factors in amniotic membrane.

Trelford and associates (1972) reported the preliminary results using amnion alone as an autograft and allograft in sheep. They reconfirmed, the Douglas observation that more consistent 'take' occurs if mesenchymal side was applied toward the host.

Robson et al (1973) on experimental rat burns, concluded that, compared to human skin, the amniotic membrane was more effective at decreasing the bacterial counts in the burn wounds. No specific antibacterial substance was found but it was 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. Similar observation was also found by Martin (1972) on experimental rats.

Robson et al (1973) treated 50 patients having open wounds with full thickness amniotic membrane. The foetal membrane was placed on the full thickness burn wound with the chorion against the granulating surface. The dressing was changed at every 48 hours. Specimens were taken for bacterial analysis at every change of dressing before and after. In partial thickness wounds, membrane were applied with chorion facing the wound and in some amnion facing the wound. They observed that foetal membrane adhered to all wound 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.

Tralfoerd and associates (1973) reported the use of amnion alone in full thickness fresh surgical wounds as biological dressing after radical volvectomies and groin dissections.

Colocho & others (1974) in the clinical and experimental studies, in 65 patients with split thickness donor sites, in 42 patients with partial thickness burns amniotic membrane was applied over the open wounds and in subcutaneous pockets in rats. He observed that if amnion was left exposed over partial thickness wound, it will disseminate and regeneration will begin by 3rd day and completed on 7th day of underlying epithelium of skin. No evidence of allergy.
rejection or neovascularization of amnion was noticed in any case. In experimental studies, amnion buried beneath the flap and continued to remain there, cellular integrity even after 5 days, suggesting the presence of plasmic and haemic circulation. None of the human donor site biopsy or India ink injection in experimental animals had shown communication with the host.

In 1974 Bapat and Kothari reported the successful use of living amniotic membrane graft for the restoration of the floor of the mouth in the patient of advanced cancer of the tongue following radical total glossectomy. Clinical observations showed that healing was rapid with induction of squamous metaplasia in 15 days without foul odour which is usually associated with skin grafts.rafted area showed hardly any scarring. The floor remained flexible and pliable.

Marilyn trelored - sauder and other (1977) reported the use of amniotic membrane to cover the raw area after pelvic extenteration, and its benefits: readily available tissue of low antigenicity, technically easy method for managing pelvic raw surface, reduced protein and fluid loss, reduced hospital stay and reduced intra-abdominal adhesions.

In 1978 they also reported the use of allograft: amniotic membrane for control of intra abdominal adhesions.

Bose B (1979) reported the use of amniotic membrane over burn wound as biological dressing and stressed that
amniotic membrane adheres more firmly than other biological
dressings. He recommended the use of amniotic membrane
especially in developing country.

Mehta N. N. et al (1983) also recommended the use of
human amniotic membrane as a biological dressing in burn
wounds.