Stem Cell Therapy In Ocular Surface Disorder

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Abstract :

Introduction: Ocular surface disorders due to various causes heal up if the limbal stem cells are adequate and healthy.In case of deficiency the disorders manifest.Present study was undertaken in two tertiary eye care centre from January 2006 to January 2015 to evaluate the result of stem cell therapy.Materials and Methods:12 cases of ocular surface disorders were included for prospective clinical trial of stem cell therapy from various sources like fresh amniotic membrane,autologous and homologous(living related donor-mother of same blood group) limbal conjunctiva,fresh umbilical cord blood.Preoperative and postoperative visual assessment was done at regular interval up to one year.Results:Amniotic membrane transplantation in Stevens Johnson syndrome and shield corneal ulcer with dry eye syndrome improved the ocular surface stability.The vision improved in two cases of partial limbal stem cell deficiency.Autologous circumferencial barrage stem cell transplantation in progressive pterygium and ocular surface limbal dysplasia was most fruitful.Probably this procedure supplemented new stem cells in the area of partial deficiency.In prevented further conjunctivalisation.But homologous stem cell therapy in a case of bilateral calcareous degeneration did not work well.Cord blood instillation in case of two cases of nonhealing corneal ulcer also supplemented embryonic stem cells and hastened healing.Conclusion:Stem cell therapy from various sources in ocular surface disorders has a promising future.

Introduction :

The term stem cell was proposed for scientific use by a Russian Scientist Alexander Maksimov in 1908 at the congress of Haematological society in Berlin. He postulated the existence of hematopoitic stem cells which was later discovered in human cord blood in 1978.

These are unspecified cells that have two defining properties: the ability to differentiate into other cells and the ability to self regenerate. They can theoretically divide without limit to replenish other cells as long as the person or animal is alive. These are present in all self renewing tissues like skin, mucous membrane, bone marrow and regenerative systems etc. These cells are long lived and have great potentiality for clonogenic cell division and are ultimately responsible for cell replacement and tissue regeneration. These are a small population of the total tissue and have been estimated to make up from 0.5% to less than 10% of the total cell population. These can be placed into either one of the following two compartments: proliferative and non-proliferative. Cells in the proliferative compartments are capableof preceding cell mitosis with DNA synthesis. This compartment includes stem cells (SC) and transient amplifying cells (TAC) that are derived from each stem cell mitosis and amplify their number by undergoing a few rounds of cell division. Cells in the non-proliferative differentiative compartment are in theory all post mitotic cells (PMC) that are committed to cellular differentiation. In the later compartment cells at different stages of differentiation can be identified during the process of tissue maturation. The terminally differentiated cells (TDC) achieve the ultimate expression of the functional aspect of the tissue. All cells except stem cells have a limited life span and are destined to die. (1)

Sources: Embryonic stem cells(ESC) are available in donated embryo cultured for about six months in a petridish until a cell line develops which contain millions of stem cells.

Cord blood is the richest source of stem cells. These are actually much more primitive in nature. When stem cells from this are used, the donor cells appear more likely to take or engraft, even when there are partial tissue mismatch. A fatal complication called Graft versus Host disease (GVHD) in which donor cells can attack the recipient tissues, appear to occur less frequently with cord blood than with bone marrow. This may be because cord blood has a muted immune system and certain cells usually active in an immune system, are not yet educated to attack recipient. Cord blood also is less likely to contain certain infectious agents, like some viruses, that can pose a risk to transplant recipients. These stem cells are taken from umbilical cord shortly after birth once the cord has been cut. This blood can be frozen and stored in a cord blood bank. When it is required the stem cells are thawed and ready to use in stem cell therapy.

Multipotent stem cells are also found in amniotic fluid. These stem cells are very active, expand extensively without feeders and are not tumorogenic. It is possible to collect amniotic stem cells without destroying the embryo.

Amniotic membrane comprises the inner most layer of the placenta. Davis in 1910 reported the use of foetal membranes as a skin substrate. Amniotic membrane transplantation became important because of its ability to diminish the occurrence of adhesions and scarring, its ability to enhance wound healing and epithelisation and its antimicrobial potential. In particular the amniotic membrane expresses incomplete HLA- A, B, C and DR or ?2-microglobulin which may account for the fact that immunological reaction after transplantation has not been observed.

Umbilical cord tissue is the richest source of mesenchymal stem cell.

An extremely rich source for adult mesenchymal stem cells is the developing tooth bud of the third molar.

Bone marrow is the richest source of adult stem cells. However, these are matured and therefore are more restricted as to what type of cells they can differentiate into. Few adult stem cells are also found in peripheral blood.

Induced pleuripotent stem cells are not adult stem cells but rather reprogrammed cells (e.g. epithelial cells) given pleuripotent capabilities by using reprogramming with protein transcription factors. These are equivalent to ESC.

Limbal stem cells:

The ocular surface is made up of two distinct types of epithelial cells constituting the conjuctival and corneal epithelia. These epithelia are stratified sqamous and non keratinized. Although anatomically continuous with each other at the corneo-scleral limbus, the two cell phenotypes represent quite distinct sub population. The stem cells for the cornea are located in the palisade of Vogt at the limbus. The micro environment of the limbus is considered to be important in maintaining potentiality of the stem cells. They also act as a barrier to conjuctival epithelial cells and prevent them from migrating on to the corneal surface. Beside this, it is postulated that stem cells are present in the conjunctiva, cilliary body and retina.(2)

In certain pathological conditions, however, the limbal stem cells may be destroyed partially or completely resulting in varying degrees of stem cell deficiency with its characteristic clinical features. These include conjuctivalisation of the cornea with vascularisation, appearance of goblet cells and an irregular and unstable epithelium. The stem cell deficiency can be managed with auto or allo transplantation of these cells; with latter option systemic immune suppression is required. The stem cells can be expanded ex vivo on a processed human amniotic membrane and

transplanted back to ocular surface with stem cell deficiency without the need of immune suppression.

The term ocular surface describes the entire mucosal epithelial lining bordered by the skin at the superior and inferior eyelid margin. This includes epithelium from the muco-cutanous junction of the eyelid margin on to the back of the lids, into the fornices and its reflections over the globe including that which covers the sclera and cornea separated by the limbus.A tear film coated and uninterrupted epithelium associated with a stable stroma with homogeneous arrangement of collagen fibers and functioning endothelium are essential for a transparent cornea. The ocular surface is the first to interact with the environment, so it is more vulnerable to injuries.

Ocular surface disorders due to various causes heal up if the limbal stem cells are adequate and healthy. In case of deficiency, the disorders manifest. This can be primary related to insufficient stromal micro environment to support the stem cells such as aniridia, erythrokeratoderma, multiple endocrine deficiencies and neurotropic keratopathy. The secondary limbal deficiencies are related to external factors that destroy the limbal stem cells such as chemical or thermal injuries, Stevens Johnson syndrome, ocular cicatricial pemphigoid, multiple surgeries or cryotherapy, contact cell wear or extensive microbial infections. Corneal stem cell deficiency can be total (diffuse) or partial (sectorial). Restoring ocular health in these eyes is frustrating. However, stem cell therapy in these cases has created a promising armamentarium (1)

A prospective clinical study on stem cell therapy in various ocular surface disorders has been undertaken to evaluate for future management.

Materials & Methods:

The study was conducted in the Department of Ophthalmology, V. S. S. Medical College, Burla (Odisha) and Sardar Raja's Medical College, Hospital & Research Centre, Kalahandi (Odisha) from January 2006 to January 2015. Twelve cases of ocular disorders were included for prospective therapeutic clinical trial of stem cell therapy from various sources.

The aim of surgery was not only to restore vision but to replenish limbal stem cells and maintain a stable ocular surface. The sources were freshly collected amniotic membrane, cord blood, auto and allo limbal conjunctiva. Fresh amniotic membrane was collected with aseptic measures from elective Caesarian section cases with informed consent from the donor and receipient. It was preserved in Ringer's Lactate solution with gentamycin injection (80mg/2ml) at 40 C. On the next day the amniotic membrane was thawed in the operation theatre to room temperature and was dissected from the chorion and transplanted on the ocular surface with epithelial side up. Intermittent incisions were given on it for better aeration.

Autologous limbal conjunctiva was retrieved from the same or other eye of the patient and transplanted in the same sitting. The size was from 2-3 clock hours from upper limbal conjunctiva. Homologus limbal conjunctiva was retrieved from the healthy eye of a living related donor (mother) of the patient. Both had same O+ve blood group.

Fresh cord blood was collected with aseptic measures in elective Caesarian section cases and a pinch of EDTA powder was mixed in it. It was preserved at 40C and instilled into the diseased eye thrice for one day.

Post operative care and evaluation from time to time was done in all cases for one year.

Results:

Prospective, non comparative interventional case series of 12 patients with ocular surface disorders underwent therapeutic stem cell therapy in this study. The cases were:-

Type of Disorder	No. of Cases
Stevens Johnson Syndrome	4
Vernal conjunctivitis with shield corneal ulcer	1
Ocular Limbal dysplasia	1
Calcareous corneal degeneration	1
Non healing corneal ulcer	2
Progresive pterygium	3

Stevens Johnson Syndrome: All the four cases were female from the age group of:-

Age Group	No. of Cases
11-20 Years	1
21-30 Years	2
41-50 Years	1

Onlay method of amniotic membrane transplantation was done in all cases with 10-0 interrupted nylon suture which were removed after 4 weeks.

Amongst them one patient aged about 13 years had a preoperative visual acuity of 6/6 in right eye and C. F. at 2 meters in her left eye. This eye had partial stem cell deficiency with corneal haziness and dry eye syndrome. The post operative vision improved from C. F. at 2meters to 6/24 after one year. All the other cases had substantial clinical improvement with a stable ocular surface such as less congestion, corneal erosion and dryness. However the visual acuity did not improve further from PL+ve and PR in all sectors.

Vernal Conjuctivitis with shield Corneal Ulcer: In this category one male aged 27 years was suffering from vernal conjunctivitis with shield corneal ulcer and dry eye syndrome in left eye. After amniotic membrane transplantation there was substantial clinical improvement with a stable ocular surface and improvement of vision from C.F. at 2 meters to 6/24 after one year.

Ocular Limbal Dysplasia :

In this category one male aged 35 years ,alife convict from jail had superior limbic jelly like dysplasia in left eye. The growth was dissected from the ocular surface and autologous limbal conjunctiva extending two clock hours from the upper limbus of the other eye was transplanted in the same sitting. The histopathological study was found to be benign in nature. After one year there was no reccurrence. However low grade superior limbic keratopathy resulted.

Calcareous Degeneration of Cornea:

This was a female case aged about 23years with bilateral calcareous degeneration of cornea. She had the visual acuity of 6/60 in right eye and HM +ve in left eye. Penetrating keratoplasty was done in left eye. The visionimproved from C.F.at 2 meters to 6/60, but there was recurrence after a year. So, in the next session after deepithelisation, the calcareous matter was removed by a needle and a swab stick soaked in EDTA solution. Then an allograft of limbal conjunctiva with a length of three clock hours from the upper limbus of her mother's healthy right eye was transplanted into upper limbus of the patient. The blood group of both was O +ve There was substantial clinical improvement of ocular surface with a visual improvement of 6/60 in that eye after 6months. But there was recurrence after a year.

Non healing Corneal Ulcer:

Two cases of non healing corneal ulcer were in the age group of 21-30 years. All were male. Fresh cord blood collected with aseptic measures and preserved with EDTA powder was instilled thrice for one day. There was substantial clinical improvement of ocular surface which hastened healing.

Progressive Pterygium:

Three cases of progressive pterygium under-went bare sclera excision with circumferential barrage limbal stem cell transplantation from the upper limbus of the

same eye. Four anchoring stitches with 10-0 nylon ware given in the affected limbal area. There was no reccurence after one year.

Discussion:

The management of ocular surface disorder is a challenging task as most of these cases lead to visual disability. Thoft & Friend proposed a "X, Y, Z hypothesis of corneal epithelial maintenance" in which the desquamated cell (Z-component) are continuously replaced not only by the basal cells (X-component) that divide but also by cells that migrate from the periphery (Y-component). Thus migration occurs centripetally from the limbus and vertically from the basal cell layer. In case of limbal stem cell deficiency this kinetic of maintenance of corneal epithelium is hampered leading to various ocular surface disorders.(3)

Stem cell therapy in the form of limbal stem cell transplantation, amniotic membrane transplantation and cord blood instillation hasbeen done in this study as a therapeutic clinical trial in various ocular surface disorders.

In this study amniotic membrane transplantation was done in Stevens Johnson Syndrome (4 cases) and one case of vernal conjunctivitis with shield corneal ulcer. In all the cases there was substantial clinical improvement such as diminution of congestion and stabilization of ocular surface. There was visual improvement in one case of partial stem cell deficiency in Stevens Johnson Syndrome with visual improvement upto 6/24 from CF at 2meters. In the case of vernal conjunctivitis with shield corneal ulcer there was also visual improvement upto 6/24 with substantial clinical improvement.

The first use of the amniotic membrane in ophthalmology was by De-Roth in 1940 who reported partial success in the treatment of conjuctival epithelial defects after symblepharon. Sorsby and Samons in 1946 found that patients with caustic burn of the conjunctiva with corneal involvement could be treated successfully using amniotic membrane . In 1995 Kim and Tseng used amniotic membrane transplantation for ocular surface reconstruction of severely damaged cornea in a rabbit model.(2)

David F. Anderson et al. (2001) studied 17 eyes of 15 patients with partial limbal stem cell deficiency whounderwent superficial keratectomy of the conjuctivalised corneal surface followed by amniotic membrane transplantation. Follow up was done for at least a year. All eyes exhibited a stable intact corneal epithelial surface after a mean follow up period of 25.8 months with no eyes developing recurrent erosion or persistent epithelial defect. The mean time for re-epithelialization was 22.8 days. Overall improvement in visual acuity was observed in 92.9%of 14 eyes with visual potential. Pain and photophobia were abolished in 86% of cases and substantially reduced in 14% with all eyes exhibiting decreased vascularisation and inflammation at final follow up.(4)

Amniotic membrane transplantation appears to be a safe and effective method of restoring a stable corneal epithelium for cases of partial limbal stem cell deficiency and can be considered as an alternative to limbal autograft or allograft.

In patients with total limbal stem cell deficiency, limbal autologous or homologous transplantation are indicated for corneal surface reconstruction. This may be combined with or followed by keratoplasty. The current technique was proposed by Kenyon and Tseng, but several modifications have been described. All these procedures aim to transplant a new source of stem cells after removal of host's diseased corneal epithelium. After successful transplantation the host's cornea is permanently covered by healthy epithelium. Donor's tissue can be obtained from the healthy fellow eye (limbal auto graft) in case of unilateral disease or from a living related donor or cadaveric eye (limbal allograft) when both eyes are involved.(3) (5)

Kazuo Tsubota et al. (1999) performed 73 stem cell transplantation in 42 patients. 3 patients did not come for follow up. Thus they evaluated 70 procedures in 43 eyes of 39 patients (26-male,13-female) with mean age of 49±23 years. The overall success rate measured as the rate of corneal epithelisation was 51%, clear cornea-35%. The patients had a final post operative visual acuity of 0.02 (1/60). Persistent epithelium defects developed in 60% which subsided with treatment. The overall rate of corneal graft-rejection in eyes into which cornea and stem cell were transplanted simultaneously was 40%.Most serious complications after surgery was ocular hypertension in 16 eyes (37%). (6)

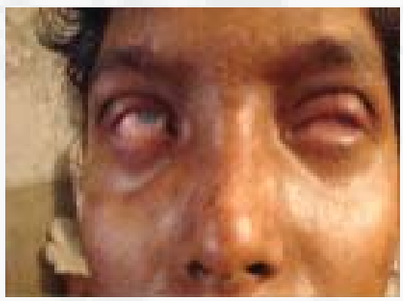
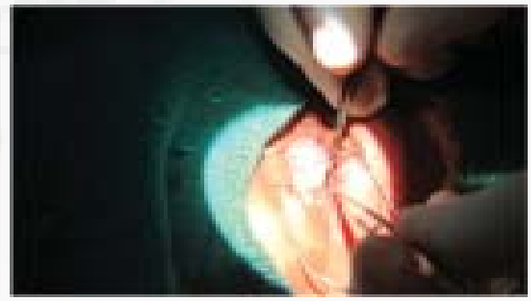
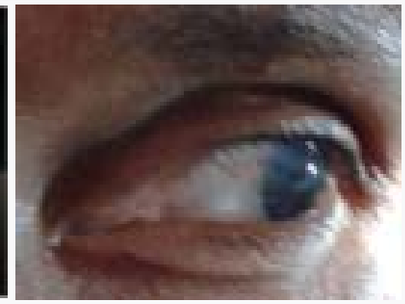
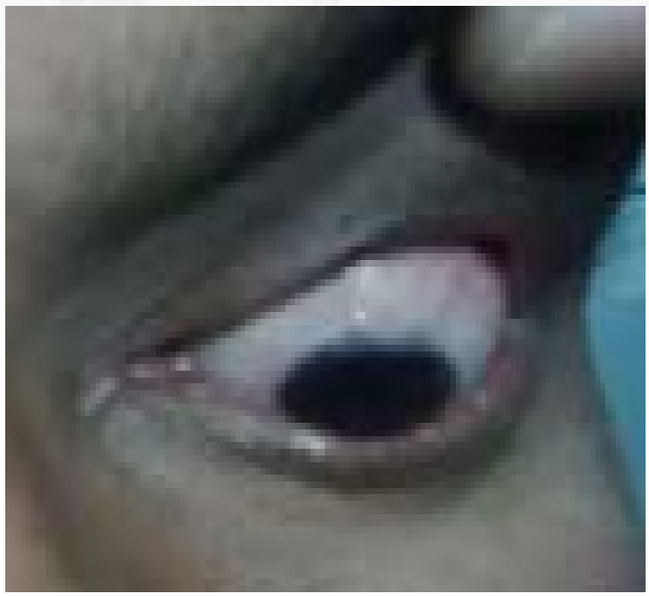
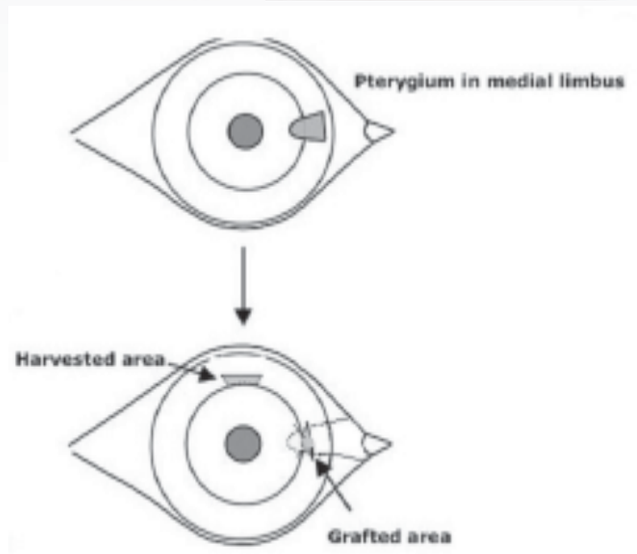
Ray Jui -Fang Tsui et al. (2000) have studied reconstruction of damaged cornea by transplantation of autologous limbal epithelial cells expanded invitro on amniotic membrane in 6 patients with partial or total limbal stem cell deficiency due to chemical burn (3 cases), pseudopterygium after excision of a cyst and congenital pterygium and recurrent chronic inflammation with phlyctenular degeneration (one case each). Four of the patients had undergone surgery like autologous conjunctival transplantation, amniotic membrane transplantation or both procedure in the damaged eye, but failed to improve. After a mean follow up period of 15± 2 months, vision improved in 5 of the 6 eyes (83%) that received transplant. There had been no recurrent neovascularisation or inflammation in the transplanted areas during the follow up period. (7)

Noriko Koizumo et al. (2001) have studied cultivated corneal epithelial transplantation for ocular surface reconstruction in acute phase of Stevens Johnson Syndrome in 2 patients. Shortly after the transplantation conjunctival inflammation rapidly subsided. After 6 months their ocular surface epithelium was stable without defects. (8)

Jun shimazaki, M.D. et al. (2002) observed 13eyes of 13 patients with severe limbal deficiency (Stevens Johnson Syndrome-8 eyes, ocular cicatricial pemphigoid-3eyes and chemical burn-2eyes) treated by transplantation of allolimbal epithelium cultivated in amniotic membraneon to the ocular surface. After the surgery the epithelium regenerated and covered the ocular surface in 8 eyes (61.5%). However 3 of 8 eyes developed partial conjunctival invasion and 2 eyes developed epithelial defect. At the last examination corneal epithelisation was achieved in 6 eyes (46.2%), 5 eyes had conjunctivalisation, 1 eye had dermal epithelisation and 1 eye was not epithelised. Complications were corneal perforation in 4 eyes and infectious keratitis in 2 eyes. (9)

Kajhi Nishida et al. (2004) reported the result of ocular surface reconstruction in 4 patients having bilateral stem cell deficiency with the use of cultivated autologous oral mucosal epithelial cells in carrier free tissue replacement sheets. Corneal transparency was restored and post operative visual acuity improved remarkably. During the follow up period the corneal surface remained transparent and there was no serious complication. They have shown that tissue engineered epithelial cell sheets fabricated exvivo from autologous oral mucosal epithelium are effective for reconstructing the ocular surface and restoring vision in patients with bilateral stem cell deficiency. (10)

In this study auto limbal stem cell transplantation was done in one case of limbal dysplasia and three cases of progressive pterygium.. In the first case there was no recurrence except mild upper limbic keratopathy and in the later cases, there was no recurrence after one year. In the pterygium cases a circumferential barrage limbal stem cell transplantataion was done. This probably prevented further conjuctivalisation of the cornea. In other forms of pterygium surgery like bare sclera excision, conjuctival transplantation from upper bulbar conjunctiva, there are chances of recurrence. But when limbal conjunctiva in barrage form is transplanted there is no recurrence. Probably partial limbal stem cell deficiency in the exposed part of inter palpebral fissure may be the cause of pterygium.

In one case of calcareous corneal degeneration , the allo graft of limbal conjunctiva from mother (living related donor) did not work satisfactorily leading to recurrence.

Virendra sangwan et al (2005) had done prospective non-comparative interventional case study in patients with limbal stem cell deficiency due to chemical injury (3 cases) and xeroderma pigmentosum (1 case). Three eyes received living related limbal conjunctival transplantation and one received harvested cadaveric kerato limbal allograft. Penetrating keratoplasty was done 3-4.5 months later for visual rehabilitation. Duration of follow up after penetrating keratoplasty ranged from 4-11 month. The ocular surface remained stable in all patients. Visual acuity improved in the early post operative period in all patients but reduced in 2 cases due to endothelial rejection and secondary glaucoma.(11)

On the whole depending on the area of limbal and corneal damage, cultured limbal epithelial cells on amniotic membrane can be used as a limbal equivalent or as a sheet covering the enire limbus and cornea.

Cord blood stem cells are much more primitive than bone marrow or peripheral blood. These cells have more advantages over those retrieved from bone marrow. It is much easier to get because they are readily obtained from the umbilical cord and placenta at the time of delivery. Harvesting stem cells from bone marrow require a surgical procedure under general anesthesia. Cord blood stem cell can be stored and transplanted back into the donor or to a family member or to an unrelated recipient. Perfect tissue matching is needed in bone marrow transplantation, where as in case of cord blood, even partial matching can be successful. Banked stem cells from cord blood can be more readily available for immediate use than a matched bone marrow.A study found that children who received a cord blood transplant from a closely matched

sibling were 59% less likely to develop graft versus host disease than children who received a bone marrow transplant from a closely matched sibling.Cord blood contains stem cells capable of giving rise to epithelial tissue, making it amenable for application in the eye (cornea), skin (wound healing). In case of the eye cornea appears to be suitable for routine clinical application.

Nichols et al. (2005), Harris et al. (2008) have used cord blood stem cells as a viable therapeutic modality for ocular surface disease, and as a source of tissue for ocular surface reconstruction. Preliminary laboratory and animal data are supportive of this hypothesis.(12)

In our study, fresh cord blood was instilled thrice for one day innon healing corneal ulcer (2 cases). This hastened healing in the follow up period.

Summary:

Twelve cases of ocular surface disorders had undergone stem cell therapy in form of fresh amniotic membrane transplantation, auto and allo limbal stem cell transplantation from living related donor (mother with the same blood group) and fresh cord blood instillation. Out of these amniotic membrane transplantation in Stevens Johnson Syndrome and shield corneal ulcer improved the ocular surface stability. Autologous circumferential barrage stem cell transplantation in progressive pterygium and ocular dysplasia was most fruitful; because probably this procedure supplemented new stem cells in the area of partial deficiency. It prevented further conjunctivalisation. Cord blood instillation in cases of non healing corneal ulcer also supplements stem cell population and hastens healing.

Reference:

1. Sangwan VS. Limbal Stem Cells in Health and Disease. Bioscience Reports 2001; 21(4): 385-404.
2. Schwan BL. Human Amniotic Membrane Transplantation For The Treatment of Ocular Surface Disease. Book Chapter Florida Medicine. www. dcms.online. org/jax/medicine 2002/journal.
3. Dua HS, Saini JS, Blanco AA, Gupta P. Limbal Stem Cell Deficiency: Concept, Aetiology, Clinical Presentation, Diagnosis and Management. Indian J. Ophthalmol 2000; 48:83-92.
4. Anderson DF, Ellies P, Pires RTF, Tseng SCG. Amniotic membrane transplantation for partial limbal stem cell deficiency. Br. J. Ophthalmol. 2001; 85:567-75.
5. Fernades M, Sangwan VS. Cultivated Limbal stem cell transplantation; Promise and Practice. DOS times 2006; 12(4):320-329.
6. Tsubota K, Satake Y, Kaido M, Shinozaki N, Shimurra S, Miyojima HB, Shimajaki J. Treatment of severe ocular surface disorders with corneal epithelial stem cell Transplantation. N. Engl J Med 1999; 340: 1697-1703.
7. Tsai RJF, Li LM, Chen JK. Reconstruction of Damaged Corneas by Transplantation of Autologous Limbal epithelial cells. N Engl J Med 2000; 343:80-93.
8. Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. Cultivated Corneal Epithelial Transplantation for Ocular Surface Reconstruction in Acute phase of Stevens-Johnson Syndrome. Arch Ophthalmol 2001; 119: 298-300.
9. Shimazaki J, Aiba M, Goto E, Kato N, Shimmura S, Subota K. Transplantatipon of Human Limbal Epithelium Cultivated on Amniotic Membrane for the Treatment of Severe Ocular Surface Disorders.Ophthalmology 2002;109:1285-1290.
10. Nishida K, Yamato M, Hayashida Y, Watanabe K,Yamamoto K, Adachi E, Nagai S, et al. Corneal reconstructionwith Tissue Engineered Cell Sheets composed of Autologous Oral Mucosal Epithelium. N Engl J Med 2004; 351:1187-1196.
11. Sangwan VS, Fernades MS, Bansal AK, Vemuganti GK, Rao GN. Early results of penetrating keratoplasty following limbal stem cell transplantation. Indian J Ophthalmol 2005; 53(1): 320-329.
12`. Harris DT. Non haematological uses of cord blood stem cells. British Journal of Hematology2009; 147: 177-184