

三次元生体組織構築への期待と課題

国立医薬品食品衛生研究所 安全性
生物試験研究センター薬理部新規試
験法評価室
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11 March 2013 Last updated at 17:23 GMT



EU bans sale of all animal-tested cosmetics

A complete ban on the sale of cosmetics developed through animal testing has taken effect in the EU.

The ban applies to all new cosmetics and their ingredients sold in the EU, regardless of where in the world testing on animals was carried out.

The 27 EU countries have had a ban on such tests in place since 2009. But the EU Commission is now asking the EU's trading partners to do the same.

Animal rights lobbyists said EU officials had "listened to the people".

The [anti-vivisection group BUAV](#) and the [European Coalition to End](#)



The search for alternatives to animal testing goes on

Related Stories

[UK retains strict animal test law](#)

Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals

Harry Olson,¹ Graham Betton,² Denise Robinson,³ Karluss Thomas,³ Alastair Monro,¹ Gerald Kolaja,⁴
Patrick Lilly,⁵ James Sanders,⁶ Glenn Sipes,⁷ William Bracken,⁸ Michael Dorato,⁹ Koen Van Deun,¹⁰
Peter Smith,¹¹ Bruce Berger,¹² and Allen Heller¹³

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Received January 22, 2000

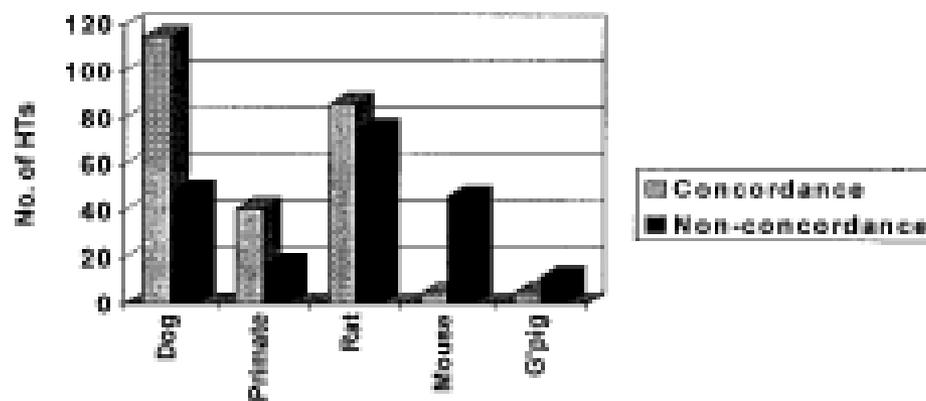
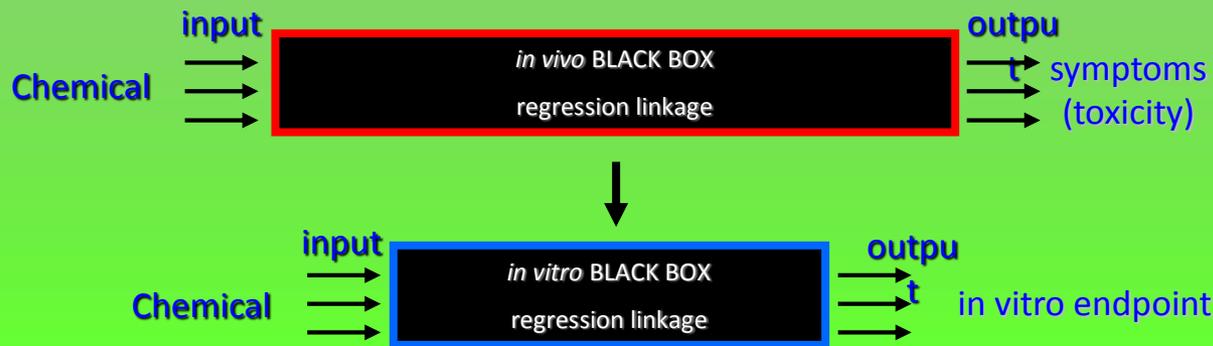


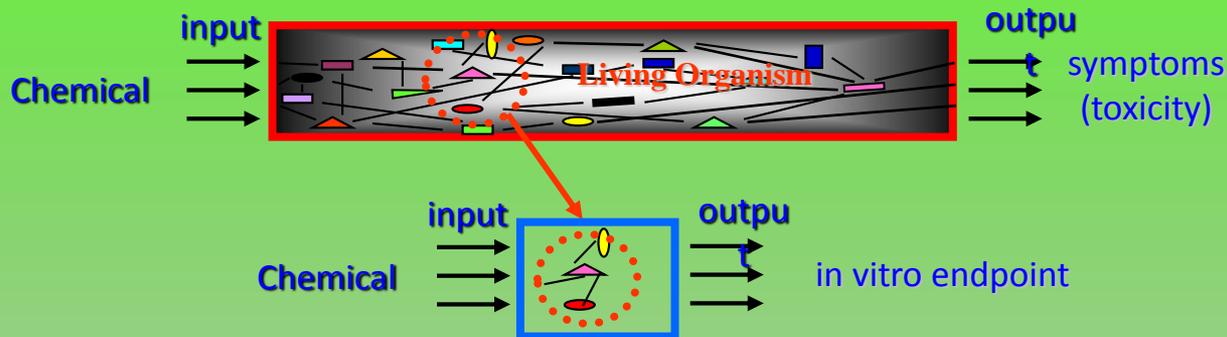
FIG. 4. Concordance rates versus species.

Animal model and *in vitro*

(1) Miniature Black Box Approach



(2) *In vivo* mechanism Excision Approach

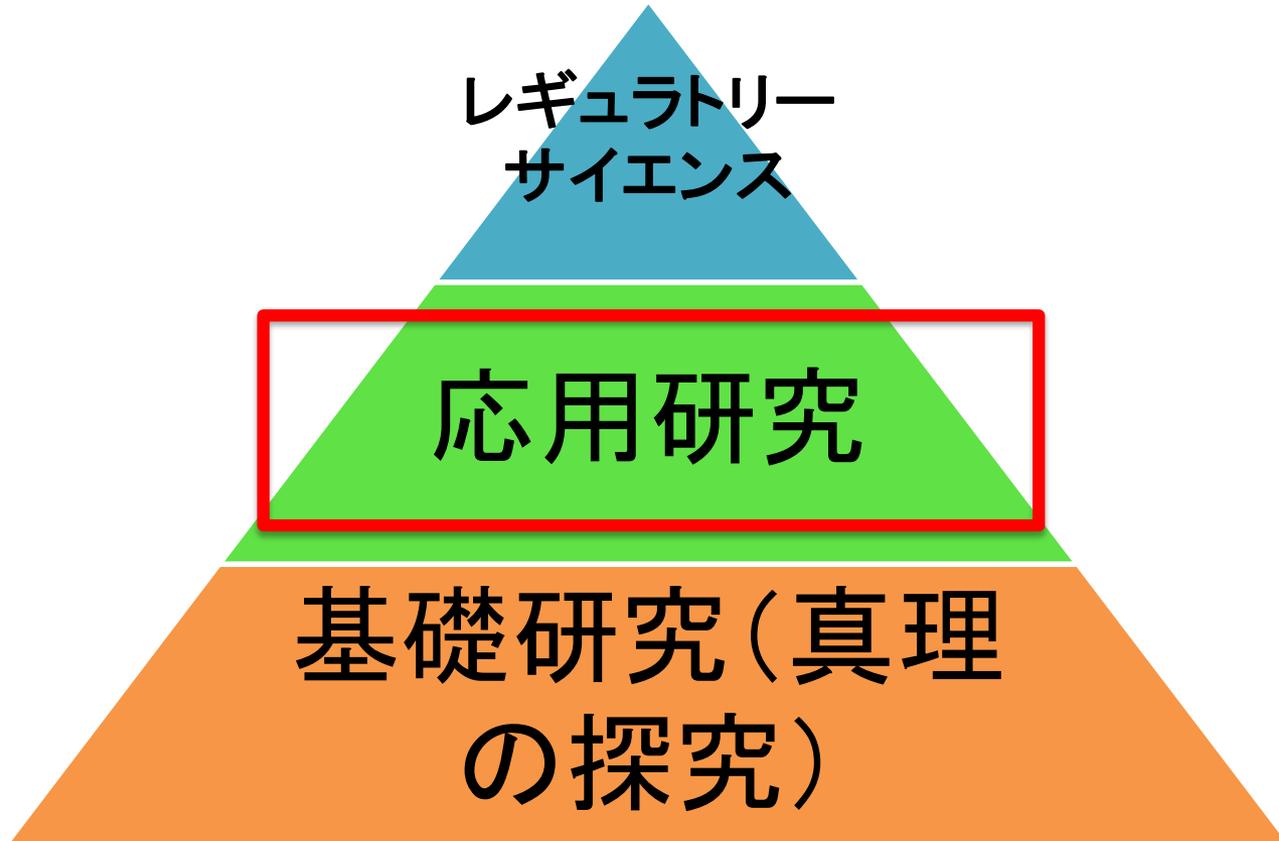


- (1) Miniature BlackBox Approach requires “diagnostic” process similar to *in vivo* studies. Until cellular symptoms are well understood to the level of *in vivo* diagnosis, the validation process will be virtually endless.
- (2) Mechanism-excision type methods always have Positive controls and Negative controls. With which the validation process is easy and concise.

内容

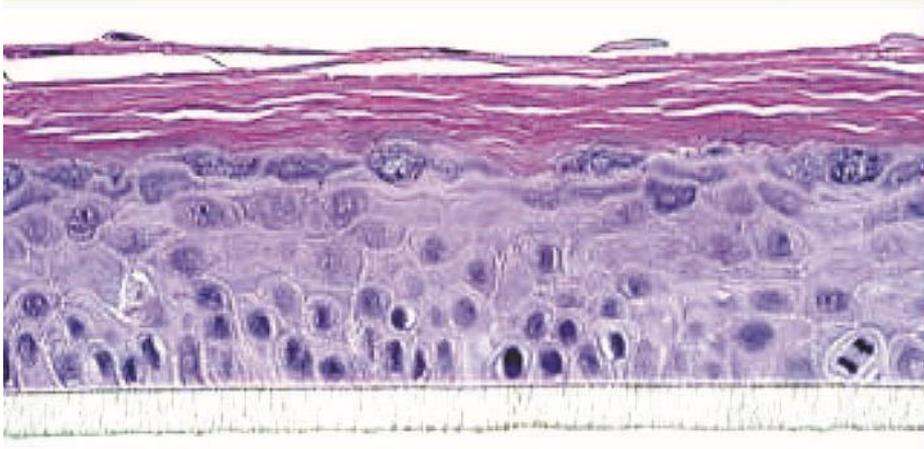
- 市販されている3次元培養モデルの種類
- 三次元生体組織を用いたテストガイドライン
- 標準化の手順
- 今後への提言

試験法開発の背景

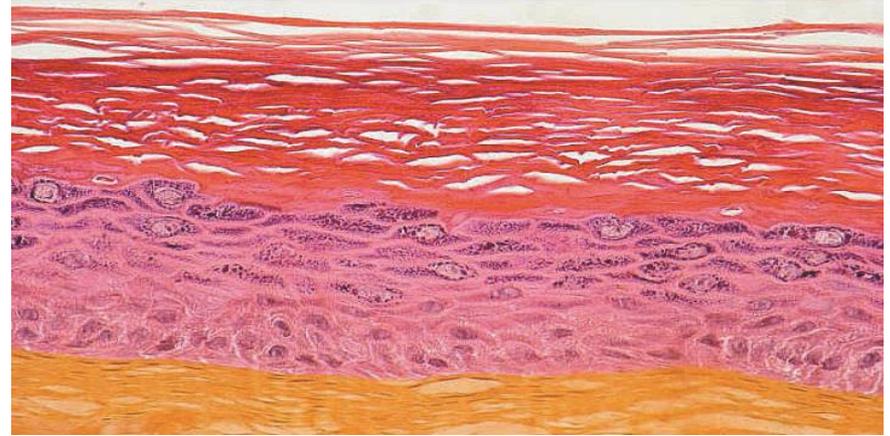


市販されている3次元培養モデルの 種類

再生ヒト表皮モデル



SKinEthics



EPISKIN

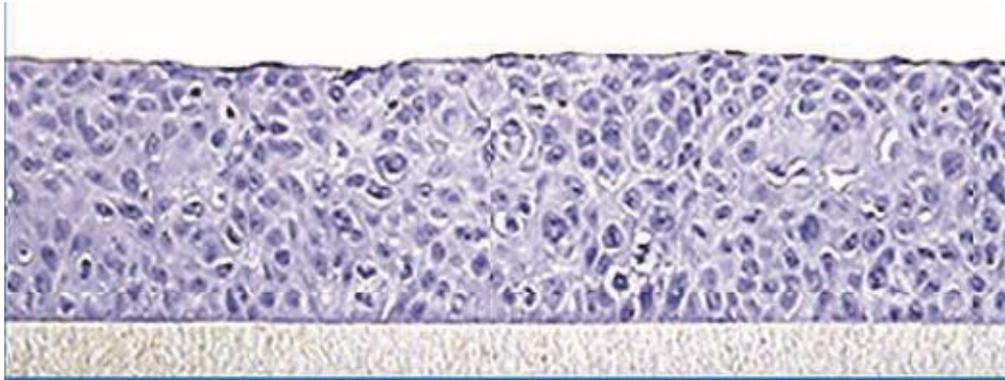


in vivo

利用方法：
皮膚腐食性試験、
皮膚刺激性試験、
光毒性試験、
経皮吸収試験および代謝など

ニコダーム提供

再生ヒト角膜上皮モデル



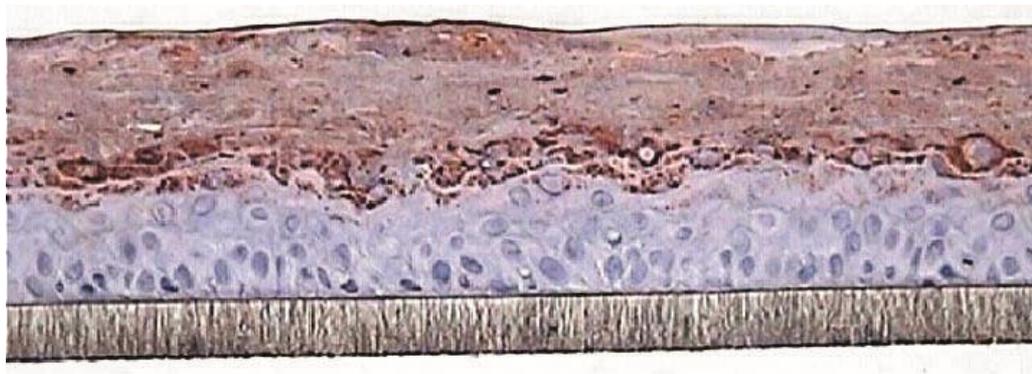
SkinEthic™ HCE



in vivo

利用方法：
眼性試験、角膜透過性試験および代謝など

再生ヒト角膜上皮モデルの免疫染色像

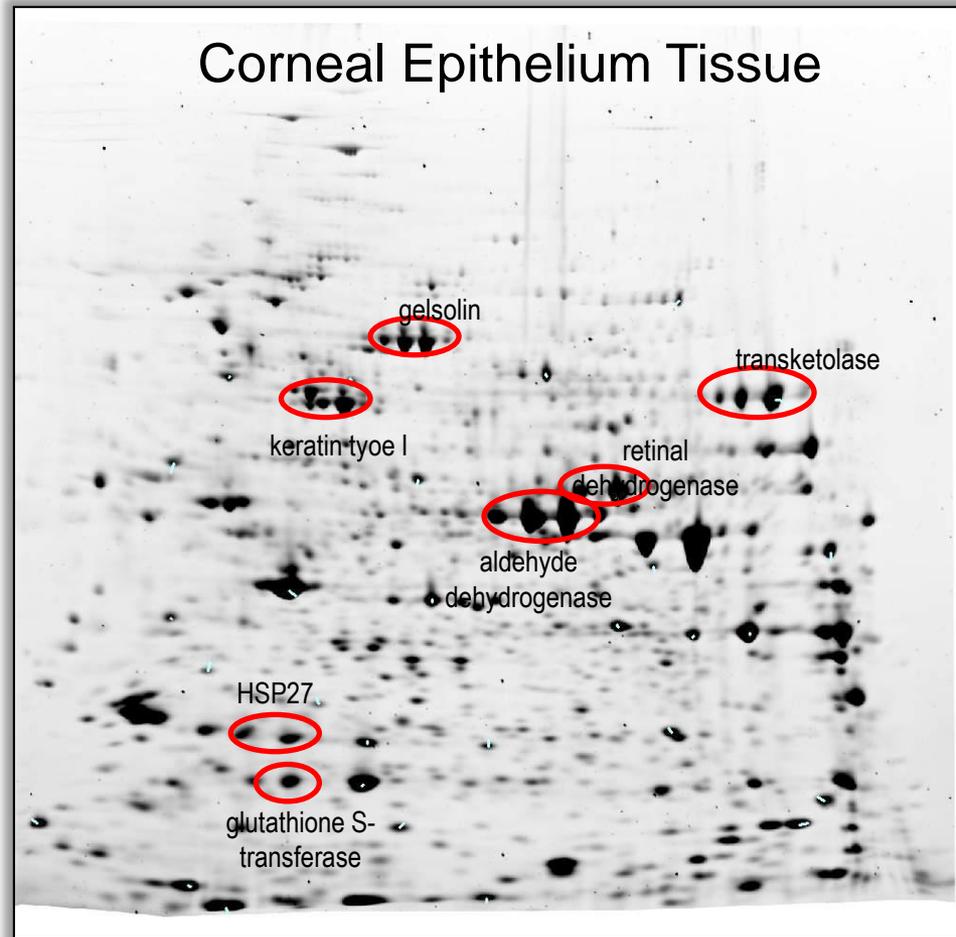
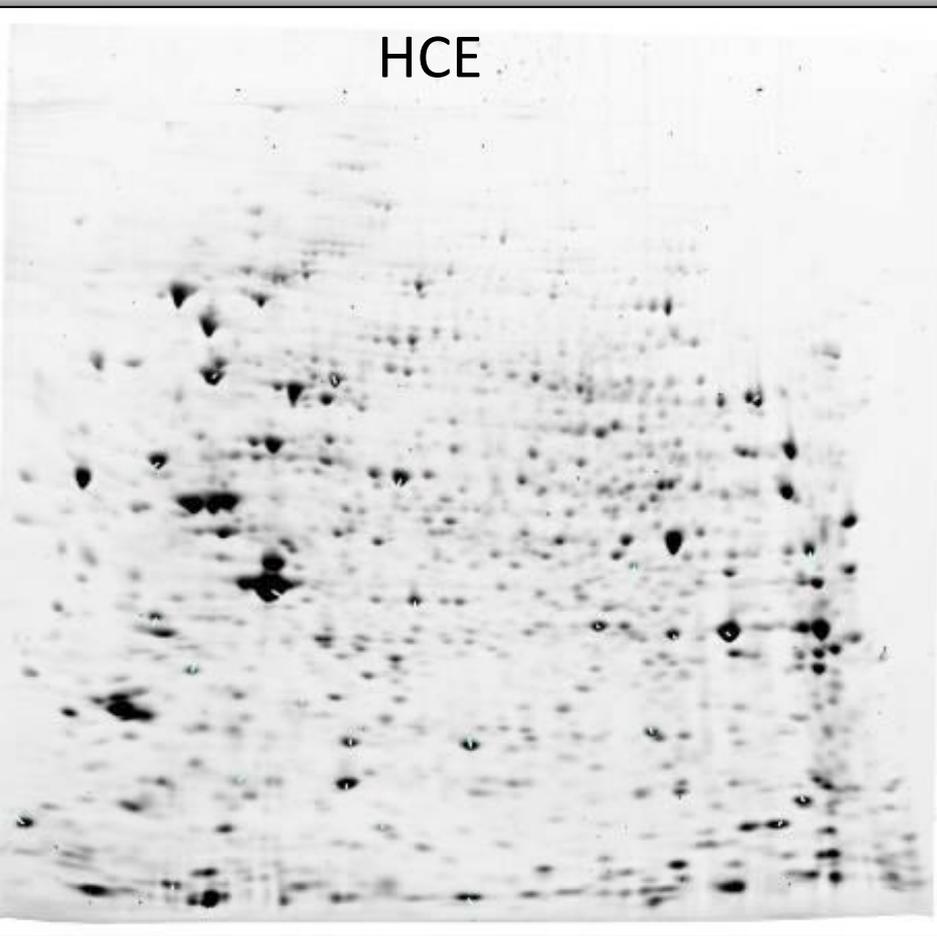


Filaggrin



Keratin 10

二次元と三次元培養での発現物の比較



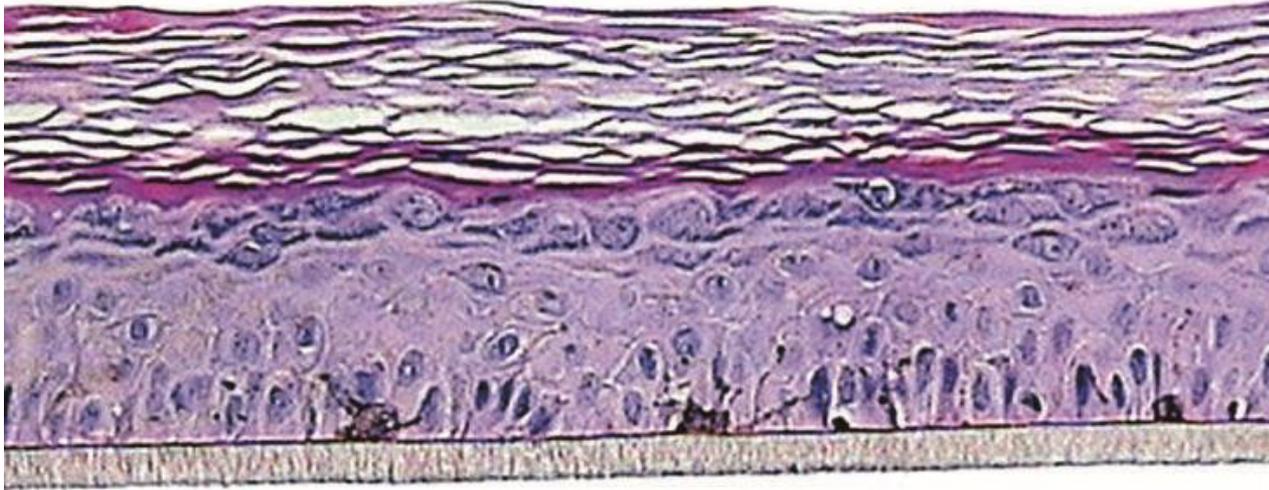
メラノサイト入り再生ヒト表皮モデル



利用方法:メラニン生成抑制効果の検証、黒化作用機構の研究

ニコダーム提供

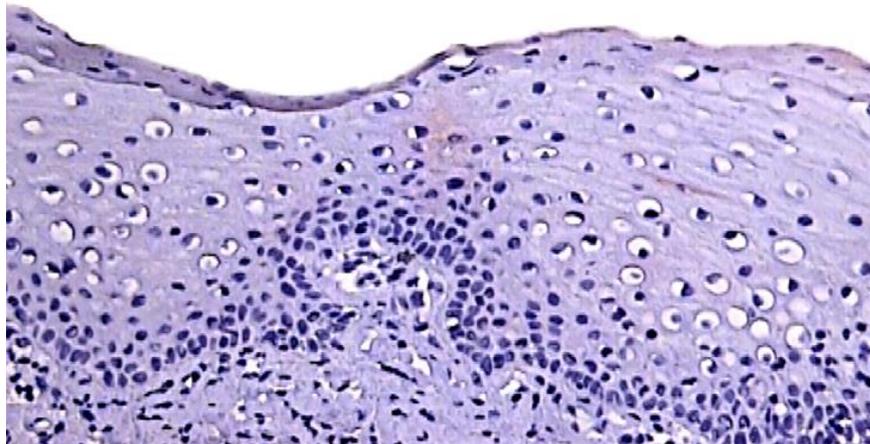
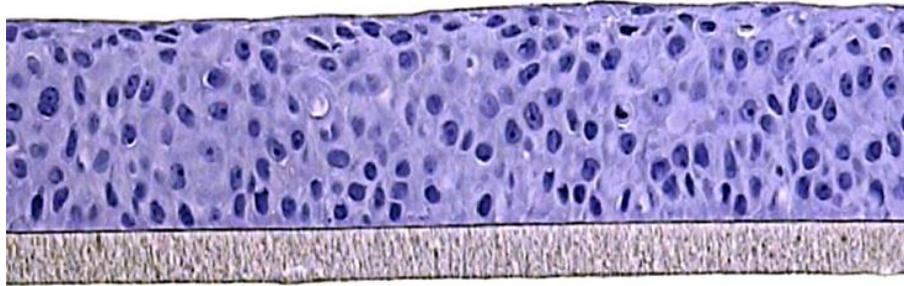
メラノサイト入り再生ヒト表皮モデル



Fontana Masson染色

ニコダーム提供

再生ヒト口腔粘膜モデル

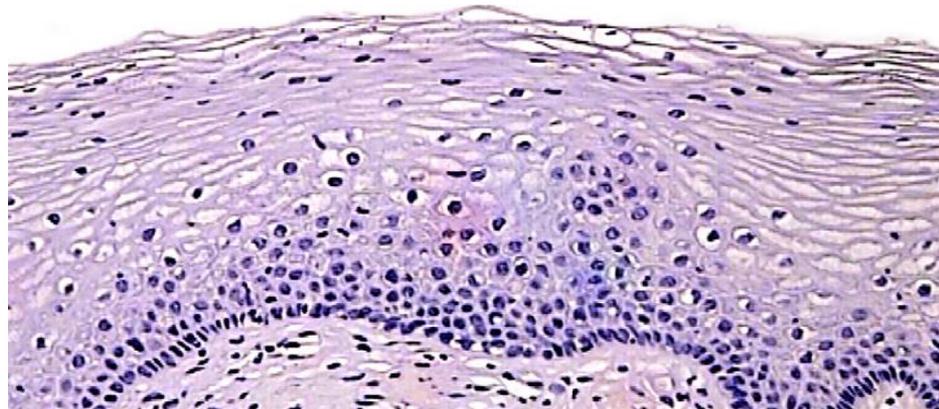
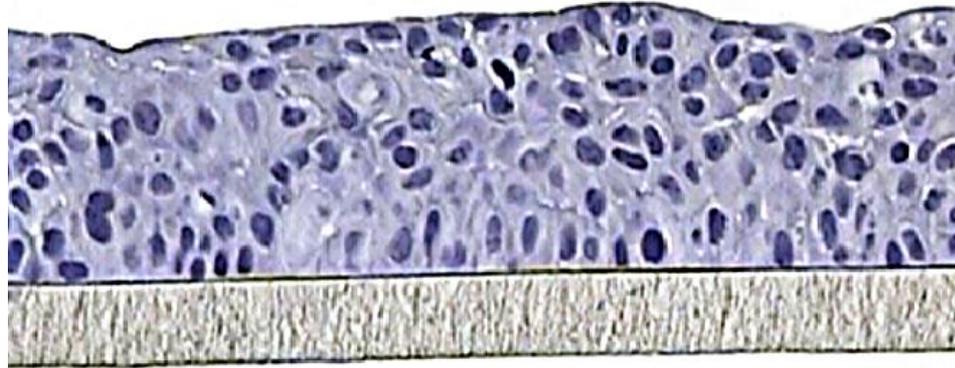


in vivo

利用方法:オーラルケア用品・歯科材料の刺激性評価、
口腔粘膜への浸透性と代謝、オーラルケア用品の抗炎症評価など

ニコダーム提供

再生ヒト膣粘膜上皮モデル

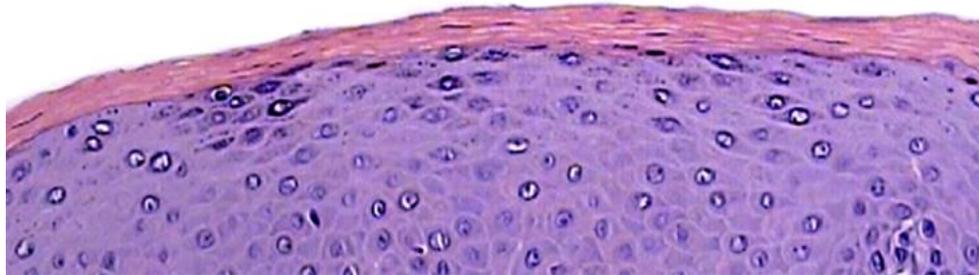
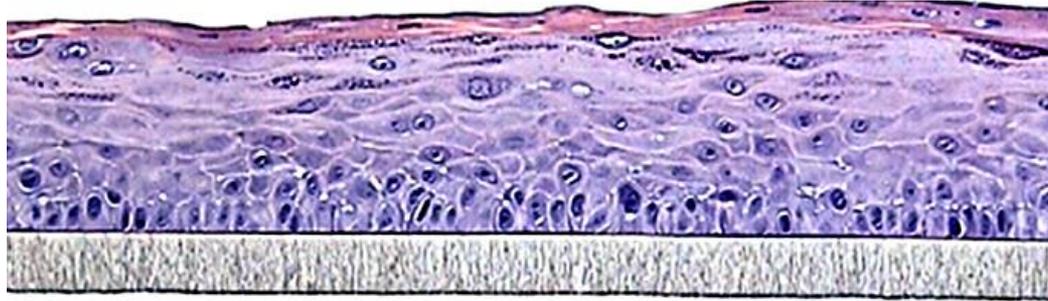


in vivo

利用方法: 婦人衛生用品・入浴剤・ボディソープなどの膣粘膜刺激性評価、
膣粘膜への浸透性と代謝など

ニコダーム提供

再生ヒト歯肉上皮モデル



in vivo

利用方法:オーラルケア用品・歯科材料の腐食性、刺激性評価、
歯肉への透過性と代謝など

皮膚3次元モデル

▶ 製品・サービス

▶ 核酸自動分離装置

▶ 病理研究製品

▶ バイオイメージング製品

▶ 汎用実験機器

▶ 細胞・組織製品

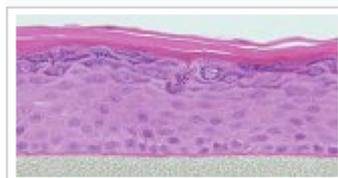
▶ 皮膚3次元モデル

▶ 遺伝子受託サービス

▶ 癌・薬物動態関連

▶ ペプチド関連製品

▶ フィルター



皮膚3次元モデル EPI-200関連製品 EPI-200・212・200X・606・606X・201・296キット

- ・EPI-200,212 NHEKから成る表皮モデル(8mm)
- ・EPI-200SIT,212SIT OECD承認の皮膚刺激性試験専用
- ・EPI-606,296 サイズが異なるモデル
- ・EPI-606X,200X,212X バリア能亢進モデル
- ・EPI-201 角層未熟モデル

▶ 製品の詳細を見る

▶ 製品購入・見積り依頼へ



皮膚3次元モデル OCL-200関連製品 OCL-200・212・200EIT・212EIT・606キット 角膜3次元モデル

- ・OCL-200,212 角膜モデル(8mm)
- ・OCL-200EIT,212EIT 欧州validation実施中の眼刺激性試験専用キット
- ・OCL-606 サイズが大きなモデル

▶ 製品の詳細を見る

▶ 製品購入・見積り依頼へ

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- 学術論文・研究報告
- ラボサイトQ&A
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EPI-MODEL24
皮膚刺激性試験セット
データシート

皮膚刺激性試験プロトコール
手技ビデオ

ラボサイト
取扱講習会

皮膚基礎研究

研究開発支援事業

R&D SUPPORT



J-TECでは、医療用培養表皮や培養軟骨の開発で蓄積した高度な培養技術を応用して、研究用ヒト培養組織を開発し、販売しています。研究用ヒト培養組織とは、ヒトの細胞を用いて体外で培養し、再構築させた組織モデルのことです。ヒト組織に極めて近い構造を再現できるため、動物や単純な培養細胞の代替となる種々の実験への適用が可能です。この製品は外用医薬品や化粧品の開発、皮膚を用いた各種研究に使用することができます。

研究用ヒト培養組織ラボサイトシリーズ LabCyte

ヒト3次元培養表皮

EPI-MODEL

エピ・モデル

メラノサイト含有ヒト3次元培養表皮

MELANO-MODEL

メラノ・モデル

ヒト3次元培養角膜上皮

CORNEA-MODEL

角膜モデル

ヒト表皮モデル作製キット

EPI-KIT

エピ・キット

EPI-MODEL24 皮膚刺激性試験セット
データシート

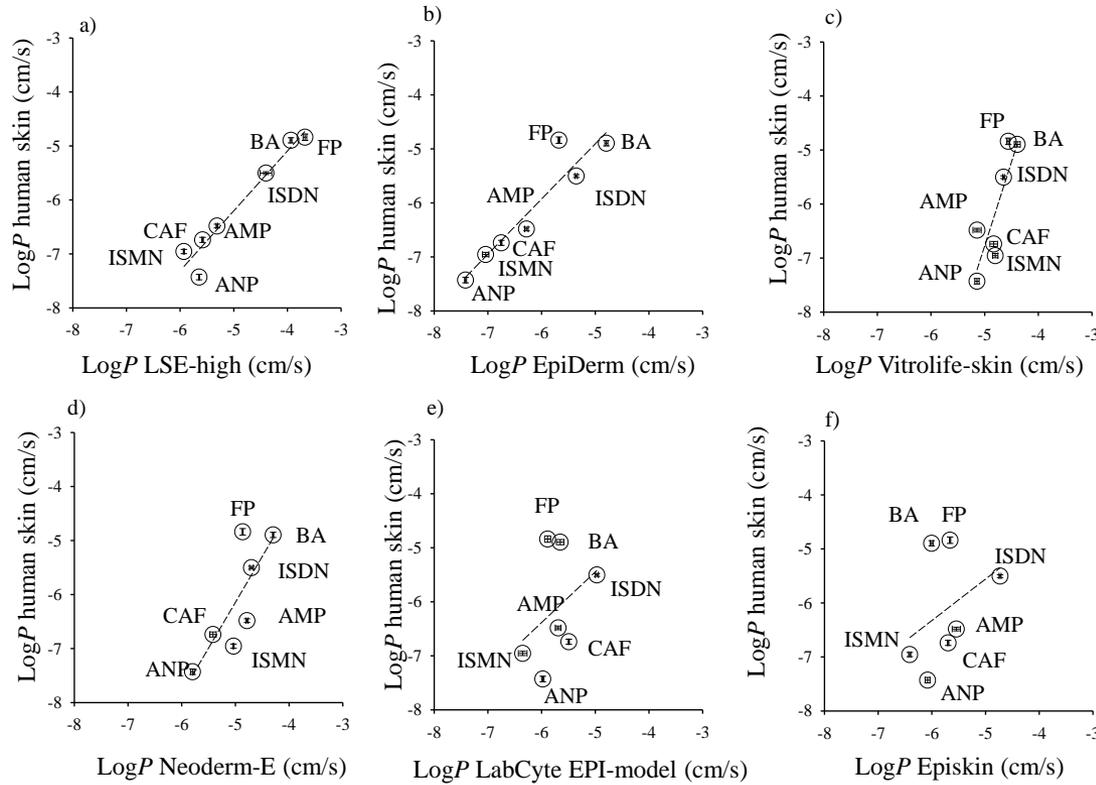
皮膚刺激性試験プロトコール
手技ビデオ

皮膚刺激性試験を体験してみませんか？

ラボサイト 取扱講習会

皮膚基礎研究

クラスターフォーラム

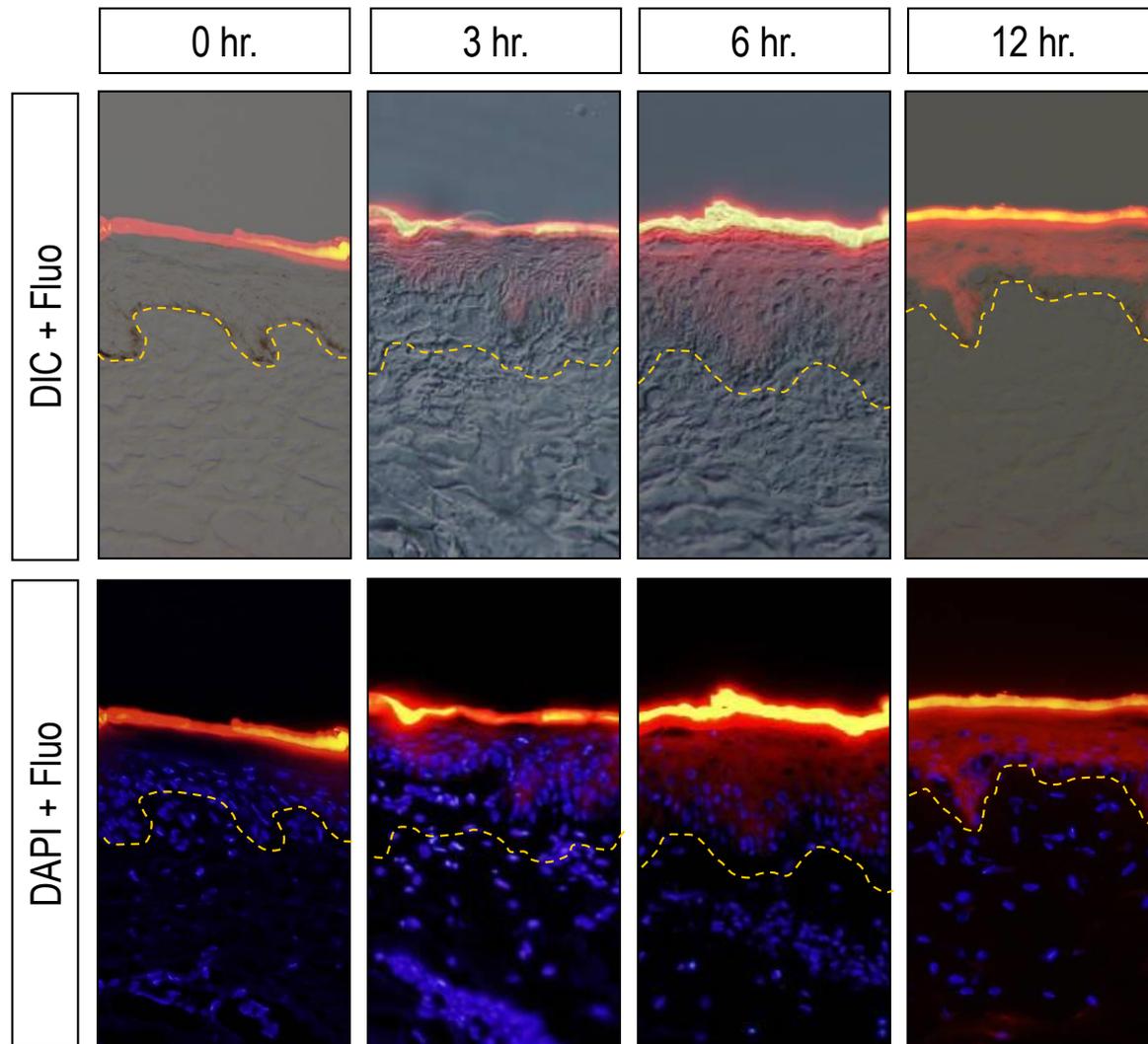


杉林堅次ら引用

Relationships between $\log P$ values in excised human cadaver skin and $\log P$ values in cultured skin models. (a): **LSE-high** versus excised human cadaver skin, (b): **EpiDerm** versus excised human cadaver skin, (c): **Vitrolife-skin** versus excised human cadaver skin, (d): **Neoderm-E** versus excised human cadaver skin, (e): **LabCyte EPI-model** versus excised human cadaver skin, and (f): **Episkin** versus excised human cadaver skin. Each point represents the mean \pm S.E. (n=4-6).

ブタ皮膚組織を用いた

経皮吸収試験の組織学的評価

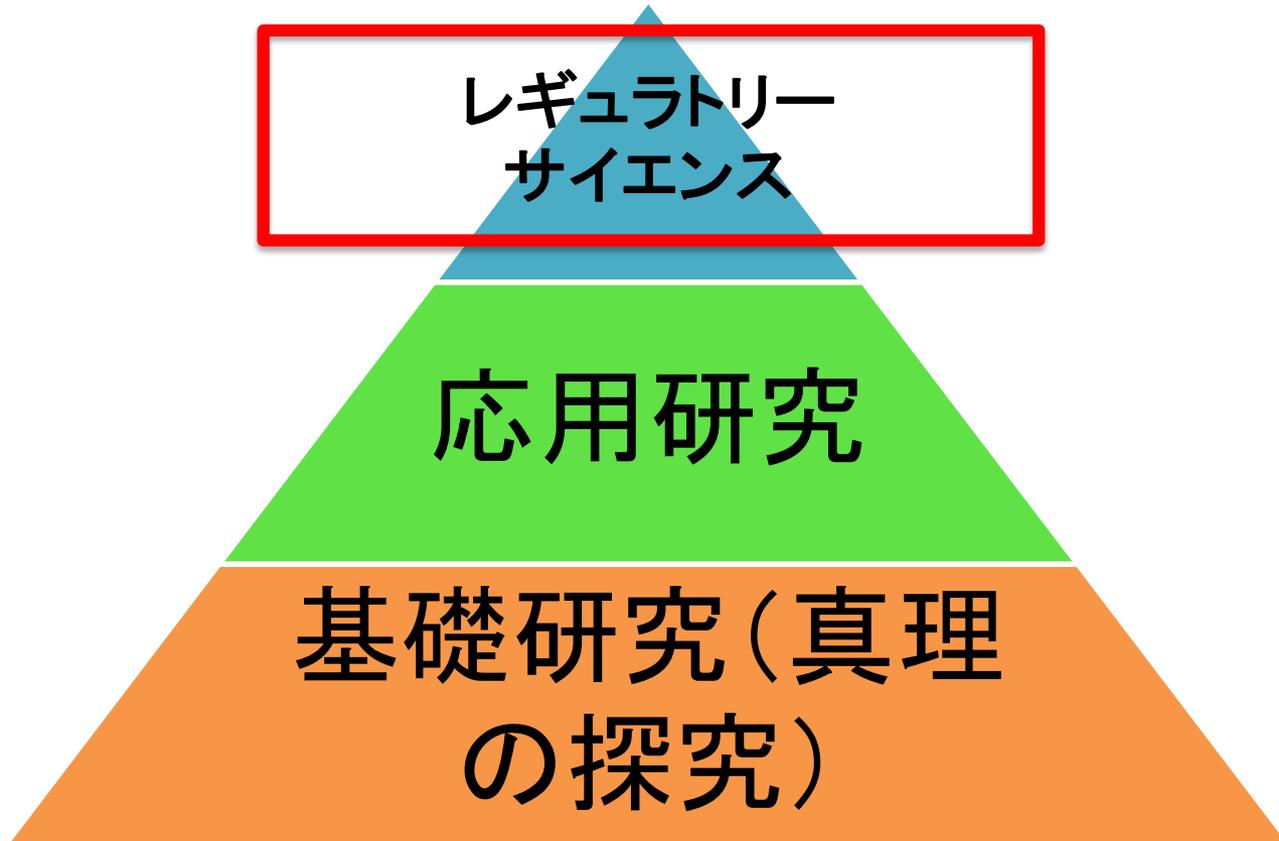


山本直樹ら引用

三次元培養モデルの利用

- 安全性評価
難水溶性物質の評価、製品の評価、暴露評価
- 作用機構の解明
二次元培養では機能発現がないバイオマーカーの検出
- 有用性評価
製品の評価

試験法開発の背景



三次元生体組織を用いたテスト ガイドライン

Table with Internationally Accepted Alternative Test Methods for Cosmetic Products/Ingredients Safety Testing, successfully worked upon by ICATM

Method	International Acceptance
<i>Dermal Corrosivity Test Methods</i>	
<i>CORROSITEX Skin Corrosivity Test</i>	OECD TG 435 (2006)
EpiSkin Skin Corrosivity Test. EpiSkin, EpiDerm,	OECD TG 431 (2004)
Rat TER Skin Corrosivity Test	OECD TG 430 (2004)
<i>Dermal Irritation Test Methods</i>	
<i>In vitro</i> reconstructed human epidermis (RhE) test methods EpiDerm; EPISKIN; SkinEthic	OECD TG 439 (2010)
<i>Dermal penetration Test Methods</i>	
Skin Absorption: In Vitro Method	OECD TG 428 (2004)
<i>Phototoxicity Test Methods</i>	
3T3 NRU Phototoxicity Test	OECD TG 432 (2004)
<i>Ocular Toxicity Test Methods</i>	
Bovine Corneal Opacity and Permeability (BCOP) Test Method for identification of ocular corrosives and severe irritants	OECD TG 437 (2009)
Isolated Chicken Eye (ICE) Test Method	OECD TG 438 (2009)
Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants	OECD TG 460 (2012)
Use of histopathology as an additional endpoint in Ocular safety testing	OECD Guidance Document 160 (2011)

Table with Internationally Accepted Alternative Test Methods for Cosmetic Products/Ingredients Safety Testing, successfully worked upon by ICATM

<i>Acute Toxicity Tests</i>	
<i>In Vitro</i> cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests	OECD Guidance document 129 (2010)
<i>Endocrine Disruptor Test Methods</i>	
Performance-Based Test for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists	OECD TG 455 (2012)
H295R Steroidogenesis Assay	OECD TG456 (2011)
BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists	OECD TG 457 (2012)
<i>Genetic Toxicity Test Methods</i>	
Bacterial Reverse Mutation Test	OECD TG471 (1997)
In vitro Mammalian Chromosome Aberration Test	OECD TG473 (1997)
In vitro Mammalian Cell Gene Mutation Test	OECD TG476 (1997)
<i>In Vitro</i> micronucleus Test	OECD TG 487 (2010)

Draft Test Guideline proposed by Japan in the OECD Work Plan

- ✓ Bhas 42 cell transformation assay
- ✓ Short time exposure (STE) assay for **eye irritation testing**
- ✓ h-CLAT assay for **skin sensitisation testing**
- ✓ Stable transfected transcriptional activation (STTA) antagonist assay for **estrogen disruptor screening**
- ✓ Stable transfected transcriptional activation (STTA) assay for **androgen disruptor screening** (AR-Ecoscreen)

来年のOECD work planに提案された試験法

No.	Lead country	Test method
1	United Kingdom	Development of a reference/characterising chemical set for testing <i>in vitro</i> metabolism systems in EAS assays
2	Korea	Androgen Receptor Transactivation Assay
3	France	MUSST for in vitro skin sensitisation
4	France	RhCE test method using SkinEthic for eye irritation
5	Japan	IL-8 Luc assay for in vitro skin sensitisation
6	Italy	In vitro Macromolecular Test Method for identifying Chemicals inducing serious eye damage
7	Slovakia	Update of TG 431 for improving predictivity of sub-categorisation by 3 test method(s)
8	Netherlands	Extension of the ICE (TG 438)

三次元培養モデルおよび摘出組織に関するテストガイドライン

分類	試験法
皮膚腐食性試験	<i>Reconstructed human epidermis (RHE) test method</i> , EpiDerm, EPISKIN, SkinEthic, epiCS
	Transcutaneous Electrical Resistance Test Method (TER)
皮膚刺激性試験	<i>In vitro</i> reconstructed human epidermis (RhE) test methods, EpiDerm, EPISKIN, SkinEthic, LabCyte EPI-MODEL
眼刺激性試験	Bovine Corneal Opacity and Permeability (BCOP) Test Method
	Isolated Chicken Eye (ICE) Test Method

OECD GUIDELINES FOR THE TESTING OF CHEMICALS***In vitro* skin corrosion: reconstructed human epidermis (RHE) test method****Table 3: QC batch release criteria**

	Lower acceptance limit	Upper acceptance limit
EpiSkin™ (SM) (18 hours treatment with SDS)(35)	IC ₅₀ = 1.0 mg/mL	IC ₅₀ = 3.0 mg/mL
EpiDerm™ SCT (EPI-200) (1% Triton X-100)(36)	ET ₅₀ = 4.0 hours	ET ₅₀ = 8.7 hours
SkinEthic™ RHE (1% Triton X-100)(37)	ET ₅₀ = 4.0 hours	ET ₅₀ = 10.0 hours
epiCS®(1% Triton X-100)(38)	ET ₅₀ = 2.0 hours	ET ₅₀ = 7.0 hours

OECD GUIDELINES FOR THE TESTING OF CHEMICALS***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method****Table 3: QC batch release criteria of the test methods included in this TG**

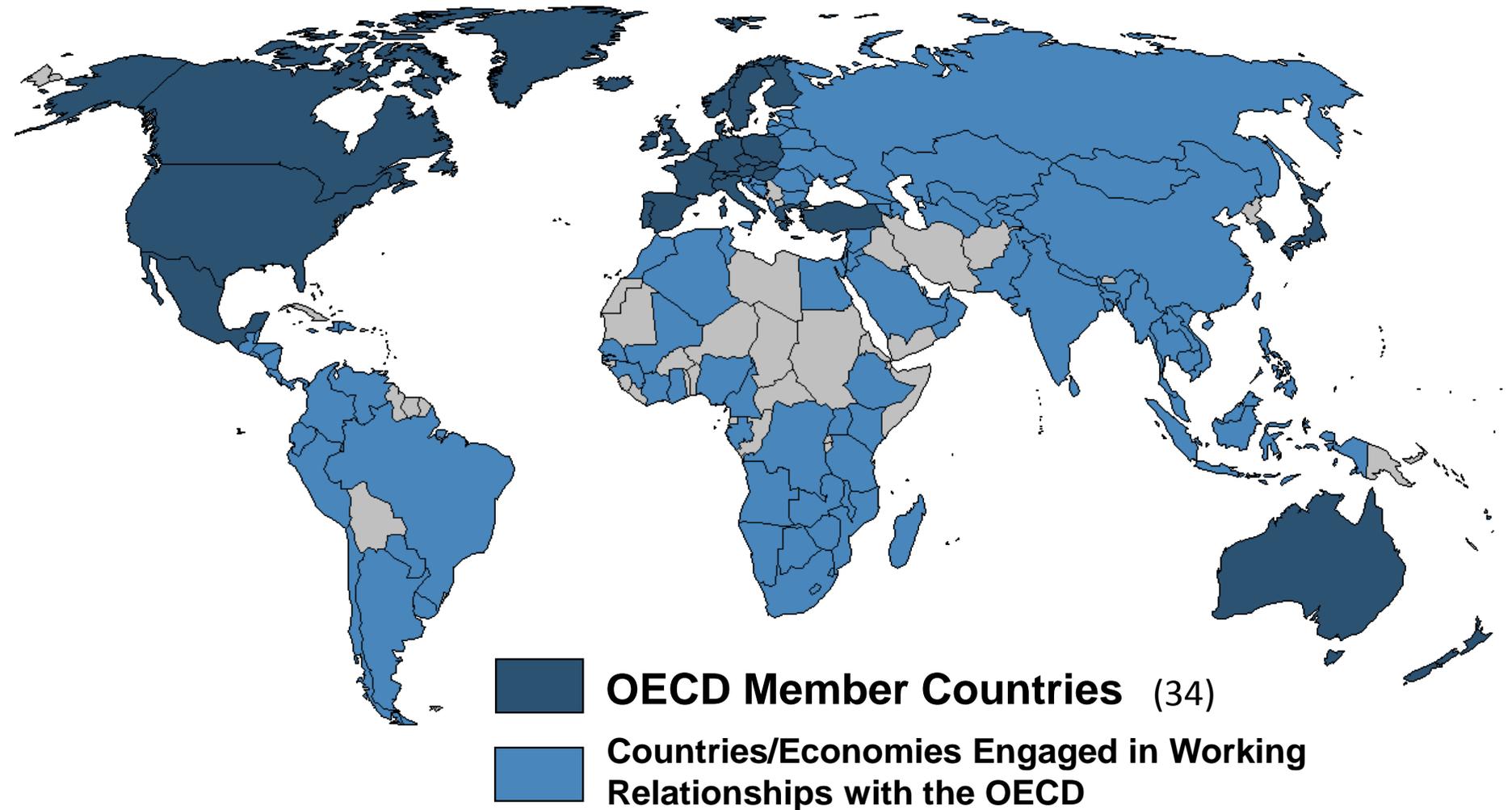
	Lower acceptance limit	Upper acceptance limit
EpiSkin™ (SM) (18 hours treatment with SDS) (27)	IC ₅₀ = 1.0 mg/ml	IC ₅₀ = 3.0 mg/ml
EpiDerm™ SIT (EPI-200) (1% Triton X-100) (28)	ET ₅₀ = 4.0 hr	ET ₅₀ = 8.7 hr
SkinEthic™ RHE (1% Triton X-100) (29)	ET ₅₀ = 4.0 hr	ET ₅₀ = 10.0 hr
LabCyte EPI-MODEL24 SIT (18 hours treatment with SDS) (40)	IC ₅₀ = 1.4 mg/ml	IC ₅₀ = 4.0 mg/ml

培養表皮モデルを用いた試験法の比較

	TG431	TG439
対象毒性	腐食性	皮膚刺激性
記載キット	EpiSkin™, EpiDerm™ (EPI-200), SkinEthic™ RHE1, epiCS®	EpiSkin、SkinEthics、EpiDerm、LabCyte-Epi Model
指標	MTTアッセイによる細胞毒性	MTTアッセイによる細胞毒性
処理時間	3分、1時間、4時間 (EpiSkinのみ)	15～60分
回復時間	なし	42時間
予測モデル	生存率15及び50%で腐食性評価、生存率35%で評価 (EpiSkinのみ)	生存率50%でGHS基準2.3を区分
習熟用物質	12	10

試験法の標準化

A global outreach



The work plan includes 5 sections for specific projects:

Section 1 (Projects related to Test Guidelines on physical–chemical properties)

Section 2 (Projects related to Test Guidelines on effects on biotic systems)

Section 3 (Projects related to Test Guidelines on environmental fate)

Section 4 (Projects related to Test Guidelines on health effects)

Section 5 (Projects related to other Test Guidelines)

Projects of a general nature are included after Section 5. *Projects remain in the work plan until the publication of the Test Guideline or other Test Guideline-related document.* Each project keeps the same identification number until it is completed. If a project is no longer supported by lead countries, it is moved to [Annex 1](#) for two years and then deleted.

Abbreviations used:

TG: Test Guideline

GD: guidance document

DRP: detailed review paper

Joint Meeting: Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology

EDTA AG: Endocrine Disrupters Testing and Assessment Advisory Group

EPOC: Environmental Policy Committee

NC: national coordinator

SPSF: standard project submission form

SSD: Streamlined Summary Document

VMG-eco: Validation Management Group for Ecotoxicity Testing

VMG-non animal: Validation Management Group for Non Animal Testing

VMG-mammalian: Validation Management Group for Mammalian Toxicity Testing

WNT: Working Group of the National Coordinators for the Test Guidelines Programme

WGP: Working Group on Pesticides

WPMN: Working Party on Manufactured Nanomaterial

TF Biocides: Task Force on Biocides

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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the OECD Secretariat at
ehscont@oecd.org

Chemical Safety and Animal Welfare



Progress made at the OECD



www.oecd.org/env/ehs

ICH安全性試験ガイドライン

2006年より、見直し

動物実験数削減の可能性もテーマの一つ

各国・地域における規制上の不調和

公衆衛生上の問題

科学技術の進展への対応

ISO and medical devices

- This concept is incorporated in the ISO 10993 standards.
- ISO 10993 part 2 states, “The protection of humans is the primary goal of the ISO 10993 series of standards. A second equally important goal is to ensure animal welfare and to minimise the number and exposure of the laboratory animals” and that the standard was “developed to ensure the welfare of animals used in biological evaluation testing.”

国際規約の内容

- 実際に動物実験、試験を行う際の考え方、方法が記載されている
- 動物実験規制とする意見もある
- しかし多くの国際的標準、指針は原則、考え方、体制整備を定めているだけで、具体的な方法は記載されていない
- 具体的な動物(実験)試験方法はOECDの指針が最上流にあり広く引用される
 - ICHの医薬品試験方法
 - ISO10993の医療機器試験法
 - 各国の行政庁の指導

今後の提言

ECVAM-LED VALIDATION STUDIES – EYE IRRITATION (EIVS)

Objective: stand-alone test methods to identify chemicals not classified as eye irritant under GHS for use in a bottom-up testing strategy

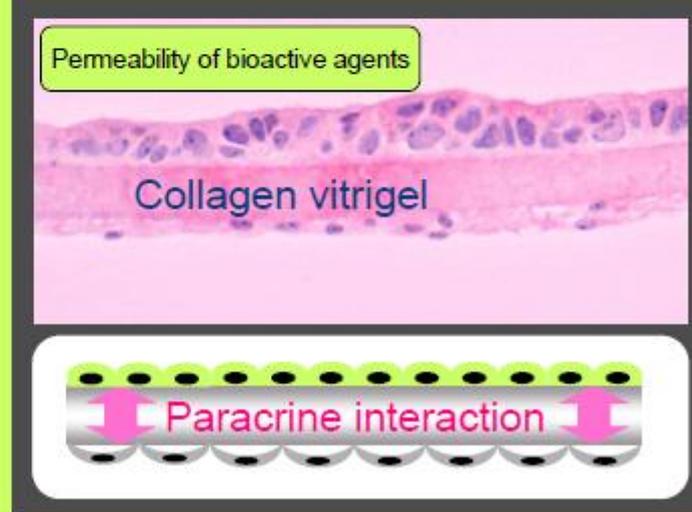
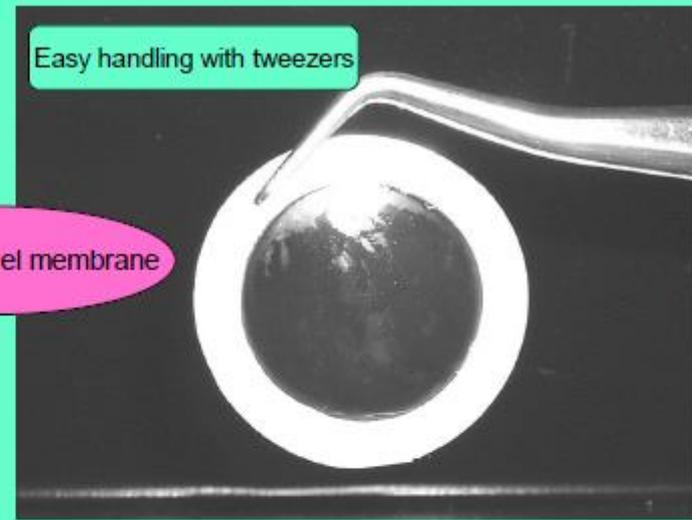
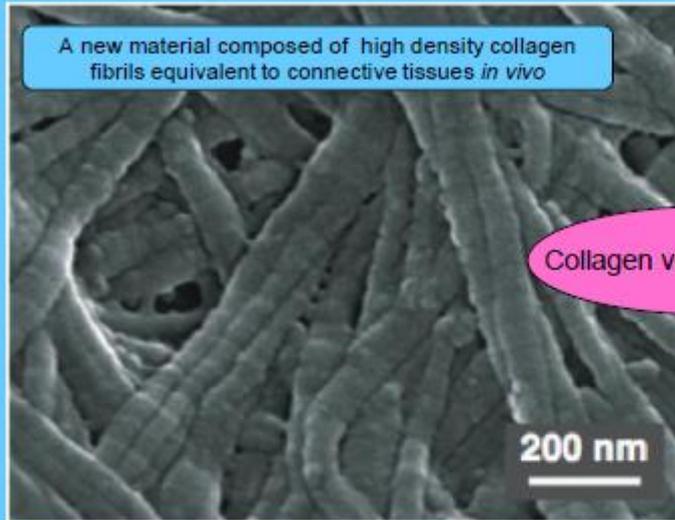
Test systems: EpiOcular™ EIT and SkinEthic™ HCE

Status:

- 104 chemicals selected and undergoing testing in 3 laboratories
- Testing phase **finished, no additional tests needed**
- Analysis of data thereafter, Validation Report possibly to ESAC for peer review in March 2014

Note: The test methods are not intended to differentiate between GHS Category 1 (irreversible effects) and 2A-B (reversible effects). This differentiation would be left to another tier of the Bottom-up/Top-down testing strategy (ECVAM Workshop 2005; Scott et al., 2009).

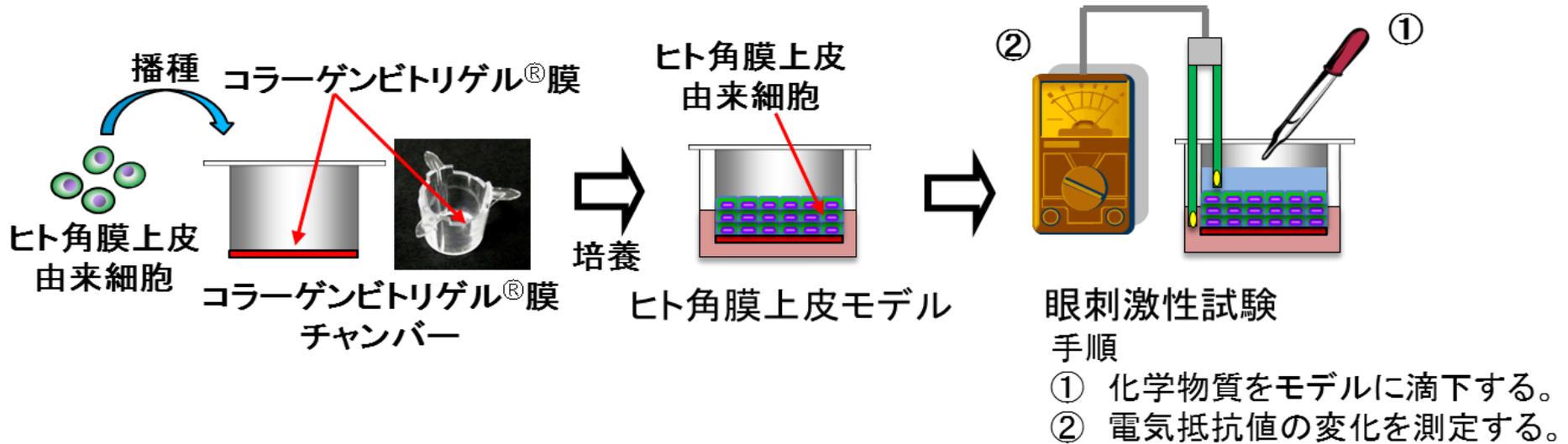
Background- 1 : Collagen vitrigel membrane (CVM)



Takezawa T, *et al.*, Cell Transplantation, 13: 463-473, 2004
Takezawa T, *et al.*, Tissue Engineering, 13: 1357-1366, 2007

Takezawa T, *et al.*, Cell Tissues Organs, 185: 237-4241, 2007
Takezawa T, *et al.*, Yakugaku Zasshi, 130: 565-574, 2010

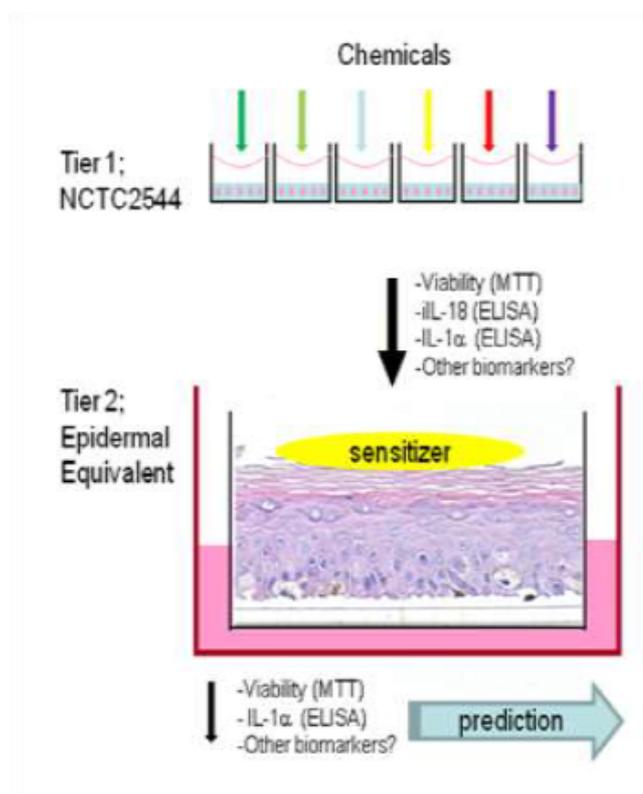
コラーゲンビトリゲル[®]膜チャンバーを用いた眼刺激性試験法



A 2-tiered approach for identification and classification of skin sensitizers

3RsMC

3Rs Management
and Consultancy
www.3RsMC.eu



• Identification of contact sensitizers in Tier 1

-IL-18 expression

- Identification of potency of sensitizers in Tier 2

- EC50, MTT

- IL-1 α expression

NCTC2544/IL-18:

- 30 chemicals tested
- WLR: >95%
- Transferable
- BLR: >95%
- Accuracy: 97% (labeling)

RHE potency test:

- 16 chemicals tested
- WLR: >95%
- Transferable
- BLR: >95%
- Concordancy: 92% (classification)

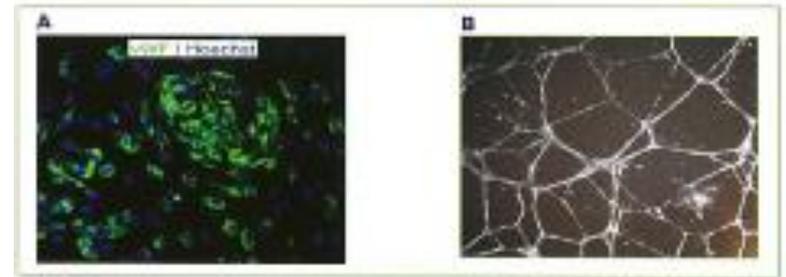
Assessed by Cosmetics Europe

- 10 coded compounds

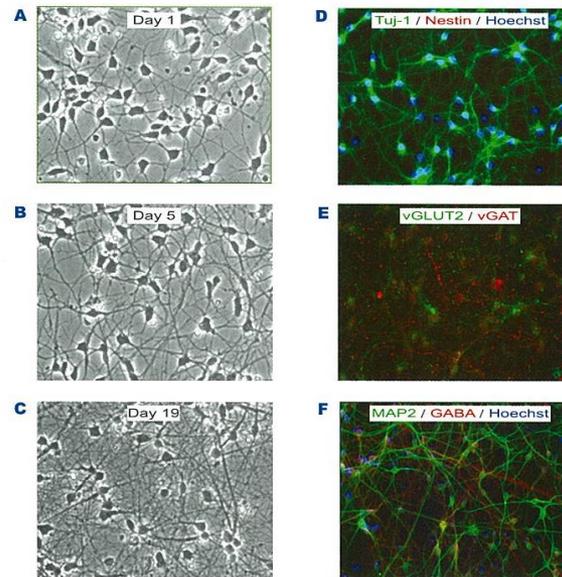
iCell® Cardiomyocytes



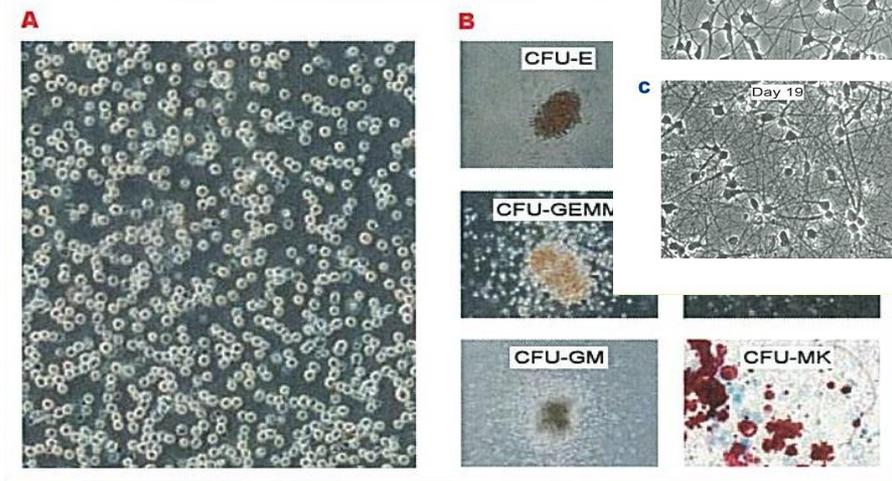
iCell® Endothelial Cells



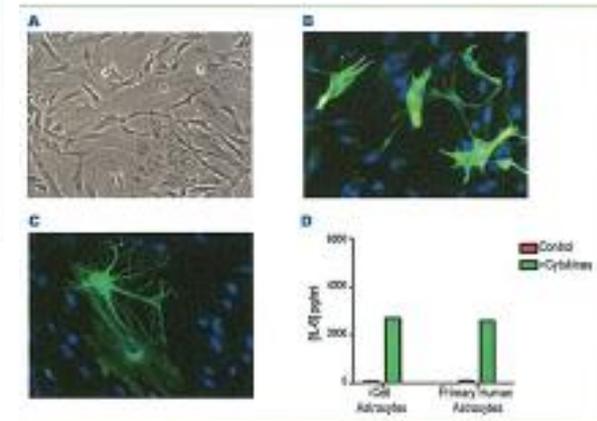
iCell® Neurons



iCell® Hematopoietic Progenitor Cells



iCell® Astrocytes

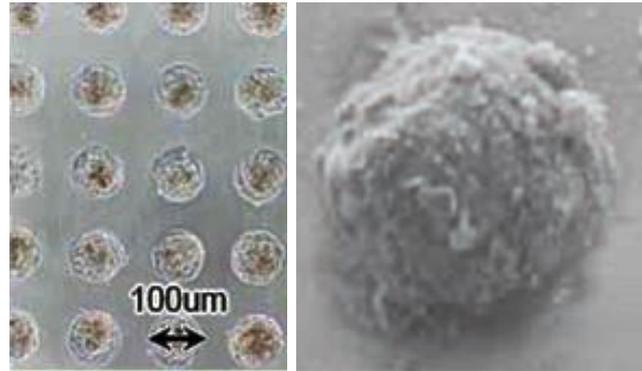


三次元培養／幹細胞分化誘導を用いた臓器モデル



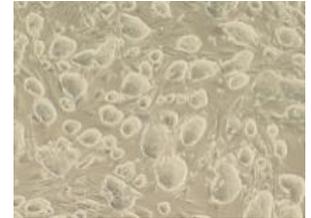
肝臓

肝スフェロイド

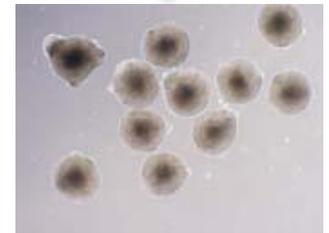


トランスパレント製の三次元培養法

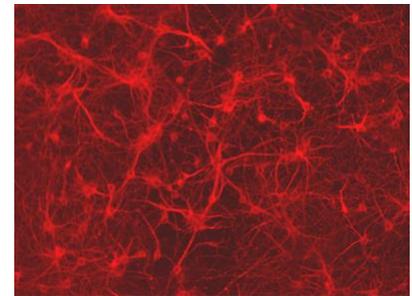
マウスES細胞



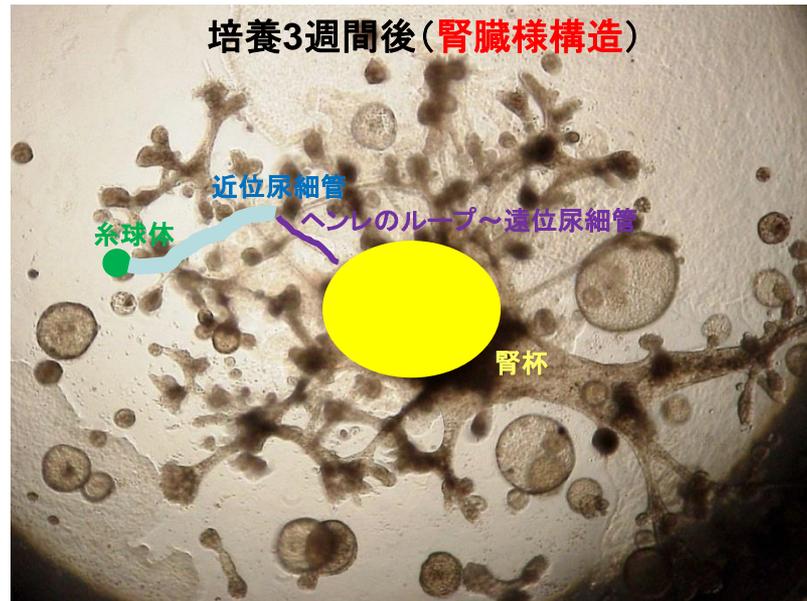
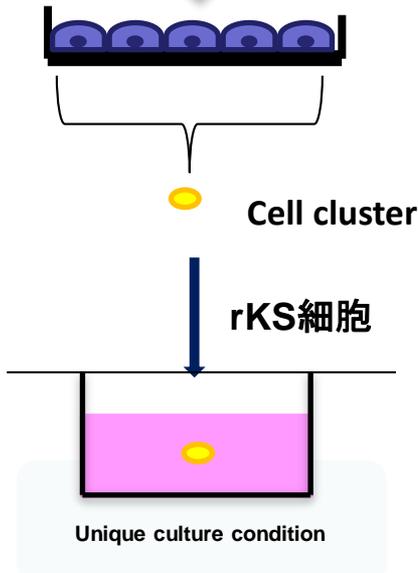
分化誘導



神経



腎臓



【Kitamura S et al. FASEB J, 2005】。

岡山大学 喜多村

ラットよりrKS細胞を樹立、試験管内で腎臓様構造体の作製に成功

最後に

- 時流にのった生体組織モデルの構築が待たれる。
 - 1) 局所から生体へ
 - 2) Human on-chipへ
 - 3) 難水溶性物質の対応へ
- 開発研究を進めるには魅力あるネーミングも必要である。
- 標準化すなわち行政的な受入れを目指すためには、ユーザーのニーズをつかむことである。



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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☞news texts dummy texts news texts (2009.7.3)

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texts dummy texts (2009.7.3)

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