Ex vivo expansion of Limbal Stem Cells : RIO Experience

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Stem cell Lab

- The lab was established as a part of the Cornea Unit headed by Prof. Dr. Ahmed Atef.
- The tremendous support and continuous follow up of Prof. Dr. Sherif Karawya, President of RIO, has a crucial role in launching the project.

Stem cell Lab Team

- Prof Dr Tarek ElSergany
- Prof Dr Azza Khalil
- Assisst Prof Nervana Anwar
- Dr Mona Abdel Rasol
- Dr Ragda Nagaty
- Dr Ahmed Mostafa
- Dr Essam ElEraky
- Dr Arwa Mohamed
- Prof Dr Maisa Nour Eldin
- Prof Dr Eman Zaki
- Prof Dr Maha Hagag
- Prof Dr Eman ElShabrawy
- Dr Shady Soliman
- Dr Mehry ElSobky
- Dr Mey Hasan
- Dr Nesrin Saleh

Stem cell Lab

- It is a Clean Room facility, using gradient air pressure and HEPA filters. It is comprised of 2 rooms (4 sections) and is equipped with:
  - Laminar Airflow
  - CO2 Incubator
  - Refrigerated Centrifuge
  - Inverted Microscope

Equippment




Historical Background

- *Ex vivo expanded human limbal* epithelium was introduced by Pellegrini *et al in 1997* as a treatment for human patients with LSCD.
- This procedure has now become a treatment of choice for LSCD in many countries.

Technical Challenges

- Since then, various protocols have been developed for expanding limbal SCs.
- They differ in a number of aspects:
  a) whether limbal biopsy tissue is used as explants and/or rendered into single cells,
  b) whether and how AM is prepared and used as a carrier,
  c) whether murine 3T3 fibroblasts or human mesenchymal stem cell-derived feeder layers are used, and
  d) use of air-lifting to promote stratification

RIO Research Plan

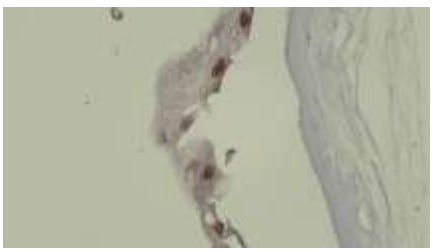
- Considering these concerns, we started with experiments of limbal stem cell expansion.
- In addition, other experiments with mesenchymal stem cells and oral mucosal epithelial cells are planned.

Limbal Stem Cell Expansion

- Culture of minced pieces of limbal biopsy (explant method) on amniotic membrane was done. Histological examinations showed growth of many layers of cells.


Characteization

- Immunohistochemical staining of expanded limbal cells using monoclonal antibodies p63α showed:



Current Experiments

- Current experiments compares culture of cell suspension prepared by enzymatic digestion to culture using limbal explants.
- Different concentrations of EGF are evaluated.
- IHC staining using ABCG2 and CK3 will be performed.

Future Experiments

- Management of bilateral LSCD by LESC-enriched cultures is not feasible, hence, alternative cell sources are needed.
- A study of the potential use of bone marrow MSC is planned. Damaged corneal epithelial cells induced by alkali burn in rat model will be treated with cultured MSCs.

Future Experiments

- Ex-vivo cultivated oral mucosal epithelial cells will be studied in rabbits as a second alternative cell source for cultivated limbal epithelial cells.