

4D CELL CULTURE SYSTEMS

Reproducible, scalable, validated and real-time

Professor Sally McArthur

MANUFACTURING AND PROBING BIOSYSTEMS FUTURE SCIENCE PLATFORM

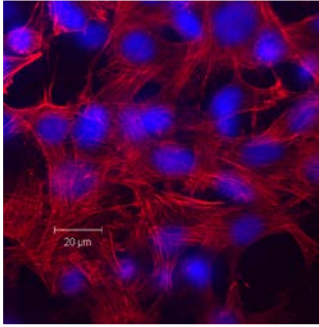
www.csiro.au



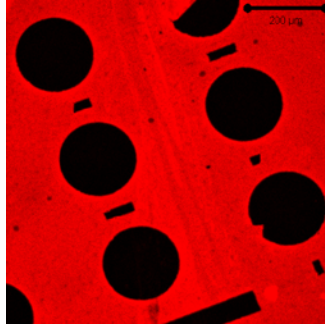
CSIRO Research+ Science Leader



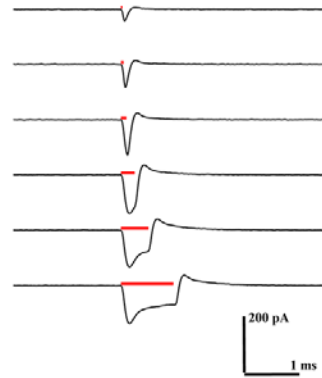
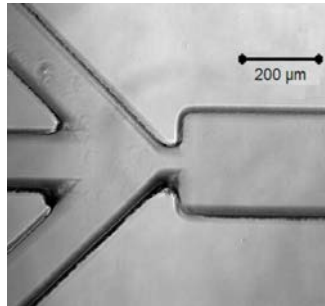
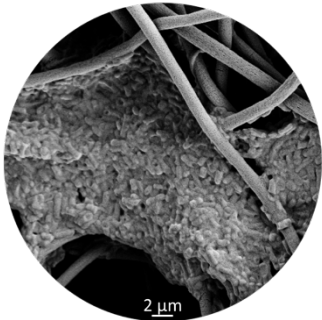
Biointerface Engineering – connecting biology & technology



Proteins, lipids, cells and bacteria on biomaterials



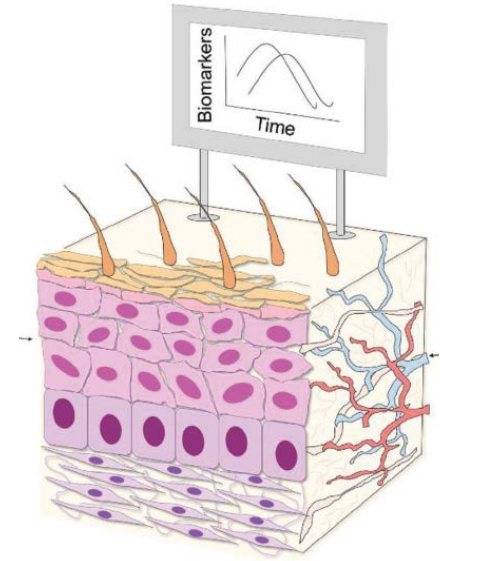
Bioarrays, microfluidics



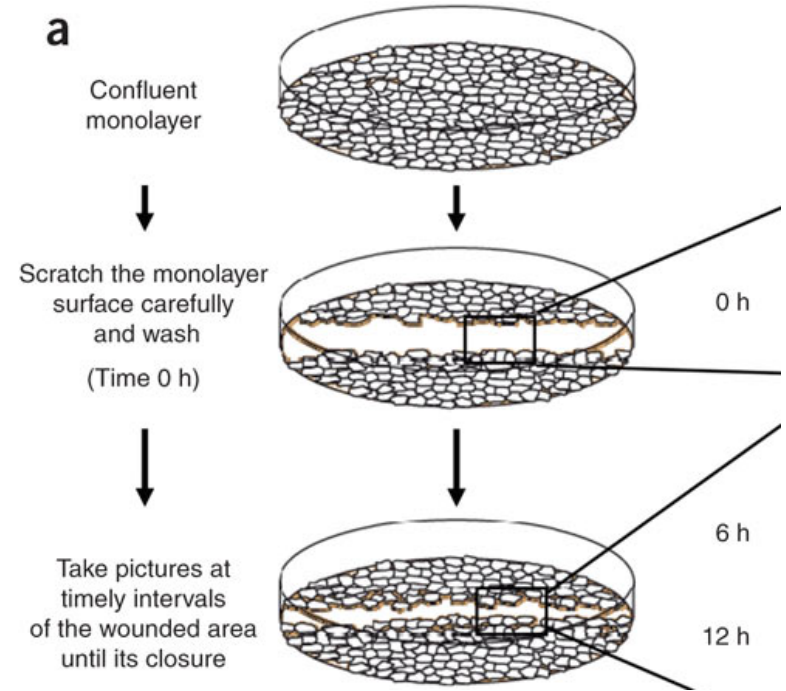
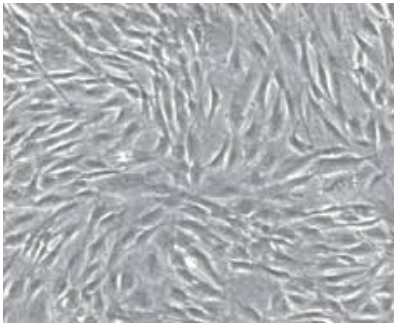
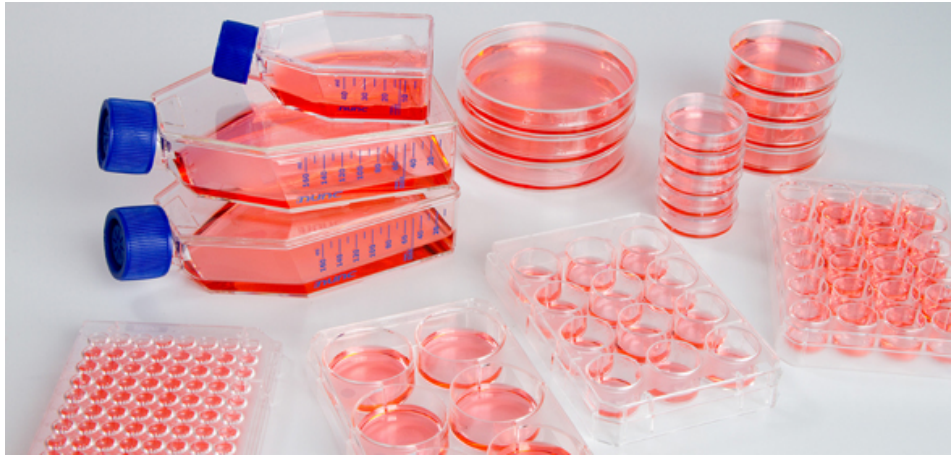
Au NP assisted NIR Stimulation

Characterisation

Biological
Physical
Chemical
XPS, ToF-SIMS, QCM-D,
AFM, Ellipsometry...



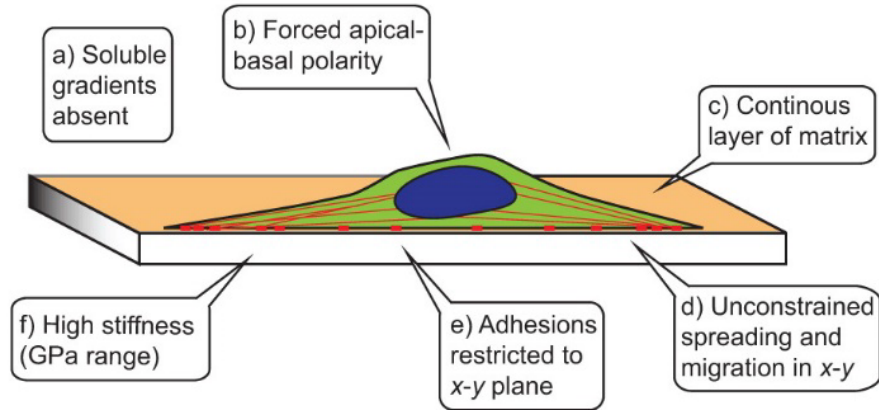
4D Cell Culture Systems



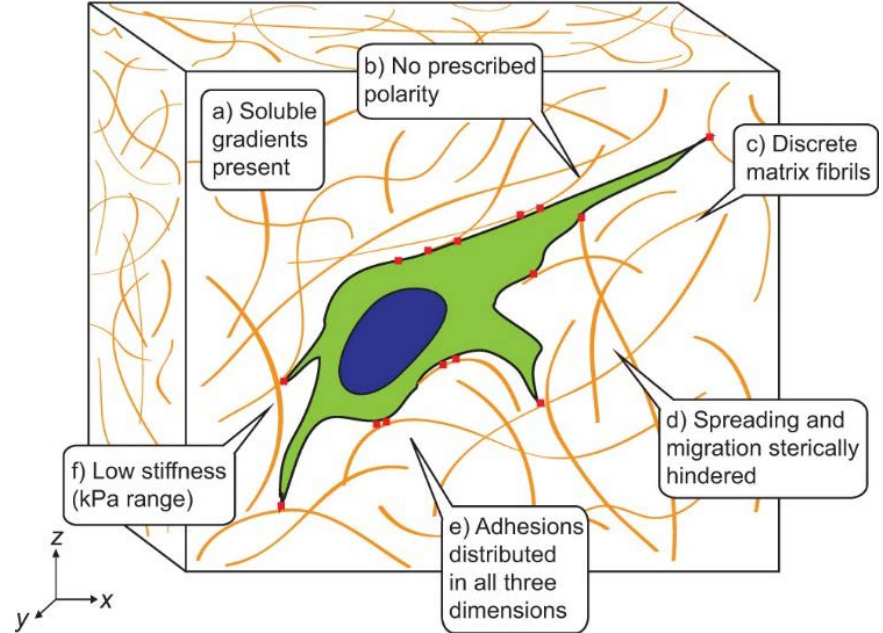
<http://www.nature.com/nprot/journal/v4/n11/images/nprot.2009.152-F5.jpg>

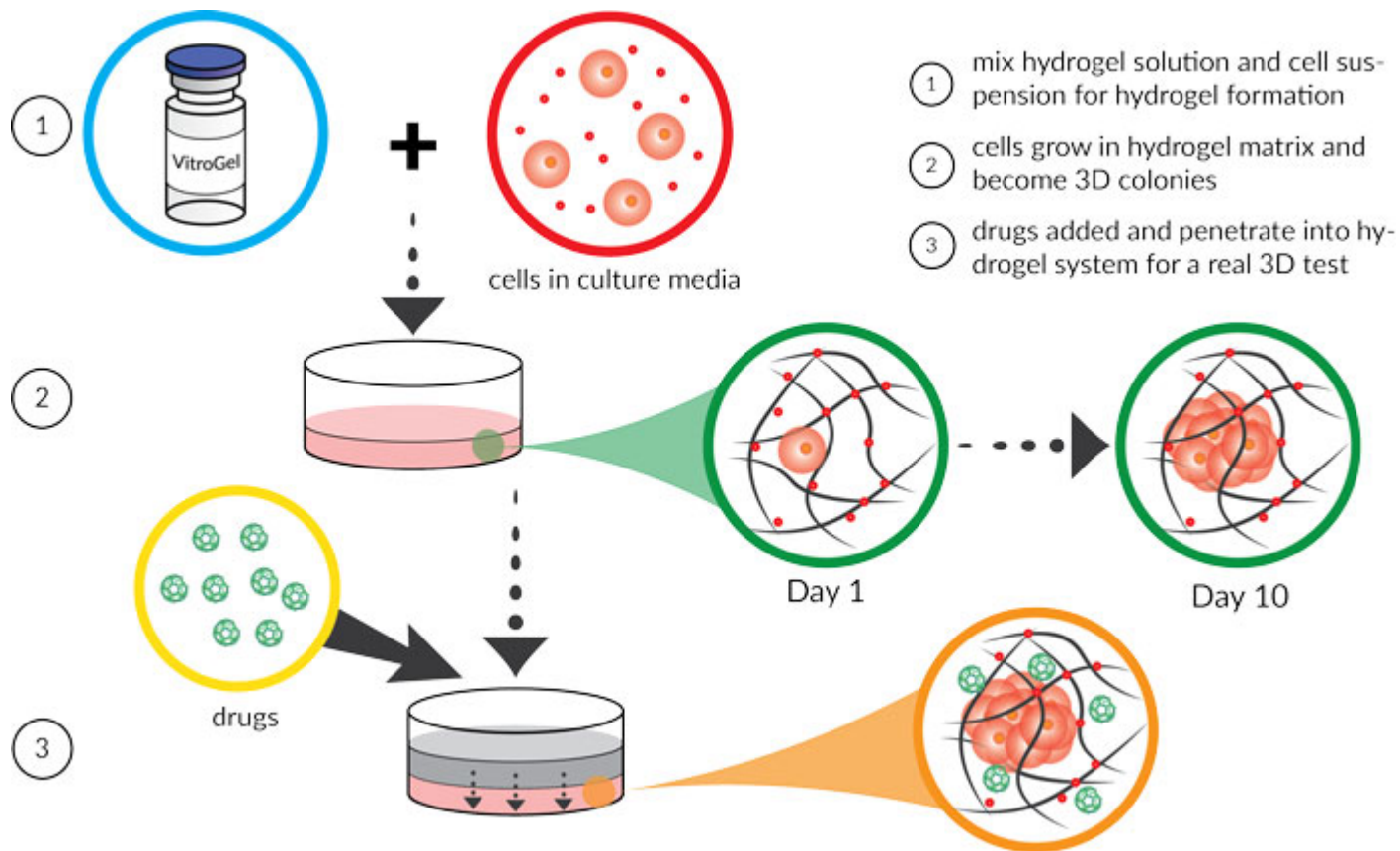
Adhesive, topographical, mechanical, and soluble cues in 2D and 3D.

Collagen-coated glass (2D)



Collagen gel (3D)

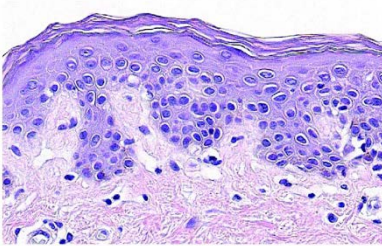




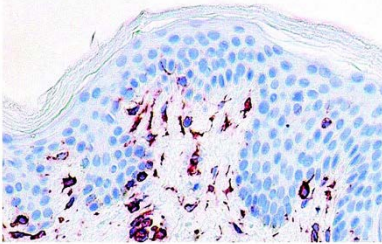
Skin is more than a monolayer of cells

Native skin

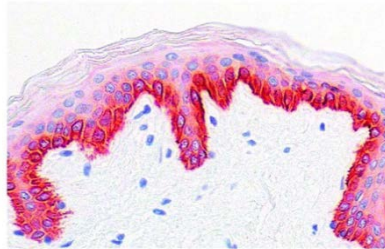
H&E



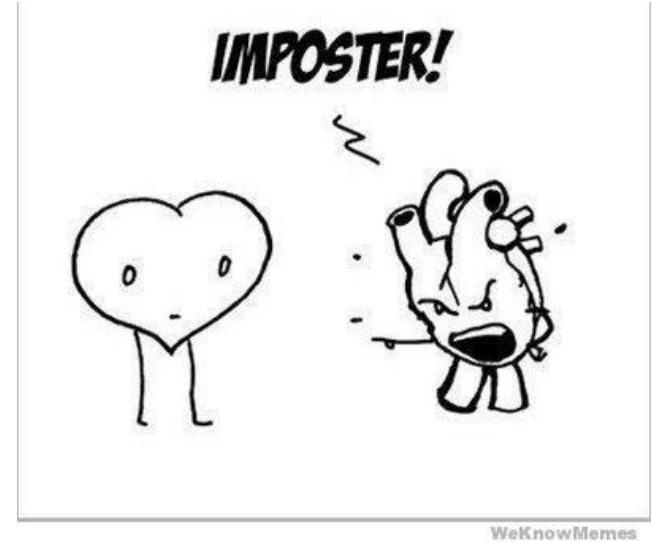
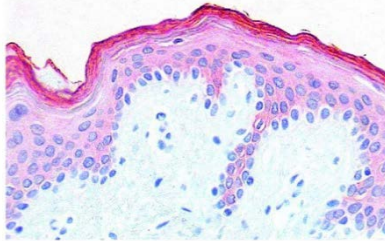
Vimentin



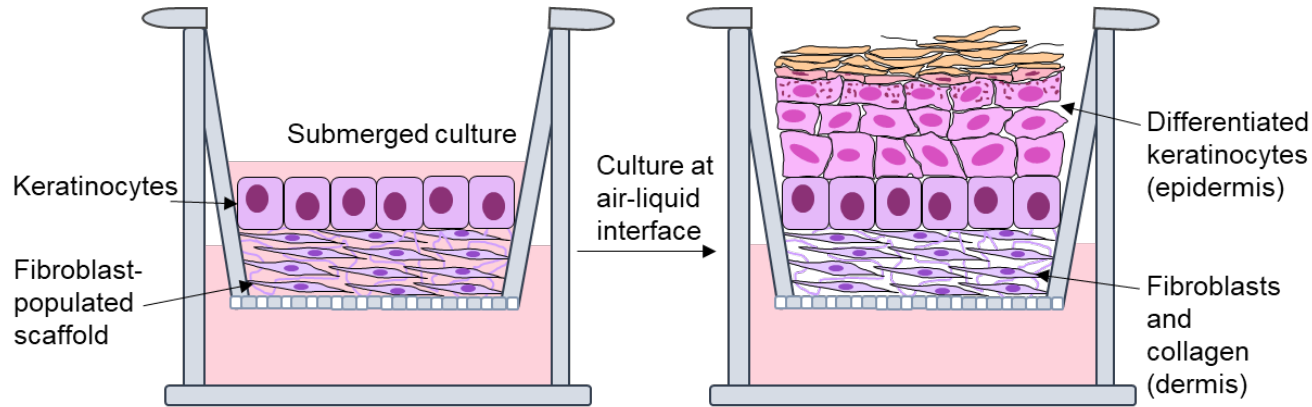
Keratin 5



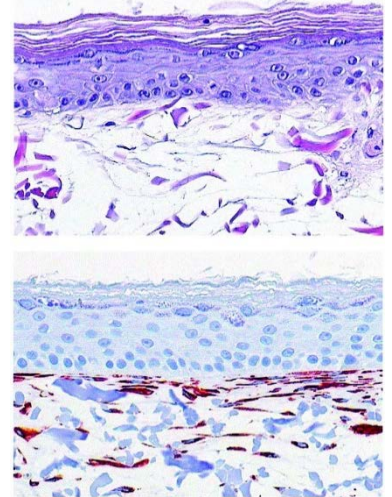
Keratin 10



3D Skin Cultures

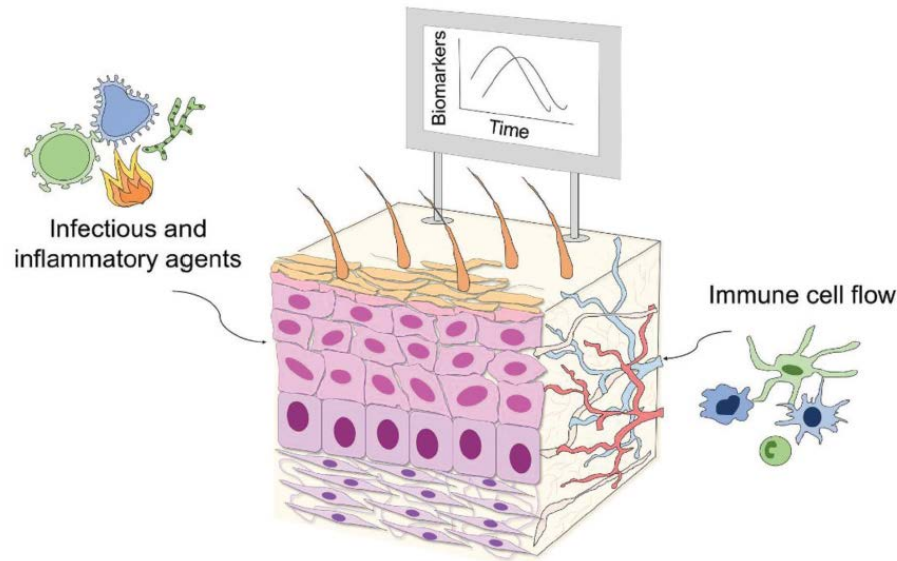


Cell line human skin equivalent



Toward Immunocompetent 3D Skin Models

Aleta Pupovac, Berna Senturk, Chiara Griffoni, Katharina Maniura-Weber, Markus Rottmar, and Sally L. McArthur**

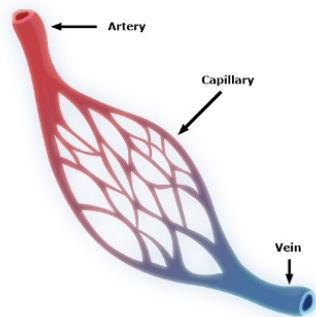


DOI: 10.1002/adhm.201701405

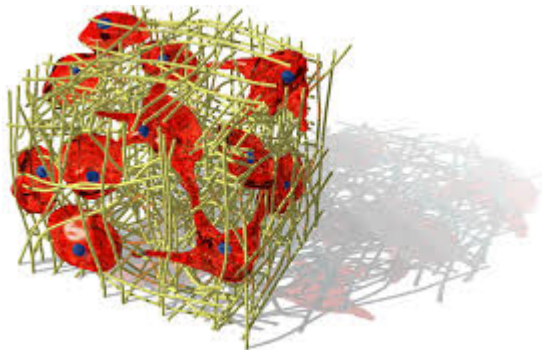
Applications

- New models for biomaterials testing
- Fundamental biology
- Drug and gene delivery systems
- Studying stimulation systems - Nanoparticle enhanced NIR etc

Perfusion systems



Scaffold and cell system



Fluidics and sensing

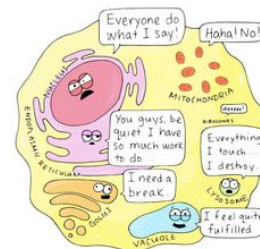
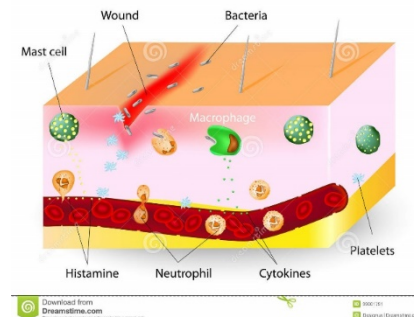


Curating 4D Cell Culture Systems



Cell-fie Imaging systems

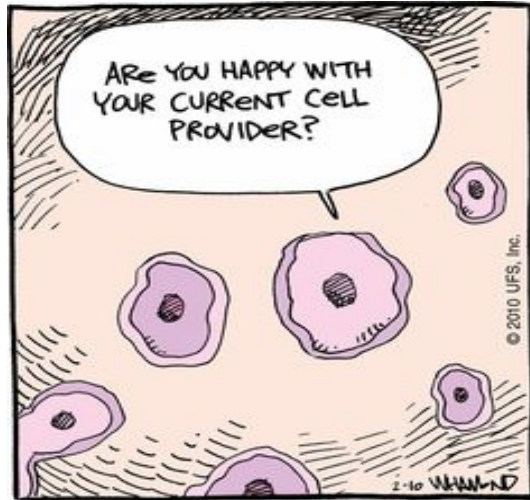
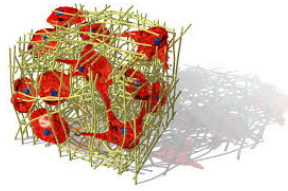
Host Responses



If organelles could talk
Beatrice the Biologist

Functional Readouts

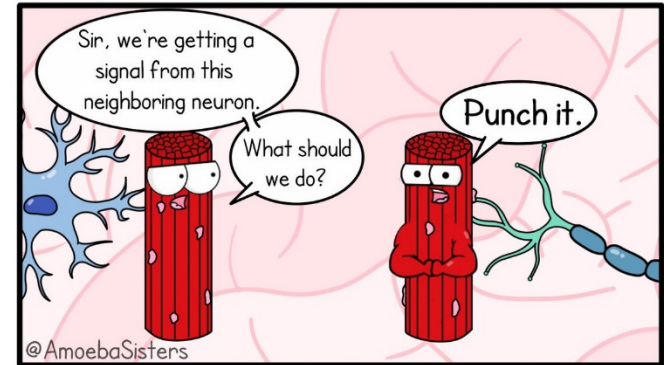
Toolbox 1: Materials, Cells, and Devices



Selection of cells for:

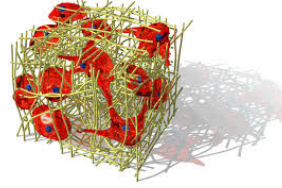
- appropriateness (age, source, primary vs secondary)
- reproducibility
- scalability
- tissue structure
- functionality
 - inflammation
 - infection
 - Signaling

If muscle cells tried to be interneurons...



CELL SPECIALIZATION IS IMPORTANT.

Toolbox 1: Materials, Cells, and Devices



Scaffolds

- Adaptable
- Decellurised tissues
- Biological or synthetic hydrogels
- Signalling and attachment cues
- Mechanical properties



3D Printing



Engineered
Tissue

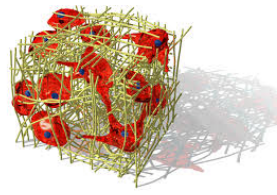


Spheroid



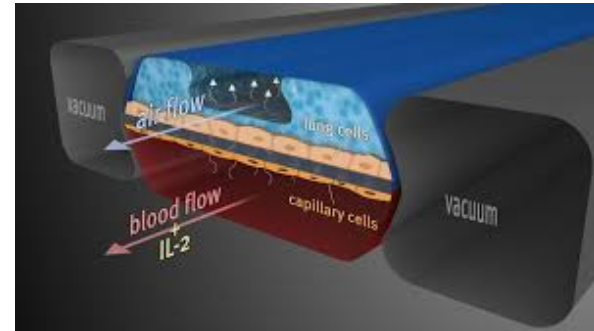
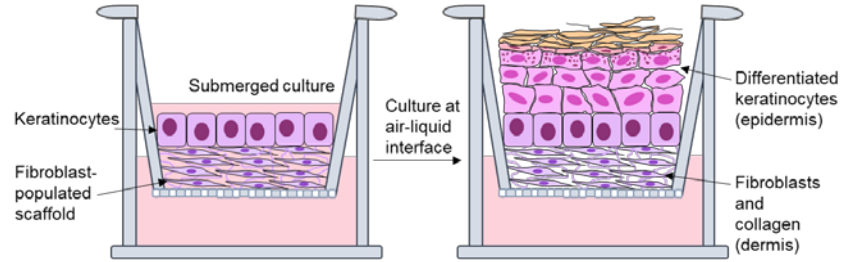
Organoid

Toolbox 1: Materials, Cells and Devices



Devices requirements

- High throughput platforms
- Aid perfusion
- Integrate with analysis
- Accommodate 3D tissues, enable in situ growth
- Allow integration of test materials etc

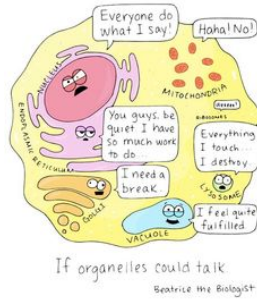
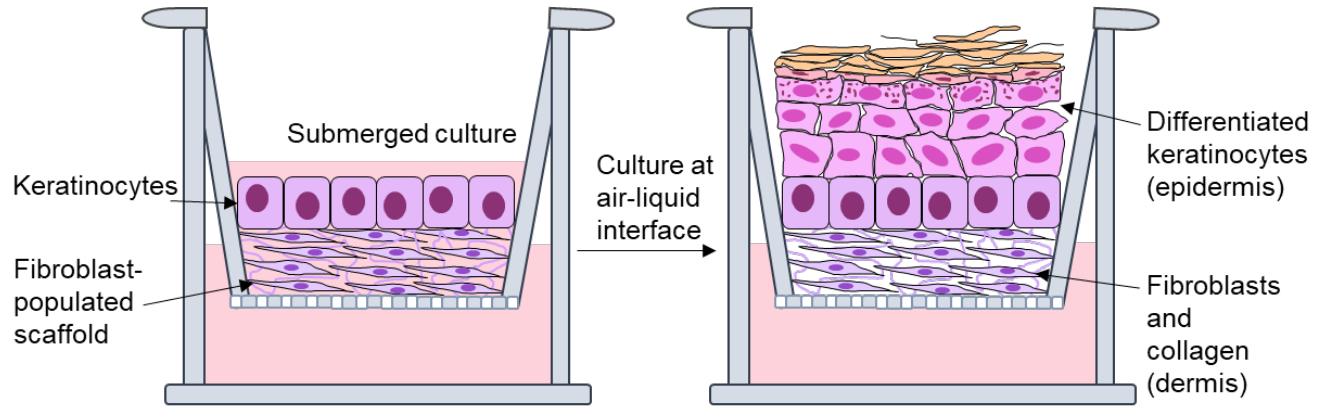


Wyss Institute, Harvard

Toolbox 2: Functional Reporting and Sensing

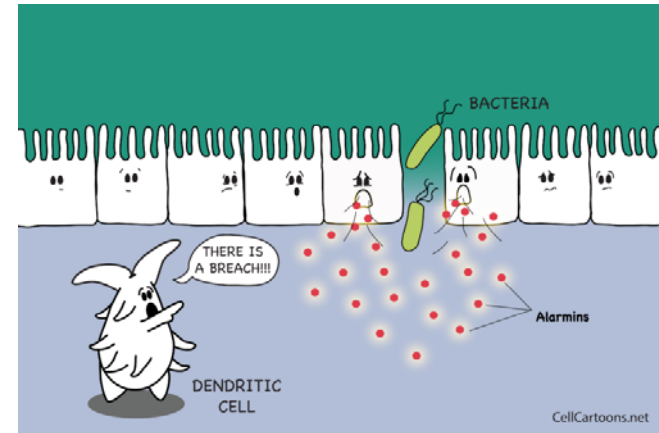
Interrogate

- Cells
- Matrix
- Media

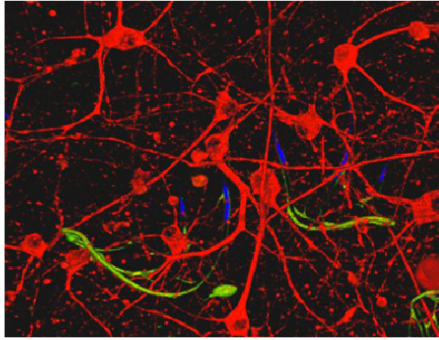


Toolbox 2: Functional Reporting and Sensing - Media

- Downstream, in line biochemical monitoring
- Cell viability and cytokine activities
- Local pH, glucose levels, electrical properties

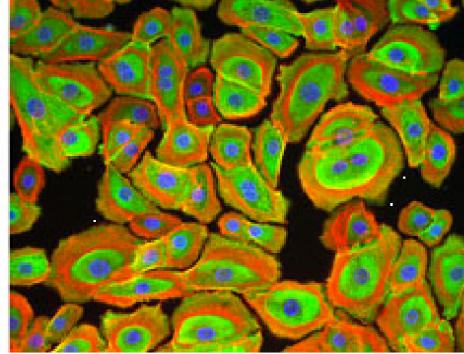


Toolbox 2: Functional Reporting and Sensing – Cells and Media



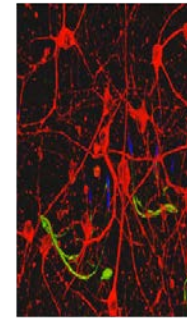
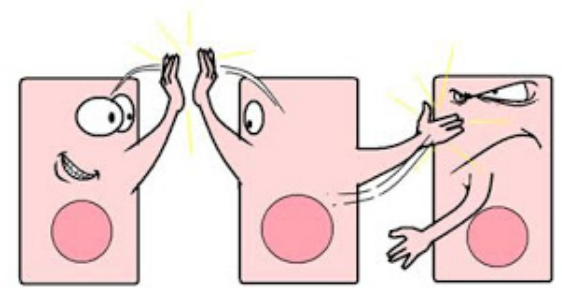
Scaffold integrated sensors

<http://images.iop.org/objects/phw/news/thumb/16/8/42/PW-2012-08-29-sensors-tissue.jpg>



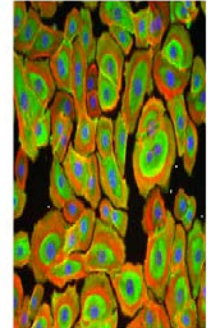
Reporter cell and molecule integration

<https://www.systembio.com/services/lentiviral/custom-reporter-cell-lines>



Scaffold integrated sensors

<http://images.iop.org/objects/phw/news/thumb/16/8/42/PW-2012-08-29-sensors-tissue.jpg>



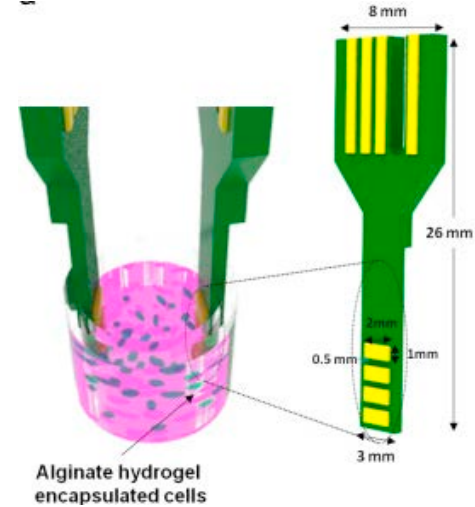
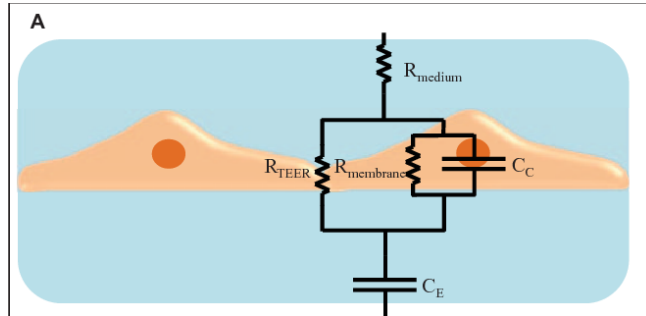
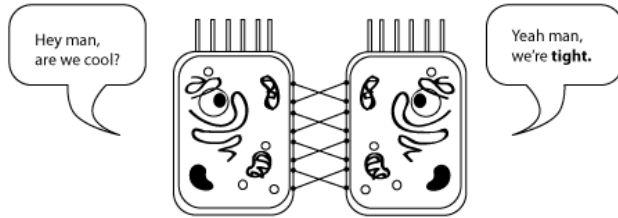
Reporter cell and molecule integration

<https://www.systembio.com/services/lentiviral/custom-reporter-cell-lines>

- Cell reporter systems with long lifespans, >10 days
- Translation of 2D assays into 3D in situ assays
- Spectroscopy within the 3D culture

Toolbox 2: Real-time Reporting – Cells and Matrix

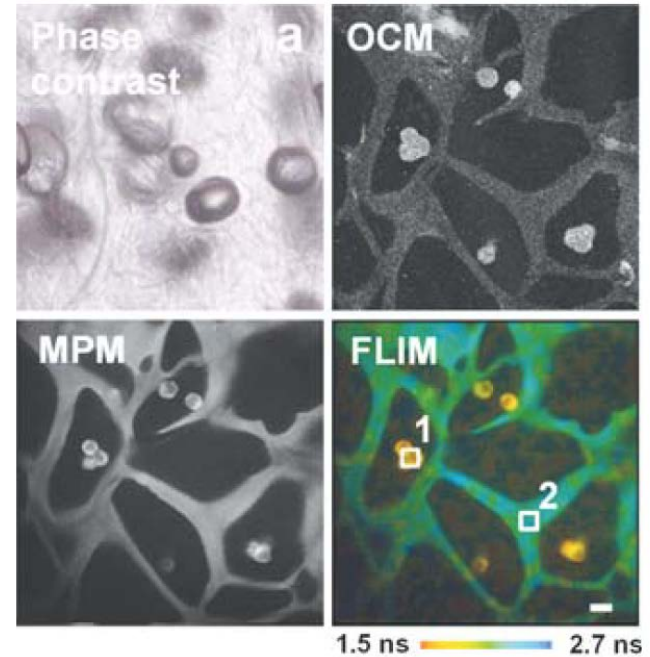
- Electrical monitoring – cell-cell junctions, cell migration, proliferation...



Toolbox 2: Real-time Imaging – Cells and Matrix

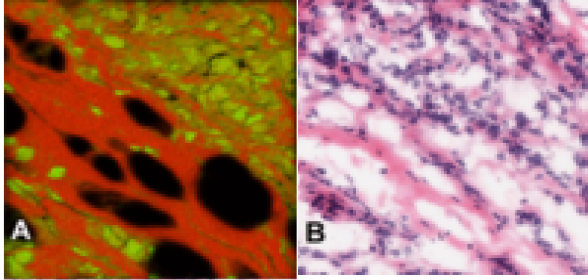


- Need depth of field
- Non-linear optical techniques
- Use native fluorescence
- Non-transparent systems
- Matrix reorganization
- Cell behavior/signaling



Boppart Group - J. Biophotonics 5, No. 5–6, 437–448 (2012)

Verify, Validate...collaborate



ToF-SIMS Tissue Imaging

Gamble et al Biointerphases 10, 019008 (2015)



Gold standard techniques

- Histology/immunostaining
- ELISA
- Flow cytometry

Probing Biosystems Future Science Platform

Technology pipeline

Organ-on-a-chip

- Disease mechanisms
- Biomarker development
- High throughput drug screening



Brain - functioning BBB

- Improved drug delivery to the CNS
- Mechanisms of viral infection
- Drug pharmacology and screening

Respiratory and vascular

- Mechanisms of viral infection
- Improved delivery of antivirals

Gut organoid

- Gut microbiome and health
- Testing advanced nutritional / bacterial foods
- Drug toxicology testing

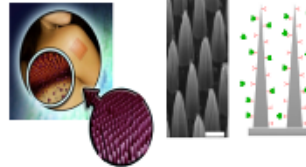
3D tissue models

- Wound healing / infection control



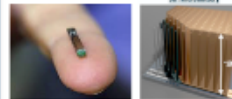
Point-of-care diagnostics

- Blood biomarkers
 - cancer biomarkers (metastatic disease)
 - neuronal ccDNA - head injury
- Saliva (premature ageing)
 - DNA damage, mitochondrial dysfunction
- Breath (asthma management – air quality)
 - novel nitric oxide sensing
- Nanochannels (virus detection)



Wearable biosensors - diagnostics

- Nanoneedle array (monitoring and drug administration)



Implantable biosensors

- Neurocybernetics
- Cardiac monitoring



Precision nanomedicine

- Improved drug delivery



Health surveillance and Biosecurity

The 4D team

- Dr Sally McArthur – materials engineer with biological tendencies and faking it until she makes it
- Dr Aleta Pupovac – immunologist, FSP project Inflammation and infection models in skin
- Dr Zay Yar Oo – cell biologist, focus on biomolecular systems, 2D to 3D translations
- Dr Daniel Langley – physicist with specialisation in instrumentation, microfluidics devices and integration
- Sorel De Leon – biosensing expert, electrical engineer
- Charlie Wilson – neuroscientist, cell biologist with engineering tendencies.

Collaboration is Key

- CSIRO Cell Biology Group, Veronica Glattauer, David Haylock.
- Sheila McNeil – U Sheffield
- Katharina Maniura and Markus Rottmar – Empa Switzerland, Biointerface Group
- Prof Heike Walles – Translation Centre, U Wurtzburg, Fraunhofer IGB
- Lara Gamble – NESAC-Bio, University of Washington, Seattle
- AFOSR Biophysics Program colleagues?

NCRIS
National Research
Infrastructure for Australia

An Australian Government Initiative



Thank you

Manufacturing/Probing Biosystems FSP

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Manufacturing

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