Proposal Name: Limbal Stem Cell Bioengineering

Submitted by: Dr. Lional Raj, Mr. Heber Anandan

Please provide a description of the proposal (up to 500 words):

The integrity of the self-renewing corneal epithelium relies on the existence of stem cells, which are located in the limbal basal layer. Destructive loss of limbal stem cells or dysfunction of their stromal niche causes what is clinically referred to as limbal stem cell deficiency (LSCD), characterized by conjunctivalization of the cornea, vascularization, chronic inflammation, and persistent epithelial defects. Severe ocular surface disease resulting from LSCD as in chemical injury, Stevens-Johnson syndrome (SJS) and ocular cicatricial pemphigoid are devastating conditions that represent a major clinical challenge. Conventional corneal transplants alone in these conditions are destined to fail. Ocular surface reconstruction by means of amniotic membrane transplantation and limbal transplantation has been effective to some extent.

The successful use of cultivated limbal stem cell transplantation to treat severe ocular surface disease may have an important advance in the pursuit of completely xenobiotic-free bioengineered ocular equivalent for clinical transplantation.

Patients having ocular surface disease unresponsive to standard treatments would be chosen for transplantation after getting informed consent.

Postoperative follow-up would include serial slit-lamp examinations with fluorescein staining, as well as photographic documentation. The success of the procedure would be defined by a stable ocular surface and subjective improvement in the symptoms of the patient at 6 months follow up. Secondary outcome measures would be visual acuity and post treatment complications.

We would develop a primary cell line against LSCD.

Describe and justify the potential public health impact\(^1\) of the proposal:

The essence of our idea is to investigate the clinical outcome of ocular surface reconstruction in limbal stem cell deficiency (LSCD) using cultivated limbal stem cell transplantation and to develop a limbal stem cell bank for South Indian population.

Describe and justify the technical feasibility\(^2\) of the proposal:

Ex vivo expansion of cultured limbal epithelial stem cells seeded on a matrix derived

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\(^1\) Principally CEWG criterion 1 but others may be relevant e.g. Equity/distributive effect including on availability and affordability of products and impact on access and delivery.

\(^2\) Principally CEWG criterion 4 but others may be relevant e.g. Rational and equitable use of resources/efficiency considerations
from human amniotic membrane would be bioengineered to circumvent potential complications related to conjunctival limbal autograft transplantation.

Confirmation of growth can be done by various methods including direct observation, whole mount stained preparation, histopathology, immunohistochemistry, thymidine incorporation and by flow cytometry using markers for cell cycle.

Surgery would be done after two weeks. At the time of surgery, complete removal of the corneal pannus and conjunctiva up to 3 mm from the limbus would be performed. Allogeneic and autologous cultivated corneal epithelial equivalents would be transplanted onto the ocular surface.

**Describe and justify the financial feasibility**¹ of the proposal:

<table>
<thead>
<tr>
<th>Equipments</th>
<th>- 75,000 USD</th>
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<tbody>
<tr>
<td>(Limbal stem cell cultivation, cell line development)</td>
<td></td>
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<tr>
<td>Clinical Testing (Up to 6 months)</td>
<td>- 25,000 USD</td>
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<tr>
<td><strong>Total</strong></td>
<td>- 100,000 USD</td>
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</table>

Development of stem cell bank would require further funding.

**Describe in what way the proposal addresses cross-cutting issues**²:

We have no product thereby no dislinking between R&D cost and price of product.

**Identify key steps necessary to begin implementation and key issues to be resolved for implementation to begin**:

A stem cell bank would be established for limbal stem cell transplantation for south Indian population.

**Provide the evidence base for the proposal including literature references and other relevant information**:

Given below.

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**Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease.**

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¹ Principally CEWG criterion 5 but others may be relevant e.g. Cost-effectiveness.
² “Cross-cutting Issues” refers principally to CEWG criteria 7-12, if not addressed elsewhere in the submission e.g. Potential for delinking R&D costs and price of products.
Schwab IR, Reyes M, Isseroff RR.

Source
University of California, Davis Medical School, Department of Ophthalmology, USA.

Abstract

PURPOSE:
To bioengineer a corneal surface replacement using ex vivo expanded, cultured corneal epithelial stem cells seeded on a matrix derived from amniotic membrane and use this bioengineered graft to manage difficult ocular surface disease.

METHODS:
Fourteen patients with ocular surface disease unresponsive to standard medical and surgical treatments, including seven patients with presumed limbal stem cell deficiency were chosen for transplantation of a bioengineered composite corneal surface in eye each. Presumed corneal stem cells were harvested from either the patient's or related donor's limbus, expanded ex vivo, and cultivated on a carrier of modified human amniotic membrane. The resulting composite cultured tissue was transplanted to the ocular surface of the diseased eye, from which the abnormal tissue had been surgically removed. Ten patients received autologous grafts, and four received allogeneic grafts.

RESULTS:
A successful outcome, defined as restoration or improvement of vision, along with maintenance of corneal re-epithelialization and absence or recurrence of surface disease was obtained in 6 of the 10 patients with autologous procedures and in all 4 allogeneic transplants. Follow-up ranged 6-19 months with a mean of 13 months.

CONCLUSIONS:
This novel technique documents that presumed corneal epithelial stem cells can be harvested safely from the limbus, expanded successfully in vitro, and grown on denuded amniotic membrane. The resultant composite cultured tissue can be transplanted and appears to successfully manage eyes with difficult ocular surface disease, including those with stem cell deficiency. This technique minimizes the threat of damage or depletion to the contralateral or donor limbus.

PMID: 10928750

[Research and development for treating devastating corneal diseases].

Kinoshita S.

Source

Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan. shigeruk@koto.kpu-m.ac.jp

Abstract

In order to develop new therapeutic modalities for corneal diseases, it is essential to combine cutting-edge translational research based upon liberal original ideas obtained from clinical experience with state-of-the-art basic science and technology. Here, I describe seven important research projects on which our group has been working. 1. Elucidation of the pathogenesis in gelatinous drop-like corneal dystrophy (GDLD). Due to loss of function of the tumor-associated calcium signal transducer 2 (TACSTD2), a responsible gene for this dystrophy, tight-junction-related proteins cease to function, resulting in severe corneal epithelial barrier impairment. As a result, various proteins contained in tear fluid continuously penetrate into the corneal stroma, promoting the development of massive amyloid deposits. 2. The development of cultivated mucosal epithelial transplantation: A landmark surgery, involving the transplantation of cultivated mucosal epithelial cells from in vitro to in vivo, now recognized as the next generation of ocular surface reconstruction. We began performing cultivated allocorneal epithelial transplantations in 1999, and cultivated auto-corneal and auto-oral mucosal epithelial transplantations in 2002. These proved to be very effective in the reconstruction of both the corneal surface and the conjunctival fornix. 3. Elucidation of the pathogenesis of Stevens-Johnson syndrome: Studies have shown that there is a close relationship between corneal epithelial stem cell loss and the associated degree of visual impairment. We discovered that a steroid pulse therapy at the acute phase aimed at minimizing stem cell loss is very effective in restoring visual acuity. This implies that inhibition of the cytokine storm is essential for the treatment of acute-phase Stevens-Johnson syndrome. The innate immunity abnormality seems to be heavily involved at the onset of this devastating disease. 4. Elucidation of the involvement of EP3 and toll like receptor 3 (TLR3) in inflammatory ocular surface reactions: We discovered that EP3, one of the prostanoid receptors expressed by ocular surface epithelium, has a dramatic inhibitory effect on ocular surface inflammation in a mouse model. Since EP3 is also expressed in human ocular surface epithelium, and since abnormality of its single nucleotide polymorphisms (SNPs) is involved in some ocular surface inflammatory diseases, we theorized that an allergic reaction may be negatively regulated by EP3 which is predominantly expressed by the ocular surface epithelium. Our findings show that this is similarly true for TLR3, which, conversely, upregulates ocular surface inflammation. 5. Functional regulation of the ocular surface epithelium: Our findings show that intracellular glutathione (GSH) content in the ocular surface epithelium regulates its intracellular redox state. For instance, the GSH content of the conjunctival epithelium decreases in dry eye diseases, yet recovers after the surgical insertion of a punctal plug. Since various amino acids are also heavily involved in the regulation of cellular functions, we investigated the profile of amino acids contained in tear fluids. Our results indicate that there is a marked difference in amino acid profiles between tear fluids
and plasma. Furthermore, we found that several amino acids are up-regulated in inflamed eyes, probably due to an oxidative redox response. 6. The development of new therapeutic modalities for corneal edema: We are developing a new therapeutic modality of cultivated corneal endothelial transplantation using methods based on regenerative medicine. For instance, our findings show that cultivated corneal endothelial sheet transplantation in monkeys maintains corneal transparency for at least four years after transplantation. The supplementation of a Rho kinase (ROCK) inhibitor in the culture media produces an excellent result in culturing human corneal endothelium, maintaining a normal-looking endothelial cell morphology. The use of a ROCK inhibitor, both for cultivated endothelial cell injection into the anterior chamber and for use as a topical application, may prove to be a potential tool for the treatment of corneal endothelial dysfunction. 7. The development of a new type of tear function test: The results of our investigations show that the time-dependent changes of tear film lipid layer (TFLL) spread are compatible with the Voigt model of viscoelasticity, and that the initial velocity of the TFLL spread after a blink decreases in proportion to the decrease in tear volume. Thus, a lipid-layer analysis will become an important tear analysis tool. The above are projects representing the way we believe new treatments for severe corneal diseases are heading.

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[PubMed - indexed for MEDLINE]


Matrix revolution: molecular mechanism for inflammatory corneal neovascularization and restoration of corneal avascularity by epithelial stem cell transplantation.

Ma DH, Chen HC, Lai JY, Sun CC, Wang SF, Lin KK, Chen JK.

Source

From the Limbal Stem Cell Laboratory, Department of Ophthalmology, Chang Gung Memorial Hospital, Taoyuan, Taiwan; Department of Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan.

Abstract

ABSTRACT Corneal neovascularization (CNV) associated with severe limbal stem cell (LSC) deficiency remains a challenging ocular surface disease in that corneal inflammation may persist and progress, and the condition will not improve without LSC transplantation. A
prominent feature after successful LSC transplantation is the suppression of corneal inflammation and CNV, which is generally attributed to the endogenous anti-angiogenic/anti-inflammatory factors secreted by corneal epithelial cells. In addition, corneal epithelial basement membrane (EBM) plays a unique role in the regulation of angiogenesis; several potent anti-angiogenic factors are derived from the matrix component of EBM, such as endostatin (from collagen XVIII) and restin (from collagen XV). Also, angio-inhibitory thrombospondin and tissue inhibitor of metalloproteinase-3 are deposited in EBM. Moreover, the heparan sulphate proteoglycan in EBM can bind and sequester VEGF and FGF-2 from activation. Recently, cultivated corneal epithelial transplantation (CCET) and cultivated oral mucosal epithelial transplantation (COMET) have emerged as promising techniques for the treatment of LSC deficiency. When human limbo-corneal epithelial (HLE) cells are cultivated on cryopreserved amniotic membrane, production of endostatin, restin, and IL-1ra is enhanced. This highlights the significance of delicate epithelial-matrix interactions in the generation of anti-angiogenic/anti-inflammatory factors by HLE cells, and this may, in part, explain the rapid restoration of corneal avascularity following CCET. In addition, whether epithelial stem cells can persist after transplantation is the key for CCET and COMET. Emerging evidence of long-term survival of cultivated epithelial cells after transplantation suggest that epithelial stem cells can be isolated and cultivated in vitro, and can re-establish the epithelial phenotype in vivo. Taken together, the merits of enhanced anti-angiogenic activity and the preservation of corneal epithelial stem cells encourage further application of this tissue engineering technique for ocular surface reconstruction.

PMID: 19635246