



Maedeh Ghasemi

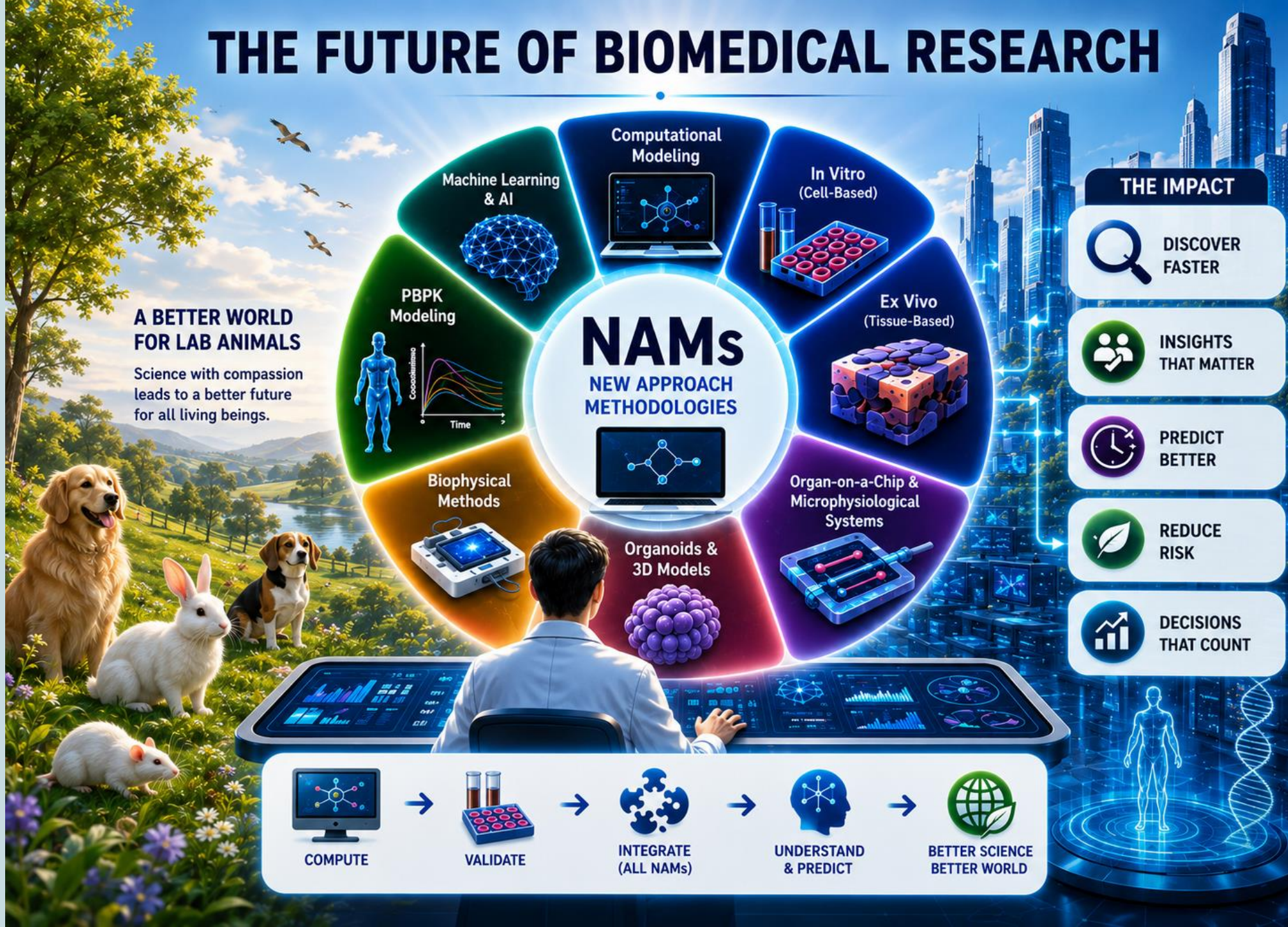
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Animal Replacement Methods



THE FUTURE OF BIOMEDICAL RESEARCH



LEARNING OBJECTIVES

Conceptual understanding

- Why is the world moving towards NAMs?
- Describe the 3Rs framework?

Technical understanding

- what types of research questions is each technology suitable for?
- differentiate between:
 - cell culture
 - organoids
 - organ-on-chip systems
 - in silico models

Critical evaluation

- what are the limitations of each method?
- can technology completely replace animal models or are they more complementary?
- how can a researcher choose the most appropriate model for their project?



If you were to answer this research question, is a new pancreatic cancer drug effective in humans?
which model would you use:



mice?
cell culture?
organoid?
organ-on-a-chip?
artificial intelligence?

Animal Research Statistics Great Britain, 2022

Research facilities in Great Britain record the number of scientific procedures carried out on animals each year. Procedures are categorised by severity, they can be as mild as an injection, or as severe as an organ transplant.

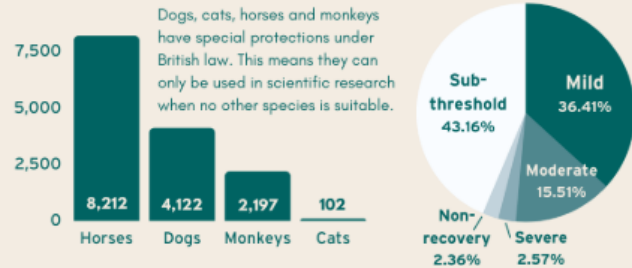


Most commonly used animals

Mice	Fish	Rats	Birds
1,971,262	371,237	185,749	136,190

Animals with special protection

Severity of experiments



ANIMAL SPECIES USED

Mice	Fish	Rats	Birds	Monkeys	Other
83.8%	13.8%	1.6%	0.3%	0.01%	0.4%

Animal Research Statistics Great Britain, 2024

Research facilities in Great Britain record the number of scientific procedures carried out on animals each year. Procedures are categorised by severity, they can be as mild as an injection, or as severe as an organ transplant.



Most commonly used animals

Mice	Fish	Rats	Birds
1,869,064	381,898	150,221	115,369

Animals with special protection

Severity of experiments



ANIMAL SPECIES USED BY THE TEN ORGANISATIONS

Mice	Fish	Rats	Birds	NHPs	Dogs	Other
76%	20%	2.2%	0.5%	0.01%	0.0008%	0.6%

Why Are Alternatives Needed?



ETHICAL

Reduce and replace animal use.



COST

Lower costs, faster results, more efficient R&D.



SCIENTIFIC

More human-relevant, mechanism-based, reproducible and predictive.

THE TRANSLATIONAL CRISIS

~90% of drugs entering human clinical trials fail.



Therefore, **NAMs** are a response to a **scientific**, **technological**, and **ethical need**, not simply a change in the way studies are conducted.

WHY DO THESE FAILURES OCCUR?



Toxicity not detected in animals



Lack of efficacy in humans



Pharmacokinetic differences



Immunological differences



WHY ANIMAL MODELS OFTEN FAIL?



Gene regulation differs



Protein expression differs



Immune responses differ



Disease progression differs



This does not mean that animal models are ineffective, but rather that each model has **its own inherent limitations**.



HUMAN-RELEVANT SCIENCE

Moving beyond animal models toward human biology



ANIMAL-CENTERED
RESEARCH



HUMAN-RELEVANT RESEARCH



THE GOAL

to achieve research that is both ethically
responsible and more predictive of human
biology



Improved
human health

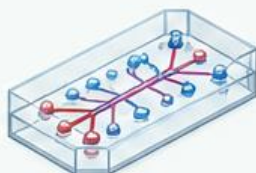
— HUMAN-DERIVED SYSTEMS MAY BETTER REPRESENT:



Human
organoids



Human
primary cells



Organ-on-chip
systems



Digital
twins



Human genetics



Human physiology



Human pathology

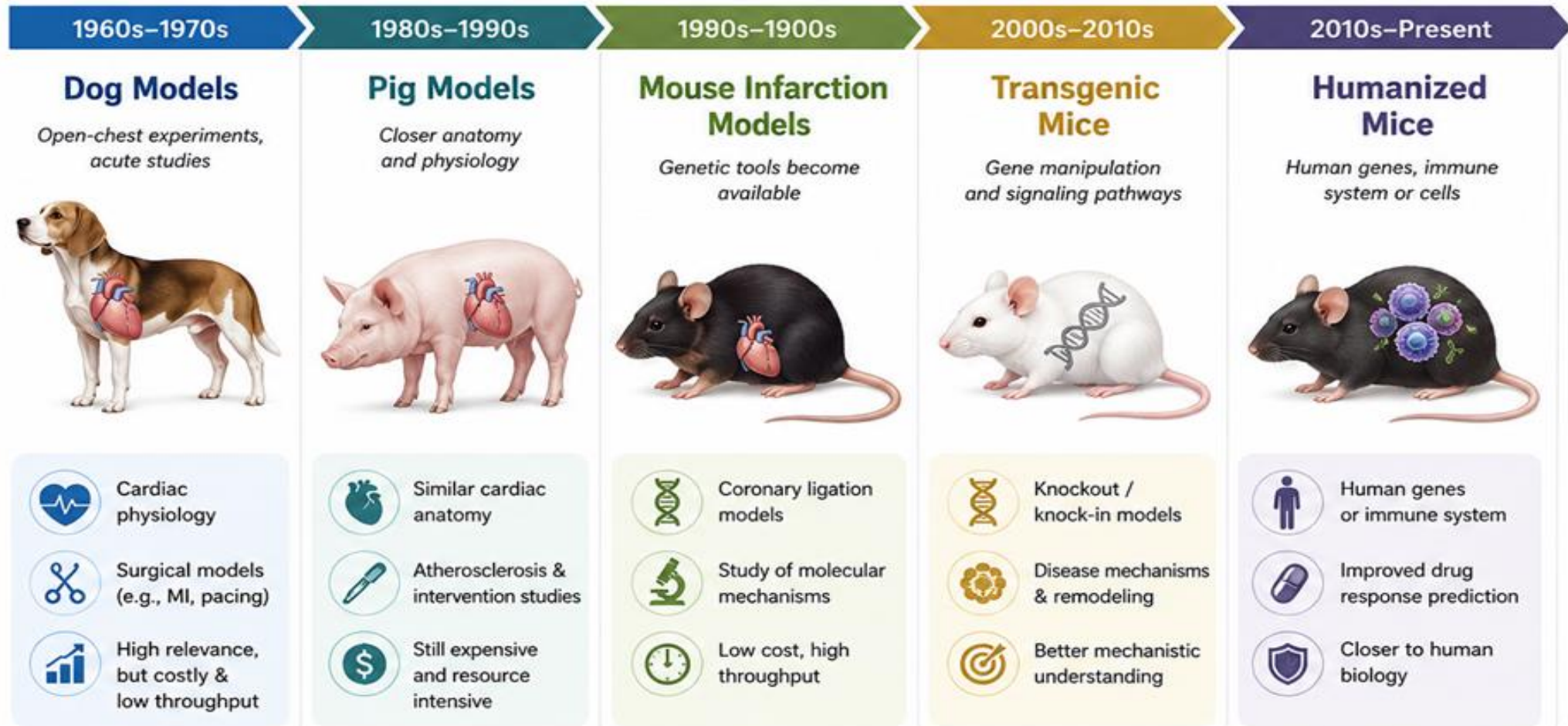


Recent FDA roadmaps emphasize that **HUMAN-RELEVANT METHODS**
may **IMPROVE PREDICTIVE PERFORMANCE** while reducing animal use.



Evolution of experimental models

in Cardiovascular Research



Whole-organism models
From large animals to mice

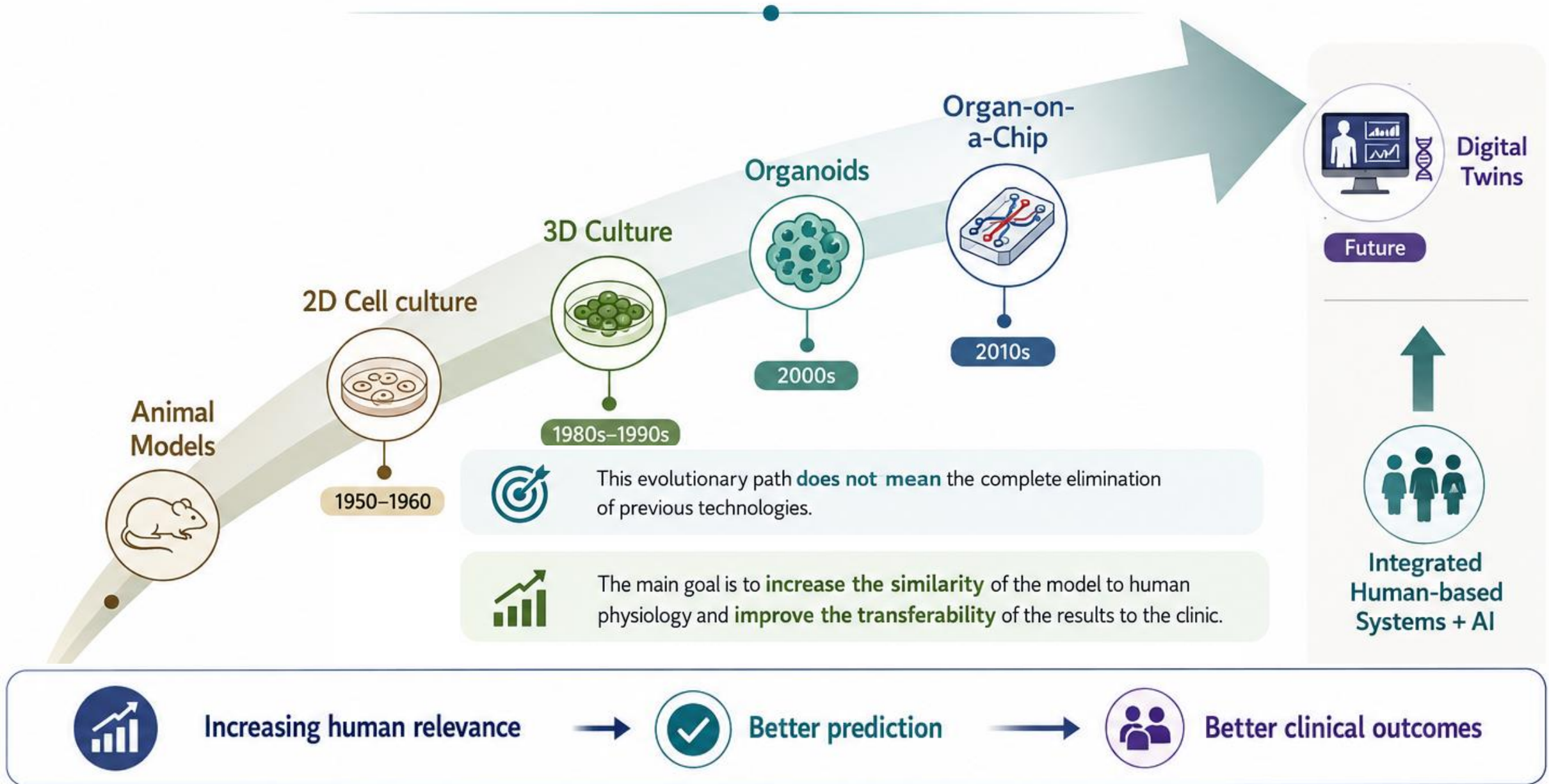


Genetic & molecular refinement
Understanding mechanisms



Human relevance
Better translation to patients

Evolution of experimental models: from animals to human models



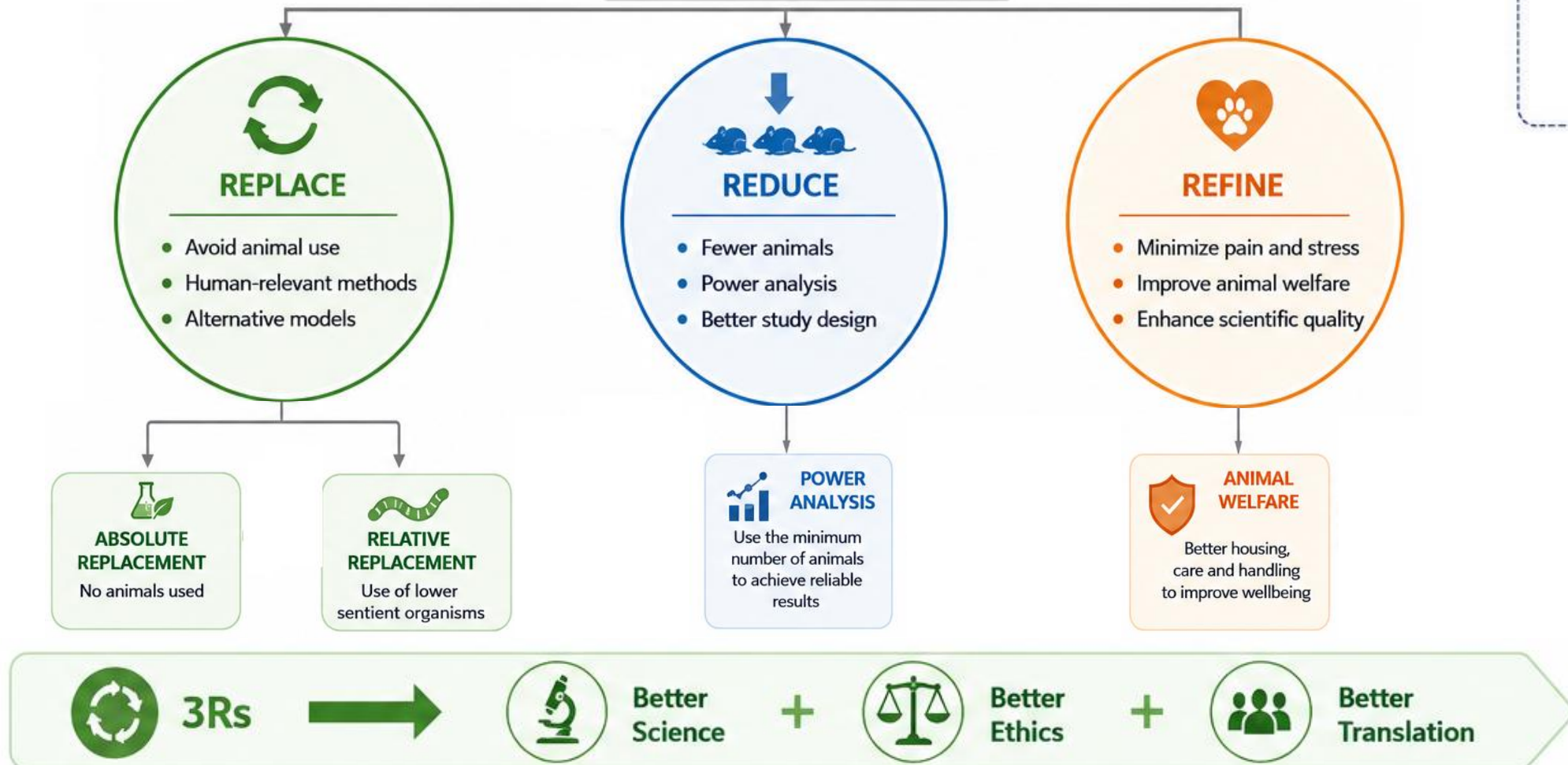
The 3Rs Principle in Animal Research

The Foundation for Ethical and Scientific Innovation

<https://nc3rs.org.uk>



RESEARCH USING ANIMALS



**Russell and Burch
(1959)**

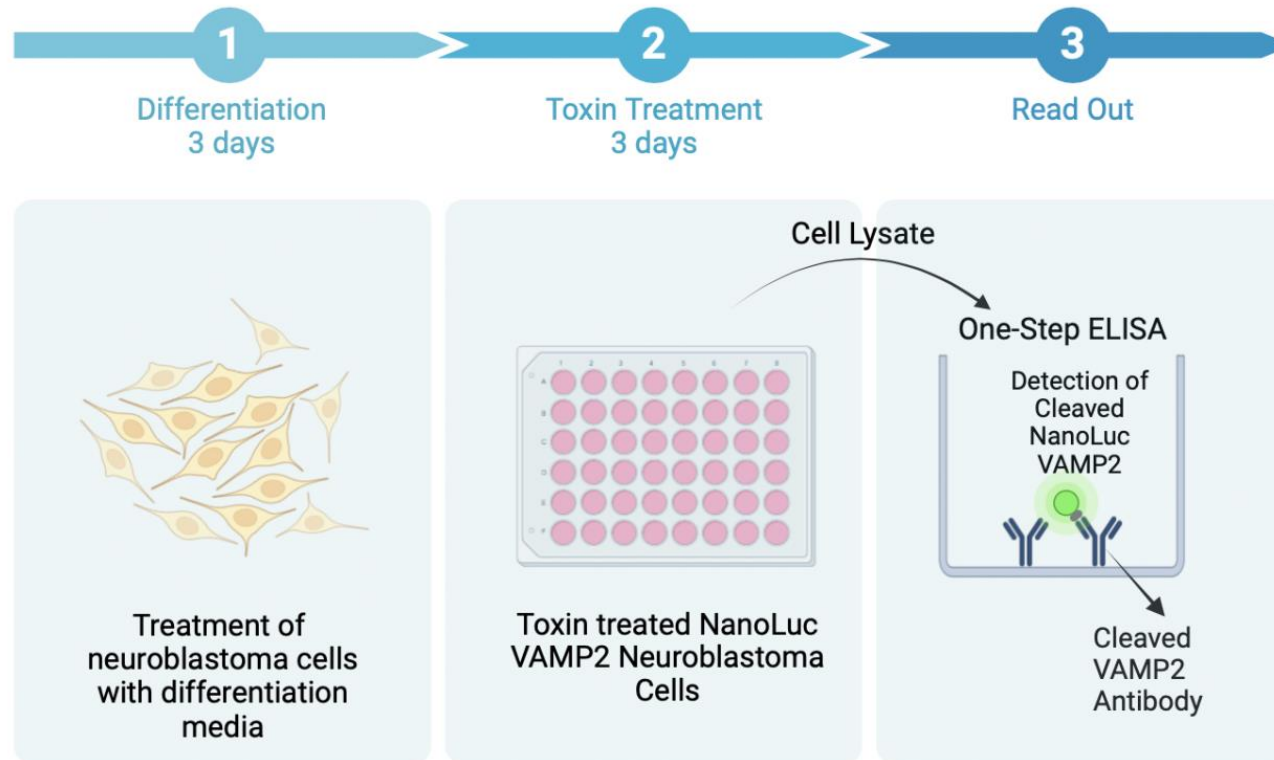
*The Principles of
Humane
Experimental
Technique*

OECD • NIH •
ARRIVE
Guidelines and
FELASA

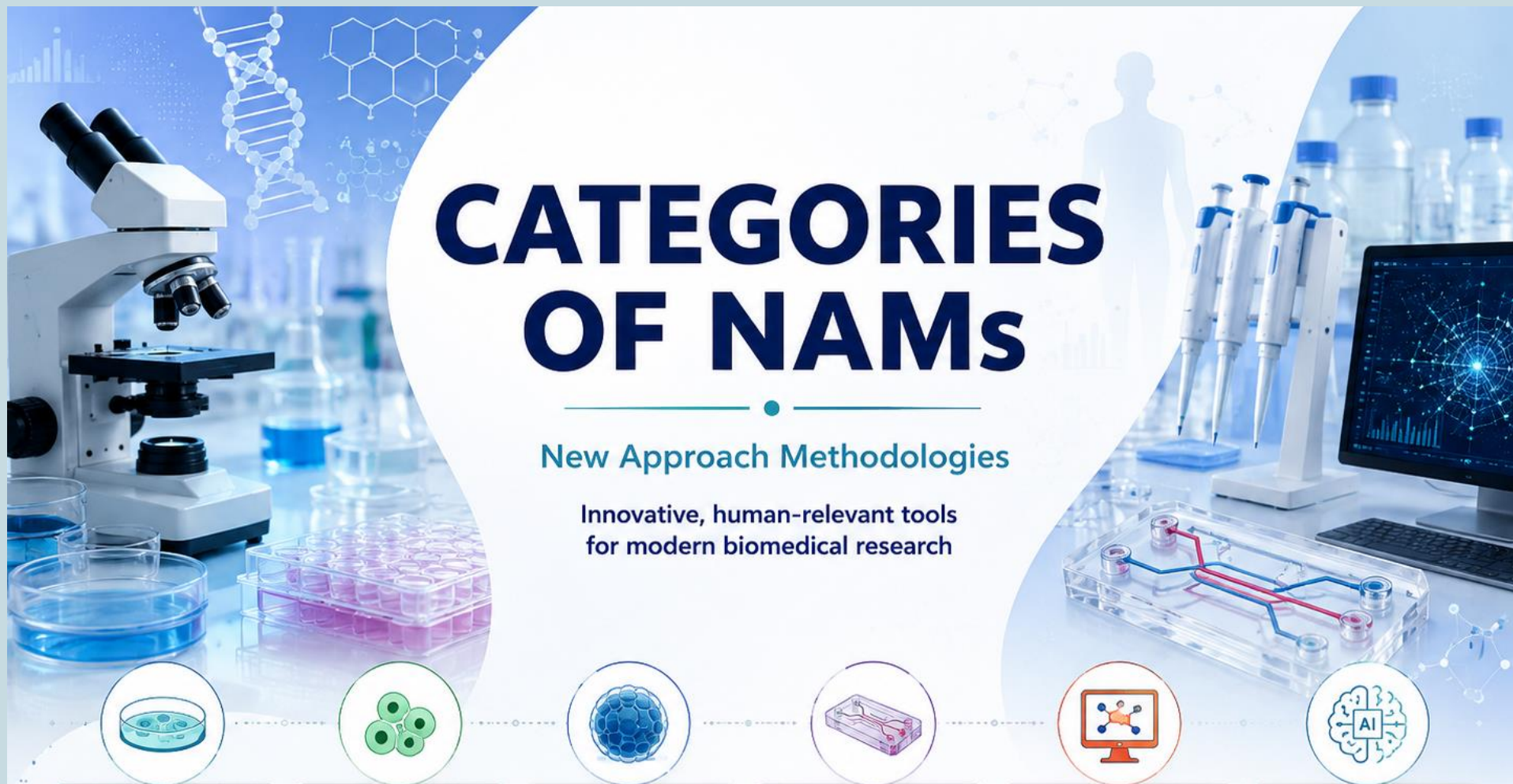
Replacement

Pharmaceutical toxin safety tests without severe suffering

Clostridium toxins







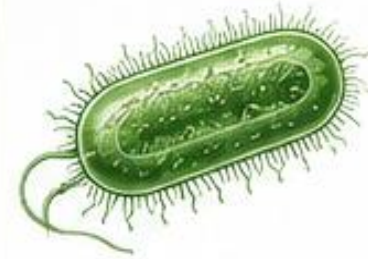
NAMs advance human relevance, improve predictivity, and reduce animal use.



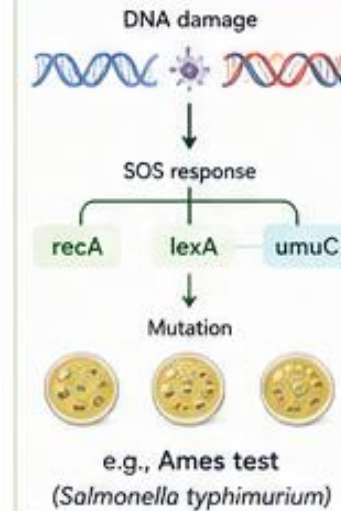
- Bacteria
- Yeast
- Amoeba
- Hydra
- *C. elegans*
- Drosophila
- Ascaris
- Daphnia
- Artemia

1 BACTERIA

Simple prokaryotes, powerful insights



Mechanistic evidence



Key applications

-  Mutagenicity (Ames test)
-  Antibiotic discovery
-  Toxicity screening
-  Metabolic engineering
-  Recombinant protein production

Alternative Biological Models

1970s-Present



4

CAENORHABDITIS ELEGANS
Transparent, tractable, and translatable

Mechanistic e

✓ ~40% of human genes have *C. elegans* orthologs

✓ Conserved aging, insulin, MAPK & dopaminergic pathways

✓ Genetic & RNAi screens predict human drug targets

Key applications

Aging & longevity

Neurodegenerative diseases

Metabolic disorders (diabetes, obesity)

Developmental genetics

Drug screening & neurotoxicity testing

Chemical Inducers

Paraquat
Methyl viologen
Juglone
Hydrogen
Glucose
.....

Microorganism Inducers

Bacteria
Fungi
Spores
Toxin
Viruses
.....

Physical External Stimulus

Heat
Hypoxia
UV radiation

Genetic Modification

Phenotypes

Lifespan

Life cycle

Body size

Locomotion

Behavior

Intestinal Lipofuscin

Fluorescence label

Biochemical indexes

Fertility rate

Hatchability

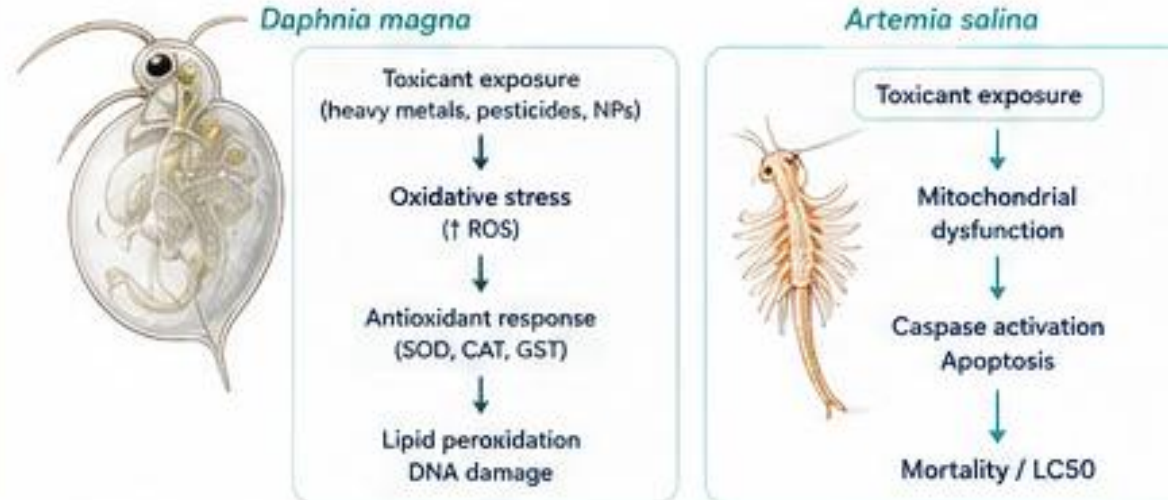
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Current Opinion in Food Science



6 AQUATIC INVERTEBRATES

Sentinels for environmental safety



Key applications

- 🌿 Ecotoxicology & water quality assessment
- ⚙️ Pesticide, heavy metal & nanoparticle toxicity
- ⚙️ Endocrine disruption & developmental toxicity
- 🧠 Preliminary toxicity screening of chemicals & natural products



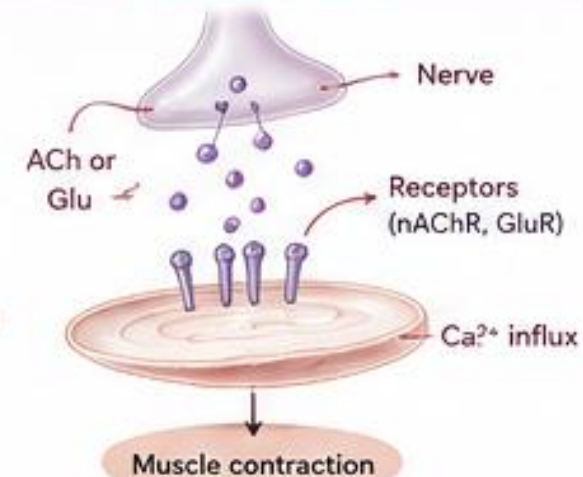
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ASCARIS (PARASITIC NEMATODE)

A neuromuscular model for parasitic diseases



Mechanistic evidence



Key applications

-  Anthelmintic drug discovery & screening
-  Neuromuscular physiology & ion channel pharmacology
-  Electrophysiology & receptor characterization
-  Drug resistance & parasite biology



Ex Vivo Human Models

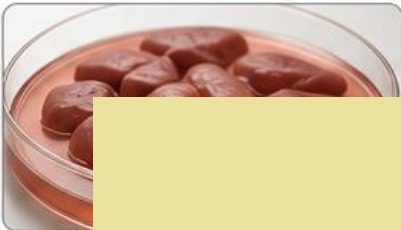


Provide physiologically relevant human tissue context for toxicity testing, disease modeling, and mechanistic studies—bridging *in vitro* and *in vivo*.

1970s–
Present



HUMAN TISSUES



- Fresh
- shortl
- Prese
- divers



PRECISION-CUT
TISSUE SLICES (PCTS)



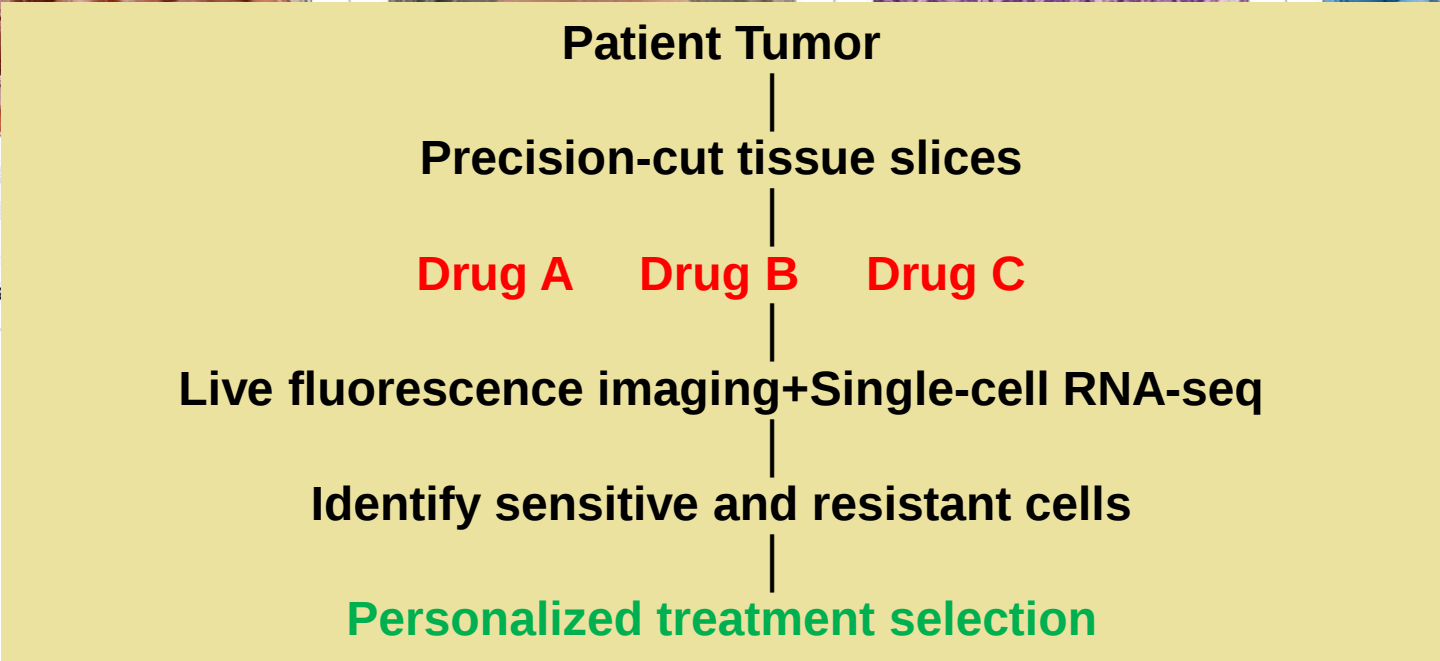
HUMAN BIOPSIES



BLOOD-BASED ASSAYS



- whole blood, plasma,
- or isolated cells
- high-throughput and
- er analysis





IN VITRO HUMAN-BASED MODELS

Human cells and 3D systems for better biological relevance

- In chemico assays
- Cell cultures(2D and 3D)
- Organoids
- Engineered tissues
- Organ-on-a-Chip



Primary cells



iPSC-derived cells



Organoids



3D cell culture



Organ-on-a-Chip



MPS

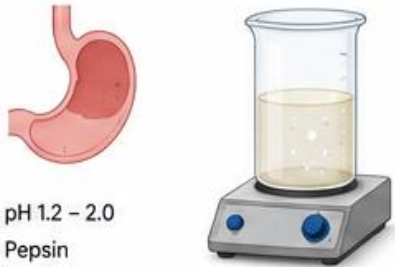
IN CHEMICO ASSAYS

Simulating Physiological Conditions to Evaluate
New Drugs Without Animals



1-Simulating gastrointestinal Condition

1. Gastric Simulation (Stomach Condition)



pH 1.2 – 2.0
Pepsin
37 °C

Purpose:
Evaluate drug stability
in acidic environment

2. Duodenal Simulation (Small Intestine Condition)



pH 6.5 – 6.8
Pancreatin, Bile salts
37 °C

Purpose:
Assess drug stability and
enzymatic degradation

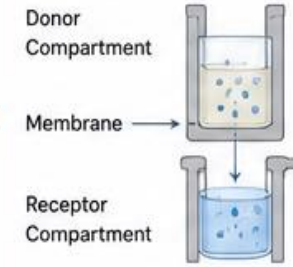
3. Intestinal (Jejunal/Ileal) Simulation



pH 7.0 – 7.4
Pancreatin, Bile salts
37 °C

Purpose:
Evaluate drug release and
solubility in intestinal fluid

4. Absorption Assessment



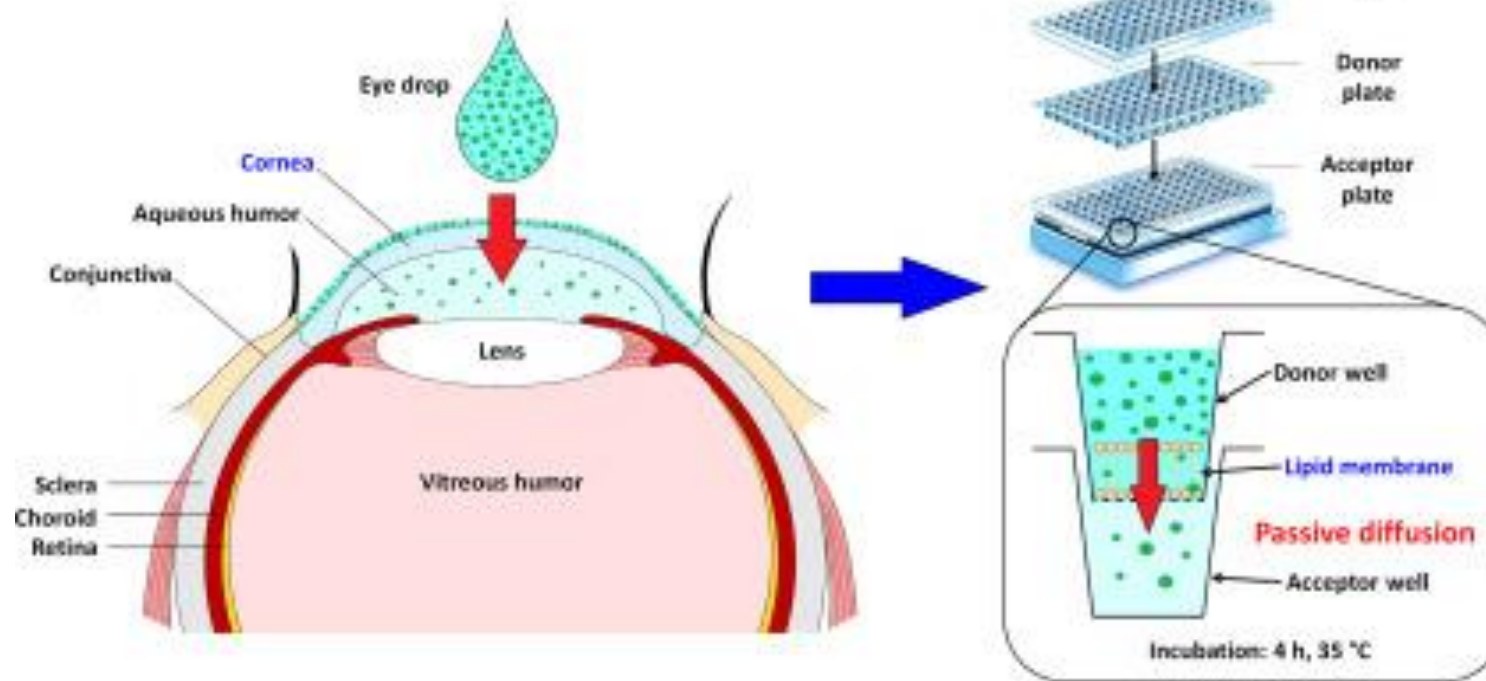
Purpose:
Predict drug permeability and
absorption potential

In chemico assays provide accurate, reproducible, and ethical data to support the development of safe and effective drugs—without the use of animals.

IN CHEMICO ASSAYS

Simulating Physiological Conditions to Evaluate
New Drugs Without Animals

2-PAMPA: Parallel Artificial Membrane Permeability Assay



Corneal-PAMPA: A novel, non-cell-based assay for prediction of corneal drug permeability-DOI: [10.1016/j.ejps.2018.12.012](https://doi.org/10.1016/j.ejps.2018.12.012)

3-Franz Diffusion Cell: Skin and mucosal Permeation





IN VITRO HUMAN-BASED MODELS

Human cells and 3D systems for better biological relevance

- In chemico assays
- Cell cultures(2D and 3D)
- Organoids
- Engineered tissues
- Organ-on-a-Chip



Primary cells



iPSC-derived cells



Organoids



3D cell culture



Organ-on-a-Chip



MPS

CELL CULTURE

The process of growing cells in a controlled *in vitro* environment for research, drug discovery and therapeutic applications.

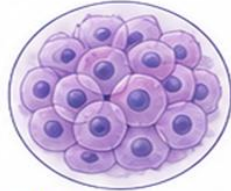


KEY APPLICATIONS

- Basic research
- Disease modeling
- Drug discovery & toxicity testing
- Regenerative medicine
- Cell therapy

HUMAN CELL LINES

Immortalized cells derived from human tissues or cancers that can proliferate indefinitely in culture.



Examples:

HeLa (cervical cancer)
HEK293 (embryonic kidney)
A549 (lung carcinoma)

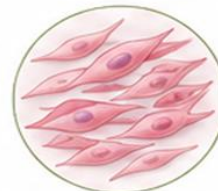


Researchers can study:

- Toxicity
- Drug mechanisms
- Cell signaling
- Gene expression

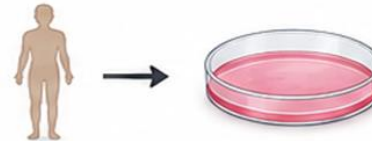
PRIMARY CELLS

Cells isolated directly from human tissues; retain normal properties but have a limited lifespan in culture.



Examples:

Human dermal fibroblasts
Human keratinocytes
Hepatocytes



- ✓ More physiologically relevant
- ✓ Maintain native functions
- ✓ Limited lifespan
- ✓ Donor variability

Although cell lines are very suitable for initial screening, they are not always the best model for predicting human response.

IN VITRO

Primary Human Cell Cultures

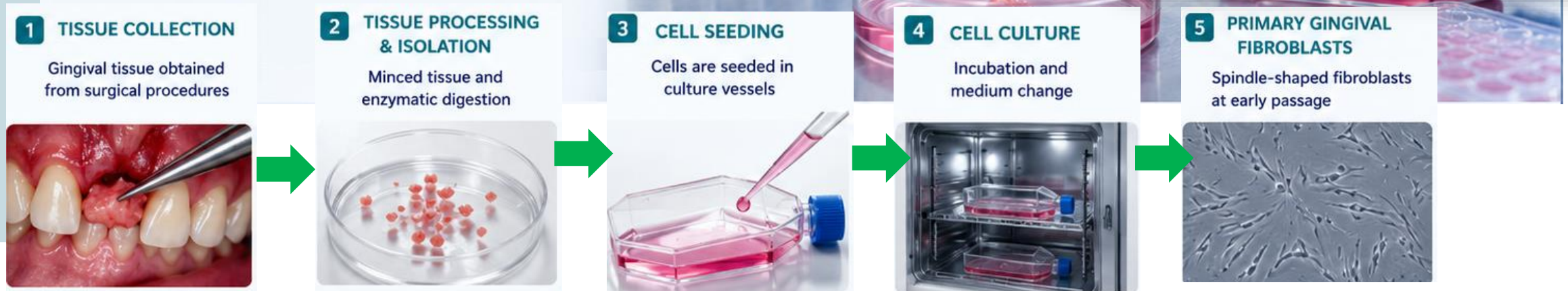
EXAMPLE IN DENTISTRY

Human gingival fibroblasts can be isolated from surgical specimens. Researchers use them to evaluate:

KEY POINTS

- ✓ High physiological relevance
- ✓ Patient-specific potential
- ✓ Maintain native cell functions
- ✓ Essential for translational research

PRIMARY HUMAN CELL CULTURE: WORKFLOW



APPLICATIONS



Implant Materials



Dental Cements



Regenerative Biomaterials



Drug Screening & Toxicity Testing



Regenerative Medicine

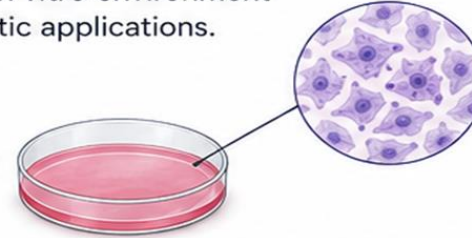
Li et al., 2024

Osteogenic mechanism of deciduous teeth periodontal ligament stem cells in inflammatory environment

Dental stem cell dynamics in periodontal ligament regeneration (Stem Cell Research & Therapy, 2024)

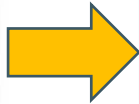
CELL CULTURE

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PRIMARY CELLS

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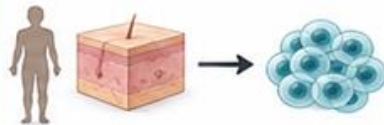


iPSC (INDUCED PLURIPOTENT STEM CELLS)

Adult somatic cells reprogrammed to a pluripotent state; similar to ESCs in their capabilities.



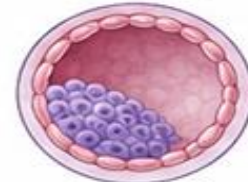
Source: Reprogrammed adult somatic cells (e.g., skin fibroblasts)



- ✓ Pluripotent
- ✓ Patient-specific
- ✓ Avoids ethical issues
- ✓ Valuable for disease modeling

ESC (EMBRYONIC STEM CELLS)

Derived from early-stage embryos; pluripotent and can differentiate into all cell types of the body.



Source: Inner cell mass of the blastocyst



- ✓ Pluripotent

MESENCHYMAL STEM CELLS (MSCs)

Multipotent stromal cells typically isolated from bone marrow, adipose tissue, umbilical cord, etc.



Examples:

Bone marrow-derived MSCs
Adipose-derived MSCs
Umbilical cord-derived MSCs



- ✓ Multipotent (e.g., bone, cartilage, fat, muscle)



CELL CULTURE IS A POWERFUL TOOL that enables scientists to study human biology, model diseases, and develop innovative therapies to improve human health.

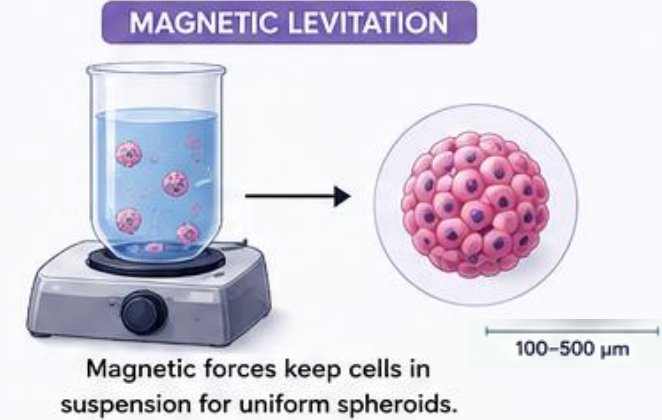
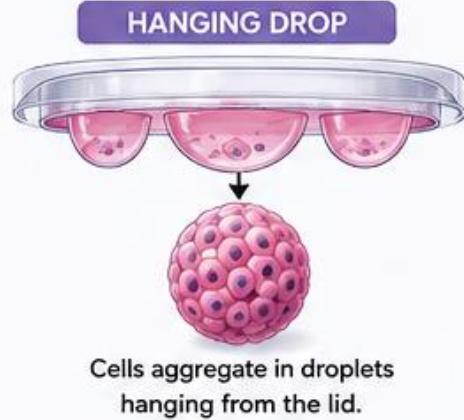
PART 1: 3D CELL CULTURE MODELS & THEIR APPLICATIONS

Recreating the Human Tissue Microenvironment

1. SCAFFOLD-FREE SYSTEMS



The output of these methods is usually a spheroid.



1. SPHEROIDS



100–500 μm

- Self-assembled 3D cell aggregates
- Enhanced cell–cell interaction
- Gradient formation (O_2 , nutrients, drugs)
- Tumor spheroids & normal spheroids

Key Applications



Tumor biology



Drug penetration studies



High-throughput screening

Spheroids are the simplest 3D model, but they play a very important role in **cancer research** and **pharmacology** due to their ability to recreate: key tumor features such as hypoxia, metabolic gradients, and drug resistance.

PART 1: 3D CELL CULTURE MODELS & THEIR APPLICATIONS

Recreating the Human Tissue Microenvironment

2. SCAFFOLD-BASED SYSTEMS

 The goal of this scaffold is to mimic the natural ECM (Extracellular Matrix).

COLLAGEN



Natural ECM protein supports cell adhesion, proliferation and tissue remodeling.

MATRIGEL



Basement membrane matrix rich in growth factors; supports organoid growth.

FIBRIN



Fibrin fibers support cell migration, angiogenesis and tissue regeneration.

ALGINATE



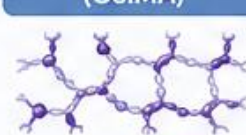
Natural polysaccharide forms gels; useful for encapsulation of cells and spheroids.

GELATIN



Denatured collagen supports cell adhesion and is enzymatically degradable.

METHACRYLATE (GelMA)



Photocrosslinkable hydrogel with tunable mechanical properties; mimics ECM.

HYALURONIC ACID



Key ECM component promotes cell survival, migration and differentiation.

HYDROGEL-BASED 3D CULTURES



- Cells embedded in biomimetic hydrogels
- Tunable stiffness & biochemical cues
- Support cell survival, proliferation & function

Key Applications



Cell behavior studies



Drug screening



Immuno-oncology



Matrix biology

PART 1: 3D CELL CULTURE MODELS & THEIR APPLICATIONS

Recreating the Human Tissue Microenvironment

Mini-organs in vitro

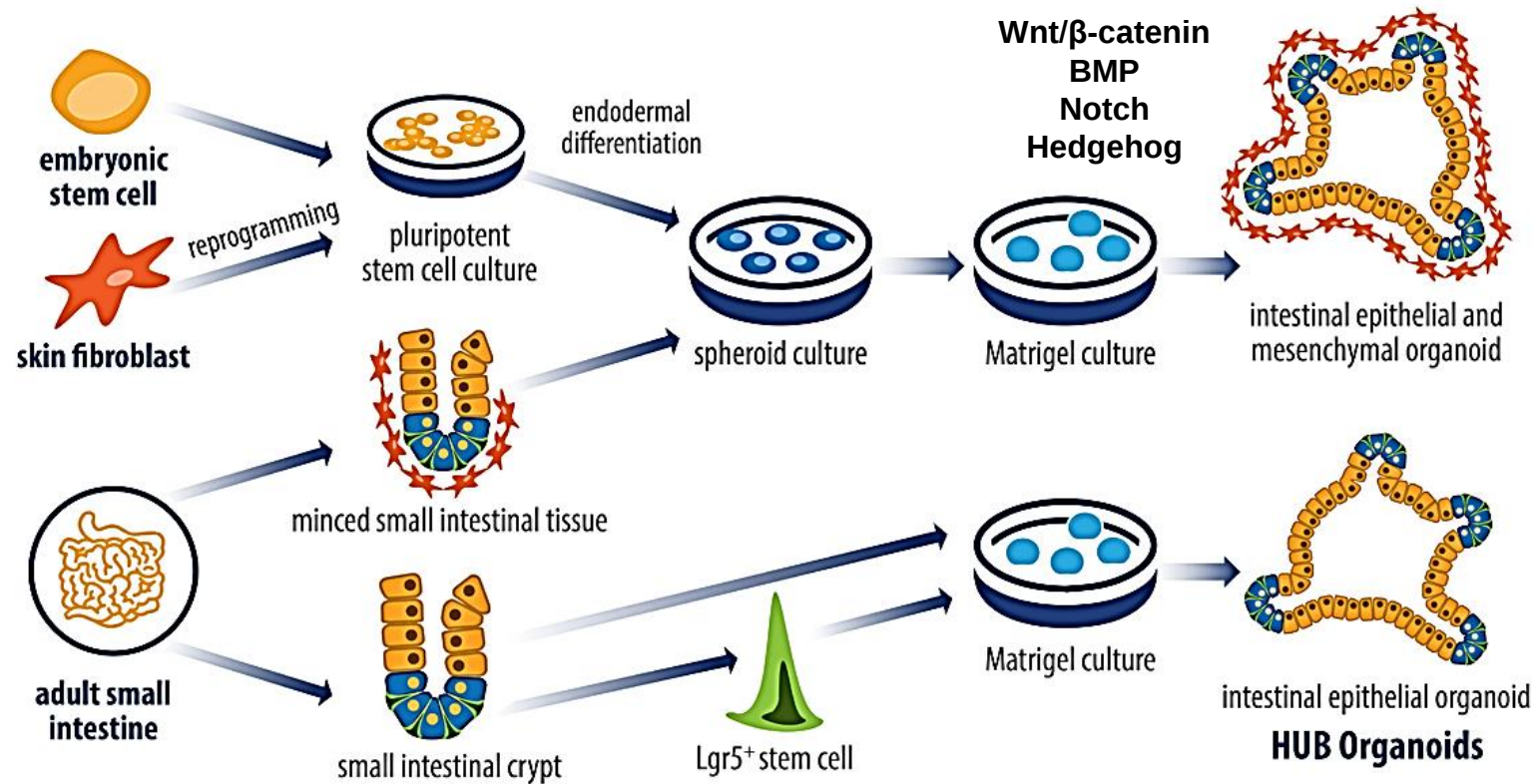
iPSCs, ESCs

2. ORGANOID



50 μ m – 2 mm

- Stem cell-derived 3D structures
- Self-organized tissue architecture
- Recapitulate key organ functions
- Patient-specific & disease models



Organoids (Part 2): Applications in Disease Modeling from genetics to personalized medicine



✓ Powerful tools for disease modeling and drug testing



Cystic Fibrosis (CF)

CFTR-deficient intestinal or airway organoids are used to study disease mechanisms and test CFTR-modulating drugs.



Example:

CF airway organoids treated with ivacaftor show restored CFTR function and improved chloride secretion. (Dekkers et al., Cell Stem Cell, 2013)



Inflammatory Bowel Disease (IBD)

Patient-derived intestinal organoids help investigate intestinal inflammation and host-microbe interactions.



Example:

IBD organoids exposed to patient microbiota reproduce disease-specific inflammatory responses. (Monteleone et al., Cell, 2019)



Pancreatic Cancer

Tumor organoids recapitulate genetic heterogeneity and are used to test targeted therapies.



Example:

Patient-derived pancreatic tumor organoids predict response to chemotherapy and targeted drugs. (Boj et al., Cell, 2015)



Microcephaly

Cerebral organoids are used to study neurodevelopmental defects.



Example:

Patient-derived organoids reveal defects in neural progenitor proliferation that cause microcephaly. (Lancaster et al., Nature, 2013)



Alzheimer's Disease (AD)

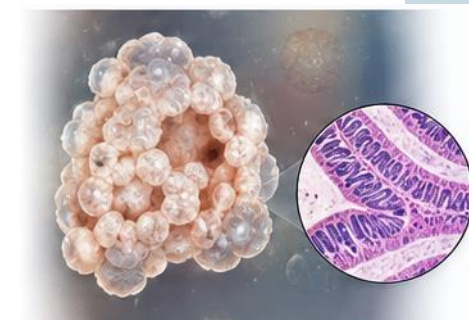
Brain organoids derived from AD patients show amyloid-beta accumulation and tau pathology.



Example:

Brain organoids treated with anti-amyloid compounds show reduced plaque burden and improved neuronal survival. (Karch et al., Cell Stem Cell, 2019)

Neurological Disorders



Human intestinal organoid
(microscopy image)

Organoids (Part 2): Applications in Disease Modeling from genetics to personalized medicine

Organoids in Oncology (Cancer Modeling)

Types of Cancer Organoids

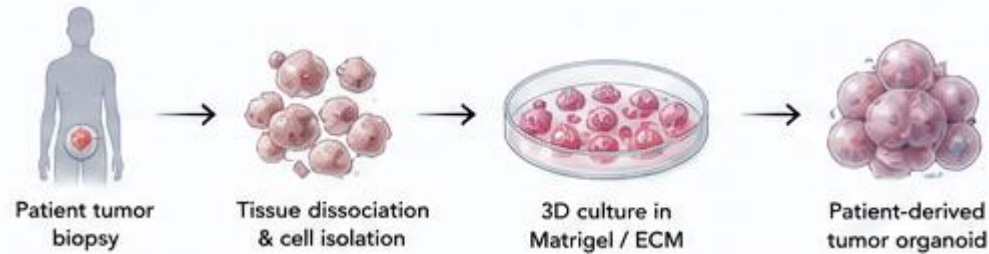


Patient-Derived Tumor Organoids (PDTOs)

Organoids from Normal Tissue

Organoids from Metastatic Sites

From Patient Tumor to Organoid



Liver Cancer Organoids

Used to study tumor biology, drug metabolism, and chemoresistance.



Breast Cancer Organoids

Capture tumor diversity and are used for drug screening.



Prostate Cancer Organoids

Model androgen dependence and therapy resistance.

Applications



Drug screening



Biomarker discovery



Mechanism of resistance

example:

Prostate cancer organoids help identify effective drug combinations for castration-resistant disease. (Gao et al., Nat Commun, 2014)



Metastasis modeling



Personalized treatment strategies

Organoids (Part 2): Applications in Disease Modeling from genetics to personalized medicine

Organoids in Infectious Disease Research



SARS-CoV-2 (COVID-19)

Lung organoids recapitulate viral entry, replication, and host responses.

Example:

Lung organoids infected with SARS-CoV-2 are used to test antiviral drugs such as remdesivir. (Hui et al., Cell Stem Cell, 2020)



Hepatitis B Virus (HBV)

Liver organoids support HBV infection and cccDNA formation.

Example:

HBV-infected liver organoids allow screening of drugs targeting viral replication. (Ding et al., Cell Stem Cell, 2018)

ارگانوئیدها ابزار قدرتمندی برای مدل‌سازی بیماری‌ها هستند که امکان مطالعه اختلالات ژنتیکی، سرطان، بیماری‌های عفونی و پاسخ دارویی در سطح بافت انسانی را فراهم می‌کنند. .
مهم‌ترین ارزش آن‌ها در این است که می‌توانند فاصله بین آزمایشگاه و بیمار را کاهش دهند و مسیر حرکت به سمت پزشکی شخصی را تسریع کنند. با این حال، نبود سیستم ایمنی، عروق و بلوغ کامل باعث می‌شود که هنوز نیاز به ترکیب با سایر مدل‌ها وجود داشته باشد.

Bioprinting

Building living tissues and organs layer-by-layer

WHAT IS BIOPRINTING?

A technology that uses 3D printing to precisely place living cells and biomaterials to create functional tissue constructs.



BIOPRINTING TECHNOLOGIES



EXTRUSION-BASED BIOPRINTING



- Widely used
- Suitable for viscous bioinks
- High cell viability



LASER-ASSISTED BIOPRINTING



- High precision
- No nozzle contact
- Ideal for fragile cells

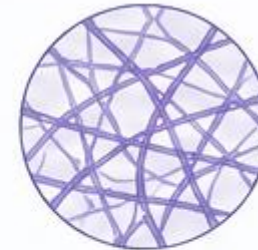
BIOINK MATERIALS



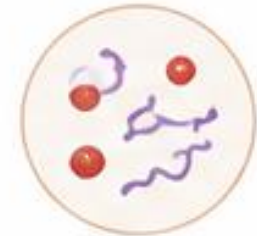
Cells
(Primary cells /
Stem cells)



Natural Polymers
(Collagen, Alginate,
GelMA)



Mimics ECM
(Extracellular
Matrix)



Bioactive Molecules
(e.g., growth factors,
peptides)

IRANIAN CONTRIBUTIONS TO BIOPRINTING-BASED NAMs



Shiraz University of Medical Sciences

- Tissue Bioprinting
- Bioinks
- Regenerative Medicine



Sharif University of Technology

- Smart Biomaterials
- Bioinks
- Tissue Engineering



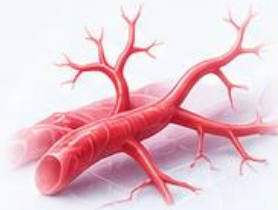
Materials and Energy Research Center (MERC)

- Scaffolds
- 3D Bioprinting
- Cartilage and Bone



Iran University of Medical Sciences

- Blood Vessel Bioprinting
- Amniotic Membrane Bioinks



Isfahan University of Medical Sciences

- Cartilage Bioprinting
- Scaffolds
- Regenerative Medicine



Tarbiat Modares University

- Biopolymers
- Bioinks



NEW IRANIAN TRENDS FOR IN VITRO RESEARCHES (2024-2025)



Decellularized ECM Bioinks

Using decellularized extracellular matrix to create bioinks that better mimic the natural microenvironment.



4D Bioprinting

Scaffolds that respond to stimuli such as temperature or pH after printing.



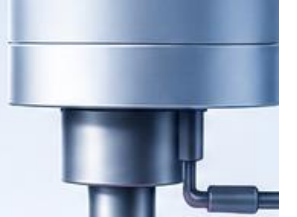
Vascularized Bioprinting

Bringing printed tissue closer to natural tissue through integrated vascular networks.



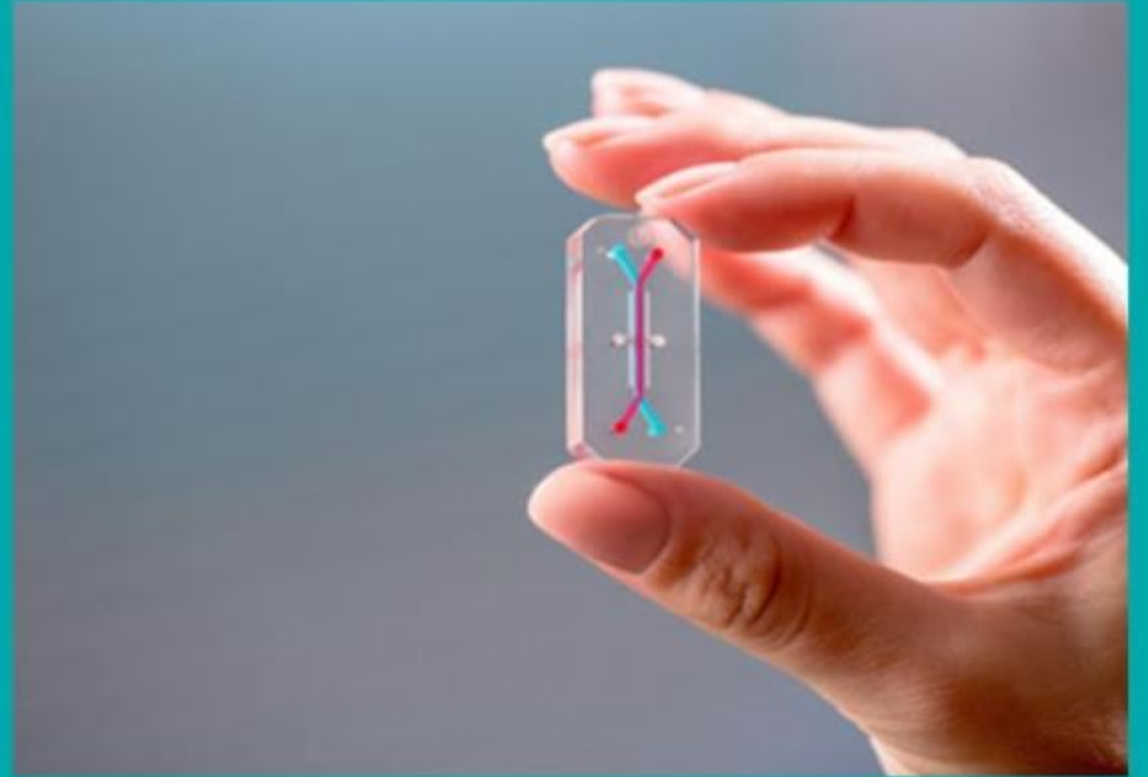
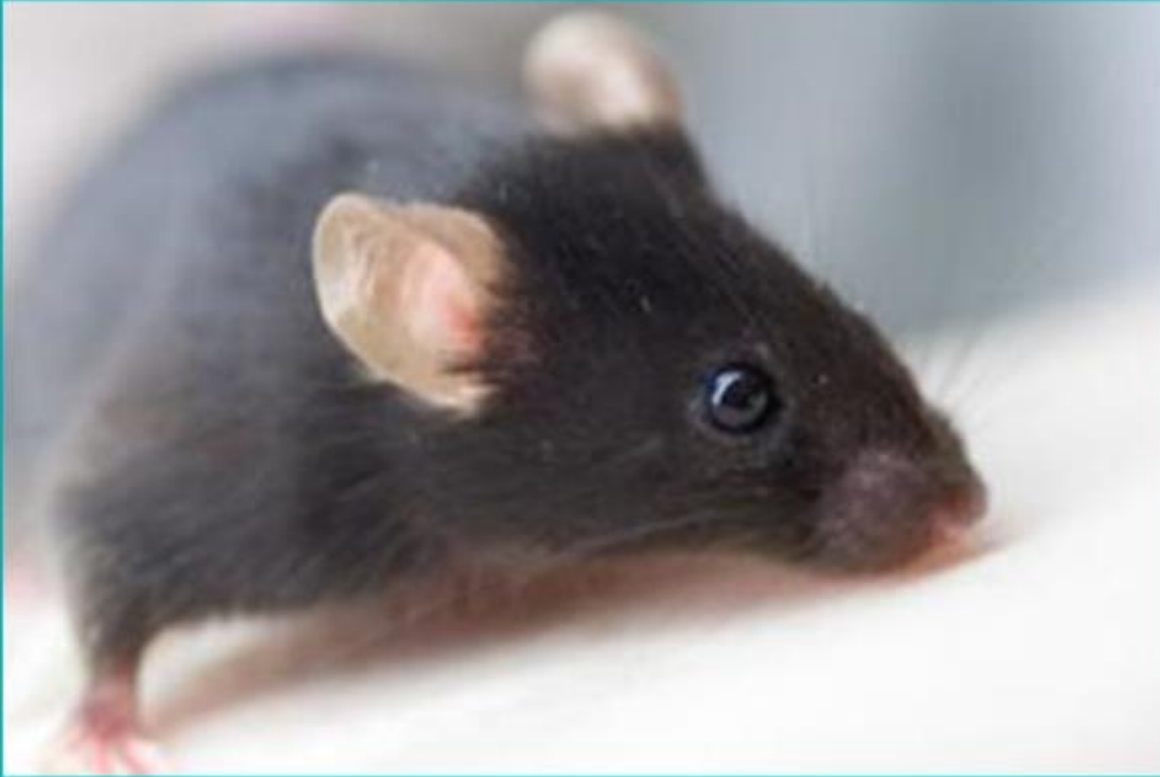
Digital Light Processing (DLP) Bioprinting

DLP bioprinting with very high precision to create more complex structures.

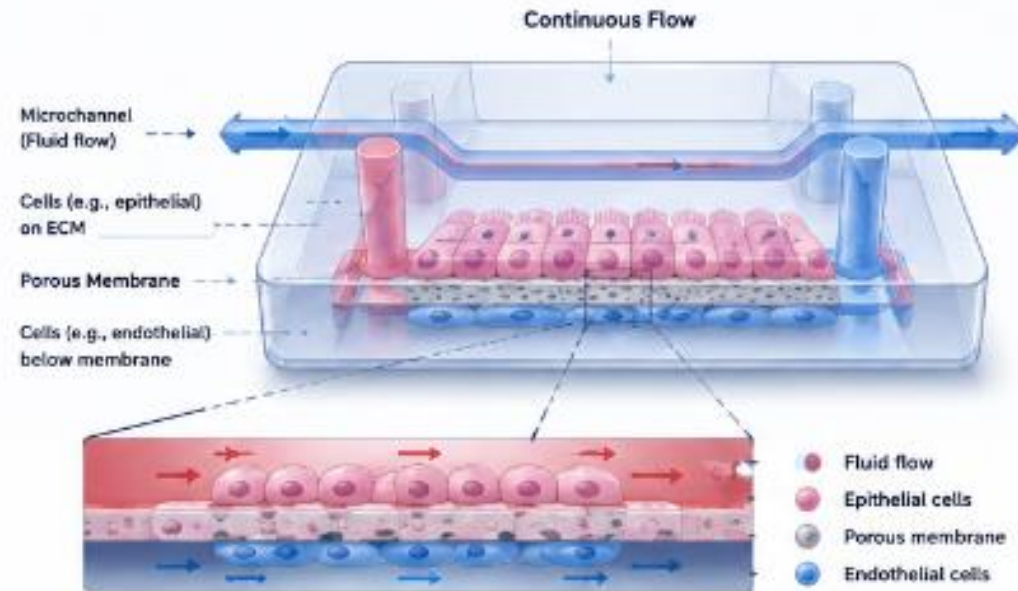


Organs on a Chip (Ooc)

One of the most advanced animal replacement technologies



Organs on a Chip; Recreating the Dynamic Function of Human Organs



4 KEY COMPONENTS OF ORGANS-ON-A-CHIP

1 MICROFLUIDIC CHANNELS

Microscopic channels that direct the flow of fluid.



- ✓ Precise control of flow rate
- ✓ Nutrient delivery and waste removal
- ✓ Establishment of gradients

2 CELL CULTURE CHAMBERS

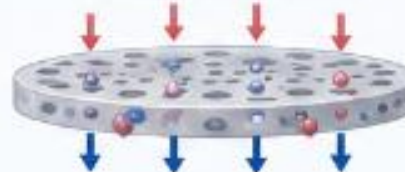
Place human cells (often on ECM or semipermeable membranes).



- ✓ Organ-specific cell types
- ✓ 3D-like microenvironment
- ✓ Cell-cell and cell-ECM interactions

3 POROUS MEMBRANES

To separate two types of cells while allowing the exchange of materials.



- ✓ Selective permeability
- ✓ Mimics natural barriers (e.g., alveolar, intestinal, blood-brain barrier)
- ✓ Enables co-culture of cell types

4 MECHANICAL ACTUATORS

To apply mechanical forces such as:

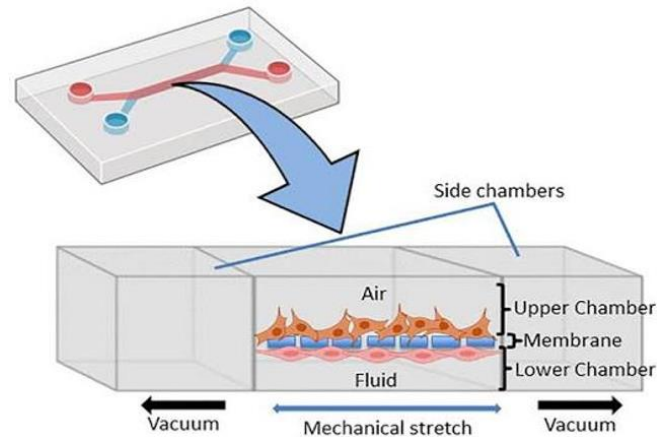
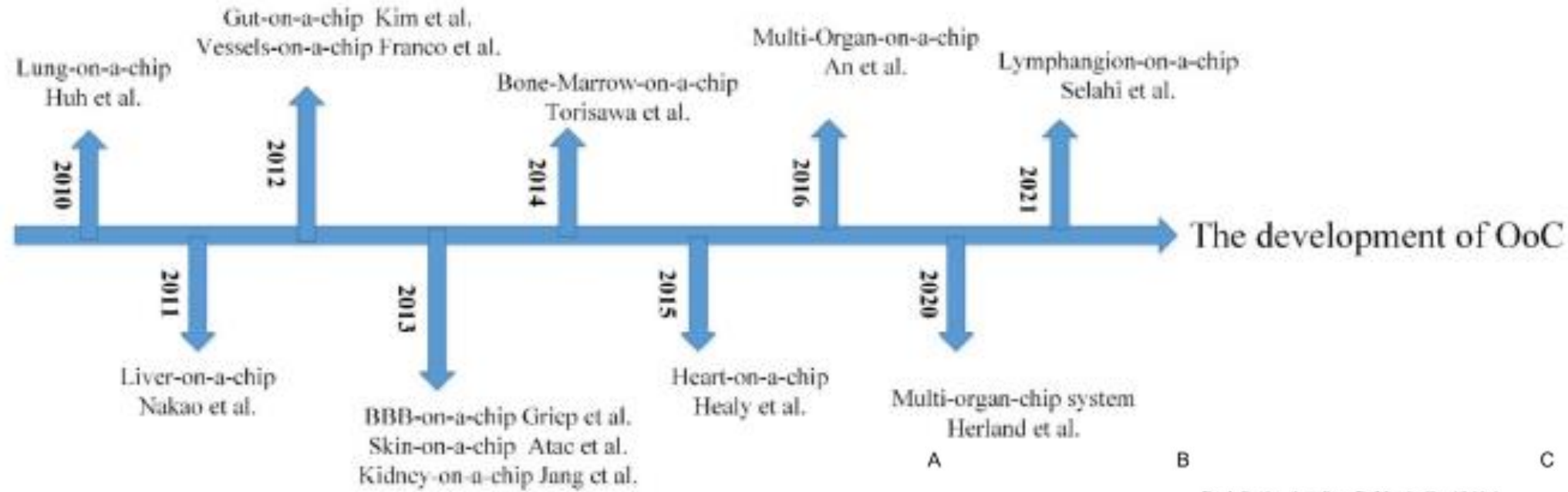
- ↔ Stretching
- ⊗ Compression
- 🌀 Air or fluid flow



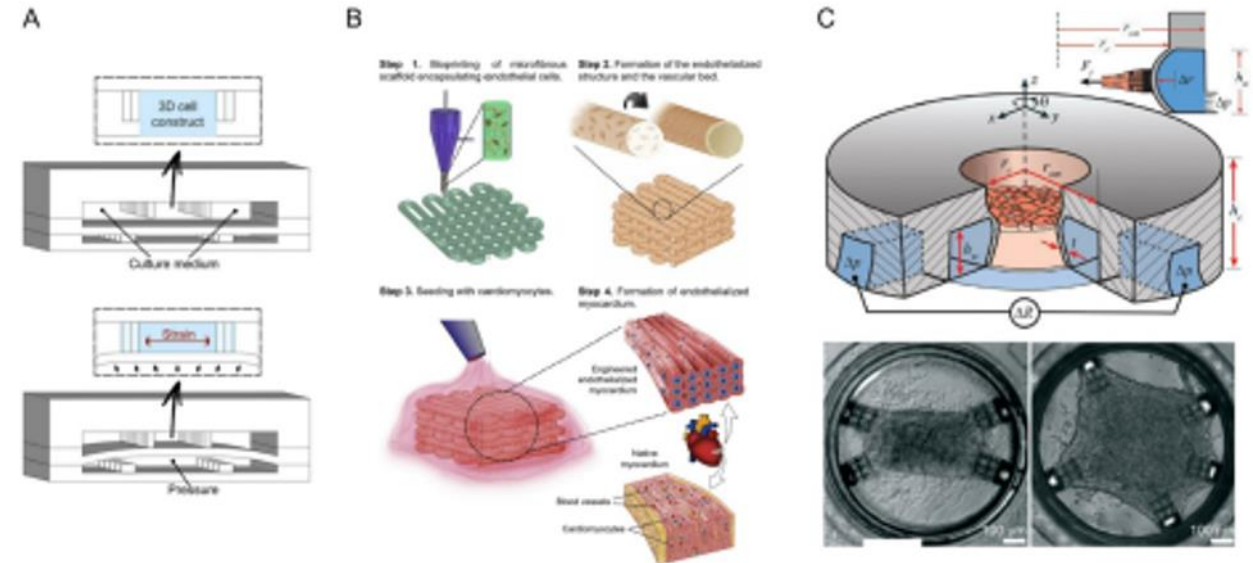
- ✓ Simulate breathing, peristalsis, blood flow
- ✓ Influence cell behavior and function
- ✓ Improve physiological relevance

EXAMPLES OF ORGANS-ON-A-CHIP

Organ-on-a-chip models recapitulate key structural, functional, and mechanical features of human organs, enabling more physiologically relevant research and drug testing.

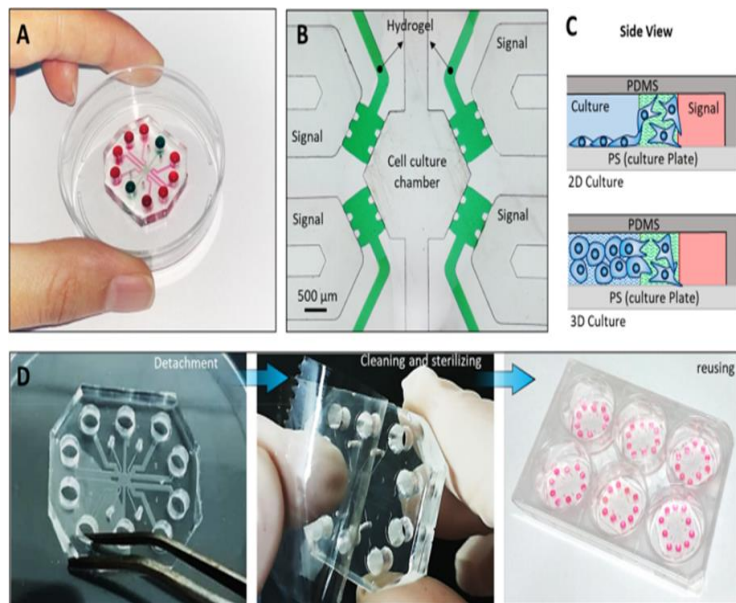


development of OoC.



مرکز تحقیقات فیزیولوژی کاربردی اصفهان

این مرکز با بهره‌گیری از دستگاه لیتوگرافی اختصاصی و همکاری با افراد دارای توانایی طراحی توانایی ساخت چیپ‌های میکروفلوئیدی سفارشی برای پژوهش‌های زیست‌پزشکی را داراست. تمرکز اصلی بر کاربردهای تشخیصی، کشت سلول و مدل‌سازی بیماری‌ها است.



Migration microfluidic
chip

🔬 زیرساخت

- دستگاه لیتوگرافی نوری، اتاق تمیز و تجهیزات ریخته‌گری

🧪 تخصص

- بهینه‌سازی پارامترهای ساخت و تست عملکرد

⚙️ طراحی چیپ میگزیشن

- انجام مطالعات مهاجرت سلولی با چیپ طراحی و ثبت اختراع شده مرکز

🤝 همکاری

پروژه‌های مشترک با گروه‌های فیزیولوژی، ایمونولوژی و بیوتکنولوژی

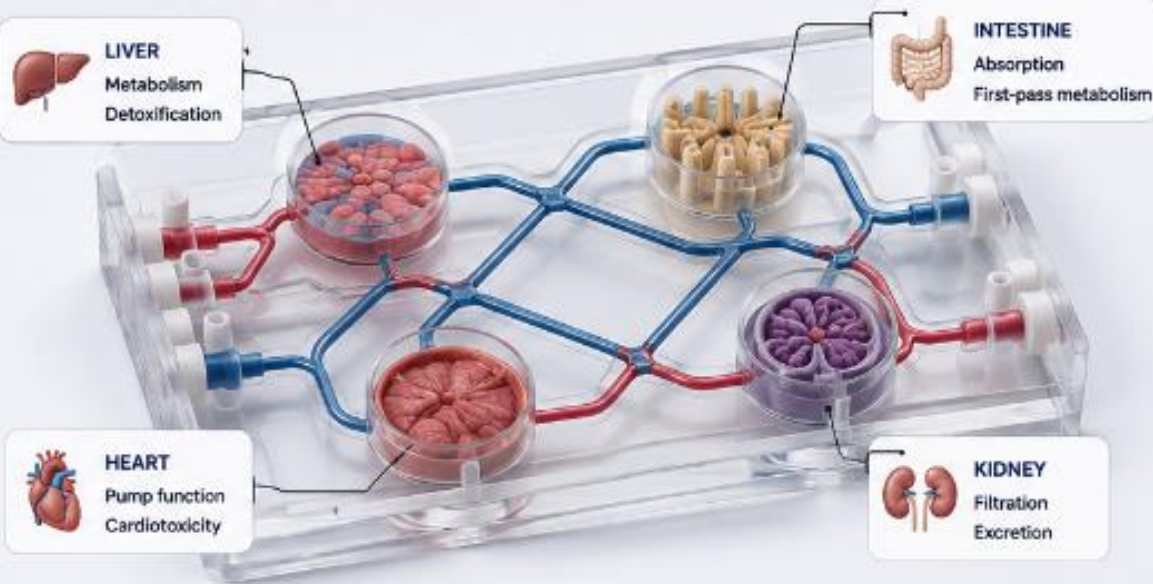


Multi-Organ-on-a-Chip (MOC)

Integrating multiple human organs in microfluidic systems to better mimic whole-body physiology

HOW IT WORKS

Human organ modules are connected by microfluidic channels that allow continuous flow of culture medium, replicating physiological transport and communication.



MAIN APPLICATIONS

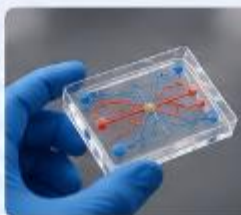
- 1**  **Pharmacokinetics (ADME)**
Simulates complete drug pathway: Absorption, Distribution, Metabolism, Excretion.
- 2**  **Systemic Toxicology**
Investigates hepatotoxicity, toxic metabolites, and drug accumulation.
- 3**  **Organ Interactions in Diseases**
Models complex diseases such as diabetes, inflammation, and infections.
- 4**  **Drug Development**
Predicts clinical failure, reduces animal testing, and helps select optimal doses.



More accurate prediction of human responses



Reduction in animal use



Better selection of drug candidates and doses



Advancing towards personalized and systems medicine

LIMITATIONS

- ✗ High complexity and cost
- ✗ Limited scalability and throughput
- ✗ Lack of standardization across platforms
- ✗ Does not fully replicate immune, hormonal, and nervous systems

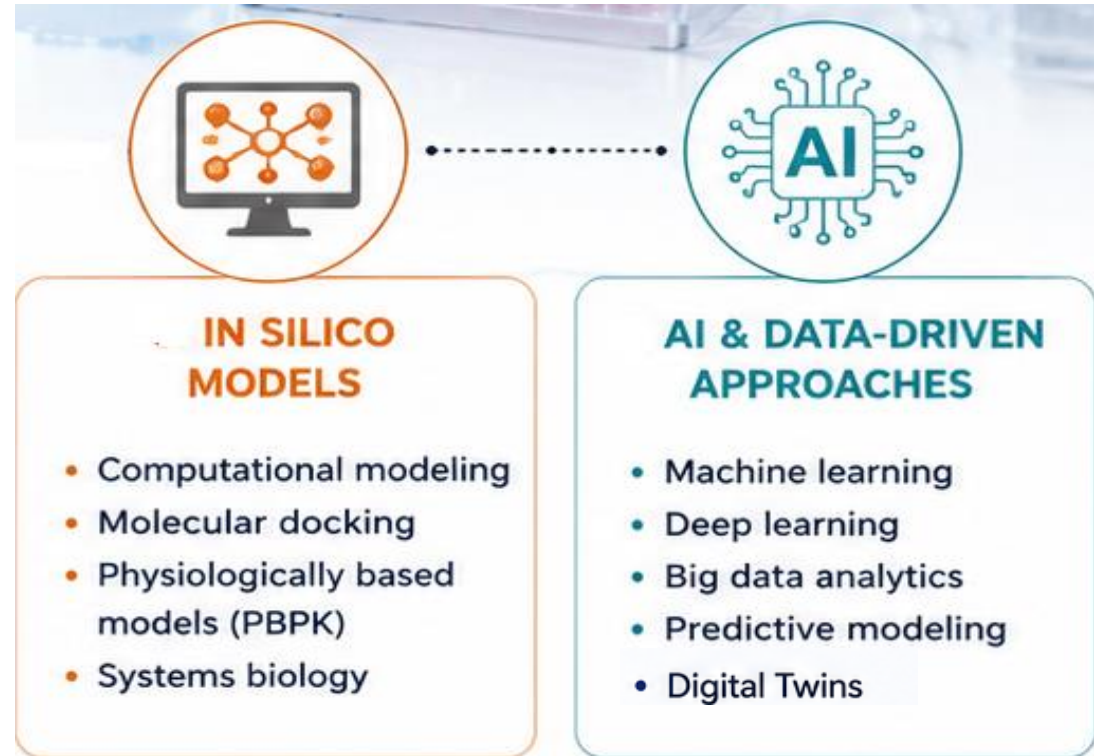
BIMMOH : BIOMEDICAL MODELS HUB

<https://bimmoh.eu/>

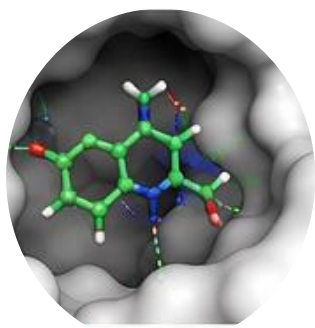


IN SILICO MODELS

Computational approaches that use data and algorithms to predict biological responses



IN SILICO MODELS



Protein Structure Prediction



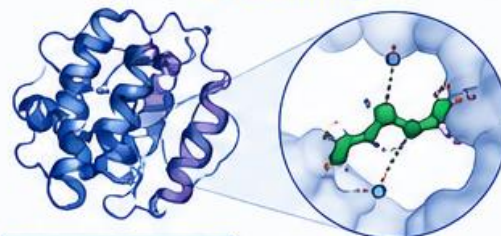
Representative Tools

AlphaFold, RoseTTAFold

Major Applications

- Protein 3D structure prediction
- Target identification and validation
- Mechanistic toxicology
- Structure–function analysis

Molecular Modeling & Docking



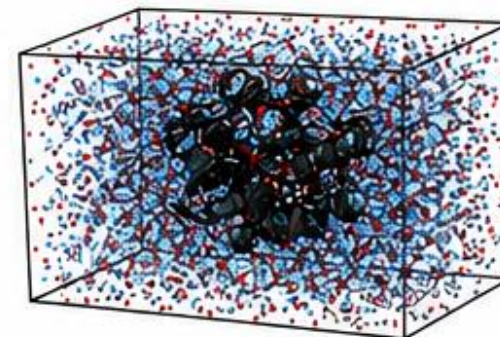
Representative Tools

AutoDock Vina, Schrödinger, MOE

Major Applications

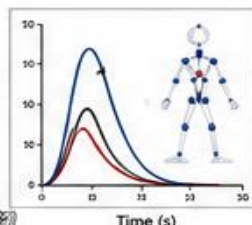
- Ligand–protein binding prediction
- Virtual screening and hit identification
- Drug discovery and repurposing
- Toxicity mechanisms (off-targets)

GROMACS (Molecular Dynamics)



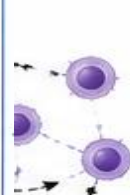
Molecular dynamics simulations to evaluate the stability of protein–ligand interactions over time

OpenSim (SimTK) (Musculoskeletal Modeling)



Simulation of movement, muscle forces and biomechanical analysis of the human body

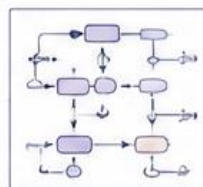
Modeling
(Simulation)



tune cell
 cancer models

PhysiCell

Systems Biology & Network Modeling



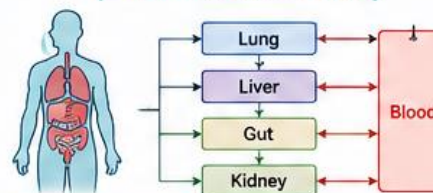
Representative Tools

CellDesigner, COPASI

Major Applications

- Biological pathway modeling
- Signaling and gene regulatory networks
- Systems toxicology
- Hypothesis generation

PBPK Modeling (Pharmacokinetics)



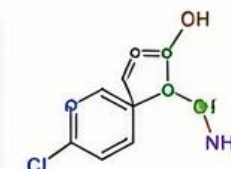
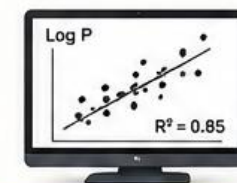
Representative Tools

PK-Sim, GastroPlus, Simcyp, MoBi

Major Applications

- ADME prediction
- Dose extrapolation (interspecies/intraspecies)
- Chemical risk assessment
- Optimization of study design

QSAR & Chemical Informatics



Representative Tools

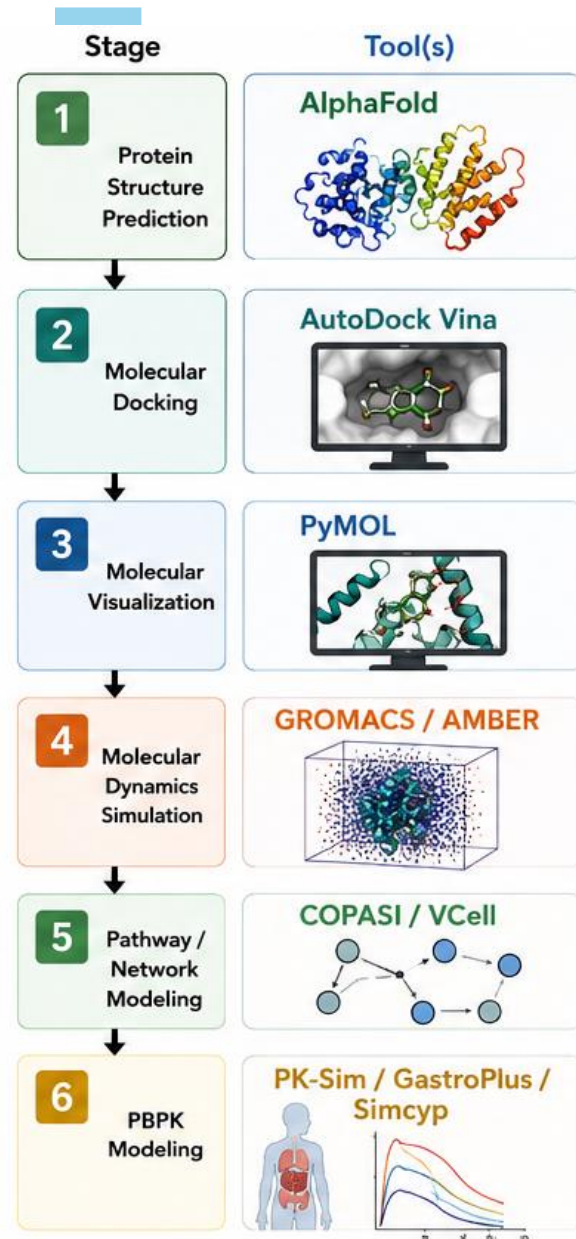
OECD QSAR Toolbox, VEGA, QSAR Toolbox

Major Applications

- Hazard and toxicity prediction
- Read-across and category approaches
- Chemical prioritization
- Regulatory decision support

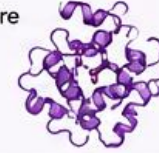
Quantitative Structure–Activity
Relationship

Designing a drug that inhibits the EGFR protein (a receptor that plays a role in cancer cell growth).



Key Outputs

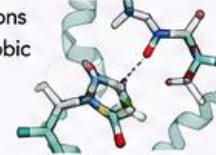
High-confidence 3D structure model of EGFR.



Predicted binding pose and binding affinity (score).



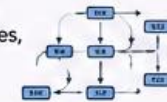
3D visualization of interactions (hydrogen bonds, hydrophobic contacts, etc.).



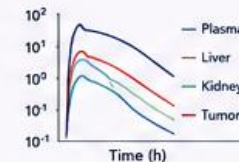
Trajectory data, RMSD, RMSF, radius of gyration, hydrogen bonds, binding free energy.



Dynamic simulation of pathway activation, downstream responses, and crosstalk.



Absorption, distribution, metabolism, excretion, and plasma concentration over time.



Human In Vivo NAMs



Microdosing
(Phase 0)

PET / MRI
imaging



Biomarker &
Omics studies



Wearable
monitoring

- Microdosing (Phase 0 studies)
- Clinical imaging (PET, MRI)
- Biomarker studies
- Wearable digital monitoring
- Omics-based clinical studies

HUMAN IN VIVO NAMs

Non- or minimally invasive approaches that generate data directly in humans

MICRODOSING (PHASE 0)



- Administration of sub-therapeutic doses (e.g., $\leq 100 \mu\text{g}$)
- Enables early PK, target engagement and ADME data
- Minimal risk to participants

Intra-Target-Microdosing (ITM)

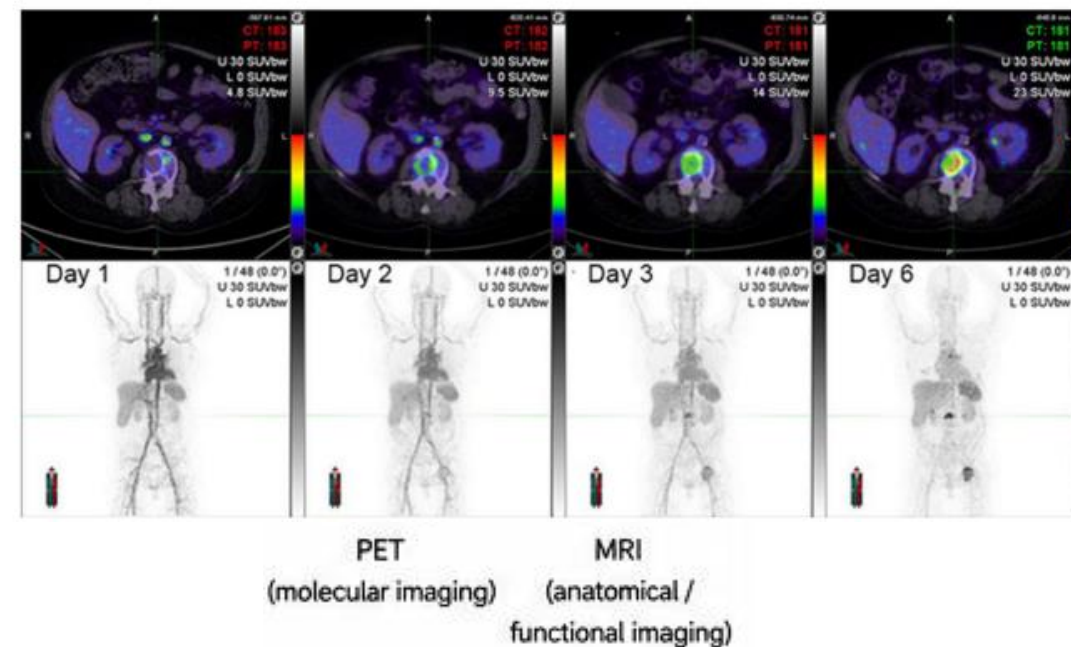
Microdosing with **psilocybin** mushrooms: a double-blind placebo-controlled study



IMAGING (PET / MRI)



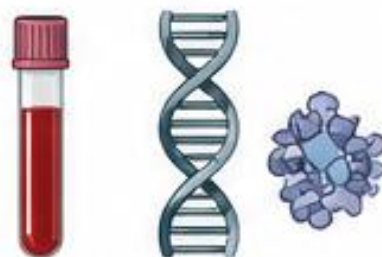
Human Subject PET/CT



HUMAN IN VIVO NAMs

Non- or minimally invasive approaches that generate data directly in humans

BIOMARKERS & OMICS



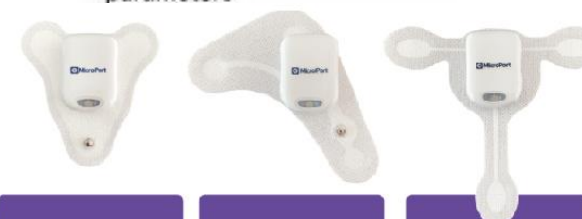
- Measurement of molecules in blood, urine or other fluids
- Genomics, transcriptomics, proteomics, metabolomics
- Enables early detection of response and toxicity

- ✓ Cell-free DNA (cfDNA)
- ✓ Circulating tumor DNA (ctDNA)
- ✓ miRNA
- ✓ exosome
- ✓ proteins
- ✓ metabolites
- ✓ cytokines
- ✓ immune cells
- ✓ circulating tumor cells (CTCs)

DIGITAL BIOMARKERS & WEARABLES



- Continuous monitoring of physiological and behavioral parameters



Pediatric
2/3 channels electrode
1-5 years of age

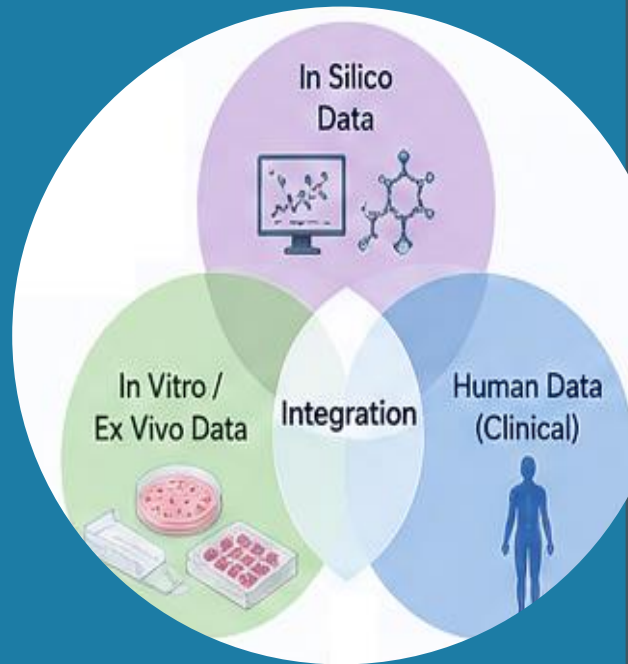
Adult boomerang shape
2/3 channels electrode

Adult T-shape
3 channels electrode



Blood-based biomarkers of Alzheimer's disease and incident dementia in the community- plasma p-Tau217, p-Tau181





Integrated Approaches

- IATA (Integrated Approaches to Testing and Assessment)
- Defined Approaches (DA)
- Adverse Outcome Pathways (AOPs)
- Read-Across
- Weight-of-Evidence (WoE)
- AI-assisted Data Integration

Data integration

Digital Human Models & Virtual Humans

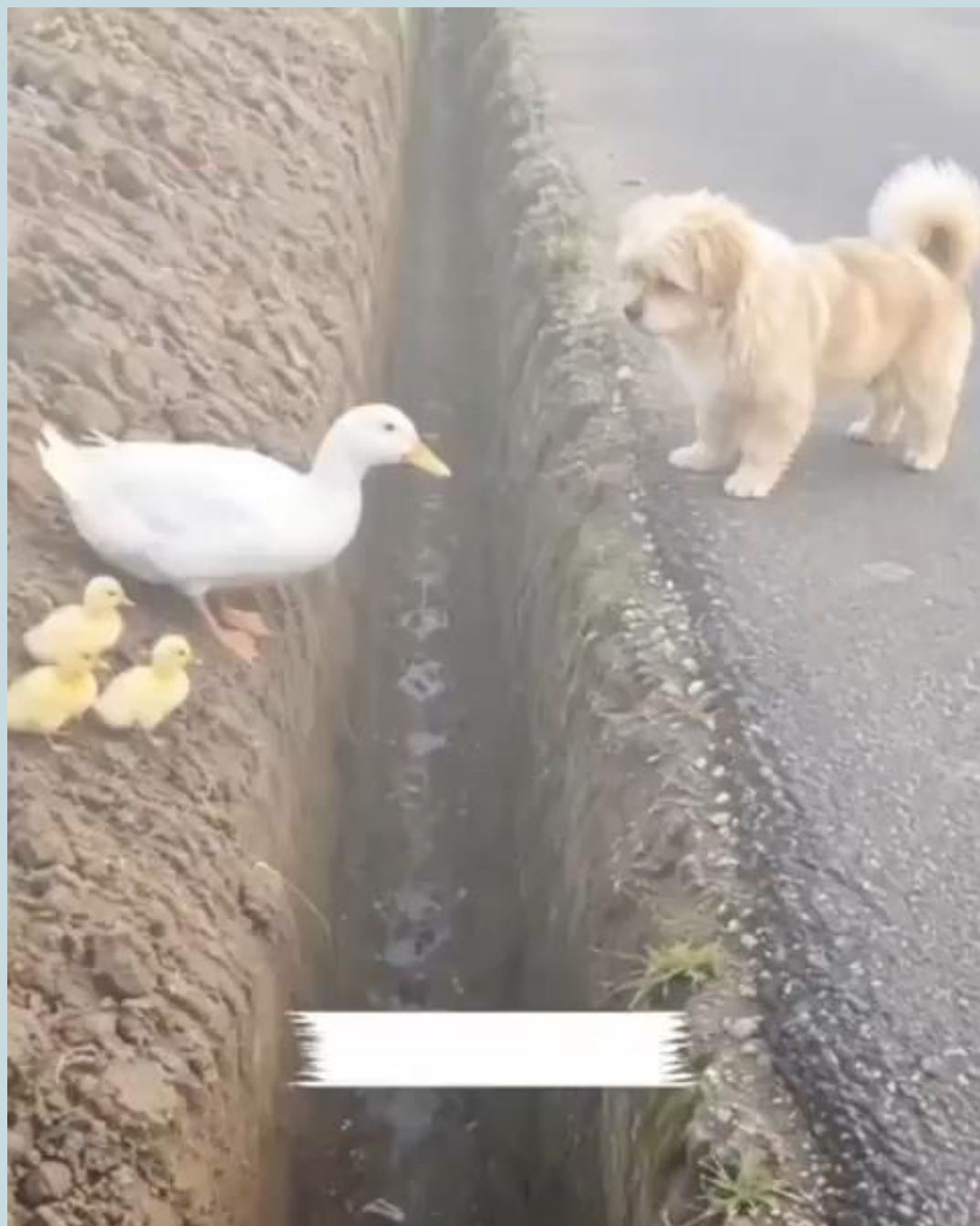


Representative Tools

Virtual Physiological Human (VPH),
Digital Twins

Major Applications

- Human physiology simulation
- Personalized medicine
- Virtual clinical trials
- Risk assessment and decision making





THANK YOU
