PROFORMA FOR SUBMISSION AND APPROVAL OF RESEARCH PROJECT

Title of the Project:

Ex-vivo Cultivated Limbal Stem Cell Transplantation for Treatment of Superficial Corneal Pathologies

Objectives of the Project:

To evaluate the safety of ex-vivo cultivated limbal stromal stem cells for the treatment of visually significant superficial corneal stromal scarring and other pathologies.

Names of the Principal and Co-investigators with designation and qualifications:

Principal Investigator Sayan Basu, MBBS, MS Consultant Corneal Surgeon and Scientist, L V Prasad Eye Institute, Hyderabad, India.	**Co-Investigator** Virender S Sangwan, MS Director, Center for Ocular Regeneration(CORE), Director, Srujana-Center for Innovation, Dr. Paul Dubord Chair in Cornea, L V Prasad Eye Institute, Hyderabad, India.
Co-Investigator Dr. Vivek Singh, Ph.D., (Scientist) Prof. Brien Holden Eye Research Center, Champalimaud Translational Centre for Eye Research, HERF, L V Prasad Eye Institute, Hyderabad, India.	**Co-Investigator** Mukesh Damala, MSc (Biochemistry) Prof. Brien Holden Eye Research Center, Champalimaud Translational Centre for Eye Research, HERF, L V Prasad Eye Institute, Hyderabad, India.

Outline of Previous Work in the field with relevant References:

Background:

Keratocytes, normally quiescent cells of the corneal stroma, transform into fibroblasts in response to injury or inflammation and repair the damaged cornea by laying down scar tissue (1, 2). The presence of altered extracellular matrix components in such stromal scars renders the cornea optically opaque, resulting in visual loss (3). Visual impairment and blindness due to corneal scarring affects millions worldwide (4, 5) and is the commonest indication for corneal transplantation in the developing world (6). Although replacing the scarred tissue with a clear corneal allograft is usually effective in improving vision (7), the global demand for donor corneal tissue vastly exceeds its availability. Moreover, post-operative complications like immune-rejection, astigmatism and infection often restrict the functional survival of corneal allografts (8), especially in developing countries (9). As such, there is increasing interest in the development of therapeutic alternatives to corneal transplant, including stem cell therapy, cell-free collagen scaffolds, and bioengineered constructs (10-12).The recent discovery of multi-potent stem cells in the

corneal stroma has opened up the possibility of developing a cell-based approach to treating corneal scars as an alternative to keratoplasty (13, 14). In a murine model of corneal opacity, human stromal stem cells were effective in regenerating normal corneal extra-cellular matrix and repairing collagen fibril defects (15). Subsequently other groups have independently confirmed the existence of these adult stem cells of mesenchymal lineage in the human peripheral corneal and limbal stroma (16-18). These stromal stem cells are immune-suppressant and may have a role not only in remodeling but also in preventing corneal stromal scars (19).

As the next step towards translating this research into clinical therapy, we proposed to investigate the transplantation of ex-vivo cultivated allogenic limbal stromal cells for the treatment of the corneal pathologies. The limbus is an ideal source as the stem cells are numerous and located very superficially in the tissue (17). Pre-clinical work suggests human corneal stromal stem cells can be isolated from the cadaveric tissues, cultivated in conditions suitable for cell based therapy and used to prevent fibrosis in a murine model of corneal stromal scarring. Further, these cells are able to successfully engraft, differentiate, and mediate wound healing in the corneal stroma such that the tissue remains healthy, free of fibrotic tissue, and optically transparent. The clinical implications of these findings are substantial in that it represents the potential to lessen the burden on donor tissue necessary for corneal allografts by using cultured cells to regenerate tissue. We foresee the ability of a clinician to and grow and expand the cells in number and after surgically removing the scar tissue from the wounded eye, apply the cultured limbal stem cells to regenerate healthy, transparent tissue.

References:

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- **Brief description of the present work:**

In this prospective interventional study patients with unilateral superficial corneal scars will undergo a surgical procedure. Limbal ring from a cadaveric donor tissue, which is therapeutically accepted and serologically tested, is collected. This tissue will then be cultivated in the stem cell biology laboratory using standardized culture technique. Briefly the limbal tissue will be cut up into small pieces and digested overnight using an enzyme (Collagenase L). The cells obtained from the digest will be cultured on a petri-dish using 2%

serum and growth factors. The cultured cells will be passaged three times to remove all epithelial cells from the culture. In the second procedure, the central corneal epithelium will be removed using a surgical sponge. 0.1ml of stromal cells in a concentration of $5x10^3$ cells/uL diluted in the thrombin component of fibrin glue (TISEEL, Baxter) will be applied to the debrided corneal stroma. A soft bandage contact lens will be placed over the cornea at the end of the procedure. The patient will receive topical antibiotic and steroid eye drops in the post-operative period. Periodic comprehensive ophthalmic evaluation along with anterior segment optical coherence tomography (ASOCT) scanning and slit-lamp photography will be done at day 1, day 7, day 45 and day 90. The primary outcome measure of this study is to note any ocular or systemic adverse effects of this intervention at the various post-operative time points. The secondary outcome measures are visual improvement and change in the density and appearance of the corneal scarring and other pathologies after treatment.

- **Justification for use of human volunteers / patients:**

This is a pilot study to ascertain the safety of application of ex-vivo cultivated limbal stromal cells in human corneas. Pre-clinical work in murine models have already demonstrated efficacy of this technique in curing murine corneal scars.

- **Patients - state the type of patients who will be studied and how they will be selected? How often and how they will be selected? How often and how many will be studied? Which consultant(s) will be responsible for the patients?**

This study will include 20 patients with unilateral blindness due to superficial (defined as involving the anterior 200 microns of the corneal stroma on ASOCT imaging) corneal stromal scars that are post-bacterial/fungal keratitis or post-traumatic with normal intra-ocular structures (clear lens and normal fundus/Bscan) and healthy fellow eye. The following exclusion criteria will be applied:

1. Bilateral corneal disease.
2. Corneal scars with limbal dysfunction (clinically defined as absent limbal palisades or conjunctivalization of the cornea) or ocular surface disease including dry eye disease (defined as a Schirmer's test of less than 10mm at 5 minutes).
3. Unknown etiology, post-herpetic eye disease or eyes with active intra-ocular inflammation.
4. Children (<18 years of age).
5. Less than 3 months after documented clinical resolution of acute disease.
6. Inability/refusal to give written informed consent or to undergo any of the anterior segment imaging tests.

- **Normal volunteers - From where will they be recruited? How many?**

No normal volunteers are needed for this study.

- **At the time of their participation in the study, will the subjects be involved in any other experimental work? Please give details.**

  No.

- **List the exact procedures and how often the subjects will be submitted to such procedures, i.e collection of urine and faeces, vene-puncture, intubation, special diet, drugs administered with dose excluding radio-isotopes or irradiation.**

At the time of the first procedure 10 ml of blood will be drawn from the patient. After the second surgical procedure the patients will undergo slit-lamp photography, anterior segment optical coherence tomography imaging (non-contact) and confocal microscopy imaging of the operated eye. All these imaging procedures are non-invasive.

- **State any potential or known hazards of the procedures listed above. How does the investigator intend to overcome these?**

Cultivation of epithelial and stromal cells from limbal biopsies is being performed at our stem cell biology laboratory for the past 13 years. These cultivated cells have been used for clinical therapy and no hazards have been encountered till date.

- **Give any details of any procedures involving radio-isotopes or irradiation.**

No radio-isotopes will be used.

- **What is the investigator's personal experience with the proposed technique?**

The clinical team involved in this study has been performing ex-vivo cultivated limbal stem cell transplantation in human eyes for the past 13 years.

- **How will the informed consent be taken and by whom?**

Written Informed Consent will be taken by the PI and Co-PI's before the patients are enrolled for the study.

- **Please details whether the subjects are to be reimbursed expensed incurred during participation in the study and also whether or not it is contemplated giving them a gift or token of any sort. If so, please state the exact amount.**

The subjects participating in this study will not be charged for the surgical interventions or imaging tests. In the event of any unforeseen adverse event the Institute will provide free treatment for the same.

- **Is the protocol, copy of the patient's information and consent form appended with the application.**

Yes.

- **Action plan for reporting adverse reaction to Ethics Committee and other participants of the study.**

  Yes.

- **In the event of any adverse reacting arising directly or indirectly during the course of the trial, who will bear the financial responsibility for the on-going care of the patient?**

  The L V Prasad Eye Institute will bear the financial responsibility.

- **Any other information that you would like to provide to the Ethics Committee.**

  No

Signatures of the Investigators and Co-investigators.