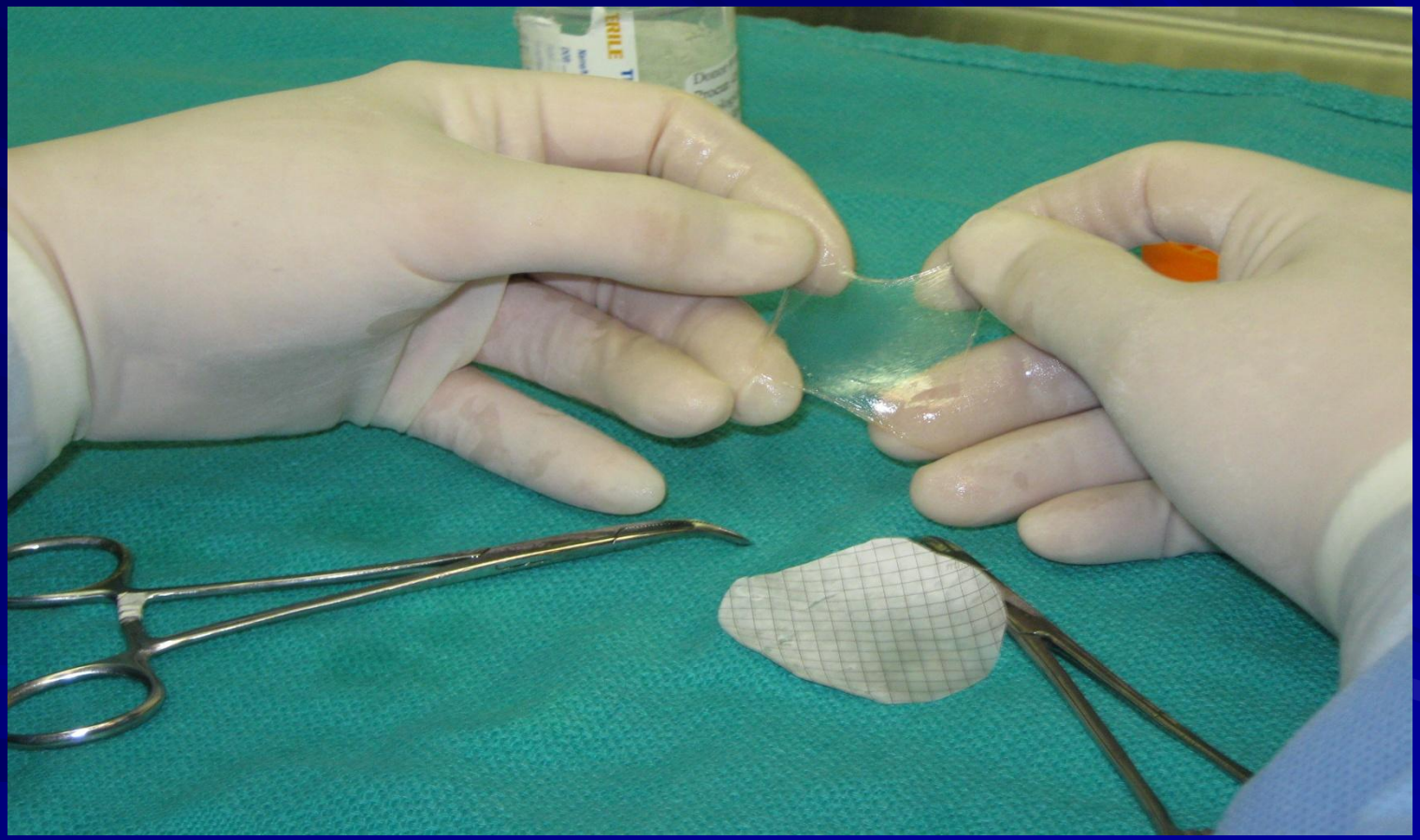
Limbal stem cell culture

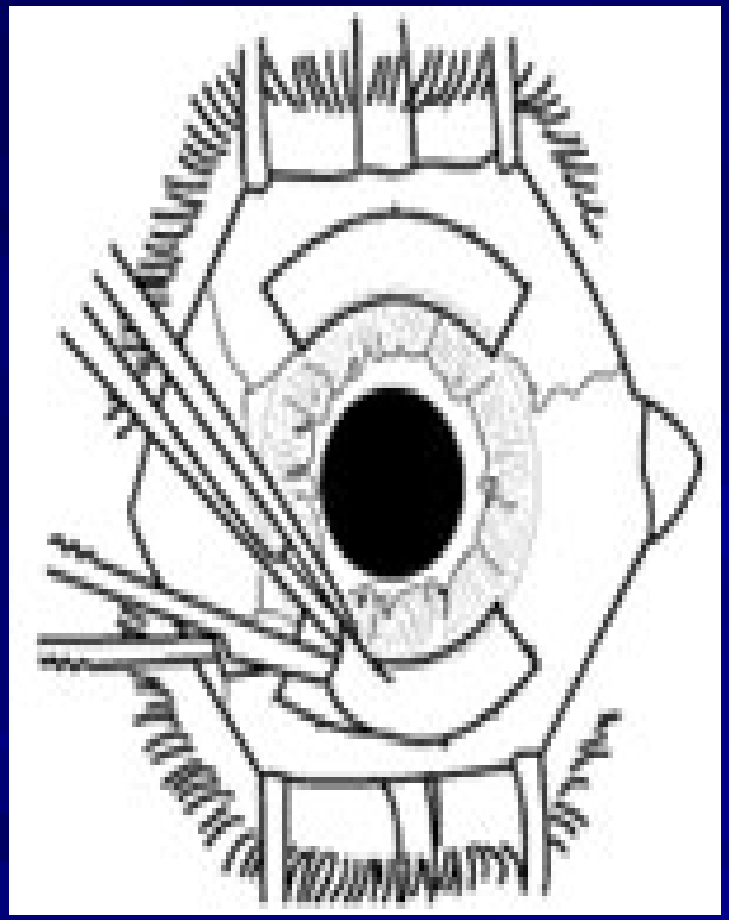
- No standardised method exists for expanding LSC's.
- Methods include:
  - the use of limbal explants culture on amnion membrane with and without epithelial cells;
  - cell suspension culture and air lifting, 3T3 fibroblast feeder layer

Ex vivo culturing LSC's

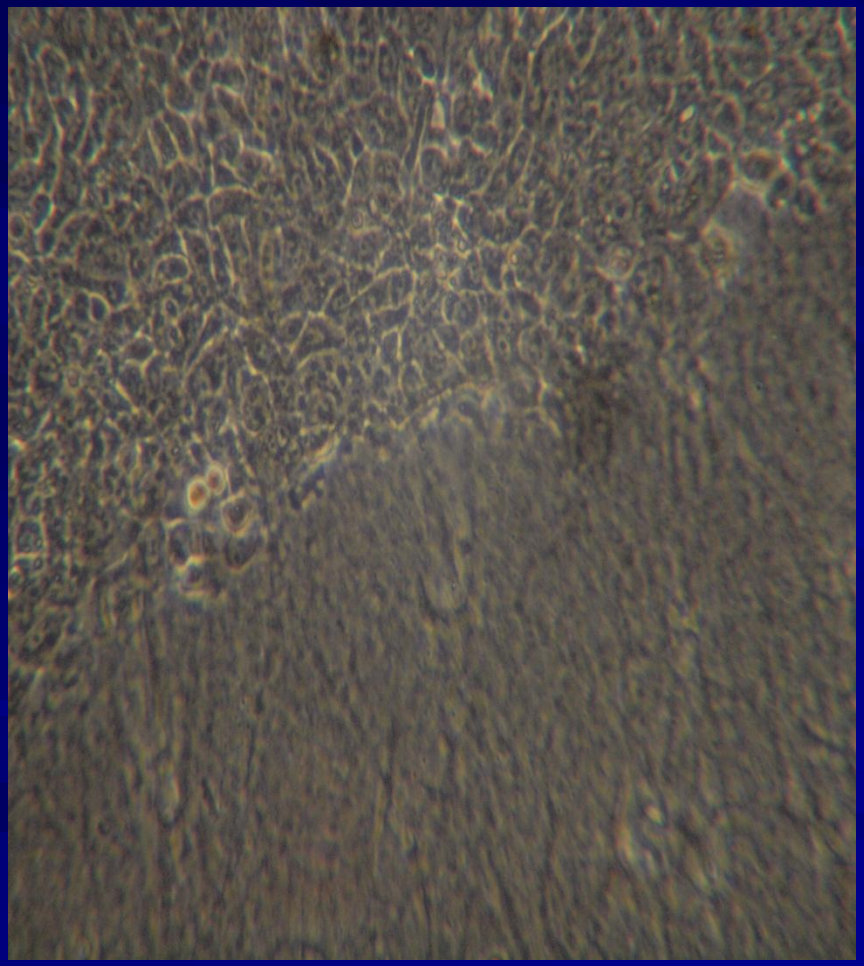


Multiple pieces of AMT (36) from one donor.

Culturing limbal stem cells



LSC culture



- Morphology
- H & E
- ΔnP63ά
- CK3
- PAS

ATMP EC No 1394/2007

- Hospital exemption – Article 28
  - Products prepared on a non routine basis according to specific quality stds;
  - within the member state, in a hospital;
  - Under the exclusive professional resp of a medical practitioner;
  - Individual prescription;
  - custom made product for an individual.

Challenges

- Research to GLP to GMP.

  -Documentation, forms, SOP's:

  standardize the procedure, Validation plans,

- Consumables – small volumes

  - CE marked consumables

  - Clinical grade materials not always

    available - risk assessment;

Challenges

- Substrate
  - Avoid use of cell lines - Xeno free
  - Source of amnion; Regea in Finland.
  - Biological variability of the carrier (amnion) and the corneal / scleral rim.

Challenges

- Media preparation

  - Challenges to find clinical grade reagents;

    PAA in Austria – ATMP ready.

  - EDQM certificate of suitability for bovine serum

Challenges

- Time consuming working in the clean rooms;

  - EM; preparation of consumables prior to entry; feeding every 2 days;
- Media fills
- Staff / training
- Difficulties in standardizing products made from living cells
- Cost

The end goal