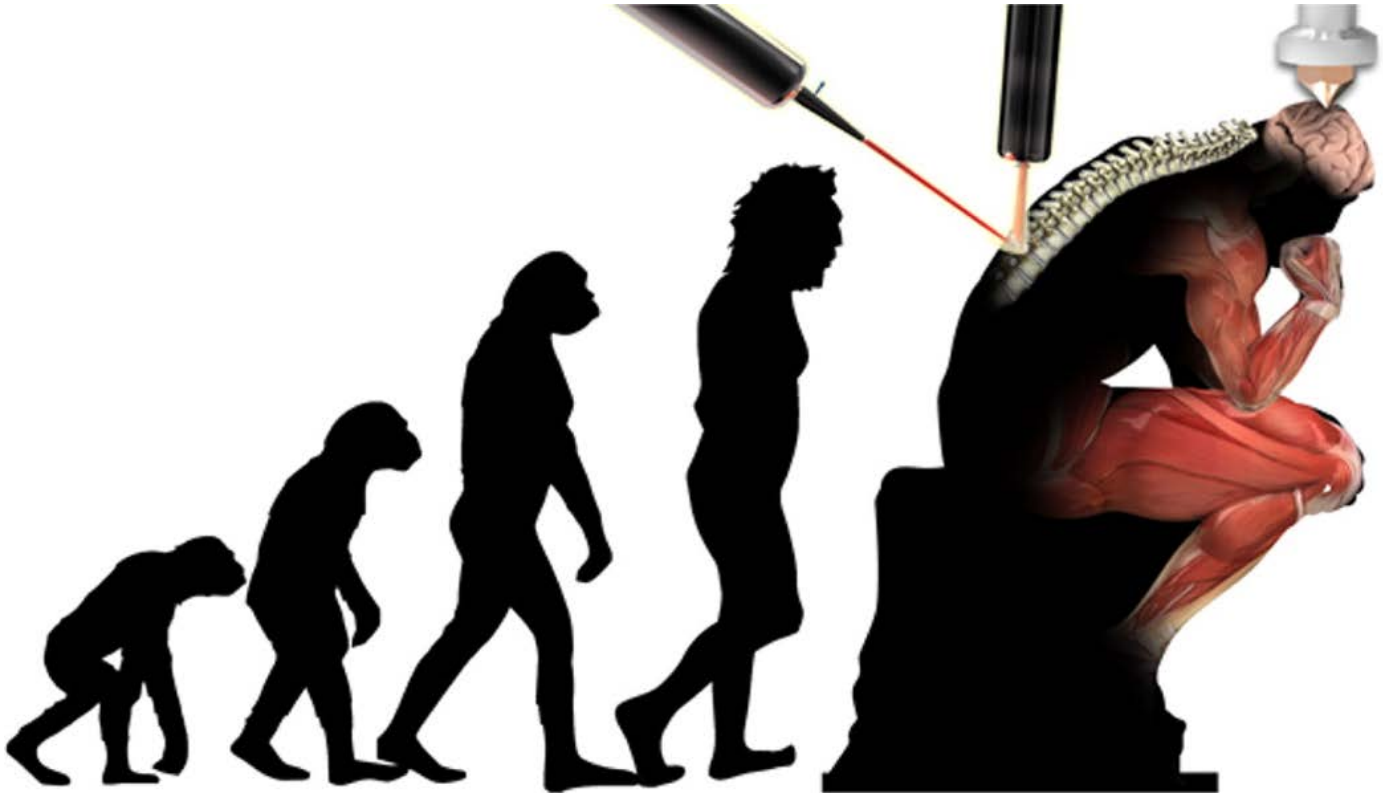
Tissue engineering by Bio-printing:

Current state of the technology:



Vijay Chandrasekar
Special series Journal club
7.2.2017

Vacanti Mouse (1997)

3D Bio-printing for tissue/organ regeneration/replacement:

The ability to print various biological materials/cells for the free-form fabrication of complex living architectures along with various tissue scaffold materials for the design and engineering of human organs and tissues.

Replacement of injured/diseased organs

Biomedical Tissue engineering

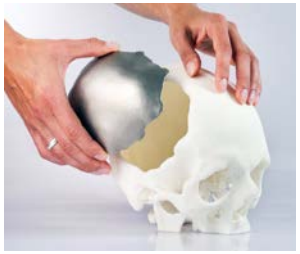
3D printing

Different approaches

Cell free 3D printed scaffold

for endogenous cells & tissue to regenerate

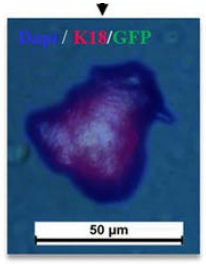
-Growth factor additives to promote the process



3D bioprinting of Cells along with biosynthetic scaffold inks

Either patient specific somatic cells

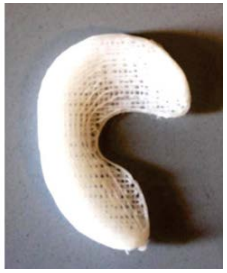
Or Patient specific stem cells, Adult or iPS derived specialized cells

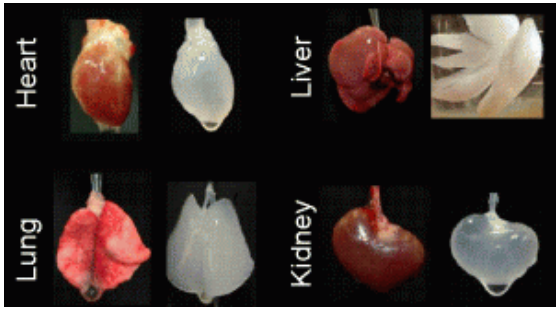


Decellularized tissue matrix

Cells seeded onto Decellularized tissue matrix (Heart, kidney, baldder, liver, etc.,

Patient specific somatic cells, ex., Fibroblasts
or Patient specific stem cells, resident Adult or iPS derived cells





Article

Chimeric organs

Interspecies Chimerism with Mammalian Pluripotent Stem Cells

Jun Wu,[1] Aida Platero-Luengo,[1] Masahiro Sakurai,[1] Atsushi Sugawara,[1] Maria Antonia Gil,[2] Taka
Keiichiro Suzuki,[1] Yanina Soledad Bogliotti,[3] Cristina Cuello,[2] Mariana Morales Valencia,[1] Daiji C
Jingping Luo,[1] Marcela Vilariño,[3] Inmaculada Parrilla,[2] Delia Alba Soto,[3] Cristina A. Martinez,[2] To
Sonia Sánchez-Bautista,[4] M. Llanos Martinez-Martinez,[4] Huili Wang,[3] Alicia Nohalez,[2] Emi Aizaw
Paloma Martinez-Redondo,[1] Alejandro Ocampo,[1] Pradeep Reddy,[1] Jordi Roca,[2] Elizabeth A. Ma
Concepcion Rodriguez Esteban,[1] W. Travis Berggren,[1] Estrella Nuñez Delicado,[4] Jeronimo Lajar
Pedro Guillen,[4,5] Josep M. Campistol,[6] Emilio A. Martinez,[2] Pablo Juan Ross,[3] and Juan Carlos
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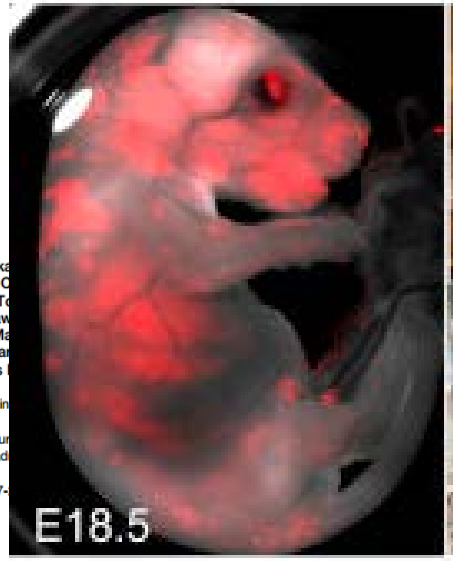
http://dx.doi.org/10.1016/j.cell.2016.12.036

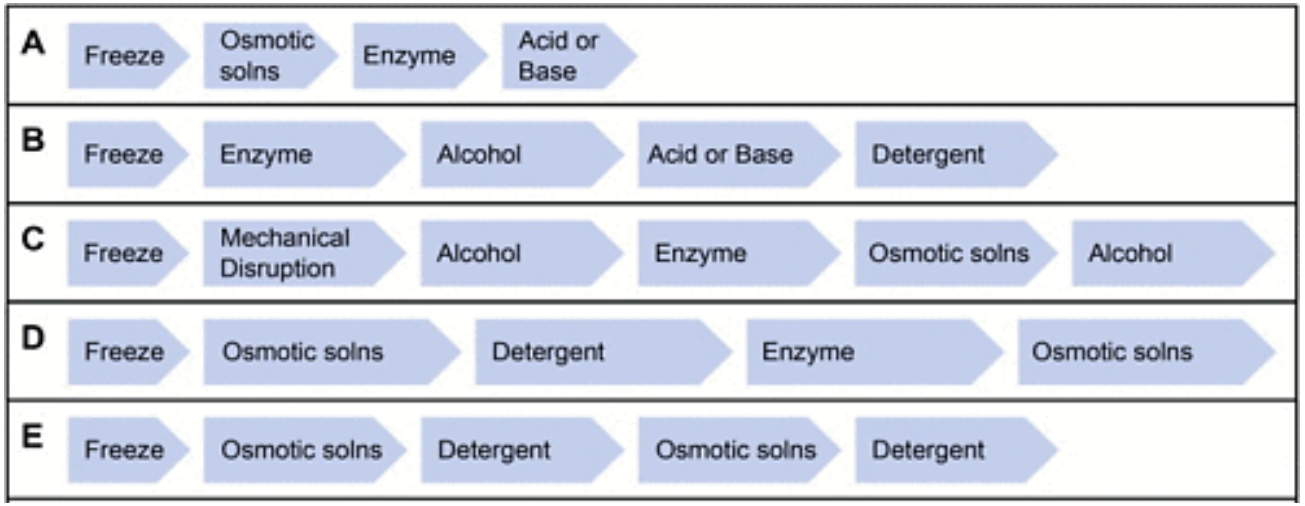


Decellularized tissue matrix

Process to isolate the extracellular matrix (ECM) of a tissue from its inhabiting cells, leaving an ECM scaffold of the original tissue intact, which can be used in artificial organ and tissue regeneration.

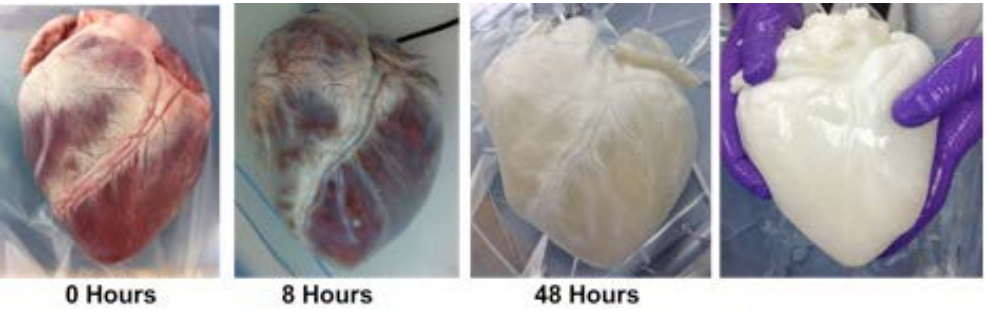
Stephen F. Badylak (University of Pittsburgh) pioneered the process of decellularization.

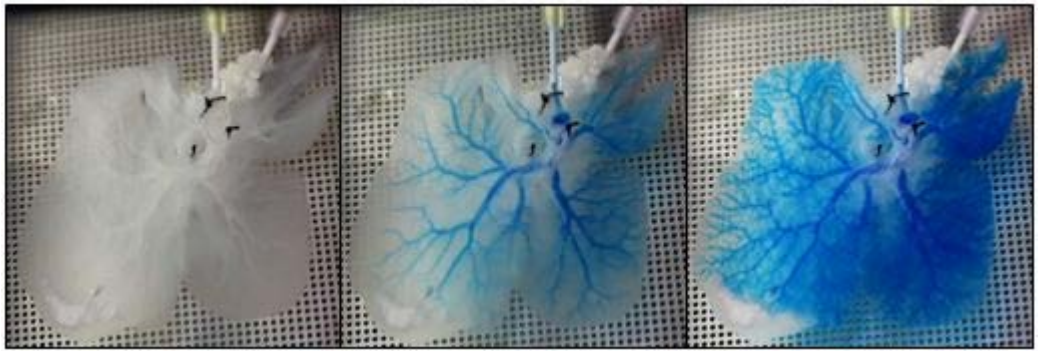
Recellularizing an ECM scaffold with a patient's own cells, the adverse immune response is eliminated



(A) thin laminate tissues, (B) thicker laminate tissues, (C) adipose tissues, (D) whole simple organs, (E) essential and complex organs (Crapo et al., 2011)

Decellularization of tissues and organs **Thomas et al., 2006**

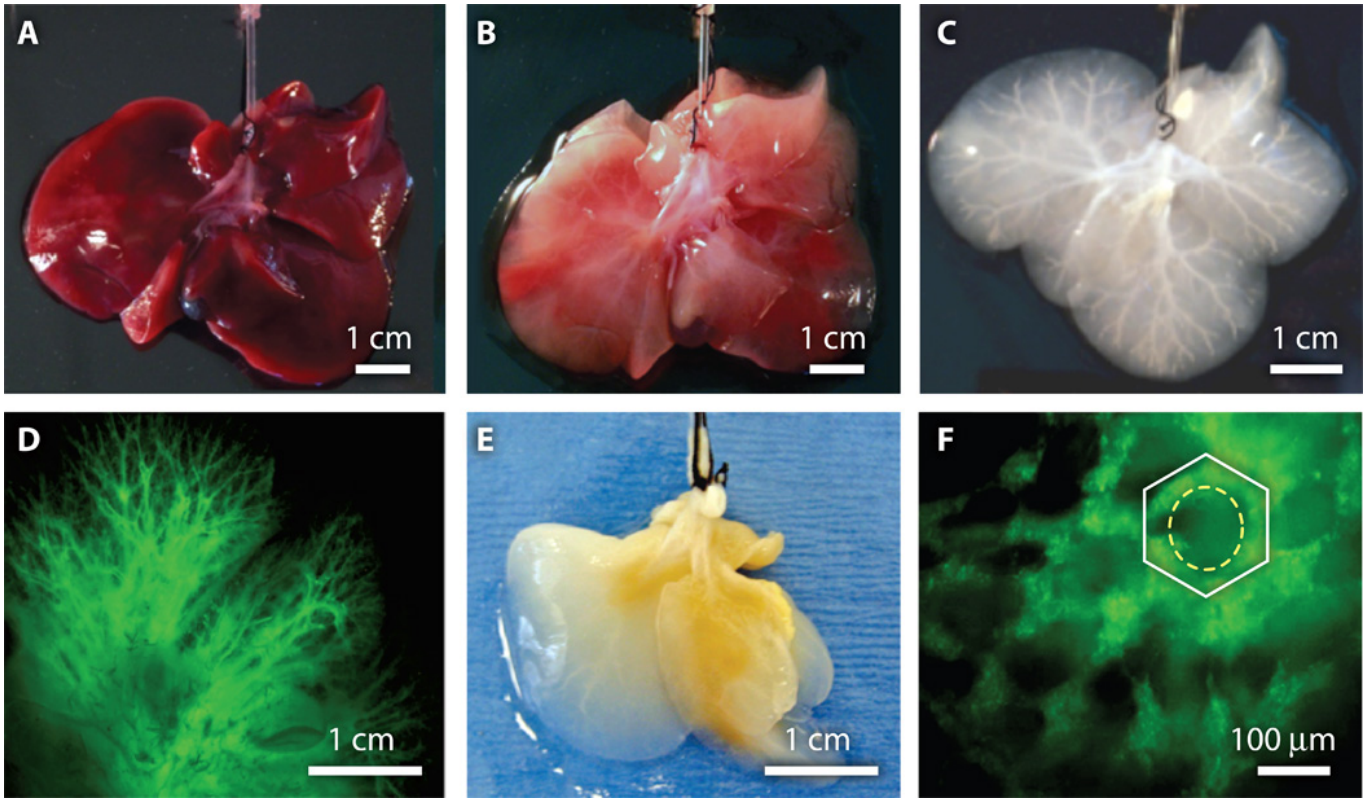




Snapshots of blue dye flowing through the vasculature of a three-dimensional rat liver ECM scaffold.

Engineering liver tissue with decellularized donor organs

The cells are reseeded both in the vasculature and in the parenchyma leading to adequate tissue architecture and function



Ferret liver scaffold vascular tree infusion with human endothelial cells and parenchymal infiltration with liver progenitor cells

Anthony Atala et al., Sci Transl Med 2012;4:160rv12

nature COMMUNICATIONS

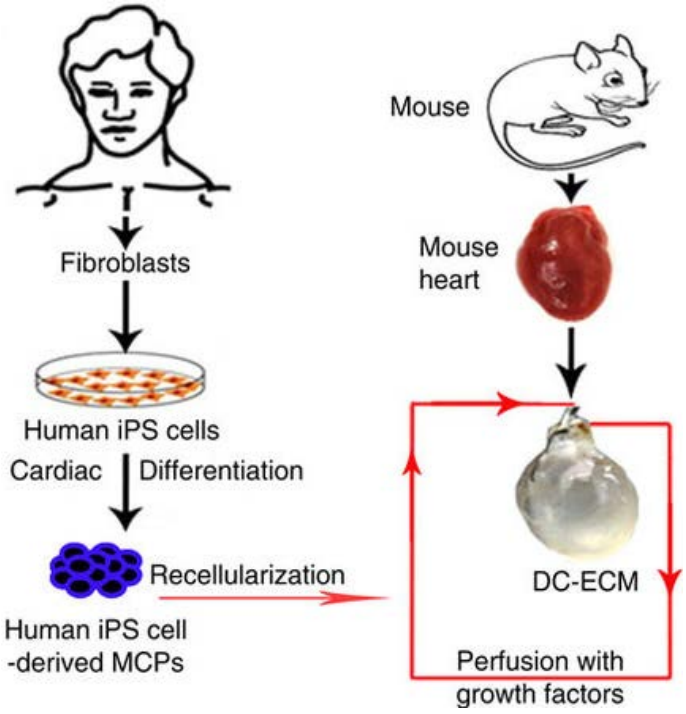
ARTICLE

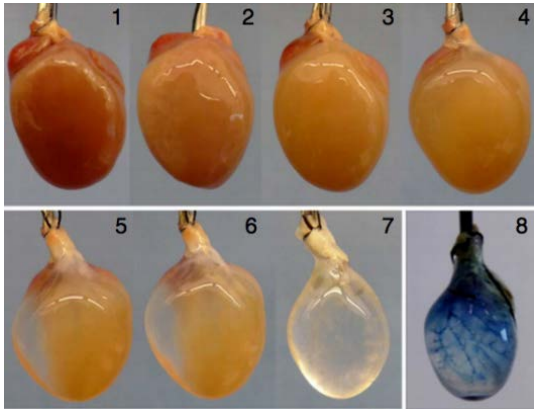
Received 18 Mar 2013 | Accepted 15 Jul 2013 | Published 13 Aug 2013

DOI: 10.1038/ncomms3307

Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells

Tung-Ying Lu[1,*], Bo Lin[1,*], Jong Kim[2], Mara Sullivan[3], Kimimasa Tobita[1], Guy Salama[2] & Lei Yang[1]





iPS-MCPs are introduced to a decellularized mouse heart sitting in a Petri dish, the cells latch onto the heart scaffold.

Cardiomyocytes, Smooth muscle cells, Endothelial cells

After 20 days the heart starts beating again at 40 to 50 beats per minute.

https://www.youtube.com/watch?v=IxzL5nFOZFw

Bioprinting may ultimately be able to fabricate solid organs, including the vascular network and functional parenchyma components

Short History:

In 2000, Dr. Thomas Boland used a modified regular inkjet printer to print cells into a petri dish.

In early 2000, Urinary baldder augmentation from 3D printed bladder scaffold (cell seeded) for transplantation in patients

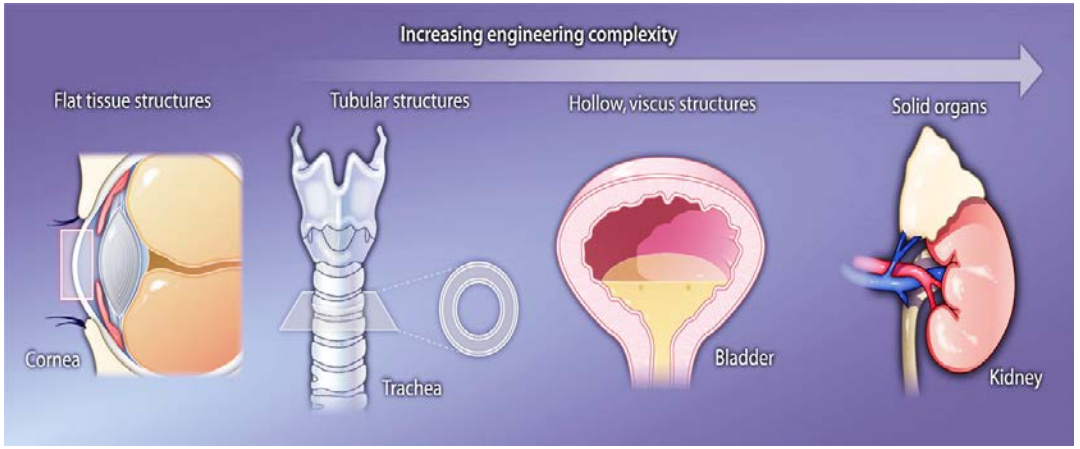
In 2002, a miniature human kidney was created at Wake Forest Institute for Regenerative Medicine

In 2008, Australian automation specialist Invetech developed the first 3D printer capable of making human organs and tissues, specifically for its partner Organovo.

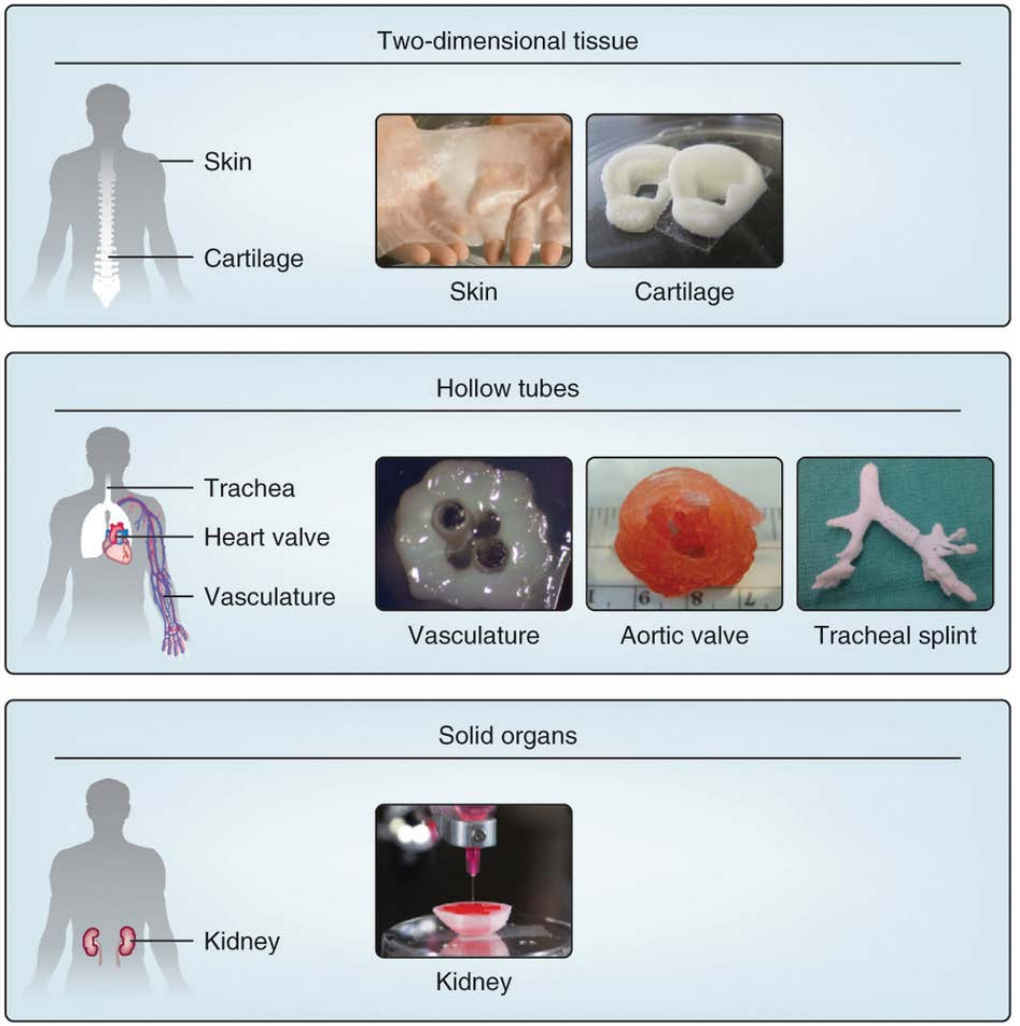
3D Bio-printing for tissue/organ regeneration/replacement:

The ability to print various biological materials and cells along with various tissue scaffold materials

Four structural levels of complex tissues and organs.

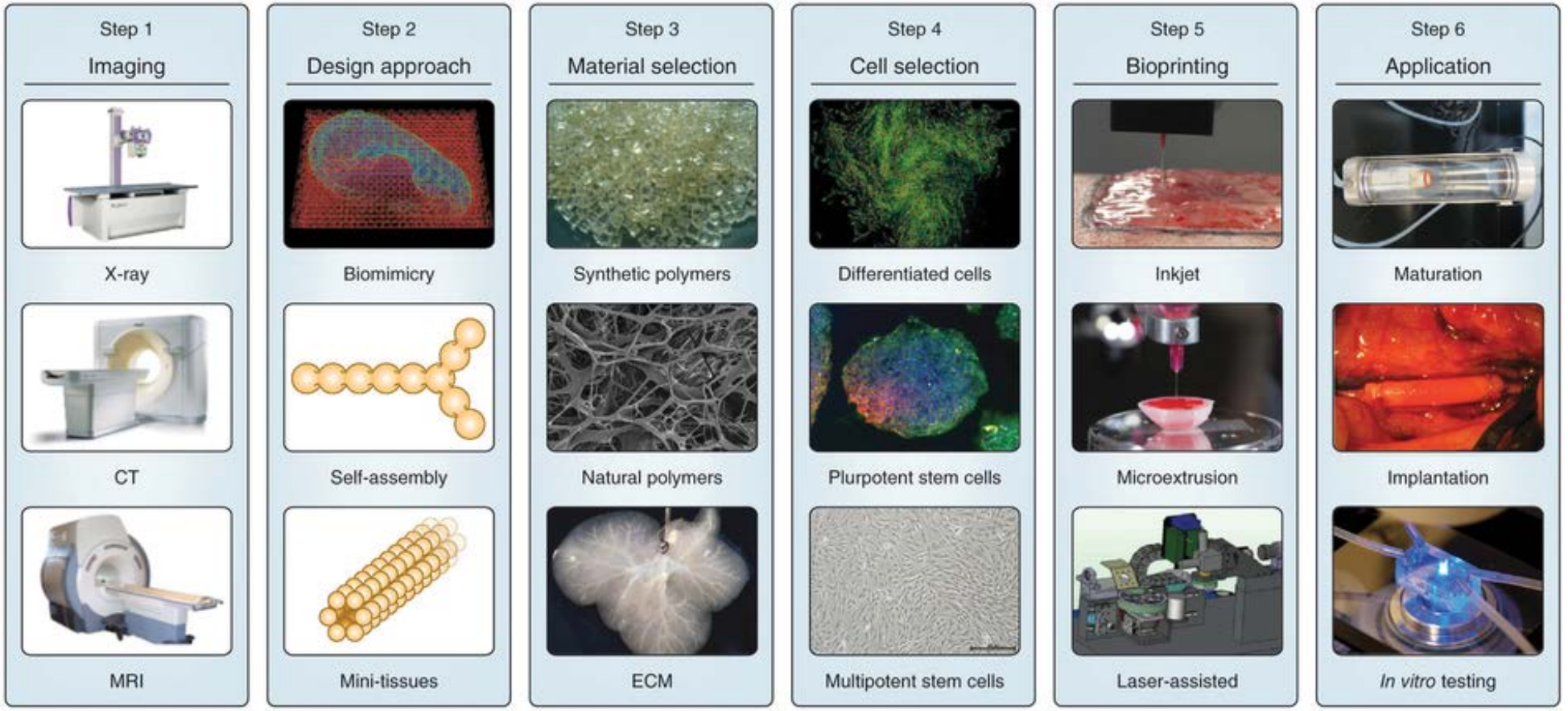


Anthony Atala et al., Sci Transl Med 2012;4:160rv12



Wake Forest Institute for Regenerative Medicine

Schematic of different stages of the 3D Bioprinting



Imaging of the damaged tissue and its environment can be used to guide the design of bioprinted tissues.

3D bioprinting of tissues and organs

Sean V Murphy & Anthony Atala
Nature Biotechnology 32, 773–785 (2014) doi:10.1038/nbt.2958

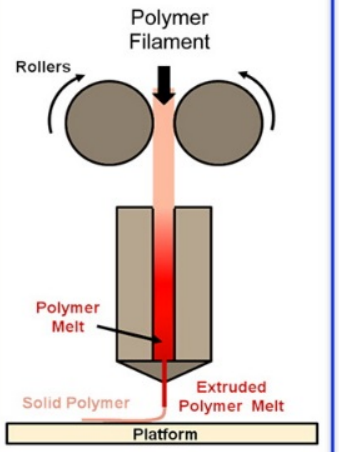
Schematics depicting 3D printers and Printing techniques

Extrusion-based methods

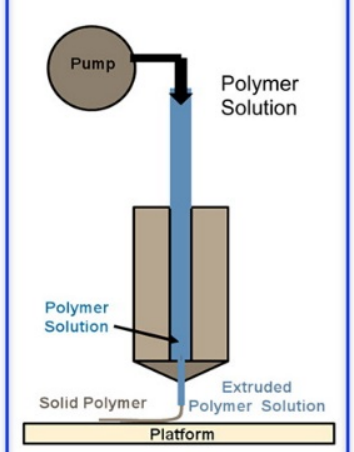
particle fusion-based method

light-based method

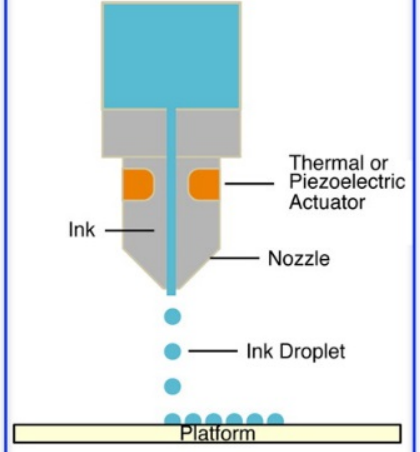
Fused Deposition Modeling (FDM)



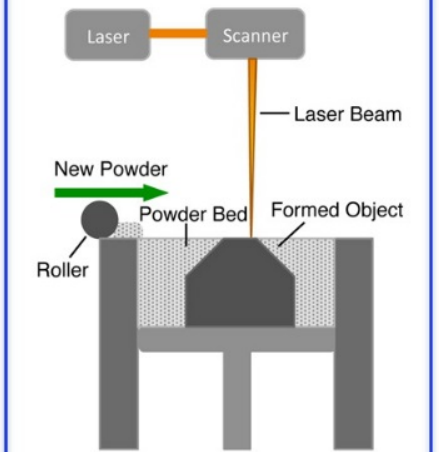
Direct Ink Writing (DIW)



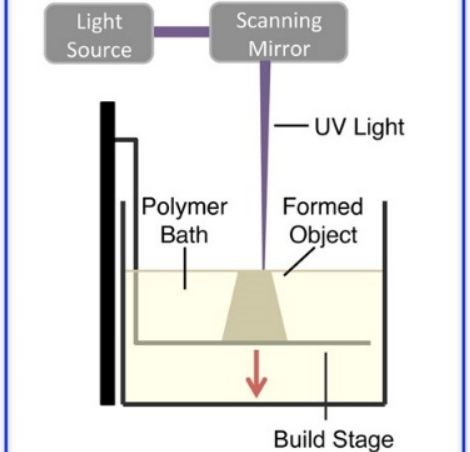
Inkjet Printing



Selective Laser Sintering (SLS)



Stereolithography (SLA)



Published in: Murat Guvendiren; Joseph Molde; Rosane M.D. Soares; Joachim Kohn; *ACS Biomater. Sci. Eng.* 2016, 2, 1679-1693.
DOI: 10.1021/acsbiomaterials.6b00121

Table 1: Comparison of bioprinter types

	Bioprinter type		
	Inkjet	**Microextrusion**	**Laser assisted**
Material viscosities	3.5–12 mPa/s	30 mPa/s to >6 × 10^7 mPa/s	1–300 mPa/s
Gelation methods	Chemical, photo-crosslinking	Chemical, photo-crosslinking, sheer thinning, temperature	Chemical, photo-crosslinking
Preparation time	Low	Low to medium	Medium to high
Print speed	Fast (1–10,000 droplets per second)	Slow (10–50 μm/s)	Medium-fast (200–1,600 mm/s)
Resolution or droplet size	<1 pl to >300 pl droplets, 50 μm wide	5 μm to millimeters wide	Microscale resolution
Cell viability	>85%	40–80%	>95%
Cell densities	Low, <10^6 cells/ml	High, cell spheroids	Medium, 10^8 cells/ml
Printer cost	Low	Medium	High

3D bioprinting of tissues and organs

Sean V Murphy & Anthony Atala
Nature Biotechnology 32, 773–785 (2014) doi:10.1038/nbt.2958

Choice of the scaffold/Bioink:

Based on several desired features

Printability

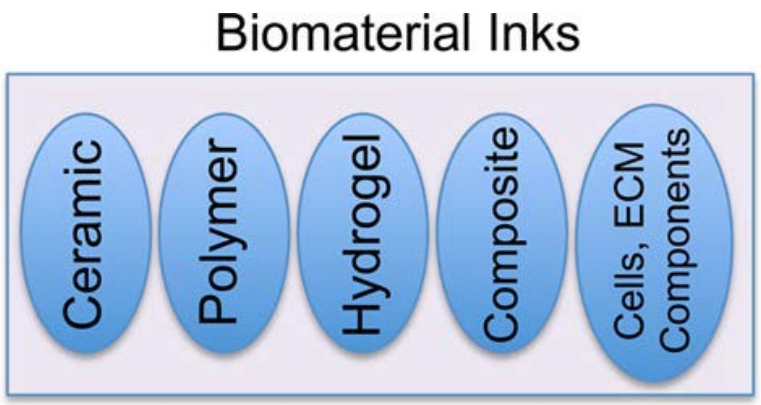
Bio-compatibility

Degradation Kinetics and byproducts

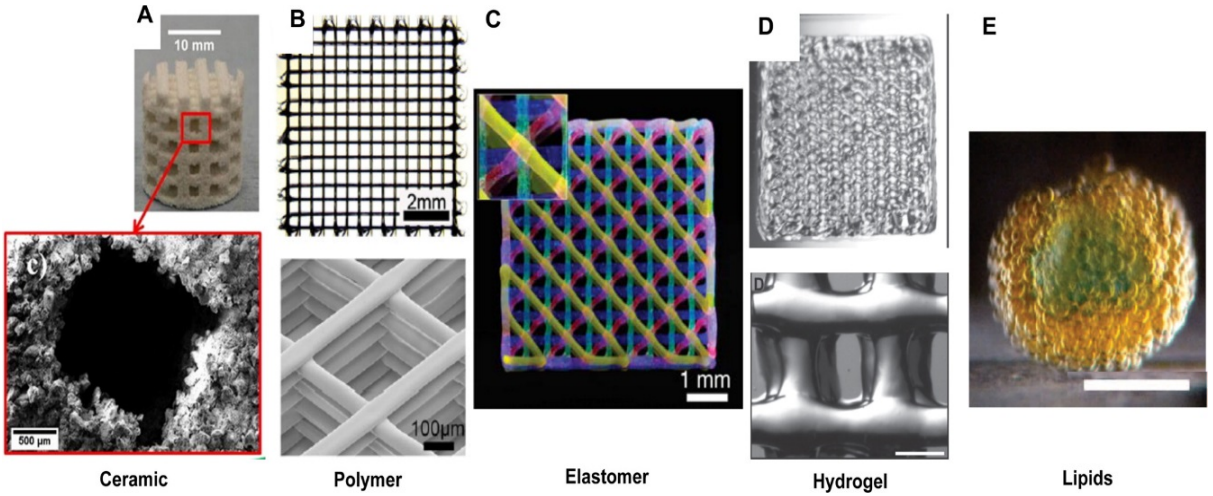
Structural and mechanical properties

Material biomimicry

Choice of the scaffold/Bioink:



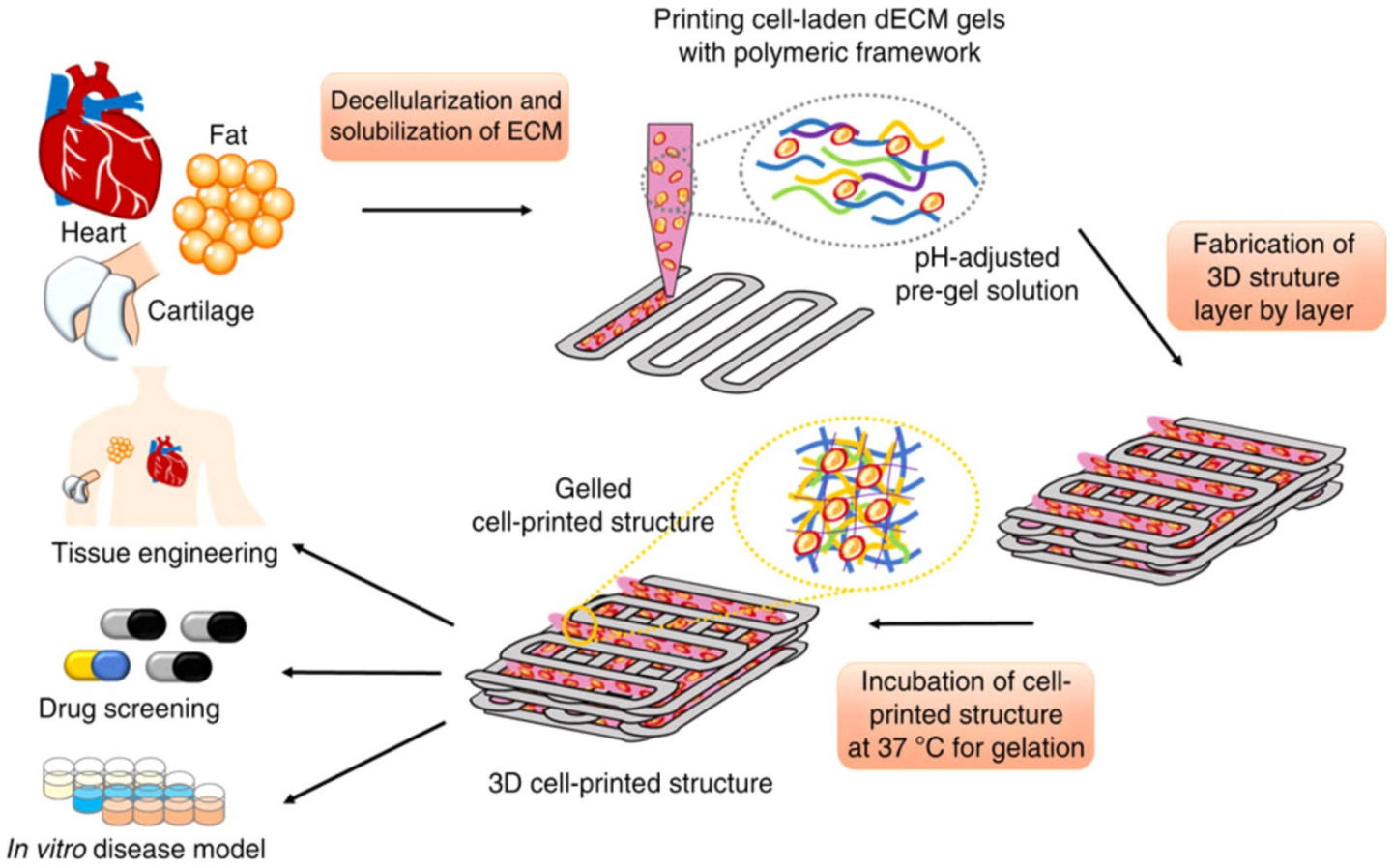
3D printed constructs



(A) hydroxylapatite (HA) scaffold, (bottom)
(B) Polycaprolactone(PCL) scaffold (solvent-cast three-dimensional printing of multifunctional microsystems.
(C) Fluorescent image of 4-layer lattice printed by sequential depositing of four PDMS inks each dyed with a different fluorophore.
(D) PEG-based hydrogel with gelatin (15 mm × 15 mm), bottom structure. 500 µ
(E) Picture of a hollow sphere-shaped lipid droplet network (printed in bulk aqueous solution). 200 mm

Murat Guvendiren; Joseph Molde; Rosane M.D. Soares; Joachim Kohn; *ACS Biomater. Sci. Eng.* 2016, 2, 1679-1693.
DOI: 10.1021/acsbiomaterials.6b00121

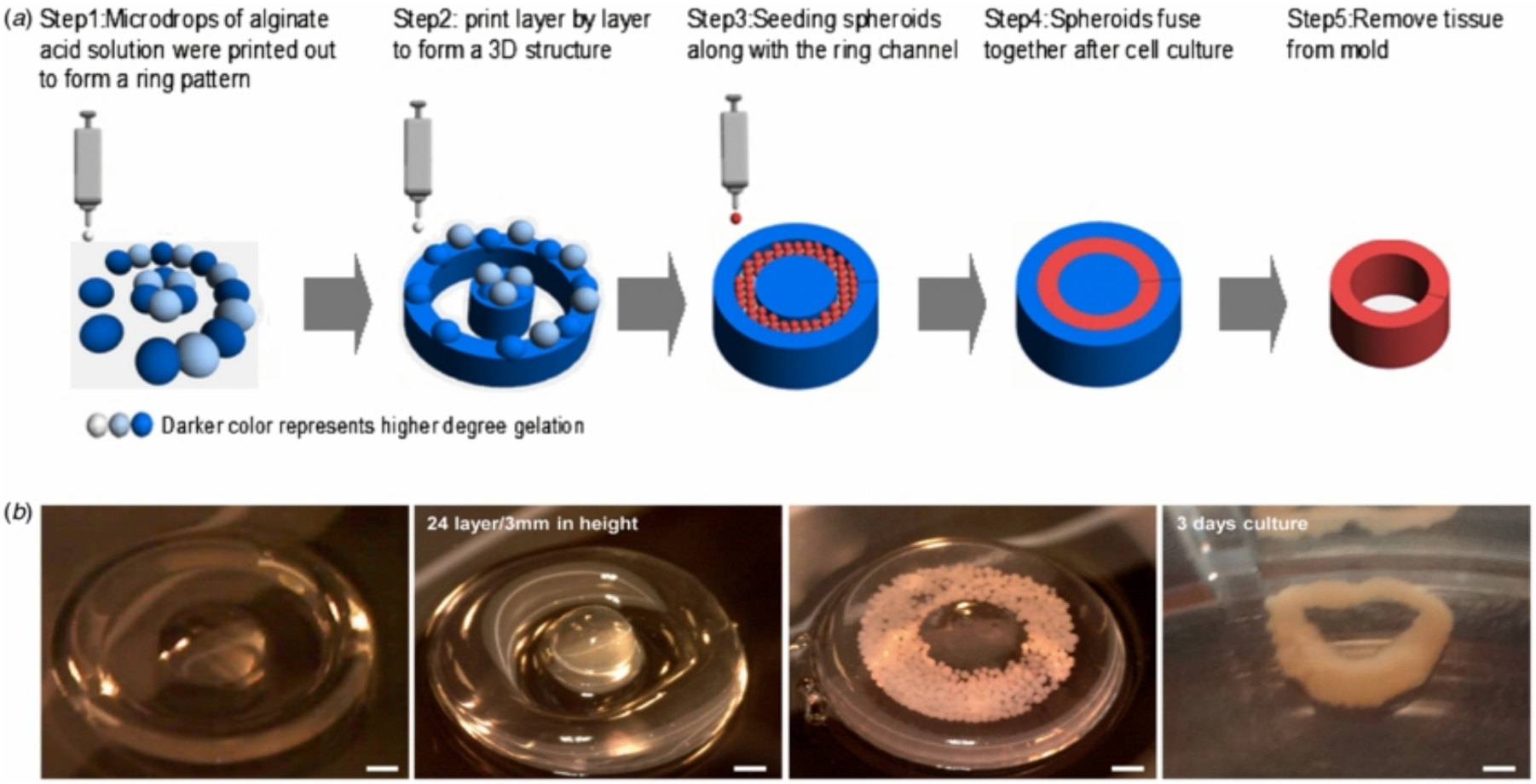
Fabrication of 3D constructs from ECM based bioinks



Bioinks were developed from decellularized tissues after harvesting. Cell-laden ECM bioinks were printed in combination with polymeric framework.

Published in: Murat Guvendiren; Joseph Molde; Rosane M.D. Soares; Joachim Kohn; *ACS Biomater. Sci. Eng.* 2016, 2, 1679-1693.
DOI: 10.1021/acsbiomaterials.6b00121

3D printing facilitated scaffold-free tissue unit fabrication
Yu Tan et al 2014 Biofabrication 6 024111 doi:10.1088/1758-5082/6/2/024111



Schematic presentation (a) and actual product (b) of 3D alginate hydrogel printing for tissue unit fabrication using vascular spheroids (i.e., containing smooth muscle cells and endothelial cells (50% hSMC media and 50% HUVEC)). Scale bar is 1 mm.

3D Bio-printing

Practical difficulties

(i) Identifying the type of cells that, establish itself and grow in the microenvironment; will not be rejected by the host's immune system

(ii) Organising the specialized cells into the 3D tissue architecture using suitable scaffolds

(iii) Keeping the 3D assembled cells viable and functional

(iv) Making them in Clinical grade and GMP compliant for transplantations

Cell free 3D printed scaffold (seeded with Bio-active molecules)

RESEARCH ARTICLE

TISSUE ENGINEERING

Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep

Chang H. Lee,[1] Scott A. Rodeo,[2] Lisa Ann Fortier,[3] Chuanyong Lu,[1] Cevat Erisken,[1] Jeremy J. Mao[1]*

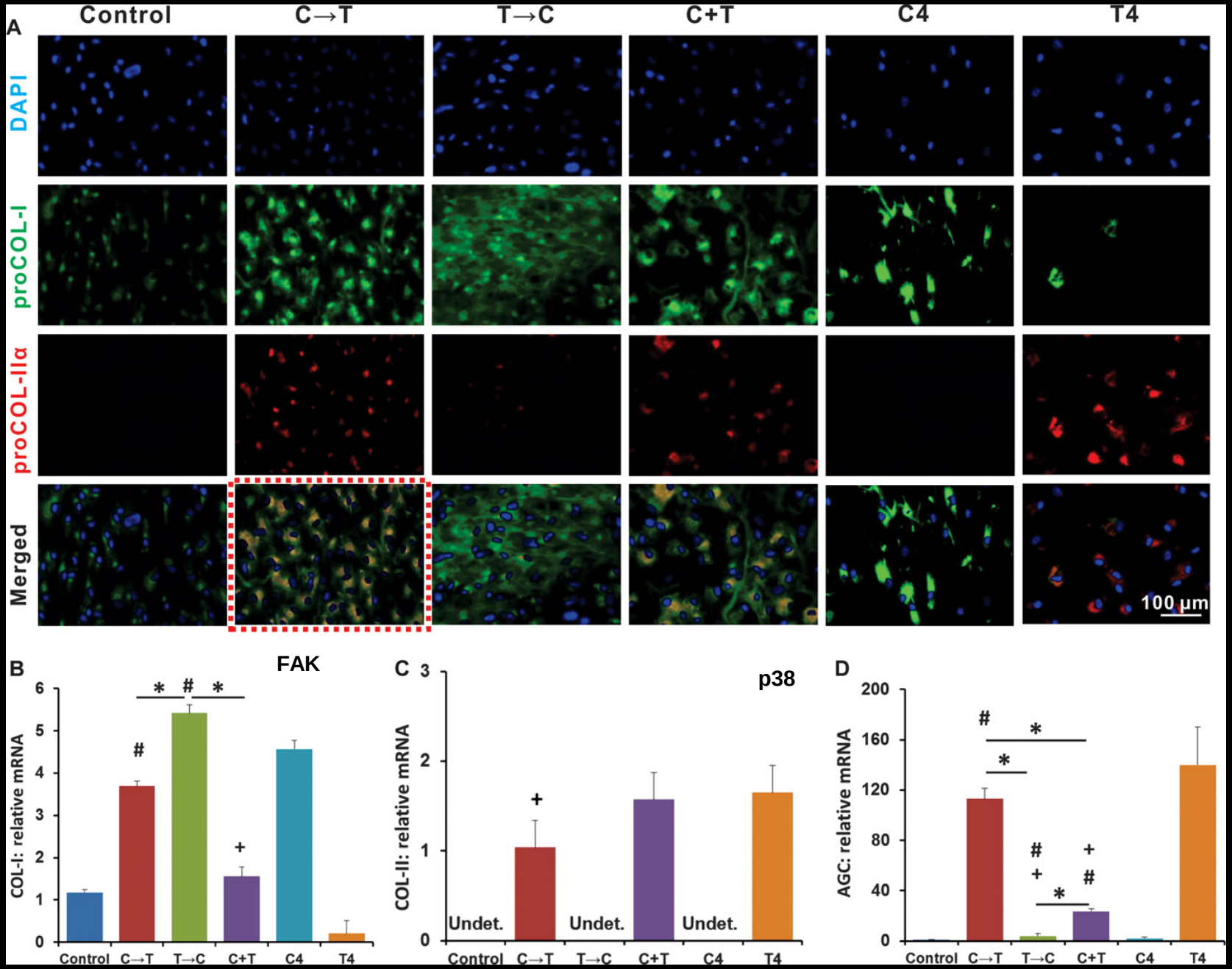
Meniscus in the knee joint is a crescent-shaped connective tissue between the distal femoral and proximal tibial condyles that provides structural congruence and absorbs mechanical forces, without which the patient is likely to develop arthritis

Meniscus replacement with allografts

Chang H. Lee et al., Sci Transl Med 2014;6:266ra171

Science Translational Medicine AAAS

Sequential rhCTGF and rhTGFβ3 treatment of bone marrow MSCs induces fibrochondrocyte differentiation.



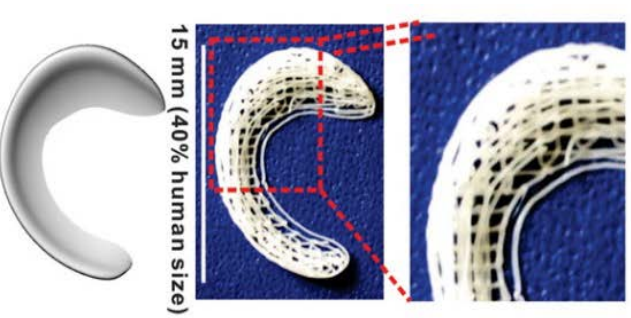
Chang H. Lee et al., Sci Transl Med 2014;6:266ra171

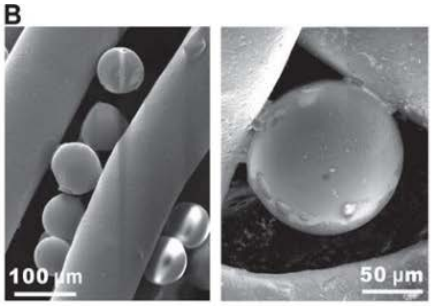
Science Translational Medicine
AAAS

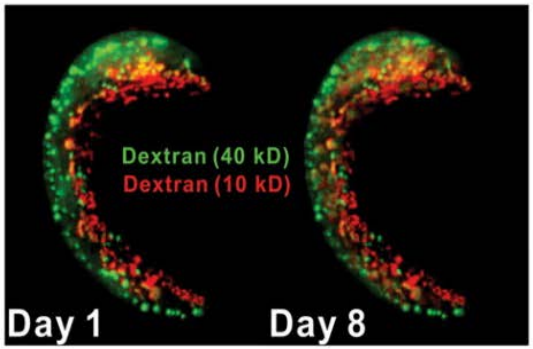
Spatiotemporally released rhCTGF and rhTGFβ3 induced fibrocartilage-like matrix formation in 3D-printed porous scaffolds.

~40% of the real size of a human cadaver meniscus

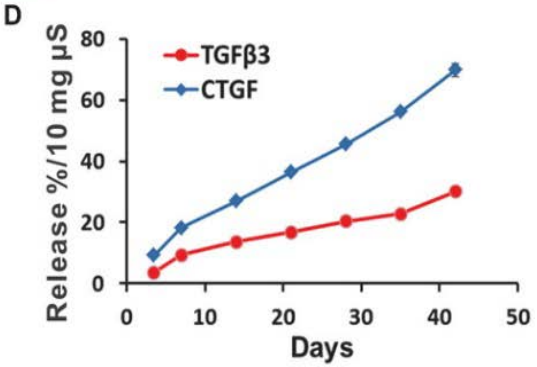
poly-ε-caprolactone (PCL)





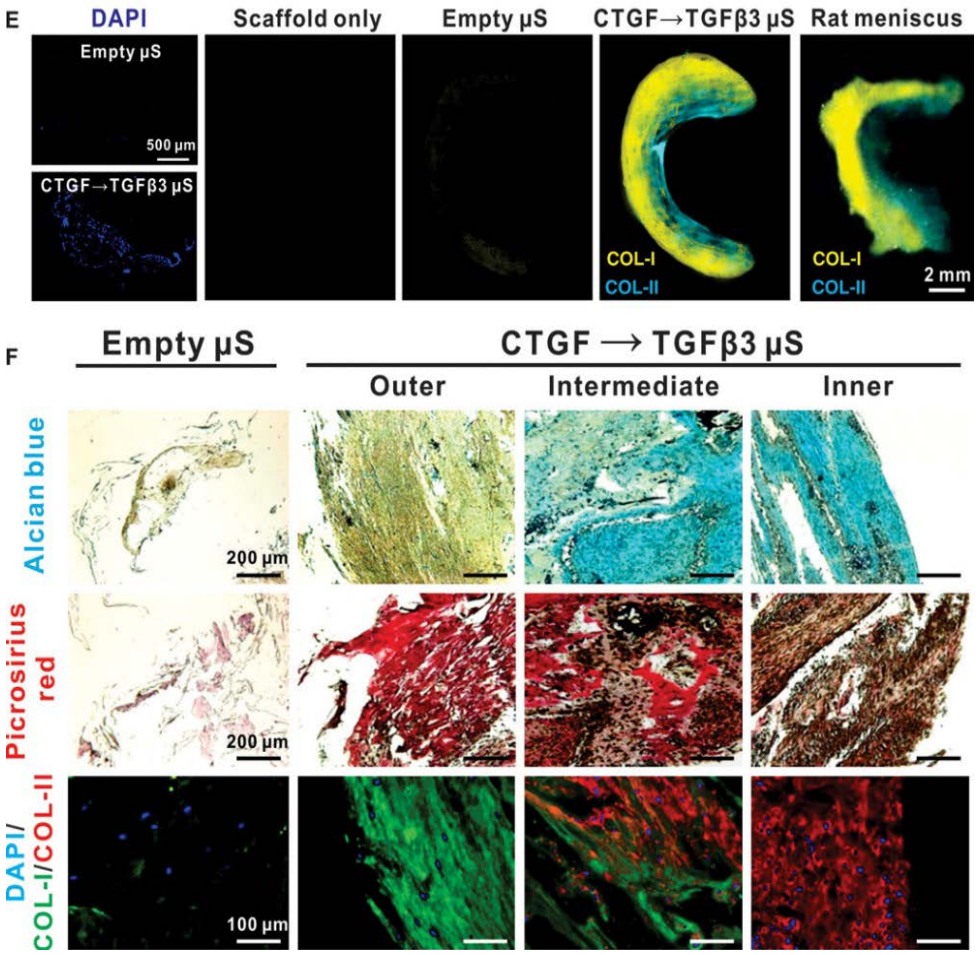


multiphase μS distribution



rapid rhCTGF release (outer layer) & slower release of rhTGFβ3 (inner layer) were sustained over 42 days

6-week incubation period *in vitro*

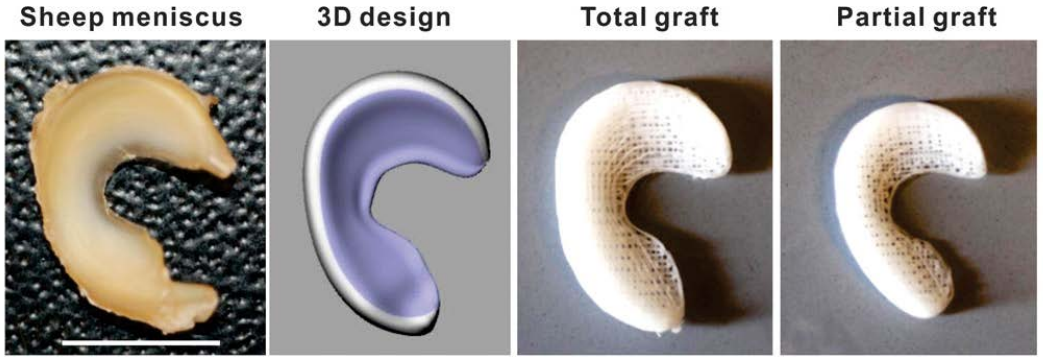


Microencapsulation in PLGA μS, with rhCTGF in 50:50 PLA/PGA μS and rhTGFβ3 in 75:25 PLA/PGA μS.

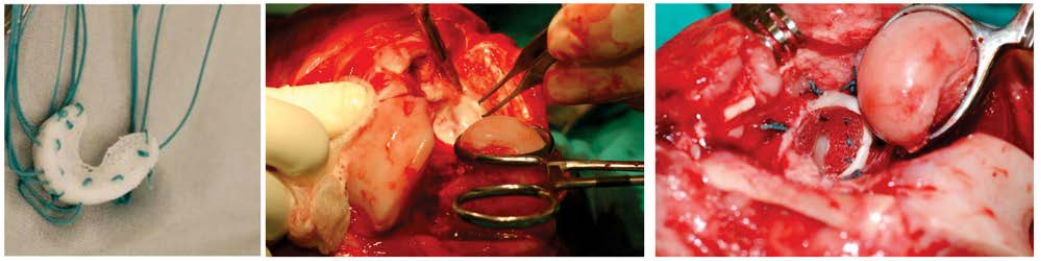
Chang H. Lee et al., Sci Transl Med 2014;6:266ra171

Scaffold implantation in sheep meniscus in vivo

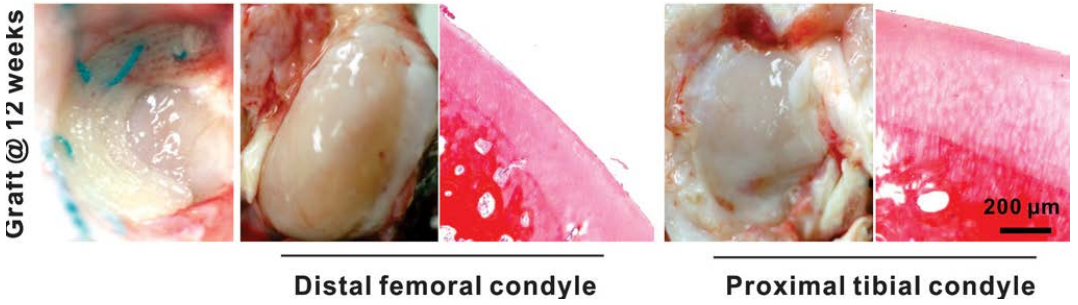
3D laser scanning of a sheep cadaver meniscus



11 skeletally mature (2- to 5-year-old) sheeps into control (scaffold only with empty μS; n = 4) & treatment (spatiotemporal rhCTGF and rhTGFβ3 delivery; n = 7) groups.



meniscus scaffold was sutured to the remaining ~20% outer rim of the native meniscus.

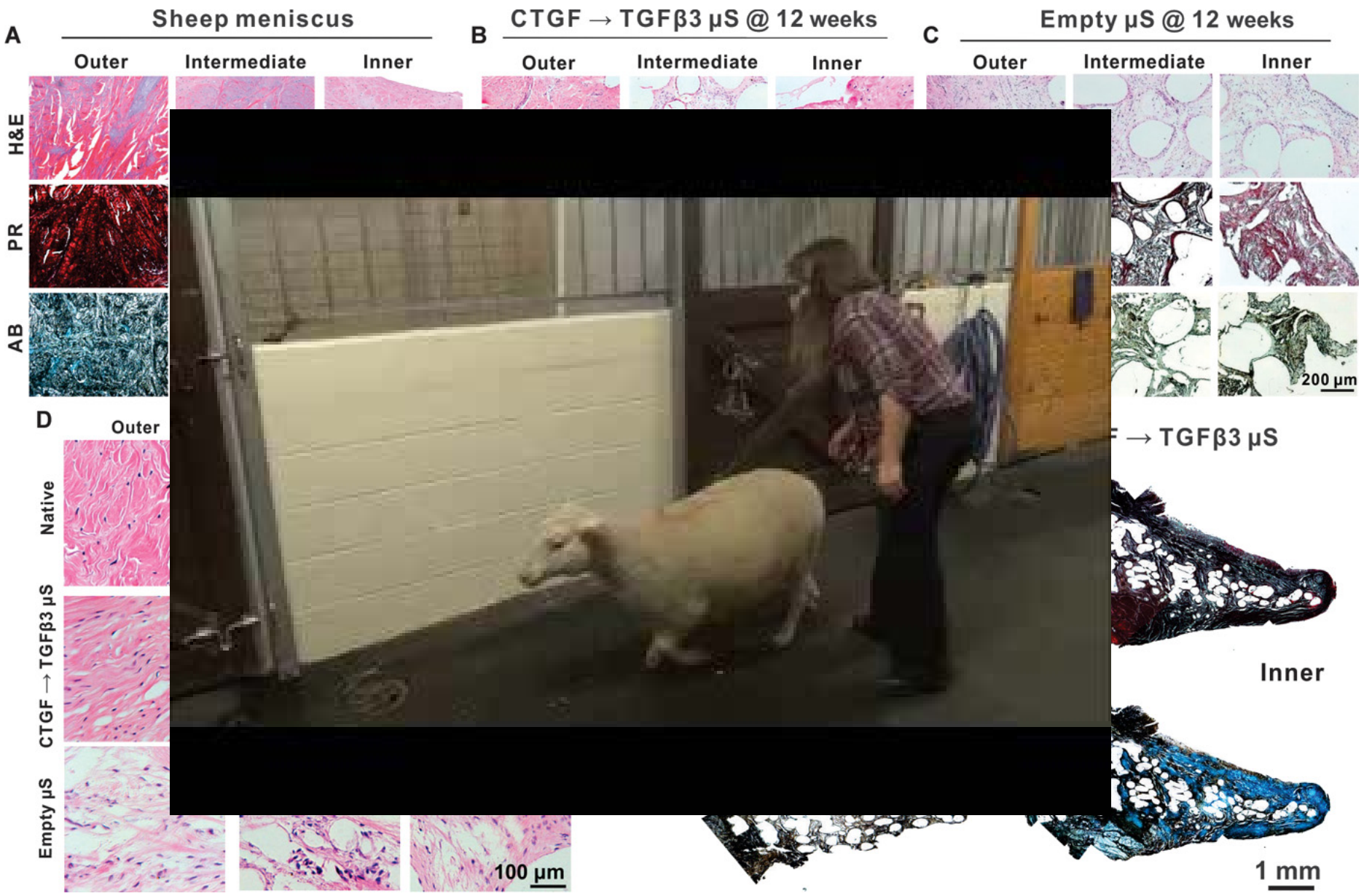


scaffold integrated fully into the remaining outer rim of the meniscus, with no notable damage to articular cartilage in either the femoral or tibial condyle

Chang H. Lee et al., Sci Transl Med 2014;6:266ra171

Science Translational Medicine AAAS

Sheep meniscus regeneration and quality of the regenerated tissue are shown.



Characteristic pattern of extracellular matrix distribution with fibroblast-like cells among collagen bundles in the outer zone, cartilage-like tissue in the inner zone, and a mixed fibrous and cartilage tissue in the intermediate zone

Chang H. Lee et al., Sci Transl Med 2014;6:266ra171

Outcome from the study:

Sheep menisci replaced with empty μS showed amorphous fibrous tissue throughout, NO zone-specific tissue phenotypes

CTGF→TGFβ3 Ms: The outer zone of the regenerated meniscus was populated by fibroblast-like, spindle-shaped cells; chondrocyte-like cells in the inner zone; and mixed fibroblast- and chondrocyte-like cells in the intermediate zone, similar to the native meniscus.

Meniscus regeneration showed the capacity to exert mechanical functions

Regeneration by cell homing

Meniscus regeneration with a protein-releasing, acellular biomaterial scaffold.

In contrast to cell transplantation, the strategy of endogenous regeneration upon spatiotemporal release of two recombinant human proteins offers a ready-to-implant graft that may serve as a therapeutic prototype.

Cell free 3D printed scaffold (seeded with Patient cells)

THE LANCET
Volume 367, Issue 9518, 15–21 April 2006, Pages 1241–1246

7 patients, aged 4–19 years, with high-pressure or poorly compliant bladders, were identified as candidates for the cytoplasty.

Fast track — Articles

Tissue-engineered autologous bladders for patients needing cystoplasty

Dr Anthony Atala, MD[a], Stuart B Bauer, MD[b], Shay Soker, PhD[a], James J Yoo, MD[a], Alan B Retik, MD[b]

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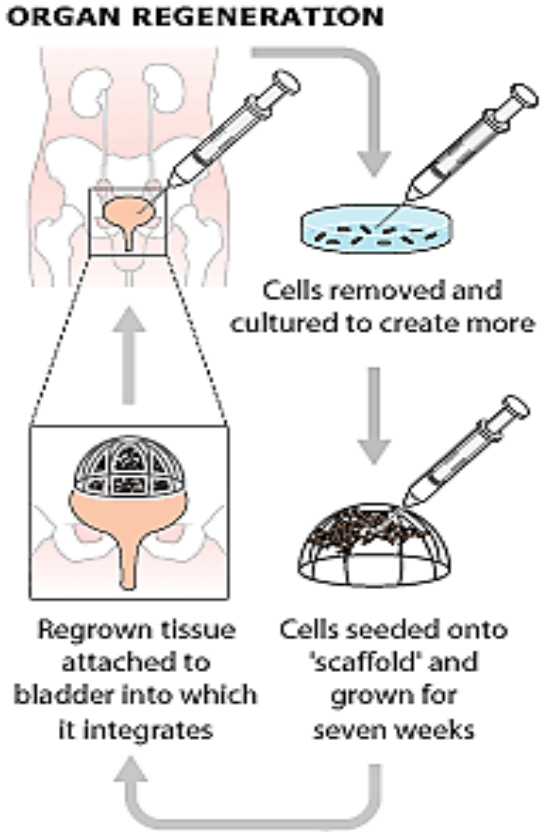
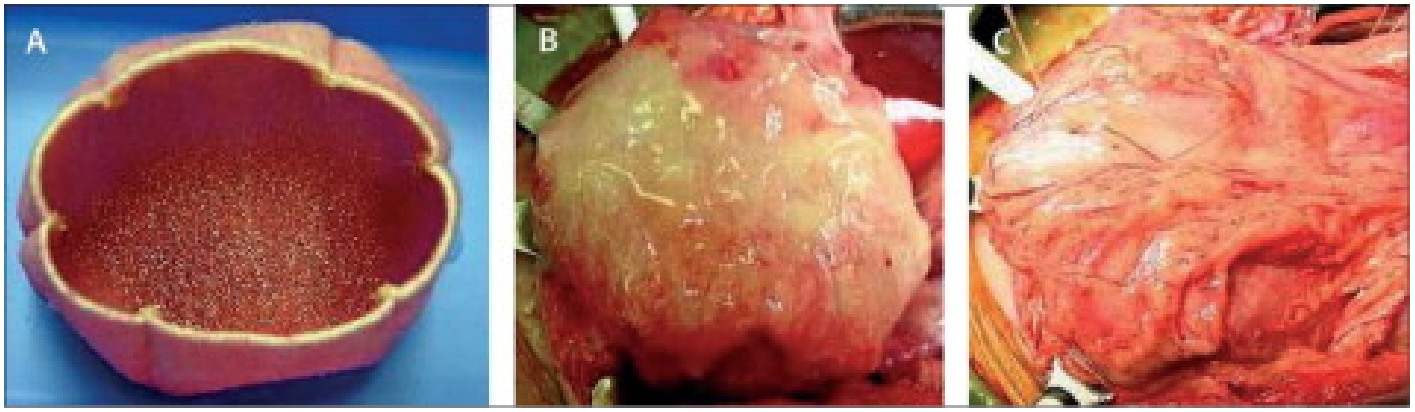


Biodegradable bladder-shaped scaffold made of a composite of collagen and polyglycolic acid (PGA)

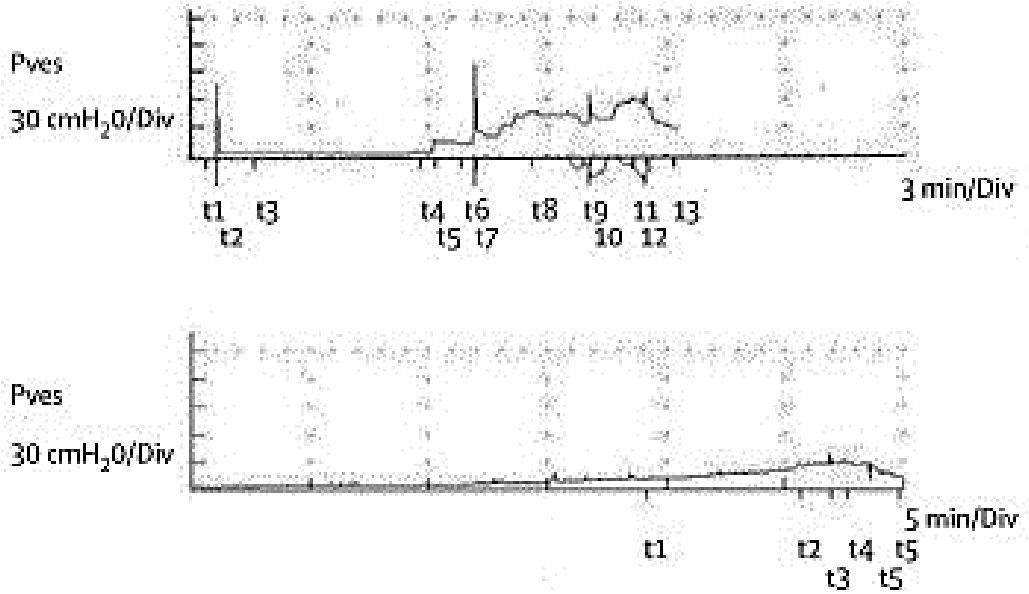
50×10^6 cells per cm3 seeding

Exterior surface-the smooth muscle cells
Inside of the scaffold-urothelial cells

Collagen-PGA scaffold engineered bladder



Construction of engineered bladderScaffold seeded with cells (A) and engineered bladder anastamosed to native bladder with running 4–0 polyglycolic sutures (B). Implant covered with fibrin glue and omentum (C).



Serial urodynamics, cystograms, ultrasounds, bladder biopsies, serum analyses were done.

Preoperative (A) and 10-month postoperative (B) urodynamic findings in patient with a collagen-PGA scaffold engineered bladder. Abnormal bladder pressures on urodynamic study preoperatively, and findings postoperatively

Tissue-engineered autologous bladders for patients needing cystoplasty

Volume 367, Issue 9518, 2006, 1241–1246

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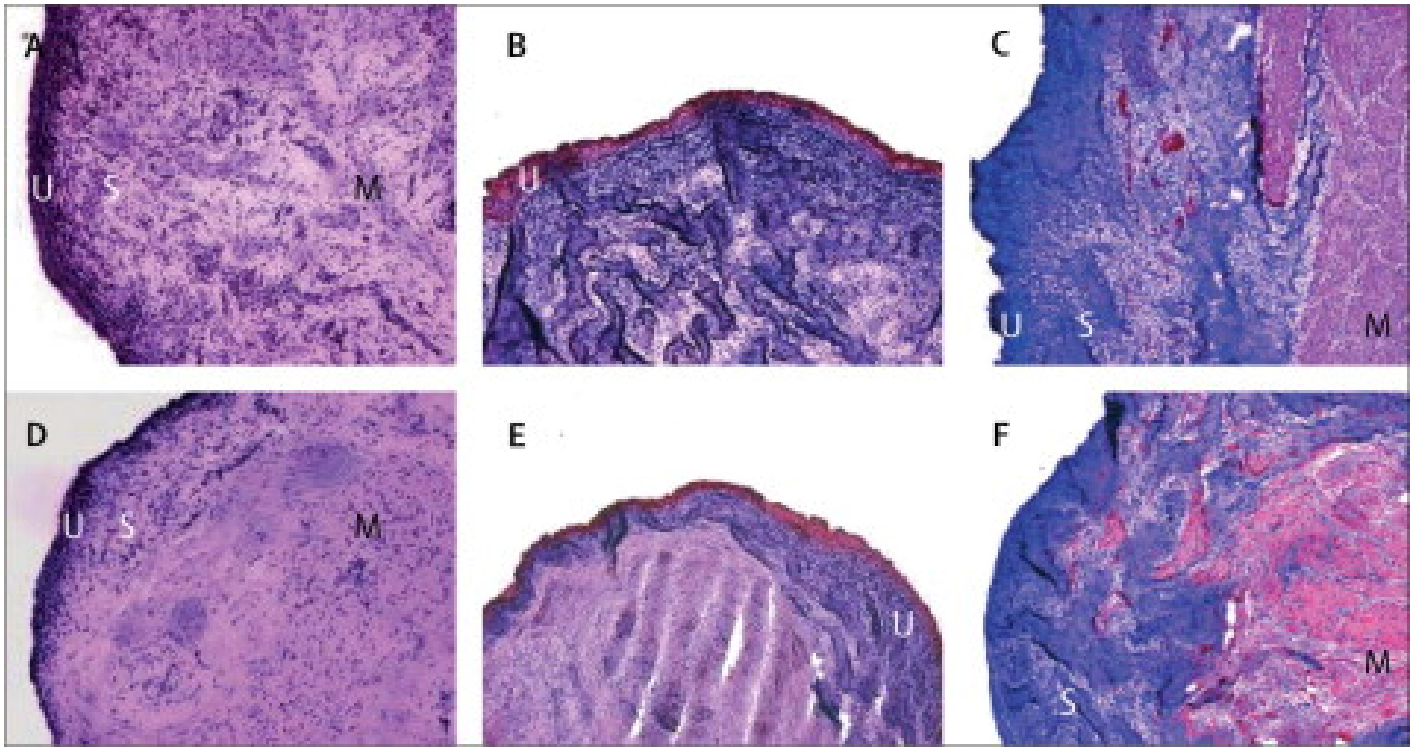
http://dx.doi.org/10.1016/S0140-6736(06)68438-9

Morphological analysis of implanted engineered bladders



Cystoscopic biopsies of implanted engineered bladders 31 months after augmentation shows extent of regeneration Engineered bladder tissue showed tri-layered structure, consisting of lumen lined with urothelial cells (U) surrounded by submucosa (S) and muscle (M).

Approx 7 Years after surgery the engineered bladder biopsies showed an adequate structural architecture and phenotype and functional recovery

3D Bioprinting of Skin

Design and Fabrication of Human Skin by Three-Dimensional Bioprinting

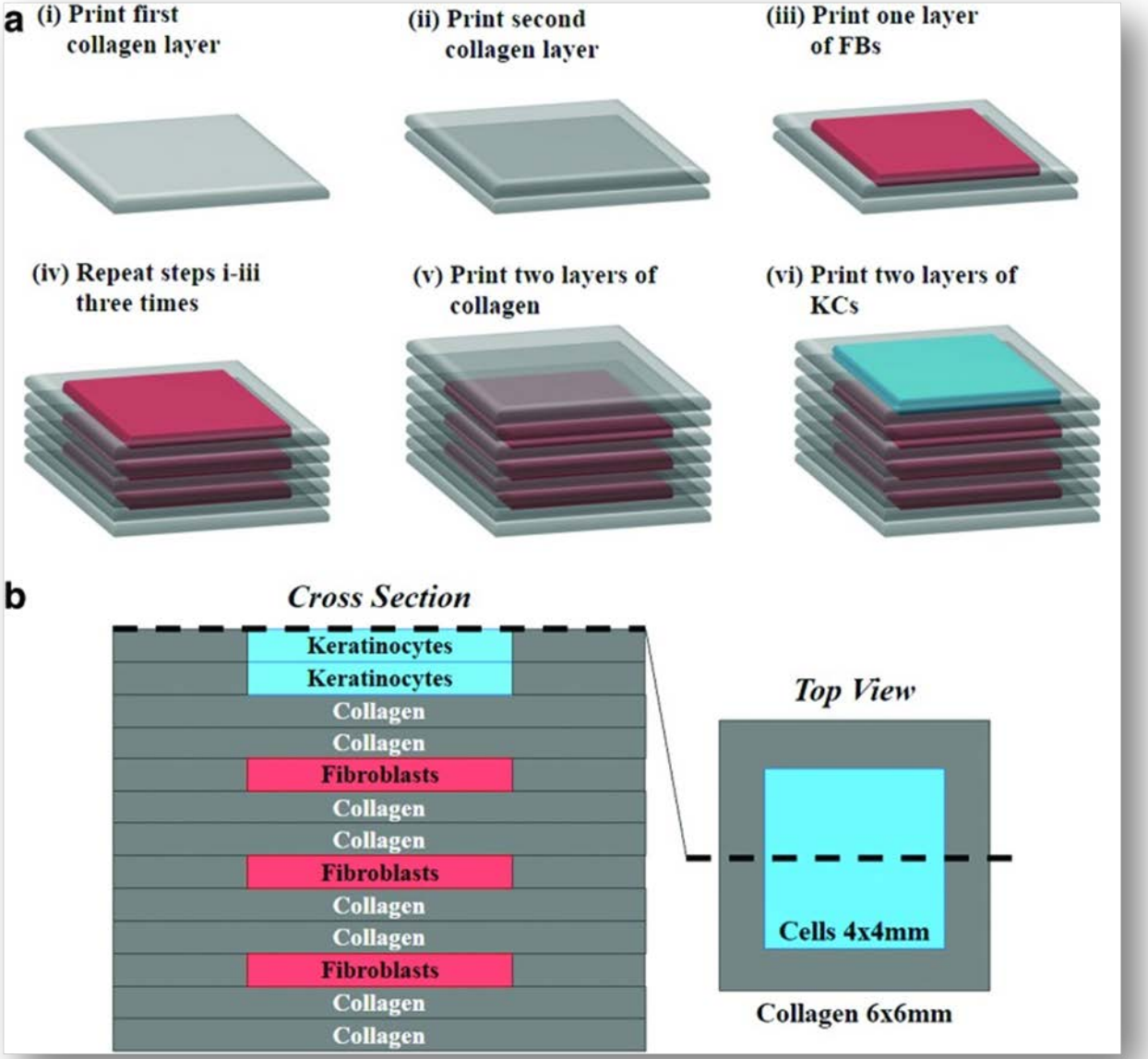


TABLE 1.

SUMMARY OF PRINTING PARAMETERS USED IN THIS STUDY

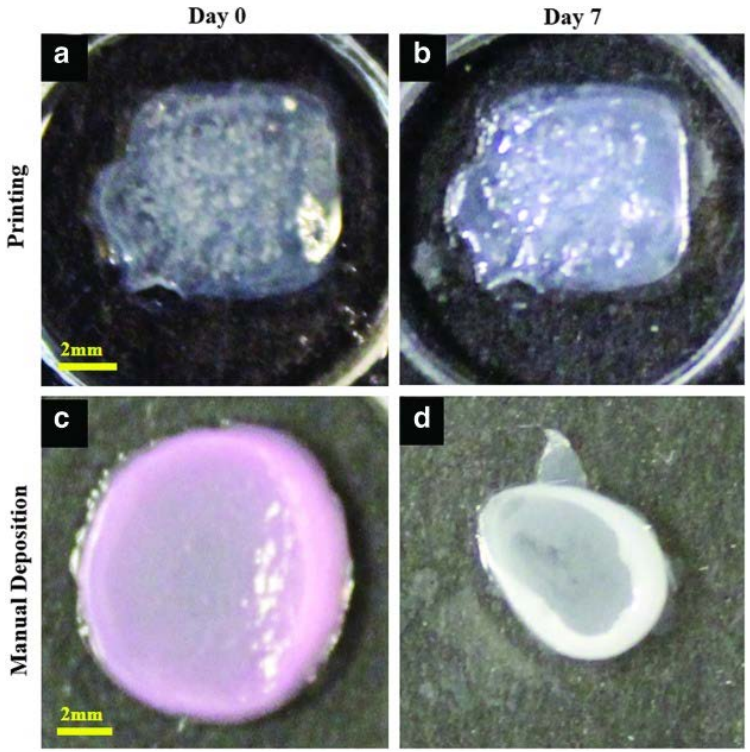
Printing parameters	*Collagen*	*Cell suspension*
Air pressure	2.5–2.7 psi	1.4–1.5 psi
Valve opening time (pulse duration)	750 μs	750 μs
Droplet volume	52.77±3.81 nL	28.53±3.15 nL
Droplet spacing (resolution)	500 μm	500 μm
Pattern size	6×6 mm	4×4 mm
Concentration/density	3.0 mg/mL	0.5–5 million cells/mL

Optimized printing parameters for maximum cell viability, optimization of cell densities in the epidermis and dermis to mimic physiologically relevant attributes of human skin.

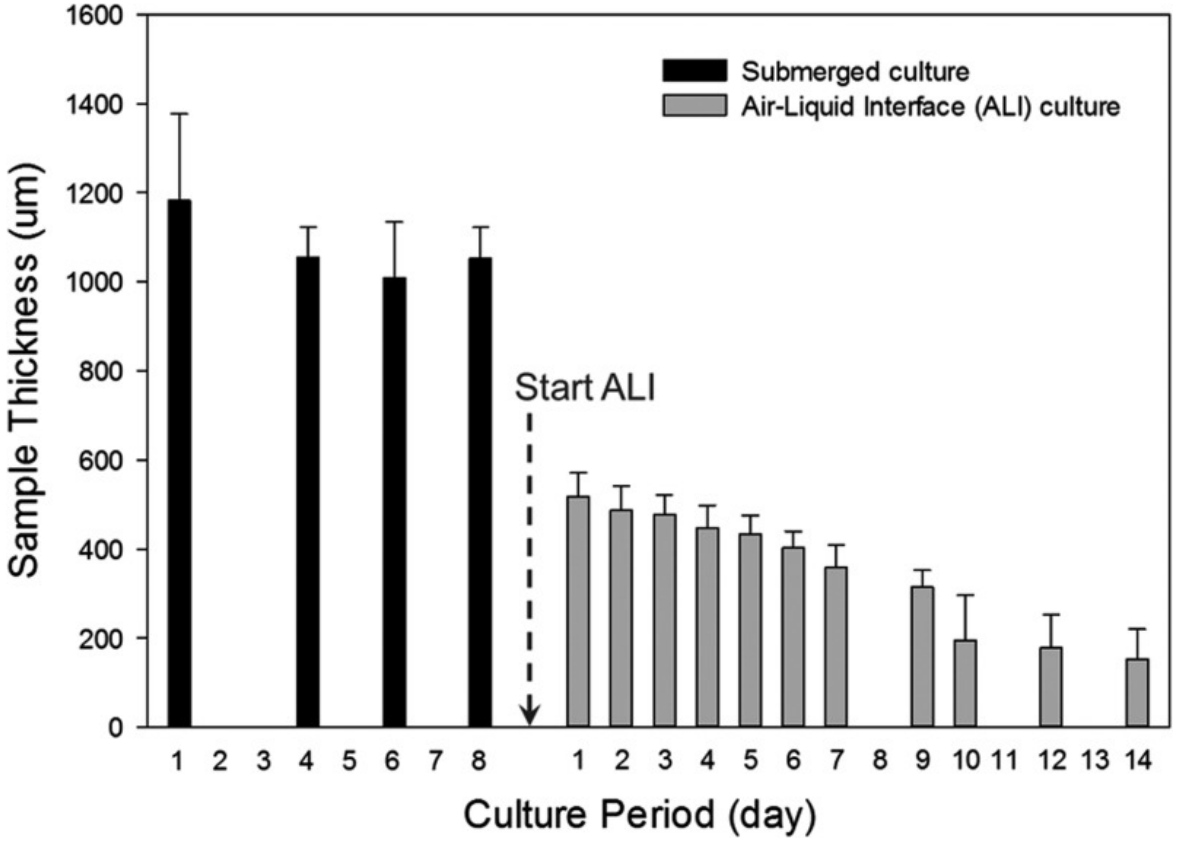
Lee et al., Tissue Eng Part C Methods. 2014 Jun 1; 20(6): 473–484.
doi: 10.1089/ten.tec.2013.0335

Design and Fabrication of Human Skin by Three-Dimensional Bioprinting

3D printed skin samples retain their form (dimensions) and shape under submerged culture condition after 7 days



Reduction (2 to 6-fold) in skin thickness on transition from submerged culture conditions to ALI



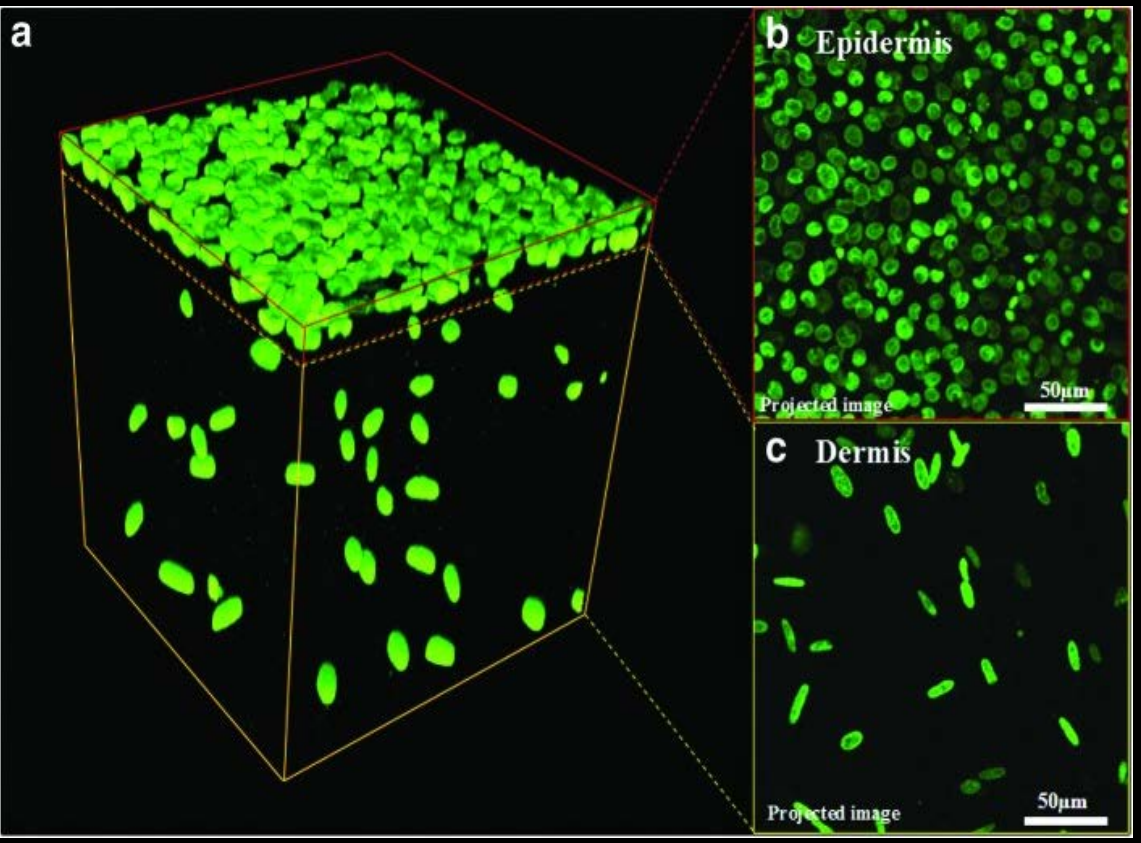
Culture conditions:
exposure of the epidermal layer to the air–liquid interface to promote maturation and stratification

KC into corneocytes and the formation of the stratum corneum (translucent appearance)

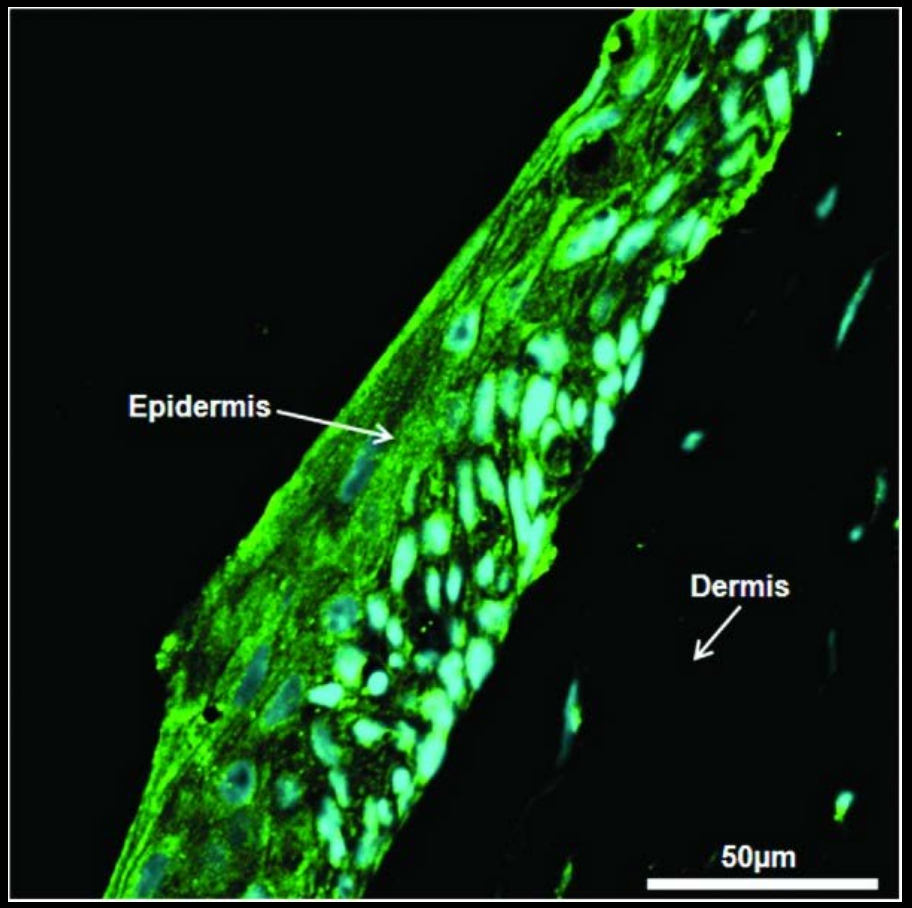
Factors:

Hydrocortisone
hEGF
bovine transferrin
Insulin
bovine pituitary extract

Design and Fabrication of Human Skin by Three-Dimensional Bioprinting



Histology of skin tissue.
Printed skin cultures H&E and nuclear staining



Immunofluorescence of skin tissue.
Printed skin structures stained for N-cadherin tight junctions (day 14 of ALI culture)

Punctate pattern in the epidermal compartment indicating the formation of junctions between KCs cell layers.

Advantage and potential use:

Several advantages in terms of shape- and form retention, flexibility, reproducibility, and high culture throughput

Broad range of potential applications in: (mimicking human skin physiology)

Transdermal and topical formulation discovery,

Dermal toxicity studies,

Designing autologous grafts for wound healing

Inclusion of diseased cells to serve as a model for studying the pathophysiology of skin diseases (psoriasis, atopic dermatitis, allergic contact dermatitis, and vitiligo and models of skin malignancies such as melanoma, etc.,)

Current limitations and future possibilities

Still consists of two cell types

Enhancing the complexity of the skin model via the incorporation of secondary and adnexal structures

Complete generation of these whole organs requires incorporation of extensive vascular networks to support the viability of cells throughout the organ as well as precise organization of multiple cell types

two challenges traditionally not faced in the biofabrication process of simpler tissues like the skin.

3D-printing: Current state & limitations:

Challenges, including cell and material requirements, tissue maturation and functionality, and appropriate vascularization and innervation.

Almost all human tissues have complex combinations and gradients of ECM components, each with specific biological and mechanical influences.

The ability to reproduce the heterogeneous spatial arrangement of biologically complex structures is yet to be achieved

Ensuring sufficient vascularization of the engineered construct is essential for the long-term viability of any bioprinted tissue construct.

3D-printing: Future & possibilities:

The ability to image, map and reproduce complex 3D structures composed of biologically relevant ECM proteins would be a major advancement for the field.

Use of decellularized tissues to gain a greater understanding of physiological ECM compositions, ECM derived from decellularized tissues may serve as a useful biomaterial.

3D-bioprinted tissue constructs: not only for transplantation but also for use in drug discovery, analysis of chemical, biological and toxicological agents, and basic research.

Bioreactors can help to maintain viability of tissue constructs and 'buy' time necessary for post-processing tissue fusion, remodeling and maturation, in combination with factors that promote angiogenesis and innervation along with maintaining cell viability

In vivo 3D bioprinted skin directly into wound/burn and bioprint bone into defects in mice

3D printing of tissue *in vivo* using robotic arms inside the patient body

NASA contractor Techshot

3D bioprinter developers nScrypt and bio-ink specialists Bioficial Organs, successfully 3D print cardiac and vascular structures in a zero gravity environment using adult human stem cells

Thank you for your attention