***SHERIEF HOSNY AHMED*, M.D, FRCSEd**
Head of Cornea & Refractive Department,
Magrabi hospital Cairo.
***MERVAT El –DEFTAR*,M.D**

The LESCs are located at

LIMBUS

The transitional zone between cornea and conjunctiva

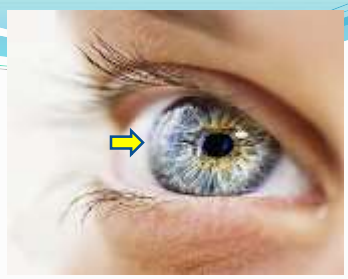


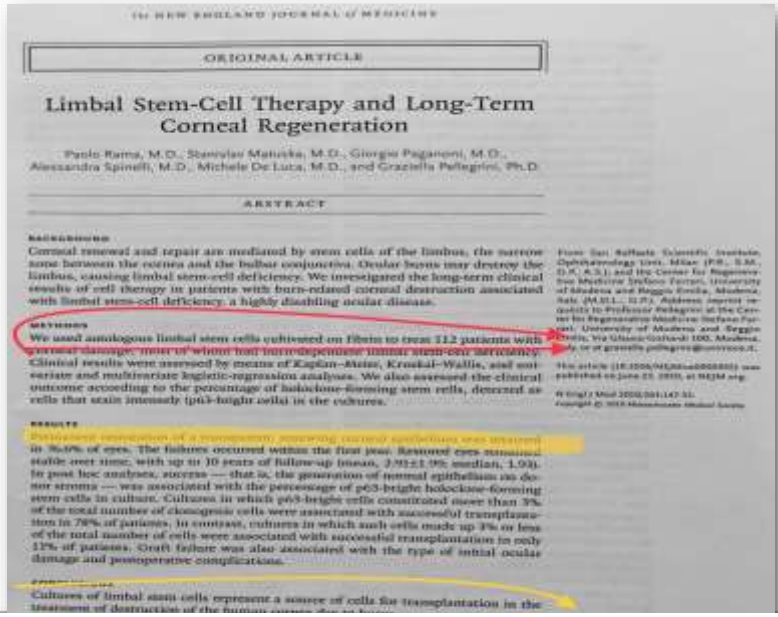
Fig. 1: Limbal ring between cornea and conjunctiva

LIMBAL STEM CELL DEFECIENCY (LSCD)

May occur as a result of depletion of stem cells or destruction of their stromal niche such as in:

- Congenital: Aniridia
- Idiopathic conditions
- Chemical/thermal burn
- Iatrogenic: surgery or contact lens use
- Autoimmune: Stevens Johnson syndrome and OCP

LEADING ARTICLES



THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration

Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D., Alessandra Spinelli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D.

ABSTRACT

MATERIAL & METHODS

Design of study

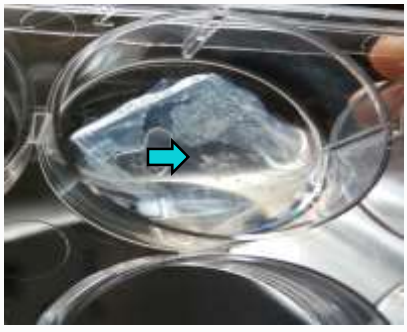
- In this pilot study we have chosen to deal with one etiology of ***LSCD***, which is ***chemical burns***.
- Five patients were selected for this study , all of them have previous occular ***chemical burns.*** Other new patients were added consecutively.
- Study started 2014
- In all affected eyes the ***limbus was totally damaged with conjunctivalization.***
- Vision was ***hand movement*** in these eyes.
- The surgical procedure of (Implanting cultured limbal cells) was started after receiving the usual medical treatment for chemical burns for 6 months .

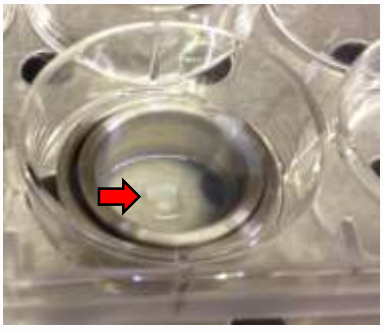
Preparing explants

a. Auto explants 2x1 mm were excised from other eye limbus , transferred to the cell culture lab in collection medium.

b. Allo grafts are transferred ,in the same way from fresh excised limbal corneal button rings. Superficial epithelial layer was freed from underlying stroma and endothelium mechanically.

Feeder-free Limbal Culture Procedure





- Fresh frozen amniotic membrane (AM) 5x5 cm was prepaired in Eye Bank. AM was screened for infectious diseases e.g. HCV, HBV and HIV.
- AM was denuded by **thermolysin enzme** (125 µg/mL) in phosphate buffered saline (D-PBS).
- AM was **stretched** and limbal explants were cultured over epithelial side.

Culture was **submerged** in growth medium for 10-14 days in CO2 incubator at 37 ˚C, 98% humidity and 5% CO2.

- **Human Corneal Epithelial (HCE medium)**
- **DMEM/F12**
- **Fetal calf serum (FCS) 10% or autologous serum**
- **Epidermal growth factor (EGF) 10 ng / ml**
- **Cholera toxin 100 nm/ml**
- **Insulin 5µg / ml**



CELL CULTURE FACILITY

Biosafety cabinet type II A

CO2 incubator

Inverted phase contrast microscope

In all experiments:

Stem cells commenced to grow from the edges of explants by day 1-3. Cells were large, rounded with high N/C ratio.

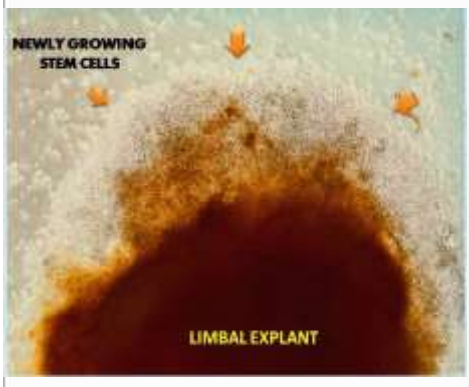


Fig . : Phase contrast microscope showing SC growing from edge of imbal explants Day 4 200x

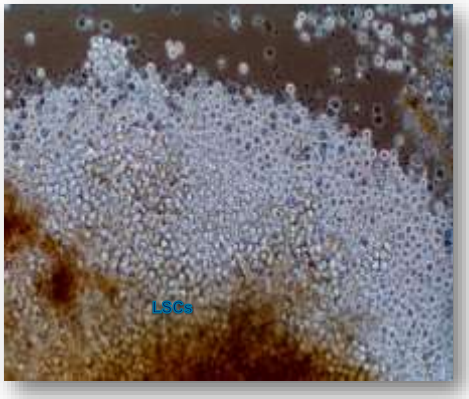


Fig. : Phase contrast microscope showing SC exhibiting rounded shape with high N/C ratio growing from edge of limbal explants Day 6, 400 x

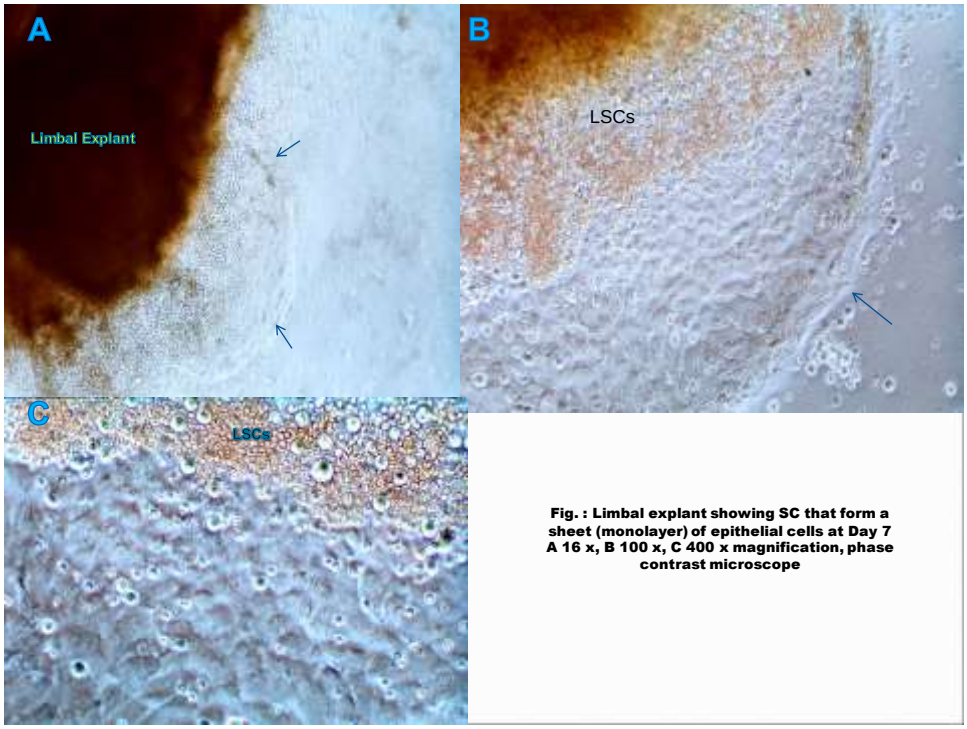
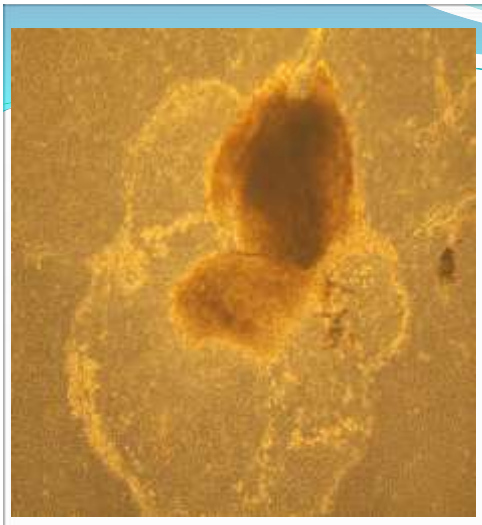
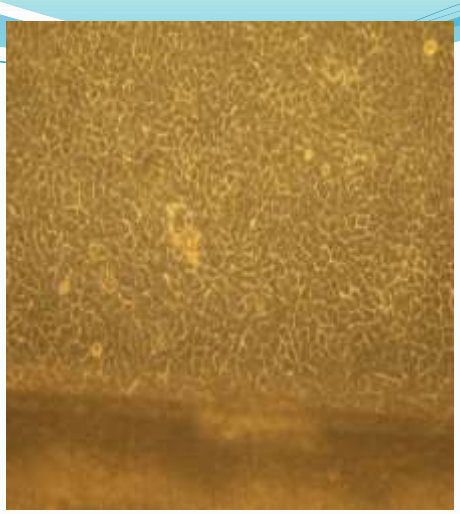


Fig. : Limbal explant showing SC that form a sheet (monolayer) of epithelial cells at Day 7 A 16 x, B 100 x, C 400 x magnification, phase contrast microscope

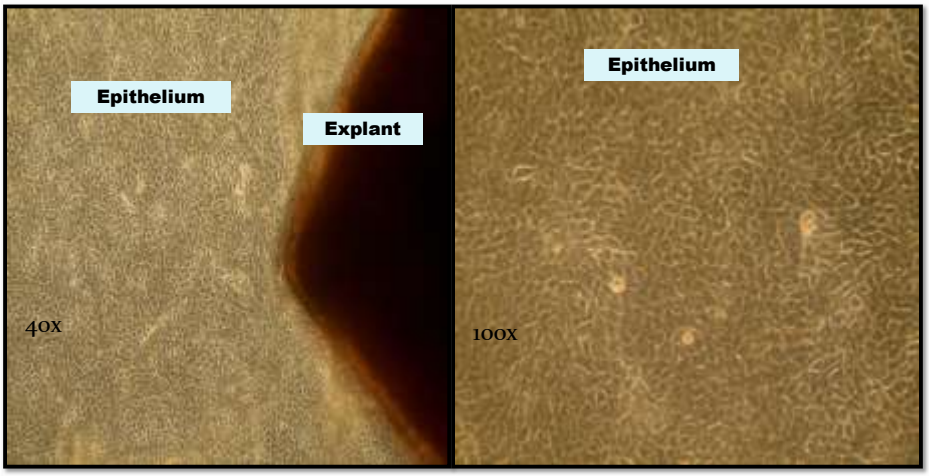


Day 2 of culture : a sheet of epithelial cells started to expnad from explant

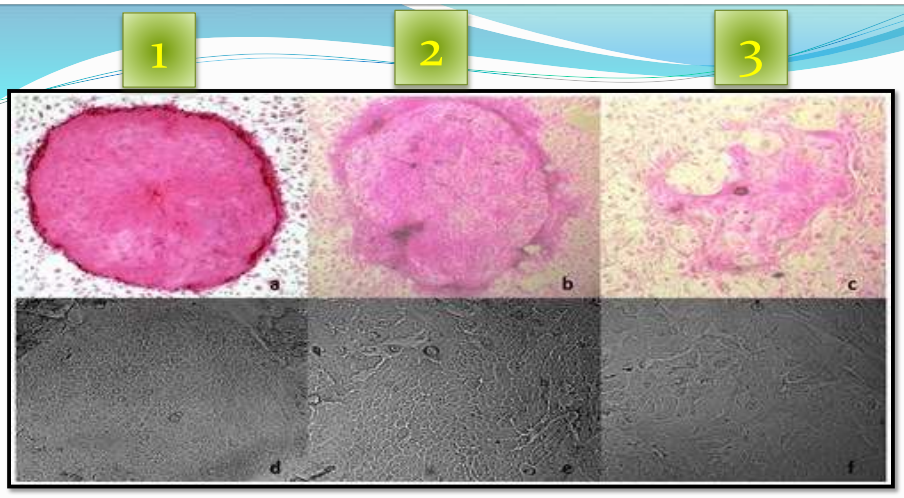


Day 12 of culture : a sheet of corneal epithelial cells covered AM

Auto & Allograft (cadaveric rim) explant culture showing confluent multilayered epithelium at Day 14 of culture ,corneal phenotype was proven by RT-PCR using P 63 & CK3/12(moclecular test)



When the AM is **sufficiently** covered by expanded limbal epithelial cells,within 14 days, it is transplanted to the eye affected by LSCD.



1- **Holoclones** : Diameter of 6-10 µm. These cells have a high proliferating capability with ≤5% aborted colonies and ≥100 cell doublings;
2- **Meroclones** : Young TA cells with intermediate proliferating capacity having a diameter of 10-18 µm. These cells usually have 5-95% aborted colonies;
3- **Paraclones** TD cells with 15-20 cell doublings and very low proliferative capability. These cells are 18-26 µm long in diameter

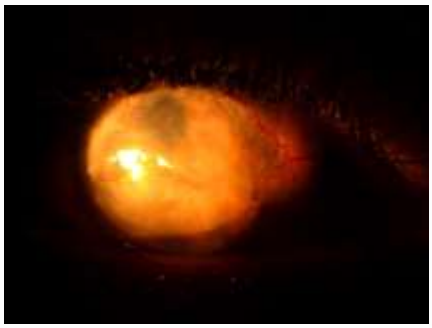
Lost cultres

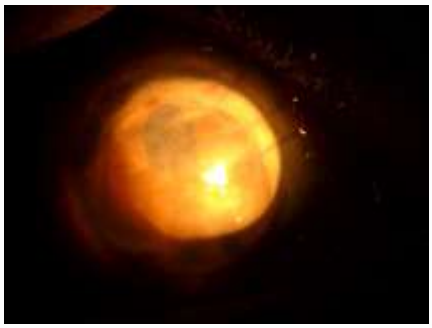
Surgical steps

- Conjuntiva is dissected through a 360 degree periotomy, peeled from underlying cornea
- The cultured amniotic membrane is transferred with cell facing up and sutured to corneal limbus with 8/0 nylon
- Cultered amniotic membrane was left to stablize and enrich the corneal surface with cultered limbal cells
- Pkp with a secondry cultured amniotic membrane was done to restore vision ,after 6 months
- Patients who had Allografts ,received cyclosporin 1% eye drops 3 times daily

FIRST CASE- ALLOGRAFT TRANSPLANTATION

- PRE OP VA : HM
- POST OP VA: CF 1 M

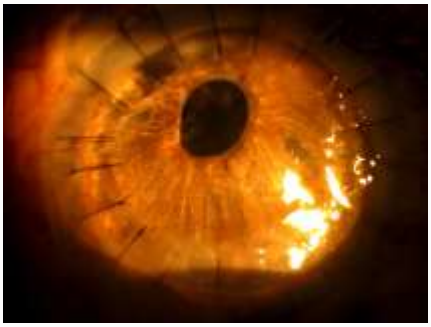


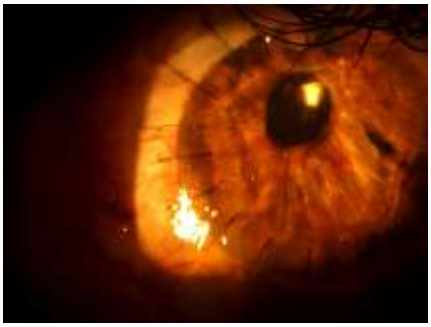


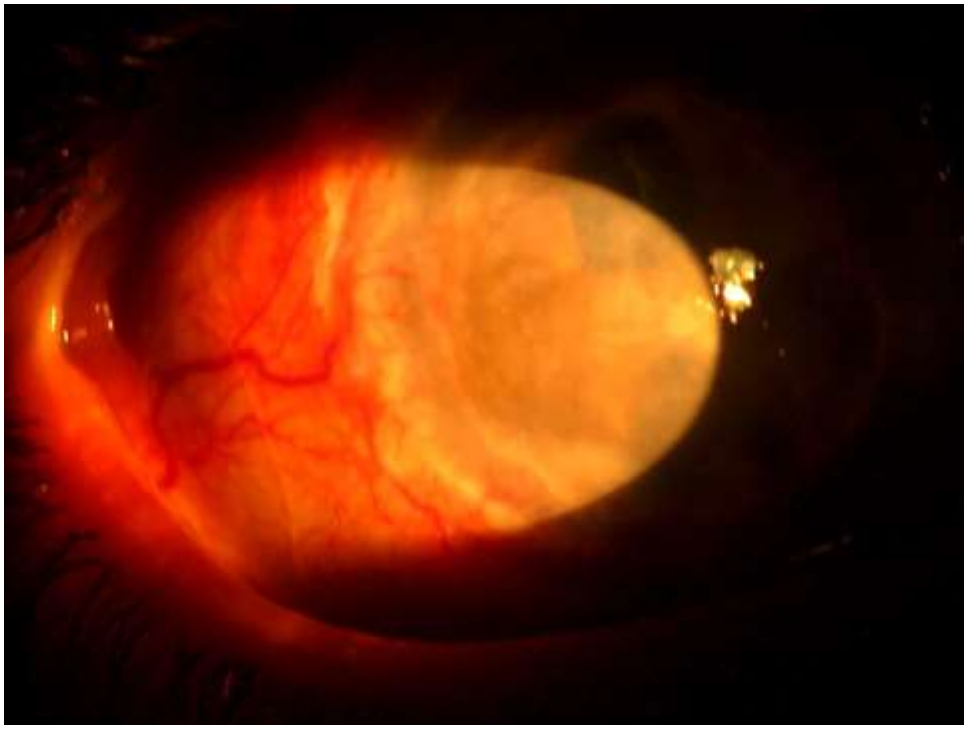
POST KERATOPLASTY AND STEM CELL GRAFT

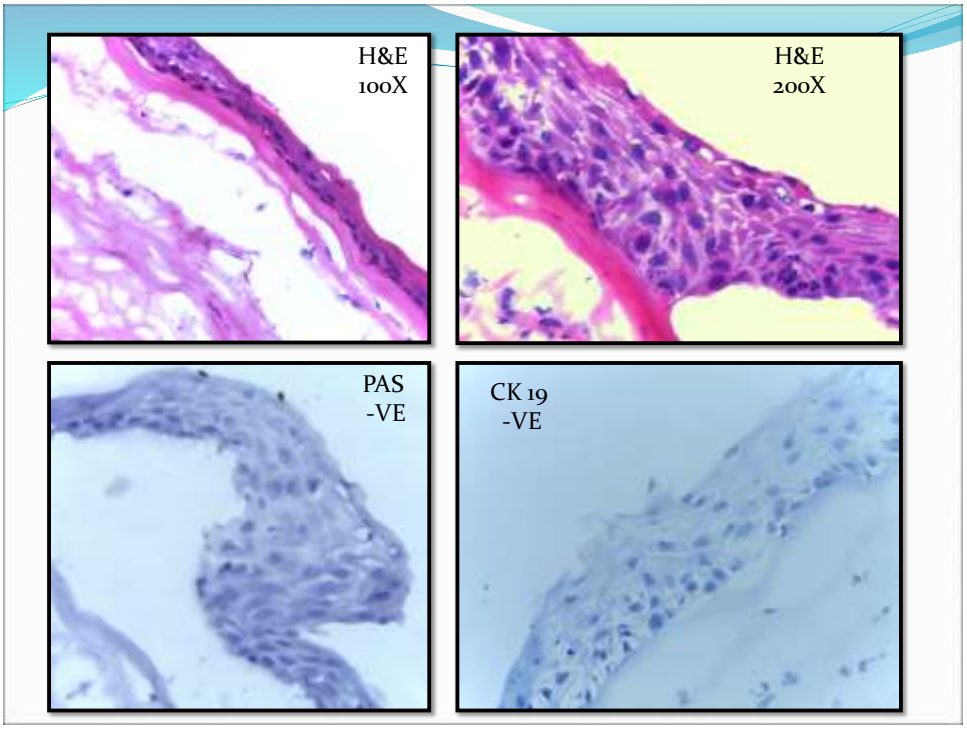
THREE MONTHS V.A : CF3M

SIX MONTHS V.A: 0.1



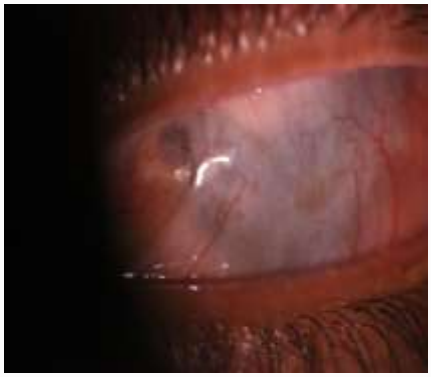





H&E
100X
H&E
200X
PAS
-VE
CK 19
-VE

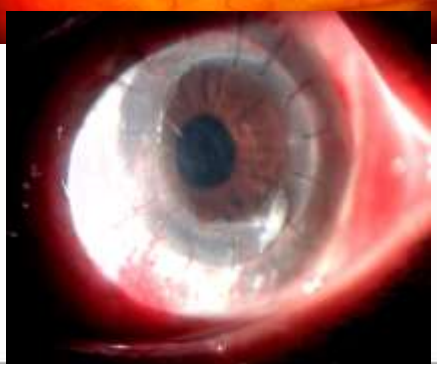
Second case (allograft)

Preop V.A : H.M



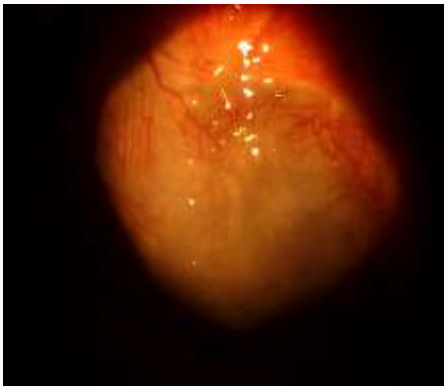
6 month post op V.A : C.F 2M

Post keratoplasty condition



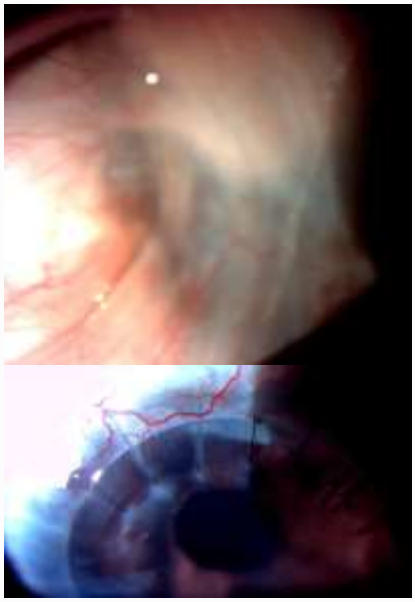
Third case (Auto grafting)

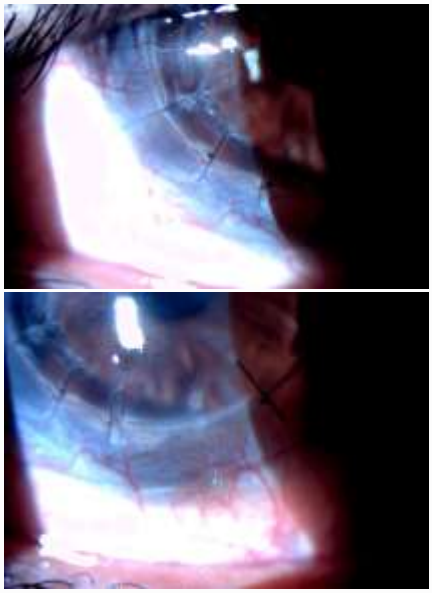
Pre op vascularized surface VA: H.M



Six month post op Avascular surface VA:CF 1 M

Fourth case(Allograft)





Fifth patient
Auto graft failure

RESULTS

- Three allografts and two autografts were done
- Two patients gained unaided vision 0.1
- One patient gained unaided vision 0.2
- Fourth patient gained unaided vision 0.2, maintaned for four years then followed by graft failure after phaco surgery
- Regarding surface vascularization, 3 cases maintained graft clarity till now.
- One patient had graft vascularization in 2 quadrants
- Fifth one suffered from total vascularization and graft failure

Conclusion

- Patients with conjunctival chemical burns have a new hope, with an overall success rate 80%
- Ex vivo limbal cell implants are a good solution for restoring a new avascular medium , ready to receive a new corneal implant
- The question is how long are these cells going to last ?
- What should we do to potentiate the residual limbal cells to multiply and produce extra cells
- We should gain local ethical committees approval for this technique, to continue with large volume studies.

3D Corneal printing

3D bioprinting of a corneal stroma equivalent.
(PMID:29772228 PMCID:PMC6083436)

Isaacson A[1], Swioklo S[1], Connon CJ[1]

Affiliations

Experimental eye Research (30 May 2018; 173:188-193)

Type: brief-report, journal Article
DOI: 10.1016/j.exer.2018.05.010

Abstract

Corneal transplantation constitutes one of the leading treatments for severe cases of loss of corneal function. Due to its limitations, a concerted effort has been made by tissue engineers to produce functional, synthetic corneal prostheses as an alternative recourse. However, successful translation of these therapies into the clinic has not yet been accomplished. 3D bioprinting is an emerging technology that can be harnessed for the fabrication of biological tissue for clinical applications. We applied this to the area of corneal tissue engineering in order to

Graphical abstract

THANK YOU