# Clinical Study Protocol

**1. Study Title:**

Comparative Evaluation of Autologous Tissue-Engineered Ocular and Oral Mucosal Tissue Grafts: A Prospective Randomized Controlled Trial.

**2. Trial Registration:** ISRCTN registry

**3. Protocol Version:** CSP001A

**4. Funding:** Supported by research grant-in-aid from the Department of Biotechnology, Ministry of Science and Technology, Government of India, vide release order No: BT/01/COE/07/03 for R&D Project 1 dated Dec 29, 2017

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## 6. Introduction:

### 6.1 Introduction:

The normal ocular surface is covered with highly specialized corneal, limbal and conjunctival epithelial cells (EC) that, together with the tear film, maintain surface integrity. Severe ocular surface damage (OSD) caused by thermal and chemical burns or Stevens - Johnson syndrome (SJS) represents serious clinical challenge.[1] Many conditions of corneal blindness are due to damage or loss of the corneal epithelium, and/or the conjunctival epithelium, both of which are considered as the Ocular Surface. These diseases are termed Ocular Surface Diseases (OSD), and blindness occurs due to limbal or conjunctival stem cell deficiency. These include acute chemical and thermal injuries to the eye, which occur in burns victims and industrial chemical accidents (usually acid or alkali splashed into the eye). Chemical injuries also occur in acid attacks, and also in various forms of chemical weapons, such as mustard gas. Other forms of ocular surface injury that occur include severe eye corneal infections (e.g. related to contact lens ulcers, or trachoma) and severe eye and skin conditions such as Stevens-Johnson syndrome, in which an allergy causes extensive skin and eye blisters, leading to blindness. Stevens-Johnson syndrome is a potentially deadly skin disease that usually results from a drug reaction. The disease can be deadly as well as very painful and distressing. In most cases, the disorders are caused by a reaction to a drug, and one drug that has come under fire lately is the cox-2 inhibitor Bextra, which is already linked to the disorders. There are other drugs that have been linked to Stevens-Johnson syndrome, and these include some other NSAIDS (non-steroid anti-inflammatory drugs), Allopurinol, Phenytoin, Carbamazepine, barbiturates, anticonvulsants, and sulfa antibiotics. The condition can sometimes, although not very often be attributed to a bacterial infection, and in some cases there is no known cause for the onset of Stevens-Johnson syndrome. However, the most common cause is through drug related reactions. Stevens-Johnson syndrome can affect any age group. However, it occurs most commonly in older people, and this could be because older people tend to use more of the drugs associated with the disease and are therefore collectively more at risk from the disease. People that have AIDS are also at an increased risk of contracting Stevens-Johnson syndrome. Those in the higher-risk groups are urged to remain vigilant for any signs of these skin diseases and are also advised to remain well informed about the symptoms that can indicate the presence or onset of Stevens-Johnson syndrome. In Symptoms, Stevens-Johnson syndrome can start with non-specific symptoms such as cough, aching, headaches, and feverishness. This may be followed by a red rash across the face and the trunk of the body, which can continue to spread to other parts of the body. The rash can form into blisters, and these blisters can form in areas such as the eyes, mouth and vaginal area. The mucous membranes can become inflamed, and with Toxic Epidermal Necrolysis layers of the skin can also come away with ease and often the skin peels away in sheets. The hair and nails can also come away in some cases, and sufferers can become cold and feverish. Ocular or thermal burns account for 7.7% to18% of ocular trauma. Most victims are young people. Burns occur in accidents on work premises, at home, or during physical attacks. The most serious injuries are due to chemical burns by strong acids or bases. Associated with the destruction of limbal stem cells, they generate recurrent epithelial ulcerations, chronic stromal ulcers, deep stromal revascularization, conjunctival

overlap, or even corneal perforation. Acids and bases are the most frequently involved chemical agents. Burns by acids and bases. Respectively account for 1.6 % and 0.6 % of all ocular trauma.The gravity of lesions is influenced by the nature, concentration, quantity, time of exposure and pH of the chemical. Mainly bases are ammonia (NH3) used as cleaning, freezing or fertilizing agent, bleach (sodium hypochlorite), soda (NaOH) used as home detergent, potassium hydroxide (KOH) use as a fertilizer and lime (Ca(OH)2) used as cement. Particles of soda and lime particularly stick to the conjunctiva, and doing so constitute a stock of toxic product. Previously, treating these diseases with conventional corneal transplants had a very high failure rate, because we were not transplanting stem cells at the time of the transplant. Over the last few years, we, and others in the field have pioneered the surgical technique of transplanting both limbal and conjunctival stem cells, and these stem cell transplants are now routine procedures within our corneal transplantation program.[2] Recently, transplantation of cultivated autologous oral mucosal epithelium has been used in the treatment of LSCD. However, oral mucosa is non-ocular tissue, and it retains the characteristics of the tissue of origin. The use of conjunctiva would therefore have obvious advantages for ocular surface epithelial replacement. We have previously demonstrated the effective use of cultivated conjunctival transplantation for conjunctival epithelial replacement in various ocular surface conditions. The uses of cultivated conjunctival transplantation for corneal epithelial replacement would be a novel method for treating severe ocular surface disease.[3] The study aims to conduct a prospective randomized controlled trial to compare the clinical outcomes of autologous tissue-engineered ocular and oral mucosal tissue grafts in patients with severe ocular surface disorders. This study will evaluate the safety, efficacy, and patient-reported outcomes of both types of grafts over a defined follow-up period. By providing a rigorous comparative analysis, the trial seeks to inform clinical decision-making and optimize treatment strategies for ocular surface reconstruction.

This trial is poised to make a significant contribution to the field of ophthalmic surgery and tissue engineering, offering new insights into the relative benefits and limitations of autologous ocular and oral mucosal tissue grafts.

**6.2 Rationale of the study:**

Damage to the ocular surface, resulting from conditions like limbal stem cell deficiency (LSCD), chemical burns, and Stevens-Johnson syndrome, poses significant clinical challenges. Traditional treatments, such as autologous conjunctival grafts and amniotic membrane transplantation, often fail in severe cases due to issues like donor tissue scarcity, immunological rejection, and limited long-term success.

Tissue engineering offers a promising alternative by using autologous grafts derived from the patient's own epithelial cells, thereby reducing risks of rejection and disease transmission. Autologous ocular surface grafts, sourced from limbal epithelial cells, and oral mucosal grafts, cultivated from buccal mucosa, have shown potential in restoring the ocular surface. However, their comparative efficacy and safety in randomized controlled trial have not been thoroughly evaluated.

The study aims to address this gap by conducting a prospective randomized controlled trial to compare these two types of autologous tissue-engineered grafts. The study will assess clinical efficacy and their outcomes, including corneal clarity, visual acuity, graft integration, and incidence of complications. Additionally, patient-reported outcomes on comfort and quality

of life will be evaluated. This study seeks to provide evidence-based guidance for researcher and clinicians, optimizing treatment strategies for ocular surface reconstruction and improving patient care in ocular surface reconstruction.

7. **Study Objectives:**

- To compare the efficacy and safety of autologous tissue-engineered ocular (COMET) and oral (CCET) mucosal tissue grafts in treating patients with bilateral limbal stem cell deficiency (LSCD).
- To evaluate the primary outcomes of complete epithelization, vascularization, and conjunctivalization.
- To assess secondary outcomes including improvement in Best Corrected Visual Acuity (BCVA) and corneal transparency.

**8. Study Design:**

- Prospective, interventional, randomized controlled clinical trial.
- Conducted from January 2018 to April 2023 at our institute.

**9. Ethical Compliance:**

- Conducted in accordance with the Declaration of Helsinki.
- Ethical clearance obtained from the Institute Ethics Committee.
- Informed consent obtained from all patients; LAR consent obtained for minors.

**10. Study Population:**

- **Inclusion Criteria:**
  - Paediatric patient from age group 5-18years
  - Adult patients from age group above 18 years
  - Either sex
  - Bilateral ocular burns i.e. Steven Johnson syndrome and chemical burns
  - Schirmer test value of at least 5mm
  - Willing to follow-up for at least 6 months
  - No systematic disorder contradicting surgical intervention.
  - Patients in whom primary insult occurred at least >4 months ago
- **Exclusion Criteria:**
  - The patients whose characteristics do not confirm to the above criteria will be excluded from the study and patients with untreated concurrent problems, such as adnexal problems, glaucoma and infection.

**11. Sample Size:**

- Total of 50 patients.
- 25 patients in the COMET group.
- 25 patients in the CCET group.
- Sample size calculated using a clinical superiority design.

**12. Randomization:**

- Participants randomized into two groups using SPSS version 15.0.

**13. Intervention Groups:**

- **COMET Group (Cultivated Oral Mucosal Epithelial Transplantation):**
  - Harvesting a 4 mm x 4 mm tissue strip from the buccal mucosa.
  - Culturing over a denuded amniotic membrane with DMEM/F12, 10% autologous serum, and antibiotics.
  - Transplantation into the recipient's eye after two weeks of culture.
- **CCET Group (Conjunctival Cultivated Epithelial Transplantation):**
  - Harvesting a 4 mm x 2 mm tissue strip from the conjunctival fornix.
  - Culturing over a denuded amniotic membrane.
  - Transplantation into the recipient's eye after two weeks of culture.

**14. Surgical Technique:**

- Detailed surgical steps for both COMET and CCET are included in the methodology section.

**15. Post-Operative Management:**

- Standard post-operative care for donor and recipient eyes.
- Specific medications prescribed for both COMET and CCET groups.

**16. Follow-Up Schedule:**

- Post-operative day 1, week 1, week 2, month 1, month 2, month 3, and month 6.
- Comprehensive ophthalmic examinations at each visit.

**17. Outcome Measures:**

- **Primary Outcomes:**
  - Complete epithelization and clinically stable corneal surface.
  - Vascularization: Complete success (avascular cornea) and partial success (mild vascularization).
  - Conjunctivalization: Complete success (absence of conjunctivalization) and partial success (mild conjunctivalization).
- **Secondary Outcomes:**
  - Improvement in BCVA.
  - Transparency of the cornea.

**18. Data Collection and Analysis:**

- Data on primary and secondary outcomes collected at each follow-up visit.
- Statistical analysis using SPSS version 15.0.
- Comparison of outcomes between COMET and CCET groups.

**19. Safety Monitoring:**

- Regular monitoring for adverse events and complications.

- Immediate intervention for any adverse outcomes.

**20. Documentation and Reporting:**

- Detailed documentation of surgical procedures, post-operative care, and follow-up assessments.
- Comprehensive reporting of results, including any deviations from the protocol.

**21. Consent Forms:**

- Written informed consent form for adult patients.
- LAR consent obtained form the guardian/parent for minors.

**22. Ethical Considerations:**

- Respect for patient autonomy and confidentiality.
- Minimization of risk and maximization of benefits.
- Adherence to ethical guidelines and regulatory requirements as per ICMR and GCP guidelines.

**References:**

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