



Human Hepatocyte Transplantation-Story so far !

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Liver Transplantation is now the standard of care with good long term patient and graft survival

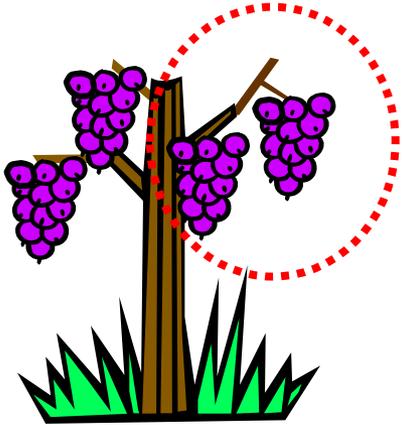
So why not continue with liver replacement ?

Because Liver transplant is a disease and not a cure!

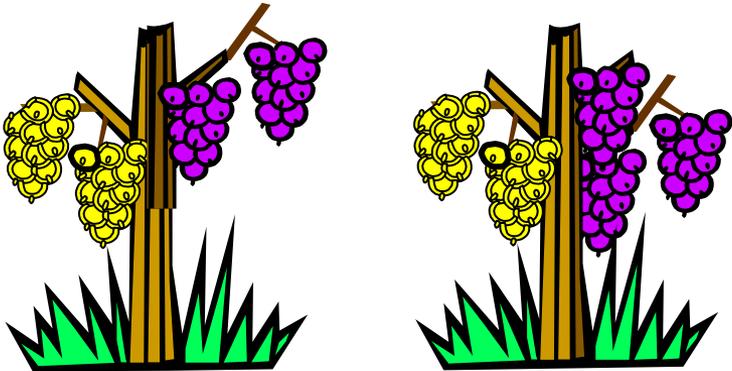
➤ *We need to be innovative*

Auxiliary Liver

Donor

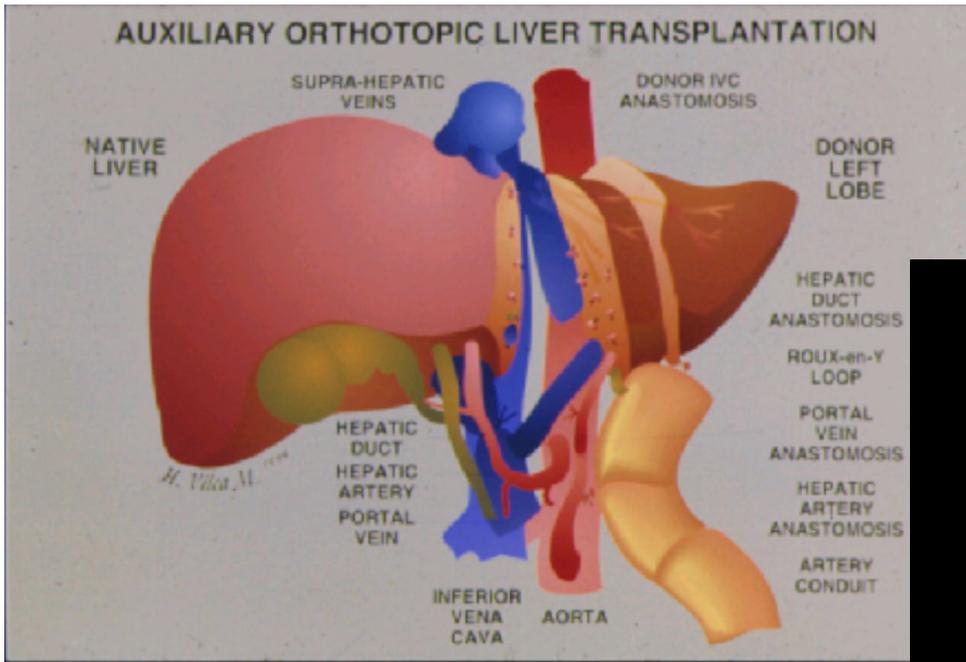


Recipient

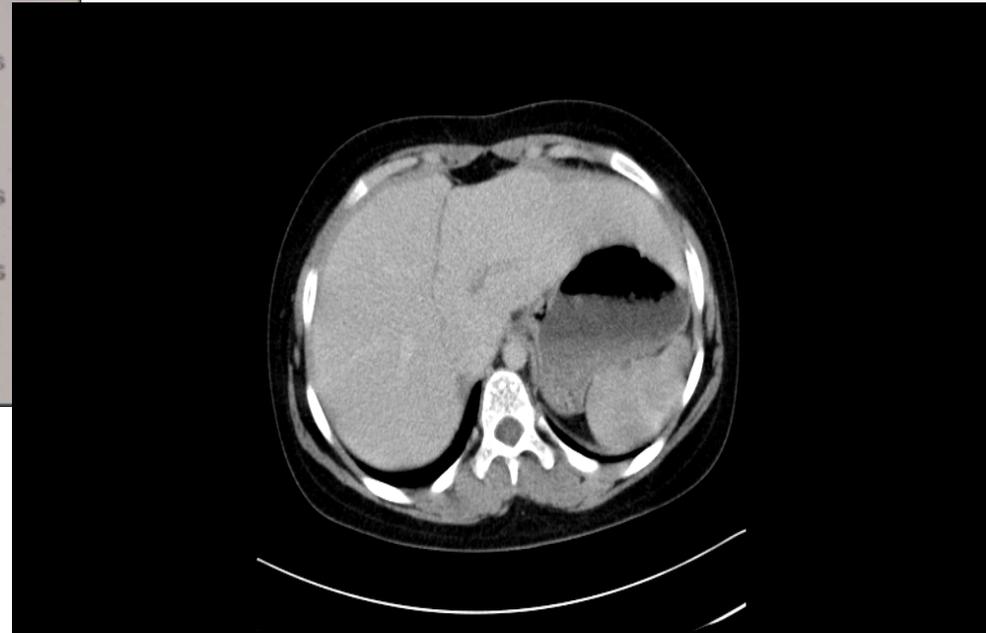


Regeneration

Auxiliary liver transplantation for non-cirrhotic metabolic diseases



Auxiliary liver tx for Propionic acidemia
16 yrs follow up (Puppi, Dhawan et al AJT 2007)



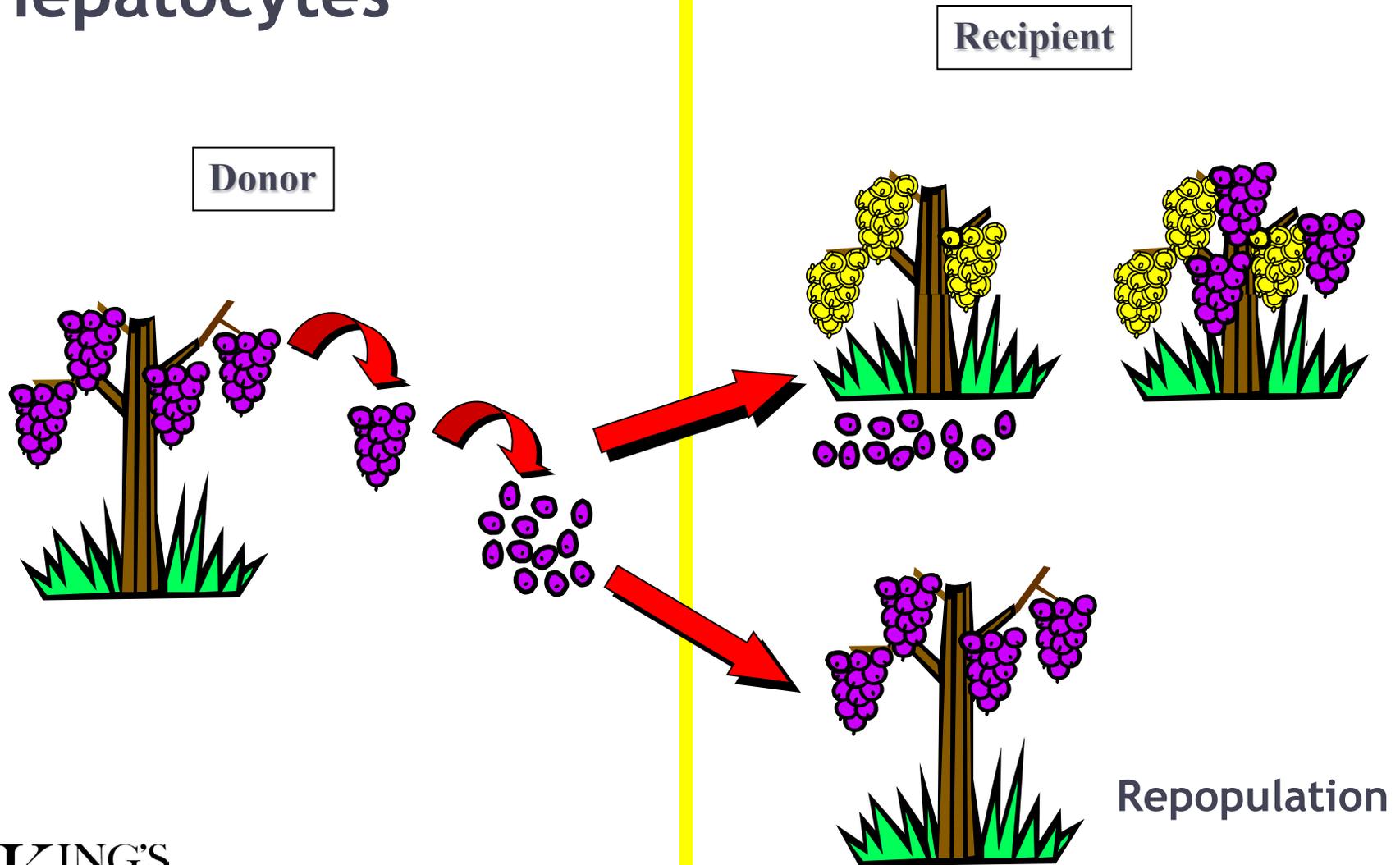
Future option of gene therapy

Is there an alternative ?

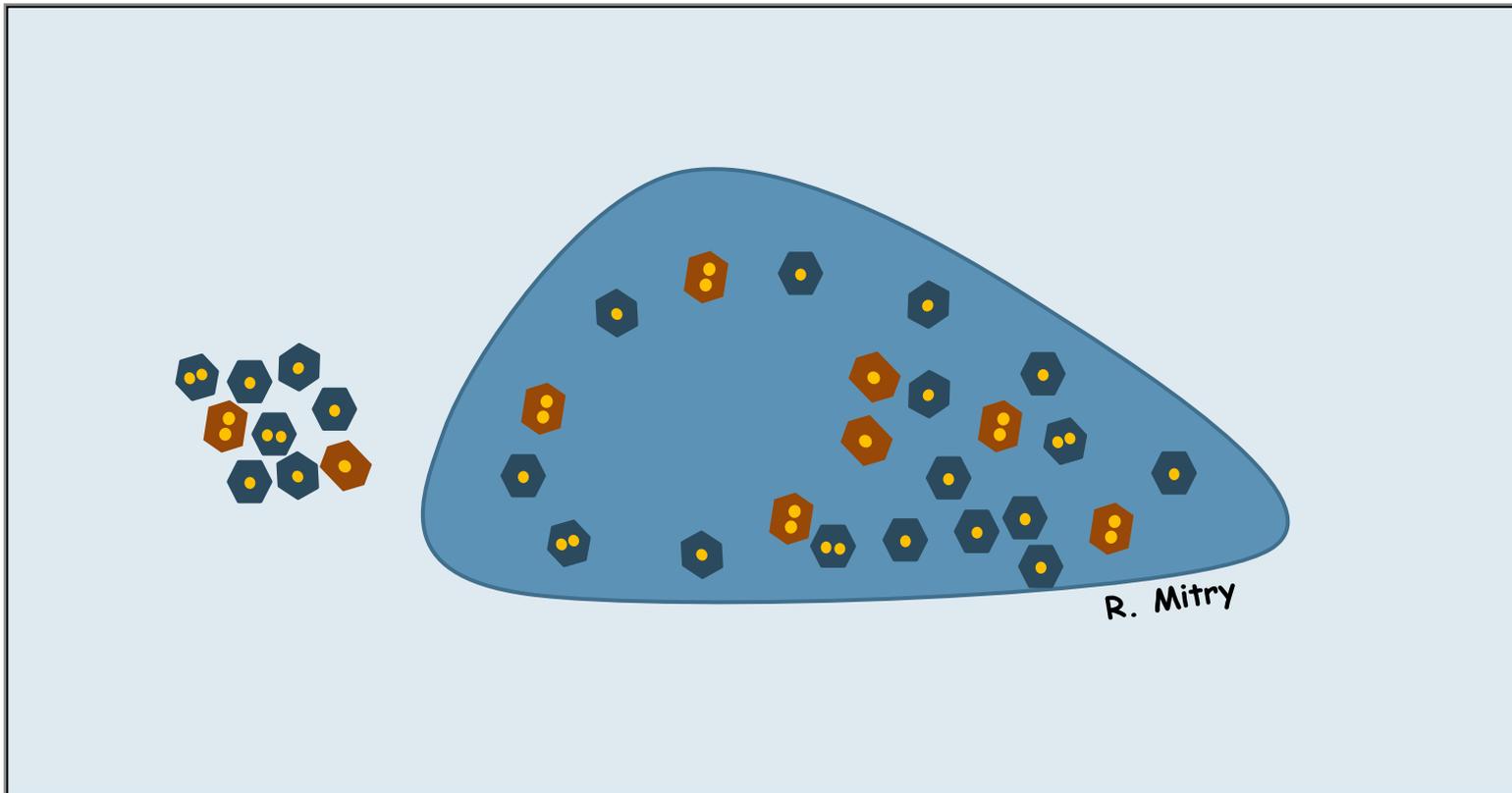
- For non cirrhotic liver based metabolic disorders whole liver replacement is un-necessary as up to 5% enzyme function is enough to change the phenotype
- Some patients with ALF survive with native liver

Success of Auxiliary liver transplantation is more than a proof of concept to believe that hepatocyte transplantation will be a success

Hepatocytes



Repopulation



Animal models of metabolic disorders-Successful outcomes of hepatocyte Tx

- Gunn rat (Crigler Najjar)
- Spf-ash mice (OTC)
- Long-Evans Cinnamon rat (Wilson disease)
- Nagase analbuminaemic rat (hypoalbuminaemia)
- Fumarylacetoacetate hydroxylase KO (Tyrosinaemia type 1)
- Mdr2 KO (PFIC1)
- Histidinemic mice
- Watanabe rabbit (hypercholesterolaemia)
- Vincent mouse (acute intermittent porphyria)
- MSUD mouse model
- Dalmation dog hyperuricaemia

Animal models of ALF - rescue with HTx

- Hepatectomy with additional liver injury
- Vascular Ligation
- Drugs
- Toxins

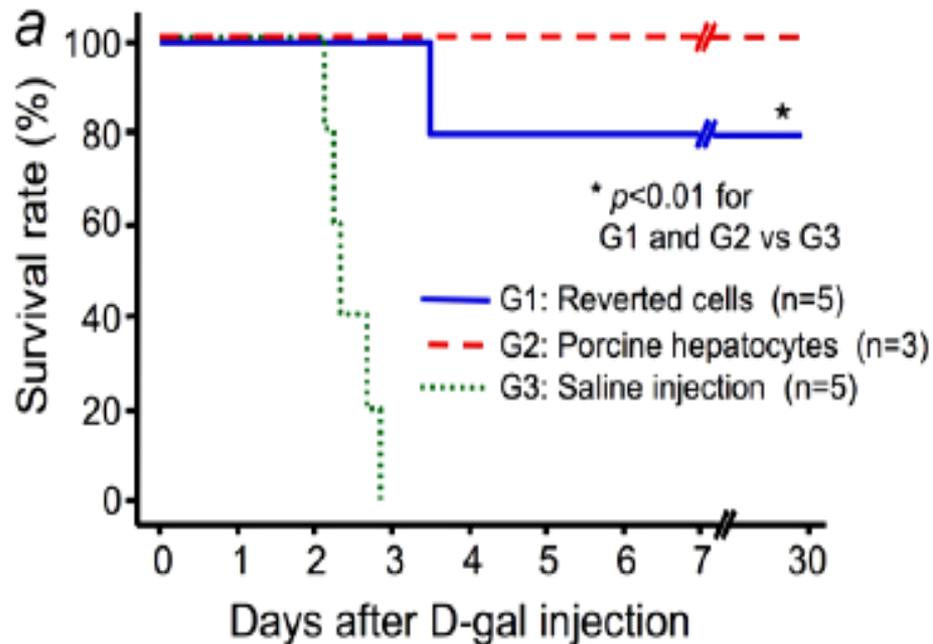
Small and large animal models

Large animal experience

Survival of liver failure pigs by transplantation of reversibly immortalized human hepatocytes with Tamoxifen-mediated self-recombination

Toshinori Totsugawa¹, Chen Yong¹, Jorge David Rivas-Carrillo¹, Alejandro Soto-Gutierrez¹, Nalú Navarro-Alvarez¹, Hirofumi Noguchi², Teru Okitsu³, Karen A. Westerman⁴, Michinori Kohara⁵, Michael Reth⁶, Noriaki Tanaka¹, Philippe Leboulch⁴, Naoya Kobayashi^{1,*}

Journal of Hepatology 47 (2007) 74–82



Hurdles to wider application and success of human hepatocyte transplantation

Availability of good quality GMP quality human hepatocytes

Engraftment

Monitoring for in vivo- cell viability and function

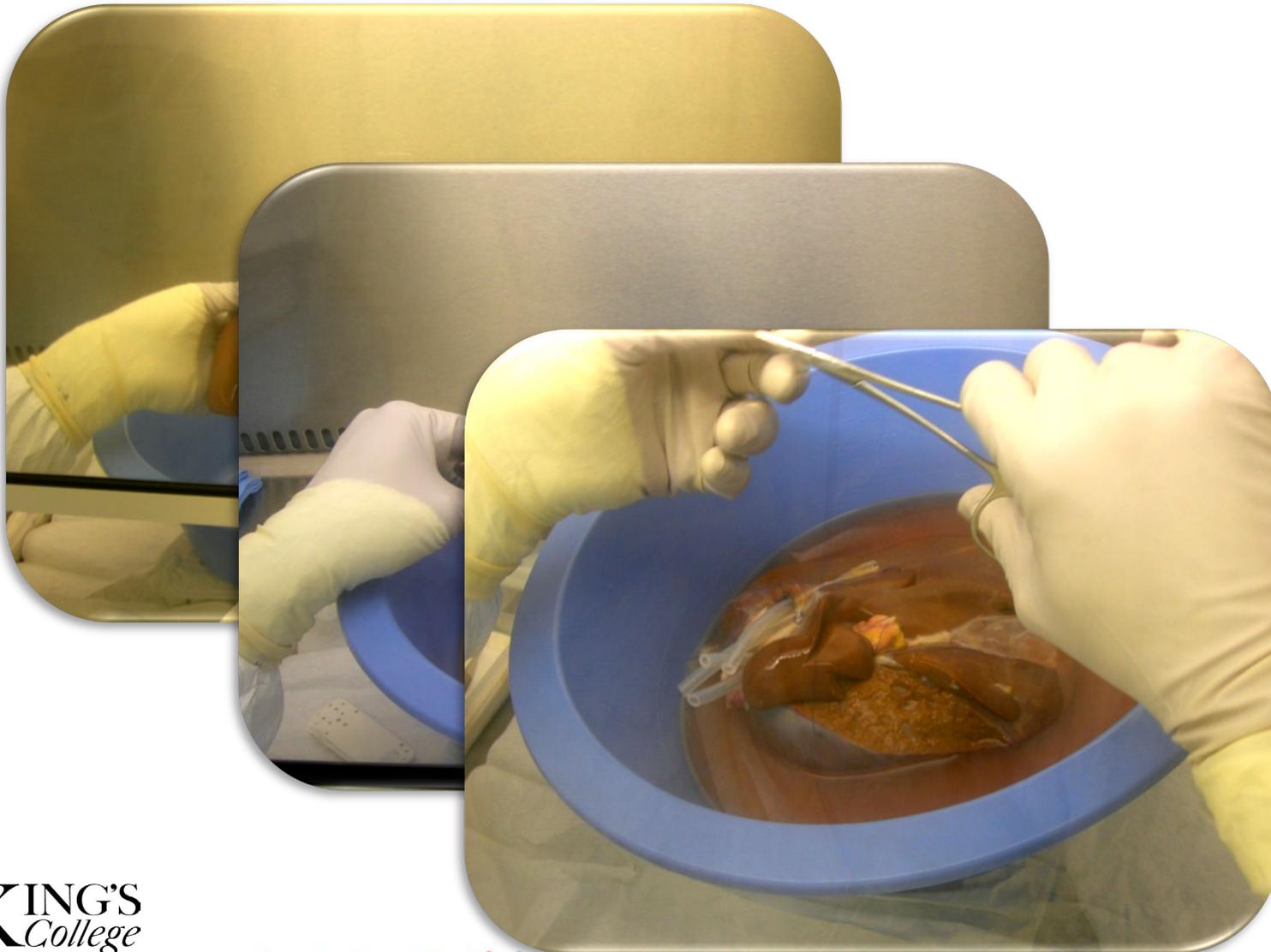
Regulatory requirements and lack of clinical trials



GMP production of hepatocytes- a prerequisite for human use



Cannulation



Cannulated liver segment



Perfusion pump

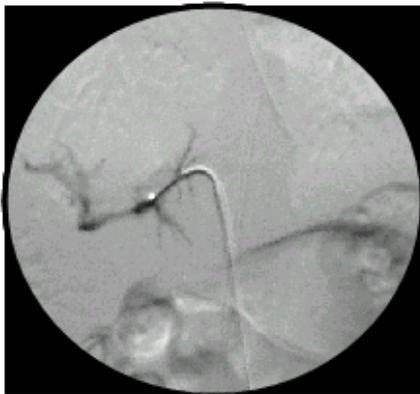
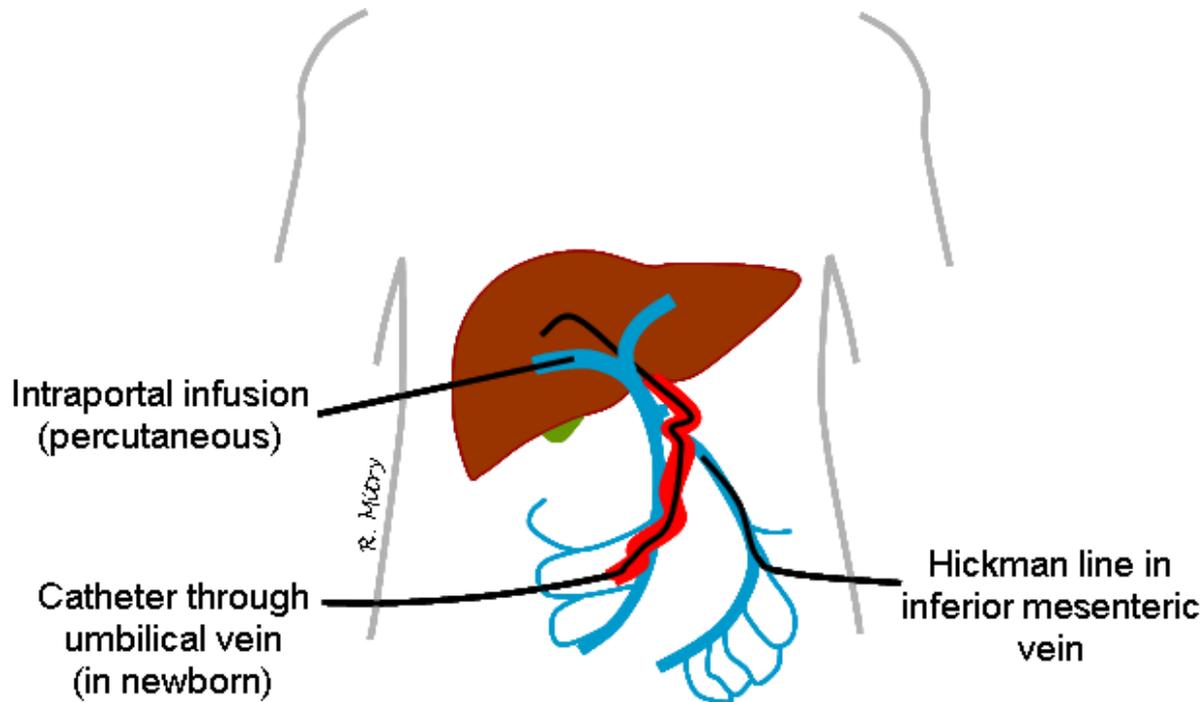


“Mincing” Digested Tissue





Intraportal hepatocyte transplantation- For Liver based metabolic defects

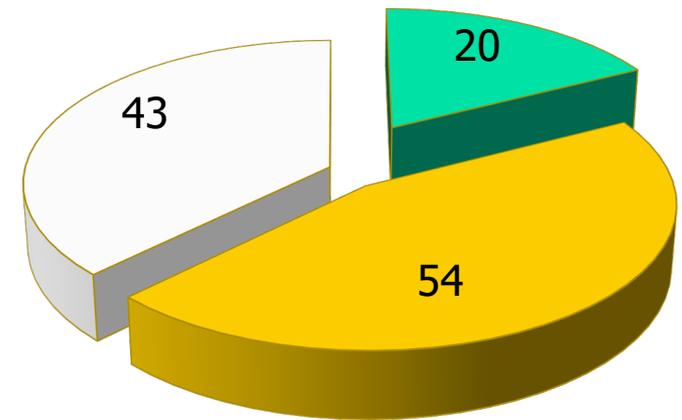


Main Criteria

- Fresh or cryopreserved hepatocytes
- Cell viability should be >60%
- ABO blood group compatible
- Up to 10^9 cells per infusion
- Portal pressure monitoring
- Repeated up to 5-10% liver mass
- Immunosuppression – tacrolimus & steroids

Clinical Hepatocyte Transplants 117 Reported

- Sustain patients with end-stage liver disease (cirrhosis) (20)
- Cell therapy for ALF without liver transplant (OLT) (5)
- or “bridging” patients to OLT (38)
- Metabolic defects (54)



Urea cycle defects

Disease	Age	Reference	No. cells	Outcome
OTC	5 d	Strom et al	1×10^9 IP	Died day 43
OTC	2 d	Mitry et al	1.8×10^9	Decreased ammonia, OLT 6m
OTC	1 d	Horlsen et al	4×10^9	11 days ammonia controlled OLT 6m
OTC	14 m	Stephenne et al	2.4×10^9	Decreased ammonia, OLT 6m
OTC	10 wk	Meyburg et al		Some stabilisation
OTC	3 yr	Meyburg et al	3×10^9	Some stabilisation
OTC	12 yr	Ribes-Koninckx	0.9×10^9	Initial stabilization, died d30
Citrullinae	2 yr	Fisher and		Decreased ammonia

Crigler Najjar syndrome

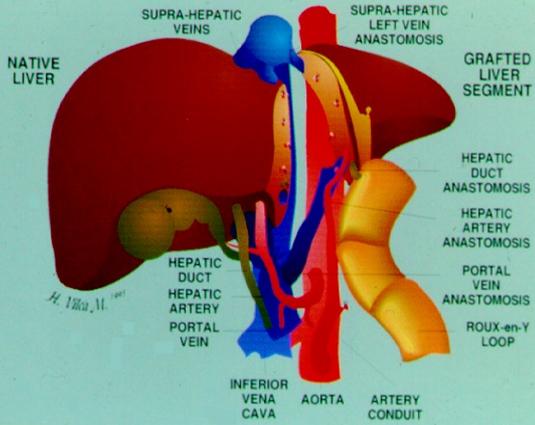
Disease	Age	Reference	No. cells	Outcome
CN1	10 yr	Fox et al	6×10^9	60% decrease in bilirubin to 11 months
	8 yr	Darwish et al	7.5×10^9	40% decrease in bilirubin to 6 months. OLT 20 mo
	9 yr	Ambrosiono et al	7.5×10^9	50% decrease in bilirubin OLT
	18 m	Dhawan et al	4.3×10^9	50% decrease in bilirubin OLT 8 months
	3 yr	Dhawan et al	2.1×10^9	30% decrease in bilirubin OLT 18 months
	8 yr	Allen et al	1.5×10^9	30% decrease in bilirubin 5 months
	2 yr	Khan et al	15 billion HFH	50% decrease in bilirubin 2 months
	1 yr	Lysy et al	1.5×10^9	50% decrease bilirubin 5 months

Promethius, a first human model of liver regeneration

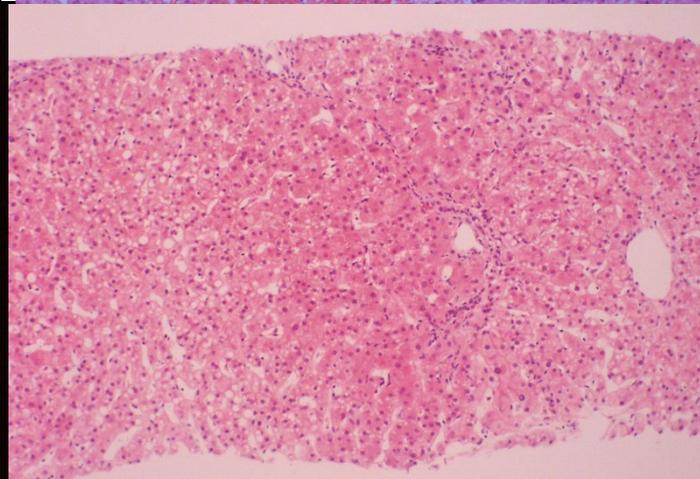
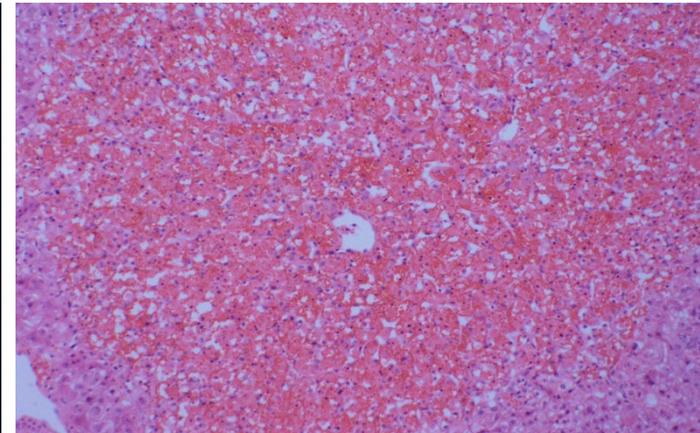
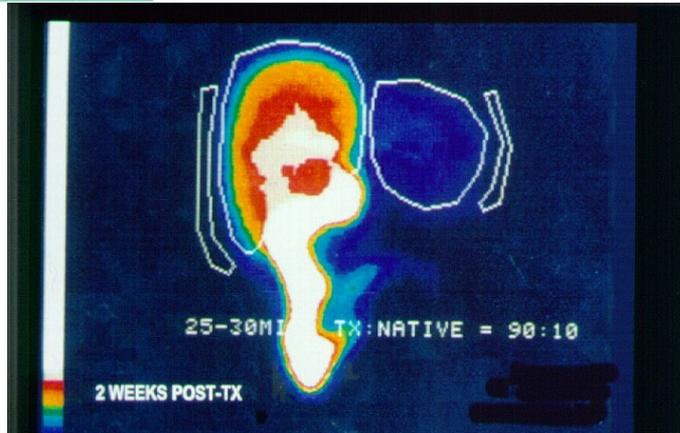


Prometheus Bound (Peter Paul Rubens, 1618)

AUXILIARY ORTHOTOPIC LIVER TRANSPLANTATION



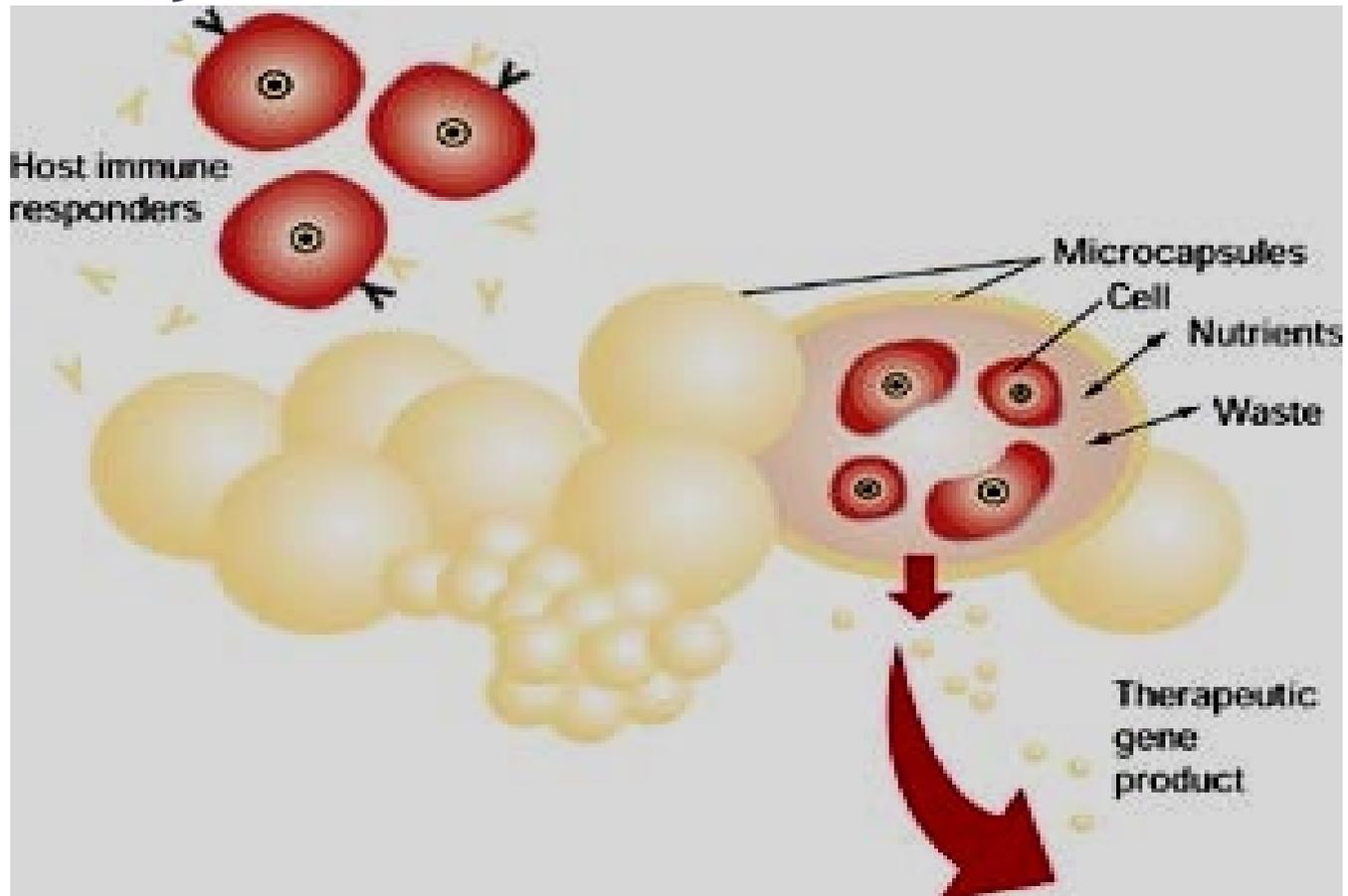
70% successful weaning of Immunosuppression



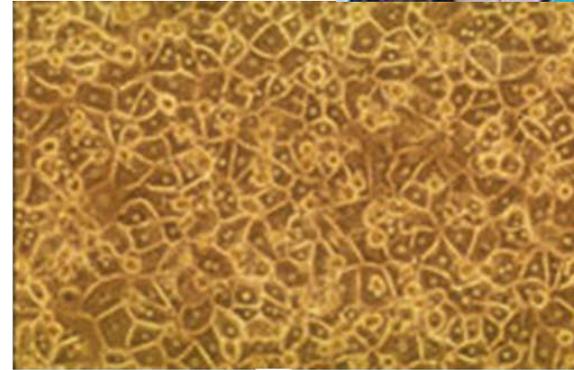
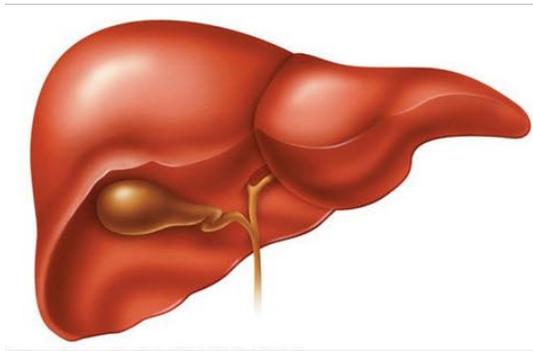
Hepatocyte Tx For ALF

- Synthetic and detoxifying function for few weeks
- No immunosuppression !
- Site that can be accessed in a coagulopathic patient

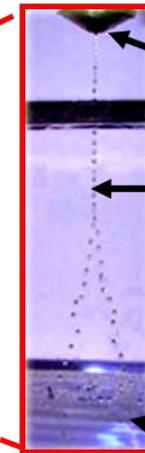
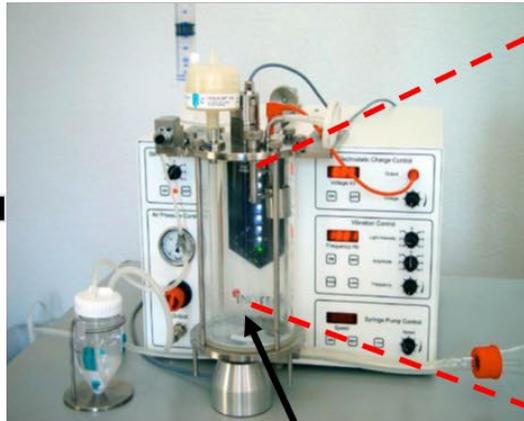
Hepatocytes in Beads



METHODS



2.5×10^6 cells/ml



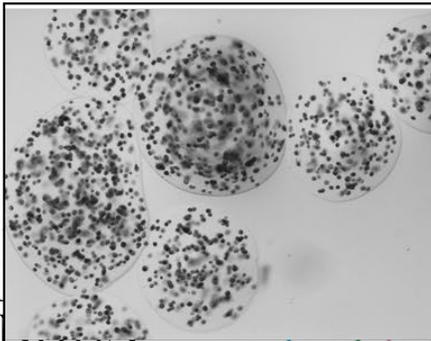
250 μ m nozzle

HCs/1.5% alginate

1.2% CaCl₂

Reaction vessel

K
LO



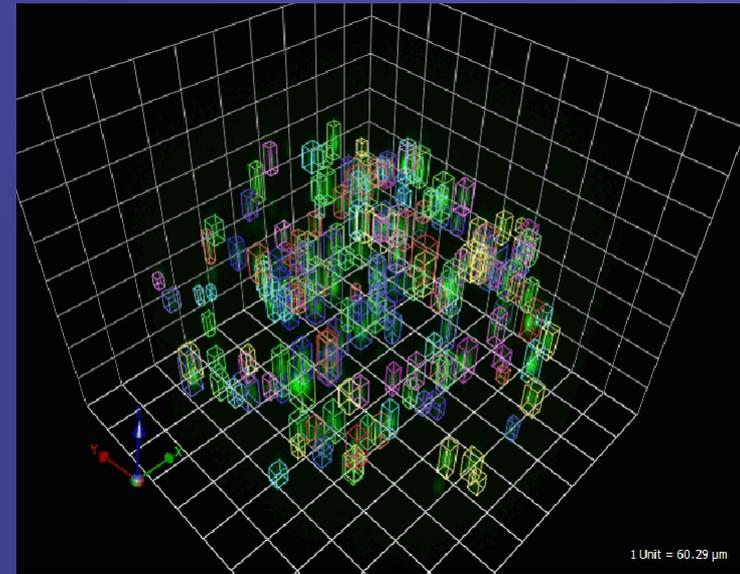
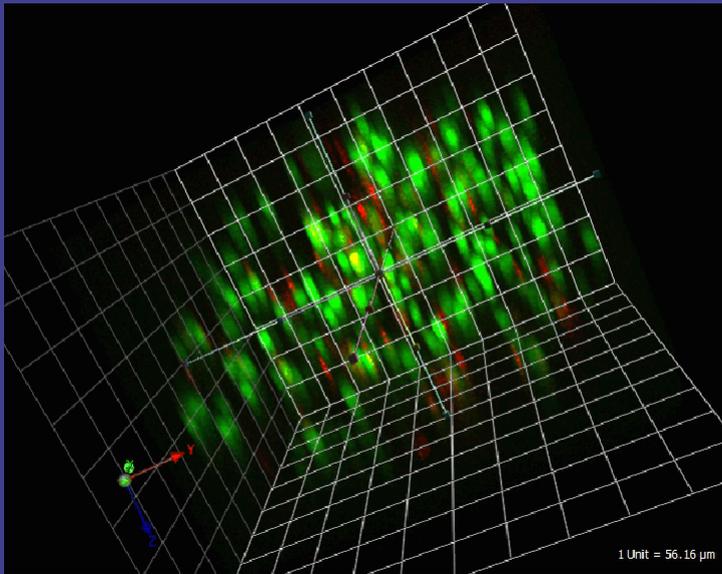
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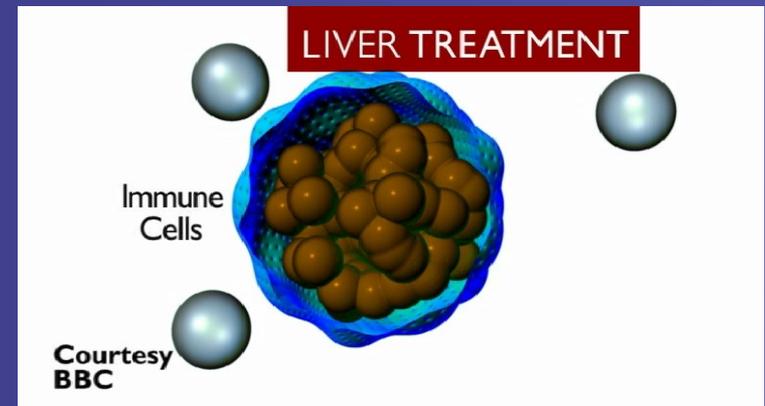
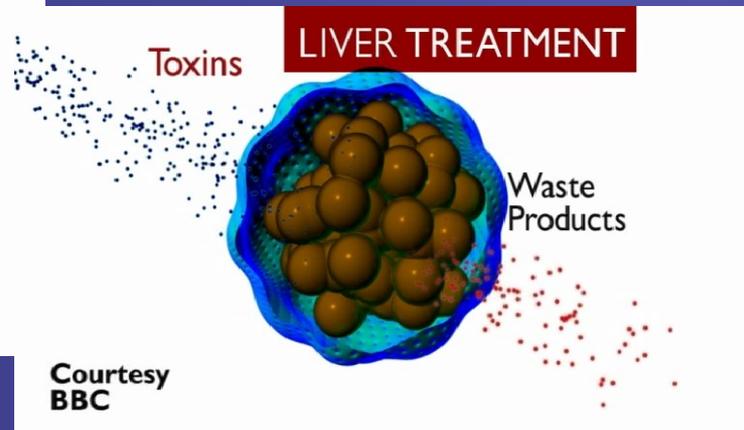


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- Microbeads density
 - Effect of hepatocyte density (2.0 to 3.5 million/ml) on cell viability and function in microbeads



First in man hepatocytes in alginate beads for ALF



8 patients to date

16G cannula



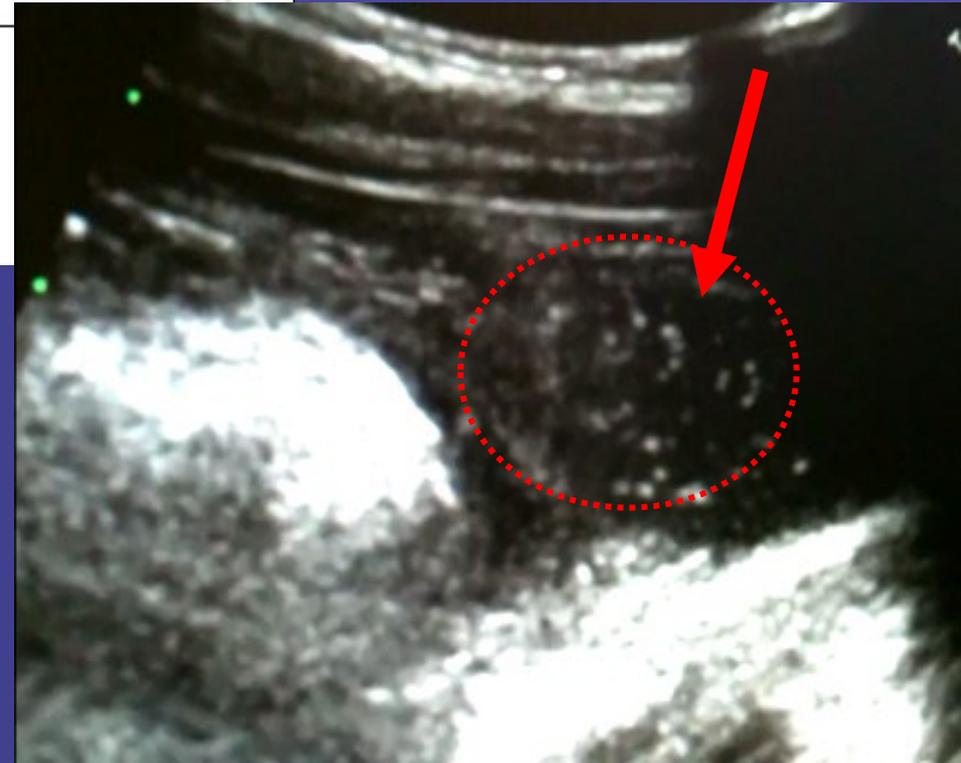
Syringe containing hepatocyte microbeads

Microbeads passing through tip of cannula into peritoneal cavity



N= 8

Infants with acute liver failure
Full filling Tx. Criteria
Awaiting liver Tx



50% Native liver recovery

Story so far..

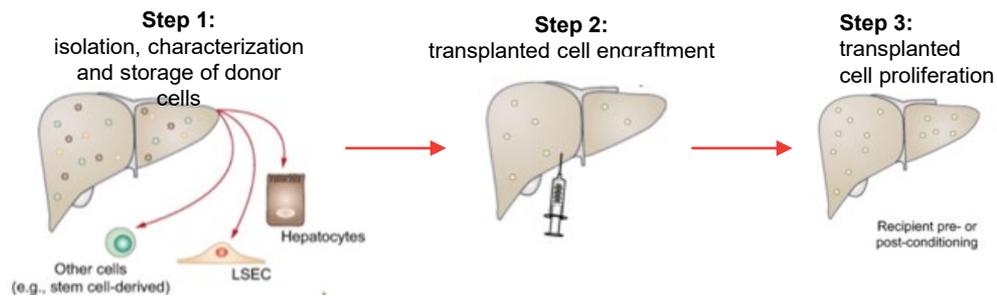
Metabolic disorders – Evidence of function under 2 years

ALF- 50% native liver survival

Hepatocytes transplantation and current barriers

To overcome these limitations:

Using alginate-encapsulated cells as a cell transplantation strategy



Major barriers

- Inferior donor quality
- Low viability and number
- Freeze- thaw damage

Most transplanted cells are cleared rapidly from the liver

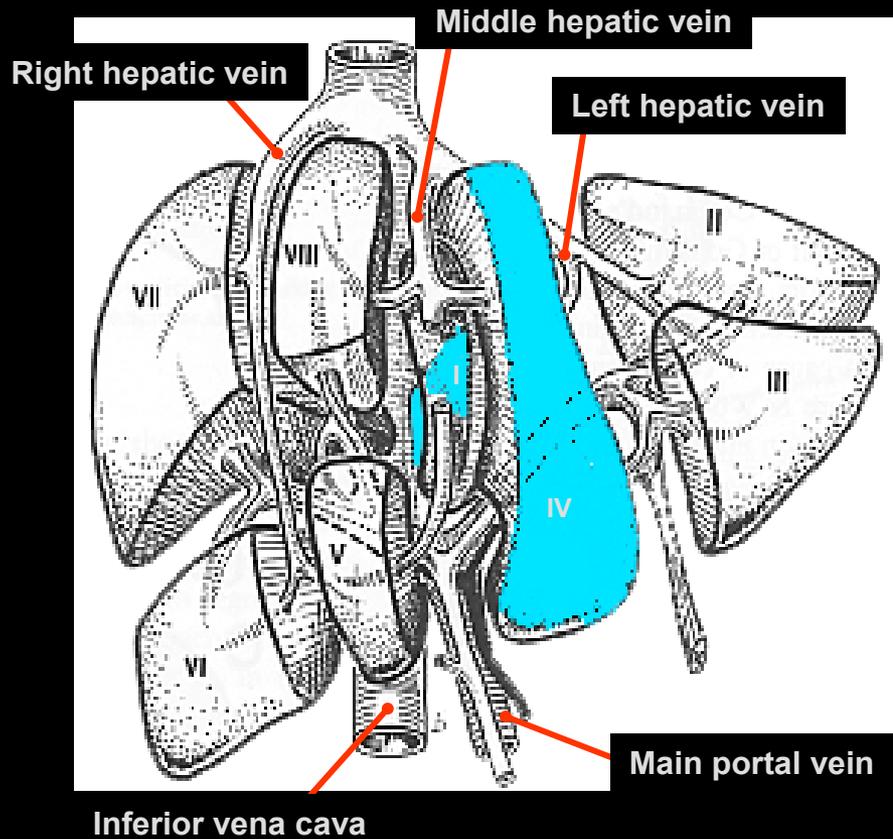
- Transplanted cells do not proliferate in the liver
- Allograft are rejected

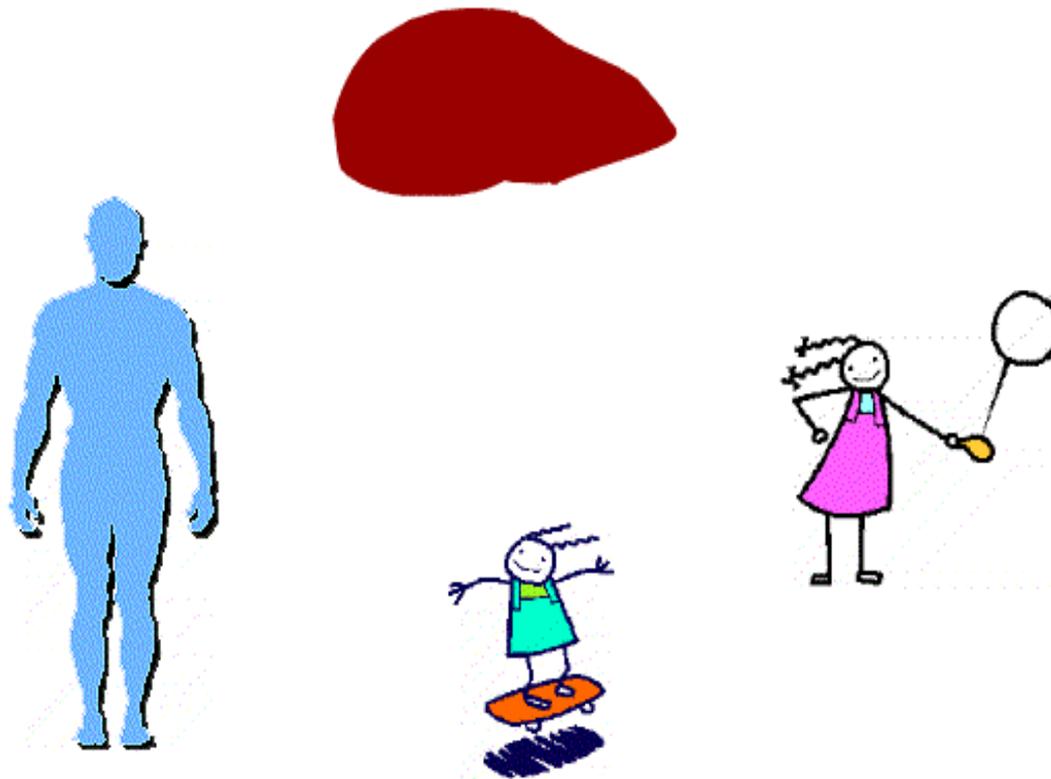
Forbes, S., Gupta S and Dhawan A Journal of Hepatology, 2015

Improving the donor pool

- 3 transplants from one liver (Segment 4)
- Non heart beating donors
- Foetal Hepatocytes
- Marginal donors
- Neonatal livers
- Stem cells

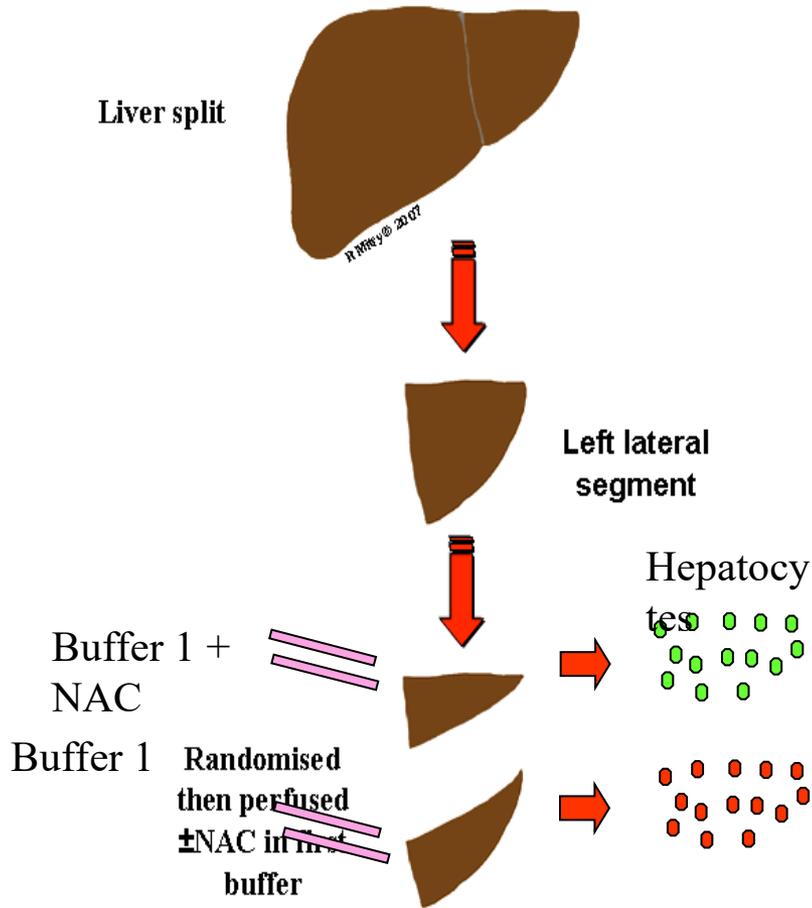
The Liver Segments



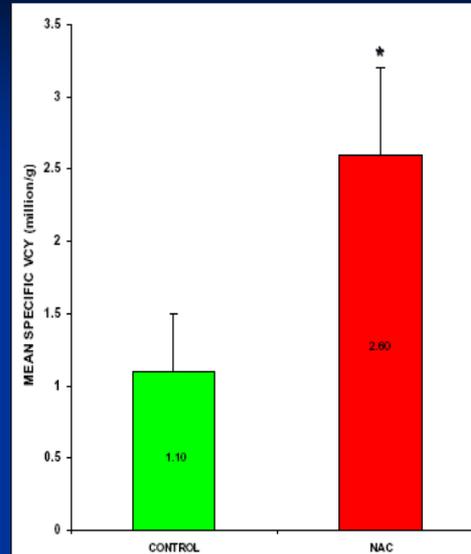


N-acetylcysteine Improves the Metabolic Function of Steatotic Human Hepatocytes for Cell Transplantation., *Cell Trans 2011*

Human Hepatocyte Isolation Perfusion with NAC

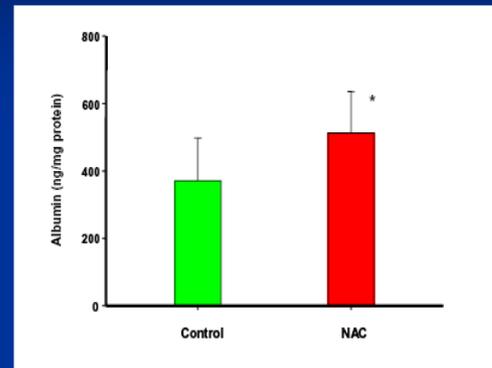


Cell Yield



Median speci
2.6 (range: 0.5-5

Albumin Content



NAC median **339**ng/mg protein/well (range: 180-1227)
vs **197**ng/mg protein/well (124-1144); **p=0.012**



- Neonatal livers often not used for solid organ transplantation
- Great Ormond Street Children's Hospital identified 45 out of 84 infants that died as potential organ donors

Percentage breakdown of infants

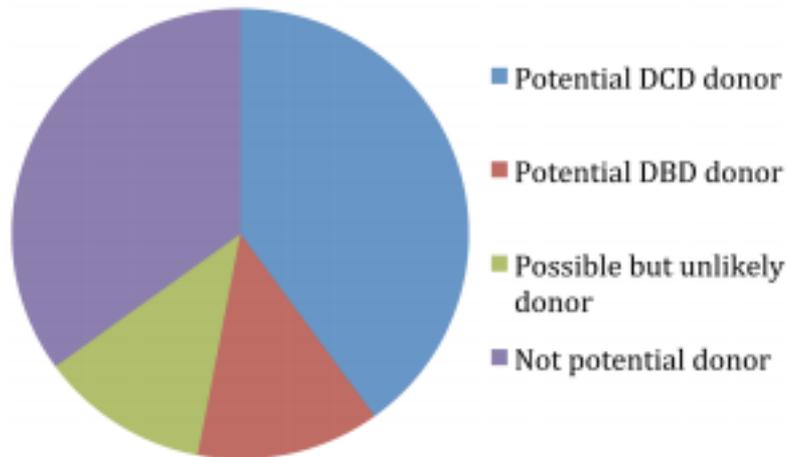


Figure 1 Pie chart showing percentage breakdown of infants as donors/non-donors.

The diagnosis of death by neurological criteria in infants less than two months old

April 2015

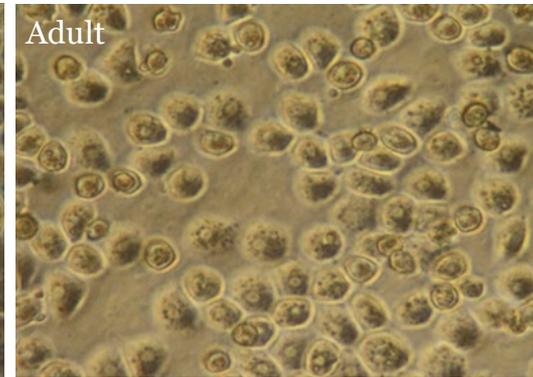
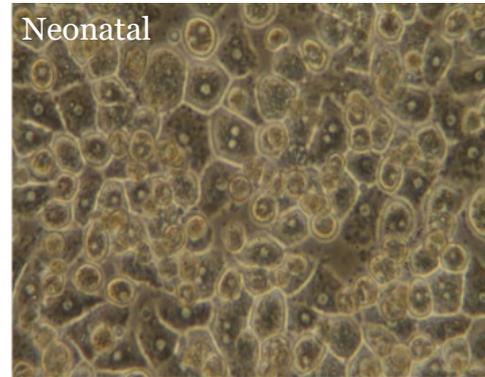
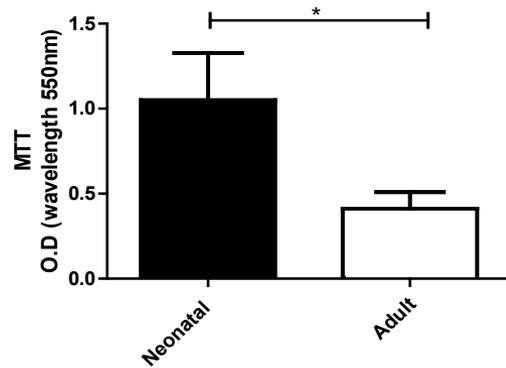
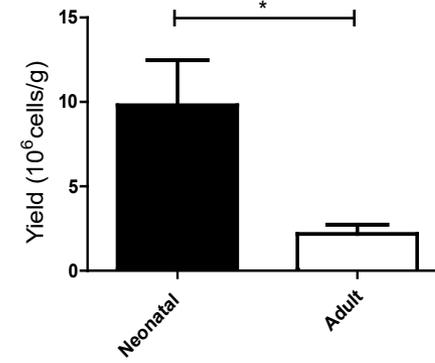
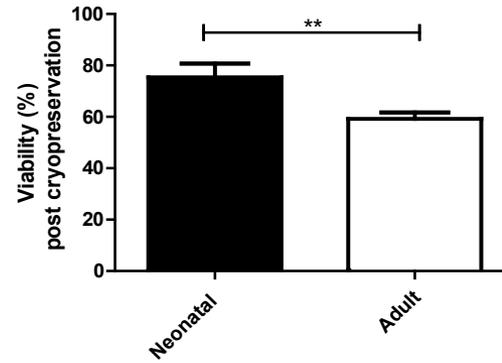
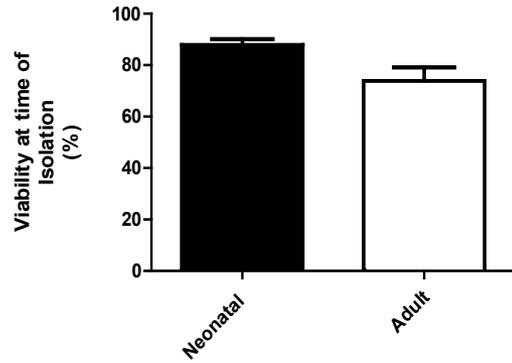


RCPCH

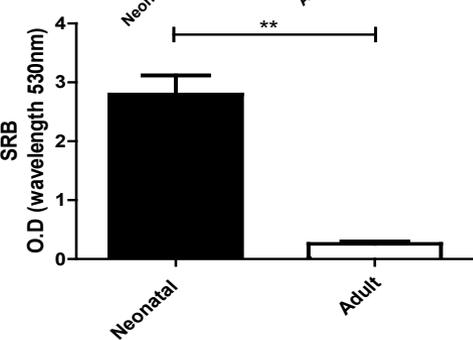
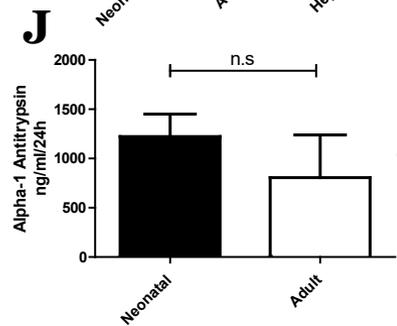
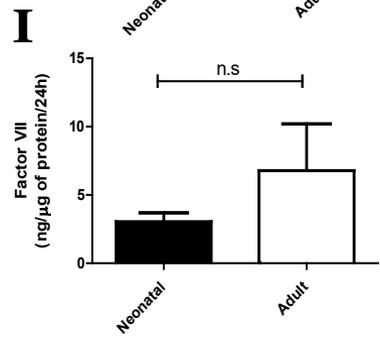
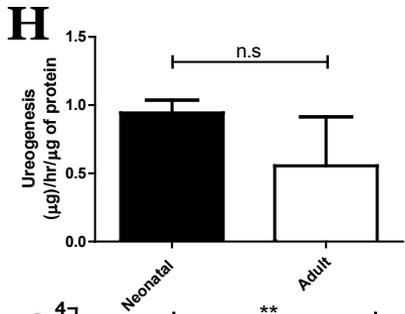
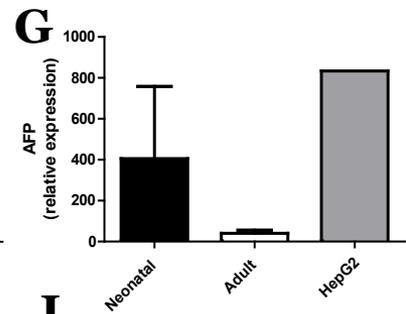
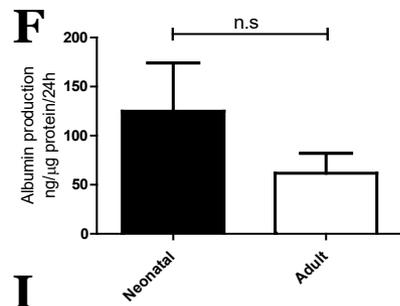
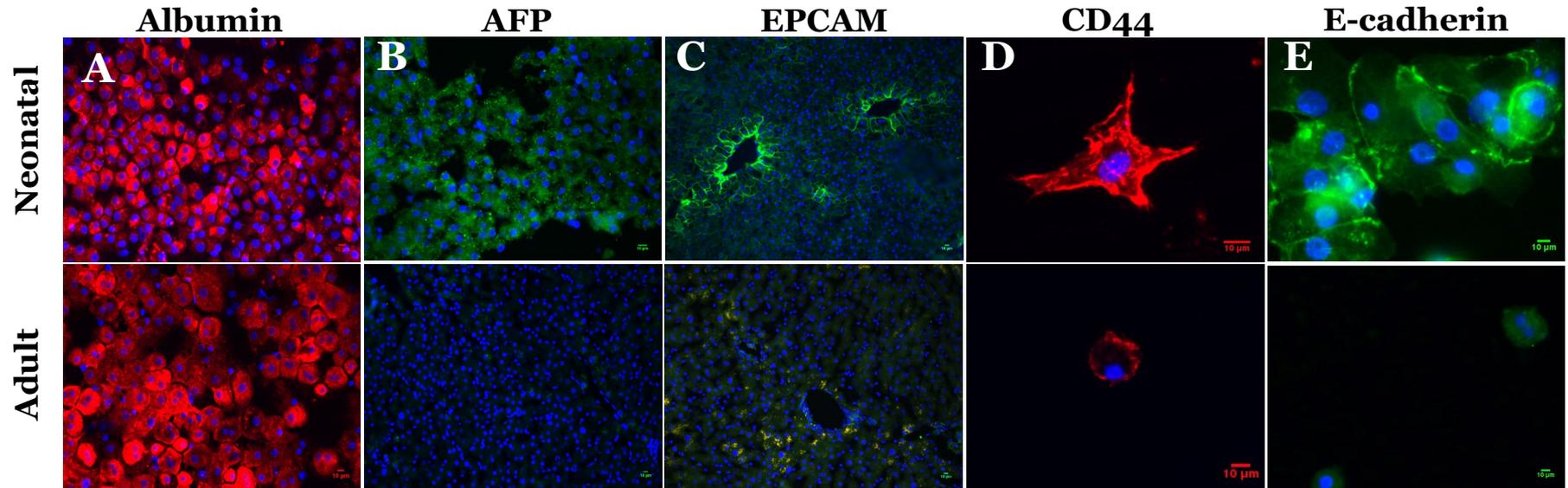
Royal College of Paediatrics and Child Health
Leading the way in Children's Health



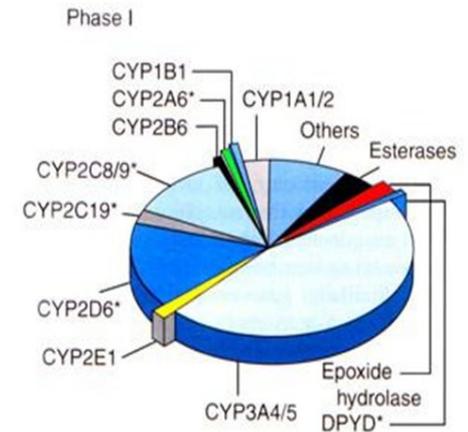
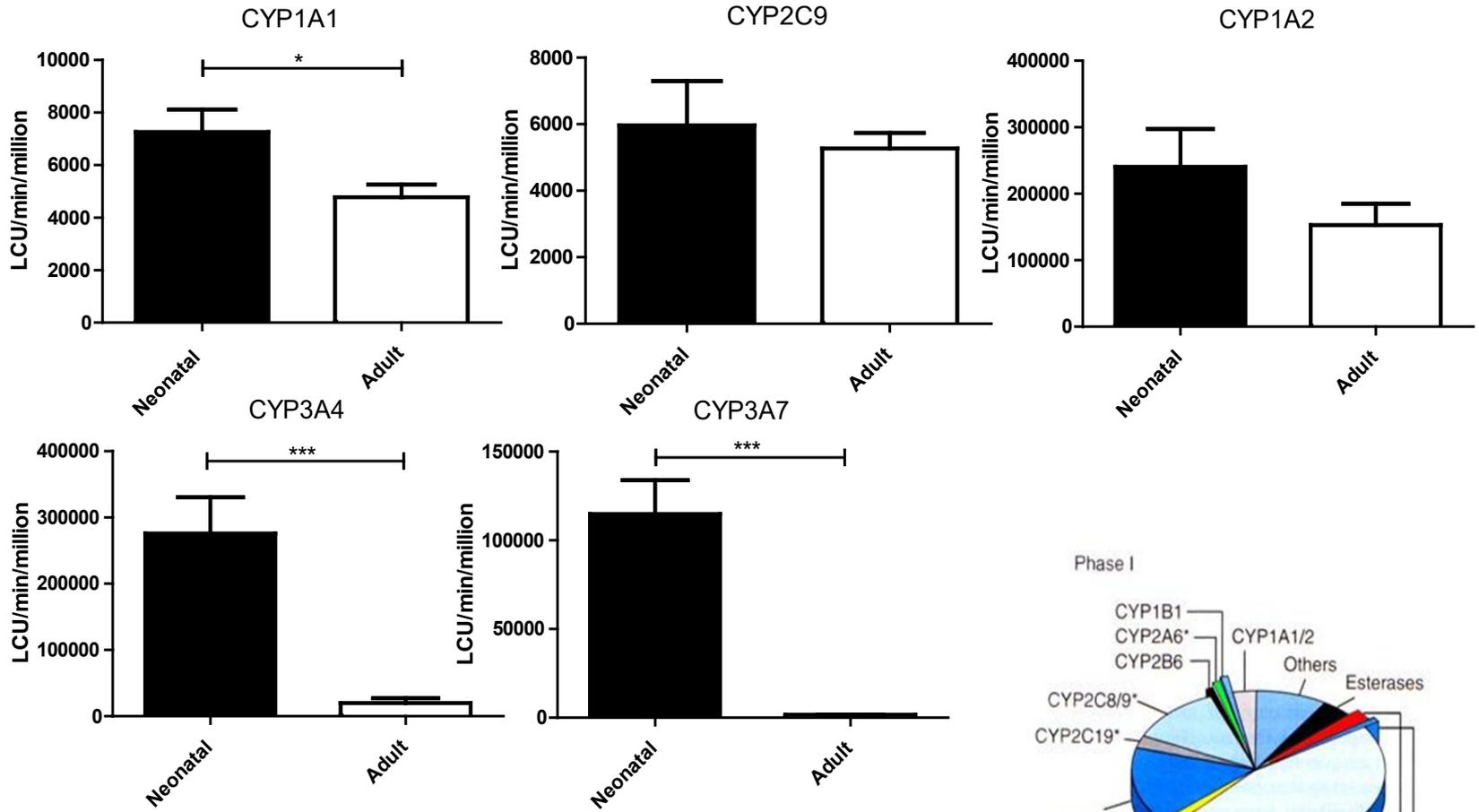
Viability and Yield



Cell attachment and Function



Function: Cytochrome P450



Immunology of cell Tx



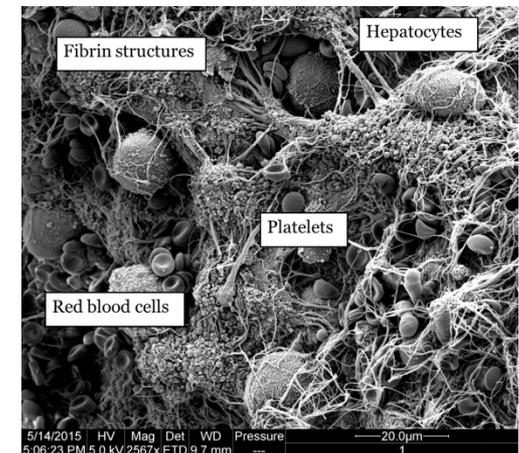
Charlotte Lee



Emer Fitzpatrick

Suppression of the Innate Immune Response following Hepatocyte Transplantation

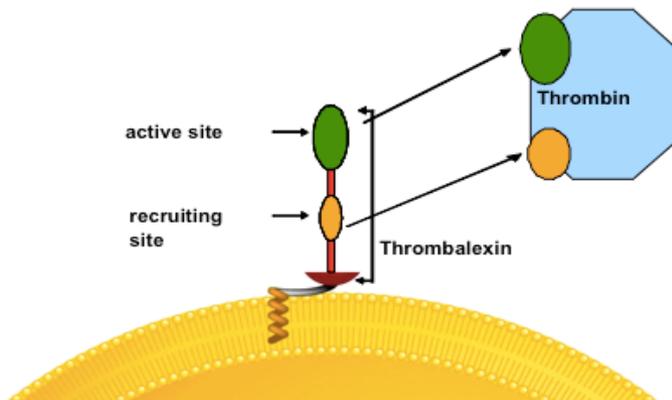
- 1) Develop an *in vitro* model of IBMIR to study the innate immune response of hepatocytes
- 2) Investigate the potential of cytotoxic and systemic therapeutic agents/drugs to inhibit the IBMIR
- 3) Use small animal models to investigate the potential of these drugs/agent for clinical use



Suppression of the innate immune response following hepatocyte transplantation

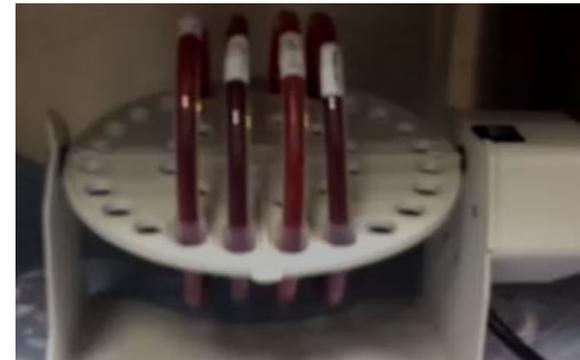
Thrombalexin

- Thrombalexin is a novel cytotoxic therapeutic agent
- Direct thrombin inhibitor
- Reduced coagulation activation in an *in vitro* model of hepatocyte transplantation



Alpha-1 Antitrypsin

- Serine protease inhibitor that primarily targets elastase
- Anti-inflammatory properties
- Successfully inhibited the IBMIR in a monkey model of islet transplantation with allograft function lasting up to 468 days post transplantation (Koulmanda et al. 2014).

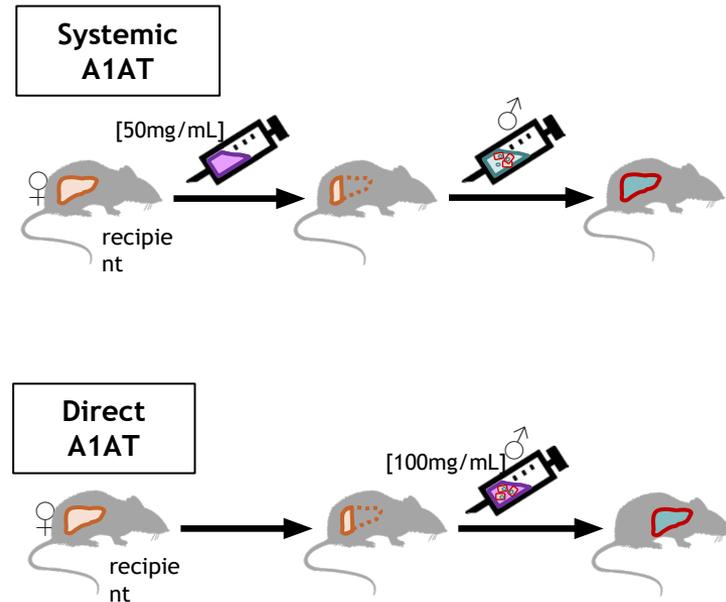
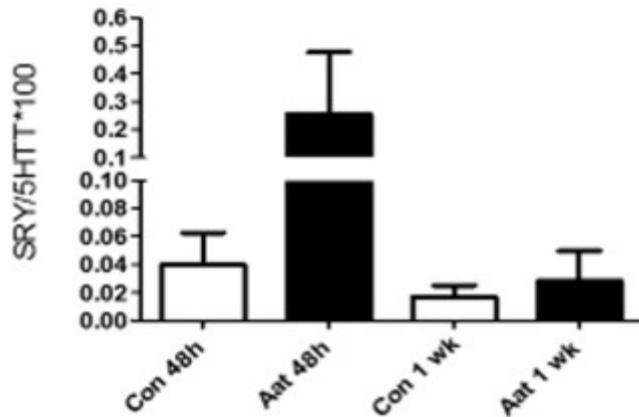


Chandler Loop model



Liver repopulation through another rat model

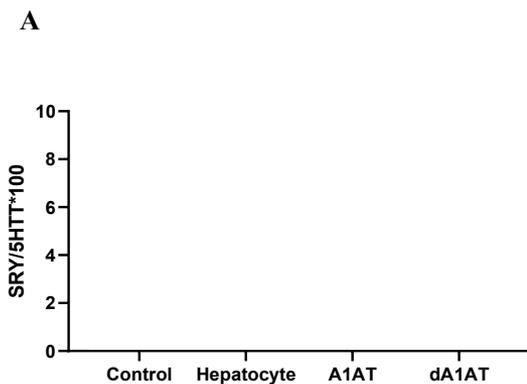
- New model: Retrorsine inhibition + partial hepatectomy
- Hepatocyte transplantation → syngeneic
- Dampen IBMIR: α1-Antitrypsin (Lee, 2017)



Syngeneic hepatocyte transplantation with α 1-Antitrypsin and partial hepatectomy under native hepatic inhibition



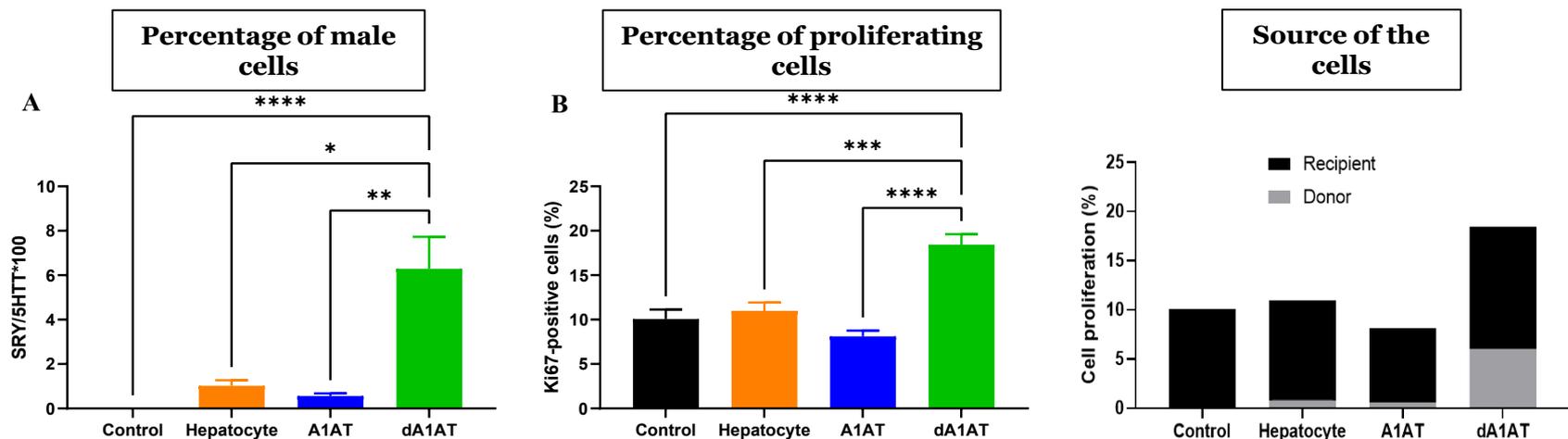
Results – liver resection with HT and A1AT (systemic & direct)





Results – liver resection with HT and A1AT (systemic & direct)

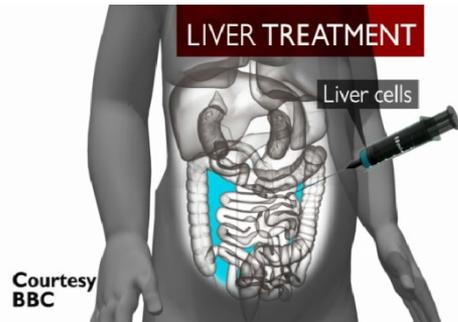
- 10-fold increased engraftment with direct A1AT vs systemic
- Combined effect of A1AT and cell viability/quality
- Over 2-fold enhanced proliferation



Hepatocyte and MSC encapsulation - alginate chemistry modifications

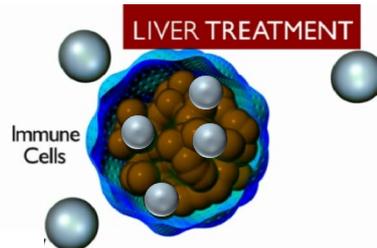


Valeria Iansante Celine Filippi

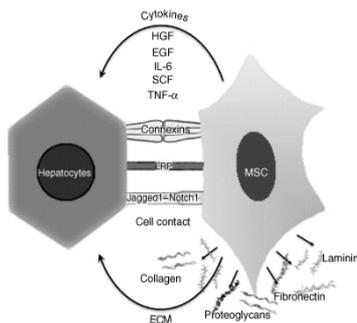


Anti-inflammatory cytokines

MSCs



NIHR i4i funding



Gomez-Aristizabal, *Mol Ther*, 2009

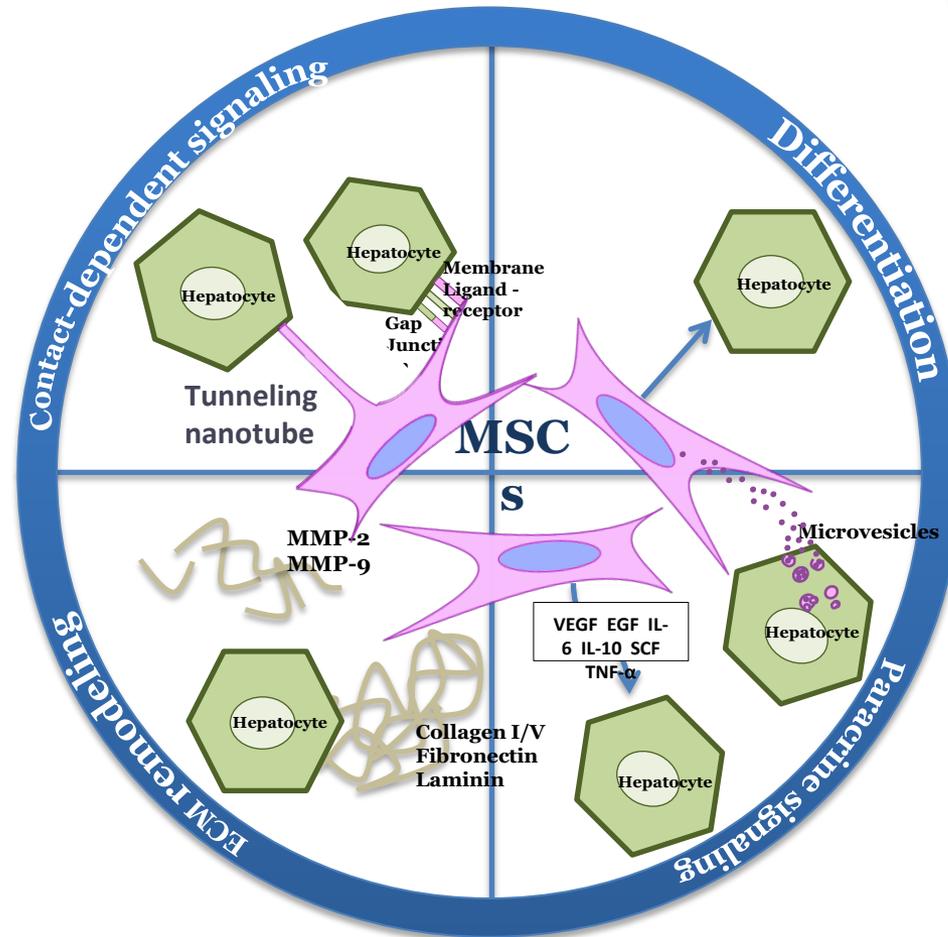
➤ Aims of the project:

1. To identify the best alginate for HC/MSC co-encapsulation.
2. To develop HC/MSC co-encapsulation procedure for clinical transplantation
3. To characterise how the best alginates improve HC/MSC performance

Khosla Fellowship - New MSC/Hep interactions

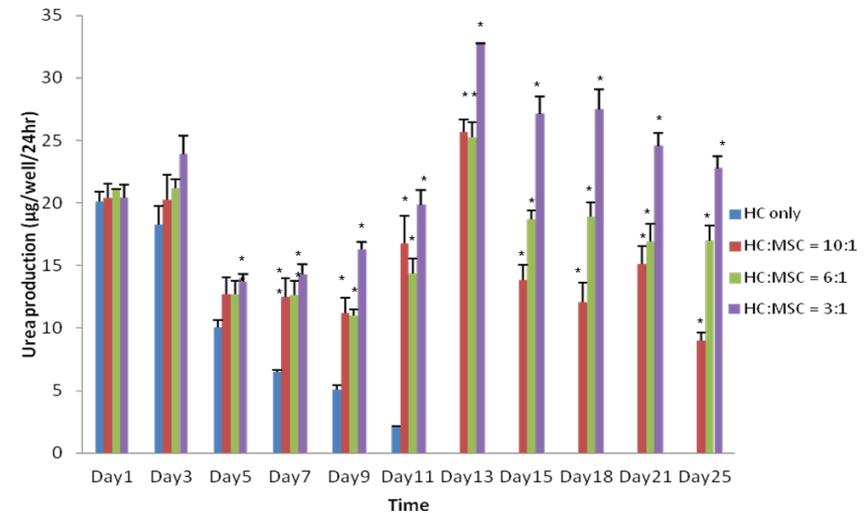
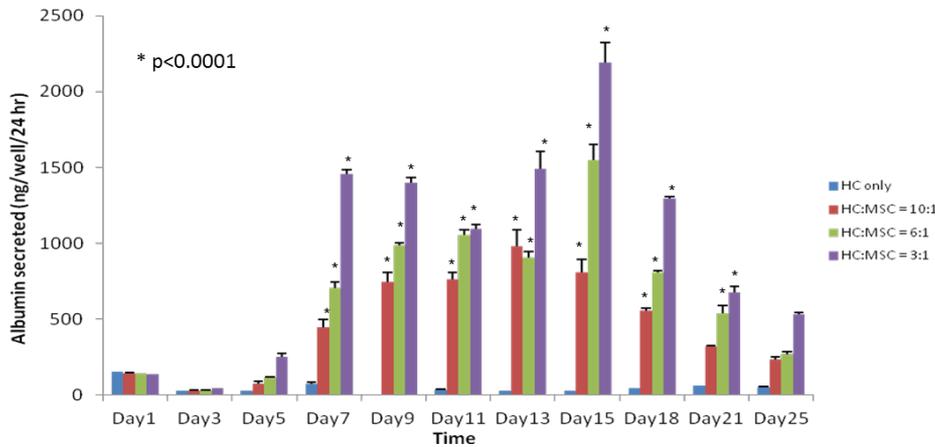


Raquel Fernandez-Dacosta
Celine Filippi





Improving the viability and function of Hepatocytes - Use of MSCs

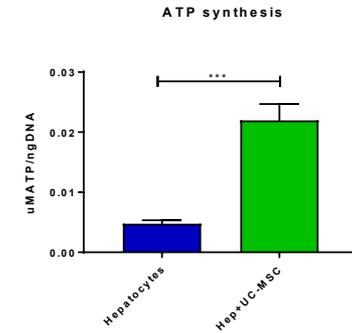
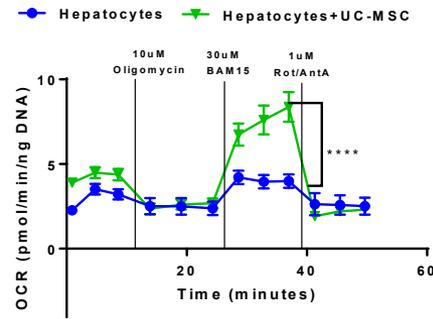
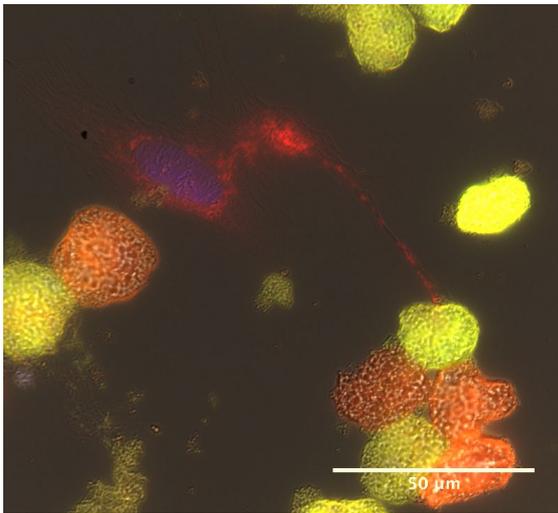


Fitzpatrick et al. *Cell Transplantation* 2013

- UC-MSC coculture not only prevents metabolism decrease but improves it
- UC-MSC co-culture also improves cell attachment and function in ALF serum



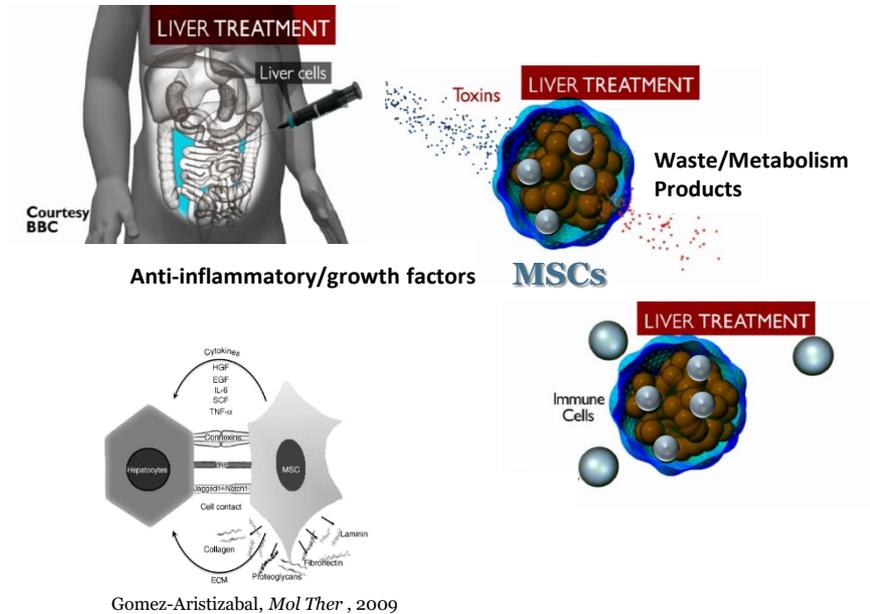
TNTs formation between UC-MSCs and hepatocytes



Fernandez-Dacosta, R. et al, unpublished data



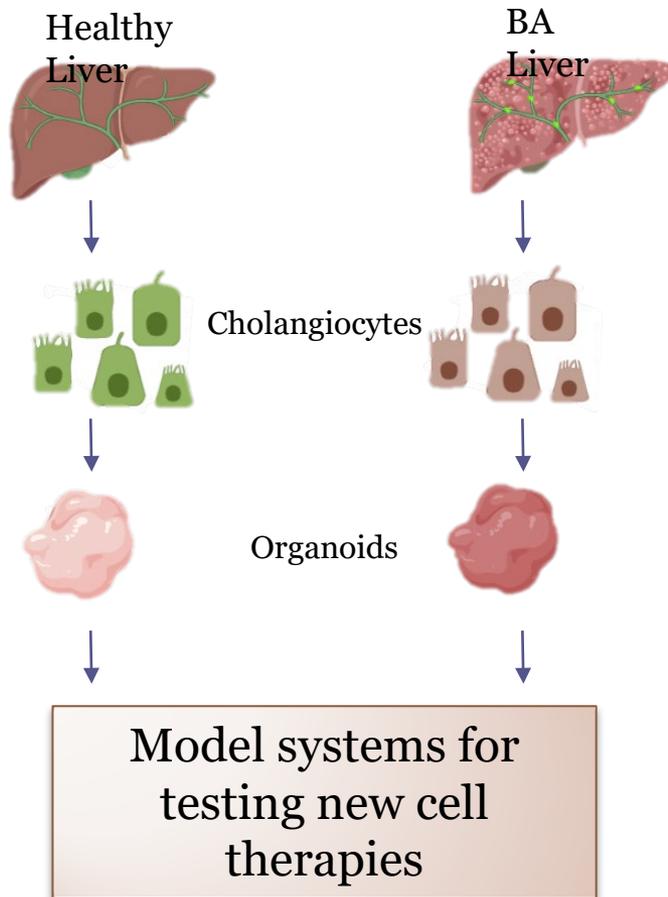
Hepatocyte and MSC encapsulation - alginate chemistry modifications



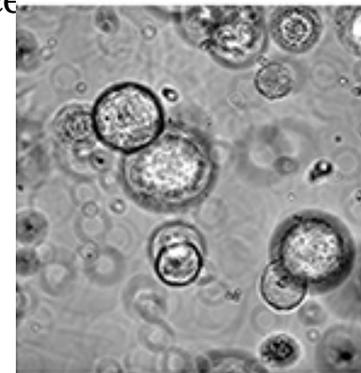
First in human clinical trial
MRC funded trial Sep 2022



In Vitro Model of Biliary Atresia



- Organoids are complex clusters of organ-specific cells which self-assemble when given a scaffolding extracellular environment, such as Matrigel or collagen. When that happens, they grow into microscopic versions of parent organs viable for 3D study.
- Using explant tissue, we are creating cholangiocyte organoids which can be used as a model system to further study Biliary Atresia and to test our new cell therapies.

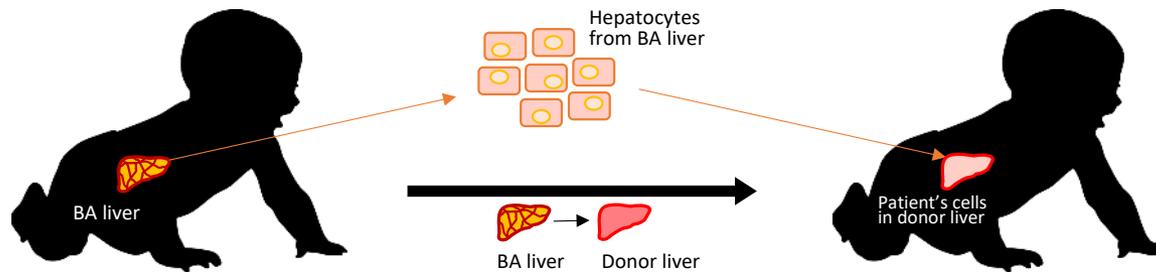


Investigation of autologous hepatocyte transplantation, after allogenic liver transplantation, to repopulate the allograft with native hepatocytes



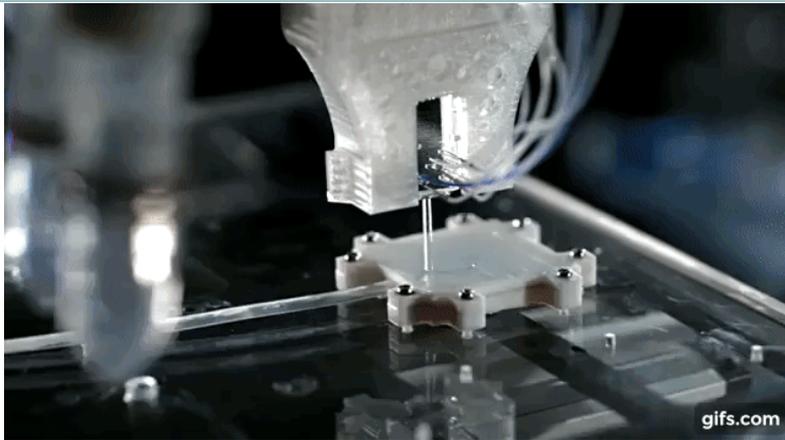
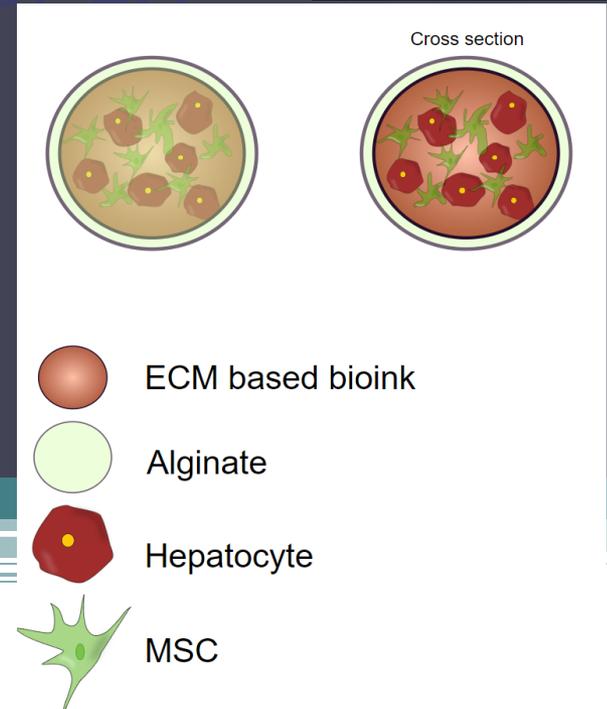
Hypothesis

- Autologous hepatocyte transplantation after the allogenic donor liver implantation could lead to the graft repopulation with patient's own cells (new treatment for Biliary Atresia)



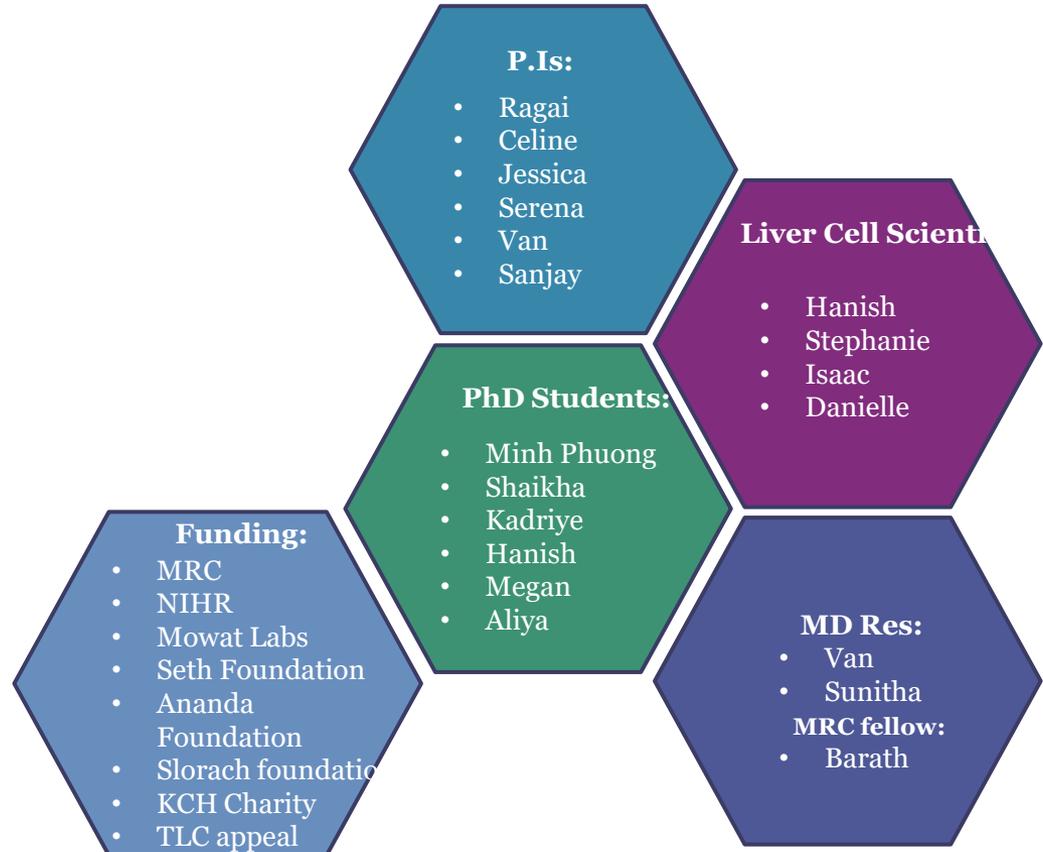
Aims:

- We have an exciting new collaboration with Aspect Biosystems.
- Building on our success with HMB002, we plan to use 3D bioprinting to further improve the interaction between MSCs and Hepatocytes.
- Using the immune protective properties of alginate encapsulation combined with the biomimetic properties of an ECM-based bioink, we can “print” constructs of controllable size and architecture.



Dhawan Lab

- Human Hepatocyte Transplantation:
 - Cellular Therapies for Biliary Atresia
 - Alginate microbeads for ALF
 - Hepatocyte Tx for liver based metabolic defects
- Wilson's Disease Clinical trials
- Liver disease in congenital myopathies
- Paediatric Fatty Liver disease
- Long-term liver transplant outcomes
- Neurodevelopmental outcomes of Biliary Atresia



Acknowledgements

Dhawan Lab

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- Dr. Robin Hughes
- Dr. Juliana Puppi
- Dr. Claire Terry
- Dr. Suttiruk Jitraruch
- Ms. Fabiane Klem
- Dr. Song Sun
- Dr. Yue Wu
- Dr. Harry Qin



LIVER TRANSPLANT SURGEONS

- Prof. Nigel Heaton
- Prof. Mohamed Rela
- Mr Hector Vilca-Melendez
- Transplant coordinators



King's College Hospital 
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Alex Mowat
PhD Studentship



Guy's and St Thomas' NHS Foundation and King's College London's
Biomedical Research Centre



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