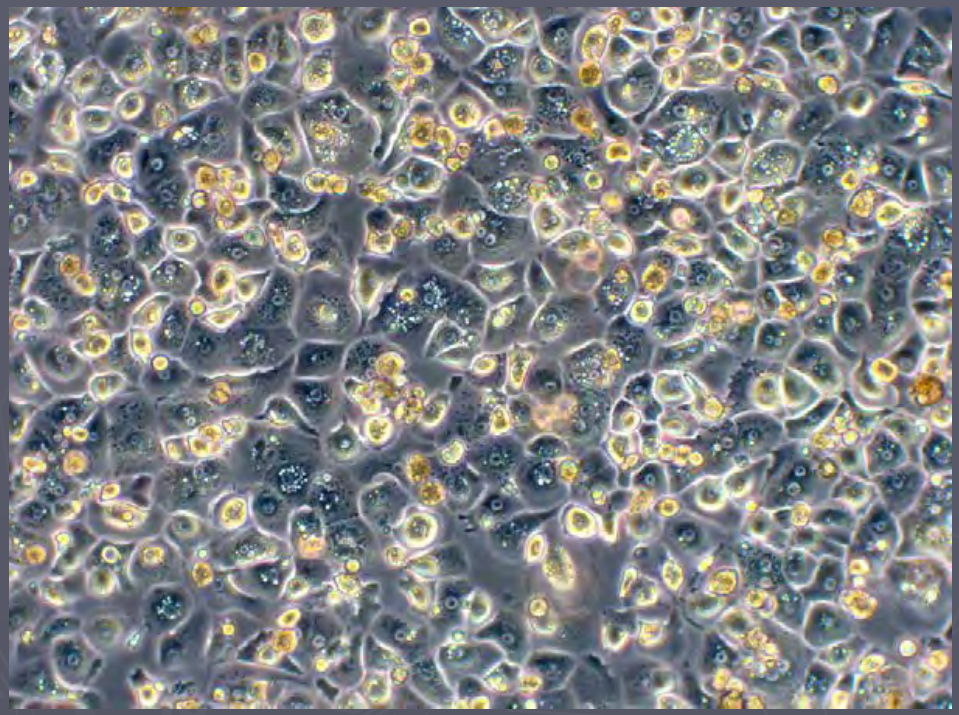
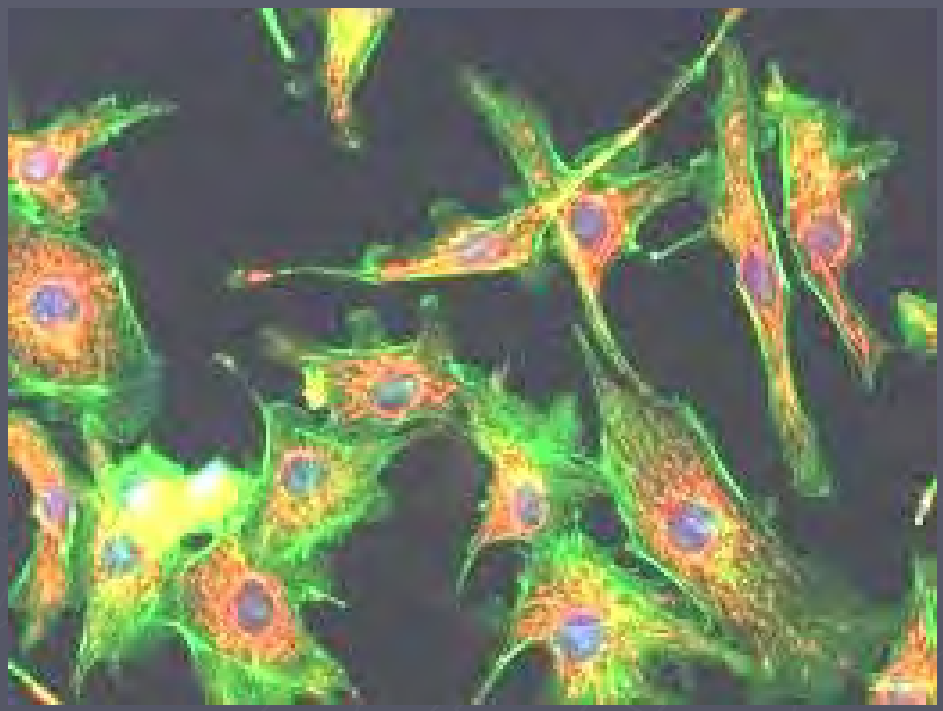
ALTERNATIVES TO ANIMAL EXPERIMENTS





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BDU

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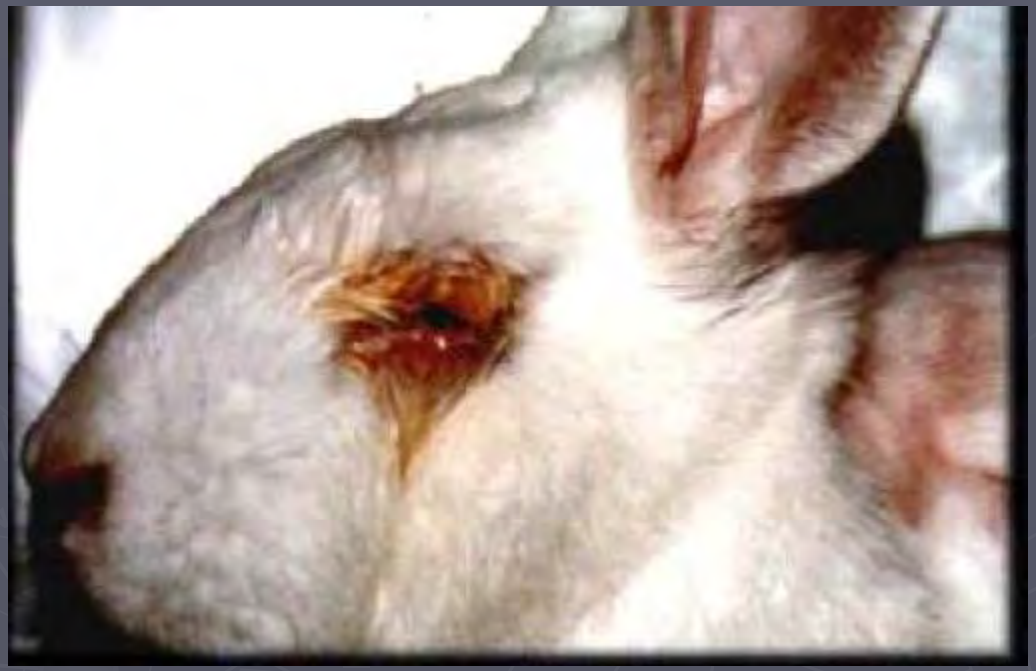
MAHATMA GANDHI DOERENKAMP CENTRE FOR ALTERNATIVES
TO ANIMAL USE IN LIFE SCIENCE EDUCATION

Experiments on animals are expensive, ethically controversial, and not always predictive of the human response

Why not on Humans!!!!!!!



Why animals testing should be restricted?

Professor Charles R. Magel states:
"Ask the experimenters why they experiment on animals, and the answer is: 'Because the animals are like us.' Ask the experimenters why it is morally OK to experiment on animals, and the answer is: 'Because the animals are not like us.'

crueltyfree

The 3 R's Concept

William M.S. Russell & Rex Burch:
The Principles of Humane Experimental Technique
1959
http://altweb.jhsph.edu/publications/humane_exp/het-toc.htm

"Humane science is good science, and the best way to achieve that is rigorous application of the 3Rs", Rex Burch once said

- The book changed the tone of Biomedical research and left scientists to reconsider the use of animal experimentation
- In 1978 D.H. Smith used the term "alternatives" to define the three R's

3R Principle and “Alternatives”

- Replacement
- Reduction
- Refinement

The question about relevance is scientific

Substances that are therapeutic in animals can get approved, later harming humans:

- **Vioxx - The arthritis pain-killer withdrawn in 2004, after causing 27,785 heart attacks and 7,000 deaths**
- **Thalidomide- Teratogenicity & neuropathy**
- **DES (Diethystilbestrol)- Clear cell adinocarcinoma of cervix & vagina in "DES daughters; genital abnormalities leading to male infertility & prostate cancer in DES sons"**
- **The polio vaccine – often cited by researchers as an example of the necessity of animal experiments- was long delayed due to misleading results from primate experiments.**

Animal testing failed to predict these tragedies

Genentech

IN BUSINESS FOR LIFE

April 2007

IMPORTANT DRUG WARNING
Regarding AVASTIN® (bevacizumab)

Dear Healthcare Provider:

Genentech, Inc. would like to inform you of important new safety information regarding AVASTIN® (bevacizumab). This information concerns **tracheoesophageal (TE) fistula** that occurred in a study combining concurrent chemotherapy and radiation plus AVASTIN in patients with limited-stage small cell lung cancer (SCLC). AVASTIN is not indicated for use in SCLC.

Five Withdrawn Drugs

Fen-Phen, Redux

Weight loss
Wyeth
Approved: 1973 (fenfluramine), April 1996 (Redux)
Withdrawn: September 1997

Fenfluramine and Redux were diet drugs made by Wyeth. Doctors began combining them with another diet medicine, phentermine, in order to get a bigger effect. But then a deadly side effect--damage to heart valves--turned up, partly as a result of David Graham's work. Both drugs were pulled from the market, and Wyeth has reserved $16 billion to cover the cost of lawsuits.

Recently Withdrawn Drugs

Ximelagatran (Exanta)	2006	Withdrawn because of risk of hepatotoxicity (liver damage).
Pergolide (Permax)	2007	Voluntarily withdrawn in the U.S. because of the risk of heart valve damage. Still available elsewhere.
Tegaserod (Zelnorm)	2007	Withdrawn because of imbalance of cardiovascular ischemic events, including heart attack and stroke. Was available through a restricted access program until April 2008.
Aprotinin (Trasylol)	2007	Withdrawn because of increased risk of complications or death; permanently withdrawn in 2008 except for research use
Inhaled insulin (Exubera)	2007	Withdrawn in the UK due to poor sales caused by national restrictions on prescribing, doubts over long term safety and too high a cost
Lumiracoxib (Prexige)	2007-2008	Progressively withdrawn around the world because of serious side effects, mainly liver damage
Rimonabant (Acomplia)	2008	Withdrawn around the world because of risk of severe depression and suicide
Efalizumab (Raptiva)	2009	Withdrawn because of increased risk of progressive multifocal leukoencephalopathy; to be completely withdrawn from market by June 2009
Sibutramine (Reductil)	2010	Withdrawn in Europe, Australasia, and the U.S. because of increased cardiovascular risk
Gemtuzumab ozogamicin (Mylotarg)	2010	Withdrawn in the U.S. due to increased risks of veno-occlusive disease and based on results of a clinical trial in which it show no benefit in acute myeloid leukemia (AML)

The Hindu
Online edition of India's National Newspaper
Saturday, Mar 19, 2011

Two drugs banned

Special Correspondent

New Delhi: Union Health and Family Welfare Ministry on Friday banned the manufacture, sale and distribution of Gatifloxacin and Tegaserod as they caused certain adverse side effects. The decision follows recommendations of the Drug technical Advisory Committee last month.

Gatifloxacin sold under brand names Gatiflo, Tequin and Zymar is an antibiotic that inhibits the bacterial enzymes DNA gyrase and topoisomerase IV. It was introduced in 1999 under the proprietary name of Tequin for the treatment of respiratory tract infections having licensed the medication from Kyorin Pharmaceutical Company of Japan.

In many countries, Gatifloxacin is available as tablets and in various aqueous solutions for intravenous therapy. It is said to cause diabetes. Tegaserod is sold under the name Zelnorm for the management of irritable bowel syndrome and constipation. Its use has been associated with an increased risk of heart attacks or stroke.

Safety Alerts (2005-2007)

- Avandia, Celebrex, Bextra, Vioxx: Increased CV risks
- Cialis: NAION (nonateritic ischemic optical neuropathy)
- Epogen: Severe anemia
- Herceptin: Cardiotoxicity
- Interferon beta-1b: Liver failure
- Zevalin: severe, fatal cutaneous and mucotaneous reactions
- Cymbalta: Hepatic injury
- Sustiva: Fetal toxicity
- Novantrone: Cardiotoxicity; secondary leukemia
- Aptivus: Intracranial hemorrage
- Ketek: Rare severe liver failure
- ACE Inhibitors: Fetal malformation
- Ontak: Persistent visual impairment
- Tracleer: Hepatotoxicity
- Trasylol: Renal dysfunction
- Rosiglitazone maleate: Macula edema

www.fda.gov/medwatch

You know why drugs tested in animals came to fail later?

MAHATMA GANDHI DOERENKAMP CENTER

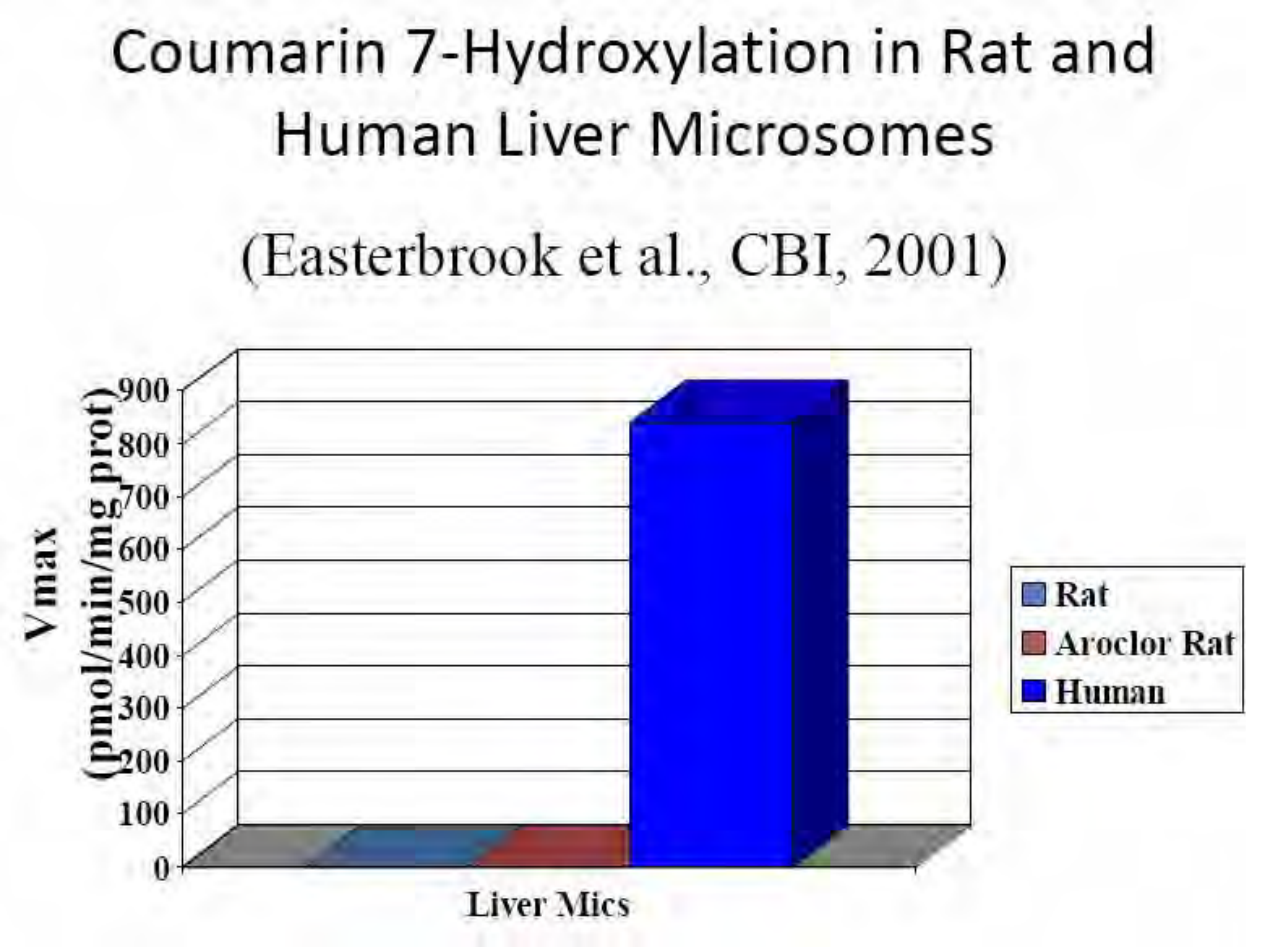
Lewis DF, loannides C, and Parke DV (1998) Cytochromes P450 and species differences in xenobiotic metabolism and activation of carcinogen. *Environmental Health Perspectives* 106, 633-641.

Table 1. P450 isoforms in various mammalian species[a]

CYP	Human	Monkey	Rabbit	Rat	Mouse
1A	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2
2A	2A6, 2A7	–	2A10, 2A11	2A1, 2A2, 2A3	2A4, 2A5
2B	2B6	2B17	2B4, 2B5	2B1, 2B2, 2B3	2B9, 2B10, 2B13
2C	2C8, 2C9, 2C18, 2C19	2C20, 2C37	2C1, 2C2, 2C3, 2C4, 2C5, 2C14, 2C15, 2C16	2C6, 2C7, 2C11, 2C12, 2C13, 2C22, 2C23, 2C24	2C29
2D	2D6	2D17	–	2D1, 2D2, 2D3, 2D4, 2D5	2D9, 2D10, 2D11, 2D12, 2D13
2E	2E1	2E1	2E1, 2E2	2E1	2E1
3A	3A4, 3A5	3A8	3A6	3A1, 3A2, 3A9	3A11, 3A13, 3A16
4A	4A9, 4A11	–	4A4, 4A5, 4A6, 4A7	4A1, 4A2, 4A3, 4A8	4A10, 4A12, 4A14

CYP, cytochrome P450 subfamily; –, no information available to date. Data from Nelson et al. (*22*).

[a]These are found predominantly, but not exclusively, in the liver of the relevant species.



- For coumarin, the major route of metabolism in humans, i.e., the 7-ydroxylation, is catalyzed by P4502A6 (CYP2A6)
- The analogous mouse ortholog is CYP2A5, which mediates the same metabolic pathway
- However, in the rat, the CYP2A1 isoform facilitates formation of the carcinogenic 3,4-epoxide

- Tamoxifen is generally regarded as being largely safe in humans, but genotoxic and carcinogenic in rodents.
- The reason for the species difference is mainly due to the way in which tamoxifen is metabolized in humans as opposed to the situation in rats and mice.
- Tamoxifen is readily metabolized by CYP2C9 and CYP3A4 isoforms in *Homo sapiens*, which leads to its relatively rapid elimination in human subjects.
- Different P450 families and subfamilies appear to be involved in the metabolic activation of tamoxifen in rodents; this may involve the formation of an epoxide (a highly reactive electrophilic species), which are also P450-mediated, such as ethyl group hydroxylation and subsequent formation of a carbonium ion intermediate that is able to interact covalently with DNA, thus giving rise to genotoxicity and, eventually, to carcinogenesis

In vitro and Bio-informatics tools are the principal alternatives

In vitro tools: Used principally for screening purposes, for generating comprehensive toxicological profiles, to obtain mechanism-derived information, and to provide important non-invasive tools to enhance the extrapolation from *in vitro* to *in vivo* in humans.

Increasing number of test systems for evaluating the possible toxicological hazard of chemical compounds have been developed, which do not rely on the use of intact animals and have been extremely useful in studying the molecular basis of a chemical's biological activity, including its mechanism(s) of toxic action.

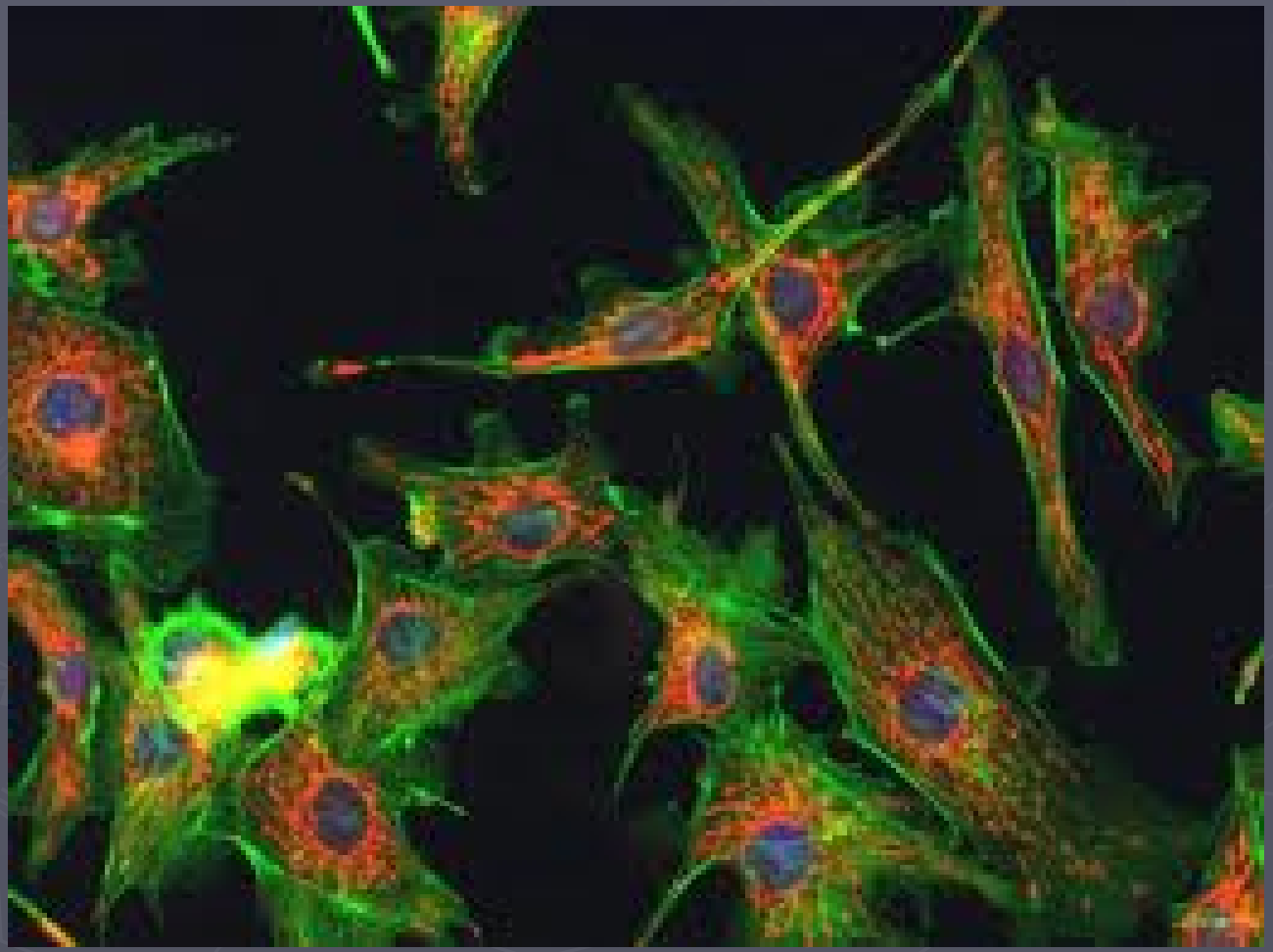
Areas of in vitro toxicology include genotoxicity, cellular responses, toxicokinetics, toxicodynamics, tissue modeling, mutagenesis, developmental toxicity (teratology), prediction of allergenicity, development and application of biomarkers, etc.

Cell culture models

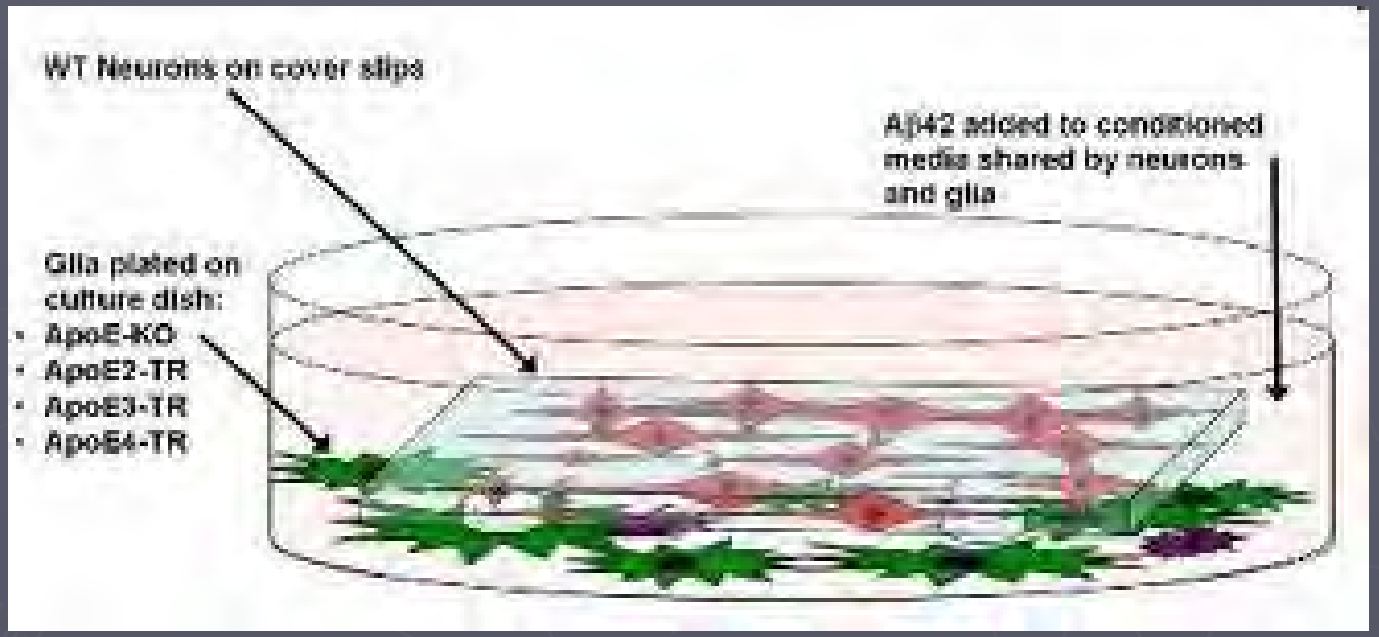
Advantages

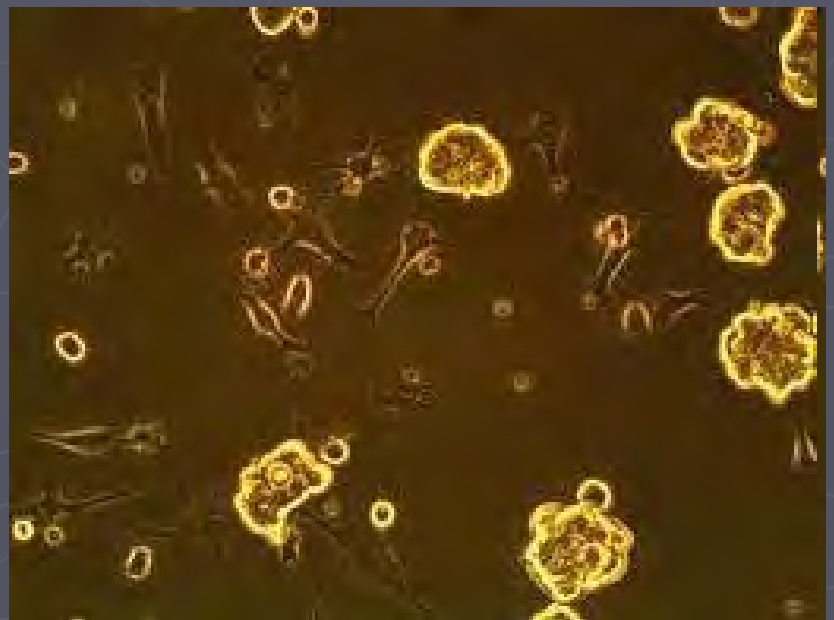
- Cost-effective
- Non-invasive
- Quick results
- Uniform chemical and physical environment
- Human materials available
- Generation of large volumes of data
- Mechanistic understanding
- Humane testing method
- Requires less lab space
- Saves number of animals
- Easy to design experiments and look comfortably at molecular targets

What should be the system of choice?
Monoculture?



Is Co-culture the Solution?

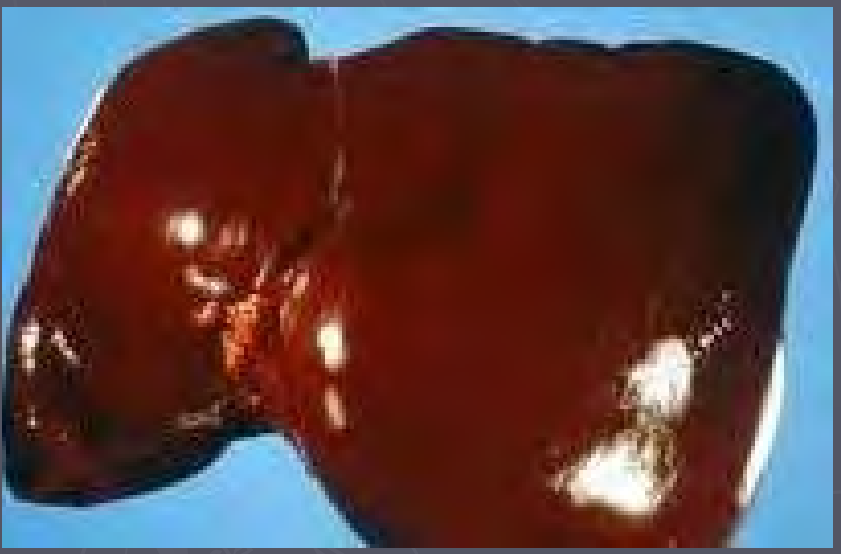


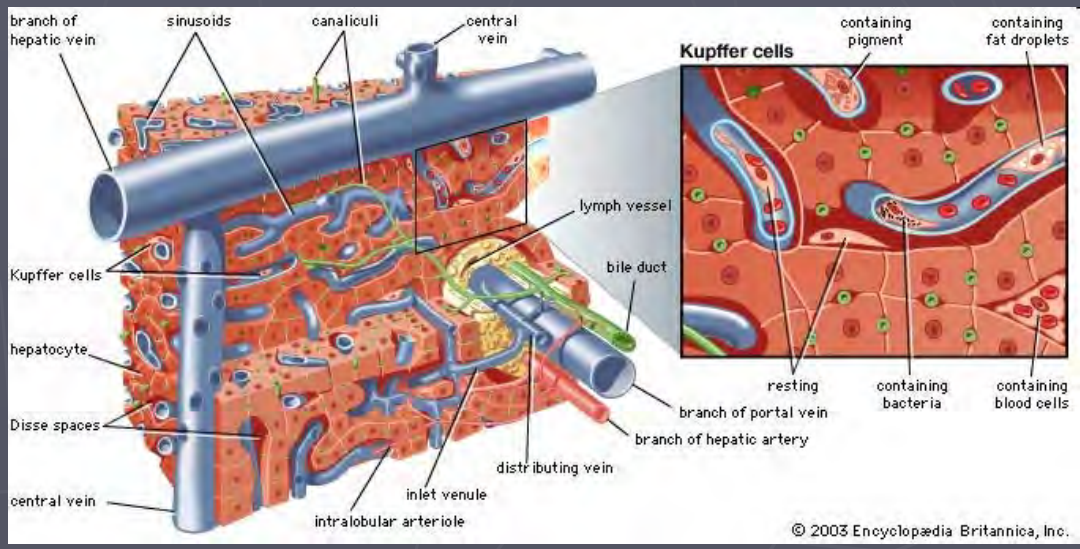


What should be the cell of choice?

- Human-specific xenobiotic toxicity cannot be accurately evaluated with nonhuman animal models.
- Experimentation in humans *in vivo* is not practicable.
- Toxicity testing *in vitro* represents the only practical preclinical approach to derive human-specific information for the accurate prediction of human xenobiotic toxicity.
- The use of dedifferentiated cell lines such as transformed or immortalized mouse or human fibroblasts or other transformed cells may not be useful, as neither of the above critical properties is present.
- The use of human organ-derived primary cells as monogenic culture (single cell type cultures) will allow the evaluation of the effect of xenobiotics.

- As the liver represents the major organ for drug metabolism, human liver-derived experimental systems must be used for the evaluation of human-specific drug/toxicological properties





Human hepatocytes

- One cell system that represents the target cells and has human metabolism capacity is human hepatocytes. This cell system has the several advantages:
- **Human xenobiotic metabolism:** Isolates of human hepatocytes are known to contain most, if not all, of the *in vivo* hepatic xenobiotic metabolism capacity.
- **Human target cells:** The hepatocytes are the cells in the human liver that are damaged by hepatotoxicants, leading ultimately to liver failure.
- However, a commonly used cell line, the HepG2, does not have these properties and, therefore, would not represent a relevant *in vitro* model for the investigation of hepatotoxicity. More importantly, is derived from a human adenocarcinoma of the liver, not the parenchymal cells which are the *in vivo* target of hepatotoxicants.

- Experimental systems derived from the liver include systems with intact, viable cells (intact cell systems) such as hepatocytes and liver slices, as well as cell-free systems such as liver homogenates, post-mitochondrial supernatants, and microsomes.
- The intact cell systems, with full complements of enzymes and cofactors at physiological levels and natural orientations, should be more representative of the liver *in vivo* than cell-free systems with disrupted membranes and incomplete cofactors and enzymes.
- The presence of the intact plasma membrane allows the modeling of differences between intracellular and extracellular concentrations potentially resulting from active uptake and excretion.
- Further, cytotoxicity studies can be performed with the intact cells, allowing investigations on toxic mechanisms, including the relationship between metabolism and toxicity.

Application of human hepatocytes

- Applications of human hepatocytes in drug development includes evaluation of
  - metabolic stability
  - metabolite profiling and identification
  - drug–drug interaction potential
  - hepatotoxic potential
- Hepatocytes in vivo have both uptake and efflux transporters, with the uptake transporter responsible for the active uptake of biomolecules and xenobiotic, and the efflux transporters for the bile excretion of bile salts and conjugated xenobiotics. Hepatocytes can be useful in evaluation of transporters.

What is the source of human hepatocytes?

- In the United States, livers procured but not used for transplantation are allowed to be used in research.
- The major reasons that procured livers are not used for transplantation are as follows:
  1. Unavailability of a matched recipient
  2. Physical damage to the liver
  3. Pre-existing liver diseases
  4. Breach of sterility during the procurement process
  5. High liver fat content
  6. Inappropriate age (too young or too old)
  7. Inappropriate warm ischemic time
  8. Inappropriate cold storage time

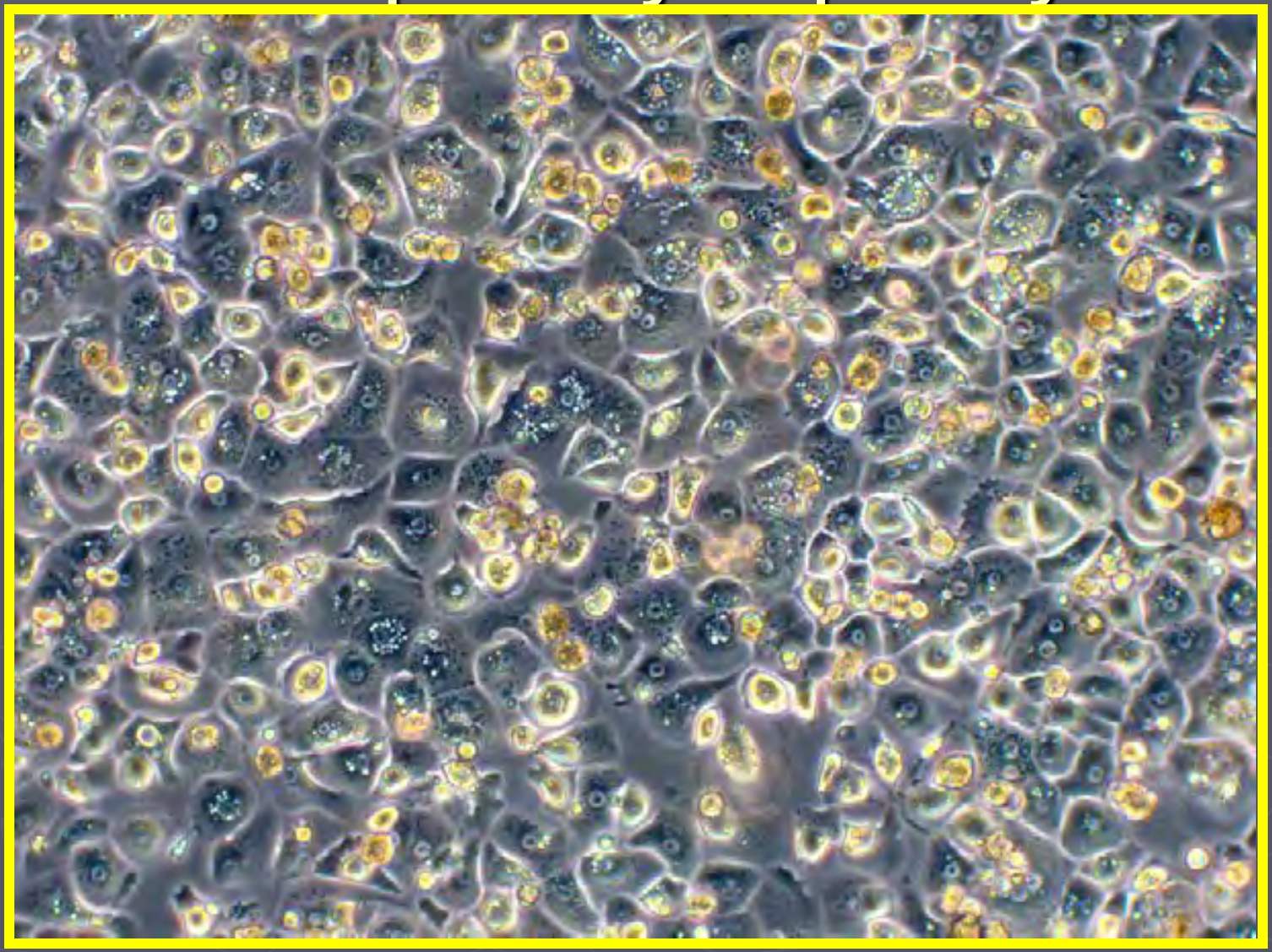
How to overcome the limitation of availability of fresh human livers?

- Relatively few laboratories have access to fresh human livers for hepatocyte isolation.
- Hence, human hepatocytes are not yet a universally available experimental system.
- Cryopreservation, if successful, would greatly enhance the availability of human hepatocytes.
- For instance, hepatocytes can be routinely prepared from one laboratory that has access to fresh human livers, stored cryopreserved, and shipped to other laboratories for experimentation.

The strengths of the cryopreserved human hepatocytes over freshly isolated hepatocytes

- 1. *Ease of experimentation*: Unlike freshly isolated hepatocytes which is dependent on the availability of a human liver for research, experimentation with cryopreserved human hepatocytes can overcome this limitation.
- 2. *Repeat experimentation*: Cryopreserved hepatocytes from a single donor (or combination of multiple donors) can be used at different times to allow the performance of repeat studies. This is not possible with freshly isolated hepatocytes.
- 3. *Choice of donors*: Experimentation with freshly isolated cells are performed with the liver from the donor available at a specific point of time. Limiting the choices of donors may lead to a prolonged waiting period for experimentation. Experimentation with cryopreserved human hepatocytes allows the researcher to select hepatocytes from donors with properties most appropriate to the experimental objectives.

Cryopreserved, thawed and plated human primary hepatocytes



A major criticism of in vitro systems

- Lack of multiple organ, systemic interactions
  - A toxicant and its metabolites may have multiple organ effects
  - A toxicant may be biotransformed by multiple organs, with metabolites from one organ may have effects on other organ(s)
  - Physiological changes (e.g. cytokine activation) in one organ may have effects on the toxicity of a toxicant on other organs

In Vitro In Vivo Strategy (IVIVS)

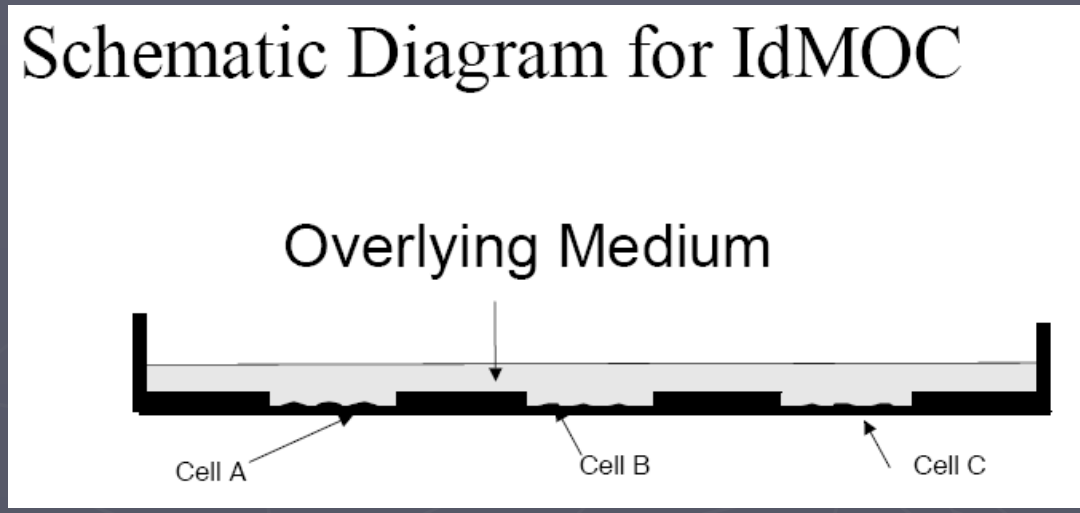
Li AP (2007). Current Drug Safety

Integrated Discrete Multiple Organ Co-Culture (IdMOC)

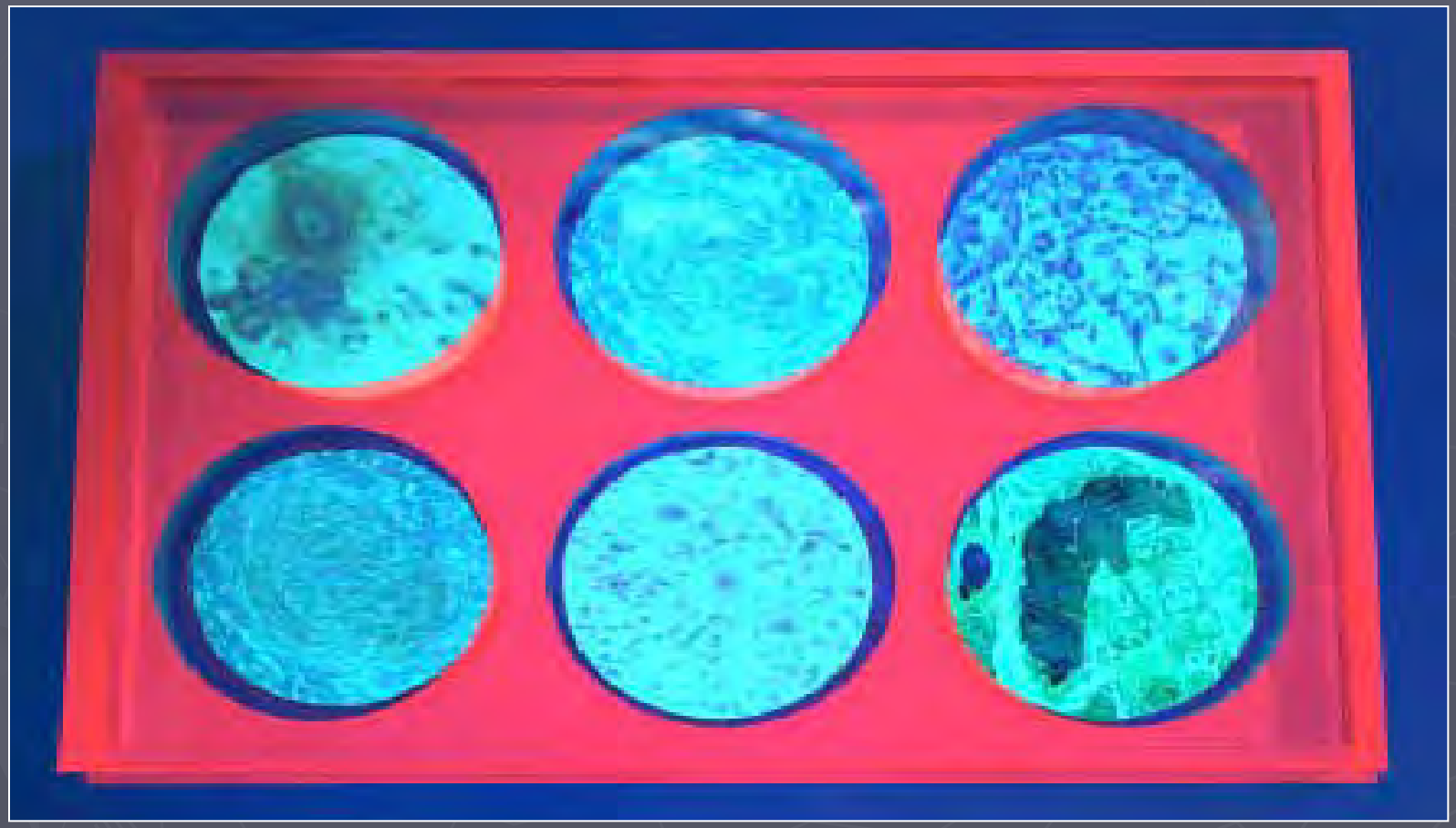
- IdMOC is a novel cell culture system that allows multiple organ interactions
- IdMOC was developed by Dr. Albert P. Li and Colleagues
- The technology is patented by A.P. Sciences, MD, USA
- The IdMOC represents an improved *in vitro* experimental system for routine screening of ADMET drug properties
- Novel technology allowing the culturing of cells from multiple organs in the same culture dish
- The IdMOC models *in vivo* multiple-organ interaction, thus allowing the evaluation of organ- specific effects of a drug and its metabolites

IdMOC: Principle

- The IdMOC involves the “wells-in-well” concept.
- The typical IdMOC plate consists of a chamber within which are six wells.
- Cells of different organs (e.g., from different organs) are initially cultured, each in its specific medium, in the wells.
- When the cells are established, the wells are flooded with an overlying medium, there-by connecting all the wells.
- The multiple cell types now can interact via the overlying medium, akin to the multiple organs in a human body interacting via the systemic circulation.



Photograph of an IdMOC plate based on the format of a standard 96-well plate (IdMOC-96). The plate consists of six individual wells inside a containing chamber. The wells are filled with colored liquids (left) to show that they are physically separated. The chambers are filled (right) to show the connection of the wells. Each chamber therefore represents a single experimental unit, allowing each plate to be used for multiple treatment conditions. For instance, one IdMOC-96 plate can be used to evaluate the effect on six cell types for 15 test articles (using one chamber as control) at a single concentration, two test articles at four concentrations in duplicates, or a single test article at four concentrations in quadruplicates.



Cell Types Co-cultured in IdMOC

- Human Hepatocytes (Liver)
- Human Aortic Endothelial Cells (Blood Vessel)
- Human Astrocytes (CNS)
- Human Renal Proximal Tubule Cells (Kidney)
- Human Small Airway Epithelial Cells (Lung)
- MCF-7 (Human Breast Adenocarcinoma)

"*In Vitro* Toxicology Adopting Integrated Discrete Multiple Organ Co-culture" (IdMOC)

Sponsored by

UGC- SAP, CSIR, University of Madras, Doerenkamp-Zbinden Foundation, Switzerland & MGDC, Bharathidasan University, Tiruchirappalli

at

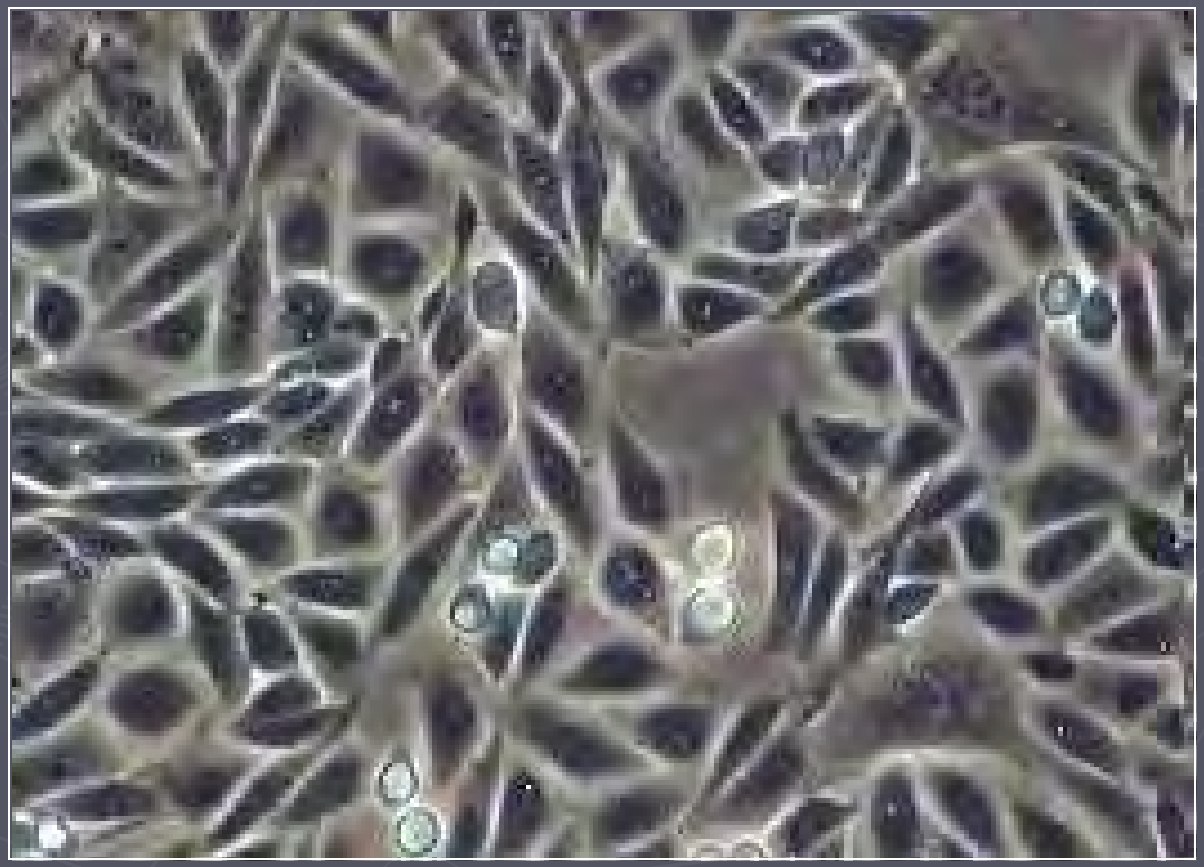
Dr. A.Venugopal Auditorium

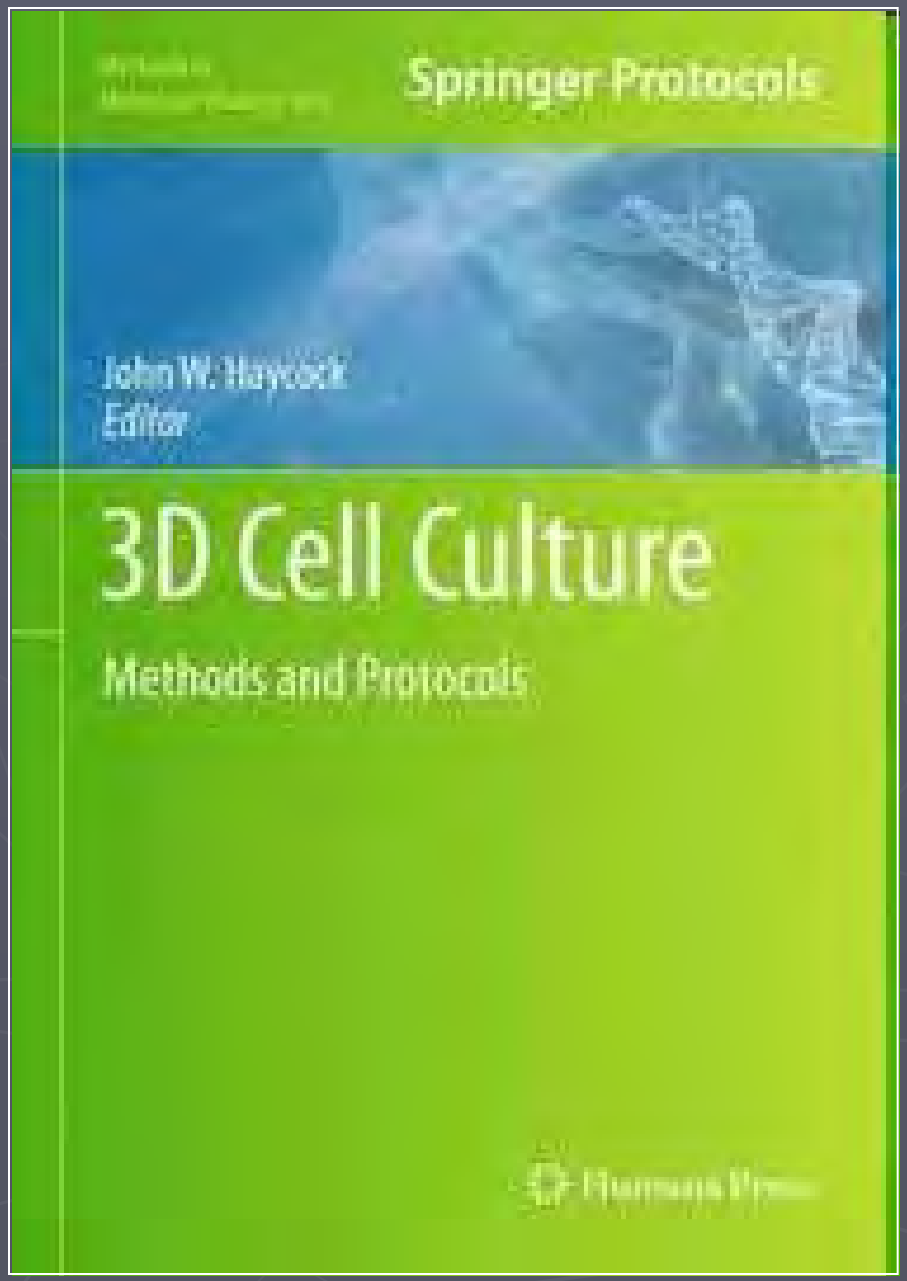
Dr. ALM Post Graduate Institute of Basic Medical Sciences, Sekkizhar Campus, Taramani, Chennai-113

on

Wednesday 16th February, 2011 at 9.30 a.m.

However, conventional two-dimensional cell cultures do not reproduce the tissue architecture *in vivo,* and do not forecast organ-specific toxicity




Springer Protocols
John W. Haycock
Editor
3D Cell Culture
Methods and Protocols
Humana Press

- Three-dimensional cultures emulate the biochemistry and mechanics of the microenvironment in tissues more closely.
- Therefore, they address the limitations of both animals and two-dimensional cultures, and provide more accurate data on the effects of short- and long-term exposure to toxicants.

Hepatpospheres: A much more Advanced Technology

Biotechnology and Genetic Engineering Reviews - Vol. 26, 129-150 (2009)

Three-Dimensional Cell Cultures in Toxicology

FRANCESCO PAMPALONI[1*] AND ERNST H. K. STELZER[1]

World Journal of Hepatology

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wjh@wjgnet.com
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World J Hepatol 2010 January 27; 2(1): 1-7
ISSN 1948-5182 (online)

EDITORIAL

Hepatospheres: Three dimensional cell cultures resemble physiological conditions of the liver

Franziska van Zijl, Wolfgang Mikulits

- The culture of primary hepatocytes as spheroids creates an efficient 3-dimensional tissue construct for hepatic studies *in vitro.*
- Spheroids possess structural polarity and functional bile canaliculi with normal differentiated function.
- Thus, hepatocyte spheroids have been proposed as the cell source in a variety of diagnostic, discovery, and therapeutic applications, such as a bio-artificial liver.

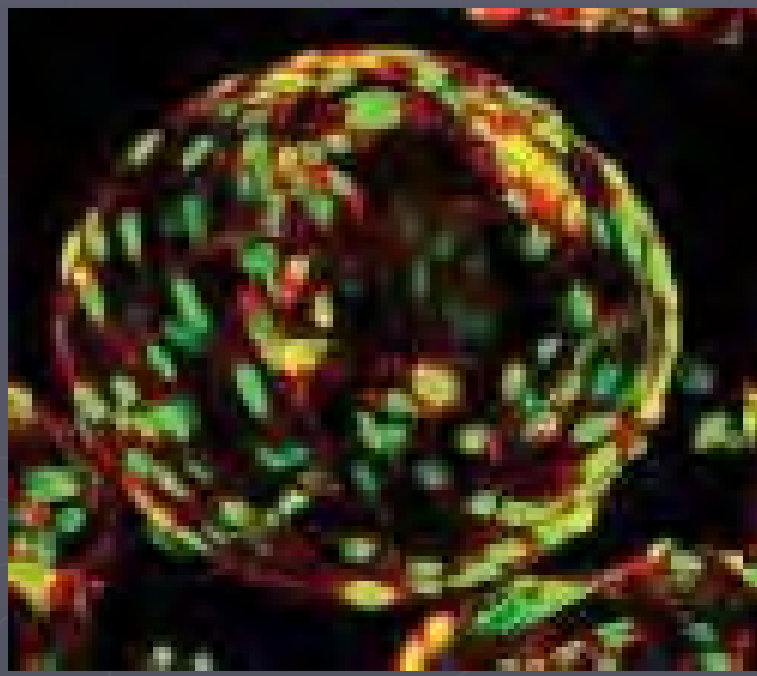


Table 1 Characteristics of hepatospheres

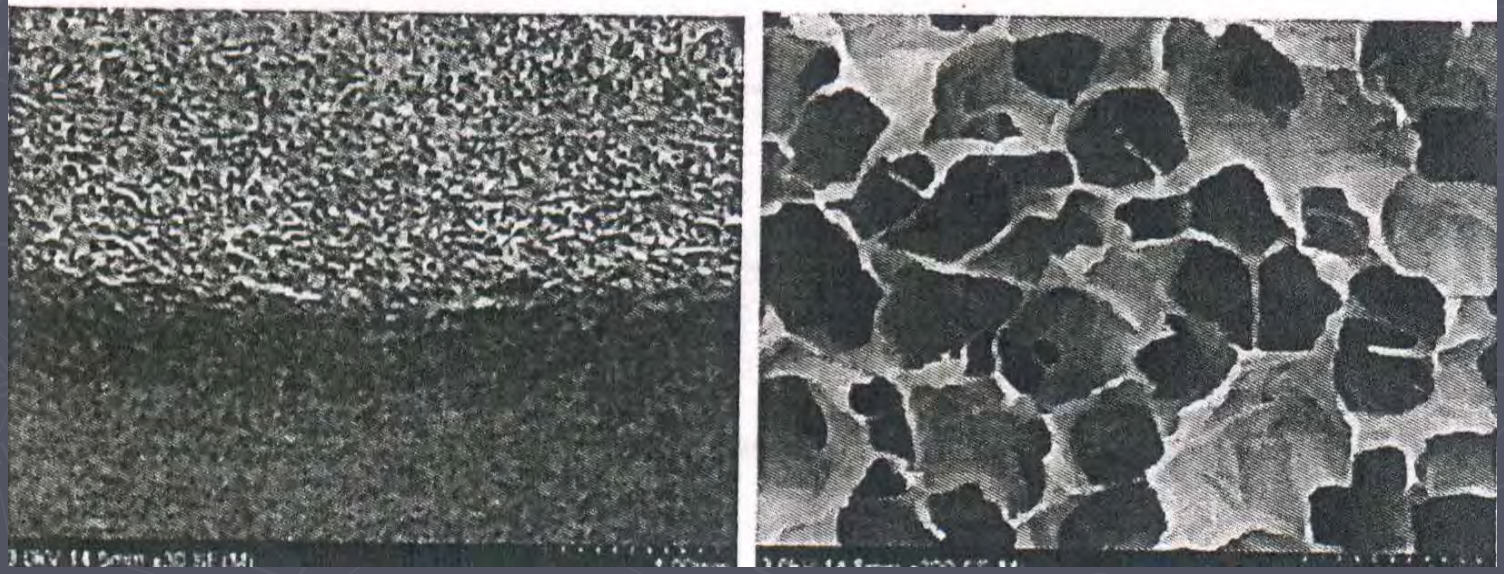
Classifications
Maintenance of physiological structure
Reassembling of liver cells in a physiological pattern, formation of ducts
Polarity, apical secretion and function of CD26
E-cadherin: Induction of spheroid formation
Integrin signaling
Elevated ECM production
Prolonged survival
Physiological expression pattern
Prolonged metabolic functions
Secretion of albumin
Tyrosine aminotransferase
Glutathione S transferase
Detoxification of urea
Biotransformation of xenobiotics (CYP)
Induction of stemness
AFP
CK19
Resembling of HCC
Angiogenesis

Do hepatospheres mimic liver *in vivo*?

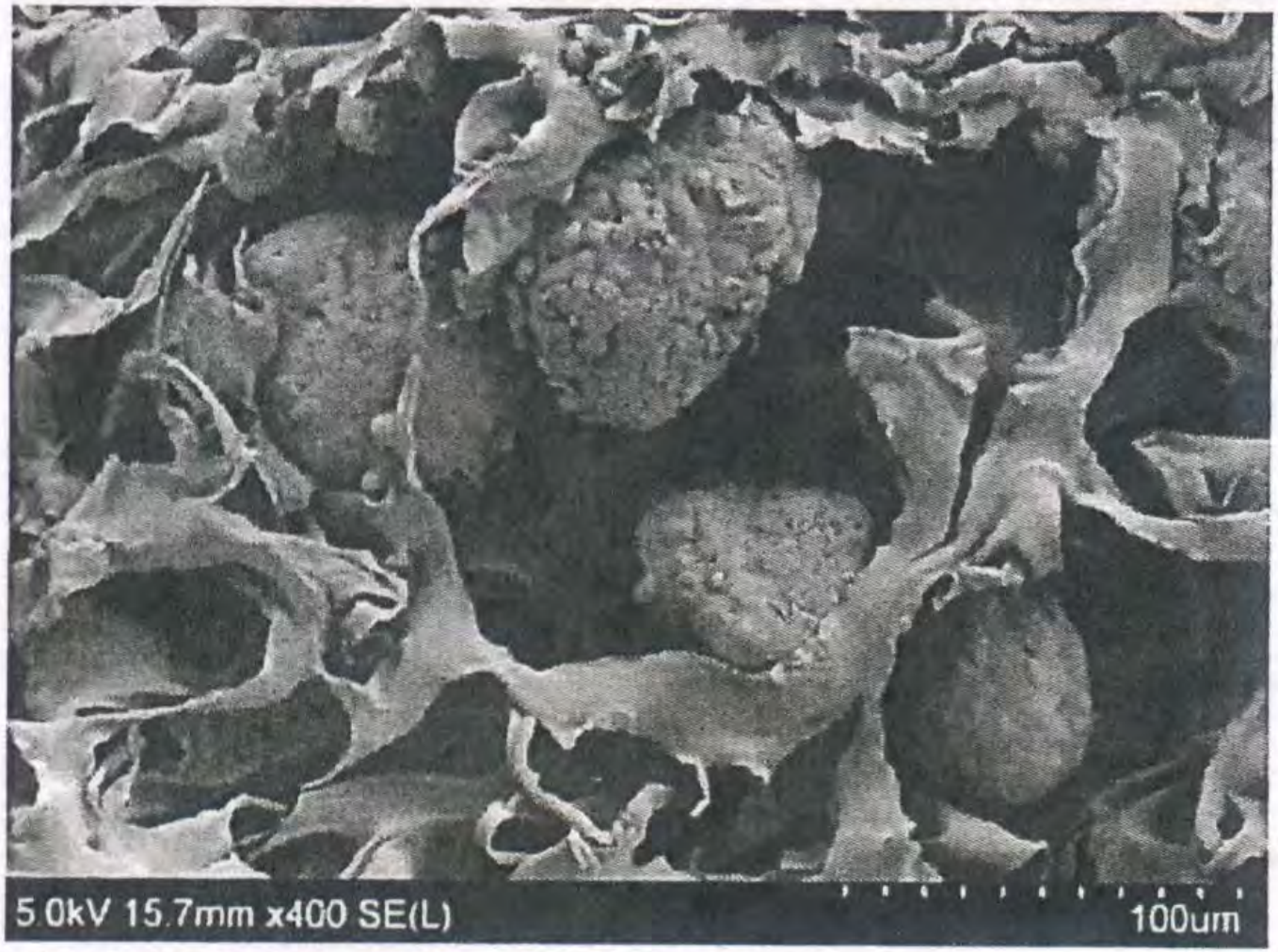
Coherent spheroid cultures

What is AlgiMatrix ?

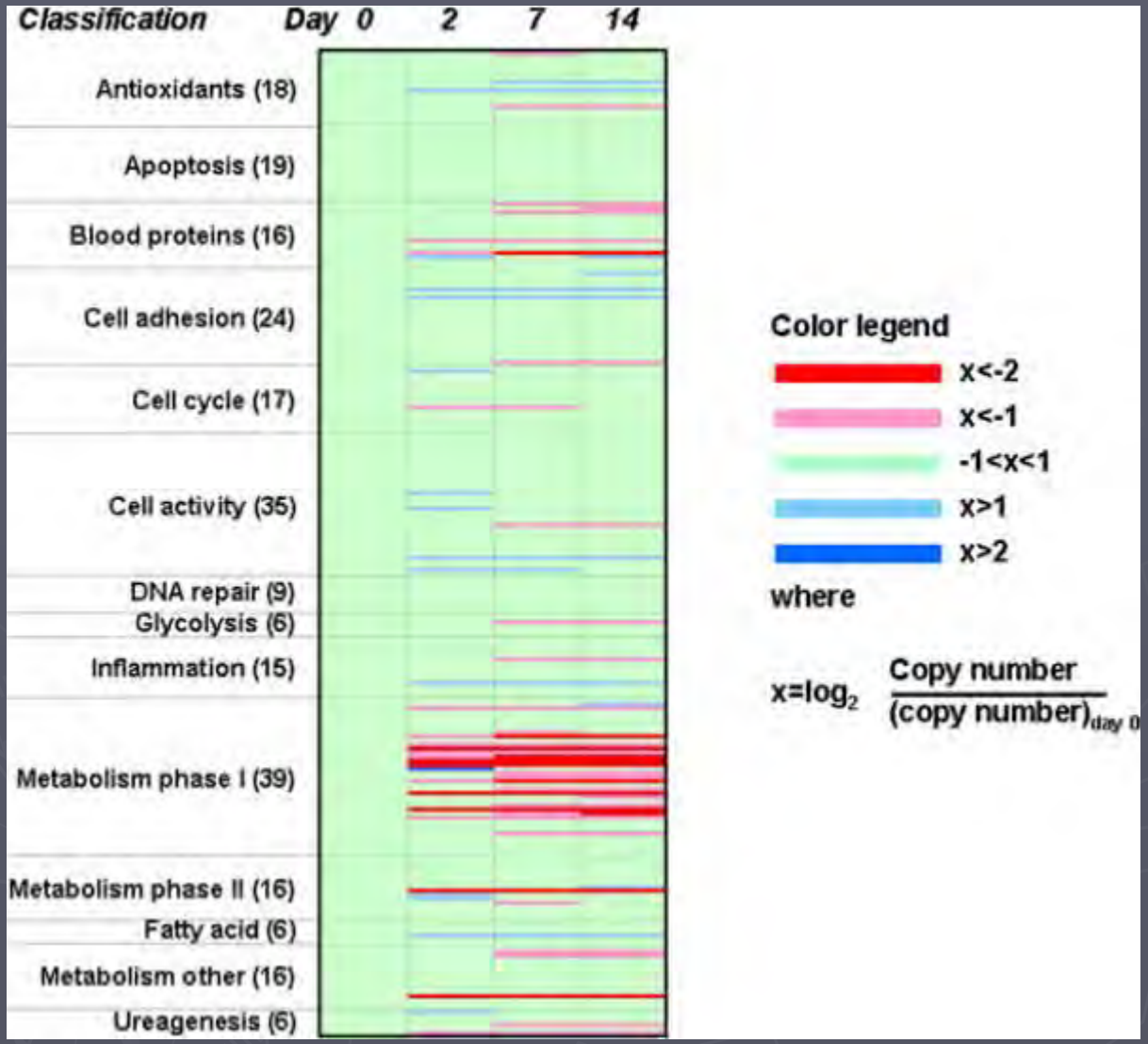
- Freeze dried alginate sponge in 6, 24, 96 well plates
- Polysaccharide derived from seaweed
- 50-200 μm pore size
- Inert – does not specifically interact with cells
- Drives intercellular interaction via pore structure



Formation of Coherent Spheroidal Structures



C3A Cells, 3 week culture

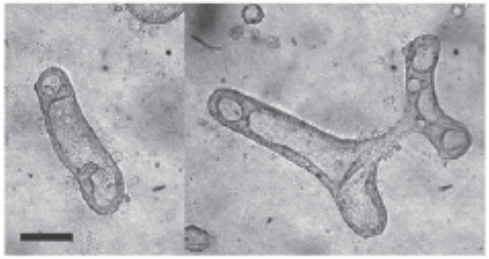


Expression of 242 liver specific genes by custom microarray in rocked spheroid culture over 14 days duration. Average expression of over 80% of these genes was stable over time (green color). A reduction in gene expression from baseline (freshly isolated rat hepatocytes) is indicated in pink or red, while an increase in expression is labeled blue. Genes of phase I metabolism showed the greatest level of fluctuation in rocked spheroid culture.

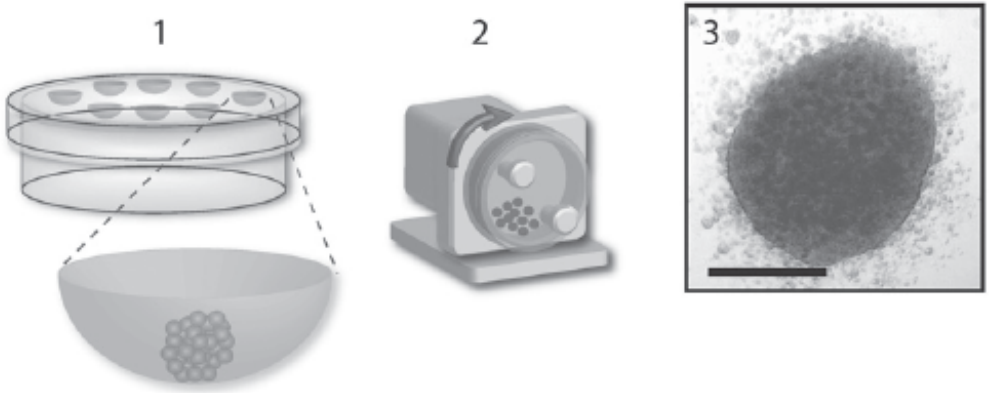
Hydrogels and Microscale Technologies

- Collagen I is the prevalent ECM component of the connective tissue (stroma).
- Collagen is extracted from animal tendons and is commercially available as an acidic solution.
- A 3D collagen fibrillar hydrogel can be easily reconstituted *in vitro* by neutralizing the solution.
- A highly ordered fibrillar architecture, similar to the one *in vivo*, can obtained by controlling the collagen concentration and sonicating the solution before gelation.
- Collagen hydrogels can be employed for 3D cultures.

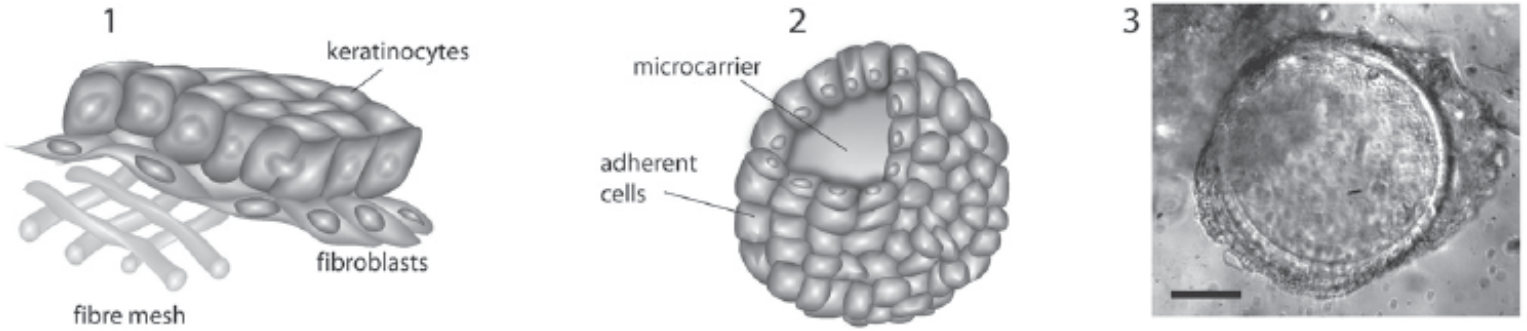
a) 3D cultures in ECM hydrogel



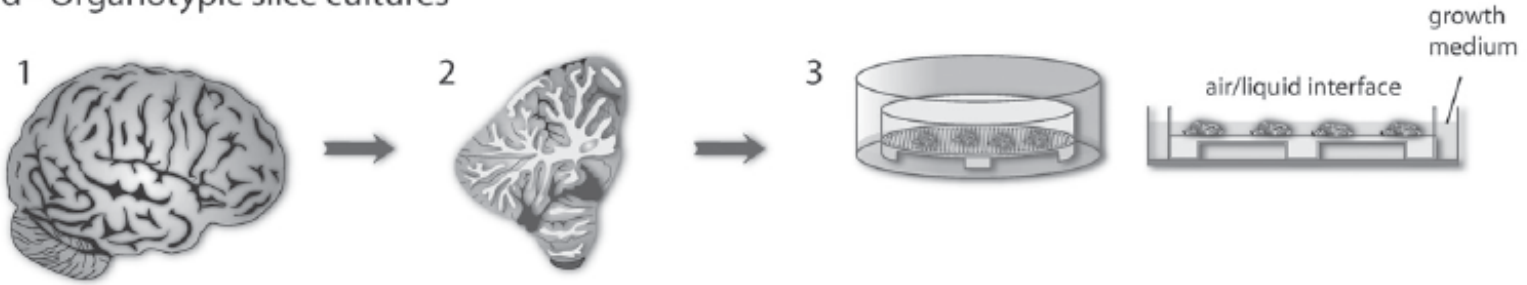
b) Cellular spheroids



c - Cultures on porous substrates and microcarriers



d - Organotypic slice cultures



Emerging Applications of Hydrogels and Microscale Technologies in Drug Discovery

a report by

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1. International Center for Young Scientists (ICYS), National Institute for Materials Science (NIMS), Japan.

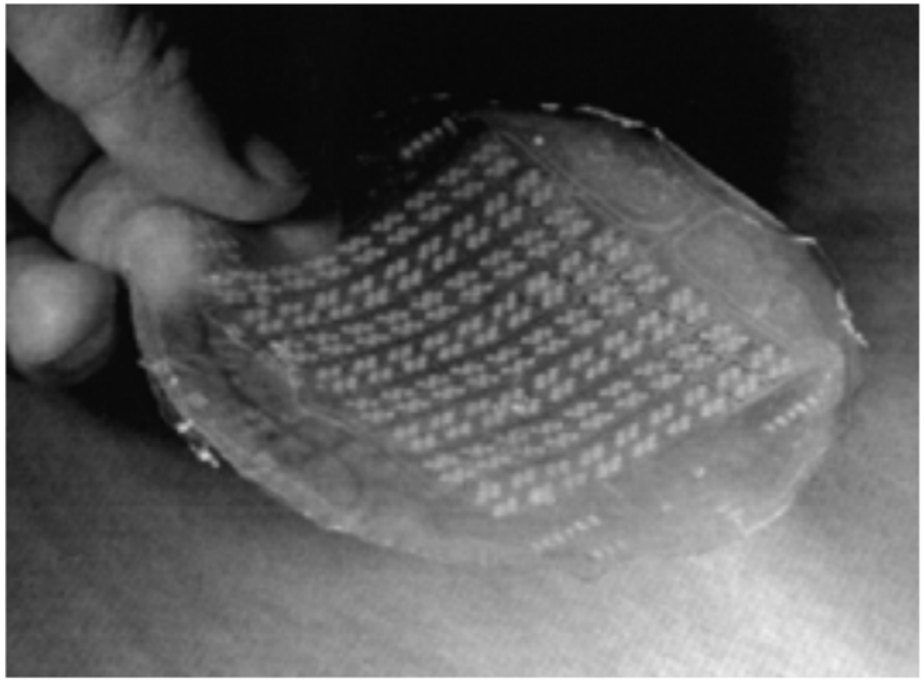
2. Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University Hospital.

3. Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology (MIT).

4. Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School

Cell-seeded microchannels

In Vitro Living Organ



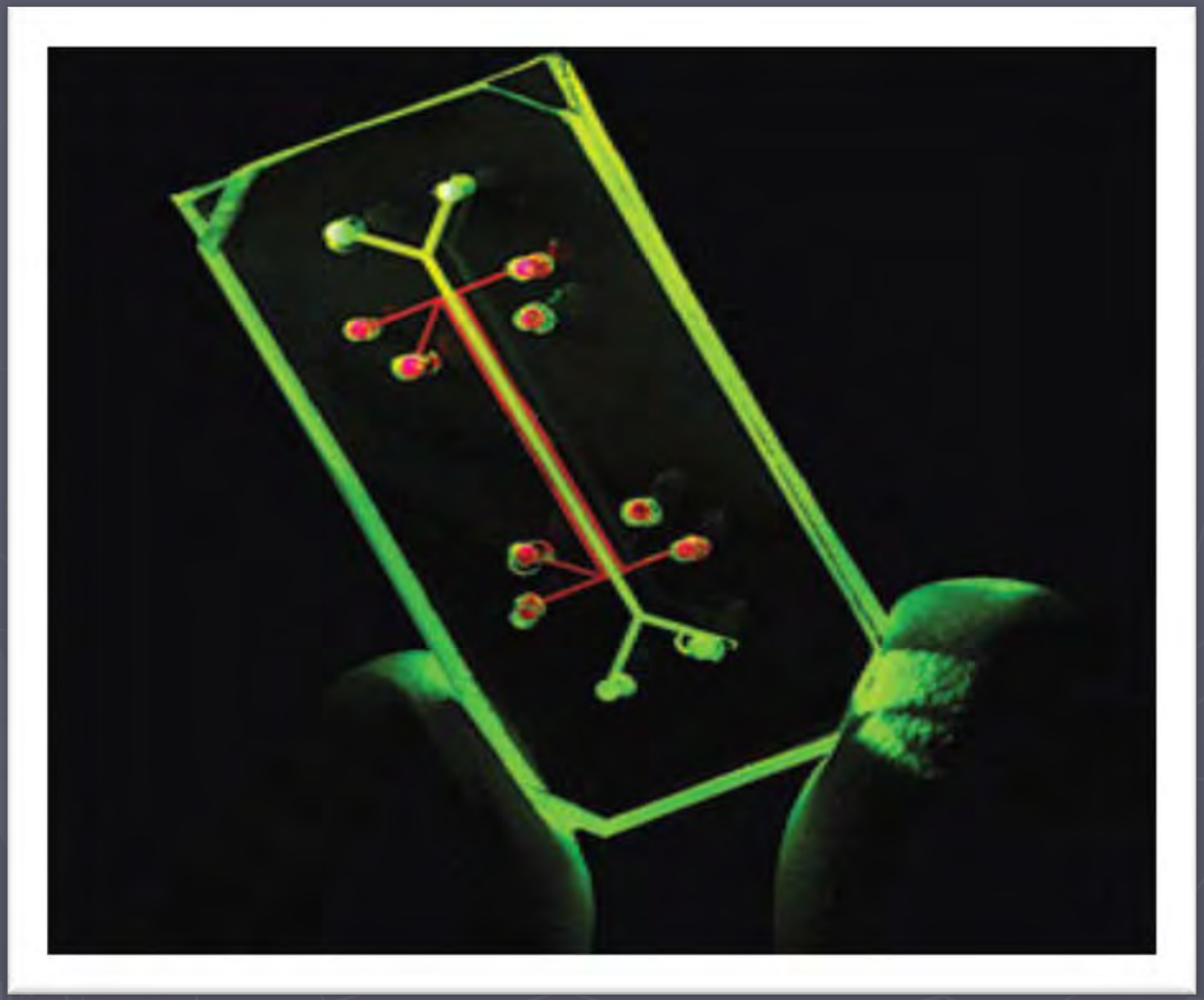
Silicon Chip

Tissue-engineered 3D in vitro culture systems applicable for:
Biological Labs, Hospitals, Research Centers, Tools and Assays

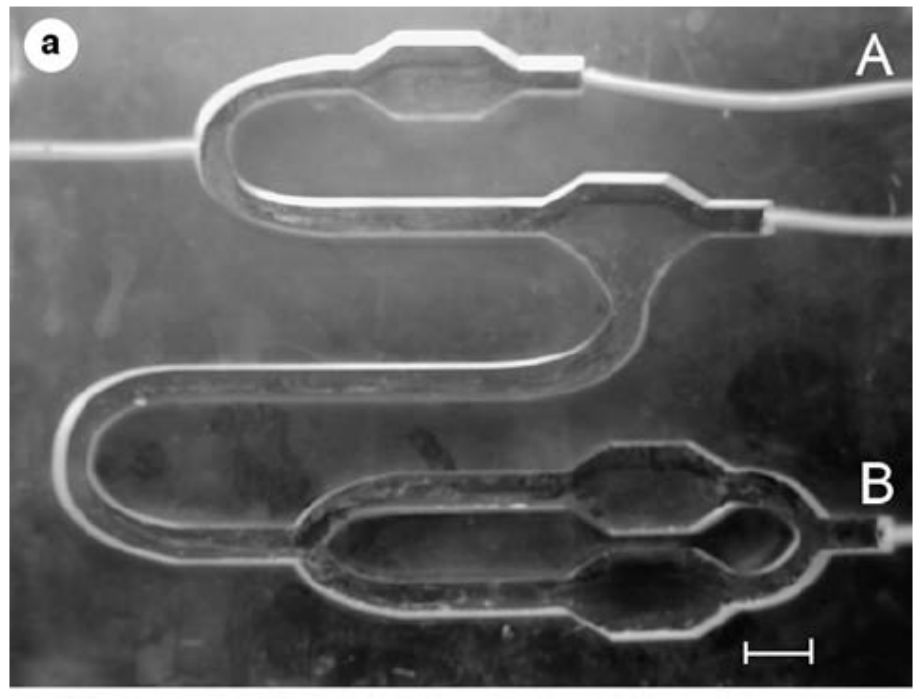
Regenerative Medicine, Drug discovery, Diagnostics

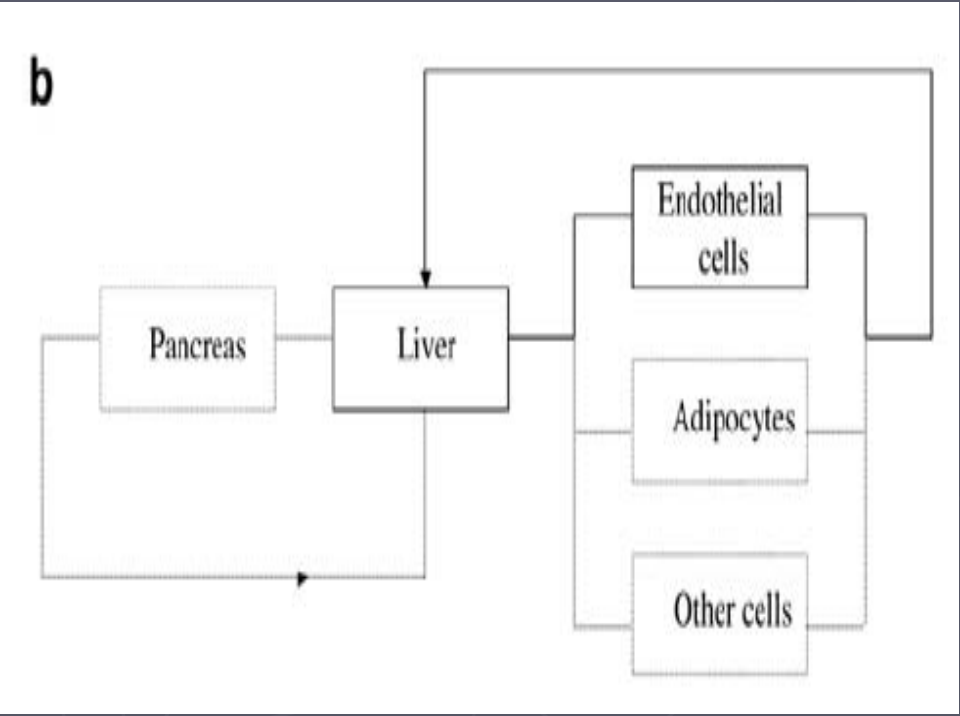
Scientific American» March 2011

Organs-in-Chip Technology



Bio-inspired Engineering

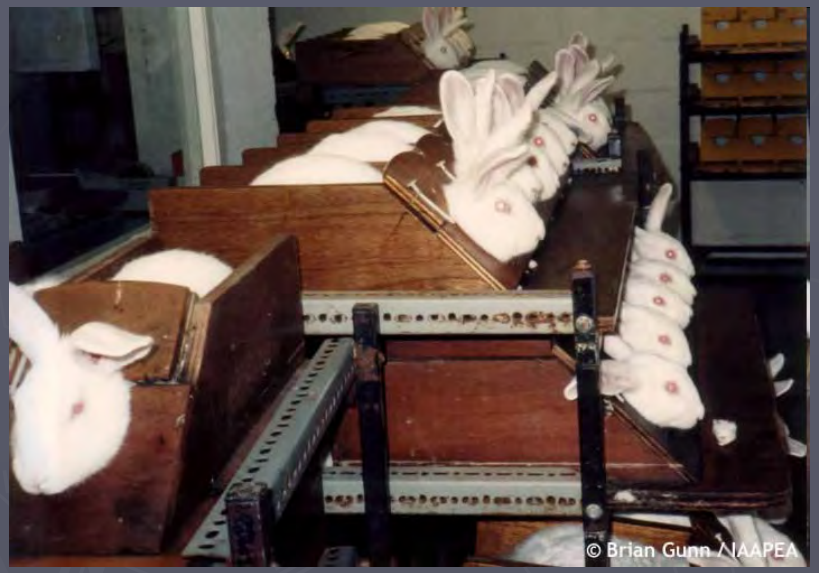

a
A
B


b
Endothelial cells
Pancreas
Liver
Adipocytes
Other cells

Pyrogen test

In vivo

Rabbit pyrogen test



In vitro

Limulus Amebocyte Lysate Test

Human whole blood test – In Vitro Pyrogen Test- Fever in the Test Tube

In vitro pyrogen test introduced into the European Pharmacopoeia. The test, devised by T. Hartung und A. Wendel, was developed at the University of Konstanz, Germany, and now helps to avoid the suffering of 200.000 rabbits per annum in Europe.

This test itself is available in different variants

- Human whole blood (IPT) (IL-1β) (University of Konstanz, D)
- Human cryopreserved blood (cryo-IPT) (IL-1β) (University of Konstanz, D)
- Human whole blood (IL-6) (NIBSC, London, UK),
- PBMC (IL-6) (Novartis, Basle, CH)
- Monomac6 (IL-6) (RIVM, Bilthoven, NL).

These tests have been validated by ECVAM and ICCVAM

MIMIC, or **modular immune *in vitro* construct**

- An artificial system imitating the human immune system
- Has applications in vaccine development
- White blood cells, specifically peripheral blood mononuclear cells including T cells and B cells, from human donors are placed in standard tubes containing specially designed tissue constructs made out of collagen, where they develop into small but functioning immune systems
- Up to ninety-six individual tubes can be carried, allowing scientists to use cells from almost a hundred different donors at any one time
- Replaces some steps in the vaccine development process that would otherwise be performed on animals and offers scientists better speed and flexibility than traditional methods.

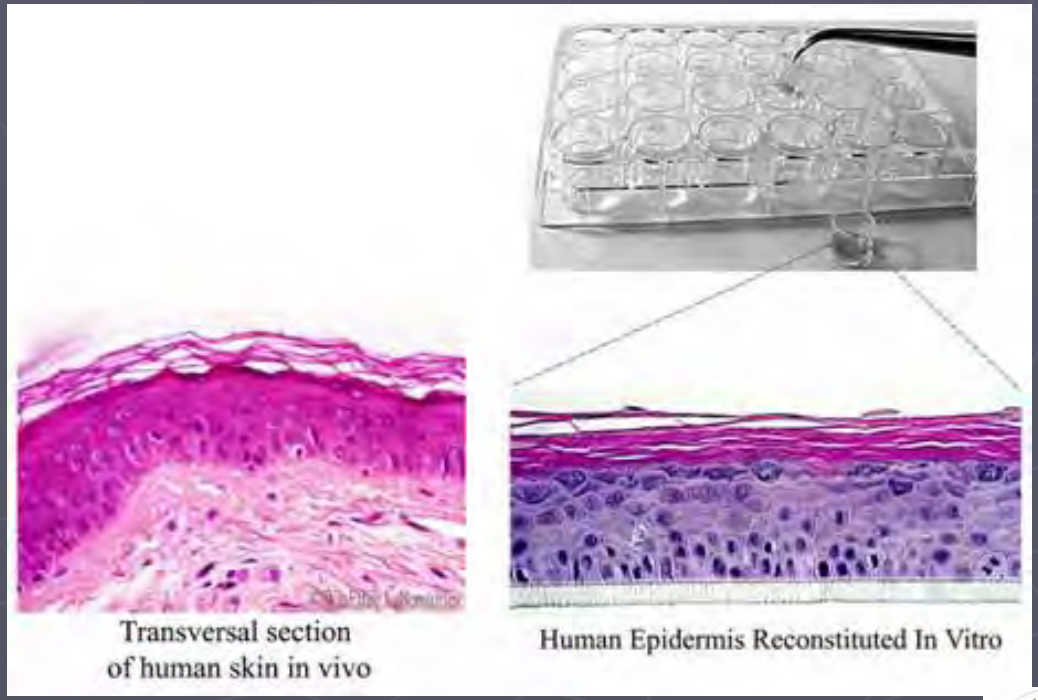
Cosmetics Testing: Consequences of 7th Amendment to the Cosmetics Directive (76/768/EEC)

Innovation Technology to Engineer 3D super intelligent diagnostic tools

Skin Corrosivity: Human Tissue Models (OECD 431)

Human Tissue Equivalents e.g., Episkin, SkinEthic RHE, MatTek Epiderm, Cellsystems EST-1000

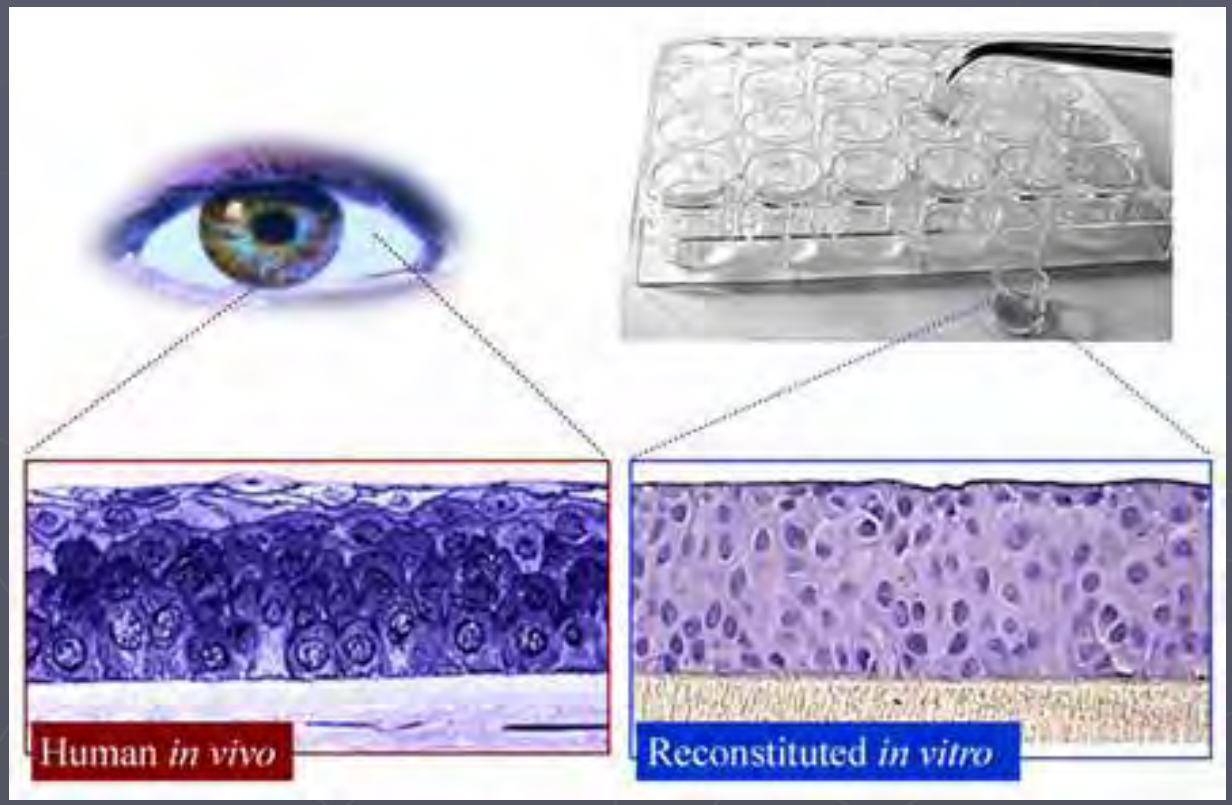
- Three dimensional tissue
- Cultured from normal, human epidermal keratinocytes
- Forms a highly differentiated tissue that closely resembles human epidermis



Eye Irritation: Human Tissue Models

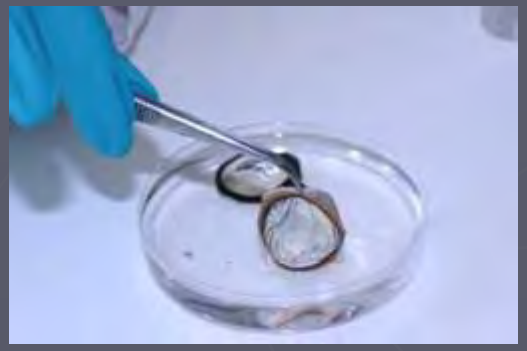
SkinEthic Human Reconstituted Corneal Epithelium (RHCE)

- Transformed human keratinocytes of the cell line HCE
- Forms a three dimensional corneal epithelium
- MEA approach



Other models are available e.g., MatTek Epiocular

Bovine Corneal Opacitiy & Permeability Test (BCOP)



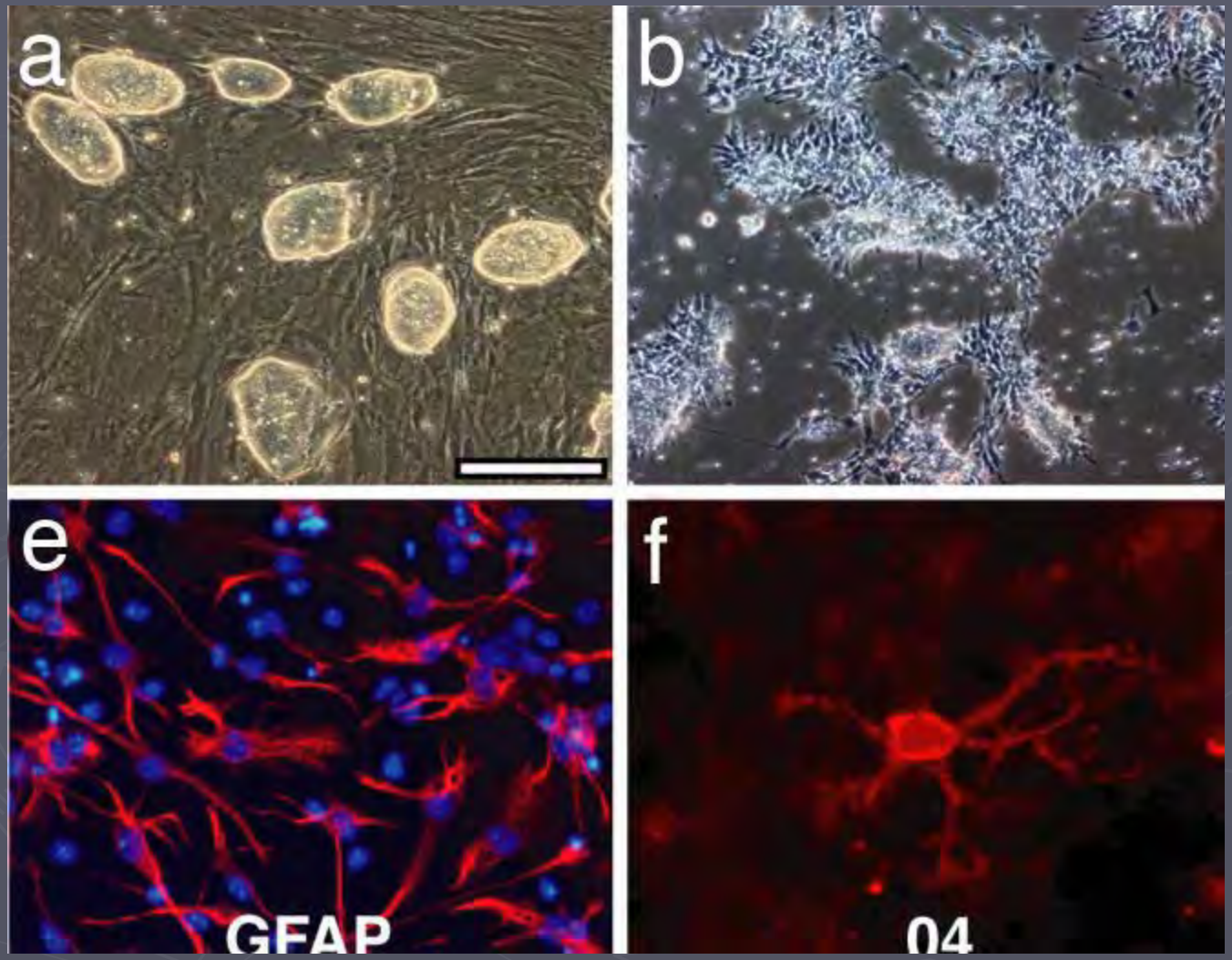
Bovine Cornea

Full thickness corneal model

In vitro Score

Endpoints: Corneal Opacity & Permeability

Wernig M et al. (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *PNAS* 105: 5856-5861.



Bio-informatics Tools

(Q)SAR

Physico-chemical properties

- Acid-base properties (pKa)
- Octanol-water partition coefficient (logPow)
- Effective logPow for ionisable substances (logD)
- Water solubility
- Lipid solubility

Pharmaco- / toxicokinetic properties (ADME)
Pharmacodynamic properties
Pharmacological properties
Envoironmental toxicological properties

ebTrack: an environmental bioinformatics system built upon ArrayTrack™ providing for toxicogenomics and interpretation of micro-array data

EbTrack is being developed as an integrated bioinformatics system for environmental research and analysis

Addresses the issues of integration, curation, management, first level analysis and interpretation of environmental and toxicological data from diverse sources.

The ToxCast Program for Prioritizing Toxicity Testing of Environmental Chemicals

The National Research Council (NRC) recently released a report titled *Toxicity Testing in the 21st Century: A Vision and a Strategy* that outlines a much more ambitious and long-term vision for developing novel *in vitro* approaches to chemical toxicity characterization and prediction (NRC 2007) that would largely eliminate animal testing.

Toxicity Testing in the 21st Century: A Vision and a Strategy

- **Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment.**

- **These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures.**

- **They could also reduce the need for animal testing by substituting more laboratory tests based on human cells.**

Systems Biology

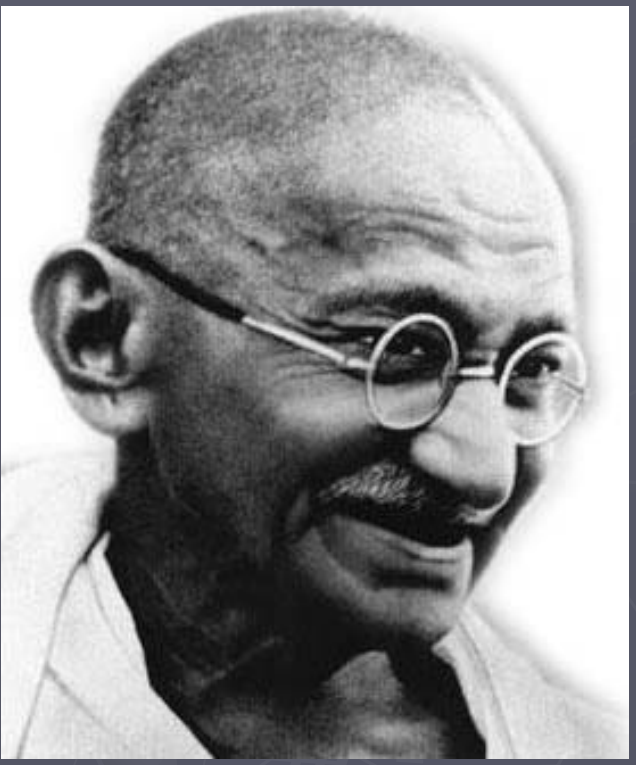
- Integration of data from all levels of complexity (genomics, proteomics, metabolomics, and other molecular mechanisms) "into a systems view of biological and pathological processes".
- The goal is to create overall computational models of the functioning of the cell, multicellular systems, and ultimately the organism.
- These *in silico* models will provide virtual test systems for evaluating the toxic responses of cells, tissues, and organisms.
- Compounds will be tested in simulation studies before being applied to cells and tissues to obtain comparative results and validation of the system.

OECD GUIDELINE FOR THE TESTING OF CHEMICALS
The Up-and-Down Procedure for Acute Oral Toxicity: Proposed Test Guideline

- It is used in the Series 870 Health Effects Test Guidelines for acute toxicity testing and by the Organization for Economic Co-operation and Development (OECD) member Nations.

- It replaces the traditional acute oral toxicity test formerly used to characterize industrial chemicals, pesticides, and their mixtures.

- This method maintains the performance of acute testing for applications that use the median lethal dose (classic LD50) test while achieving significant reductions in animal use.



- "The greatness of a nation and its moral progress can be judged by the way its animals are treated.“
- “I hold that the more helpless a creature, the more entitled it is to protection by man from the cruelty of man.”
- “I abhor vivisection with my whole soul. All the scientific discoveries stained with innocent blood I count as of no consequence”
- “I do feel that spiritual progress does demand at some stage that we should cease to kill our fellow creatures for the satisfaction of our bodily wants.”

- Mahatma Gandhi

Charles Darwin, who wrote to Ray Lankester in March 1871:
"You ask about my opinion on vivisection. I quite agree that it is justifiable for real investigations on physiology; but not for mere damnable and detestable curiosity. It is a subject which makes me sick with horror, so I will not say another word about it, else I shall not sleep to-night."

W. H. Stone. "One might think that with the advent of cell and molecular biology, animal experimentation will no longer be necessary. Quite the contrary, in the final analysis cells and molecules must be tested in living animals for such research to improve human health. Current thinking expresses the translation of laboratory research to the clinic as "*from bench to bedside".* We dare say that this should be changed to read, "*from bench to animals to bedside*".

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