Wake Forest™
School of Medicine
Institute for Regenerative Medicine

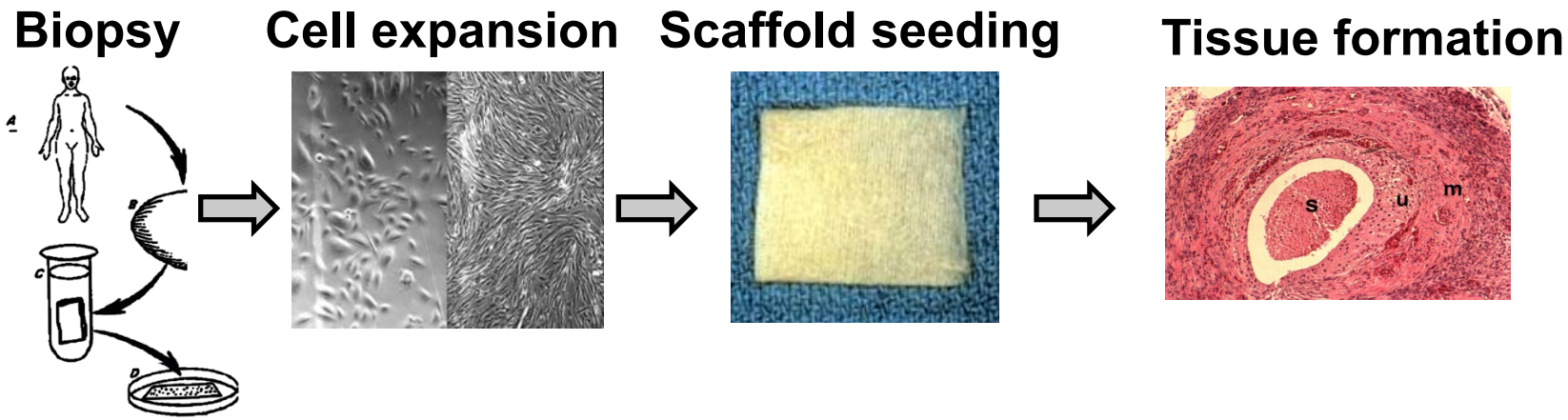
Use of Regenerative Medicine and Bioprinting Techniques for Human Tissue Testing

Fourth Workshop on Validation and Qualification of New In Vitro Tools and Models for the Pre-Clinical Drug Discovery Process
March 7, NIH Campus, Bethesda, MD

Anthony Atala / Shay Soker
Institute for Regenerative Medicine
Wake Forest School of Medicine

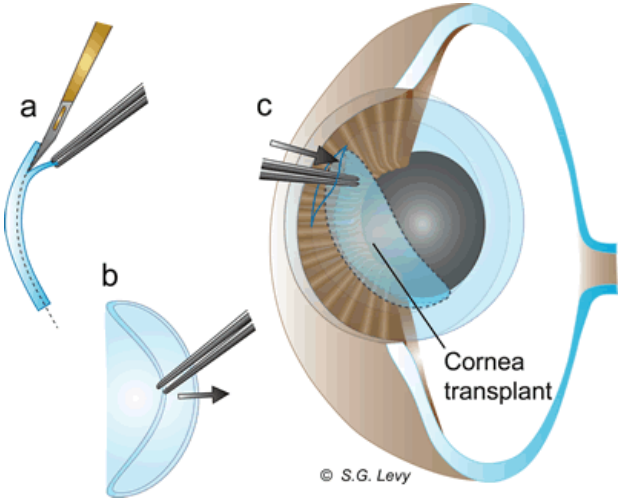
Tissue Engineering Scheme

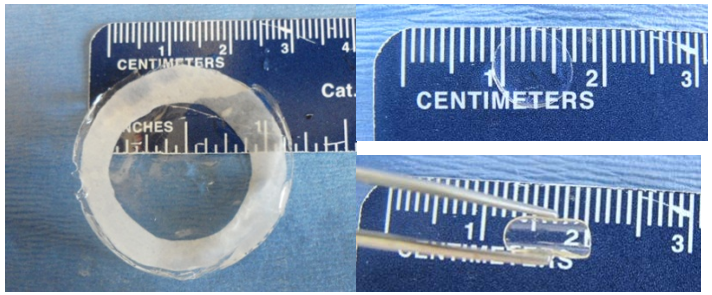
The ultimate goal of Tissue Engineering is to replace damaged and non-functioning tissues or organs

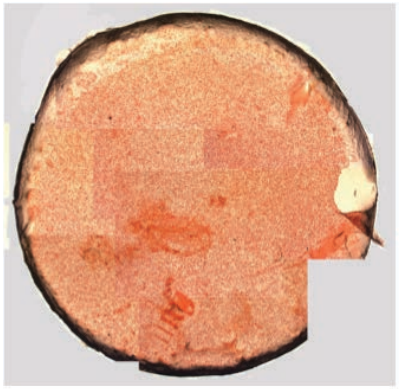


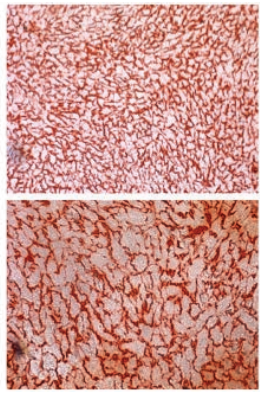
Current "state of the art":
Simple, thin, tissues and organs

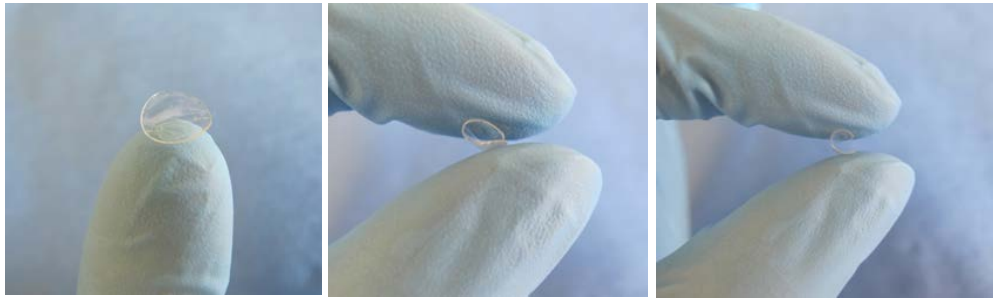
Development of gel-based scaffolds for cornea transplantation







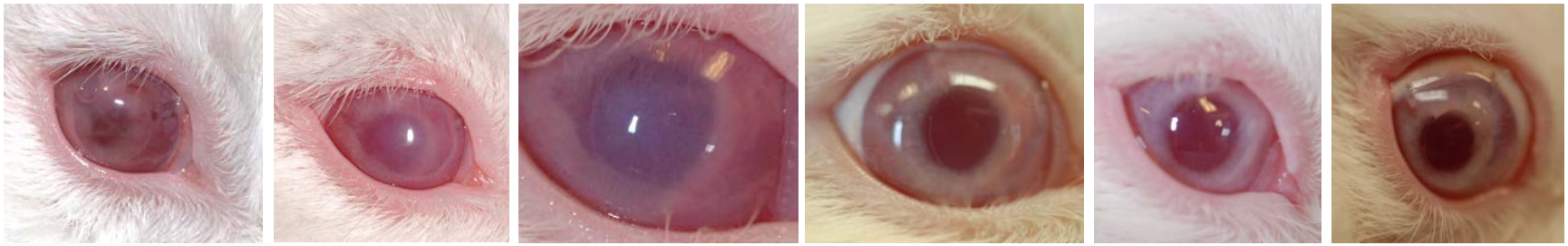




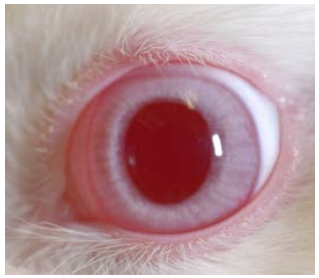
Human Corneal Endothelial Cells
(3,000 cells/mm2; 7days after cell seeding)

6"

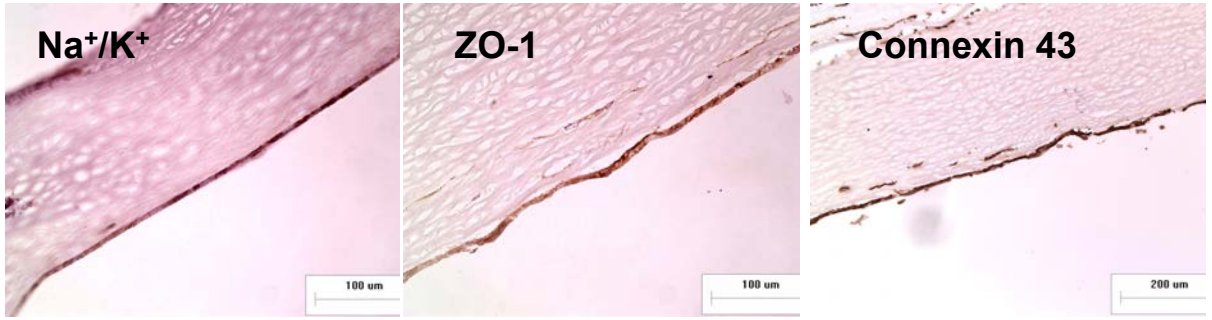
Bioengineered cornea transplantation



1 day | 1 week | 2 weeks | 3 weeks | 4 weeks | 5 weeks

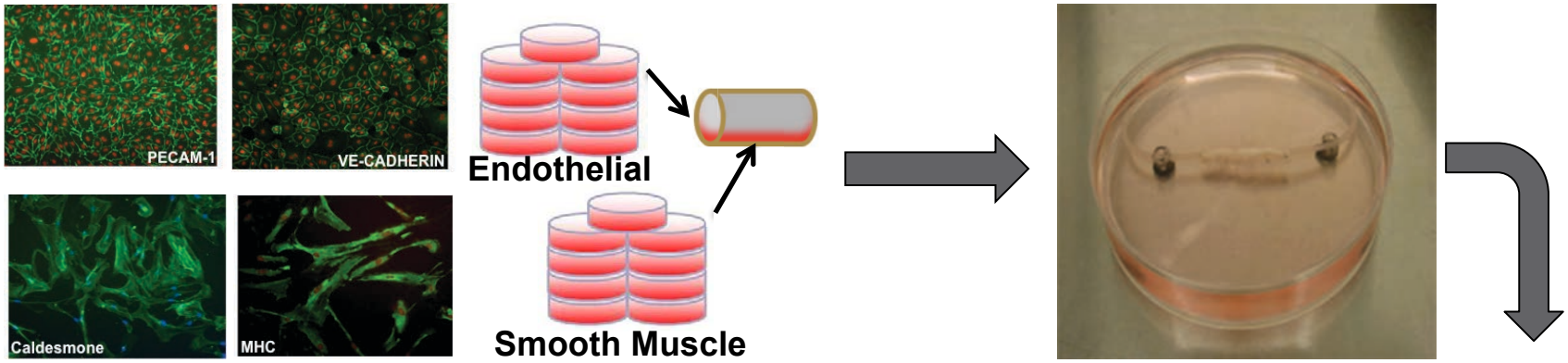


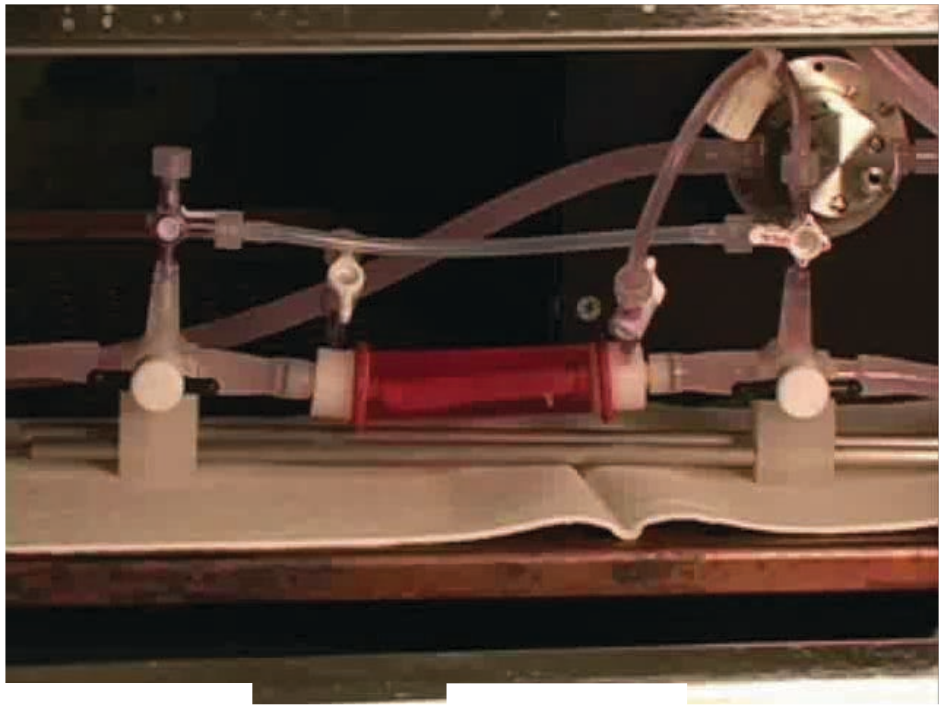
Normal



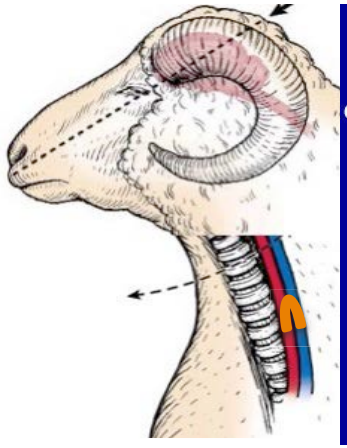
- New Zealand white rabbit (male)
- Cell seeded scaffold implanted
- DSEK
- Observation at 1, 2, 3, 4 and 5 weeks after operation
- Harvest and evaluation with H&E and IHC

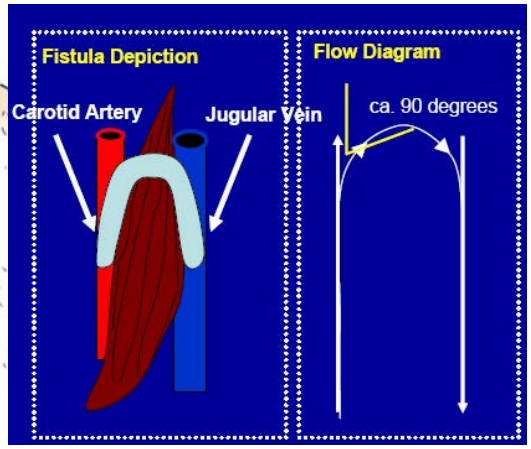
Blood Vessel Engineering

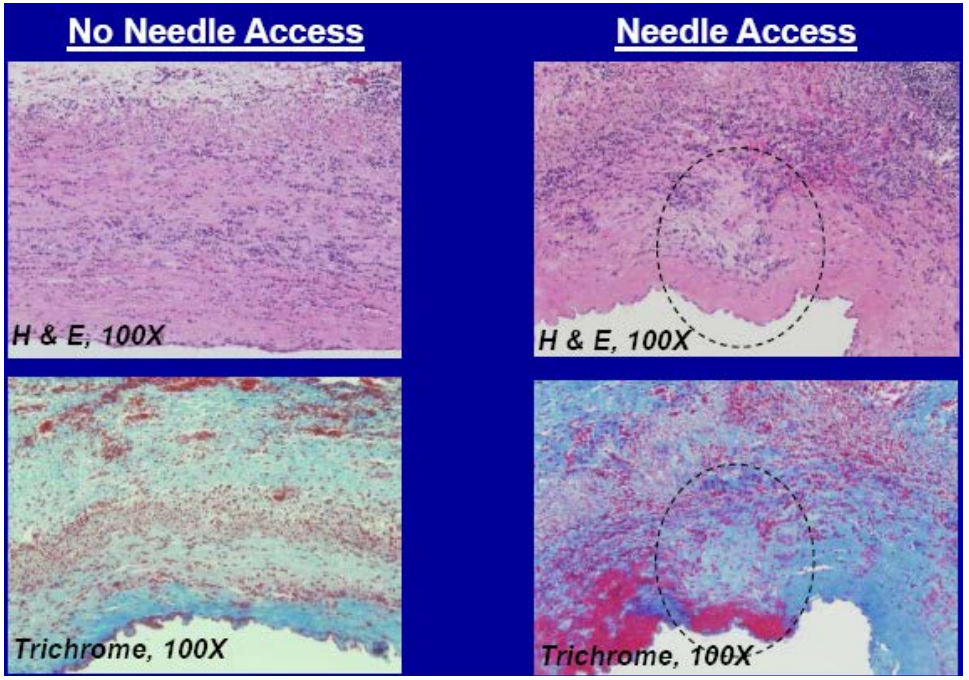


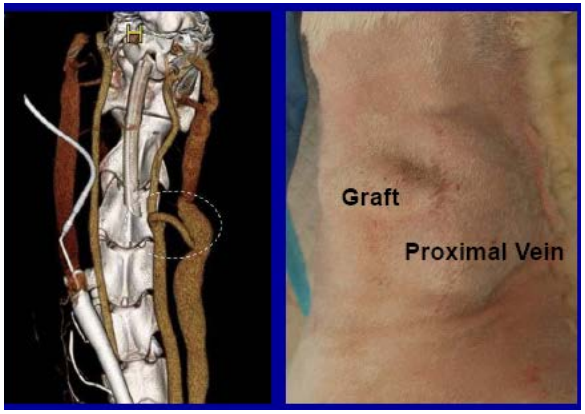


In vivo testing (AVF graft in sheep)



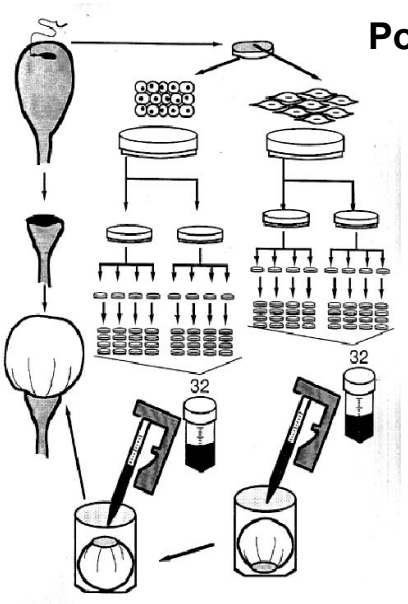




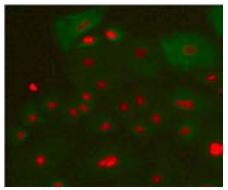


Bladder Tissue Engineering

Biopsy



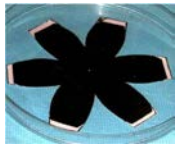
Cell expansion



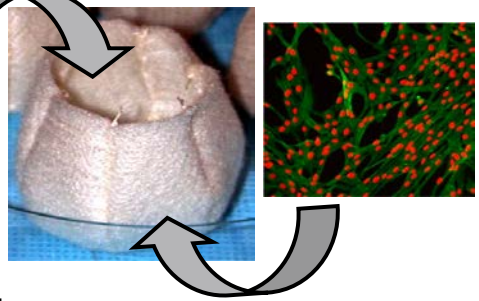
Urothelial Cells

Scaffold seeding

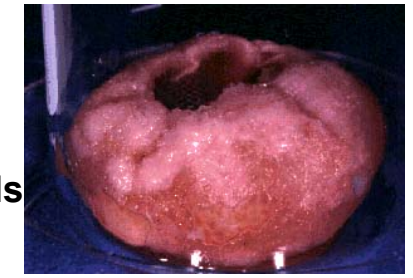
Polymer Template

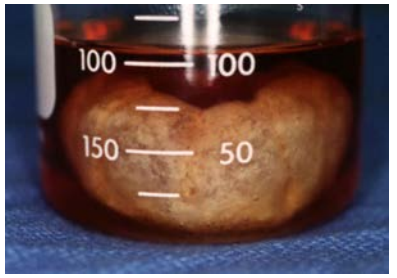


Smooth Muscle Cells

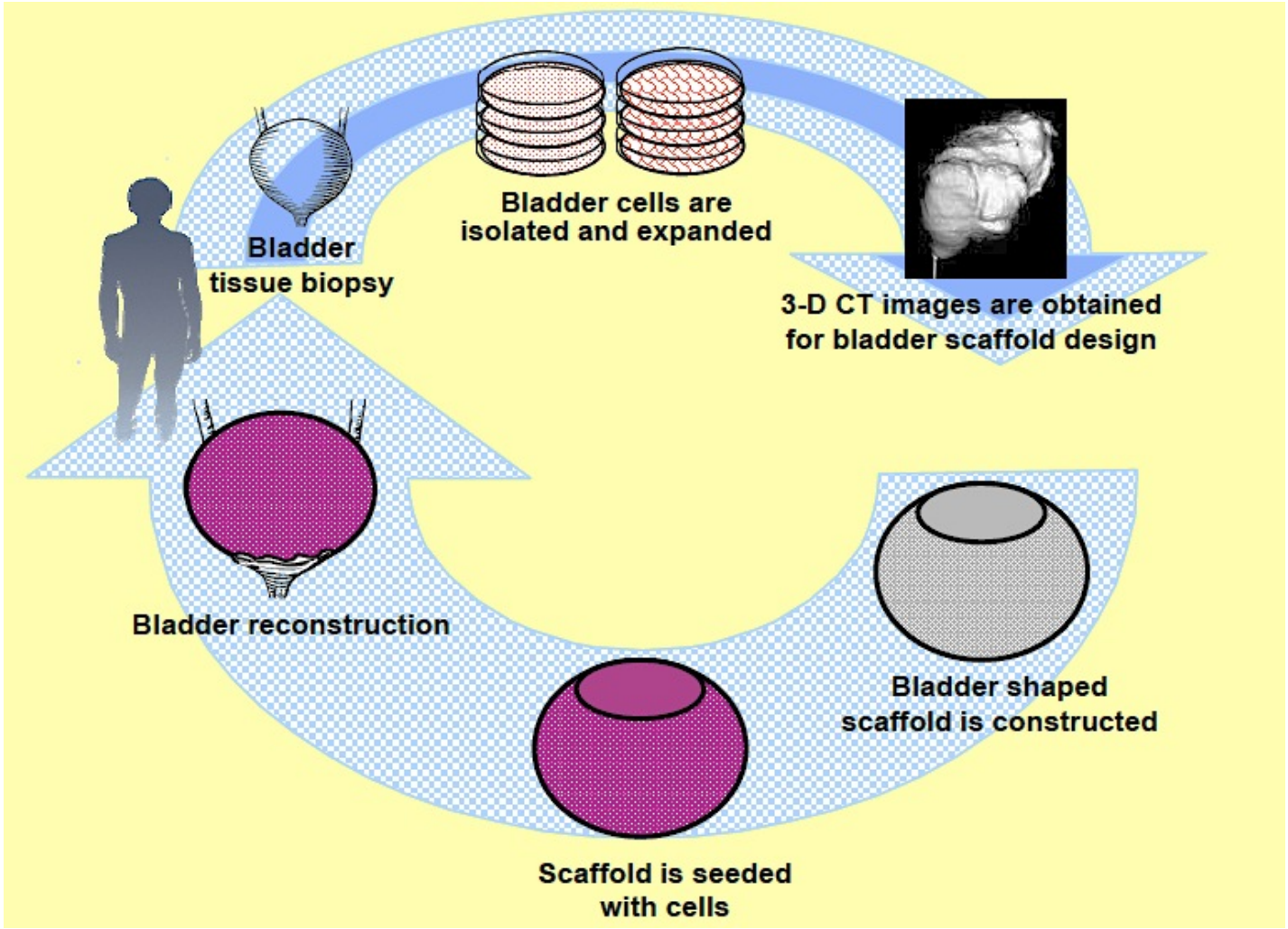


Tissue formation

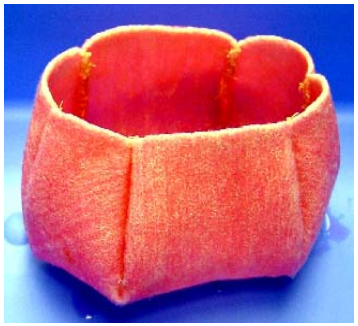


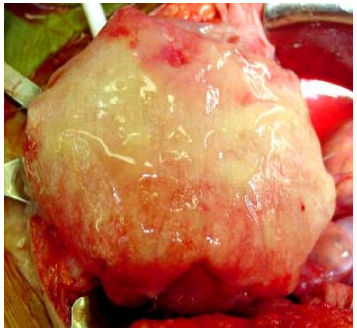


Clinical Translation of Bioengineered Bladders



Clinical experience





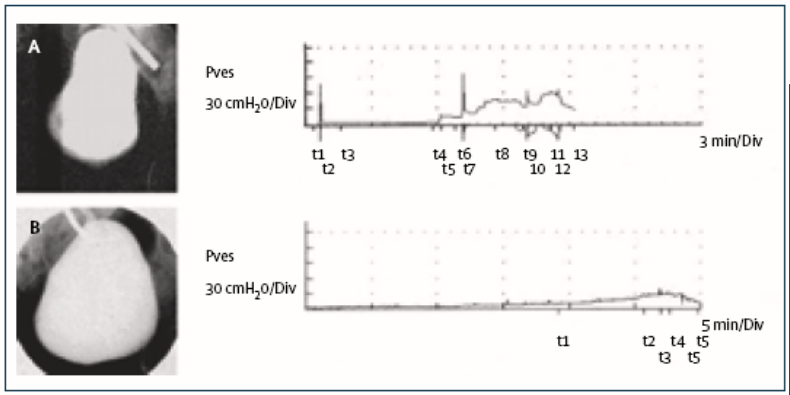


Figure 2: Preoperative (A) and 10-month postoperative (B) cystograms and urodynamic findings in patient with a collagen-PGA scaffold engineered bladder
Note irregular bladder on cystogram, abnormal bladder pressures on urodynamic study preoperatively, and improved findings postoperatively.

Phase 1, 2 trials completed
Phase 3 in progress
Over 8 year follow-up
Work still in progress

THE LANCET.

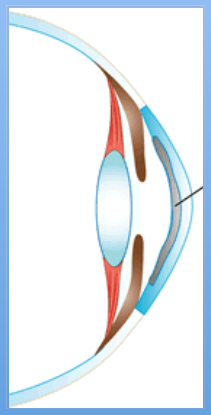
"Tissue-engineered autologous bladders for patients needing cystoplasty"

April 2006

Progress in tissue engineering

Increasing engineered complexity

Flat structures

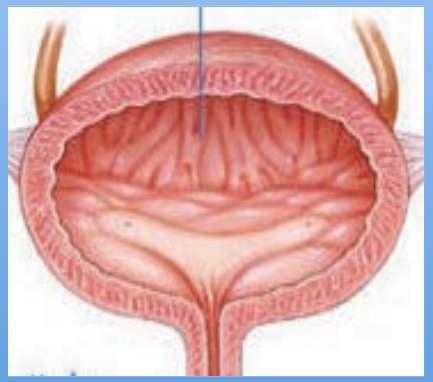


Cornea
Skin,

Tube structures

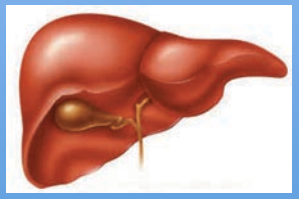
Blood vessel
Urethra, Esophagus, Intestine,

Hollow structures



Bladder

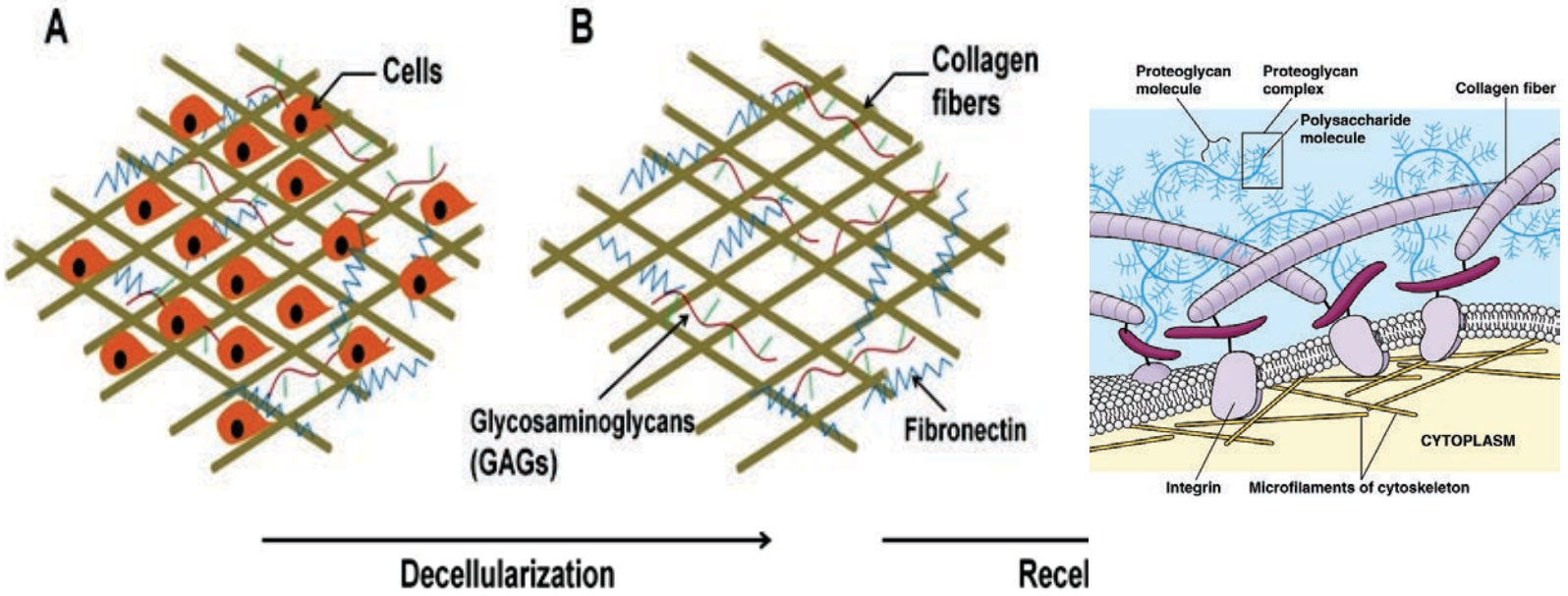
SOLID ORGANS

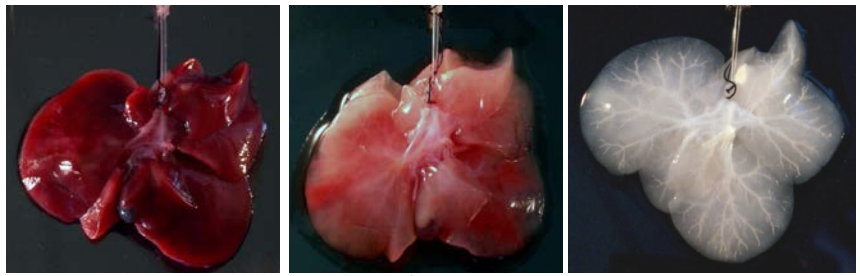


Kidney, Liver, Pancreas, etc.

Using native tissue as a scaffold

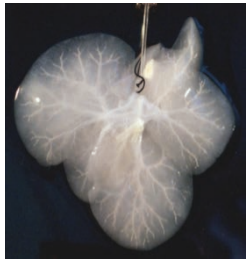
- Provides authentic structural support to cells.
- Contains several bioactive molecules which, in their unique spatial distribution, provide a reservoir of biologic signals that are difficult to artificially replicate.

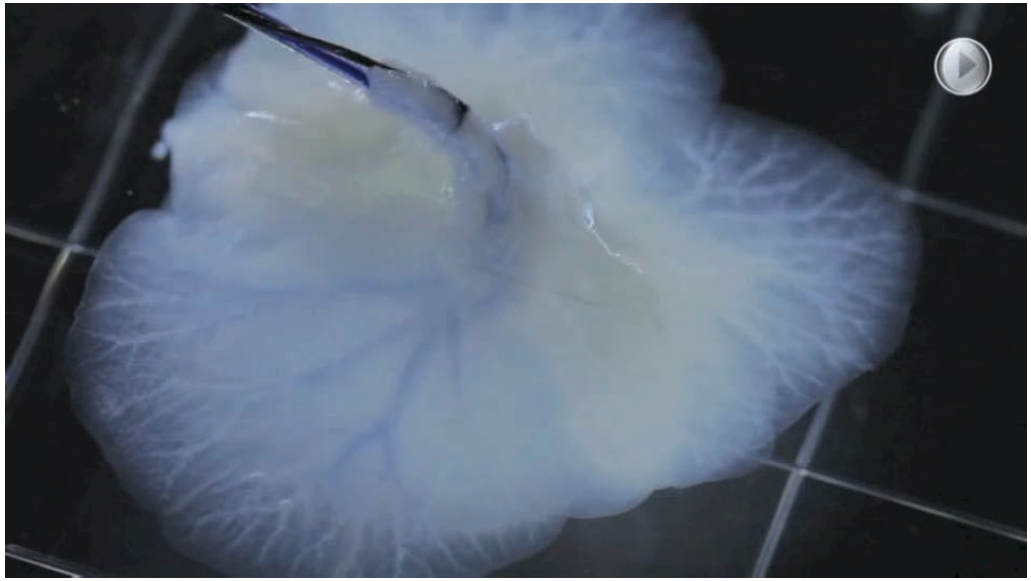


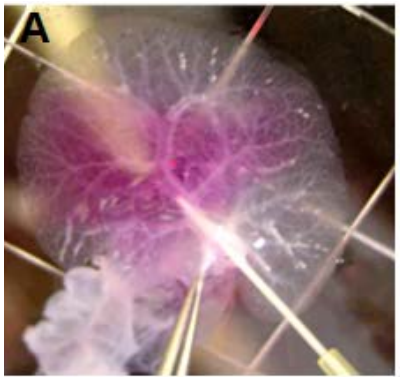


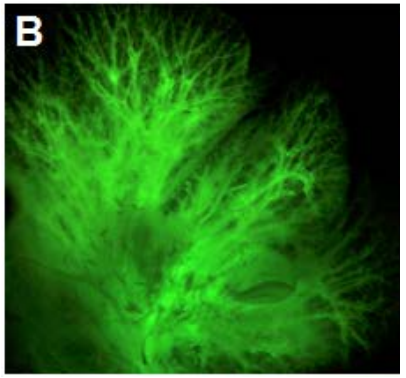
Pre-wash H_2O Detergent (2 hrs)

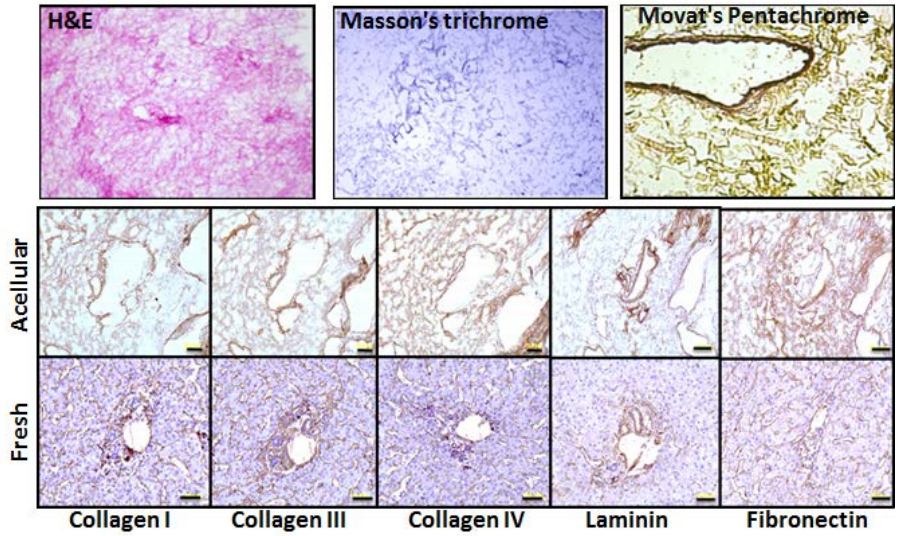
Intact vascular network and native ECM







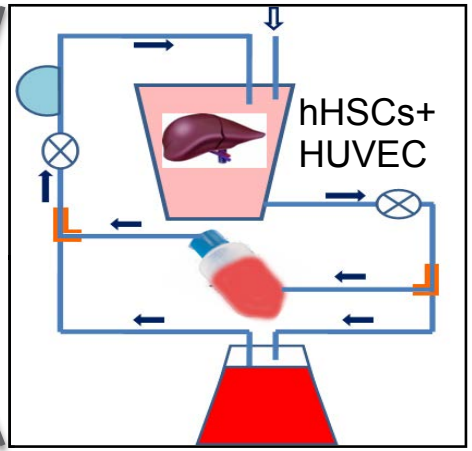




Baptista et al Hepatology 2011

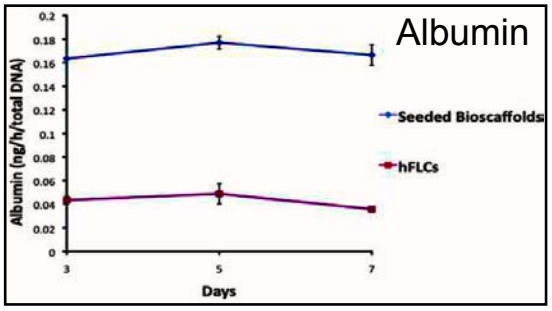
Recellularization of the liver scaffold

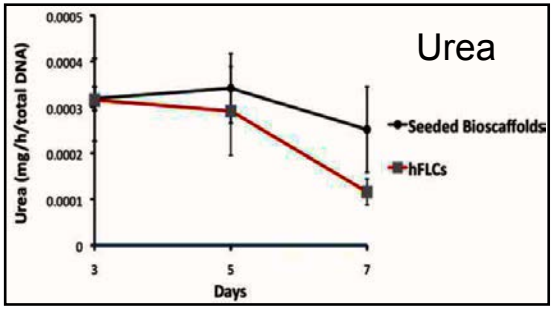
hHSCs-Human hepatic stem cells

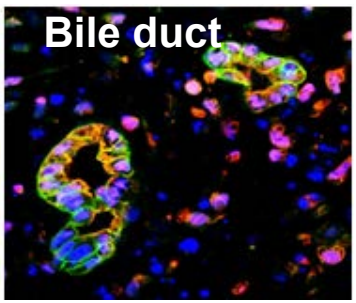


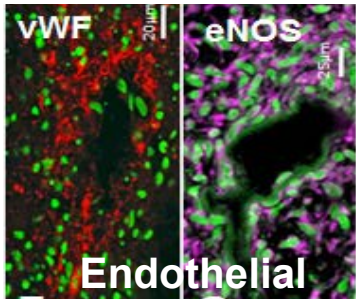
Before

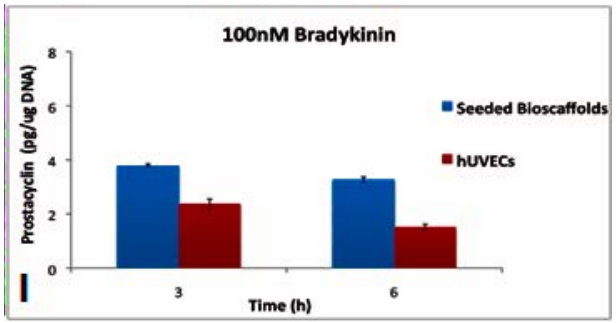
After



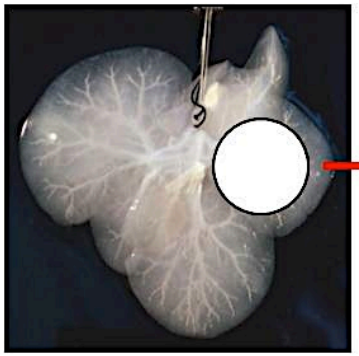


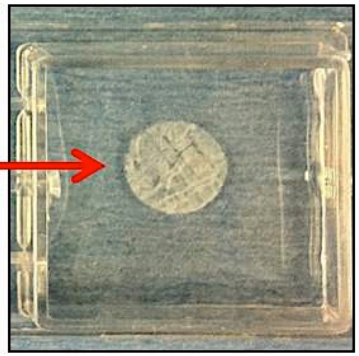






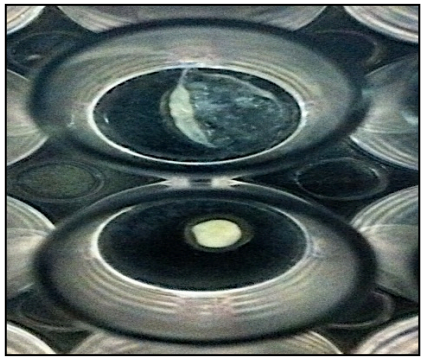
Bioengineered liver tissue for drug screening; Acellular Liver Discs

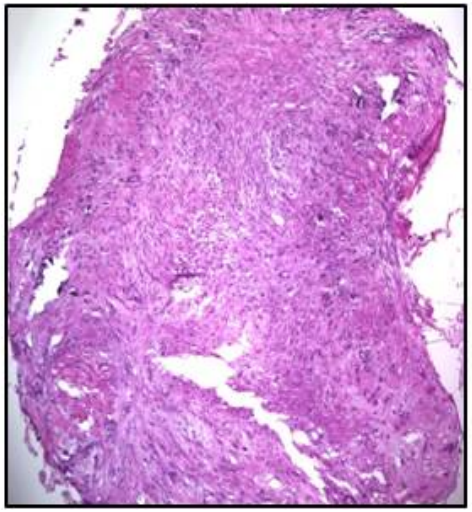


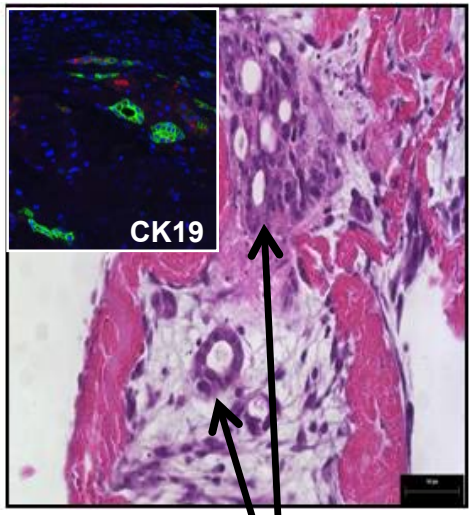


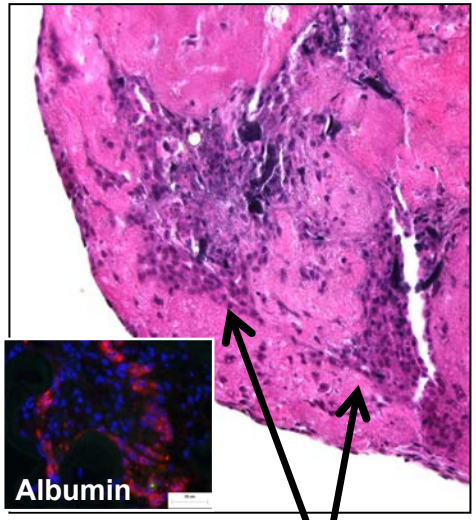
0.5 – 1 X 10^6 hFLPCs

3 week differentiation







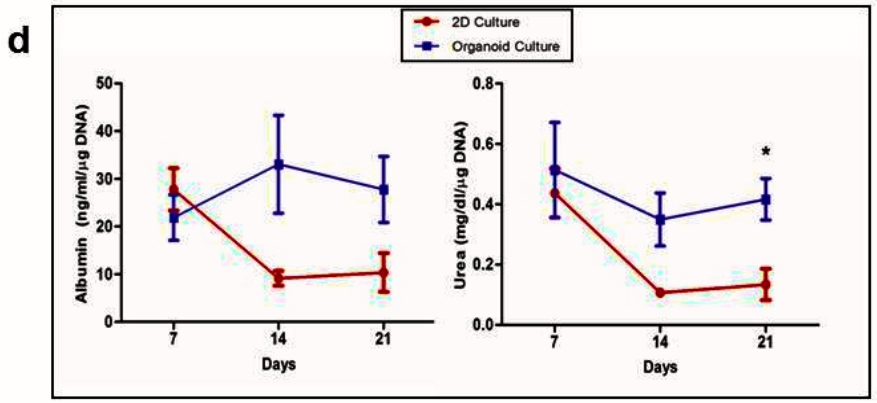


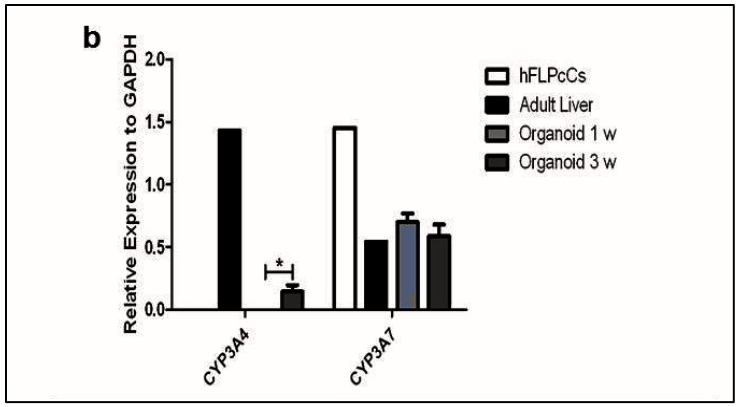
Biliary ducts

Hepatocyte clusters

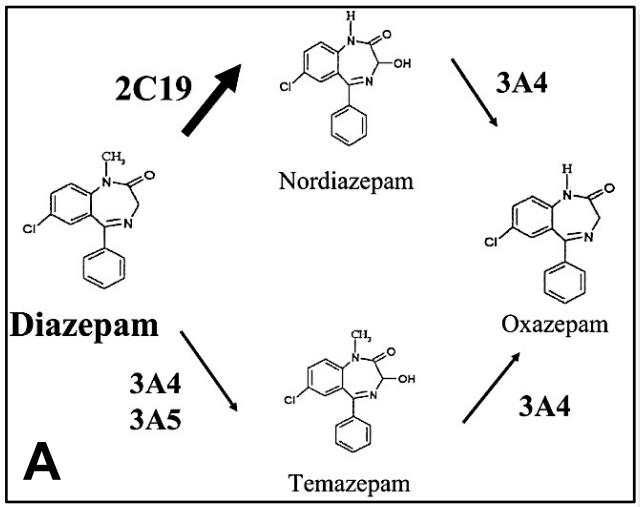
Dipen Vyas, Pedro Baptista

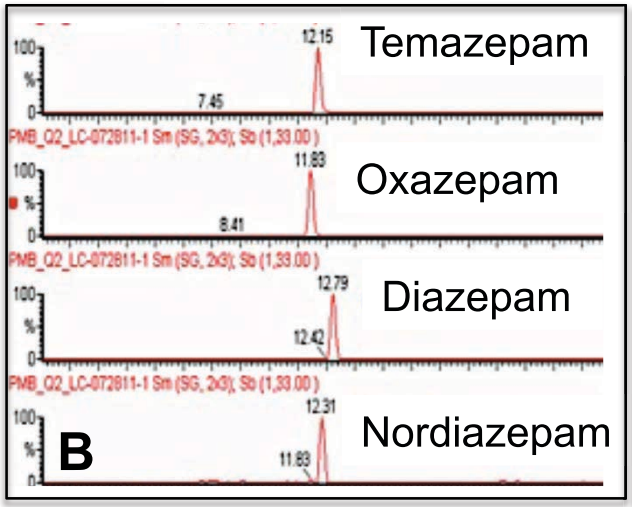
Shay Soker PhD *Wake Forest Institute for Regenerative Medicine*

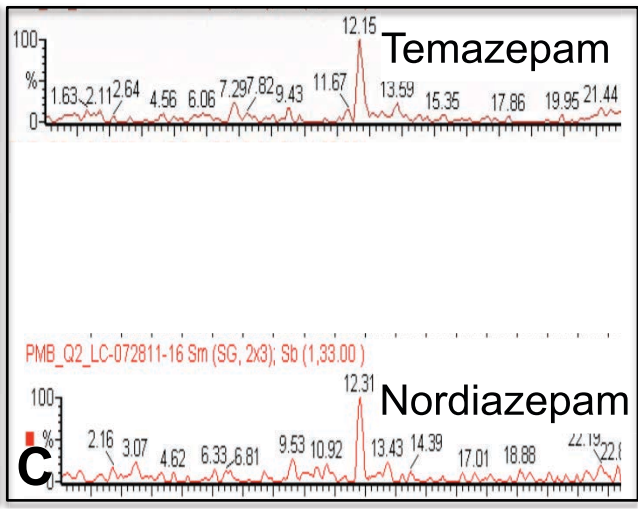


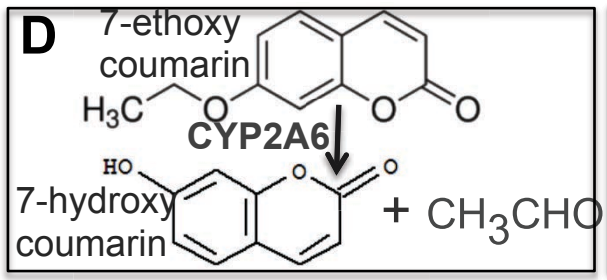


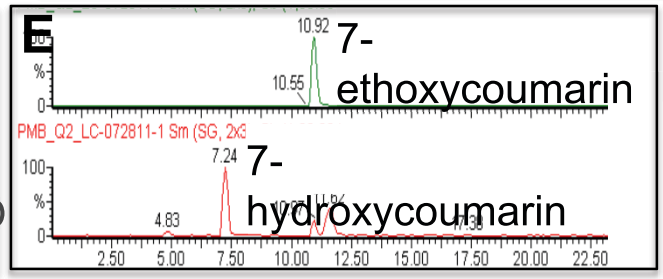
Standards **Liver Discs**

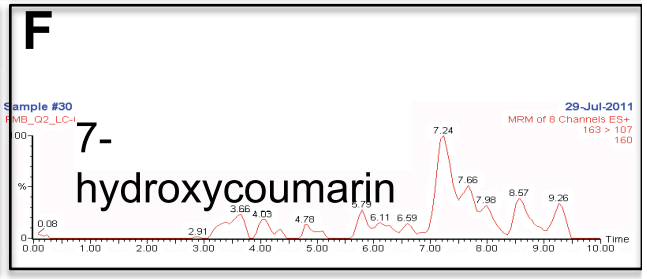








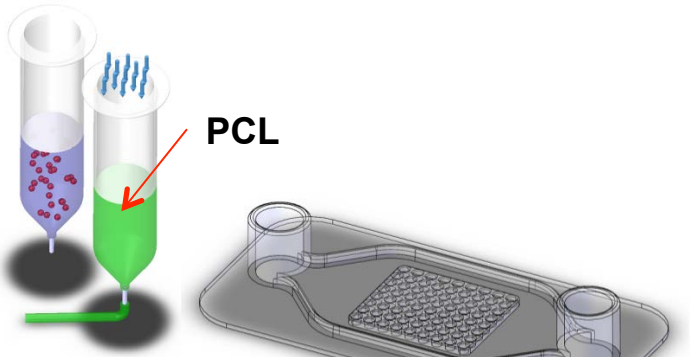




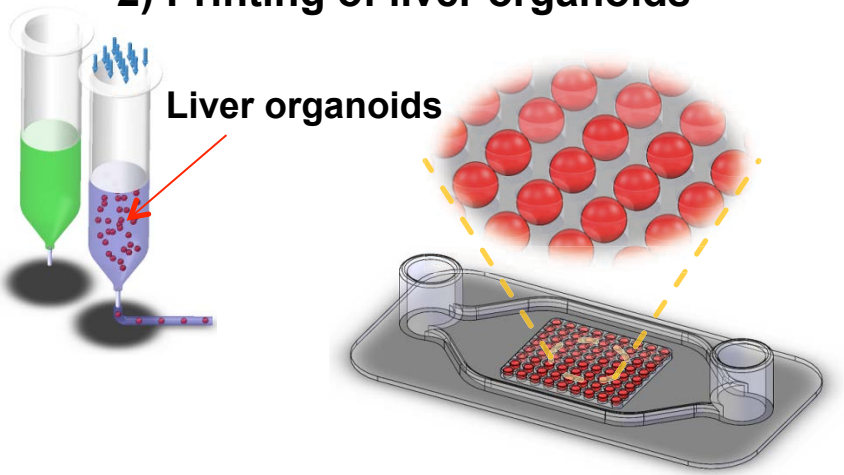
Dipen Vyas, Pedro Baptista

Bioprocessing: Bioprinting of Multi-Organoid Fabrication

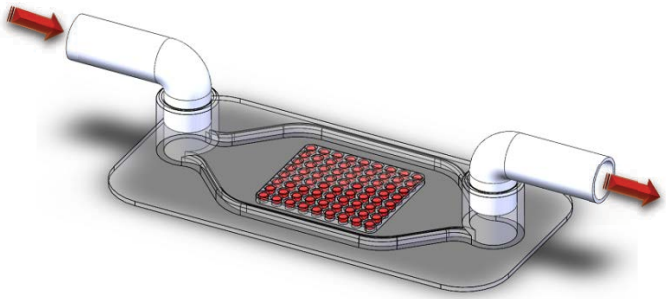
1) Printing of structural material

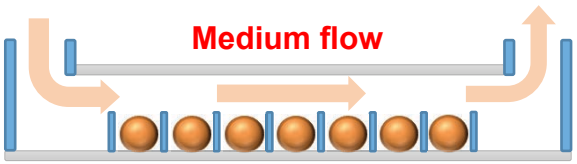


2) Printing of liver organoids

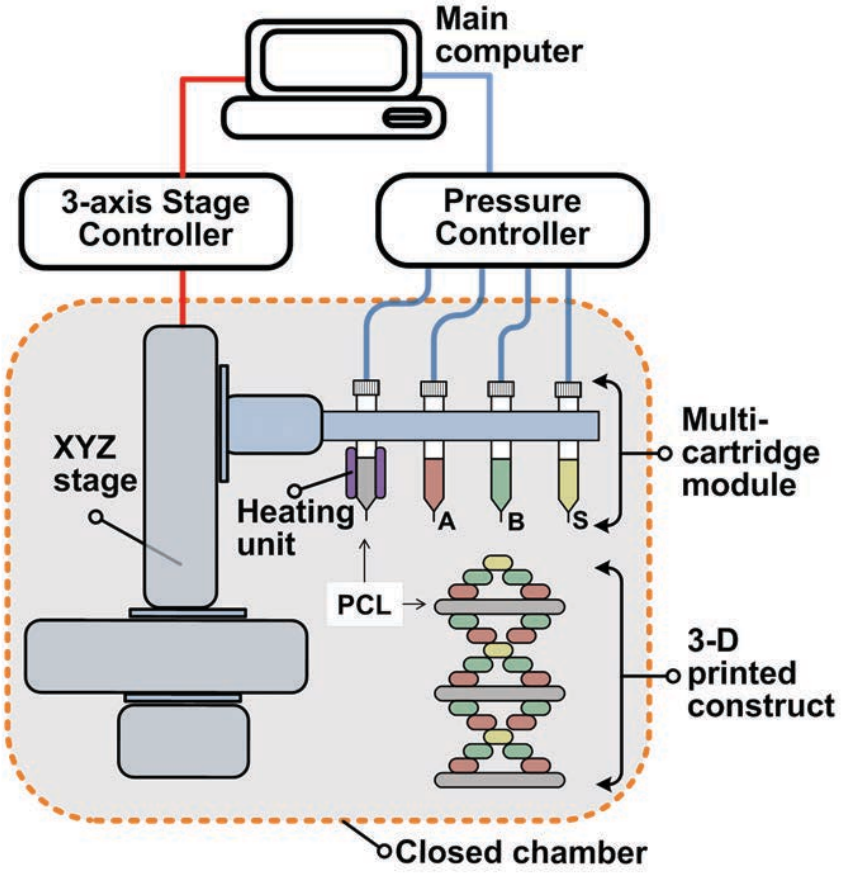


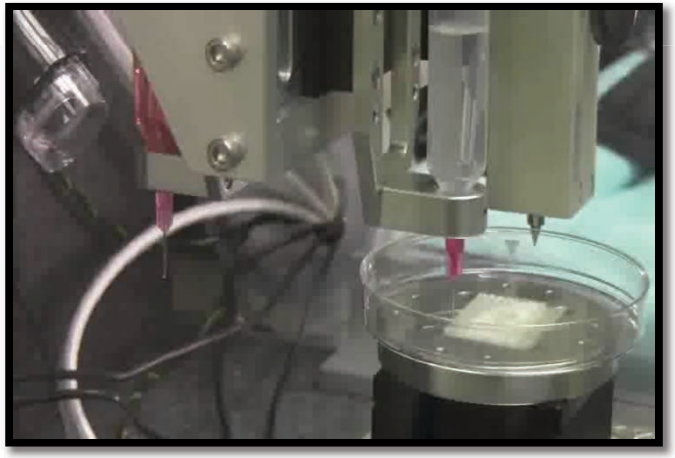
3) Microfluidic device

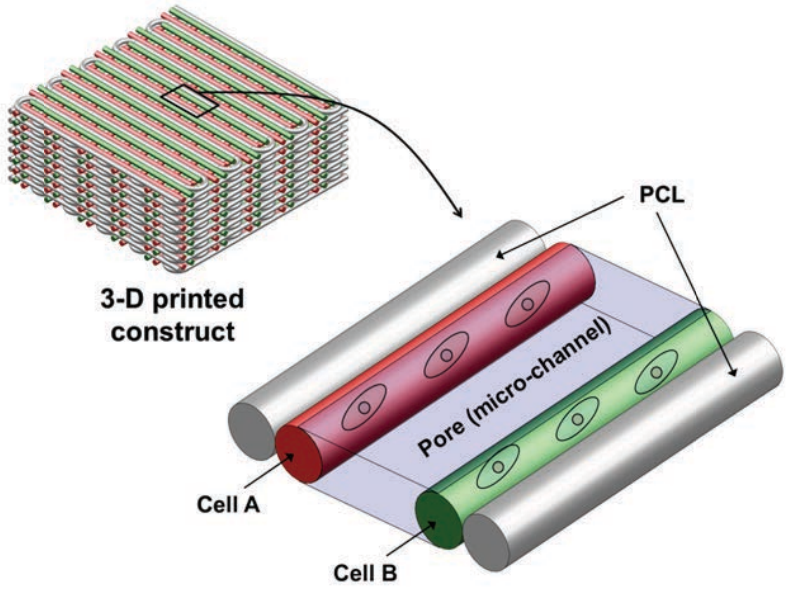




Bioprinting: Working Principle – Cells and Biomaterials
Cells and Biomaterials

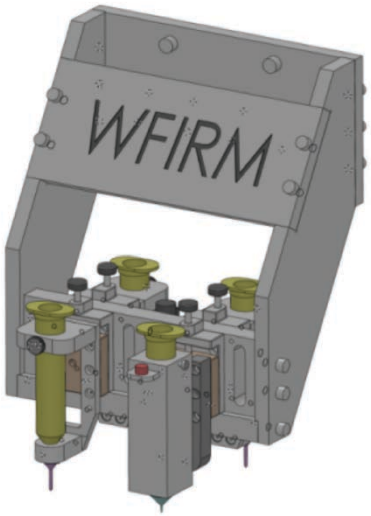






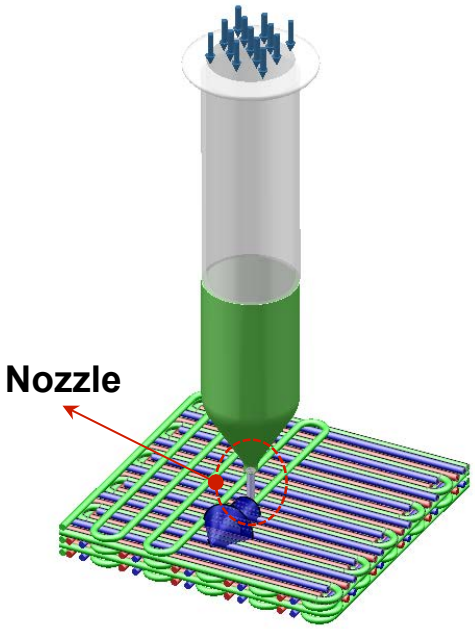
Bioprinting: Resolution

Dispensing Module and Nozzle



Cell Printing

Pneumatic pressure

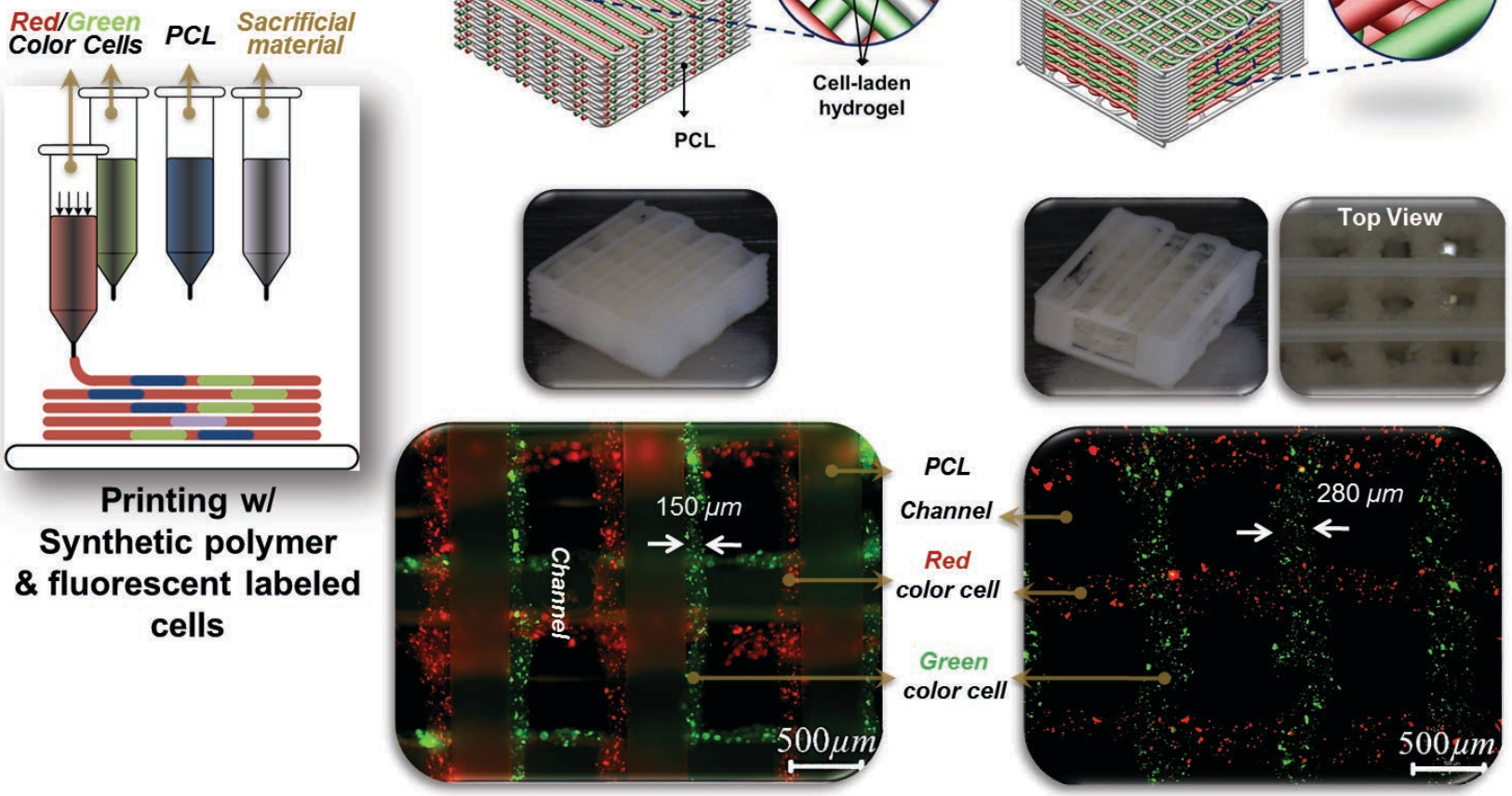


Material Printing

Pneumatic pressure

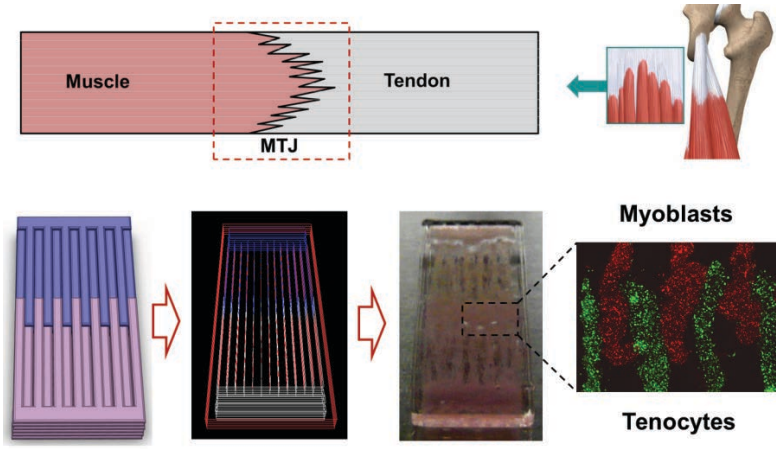
Syringe heater

Bioprinting: 3-D Patterning with Multiple Cell Types

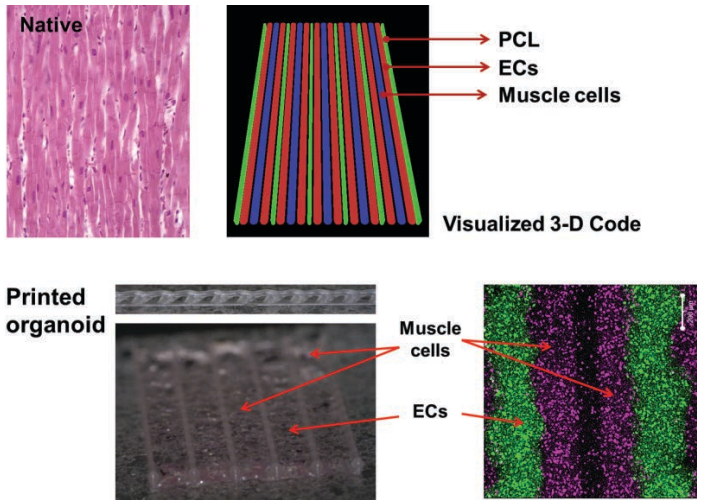


Bioprinting: 3-D Bioprinted Tissue Structures

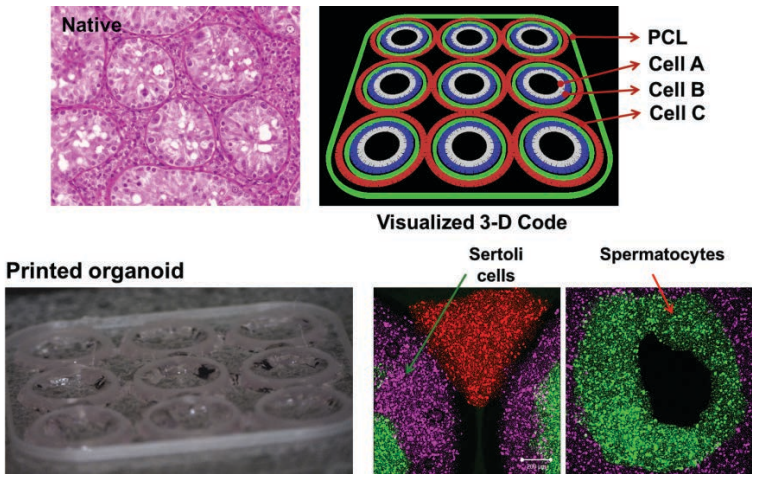
MTJ



Muscle



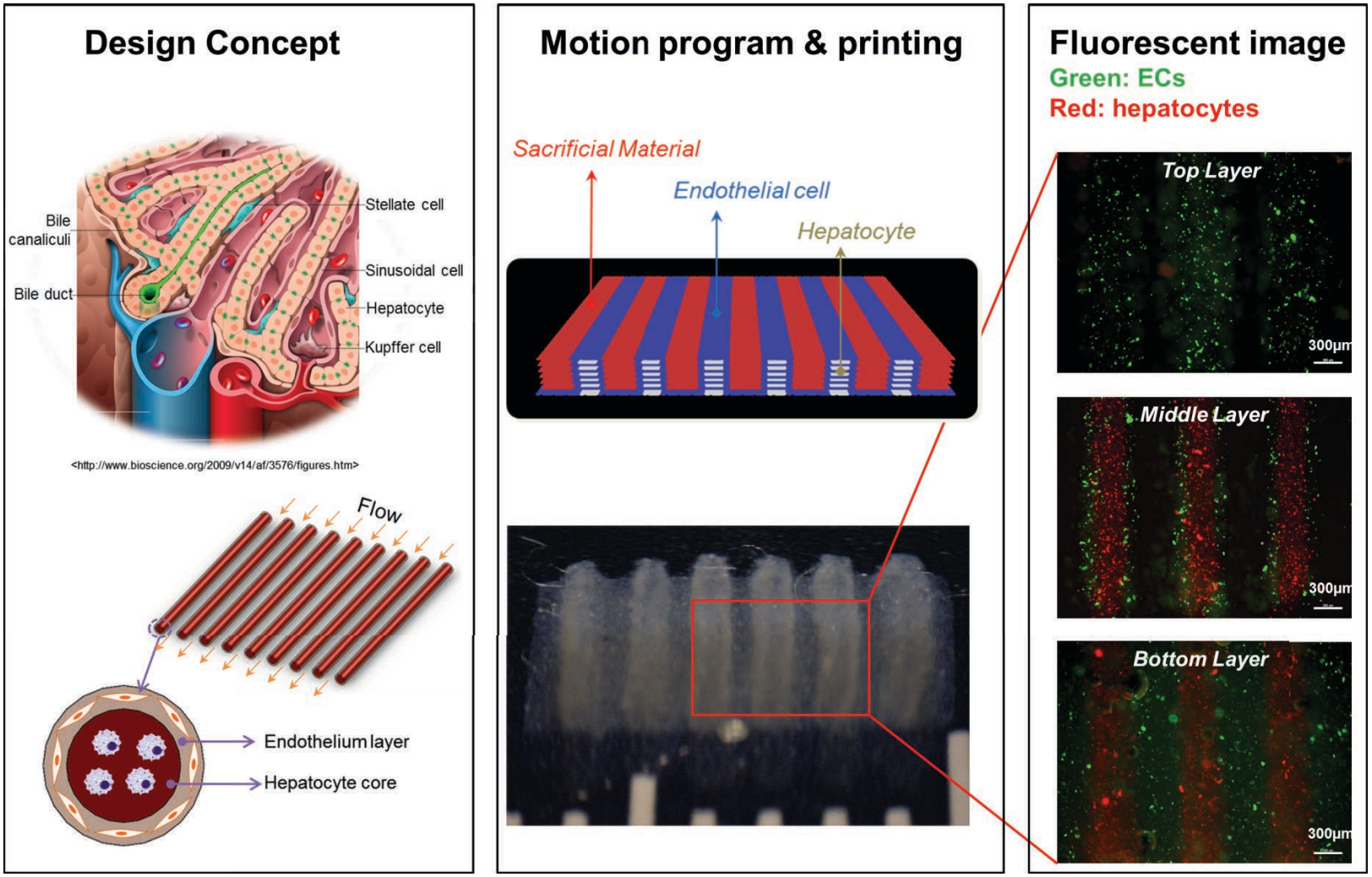
Testis



5="

Bioprinting: 3-D Bioprinted Liver Structure

Rudimentary Architecture Achievable Through Bioprinting



Tissue-Specific Biogel

Biomaterials 33 (2012) 4565–4575

Contents lists available at SciVerse ScienceDirect

Biomaterials

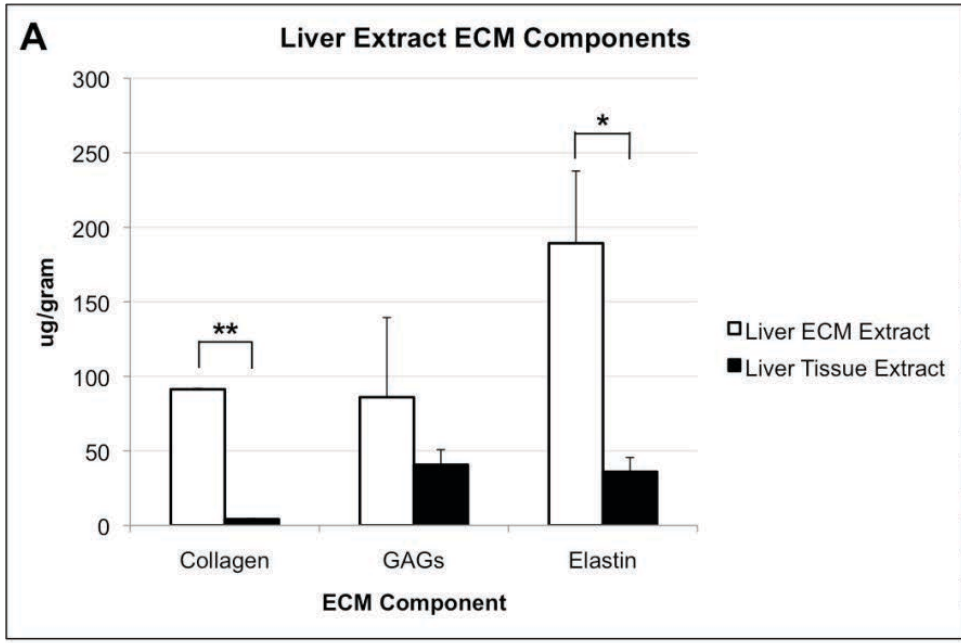
journal homepage: www.elsevier.com/locate/biomaterials

Tissue specific synthetic ECM hydrogels for 3-D *in vitro* maintenance of hepatocyte function

Aleksander Skardal, Leona Smith, Shantaram Bharadwaj, Anthony Atala, Shay Soker*, Yuanyuan Zhang**

Wake Forest Institute for Regenerative Medicine, 391 Technology Way, Winston-Salem, NC 27101, USA

Decellularized liver tissue is frozen, lyophilized and cryo-milled into a powder. The compound is dissolved in HCl with pepsin prior to incorporation into HA-heparin gel (HP).



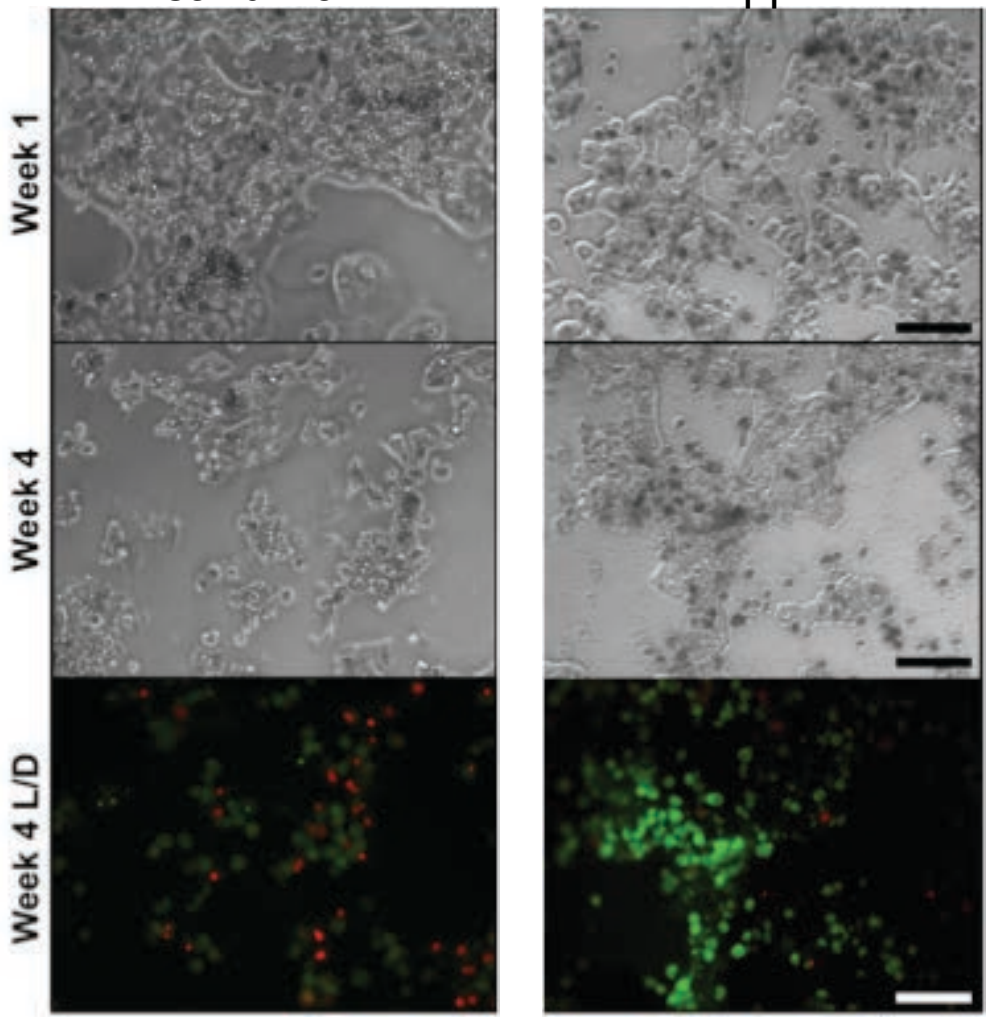
B Growth Factor C

(pg/ml)	LEE
AR	0.00
BDNF	105.56
bFGF	6439.33
BMP-4	0.00
BMP-5	189712.69
BMP-7	309.58
EGF	17.74
EGF R	27.34
FGF-4	10471.61
FGF-7	115.86
GDNF	0.00
GH	100.80
HB-EGF	7.79
HGF	17.45
IGFBP-2	95.45
NT-3	176.74
SCF R	382.08
TGFb1	510.85

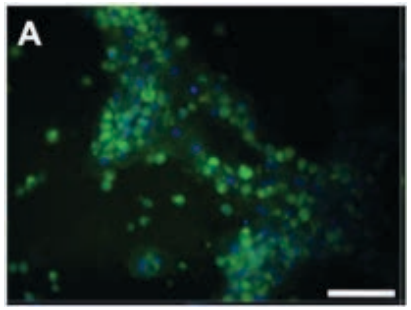
Liver biogel supports hepatcyte culture

Standard collagen I "sandwich"

Extracel-HP with Liver ECM Supplement



Albumin secretion

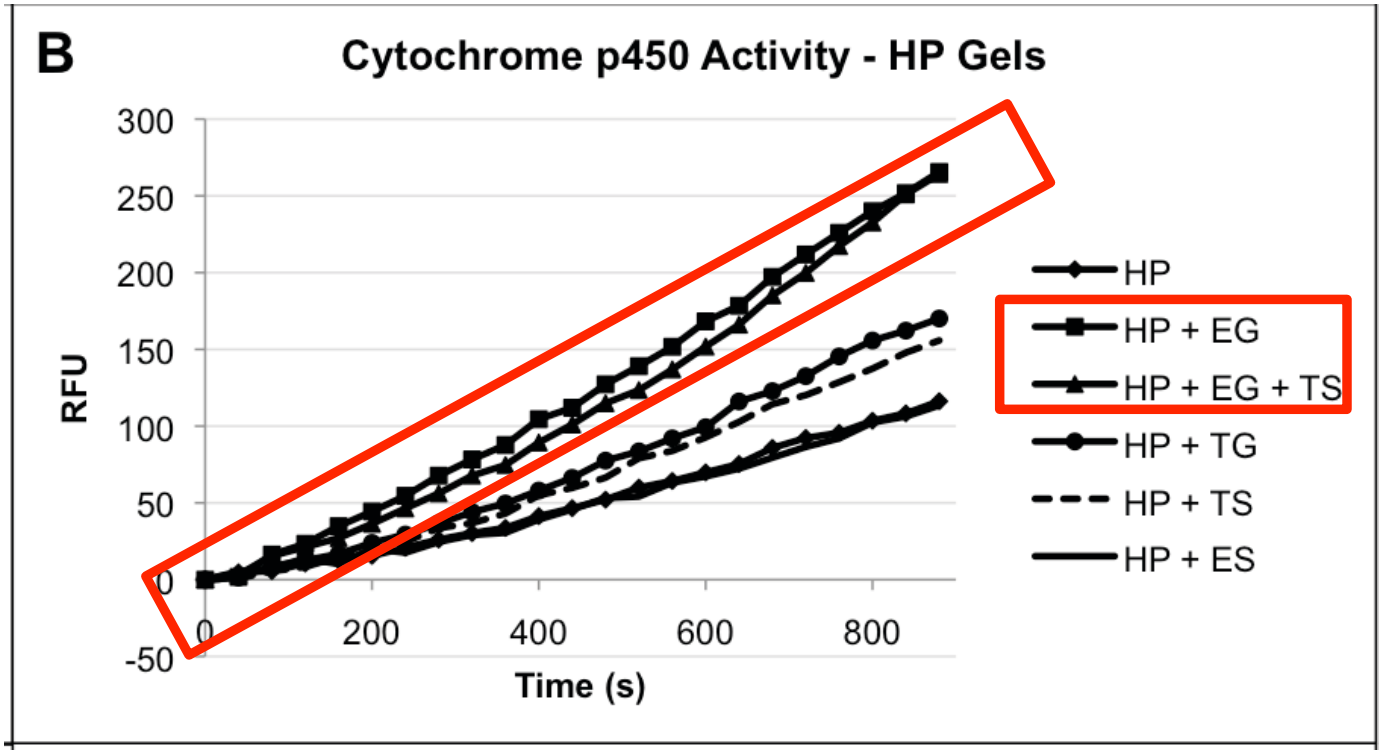


← Cell interconnections

← >3.?"03/@3A3'B"

Liver biogel Improves Phase I Metabolism

Metabolism of 3-cyano-7-ethoxycoumarin results in a fluorescent byproduct and is increased in primary hepatocytes cultured with ECM supplement.



EG = ECM digest
TS = Whole tissue digest

Thank You

Funding

N66001-13-C-2027 DTRA XCEL

DEFENSE THREAT REDUCTION AGENCY

R01CA180149 (Agus, Atala, Soker)
(PQB6) An Integrative Computational and Bioengineered Tissue Model of Metastasis

