

Use of Amniotic Membrane, Amniotic Fluid, and Placental Dressing in Advanced Burn Patients

Niranjan Bhattacharya

37.1 Introduction

Fire-related deaths are a neglected public-health issue in India. These deaths are mostly due to kitchen accidents, self-immolation, domestic violence, and even dowry-related abuses. Death associated with burn injuries primarily depends on the age of the patient, the percentage of the body surface burned, and associated smoke-inhalation injury apart from the role of preexisting diseases such as diabetes, hypothyroid, clinical and subclinical tuberculosis, arthritis, nutrition level, etc.

In a report published in *Lancet*, 163,000 fire-related deaths were reported in 2001 in India, and 106,000 of these were of women, mostly between 15 and 34 years of age. The average ratio of fire-related deaths of young women to young men was 3:1.¹

Wound sepsis and chronic wound formation are two frequent associates of burn injuries, which enhance the mortality and morbidity of burn patients. Chronic wounds are defined as wounds that have not proceeded through orderly and timely reparation to produce anatomic and functional integrity after 3 months. Burns cause dynamic injuries that may progress over the first 2–3 days to months; therefore, frequent reassessment of the wound is required to ensure optimal management. Many burns are not uniform; the depth of the burn varies from one area to another. Proper surgical management of burn wounds before colonization of the eschar by bacteria and septic liquefaction, which otherwise are inevitable, is very important.

All burn wound types have the potential to become chronic; additional modifying factors such as venous or arterial insufficiency, local-pressure effects, and existence of preexisting diseases as mentioned earlier may retard the process of healing.

Controlling wound infection and covering the burn wound with bio-friendly substances are two essential parts of burn treatment. An ideal skin substitute should be used to cover the wound for dressing purposes. This skin-covering agent for burn wounds should be easily available, nontoxic in nature with elastic and adherence properties, cost little so that it is affordable to all sections of the society, and be universally available. It should act as a semipermeable membrane for essential substances such as oxygen and other micronutrients, and ideally, prevent the entrance of bacteria.

Unfortunately, none of the available artificial skin substitutes, in a truly global perspective, fulfills the ideal requirements for burn injury. The objective of the present study was to examine whether a readily available hospital waste, that is, pregnancy-specific biological substances (PSBS), could be used as a suitable and effective biological dressing in reducing burn wound sepsis in the treatment of thermal burns among all age groups.

37.2 Materials and Methods

Initially, 97 patients were randomly recruited for the present study of the utility of PSBS in the burn management. Of these, 33 patients, who did not agree to the PSBS protocol, were transferred to the burn unit of a tertiary-care hospital for treatment.

The rest, i.e., 64 burn patients with 26–76% of total body surface area burnt calculated on the basis

N. Bhattacharya
Department of General Surgery, Obstetrics and Gynaecology and Clinical Immunology, Advanced Medical Research Institute, Gol Park, B.P. Poddar Hospital and Vidyasagore Hospital, Kolkata 700034, India

of the famous rule of nine, were enrolled in the present study for PSBS treatment (1999–2009).

The patients were asked for a voluntary written consent before carrying out the proposed procedure. The ethical committee of the hospital had approved this research project earlier.

The area affected included both partial to complete thickness and at times the adjacent soft tissue injury in case of thermal burns. Patients suffering from chemical burns or burn injuries in sensitive parts of the body such as the genitals or face were also included in this study from 1999 to 2009.

After admission, all the cases were treated with (1) normal saline for initial removal of the dirt and debris followed by (2) application of freshly collected placenta's raw surface at the site of the wound (Fig. 37.2), after which there was (3) sprinkling of copious amounts of freshly collected clear amniotic fluid at the site of the burn injury (after 5–10 min), and lastly, the application of amniotic membrane (4) at the burned wound site (amniotic or the fetal side of the amniotic membrane in case of superficial or partial thickness skin burn for early epithelialization, and maternal attachment site or the chorionic site in deep burn to improve circulation through angiogenesis-supporting cytokine content of the chorionic site of the membrane) (Figs. 37.3 and 37.4). This amniotic membrane was kept in the amniotic fluid, which was freshly collected from consenting donor mothers who were VDRL, hepatitis B and C, and HIV (1&2)-negative and had undergone cesarean section.

Routine prophylactic antibiotics (ceftriaxone 1 g twice daily intravenously and gentamycin 60–80 mg twice daily through intramuscular route, metronidazole 200 mg thrice daily initially through intravenous route with intravenous isolyte fluid) were routinely given to all patients who consented for the present study 1999–till date. Subsequently, there was switching to oral broad spectrum antibiotics for all cases. Routine multivitamins, minerals, and trace elements were also prescribed to all the patients. Other drugs such as long-acting beta-blocker (Inderal) 40 mg/day in case of adults were also routinely prescribed. Weight is considered as the basic guideline for the dosage calculation of drugs in different age groups. In case of diabetes, or hepatic and renal or cardiac problems, appropriate additional drugs and insulin (Act Rapid variety in standard sliding scale is followed till the healing process is stable) was prescribed in consultation with a senior physician along with stoppage of the aminoglycoside antibiotic in general.

Once the healing process started stabilizing, physiotherapeutic protocol was prescribed by the senior physiotherapist of the hospital. The wounds were inspected regularly to look for exudation, offensive odor, or any other clinical local or systemic sign of infection.

Amniotic membrane dressings, however, have one major drawback, i.e., of rejection of the graft, and hence only serve as a temporary dressing unless the different stem cell components of the freshly collected amniotic fluid, amniotic membrane, placenta, and their intrinsic cytokine network participate; they participate and assist the healing process of the host. All the steps of clinical improvement were meticulously noted with photographic evidences and histological evidences to understand the steps and etiopathogenesis of the reparative process involved with the application of PSBS in burn wounds.

37.3 Results and Analysis

There are many treatment options available for burns and the resulting wounds.² In the present study, as mentioned, human pregnancy-specific biological waste materials alone were used; these include the placenta, the amniotic fluid, and the amniotic membrane. These materials are normally discarded and go to the incinerator, but can easily be collected from the obstetric/labor room/operation theater and effectively used in case of burn wound.

In the present series, 64 burn patients (male 24, age 2–96 years, mean 36 ± 5.4 years and female 40, age 7–68 years, mean 32 ± 5.7 years) with 26–76% of total body surface area burned, as mentioned earlier, were enrolled for PSBS treatment (1999–2009).

All the patients were treated with prophylactic antibiotic (splint as and when necessary depending on the site and proximity of the joint) and other support regime including early ambulation and physiotherapy in addition to the specific treatment with the pregnancy-associated biological substances (PABS) as mentioned earlier in the material and method section. The membrane prevents heat and water loss from the wound surface and acts as a barrier against bacterial contamination, thus aiding the healing process and reducing morbidity. Another clinically significant and important property

of the amniotic membrane is its ability to offer marked relief from pain.

Among the 64 cases enrolled for the study, 24 (37.5%) were males and the remaining 40 (62.5%) were females. The burnt area varied from 26% to 76% of the surface area as per standard calculation of the rule of 9. The prevalence is noted below. There were six patients in the less than 10 years (9.37%) age group, while 16 cases (25%) were in the range of 11–20 years. There were 22 (34.37%) in the age group of 21–30, 11 (17.18%) in the age group of 31–40, and 4 (6.25%) in the age group of 41–50 years respectively, while 2 (3.12%) were in the 51–60 age range, 2 (3.12%) in the age group of 61–70, and 1 (1.56%) in the 71 and above age group.

The thermal injuries were caused by hot water in 20 (31.25%) cases, followed by exposure to direct flame (kerosene or other cooking material), comprising 28 (43.75%) cases. Suicide attempts were the cause in 10 (15.62%) cases. Burn was caused in the other six cases (9.38%) due to accidents involving coal or cigarettes (smoking inside mosquito nets, after the intake of alcohol with the risk increasing with drug addiction), attempts at dowry killing or other attempted murders.

In the present group, no fatality was encountered. Follow-up for (up to) 6 months continued after the total healing of the scar. Problems of keloid and hypertrophic scars were noted in six (9.38%) cases only. Contrary to our expectations, post-burn leucoderma was not encountered in a single case. Some degree of hypopigmentation at the burn scarring site was seen in 14 cases (21.87%), which gradually reverted to the normal skin color and architecture within the 6 months follow-up period. Another important complication was post-burn contracture, which was noted in six cases (9.38%). Again, this problem was treated with appropriate release incision and fresh amniotic membrane application. Partial thickness skin autograft was avoided in the treatment regimen. The most difficult patient, an attempted suicide victim with 76% burns, who was treated with pregnancy specific biological substances only, is presented here with photographic and histological evidences. The clinical photographs of improvement are shown in Figs. 37.1–37.7.

Wound healing is a very complex process that is tightly regulated to achieve wound repair. The process has three important components, i.e., inflammation, proliferation, and maturation. Following the initial tissue injury, inflammatory mediators known as cytokines are released from the injured tissue cells and wound



Fig. 37.1 Suicidal attempt with kerosene resulting 76% partial and complete thickness skin burn treated with pregnancy-specific biological substance dressing



Fig. 37.2 Photograph showing the application of placenta at the burnt site



Fig. 37.3 Photograph showing the application of amniotic membrane at the burnt site



Fig. 37.4 Photograph showing the application of amniotic membrane at the burnt site



Fig. 37.5 Photograph showing the post-amniotic membrane application of Vaseline gauge to cover the site and to keep it moist



Fig. 37.6 Photograph of the same patient after 6 weeks



Fig. 37.7 The same patient after 6 months of periodic dressing with the pregnancy-specific biological substances as per text schedule

blood clots, after which the inflammatory phase initiates. Then, the proliferation stage begins several days after injury. In this stage, the platelet degranulation activates the coagulation cascade, and the resultant fibrin clot serves as a scaffold for the proliferation phase of wound healing. During the proliferative phase, fibroblasts in the extracellular matrix increase and synthesize the tissue components, such as proteoglycans, fibronectin, and collagen. New vessels and epithelium are formed as rapidly as possible to maximize the tissue replacement dynamics. All wound cells are maximally active and are sensitive to factors that regulate cell proliferation and protein biosynthesis. All the cells proliferate, and metalloproteinases are simultaneously released into the extracellular fluid to activate a matrix breakdown process. The balance between tissue degradation and biosynthesis permits remodeling of the provisional tissue and its ultimate repair as seen in Figs. 37.8–37.11.

In order to augment the complex process of wound healing, investigators who have used amniotic membrane as a temporary dressing material with the belief

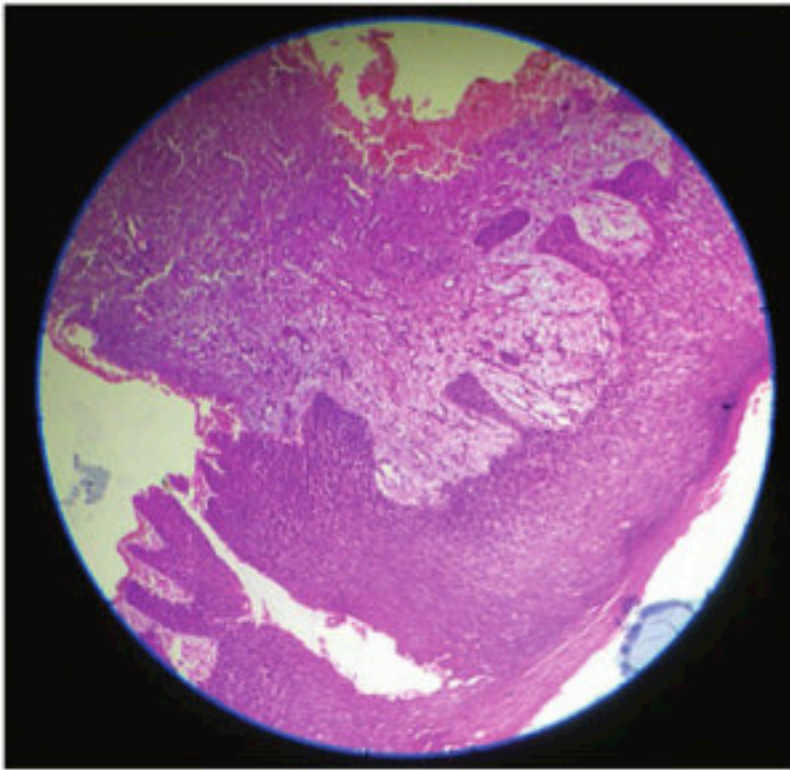


Fig. 37.8 Showing the histological features of the skin stained with hematoxylin and eosin of the same patient under scan power. Wound healing is a very complex process that is tightly regulated to achieve wound repair. The process has three important components, i.e., inflammation, proliferation, and maturation. Following the initial tissue injury, inflammatory mediators known as cytokines are released from the injured tissue cells and wound blood clot, after which the inflammatory phase initiates

that human amniotic epithelial cells do not express HLA-A, -B, -C, and -DR antigens, or beta 2-microglobulin on their surfaces.³ However, there has been some recent controversy regarding this topic. It has been noted that amniotic membrane epithelial cells display some degree of antigenicity and immunogenicity as allografts due to the presence of some (though definitely less than adult) MHC class I and II antigens.

On the other hand, viable human amniotic epithelial cells (HAECs) have been shown to elicit beneficial effects on secretion of anti-inflammatory factors. It has been seen that topical application of culture supernatant from HAECs leads to profound suppression of suture-induced neovascularization in the cornea. In addition, expression of interleukin (IL)-1 beta mRNA was suppressed in cauterized cornea where amniotic membrane was applied. These results suggest that amniotic cells are a source of soluble anti-inflammatory factors that suppress inflammation.⁴

In spite of the scientific controversy, the clinical and practical impression suggests that this dressing is extremely effective. It speeds up the healing process and reduces pain. The relative ease of procurement

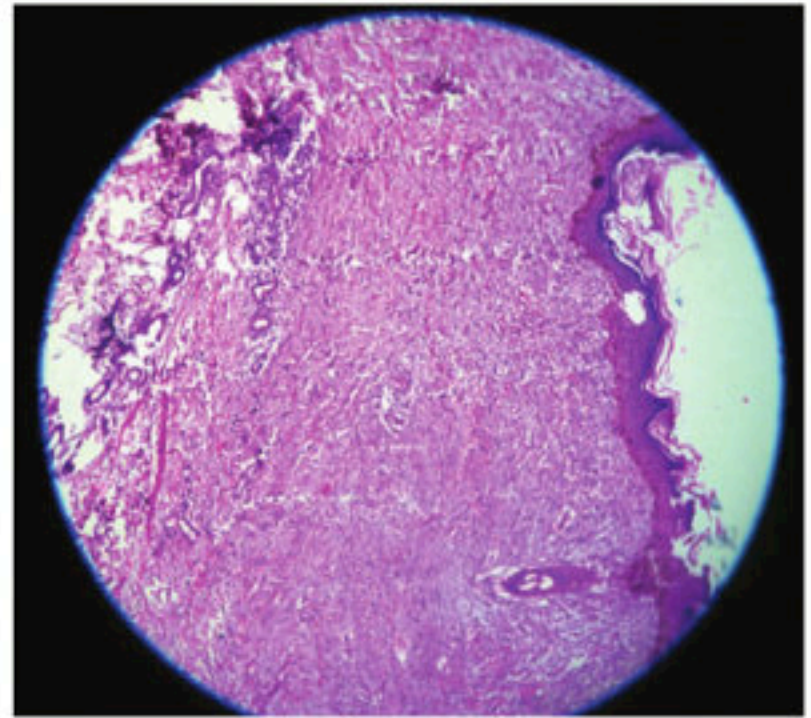


Fig. 37.9 Showing the histological features of the skin stained with hematoxylin and eosin of the same patient under low power suggesting steps of regeneration. During the proliferative phase, fibroblasts in the extracellular matrix increase and synthesize the tissue components, such as proteoglycans, fibronectin, and collagen. New vessels and epithelium are formed as rapidly as possible to maximize the tissue replacement dynamics. All wound cells are maximally active and are sensitive to factors that regulate cell proliferation and protein biosynthesis. All the cells proliferate, and metalloproteinases are simultaneously released into the extracellular fluid to activate a matrix breakdown process. The balance between tissue degradation and biosynthesis permits remodeling of the provisional tissue and its ultimate repair as seen

and preparation and its low cost and easy availability projects the amniotic membrane as an ideal temporary skin substitute for burn wounds. Amniotic membrane has been utilized in various studies to cover the burn wound dressing for less exudation of protein and electrolyte, as well as for its bio-friendly nature and hypoantigenic qualities apart from the cytokine support it lends to wound healing.

This substance when used as a biological dressing not only lowered the hospital's and patients' costs, but also significantly reduced the rate of infection and pain. Sometimes recipients experienced a problem of odor. This was a normal response of amniotic membrane sloughing due to rejection by the host immune system or this may be due to *Pseudomonas aeruginosa* or *Staphylococcus aureus* infection.⁵

The odor becomes offensive at times depending on the type and concentration of the bacteria and the condition of the wound. This is a condition suggesting a change of the dressing with a fresh one. After the first

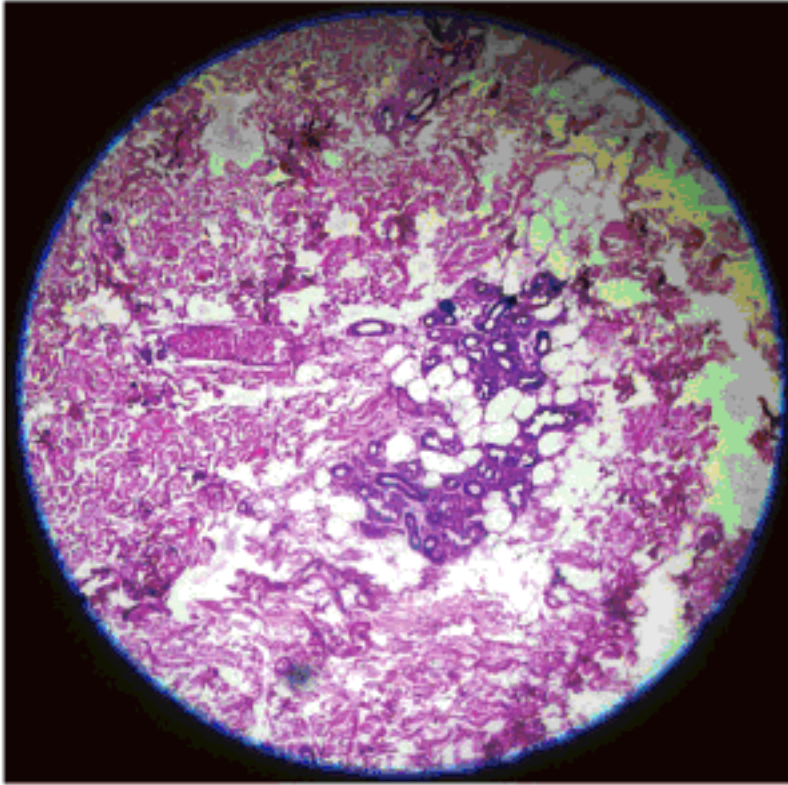


Fig. 37.10 Showing the histological features of the skin-stained examination with hematoxylin and eosin of the same patient under low power suggesting steps of regeneration with cell nest-like structure of fetal origin, which is actively helping the regeneration process

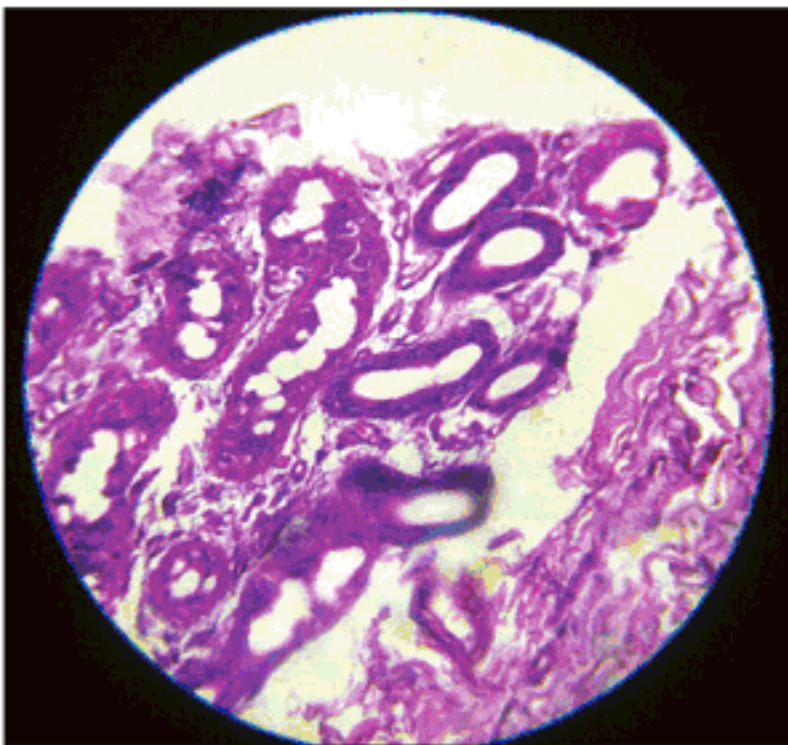


Fig. 37.11 Showing the histological features of the skin stained with hematoxylin and eosin of the same patient under high power. The slide is suggesting steps of regeneration of the skin

dressing on admission till the period when the acute infection of the wound is reasonably controlled, patients were advised to keep the burn wound open and to stay inside mosquito nets. Since the wounds were visible, the amniotic membrane was changed when necessary,

i.e., when the integrity of the dressing was lost, which was approximately every fourth day to the seventh day. In infected cases, the same procedures of inspection, debridement, antibiotic treatment, and change of biological dressings were carried out whenever necessary. After recovery, the patients were followed up on a weekly basis for up to 4 weeks, and on a monthly basis for up to 3 years. Any signs of infection or abnormal scar formation were recorded.

Another investigator, Dr. B. Bose, in 1979 claimed that the use of amniotic membranes was cost-effective, even better than other allografts and heterografts, and a useful temporary biological dressing.⁶ Further, it does not need special facilities, and can be easily employed even in peripheral hospitals with modest infrastructure facilities. The use of such material has been advocated for use in developing countries because of its promising good results.⁷ Amniotic membrane patch has even been found to be effective in the treatment of acute corneal alkali burns.⁸ The mechanisms underlying the effectiveness of amniotic membrane as an aid in the treatment of burn wounds have been postulated by a number of researchers. Apart from its physical properties in reducing water and heat loss, Kim et al.⁹ have suggested that the mechanism responsible for the rapid healing observed is due to the inhibition of proteinase activity, thus reducing the inflammatory responses by reducing the infiltration of polymorphonuclear leukocytes. Benefits claimed for this procedure of biological dressings include rapid healing with cosmetically satisfactory results (i.e., lack of scarring) and the avoidance of autografting. The success of the procedure is ascribed at least in part to the biological activities of growth factors secreted by the donor fibroblasts that are transiently present in the wounds. The basis for use of all of these biological and artificial skin substitutes is that they create a moist environment, which has been shown to improve the rate of healing under a variety of clinical and experimental conditions. Another renowned plastic surgeon and author, Dr. JB Lynch, strongly justified the use of amniotic membrane as a suitable biological skin dressing in 1979.¹⁰

Another researcher of repute Dr. Eaglestein reviewed the role of a variety of occlusive and semi-occlusive artificial skin substitutes for control of wound sepsis and early healing.¹¹ Despite their wide use in many developed countries, none of these artificial skin substitutes serve as ideal dressings besides being very expensive, especially for routine use.

It should be mentioned here that amniotic membrane was actually used as a burn dressing for many years before anyone thought of the stem cell components of the amniotic membrane and other pregnancy-specific biological substances. However, no investigator had ever used freshly collected bacteria-free amniotic fluid, which is a rich source for mesenchymal stem cells and as known, has a bacteriocidal property due to the presence of properdin-like materials embedded in it.

37.4 Discussion

Burn is an injury vis-à-vis inflammation at the site of contact of heat, cold, electricity, chemicals, light, radiation, or friction. The result and the degree of burn depend on the involvement of the underlying skin and the subcutaneous tissue, muscle, bone, blood vessel, and other adjacent tissues.

Pain is severe in case of burn due to exposure of the free nerve endings and also because of the cytokines and other toxins produced at the site of the burn where vascularity and tissue autoregulation are affected. Apart from local metabolites' participation, direct and profound injury of the nerves also plays an important role. In general, thermal injuries to living tissues occur as a function of temperature and duration of exposure to a heat source,¹² which leads to complications such as shock, infection, electrolyte imbalance, and respiratory distress. All these complications cumulatively may at times have a fatal implication.

The survival of a burn patient is largely dependent upon prompt and efficient wound healing and control of infection, which also prevents wound contracture. Sequel due to infection by deadly *Pseudomonas* plays a very negative role in the mortality of burn patients after the first week of the initial burn injury. Appropriate first aid limits progression of the burn depth and influences outcome. Therefore, assessment of area and depth is crucial to formulating a management plan. Burn depth may progress with time, so reevaluation is essential.

Different biological dressings to cover the burn wounds in order to assist the healing process have been attempted to treat burns over the centuries. Biological dressings are classified as Group a: *Biological* (homologous skin) – glycerinized pigskin with or without silver or aldehyde treatment variety, human amniotic membrane (amniotic side for epithelialization and chorionic

side for neovascularization). A variety of techniques utilizing different heterografts from lizard to frog skin, then to homografts, and ultimately autografts were used to cover the wound in case of burn and to prevent infection. Further developments made possible by improvement of storage techniques in the 1970s, involved the wider employment of preserved allografts; Group b: *Synthetic skin substitutes* like polyurethane and hydrocolloids, polyurethane film, acrylamide film, and hydroxyethyl methacrylate with polyurethane films; and finally Group c: *Bioengineered skin substitutes*, which are derived from and with the association of biological materials such as collagen and silicone sheets, also used in different centers of the world to cover the burn wound and assist the healing process as a whole. Covering the burn wound prevents loss of water, electrolytes, and proteins and also prevents the dispersion of heat etc.

The Chinese system of traditional medicine has appreciated the wound healing property of the placenta or its different extract preparations, apparently for thousands of years. In a relatively recent published article, investigators have suggested that the application of human placental extract (HPE) in rats both at topical and intramuscular routes (2.5 mL/kg) caused significant lowering of wound size ($P < 0.05$), wound index ($P < 0.05$), and the number of days required for complete healing ($P < 0.01$); significant gain in tensile strength ($P < 0.01$); appreciable increase of tissue DNA, total protein, and collagenesis were observed in the HPE-treated group. The authors concluded that human placental extract systematically helps collagenesis leading to potent healing of wounds.¹³ In another article, it was shown that placental-extract gel and cream were effective topical agents for chronic non-healing wounds. In addition, there was less pain and discomfort during dressing change with the placental-extract cream, which the investigators therefore recommended for topical application in chronic non-healing wounds.¹⁴ In a different study, another group of investigators conducted a comparative study on the efficacy and safety of topical application of a purified extract of human placenta (placentrex gel) versus povidone iodine for its wound-healing potential after orthopedic surgeries. The authors concluded that purified placental extract and povidone iodine have comparative wound-healing effects.¹⁵

Apart from the biological healing property of the placenta and its extract, another pregnancy-specific

biological substance, i.e., amniotic fluid, also plays a very important role if used in burn injuries. It has to be remembered in this connection that nature washes the vaginal canal of the mother with the amniotic fluid before the birth of a baby in all species in order to prevent infection to the baby. This is Nature's proof of the sterile and bacteriocidal properties of the amniotic fluid.

In addition, the amniotic fluid possibly possesses a lubricating effect due to its higher viscosity and protein and cellular composition, and may also have a reparative effect due to the progenitor cell/stem cell component in it, i.e., the epithelial and the mesenchymal stem cell population. The stem cells of the amniotic fluid are capable of differentiating into multiple lineages; this may be valuable for therapy. In this context it is relevant to mention that a group of renowned investigators have reported in *Nature Biotechnology* on the isolation of human and rodent amniotic fluid-derived stem (AFS) cells that express embryonic and adult stem cell markers. Undifferentiated AFS cells expand extensively without feeders, double in 36 h and are not tumorigenic. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells were noted to be broadly multipotent.¹⁶

Use of amniotic membrane for covering the burn wound decreases oozing from the wound site after debridement and thus decreases the need for blood and albumin transfusion, and causes less electrolyte imbalance. Amniotic membrane also possesses some antibacterial characteristics, namely, bacteriostatic effect on gram-positive bacteria due to the lysozyme content. Amniotic membrane also helps in the epithelialization of the wound.

One important contributor to this book, Prof Andrew Burd of the Chinese University of Hong Kong, a renowned plastic and reconstructive surgeon, has calculated the global production of placentas, amniotic fluid, and the amniotic membrane which are noted in his article in this book. This will convey a picture of the massive global wastage of such materials when we throw them into the dustbin for eventual destruction through incinerators. Before analyzing the utilization potential of pregnancy-specific biological substances in burn wound, it is necessary to give a brief account of the substance-compositions and properties, and the contemporary scientific advances in the field of stem cell research which relate to these important substances.

Placenta:	Weight	50 million kg
	Volume	60,000 m ³
Amniotic membrane:	Area	15 million m ²
Amniotic fluid:	Volume	60 million L

37.4.1 Amniotic Epithelial Cells

Amniotic epithelial cells are isolated following the stripping of amniotic membrane from the chorion by trypsin digestion.¹⁷ Such procedure allows for selection of relatively homogeneous cell suspension, which attach to plastic in in vitro culture. Contrary to mesenchymal stromal cells (MSC-type cells), amniotic epithelial cells need the addition of epidermal growth factor (EGF) to Dulbecco's Modified Eagle's Medium (DMEM) supplemented with forward scatter (FSC). Cells grow throughout two to six passages, displaying typical epithelial morphology. Both the expression of CD90 antigen and HLA-A, -B, -C (human leukocyte antigens) increase in culture – the initial expression levels are too low for using these antigens as identification/selection markers for freshly isolated epithelial cells. Among the other markers, cells express molecular markers of pluripotent stem cells (SRY-related HMG-box gene SOX-2, octamer-binding protein 4 Oct-4, and Nanog). Contrary to placental MSCs, epithelial cells do not express the Cd49d marker.

Both the molecular markers, and differentiation experiments suggest that amniotic epithelial cells are pluripotent, having not only adipogenic,¹⁸ osteogenic¹⁹ chondrogenic, but also myogenic, and cardiomyogenic, neurogenic,²⁰ pancreatic,²¹ and hepatogenic²² potentials.

37.4.2 Mesenchymal Stromal Cells from Amniotic and Chorionic Regions

Amniotic MSC are isolated from amnions at any gestation stage when the placenta is fully developed. The amnion must be carefully dissected from fetal membranes to avoid the presence of maternal cells. The most popular isolation protocol is based on the two-step digestion procedure, first with trypsin, and subsequently

with collagenase. The resulting plastic-adherent cells can be expanded in in vitro culture similarly as in adult bone marrow MSCs.²³ Cells at the second passage stage (at least) express mesenchymal, but not hematopoietic markers. Animal in vivo experiments confirmed that human placental MSC, transplanted into animals, are able to migrate into various organs: bone marrow, thymus, spleen, lung, liver, brain and kidney. In vitro, these cells are able to differentiate into cartilage, bone,²⁴ fat tissue,²⁵ skeletal muscles,²⁶ heart muscle,²⁷ epithelium, nerve cells, or pancreatic islets.²⁸

37.4.3 Placental Tissue and the Umbilical Cord is an Important Source of Fetal Mesenchymal Stem Cell

Placental tissues are considered an attractive source of cells with considerable phenotypic plasticity as well as immunomodulatory properties, including mesenchymal stem cells and amniotic membrane-derived epithelial cells.

Mesenchymal stem cells or mesenchymal stromal cells have been isolated from the amniotic and chorionic regions of the fetal placenta, as well as from the umbilical cord.

Amniotic-, chorionic-, and umbilical cord MSC are usually isolated by mechanical peeling or removal of the tissue followed by enzymatic digestion. After primary culture and sub-cultivation of fibroblast colony-forming units, evolved adherent cells exhibit a pattern of antigen expression (including CD105+, CD90+, CD73+, CD34-, CD45-, Oct-4+), which is not different from that expressed by UCB-MSC, with the exception of the embryonic marker, Oct-4. Placental MSC has the intrinsic property to differentiate into cells of the adipogenic, chondrogenic, osteogenic, and skeletal myogenic lineages after exposure to the appropriate stimuli. These cells also exhibit a robust hematopoiesis-supportive function.^{29,30} Moreover, UCB-MSC exhibit a hematopoietic-supportive function.³¹

Having given the background, it is important to point out that fetal stem cells, like their adult counterparts, are able to differentiate into several tissues, and migrate to the site of tissue injury. They may form the new cells replacing those destroyed by injury or illness, and also modify the healing process. Fetal cells have greater proliferation and differentiation potential

(longer telomeres and telomerase activity). The cells collected from the placenta depend on the region of collection: amniotic epithelial cells from the amniotic epithelial region or amniotic mesenchymal stromal cells (MSC) from the amniotic mesenchymal region, to name a few.

Of these cells, amniotic and chorionic mesenchymal stromal cells represent characteristics similar to in vitro growth characteristics, exhibit surface antigen expression, and differentiation potential. Both cell types are hematopoietic markers – negative (CD34⁻, CD45⁻), HLA-DR⁻, and positive for markers attributed for MSC: CD73, CD90, and CD105. The characteristics of the amniotic epithelial cells are somewhat more complex – they are able to proliferate shorter than MSCs in in vitro culture, proliferate only in higher densities in presence of epidermal growth factor (EGF), and change the expression of selected markers (HLA-A, -B, -C, -CD90) depending on the culture time. This latter phenomenon may suggest that the amniotic epithelial cells are a heterogeneous population being subsequently selected to higher homogeneity by culture conditions.

37.4.4 Feto-Maternal Cell Traffic in Pregnancy and Its Long-Term Consequences

Whether any transplacental cell traffic is a cause of inflammation or is just a bystander in the regeneration of damaged tissue, is a matter of ongoing debate.³²⁻³⁴ Analyzing murine syngeneic and allogeneic pregnancies, Khoshroterhani and colleagues showed that fetal microchimerism is a naturally occurring phenomenon leading to detectable levels of mononuclear cells in several maternal tissues, such as lungs, heart, spleen, kidney, and bone marrow.^{33,34} It has been shown for human and murine pregnancies that levels decrease after delivery. This phenomenon indicates that the maternal immune effector cells shift back to their normal allo-response. Although the Khoshroterhani group was unable to detect microchimeric fetal cells in maternal mouse brain during pregnancy, other researchers have found a relevant proportion of fetal progenitors that obviously were able to cross the blood-brain barrier during pregnancy.³⁵ The cells even increased in number during a period of 4 weeks post partum and adopted

a local phenotype such as perivascular macrophage-, neuron-, astrocyte-, and oligodendrocyte-like cell type. The levels of fetal microchimerism were increased in brain injuries suggesting an active role in tissue regeneration. Whether this is just associated with inflammation and phagocytosis of damaged tissue, or the formation of truly new neurons, needs further attention. Similar uncertainties exist for an animal model on murine maternal hepatic injury.³⁶ Here too, higher levels of microchimerisms were detected after chemical compared to surgical laceration, with increasing levels between 4 and 8 weeks after injury.

It has also been shown that fetal progenitors could persist within the maternal blood and tissue over decades.³⁷ These CD34+ and CD34+/CD38+ fetal progenitors are capable of differentiating into functional T and B cells, and may help in the regeneration process on the maternal system.

If we come back to our study, it is worth remembering that the skin offers a perfect model system for studying the wound-healing process, which involves a finely tuned interplay between several cell types, pathways, and processes. The dysregulation of these factors may lead to wound-healing disorders, resulting in chronic wounds, as well as abnormal scars such as hypertrophic and keloid scars.

In the present work, the possibility that mesenchymal and epithelial stem cells supplied by the amniotic fluid and placental and membrane sources, are acting as a cell therapy source in combination to augment the process of healing, is strongly postulated. This is possibly the basis for the augmented strategy of healing with the application of pregnancy-specific biological substances as dressing material. The therapeutic effect of the stem cell seems consistent with both the paracrine function and the transdifferentiation. Systemic and micro-milieu factors appear to dictate the fate of implanted stem cells.

Researchers must begin to focus upon a few basic critical issues: the modulation of the systemic and microenvironment for stem cells in order to augment stem cell survival and transdifferentiation; the underlying mechanisms of stem cell therapy and the fate of stem cells; and the differentiation into specific cell types as per the local demand or other terminal cell populations with synchronizing and favorable paracrine functions.

Earlier scientists used stem cells in different routes, namely, subcutaneous or intravenous route. In the

present study, the transdermal route, which is seen as deficient, was used for fetal and neonatal-studded PSBS (as burn dressing) with good effect in burn victims. This is the clinical validation of the fetal and neonatal stem cells-studded pregnancy specific biological substances, which is used through the deficient transdermal route in burn victims.

In the earlier paragraphs, we have discussed in brief the major advances in the field of PSBS and its potential regenerative impact through the progenitor or stem cells found in it. This property has made them a unique category on the tissue used for regenerative biology.

The present article is a report on the simultaneous and judicious utilization of the chorionic (for angiogenesis) and amniotic membrane (for epithelization) and is a clinical validation of molecular advances in the field of stem cell biology in case of burn victims. The utilization of bio-friendly amniotic fluid with intrinsic bacteriocidal property for the preservation of the fresh placenta and membrane instead of traditional normal saline, and also dressing with amniotic fluid, not only reduces the unnecessary utilization of costly antibiotic cream in dressing or tulle formation, but also helps in the prevention of the emergence of bacterial resistance and mixed infection due to opportunistic fungi and resistant bacterial infection including anaerobes, bacterioids, and other trouble-making organisms.

Furthermore, it may be noted that there has not been a single case of mortality among all the patients treated with PSBS so far in our study. In the quest to understand the mechanism for this positive outcome, histology and hematoxylin eosin staining was done from the burn patient's skin, which revealed groups of cells that looked as though a cell nest was migrating to the site of burn injury to assist proper epithelization. There was no unusual inflammatory cellular infiltrate or feature suggesting graft versus host reaction.

The present research group has had similar experiences vis-à-vis different sets of experiments on the survival of the fetal human leucocytic antigen (HLA) randomized tissue graft in subcutaneous space of different hosts, without the help of immunosuppressives.³⁸⁻⁴³

This study possibly hints at the transdermal route of attachment, migration, and subsequent assistance on the host's reparative process by the stem cells supplied to the site of burn wound by the freshly collected amniotic membrane, amniotic fluid, or the placenta, collectively known as PSBS for positive outcome.

37.5 Conclusion

The skin offers a perfect model system for studying the wound-healing process, which involves a finely tuned interplay between several cell types, pathways, and processes. As the contribution of stem cells toward tissue regeneration and wound healing is increasingly appreciated, a rising number of stem cell therapies for cutaneous wounds are currently under development, encouraged by emerging preliminary findings in both animal models and human studies.

The present work shows that the proper and judicious use of naturally occurring pregnancy waste material's fluids and membranes not only helps in early recovery and proper epithelization of the wound, but also prevents wound contracture, keloid, and ugly hypertrophic scars in most cases as noted in our preliminary observation.

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