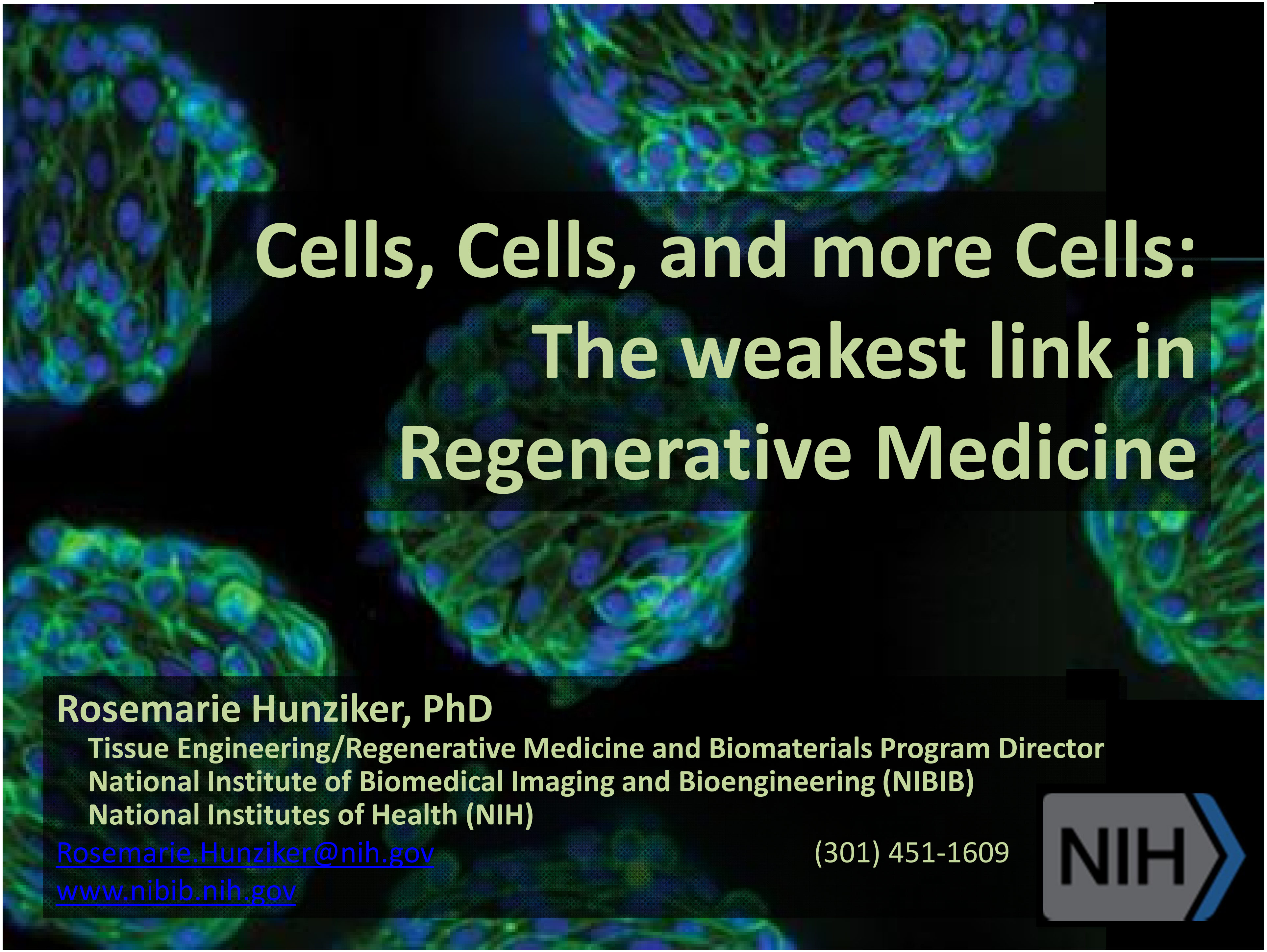


Cells, Cells, and more Cells: The weakest link in Regenerative Medicine

Rosemarie Hunziker, PhD
Tissue Engineering/Regenerative Medicine and Biomaterials Program Director
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
National Institutes of Health (NIH)
Rosemarie.Hunziker@nih.gov
www.nibib.nih.gov
(301) 451-1609

Six Take Aways

- NIH directs $$ into the possible outcomes of Additive Manufacturing approaches, not in the methods development
- Federal funding for Regenerative Medicine is concentrated on discovery science. Technology development is secondary
- Key challenges for clinical deployment of cell-based therapy include Bioprocessing (Bioprinting + Cell manufacturing)
- Scaling-Up/Scaling-Out (i.e. cell manufacturing) are urgent bottlenecks in translating Regenerative Medicine solutions
- Federal Programs in Cell Manufacturing are emerging, with greatest interest at NSF, NIST, and DoD
- Any specific NIH investments will likely be follow-ons, and address cell differentiation and phenotype maintenance

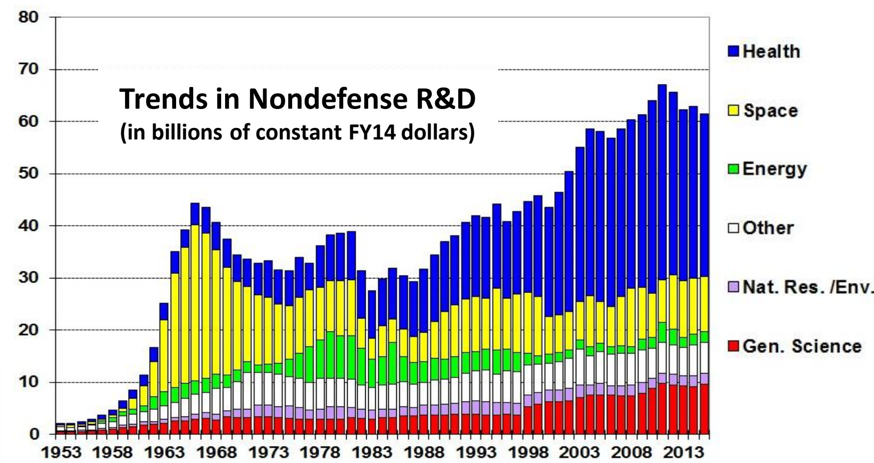
Know Your Target

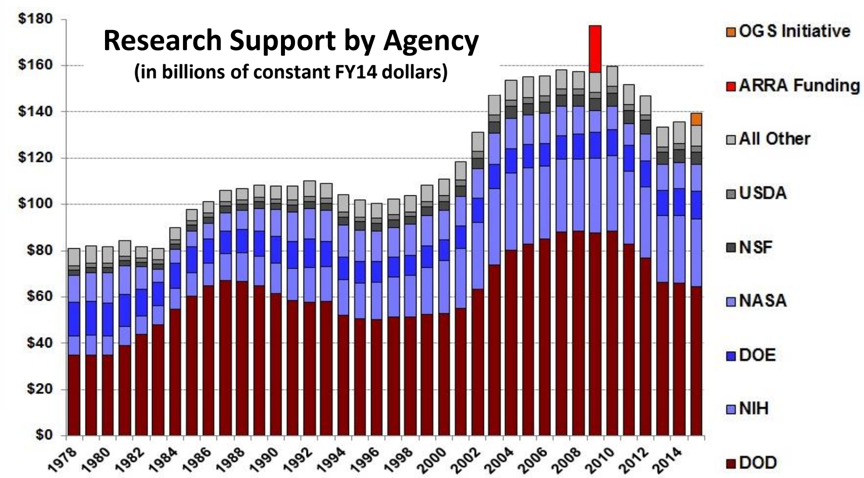
Federal Agencies in Science and Technology have different

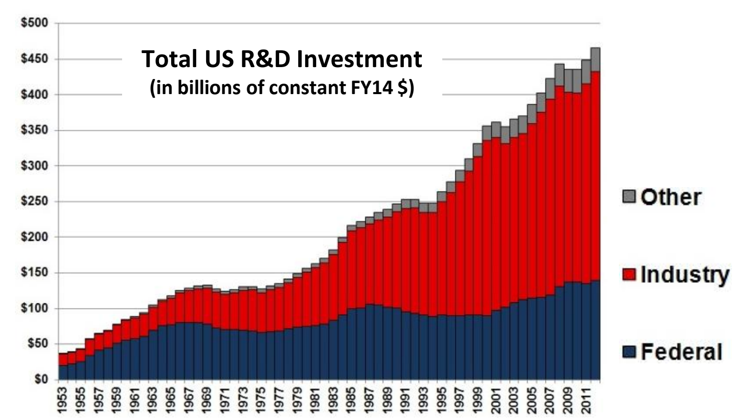
- ✓ missions
- ✓ cultures
- ✓ rules
- ✓ levels of support
- ✓ expectations

But the same overall goal

- ✓ protect the security, health, and well being of Americans
- ✓ maintain knowledge and application superiority
- ✓ fuel the engine of US economic growth







Source: AAAS http://www.aaas.org/page/historical-trends-federal-rd

What is the National Institutes of Health?

Overview

- world's largest biomedical research agency
- Organized into 27 Institutes and Centers
- Supports efforts by 300,000 people at 2,500 universities and research institutions
- 6,000 scientists work in NIH's own research laboratories in Bethesda, MD
- FY2015 budget = $30 B

Mission

seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability

Accomplished via understanding/translating

- causes, diagnosis, prevention, and cure of human diseases
- processes of human growth and development
- biological effects of environmental contaminants
- mental, addictive and physical disorders
- collection, dissemination, and exchange of information in medicine and health

NIH does NOT pursue discovery science for it's own sake, but to address the underlying biology of health

NIH and Bioprinting

- Search of the NIH RePORTer database for "Bioprinting" or "Additive Manufacturing" yields 5 grants

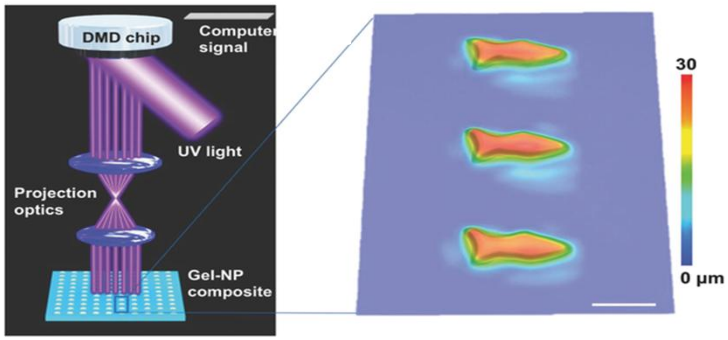
NIH RePORTER

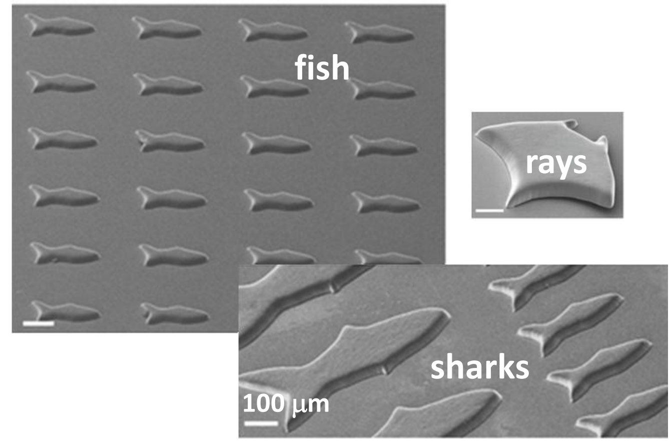
- RePORTer search for
  - "Scaffold" yields 1294 active grants
  - "Tissue Engineering" gives over 3500

NIH is an active supporter of research using (and developing) bioprinting, without specific programs focused on this approach

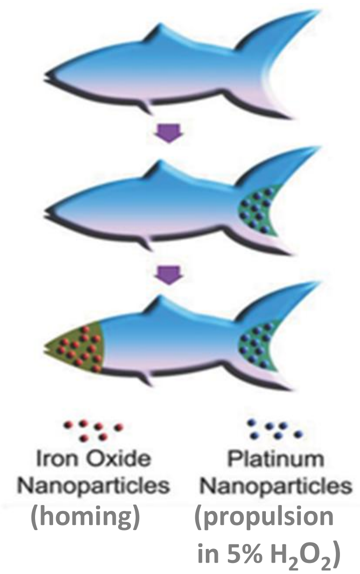
Two examples...

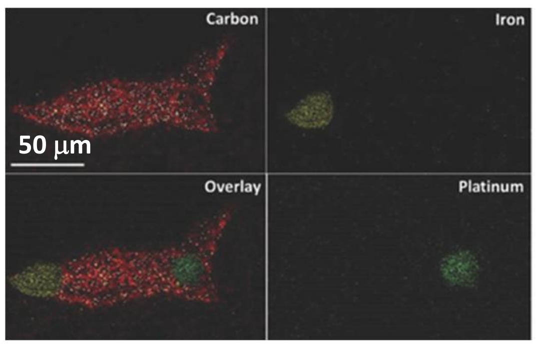
3D-Printed Microfish: Scalable Delivery Systems





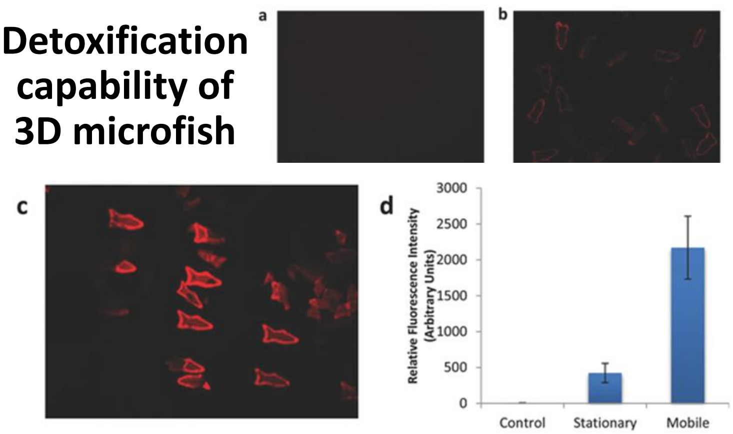
Micromirror-spread UV light generates reproducible, high resolution shapes in photosensitive hydrogels





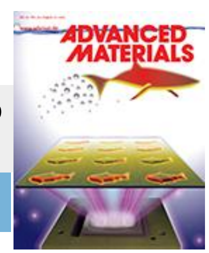
Layer-by-layer approach localizes functionality

Detoxification capability of 3D microfish



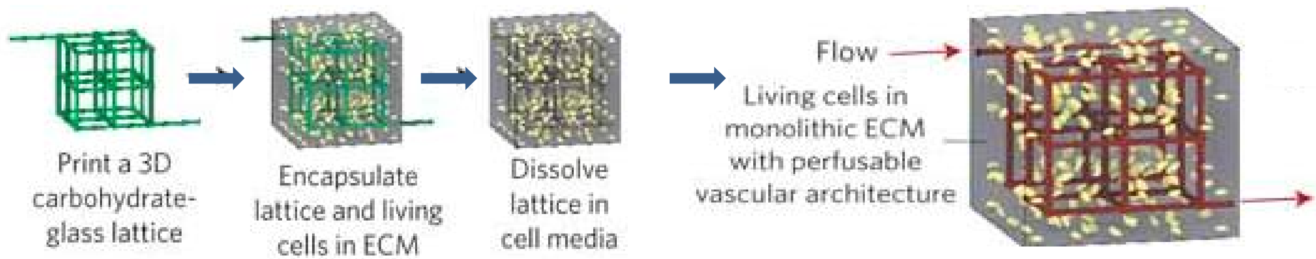
Control in H_2O_2 without toxin (a). Microfish incubated in 2.5 mg mL^{-1} melittin toxin , 5% H_2O_2 either stationery (b) or mobile (c). Relative fluorescence (d) indicates melittin absorbed by the microfish.

Shaochen Chen, PhD
EB 12597, EB 17876
UCSD Jacobs School of Engineering



Zhu, et al (2015) Adv Mater *27*: 4411

Rapid, robust casting of patterned vascular networks for engineered tissues

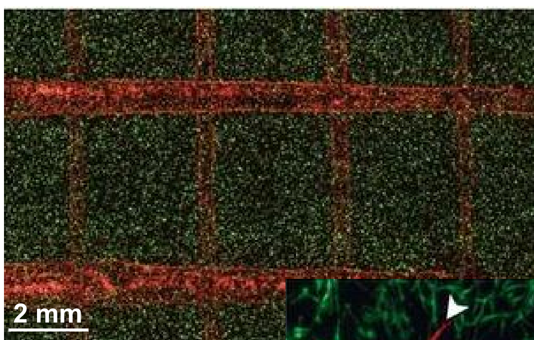


R01 EB 8396
(Chen and Bhatia)

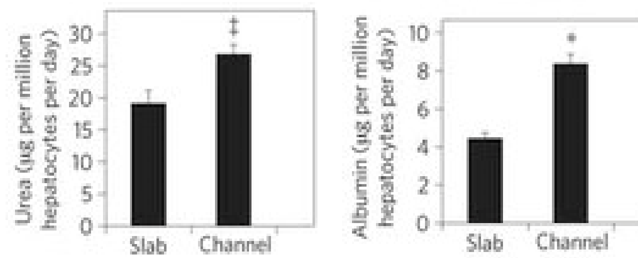
Nature Materials (2012)
doi: 10.1038/NMAT3357

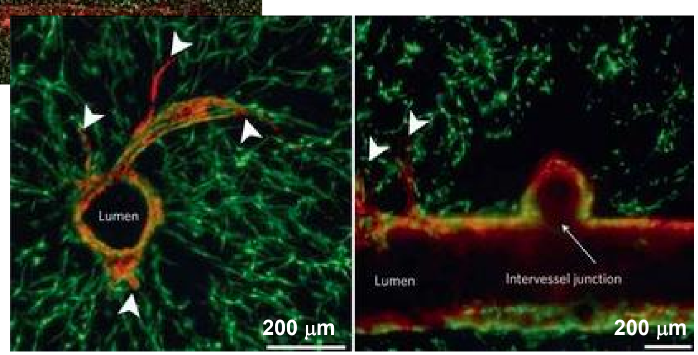
Open, interconnected carbohydrate-glass lattice is printed as the sacrificial element and encapsulated along with hepatocytes. Flowing cell media dissolves lattice (without damage to nearby cells) yielding a monolithic tissue construct with a continuous vascular architecture.

10T1/2 (EGFP)
Interstitium
HUVEC (mCherry)
Vascular wall

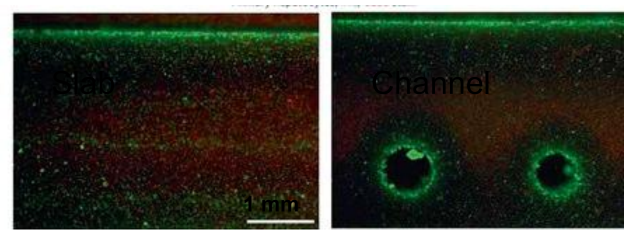


Interstitial stem cells surround open channels lined with **endothelial cells**. Scale bar = 2 mm.



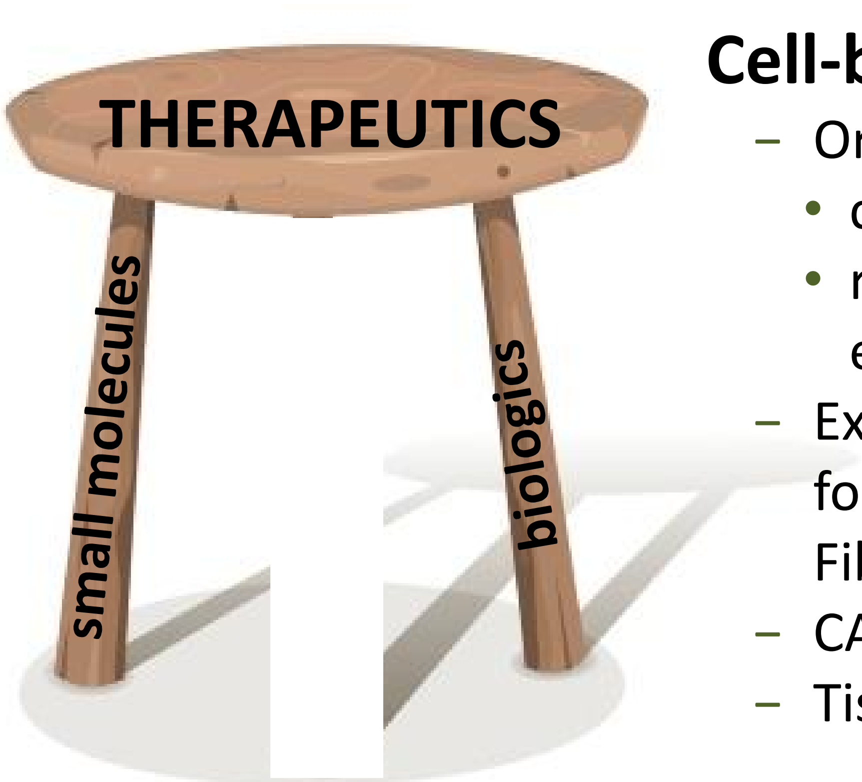


Neovascularization via endothelial cell sprouting (arrowheads) from patterned channels.



Hepatocytes in agarose gels without (slab) or with (channel) vascularization. Metabolic state shown by urea (a) and albumin (b) secretion.

Evolution of Medicine



Cell-based "Killer Apps"

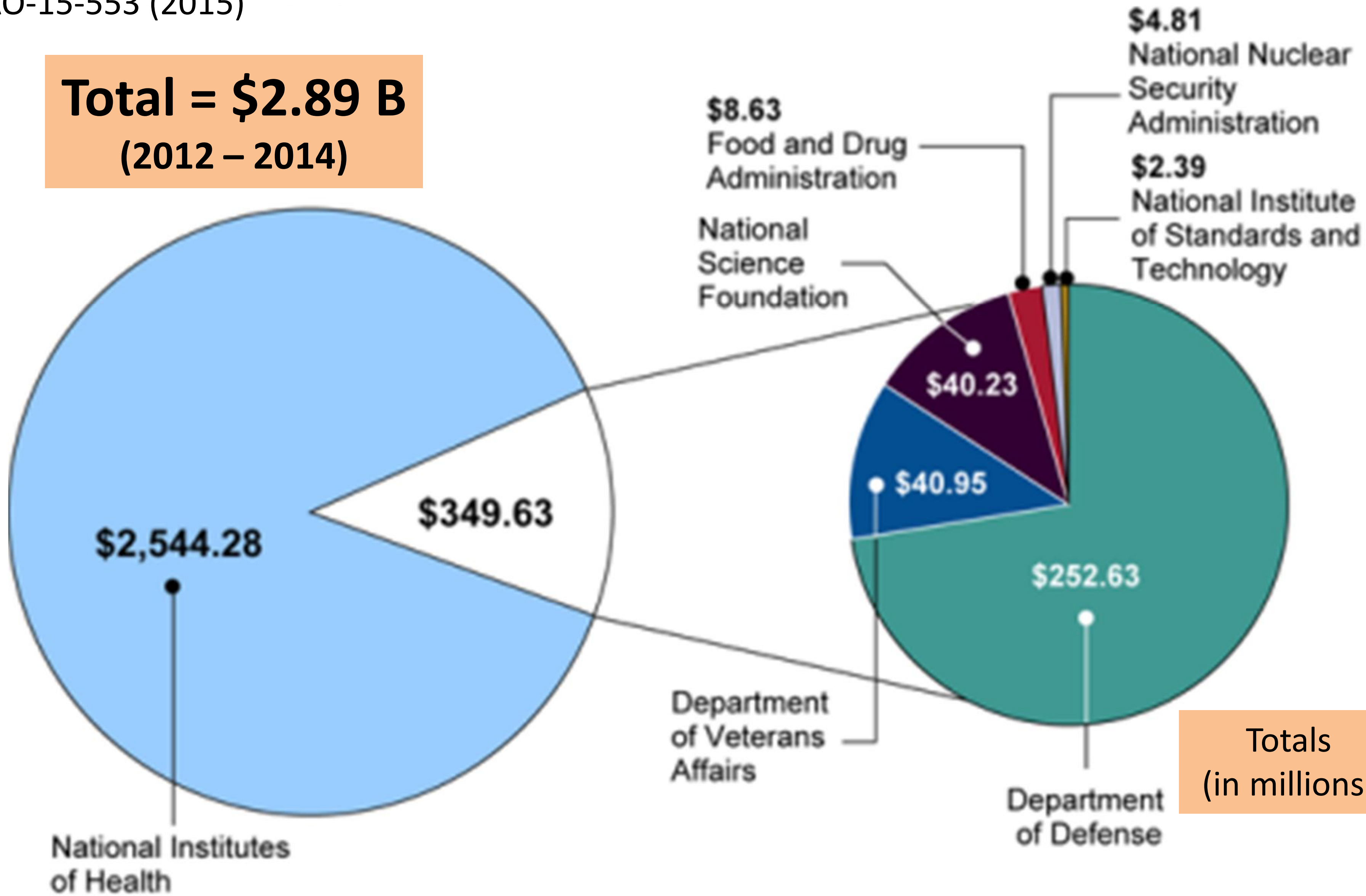
- Organ/tissue replacement via
  - cell transplantation
  - recruitment/mobilization of endogenous cells
- Ex vivo gene therapy and implantation for genetic deficiencies (e.g. Cystic Fibrosis, SCID)
- CAR-T cell therapy to kill tumors
- Tissue Chips/MicroPhysiological Systems

Fischback, Bluestone, and Lim (2013) Science Trans Med *5*: 179.

Regenerative Medicine: Federal Investment, Information Sharing, and Challenges in an Evolving Field

GAO-15-553 (2015)

Total = $2.89 B
(2012 – 2014)



Totals
(in millions)


Discovery Science
Proof-of-Principle
Road Less Traveled
Manufacturing
Federal Investment Highway for Regenerative Medicine

If I had asked people what they wanted, they would have said "faster horses". - Henry Ford

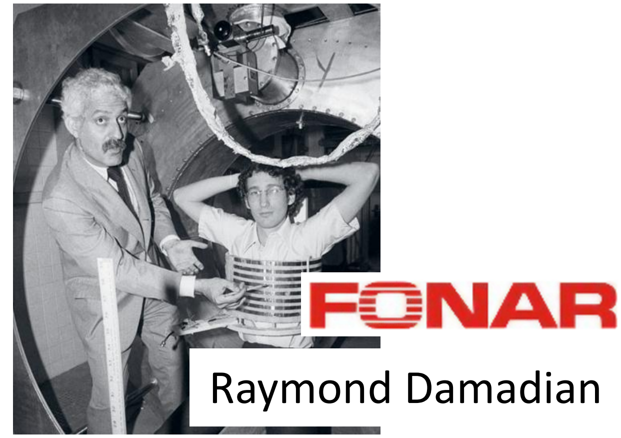
Disruptive technologies typically enable new markets to emerge.

- Clay Christensen

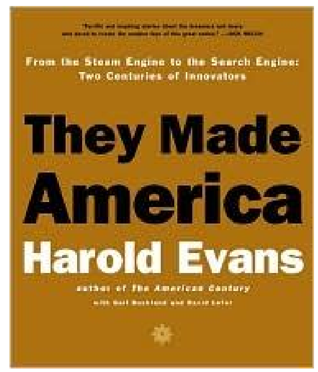
Largest Impact?

Paul Lauterbur and Peter Mansfield

Magnetic Resonance Imaging

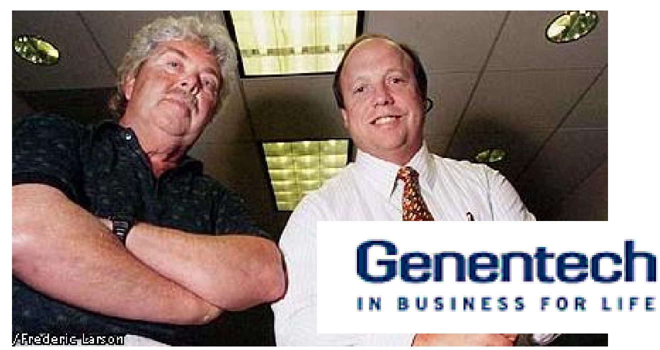


Raymond Damadian



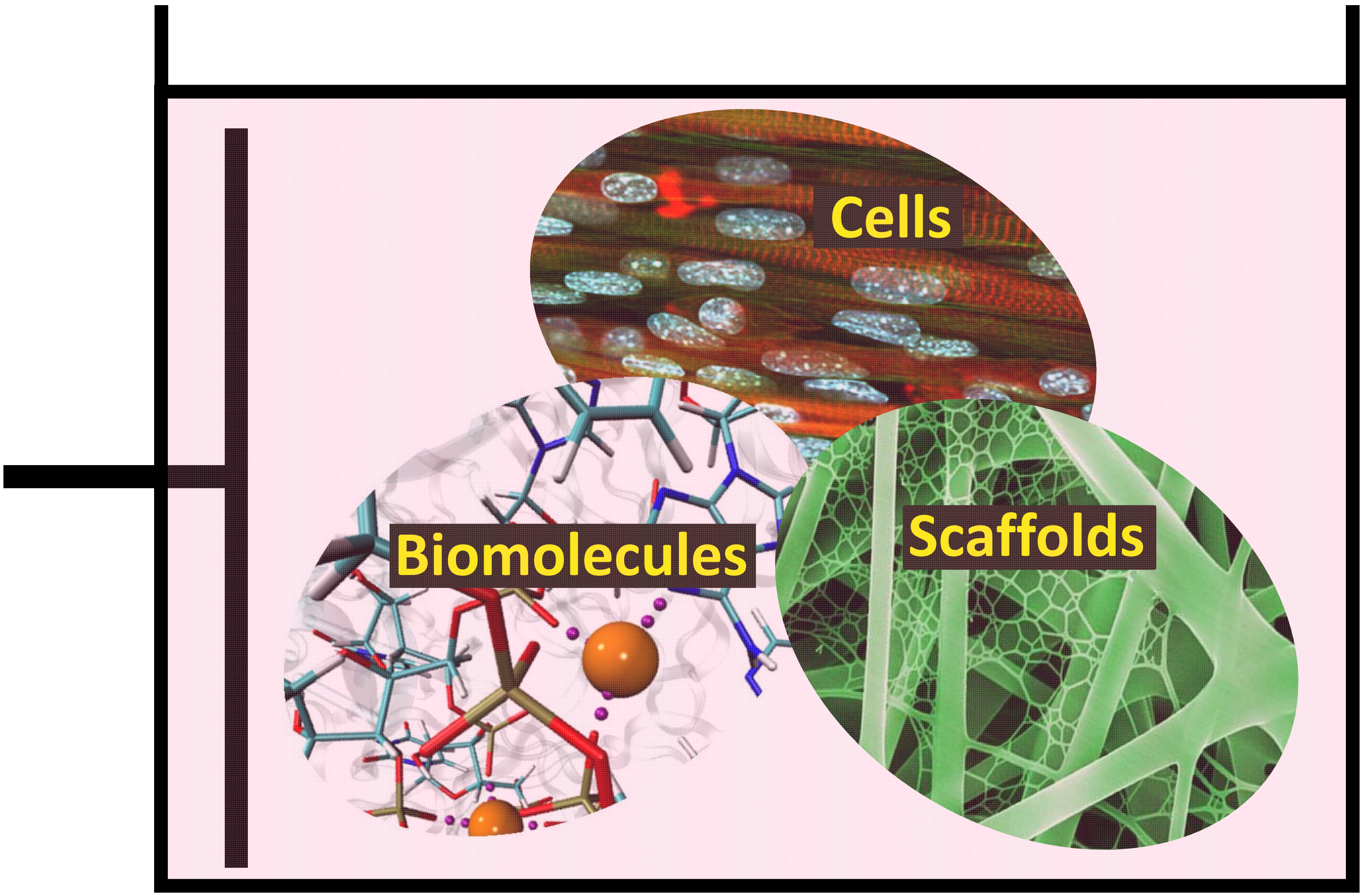
Paul Berg, Walter Gilbert, and Fred Sanger

Recombinant DNA



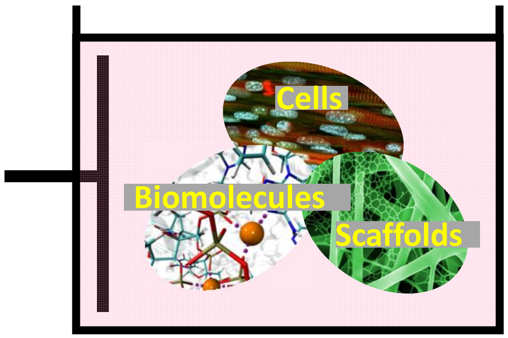
Herb Boyer and Bob Swanson

Key Elements of Engineered Tissues



Life Cycle of a Cell-Based Therapeutic

Discovery:
Designing a construct



Utility:
Wide-spread use and monitoring

lots of grant $$ here

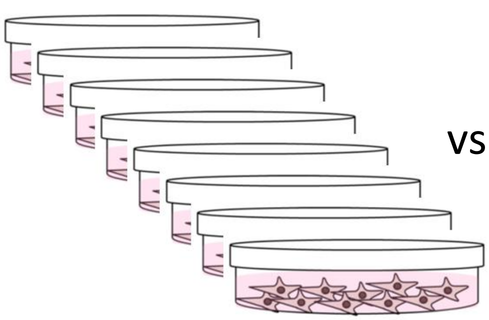
lots of attention here

Where are the
Gaps?

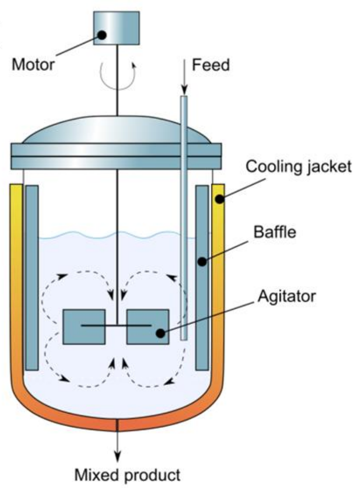
??

good models here

Development:
Scale up or scale out



vs



Deployment:
Clinical trials, market approval

State-Of-the-Art:
BIOLOGICS Production Systems

SCALE UP: Batch processing

SCALE OUT: GMP incubator

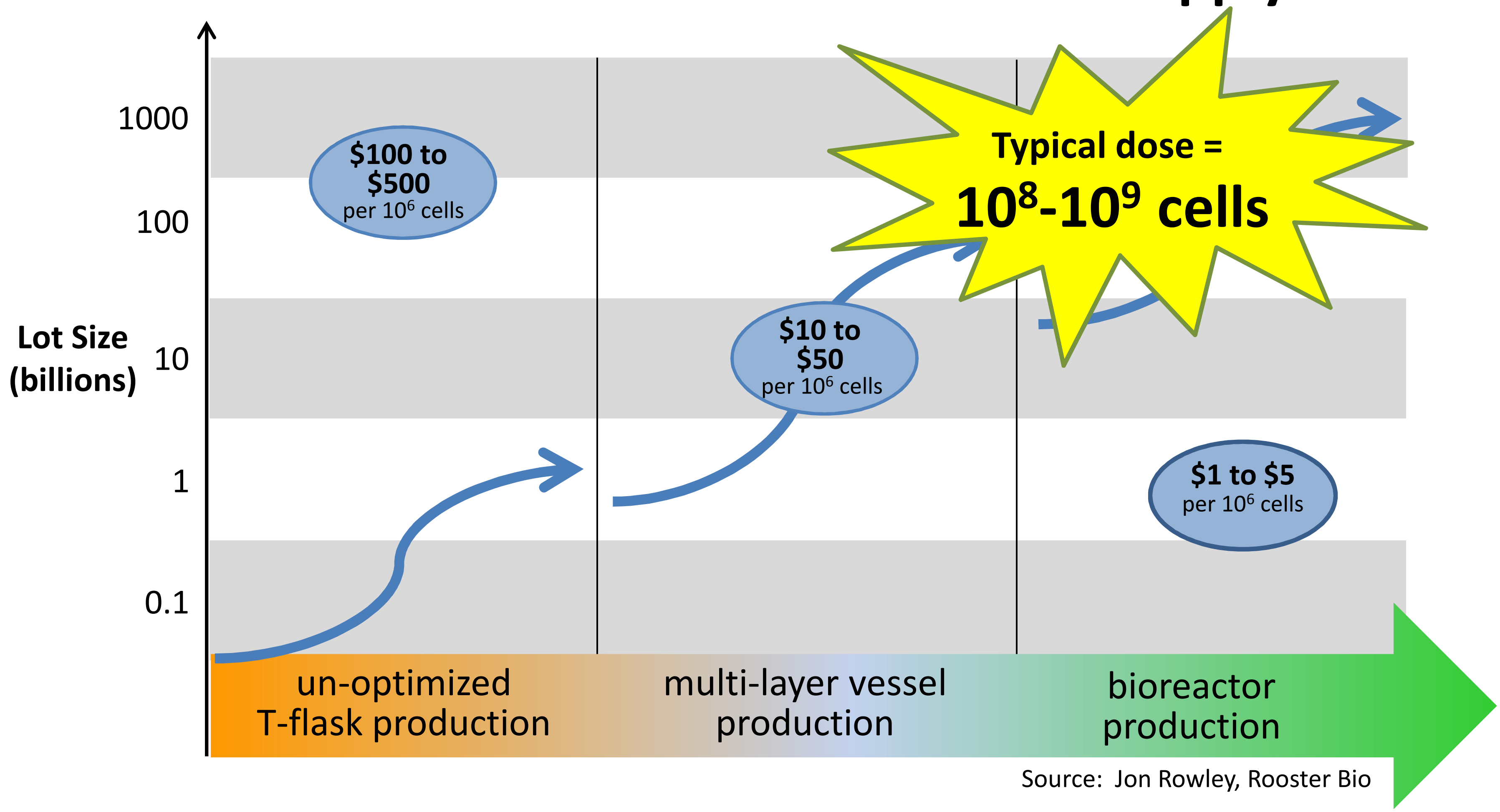
Requirements

- well-characterized cell lines synthesizing secreted molecules at high productivities
- low cost, high quality, readily available, traceable materials and supplies
- in-line sensors/monitors and fast, easy off-line assays
- effective, efficient separations process
- maximizing automation and minimizing process transitions along the production chain

How can this be adapted for CELL-BASED Therapies?

Manufacturing Platforms MUST Evolve

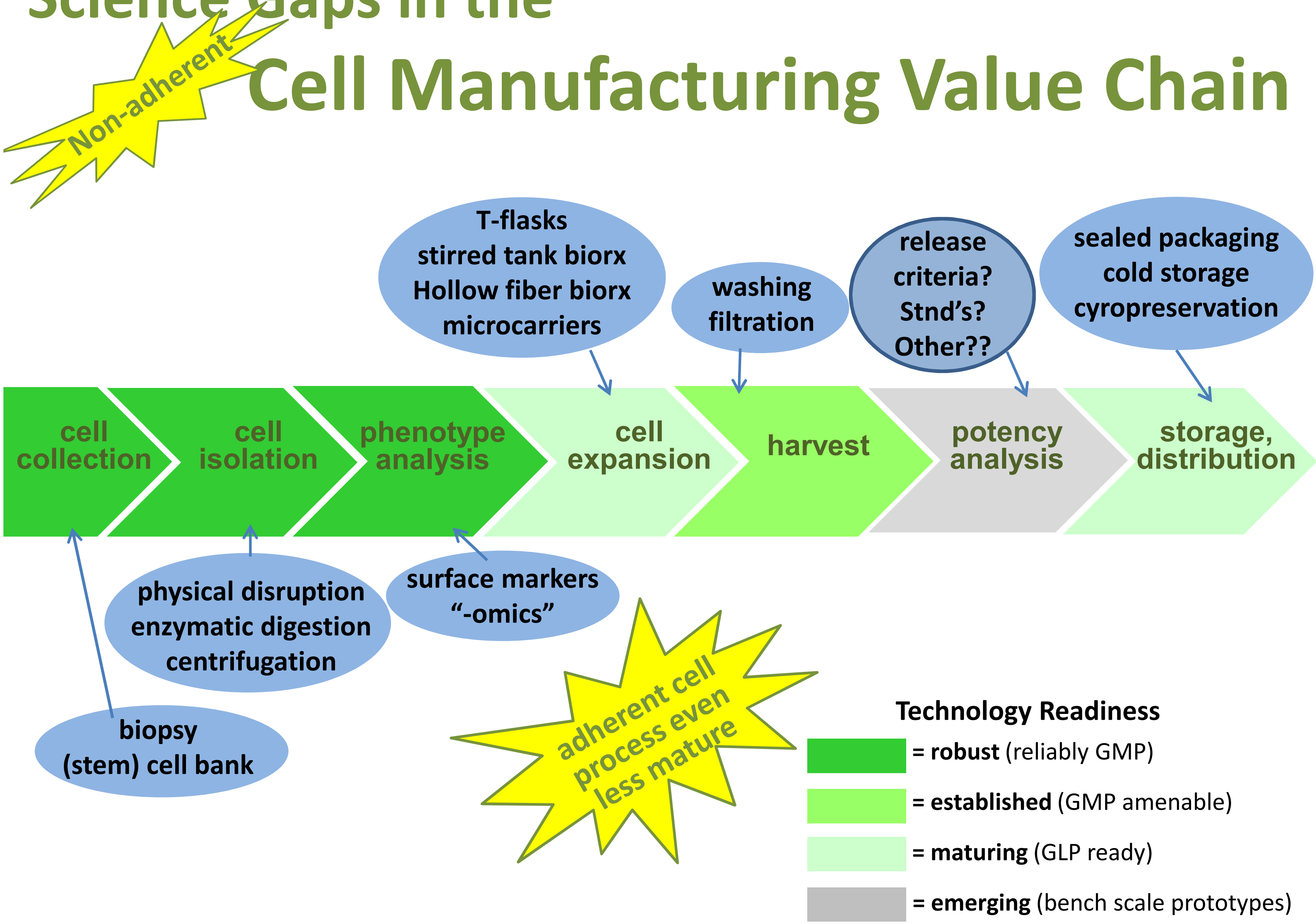
Lower costs → Drive Innovation → Supply Cells



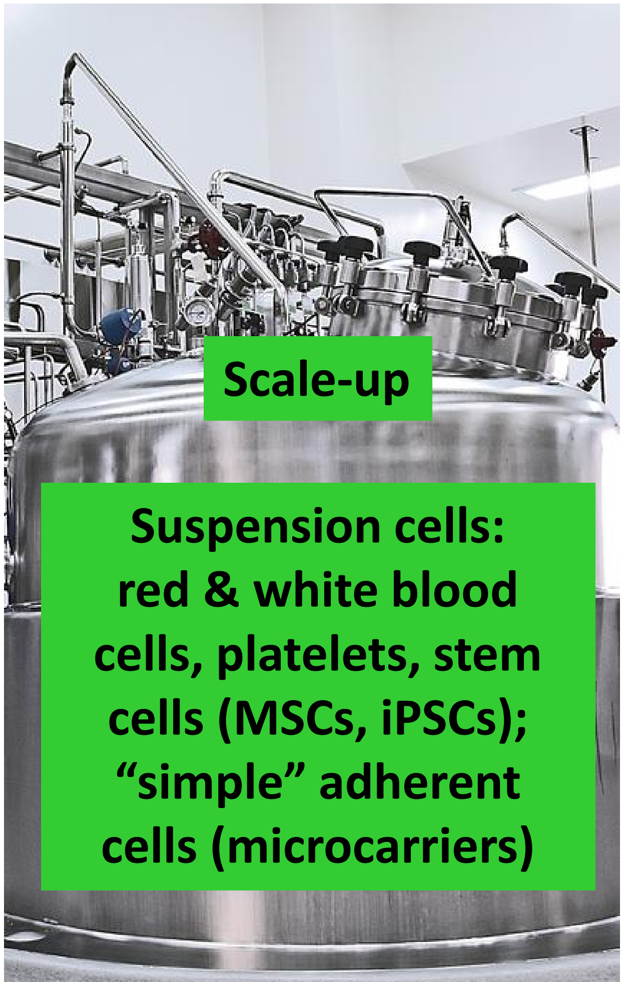
Source: Jon Rowley, Rooster Bio

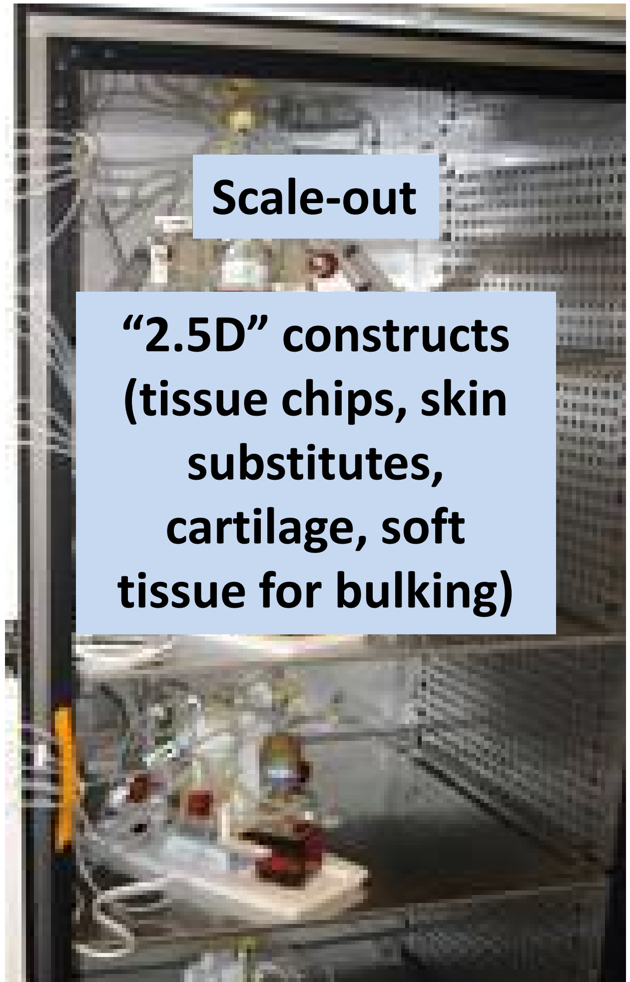
See also: Brindley, et al (2012) "Peak serum: implications of serum supply for cell therapy manufacturing" Regen Med *7*: 7.

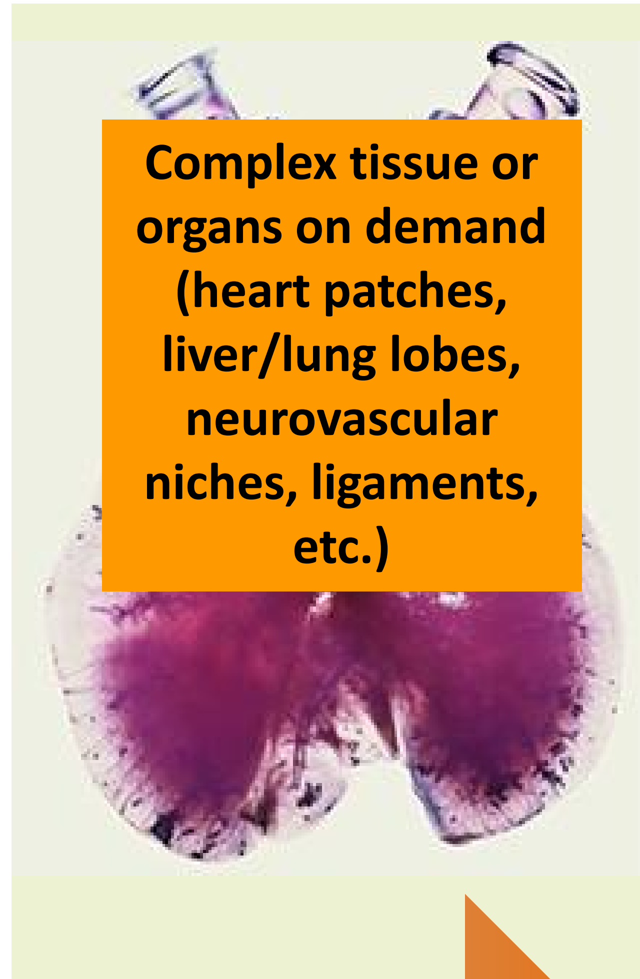
Science Gaps in the Cell Manufacturing Value Chain



Cell Manufacturing: Readiness Levels







Now
Optimize/Re-engineer existing technologies

Soon
Streamline/harden microfluidic platforms

??
Invent new processes

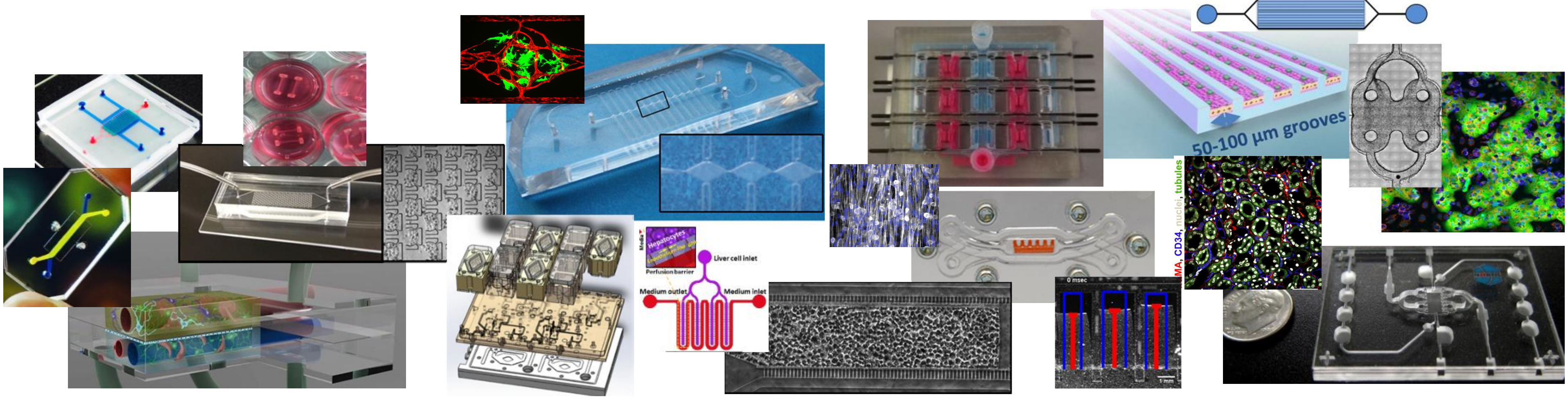
What keeps bioprocess engineers up at night?

The lack of sufficient

- Scale-up, quality control for batch processing
- Scale-out of 3D tissue units (e.g. “tissue chips”)
- Separations technologies for product purification
- Tractable induction of tissue morphogenesis
- Validated biomarkers for pluripotency and/or differentiation along the entire production line
- Additive manufacturing (bioprinting) of 3D constructs containing cells and supporting matrices
- Built-in, continuous, on-board, in-line, non-destructive monitoring
- Bioreactors with chemical and physical (i.e. electrical, mechanical) spatio-temporal gradient control
- Release standards
- Preservation technologies to sustain fragile constructs under biologically optimized conditions

Basic Science Challenges for Tissue Chips

- Substitution for polydimethylsiloxane (PDMS) which absorbs small and large hydrophobic species
- Platform compatibility and/or connectivity
- Universal media that “connects” all tissues on a platform
  - Interface circulating “blood substitute” and local, tissue-specific medium
- **Scaling and translating to physiologically based pharmacokinetic (PBPK) models**



Federal Manufacturing-related Efforts

Discovery → Development → Production

NSF

- Bio-printing -> additive manufacturing
- Cellular Biomanufacturing Initiatives (EAGERs)
- Global Assessment(s) of Biological Engineering & Manufacturing

NIH (National Institutes of Health)

- Bio-printing portfolio(s)
- Tissue on a chip
- Production Assistance for Cellular Therapies
- Biopharmaceutical Development Program

NIST (National Institute of Standards and Technology)

- AmTech Cell Manufacturing Consortium
- Manufacturing Innovation Institutes
- Metrics, standards, measurements for product characterization

FDA (U. S. Food and Drug Administration)

- Manufacturing quality control development and research
- Regulatory oversight

DHA (Defense Health Agency)

- Regenerative Medicine Manufacturing Technology
- Manufacturing Innovation Institutes

Department of the Army, United States of America

- Medical Technology Enterprise Consortium (MTEC)

DARPA

- Tissue on a chip
- Living Foundries

Thanks to: Kristy Pottol,USAMMDA

Cell Manufacturing Consortium

National Institute of Standards and Technology

Advanced Manufacturing Technology Consortia

Competitive Planning Grants to:
establish and strengthen industry-focused research consortia,
develop shared vision technology roadmaps of industry's needs.

… aims to position the United States as the leading developer of cell-manufacturing technologies and the chief authority on cell manufacturing standards, worldwide.

Georgia Research Alliance

Aruna Biomedical, Celgene Cellular Therapeutics, Cellular Dynamics International, Georgia Inst of Technology, North Carolina State Univ, RoosterBio, Univ of California Berkeley, Univ of Georgia, Univ of Wisconsin

EArly Concept Grants for Exploratory Research (EAGER) for Cellular Biomanufacturing

Topics

- **increase efficiency, rate of stem cell tissue-specific differentiation**
- **rapid, non-destructive cell phenotyping and potency analysis**
- **bioreactors for reproducible cell expansion/differentiation**
- **separation technologies (for starting biopsy or final product)**
- **robust correlations between biomarkers and cell functionality**
- **cell source variability in autologous cells from various subjects**
- **stable cell lines better suited to protein therapeutics manufacture**
- **computational models of stochastic cell variability**

13 awards, $3.7 M

Even more EAGERs

Advanced Cellular Biomanufacturing

Topics

- Culture configurations/bioreactors for reproducible cell expansion
- Expand T-cells that are tumor specific and have central memory
- Scalable, cost-effective cell separation and purification
- Reduce or eliminate cell product variability in manufacturing
- Rapid, non-destructive characterization of cellular potency
- Mathematical methods and computational models to characterize variability (e.g. cell subtypes during biomanufacture) which could be used for process tracking and/or validation.

Proposals due June 1, 2016!

http://www.nsf.gov/pubs/2016/nsf16053/nsf16053.jsp

U.S. Army Medical Research and Materiel Command

Medical Technology Enterprise Consortium

Defense Health Agency

501(c)(3) non-profit corporation to organize and conduct advanced applied research for prototype development addressing military capability needs.

SCRA Technology Ventures

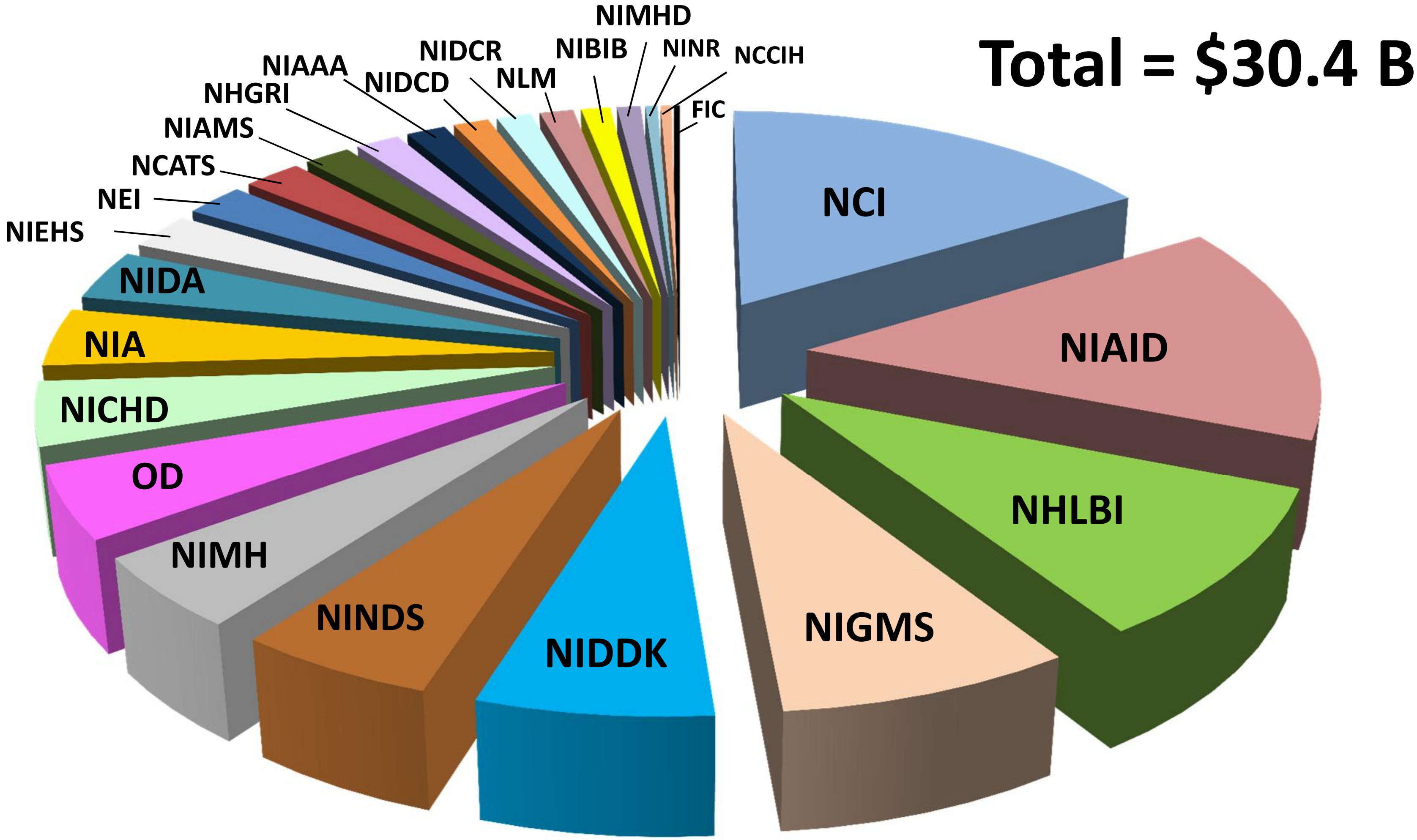
- **Biomedical prototype advanced development**
  - Infectious diseases
  - Combat casualty care
  - Military operational medicine
  - Clinical and rehabilitative medicine
  - Medical chemical, biological, radiological defense
- **Capitalization of private sector technology opportunities**
- **Technology transfer**
- **Commercialization of Government intellectual property**
- **Follow-on production**

Emerging topic: Regenerative Medicine Manufacturing

http://www.army.mil/article/154173/USAMRMC_Awards_New_Medical_Technology_Enterprise_Consortium/

NIH FY15 Budget

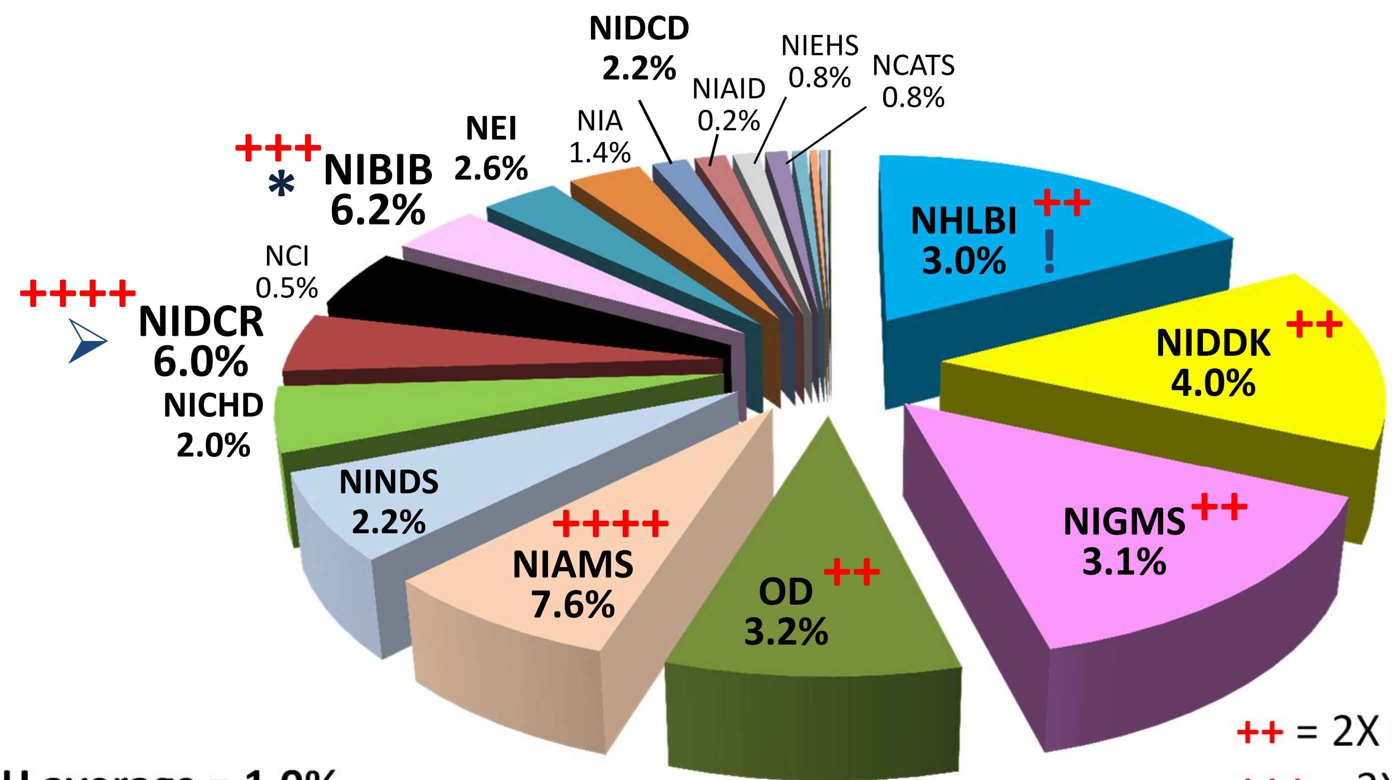
NIH Divides most of its investment according to the interests of the component parts (i.e. Institutes or Centers), with <5% allocated to trans-NIH initiatives.



About 85% distributed via Extramural grants, contracts, cooperative agreements

NIH FY15 Regenerative Medicine Funding

Wedge size = $$ as fraction of NIH total
% = $ as % of IC total budget



++ = 2X NIH average
+++ = 3X NIH average
++++ = 4X NIH average
➢ = longest involvement
* = lead IC
! = most programs

NIH average = 1.9%

- < average: NCATS, NCI, NHGRI, NIA, NIAAA, NIAID, NIDA, NIEHS, NIMH, NIMHD
- none: FIC, NCCIH, NINR, NLM

Filling the Production Gap in Translational Science

Production Assistance for Cellular Therapies

National Heart Lung and Blood Institute

National Institutes of Health

- **Establish Master Cell Banks**
- **Manufacture clinical grade product(s)**
  - IND-enabling pre-clinical studies
- **Work with FDA to facilitate translation**
- **Cell characterization for clinical use**

PACT III **PACT I &II**

***Manufacturing**
- Scale up
- Validation
- Release Criteria
- CMC

***Animal Studies**
- GLP/GMP product
- Efficacy
- Toxicity

Dose escalation; safety and toxicity studies; small trial size

IND Filing

Discovery; Proof of concept; Therapeutic mechanism; cell and disease interaction

Dose ranging; Safety studies; escalating trial size

Efficacy and safety studies; full product characterization; potency; scale up; full GMP

Basic Research → **Pre-clinical** → **Phase I** → **Phase II** → **Phase III**

Previous contract (2010 – 2015) delivered > 650 cell preparations (WBCs, MSCs, cardiac precursors).
PACT III contract proposals under evaluation.

RFA-15-004/008/017:
Bioreactors for Reparative Medicine

National Heart, Lung, and Blood Institute

NHLBI, NIBIB, NCATS

Small business opportunities (FY15 – 17) $21M available

Potential topics—REVOLUTIONARY bioreactors incorporating

- Biomarkers, probes to effectively detect and measure evolving phenotype
- Increased efficiency, reduced expense of growing 3D cell aggregates
- Controlled mechanical and electrical loading
- Additive 3D tissue printing using natural and/or artificial materials +/- cells
- Complex lung tissue (multiple cell types, mimicking breathing, air/liquid interfaces)
- GMP standards for bioreactors, production process, scale-up, test methods
- Growing human tissues from pluripotent cells in a suitable animal model
- Extended storage or transport of transplant tissues or engineered tissues
- Efficient, low-cost clinical production of human blood cells from progenitors

http://grants.nih.gov/grants/guide/rfa-files/RFA-HL-15-004.html

NIDCR's Translational Regenerative Medicine Pipeline

Stage 1: PLANNING (1 year)

- articulate a vision, roadmap, organizational structure and operating procedures

Stage 2: RESOURCE CENTER (3 Years)

- develop robust infrastructure to deliver technical support and research capacities
- organize, recruit and integrate interdisciplinary teams
- improve, optimize, validate and standardize tools/technologies

Stage 3: CONSORTIUM (4-5 years)

- complete validation, manufacturing, and preclinical testing
- develop INDs/IDEs and initiate Phase I trials

NIH National Institute of Dental and Craniofacial Research

RFA-DE-15-005: Planning Grant Awardees

- Dan Gazit (Cedars-Sinai Medical Center)
- Michael Yaszemski (Mayo Clinic)
- Pamela Yelick (Tufts University)
- Benjamin Wu (UCLA)
- Jeffrey Lotz (UCSF)
- David Kohn (University of Michigan)
- Anh Le (University of Pennsylvania)
- Charles Sfeir (University of Pittsburg)
- Yang Chai (USC)
- George Christ (University of Virginia)

NCI Advanced Technology and Research Facility *- a unique collaboration*

- **Accelerate cancer and AIDS translational research**
- **Frederick National Lab facilities and expertise**
  - 330,00 ft^2 production, testing space
  - genomics, proteomics, nanotechnology, molecular diagnostics, bioinformatics…
- **cGMP grade biologics**
- **Cooperative, co-located R&D partnerships: government, academia, industry**

Bioreactors

1000-L bioreactor for eukaryotic cells in suspension

Protein Purification

Orthogonal column chromatography to purify proteins, viral products

Product Testing and Characterization

Wide array of techniques: HPLC, UPLC, IEF, AAA, FACS, DLS, DSC, CD, TOC, BIAcore, electrophoresis, microfluidics, fluorometry, cell-based assays…

Fill and Finish

Automatic, fully enclosed vial filling

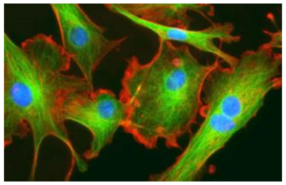
Lyophilization suite

NATIONAL CANCER INSTITUTE®

Bioprinting Dermal Tissue

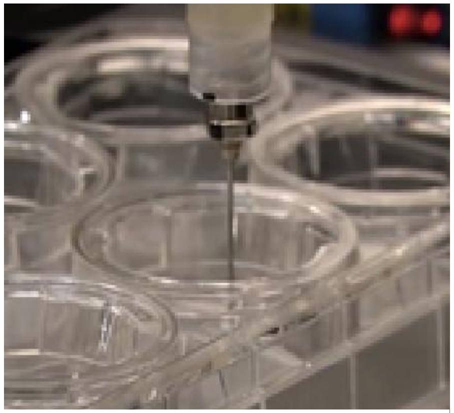
polymer hydrogel

+



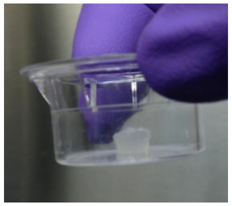
mixed skin cells

printer

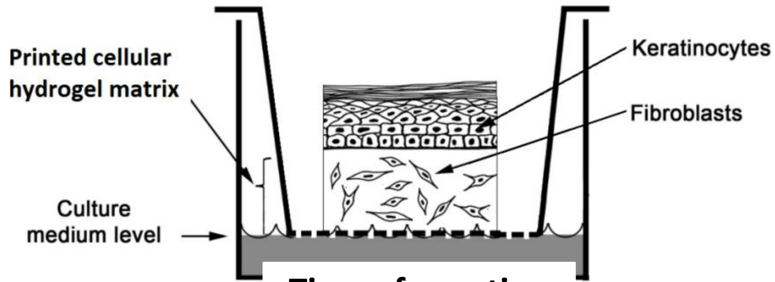


Layer by layer deposition of the cell/gel mixture

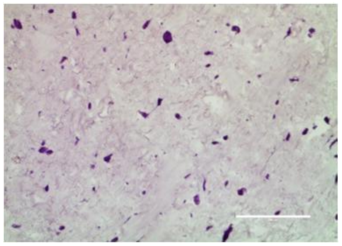
Printed construct incubated to allow cell differentiation and tissue formation.



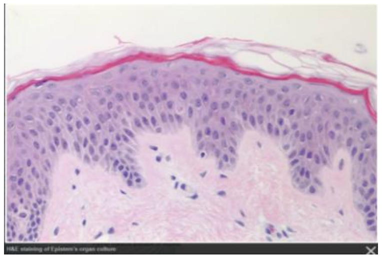
Construct in transwell



Tissue formation during culture



Printed dermal region



Native skin

Histology

NIH Center for Regenerative Medicine *(version 2.0)*

Same Mission
New Director
New Home
New Facilities

Ilyas Singeç, M.D., Ph.D.
Head, Stem Cell Research
Division of Pre-Clinical Innovation
NCATS

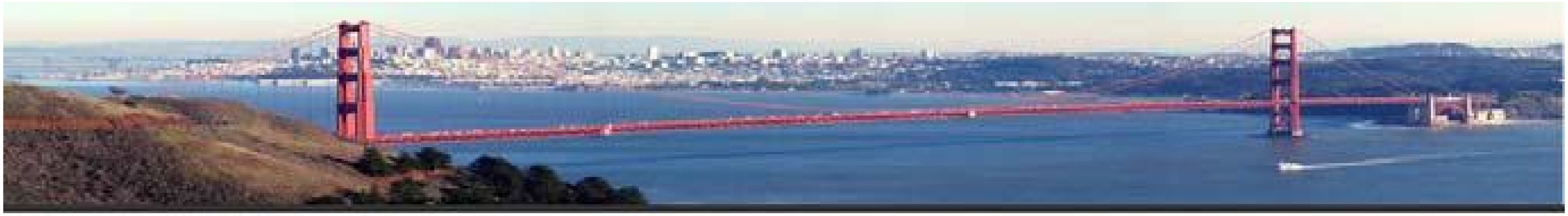
Goals

- industrial-scale cellular engineering for regenerative medicine
- detailed quality control (QC) standards to define
  - differentiated cell identity
  - pluripotency
- Methods (especially computational) to assess variations in cells derived from iPSCs
- standards for producing mature cells meeting GMP standards

NIH Contract Resources for Pharmaceutical Manufacturing

Bridging Interventional Development Gaps (BrIDGs)

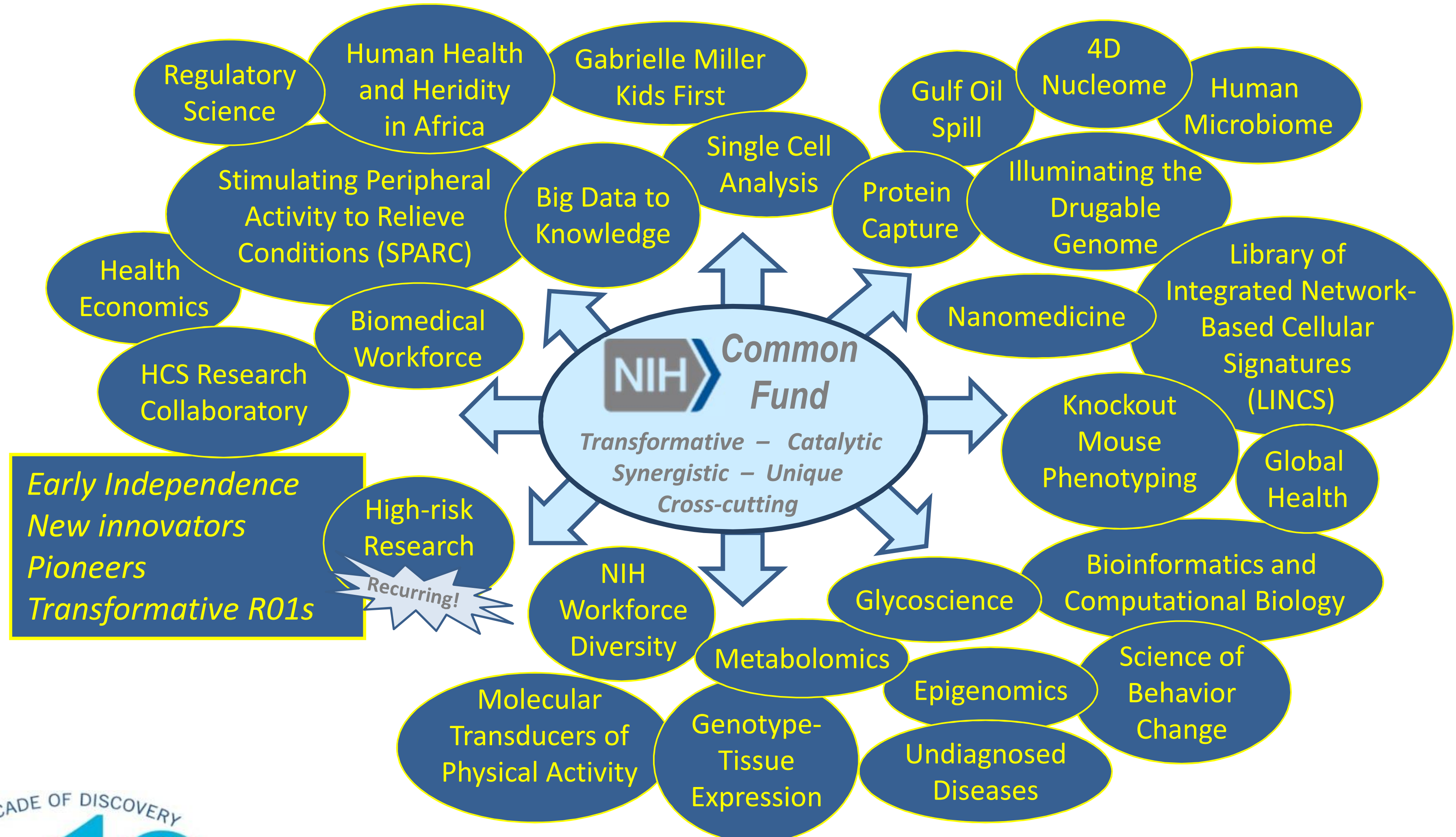
- Access to contract resources for formulation and GMP Manufacturing
- Assay Development for ADMET, PK/PD
- Drugs (small molecules, biologics) and delivery systems (no vaccines, devices or diagnostics)
- Product Development and Regulatory Advice



Therapeutics for Rare and Neglected Diseases (TRND)

- New chemical entities and repurposed drugs
- Access broad scientific, translational, clinical expertise
- In-kind and collaborative research to accelerate development
- Spans spectrum from lead optimization to IND filing
- Integrated approach: underlying mechanisms thru technology platform development

Trans-NIH Programs



A DECADE OF DISCOVERY
NIH COMMON FUND 10 Years 2004-2014

http://commonfund.nih.gov/

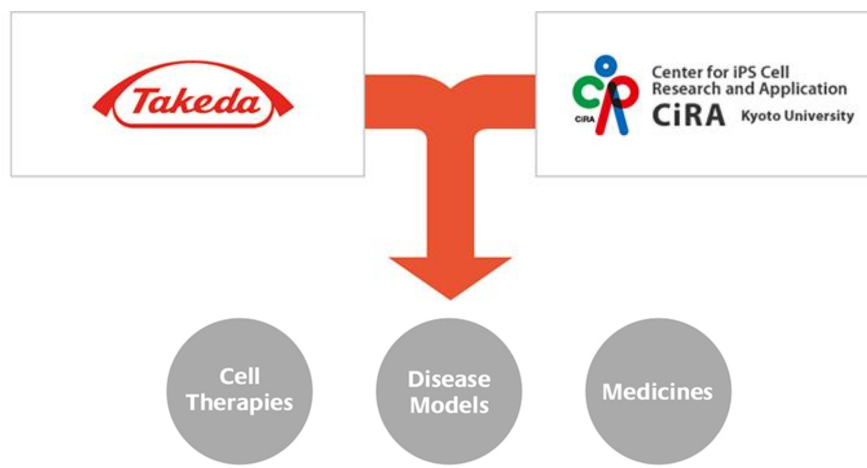
Cell-Based Manufacturing Efforts: Competition Abroad

- £70M, 5 year effort (Innovate UK)
- "growing a UK cell therapy industry delivering health and wealth"
- Create innovation ecosystem
- Translate early stage research into commercially viable therapies

- $20M Australian government grant leverage $59M investment
- Facilitate cost-effective manufacture and rapid translation

Toronto, Canada

- Integrates Canada's stem cell, biomaterials science with business leadership
- Six academic centers, 40 companies
- GMP Bioprocess optimization facility



- Led by Nobel Laureate and iPSC discoverer Shinya Yamanaka
- Takeda to provide ¥20B over 10 years

The future is not made… it is manufactured.

Rosemarie Hunziker, PhD

Tissue Engineering/Regenerative Medicine and Biomaterials Program Director
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
National Institutes of Health (NIH)
hunzikerr@mail.nih.gov **301-451-1609**