Queen Victoria Hospital
NHS Foundation Trust

East Grinstead Eye Bank

Cell culture for treating LSCD

January 2025

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Limbal Stem Cell Deficiency

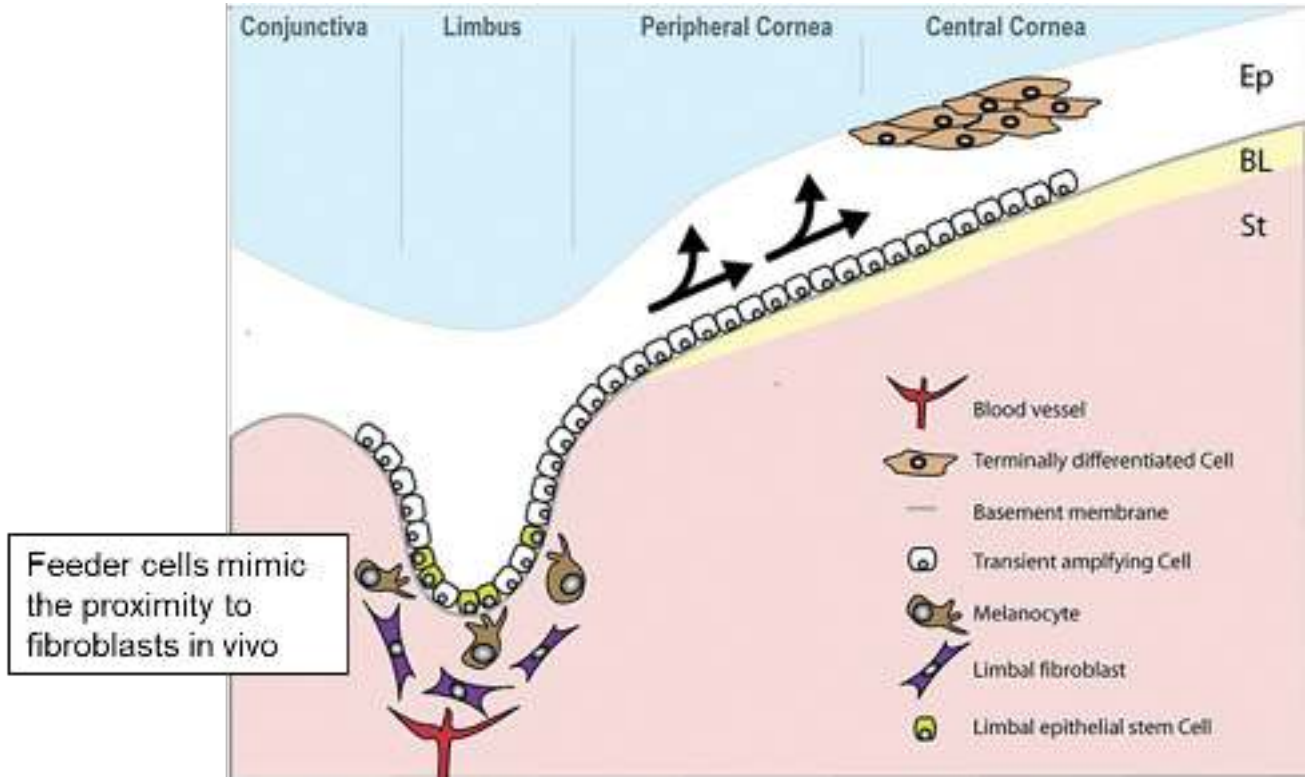
Severe LSCD which requires surgical intervention

Limbal stem cells are slow cycling cells with low mitotic activity and a long life span under the normal steady state.

Limbal stem cells are supported by a unique stromal microenvironment or stem cell niche, which consists of extracellular matrix components, cell membrane associated molecules, and cytokine dialogues.

They may be activated on demand for tissue regeneration to increase their own population or to differentiate into early and then late transient amplifying cells (TAC), which are located in the corneal basal epithelium.

Damage to the limbus results in loss of barrier function and vascularized conjunctival invasion of the cornea and subsequent epithelial defects.



Limbal Stem Cell Deficiency

Aniridia, chemical/thermal burns, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, and severe contact lens-induced keratopathy or as a result of multiple surgical procedures.

Leading cause of untreated blindness in the US, estimated at 1-5 per 10,000 prevalence in US

Holland et al. Cornea. 2023, 42 ,9

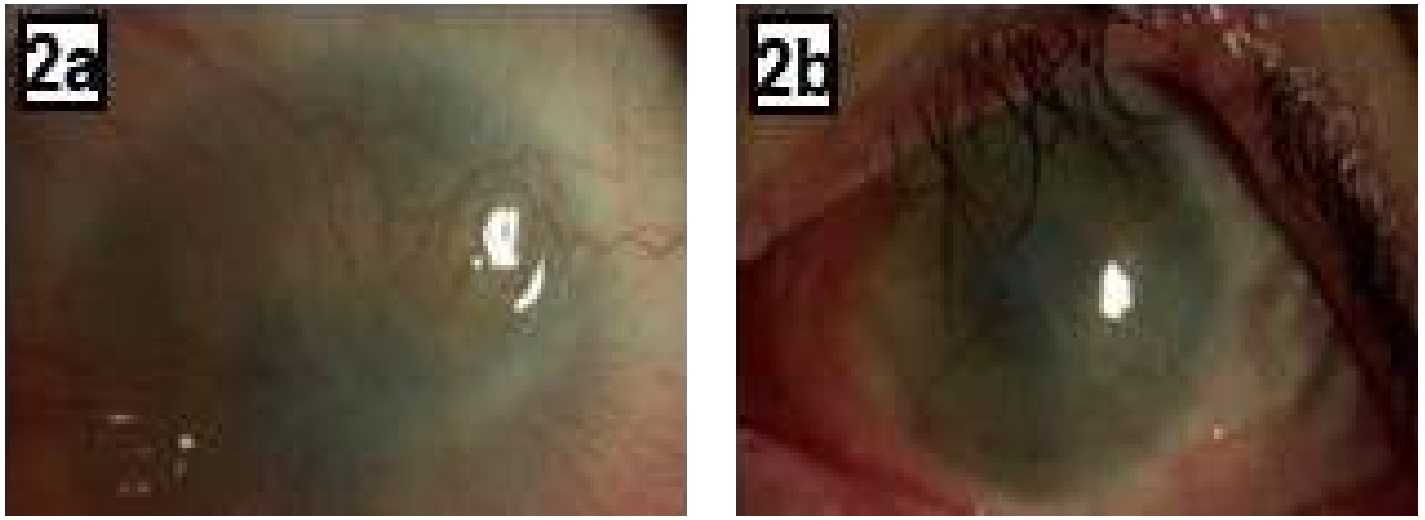
10 million people globally

Marta Cadenas-Martin et al. Int. J. Mol. Sci. 2023, 24, 2350

Management of this disease has really accelerated over the last 30 years and there have been many developments. Initial surgical treatment involved keratolimbal or conjunctival limbal auto graft and following along from this the use of auto slet

Auto grafts are limited to unilateral disease so for bilateral disease and if damage to the fellow eye is a concern the options have been keratolimbal allograft and allo slet.

Cell culture therapies are less common due to lack of specialist facilities based on cGMP but the process began in the 1990s



a) LSCD in a patient suffering from a thermal burn
b) 8 weeks post ex-vivo stem cell graft

Clinical Cell Culture at East Grinstead Eye Bank

Our Limbal stem cell culture program started in 1999 surgical application taking place in early 2004

Limbal cell culture – came out of skin keratinocyte culture

Use at QVH was first published by Daya et al. 2005. Ophthalmology, 112,3

Outcomes of first 10 patients published in 2005. This led to National Institute for health and Clinical Excellence (NICE) approval moving it to a standard treatment in the NHS

COMET Cell Culture treatments began at QVH 2014 –

Oral mucosal Cells - Alternative cell type with a similar cell structure – no requirement for immunosuppression

Cornea availability and Immunosuppression not required

QVH

Cell culture requirements – Laws, standards and regulation

UK Regulation for Cell Culture – when we started there was limited regulation and changing regulators

Starting material – consent and procurement HTA

We are in a fortunate position in the UK -

Processing through to issuing authorised by MHRA under a hospitals specials licence – does not have marketing authorisation so used on individual request basis

MHRA grouped Oral Mucosal cell culture in with Epithelial (skin) culture process from an existing trial into paediatric burns

Introduced by a change in practice submission agreed at hospital level and followed up by an initial audit

As with other countries there is requirement for compliance with cGMP

Culture facility requirements – facilities and services

UK / EU requires cleanroom for processing cGMP Grade A safety cabinets + Grade B back ground air quality (approx. ISO grade 5)

Bi annual validation + constant viable and non viable particle monitoring during critical processes

Access virology testing laboratories

Starting material – pathogen free for Limbal cell culture donor free from any previous malignancies

Documented maintenance program and monitoring of storage equipment

Use of defined products all sterilised before use

Sterility testing throughout process any contamination followed up by Species level identification

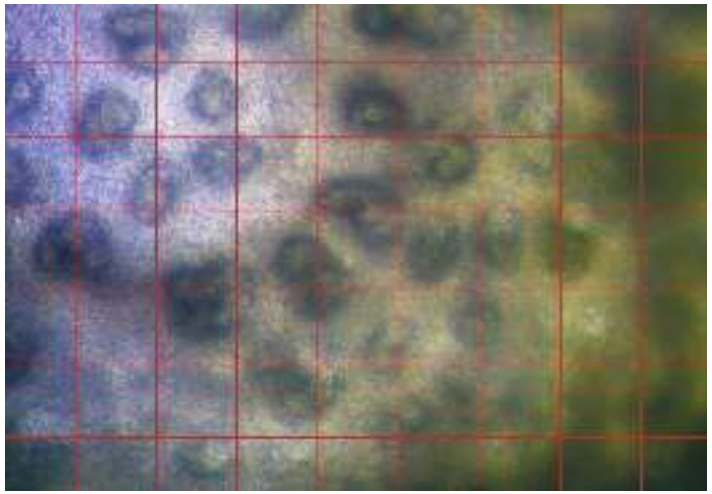
Clinical cell culture starting materials

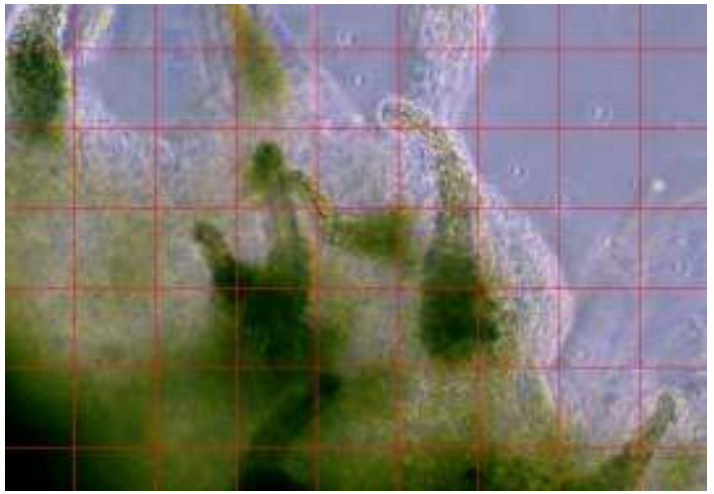
COMET Cell Culture

Punch biopsies taken from inside the lower lip

Non keratinized stratified squamous epithelium
Undulating epithelium rete pegs attach to the underlying projections of the lamina propria

Prepared up to one day after procurement



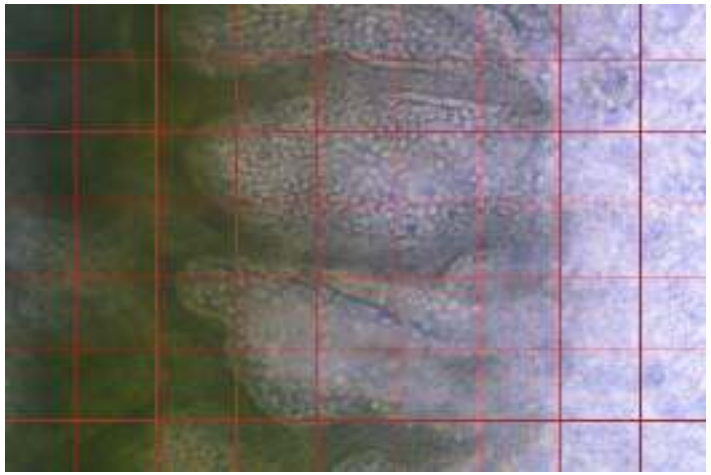


Limbal Stem Cell culture

Whole corneas used from non malignant donors

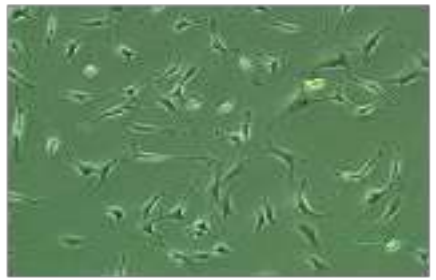
Possible to use rims remaining from PK
Sterility issues if opened for a prior procedure

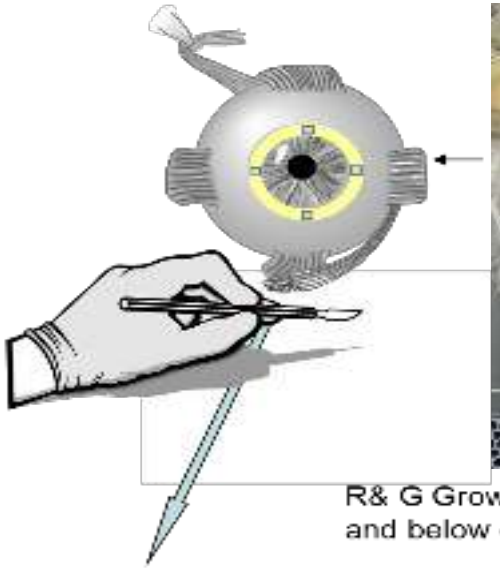
Optisol or Organ Cultured corneas used



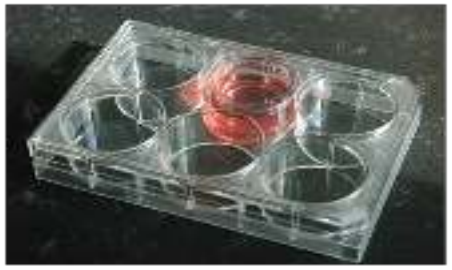
Clinical Cell Culture at East Grinstead Eye Bank

Murine fibroblasts





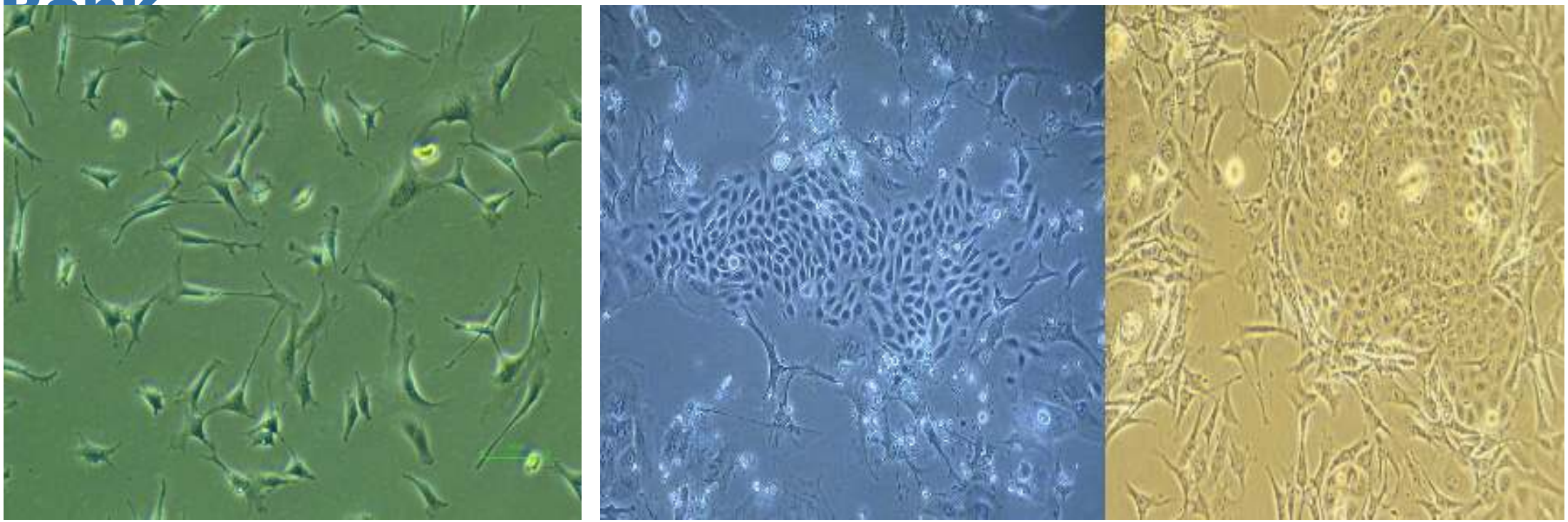
R& G Growth media above and below cell support



Cell sheet applied to patients after 21 day culture period

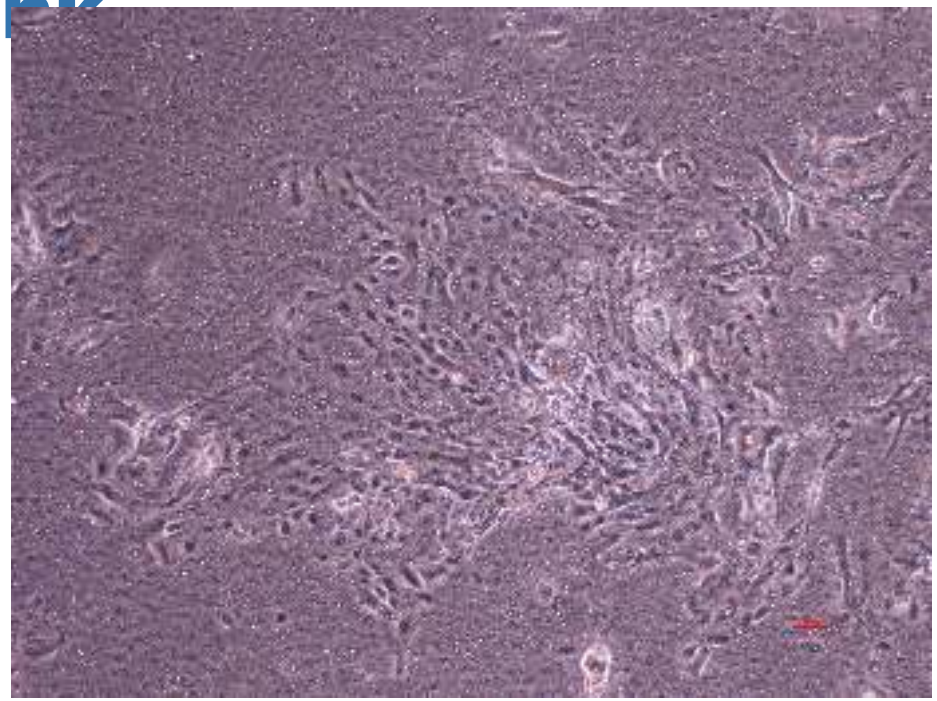
- DMEM
- F12
- FBS
- EGF
- Hydrocortisone
- Cholera toxin

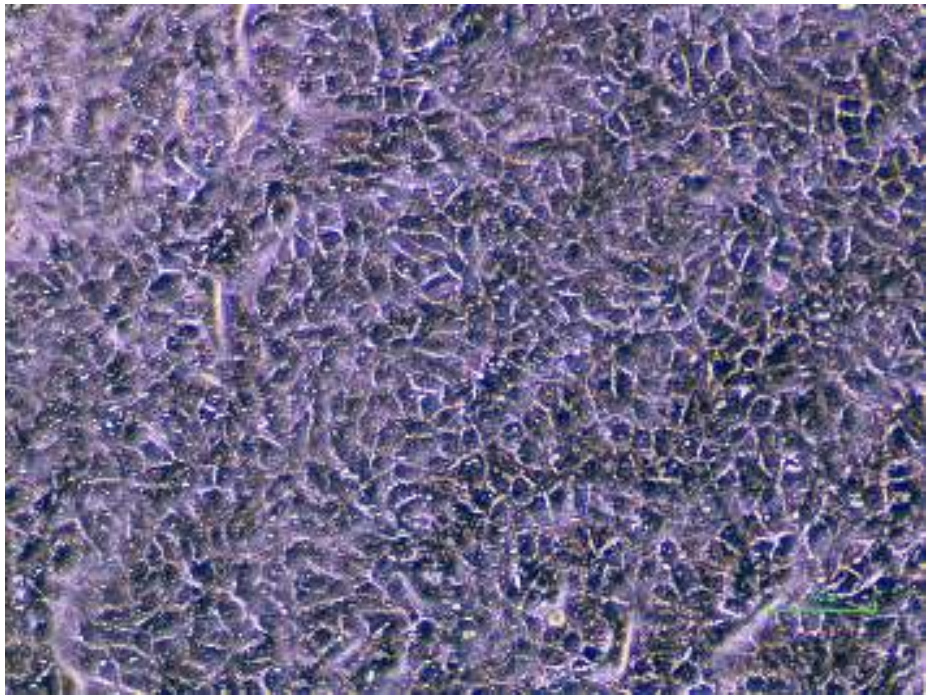
Clinical Cell Culture at East Grinstead Eye Bank



Co Culture with X-Ray irradiated murine fibroblasts

Clinical Cell Culture at East Grinstead Eye Bank





Clinical Cell Culture at East Grinstead Eye Bank

Alternative Culture Systems

Explants or cell suspensions as starting points
De-epithelialized or intact amniotic membrane
Fibrin, Collagen, Chitosan substrates
Hyaluronic acid hydrogels
Nano cellulose
Electrospun substrates
Temperature responsive polymers

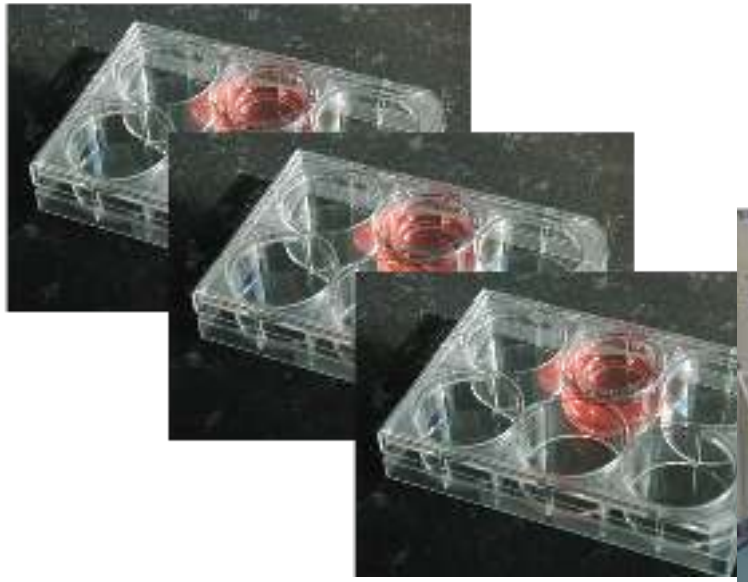
Each may have different advantages, create better microenvironments, allow different cell types to be incorporated, increase ease of transport / application

Remove the need for enzymatic release of cell sheets and possible cell or cell adhesion damage

Our methodologies work for our establishment set up – eye bank adjacent to theatres

Clinical Cell Culture at East Grinstead Eye Bank

Samples are taken for microbiological sterility testing at 3 stages during the cell culture process - set up, mid process and at cell harvest.



3 Week culture period
Media change 2/3 days
2 sheets of cells issued
Autologous Plasma Eye Drops depending on treatment strategy

Clinical Cell Culture at East Grinstead Eye Bank

Outcomes

Elalfy et al. January 2025. Ophthalmology and Therapy.
Retrospective study

40 eyes treated with COMET cells
54 eyes treated with ACLET cells

Majority of patients – Aniridia or Chemical Injury

Graft – (surface) survival 81.7% in ACLET vs 60.7% COMET

Improvements in VA shown in ACLET group but not COMET

However, main purpose is surface stabilisation – particularly when additional graft procedures are required

COMET does not require immunosuppression 13% associated post op complications with ACLET

COMET and ACLET both effective for managing advanced and bilateral LSCD.

Future developments

Limited commercially available products + limited use case.

Holoclar® – EU Italy. Autologous limbal cells
Nepic® – Japan. Autologous Limbal epithelial cells
Ocural® – Japan. COMET oral mucosal cells
Sakracy®– Japan. COMET oral mucosal cells with AMT
Toshida et al. Int. J. Mol. Sci. 2023, 24, 8926

All expensive -more cost effective cell culture solutions required.

Wider access to autologous limbal and COMET cells more difficult as it is a 2 stage production process requiring specialist facilities which come with many licencing and regulatory requirements.

Banked expanded Limbal cells more work needed to maintain LSC characteristic

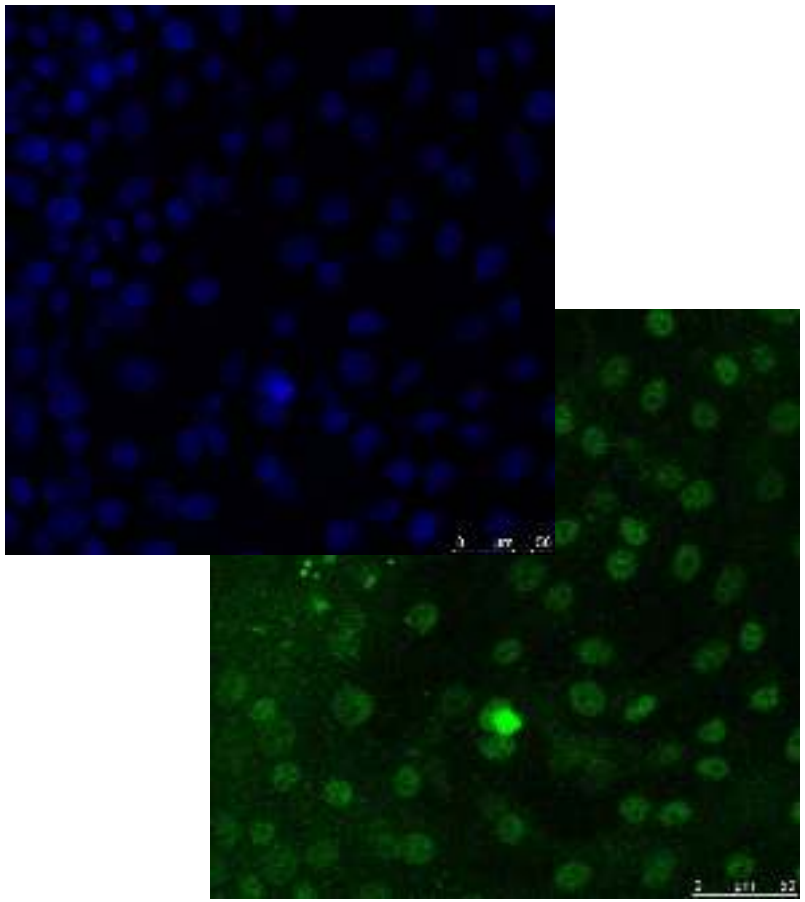
For wide scale roll out cells need to be produced in bulk and be transportable

Xeno free culture – possible disease reduction. Defined media - removing the use of animal serum (difficult with our methodology due to sheet formation issues)

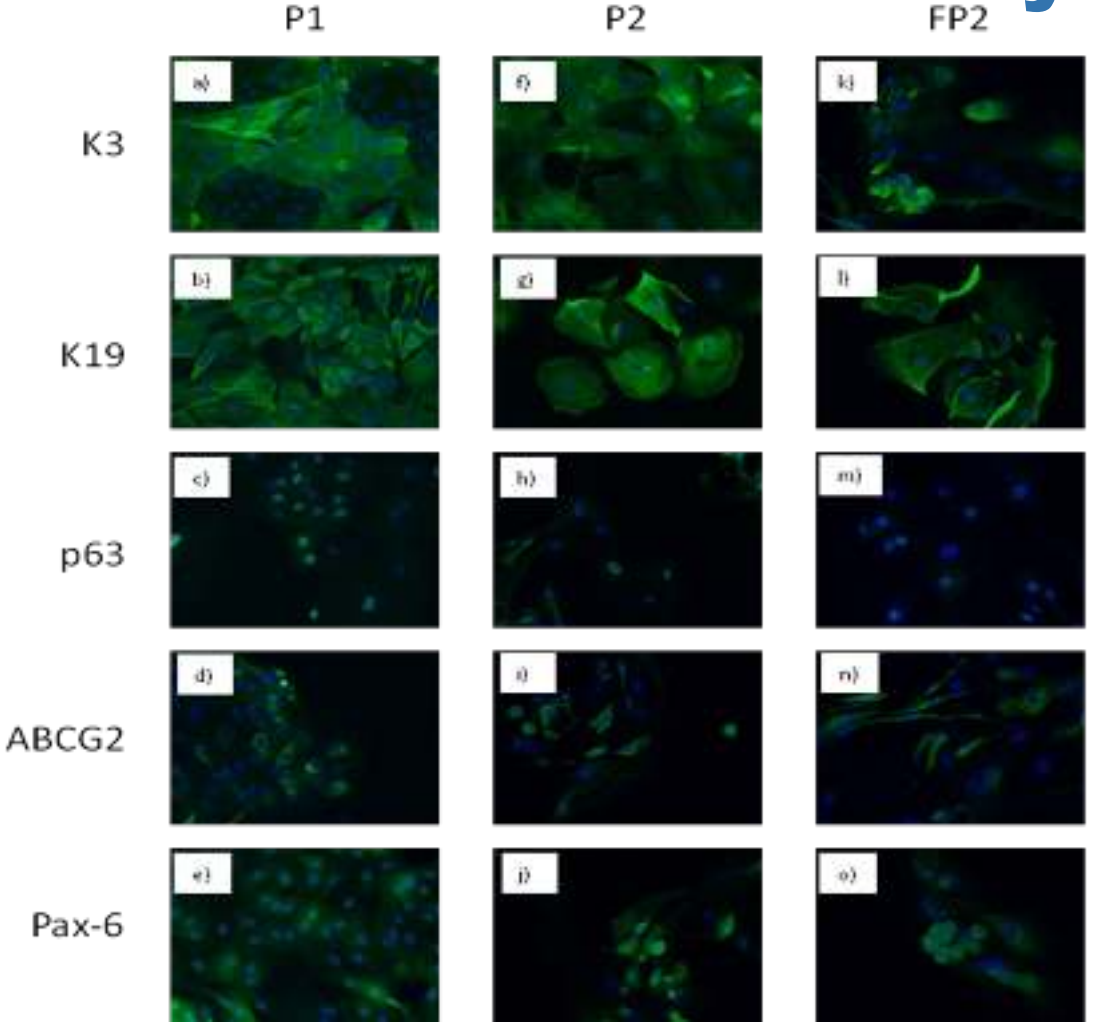
3t3 replaced with other feed cell types i.e. human processed lipoaspirate cells (PLA)
Nieto-Nicolau et al. Stem Cell Research & Therapy. 2019, 10.

QVH

Clinical Cell Culture at East Grinstead Eye



Confocal images blue stain (DAPI) – nucleus staining and green +ve p63 marker.



- The Immunofluorescence images show positive staining for the putative stem cells markers K19, p63, ABCG2 and Pax-6
- There was some (possible background) staining for K3 at P1
- This indicates the probability of there being a mixed population of cells but shows that we are culturing cells originating from the basal layer.
- As such the isolation and culture techniques used are supportive of LESC.

Future developments

Better understanding of the niche environment and functionality – improving the host environment means that cell transplanted cells will more likely perform as required

Better understanding of the mode of action – stimulatory effect on pre-existing dormant / limited number of cells or wound healing response recruiting circulating cells – development of biologics

Delivery mechanisms - Improving the environment for transplanted cells to increase the stemness of cultured cell population. i.e. collagen scaffolds seeded with other cells types – e.g. keratocytes - replacing HAM and 3t3 cells.

Other cell sources for repopulating of the corneal epithelium such as mesenchymal stem cells (MSCs) and induced pluripotent stem cells are being investigated. IPCs a newer emergence - possible increase risk of tumorgenicity.

Derived stem cells from Human Adult Mesenchymal Stem Cells / Adipose cells These offer a greater source of cells which ultimately could be produced with clearly defined properties. Adipose cells more differentiated may not be as effective

Suggested direct injection of MSCs

Future – Gene therapies / Micro RNAs as modulators of cellular activities

Future developments

From a clinic / medical public health perspective: -

Pharmacologic therapies. Limited currently – corticotrophin immunomodulator for Stage II LSCD. Topical growth factors increased use / availability of plasma / serum eye drops

Cho et al. Invest Ophth Vis Sci. 2023, 64

Prevention - Education Public health messaging chemical / thermal injuries – a significant percentage of male subjects

Surgical – Better diagnosis, diagnostic tests, confirmation of LSCD - removing inappropriate surgical interventions

Better training + understanding of management of LSCD e.g. use of SLET + use of systemic immunosuppression - earlier referral to specialist centres

Wider adoption of internationally agreed classification, diagnosis and management policies

Deng et al. Cornea. 2019, 38(3) Deng et al. Cornea 2020, 39, 10

Awareness of the adverse impacts other treatments can exert on the limbus / stem cell niche, preservative drops, other surgical procedures

Cheung et al. Cornea. 2021, 40, 12

Summary

There is a wide range of treatments being looked at currently in clinical trials.

Cell based products need to be scalable, and transportable for widespread uptake in their use

The development of cell based therapies and biologics for example will need to follow the development path of other pharmaceutical products to become widely commercially available other wise they will remain restricted to a few specialist centres

All these promising areas will however need significant further funding, collaboration between scientists, clinicians and Eye Banks, involvement of multi disciplinary teams and engagement with industry to improve on existing strategies and make these more widely available and improve the treatment of LSCD.