Amniocytes:
cells, neuronal cells and epithelial tissues following established in vitro cell culture protocols. Mesenchymal stem cells that can transdifferentiate into cartilage, bone, heart and skeletal muscles. Inhibitors (TIMP's) to counterbalance the excessive growth. Amniotic membrane additionally contains matrix metalloproteinases and their inhibitors (TIMP's) to counterbalance the excessive growth. Amniotic membrane is a liquid which forms after 2 weeks of fertilization in the amniotic cavity and exists as a bag of water. It is composed of water, protein, lipid, fetal urine and electrolyte. Its main role is to help in the active transport of sodium and chloride, proteins and water through the chorion amniotic membrane and the embryo’s skin. The volume of the fluid increases to around 270 to 400 ml between 16 to 20th week of pregnancy and about 800 ml in the 24th week. At times, the volume reduces to around 600 ml. This fluid may be used for karyotyping and genetic or molecular prenatal diagnosis.

Important properties of PSBS:
Amniotic Membrane:
The use of amniotic membrane in cases of burn injuries has been documented since 1910 with the variable degree of success. The advantages of amniotic membrane are mainly its water retention capacity, large in size to cover large wound areas, easy availability, requires simple preparation for wound dressing, can be easily sterilized and is hypo antigenic due to poor expression of HLA-A, B, C DR antigens on their surface. However it is not fully regarded as full immune privileged tissue as some degree of antigenicity and immunogenicity is observed due to the presence of MHC Class I and II antigens. The further amniotic membrane has a structure quite similar to that of skin.

Amniotic fluid is a fluid which forms after 2 weeks of fertilization in the amniotic cavity and exists as a bag of water. It is composed of water, protein, lipid, fetal urine and electrolyte. Its main role is to help in the active transport of sodium and chloride, proteins and water through the chorion amniotic membrane and the embryo’s skin. The volume of the fluid increases to around 270 to 400 ml between 16 to 20th week of pregnancy and about 800 ml in the 24th week. At times, the volume reduces to around 600 ml. This fluid may be used for karyotyping and genetic or molecular prenatal diagnosis.

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The human amniotic fluid contains amniocytes which are a large pool of self-renewal cells, mainly fetal in nature with distinct pluripotent stem cell markers like SSEA-1, 3, 4 TRA-1-60, TRA 1-81.(11, 12) These amniocytes have an extremely complex molecular behaviour and express trophoblastic, ectodermal, endodermal and mesodermal cell, specific regulators.(12) These are embryonic pluripotent like stem cells which are distinct from both embryonic and induced pluripotent stem cells have the propensity to be reprogrammed into a primitive fully functional pluripotent stem cell. (13,14,15,16,17) Amniocytes have a high proliferative capacity like human embryonic stem cells but do not form any tumours and are not immortal. (13)

Amniotic fluid has a rich source of stem cells also known as amniotic fluid-derived stem cells which can grow without feeder layers, has a doubling time in about 36 hours, not tumorigenic in nature including greater self-renewal potency.(19, 20, 21) Lines maintained over 250 populations shows the AFSC’s pluripotent nature which is due to the presence of long telomere ends in these AFSC’s found after normal karyotyping. (22)

Cost and affordability are another important major concern, especially in poor and developing nations. Freshly collected and properly screenred amniotic membrane can be easily applied. In terms of hospital and patient economics, amniotic membrane & amniotic fluid due to its several unique properties can significantly reduce both patient infection control and cost to the hospital.

**Mechanism:**

Wound healing is a complicated process accompanied by three important stages of inflammation, proliferation and maturation with an initial release of different cytokines from the injured tissue. (23) The four distinct and most important features of amniotic membrane are (a) Rapid adherence to the wound bed (b) Balance between angiogenesis by control of mesenchymal stem cells and TIMP’s (c) rapid re-epithelisation process due to the presence of amniotic epithelial cells and (d)Inhibition of protease enzyme and PMN infiltration and secretion of growth factors by the donor fibroblasts. (3)

**Application of PSBS in Ophthalmology:**

Roth and Sorsby (24, 25) in 1940 suggested the role of amniotic membrane application in ocular surgery in the management of second-degree chemical burns of the eye with extremely encouraging results. (26)

Acute or chronic loss of stem cells results in cicatrizizing diseases of the eye including chemical & thermal burns and Stevens-Johnson syndrome (SJS). In such cases, an amniotic membrane in rabbit models has shown reduced vascular growth and preserved corneal transparency. (27, 28) Tsubota and colleagues reported positive results with an amniotic membrane in 7 treated eyes with cicatrical pemphigoid and 2 of the four eyes with Stevens-Johnson syndrome. (29, 30)

Further, Lee and Tseng reported the clinical use of amniotic membrane transplantation in 11 consecutive patients suffering from cornea ulceration between 14 to 17 weeks. Amniotic membrane grafts were sutured at the area of the defect and covered with a bandage. Ten (10) patients healed without any recurrence for the next 6 to 9 months with the exception of one patient due to a presence of other background diseases. (31)

Kruse in 1999, conducted studies with the use of multiple layers of amniotic membrane for reconstruction of deep corneal ulcers where 9 out of 11 patients healed after 1-year follow-up. (32) Hanada et al. used amniotic membrane as space fillers to cover the defect and let it act as a scaffold for the epithelium to grow. The study confirmed successful results in 8 patients with complete re-epithelialisation within 3 weeks time. (33) Chenel used 3 layers of the amniotic membrane including an additional patch to treat neurotrophic ulcers secondary to diabetes, keratoplasty, radiation, HSV keratitis, and neuro-surgery. Twelve (12) patients showed improvement within 3 weeks. (34) Similarly, Rakowska reported prompt healing in 7 patients with perforated corneal ulcers after application of amniotic membrane in 18 patients. (35)

The amniotic membrane in conjunction with beta irradiation and mitomycin has been also used to cover wide areas destroyed after excision of large conjunctival tumors and other lesions. Meller used amniotic membrane to cover such large defects in 47 eyes of 40 patients with observable improvements. (36) The first successful use of amniotic membrane in pterygium surgery was reported by Prabhasawat. (37) Shimazaki used the amniotic membrane in 4 patients with primary and recurrent pterygium with satisfactory results. (38) Further applications of the amniotic membrane along with steroid injection for primary and recurrent pterygia have shown encouraging results thereby promoting it as a useful alternative therapy to conjunctival autograft in pterygium surgery. (39) Amniotic membrane has also shown to exert the positive therapeutic effect on symptoms of bulous keratopathy by Pires. (40) Follow-up of the clinical study further revealed post-operative pain-free post compared to intolerable preoperative application of amniotic membrane. (40)

**Application of PSBS in Burn:**

Fire-related deaths in India are a common phenomenon with Lancet reporting more than 163,000 deaths in 2001 of which 106,000 are young women. Most of these deaths are due to kitchen accidents, domestic violence, self-immolation or even dowry-related issues. (41, 42) In burn injuries, death normally depends on the age of the patient, a percentage of the body surface burnt in the background of other diseases such as diabetes, hypothyroid, tuberculosis, nutrition level, and arthritis. (10, 42) Burn injuries in the background of
other diseases like diabetes or Tuberculosis can also result in the formation of a chronic wound where high morbidity and mortality are observed due to wound sepsis, a disintegration of anatomic and functional integrity. (10) After 3 months, if a wound has still not healed in a timely fashion with a functional integrity then it is termed as a chronic wound. (10) Infection is also a major problem associated with burn-related injuries and can be controlled by dressing with artificial skin substitutes which should be ideally nontoxic, elastic with good adherence property and affordable. (10) It should also facilitate the exchange of micronutrients, oxygen and prevent bacteriological contamination. Unfortunately, none of the skin grafts fulfills the ideal parameters for an ideal requirement for burn injury. (10)

Between 1999-2009, 97 burn patients based on the famous rule of nine were randomly recruited for a study conducted by a research group at Calcutta School of Tropical Medicine. (10) Only 64 (24 males and 40 females, age range: below 10 years to 71 years) patients with 26-76% of burn were treated after satisfying the inclusion-exclusion criteria. These included chemical and thermal burn patients. (10, 42) The process included treating all burn injuries with normal saline for removing the dirt and debris followed by the sprinkling of freshly collected and screened for Syphilis, CMV, Hepatitis B & C, VDRL, HIV 1 & 2 amniotic fluid at the site of the burn injury. (10) This was followed by the application of the amniotic membrane as a biological dressing material. The amniotic or the fetal side is normally used in case of superficial or partial thickness skin burn for early epithelialisation and maternal or chorionic side for improving circulation via angiogenesis. (10, 42) The patients were initially given antibiotics routinely via intravenous injection & later on supplemented with oral antibiotics. In cases of diabetic patients oral anti-diabetic drugs including insulin was prescribed in consultation with senior physicians. (10, 42)

Stabilization of the healing procedures was followed by physiotherapy and rehabilitation. Routine follow-ups were conducted religiously in order to look for any offensive odour, exudation or any other clinical, local or systemic sign of infection. (10) In long-term follow-up studies up to 6 months, no mortality was observed in any of the patients. Six (6) patients reported problems of keloid and hypertrophic scars and the others reported complete healing who were further treated with amniotic membrane and appropriate release incision. (10, 42) Post leucoderma was not encountered in any of the cases. (10, 42) Hypo pigmentation was seen in 14 cases but further follow-up studies showed normal skin colour and architecture.

**Application of PSBS in Leprosy induced gangrene:**
The same group of researchers from Calcutta treated leprosy patients with gangrene by an amniotic membrane in 1999. (6) All the cases were treated with removal of maggots, washed with normal saline for clearing the debris followed by the amniotic fluid as an antiseptic agent after 5 to 10 mins. (6) The superficial or partial thickness wounds were dressed with freshly collected, full screened for HIV-1, 2, VDRL, Syphilis, Hep B,C & CMV amniotic membranes. (6) Patients followed anti-standard leprosy treatment in the meantime. (6) In all the cases healing was observed within 3 months of a follow-up study by a formation of granulation tissues and by re-epithelialisation followed by a further 6 months follow-up. (6)

**Application of PSBS Osteoarthritis:**
The osteoarthritis-related knee problem is a common morbidity observed among older patients. (43) Freshly collected, properly screened amniotic fluid after necessary ethical committee clearance and patient informed consent was injected in 52 OA patients’ between 39 to 82 years of age who had not responded to any standard non-pharmacological and pharmacological treatment before. (44) In this study, amniotic fluid was injected into the joint space with adequate antiseptic and aseptic preparations. It was a 2 arm study where one group received steroid treatment and the other amniotic fluid treatment completion. (45) This better therapeutic efficacy with amniotic fluid was observed till the 4th month. (44, 45) Also, while comparing joint effusion and non-joint effusion groups; it was revealed that in both cases where amniotic fluid was applied, a better positive outcome was observed when compared with intraarticular steroid injection. (44, 45)

**Application of PSBS in paediatric burn:**
5 paediatric cases with varying degrees of burn (age range from 8 to 17 years) were also treated with freshly collected and screened amniotic fluid and fetal skin. (46) All these cases responded positively except two patients who had other conventional treatments from different hospitals. (46)

**PSBS & Cell Therapy:**
Both amniotic fluid and the amniotic membrane have a rich content of different types of stem cells. In myocardial infarction rats, engraftment of amniotic fluid and membrane-derived stem cells has shown to differentiate into myocardial-like cells with increased venous ejection faction. (47, 48) In restoring liver function, AMSC and AF-MSC’s have shown the positive therapeutic effect after local and intravenous injection in animal models. Engrafted AM and AF derived mesenchymal stem cells have shown the increase in serum albumin level in rat models with severe lung disease. (49, 50) 8 weeks engraftment of AMSC’s has also been observed in liver tissue in a hepatic cirrhosis model. (51)

Decellularized AM has been successfully used as a nerve conduit for improving peripheral nerve regeneration in different animal models. AMSC’s have both reported to secrete different brain derived and
neuro-derived trophic factors for regeneration of the central and peripheral nervous system. (52, 53) AM and AF stem cells can help in rescuing ovarian function in rats. The ovarian function improved after injection with AM and AF derived MSC’s through up-regulation of endogenous cytokines and creation of a perfect niche for follicular development. The level of E2 was found to be normal and up regulated with a normal down regulation of the FSH when compared to a normal mice ovary including follicular development was also observed to be normal. (54) In cases of autoimmune diseases like rheumatoid arthritis, human AMSC’s may play a functional role by inhibiting the deposition of collagen-induced arthritis. AMSC treatment in animal models have shown to alleviate pathological damage to joints with a lower arthritis index score and decreased a volume of collagen. Also, decrease in the level of Th1, 2, 17 and NKT cells was observed (55)

**Potentialities of PSBS:**

AFSC’s has the ability and has shown to enhance repair of damaged intestine in necrotising colitis via COX-2 dependent mechanism with improved clinical status, gut structure, and function.(56)

Research on human amniotic MSC’s has shown that different chemotherapeutic drugs like Paclitaxel can be safely loaded onto these cells without much toxic effect. (57) Therefore, these cells can be manipulated as anti-cancer bio-delivery vehicles which can initiate specific cancer cell death by a release of chemotherapeutic drugs with minimal tissue toxicity otherwise observed in chemotherapy and radiotherapy.

**Conclusion:**
The use of properly screened amniotic membrane and its fluid and its fresh application within 4 hours of its collection was first attempted in 1999. Some believe that freshly collected amniotic membrane might have a chance of bacterial and HIV contamination. However, this can be avoided if an amniotic membrane is properly screened for all serological and bacterial tests before its application. This process of freshly collected properly screened amniotic membrane has been utilized for a therapeutic process without any mortality since 1999. The main reason behind the immediate use of the properly screened amniotic membrane is the utilization of its diverse biological properties. Both amniotic membrane and amniotic fluid are rich in growth factors, cytokines, and anti-inflammatory properties and it has been seen used within 4 hours of its collection after proper screening it can enhance and aid the rate of wound closure and healing. A very small number of graft rejection cases, accompanied with the foul odour, were observed which can be attributed due to the amniotic membrane sloughing and rejection of the host's immune system or due to Pseudomonas aeruginosin.(58) Normally in such cases, the patients are often dressed with a new one. (10) The use of amniotic fluid is essentially a new concept in the field of wound healing and is a rich hub of stem cell, cytokines and growth factors which play an important role in wound healing.

**References:**


External Links:
Amniotic fluid
https://en.wikipedia.org/wiki/Amniotic_fluid
Amniotic stem cells
https://en.wikipedia.org/wiki/Amniotic_stem_cells

Dressing (medical)

See Also:
Amniotic membrane in Ophthalmology
http://eyewiki.aao.org/Amniotic_Membrane_Transplant