ENGINEERING 3D TISSUE SYSTEMS TO BETTER MIMIC HUMAN BIOLOGY

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The Scientific Method is GREAT!

Observation

Hypothesis

Test

Accept Hypothesis

Reject Hypothesis

$$F = \frac{GMm}{r^2}$$

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Except when it could kill you!

Observation

Hypothesis

Test

Accept Hypothesis

Reject Hypothesis

$F = \frac{GMm}{r^2}$

This drug is toxic to humans

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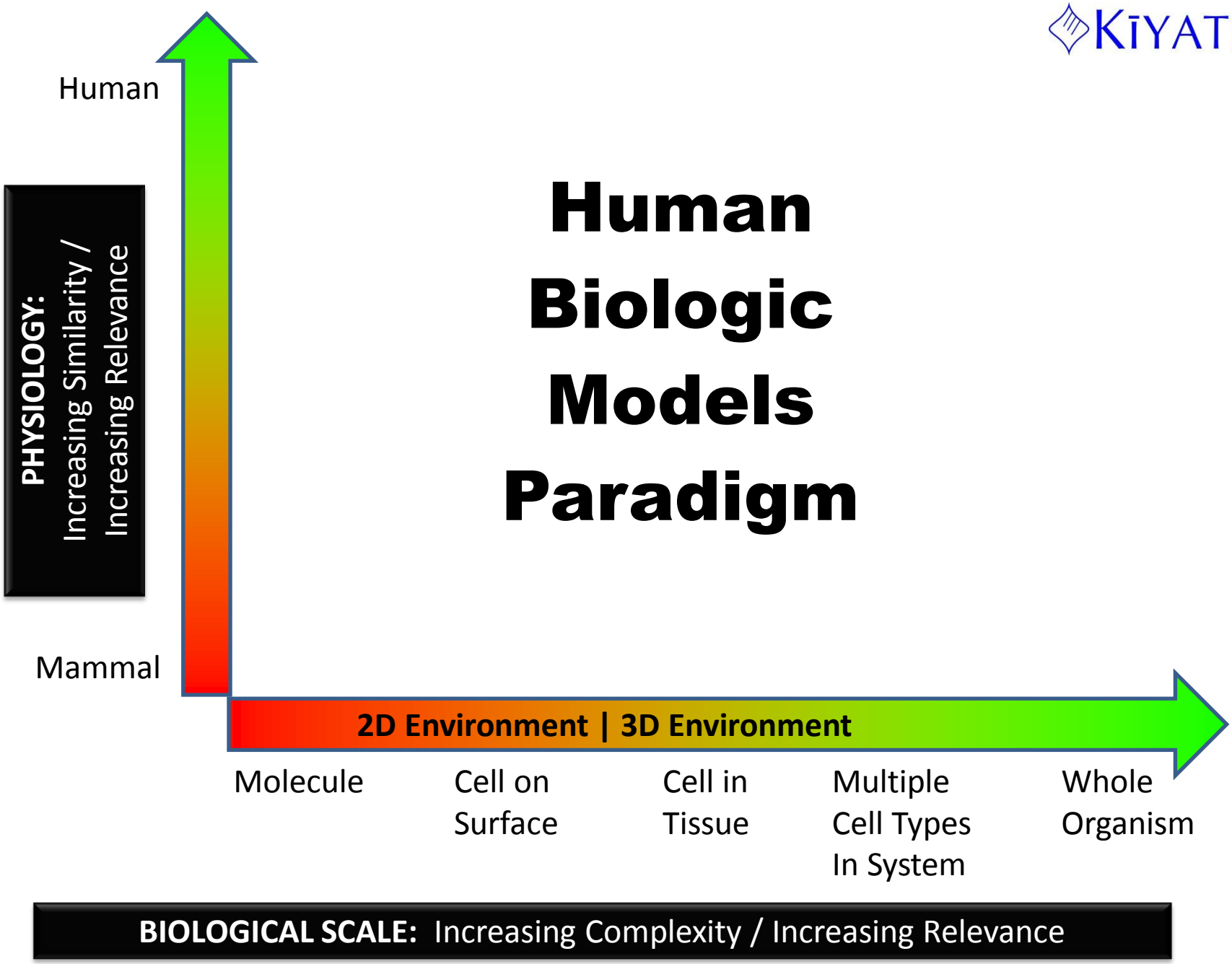
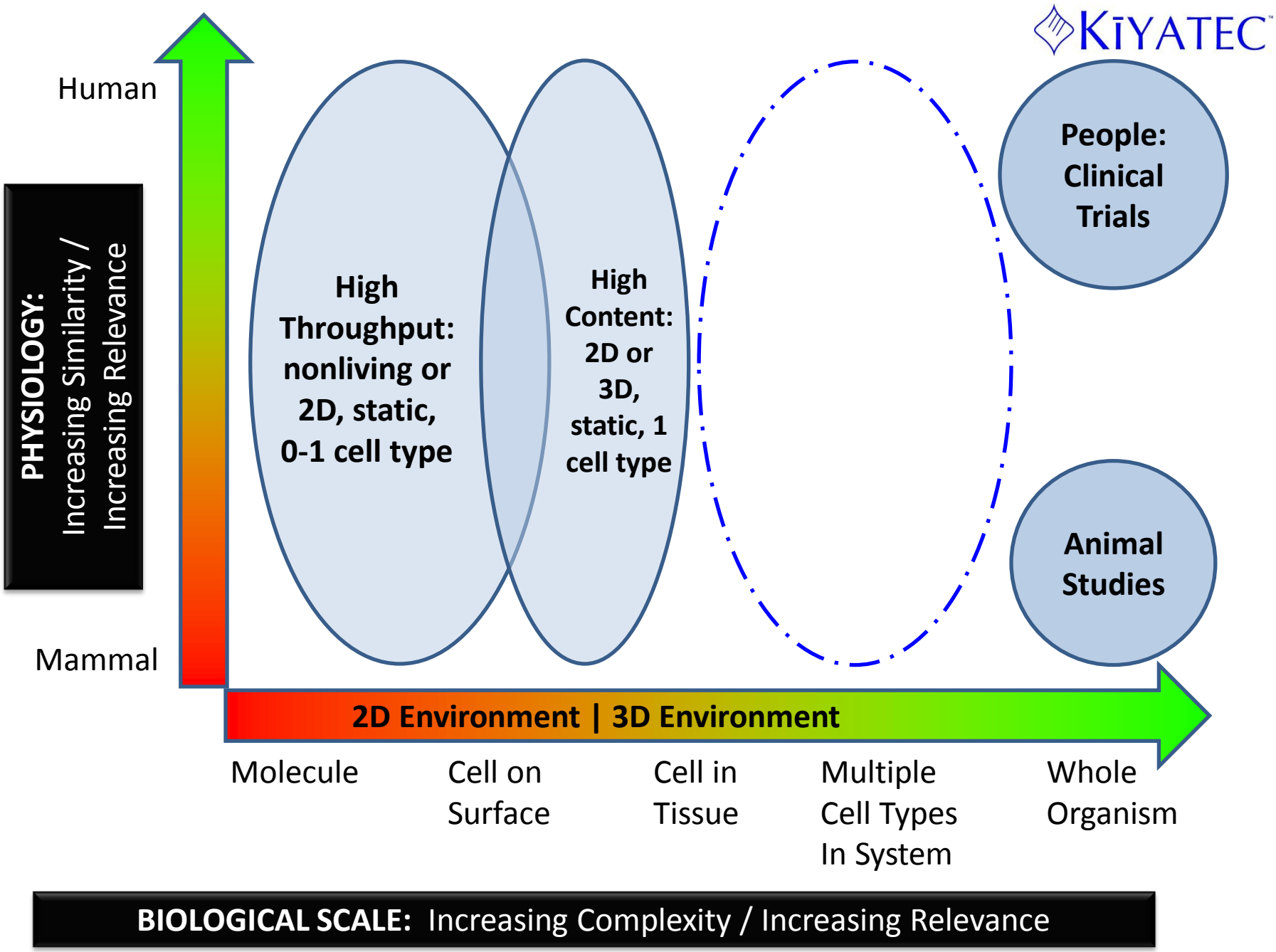
Human Biologic Models Paradigm

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Human
PHYSIOLOGY: Increasing Similarity / Increasing Relevance
Mammal
High Throughput: nonliving or 2D, static, 0-1 cell type
High Content: 2D or 3D, static, 1 cell type
People: Clinical Trials
Animal Studies
2D Environment | 3D Environment
Molecule
Cell on Surface
Cell in Tissue
Multiple Cell Types In System
Whole Organism
BIOLOGICAL SCALE: Increasing Complexity / Increasing Relevance

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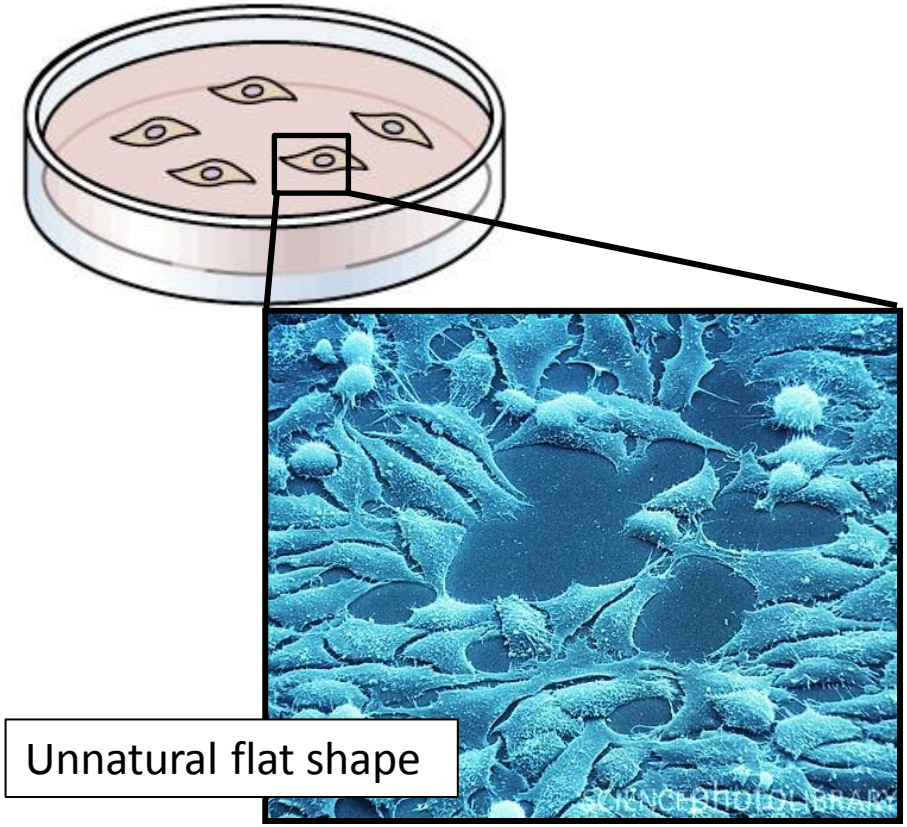
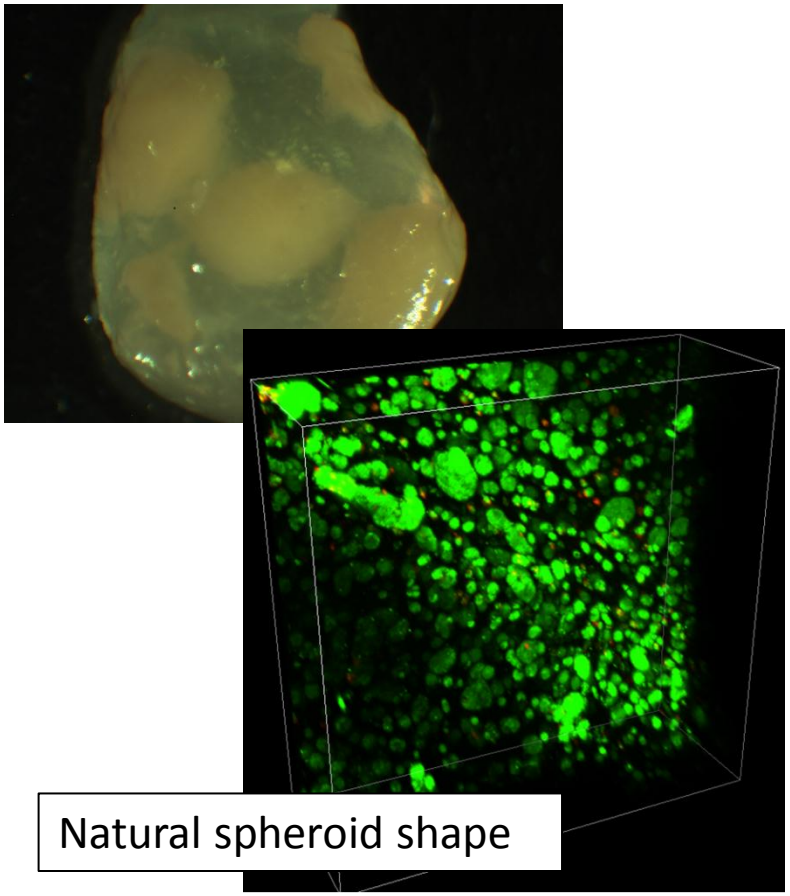
Cells Are Alive and Interact

Cells in 2D culture



Unnatural flat shape

Cells in 3D culture



Natural spheroid shape

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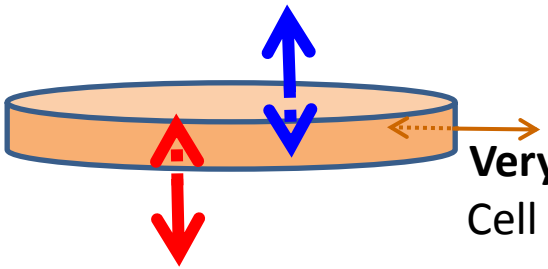
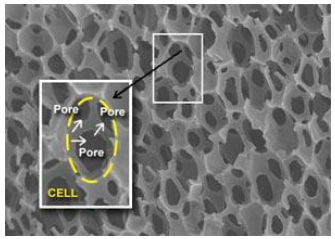
Cell Surface Interaction Contrast

~ 50%:
Soluble
Environment

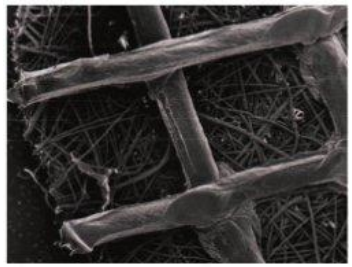


Very Little
Cell to Cell

~ 50%: Rigid
Plastic or
Mediating
Layer



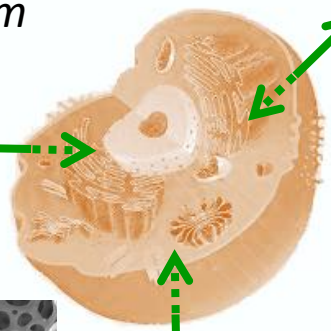
biomerix.com



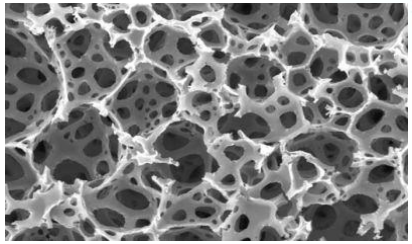
nbsc.com

Cell + Matrix +
Soluble

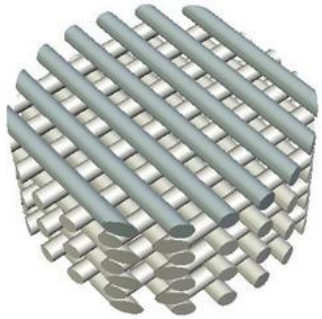
Cell + Matrix
+ Soluble



Cell + Matrix
+ Soluble



reinnervate.com



3dbiotek.com

Cell in 2D

Cell in 3D

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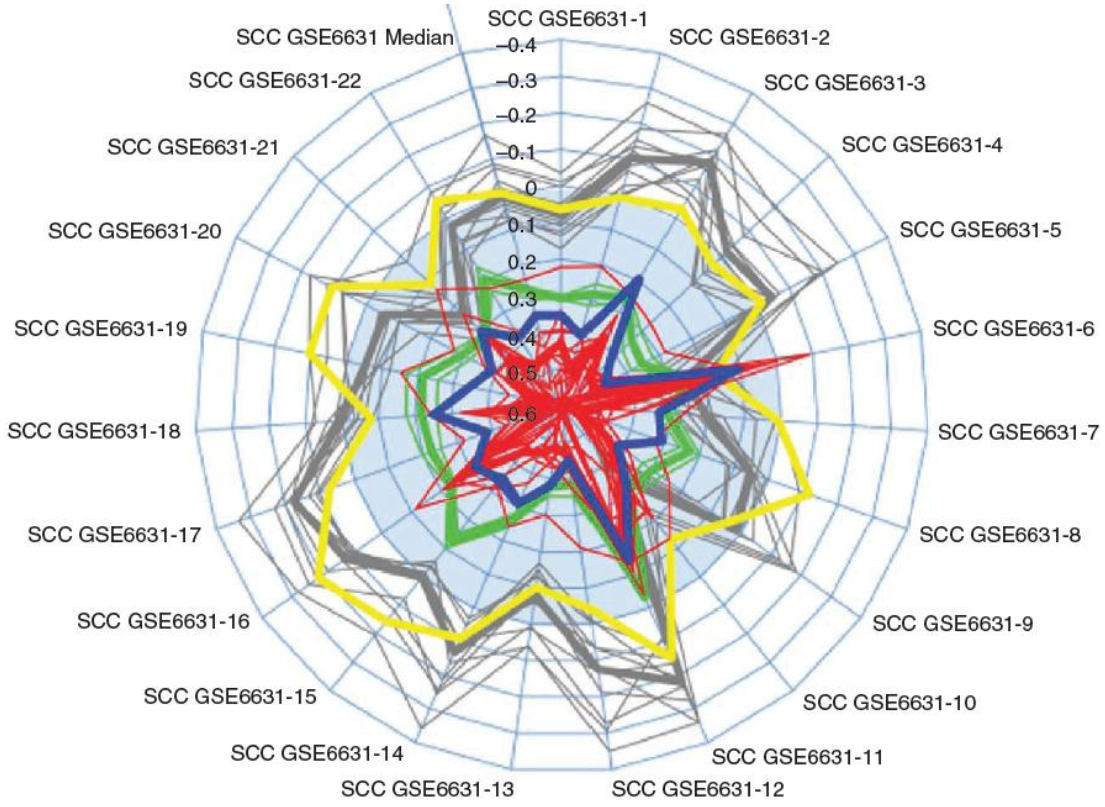
Superior Clinical Relevance of 3D Models



Human

3D Cell Culture

Mouse

2D Cell Culture

Pearson Correlation Coefficient

Most Relevant

Human – Human = 0.45

3D – Human = 0.34

Mouse – Human = 0.28

2D – Human = 0.0

Least Relevant

3D: better than mouse!
2D: ZERO correlation!

Ridky TW, Chow JM, Wong DJ, Khavari PA. Invasive three-dimensional organotypic neoplasia from multiple normal human epithelia. ***Nature Medicine*** 16(12):1450-55, **2010**.

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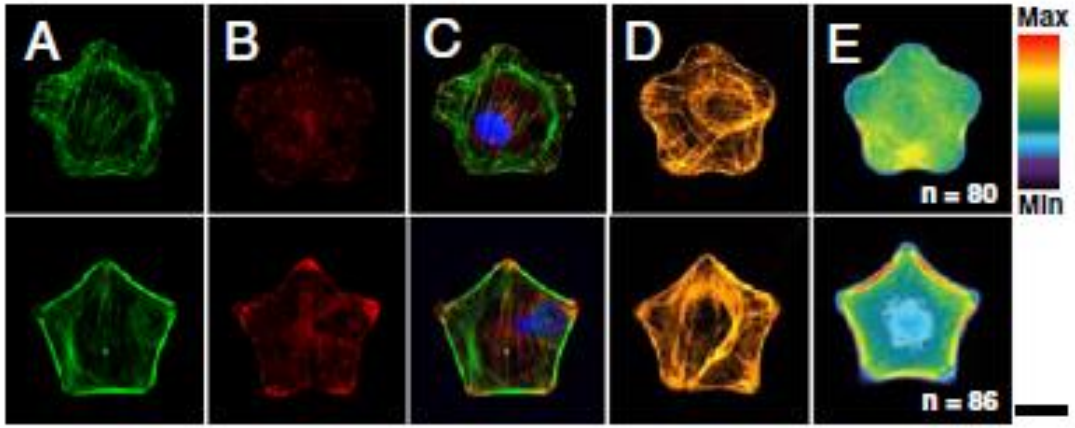
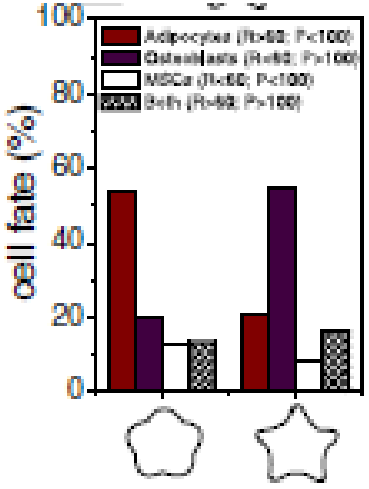
Shape-Function Paradigms

- "Shape Happens"
  - Maximize cell opportunity to form natural (*in vivo*) shape to maximize *in vivo* correlation
- Engineered Control
  - Force cell shape to force cell function





Kilian, (2010). PNAS **107**(11): 4872-4877

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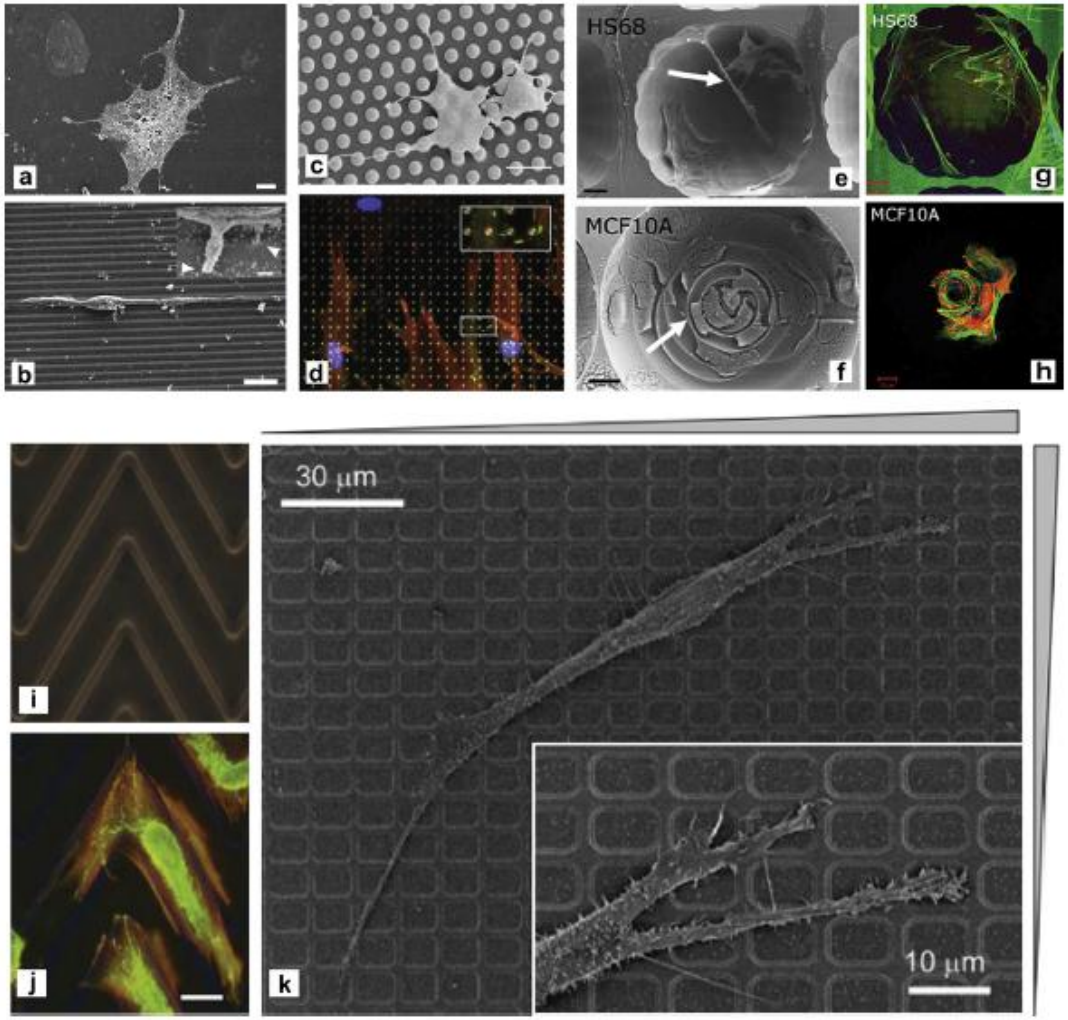
Surface Topology – “On” vs. “In”

- Surface fabrication of grooves, pillars, posts, pits, pyramids, etc. fabricated
- Cells grown on modified 2D surface
- Measurable cell response in many attributes



Nikkhah, (2012), Biomaterials **33**(21): 5230

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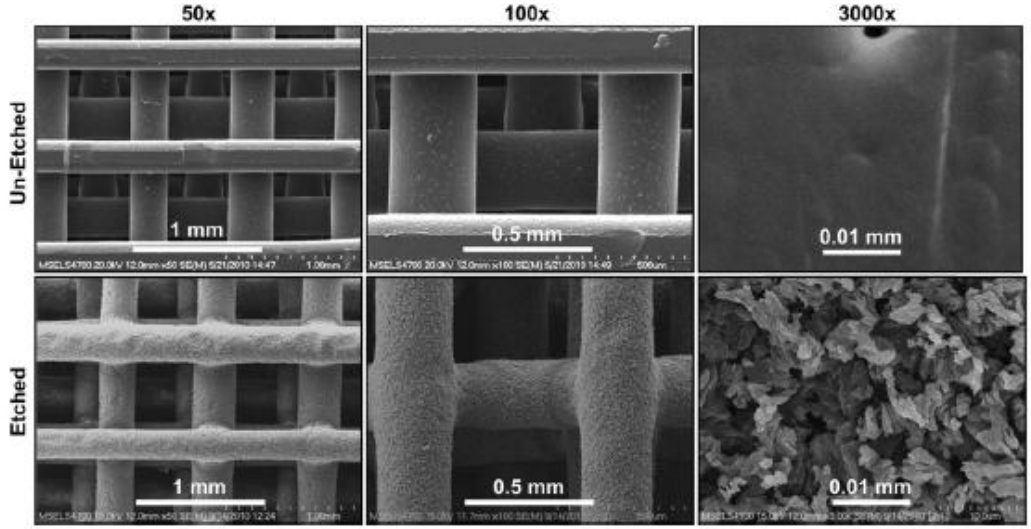
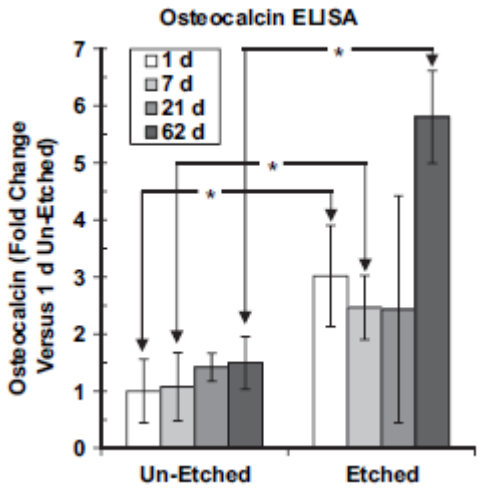
Surface Topology – “On” vs. “In”

- Topology on interior surfaces of 3D construct
- Cells grown in scaffold
- Measurable cell response in many attributes





Kumar, (2012), Biomaterials **33**(16): 4022

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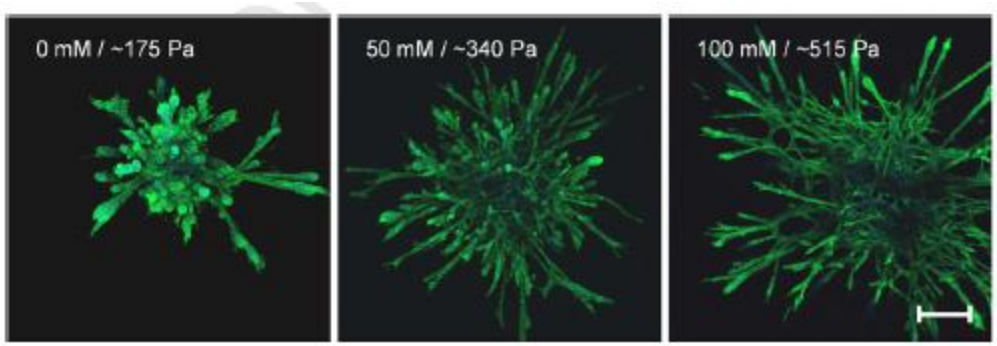
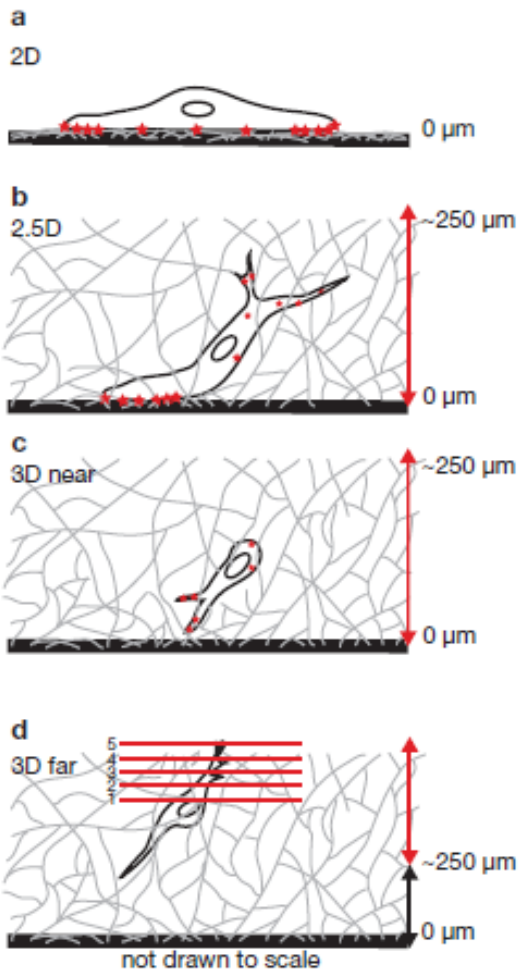
Size Does Matter - Edge Effect

- Cells respond to different matrix stiffness
- In many matrices internal stiffness varies with distance from edge
- Creates minimum real matrix dimensions to achieve natural (*in vivo*) cell micro-environment



Mason BN, (2012). Acta Biomater



Fraley, (2011), Nature Cell Bio **13**(1): 5

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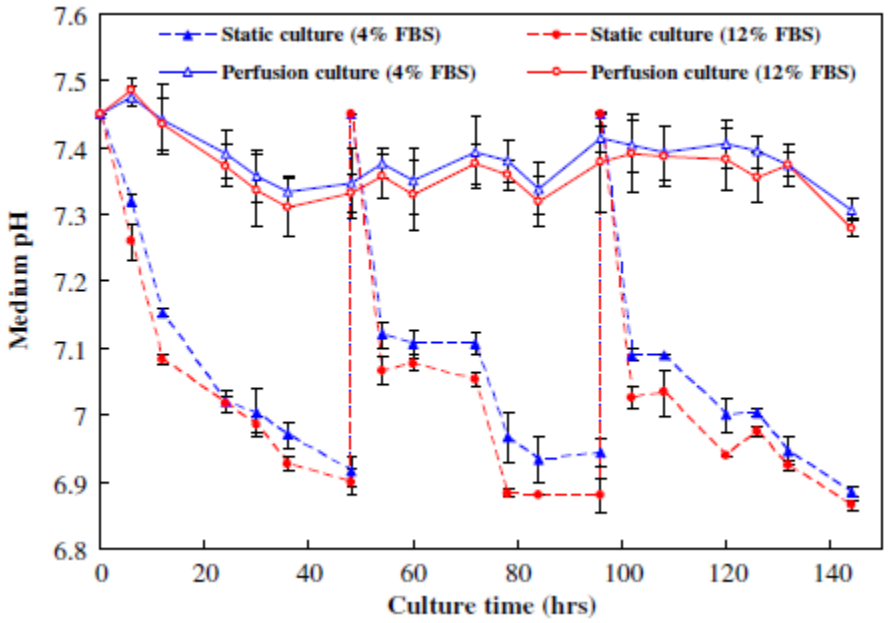
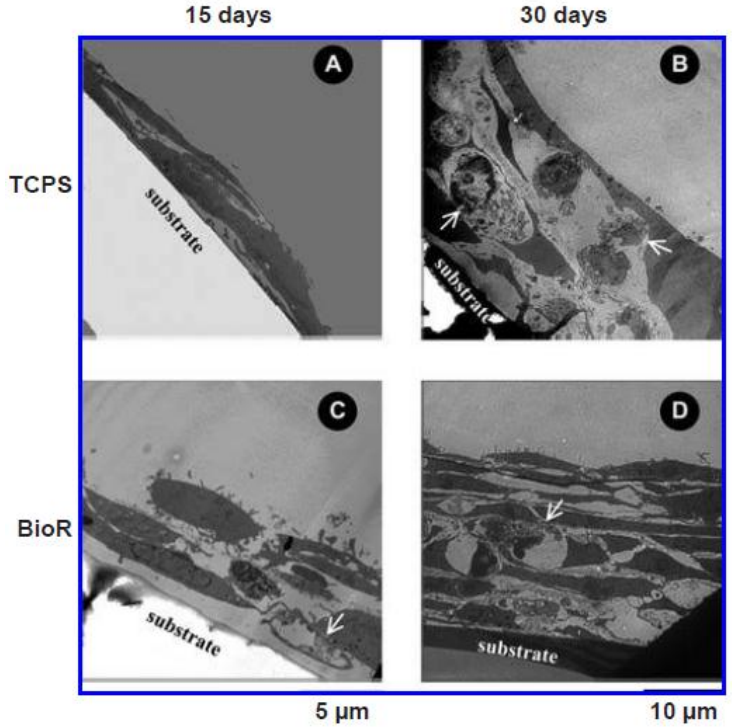
Soluble Environment Effects

- Typical: “feast vs. famine” accepted trade-off
- Better: gradual replacement according to needs



Wu, (2011), Biomed Microdevices **13**(1): 131



Dhurjati, (2006), Tissue Engineering **12**(11): 3045

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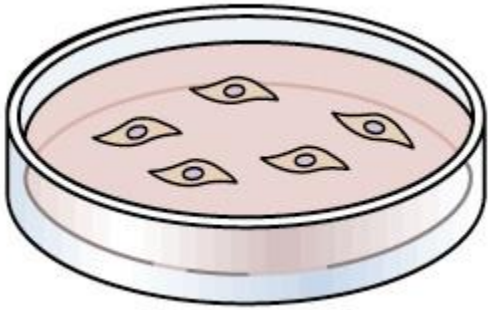
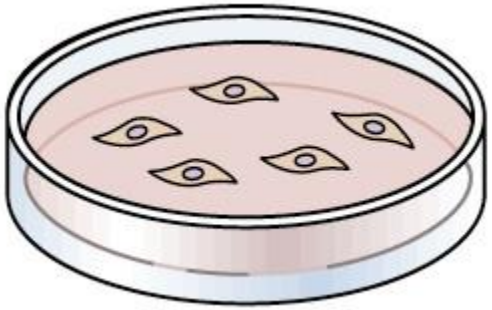
Perfusion – Over and Through

Static

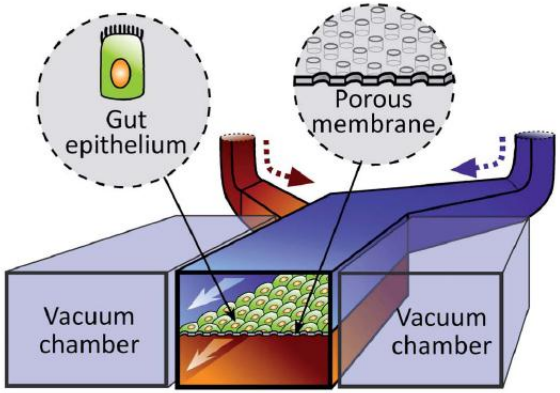


Perfusion – Over and Through

Static



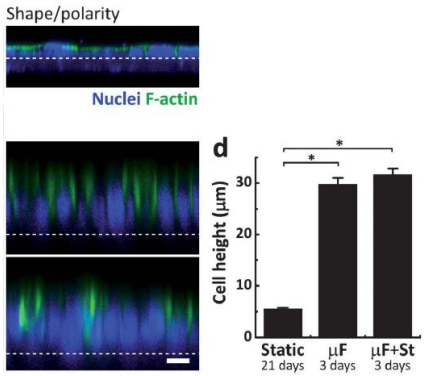
Perfusion "over" (flat)



wyss.harvard.edu

"Over"

- Dense
- Luminal
- Parallel



Kim, (2012), Lab Chip **12**: 2165

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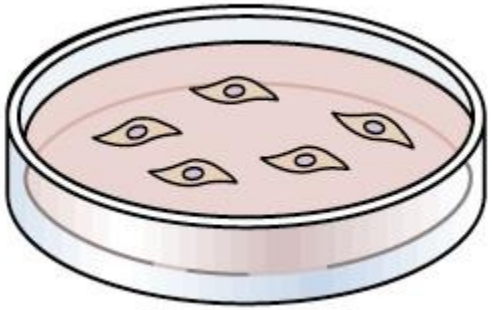
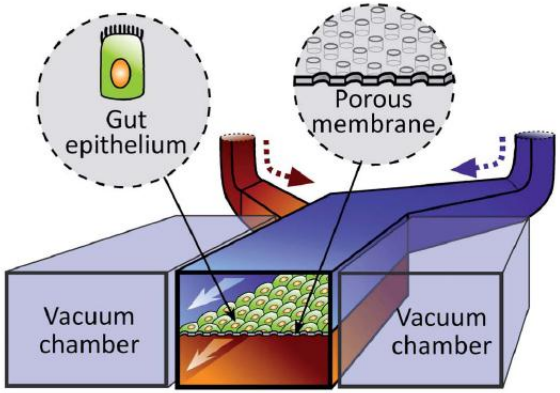
Perfusion – Over and Through

Static

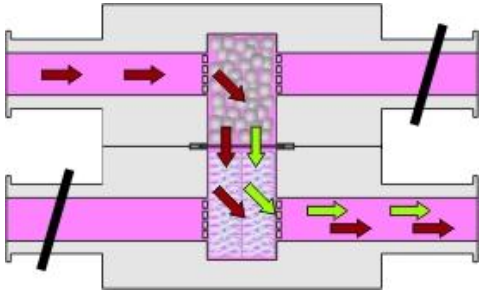


Perfusion "over" (flat)



wyss.harvard.edu

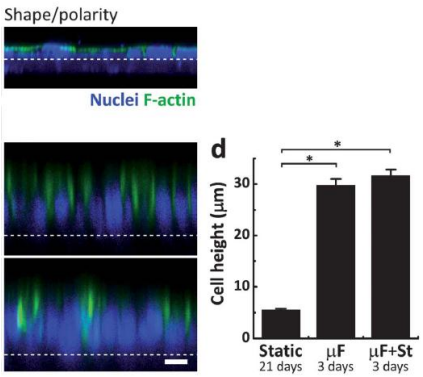
Perfusion "through"



kiyatec.com

"Over"

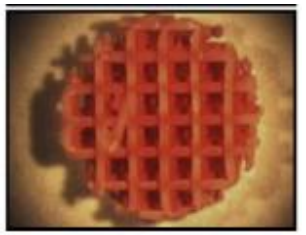
- Dense
- Luminal
- Parallel



Kim, (2012), Lab Chip **12**: 2165

"Through"

- Porous
- Interstitial
- Perpendicular



Kumar, (2012), Biomaterials **33**(16): 4022

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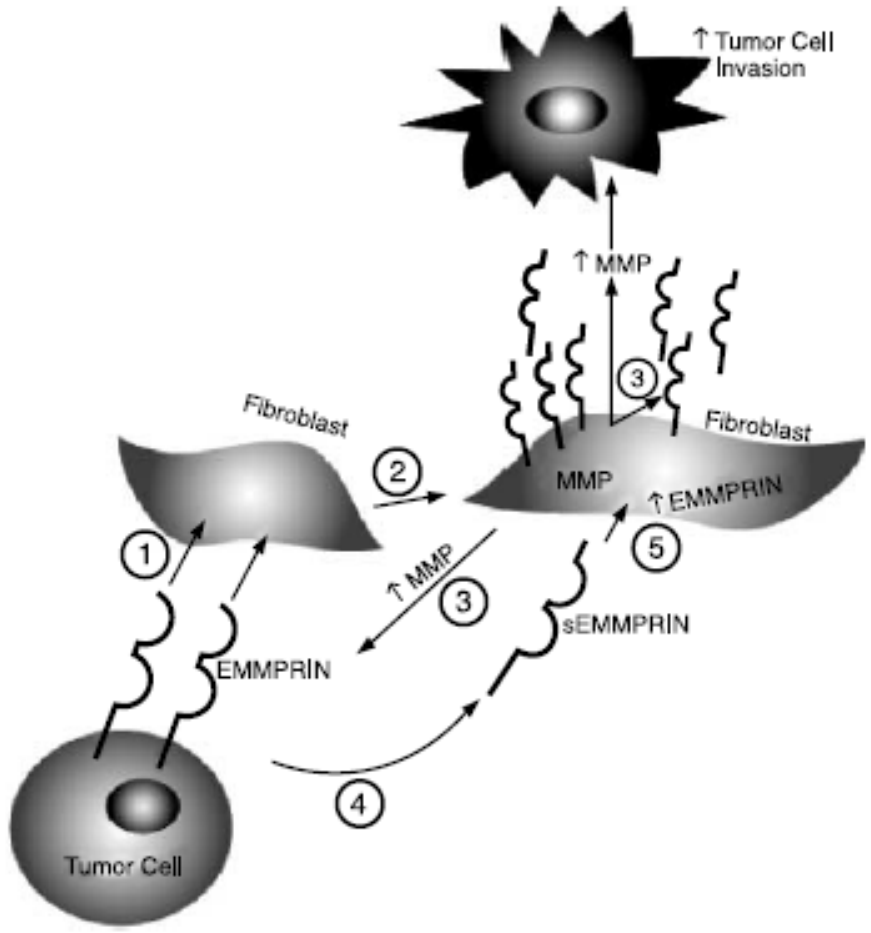
The Need For Co-cultures



① Tumor Fibroblast contact via EMMPRIN
② ↑ MMP and EMMPRIN
③ Secreted MMPs cleave EMMPRIN generating sEMMPRIN
④ sEMMPRIN acts on local or distant cells to
⑤ ↑ MMP and ↑ EMMPRIN
⑥ MMP ↑ Invasion

Tang, (2004), Mol Canc Res **2**:73

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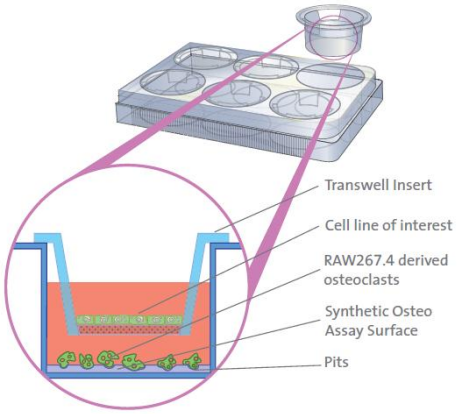
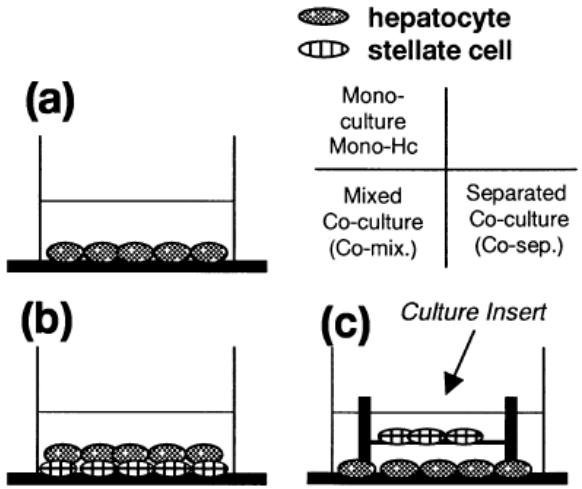
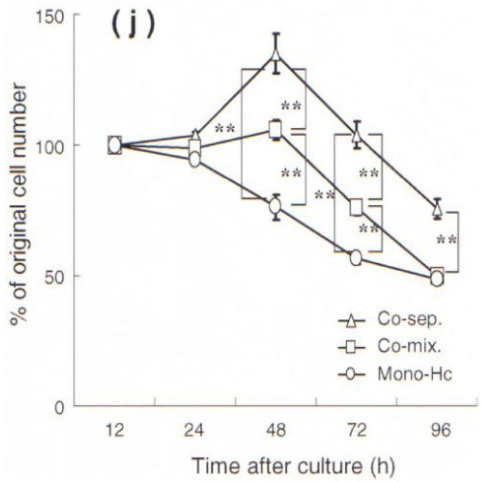
Traditional Co-Culture Models (2D)



corning.com





Uyama, (2002), J Hepatology **36**: 590

Mixed

- Cell-to-cell contact
- Same Media
- Competition

Segregated (Insert)

- Soluble factor mediated
- Different Media
- Non-competitive

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Putting It All Together

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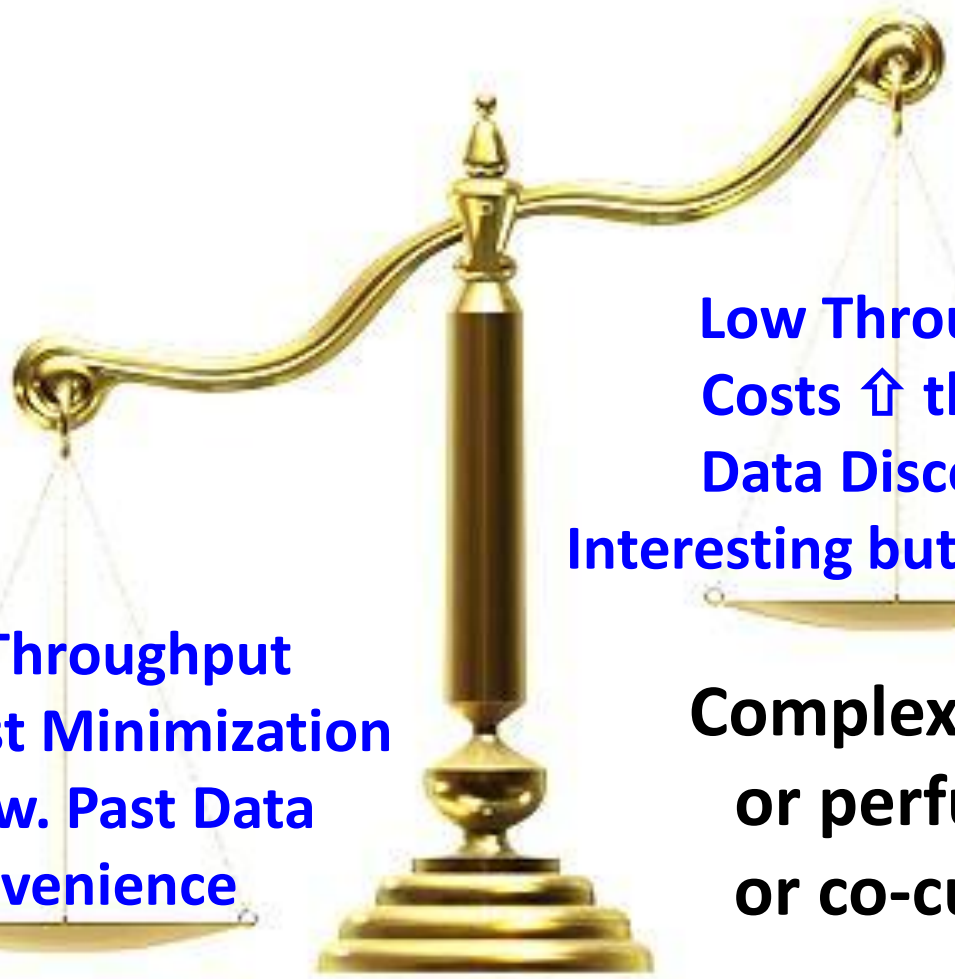
Old Paradigm - *in vitro* Models' Value



High Throughput
Acute Cost Minimization
Synch w. Past Data
Convenience

2D static monoculture

Low Throughput
Costs ⇧ than 2D
Data Disconnect
Interesting but Impractical

Complexity: 3D or perfusion or co-culture

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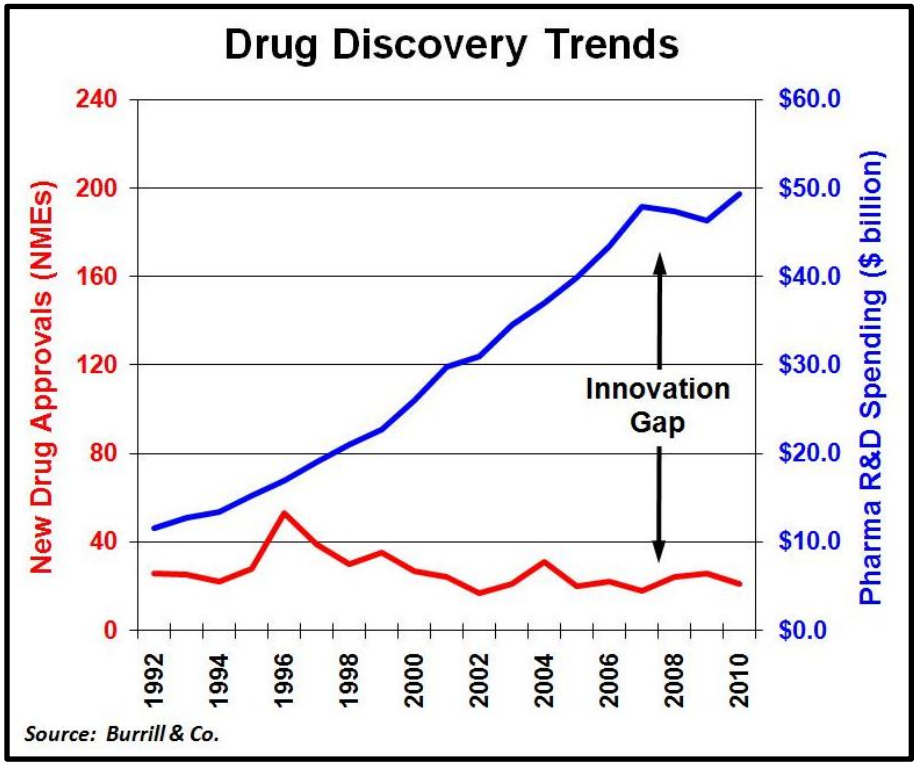
Old Paradigm - Effectiveness

Poorly Predictive

Only 12% of new drug candidates (NMEs) entering into clinical trials will successfully make it to launch – **88% failure rate**!

Source: Nature Reviews Drug Disc 9, 203 (2010)

Increasingly Expensive



- **Little to no "complex" (3D, …) cell-based data!**

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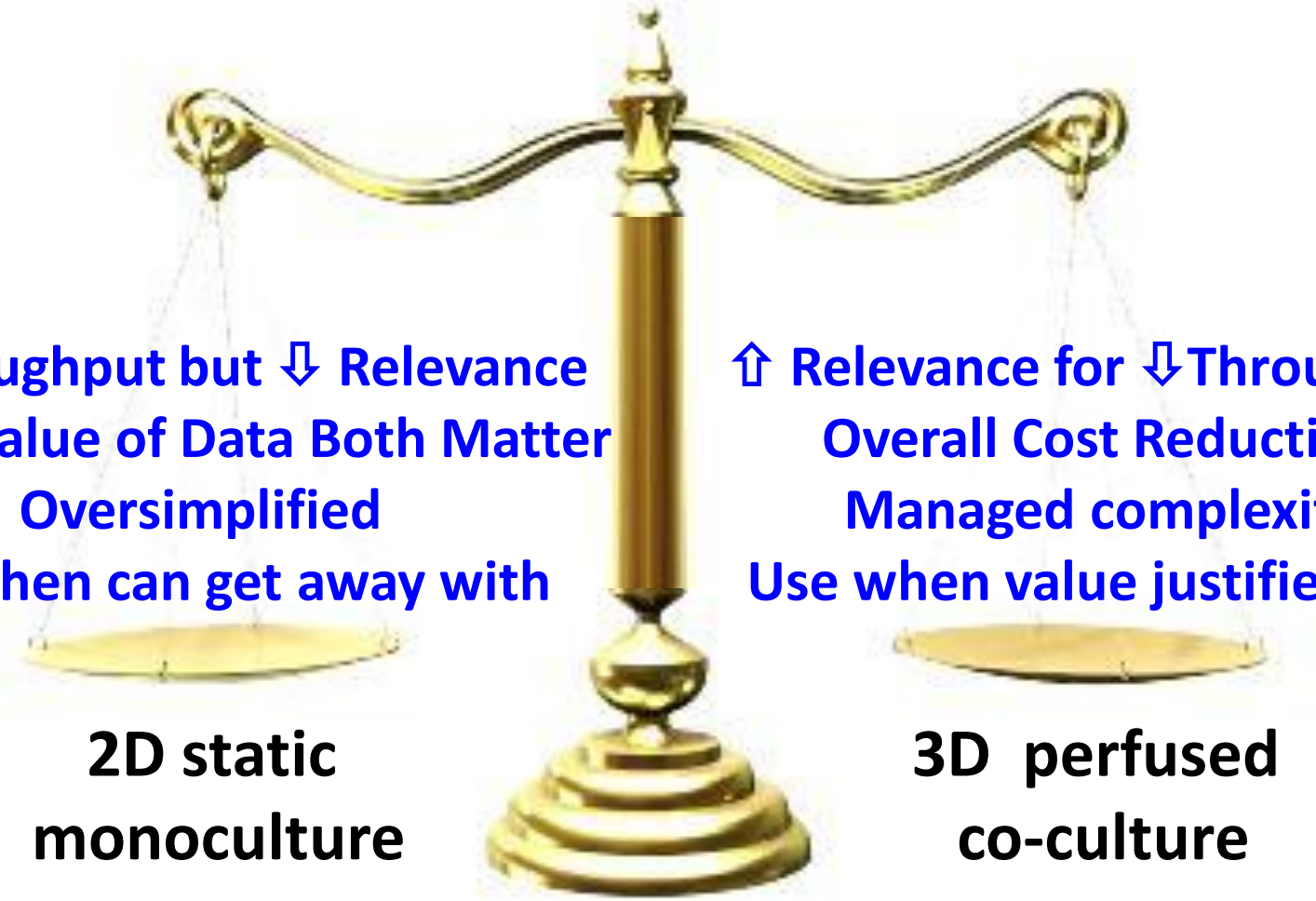
New Paradigm - *in vitro* Models' Value

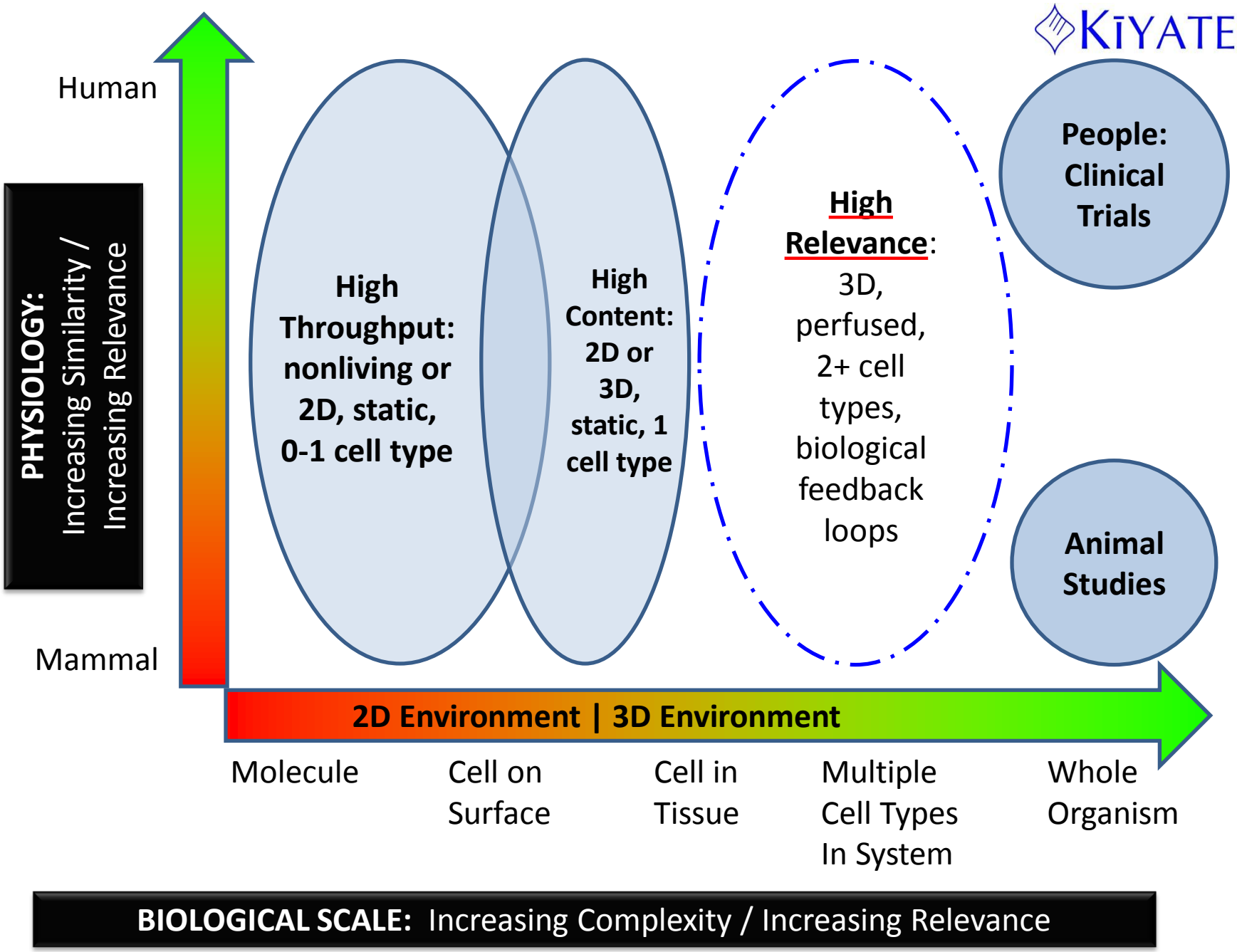


⇧ Throughput but ⇩ Relevance
Cost & Value of Data Both Matter
Oversimplified
Use when can get away with

2D static monoculture

⇧ Relevance for ⇩Throughput
Overall Cost Reduction
Managed complexity
Use when value justifies cost

3D perfused co-culture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Mammal
High Throughput: nonliving or 2D, static, 0-1 cell type
High Content: 2D or 3D, static, 1 cell type
High Relevance: 3D, perfused, 2+ cell types, biological feedback loops
People: Clinical Trials
Animal Studies
2D Environment | 3D Environment
Molecule
Cell on Surface
Cell in Tissue
Multiple Cell Types In System
Whole Organism
BIOLOGICAL SCALE: Increasing Complexity / Increasing Relevance

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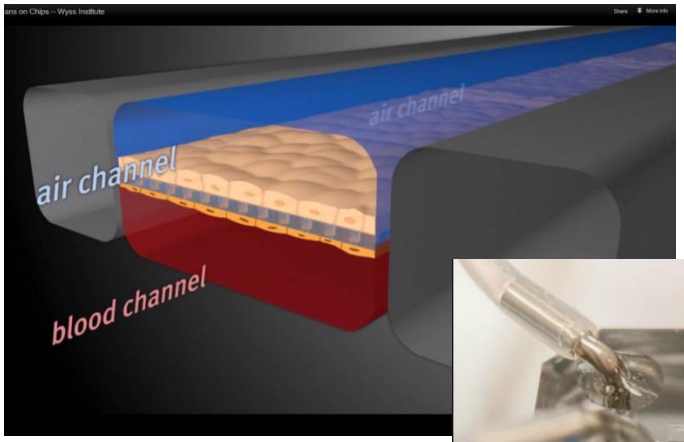
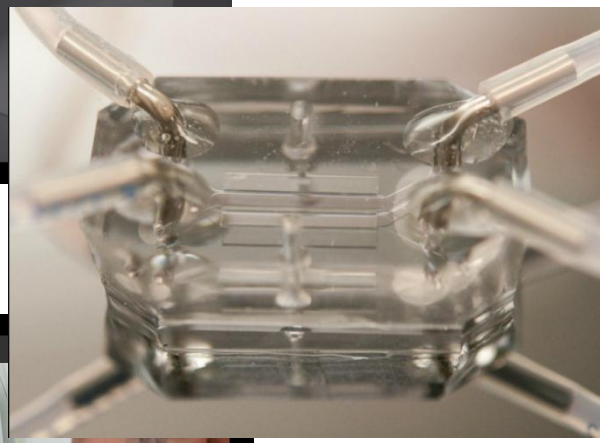
Layered Approaches: Micro-fluidics

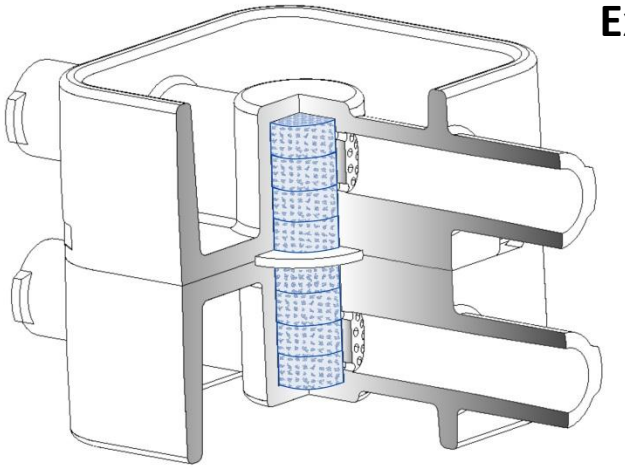




Example research system: Wyss "Organ On A Chip"

- 2D? 3D?
  - μm dimensions
  - Barrier / lumen emphasis
- Perfused
  - Focus on "over" flat
- Co-culture
  - Segregated & mixed
- Now
  - Commercialized passive plates; single organ models
- Future
  - Linked system models

Layered Approaches: Meso-fluidics



Example commercial platform: KIYATEC 3DKUBE™

Easy loading and removal of cell-scaffold construct

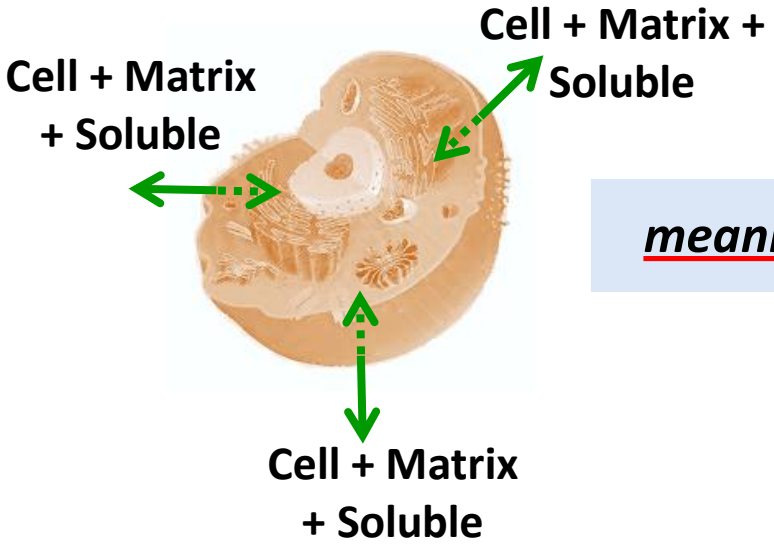
- 3D
  - mm dimensions
  - Tissue emphasis
- Perfused
  - Focus on “through”
- Co-culture
  - Segregated & mixed
- Now
  - Commercialized pumped platforms; single organ models
- Future
  - Linked system models; crossover to regen. med. / tissue eng.

Engineered 3D environments for living, human cells in laboratory culture

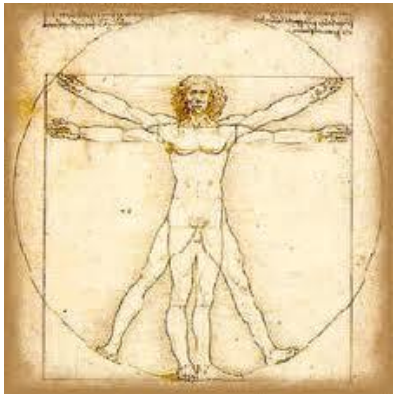
Accurate recreation of living, interacting physiologic systems in human being

prediction

- fewer drug clinical trial failures
- best patient therapy chosen first
- RRR of animal research



meaningful *output*



construction

- better cell therapy process
- biomanufacturing (tissue eng)
- harnessed biology (regen med)

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