

培養組織モデルの国際標準化の状況

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安全性予測評価部



本発表は、個人的な見解であり、必ずしも
OECDや国立衛研の公式見解ではありません。
また、発表に利益相反はありません。

本発表の内容

- 培養組織モデルの行政的受入れ
- 培養組織モデルの今後

背景・目的

動物実験の 3 Rs の普及、種差を越えた安全性評価を求める国際動向もあり、経済協力開発機構（OECD: Organisation for Economic Co-operation and Development）では、多くの培養組織モデル（再構築および摘出モデル）が試験法ガイドラインで採択されている。創薬開発においても、ヒト細胞を用いた多くの *in vitro* 試験の研究が進んでおり、世界的にも、*in silico* も含め益々、非動物実験による安全性および薬効評価は加速していく傾向にある。

培養組織モデルの行政的受入れ

APPLY INTEGRATED APPROACHES TO TESTING AND ASSESSMENT

All of the work on alternative methods is undertaken at the OECD with the objective of contributing to more integrated approaches to testing and assessment. In practice, integrated approaches, which take into account the tools outlined above, are used in the OECD Existing Chemicals Programme which generates internationally agreed initial hazard assessments of chemicals.

This practical application of integrated approaches improves their regulatory acceptance and facilitates their implementation into national and regional chemical assessment schemes in OECD member countries.

AVOID DUPLICATION OF TESTING

The OECD Mutual Acceptance of Data (MAD) framework has had a major impact on testing practices. MAD guarantees that data generated in the testing of chemicals in an OECD member country, or adhering non-member country, in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice shall be accepted in other member or adhering countries for purposes of chemical assessment and other uses relating to the protection of man and the environment. This proactive framework saves thousands of animals every year and its impact increases as non-OECD economies join the MAD system.

Furthermore, the OECD has developed the **Global Portal to Information on Chemical Substances** (eChemPortal). eChemPortal offers free public access to information on properties of chemicals through a simultaneous search of multiple databases, thereby improving the access to existing test results and reducing the risk of unnecessary testing.

WHERE CAN I FIND OECD TOOLS RELATED TO CHEMICAL SAFETY AND ANIMAL WELFARE ?

(Q)SARs, Grouping of Chemicals and the (Q)SAR Application Toolbox

www.oecd.org/env/existingchemicals/qsar

Test Guidelines, in vitro test methods, molecular screening and toxicogenomics

www.oecd.org/env/testguidelines

Integrated Approaches to Testing and Assessment

www.oecd.org/env/existingchemicals

Mutual Acceptance of Data

www.oecd.org/env/glp

Global Portal to Information on Chemical Substances

www.oecd.org/ehs/eChemPortal

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For more information contact
the OECD Secretariat at
ehscont@oecd.org

Chemical Safety and Animal Welfare



Progress made
at the OECD



www.oecd.org/env/ehs

OECD 試験法ガイドラインに採択されている非動物実験(2019)

Class	Test methods
Corrosion	<i>In vitro</i> Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) :TG430
	<i>In vitro</i> Skin Corrosion: Reconstructed Human Epidermis (RHE) test method :TG431
	CORROSITEX Skin Corrosivity Test :TG435
Skin irritation	<i>In vitro</i> Reconstructed Human Epidermis (RhE) Test methods, EpiDerm, EPI-SKIN, SkinEthic, LabCyte EPI-Model: TG439
Phototoxicity	3T3 NRU Phototoxicity Test :TG432
	ROS(Reactive Oxygen Species) Assay for Photoreactivity: TG495
Eye irritation	Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage: TG437
	Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage: TG438
	Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants : TG460
	Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage : TG491
	Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage : TG492
	Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage: TG494
Skin sensitisation	<i>In chemico</i> Skin Sensitisation: TG442C
	<i>In vitro</i> Skin Sensitisation: TG442D
	<i>In vitro</i> Skin Sensitisation: TG442E
Endocrine disrupter screening	Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists : TG455
	H295R Steroidogenesis Assay :TG456
	Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals: TG458
	Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity : TG493
Genotoxicity	Bacterial Reverse Mutation Test : TG471
	<i>In vitro</i> Mammalian Chromosome Aberration Test : TG473
	<i>In Vitro</i> Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes : TG476
	<i>In vitro</i> Micronucleus Test : TG487
	<i>In Vitro</i> Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene : TG490
Skin absorption	Skin Absorption: <i>In vitro</i> Method :TG428

腐食性および皮膚刺激性評価のための培養組織モデル

Class	Test methods
皮膚腐食性	<i>In vitro</i> Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) :TG430
	<i>In vitro</i> Skin Corrosion: Reconstructed Human Epidermis (RHE) test method :TG431
皮膚刺激性	<i>In vitro</i> Reconstructed Human Epidermis (RhE) Test methods, EpiDerm, EPISKIN, SkinEthic, LabCyte EPI-Model: TG439



Section 4 Health effects

Test Guideline No. 431

In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method

Table 2. Acceptability ranges for negative control OD values to control batch quality

	Lower acceptance limit	Upper acceptance limit
EpiSkin™ (SM)	= 0.6	= 1.5
EpiDerm™ SCT (EPI-200)	= 0.8	= 2.8
SkinEthic™ RHE	= 0.8	= 3.0
epiCS	= 0.8	= 2.8
LabCyte EPI-MODEL24 SCT	= 0.7	= 2.5

18 June 2019



Section 4
Health effects

Test Guideline No. 439

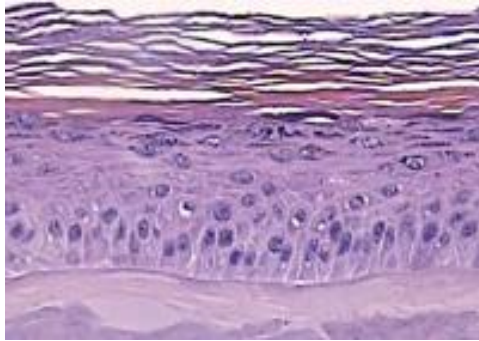
In Vitro Skin Irritation: Reconstructed
Human Epidermis Test Method

Table 2. Acceptability ranges for negative control OD values in the MTT assay of the test methods included in this TG

	Lower acceptance limit	Upper acceptance limit
EpiSkin™ (SM)	≥ 0.6	≤ 1.5
EpiDerm™ SIT (EPI-200)	≥ 0.8	≤ 2.8
SkinEthic™ RHE	≥ 0.8	≤ 3.0
LabCyte EPI-MODEL24 SIT	≥ 0.7	≤ 2.5
epiCS®	≥ 0.8	≤ 2.8
Skin+®	≥ 0.8	≤ 2.5

18 June 2019

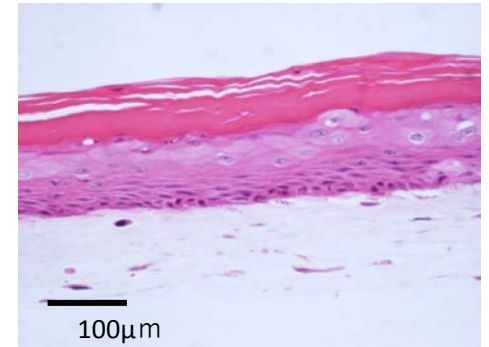
培養表皮モデルの例



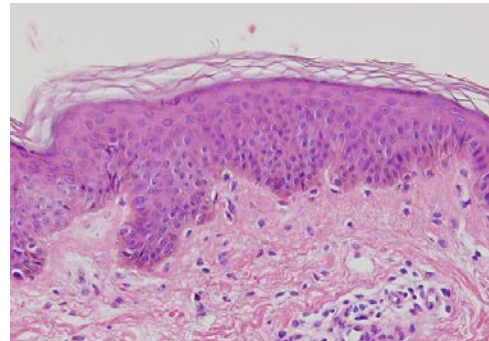
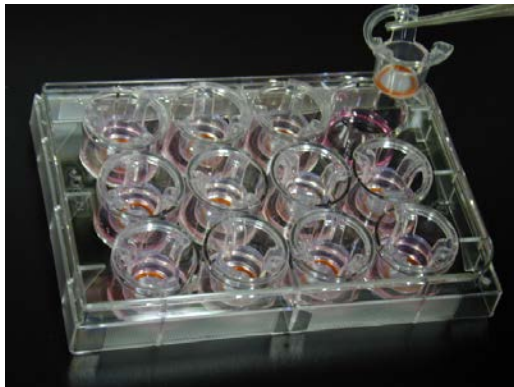
EpiSkin



EpiDerm(EPI-100)



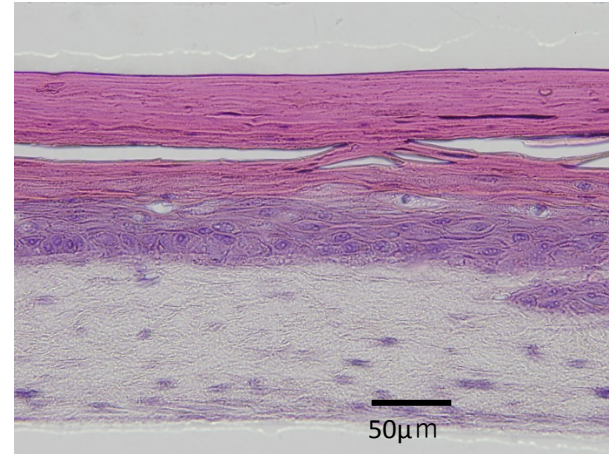
LabCyte



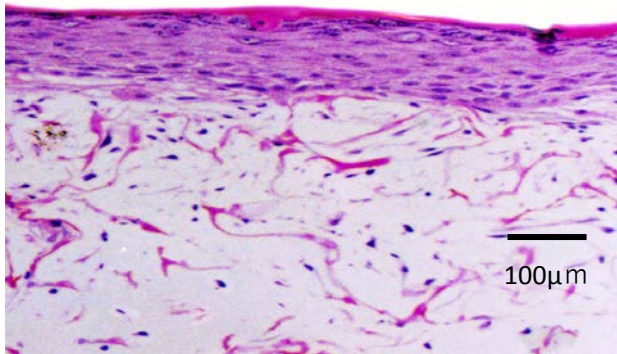
Human skin



Skin²



TESTSKIN



Vitrolife-Skin

培養皮膚モデルの例

日本で進行中の培養組織モデルを用いた 試験法のバリデーション研究

Dermo–Epidermal Skin
Equivalents (**LbL-3D Skin**)
for skin irritation testing

実験終了

EpiSensA for skin
sensitization testing

Phase IA終了

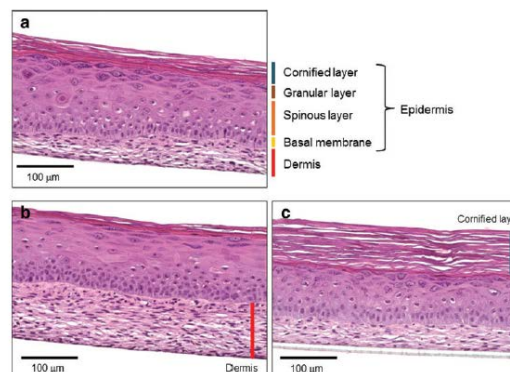
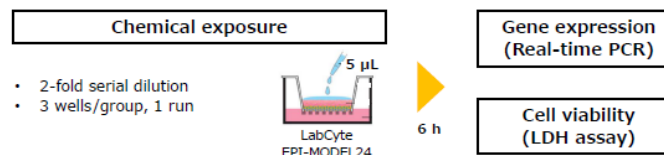


FIG. 2. HE-stained images of DESEs consisting of (a, c) NHDF 1×10^6 cells+KC 6×10^5 cells and (b) NHDF 2×10^5 cells+KC 6×10^5 cells after differentiation for (a, b) 7 days and (c) 14 days of airlift culture. Color images available online at www.liebertpub.com/tea

EpiSensA (Epidermal Sensitization Assay)

Saito et al., Toxicol. In Vitro 2017

- Vehicle : Acetone:Olive oil = 4:1(AOO), Distilled water(DW), 50%EtOH
 - selection criteria : a chemical is soluble at highest concentration
 - selection priority : AOO $\Rightarrow$ DW $\Rightarrow$ 50%EtOH
- Max. applied concentrations. : Solid; 50(w/v)%, liquid; neat
- Min. applied concentration : 0.02%

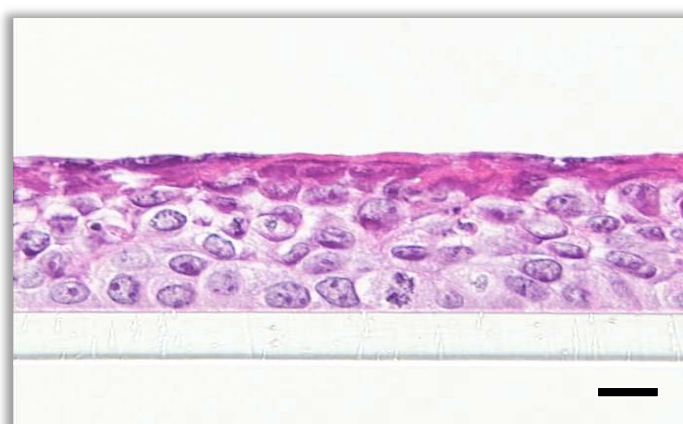
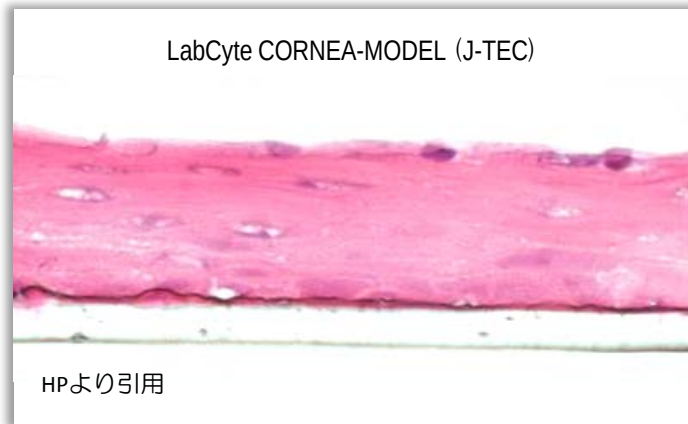
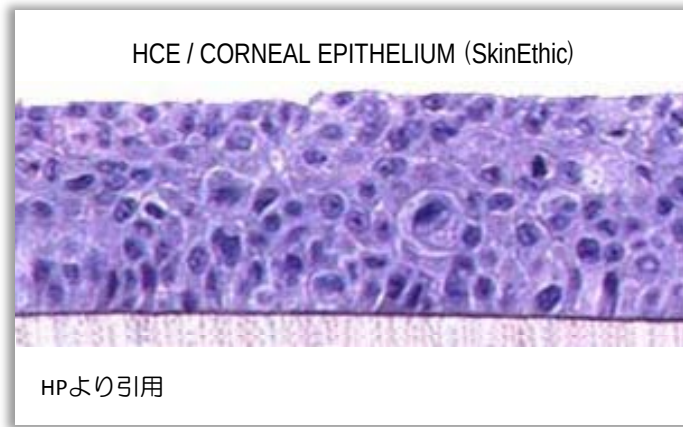
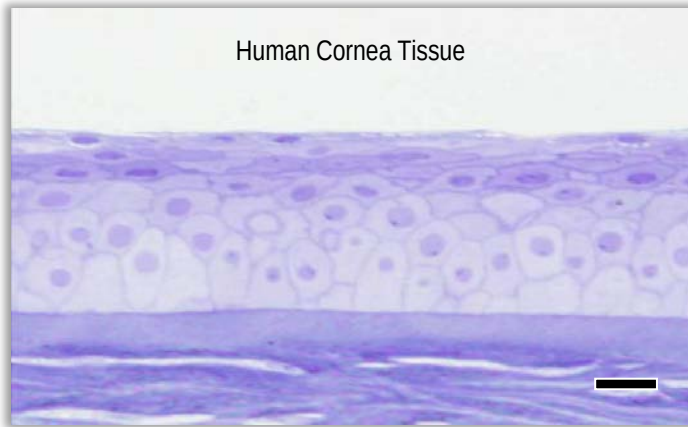


いずれもOECDでの標準化を目指す

眼刺激性評価のための培養組織モデル

眼刺激性試験	Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage: TG437
	Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage: TG438
	Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage : TG492
	Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage: TG494

培養角膜モデルの例



bar = 20 μ m



Section 4 Health effects

Test Guideline No. 492

Reconstructed human Cornea-like
Epithelium (RhCE) test method for
identifying chemicals not requiring
classification and labelling for eye
irritation or serious eye damage

18 June 2019

Table 2. Acceptability ranges for negative control OD values (for the test method users)

TestMethod	Loweracceptancelimit	Upperacceptancelimit
EpiOcular™EIT(OCL-200)– VRM1 (forboththeliquidsandthesolidsprotocols)	>0.8 ¹	<2.8
SkinEthic™HCE EIT(HCE/S)– VRM2 (forboththeliquidsandthesolidsprotocols)	> 1.0	≈2.5
LabCyteCORNEA-MODEL24EIT (forboththeliquidsandthesolidsprotocols)	≈0.5	≈1.6
MCTT HCE™EIT (forboththeliquidsandthesolidsprotocols)	≈1.6	≈3.0

¹This acceptance limit considers the possibility of extended shipping/storage time (e.g. > 4 days), which has been shown not to impact on the performance of the test method (44).



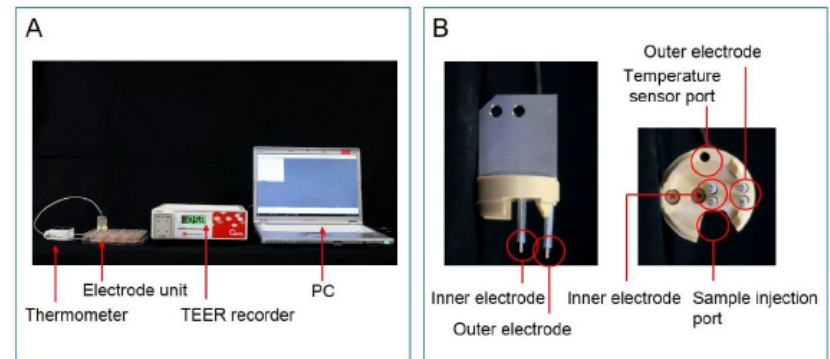
Section 4
Health effects

Test Guideline No. 494

Vitrigel-Eye Irritancy Test Method for
Identifying Chemicals not requiring
Classification and Labelling for Eye
Irritation or Serious Eye Damage

18 June 2019

ANNEX 3 - PHOTOGRAPHIC IMAGES OF THE TEER MEASURING SYSTEM



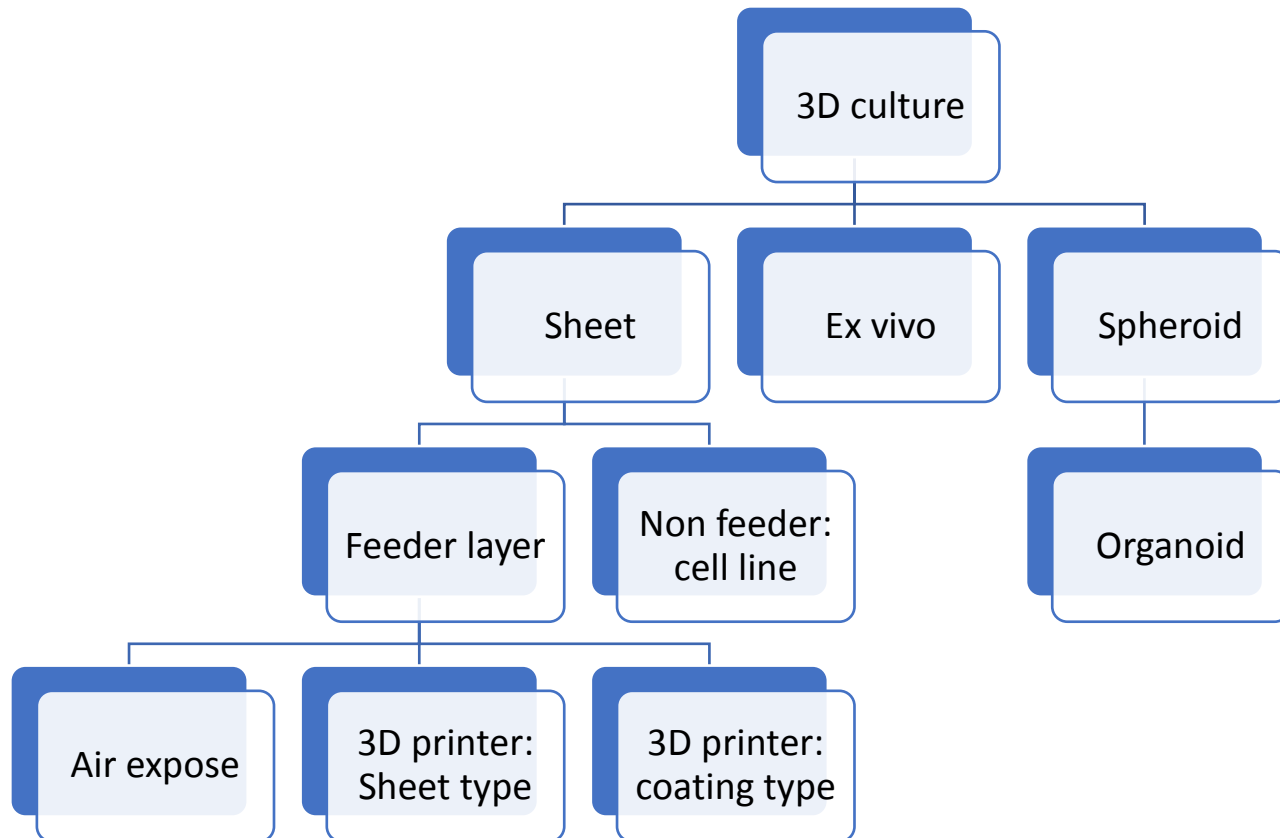
The TEER measuring system (A) and the electrode unit (B).

培養組織モデルを用いた試験法として、OECD では
皮膚腐食性試験 (OECD Test No. 431),
皮膚刺激性試験 (OECD Test No. 439),
眼刺激性試験 (OECD Test No. 492)などが採択されている。

欧米以外の国々も表皮モデルや角膜モデルの開発に興味
を持っており、TGの中に採択されるモデルは増えていく
と予想している。

培養組織モデルの今後

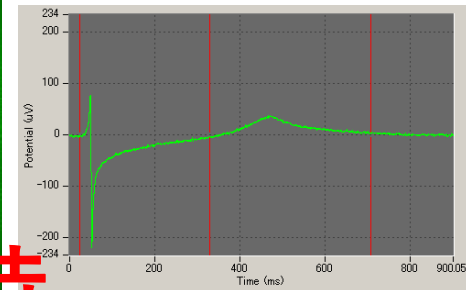
培養組織モデルの分類



Pharmacological and toxicological tests using human iPS cells

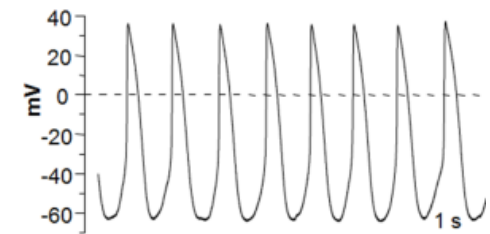
Cardiomyocytes

- Action potential recording from single cells
- Field potential recording



LbL-3D Heartに改善を期待

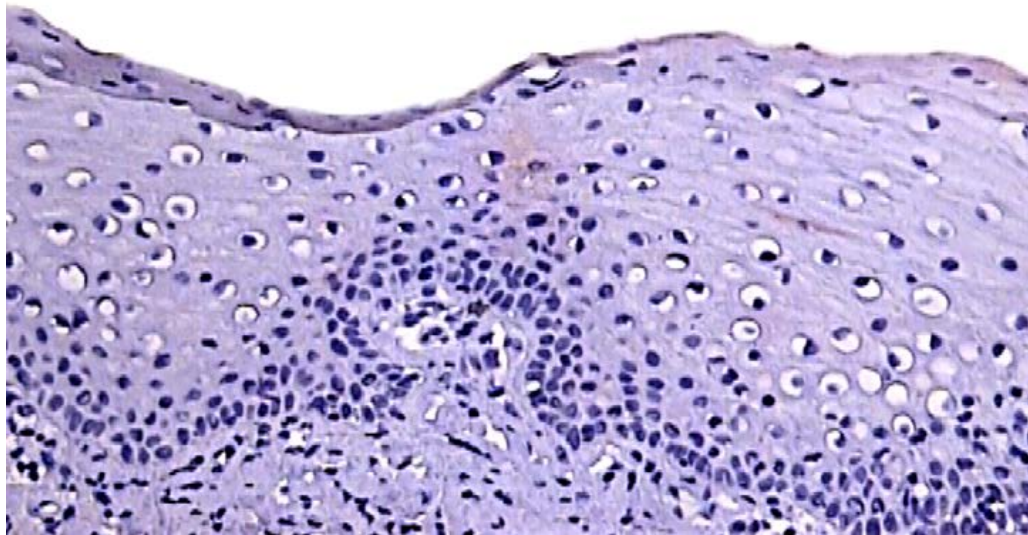
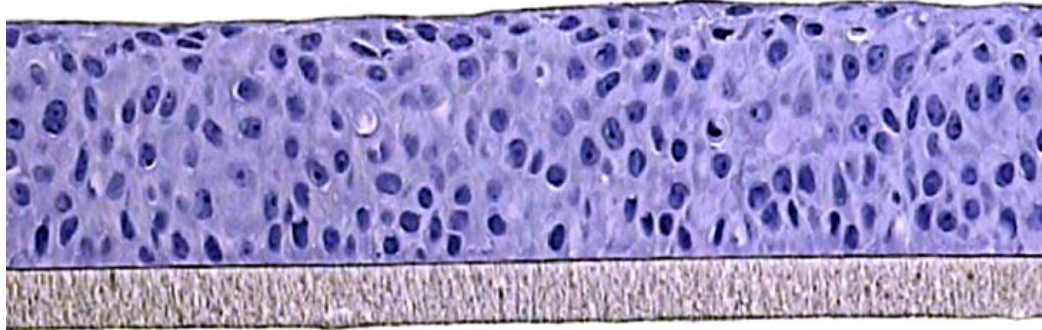
	CredibleMeds		
	Known risk of TdP	Other Categories	
High risk	8	1	9
Low risk	2	8	10
	10	9	19
	Sensitivity 80%		
	Specificity 88.9%		
	Accuracy 100%		



Shared by Y. Sekino (Tokyo Univ.)

Journal of Pharmacological Sciences 136 (2018) 249–256

EpiOral

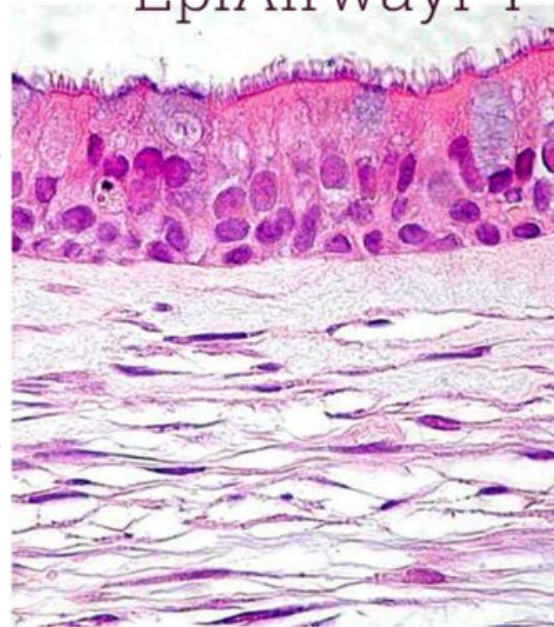


Human

EpiAirwayFT

Mucociliary Epithelium -

Stromal Fibroblasts -

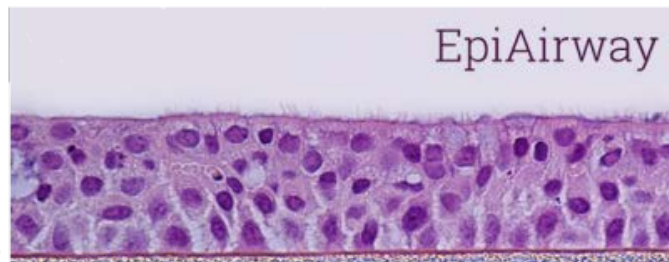


EpiAirway

Ciliated Apical Surface -

Mucociliary Epithelium -

Microporous Membrane -



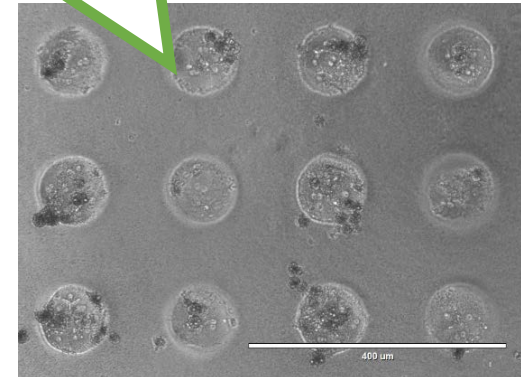
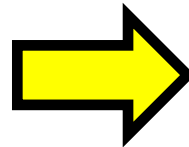
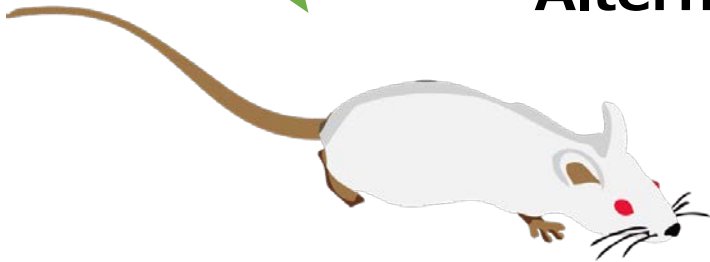
肝スフェロイド培養

Rat
LD₅₀ (Oral)

IVIVE

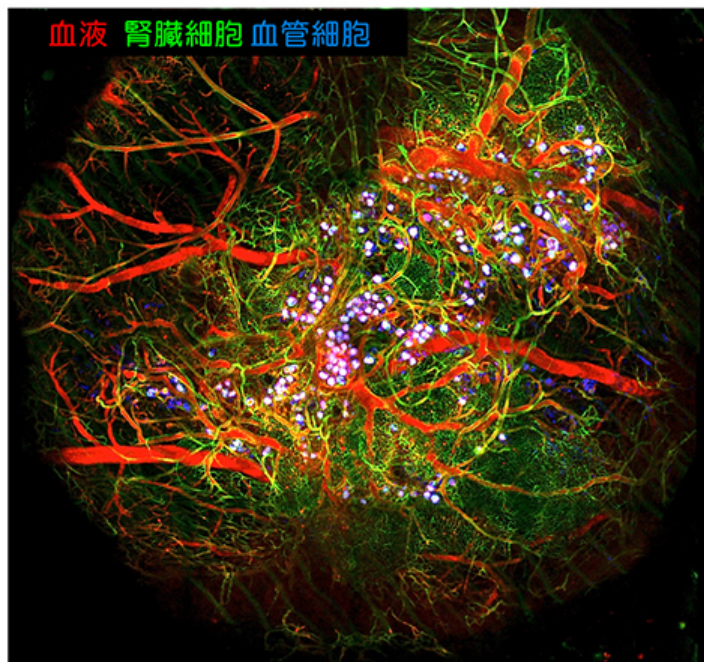
Primary human hepatocyte
spheroids

Alternative Method

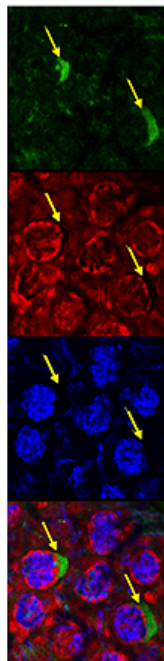


オルガノイド

多数の腎組織（糸球体）の形成



尿産生（黄矢印）



NCATS-Supported Scientists Model Aging-Related Conditions in Space to Improve Human Health on Earth



- Lung and bone marrow chips:
- Kidney chips:
- Chips modeling the blood-brain barrier
- Bone and cartilage chips



Organ-on-chip Consortium

Since 2015

Expertise

Stem cell technology

- iPSC
- Adult stem cells (organoids)
- Tissue slices

Read-out technology

Microfluidics

Microelectronics

Microfabrication

Polymer chemistry

Computational modeling

Metabolism

Food toxicology

Drug development expertise

Validation/valorization

Focus

- Human organ and

ORCHID (ORgan-on-C

H2020 Future and Emerging Technologies

CSA Coordination and support action



EUROOCS

EUROPEAN ORGAN-ON-CHIP SOCIETY CONFERENCE 2019

Graz, Austria, 2-3 July 2019

eurooc2019.eu

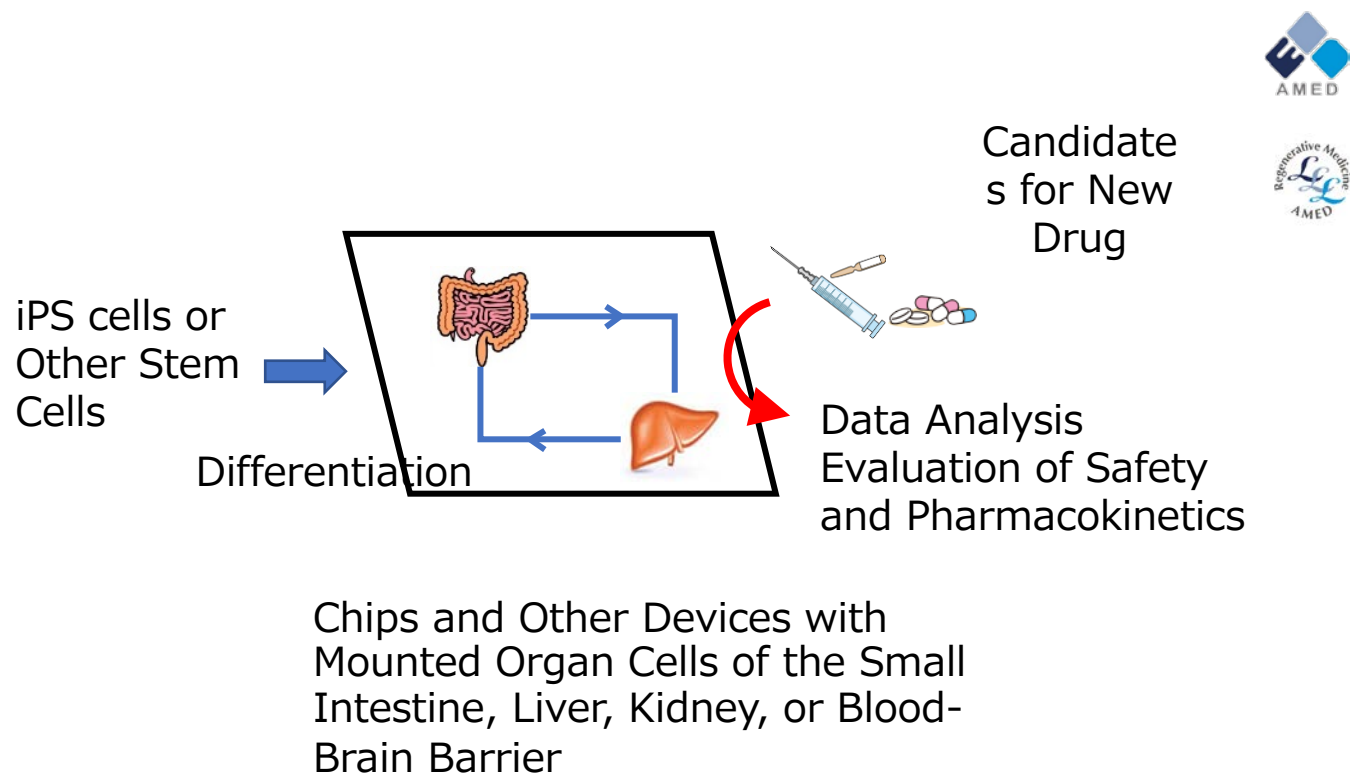
organ-on-chip technology

- Build a network of academia, research institutes, industry, regulatory bodies, patient organizations etc.
- Raise awareness by dissemination and communication



Universidad
Zaragoza

再生医療技術を利用した創薬開発プロジェクト



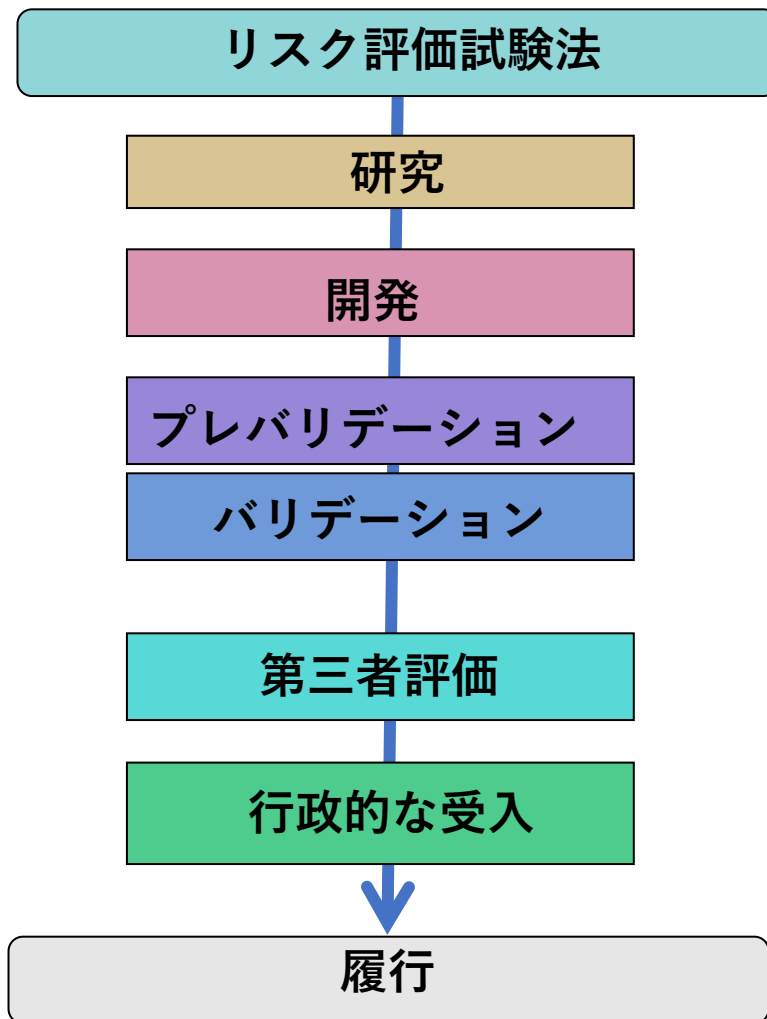
大阪大学より、松崎先生や高山先生が参画

培養組織モデルは、表皮や角膜中心から、新しい世代モデルの開発に進みつつある。

また、単一臓器モデルだけでなく、organ-on-a-chipの開発競争に欧米がしのぎを削っており、日中も参画できる機会を伺っている。

これらのモデルを用いた試験法の、行政的な受け入れ議論が世界的にも進むと予測される。

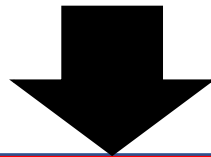
OECDの定める試験法開発の手順



OECD GD34 より

バリデーションで明確にすべき事項

- 試験法の科学的妥当性を明確にする。
- 試験法の再現性（施設内・施設間）を確認する。
- 試験法の予測性を明確にする。
- 試験法の適用限界を明確にする。



プロトコル、SOPを開発する。

LbL技術を用いて創薬開発において実用性の高いモデルが開発され、大阪大学ベンチャーとして市場を確保できるビジネスプランを期待したい。

ただし、皮膚および角膜モデルの市場は飽和しつつある。あくまでも、LbL-3D Skinの標準化は目的でなく、過程である。この標準化の経験を活かし、国際市場で勝負できる斬新なLbLモデルおよびそのモデルを用いた試験法の開発を期待している。



About JaCVAM



Update on JaCVAM



Academic activities



Submission of Alternative
Methods to JaCVAM



International Cooperation

御静聴ありがとうございました

Policy and Mission: JaCVAM's policy and mission is to promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and establish guidelines for new alternative experimental methods through international collaboration.

the 3Rs in animal experiments—Reduction (of animal use)

Refinement (to lessen pain or distress and to enhance animal well-being)

Replacement (of an animal test with one that uses non-animal systems or phylo-genetically lower species)
(OECD GD34)

News

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Contents

☞About JaCVAM

Message from JaCVAM / Policy and Mission of JaCVAM /

Organization of JaCVAM / Glossary /

Proposal for Engagement Rules

☞JaCVAM Activities