## Role of Pregnancy-Specific Biological Substances in wound healing

Placenta, Umbilical cord ,Amniotic fluid and amniotic membrane are classified as **Pregnancy Specific Biological Substances (PSBS) because they appear during pregnancy to support the pregnancy process and is discarded to the trash/incinerator after the birth of the baby**, everywhere in the world The process of its aseptic collection is, however, easy and can be obtained on a regular basis by an obstetrician/trained sister or any other specific health personnel .After its proper screening for HIV (1& 2),Hepatitis(B&C)Cytomegalovirus(CMV), Syphilis, Malaria etc these Pregnancy Specific Biological Substances (PSBS) may be extremely effective if used as biological dressings in cases of cornea and pterygium degeneration, chronic burn injuries and diabetic foot ulcers, just to name a few, and many other illness.

**Ontogeny of PSBS:**

Gross examination of the amniotic membrane reveals three important layers:(a) amnion, the innermost thin translucent layer which covers the embryo (b) lines of the amniotic cavity along with the amniotic fluid and(c) a middle collagen-rich connective tissue forming layer which in turn is connected with an outer collagen-rich reticular chorionic layer.(1,2)The amnion consists of the mesenchymal stromal cells, amniotic epithelial cells including embryonal-like stem cells. Both the amnion and the chorion are composed of a stromal layer and a basement membrane.(3)

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 chorio amniotic membrane and the embryo's skin.(2) The volume of the fluid increases to around 270 to 400 ml between 16 to 20$^{th}$week of pregnancy and about 800 ml in the 24$^{th}$ week. At times, the volume reduces to around 600 ml. (2) This fluid may be used for karyotyping and genetic or molecular pre natal diagnosis. (4)

**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. (5)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.(6) The further amniotic membrane has a structure quite similar to that of skin.(7, 8)

Amniotic membrane has anti-microbial properties due to the presence of Beta-defensins and elafin, storage of lysozyme enzymes which can produce the bacteriostatic effect on gram-positive bacteria. (6, 9) It can also help in preventing the loss of proteins, water, electrolytes, and fluids by forming a biological dressing and cover the wound thereby giving a moist environment which is extremely essential for healing. (6)

**Amniotic fluid:**

Amniotic fluid is another important component of the Pregnancy Specific Biological Substances (PSBS) that has the ability to prevent infection and help in regeneration through cell therapy. In a pregnant mother, amniotic fluid normally washes the vaginal canal before the birth of the baby so as to destroy any pathogenic environment. (10)Therefore, this phenomenon explains the extreme sterile and bactericidal nature of the amniotic fluid. The viscosity of the amniotic fluid is an important property in topical application for burn injuries.(10)

**Cellular composition:**

Both the amniotic fluid and the amniotic membrane has the rich source of the progenitor, multipotent, fetal and embryonal like stem cells. Epithelial stem cells from the epithelial layer of the amniotic membrane help in re-epithelialisation and closure of large wounds. (3)Apart from epithelial cells, fibroblasts are also present in the amniotic membranes providing lining and strengthening of the tissues and acting as a biological scaffold. (3) Other important extracellular components of the amniotic membrane include fibronectin, proteoglycans, glycosaminoglycans, laminins, collagen type IV, V and VII which helps in maintaining the structural integrity and as a biological substrate for migration of cells in advanced wound healing. (3)Human amniotic epithelial cells or HAEC's like amniotic membrane are also hypo antigenic due to the down-regulation of HLA-A,B,C,DR antigens with an increased suppression of neovascularization. (10)

Both amniotic fluid and the amniotic membrane have a cocktail of cytokines and growth factors like FGF, PDGF, EGF, TGF-Beta. (3) Amniotic membrane additionally contains matrix metalloproteinases and their inhibitors (TIMP's) to counterbalance the excessive growth. (3) The amnion has also shown to contain mesenchymal stem cells that can transdifferentiate into cartilage, bone, heart and skeletal muscles, fat cells, neuronal cells and epithelial tissues following established in vitro cell culture protocols. (11)

**Amniocytes:**

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 screened 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 cicatrizing 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 cicatricial 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 bullous 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 bacterial contamination. Unfortunately, none of the skin grafts fulfils 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. (44)The amniotic fluid treated group showed a better improvement when compared with steroid treated group after 2 months of 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:


1.Harminder S. Dua,Jose A.P. Gomes,Anthony J. King, V. SenthilMaharajan.The diagnostic and surgical technique, Survey of Ophthalmology, volume 49, Number 1, Jan-Feb, 2004.

2. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Non-fetal sources of stem cells, Springer-Verlag London Limited 2015,Chapter No: 1,A Massive wastage of the global resources, Page No:4-5.

3. Donald E. Fetterolf& Robert J. Snyder, Scientific and Clinical Support for the Use of Dehydrated Amniotic Membrane in Wound Management, Wounds 2012;24 (10):299–307.

4. Brian E. Future of prenatal cytogenetic studies: rapid aneuploidy testing or full karyotype. Ginekol Pol. 2007;78(11):881–7.

5.Robson MC, KrizekTJ, Koss N, Samberg JL. Amniotic membrane as a temporary wound dressing. SurgGynaccol Obstet.1973; 136: 904-6.

6. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Non-fetal sources of stem cells, Springer-Verlag London Limited 2015, Chapter No: 26 , Freshly collected Amniotic fluid and Amniotic membrane as Dressing material. Page No: 257-260.

7.HadjiiskiO,Anatassov N. Amniotic membranes for temporary burn coverage.Annals of Burns and Fire Disasters. 1996;10: 2-7.

8.Lorusso R, Geraci V, Masellis M. The treatment of superficial burns with biological and synthetic material: frozen amnion and bio brand .Annals of the MBC. 1989; 2: 2 -5.

9. Ana CatarinaMamede, Maria FilomenaBotelho: Editors, Amniotic Membrane: Origin, Characterization and Medical Applications, Springer; 1st ed. 2015 edition. Chapter 2: Biochemical properties of Amniotic Membrane, Sandra Catarina, Moreira Rocha and Claudio Jorge Maria Baptista. Page no: 20.

10. Niranjan Bhattacharya and Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy-specific biological substances, Springer-Verlag London Limited 2009, Chapter No: 37,Use of Amniotic Membrane, Amniotic Fluid, and Placental dressing in Advanced Burn Patients. Page No:383-393.

11.Niranjan Bhattacharya and Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy-specific biological substances, Springer-Verlag London Limited 2009, Chapter No: 36,Amniotic fluid and placental stem cells, Anthony Atala, Page No: 375-380.

12. Colin T. Maguire, Bradley L. Demarest, Jonathon T. Hill, James D. Palmer, Arthur R. Brothman, H. Joseph Yost, Maureen L. Condic. Genome-Wide Analysis Reveals the Unique Stem Cell Identity of Human Amniocytes. Volume 8, January 2013, PLOS ONE.

13.Moschidou D, Mukherjee S, Blundell MP, Drews K, Jones GN, et al. (2012)Valproic Acid Confers Functional Pluripotency to Human Amniotic Fluid StemCells in a Transgene-free Approach. MolTher 20(10):1953–67.

14. Li C, Zhou J, Shi G, Ma Y, Yang Y, et al. (2009) Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells. Hum Mol Genet18: 4340–9.

15. Wolfrum K, Wang Y, Prigione A, Sperling K, Lehrach H, et al. (2010) TheLARGE principle of cellular reprogramming: lost, acquired and retained gene expression in the foreskin and amniotic fluid-derived human iPS cells. PLoS One 5:e13703.

16. Liu T, Zou G, Gao Y, Zhao X, Wang H, et al. (2012) High-Efficiency ofReprogramming CD34(+) Cells Derived from Human Amniotic Fluid intoInduced Pluripotent Stem Cells with Oct4. Stem cells and development 21(12):2322–32.

17. Easley Cat, Miki T, Castro CA, Ozolek JA, Minervini CF, et al. (2012) HumanAmniotic Epithelial Cells are Reprogrammed More Efficiently by InducedPluripotency than Adult Fibroblasts. Cellular reprogramming 14: 193–203.

18. De Coppi P, Bartsch G, Jr., Siddiqui MM, Xu T, Santos CC, et al. (2007)Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol25: 100–6.

19. A. R. Prusa, E. Marton, M. Rosner, G. Bernaschek, and M. Hengstschläger, “Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research?” Human Reproduction, vol. 18, no. 7, pp. 1489–1493, 2003.

20.A. R. Prusa, E. Marton, M. Rosner, A. Freilinger, G. Bernaschek, and M. Hengstschläger, “Stem cell marker expression in human trisomy 21 amniotic fluid cells and trophoblasts,” Journal of Neural Transmission, vol. 67, supplement, pp. 235–242, 2003.

21.Margit Rosner, Katharina Schipany, BharanidharanShanmugasundaram, GertLubec, and Markus Hengstschläger, Amniotic Fluid Stem Cells: Future Perspectives, Stem Cells International, Volume 2012.

22. Mara Cananzi and Paolo De Coppi, CD117$^{+}$ amniotic fluid stem cells, State of the art and future perspectives. Organogenesis. 2012 Jul 1; 8(3): 77–88.doi: 10.4161/org.22426.

23.Niranjan Bhattacharya and Phillip Stubblefield: Editors, Regenerative Medicine using Non-fetal sources of stem cells, Springer-Verlag London Limited 2015, Chapter No: 26, Freshly collected Amniotic fluid and Amniotic membrane as Dressing material for leprosy patients: A preliminary case report on an Experience with six cases. Page No: 257-260.

24. Sorsby A, Hawthorne J, Reed H: Further experience with amniotic membrane grafts in caustic burns of the eye. BrJ Ophthalmol 31:409–18, 1947.

25. Sorsby A, Symmons HM: Amniotic membrane grafts in caustic burns of the eye (burns of the second degree). Br J Ophthalmol30:337–45, 1946.

26. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy-specific biological substances, Springer-Verlag London Limited 2009, Introduction, page no: vii.

27. Kim JC, Tseng SC: The effects on inhibition of corneal neovascularization after human amniotic membrane transplantation in severely damaged rabbit corneas. Korean JOphthalmol 9:32–46, 1995.

28. Kim JC, Tseng SCG: Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. Cornea 14:473–84, 1995.

29. Shimazaki J, Yang HY,Tsubota K: Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. Ophthalmology 104:2068–76, 1997.

30. Tsubota K, Satake Y, Ohyama M: Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Am J Ophthalmol122:38–52, 1996.

31. Lee SH, Tseng SC: Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am J Ophthalmol123:303–12, 1997.
32. Kruse FE, Rohrschneider K, Volcker HE: Multilayer amniotic membrane transplantation for reconstruction of deep corneal ulcers. Ophthalmology 106:1504–10, 1999.

33. Hanada K, Shimazaki J, Shimmura S, Tsubota K: Multilayer amniotic membrane transplantation for severe ulceration of the cornea and sclera. Am J Ophthalmol 131:324– 31,2001.
34. Chen HJ, Pires RT, Tseng SC: Amniotic membrane transplantation for severe neurotrophic corneal ulcers. Br JOphthalmol84:826–33, 2000.

35. Rakowski E, Zagorski Z, Kardaszewska A, Durakiewicz D:Application of amniotic membrane transplantation in severe corneal diseases. KlinOczna 101:417–21, 1999.

36. Meller D, Maskin SL, Pires RT, Tseng SC: Amniotic membrane transplantation for symptomatic conjunctivochalasis refractory to medical treatments. Cornea 19:796–803, 2000.

37. Prabhasawat P, Barton K, Burkett G, Tseng SC: Comparison of conjunctival autografts, amniotic membrane grafts, and primary closure for pterygium excision. Ophthalmology104:974–85, 1997.

38. Shimazaki J, Shinozaki N, Tsubota K: Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. Br JOphthalmol 82:235–40, 1998.

39. Solomon A, Pires RT, Tseng SC: Amniotic membrane transplantation after extensive removal of primary and recurrent pterygia. Ophthalmology 108:449–60, 2001.

40. Pires RT, Tseng SC, Prabhasawat P: Amniotic membrane transplantation for symptomatic bullous keratopathy. ArchOphthalmol 117:1291–7, 1999.

41. Sanghavi P, Bhalla K, Das V. Fire Related Deaths in India in2001 A Retrospective Analysis of Data. Lancet. 2009; 373(9671):1282–1288.

42. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Non-fetal sources of stem cells, Springer-Verlag London Limited 2015,Chapter No: 26, Page No: 257-260.

43. Ref: Royal College of General Practitioners. MorbidityStatisticsfrom General Practice 1991–92. London, U.K.:HMSO; 1995.

44. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy-specific biological substances, Springer-Verlag London Limited 2009, Chapter No: 38, Clinical use of Amniotic Fluid in Osteoarthritis: A source of Cell Therapy by Niranjan Bhattacharya, Page No:395-400.

45. Niranjan Bhattacharya, Clinical Use of Amniotic Fluid in Osteoarthritis: A Source of Cell Therapy. Transplantation 90 · January 2011.

46. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Non-fetal sources of stem cells, Springer-Verlag London Limited 2015,Chapter No: 27, Page No: 263-265.

47. Fang CH, Jin J, Joe JH, Song YS, So BI, Lim SM, Cheon GJ, Woo SK, Ra JC, Lee YY, Kim KS.In vivo differentiation of human amniotic epithelial cells into cardiomyocyte -like cells and cell transplantation effect on myocardial infarction in rats: comparison with cord blood and adipose tissue-derived mesenchymal stem cells. Cell Transplant. 2012;21(8):1687–96.

48. Wang YY, Fang N, Chen DX, Yu LM, Liu ZL, Wan X. Human amnion-derived mesenchymal
stem cells engraftment and differentiate in rats with acute myocardial infarction. ZhongGuo Lao NianXueZaZhi. 2013a;4(33):1583–6.

49.Gong LM, Fang N, Chen DX, Wan EH, Zhang T, Liu ZL, Yu LM, Zhao CH. ZhongGuoZuZhi
Gong Cheng Yan Jiu Yu Lin Chuang Kang Fu. 2011;15(36):6709–12.

50.Zheng YB, Zhang XH, Huang ZL, Lin CS, Lai J, Gu YR, Lin BL, Xie DY, Xie SB, Peng L, Gao ZL. Amniotic-fluid-derived mesenchymal stem cells overexpressing interleukin-1 receptor antagonist improve fulminant hepatic failure. PLoS One. 2012;7(7):e41392.

51. Zhang D, Jiang M, Miao D. Transplanted human amniotic membrane- derived mesenchymal
stem cells ameliorate carbon tetrachloride-induced liver cirrhosis in a mouse. PLoS One.2011;6(2):e16789.

52.Banerjee A, Nürnberger S, Hennerbichler S, Riedl S, Schuh CM, Hacobian A, Teuschl A, Eibl
J, Redl H, Wolbank S. In toto differentiation of human amniotic membrane towards theSchwann cell lineage. Cell Tissue Bank. 2014;15(2):227–39.

53. Liang H, Liang P, Gao A, Qi Q, Liu E, Wu J, Xu X. A new composite matrix bridging both
stumps of spinal cord transection in rats to promote recovery of motor function. Zhongguo Xiu Fu Chong JianWaiKeZaZhi. 2009;23(11):1376–81.

54. Wang F, Wang L, Yao X, Lai D, Guo L. Human amniotic epithelial cells can differentiate into
granulosa cells and restore folliculogenesis in a mouse model of chemotherapy-induced premature
ovarian failure. Stem Cell Res Ther. 2013b;4(5):124.

55.Xiao Y, Fang N, Chen DX, Yu LM, Zhang T, Zhao CH. Therapeutic effect and immunological
regulation of human amniotic mesenchymal stem cells on the rat with collagen-induced arthritis.
Man Yi XueZaZhi. 2013;29(6):461–6.

56. Zani A, Cananzi M, Fascetti-Leon F, Lauriti G, Smith VV, Bollini S, Ghionzoli M, D'Arrigo A,
Pozzobon M, Piccoli M, Hicks A, Wells J, Siow B, Sebire NJ, Bishop C, Leon A, Atala A Lythgoe MF, Pierro A, Eaton S, De Coppi P. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut. 2014;63(2):300–9.

57. Arianna Bonomi, AntoniettaSilini, Elsa Vera, PatriziaBonassiSignoroni, Valentina Coccè, LoredanaCavicchini,FrancescaSisto, GiulioAlessandri, Augusto Pessina, and OrnellaParolini. Human amniotic mesenchymal stromal cells (hAMSCs) as potential vehicles for drug delivery in cancer therapy: an in vitro study. Stem Cell Res Ther. 2015; 6(1): 155.

58. Pruit BA, Lindberg RB. Pseudomonas aeruginosin infection in burn patients. In: Doggett RG, editor. Pseudomonas aeruginosin: clinical manifestations of infection and current therapy. 1st ed. New York:Academic; 1979. p. 339–66.

**External Links:**
Amniotic fluid
https://en.wikipedia.org/wiki/Amniotic_fluid

Amniotic stem cells
https://en.wikipedia.org/wiki/Amniotic_stem_cells

Dressing (medical)
https://en.wikipedia.org/wiki/Dressing_(medical)

**See Also:**
Amniotic membrane in Ophthalmology
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