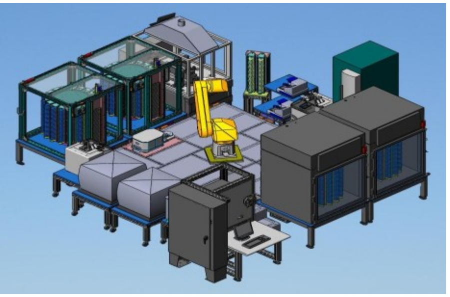
High-throughput screening to advance *in vitro* toxicology

Anton Simeonov, Ph.D.

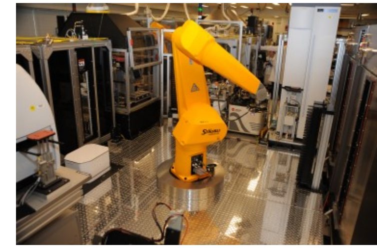
Scientific Director, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH)

Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology

November 15, 2021



The Tox21 Screening Project



Collection of diverse chemicals

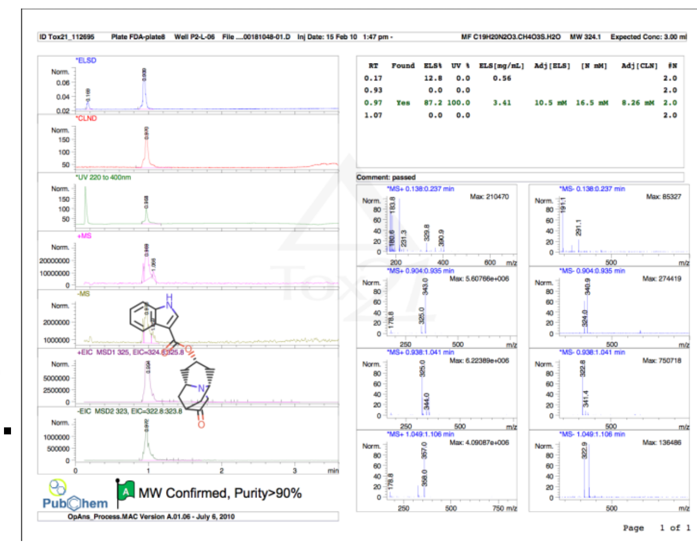
In vitro test methods, screening

High quality bioactivity data

Predictive models

(of bioactivity of a new chemical *in vitro* and, one day, *in vivo*)

>70 screening campaigns of the 10K Collection



Tox21 10K Chemical Collection: ~10,000 chemicals (nominated and procured by EPA, NIEHS, and NCATS) comprising approved drugs, failed drugs, pesticides, industrial chemicals, etc. Extensive Quality Control →

- Deposition into the public domain of the largest-ever toxicology dataset (100M datapoints), >100 publications.
- Using crowdsourcing to move from data to knowledge.

National Center for Advancing Translational Sciences

Tox21 10K Compound Library

EPA

- ToxCast I and II compounds
- Antimicrobial Registration Program
- Endocrine Disruptor Screening Program
- OECD Molecular Screening Working Group
- FDA Drug Induced Liver Injury Project
- Failed Drugs

NTP

- NTP-studied compounds
- NTP nominations and related compounds
- NICEATM/ICCVAM reference compounds for regulatory tests
- External collaborators (e.g., Silent Spring Institute, U.S. Army Public Health Command)
- Formulated mixtures

NCATS

- Approved Drugs
- Investigational Drugs

- *88 internal standards.*
- *Three library replicates.*
- *Each sample arrayed in 15 doses.*

The Tox21 10K Compound Library: Collaborative Chemistry Advancing Toxicology

Ann M. Richard,* Ruili Huang, Suramya Waidyanatha, Paul Shinn, Bradley J. Collins, Inthirany Thillainadarajah, Christopher M. Grulke, Antony J. Williams, Ryan R. Lougee, Richard S. Judson, Keith A. Houck, Mahmoud Shobair, Chihae Yang, James F. Rathman, Adam Yasgar, Suzanne C. Fitzpatrick, Anton Simeonov, Russell S. Thomas, Kevin M. Crofton, Richard S. Paules, John R. Bucher, Christopher P. Austin, Robert J. Kavlock, and Raymond R. Tice

Cite This: *Chem. Res. Toxicol.* 2021, 34, 189–216

Read Online

Chem Res Toxicol 2021 34(2):189-216

National Center for Advancing Translational Sciences

Entire-Library QC Project

- Multi-year undertaking using a range of LC-/GC-MS and NMR methods.
- >10,000 analytical chromatograms in PDF format available through PubChem: http://www.ncbi.nlm.nih.gov/pcsubstance

Identification

Depositor-Supplied Synonyms

DOLASETRON MESYLATE
DSSTox_CID_26827
DSSTox_RID_81939
DSSTox_GSID_46827
Tox21_112695
NCGC00181048-01
CAS-115956-13-3

... see more options

Substance Information

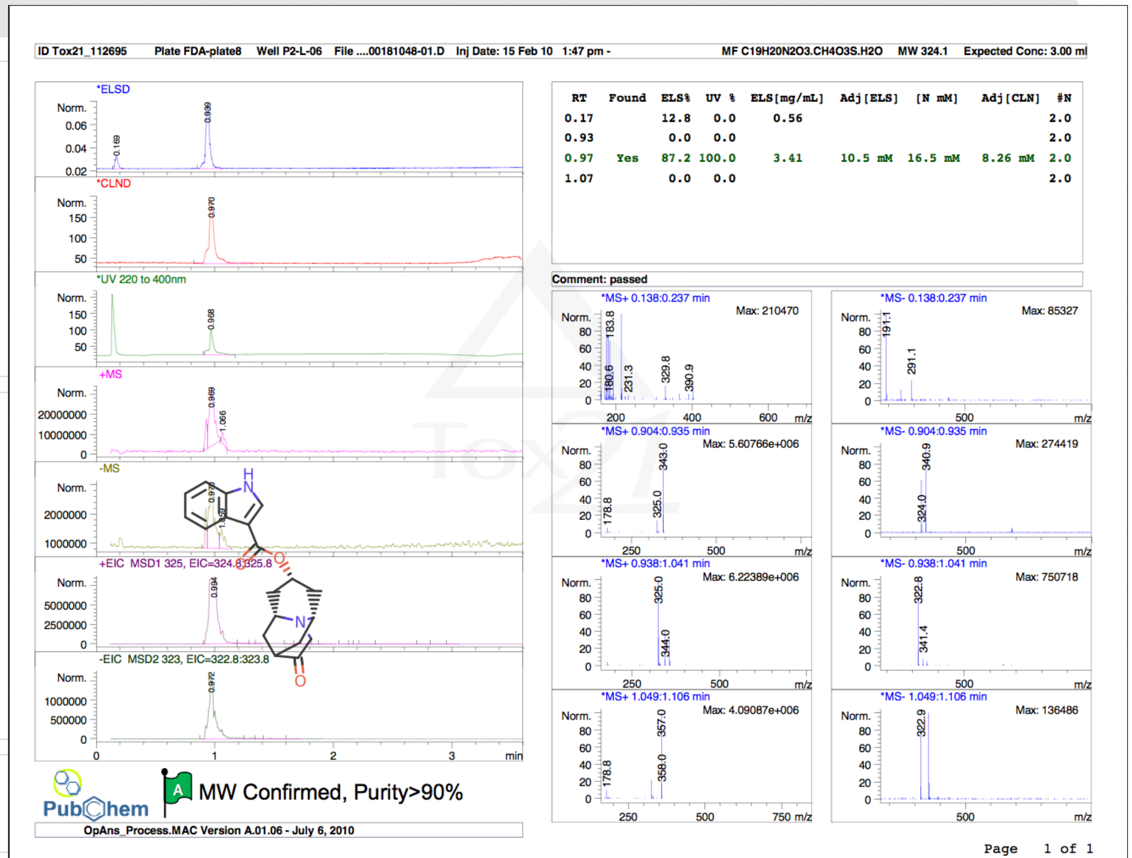
SID 144206248
Deposit Date: 2012-10-06
Modify Date: 2014-12-12
Substance Version: 2

Data Source:
Depositor: Tox21
External ID: NCGC00181048-01

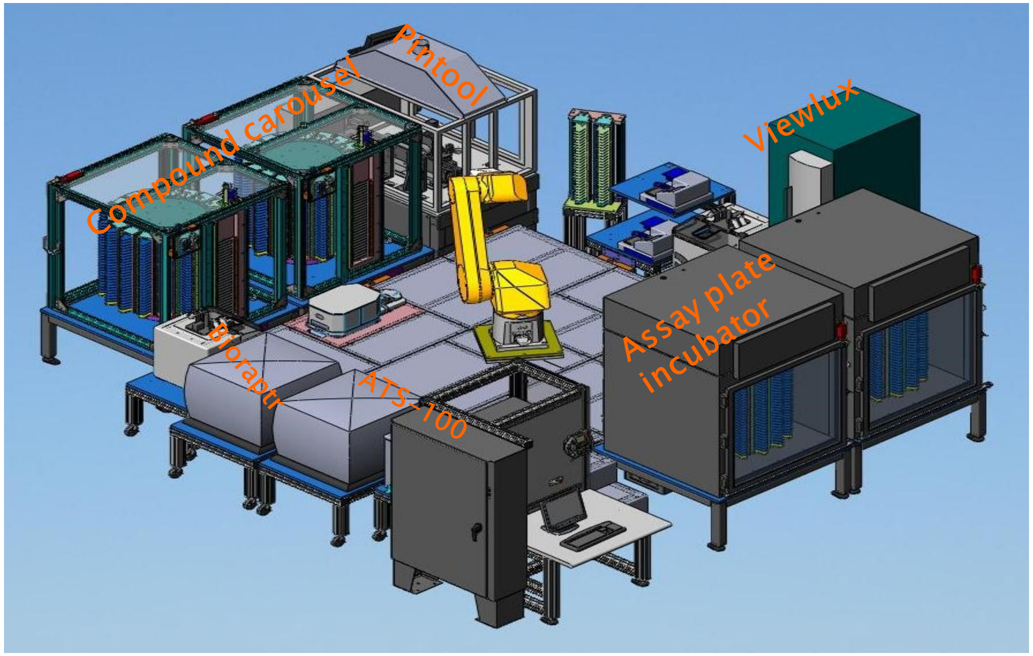
Compound CID: 3033817

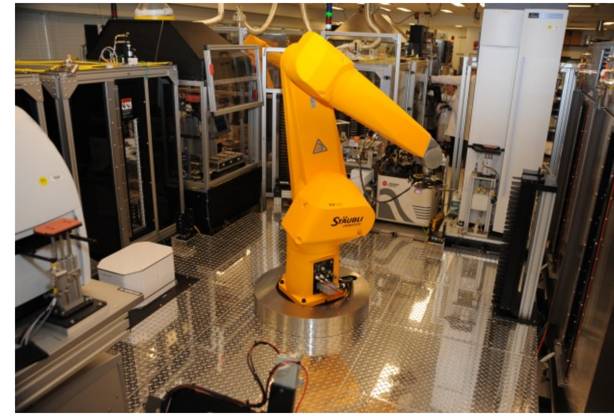
Depositor Comments

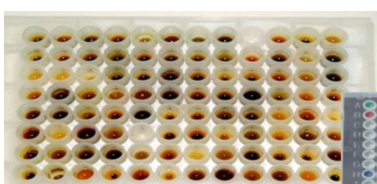
TOX21S_v5a



Tox21 Robot Platform

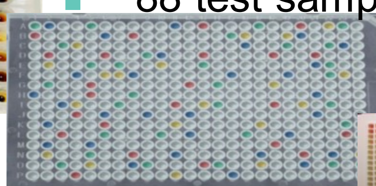






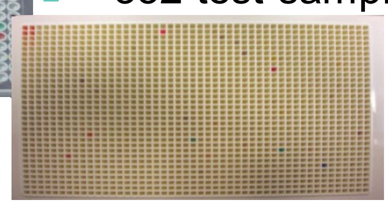
- 8 rows x 12 columns
- 88 test samples

96-well plate



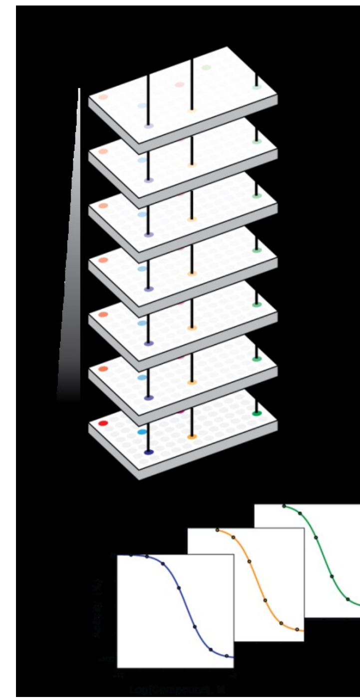
- 16 rows x 32 columns
- 352 test samples

384-well plate
4 x 96-well plates



- 32 rows x 48 columns
- 1,408 test samples

1536-well plate
16 x 96-well plates

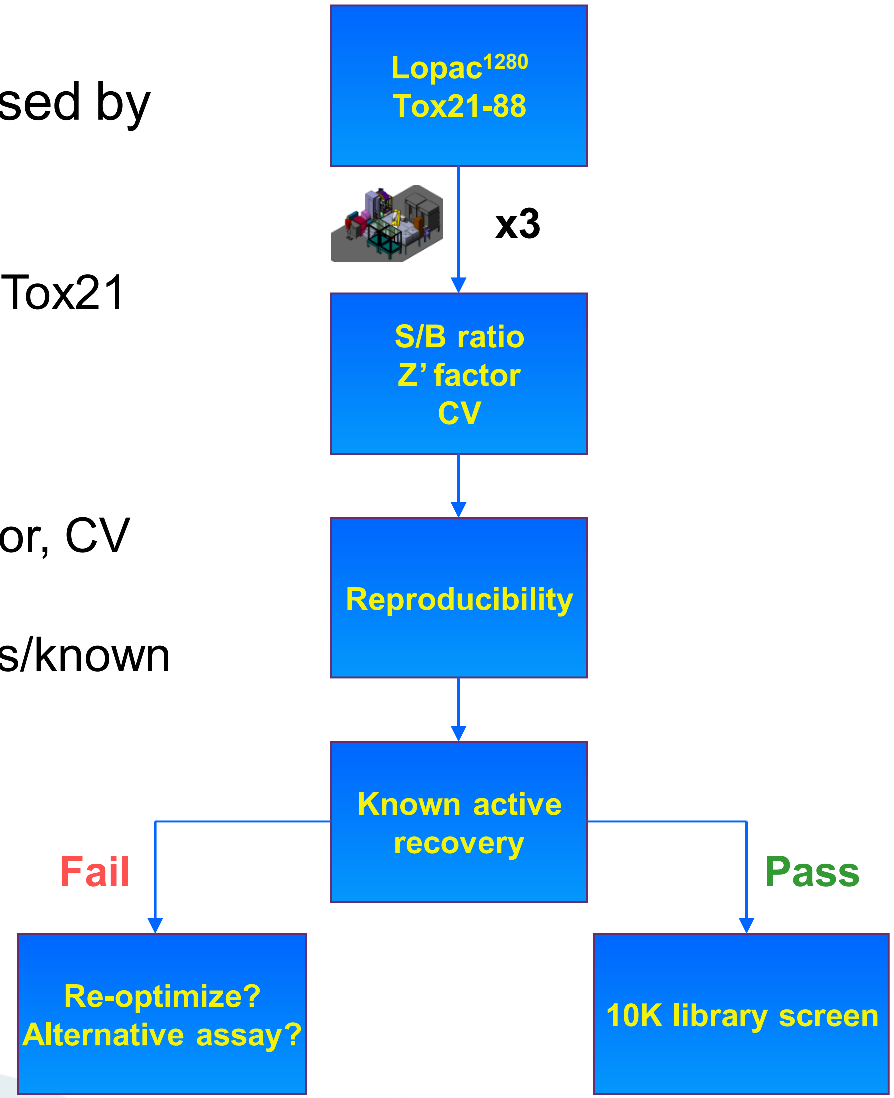


Dose-response-based screening
Proc Natl Acad Sci 103:11473

National Center for Advancing Translational Sciences

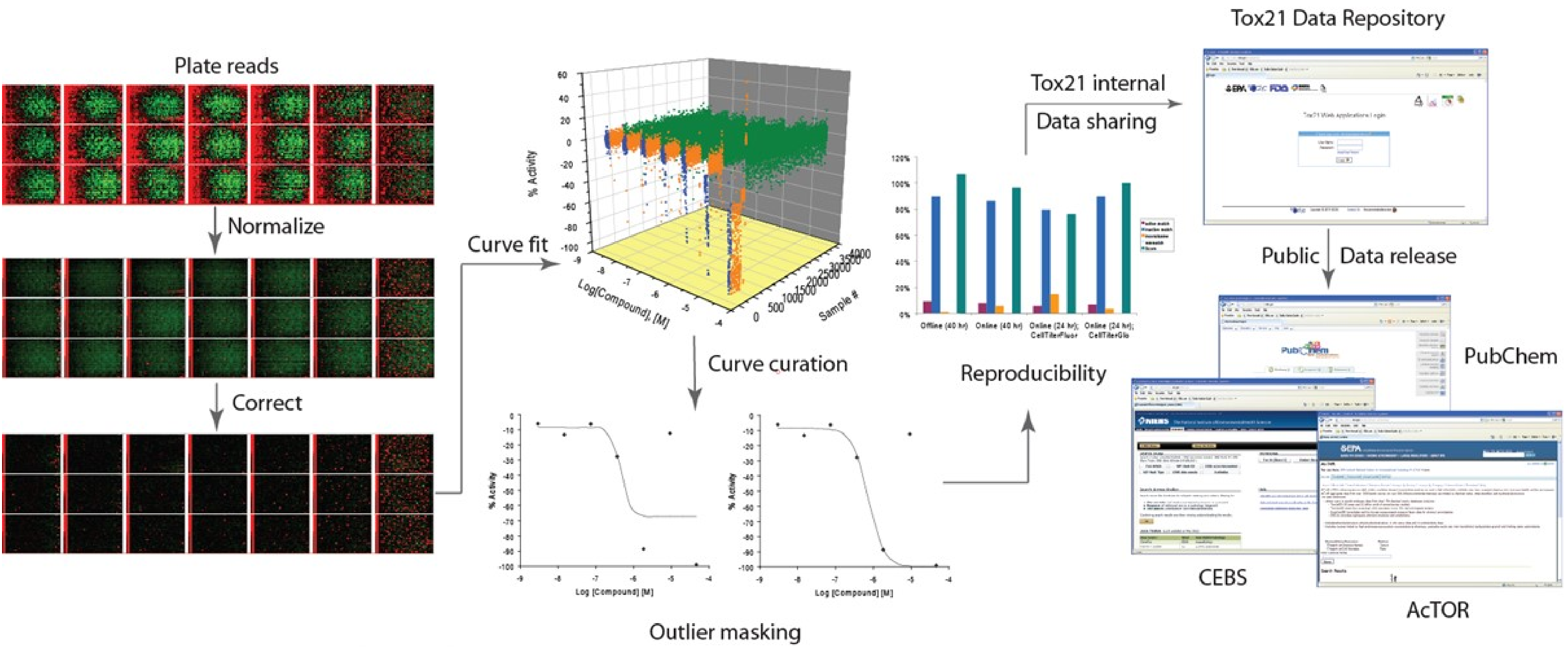
Assay Nomination and Validation Process

- Screening assay proposed and discussed by Assays and Pathways WG.
- Online validation on Tox21 Robot
  - Tox21 validation plate: Lopac^{1280} + 88 Tox21 replicates
  - Triplicate runs
- Acceptance criteria
  - Performance metrics: S/B ratio, Z' factor, CV
  - Reproducibility
  - Ability to recover reference compounds/known actives
- Pass
  - Proceed to 10K library screening
- Fail
  - Go back to optimization?
  - Select alternative assay?



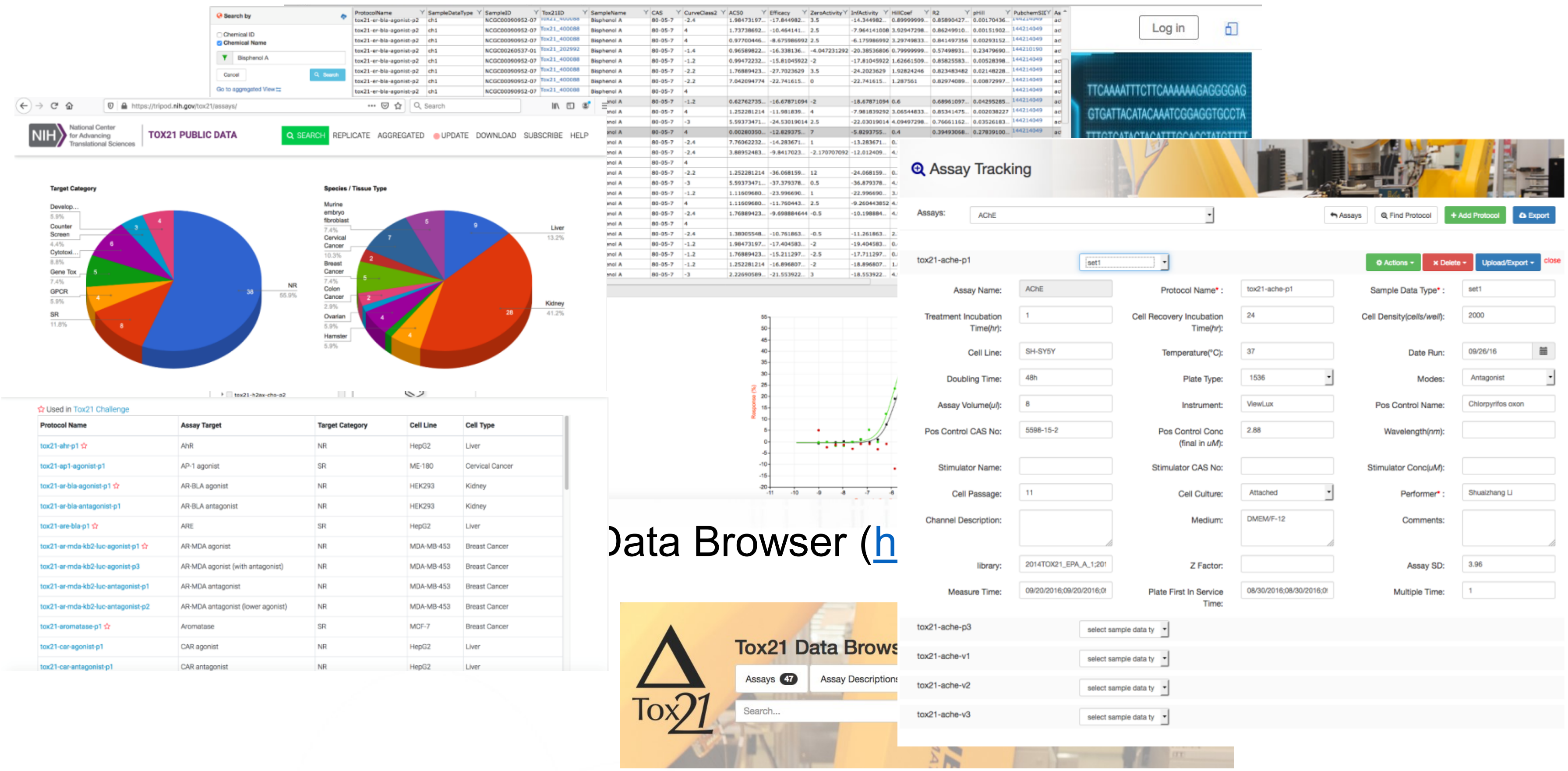
National Center for Advancing Translational Sciences

Informatics Analysis Process



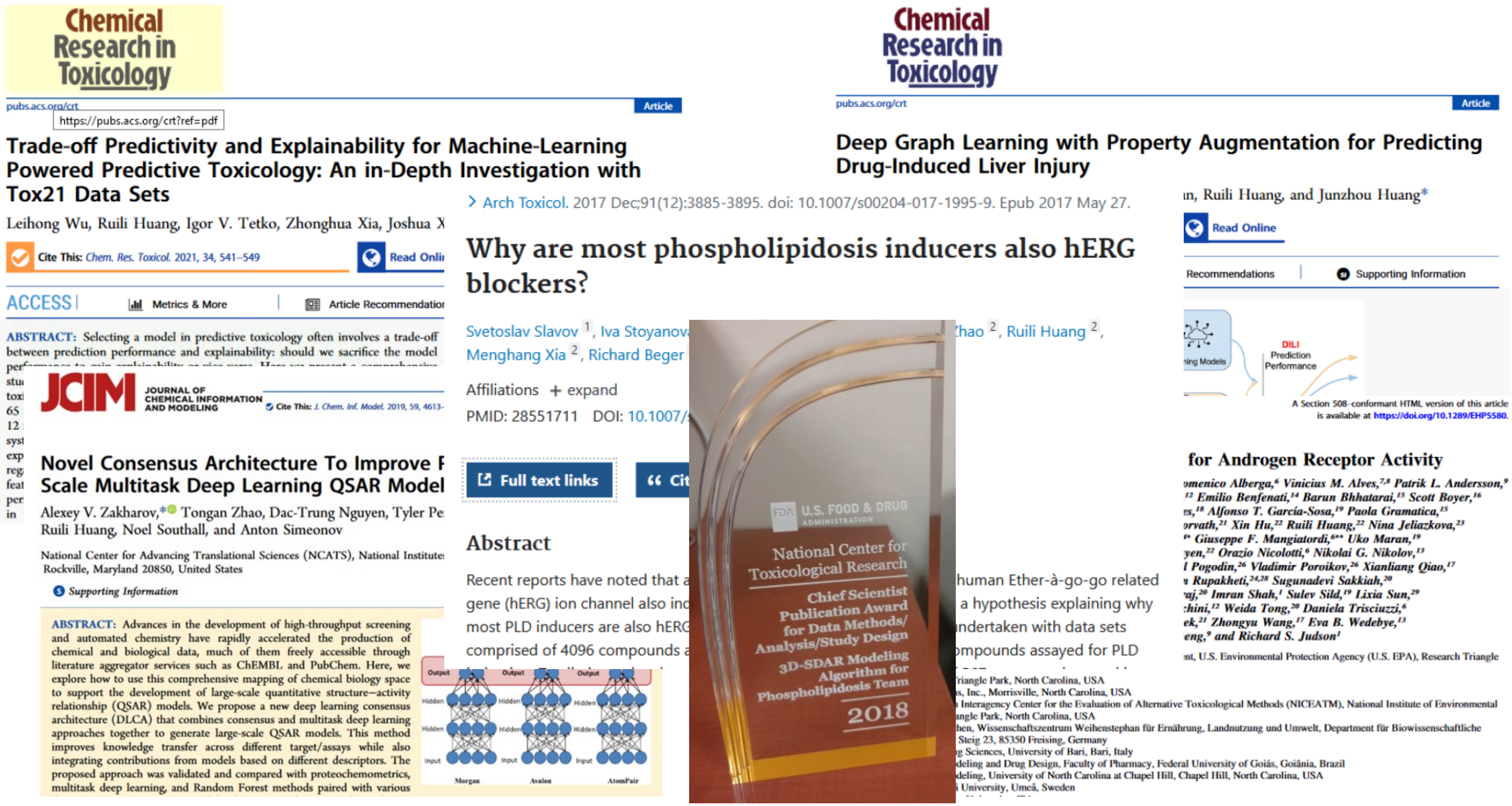
Tox21 Applications Gateway

http://tripod.nih.gov/tox/



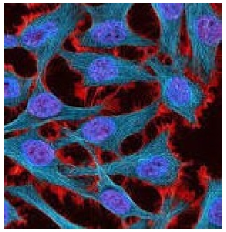
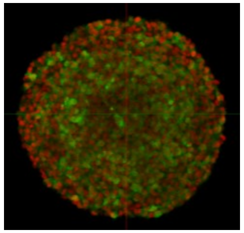
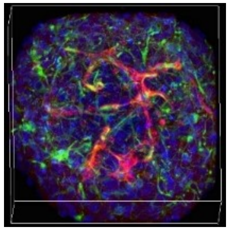
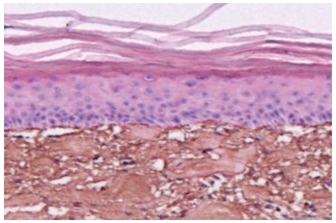
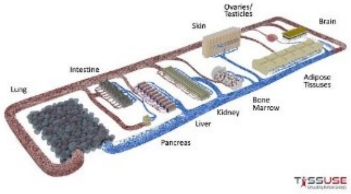
National Center
for Advancing
Translational Sciences

Predictive modeling with Tox21 data: collaborations with the scientific community

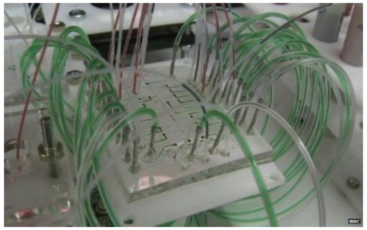


National Center for Advancing Translational Sciences

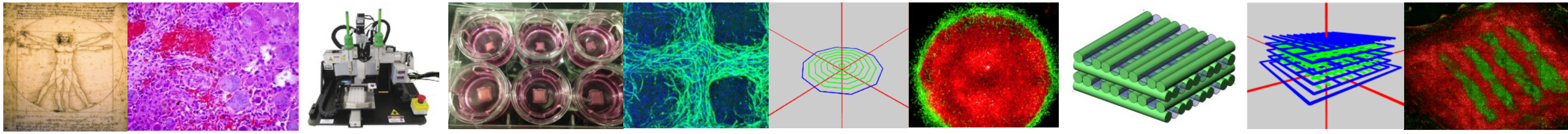
Increasing the predictivity of *in vitro* assays: a continuum of 3D models of healthy and diseased tissues

2D	Spheroids	Organoids	Printed Tissues	Organ-on-a-chip
				

Physiological complexity

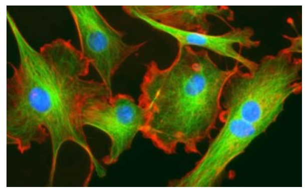
				

3D Tissue Bioprinting

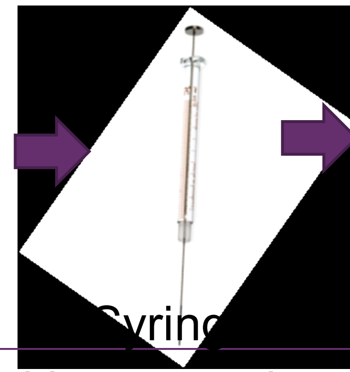


Gel

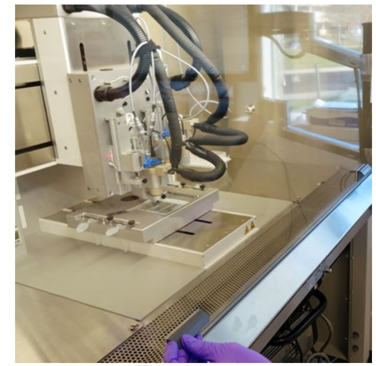
+



Cells

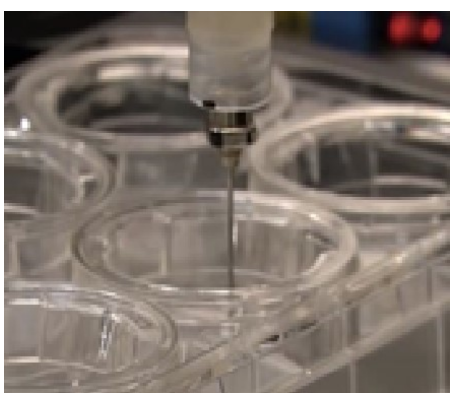


Syring

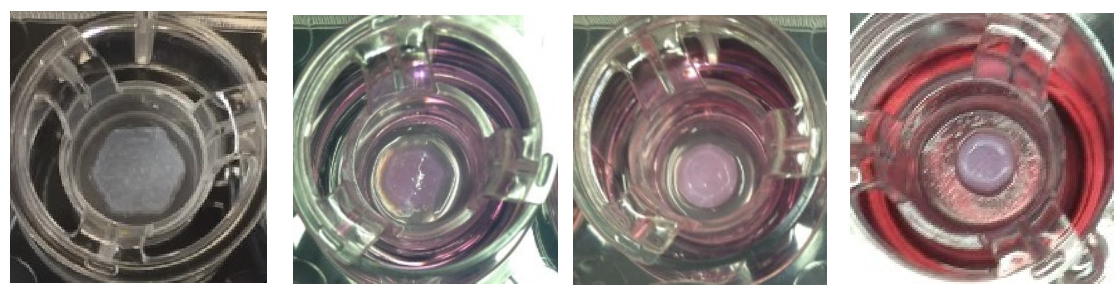


Printer

Hydrogel polymer is mixed with cells and loaded into syringe.



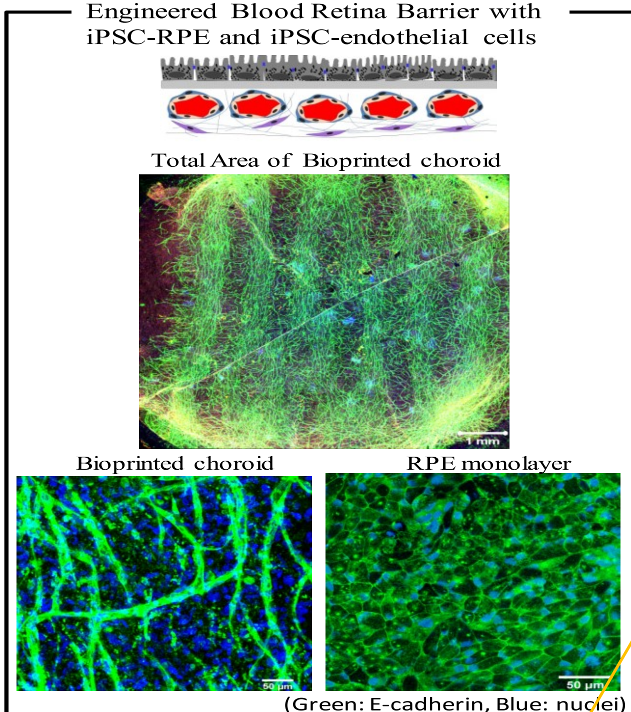
The printer "3D prints" the cell/gel mixture in a layer by layer approach.

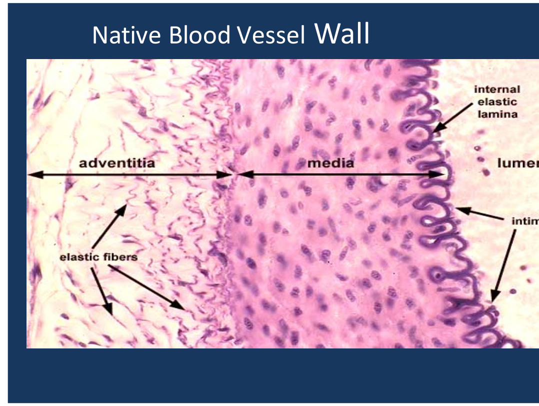


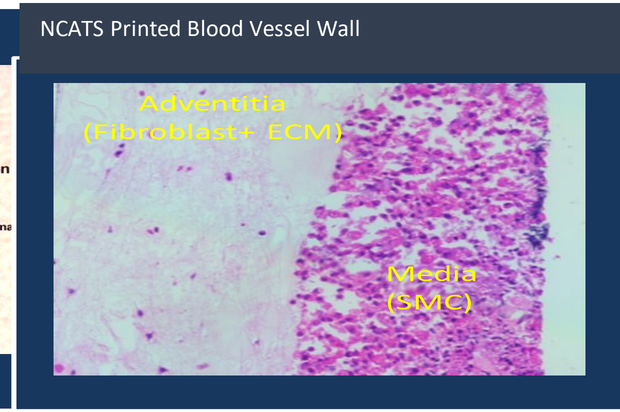
Printed construct | 1 day | 1 week | 2 weeks

The printed construct is incubated to allow the cells to form a tissue, and to enable proper cell differentiation.

Examples of 3D Bioprinting Projects

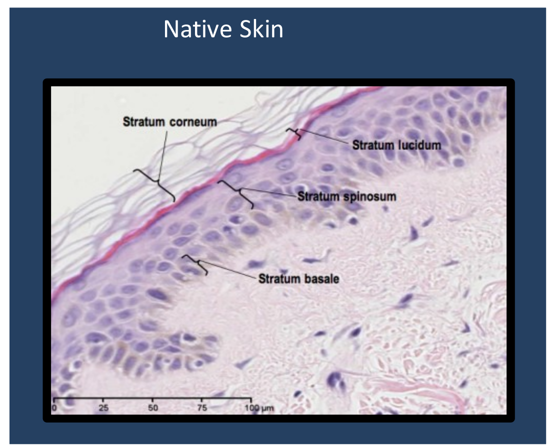


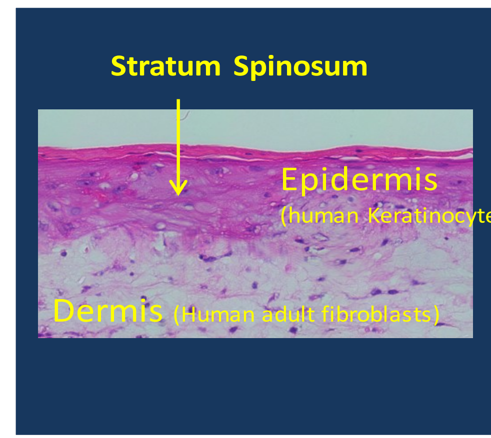




Blood vessel wall

Retina



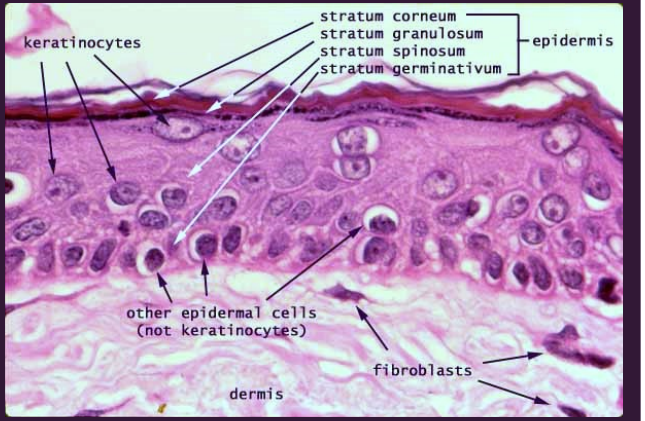


Skin

Recently initiated: lung and neuronal models for COVID-19 and pain, respectively.

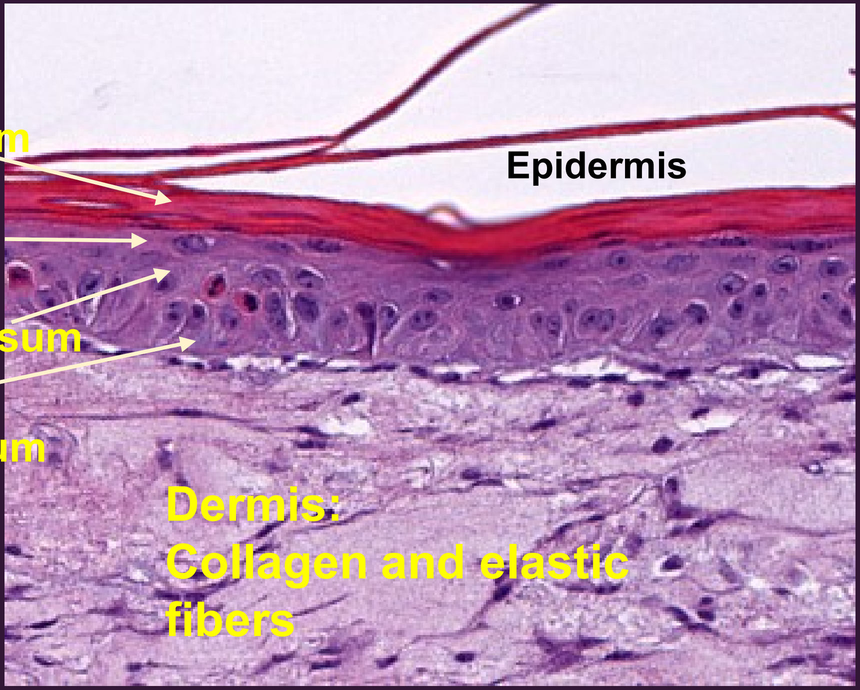
Skin biofabrication

Native Skin

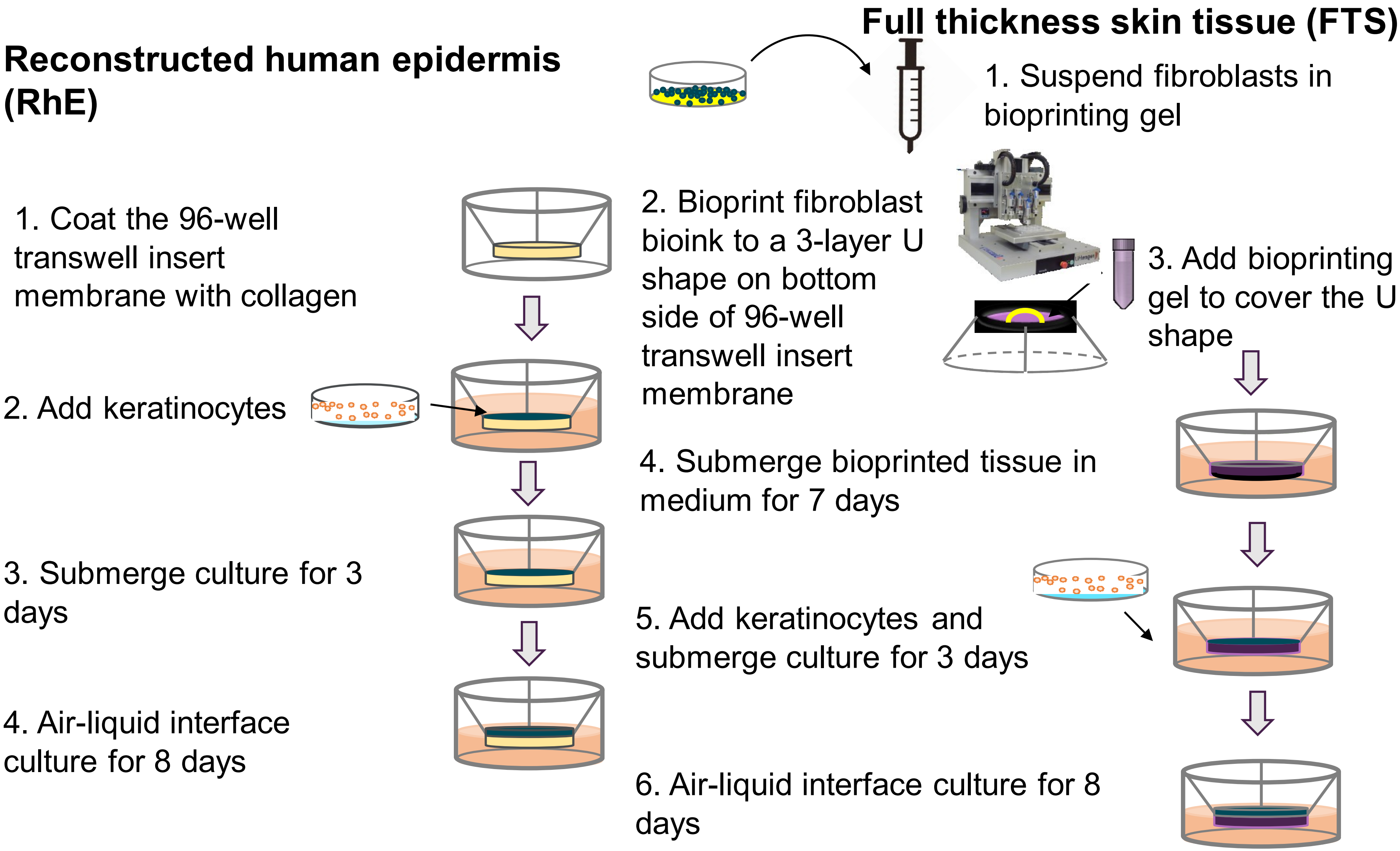


http://www.siumed.edu/~dking2/intro/IN005b.htm

3D-Bioprinted Skin



Generation of bioprinted skin tissues to test for irritants and sensitizers

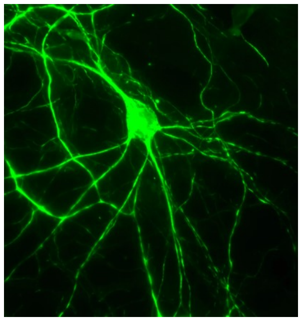


Z Wei, et al., Frontiers in Bioengineering and Biotechnology (2020)

NIH National Center for Advancing Translational Sciences

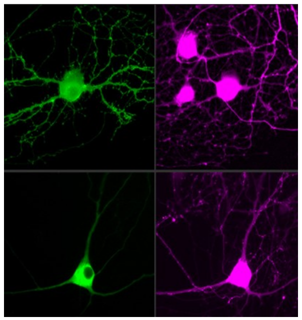
Enabling advanced 3D models through stem cell technologies

NCATS Stem Cell Translation Laboratory:



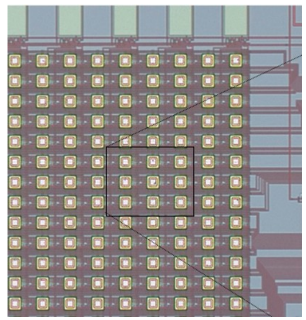
Access to relevant human cell types

Sensory neurons (nociceptors) and other neuronal subtypes, hepatocytes, etc.



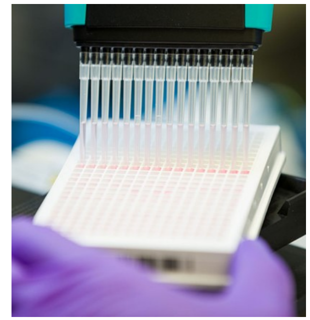
Advanced imaging technologies for functional cell characterization

High-content confocal, calcium imaging, optogenetics



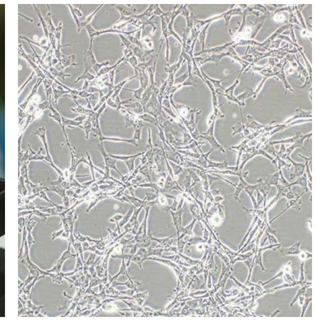
High-throughput electrophysiology methods

High-density multi-electrode arrays 26,400 electrodes/well



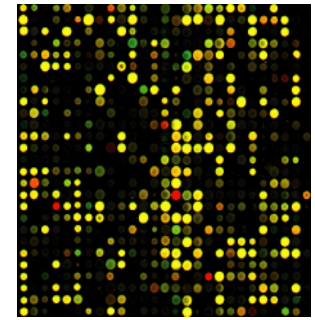
Measurement of signaling pathways, metabolism & specific targets

Cyclic AMP, PKA activity, CREB phosphorylation, energy metabolism



Longitudinal tracking of cell behavior

Multiple measurements over days, weeks or months



Combined single-cell transcriptomic & proteomic analyses

Drug response in individual cells

Example: a cheap 4-molecule cocktail that dramatically enhances survival of hPSCs and facilitates single-cell cloning, cell thawing and passaging, and CRISPR applications. Chen, *et al*, *Nature Methods* (2021)

National Center for Advancing Translational Sciences

Where do I go for more information about assay development and screening?

Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)

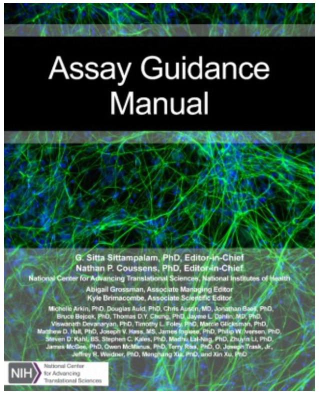


Table of Contents

Website: https://ncats.nih.gov/expertise/preclinical/agm

Email us: NCATS_AGM_Editors@mail.nih.gov

Facebook: www.facebook.com/assayguide

Linkedin: www.linkedin.com/groups/7437344

https://ncats.nih.gov/agm-video

August 7th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Strategies for Assay Selection & Robust Biochemical Assays**
3. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
4. Trask, OJ: **Assay Development Considerations for High Content Imaging**
5. Auld, DS: **Studies in Mechanisms and Methods in Assay Interferences**
6. Dahlin, JL: **Assay Interference by Chemical Reactivity**
7. Chung, TDY: **Basic Assay Statistics, Data Analysis & Rules of Thumb**
8. Devanarayan, V: **Reproducibility & Differentiability of Potency Results**
9. Sittampalam, GS: **Avoiding Artifacts & Interferences in Assay Operations**

March 26-27th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Robust Assays Define Success in Preclinical Research**
3. Lal-Nag, M: **Target Identification & Validation in Translational Discovery**
4. Foley, TL: **Development & Validation of Cell-Based and Biochemical Assays**
5. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
6. Trask, OJ: **Assay Development for HCS & Best Practices for 3D HCS**
7. Roth, KD: **Mass Spectrometry for Drug Screening and Lead Optimization**
8. Dahlin, JL: **Bioassay Interference by Aggregation and Chemical Reactivity**
9. Patnaik, S: **Lead Selection and Optimization by Medicinal Chemistry**
10. Xia, M: ***In Vitro* Toxicological Testing Using a qHTS Platform**
11. Xu, X: ***In Vitro* Assessment of ADME Properties of Lead Compounds**
12. Kahl, SD: **Statistical Design of Experiments for Assay Development**
13. Guha, R: **Pharos Application to Target Evaluation and Drug Discovery**
14. Weidner, JR: **Assay Operations: Keeping Assays Robust and Reproducible**

NIH National Center for Advancing Translational Sciences

Learn More About NCATS

Website: www.ncats.nih.gov

Facebook: facebook.com/ncats.nih.gov

Twitter: twitter.com/ncats_nih_gov

YouTube: youtube.com/user/ncatsmedia

E-Newsletter: ncats.nih.gov/news-and-events/e-news/e-news.html

Email us: info@ncats.nih.gov