



Regenerative Medicine & Tissue Engineering

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SCTIMST- BMT

Indo-Dutch Workshop on Life Sciences and Health

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Strategies for regenerative medicine



Cell-based therapy (Applied)

Cell sourcing

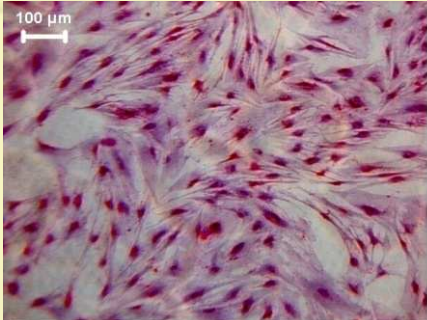
Bioartificial tissues and devices (Applied)

- Development of polymeric substrate for tissue engineering and scaffold free in vitro tissues
- Bioengineered cornea
- Bioartificial liver
- Toxicity model

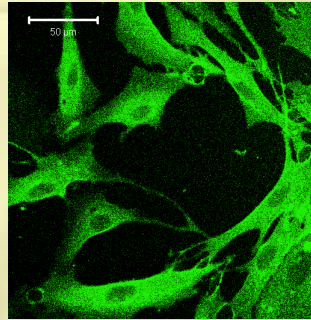
In situ induction of regeneration (Basic science)

- Understanding of stem cell to epithelial differentiation
- Mesenchymal stem cells to hepatocytes and corneal epithelium .

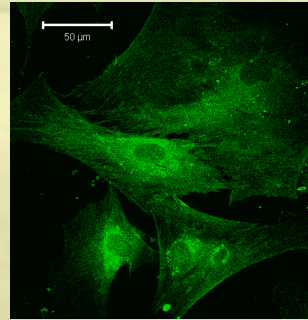
Mesenchymal stem cells



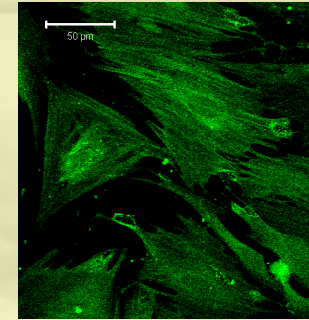
Geimsa



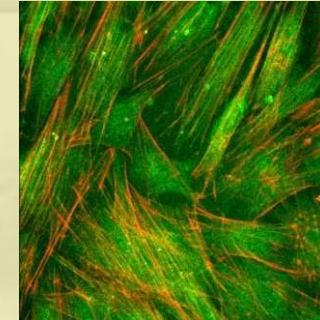
CD90



Vimentin

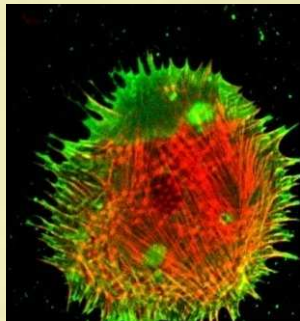


CD105

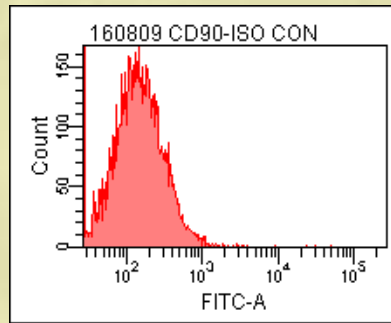


CD90 FITC Actin
Texas red

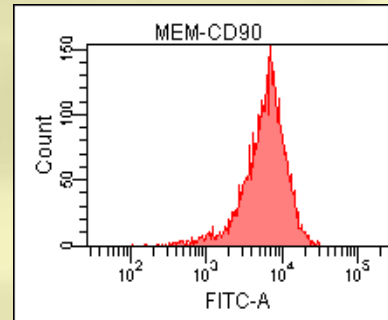
Differentiation



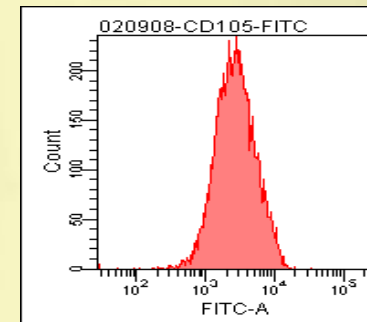
CD90 FITC
CD105 TRITC



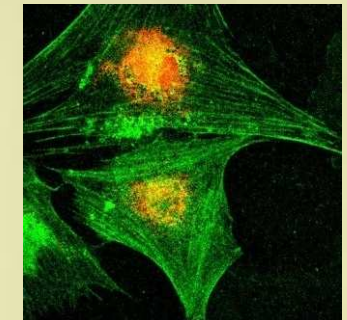
Control



CD90- 94%

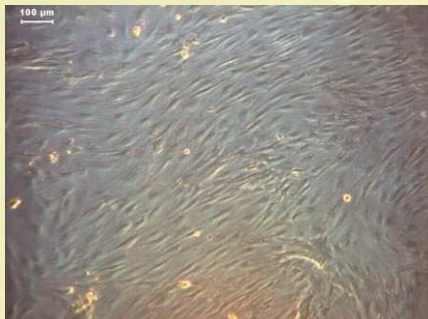


CD105- 97%



Albumin FITC + PI

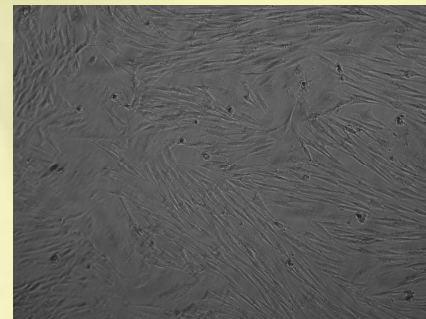
Immortalisation (RGCB)



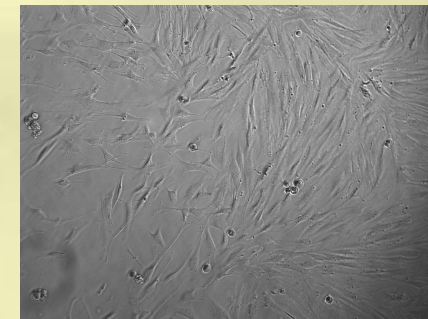
Pc DNA Neo HTERT



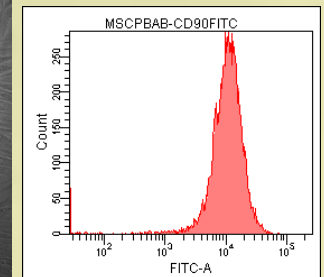
PBABE Puro HTERT



Pc DNA Neo HTERT(P15)



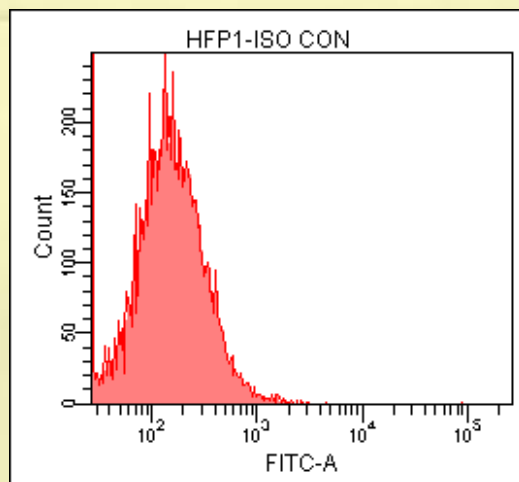
PBABE Puro HTERT(P15)



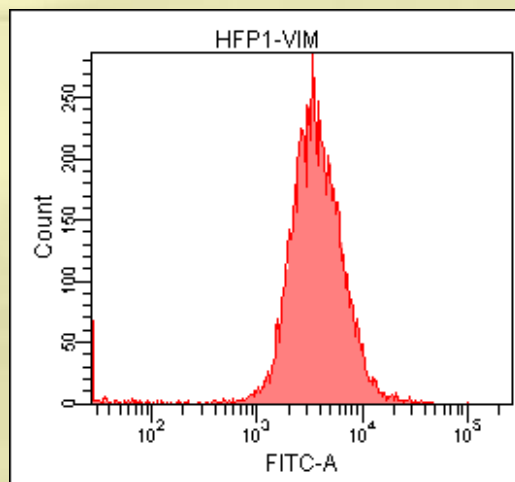
CD90- 98%



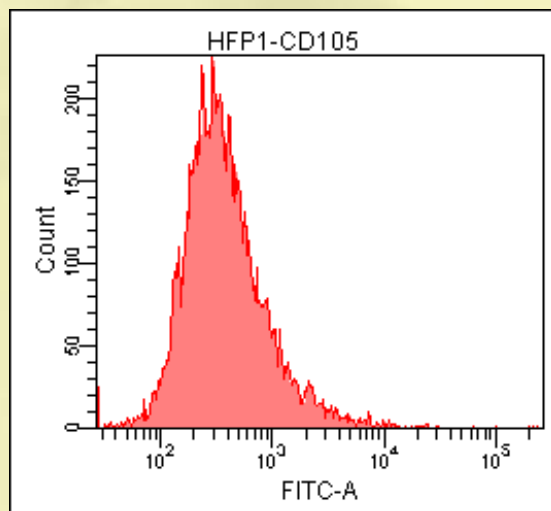
Liver progenitor cells



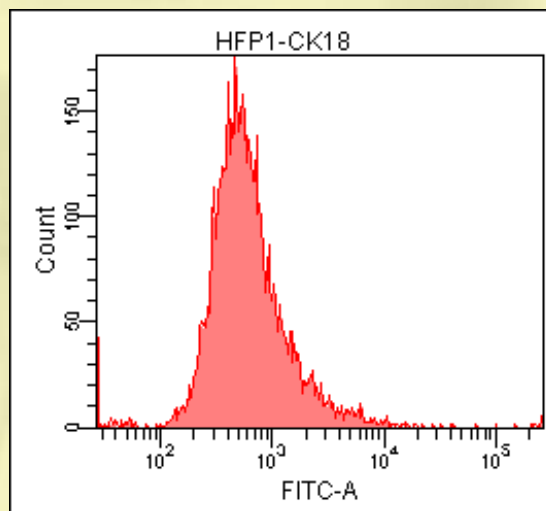
Control



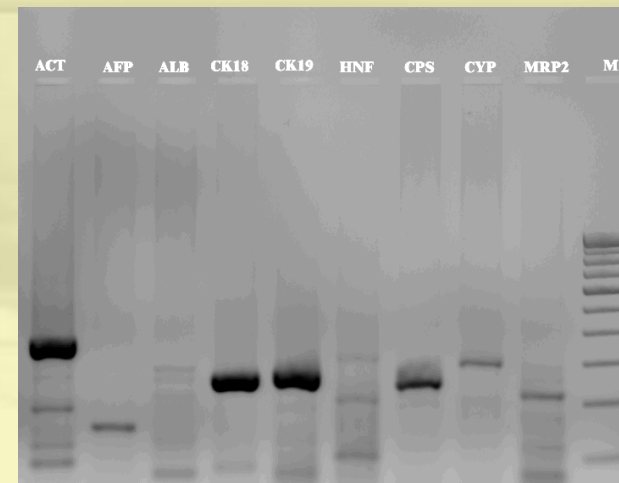
Vimentin- 98%



CD105-35%



CK18 -63%

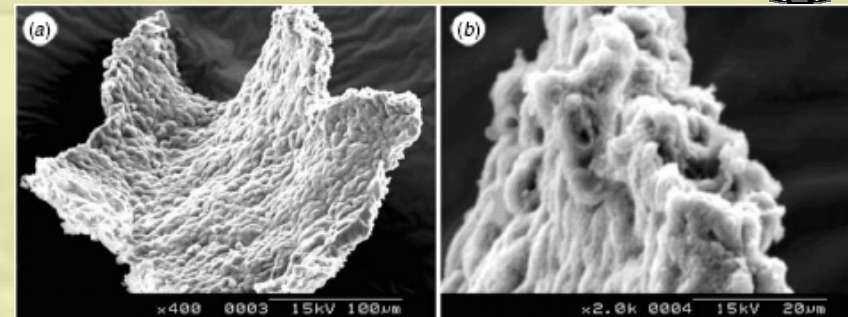
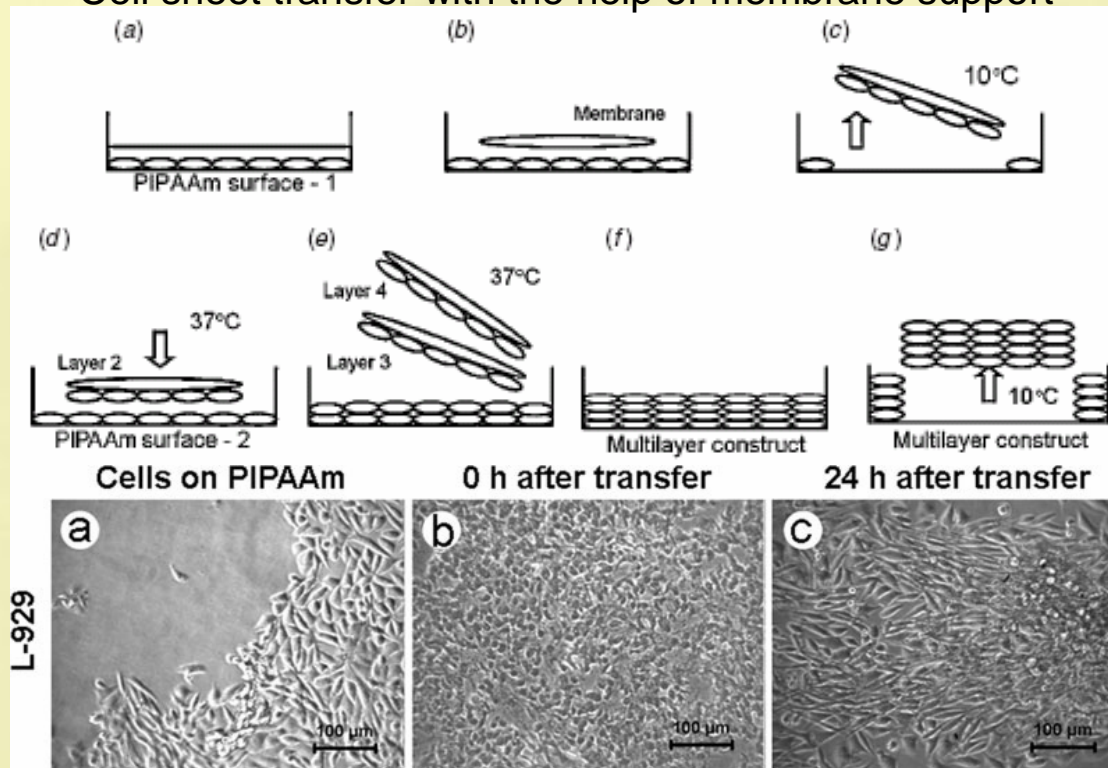


Adult stem cells- MSC, LSC
Progenitor cells
Hepatocytes
Co Culture – Hepatocytes &
SEC

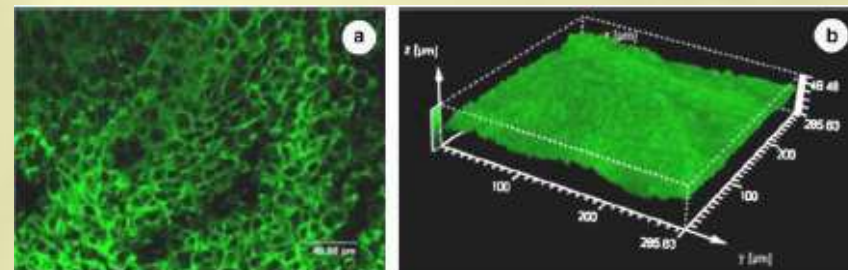
Scaffold free in vitro tissues using different NIPAAm based polymers



Cell sheet transfer with the help of membrane support



HOS tissue construct under SEM showing multilayers

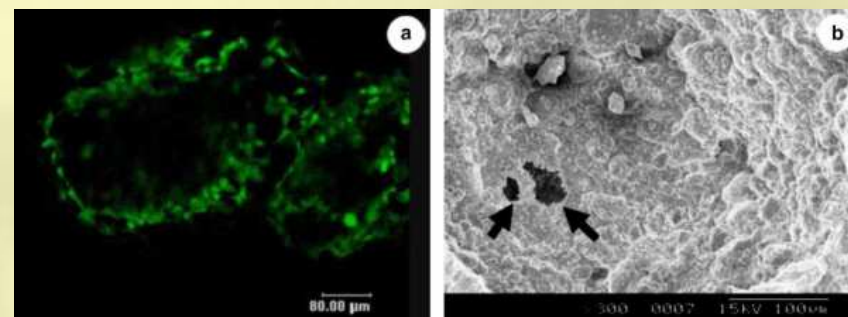


Confocal image of actin filaments of osteoblast cell sheet . 3D constructed image of cell sheet

Cell sheet transfer without membrane support



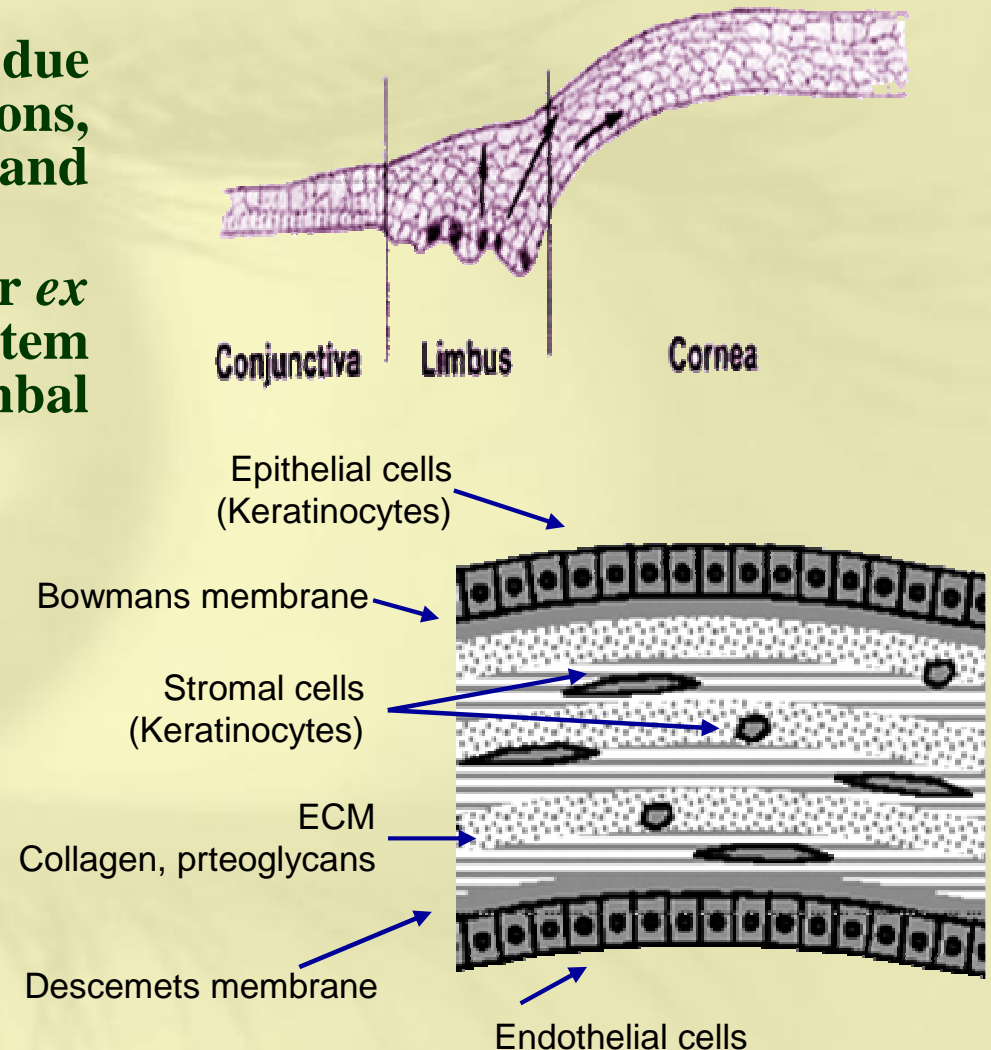
Multilayered osteoblast cell construct



Cell patch seeding improves cellularization of porous scaffold. HOS lining on porous HA

Ocular surface regeneration

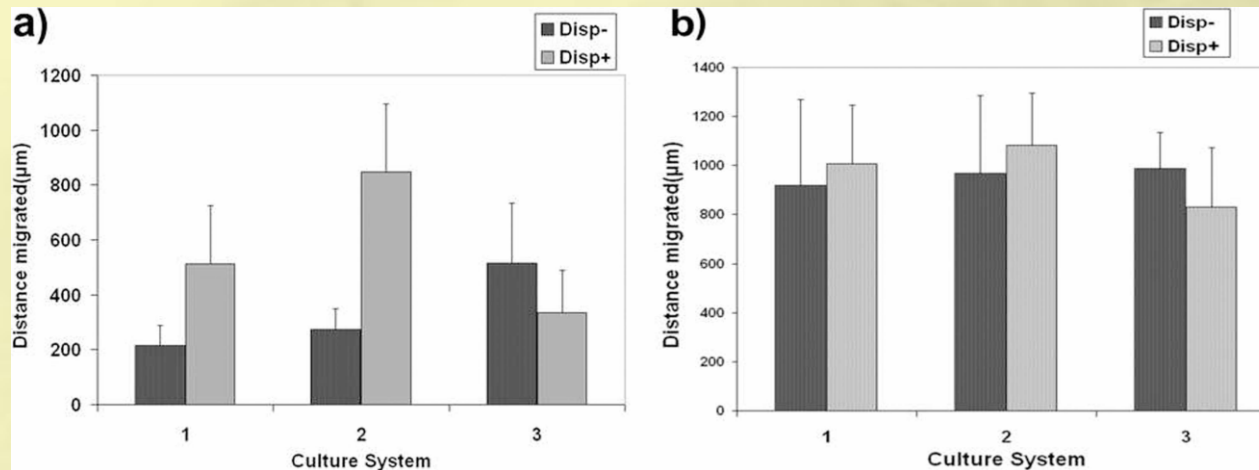
- Corneal epithelial regeneration-Limbal stem cells present at the corneoscleral rim.
- Deficiency in the Limbal stem cells, due to either inherited or acquired reasons, can result in gradual opacity and blindness.
- Limbal stem cell transplantation after *ex vivo* expansion of autologous limbal stem cells (LSC) is ideal for treating limbal stem cell deficiency
 - **Feeder layers**
 - **Xenogenic source**
 - **Immunorejection**
 - **Amniotic membranes:**
 - **Disease transmission**
 - **Extensive quality control.**



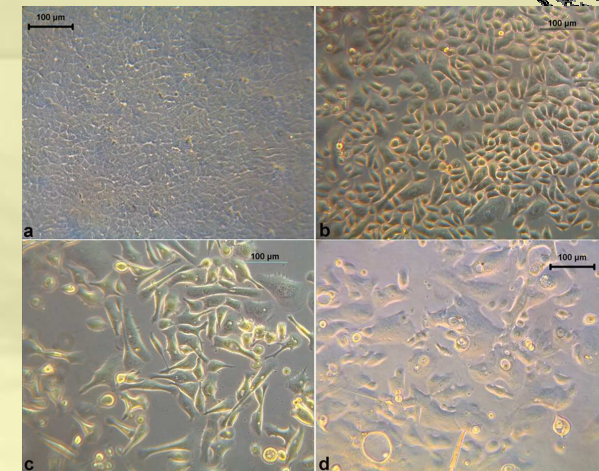
Xeno feeder free limbal cell culture system for ocular surface regeneration



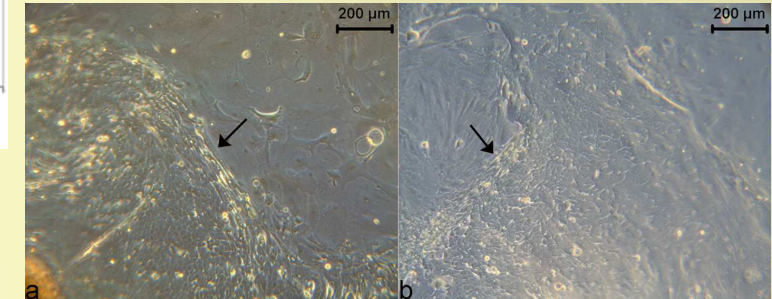
- **Culture system Constituents**
- 1- Completed Panserin 801 with 5% serum
- 2 -Iscove's Modified Dulbecco's Medium with 10% serum
- 3 -IMDM+ Pan
- 4 Culture system 2 for initial days, replaced by culture3.



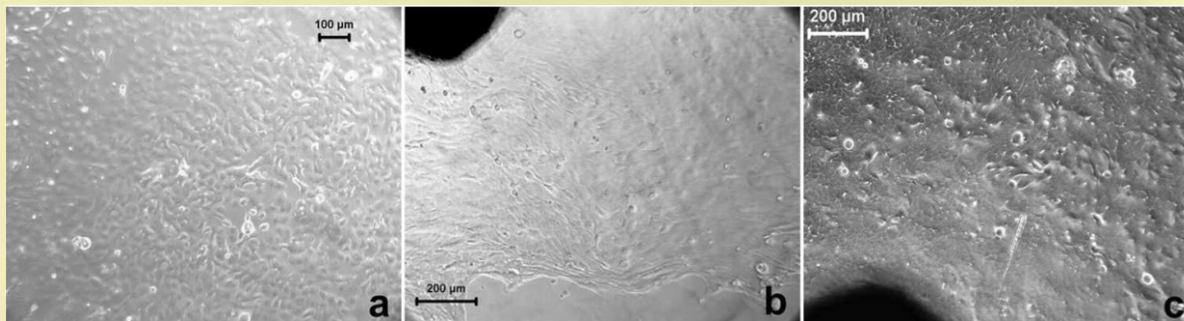
Distance migrated by cells from explants treated with and without dispase



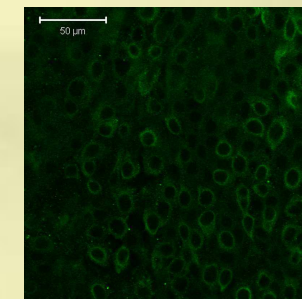
Migrated cells from limbal explants without dispase and with dispase



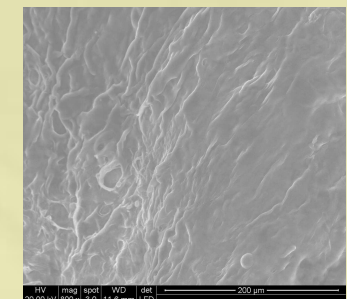
Growth of epithelial progenitor cells on a layer of cells that act as autofeeder



Morphology of cells from limbal explants in different media. a) Culture system 1 b) Culture system 2 c) Culture system 3.



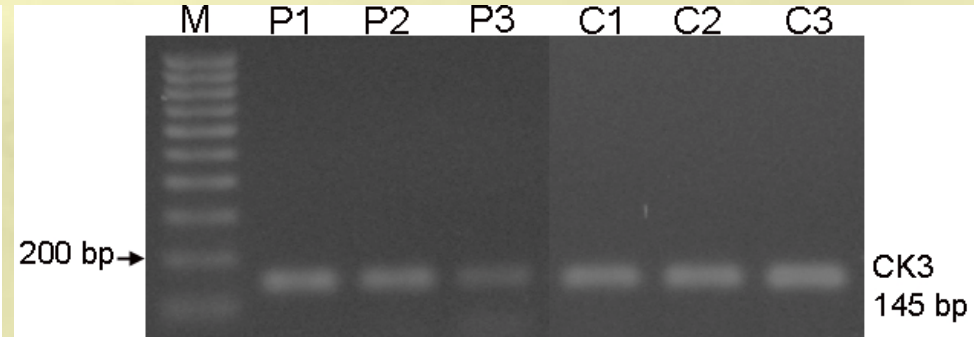
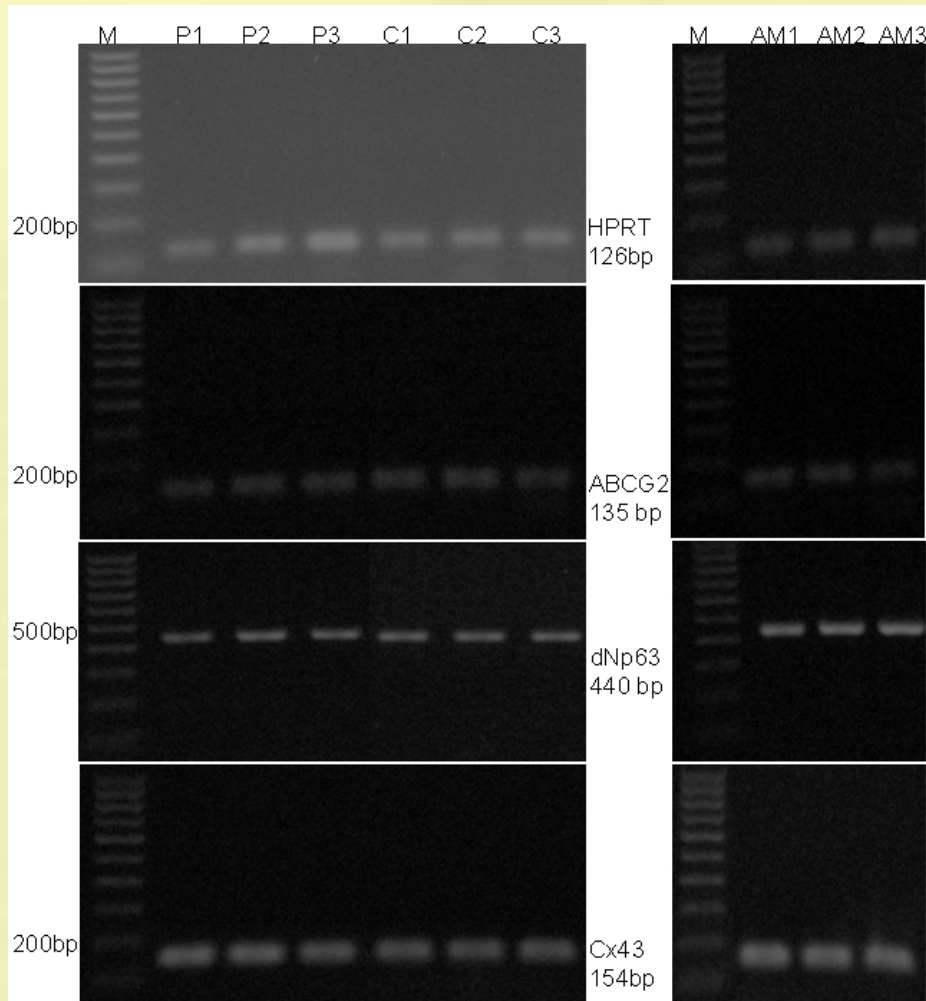
ABCG2 expression in limbal cells



The migrated limbal cells, under ESEM



Human Corneal tissue construct using Polymer as alternative to Amniotic membrane



P – NIPAAm-MMA, C - control (TCPS), AM - Amniotic membrane

Human limbal cells grown on all substrates contained progenitor as well as differentiated population

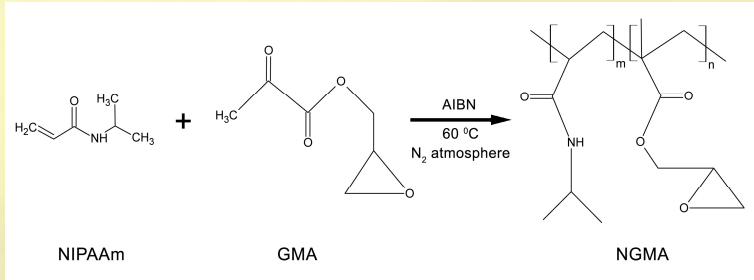
Progenitor population on copolymer increase the chance of successful transplantation

Secretome analysis showed same profile in important proteins involved in corneal regeneration

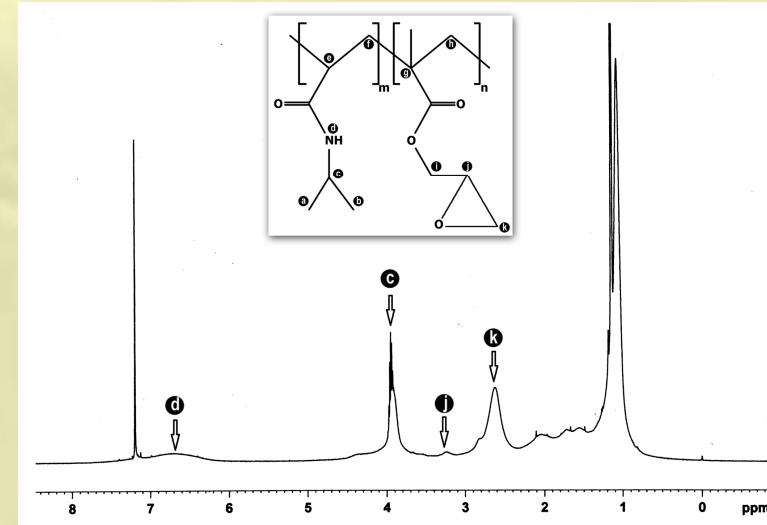
Protein	Function	TCPS	NIPAAm-MMA	AM
Mimecan	<ul style="list-style-type: none"> ➤ Role in UV response ➤ Glycosylated form required for corneal transparency 	+	+	+++
Matrix metalloproteinase-1	<ul style="list-style-type: none"> ➤ Role in migration and reepithelialisation during wound healing ➤ Degrading and remodelling ECM 	++	++	++
Kallikrein 7	<ul style="list-style-type: none"> ➤ Degradation of intercellular adhesion molecules during desquamation ➤ Might be responsible for cell shedding from corneal surface 	++	++	+
Plasminogen activator inhibitor-I	➤ Epithelial adhesion and migration during corneal wound healing	+++	+++	+++
Insulin growth factor binding protein-7	➤ Induce apoptosis and reduce growth rate	+	+	+
Serine protease HTRA1	➤ Regulate availability of insulin growth factors	++	++	+
Annexin A2	➤ Role in epithelial cell migration	++	++	++
Neutrophil gelatinase associated lipocalin	➤ Extracellular defense role	+++	+++	++
Interleukin-6	<ul style="list-style-type: none"> ➤ Increase in amount initiate wound healing ➤ Promotes epithelial migration 	+	+	+
βig-h3	➤ Mutations in this results in dominant corneal dystrophies	+	+	++

Bioengineered corneal cell sheet

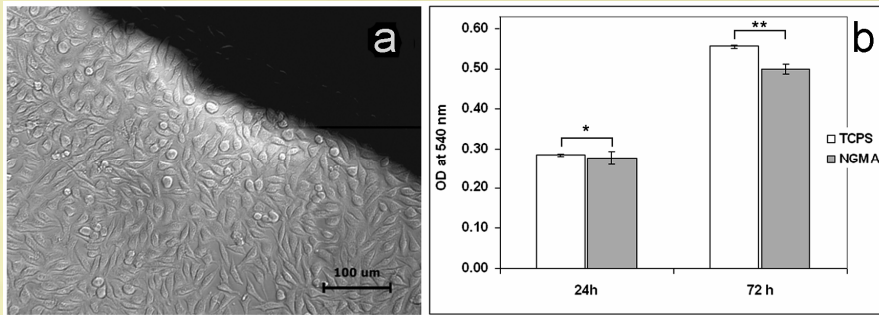
Poly(N-Isopropylacrylamide)-co-Glycidyl methacrylate



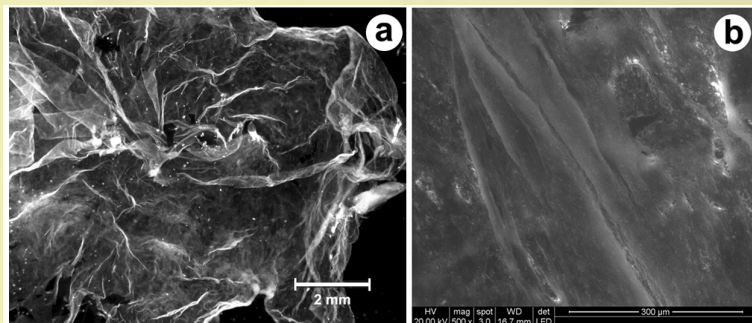
Synthesis of NGMA copolymer



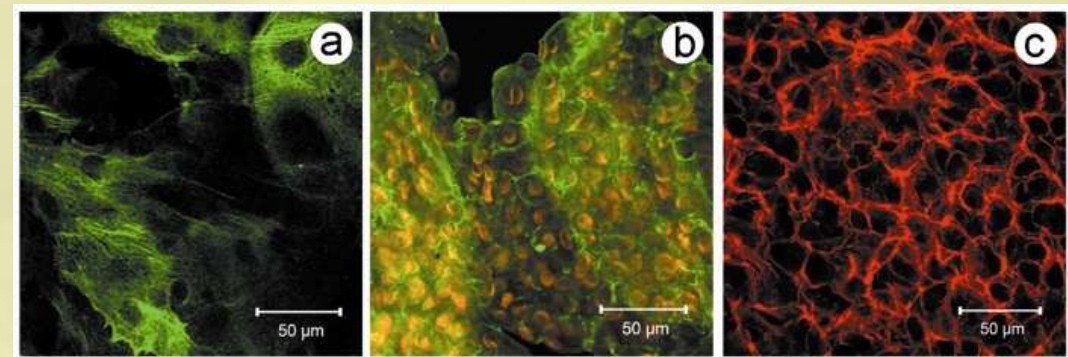
¹H NMR spectrum of NGMA copolymer



Cytotoxicity analysis of NGMA coated surface. (b) L-929 proliferation on NGMA and PSTC



(a) Retrieved corneal cell sheet remain transparent. (b) Intact tissue architecture of corneal cell sheet



Rabbit corneal cell sheet. (a) CK 3/12 (b) ZO-1 (Green) and counter stained with propidium iodide (red). (c) Actin

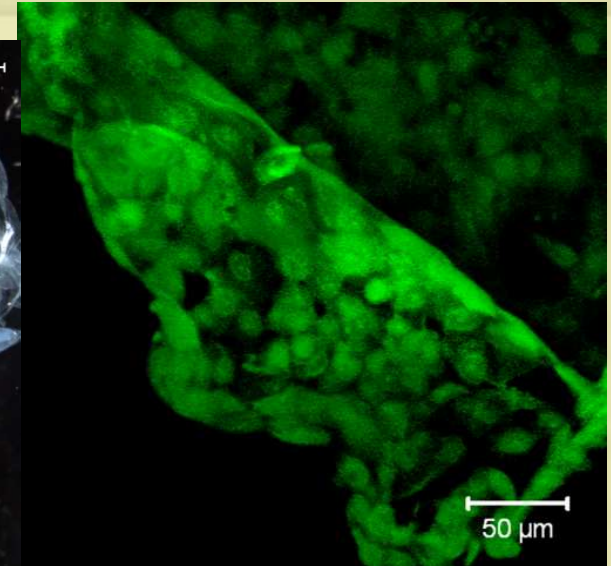
Retrieved Cell sheet



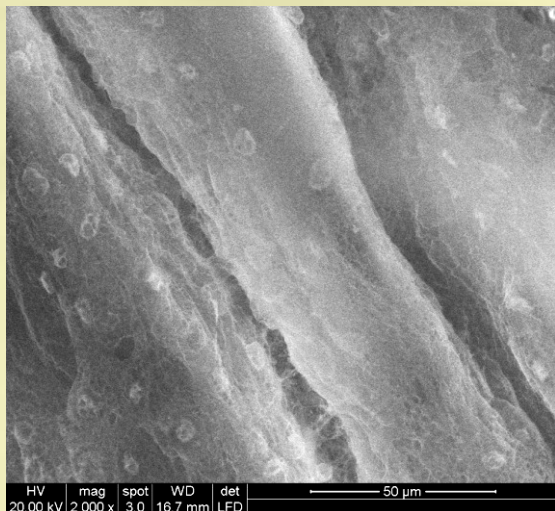
Limbal cell sheet retrieved from NGMA



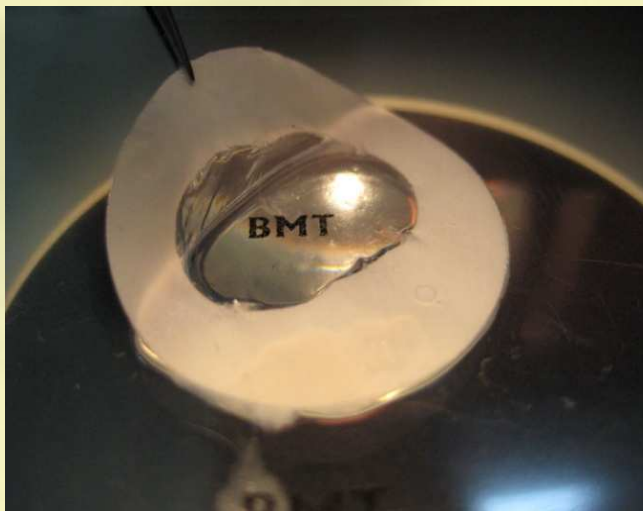
Cell sheet under 3D stereo microscope



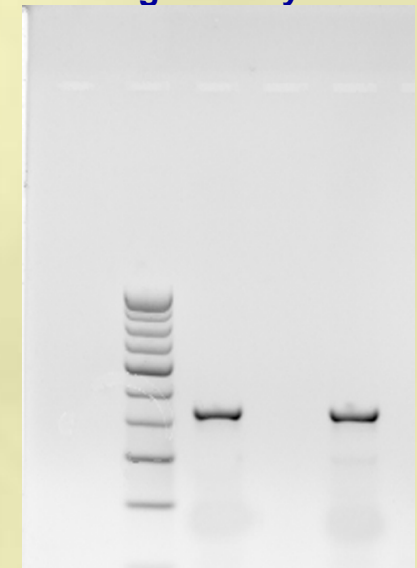
FDA stained cell sheet showing viability



ESEM image of intact cell sheet



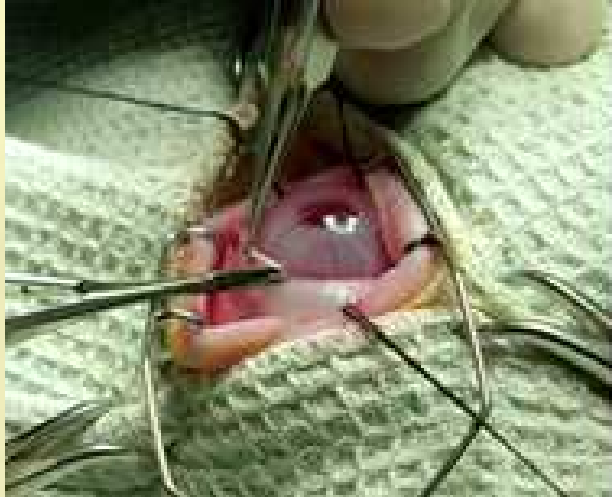
Transparency



RT-PCR Profile showing p63 gene expression

Animal experiments

Development LSCD model



360° excision of limbus



Mechanical debridement of cornea



LSCD development after 8 days



12 days



30 days



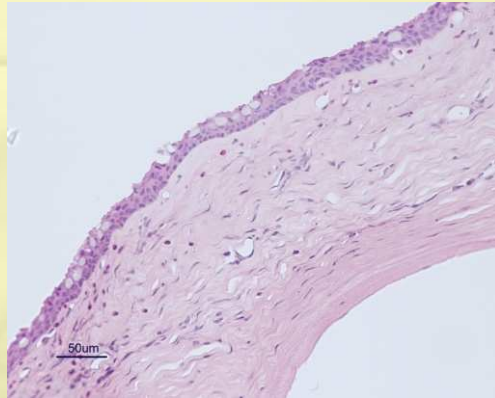
38 days

Progression of limbal stem cell deficiency in rabbit. The opacity formed initially decreases and is replaced by high neovascularization

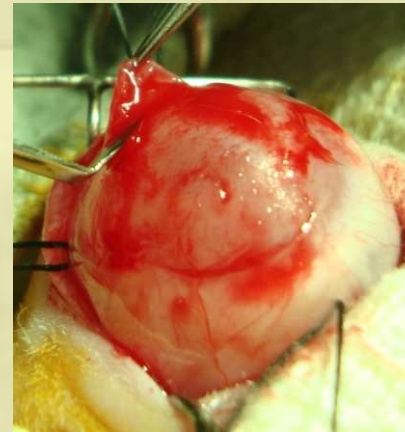
Pre clinical efficacy study in rabbits



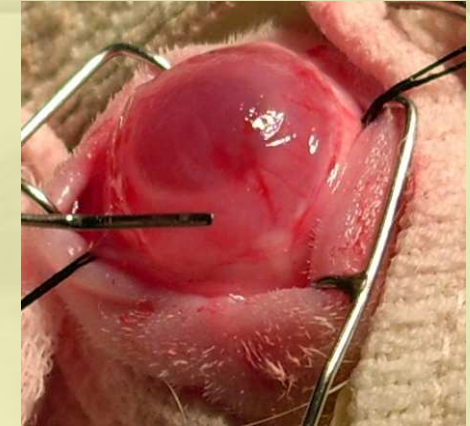
Before transplantation



LSCD



Superficial keratectomy



Rabbit eye just after cell sheet transplantation



7 Days



30 Days



After transplantation (1 year)



Control



4 months

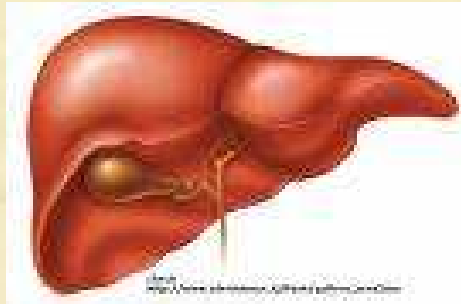


5 months

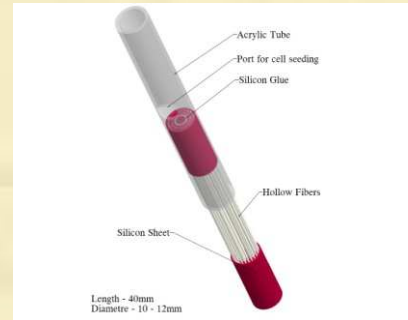
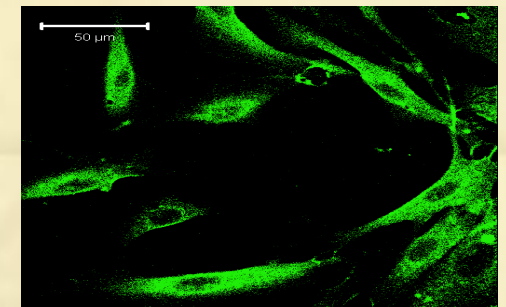
Ocular surface regeneration of rabbit LSCD model by limbal cell sheet transplantation. The opacity and vascularization observed initially was completely absent with improved corneal clarity.

Liver Tissue Engineering

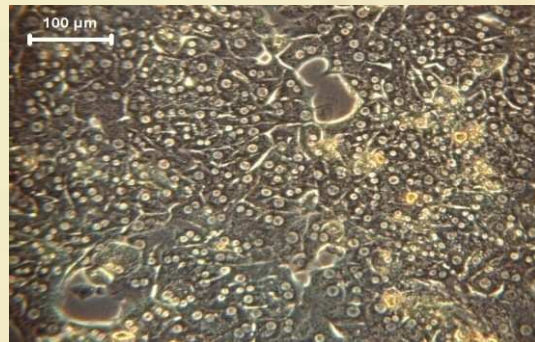
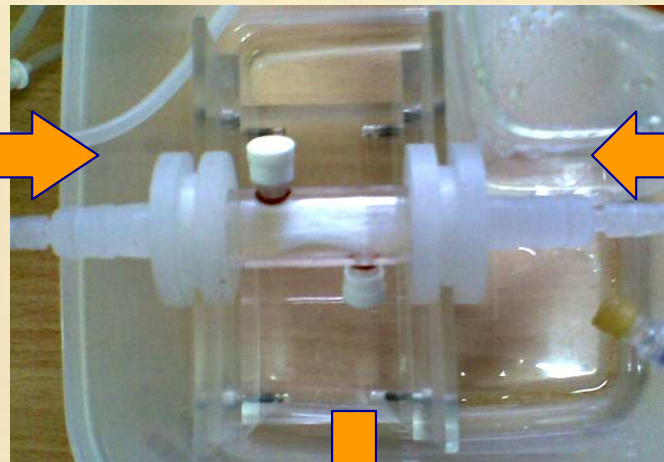
Liver



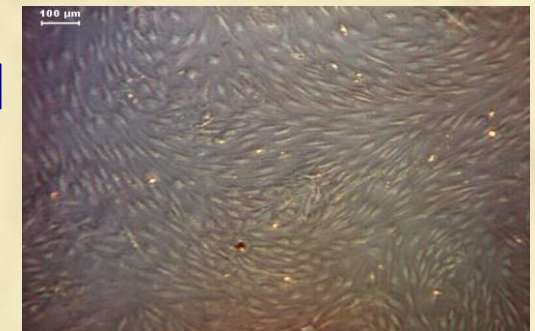
Stem cells



Prototype bioreactor



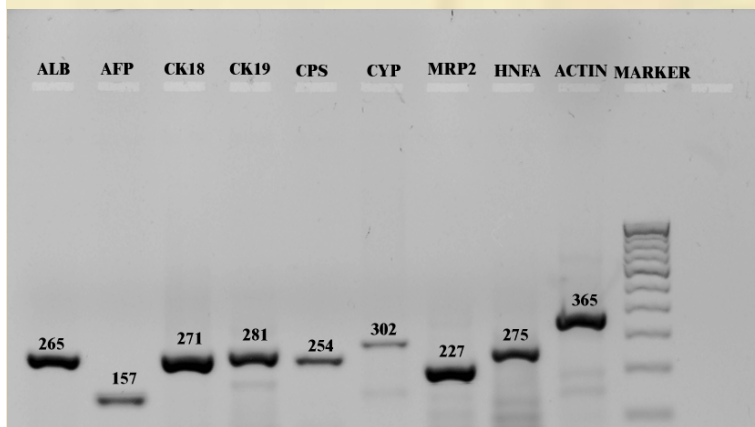
Hepatocytes/
cell lines



Immortalized
stem cells

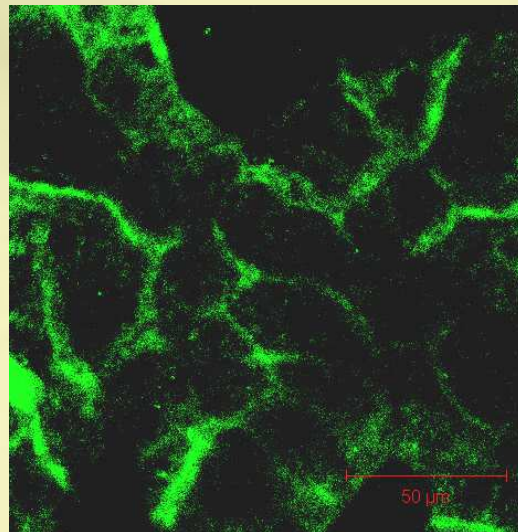
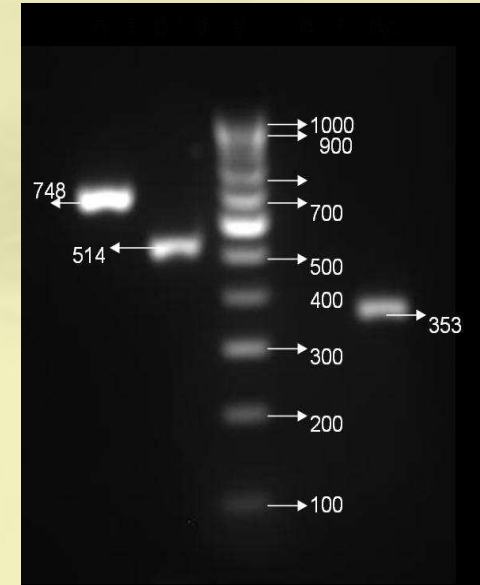
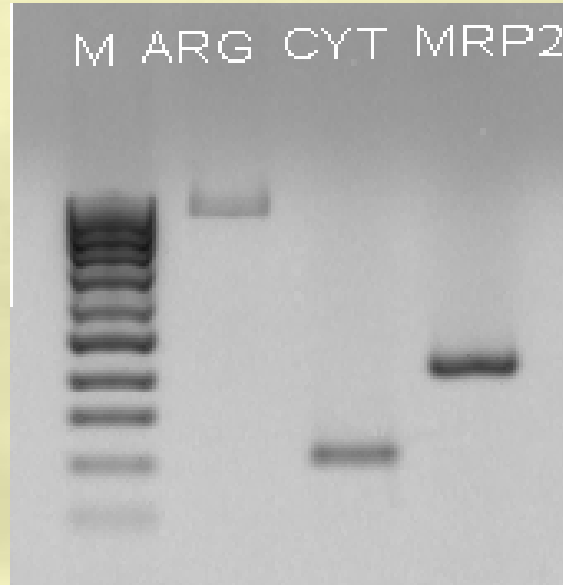
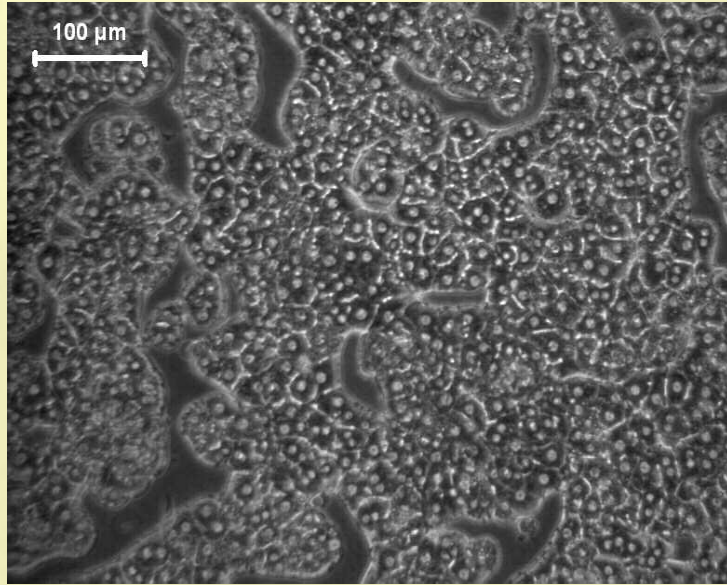


Functionality / Xenobiotic metabolism

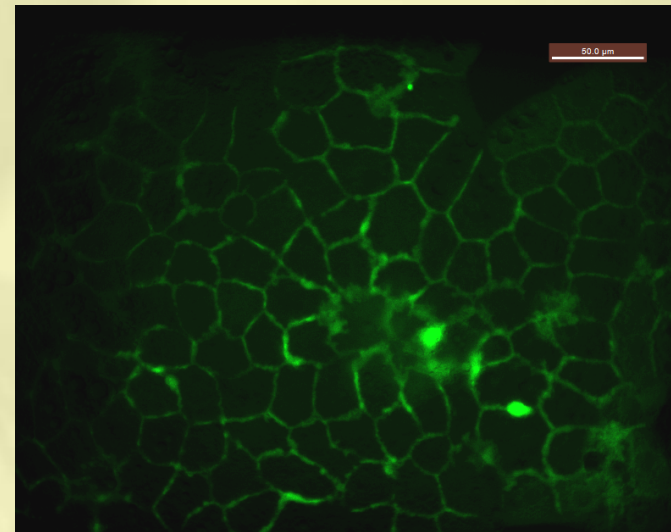


HepG2

Hepatocytes - BAL/Toxicity



MRP2 FITC



FDA localized with in bile canaliculi

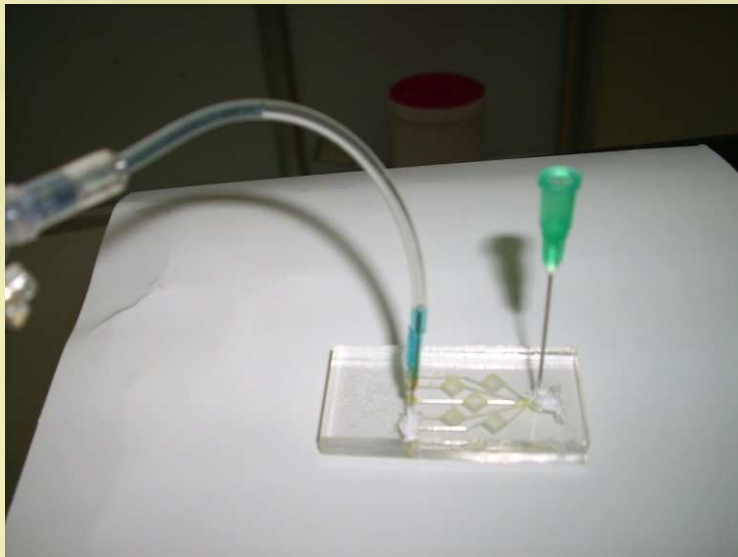
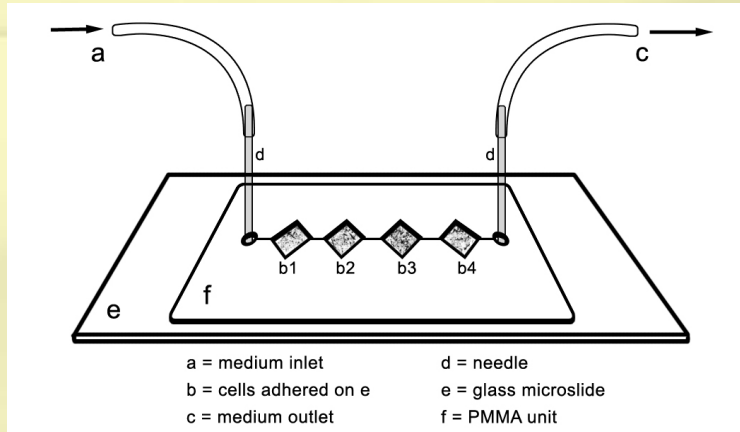


Collaborative Research

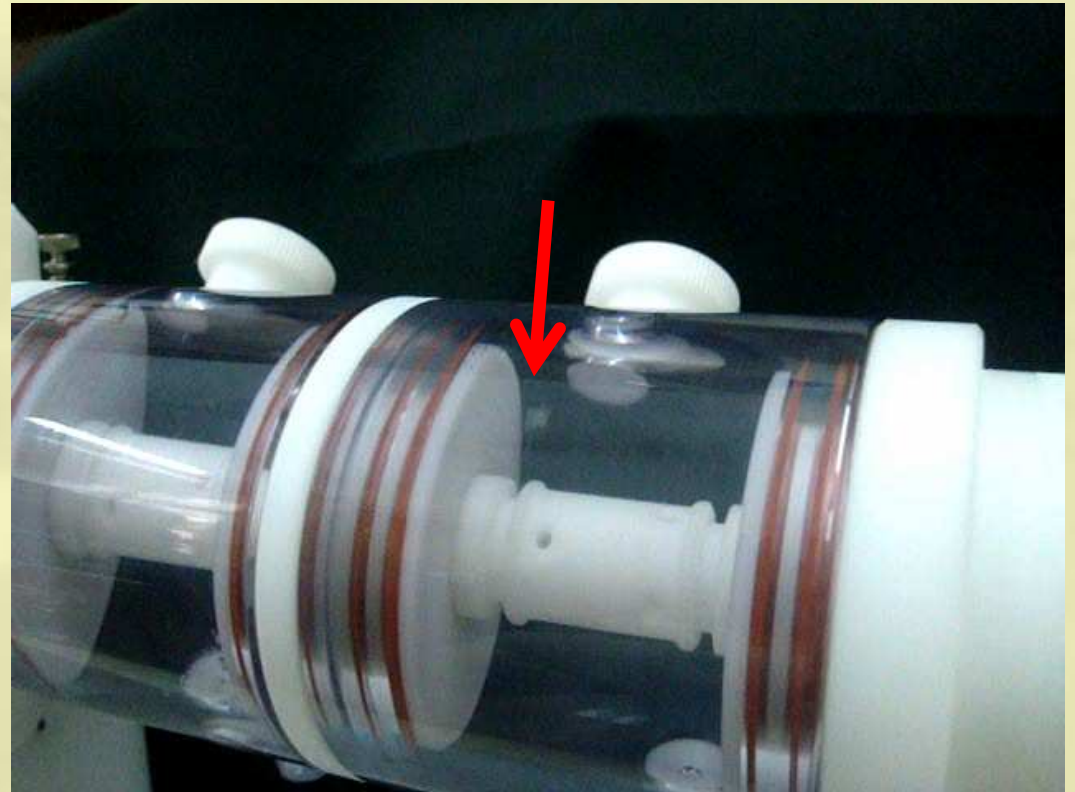
- Microfluidics (Simon Fraser University, Canada)
 - New module for toxicity screening
 - Opportunity to find alternate experiment system for stem cell research
- Microgravity (IIST)
 - Studies of the fundamental role of gravity is a central component of the human exploration of space
 - Cell culture studies under simulated microgravity can improve our understanding of gravity sensing, transduction and responses in living cells and tissues



Working systems



Microfluidics



Microgravity

Research in Regenerative Medicine & Tissue Engineering at SCTIMST



- **Cornea**
- **Bone**
- **Liver**
- **Cartilage**
- **Vascular**
- **Pancreas**
- **Skin**
- **Lungs**

COE in TE, DBT, Gov of India



THANK YOU