The On-Demand Era of Human Tissue Research : Bioprinted Skin Equivalents

ROKIT HEALTHCARE

The webinar discusses the promise of bioprinted skin equivalents in improving the accuracy of skin research with applications in toxicology, basic physiology and disease modeling. With the promise of customized selections of cells and on-demand printing of tissues, bioprinted skin equivalents offer values of reducing time and boosting the quality of research.

ABOUT PRESENTER

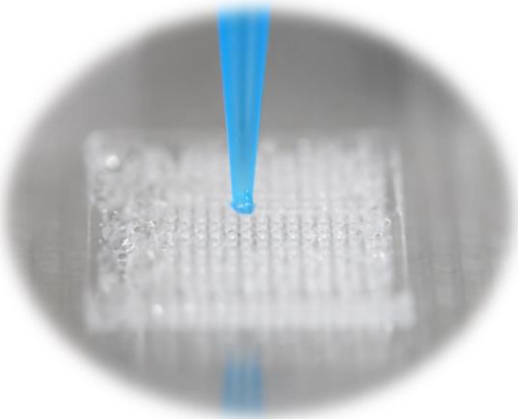
Ms. Da-Yae Lee

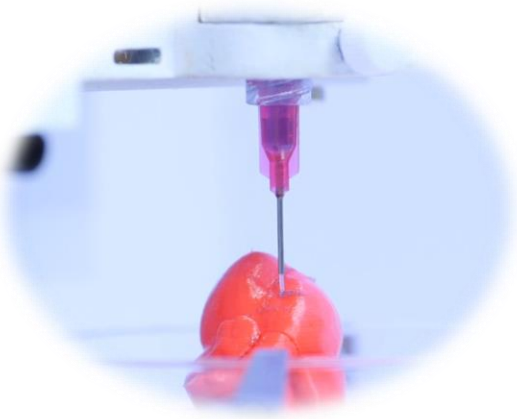
Senior Bio-Consultant
Project Leader, Global Business Development
ROKIT Healthcare

Lee is a part of the “Bioprinting Applications” team commercializing three-dimensional human skin equivalent models, aimed at supporting the animal 3Rs (Reduction, Replacement, Refinement) and improving the accuracy of human skin research.

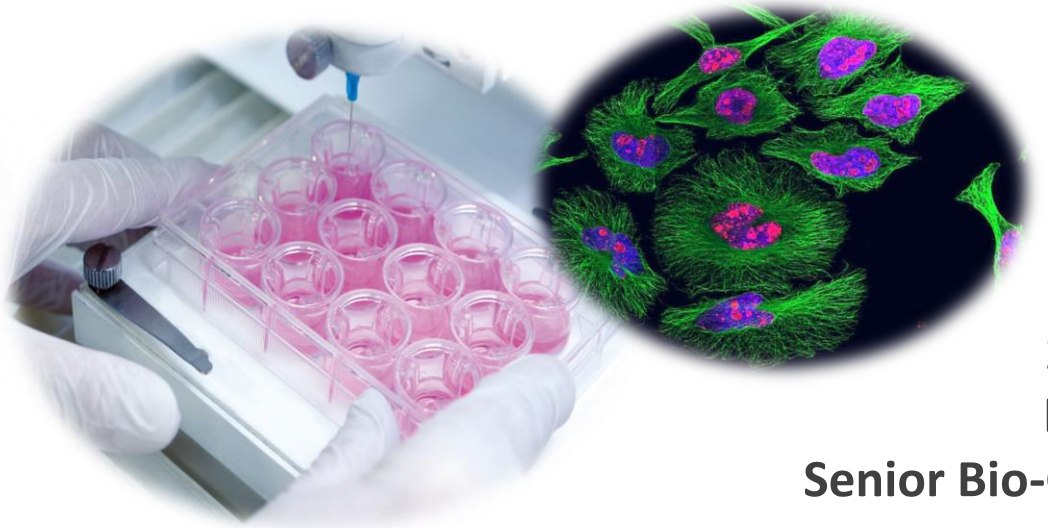
Before joining ROKIT Healthcare, Da-Yae was a research assistant at the MIT Koch Institute for Integrative Cancer Research and Brigham and Women’s Hospital Crohn’s and Colitis Center in Boston, USA, with experiences both at the bench and in clinical trial coordination.
She has a B.A. in Biochemistry from Smith College in Massachusetts, USA.

ROKIT HEALTHCARE

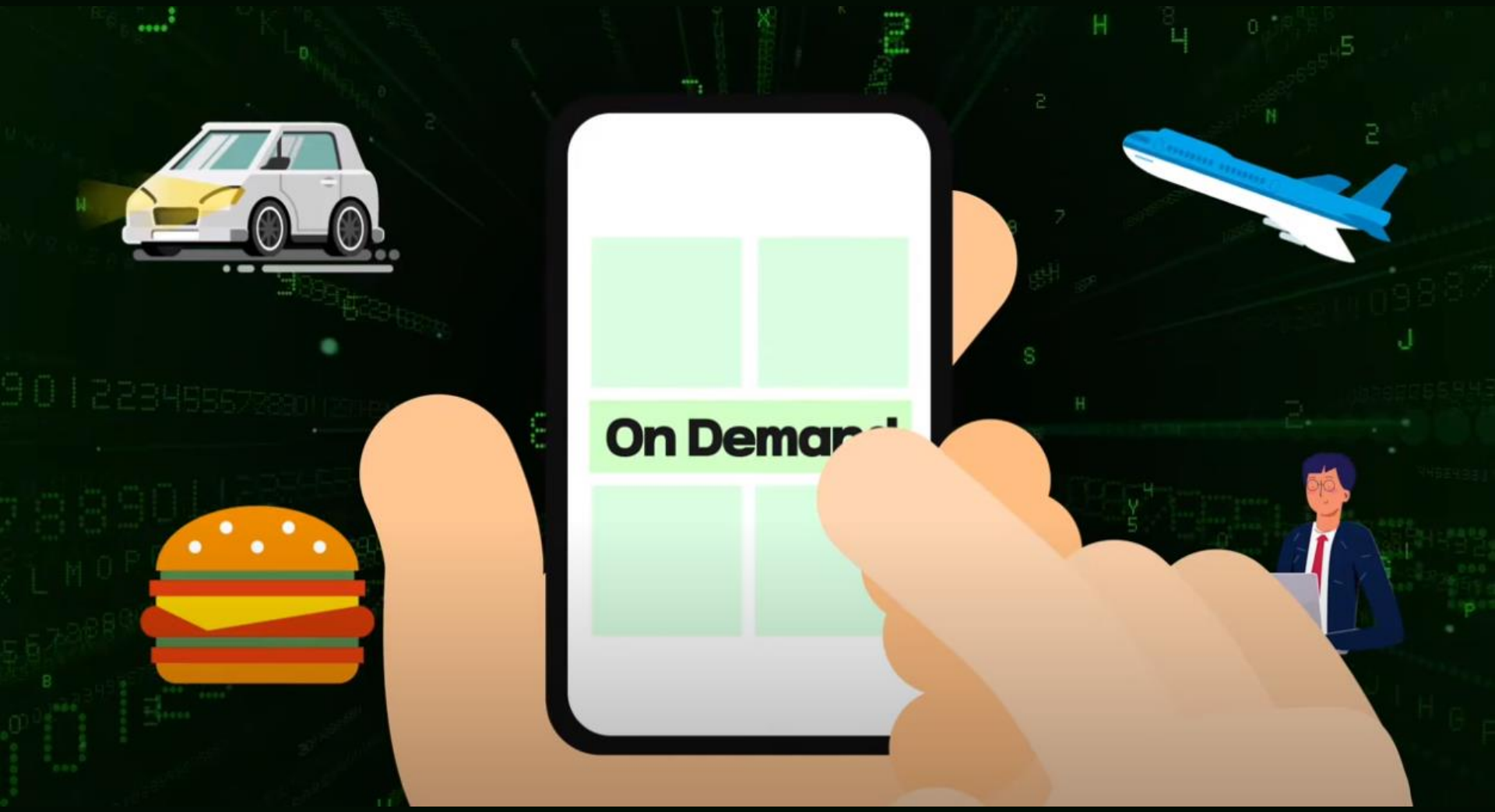




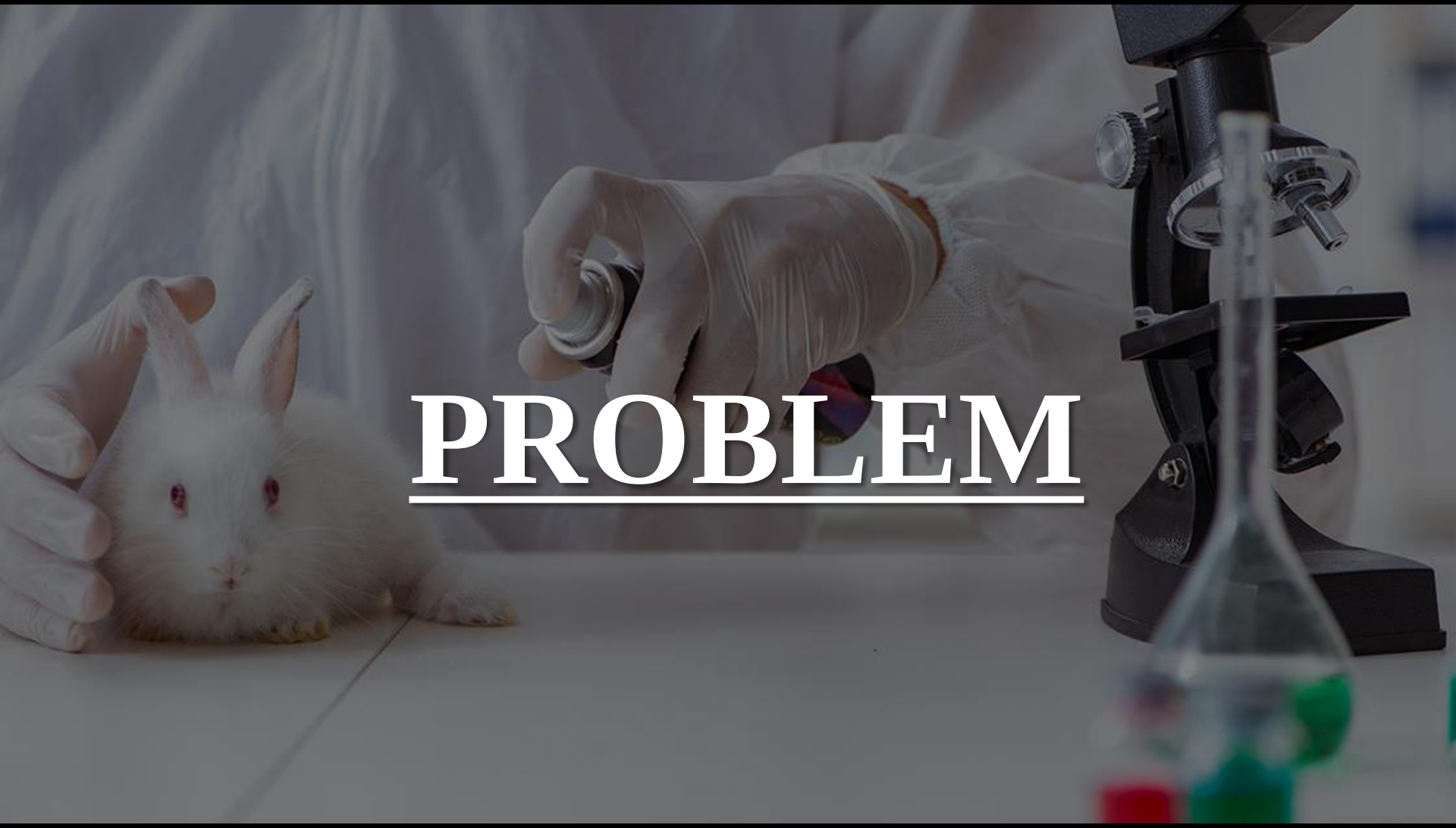
ON-DEMAND HUMAN TISSUE RESEARCH:
APPLICATIONS OF BIOPRINTING IN SKIN



2020. 06. 11
Da-Yae Lee
Senior Bio-Consultant


On Demand


Control
of the Time
Desired
Design
High
Quality


PROBLEM

End of Dependence of Animal Testings

NEWS

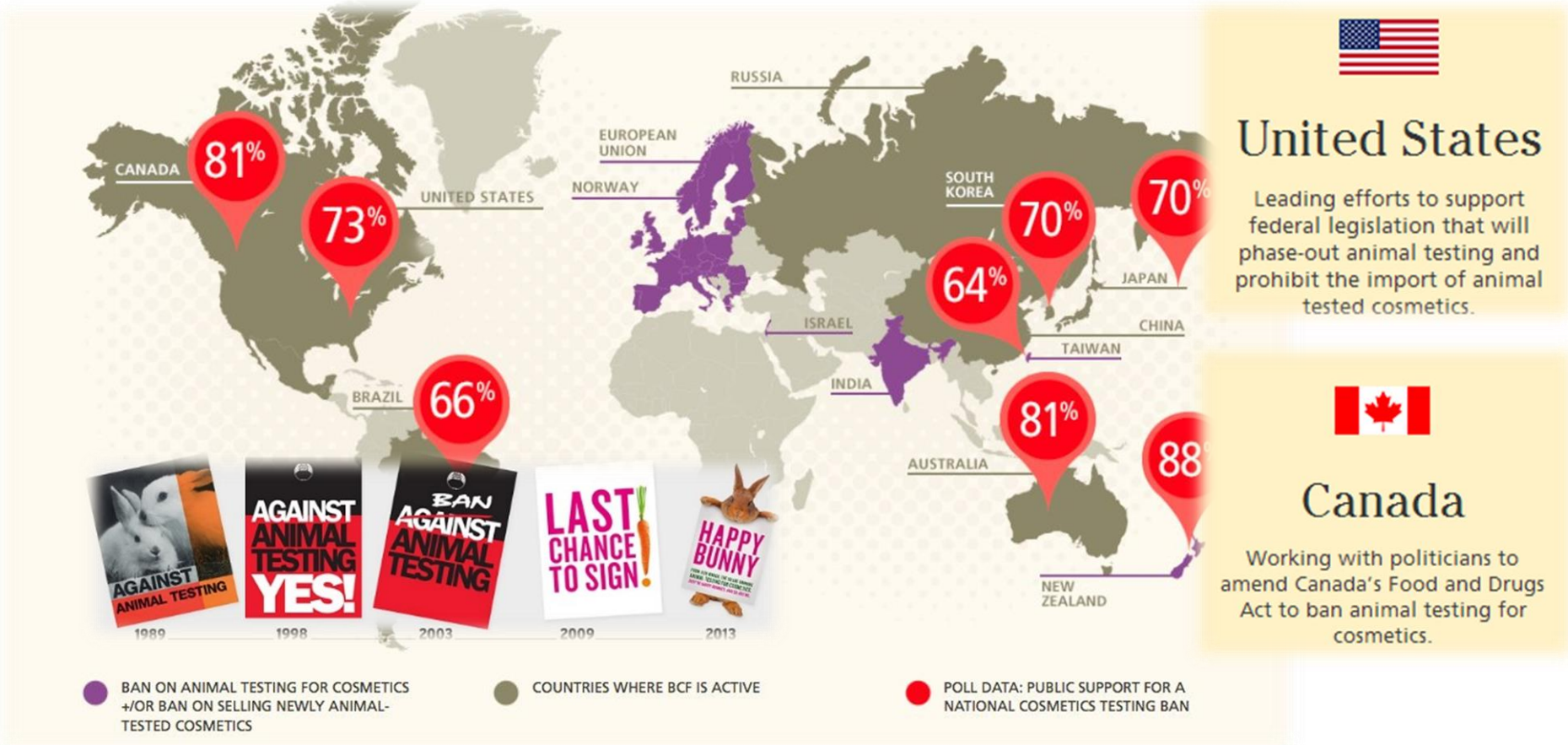
Cosmetic animal testing bill tabled in House of Commons

By Holly Lake. Published on Apr 12, 2019 9:59am

US states join global push to ban animal-tested cosmetics

By Associated Press

February 3, 2020 | 11:32am



LIMITATIONS OF ANIMAL & 2D MODELS

VS. RABBIT TEST

Comparison of human skin irritation patch test data with *in vitro* skin irritation assays and animal data

DAGMAR JÍROVÁ[1], DAVID BASKETTER[2], MANFRED LIEBSCH[3], HANA BENDOVÁ[1], KRISTINA KEJLOVÁ[1], MARIE MARRIOTT[4] AND HELENA KANDÁROVÁ[5]

[1]National Institute of Public Health, Prague, Czech Republic, [2]DABMEB Consultancy Ltd., Sharnbrook, UK, [3]ZEBET, Federal Institute for Risk Assessment, Berlin, Germany, [4]Safety and Environmental Assurance Center, Unilever Colworth Laboratory, Bedford, UK, [5]MatTek Corporation, MA, Ashland, USA

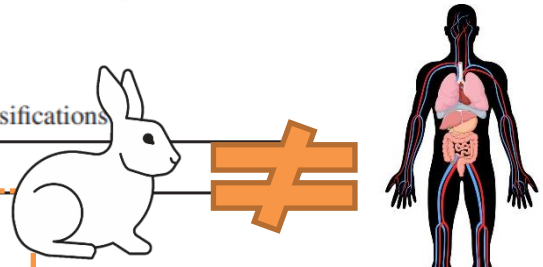


Table 4. Concordance of classifications

Classification methods
Rabbit test ▶ 4-hr HPT
4-hr HPT PT ▶ Rabbit test
15 min/42 hr EPISKIN assay ▶ Rabbit test
15 min/42 hr EpiDerm assay ▶ Rabbit test
15 min/42 hr EPISKIN assay ▶ 4-hr HPT
15 min/42 hr EpiDerm assay ▶ 4-hr HPT

Concordance of classifications is calculated on the basi

Sensitivity	Specificity	Accuracy (%)
100.0% (5/5)	45.0% (9/20)	56.0
31.3% (5/16)	100% (9/9)	56.0
50.0% (7/14)	66.7% (6/9)	56.5
43.8% (7/16)	77.8% (7/9)	56.0
100.0% (3/3)	65.0% (13/20)	69.6
80.0% (4/5)	75.0% (15/20)	76.0

f results from ECVAM SIVS study (16).

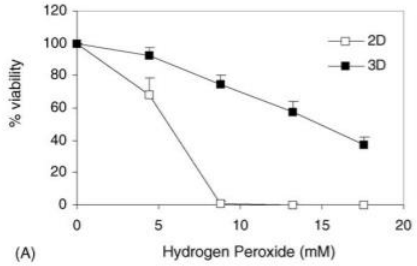
VS. 2D HUMAN MODEL

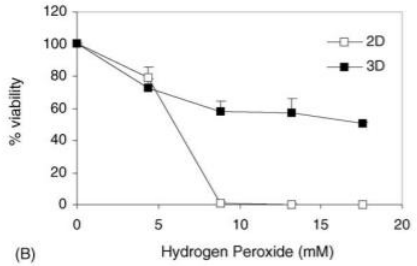
Culture of skin cells in 3D rather than 2D improves their ability to survive exposure to cytotoxic agents

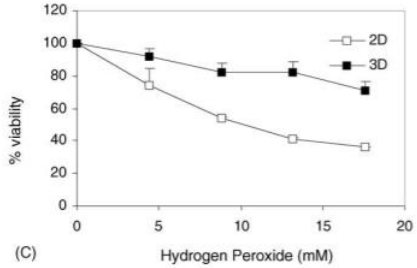
Tao Sun[a], Simon Jackson[a], John W. Haycock[a], Sheila MacNeil[a,b,*]

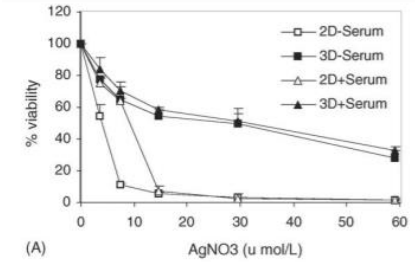
[a] *Department of Engineering Materials, Sheffield University, Kroto Research Institute, Broad Lane, Sheffield S3 7HQ, UK*
[b] *Division of Clinical Sciences, Sheffield University, Northern General Hospital, Sheffield S5 7AU, UK*

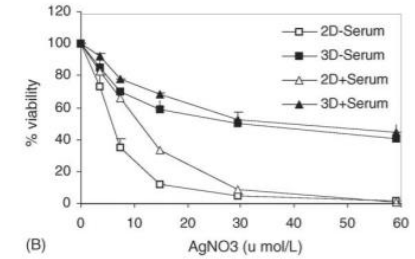
Received 6 October 2005; received in revised form 6 December 2005; accepted 12 December 2005

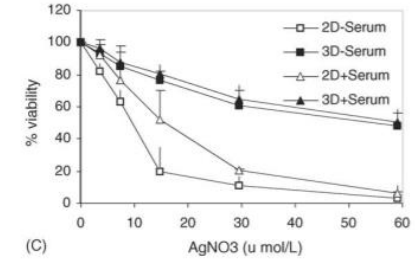






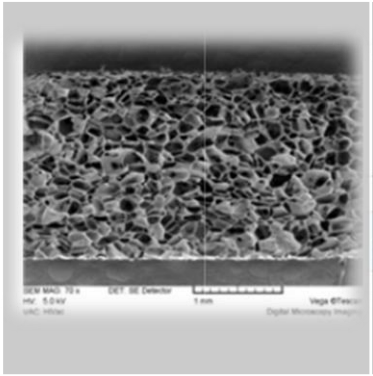
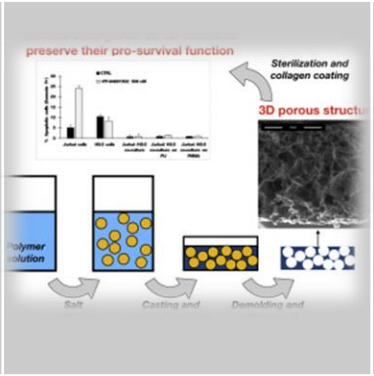
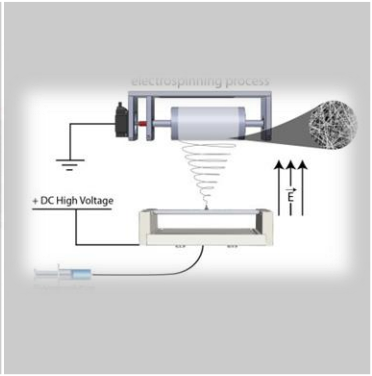
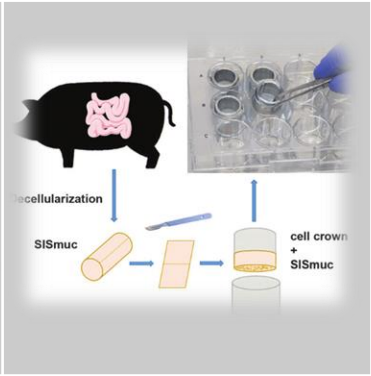
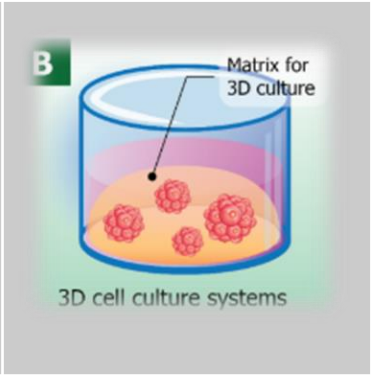






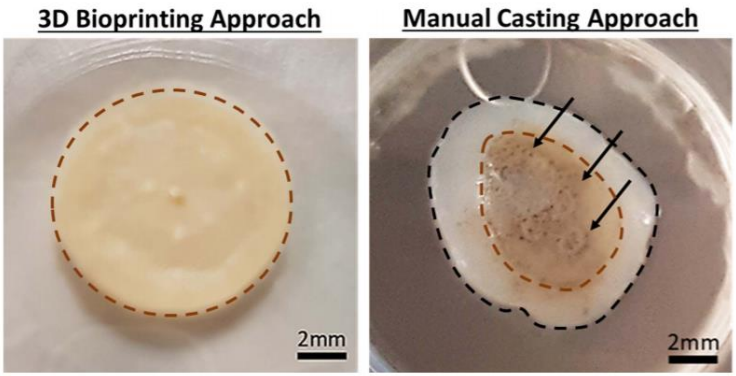
LIMITATIONS OF EXISTING 3D MODELS

Traditional 3D fabrication methods for human tissue include:

Freeze-Drying	Solvent-Casting	Electrospinning	Decell'ed Animal Skin	Spheroids
				

LIMITATIONS

- **Difficult control** over cell, material composition and pore sizes
- Adverse effects of **long pre-processing**
- **Data inconsistency** due to human error
- **Reliance on animal-derived components**
- **Necrotic cores**



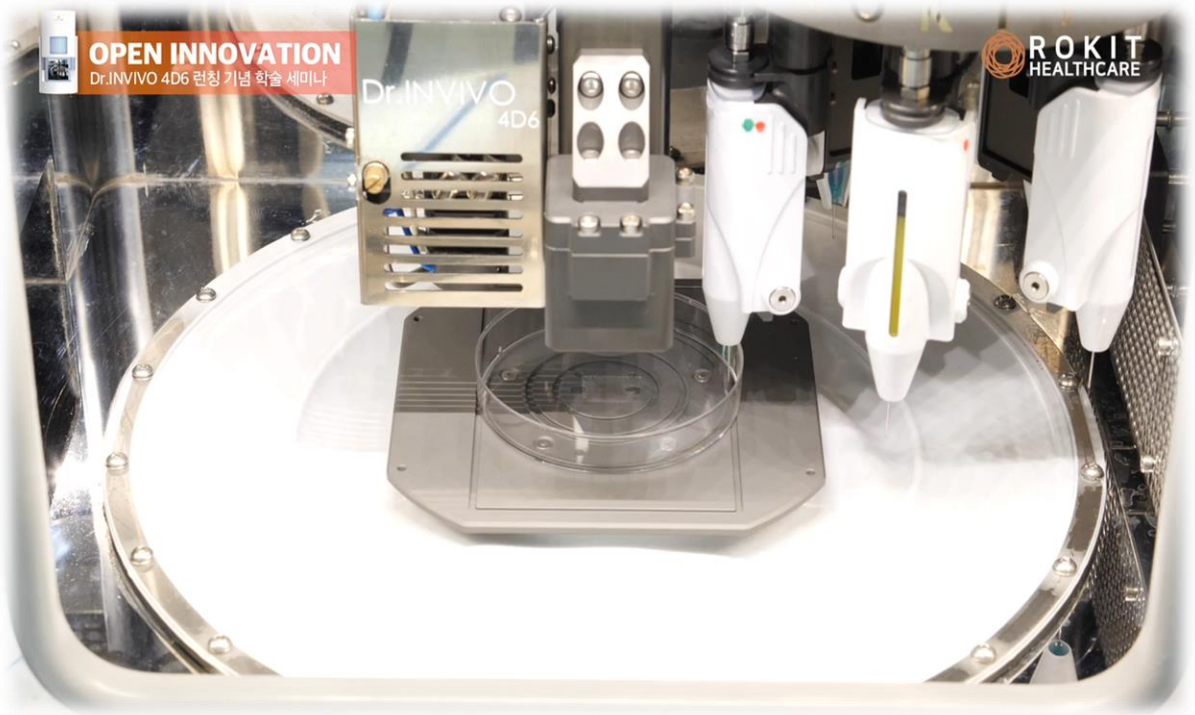
SOLUTION

DO BETTER RESEARCH WITH ROBOTIC SOLUTION

ROKIT HEALTHCARE

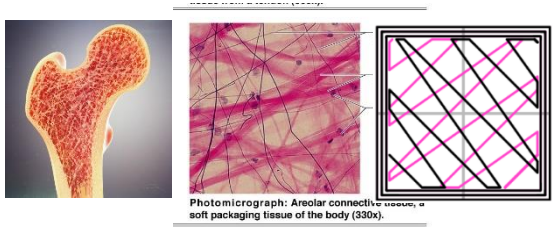
COMPLEX ARCHITECTURE

6-PRINTHEADS

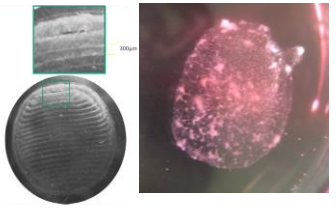


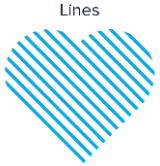
RETINA

BONE



MUSCLE & HEART



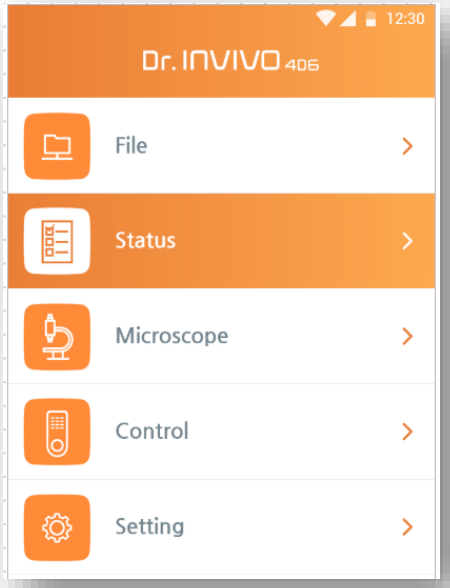


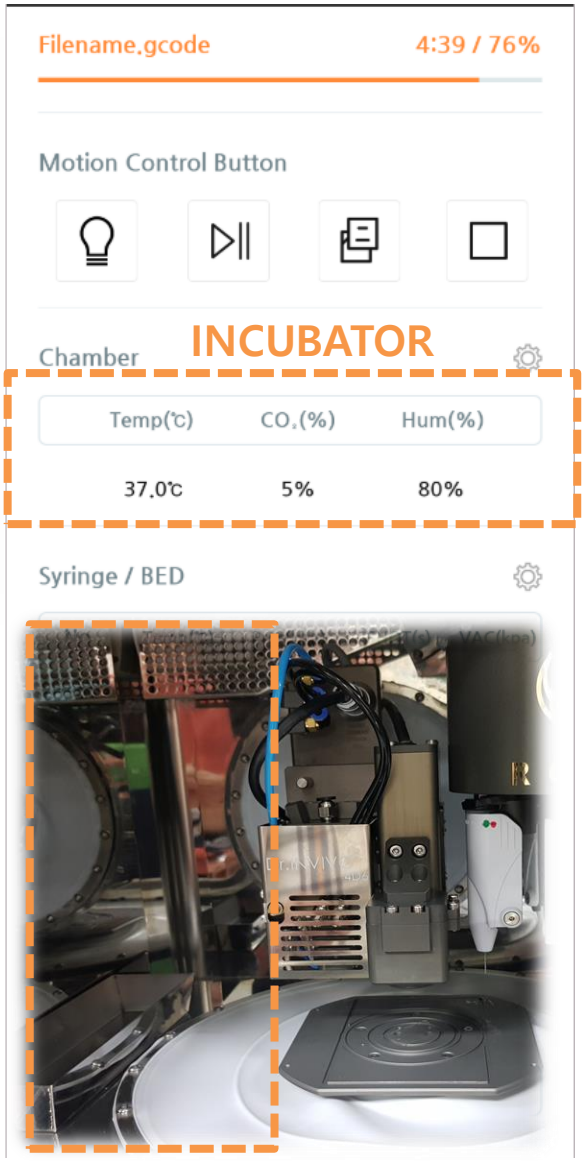
DO BETTER RESEARCH WITH ROBOTIC SOLUTION

ROKIT HEALTHCARE

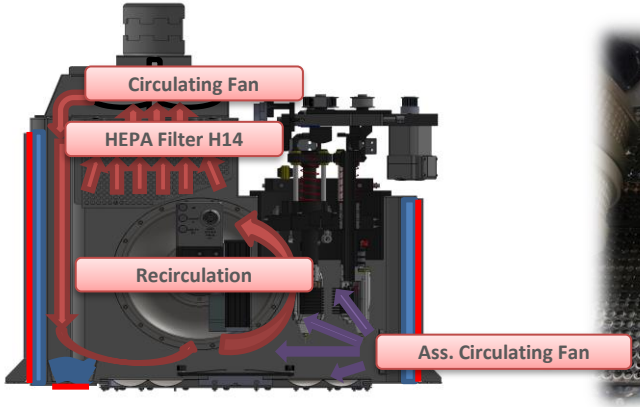
HUMAN CELLS & TISSUE

INCUBATOR & STERILIZER





PLASMA STERILIZER

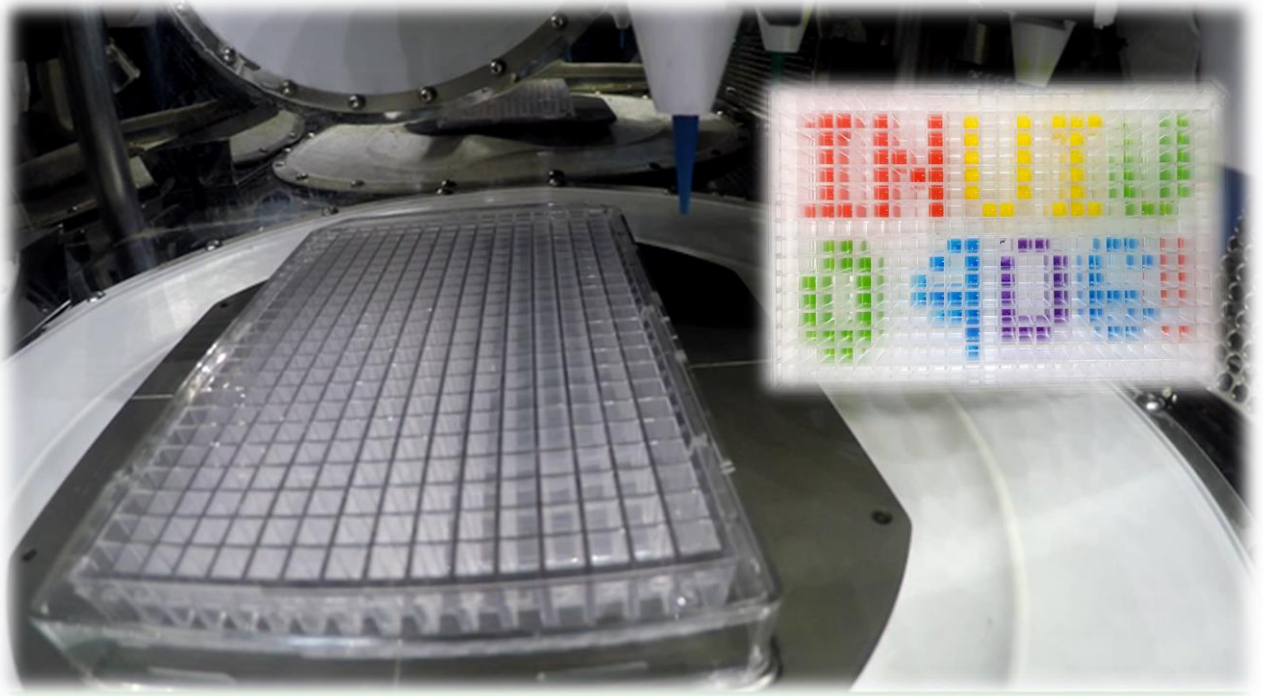


HEPA H14 FILTER

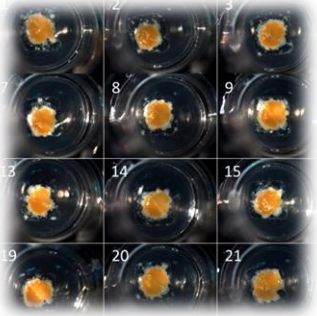
Do Better Research with Robotic Solution

HIGH-THROUGHPUT

UP TO 384-WELL

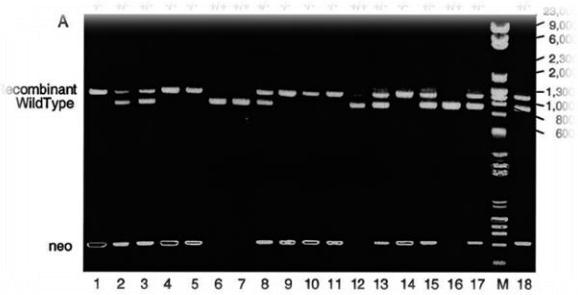


Mini-Liver Drug Development



Safer, Contained Vaccine Research

PCR Genotyping



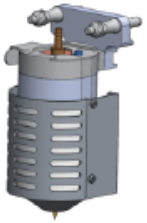
DO BETTER RESEARCH WITH ROBOTIC SOLUTION

ROKIT HEALTHCARE

DIVERSE MATERIALS

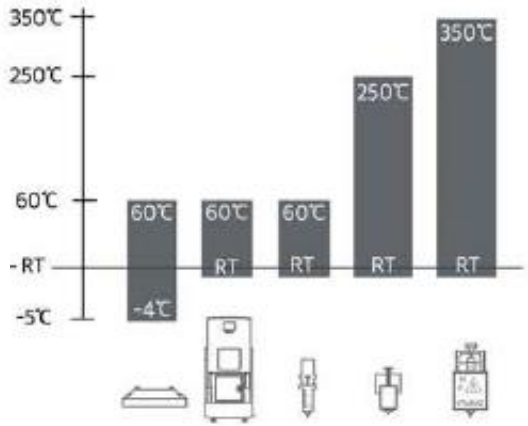
HYBRID TECHNOLOGIES

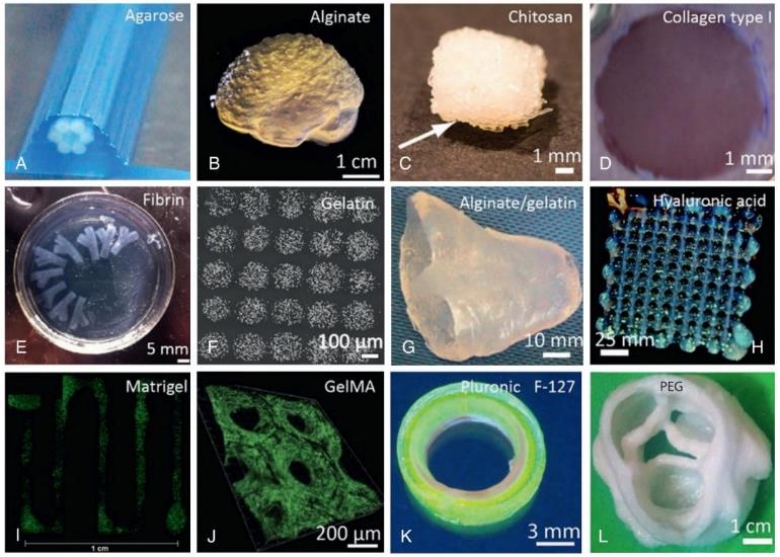
Hot Melting Module



Rotary Module

Extruder Module





SOFT	HARD
Alginate	PLA
Agarose	PCL
Chitosan	PLGA
Collagen/ColMA	PLLA
Fibrin	Nano-ceramics
Gelatin/GelMA	Polyurethane
Pluronic F-127	Tri-calcium Phosphate
Hyaluronic acid	Hydroxyapatite (HAp)
Cellulose	Bioactive glass

Fast Track to Medical Commercialization

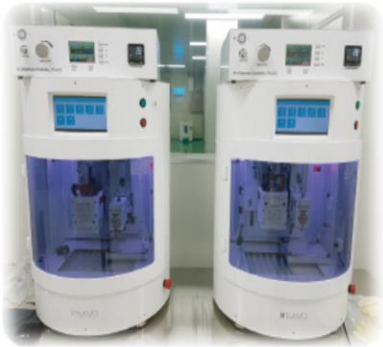
Dr. INVIVO has already been used to treat patients in the operating room!

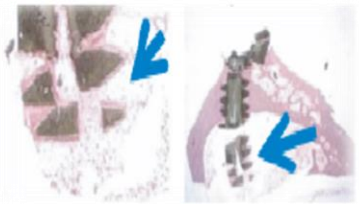
cGMP-READY

HOSPITAL-USE CASES

(2019) SOFT TISSUE: DIABETIC FOOT ULCER SKIN

(2020) HARD TISSUE: CUSTOMIZED BONE IMPLANTS





EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

MFDS KOREA

TODAY'S FOCUS: BIOPRINTING HUMAN SKIN

IN VIVO (IN SITU)

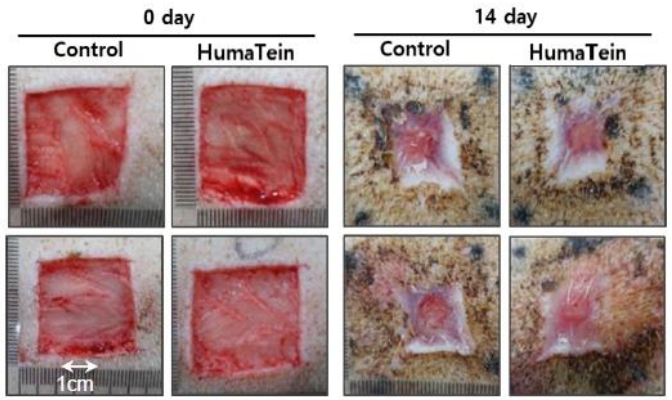
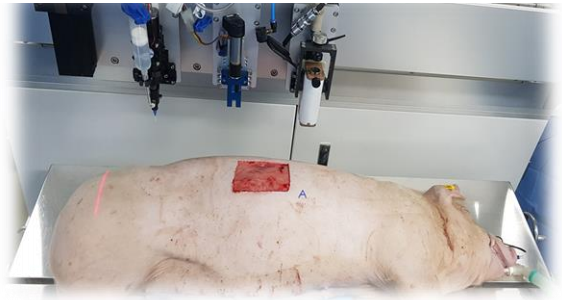
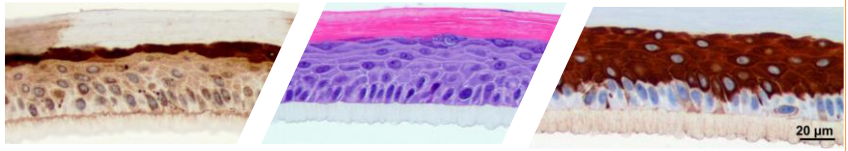


Figure 8. Wound Closure (Healing Rate) Evaluation: compared to control (showing more depth due to slow regeneration, HumaTein treated wound filled with neo-tissue and the tissue contraction is less noticeable



Wound Healing
In-Hospital Solution

IN VITRO



Toxicology
Basic Skin Research (Physiology)
Disease Modeling

WORLD'S FIRST DFU SKIN REGENERATION WITH INVIVO®

⑤ Remove the scaffold & apply only MA-ECM, a shape of dermal patch to the patient's wound

⑥ Follow up the wound size and skin regeneration

4weeks 5weeks 10weeks

④ Printing scaffold & MA-ECM

① Harvesting adipose-tissue by liposuction

Dr.INVIVO (3DBioprinter)

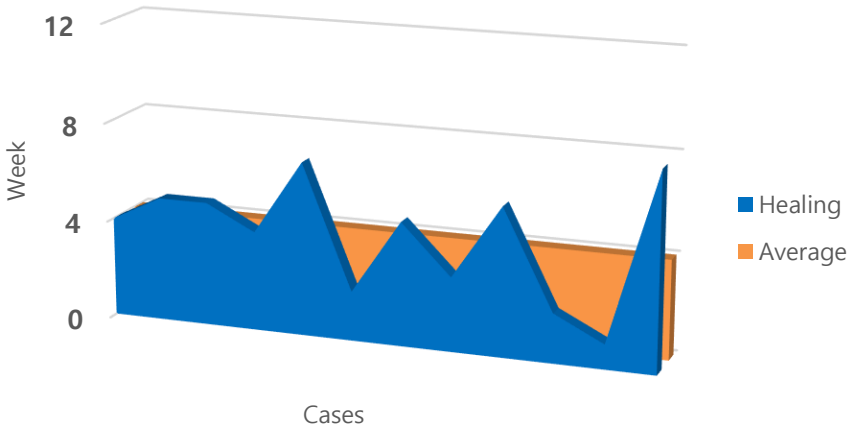
② Nano-fat processing with filters

③ Preparing bio-inks

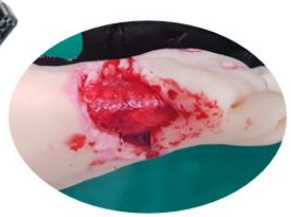
20 PATIENTS: 100% EPITHELIALIZATION

➔ **100% Epithelialization**

(Wound Healing Rate: Ave. 4week)



INTEGRATED 3D SCAN-PRINT SYSTEM

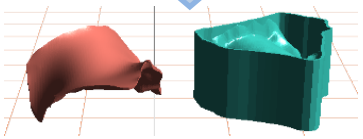


3D Scanning DFU affected area

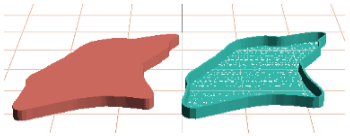
Visualizing and save 3D Scan Data
(Point, Meshing, Hole Filling)

Scanning Program

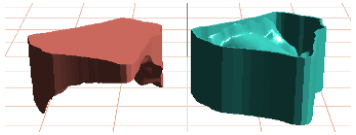
Pointing affected area by One-click
- Measuring printing time based on size, volume of affected area



① Curve Type



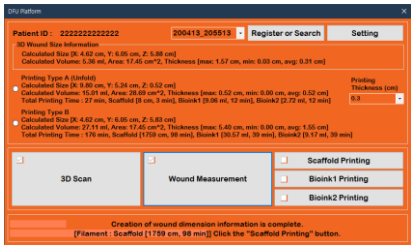
② Planar Type



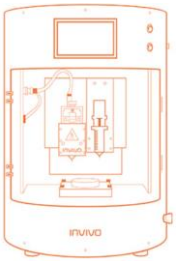
③ Flat Type

Automatic simulation of affected area and recovered simulation

Wound Recovery Program



All-in-one Program
(Scanner, Wound Recovery, Control DFU INVIVO
Volume, size, length of filamnet, Bioink)



DFU INVIVO

Scaffold Printing

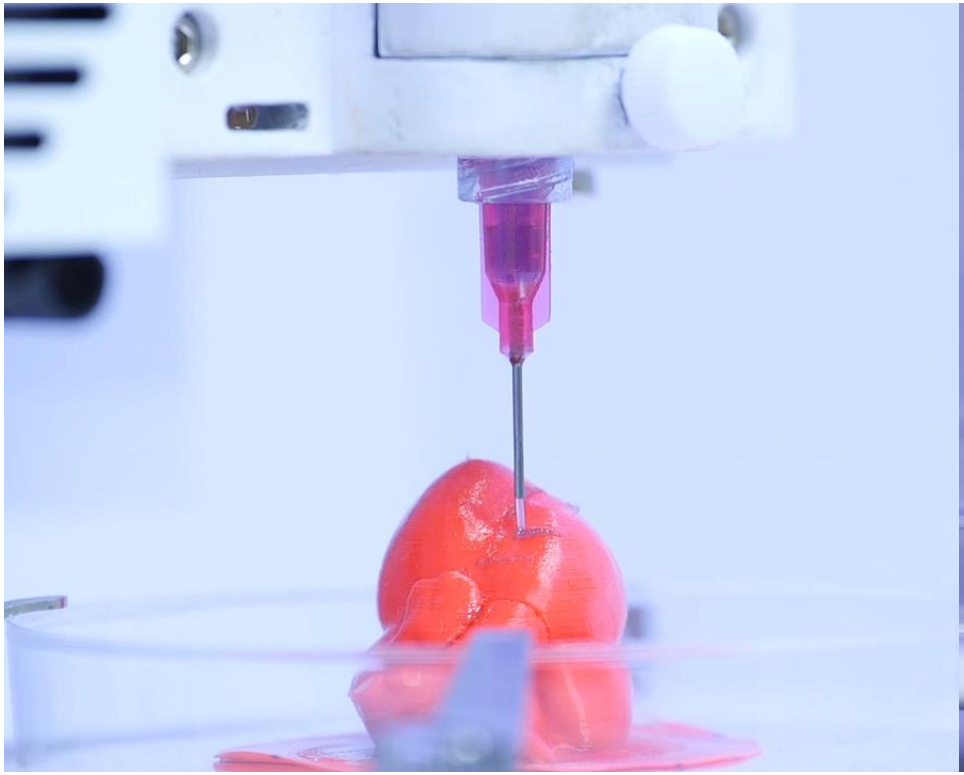
Bioink1 Printing

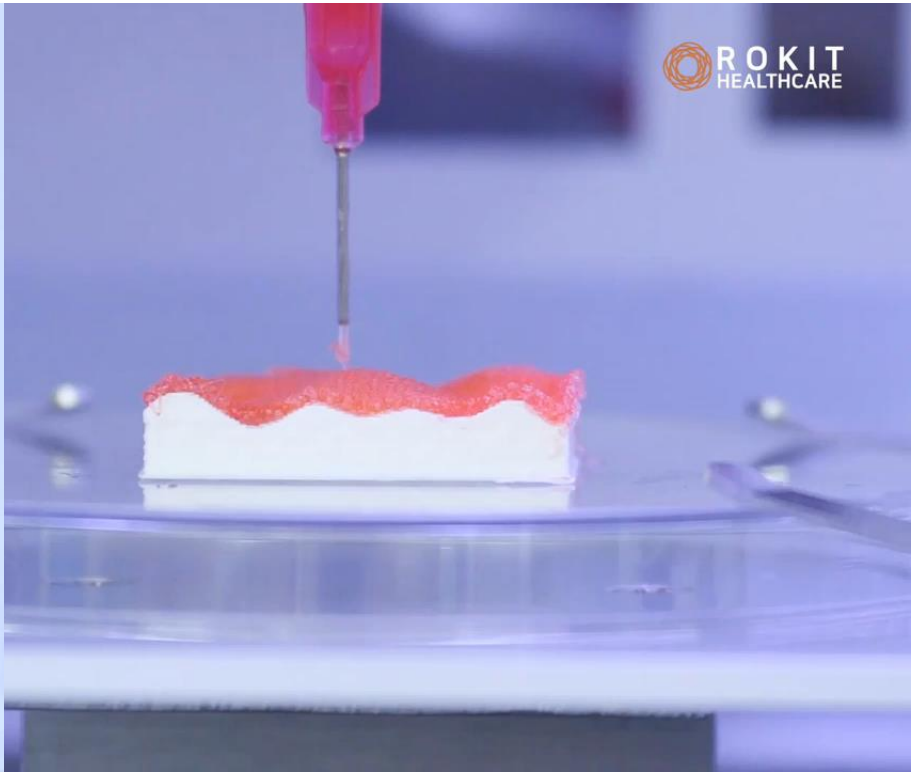
Bioink2 Printing

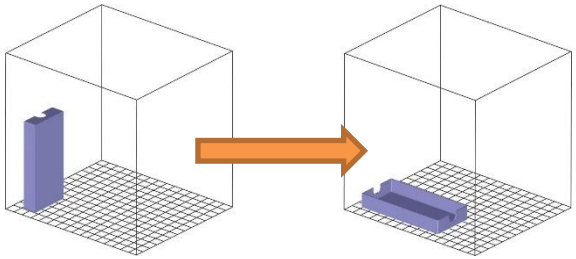
Printing

Application

In Situ – Curve Print System



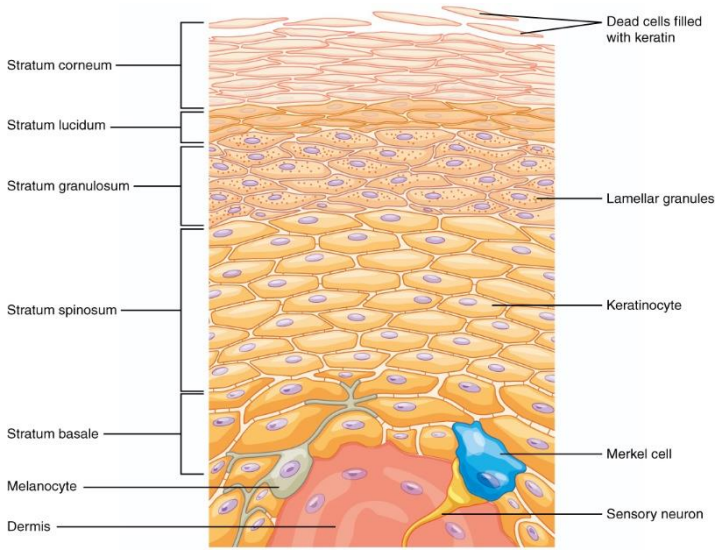




Dr. INVIVO 4D6
One-Touch
Horizontal -> Vertical Slicing

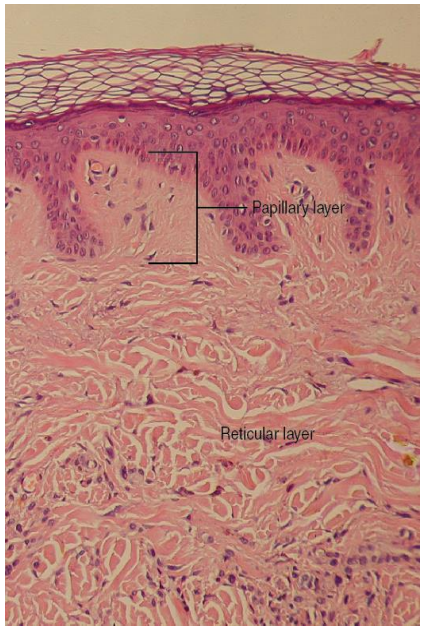
HUMAN SKIN EQUIVALENT (HSE): COMPOSITION

EPIDERMIS



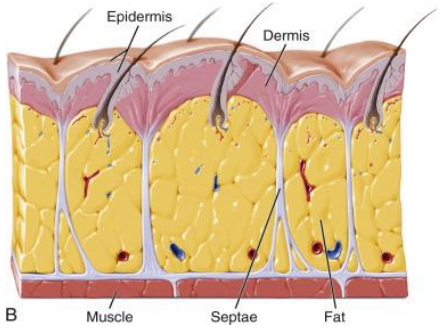
- Keratinocytes (90-95% of the components)
  - Corneocytes (terminally differentiated)
- Langerhans cells (immunological response)
- Merkel-Ranvier cells (sensory reception)
- Melanocytes (skin pigmentation, UV protection)

DERMIS



- Fibroblasts: majority, with dense fibers
- Collagen and elastin fibers: structure, tensile strength, and elasticity

HYPODERMIS



- Adipose cells: fat storage and provides insulation and cushioning

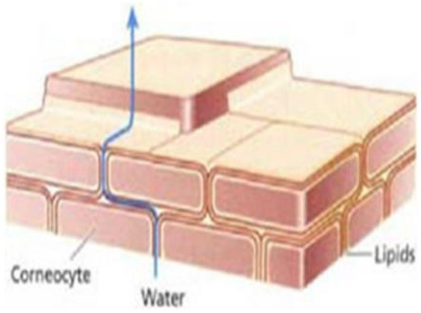
CRITERIA FOR SUCCESSFUL HSE

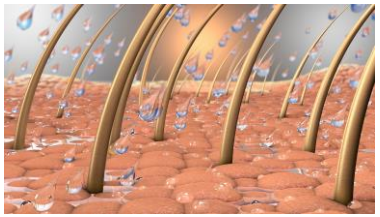
- Considerations of stratified cell crosstalk and signaling
- Considerations of diverse cell types (i.e. keratinocytes, fibroblasts, immune cells and bacteria)
- Considerations of human-to-human variations (age, race, hair, etc.)
- Adherence to regulatory guidelines for *in vitro* skin tests

APPLICATIONS OF THE HSE

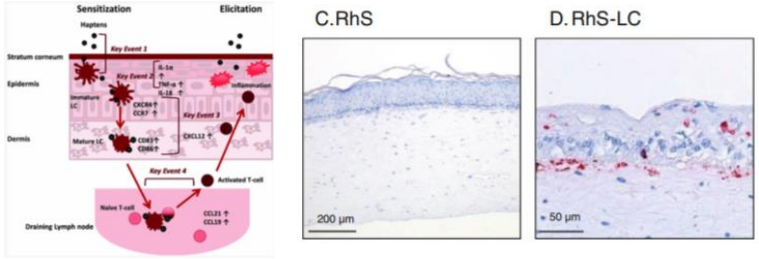
Corrosion / Irritation

Absorption / Penetration



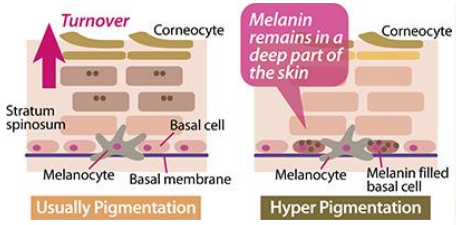


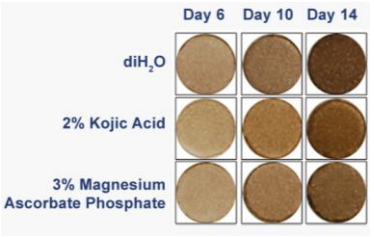
Sensitization



C. Rodrigues Neves and S. Gibbs, Curr Top Microbiol Immunol 2018

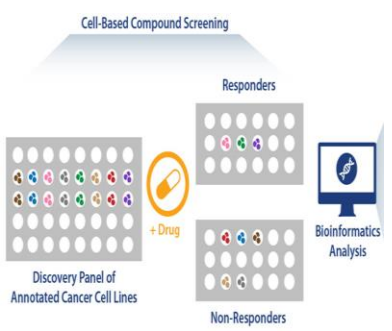
Lightening/Melanogenesis

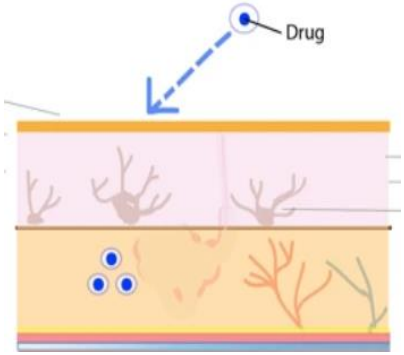




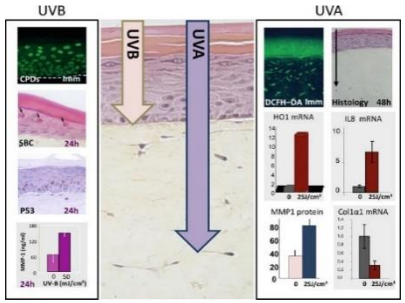
APPLICATIONS OF THE HSE

Drug Screening / Delivery

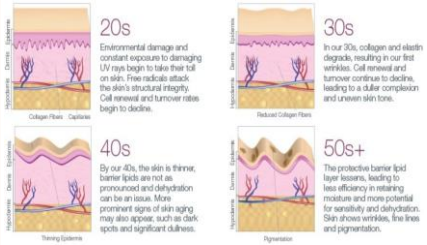




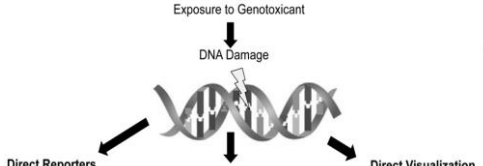
Phototoxicity / UV

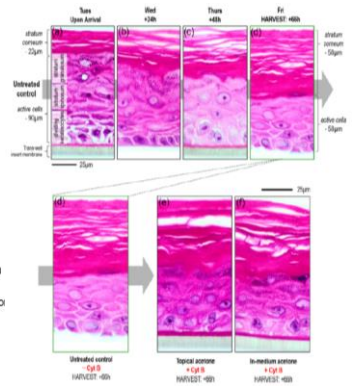


Anti-aging



Genotoxicity

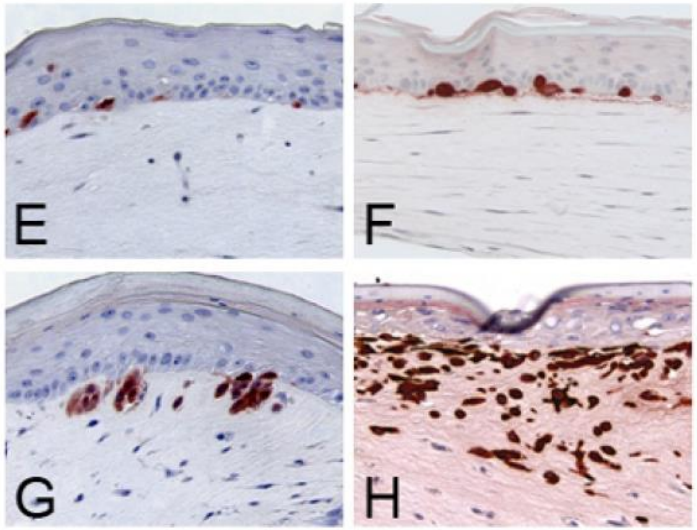




PUBLICATIONS USING HSE

RECONSTRUCTED SKIN-DISEASE MODELS

CANCER



Skin reconstructs of different stages of melanomas.
Metastatic melanoma cells grow in all directions and invade deep into the dermis as single cells or clusters.

WOUND HEALING

Figure 5: Wound healing process after injury with a laser

Wound healing process after injury with a laser.

FUTURE DIRECTION

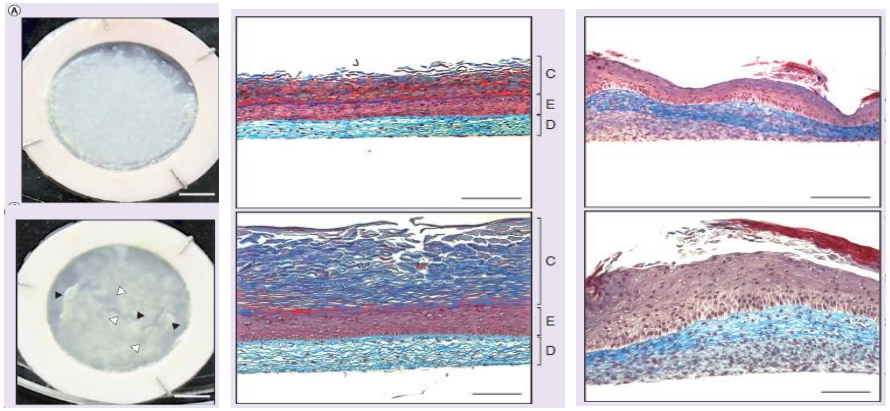
Skin reconstruct models bridge the gap between in vitro and in vivo studies. They will lead to a better understanding of which genes are involved in transformation and how stem cells contribute to that transformation.

J. Vis. Exp. (54), e2937, doi:10.3791/2937 (2011).

PUBLICATIONS USING HSE

RECONSTRUCTED SKIN-DISEASE MODELS

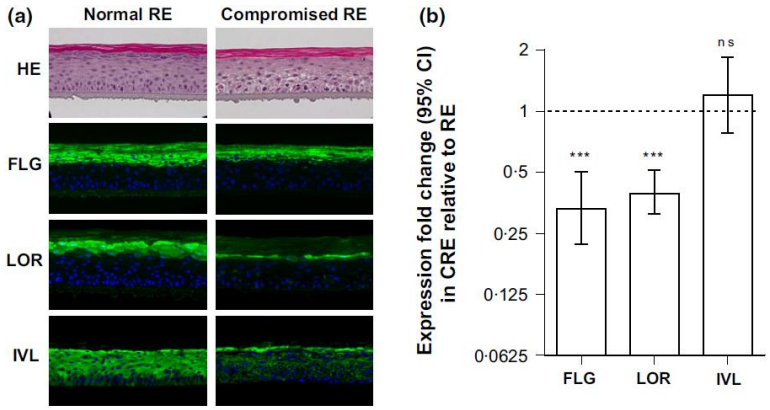
PSORIASIS



Macroscopic and histological analyses of the tissue -engineered psoriatic substitutes. the tissue-engineered psoriatic substitutes treated with cytokines display further irregular epidermis with protuberances and thinner region s, which is typical of psoriatic tissues.

Regen Med, 11 (6), 545-57 (2016)

ATOPIC DERMATITIS



Difference between normal RE and CRE model. The inflammatory cocktail alters the morphology and epidermal protein expression in reconstructe d epidermis.

FUTURE DIRECTION

Skin reconstruct models bridge the gap between in vitro and in vivo studies. They will lead to a better understanding of which genes are involved in transformation and how stem cells contribute to that transformation.

J. Vis. Exp. (54), e2937, doi:10.3791/2937 (2011).

PUBLICATIONS USING HSE

RECONSTRUCTED HUMAN SKIN VARIATION MODELS

AGING

Property characterization of reconstructed human epidermis equivalents, and performance as a skin irritation model

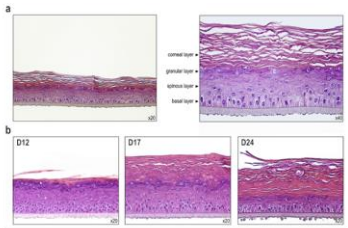
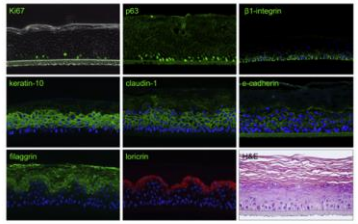


Fig1. Morphological study of human epidermis equivalent.



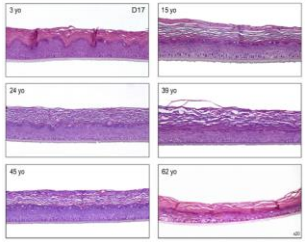


Fig2. Comparison of reconstructed human epidermises with keratinocytes isolated from donors of different ages (3, 15, 24, 39, 45 or 62 years old).

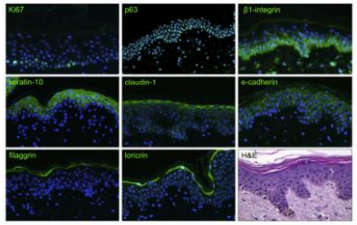


Fig3. Immunodetection of various markers in the model of epidermal equivalent (a) and in native skin (b).

- CONCLUSION

✓ The model presented in this study is a 3D reconstructed human epidermis model
developed from keratinocytes that were isolated from human skin.
✓ It is alternatively possible to select the age, the gender, or the ethnicity of the donors, and the differentiation stage of the tissue in order to develop specific models.

HAIR

Engineered building blocks to print endogenous tissues and complex organs in vitro

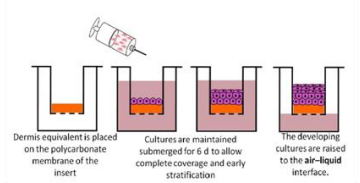


Fig1. Schematic procedure for human skin equivalent

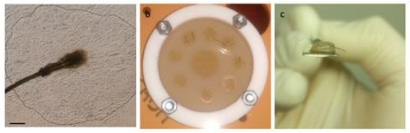


Fig2. hair implantation inside the maturation chamber (b) and 3D dermis equivalent with hair after 2 weeks of maturation time (c)

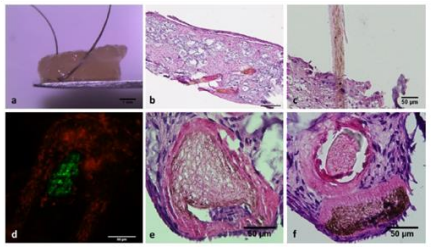


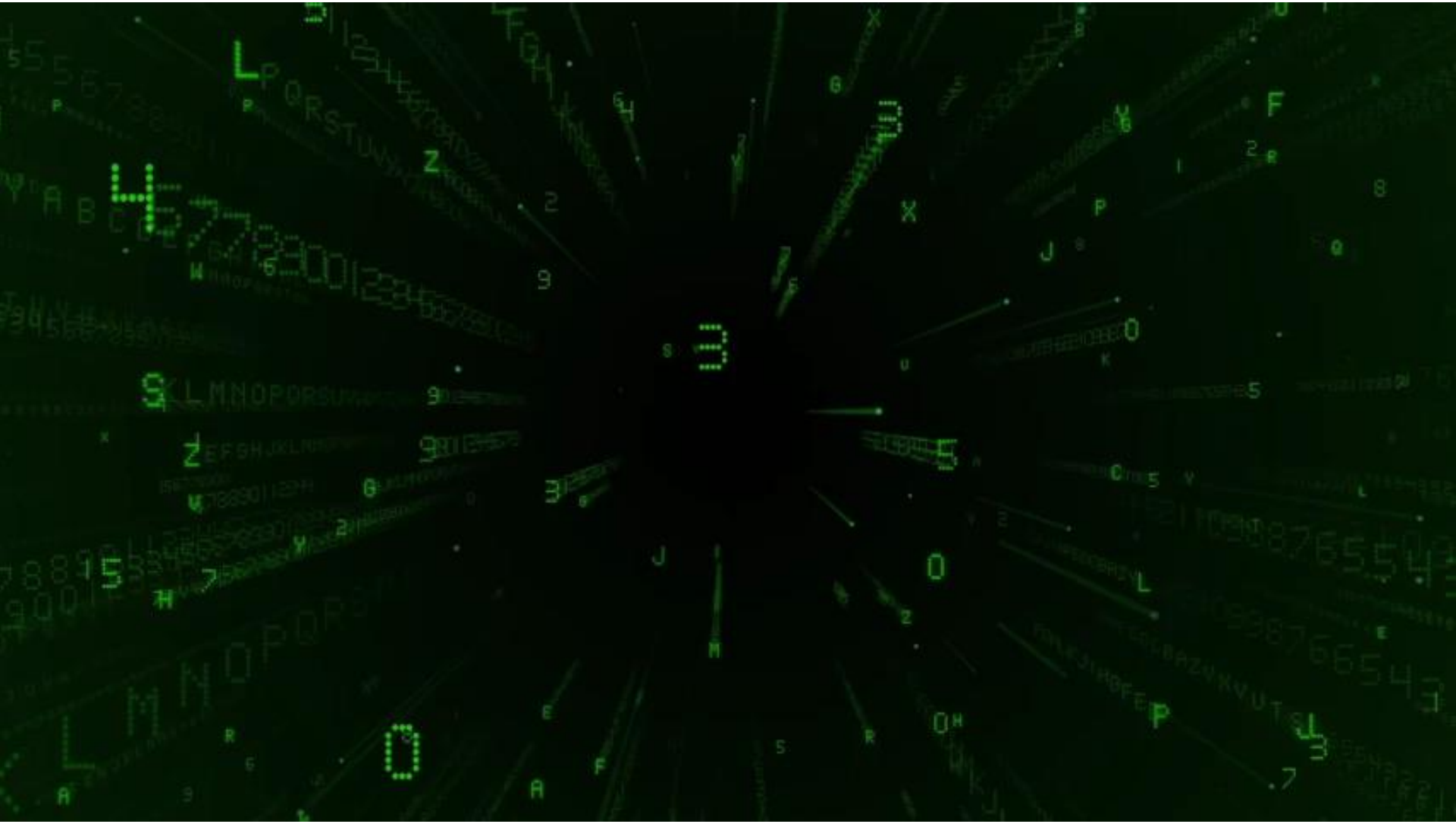
Fig3. biohybrid with exogenous hair (a); H&E staining of HSE with hair implantation (b-c); immunofluorescence analysis of hair follicle (Versican, CD133) (d); H&E staining of exogenous hair bulb (e-f).

Alessandro Garziano on 01 June 2016.
SCIENTIFIC REPORTS | (2018) 8:13434
Toxicology in Vitro(2018), S0887-2333(18)30355-2

- CONCLUSION

✓ The goal of this study is to develop skin substitutes with greater homology to native skin, in terms of dermis layer, extra cellular matrix, epidermal differentiated layer, and hair follicles.
✓ The introduction of anagen hairs in our 3D human skin equivalent, demonstrates the relevance of the endogenous ECM. The mature hair follicles are able to growth in several kind of conditions, fresh medium, conditioned medium, in exogenous ECM and fibroblasts

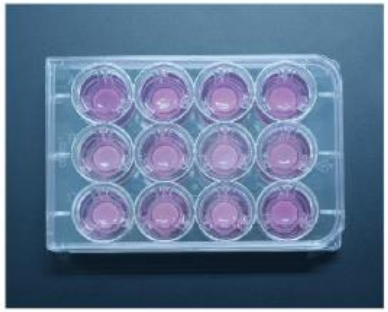
Age of On-Demand Research Solutions



EpiTem, The Human Skin On-Demand

EpiTem: 3D Bioprinted HSE

"I'd like to use ready-to-use 3D bioprinted human epidermis"



- *EpiTem: epidermis*
- *EpiTem FT: epidermis + dermis*

EpiTem Creator Kit

"I'd like to explore diverse human skin study designs on my own"

Bioprinter, Dr. INVIVO

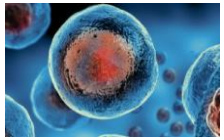
\+

Bioink KIT

\+

Media KIT

\+



Cell KIT

\+

Protocol

EpiTem Validation: Structural & Functional

Feature

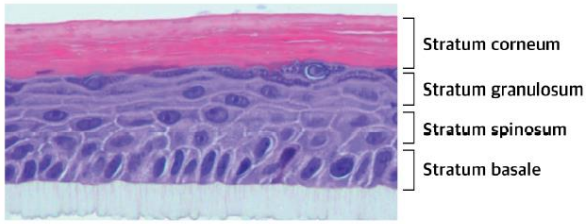


Fig 1. EpiTem had the multi-layered and highly differentiated epidermis.
Histological morphology is observed following H&E staining after 18 days of reconstruction and characterization of the localization proteins expression.

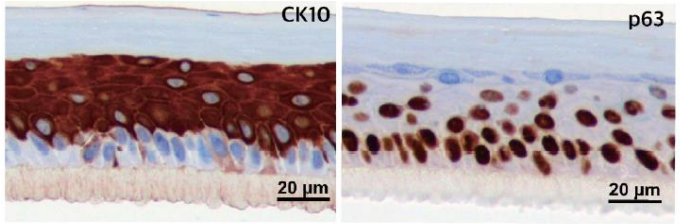


Fig 2. Immunohistochemistry results of (A) Cytokeratin10 and (B) p63.
IHC was performed by histological cross-sections of the EpiTem to characterize the expression localization of these proteins.

Barrier Function Test

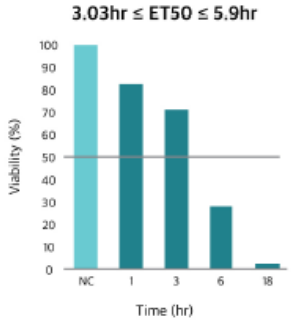


Fig 3. Barrier function result depends on time exposure.
Exposure Time 50 (ET50) corresponds to the time to observe 50% cell mortality after treatment of 1% Triton X-100 on EpiTem.

Irritation Test

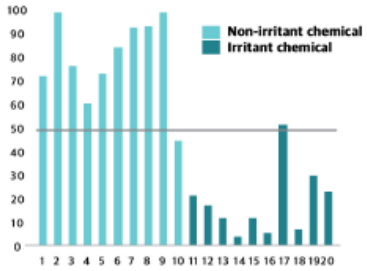


Fig 4. Irritation test results of 20 chemicals according to TG439 guidelines.
Non-irritation chemicals show over than 50% cell viability, but classified chemicals represent less than 50% viability.

OECD
BETTER POLICIES FOR BETTER LIVES

Discover EpiTem

Advanced, Customized Human Skin Equivalent

To be used in Cosmetic, Chemical & Pharmaceutical industry

BIOPRINTING: AUTOMATED, HUMAN, SMART SOLUTION

BIOPRINTING IS A PLATFORM BIO-MANUFACTURING TECHNOLOGY WITH APPLICATIONS FOR ALL MAJOR-FUNDED RESEARCH FIELDS

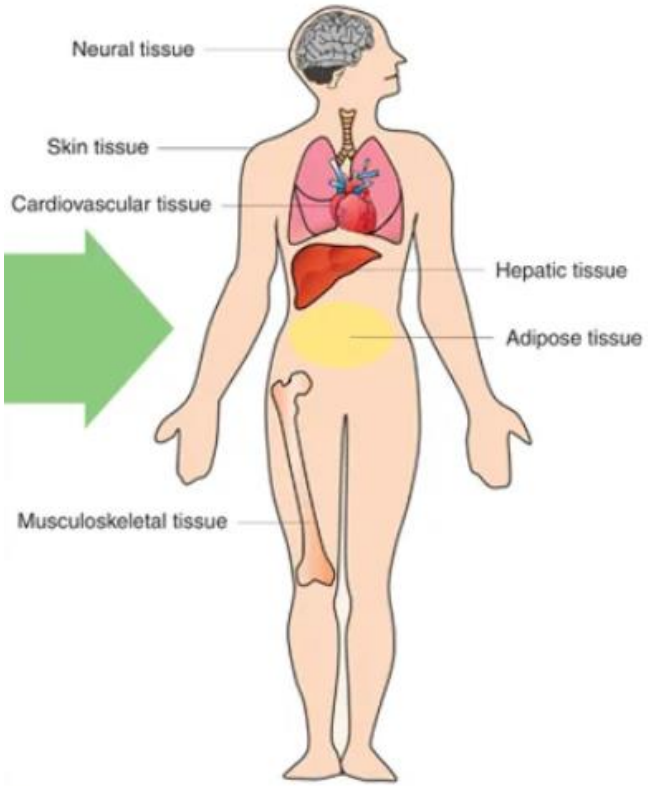
Organ systems	Cell Types used in 3D Bioprinting
Cardiovascular tissue	ESCs (8) MSCs(10, 18, 19) Cardiac progenitor cells (11, 12, 14) IPSC-derived cardiomyocytes (13, 20) Adipose-derived stromal vascular fraction cells (21) Myoblasts (15, 19)[a]
Musculoskeletal tissue	MSCs (36) MDSCs (35) Myoblasts (31, 32)[a]
Neural tissue	ESCs(41, 42) MSCs (7, 43) Glioma stem cells (40) NSCs(39, 46, 47)
Hepatic tissue	iPSC-derived (51, 54)or ESC-derived(54) hepatocyte-like cells iPSC-derived hepatic progenitor cells (57) ADSCs (57, 59) Hepatocellular carcinoma cells (52)[a]
Adipose tissue	ADSCs(61)
Skin tissue	AFSCs (65) MSCs (65, 66, 67, 70) ADSCs (70) Epithelial progenitor cells (69)

CELLS

Bioink polymers	Bioink concentration	Applications	
Collagen	0.223%	Perfusable artificial tissue	
Collagen/alginate	15 mg/mL collagen 0.1 g/mL alginate	Cartilage TE	
Gelatin	10–20%	TE, stem cell, & canc	
Gelatin/ Agarose	0.06%	Heart valve regenera	
Gelatin with transglutaminase (TG)	5% (w/v) with 2.5–20 un TG/g of gelatin	Tumor modeling & r medicine.	
Hyaluronic acid (HA) with gelatin	0.5% (w/v) HA-Ph, 3.0% (w/) Gelatin-Ph, Ru(II) & SPS	& regenerati edicine	
HA with methylcellulose (MC)	2.0 wt% HA, 5–9 wt% of MC	TE	
Fibrin	10 mg/ml, 20 U/ml	Nerve TE	
Silk/PEG	5–10% w/v	TE	[97, 122]
Decellularized the adipose (adECM), cartilage (cdECM) & heart (hdECM)	3%	TE, in vitro drug screening & tiss ue/cancer model.	[100, 103, 122]
HdECM with vitamin B2 & VEGF	20 mg/Ml	Cardiac TE	

BIOINKS

MANUFACTURED HUMAN TISSUES



- Source: Ong, C. S. (2017). 3D bioprinting using stem cells. *Nature.* & Gopinathan, J. (2018). Recent trends in bioinks for 3D printing. *Biomaterials.*

Thank You for Pioneering with Us

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Organ Regeneration Platform Company

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4D "Regenerator" Human Cell-Based BioInks Clinical Product Development

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