

**XIV SIMPÓSIO DE BIOSSEGURANÇA E  
DESCARTES DE PRODUTOS QUÍMICOS  
PERIGOSOS  
E ORGANISMOS GENETICAMENTE  
MODIFICADOS (OGMs)  
EM INSTITUIÇÕES DE ENSINO E PESQUISA**

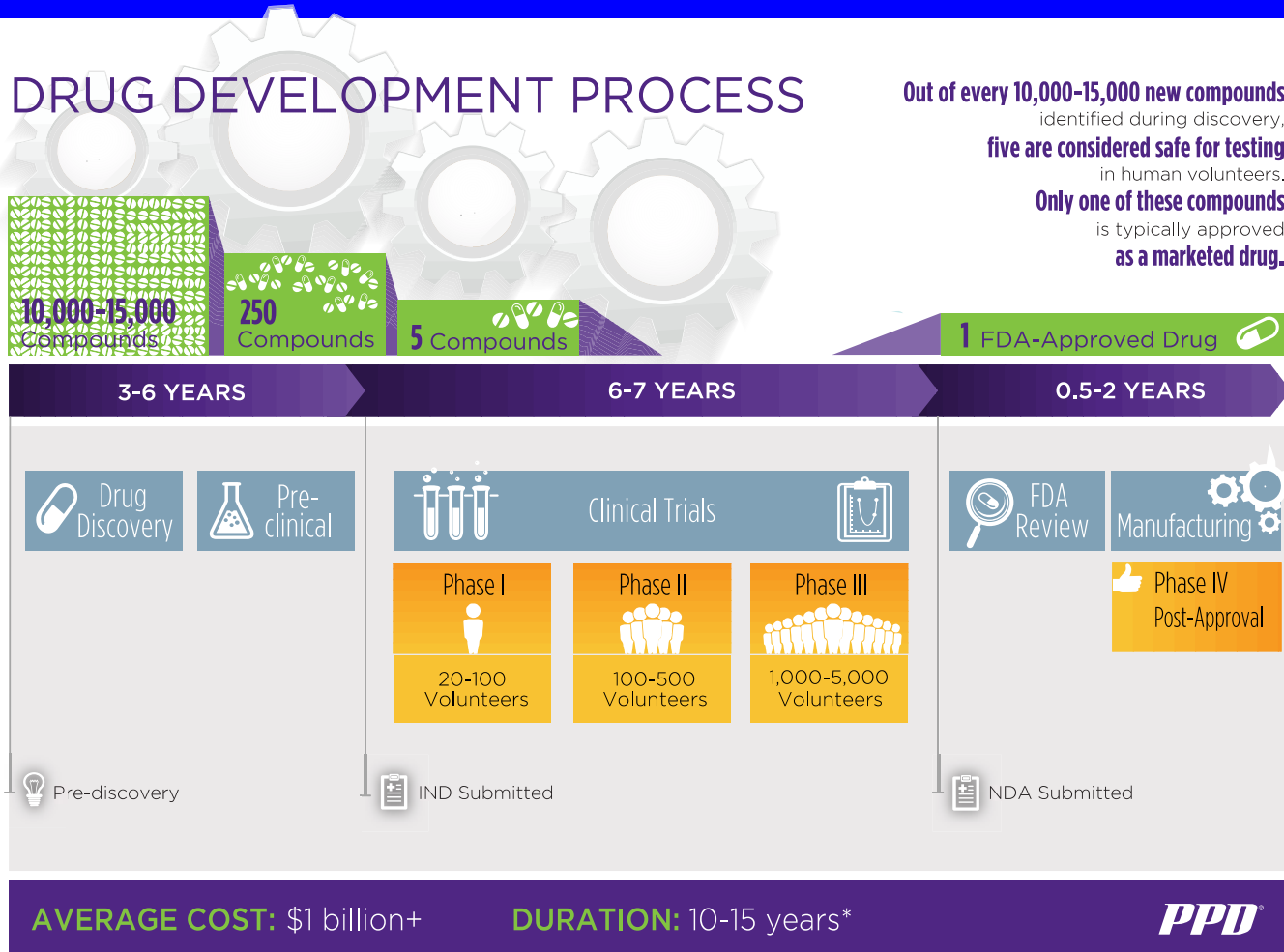


**I SIMPÓSIO DE SEGURANÇA QUÍMICA E  
BIOLÓGICA**

**MÉTODOS ALTERNATIVOS À EXPERIMENTAÇÃO ANIMAL NO  
COTEXTO DE BIOSEGURANÇA  
SILVYA STUCHI MARIA-ENGLER**

**CLINICAL CHEMISTRY AND TOXICOLOGY DEPT  
SCHOOL OF PHARMACEUTICAL SCIENCES  
UNIVERSITY OF SÃO PAULO**

# PHARMACEUTICAL SUPPLY CHAIN: BIOSAFETY MATTERS!!!!



\*Source: ACRO

ndi.com

Large-molecule **therapeutics**, which cannot be produced by chemical synthesis, are traditionally **manufactured** either through **microbial fermentation** or more commonly **via mammalian cell culture**.

# Why do companies test cosmetics, pharmaceuticals and other products on animals?

*Determination of quantitative or qualitative value of risk related to a concrete situation and a recognized threat (also called hazard).*



# Why do companies test cosmetics, pharmaceuticals and other products on animals?

*Determination of quantitative or qualitative value of risk related to a concrete situation and a recognized threat (also called hazard).*



Animal testing for research and development

80



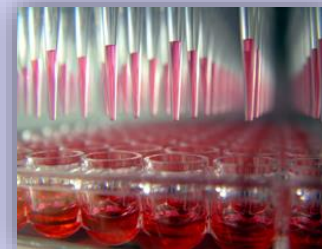
**Adopting of 3 Rs**  
- **Reduction,**  
**Refinement,**  
**Replacement**  
(Russell & Burch, **1959**).

90



**ECVAM** promotion  
and validation of  
in vitro assays

2000 -



**Seventh Amendment to the Cosmetics.**  
imposes a ban on  
marketing cosmetics  
in Europe if the  
finished product or its  
ingredients have been  
tested using animals.



**REACH - EUROPEAN  
REGULATION, 2006**  
Regulation on  
Registration,  
Evaluation,  
Authorisation and  
Restriction of  
Chemicals.

Animal research and testing has played a part in almost every medical breakthrough of the last century. It has saved hundreds of millions of lives worldwide..."

# Alternative Methods Brazil



2008 - 2009



**Publication of Law AROUCA 11.794/08**

Creation of the National Council for the Control of Animal Experimentation - CONCEA

2012



Creation of **BraCVAM** connected to the National Institute of Quality Control and Health - INCQS FIOCRUZ

Creation of **RENAMA** - National Network of Alternative Methods (MSTI)

2013



**Law 15.316:**  
Ban the use of animals in cosmetics testing. Government of São Paulo

March - 2014



**CONCEA:**  
*After the validation of the alternative methods in Brazil, companies will have up to five years to implement them*

# RUSSELL, W.M.S. AND BURCH, R.L.

*The Principles of Humane Experimental Technique* Methuen,  
London, 1959

- **Redução:** obter informações comparáveis de um número menor de animais.
- **Substituição:** uso de métodos não animais sempre que for possível alcançar os mesmos objetivos científicos.
- **Refinamento:** minimizar ou aliviar a dor potencial, sofrimento ou angústia e melhorar o bem-estar animal.



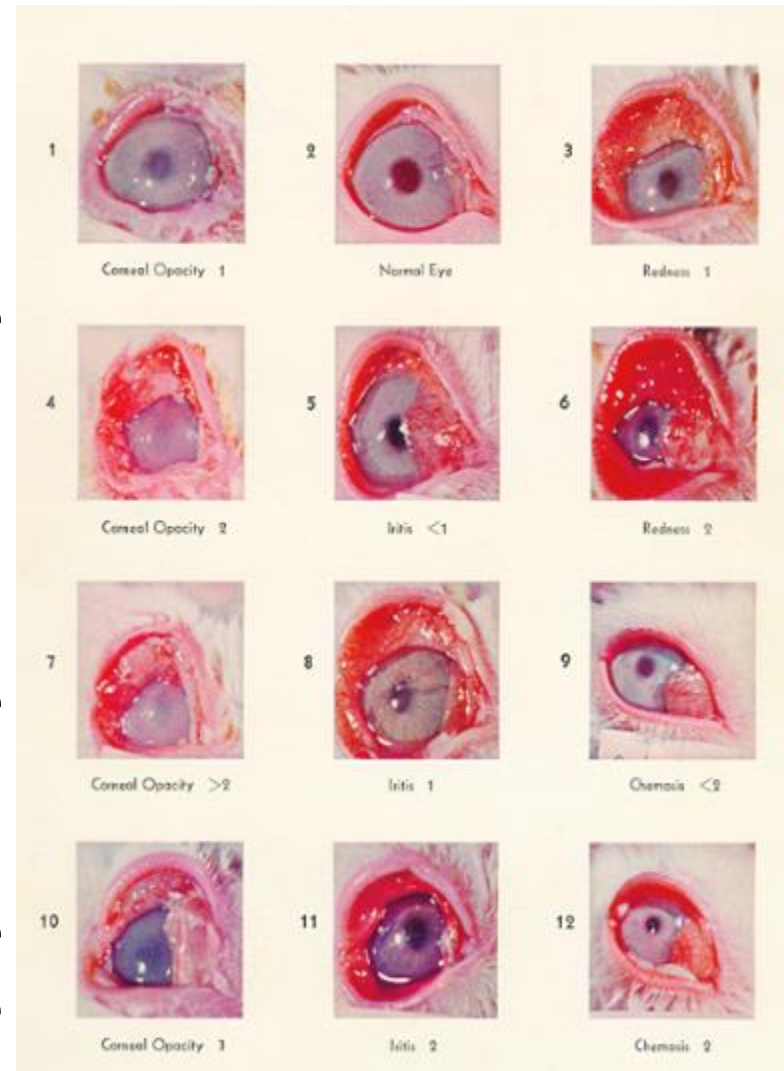
# Critérios normativos mínimos para as pesquisas que utilizam animais

- Definir objetivos legítimos para a pesquisa em animais;
- Impor limites à dor e ao sofrimento;
- Garantir tratamento humanitário;
- Avaliar previamente os projetos por um Comitê Independente;
- Fiscalizar instalações e procedimentos;
- Garantir a responsabilização pública

Hampson J. Animal Experimentation: practical dilemmas and solutions. In: Paterson D, Palmer M. The status of animals. Oxon (UK): CAB, 1989: 101.

# TESTE DE DRAIZE

- Este teste é um dos mais criticados, particularmente no que diz respeito à avaliação do potencial de irritação ocular de produtos cosméticos.
- Avaliar os efeitos da irritação de substâncias na conjuntiva, na córnea e na íris de olhos de coelhos albinos.
- Kay & Calandra (1962) incluíram parâmetros como eritema e espessura das pálpebras, abrangendo edema, lacrimejamento, opacidade, danos e neovascularização da córnea neste protocolo.





# TESTE DE DRAIZE

- **Estimativas variáveis de irritação ocular**

38 - 59% variabilidade intra laboratorial

- Variabilidade da exposição tempo e dose
- Variações das respostas entre animais

- **Diferenças entre coelhos e humanos**

- Diferenças fisiológicas
- Coelhos mais sensíveis que humanos



# TESTE DE OPACIDADE DA CÓRNEA BOVINA (BCOP)

- São testadas :
  - opacidade e
  - permeabilidade de córnea

provinda de olhos de bovinos (que seriam descartados), após a exposição à substância a ser testada.



# MICROORGANISMOS

- Geralmente os microorganismos, tais como bactérias e leveduras, são aceitos como modelos para estudo de metabolismo, genética e bioquímica.
- **Exemplo - Leveduras:** Possuem receptores de estrogênio que apresentam afinidade idêntica aos encontrados em útero de ratas.

# TESTE DA MEMBRANA CORION ALANTÓIDE (HETCAM):

- Utiliza ovos de galinha fertilizados
- Avalia a irritabilidade da membrana corion alantóide, que possui uma grande quantidade de vasos sanguíneos
- Semi-quantitativo (hiperemia, hemorragia e coagulação), após cinco minutos de aplicação do produto, puro ou diluído, sobre a membrana cório-alantóide.



# MODELOS MATEMÁTICOS

- Os modelos matemáticos podem contribuir para o trabalho experimental através da definição de variáveis e testando teorias, reduzindo o custo desses experimentos e os tornando mais eficazes.
- Um exemplo disso é a predição, através de modelos matemáticos, da estrutura de proteínas, que poderiam prever suas propriedades físicas e químicas.
- É sempre preciso lembrar que computadores processam e armazenam conhecimentos já existentes e muitos deles foram adquiridos com a utilização de animais na pesquisa.

# SISTEMAS IN VITRO

- Experimentos *in vitro* são apropriados para algumas áreas da ciência biológica. Por exemplo, vários estudos sobre o metabolismo intermediário utilizam a bioquímica para estudar a dinâmica de reações enzimáticas que ocorrem em nosso sistema biológico.

# Aceitação de metodologias alternativas pela OECD

- **1999-2001**

- **Refinamento:** Teste indolor para a sensibilização da pele
- **Redução:** O número de animais para toxicologia aguda 45-8.

- **2004-2010**

- **Substituição:**
  - Fototoxicidade
  - Irritação e corrosão ocular /cutânea

# BRASIL EM RELAÇÃO AOS MÉTODOS ALTERNATIVOS

**Lei AROUCA  
(11.794/08)**  
Regulamenta a  
experimentação animal  
em estabelecimentos de  
ensino e pesquisa.  
**CONCEA**  
Conselho Nacional de Controle  
da Experimentação Animal

2007

**BraCVAM**

Set/2012



LEI Nº 15.316, DE 23 DE JANEIRO DE 2014.  
Governador de SP promulga lei que proíbe  
o uso de animais em testes de produtos  
cosméticos, no estado de São Paulo.

Jan/2014



Prazo final para o que  
o Brasil substitua  
ensaios em animais  
por métodos  
validados

2019?

Jul/2012

**RENAMA**  
Rede Nacional de  
Métodos Alternativos



2013

Invasão e  
deprecação do  
Instituto Royal  
por ativistas  
acendem a discussão  
na sociedade brasileira  
sobre métodos  
alternativos

Set/2014

ANVISA reconhece  
17 métodos  
alternativos ao uso de  
animais

~€ 0,5  
milhões

Investimento  
brasileiro em pesquisa  
- RENAMA  
(2012-2014)

E 238MI  
2003-2011



# CONCEA & ANVISA SET/14

## **I - Para avaliação do potencial de irritação e corrosão da pele:**

Método OECD TG 430 - Corrosão dérmica in vitro: Teste de Resistência Elétrica Transcutânea;  
Método OECD TG 431 - Corrosão dérmica in vitro: Teste da Epiderme Humana Reconstituída;  
Método OECD TG 435 - Teste de Barreira de Membrana in vitro; e  
Método OECD TG 439 - Teste de irritação Cutânea in vitro.

## **II - Para avaliação do potencial de irritação e corrosão ocular:**

Método OECD TG 437 - Teste de Permeabilidade e Opacidade de Córnea Bovina;  
Método OECD TG 438 - Teste de Olho Isolado de Galinha;  
Método OECD TG 460 - Teste de Permeação de Fluoresceína.

## **III - Para avaliação do potencial de fototoxicidade:**

Método OECD TG 432 - Teste de Fototoxicidade in vitro 3T3 NRU.

## **IV - Para avaliação da absorção cutânea:**

Método OECD TG 428 - Absorção Cutânea método in vitro.

## **V - Para avaliação do potencial de sensibilização cutânea:**

Método OECD TG 429 - Sensibilização Cutânea: Ensaio do Linfonodo Local; e  
Método OECD TG 442A e 442B - Versões não radioativas do Ensaio do Linfonodo Local.

## **VI - Para avaliação de toxicidade aguda:**

Método OECD TG 420 - Toxicidade Aguda Oral – Procedimento de Doses Fixas;  
Método OECD TG 423 - Toxicidade Aguda Oral – Classe Tóxica Aguda;  
Método OECD TG 425 - Toxicidade Aguda Oral – procedimento "Up and Down"; e  
Método OECD TG 129 - estimativa da dose inicial para teste de toxicidade aguda oral sistêmica.

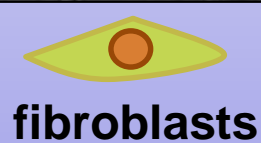
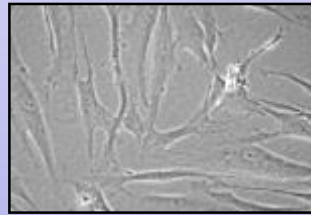
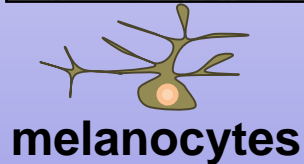
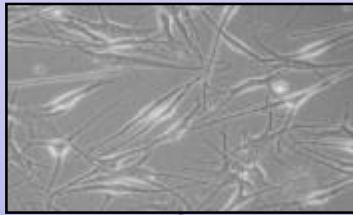
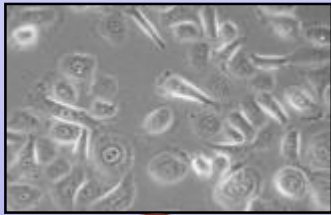
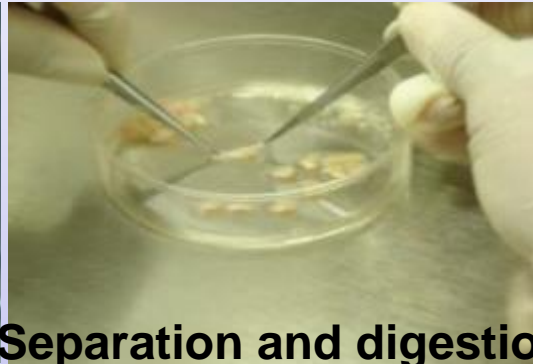
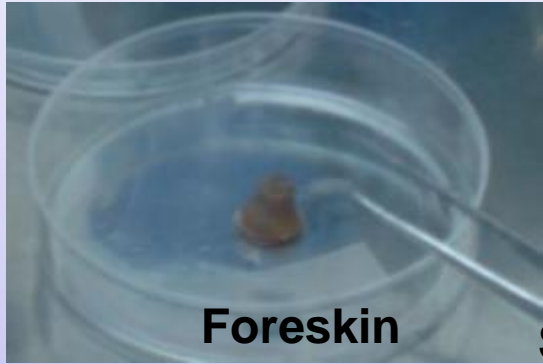
## **VII - Para avaliação de genotoxicidade:**

Método OECD TG 487 - Teste do Micronúcleo em Célula de Mamífero in vitro.

# **ALL MODELS HAVE LIMITATIONS NO MODEL CAN POSSIBLY EXPLAIN EVERY DETAIL OF A SCIENTIFIC PHENOMENA**

- **drug properties, absorption, distribution, metabolism, elimination and toxicity, are properties crucial to the final clinical success of a drug candidate.**
- **It has been estimated that nearly 50% of drugs fail because of unacceptable efficacy, which includes poor bioavailability as a result of ineffective intestinal absorption and undesirable metabolic stability.**
- **It has also been estimated that up to 40% of drug candidates have failed in the past because of safety issues.**
- **Species differences between animals and humans can cause fundamental confounders such as metabolic processes, enzymes, and membrane proteins.**
- **in vitro human cell-based drug evaluations, including drug efficacy testing, toxicology, and basic cell biology, are of great importance as an alternative to animal experiments to solve the significant issue of species differences.**

# (3R) RECYCLING SKIN



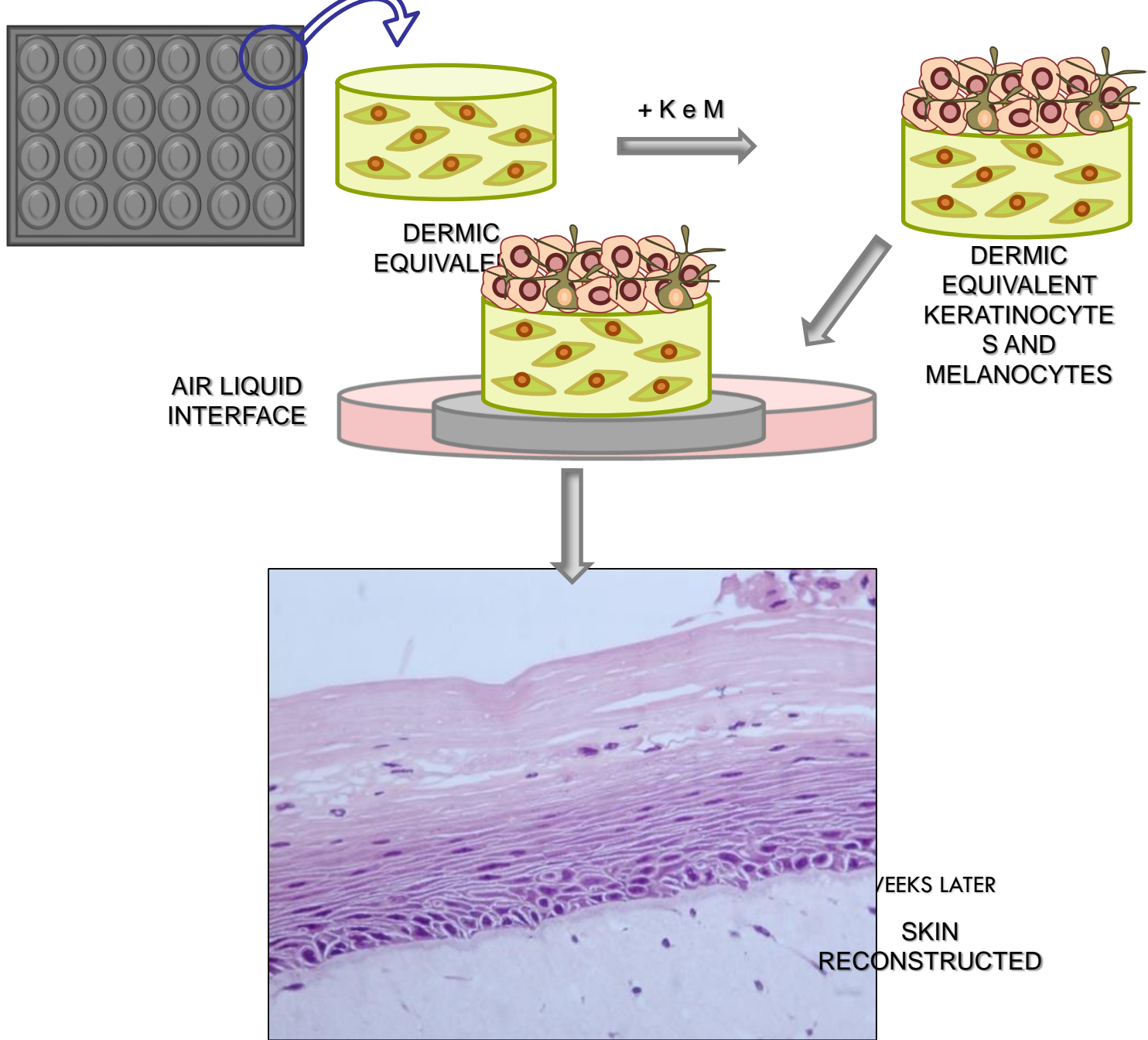
ALTERNATIVE METHOD  
FOR SCREENING OF:

- NEW COMPOUNDS BASED ON BRAZILIAN FLORA
  - PLANT EXTRATS
- NEW SYNTHETIC MOLECULES/ COMPOUNDS

PHARMACOLOGICAL POTENTIAL AND  
CLINICAL APPLICATIONS IN SKIN

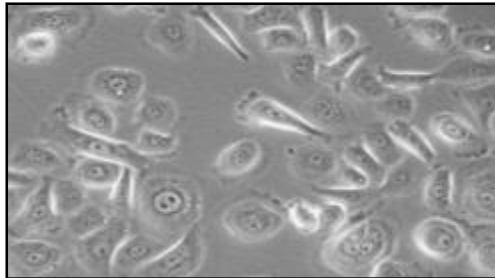
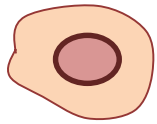
- ANTI-TUMORAL (melanoma, cervical cancer)
- SKIN DISORDERS (psoriasis)

COSMETICS INDUSTRY ( BRAZIL AS A  
HUGE MARKET)

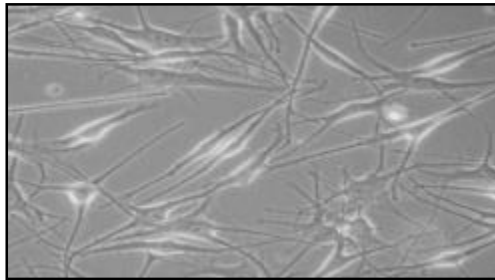
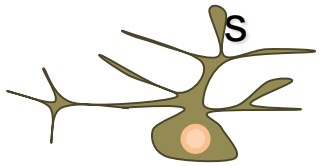


# FULL-THICKNESS SKIN MODEL AND MELANOMA INVASION IN FULL THICKNESS

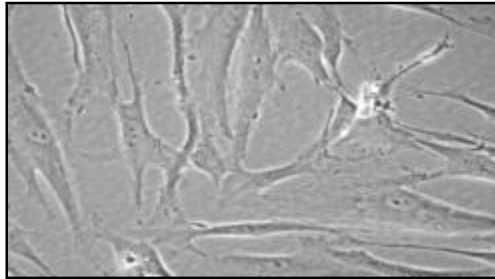
Keratinocytes



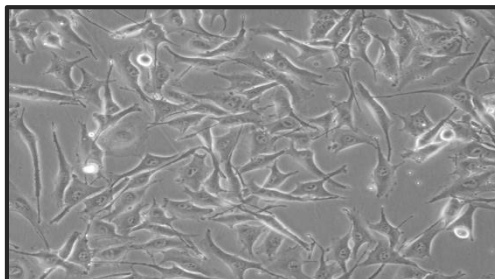
Melanocyte



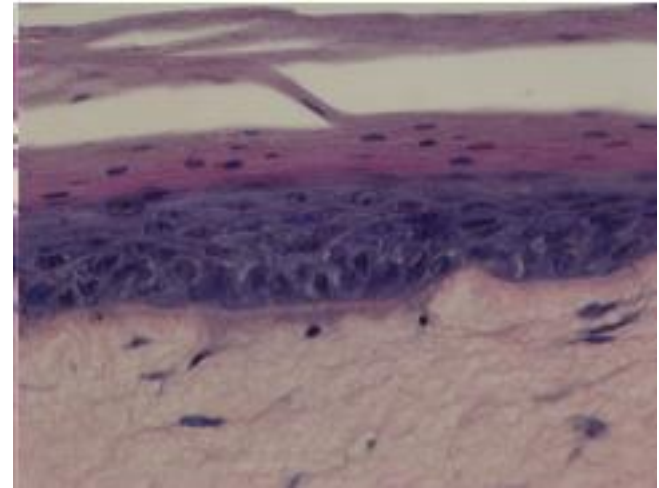
Fibroblasts



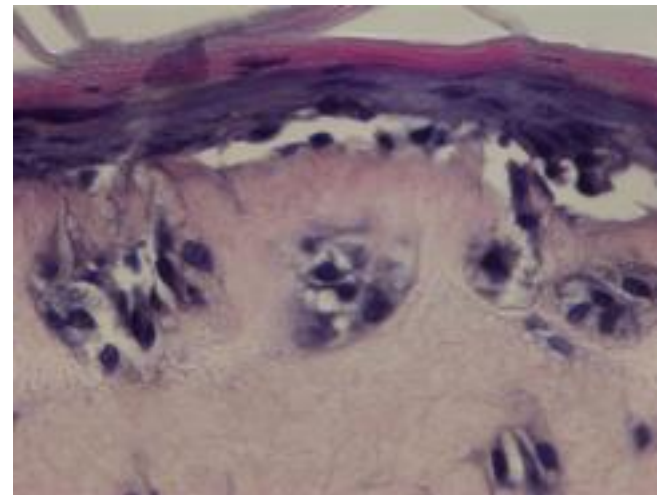
Melanoma



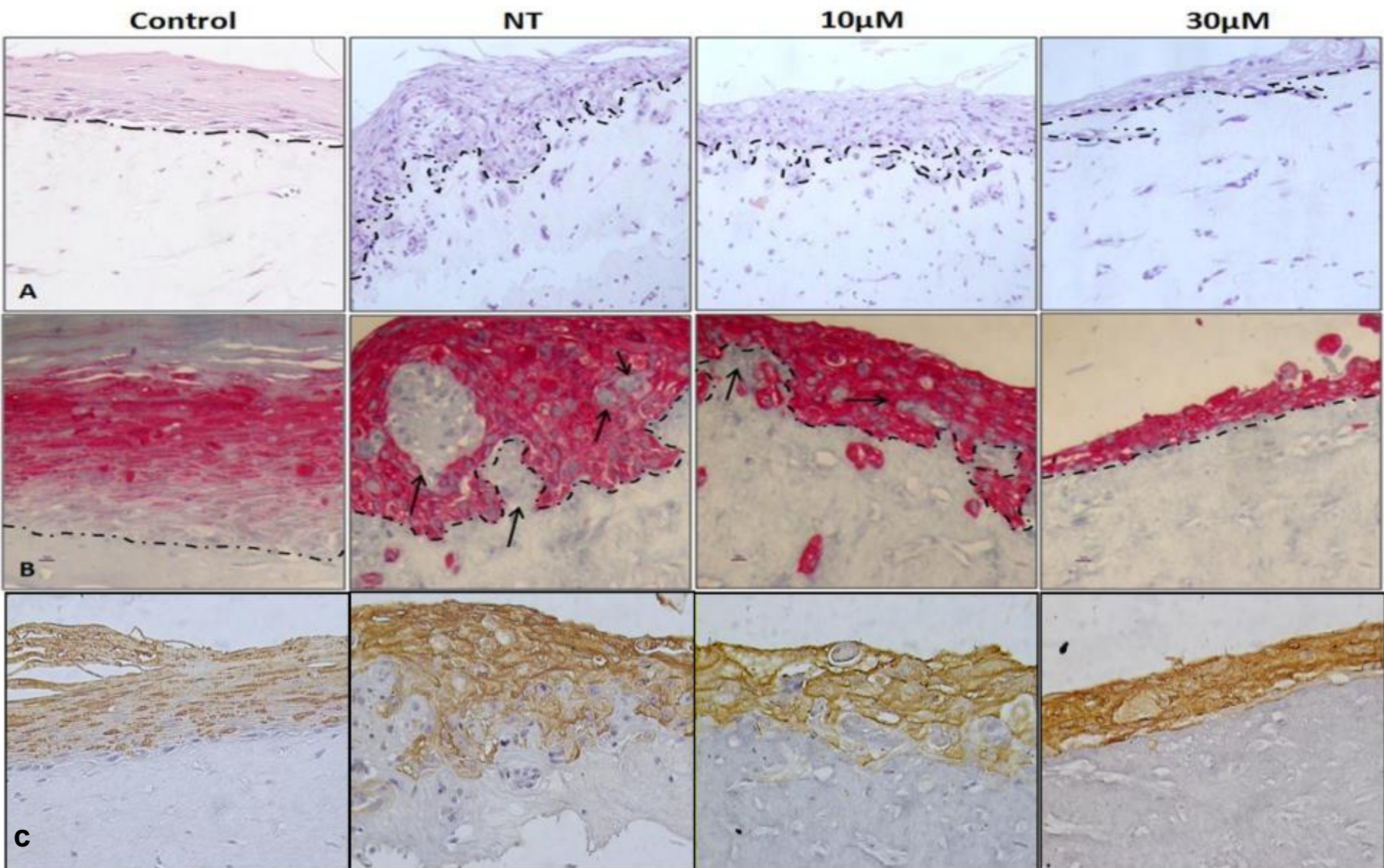
Reconstructed Skin



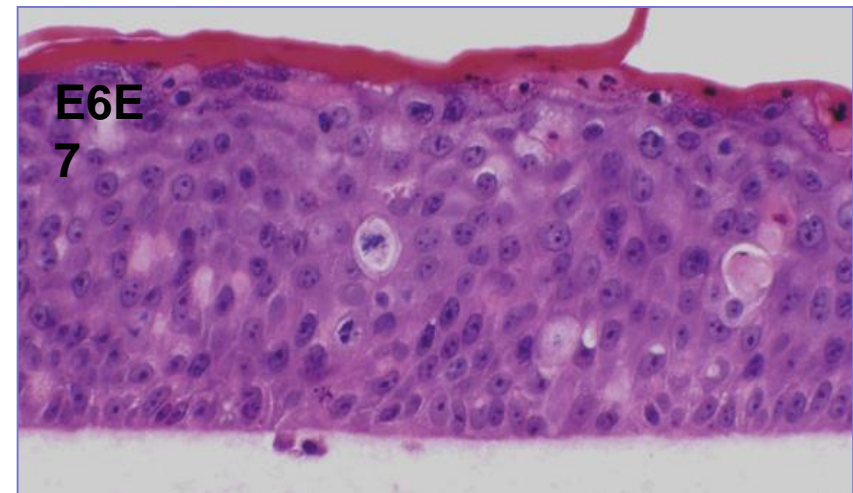
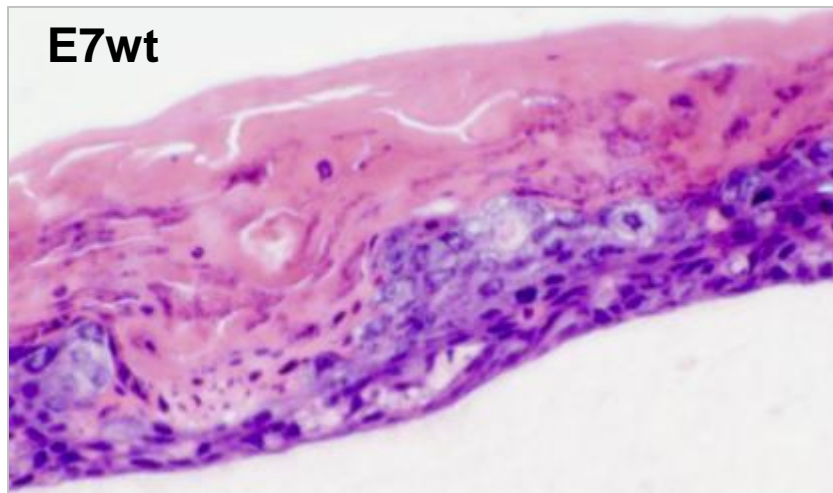
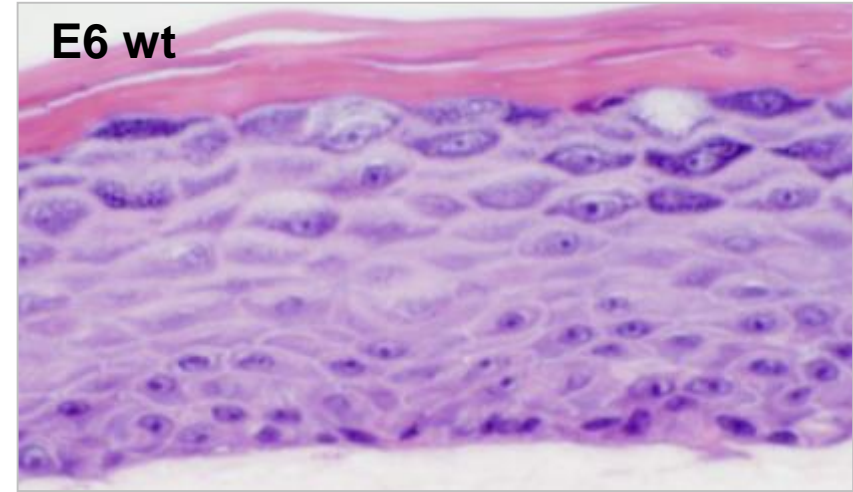
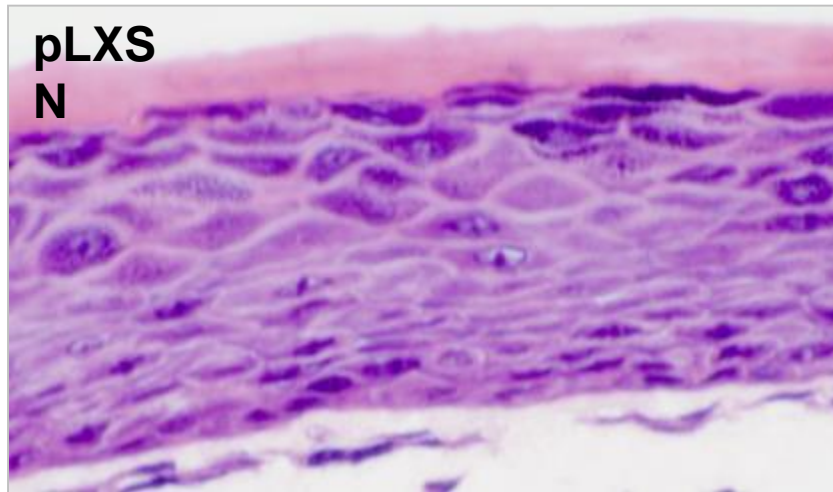
Melanoma In Reconstructed Skin



# ORGANOTYPIC CELL CULTURE MODELS PROVIDE ESSENTIAL CONTEXT-DEPENDENT INFORMATION CRITICAL FOR THE DEVELOPMENT OF NEW THERAPEUTIC STRATEGIES

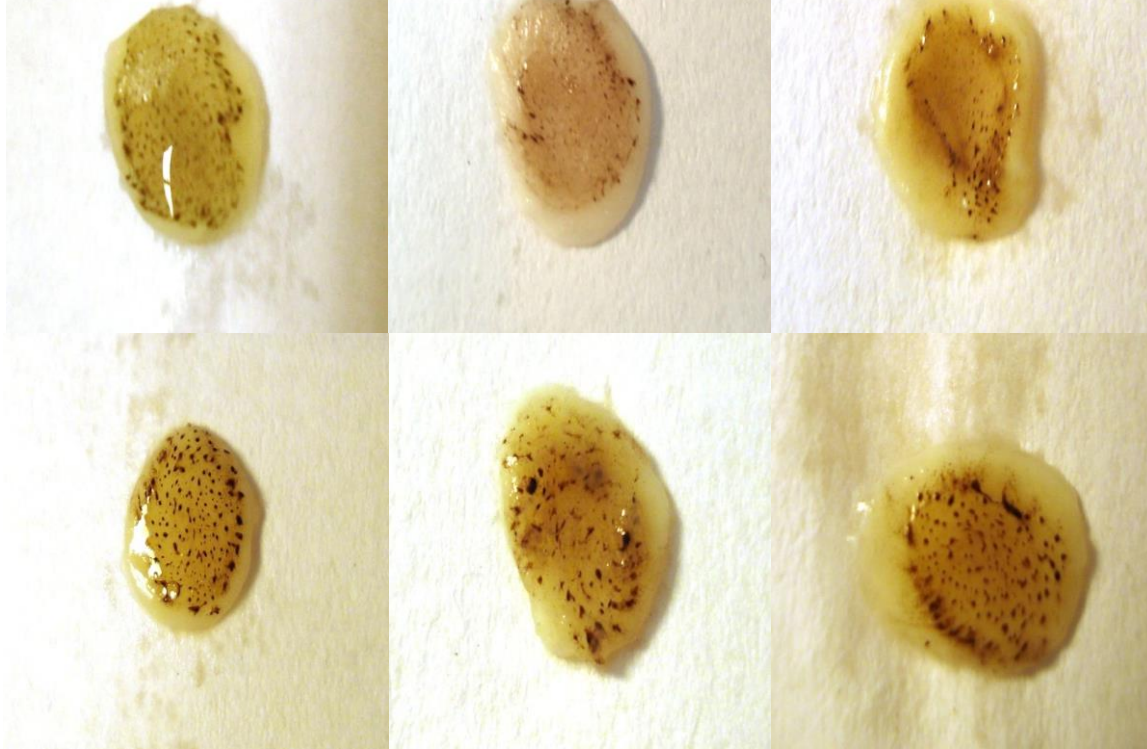


# E6 E7 HPV16 ONCOPROTEINS IN ORGANOTYPICAL CULTURE: CERVICAL CANCER IN VITRO



# Skin reconstructs: a tool for pathophysiological models

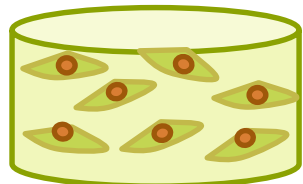
Non= treated



Glicated

- **PIGMENTED SKIN RECONSTRUCTED**
- **PHOTOPROTECTION AND PHOTOAGING MODEL (UV EFFECT)**





DERMAL  
EQUIVALENT:  
COLLAGEN+  
FIBROBLASTS

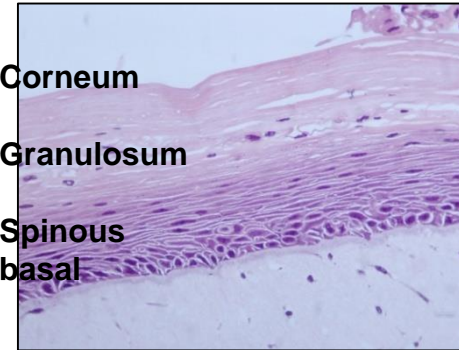


KERATINOCYTES AND  
MELANOCYTES  
DERMAL EQUIVALENT

AIR LIQUID  
INTERFACE



2 WEEKS LATER



Corneum

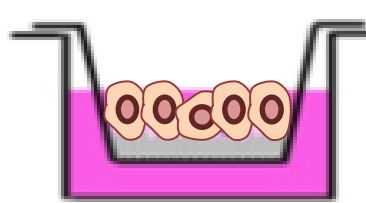
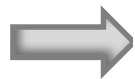
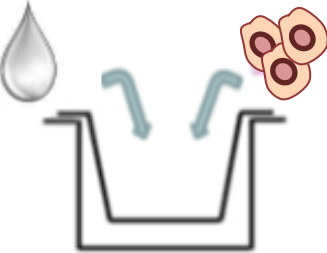
Granulosum

Spinous  
basal

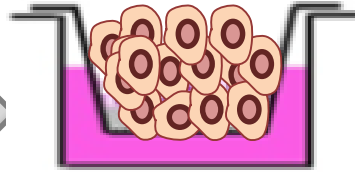
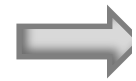
SKIN  
RECONSTRUCTED

Collagen  
IV film

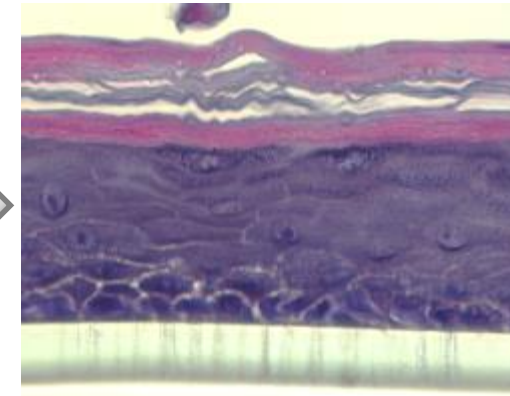
KCs



1 DAY IN  
MEDIUM

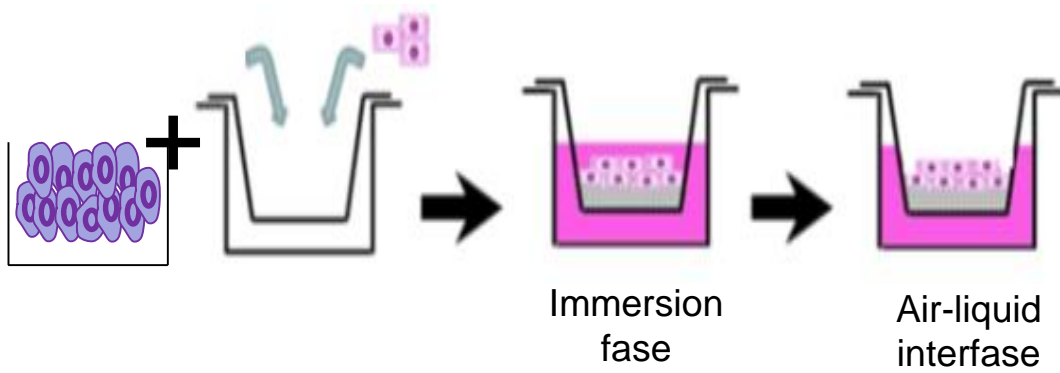


12 DAYS IN  
AIR-LIQUID  
INTERFACE

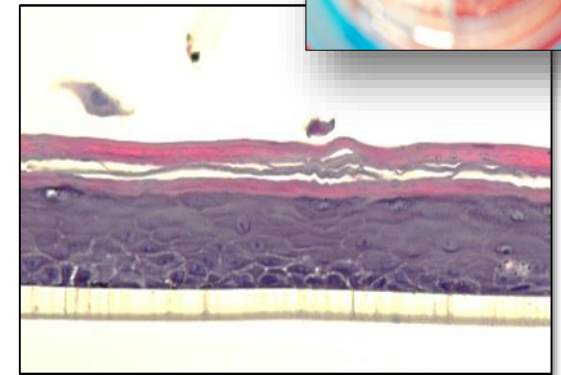
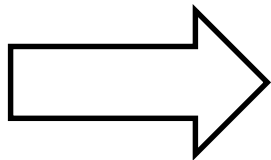


EPIDERMAL  
EQUIVALENTS

# DEVELOPMENT OF EPIDERMAL EQUIVALENT



Deep Well



# Artificial Skin In Pharmaceutical/Cosmetic productive chain in Brazil

Systematize the protocol of generation of the epidermal equivalent on *transwell* focused on future evaluation of safety and efficacy tests;

Investigate the functionality of the model evaluating corrosive and non-corrosive substances via MTT, according to the Guide 431 - OECD (“Organization for Economic Co-operation and Development”);

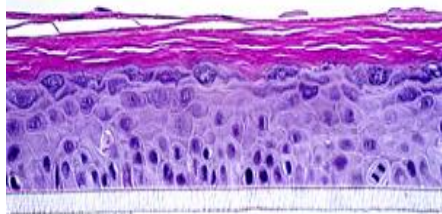
Brazil is one of the largest consumer markets of cosmetic in the world.

In Brazil, the use of artificial skins as alternative methods for cosmetics testing is still lacking;

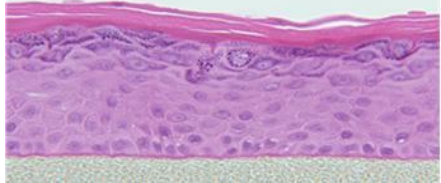
The importation of commercial kits of artificial skin is unfeasible due to customs processing issues;

**OECD Recommends epidermis models**  
**RENAMA (BRAZILIAN NETWORK FOR ALTERNATIVE METHODS) MCTI/  
CNPQ 2013**  
**AIM: SKIN RECONSTRUCTS (EPIDERMAL LAYERS)**

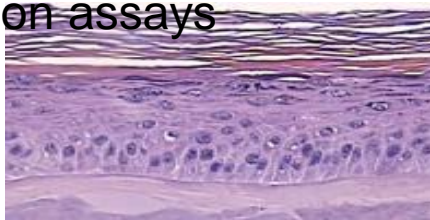
**Irritation and Corrosion assays**



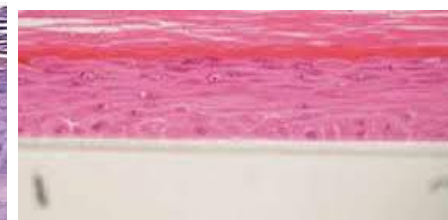
SkinEthic™ RHE (L'oreal)



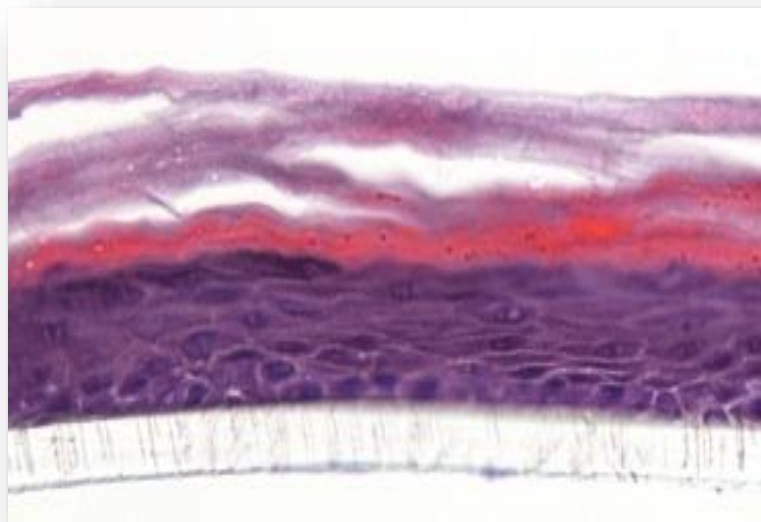
EpiDerm™ Tissue Model (MatTek)



EpiSkin model

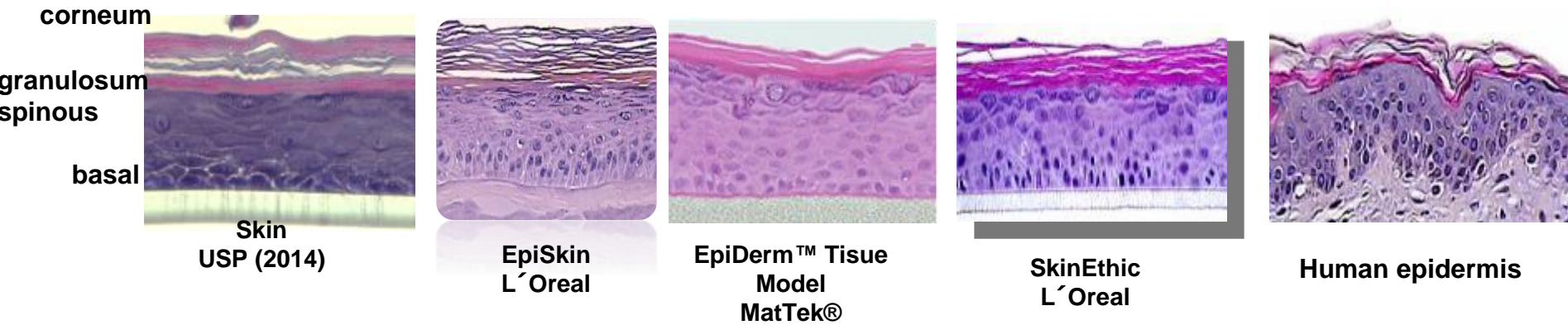


LabCyte EPI-MODEL



**in house epidermal equivalente**

- **Epidermal Equivalent in vitro**  
VMR= validated methods references

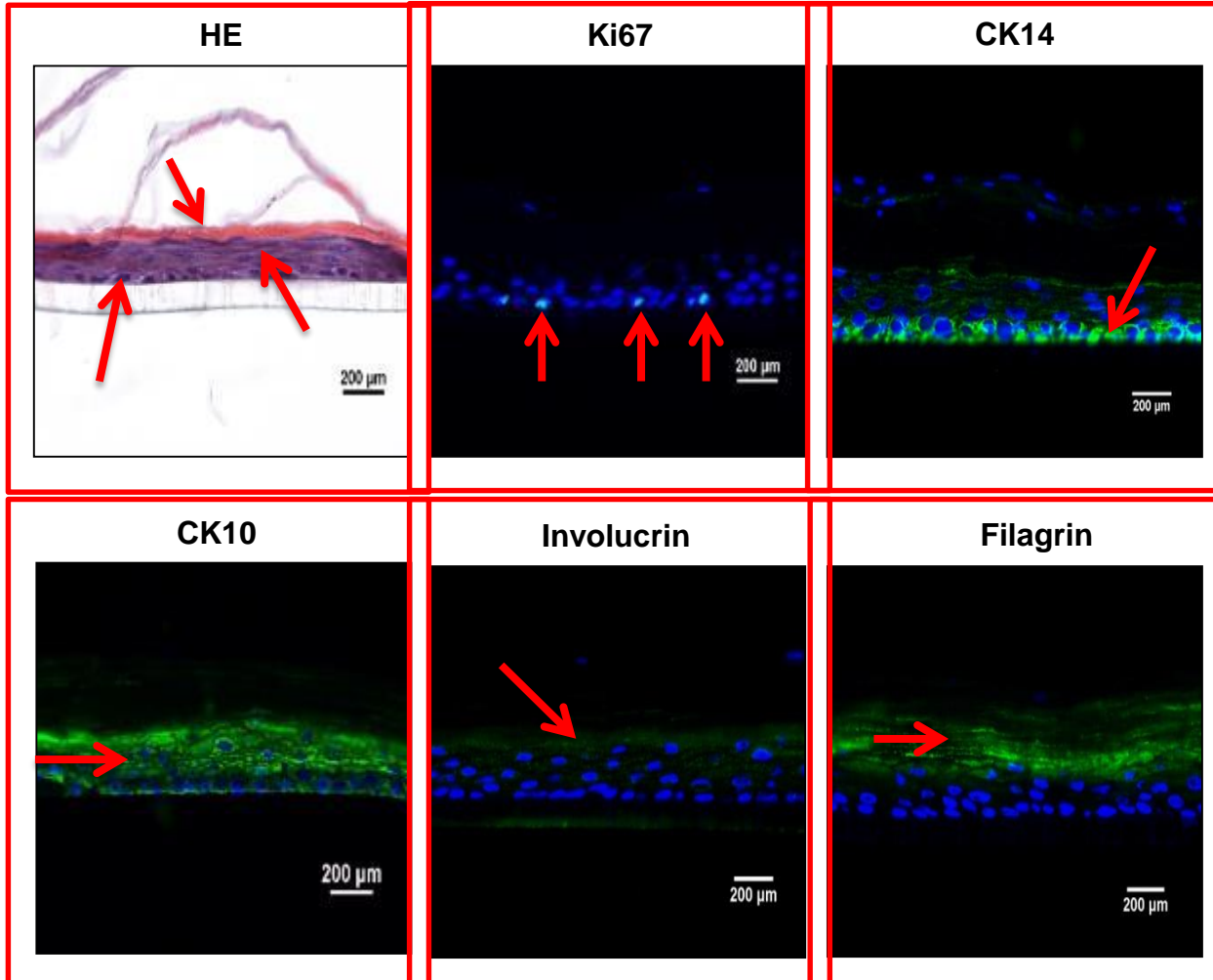


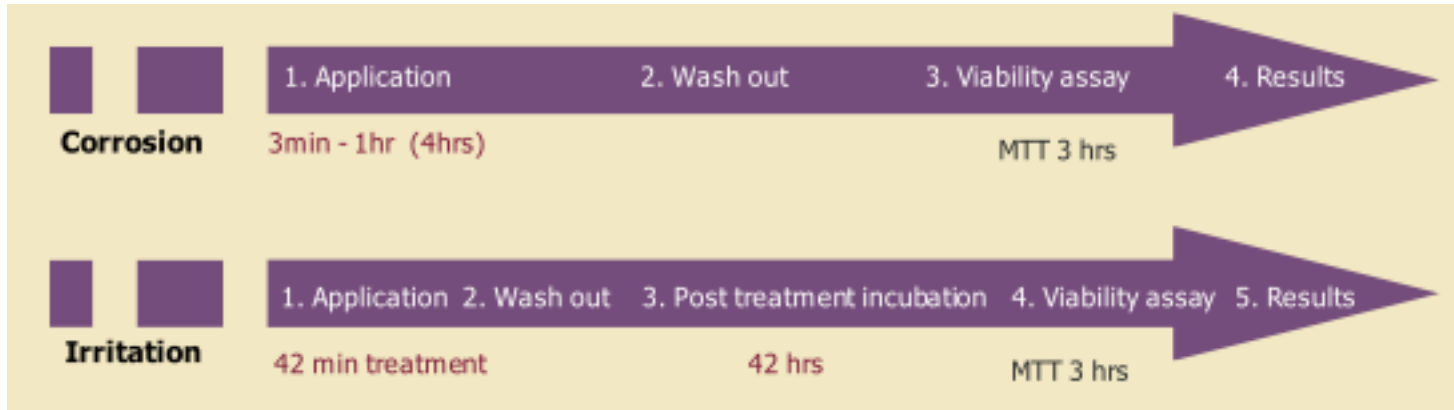
- **Draize Test**



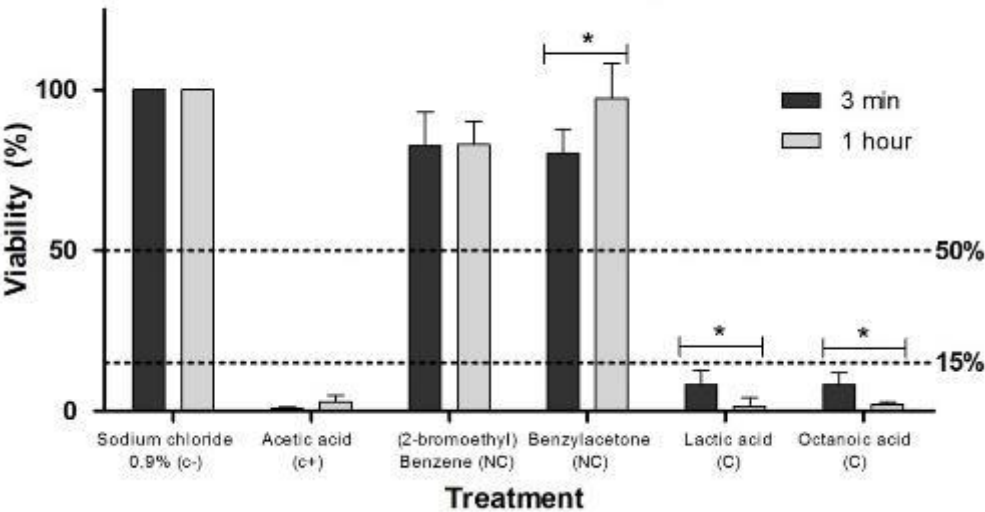
# Validation of an in house Epidermis Reconstructs (ER)

**Standardizing the protocol: ER: Morphological analysis → HE staining and Protein expression → imunofluorescence**

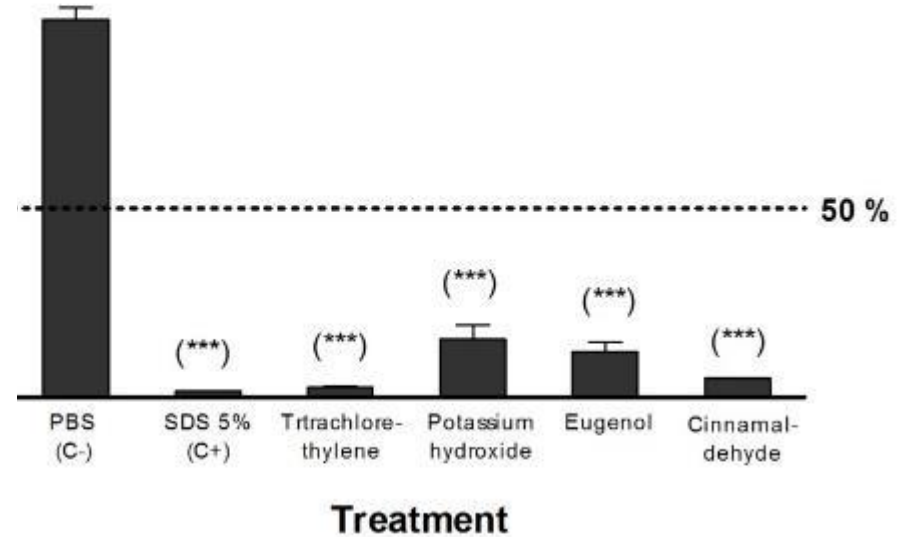




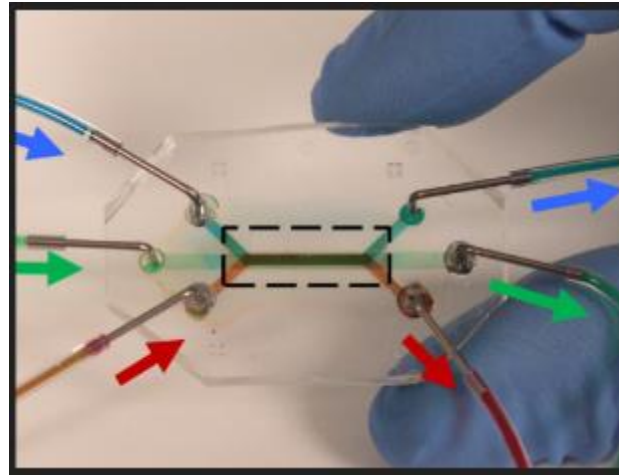
Corrosion Test - Epidermal Equivalent



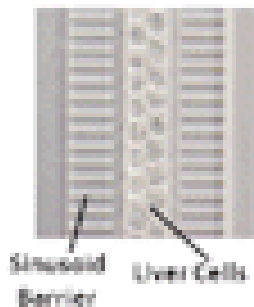
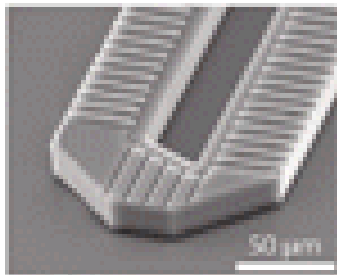
Irritation Test - Epidermal Equivalent



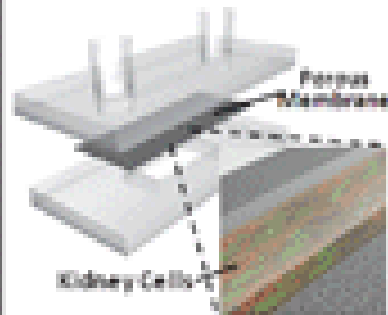
# MICROFLUIDIC SYSTEMS, ORGAN AND BODY ON A CHIP



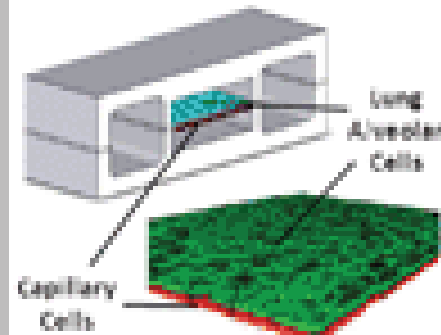
**Liver Chip**



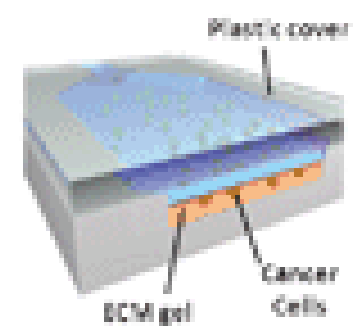
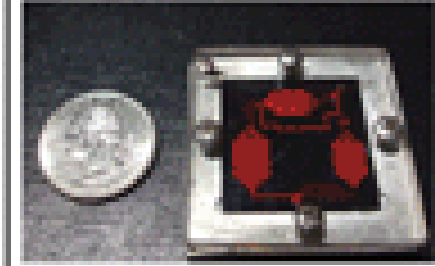
**Kidney Chip**



**Lung Chip**

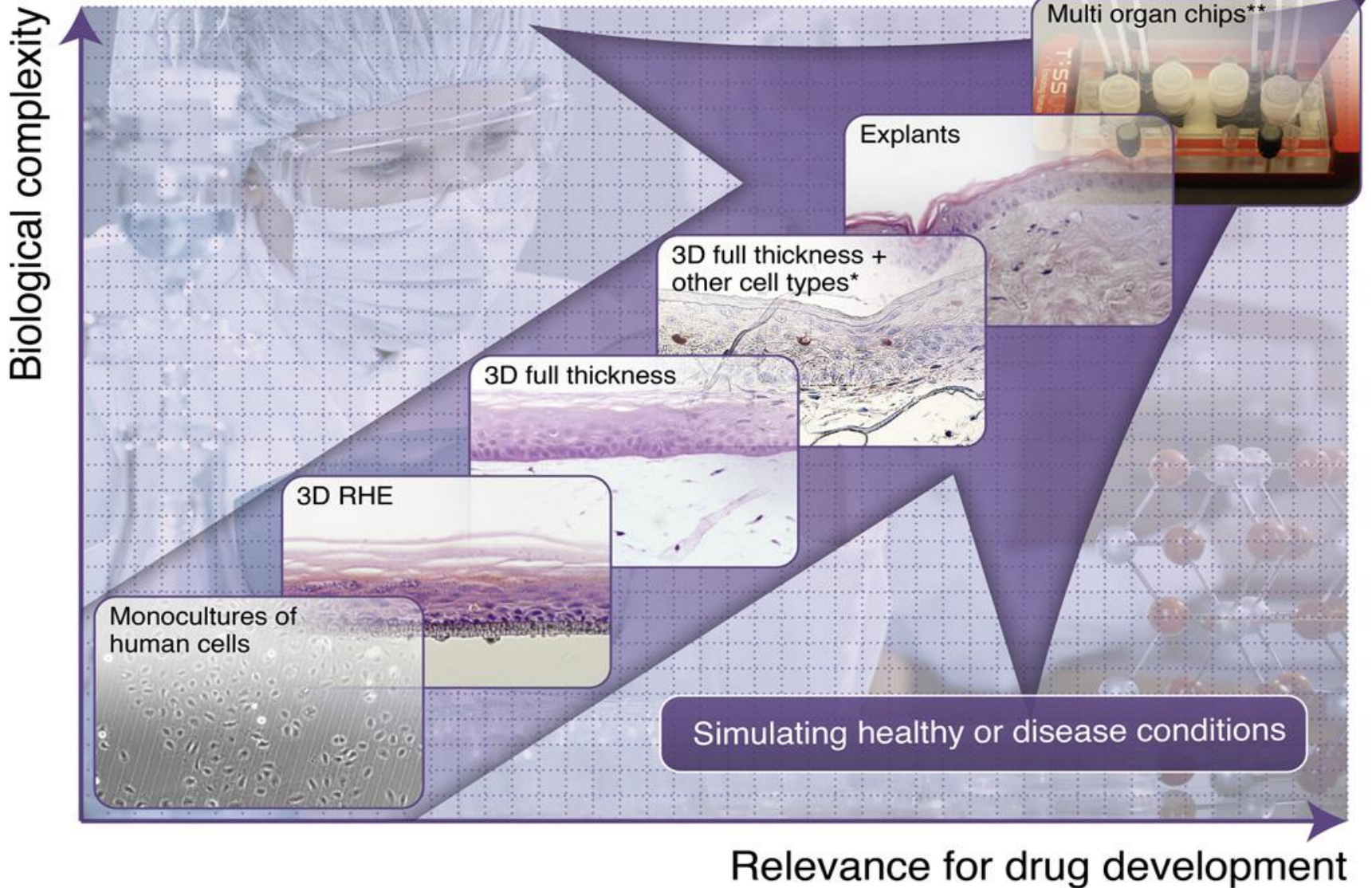


**Body Chip**





# IN VITRO METHODS 2D X 3D AS ALTERNATIVE TO ANIMAL TESTING



Relevance for drug development

# SKIN BIOLOGY GROUP -FCF -USP



Collaborators:

Silvia Berlanga M. Barros, USP  
Lorena R. G. Cordeiro, USP-RP  
Daniele Palma, USP-RP  
Luisa Villa, USP  
Enrique Boccardo, USP  
Luciana B. Lopes, USP  
Paulo Zaini, IQ-USP  
Márcia Consolaro, UEM, PR  
Carmen Ferreira, UNICAMP

K. Smalley, Moffitt, USA



Carolina Catarino  
PhD Student



Paula Pennacchi  
PhD



Tatiana Pedrosa  
PhD Student



Thalita Zanoni,  
PhD

